Assessment of the bacterial community structure in shallow and deep sediments of the Perdido Fold Belt region in the Gulf of Mexico

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The Mexican region of the Perdido Fold Belt (PFB), in northwestern Gulf of Mexico (GoM), is a geological province with important oil reservoirs that will be subjected to forthcoming oil exploration and extraction activities. To date, little is known about the native microbial communities of this region, and how these change relative to water depth. In this study we assessed the bacterial community structure of surficial sediments by high-throughput sequencing of the 16S rRNA gene at 11 sites in the PFB, along a water column depth gradient from 20 to 3700 m, including 5 shallow (20-600 m) and 6 deep (2800-3700 m) samples. The results indicated that OTUs richness and diversity were higher for shallow sites (OTUs = 2888.2 ± 567.88; $H' = 9.6 ± 0.85$) than for deep sites (OTUs = 1884.7 ± 464.2; $H' = 7.74 ± 1.02$). Nonmetric multidimensional scaling (NMDS) ordination revealed that shallow microbial communities grouped separately from deep samples. Additionally, the shallow sites plotted further from each other on the NMDS whereas samples from the deeper sites (abyssal plains) plotted much more closely to each other. These differences were related to depth, redox potential, sulfur concentration, and grain size (lime and clay), based on the environmental variables fitted with the axis of the NMDS ordination. In addition, differential abundance analysis identified 147 OTUs with significant fold changes among the zones (107 from shallow and 40 from deep sites), which constituted 10 to 40% of the total relative abundances of the microbial communities. The most abundant OTUs with significant fold changes in shallow samples corresponded to Kordiimonadales, Rhodospirillales, Desulfbacterales (Desulfococcus), Syntrophobacterales and Nitrospirales (GOUTA 19, BD2-6, LCP-6), whilst Chromatiales, Oceanospirillales (Amphritea, Alcanivorax), Methylococcales, Flavobacteriales, Alteromonadales (Shewanella, ZD0117)
and *Rhodobacterales* were the better represented taxa in deep samples. Several of the OTUs detected in both deep and shallow sites have been previously related to hydrocarbons consumption. Thus, this metabolism seems to be well represented in the studied sites, and it could abate future hydrocarbon contamination in this ecosystem. The results presented herein, along with biological and physiochemical data, constitute an available reference for further monitoring of the bacterial communities in this economically important region in the GoM.
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Abstract

The Mexican region of the Perdido Fold Belt (PFB), in northwestern Gulf of Mexico (GoM), is a geological province with important oil reservoirs that will be subjected to forthcoming oil exploration and extraction activities. To date, little is known about the native microbial communities of this region, and how these change relative to water depth. In this study we assessed the bacterial community structure of surficial sediments by high-throughput sequencing of the 16S rRNA gene at 11 sites in the PFB, along a water column depth gradient from 20 to 3700 m, including 5 shallow (20-600 m) and 6 deep (2800-3700 m) samples. The results indicated that OTUs richness and diversity were higher for shallow sites (OTUs = 2888.2 ± 567.88; $H' = 9.6 ± 0.85$) than for deep sites (OTUs = 1884.7 ± 464.2; $H' = 7.74 ± 1.02$). Nonmetric multidimensional scaling (NMDS) ordination revealed that shallow microbial communities grouped separately from deep samples. Additionally, the shallow sites plotted
further from each other on the NMDS whereas samples from the deeper sites (abyssal plains) plotted much more closely to each other. These differences were related to depth, redox potential, sulfur concentration, and grain size (lime and clay), based on the environmental variables fitted with the axis of the NMDS ordination. In addition, differential abundance analysis identified 147 OTUs with significant fold changes among the zones (107 from shallow and 40 from deep sites), which constituted 10 to 40 % of the total relative abundances of the microbial communities. The most abundant OTUs with significant fold changes in shallow samples corresponded to Kordiimonadales, Rhodospirillales, Desulfobacterales (Desulfococcus), Syntrophobacterales and Nitrospirales (GOUTA 19, BD2-6, LCP-6), whilst Chromatiales, Oceanospirillales (Amphritea, Alcanivorax), Methylococcales, Flavobacteriales, Alteromonadales (Shewanella, ZD0117) and Rhodobacterales were the better represented taxa in deep samples. Several of the OTUs detected in both deep and shallow sites have been previously related to hydrocarbons consumption. Thus, this metabolism seems to be well represented in the studied sites, and it could abate future hydrocarbon contamination in this ecosystem. The results presented herein, along with biological and physicochemical data, constitute an available reference for further monitoring of the bacterial communities in this economically important region in the GoM.

Introduction

Microorganisms are well recognized as key drivers of biogeochemical cycles in marine environments (Webster et al. 2003; Santos et al. 2011). Measuring the changes in microbial communities is of particular interest to understanding how environmental factors modulate their structure and how that, in turn, is related to the function and stability of the ecosystem (Huber et
In marine sediments, the type of substrate, energy and carbon sources, and the variables that are correlated with water depth (e.g., temperature, oxygen concentration, light penetration and hydrostatic pressure), generally describe the most important aspects of the variability amongst habitats (Miller et al. 2012).

In the Gulf of Mexico (GoM), changes in bacterial community structure have been mainly related with depth in the water column, and likely result from differences in temperature, dissolved oxygen and suspended particles (King et al. 2013) which occur across these depth differences. In marine sediments from the GoM, bacterial community composition has been determined at different depths below the seafloor (from sediment cores). It has been proposed that the bacterial community composition of these sediments likely results from the interaction between the water column and a benthic microbial population limited to the upper layer of the sediments (Reese et al. 2013). In contrast, the microbial diversity present in different sediment depths from seep systems in the GoM have been directly related to the composition and magnitude of hydrocarbon seepage (e.g., natural oils, methane, and non-methane hydrocarbons) (Orcutt et al. 2010), as well as to the presence of overlying microbial mats (Mills et al. 2004).

Knowledge about microbial communities in the GoM increased notably with the Deepwater Horizon (DWH) massive oil spill in 2010, which occurred in the north zone of the GoM across an enormous area with different environmental conditions (Kostka et al 2011; Mason et al. 2012; Liu and Liu 2013; Joye et al. 2014). During the spill, it was observed that different bacterial phyla in the deep-water plume (e.g., Oceanospirillales, Cycloclasticus and Colwellia) rapidly responded and were enriched within hours - weeks following the DWH well blow out (Redmond and Valentine 2011; Mason et al. 2012). In natural hydrocarbon seep sites sampled at the same
time, several rare taxa increased rapidly in abundance after the spill, emphasizing the importance
of specialized sub-populations and potential ecotypes during massive deep-sea oil discharges
(Kleindienst et al. 2015). The oil transported to the shoreline after the discharge also had a
profound impact on the abundance and community composition of indigenous bacteria in beach
sands, where members of the *Gammaproteobacteria* and *Alphaproteobacteria* participated as
key players in oil degradation (Kostka et al. 2011).

The precise volume of oil spilled and the trajectory of the oil slicks from the DHW are still
controversial (Salcedo et al. 2017). Based on the surface circulation models of the waters of the
GoM, it was inferred that the Mexican region of the Perdido Fold Belt (PFB), was also
susceptible to this environmental disturbance (Soto and Vázquez-Botello 2013). Knowledge of
environmental and biotic data of this region is, however, scarce despite being a geologic
province with oil reservoirs. The present study assessed changes in the bacterial community
structure of surficial sediments with respect to a depth gradient and specific environmental
variables in the PFB region, where oil exploration and extraction activities are predicted to
impact environmental conditions, and consequently the structure of bacterial communities.
Additionally, bacterial phylotypes putatively involved in hydrocarbon degradation were
highlighted in this work.

**Material and Methods**

**Sample collection and physicochemical variables from marine sediments**

The samples used in this study were collected in the Northwestern Gulf of Mexico in April 2014.
Sediment samples from a depth gradient (20-3700 m), were collected perpendicular to the
coastline at 11 sites on the parallels 25 and 25.15°N (Fig. 1, Table 1). Samples were collected with a Hessler-Sandia MK-II boxcore (40 x 40 cm) from which three different surficial (0 - 5 cm) subsamples were taken: 1) sterile 100 mL plastic containers immediately frozen at -20°C on board for further molecular analysis; 2) a 2 inch core extracted to determine total sulfur concentration (TS) and redox potential on board, with a sulfide ion selective electrode and potentiometer (Bricker 1982; Brassard 1997); and 3) approximately 400 g of sediment stored in high density polyethylene bags at 4°C until the determination of total organic matter (TOM), total organic carbon (TOC), and grain size in the laboratory. TOM was determined by the wet oxidation technique with an excess of dichromate and back titration with iron (II) (Buchanan, 1984). TOC content was quantified using the oxidation with potassium dichromate in acid medium, and titration of excess oxidant with ferrous sulfate and diphenylamine as an indicator (Buchanan 1984). The amounts of sand, lime and clay were estimated using a hydrometer (Buchanan 1984). These physicochemical variables were compared among shallow and deep sites using a one-way analysis of variance (ANOVA $p<0.05$ and $F$-values) and were correlated with the depth variable.

**DNA extraction**

Sediment samples were stored during five months at -20°C. Then, in October 2014 sediments were thawed at 4°C, homogenized mechanically under sterile conditions, and centrifuged 1 min/10,000 x g in order to separate the sediment from remaining water, which was discarded. Total DNA was extracted from 0.25 g of sediment using the PowerSoil-DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) following the manufacturer’s protocol. The quality of the DNA extractions was verified by agarose gel electrophoresis and the concentration was determined through UV absorption analysis with a NanoDrop 2000 Spectrometer (ThermoFisher
Scientific Inc., Wilmington, DE, USA). Extracted DNA was stored at -20°C for further 16S rRNA Illumina sequencing and DGGE analysis (see a detailed description in supplementary methods).

**16S rRNA gene sequencing**

Amplicons from environmental DNA were prepared for sequencing the 16S rRNA V3 and V4 variable regions by using a two PCR steps approach following the “16S Metagenomic Sequencing Library Preparation” protocol (Illumina). Briefly, the first PCR step amplified the template out of the DNA samples using the forward primer 5’-CCTACGGGNGGCWGCAG-3’ and the reverse primer 5’-GACTACHVGGGTATCTAATCC-3’ with Illumina overhang adapters attached, to obtain ~550 bp fragments (Klindworth et al. 2013). The PCR program was performed in a thermal cycler (Applied Biosystems Veriti ABI Inc., Foster City, CA, USA) with an initial denaturation at 95°C - 3 min, 25 cycles of 95°C - 30 s, 55°C - 30 s, 72°C - 30 s and a final extension at 72°C - 5 min. Each PCR reaction (20 µl) included 2 µl of environmental DNA (5 ng/µl), 0.5 µl of each primer (10 µM) and 10 µl of 2x Phusion High-Fidelity MasterMix (Thermo Scientific, Waltham, MA, USA). The correct size of the amplicons was verified on a QIAxcel Advanced system (QIAGEN, Hilden, Germany), DNA and the PCR clean-up were carried out using AMPure XP beads to discard free primers and primer dimer species. In the second PCR, eight cycles attached dual indices and the Illumina sequencing adapters using the Nextera XT Index Kit. PCR barcoded amplicons were verified and purified as previously described and quantified using a Qubit 3.0 fluorometer (Life Technology, Shah Alam, Selangor, Malaysia). The individual barcoded amplicons were diluted on 10 mM Tris (pH 8.5) and pooled in equimolar concentrations (9 pM). Paired-end sequencing (2x300 bp) was carried out using the
MiSeq platform (Illumina, San Diego, CA, USA) with a MiSeq Reagent Kit V3 (600 cycles). Sequencing was performed in the Aquatic Pathology laboratory at CINVESTAV-Mérida.

**Data analysis**

Demultiplexing of the pooled amplicons and trimming the barcode and adapter sequences was performed using the MiSeq Reporter Metagenomics Workflow (Illumina, 2014). Reads were overlapped with the fast length adjustment of short reads to improve genome assemblies (FLASH) software (Magoč and Salzberg, 2011), and processed using the QIIME (version 1.9) pipeline with the parameters q (phred_quality_treshold)=20, r (max_bad_run_length,)=3, p (min_per_read_length_fraction)=0.75, for quality filtering. The demultiplexed sequences were clustered in operational taxonomic units (OTUs) with the `pick_open_reference_otus.py` script at 97% of similarity using the usearch61 method (Edgar 2010) with a minimum OTU cluster size of 5. Chimeric sequences were removed with the `uchime2` algorithm in the reference mode (v 9.1.13) (Edgar 2016) and, the taxonomic assignment was performed by the SortMeRna (Kopylova et al. 2012) with an e-value of $3 \times 10^{-6}$ and default parameters from QIIME, using the GreenGenes database (v13.8). An OTUs alignment was performed with the Mafft algorithm (Katoh and Standley 2013) to build a phylogenetic tree using the Fasttree software (Price et al. 2009) for its subsequent use in the UniFrac (Lozupone, Hamady and Knight, 2006) distance analysis.

Community structure and composition analyses were performed by processing the OTU table in the R environment (R Core Team, 2014) with the Phyloseq (McMurdie and Holmes 2013), vegan (Oksanen et al. 2013) and ggplot2 (Wickham, 2010) packages. The data set was rarefied at the depth from the smallest library (20,400). We reported the observed OTUs, the Shannon and
Simpson diversity indexes ($H'$ and $D$, respectively) and the nonparametric richness from \textit{Chao1}.

The Good’s coverage was calculated to corroborate the adequate sampling depth.

In order to compare the microbial community composition among shallow and deep sampling sites, a non-metric multidimensional scaling (NMDS) was plotted (Lozupone et al. 2006; Giloteaux et al. 2010) with the weighted UniFrac distance metric (Lozupone, Hamady and Knight, 2006) and a test of beta significance among groups of sample was performed using a two-sided Student's two-sample t-test with the ‘make\_distance\_boxplots.py’ script of QIIME, $p$-values were calculated using 1000 Monte Carlo permutations. The physicochemical variables were fitted with the \textit{envfit} function to the NMDS ordination to correlate them with the community composition ($p$-value < 0.05 and 10 000 permutations). Physicochemical variables were also tested with a PERMANOVA analysis at a $p$-value <0.05 (Table S1). A differential abundance analysis was performed with the DESeq2 (Love et al, 2014) R library to identify the OTUs that have significant fold changes among the shallow and deep zones. The $p$-value was corrected with the false discovery rate (FDR) method (Benjamini and Hochberg, 1995) and a Log2 fold change plot was made with the significant OTUs at a $p$-value < 0.01.

All the sequences are available at SRA site from NCBI database in the BioProject PRJNA429278 and biosample accession ID’s SRR6457706 to SRR6457716 and the raw processed data in the supplementary material.

**Results**

**Physicochemical properties from marine sediments**
The physicochemical variables and textures of the sediment samples are shown in Table 1. Redox potential ranged from -204 to 236 mV. The electronegative values were found in samples from 20 to 600 m depth, while the electropositive values corresponded to the deep sediments (2800 - 3700 m). TS was constant at depths from 20 to 600 m at a concentration of 0.05 µM, while the maximum values were detected for deep sites (0.10 - 0.12 µM). The percentage of TOM and the concentration of TOC varied for all sites with averages of 0.872 ± 0.22% and 0.484 ± 0.123 µM, respectively. The percentages of sand, lime and clay in the sediment samples ranged from 20.50 - 34.5, 51.2 - 70.30 and 5.9 - 28.4%, respectively. Values of TS, redox potential, and the percentages of lime and clay showed significant correlations with depth ($p$-value <0.01) (Table 1).

**Bacterial community structure in a depth gradient**

The NMDS ordination method showed that microbial communities from shallow sites grouped separately from those from the deep sites (Fig. 2), and it was according with the test of significance were the mean of the distances of Shallow and Deep samples were different with a $p$-value < 0.05. Samples taken from shallow sites displayed greater dissimilarity distance from each other, while those derived from the deep sites displayed a closer proximity to each other (Fig. 2). The environmental variables fitted on the NMDS ordination were: depth ($r$= 0.79), redox potential ($r$= 0.77), the concentration of total sulfur ($r$= 0.73) and percentages of clay ($r$= 0.71) and lime ($r$= 0.7), at a $p$-value <0.05.

The observed OTUs, Shannon ($H'$) and Simpson ($D$) diversity indexes, and the nonparametric richness estimation from *Chao1*, were different among shallow and deep-sea sites ($p$-value
These ecological estimators showed higher values in the sediment samples from shallow sites (observed OTUs = 2888.2 ± 567.88, $H' = 9.6 \pm 0.85$, $D = 0.99 \pm 0.005$ and $Chao1 = 3791.2 \pm 737.81$) than those obtained from the deep ocean floor (observed OTUs = 1884.7 ± 464.2, $H' = 7.74 \pm 1.02$, $D = 0.97 \pm 0.02$, and $Chao1 = 2806.18 \pm 589.39$) (Table 2).

**Bacterial community composition**

Microbial community analysis resulted in the detection of 25 bacterial phyla, however only 14 were $\geq$1% in relative abundance (Table S2). *Proteobacteria*, represented by *Gamma-, Alpha- and Deltaproteobacteria* classes, were dominant in all the analyzed samples (Fig. 3). Nevertheless, *Bacteroidetes, Acidobacteria, Chloroflexi, Nitrospirae, Planctomycetes, Gemmatimonadetes* and the *NC10* phyla were also well represented in the samples. Two archaeal phyla, corresponding to the *Euryarchaeota (Thermoplasmata class)* and *Crenarchaeota* including *Miscellaneous Crenarchaeota Group (MCG)* and *Thaumarchaeota* classes (based on the GreenGene database), were also detected in low abundances (0.3 to 1.2%) (Fig. 3). At lower taxonomic levels, 51 families were detected, from which *Piscirickettsiaceae, Rhodobacteraceae, Flavobacteriaceae, Syntrophobacteraceae, Thermovibrionaceae, Desulfobacteraceae, Colwelliaceae, Marinicellaceae, Alcanivoracaceae, Colwelliaceae*, and *Cenarchaeaceae* were among the families with higher relative abundances in the samples (Fig. S1A). Moreover, 23 genera were identified at $> 1\%$ of relative abundances (Fig S4). *Desulfococcus, Alcanivorax, Fulvivirga, Amphritea, BD2-6, Shewanella, ZD0117* and *Nitrospina* were the better represented in the samples (Fig S4).
Differential abundance analysis identified 147 OTUs with significant fold changes between the deep and shallow zones, 107 from shallow and 40 from deep sites (Fig 4A). From the shallow samples, most of these OTUs belonged to *Delta-*, *Alpha- and Gammaproteobacteria* (38, 24 and 18, respectively), while the rest of the OTUs were distributed in 13 classes of 10 phyla (Fig. 4A). In deep samples, all of the 40 OTUs belonged to *Gamma-, Alphaproteobacteria, Bacteroidetes* and *Nitrospirae* (27, 6, 6 and 1, respectively) (Fig 4A). The relative abundances of the 147 OTUs with significant fold changes constituted approximately 10 to 40% of the total microbial communities (Fig 4B). *Kordiimonadales, Rhodospirillales, Desulfobacterales, Syntrophobacterales* and *Nitrospirales*, were the orders with highest relative abundances for shallow samples whilst the orders *Chromatiales, Oceanospirillales, Methylococcales*, *Flavobacterales* and *Rhodobacterales* were the best represented orders for deep samples (Fig. 4B). At genus taxonomic level, differential abundance analysis allowed to identify *Marinicella, Alcanivorax, Shewanella* and *ZD0117* with significant fold changes for deep samples; and *Nitrosopumilus, GOUTA19, LCP-6, BD2-6, Amphritea, Desulfococcus* and *Mariprofundus* for shallow sites (Fig. S5).

**Discussion**

Physicochemical properties of sediment samples

Physicochemical properties (*e.g.*, temperature, pH, redox) in marine sediments commonly change according to water depth (Miller et al. 2012). In this study, lime percentage, redox potential and total sulfur were positively correlated to depth, while the clay particles percentages were negatively correlated with depth (Table 1). The electronegative redox values measured in
the first 5 cm of the sediments in all shallow sites (Table 1), suggested that oxygen was depleted by microbial respiration as common reported for this layer in shallow marine sediments (Aranda et al. 2015). Electronegative redox values in the shallow samples were likely enhanced at higher fine-grained clay content, since this diminishes the sediment permeability as reported elsewhere for sediments in the continental platform (Probandt et al. 2017). In contrast, the electropositive redox values detected in the first 5 cm of the sediments in all the deep sites (Table 1) suggested oxidizing conditions. This condition has also been observed at different latitudes in the GoM (Deming and Carpenter 2008) due to both the circulation of deep water masses carrying oxygen (Jochens and DiMarco 2008), and low rates of oxygen consumption (Rowe et al. 2008).

Total organic matter percentage (TOM%) was determined to be below 2% for all the sediments analyzed (Table 1). These low organic contents are similar to those reported in studies of two onshore-offshore transects in the Northwestern GoM (Goñi et al. 1998). Shallow sites likely receive inputs from terrestrial organic matter (Balsam and Beeson 2003), and yet these sediments presented similar values of TOM than those detected for deep sites (Table 1). Previous studies have reported high production and consumption rates of oxygen measured for shallow sediments in Northeastern GoM (Mills et al. 2008). TOM values determined in this study for shallow sites are in concordance with previous reports for the GoM (Balsam and Beeson 2003). We hypothesize that these values are related to the consequence of high rates of microbial metabolism in those same zones.”

Microbial Ecology assessment
The effect of storage temperature on microbial community structure has been previously studied, and it is well recognized that microbial metabolism could keep on going at -20 ºC, however the survival at these subzero temperatures requires several genetic and physiological strategies that exclusively cold-adapted microorganisms (permafrost and seasonally frozen soil microbial communities) have developed (Jansson and Tas 2014; Boetius et al 2015). In the present study, microbial communities from marine sediments living at temperatures ranging from 4 to 27 ºC were analyzed. Thus, we considered that changes in the microbial community composition due to the storage temperature (-20 ºC) were unlikely, because the sediments were immediately frozen at -20ºC on board and they were never thawed during the transport neither in the laboratory before DNA extraction, as has been recommended in previous studies (Tedjo et al. 2015). The storage of the samples at -20 ºC is a standard procedure commonly used for microbial community analyses from marine sediment (e.g., Bienhold et al. 2016; Probandt et al. 2017).

Diversity indices are commonly used in microbial ecology studies to understand the links between the environmental conditions and the community (Kimes et al. 2013; Bargiela et al. 2015). It has been suggested that microbial richness and diversity could be an expression of environmental variation correlated to energy sources and temperature (Santelli et al. 2008; Rosano-Hernández et al. 2012; Bargiela et al. 2015). Our results revealed higher richness and diversity in shallow sites compared to deep sites (Table 2). This can be a result of more dynamic shallow environments, characterized by strong physical mixing and seasonal variation. In shallow environments the interaction among atmosphere, land and ocean increase environmental variation, and therefore higher microbial diversity, as reported for coastal environments elsewhere (Gobet et al. 2012). In contrast, lower richness and diversity in the deep sites could be
the result of relatively constant environmental conditions. Microbes from the abyssal plains environment are able to overcome the extreme conditions of temperature, pressure, oligotrophy and darkness (Jochens and DiMarco 2008) but are subjected to far less environmental variability.

It is well recognized that marine sediments are dynamic environments shaped by interactions among biotic and abiotic processes, including the redox reactions (Wasmund et al. 2017). In the present study, the electronegative redox values detected in the first 5 cm of the sediments for shallow sites (Table 1), suggested high microbial respiration or anoxic/reducing conditions (Valdes and Real 2004; Aranda et al. 2015). However, genera known to be both aerobic (e.g., *Marinicella* and *Nitrosopumilus*) and anaerobic (e.g., *Amphritea, Desulfococcus, GOUTA-19, LCP-6, and Pseudidiomarina*) were found in shallow samples (Fig S4). This suggested the co-occurrence of different metabolisms in these sites, in accordance with previous reports for other shallow marine sediments (Jørgensen 1977; Mills et al. 2008; Acosta-González et al. 2013; Tolar et al. 2013; Cerqueira et al. 2015).

Electropositive redox values detected in the deep sites (Table 1), suggested oxidizing conditions at those water depths, likely because the upper sediment layers remain oxygenated due to relatively high oxygen concentrations in deep waters of the GoM (Jochens and DiMarco 2008) as well as low rates of sediment community oxygen consumption previously reported in the deep GoM (Rowe et al. 2008). In these oxidizing deep sediments, genera known to be aerobic and facultative anaerobic bacteria, such as *Shewanella, Alcanivorax, Marinicella, Nitrospina* and *ZD0117 (Alteromonadaceae)* were well represented (Fig. S4). Thus, these results suggest that
aerobic (and facultatively anaerobic) lifestyles seem to be favored in abyssal sediments of the studied region.

In this study, water depth was highly correlated with microbial community structure (Fig. 2). It is known that water depth and its dependent variables, such as temperature and pressure, are among the most important factors explaining variation in the bacterial community composition of seafloor sediments (Orcutt et al. 2010; Bienhold et al. 2016). As an example of this, our results showed a relatively high abundance of psychropiezophilic organisms of the genera *Colwellia* and *Shewanella* for the deep sites (Fig. S2 and Fig. S4), where conditions are favorable for the development of these microbial metabolisms (Nogi et al. 2004).

Detection of phylotypes putatively related to hydrocarbons degradation

Due to the presence of natural hydrocarbon effluents and the oil spills which have occurred in the GoM (*e.g.*, DWH spill), the potential effects of hydrocarbons on microbial community structure has been extensively studied there (Rosano-Hernández et al. 2012; Orcutt et al. 2010; Kleindienst et al. 2015). Recent investigations have provided insights about microbial populations responding to the presence of hydrocarbons, highlighting microorganisms capable of using these organic compounds as carbon source (Kostka et al. 2011; Redmond and Valentine 2011; Mason et al. 2012). In this work, we report the occurrence of several genera, such as *Shewanella*, *Alcanivorax*, *Pseudoalteromonas* and *Phaeobacter* (Fig. S4), which have been previously associated with hydrocarbon consumption (Beazley et al. 2012; Mason et al. 2014; Barbato et al. 2016; Liu et al. 2017).
In addition, this work reports for the first time NC10 and Kordiimonadales phylotypes for sediments of the GoM (Fig. 3 and Fig S1), that based on the available information, are capable of anaerobic methane oxidation with nitrite denitrification, and aerobic degradation of alkanes and polycyclic aromatic hydrocarbons, respectively (Kwon et al. 2005; Xu et al. 2016; Math et al. 2012; Zhou and Xing 2015; Padilla et al. 2016). The Kordiimonadales phylotype was even detected by DGGE band sequencing approach (Fig. S2B), supporting their relatively high abundances in the analyzed samples.

The potential capability to degrade aromatic compounds, such as nitrotoluene, ethylbenzene, chlorocyclohexane and fluorobenzoate were predicted based on trait modeling software (PICRUSt) (Fig S3). This also suggests that microbial metabolisms related to the hydrocarbon degradation might be well distributed in the Mexican region of the PFB and could contribute with the bioremediation in case of hydrocarbon contamination due to forthcoming oil exploration and extraction activities in this area.

Conclusions

To our knowledge, this is the first study to report differences in abundances and composition of the microbial communities inhabiting surficial sediments in a water depth gradient in an important province for oil extraction in the Mexican region of the Gulf of Mexico. In this study, the combination of nucleic acid-based molecular methods and physicochemical measurements allowed the detection of changes in the structures of microbial communities which were mainly related to the redox potential, total sulfur concentration, grain size, as well as depth and the variables that change with it, such as temperature and pressure. Our findings indicate that shallow microbial communities are taxonomically richer than communities in the deep
sediments. Bacterial phylotypes putatively related to hydrocarbon degradation appear to be well represented for all the analyzed samples and could ameliorate future anthropogenic oil spills in this region of the GoM. These results contribute to the current knowledge of the environmental and biological dataset of the Perdido Fold Belt, which will be useful for further monitoring of this area in the Gulf of Mexico.

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Figure 1

Location of sampling sites in the Mexican Region of the Northwestern Gulf of Mexico.

Circles: shallow sites in the continental platform and slope from 20 to 600 m depth. Triangles: deep sites in the abyssal plain from 2800 to 3700 m depth. The names of the stations are indicated. Source credit: Abril Gamboa-Muñoz.
Figure 2

Non-metric multidimensional scaling (NMDS) of the bacterial community structure and the environmental variables.

Ordination of samples based on the weighted UniFrac distance from the community structure and their relationship with the physicochemical variables. Shallow sites included samples collected from 20 to 600 m, and deep sites from 2800 to 3700 m depth. Physicochemical variables related to the bacterial community structure are shown in green arrows. TS total sulfur concentration. NMDS stress value = 0.0684.
Figure 3

Relative abundances from bacteria inhabiting sediments in the Mexican Region in the northwestern GM.

Taxonomic diversity at phylum and class levels from shallow and deep samples. **Shallow** sediment samples retrieved from 20 to 600 m depth, and **deep** sediment samples retrieved from 2800 to 3700 m depth. Only the 20 most abundant classes are shown. The remaining classes were agglomerated in the “Others” category.
Figure 4

Differential OTUs abundance among shallow and deep communities from sediment samples in the Mexican northwestern Gulf of Mexico.

A) OTUs with significant fold changes among the community composition from shallow and deep zones. Circles in colors defines the phyla of the OTUs. B) Relative abundances of the OTUs with significant fold changes at a class-order taxonomic level.
physicochemical variables measured with no replicates. pearson coefficient showed significant correlation at a p-value <0.01 among TS ($r^2 = 0.98$), redox potential ($r^2 = 0.98$), and the percentages of lime ($r^2 = 0.78$) and clay ($r^2 = -0.78$) particles with depth. ↑ variables with positive correlation with depth. ↓ variables with negative correlation with depth.

Table 1 (on next page)

Physicochemical variables from shallow and deep sediment samples.

TS total sulfur, TOM total organic matter and TOC total organic carbon.
Table 1. Physicochemical variables from shallow and deep sediment samples.

| Site | Sample | Longitude | Latitude | Depth (m) | Redox ↑ (mV) | TS ↑ (µM) | TOM (%) | TOC (µM) | Sand (%) | Lime ↑ (%) | Clay ↓ (%) |
|------|--------|-----------|----------|-----------|--------------|----------|---------|----------|----------|------------|-------------|
| Shallow | E10 | 97°13'48"W | 25°15'N | 20 | -131.0 | 0.05 | 0.80 | 0.45 | 34.5 | 51.2 | 14.4 |
|        | E11 | 96°56'24"W | 25°15'N | 75 | -174.4 | 0.05 | 1.10 | 0.61 | 26.5 | 57.3 | 16.3 |
|        | E12 | 96°43'12"W | 25°15'N | 100 | -204.0 | 0.05 | 1.02 | 0.56 | 20.5 | 51.2 | 28.4 |
|        | E13 | 96°27'36"W | 25°15'N | 200 | -131.7 | 0.05 | 0.83 | 0.46 | 22.5 | 51.2 | 26.4 |
|        | E14 | 96°18'36"W | 25°15'N | 600 | -192.9 | 0.05 | 1.02 | 0.56 | 23.7 | 68.2 | 8.1 |
| Deep | E121 | 95°30'W | 25°00'N | 2800 | 230 | 0.10 | 1.22 | 0.68 | 33.75 | 60.3 | 6.0 |
|       | E122 | 95°00'W | 25°00'N | 3600 | 233 | 0.10 | 0.59 | 0.33 | 27.75 | 66.3 | 6.0 |
|       | E123 | 94°00'W | 25°00'N | 3700 | 225 | 0.11 | 0.44 | 0.25 | 25.7 | 66.3 | 8.0 |
|       | E124 | 93°00'W | 25°00'N | 3700 | 215 | 0.11 | 0.85 | 0.47 | 29.8 | 64.3 | 5.9 |
|       | E125 | 92°00'W | 25°00'N | 3500 | 236 | 0.12 | 0.81 | 0.45 | 23.9 | 70.3 | 5.9 |
|       | E126 | 91°00'W | 25°00'N | 3700 | 215 | 0.12 | 0.92 | 0.51 | 25.8 | 68.2 | 6.0 |

TS total sulfur, TOM total organic matter and TOC total organic carbon. Physicochemical variables measured with no replicates. Pearson coefficient showed significant correlation at a p-value <0.01 among particles with depth.

↑ Variables with positive correlation with depth.

↓ Variables with negative correlation with depth.
Alpha diversity estimations

Alfa diversity data from observed OTUs, $H'$ Shannon diversity index, $D$ Simpson diversity index, and Chao1 for nonparametric richness estimation. Statistical differences were observed among sampling sites Shallow and Deep-sea ($p$-value <0.05). Percentages of Good`s coverage shows the fraction of the OTUs subsampled more than once.
Table 2. Alpha diversity estimations.

| Site  | Sample | Depth (m) | Total counts | Observe d OTUs | $H'$ | $D$ | Chao1 | Good's coverage (%) |
|-------|--------|-----------|--------------|----------------|------|-----|-------|---------------------|
| Shallow | E10    | 20        | 48886        | 2401           | 9.3  | 0.99| 3002.2 | 96.51               |
|       | E11    | 75        | 30290        | 3262           | 10.1 | 1.00| 4141.4 | 94.83               |
|       | E12    | 100       | 21243        | 3509           | 10.4 | 1.00| 4770.5 | 93.94               |
|       | E13    | 200       | 35253        | 3081           | 9.8  | 1.00| 3925.3 | 95.09               |
|       | E14    | 600       | 42131        | 2188           | 8.2  | 0.99| 3116.4 | 95.8                |
| Deep  | E121   | 2800      | 53408        | 2238           | 8.7  | 0.99| 3087.3 | 95.75               |
|       | E125   | 3600      | 56735        | 1434           | 7.0  | 0.97| 2059.0 | 97.36               |
|       | E122   | 3700      | 20402        | 1243           | 6.7  | 0.97| 2081.6 | 97.23               |
|       | E123   | 3700      | 61025        | 2139           | 8.0  | 0.97| 3181.1 | 95.54               |
|       | E124   | 3500      | 42768        | 1848           | 6.9  | 0.94| 3009.7 | 95.80               |
|       | E126   | 3700      | 45107        | 2406           | 9.11 | 0.99| 3418.39| 95.40               |

Alpha diversity data from observed OTUs, $H'$ Shannon diversity index, $D$ Simpson diversity index, and Chao1 for nonparametric richness estimation. Statistical differences were observed among sampling sites Shallow and Deep-sea ($p$-value <0.05). Percentages of Good’s coverage shows the fraction of the OTUs subsampled more than once.