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*, *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.

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2 **Perdido Fold Belt region in the Gulf of Mexico**

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21 **Abstract**

22 The Mexican region of the Perdido Fold Belt (PFB), in northwestern Gulf of Mexico (GoM), is a

23 geological province with important oil reservoirs that will be subjected to forthcoming oil

24 exploration and extraction activities. To date, little is known about the native microbial

25 communities of this region, and how these change relative to water depth. In this study we

26 assessed the bacterial community structure of surficial sediments by high-throughput sequencing

27 of the 16S rRNA gene at 11 sites in the PFB, along a water column depth gradient from 20 to

28 3700 m, including 5 shallow (20-600 m) and 6 deep (2800-3700 m) samples. The results

29 indicated that OTUs richness and diversity were higher for shallow sites (OTUs = 2888.2 $\pm$

30 567.88; H' = 9.6 $\pm$ 0.85) than for deep sites (OTUs = 1884.7 $\pm$ 464.2; H' = 7.74 $\pm$ 1.02).

31 Nonmetric multidimensional scaling (NMDS) ordination revealed that shallow microbial

32 communities grouped separately from deep samples. Additionally, the shallow sites plotted

33   further from each other on the NMDS whereas samples from the deeper sites (abyssal plains)

34   plotted much more closely to each other. These differences were related to depth, redox

35   potential, sulfur concentration, and grain size (lime and clay), based on the environmental

36   variables fitted with the axis of the NMDS ordination. In addition, differential abundance

37   analysis identified 147 OTUs with significant fold changes among the zones (107 from shallow

38   and 40 from deep sites), which constituted 10 to 40 % of the total relative abundances of the

39   microbial communities. The most abundant OTUs with significant fold changes in shallow

40   samples corresponded to *Kordiimonadales, Rhodospirillales*, *Desulfobacterales* (*Desulfococcus*)*,

41   *Syntrophobacterales and Nitrospirales* (*GOUTA 19*, *BD2-6*, *LCP-6*), whilst *Chromatiales*,

42   *Oceanospirillales* (*Amphritea*, *Alcanivorax*), *Methylococcales*, *Flavobacteriales*,

43   *Alteromonadales* (*Shewanella*, *ZD0117*) and *Rhodobacterales* were the better represented taxa in

44   deep samples. Several of the OTUs detected in both deep and shallow sites have been previously

45   related to hydrocarbons consumption. Thus, this metabolism seems to be well represented in the

46   studied sites, and it could abate future hydrocarbon contamination in this ecosystem. The results

47   presented herein, along with biological and physicochemical data, constitute an available

48   reference for further monitoring of the bacterial communities in this economically important

49   region in the GoM.

50

51   **Introduction**

52   Microorganisms are well recognized as key drivers of biogeochemical cycles in marine

53   environments (Webster et al. 2003; Santos et al. 2011). Measuring the changes in microbial

54   communities is of particular interest to understanding how environmental factors modulate their

55   structure and how that, in turn, is related to the function and stability of the ecosystem (Huber et

56   al. 2007; Jones et al. 2012; Fuhrman et al. 2015). In marine sediments, the type of substrate,

57   energy and carbon sources, and the variables that are correlated with water depth (*e.g.*,

58   temperature, oxygen concentration, light penetration and hydrostatic pressure), generally

59   describe the most important aspects of the variability amongst habitats (Miller et al. 2012).

60   In the Gulf of Mexico (GoM), changes in bacterial community structure have been mainly

61   related with depth in the water column, and likely result from differences in temperature,

62   dissolved oxygen and suspended particles (King et al. 2013) which occur across these depth

63   differences. In marine sediments from the GoM, bacterial community composition has been

64   determined at different depths below the seafloor (from sediment cores). It has been proposed

65   that the bacterial community composition of these sediments likely results from the interaction

66   between the water column and a benthic microbial population limited to the upper layer of the

67   sediments (Reese et al. 2013). In contrast, the microbial diversity present in different sediment

68   depths from seep systems in the GoM have been directly related to the composition and

69   magnitude of hydrocarbon seepage (*e.g.*, natural oils, methane, and non-methane hydrocarbons)

70   (Orcutt et al. 2010), as well as to the presence of overlying microbial mats (Mills et al. 2004).

71

72   Knowledge about microbial communities in the GoM increased notably with the Deepwater

73   Horizon (DWH) massive oil spill in 2010, which occurred in the north zone of the GoM across

74   an enormous area with different environmental conditions (Kostka et al 2011; Mason et al. 2012;

75   Liu and Liu 2013; Joye et al. 2014). During the spill, it was observed that different bacterial

76   phyla in the deep-water plume (*e.g.*, *Oceanospirillales*, *Cycloclasticus* and *Colwellia*) rapidly

77   responded and were enriched within hours - weeks following the DWH well blow out (Redmond

78   and Valentine 2011; Mason et al. 2012). In natural hydrocarbon seep sites sampled at the same

79   time, several rare taxa increased rapidly in abundance after the spill, emphasizing the importance

80   of specialized sub-populations and potential ecotypes during massive deep-sea oil discharges

81   (Kleindienst et al. 2015). The oil transported to the shoreline after the discharge also had a

82   profound impact on the abundance and community composition of indigenous bacteria in beach

83   sands, where members of the *Gammaproteobacteria* and *Alphaproteobacteria* participated as

84   key players in oil degradation (Kostka et al. 2011).

85

86   The precise volume of oil spilled and the trajectory of the oil slicks from the DHW are still

87   controversial (Salcedo et al. 2017). Based on the surface circulation models of the waters of the

88   GoM, it was inferred that the Mexican region of the Perdido Fold Belt (PFB), was also

89   susceptible to this environmental disturbance (Soto and Vázquez-Botello 2013). Knowledge of

90   environmental and biotic data of this region is, however, scarce despite being a geologic

91   province with oil reservoirs. The present study assessed changes in the bacterial community

92   structure of surficial sediments with respect to a depth gradient and specific environmental

93   variables in the PFB region, where oil exploration and extraction activities are predicted to

94   impact environmental conditions, and consequently the structure of bacterial communities.

95   Additionally, bacterial phylotypes putatively involved in hydrocarbon degradation were

96   highlighted in this work.

97

98   **Material and Methods**

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

100   The samples used in this study were collected in the Northwestern Gulf of Mexico in April 2014.

101   Sediment samples from a depth gradient (20-3700 m), were collected perpendicular to the

102   coastline at 11 sites on the parallels 25 and 25.15ºN (Fig. 1, Table 1). Samples were collected

103   with a Hessler-Sandia MK-II boxcore (40 x 40 cm) from which three different surficial (0 - 5

104   cm) subsamples were taken: 1) sterile 100 mL plastic containers immediately frozen at -20°C on

105   board for further molecular analysis; 2) a 2 inch core extracted to determine total sulfur

106   concentration (TS) and redox potential on board, with a sulfide ion selective electrode and

107   potentiometer (Bricker 1982; Brassard 1997); and 3) approximately 400 g of sediment stored in

108   high density polyethylene bags at 4ºC until the determination of total organic matter (TOM),

109   total organic carbon (TOC), and grain size in the laboratory. TOM was determined by the wet

110   oxidation technique with an excess of dichromate and back titration with iron (II) (Buchanan,

111   1984), TOC content was quantified using the oxidation with potassium dichromate in acid

112   medium, and titration of excess oxidant with ferrous sulfate and diphenylamine as an indicator

113   (Buchanan 1984). The amounts of sand, lime and clay were estimated using a hydrometer

114   (Buchanan 1984). These physicochemical variables were compared among shallow and deep

115   sites using a one-way analysis of variance (ANOVA $p<0.05$ and F-values) and were correlated

116   with the depth variable.

117   **DNA extraction**

118   Sediment samples were stored during five months at -20°C. Then, in October 2014 sediments

119   were thawed at 4ºC, homogenized mechanically under sterile conditions, and centrifuged 1

120   min/10,000 x g in order to separate the sediment from remaining water, which was discarded.

121   Total DNA was extracted from 0.25 g of sediment using the PowerSoil®DNA Isolation Kit (Mo

122   Bio Laboratories, Carlsbad, CA, USA) following the manufacturer's protocol. The quality of the

123   DNA extractions was verified by agarose gel electrophoresis and the concentration was

124   determined through UV absorption analysis with a NanoDrop 2000 Spectrometer (ThermoFisher

125 Scientific Inc., Wilmington, DE, USA). Extracted DNA was stored at -20°C for further 16S

126 rRNA Illumina sequencing and DGGE analysis (see a detailed description in supplementary

127 methods).

128 **16S rRNA gene sequencing**

129 Amplicons from environmental DNA were prepared for sequencing the 16S rRNA V3 and V4

130 variable regions by using a two PCR steps approach following the "16S Metagenomic

131 Sequencing Library Preparation" protocol (Illumina). Briefly, the first PCR step amplified the

132 template out of the DNA samples using the forward primer 5'-CCTACGGGNGGCWGCAG-3'

133 and the reverse primer 5'-GACTACHVGGGTATCTAATCC-3' with Illumina overhang

134 adapters attached, to obtain ~550 bp fragments (Klindworth et al. 2013). The PCR program was

135 performed in a thermal cycler (Applied Biosystems Veriti ABI Inc., Foster City, CA, USA) with

136 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

137 final extension at 72°C - 5 min. Each PCR reaction (20 µl) included 2 µl of environmental DNA

138 (5 ng/µl), 0.5 µl of each primer (10 µM) and 10 µl of 2x Phusion High-Fidelity MasterMix

139 (Thermo Scientific, Waltham, MA, USA). The correct size of the amplicons was verified on an

140 QIAxcel Advanced system (QIAGEN, Hilden, Germany), DNA and the PCR clean-up were

141 carried out using AMPure XP beads to discard free primers and primer dimer species. In the

142 second PCR, eight cycles attached dual indices and the Illumina sequencing adapters using the

143 Nextera XT Index Kit. PCR barcoded amplicons were verified and purified as previously

144 described and quantified using a Qubit 3.0 fluorometer (Life Technology, Shah Alam, Selangor,

145 Malaysia). The individual barcoded amplicons were diluted on 10 mM Tris (pH 8.5) and pooled

146 in equimolar concentrations (9 pM). Paired-end sequencing (2x300 bp) was carried out using the

147   MiSeq platform (Illumina, San Diego, CA, USA) with a MiSeq Reagent Kit V3 (600 cycles).

148   Sequencing was performed in the Aquatic Pathology laboratory at CINVESTAV-Mérida.

149   **Data analysis**

150   Demultiplexing of the pooled amplicons and trimming the barcode and adapter sequences was

151   performed using the MiSeq Reporter Metagenomics Workflow (Illumina, 2014). Reads were

152   overlapped with the fast length adjustment of short reads to improve genome assemblies

153   (FLASH) software (Magoč and Salzberg, 2011), and processed using the QIIME (version 1.9)

154   (Caporaso et al., 2010) pipeline with the parameters q (phred_quality_treshold)=20, r

155   (max_bad_run_length,)=3, p (min_per_read_length_fraction)=0.75, for quality filtering. The

156   demultiplexed sequences were clustered in operational taxonomic units (OTUs) with the

157   'pick_open_reference_otus.py' script at 97% of similarity using the usearch61 method (Edgar

158   2010) with a minimum OTU cluster size of 5. Chimeric sequences were removed with the

159   uchime2 algorithm in the reference mode (v 9.1.13) (Edgar 2016) and, the taxonomic assignment

160   was performed by the SortMeRna (Kopylova et al. 2012) with an e-value of 3 e^{-6} and default

161   parameters from QIIME, using the GreenGenes database (v13.8). An OTUs alignment was

162   performed with the Mafft algorithm (Katoh and Standley 2013) to build a phylogenetic tree using

163   the Fasttree software (Price et al. 2009) for its subsequent use in the UniFrac (Lozupone,

164   Hamady and Knight, 2006) distance analysis.

165   Community structure and composition analyses were performed by processing the OTU table in

166   the R environment (R Core Team, 2014) with the Phyloseq (McMurdie and Holmes 2013),

167   vegan (Oksanen et al. 2013) and ggplot2 (Wickham, 2010) packages. The data set was rarefied at

168   the depth from the smallest library (20,400). We reported the observed OTUs, the Shannon and

169   Simpson diversity indexes (*H'* and *D*, respectively) and the nonparametric richness from *Chao1*.

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

171   In order to compare the microbial community composition among shallow and deep sampling

172   sites, a non-metric multidimensional scaling (NMDS) was plotted (Lozupone et al. 2006;

173   Giloteaux et al. 2010) with the weighted UniFrac distance metric (Lozupone, Hamady and

174   Knight, 2006) and a test of beta significance among groups of sample was performed using a

175   two-sided Student's two-sample t-test with the 'make_distance_boxplots.py' script of QIIME, *p*-

176   values were calculated using 1000 Monte Carlo permutations. The physicochemical variables

177   were fitted with the *envfit* function to the NMDS ordination to correlate them with the

178   community composition (*p*-value < 0.05 and 10 000 permutations). Physicochemical variables

179   were also tested with a PERMANOVA analysis at a *p*-value <0.05 (Table S1). A differential

180   abundance analysis was performed with the DESeq2 (Love et al, 2014) R library to identify the

181   OTUs that have significant fold changes among the shallow and deep zones. The *p*-value was

182   corrected with the false discovery rate (FDR) method (Benjamini and Hochberg, 1995) and a

183   Log2 fold change plot was made with the significant OTUs at a *p*-value < 0.01.

184   All the sequences are available at SRA site from NCBI database in the BioProject

185   PRJNA429278 and biosample accession ID's SRR6457706 to SRR6457716 and the raw

186   processed data in the supplementary material.

187

188   **Results**

189   **Physicochemical properties from marine sediments**

190 The physicochemical variables and textures of the sediment samples are shown in Table 1.

191 Redox potential ranged from -204 to 236 mV. The electronegative values were found in samples

192 from 20 to 600 m depth, while the electropositive values corresponded to the deep sediments

193 (2800 - 3700 m). TS was constant at depths from 20 to 600 m at a concentration of 0.05 µM,

194 while the maximum values were detected for deep sites (0.10 - 0.12 µM). The percentage of

195 TOM and the concentration of TOC varied for all sites with averages of $0.872 \pm 0.22\%$ and

196 0.484 ± 0.123 µM, respectively. The percentages of sand, lime and clay in the sediment samples

197 ranged from 20.50 - 34.5, 51.2 - 70.30 and 5.9 - 28.4%, respectively. Values of TS, redox

198 potential, and the percentages of lime and clay showed significant correlations with depth (p-

199 value <0.01) (Table 1).

200

201 **Bacterial community structure in a depth gradient**

202 The NMDS ordination method showed that microbial communities from shallow sites grouped

203 separately from those from the deep sites (Fig. 2), and it was according with the test of

204 significance were the mean of the distances of Shallow and Deep samples were different with a

205 p-value < 0.05. Samples taken from shallow sites displayed greater dissimilarity distance from

206 each other, while those derived from the deep sites displayed a closer proximity to each other

207 (Fig. 2). The environmental variables fitted on the NMDS ordination were: depth ($r^2 = 0.79$),

208 redox potential ($r^2 = 0.77$), the concentration of total sulfur ($r^2 = 0.73$) and percentages of clay ($r^2 =$

209 0.71) and lime ($r^2 = 0.7$), at a p-value <0.05.

210 The observed OTUs, Shannon (H') and Simpson (D) diversity indexes, and the nonparametric

211 richness estimation from *Chao1*, were different among shallow and deep-sea sites (p-value

212 <0.05). These ecological estimators showed higher values in the sediment samples from shallow

213 sites (observed OTUs = 2888.2 ± 567.88, H' = 9.6 ± 0.85, D = 0.99 ± 0.005 and $Chao1$ = 3791.2

214 ± 737.81) than those obtained from the deep ocean floor (observed OTUs = 1884.7 ± 464.2, H' =

215 7.74 ± 1.02, D = 0.97 ± 0.02, and $Chao1$ = 2806.18 ± 589.39) (Table 2).

216
217 **Bacterial community composition**

218 Microbial community analysis resulted in the detection of 25 bacterial phyla, however only 14

219 were ≥1% in relative abundance (Table S2). *Proteobacteria*, represented by *Gamma-*, *Alpha-* and

220 *Deltaproteobacteria* classes, were dominant in all the analyzed samples (Fig. 3). Nevertheless,

221 *Bacteroidetes*, *Acidobacteria*, *Chloroflexi*, *Nitrospirae*, *Planctomycetes*, *Gemmatimonadetes* and

222 the *NC10* phyla were also well represented in the samples. Two archaeal phyla, corresponding to

223 the *Euryarchaeota* (*Thermoplasmata* class) and *Crenarchaeota* including *Miscellaneous*

224 *Crenarchaeota Group* (MCG) and *Thaumarchaeota* classes (based on the GreenGene database),

225 were also detected in low abundances (0.3 to 1.2 %) (Fig. 3). At lower taxonomic levels, 51

226 families were detected, from which *Piscirickettsiaceae*, *Rhodobacteraceae*, *Flavobacteriaceae*,

227 *Syntrophobacteraceae*, *Thermovibrionaceae*, *Desulfobacteraceae*, *Colwelliaceae*,

228 *Marinicellaceae*, *Alcanivoracaceae, Colwelliaceae,* and *Cenarchaeaceae* were among the

229 families with higher relative abundances in the samples (Fig. S1A). Moreover, 23 genera were

230 identified at > 1% of relative abundances (Fig S4). *Desulfococcus, Alcanivorax, Fulvivirga,*

231 *Amphritea*, *BD2-6*, *Shewanella*, *ZD0117* and *Nitrospina* were the better represented in the

232 samples (Fig S4).

233

234   Differential abundance analysis identified 147 OTUs with significant fold changes between the

235   deep and shallow zones, 107 from shallow and 40 from deep sites (Fig 4A). From the shallow

236   samples, most of these OTUs belonged to *Delta-, Alpha- and Gammaproteobacteria* (38, 24 and

237   18, respectively), while the rest of the OTUs were distributed in 13 classes of 10 phyla (Fig. 4A).

238   In deep samples, all of the 40 OTUs belonged to *Gamma-*, *Alphaproteobacteria*, *Bacteroidetes*

239   and *Nitrospirae* (27, 6, 6 and 1, respectively) (Fig 4A). The relative abundances of the 147 OTUs

240   with significant fold changes constituted approximately 10 to 40 % of the total microbial

241   communities (Fig 4B). *Kordiimonadales, Rhodospirillales*, *Desulfobacterales,*

242   *Syntrophobacterales* and *Nitrospirales*, were the orders with highest relative abundances for

243   shallow samples whilst the orders *Chromatiales*, *Oceanospirillales*, *Methylococcales*,

244   *Flavobacteriales*and *Rhodobacterales* were the best represented orders for deep samples (Fig.

245   4B). At genus taxonomic level, differential abundance analysis allowed to identify *Marinicella*,

246   *Alcanivorax*, *Shewanella* and *ZD0117* with significant fold changes for deep samples; and

247   *Nitrosopumilus*, *GOUTA19*, *LCP-6*, *BD2-6*, *Amphritea*, *Desulfococcus* and *Mariprofundus* for

248   shallow sites (Fig. S5).

249
250   **Discussion**

251   **Physicochemical properties of sediment samples**

252

253   Physicochemical properties (*e.g.*, temperature, pH, redox) in marine sediments commonly

254   change according to water depth (Miller et al. 2012). In this study, lime percentage, redox

255   potential and total sulfur were positively correlated to depth, while the clay particles percentages

256   were negatively correlated with depth (Table 1). The electronegative redox values measured in

257   the first 5 cm of the sediments in all shallow sites (Table 1), suggested that oxygen was depleted

258   by microbial respiration as common reported for this layer in shallow marine sediments (Aranda

259   et al. 2015). Electronegative redox values in the shallow samples were likely enhanced at higher

260   fine-grained clay content, since this diminishes the sediment permeability as reported elsewhere

261   for sediments in the continental platform (Probandt et al. 2017). In contrast, the electropositive

262   redox values detected in the first 5 cm of the sediments in all the deep sites (Table 1) suggested

263   oxidizing conditions. This condition has also been observed at different latitudes in the GoM

264   (Deming and Carpenter 2008) due to both the circulation of deep water masses carrying oxygen

265   (Jochens and DiMarco 2008), and low rates of oxygen consumption (Rowe et al. 2008).

266
267   Total organic matter percentage (TOM%) was determined to be below 2% for all the sediments

268   analyzed (Table 1). These low organic contents are similar to those reported in studies of two

269   onshore-offshore transects in the Northwestern GoM (Goñi et al. 1998). Shallow sites likely

270   receive inputs from terrestrial organic matter (Balsam and Beeson 2003), and yet these sediments

271   presented similar values of TOM than those detected for deep sites (Table 1). Previous studies

272   have reported high production and consumption rates of oxygen measured for shallow sediments

273   in Northeastern GoM (Mills et al. 2008). TOM values determined in this study for shallow sites

274   are in concordance with previous reports for the GoM (Balsam and Beeson 2003). We

275   hypothesize that these values are related to the consequence of high rates of microbial

276   metabolism in those same zones.”

277

278   **Microbial Ecology assessment**

279

280   The effect of storage temperature on microbial community structure has been previously studied,

281   and it is well recognized that microbial metabolism could keep on going at -20 ºC, however the

282   survival at these subzero temperatures requires several genetic and physiological strategies that

283   exclusively cold-adapted microorganisms (permafrost and seasonally frozen soil microbial

284   communities) have developed (Jansson and Tas 2014; Boetius et al 2015). In the present study,

285   microbial communities from marine sediments living at temperatures ranging from 4 to 27 ºC

286   were analyzed. Thus, we considered that changes in the microbial community composition due

287   to the storage temperature (-20 ºC) were unlikely, because the sediments were immediately

288   frozen at -20ºC on board and they were never thawed during the transport neither in the

289   laboratory before DNA extraction, as has been recommended in previous studies (Tedjo et al.

290   2015). The storage of the samples at -20 ºC is a standard procedure commonly used for microbial

291   community analyses from marine sediment (*e.g.*, Bienhold et al. 2016; Probandt et al. 2017).

292

293   Diversity indices are commonly used in microbial ecology studies to understand the links

294   between the environmental conditions and the community (Kimes et al. 2013; Bargiela et al.

295   2015). It has been suggested that microbial richness and diversity could be an expression of

296   environmental variation correlated to energy sources and temperature (Santelli et al. 2008;

297   Rosano-Hernández et al. 2012; Bargiela et al. 2015). Our results revealed higher richness and

298   diversity in shallow sites compared to deep sites (Table 2). This can be a result of more dynamic

299   shallow environments, characterized by strong physical mixing and seasonal variation. In

300   shallow environments the interaction among atmosphere, land and ocean increase environmental

301   variation, and therefore higher microbial diversity, as reported for coastal environments

302   elsewhere (Gobet et al. 2012). In contrast, lower richness and diversity in the deep sites could be

303   the result of relatively constant environmental conditions. Microbes from the abyssal plains

304   environment are able to overcome the extreme conditions of temperature, pressure, oligotrophy

305   and darkness (Jochens and DiMarco 2008) but are subjected to far less environmental variability.

306

307   It is well recognized that marine sediments are dynamic environments shaped by interactions

308   among biotic and abiotic processes, including the redox reactions (Wasmund et al. 2017). In the

309   present study, the electronegative redox values detected in the first 5 cm of the sediments for

310   shallow sites (Table 1), suggested high microbial respiration or anoxic/reducing conditions

311   (Valdes and Real 2004; Aranda et al. 2015). However, genera known to be both aerobic (*e.g.*,

312   *Marinicella and Nitrosopumilus*) and anaerobic (*e.g.*, *Amphritea*, *Desulfococcus*, *GOUTA-19*,

313   *LCP-6*, and *Pseudidiomarina*) were found in shallow samples (Fig S4). This suggested the co-

314   occurrence of different metabolisms in these sites, in accordance with previous reports for other

315   shallow marine sediments (Jørgensen 1977; Mills et al. 2008; Acosta-González et al. 2013; Tolar

316   et al. 2013; Cerqueira et al. 2015).

317

318   Electropositive redox values detected in the deep sites (Table 1), suggested oxidizing conditions

319   at those water depths, likely because the upper sediment layers remain oxygenated due to

320   relatively high oxygen concentrations in deep waters of the GoM (Jochens and DiMarco 2008) as

321   well as low rates of sediment community oxygen consumption previously reported in the deep

322   GoM (Rowe et al. 2008). In these oxidizing deep sediments, genera known to be aerobic and

323   facultative anaerobic bacteria, such as *Shewanella*, *Alcanivorax*, *Marinicella*, *Nitrospina* and

324   *ZD0117* (*Alteromonadacea*e) were well represented (Fig. S4). Thus, these results suggest that

325   aerobic (and facultatively anaerobic) lifestyles seem to be favored in abyssal sediments of the

326   studied region.

327

328   In this study, water depth was highly correlated with microbial community structure (Fig. 2). It is

329   known that water depth and its dependent variables, such as temperature and pressure, are among

330   the most important factors explaining variation in the bacterial community composition of

331   seafloor sediments (Orcutt et al. 2010; Bienhold et al. 2016). As an example of this, our results

332   showed a relatively high abundance of psychropiezophilic organisms of the genera *Colwellia* and

333   *Shewanella* for the deep sites (Fig. S2 and Fig. S4), where conditions are favorable for the

334   development of these microbial metabolisms (Nogi et al. 2004).

335

336   **Detection of phylotypes putatively related to hydrocarbons degradation**

337   Due to the presence of natural hydrocarbon effluents and the oil spills which have occurred in the

338   GoM (*e.g.*, DWH spill), the potential effects of hydrocarbons on microbial community structure

339   has been extensively studied there (Rosano-Hernández et al. 2012; Orcutt et al. 2010;

340   Kleindienst et al. 2015). Recent investigations have provided insights about microbial

341   populations responding to the presence of hydrocarbons, highlighting microorganisms capable of

342   using these organic compounds as carbon source (Kostka et al. 2011; Redmond and Valentine

343   2011; Mason et al. 2012). In this work, we report the occurrence of several genera, such as

344   *Shewanella*, *Alcanivorax*, *Pseudoalteromonas* and *Phaeobacter* (Fig. S4), which have been

345   previously associated with hydrocarbon consumption (Beazley et al. 2012; Mason et al. 2014;

346   Barbato et al. 2016; Liu et al. 2017).

347

348   In addition, this work reports for the first time *NC10* and *Kordiimonadales* phylotypes for

349   sediments of the GoM (Fig. 3 and Fig S1), that based on the available information, are capable of

350   anaerobic methane oxidation with nitrite denitrification, and aerobic degradation of alkanes and

351   polycyclic aromatic hydrocarbons, respectively (Kwon et al. 2005; Xu et al. 2016; Math et al.

352   2012; Zhou and Xing 2015; Padilla et al. 2016). The *Kordiimonadales* phylotype was even

353   detected by DGGE band sequencing approach (Fig. S2B), supporting their relatively high

354   abundances in the analyzed samples.

355   The potential capability to degrade aromatic compounds, such as nitrotoluene, ethylbenzene,

356   chlorocyclohexane and fluorobenzoate were predicted based on trait modeling software

357   (PICRUSt) (Fig S3). This also suggests that microbial metabolisms related to the hydrocarbon

358   degradation might be well distributed in the Mexican region of the PFB and could contribute

359   with the bioremediation in case of hydrocarbon contamination due to forthcoming oil exploration

360   and extraction activities in this area.

361

362   **Conclusions**

363   To our knowledge, this is the first study to report differences in abundances and composition of

364   the microbial communities inhabiting surficial sediments in a water depth gradient in an

365   important province for oil extraction in the Mexican region of the Gulf of Mexico. In this study,

366   the combination of nucleic acid-based molecular methods and physicochemical measurements

367   allowed the detection of changes in the structures of microbial communities which were mainly

368   related to the redox potential, total sulfur concentration, grain size, as well as depth and the

369   variables that change with it, such as temperature and pressure. Our findings indicate that

370   shallow microbial communities are taxonomically richer than communities in the deep

371   sediments. Bacterial phylotypes putatively related to hydrocarbon degradation appear to be well

372   represented for all the analyzed samples and could ameliorate future anthropogenic oil spills in

373   this region of the GoM. These results contribute to the current knowledge of the environmental

374   and biological dataset of the Perdido Fold Belt, which will be useful for further monitoring of

375   this area in the Gulf of Mexico.

376

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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.

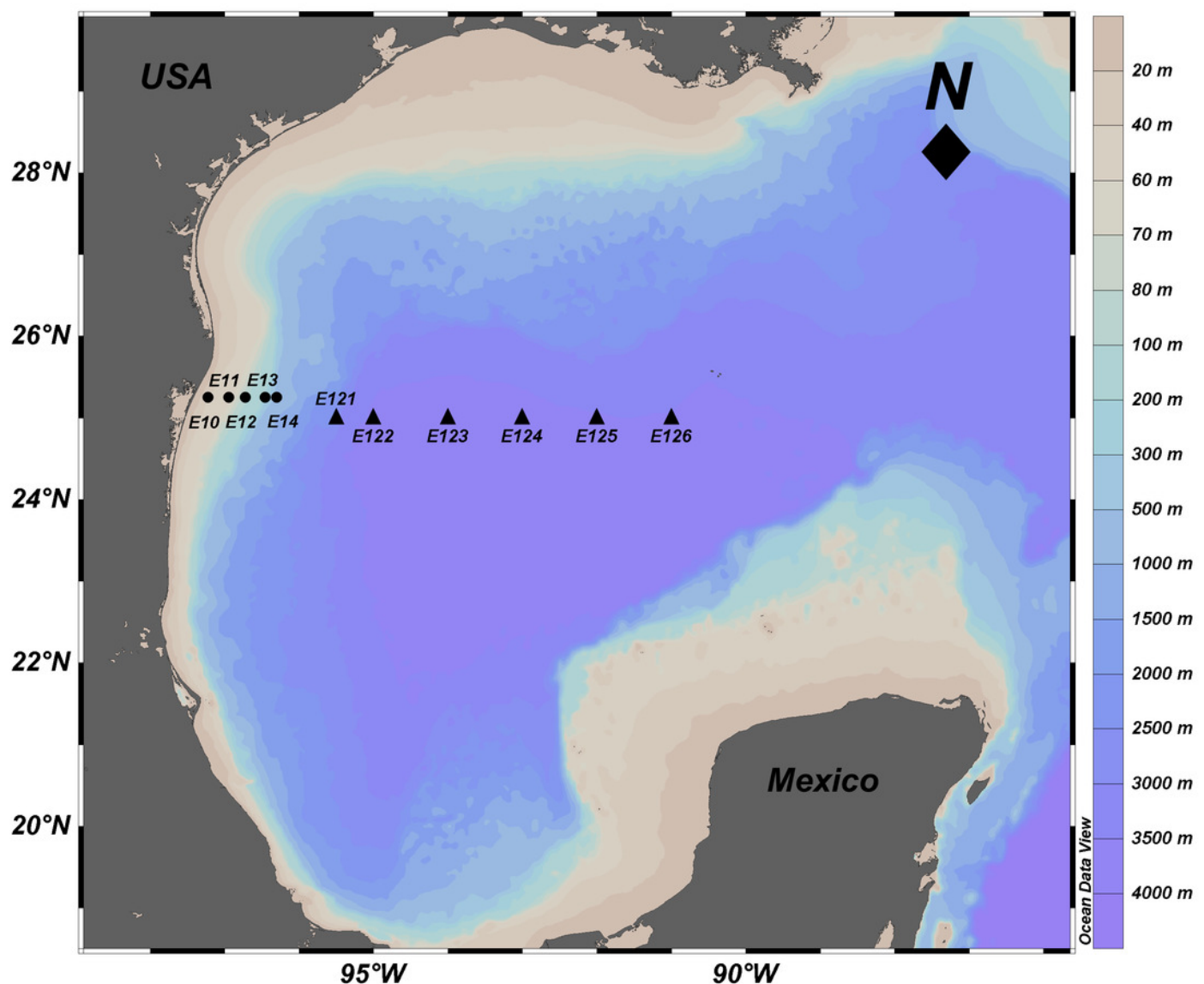



Figure 2

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.

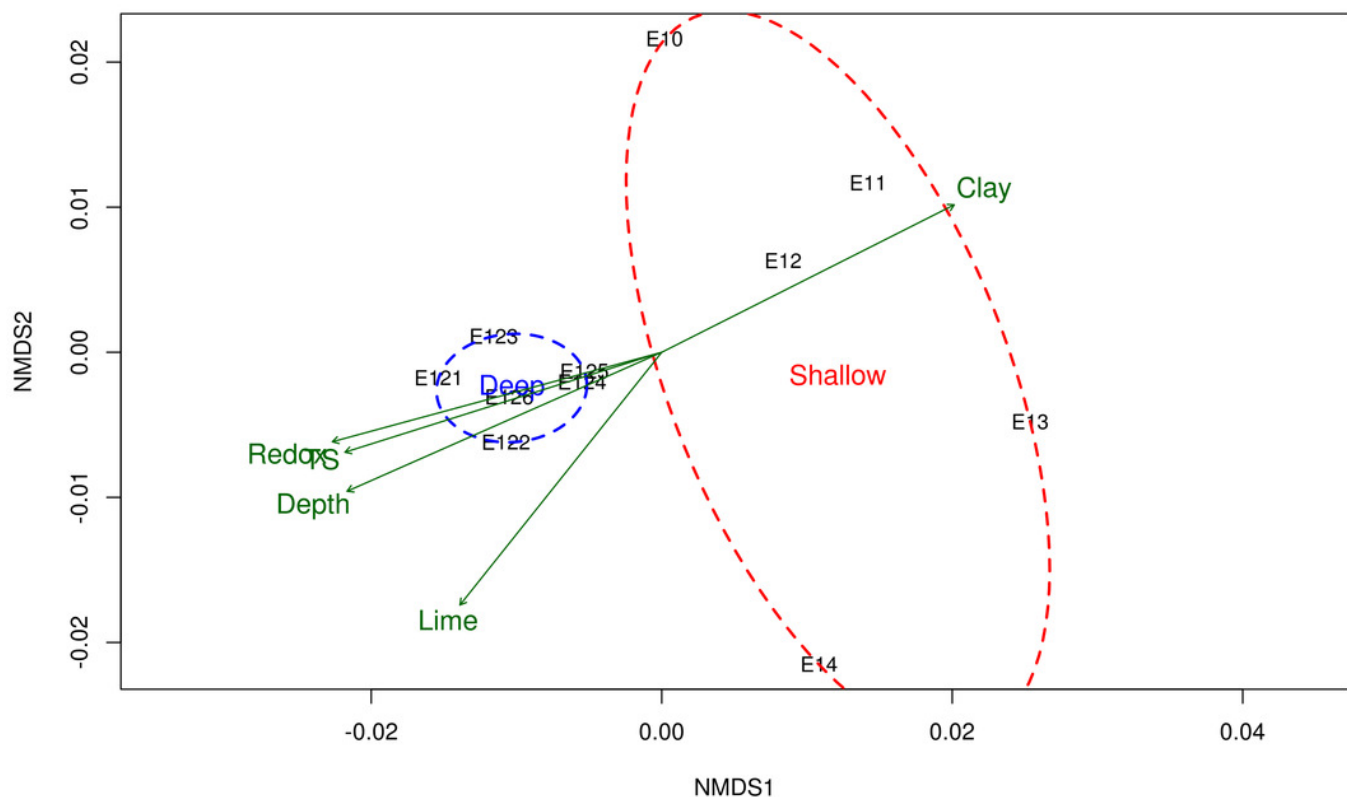



Figure 3

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.

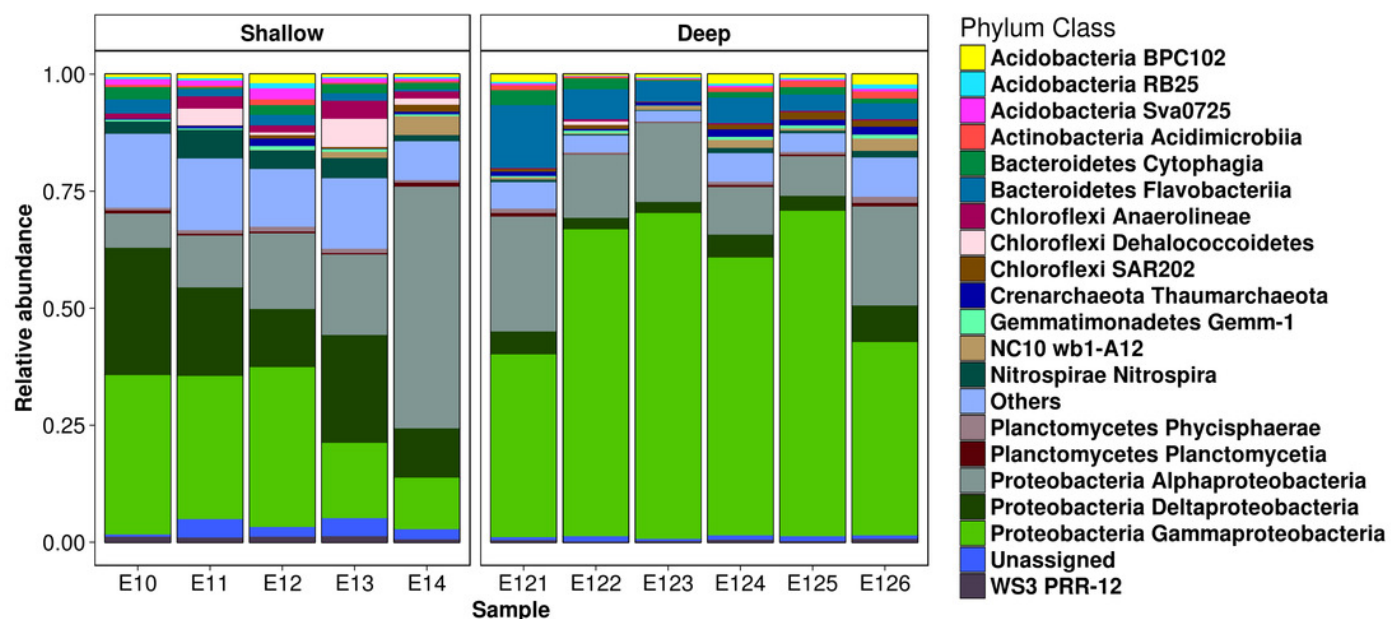
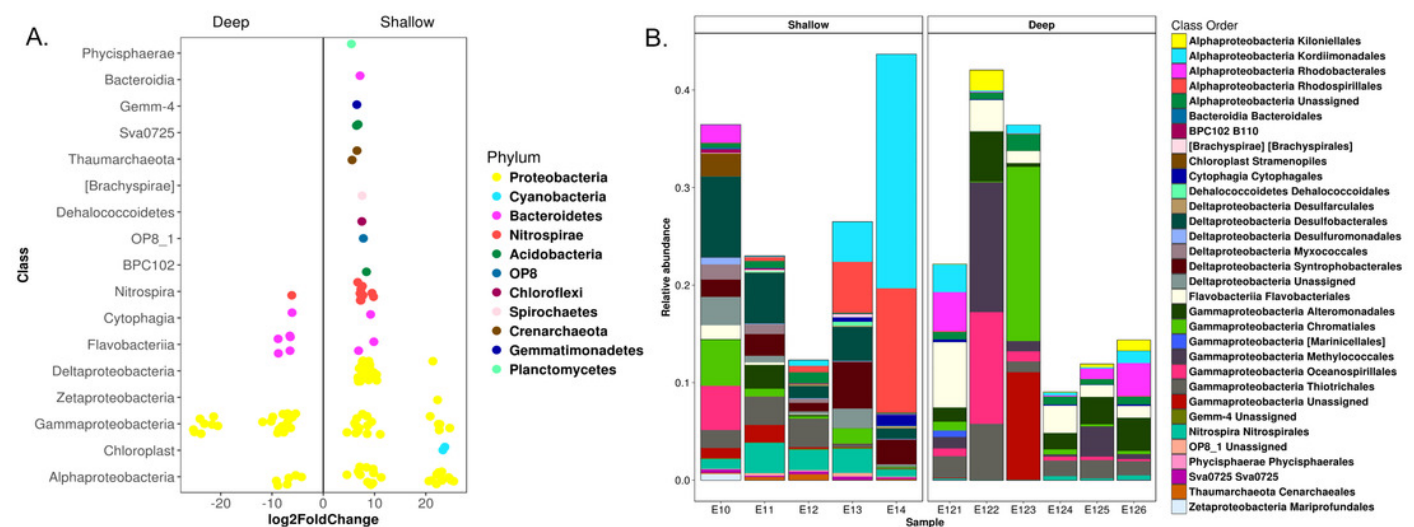



Figure 4

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 from shallow and deep sediment samples.

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 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.

1   **Table 1. Physicochemical variables from shallow and deep sediment samples**.

		Geographical location			Physicochemical variables							
Site	Sample	Longitude	Latitude	Depth (m)	Redox ↑ (mV)	TS ↑ (μM)	TOM (%)	TOC (μM)	Sand (%)	Lime ↑ (%)	Clay ↓ (%)
Shallow	E10	97⁰13'48"W	25⁰15'N	20	-131.0	0.05	0.80	0.45	34.5	51.2	14.4
	E11	96⁰56'24"W	25⁰15'N	75	-174.4	0.05	1.10	0.61	26.5	57.3	16.3
	E12	96⁰43'12"W	25⁰15'N	100	-204.0	0.05	1.02	0.56	20.5	51.2	28.4
	E13	96⁰27'36"W	25⁰15'N	200	-131.7	0.05	0.83	0.46	22.5	51.2	26.4
	E14	96⁰18'36"W	25⁰15'N	600	-192.9	0.05	1.02	0.56	23.7	68.2	8.1
Deep	E121	95⁰30'W	25⁰00'N	2800	230	0.10	1.22	0.68	33.75	60.3	6.0
	E122	95⁰00'W	25⁰00'N	3600	233	0.10	0.59	0.33	27.75	66.3	6.0
	E123	94⁰00'W	25⁰00'N	3700	225	0.11	0.44	0.25	25.7	66.3	8.0
	E124	93⁰00'W	25⁰00'N	3700	215	0.11	0.85	0.47	29.8	64.3	5.9
	E125	92⁰00'W	25⁰00'N	3500	236	0.12	0.81	0.45	23.9	70.3	5.9
	E126	91⁰00'W	25⁰00'N	3700	215	0.12	0.92	0.51	25.8	68.2	6.0

2   *TS* total sulfur, *TOM* total organic matter and *TOC* total organic carbon. Physicochemical variables

3   measured with no replicates. Pearson coefficient showed significant correlation at a *p*-value <0.01 among

4   TS ($r^2 = 0.98$), redox potential ($r^2 = 0.98$), and the percentages of lime ($r^2 = 0.78$) and clay ($r^2 = -0.78$)

5   particles with depth.

6   ↑ Variables with positive correlation with depth.

7   ↓ 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	Observed OTUs	Ecological estimators			Good's coverage (%)
					H'	*D*	Chao1	
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

Alfa diversity data from observed OTUs, *H'* Shannon diversity index, *D* Simpson diversity index, and Chao1for 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.