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ABSTRACT

Crude oil is still the dominant energy source in Brazil and that the consumption keeps rising since 2013, being responsible for 2.2% of the world's energy consumption. The recent discovery of crude oil reservoirs at the Espirito Santo basin, Campos basin and Santos basin, can be considered as an excellent opportunity to supply the country's economic and energetic demands. However, albeit the opportunity these crude oil reservoirs represent, offshore exploration offers risks to the microbiota and the whole sea life, as petroleum hydrocarbons are toxic, mutagenic, teratogenic and carcinogenic. Microbes are responsible for nutrient cycling and can degrade even very recalcitrant hydrocarbons. This work aimed to evaluate the microbial community shift (Archaea, Bacteria and Fungi) from Trindade Island coastal environment under petroleum contamination. Microcosms were assembled using Trindade Island coastal soil to create two treatments, control and contaminated (weathered crude oil at 30 g kg^{-1}). Soils were incubated during 38 days with CO_2 measurements every four hours. Total DNA was extracted, purified and submited for sequencing of 16s rRNA gene, for Bacteria and Archaea domains and Fungal ITS1 region using Illumina MiSeq platform. We compared alpha diversity, beta diversity and taxonomic shifts between controls and contaminated samples. Three days after contamination, emission rate peaked at more than 20x the control and the emissions remained higer during the whole incubation period.

Microbial alpha diversity was reduced for contaminated-samples. Fungi community of contaminated samples was reduced to almost 40% of the observed species. Taxonomy comparisons showed rise of the Actinobacteria phylum and reduction of the Archaea *Candidatus nitrosphaere*.

Keywords: Next Generation Sequencing; metagenome; alpha diversity; beta diversity; Biodiesel Co-Product (BCP); taxonomy comparison

INTRODUCTION

Brazil contributes to more than 2% of the world crude oil production (BP Statistical Review of World Energy, 2014). The British Petroleum review of 2015, states that crude oil is still the dominant energy source in Brazil and that the consumption keeps rising since 2013, being responsible for 2.2% of the world's energy consumption. The recent discovery of crude oil reservoirs at the Espirito Santo basin, Campos basin and Santos basin, can be considered as an excellent opportunity to supply the country's economic and energetic demands (Lima, 2010). These current exploration efforts are towards the so-called pre-salt reservoir, which is a geologic formation covered by a two thousand meters salt layer deposited in the ocean floor (Seabra et al., 2011). However, albeit the opportunity these crude oil reservoirs represent, offshore exploration offers risks to the microbiota and the whole sea life, as petroleum hydrocarbons are toxic, mutagenic, teratogenic and carcinogenic. (Hentati et al, 2013; Mckee et al, 2013).

Trindade Island is located at the South Atlantic Ocean, 1,160 km from the city of Vitória, capital of Espirito Santo State, Brazil. The island has 10.8 km^2 and its highest point reaches 600 m over the sea level. Its strategic location and the presence of endemic species of plants and animals justify the Brazilian interest and efforts for its protection (Alves & Castro, 2006; Mohr et al., 2009). Having control over the island allows Brazil to pledge, with United Nations (UN), the expansion of its area of Exclusive Economic Zone (EEZ), so that we can seek and explore resources in the area between Brazilian shoreline and the Trindade Island. Moreover, the expansion of the Brazilian EEZ will be beneficial for application and research of green energy technologies produced by the marine renewable energy industry, as wave energy converters (Mueller and Wallace, 2010), when energy sources based on fossil fuels become no longer available.

Microbes are responsible for nutrient cycling and can degrade even very recalcitrant hydrocarbons. They are capable of degrading crude oil hydrocarbons through a number of aerobic and anaerobic metabolic pathways, using these compounds as sources of carbon and energy (Zobell, 1946; Atlas, 1981; Haritash & Kaushik, 2009).

Our ability to describe the microbial world has increased significantly during the last 10 years, when the first next generation sequencing (NGS) methods of evaluating the microbial community were published (Edwards et al., 2006; Roesch et al, 2007; Caporaso et al., 2012). Taking advantage of the new sequencing technologies, more complete databases and more robust

68 approaches, we are now able to describe the microorganisms in a much better resolution than in
69 the time of Zobell (1946) and Atlas (1981).
70 This work aimed to evaluate the microbial community shift (Archaea, Bacteria and Fungi)
71 from Trindade Island coastal environment under crude oil contamination.
72

MATERIAL AND METHODS

Sampling site and soil analysis

Trindade Island soil was randomly sampled to the depth of 0-10 cm from the northeast shoreline of Trindade (coordinates: 20°30' S and 29° 19' W), under influence of native vegetation (*Cyperus atlanticus*). Soil cores were bulked, sieved (<2mm) and stored at 4°C until the microcosms were assembled. The sampling expedition occurred through April 2013 and was supported by the Brazilian Navy and PROTRINDADE Research Program. The National Counsel for Scientific and Technological Development (CNPq) provided all approvals and permits (project grant number 405544/2012-0 and authorization access to genetic resources process number 010645/2013-6) to conduct the study within this protected area. The field study did not involve endangered or protected species. A total of 11 chemical variables, plus physical properties, were assessed in the soil analysis. The protocol references and results are shown in Table 1.

Soil treatment with crude oil

Firstly, to simulate the ageing of crude oil exposed to environmental conditions at spillage conditions, we heated 500 mL of crude oil to 90^0 C and incubated for two hours in a fume hood. The resulting aged crude oil was a material highly viscous and difficult to work with. To obtain homogenous mixing of oil with soil, we dissolved the aged crude oil in hexane and applied to a subsample of each experimental soil. Hexane was also added to soils without crude oil to create a hexane-only contaminated control stock. These hexane (and crude oil + hexane) exposed soil stocks were kept in a fume hood until all hexane had evaporated. We then added 10 g of the control stock soil (hexane evaporated) to the flasks corresponding to 'Control', and made up to 20 grams with the corresponding soil that had not been exposed to hexane. The same procedure was repeated for the stock soils contaminated with crude oil, corresponding to the treatment 'Crude Oil'. This combination method was required to repopulate the native soil microbial community decimated by hexane. The final concentration of crude oil was 30g kg^{-1}. The flasks were incubated at 26 ^oC and the soil moisture was kept at 60% of its water holding capacity (remoistened periodically with deionized water upon reaching c. 50% water holding capacity).

Experimental design

To evaluate the effects of crude oil over the soil microbial community, we assembled six soil microcosms in respirometer flasks of 250 mL, containing 10 grams (dry weight) of each soil. The microcosms were incubated at 26 °C during 23 days, and the microbial activity was monitored by quantifying CO_2 emissions every 4 hours, using a continuous-flow respirometer coupled to an infrared CO_2 detector (TR-RM8 Respirometer Multiplex – Sable System) (HEINEMEYER et al., 1989). After this period, the flasks received 10g of either the two soil treatments upon the addition of stock soil treated with hexane for Control (Cntrl) or stock soil treated with crude oil and hexane to the crude oil contaminated treatment (Oil). The concentration of crude oil in the Oil treatment was 30g kg^{-1}. The incubation continued for more 15 days.

Molecular analyses

DNA extraction and quality check

Genomic DNA was extracted and purified from each soil sample (0.5 g) using the PowerMax® Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) following the manufacturer's instructions. The purity of the extracted DNA was checked using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) (260/280 nm ratio), DNA concentration was determined using Qubit® 2.0 fluorometer and dsDNA BR Assay kit (Invitrogen™). The integrity of the DNA was confirmed by electrophoresis in a 0.8 % agarose gel with 1 X TAE buffer.

High-throughput sequencing

Sequencing was done on the Illumina MiSeq® platform (Caporaso et al. 2012) at the High-throughput Genome Analysis Core (HGAC), Argonne National Laboratory (Illinoi, USA). Bacterial and archaeal 16S rRNA genes were amplified using primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') for paired-end microbial community. Fungal ITS1 region was amplified using primers ITS1F (5'-CTTGGCCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') using the method described by SMITH & PEAY (2014).

135

136 **Data analysis**

137 We applied the 16S and ITS bioinformatics pipeline recommended by the Brazilian
138 Microbiome Project, available at http://brmicrobiome.org (Pylro et al, 2014). This pipeline uses
139 QIIME (Caporaso et al., 2010) and Usearch 7.0 (Edgar, 2010) for filtering low quality sequences,
140 clustering sequences of high similarity, diversity analysis, diversity comparisons and graphical
141 plotting. For fungal ITS analysis we also used the software ITSx (Bengtsson-Palme, 2013) for
142 taxonomic assignment improvement. The microbial diversity changes were measured using the
143 alpha diversity metrics: PD_whole_tree (for 16s rRNA gene only), Chao (Chao, 1984) and
144 observed species. For beta diversity estimations, we generated distance matrixes using the
145 phylogenetic method weighted unifrac (Lozupone et al, 2005) for 16s rRNA gene sequences and
146 the Bray-Curtis (Bray & Curtis, 1957) method for ITS sequences. We plotted the beta-diversity
147 distance matrixes using a three-dimensional Principal Coordinates Analysis (PCoA). The
148 hypothesis testing method used to compare treatments was a two-sided Student's parametric t-test
149 with p-values Bonferroni-corrected for multiple comparisons.

150

RESULTS

Soil respiration and physicochemical characteristics

The Trindade Island soil samples were incubated at 26 °C, with no crude oil addition during 23 days, as an acclimatizing period in order to allow the microbial community to eliminate most of the immediately accessible organic carbon. At the 24th day of incubation, we split the samples in two treatments, control and crude oil 30g kg^{-1}. CO_2 emission rate by oil-contaminated samples increased 8x compared to the control in the first 4 hours (see figure 1). Three days after contamination, emission rate peaked at more than 20x the control. CO_2 emission of the oil treated samples was higher than the control during all the incubation period (38 days). At the last day of incubation (38th), CO_2 emission rate of the contaminated treatment was still almost 4 times higher than the control (figure 1).

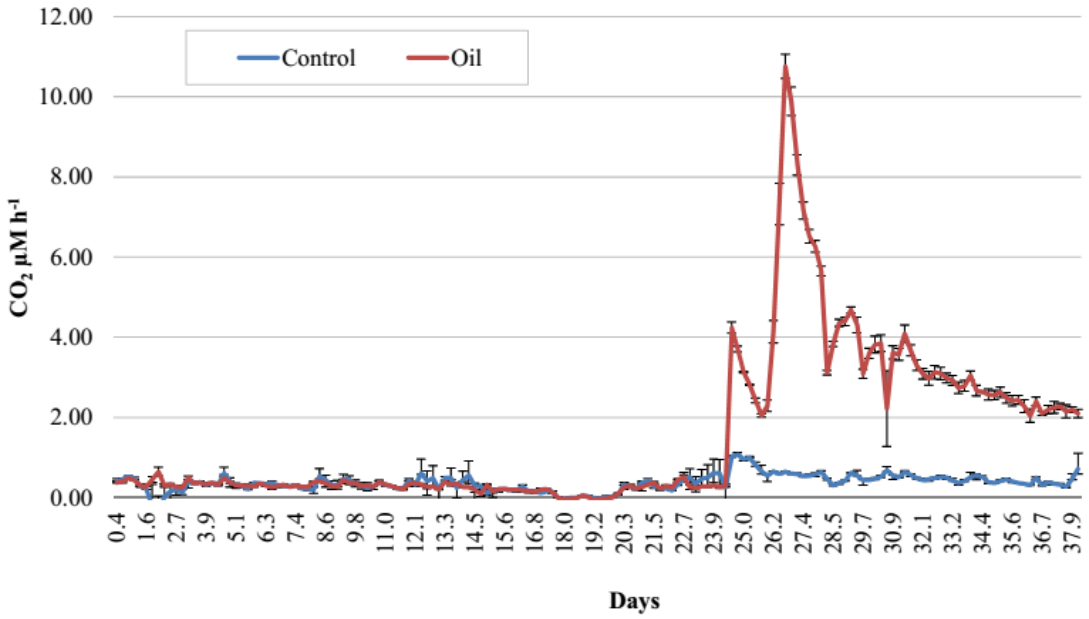


Figure 1 Respiration analysis of Trindade Island coastal soil microcosms. Average CO_2 emission rates evaluated during 38 days of incubation. Emissions until 24 days represent the acclimatizing period without oil addition. Readings after 24 days show the differences in CO_2 emissions after establishing the two treatments (Control and Oil). The microcosms were incubated at 26 °C and CO_2 emission was monitored by an automated respirometer coupled to an infrared CO_2 detector.

169 Table 1 – Summary of physicochemical data for surface soil cores (0 - 10 cm) sampled at the
170 northeast coast of Trindade Island - Brazil.

Characteristic	Unit	Value
pH – H_2O		5.6
Soil texture		Sandy Loam
P-rem [1]	mg L^{-1}	26.5
P[2]	mg kg^{-1}	1290.8
K[2]		180.33
S[3]		5.63
Ca^{+2}[4]	$cmol_c$ kg^{-1}	9.84
Mg^{+2}[4]		2.78
OM	%	0.64
N		0.19
C[5]		0.37

171 [1] Remaining phosphorus (Alvarez et al., 2000). [2] Extracted with Mehlich – 1. [3] Extracted with
172 monocalcium phosphate in acetic acid (HOEFT et al., 1973). [4] Extracted with KCl 1 mol L^{-1}. [5] Walkley
173 and Black method/OM = C.org * 1.724.

174 **Sequencing results**

175 A total of 314,748 joined and quality filtered 16S rRNA gene Illumina® barcoded reads,
176 and 424,269 single end quality filtered fungal ITS Illumina® barcoded reads were obtained from
177 the soil samples (Table S1). The contaminated treatment yielded a smaller amount of sequences.
178 To minimize the effects of sequencing depth variation on diversity analysis and taxa comparison,
179 we applied the rarefaction method (random subsampling of sequences to equalize sequencing
180 effort). Estimates of alpha- and beta-diversity were based on evenly rarefied OTU matrices (45,695
181 sequences per sample for Bacteria and Archaea and 25,315 sequences per sample for Fungi).

182 **Alpha diversity comparison**

183 The alpha diversity indexes used in this experiment represent species richness (tables 2 and
184 3). We compared treatment's effects over Bacteria/Archaea community using the estimators
185 Faith's PD (phylogenetic measure of diversity based on total branch length of phylogeny captured
186 by a sample, developed by Faith, 1992), the Chao-1 (estimator of total species richness developed

187 by Chao, 1984), and observed species (number of species detected) (table 2). The effects on Fungal
188 community was measured using only the Chao-1 and Observed species estimators, as there was
189 not an ITS1 phylogenetic tree available to use the Faith's PD estimator. All methods yielded
190 similar results for Bacteria/Archaea and Fungi. The comparison between the two treatments shows
191 a significant reduction of diversity upon the addition of oil for Bacteria, Archaea and Fungi. Fungal
192 community was the most sensitive group to the oil addition, showed a reduction of ~40% for the
193 indexes Chao1 and Observed species (table 3).
194
195 Table 2 – Average alpha diversity comparison between the treatments control and crude oil for
196 bacteria and archaea groups.

Treatments	Sequences	PD whole tree	Chao 1	Observed species
Crude Oil	45690	164.87	2796.51	2443.20
Control	45690	177.51	3107.12	2679.23
p-value*		0.012546	0.047083	0.018392

197 *Two-sample parametric t-test. Rarefaction to 45,690 sequences for each sample.
198
199 Table 3 – Average alpha diversity comparison between the treatments control and crude oil for
200 fungi.

Treatments	Sequences	Chao 1	Observed species
Crude Oil	25315	69.96	67.8
Control	25315	100.58	96.46
p-value*		0.0548	0.0681

201 *Two-sample parametric t-test. Rarefaction to 25,315 sequences for each sample.
202
203 The rarefaction analysis (figures 3 and 4), which plots the operational taxonomic unit
204 (OTU) richness as a function of sequencing deph, shows that sequencing effort was sufficient to
205 capture the Bacterial, Archaeal and Fungal diversity of samples. The analysis also confirms that
206 crude oil had a reductive effect on microbial diversity, especially for Fungi.

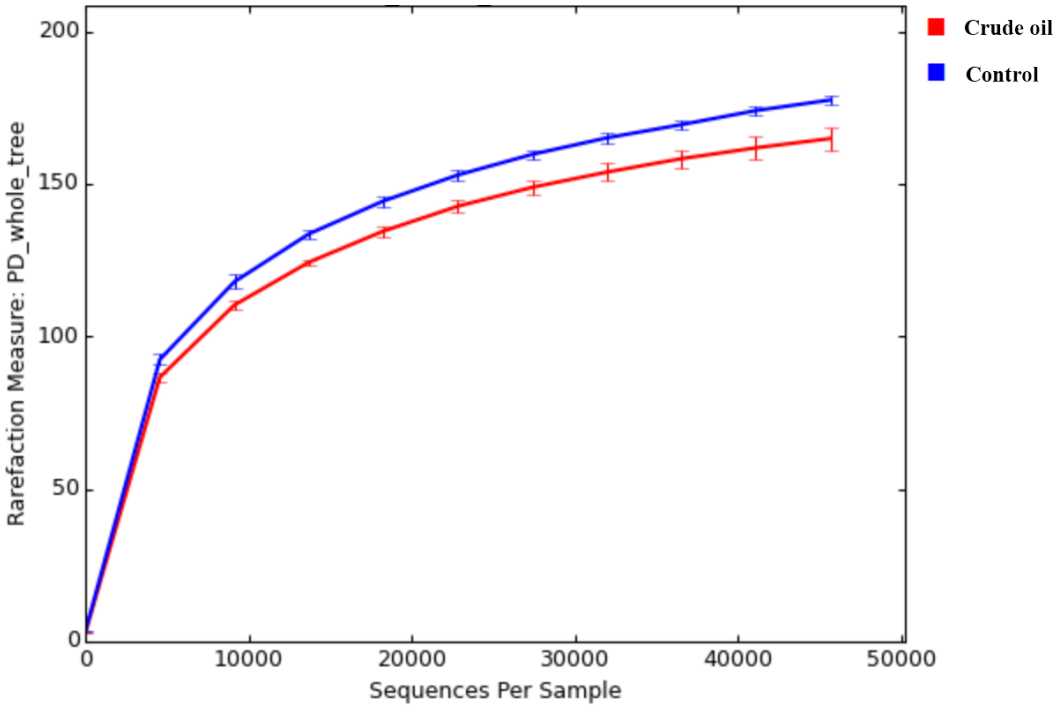
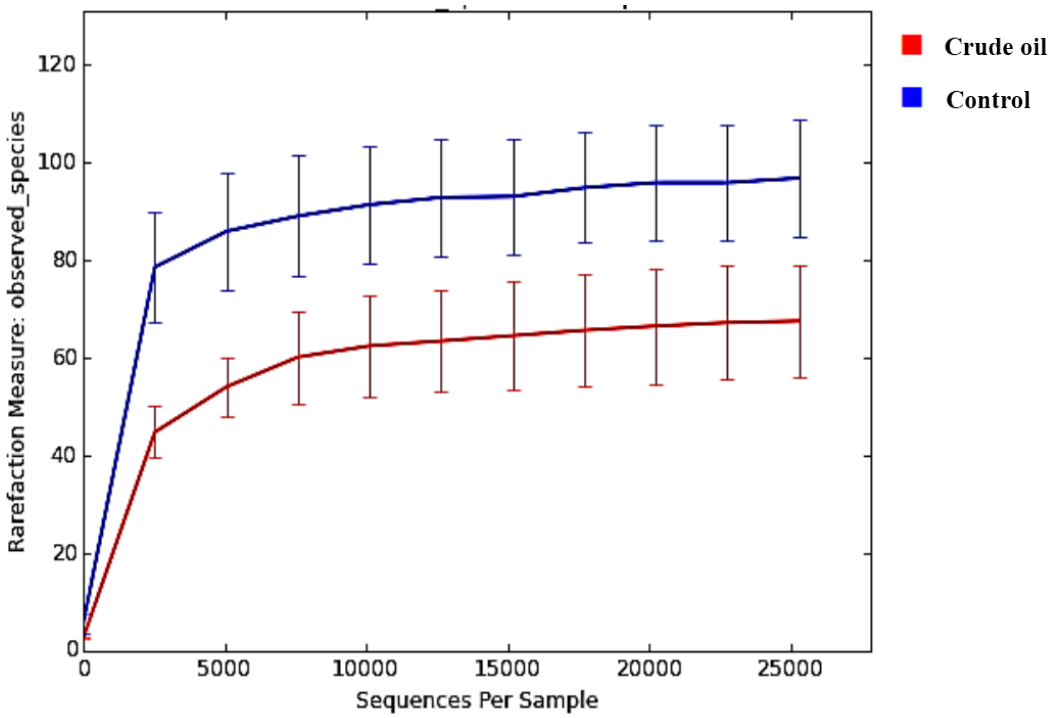


Figure 2 Average alpha diversity rarefaction plot at different rarefaction dephs, of phylogenetic diversity for Control and Oil treatment of microcosms from Trindade Island coastal soil samples, based on partial sequences of bacterial/archaeal 16S rRNA genes.



212

213 Figure 3 Average alpha diversity rarefaction plot at different rarefaction dephs. Observed species
214 for treatments Control and Oil of microcosms from Trindade Island coastal soil samples, based on
215 partial sequences of fungal ribosomal intergenic spacer ITS1.

216 **Beta Diversity comparison**

217 The beta diversity analysis was performed using two different metrics (figures 2 and 3),
218 Weighted Unifrac for 16s rRNA gene and Bray-Curtis for fungal intergenic spacer ITS1 due to the
219 lack of a phylogenetic tree for ITS1 marker. Both methods showed two very distinct clusters (q-
220 value significative under 5% of probability).

221

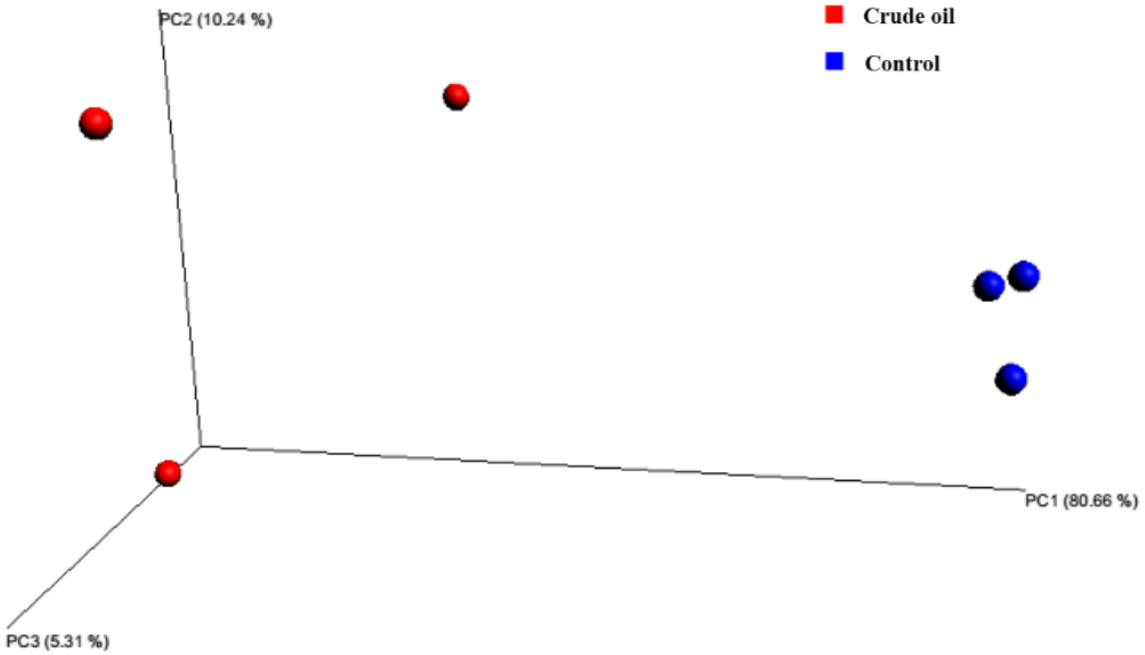


Figure 2 Principal coordinate analysis (PCoA) of Weighted Unifrac distances generated from taxa tables summarized at the genus level for bacterial and archaeal partial 16S rRNA gene. Three coordinates explain 95.21% of the data. The PC1 explains 80.66% of data distribution, the PC2 explains 10.24% of data distribution and the PC3 explains 5.31% of data distribution. The blue dots correspond to Control samples and the red dots correspond to Crude Oil contaminated samples.

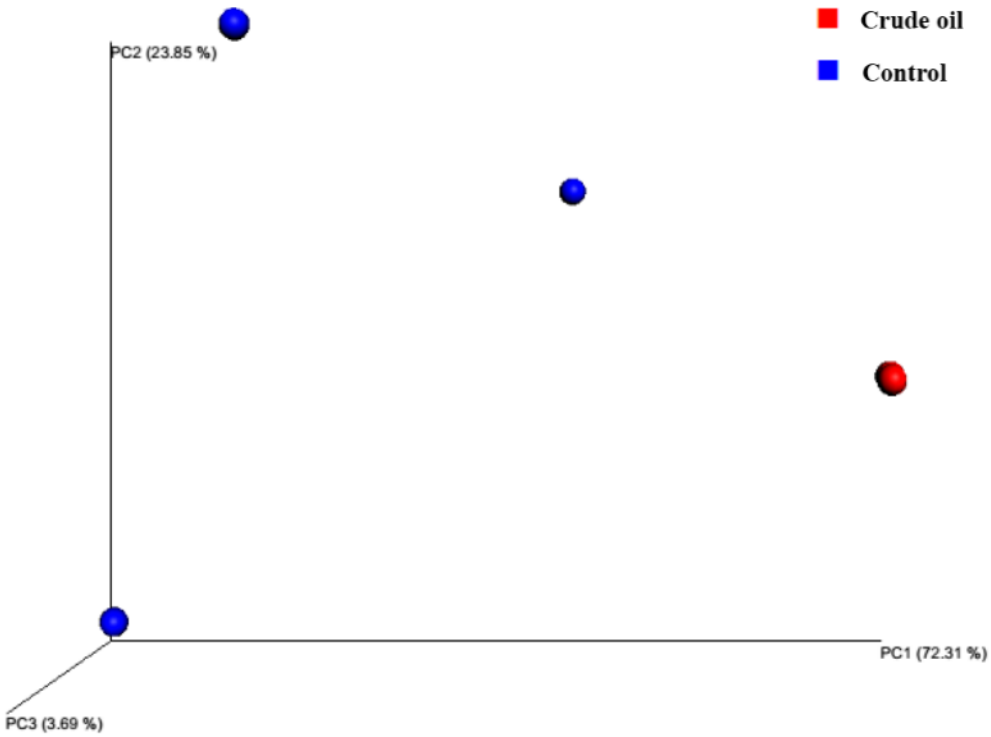


Figure 3 Principal coordinate analysis (PCoA) of Bray-Curtis distances generated from taxa tables summarized at the genus level for fungal partial ribosomal intergenic spacer ITS1, rarefied at 25315 reads per sample. Three coordinates explain 99.85% of the data. The PC1 explains 72.31% of data distribution, the PC2 explains 23.85% of data distribution and the PC3 explains 3.69% of data distribution. The blue dots correspond to Control samples and the red dots correspond to Crude Oil contaminated samples.

Taxonomic comparison

The taxonomic distributions of Bacteria/Archaea are shown in table S2 at phylum level. The control treatment show 6% of sequences to be from the Archaea domain, 93.4% from Bacteria domain and 0.5% were not assingned to any taxa from the GreenGenes database (DeSantis et al., 2006). For Archaea, we found only three representatives: the species *Candidatus nitrosphaere* belonging to the phylum Crenarchaeota, the order E2 belonging to the phylum Euryarchaeota and the order YLA114 belonging to the phylum Parvarchaeota. The addition of oil reduced the relative abundance of archaea to 2.7%.

We identified 478 genera belonging to 225 orders in the bacterial group of the control samples (93.4% of total 16s rRNA gene sequences). The most abundant bacterial orders in the control were Acidobacteria order iii1-15 (7%), Rhizobiales (6.5%), Rubrobacterales (6.3%), Nitrospherales (6.1%), Xanthomonadales (4.8%), Syntrophobacterales (4.2%), Gaielalles (4%) and Myxococcales (4%). Oil-contaminated samples presented 463 genera belonging to 224 orders, and the most abundant orders were Actinomycetales (17%), Acidobacteria order iii1-15 (8.5%), Rhizobiales (6.4%), Burkholderiales (4%), Xanthomonadales (3.9%), Chloroacidobacteria order RB41 (3.4%), Sphingomonadales (3%), Acidimicrobiales (2.9%). Abundance of 9 taxa was significantly different between control and oil-contaminated soils (table 5).

Table 4 – Bacterial/archaeal OTUs presenting an average absolute abundance significantly different between the treatments "Oil" and "Control", under the two-way Student t-test and $\alpha = 0.05$.

OTUs	q-value*	Crude Oil	Control	Taxonomy
OTU_112	0.046688	30.7	99.0	o__Myxococcales
OTU_292	0.046688	33.3	0.7	g__Burkholderia
OTU_9	0.046688	1247.7	55.3	f__Streptomycetaceae
OTU_1050	0.046688	1.7	18.3	g__Bacillus
OTU_6	0.046688	401.3	1715.7	g__Rubrobacter
OTU_231	0.046688	9.3	41.0	f__Gemmataceae
OTU_238	0.046688	7.0	31.7	g__Cellvibrio
OTU_252	0.046688	100.3	39.7	g__Streptomyces
OTU_583	0.054659	17.7	8.0	o__Solirubrobacterales

*p-values corrected by the FDR method.

Fungal taxonomy analysis (table S3) was assessed using the UNITE database (Kõljalg et al., 2005), version 7. 5% of the reads from non-contaminated soil were not assigned to any taxonomic group. For the crude oil treatment, only 0.7% of the sequences did not match to a taxum. We found 43 genera belonging to 29 orders in the fungal group of the control samples. The most abundant orders in the control were Hypocreales (41%), Mortierellales (27%) and Sordariales (7.5%). Oil-contaminated samples presented 48 genera belonging to 29 orders, and the most abundant orders were Mortierellales (70%), Hypocreales (24%) and Botryosphaeriales (1.1%). Abundance of 4 taxa was significantly different between control and oil-contaminated soils (table 5).

Table 5 – Fungal OTUs presenting an average absolute abundance significantly different between the treatments "Crude Oil" and "Control", under the two-way Student t-test and $\alpha = 0.07$.

OTUs	q-value*	Crude Oil	Control	taxonomy
OTU109	0.007762	19800.67	833.4	p__Ascomycota; g__*Fusarium*
OTU067	0.014401	2442.67	96.4	p__Ascomycota; f__Clavicipitaceae
OTU767	0.061176	68780.33	11366.7	p__Zygomycota; g__*Mortierella*
OTU636	0.061176	1048.33	63.3	p__Zygomycota; s__*Mortierella ambigua*

*p-values corrected by the FDR method.

Discussion

In this study, we characterized the microbial community of a soil from the northeast shoreline of Trindade Island. We also evaluated the effects of oil amendment on the soil microbial diversity and on microbial taxa at different taxonomic levels. We found that crude oil had a deleterious effect on microbial alpha-diversity. This result is similar to the obtained by Yang (2014), as crude oil was thought to have an eco-toxicological effect. Besides toxic effect, we also suppose that some taxa able to use oil hydrocarbons as a source of carbon and energy were favoured by oil amendment, gradually overcoming the populations without those abilities. The effects on fungal diversity were more severe than that observed on bacterial and archaeal diversity, corroborating Embar (2006), who mentioned a reduction and quick specification of fungal community in response to oil contamination. The strong effect of oil on the fungal diversity may also be explained by metabolic differences between fungi and bacteria. This effect relates to the increased toxicity of polycyclic hydrocarbons present in crude oil after metabolic activation mediated by the enzyme cytochrome P450 (CYP) of eukaryotes. Shimada and Fuji-Kuriama (2003) explain that most carcinogens in the environment are inert by themselves and require the metabolic activation by CYP, in order to exhibit carcinogenicity. The CYP genes belong to the superfamily of dioxygenases, present in all domains of life. This superfamily is so diverse that can share similarities smaller than 16% (Werck-Reichhard et al., 2000). Genes that code for dioxygenases in prokaryotes are related to toxin and xenobiotic degradation, while in eukaryotes

CYP genes may be related to a plethora of functions, ranging from biosynthesis of hormones to chemical defence in plants (Werck-Reichhard et al., 2000).

During the analysis of beta diversity of Bacteria and Archaea, (figure 2) we found that oil-contaminated samples showed a broader variation in the three-dimension PCoA. Compared to the control, whose replicates are clustered more tightly. Because of selective pressure, the taxa that resisted the contamination event and the populations able to degrade hydrocarbons will gradually outnumber the rest of the community in the curse of succession (Yang et al., 2014). Therefore, as oil presented a toxic effect, we would expect that the bacterial community of contaminated samples would show a more compact clustering, as happened with the fungal community. However, as bacterial community is several times more diverse than the fungal community is, the shifts in the bacterial community might be more related to soil microhabitats present in each replicate, than with the oil effect. This phenomenon was also observed in other works (Juck et al., 2000; Liang et al., 2011; Yang et al., 2014) and could be explained by the appearance of new niches in the contaminated soil, as a result of carbon addition (crude oil) and death of more abundant populations, while the control samples didn't suffer any changes and no addition of carbon, being then less susceptible to changes.

Soil is the most diverse environment on earth, and many of the native microorganisms possess the ability to resist and degrade crude oil hydrocarbons. These groups will have an advantage on contaminated sites. In this study, we were able to detect community shifts in Actinobacteria, Proteobacteria, Firmicutes and Planctomycetes. The phylum Actinobacteria had its abundance increased in response to crude oil addition. We detected shifts in one unidentified specie from the family Streptomycetaceae, one specie from the genre *Streptomyces* and one specie from the order Solirubrobacterales. Several studies have reported Actinobacteria as a good option for removing recalcitrant hydrocarbon, they are known for the production of extracellular enzymes that degrade a wide range of complex hydrocarbons, also, many species of Actinobacteria are able to produce biosurfactants that enhance hydrocarbons solubility and bioavailability (Pizzul et al., 2007; Kim & Crowley, 2007; Balachandram et al., 2012). The Actinobacteria phylum is recognized as the main alkane degrader in polar soils (Aislabie et al., 2006) and are known for producing multiple types of antifungals, antivirals, antibiotics, immunosuppressives, anti-hypertensives, antitumorals (Benedict, 1953; Omura et al., 2001; Khan et al., 2011; de Lima Procópio et al., 2012). Although some works have reported prevalence of gram negative bacteria

upon soils contaminated with heavily weathered petroleum (Kaplan & Kitts, 2004), our work shows a big shift on gram-positive Actinobacteria. Our results corroborate with Chikere and collaborators (2009) who reports the prevalence of Actinobacteria after oil addition. The capability of antibiotic production by Actinobacteria may have controlled the growth of other groups who also have the molecular apparatus to use petroleum hydrocarbons as a carbon source and reinforces the importance of microbial diversity analysis isolated environments as Trindade Island, showing the biotechnological potential of microbial populations from these environments.

Supporting data

Sequence data is available in the MG-RAST under the accession numbers 4643785.3 and 4643786.3

Suplementary material

Table S1 – Number of quality filtered Illumina® barcoded sequences

Treatments	16S rRNA gene	Fungal Intergenic Spacer (ITS1)
Crude Oil	45695	25315
Crude Oil	47917	46921
Crude Oil	52889	55579
Control	54458	91866
Control	55487	100736
Control	58337	103852
Total	314748	424269

Sequences of 16S rRNA gene (average length 253 bp) and Fungal Intergenic Spacer ITS1 (average length 250 bp). The DNA sequenced was extracted from three replicate microcosms contaminated with crude oil and three control replicate microcosm, assembled from Trindade Island coastal soil.

348 Table S2 – 16s rRNA gene taxonomy at the phylum level and relative abundance for each treatment
349 (n=3).

Taxa	Contaminated	Control
Unassigned	0.005413	0.005378
k__Archaea;p__Crenarchaeota	0.027973	0.061058
k__Archaea;p__Euryarchaeota	9.56E-05	8.32E-05
k__Archaea;p__[Parvarchaeota]	0.000246	0.000178
k__Bacteria;p__	4.78E-05	2.97E-05
k__Bacteria;p__Acidobacteria	0.152743	0.132438
k__Bacteria;p__Actinobacteria	0.312005	0.252267
k__Bacteria;p__Armatimonadetes	0.002751	0.003898
k__Bacteria;p__BHI80-139	2.05E-05	4.16E-05
k__Bacteria;p__BRC1	0.000287	0.000475
k__Bacteria;p__Bacteroidetes	0.027877	0.039565
k__Bacteria;p__Chlamydiae	0.000171	0.000624
k__Bacteria;p__Chlorobi	0.001372	0.001527
k__Bacteria;p__Chloroflexi	0.026314	0.031049
k__Bacteria;p__Cyanobacteria	0.000921	0.000945
k__Bacteria;p__Elusimicrobia	0.001283	0.001444
k__Bacteria;p__FBP	0.000171	0.000172
k__Bacteria;p__Fibrobacteres	0.000266	0.000535
k__Bacteria;p__Firmicutes	0.007072	0.018047
k__Bacteria;p__GAL15	7.51E-05	9.51E-05
k__Bacteria;p__GN02	2.05E-05	2.97E-05
k__Bacteria;p__Gemmatimonadetes	0.036546	0.03821
k__Bacteria;p__NKB19	1.37E-05	1.19E-05
k__Bacteria;p__Nitrospirae	0.01073	0.01674
k__Bacteria;p__OD1	0.000287	0.000309
k__Bacteria;p__OP11	2.05E-05	5.35E-05
k__Bacteria;p__OP3	0.000137	0.00016
k__Bacteria;p__Planctomycetes	0.037194	0.046226
k__Bacteria;p__Proteobacteria	0.322537	0.324432
k__Bacteria;p__SBR1093	6.83E-06	1.19E-05
k__Bacteria;p__TM6	0.00028	0.000398
k__Bacteria;p__TM7	0.000539	0.001058
k__Bacteria;p__Verrucomicrobia	0.022095	0.02043
k__Bacteria;p__WPS-2	8.19E-05	7.13E-05
k__Bacteria;p__WS2	4.10E-05	6.54E-05
k__Bacteria;p__WS3	0.002321	0.001872
k__Bacteria;p__[Thermi]	4.78E-05	7.13E-05

350

351

352 Table S3 – ITS taxonomy at the order level and relative abundance for each treatment (n=3).

Taxa	Contaminated	Control
Unassigned	0.007435	0.049978
k__Fungi	0.000236	0.004084
p__Ascomycota	0.000263	0.003028
p__Ascomycota;o__Botryosphaeriales	0.011381	0.018988
p__Ascomycota;o__Capnodiales	0.001359	0.013653
p__Ascomycota;o__Dothideales	0	0.000133
p__Ascomycota;o__Incertae sedis	3.04E-05	0.000289
p__Ascomycota;o__Pleosporales	0.001444	0.003779
p__Ascomycota;o__unidentified	0.000196	0
p__Ascomycota;o__Chaetothyriales	0.000445	0.013285
p__Ascomycota;o__Eurotiales	0.001815	0.025154
p__Ascomycota;o__Onygenales	8.43E-05	0.001252
p__Ascomycota;o__Incertae sedis	4.72E-05	7.04E-05
p__Ascomycota;o__Helotiales	0.000415	0.011086
p__Ascomycota;o__Orbiliales	0.000337	0.000915
p__Ascomycotas;o__Saccharomycetales	0.000152	0
p__Ascomycota;Other	0	0.000743
p__Ascomycota;o__Chaetosphaeriales	0	0.000415
p__Ascomycota;o__Diaporthales	0.000557	0.000344
p__Ascomycota;o__Hypocreales	0.243829	0.413457
p__Ascomycota;o__Microascales	0	0.000102
p__Ascomycota;o__Sordariales	0.006537	0.075445
p__Ascomycota;o__Xylariales	0.001366	0.005719
p__Ascomycota;o__unidentified	0.002368	0.022407
p__Ascomycota;o__unidentified	0.003643	0.019082
p__Basidiomycota;o__Boletales	0.000317	0
p__Basidiomycota;o__Cantharellales	0.000351	0
p__Basidiomycota;o__Russulales	0.000162	0
p__Basidiomycota;o__Thelephorales	0.003076	0.02887
p__Basidiomycota;o__Trechisporales	0.000557	0.002378
p__Basidiomycota;o__Sporidiobolales	9.78E-05	0.001361
p__Basidiomycota;o__Filobasidiales	0	0.001064
p__Zygomycota;o__Mortierellales	0.706818	0.268278
p__unidentified;	0.004682	0.014638

353

Additional Information and Declarations

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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