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# Bacterial interactions and implications for oil biodegradation process in mangrove sediments

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ABSTRACT

Mangrove sediment harbors a unique microbiome and is a hospitable environment for a diverse group of bacteria capable of oil biodegradation. Our goal was to understand bacterial community dynamics from mangrove sediments contaminated with heavy-oil and to evaluate patterns potentially associated with oil biodegradation is such environments. We tested the previously proposed hypothesis of a two-phase pattern of petroleum biodegradation, under which key events in the degradation process take place in the first three weeks after contamination. Two sample sites with different oil pollution histories were compared through T-RFLP analyses and using a pragmatic approach based on the Microbial Resource Management Framework. Our data corroborated the already reported two-phase pattern of oil biodegradation, although the original proposed explanation related to the biophysical properties of the soil is questioned, opening the possibility to consider other plausible hypotheses of microbial interactions as the main drivers of this pattern.



## 1. Introduction

Microbial communities in contaminated ecosystems tend to be dominated by the organisms that can degrade or tolerate the contaminant. Since contamination is a strong selection force, these communities are typically less diverse than those in non-stressed ecosystems. Several studies on oil contamination reported a drastic short-term reduction in the diversity of the bacterial communities, which could be accounted for by oil toxicity and strong selection for particular hydrocarbonoclastic bacteria, such as *Alcanivorax* spp. and *Cycloclasticus* spp. (Hazen et al., 2010; Jurelevicius et al., 2013; Kimes et al., 2012; Kostka et al., 2011; Sutton et al., 2012).

It has been reported that oil biodegradation follows a two-phase pattern, characterized by a first phase of fast petroleum degradation with high abundance of few species, followed by a second phase of slower petroleum degradation with high richness of low abundant species. This two-phase pattern has been related to the bioavailability of free total petroleum hydrocarbons (TPH) in the first phase and with a slower desorption rate of soil-sequestered TPH in the second phase (Kaplan & Kitts, 2004).

Several studies suggest that mangrove is a hospitable environment for the growth of a diverse group of bacteria capable of oil biodegradation (Brito et al., 2006; Gomes et al., 2008; Jurelevicius et al., 2013; Liu et al., 2011; Ramsay et al., 2000; Santos et al., 2010; Tian et al., 2008). Mangroves are intertidal ecosystems along the coastlines of tropical and subtropical regions, with unique features such as high primary productivity, abundant detritus, rich organic carbon content and anoxic/reduced (Ghizelini et al., 2012). In tropical mangroves, bacteria and fungi constitute 91% of the total microbial biomass, whereas algae and protozoa represent only 7% and 2%, respectively (Alongi, 1987). Microbial structure and function of mangroves are directly responsible for this ecosystem functioning (Holguin et al., 2001).

Mangrove sediments harbor a unique microbiome and metabolic reconstructions suggest that ecological processes may be modulated by the prevailing conditions found in mangrove (Andreote et al., 2011). We conducted a laboratory oil contamination experiment using sediments from two mangroves with different oil contamination histories, aiming to test the two-phase pattern of oil biodegradation hypothesis (Kaplan & Kitts, 2004). We approached this goal by performing an ecological survey (Marzorati et al., 2008) aiming at understanding bacterial community dynamics from mangrove sediments under heavy-oil contamination stress, and at looking for common patterns that may be associated with oil biodegradation is such environments. This ecological survey is a key step in the decision flow of the Microbial Resource

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Management Framework (Read et al., 2011; Verstraete et al., 2007), which was developed with the goal of finding sustainable solutions to global challenges, through the use of microorganisms.

## 2. Materials and methods

### 2.1. Sampling sites and sample collection

Four sampling sites were chosen with respect to their different hydrocarbon pollution history. Sampling sites GBA (22°41′14.5″S; 43°05′6.83″O) and GBB (22°41′1.55″S 43°05′9.21″O) were located in the Guanabara Bay, in the city of Rio de Janeiro, Brazil, and sampling sites GR (21°36′27.85″S 41°03′05.74″O) and GV (21°35′9.11″S 41°03′39.70″O) were located in Gargaú, in the city of São Francisco do Itapaboana, in the northern part of the state of Rio de Janeiro, Brazil (Fig. 1). Physicochemical parameters of the four sampling sites are shown in Table 1. The organic carbon (OC) and total nitrogen (TN) were measured in an elemental analyzer (Flash 2000) and the values were expressed in percentage of dry weight (%). Organic carbon was analyzed after direct acidification in silver vials and total nitrogen in bulk sample. Analytical coefficients of variation for elemental compositions were below 5% for individual samples and accuracy was determined using a certificate material (Low Organic Content Soil, Elemental Microanalysis) with a 97% of recovery.

Guanabara Bay is notorious for its chronically polluted conditions, with a history of oil spill accidents (Ghizelini et al., 2012). The mangrove in Gargaú is located in the estuary of Rio Paraíba do Sul, the biggest estuary in the northern region of the state of Rio de Janeiro. The degradation of this mangrove is primarily related to selective logging and deforestation for the implantation of pastures for cattle ranching, raw sewage, urban runoff, industrial waste release, and construction of roads and landfills (Bernini et al., 2010). There is no record of oil spill in this area.

For each site, three composite samples consisting of five sediment cores each (c. 10 cm of top sediment with 8 cm diameter) were randomly collected. The samples were at least 10 m apart from each other and within each sample the cores were at least 1 m distant from each other. Within each site, the composite samples were collected at the same time, during the low tide. After collection, they were transported to the laboratory in an insulated container with ice, where they were thoroughly homogenized to one representative sample per locality and immediately processed.

**Table 1**
Physicochemical parameters of the four sampling sites (GBA, GBB, GV, and GR) considered in this study.

| | | GBA | GBB | GV | GR |
|---|---|---|---|---|---|
| pH | | 7,7 | 7,6 | 6,8 | 6,1 |
| Salinity | | 24 | 24 | 4 | 3 |
| Granulometry (%) | Sand | 30 | 76 | 14 | 12 |
| | Clay | 13 | 6 | 18 | 20 |
| | Silt | 57 | 18 | 68 | 68 |
| OC (%) | | 5,72 | 0,75 | 5,86 | 7,56 |
| TN (%) | | 0,24 | 0,04 | 0,39 | 0,43 |

OC (organic carbon); TN (total nitrogen).

### 2.2. Artificial oil contamination

Heavy oil contamination was performed using fresh sediment samples from each locality and the oil biodegradation process was monitored weekly during the first month and then monthly during the four following months, when the oil was visually degraded. This strategy was based in the reported two-phase pattern of petroleum degradation, where key events in the degradation process take place in the first three weeks after the contamination (Kaplan & Kitts, 2004). Fifty grams of fresh samples were incubated at 28 °C in an Erlenmeyer flask with 450 ml of mineral medium ($K_2HPO_4$ 0,1%; $KH_2PO_4$ 0,1%; $NH_4Cl$, 0,1%; $MgSO_4 \cdot 7H2O$ 0,05%, $CaCl_2$ 0,001%, $FeSO_4$ 0,001%) and 2% oil. Samples were kept shaking at 120 rpm. Aliquots of sediment were taken weekly for DNA extraction, at days 7, 14, 21 and 28 of incubation. The samples were kept under the same conditions until the oil was visibly degraded, which happened after 5 months. Mineral medium was added monthly. Aliquots of sediment for DNA extraction were taken monthly, at 60, 90, 120, and 150 days of incubation.

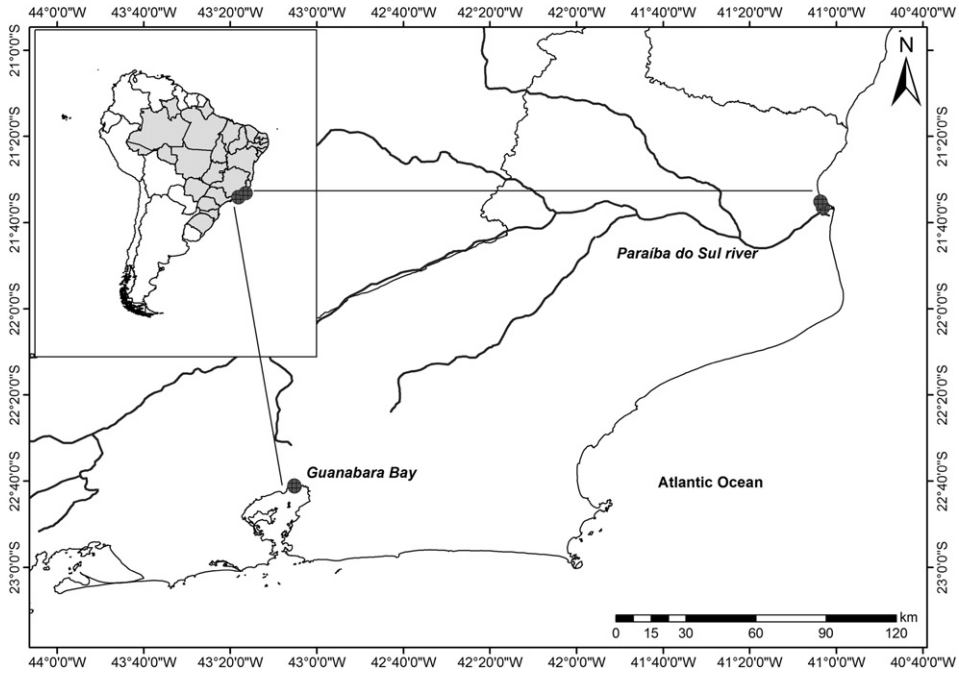


**Fig. 1.** Location of the sampling sites considered in this study.

### 2.3. Molecular analyses

Total genomic DNA was extracted from 1 g of sediments using the UltraClean Soil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA), following the manufacturer's instructions. The extractions were performed immediately after the sample collection and at the following oil-enriched intervals: 7, 14, 21, 28, 60, 90, 120, and 150 days. Primers 27F (5′AGAGTTTGATCCTGGCTCAG) labeled at the 5′ end with 6-carboxyfluorescein (6-FAM), and 1525R (5′AAGGAGGTGWTCCARCC) were used to amplify approximately 1500 bp of the 16S rRNA gene. The PCR reaction (20 μl) contained 10 ng of template DNA, 5 pmol of each primers, 10 μl do kit *HotStarTaq® Master Mix Kit* (Qiagen Inc., Valencia, CA, USA), 5 μl of purified water. The amplification conditions were 1 cycle of 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 52 °C for 30 s, with a final extension of 72 °C for 1 min and 30 s. Amplicons (20 ng) were digested using *Mnl*I, following the manufacturer's instructions. The digested DNA was ethanol-precipitated and resuspended with 14,8 μl of Hi-Di formamide mixed with 0,2 μl of *standard Gene Scan 600 Liz* (Applied Biosystems, Foster City, CA, USA). After this, the sample was separated by capillary electrophoresis in an ABI 3500 Genetic analyzer and analyzed with GeneMapper version 4.1 (Applied Biosystems, Foster City, CA, USA), using a baseline detection value of 5 fluorescence units. All T-RFs over this baseline value and with lengths from 50 to 600 bp were rounded to the nearest integer. Peak filtration and binning were performed with R software using the IBEST script suite (Abdo et al., 2006). True peaks (operational taxonomic units, OTUs) were distinguished from background noise, based on a three-fold standard deviation (IBEST default). Each peak corresponded to one OTU.

### 2.4. Data analysis

T-RFLP fingerprinting is a high-throughput, culture-independent method for community profiling originally developed for characterizing highly diverse bacterial communities (Liu et al., 1997). It is a reproducible and robust method that results in high-quality community fingerprints (Osborn et al., 2000). In spite of some technical limitations, (Schütte et al., 2008), T-RFLP results are generally consistent with the results from clone libraries (Dunbar et al., 2000; Hackl et al., 2004) and next-generation sequencing (NGS) technologies (Bokulich and Mills, 2012; Camarinha-Silva et al., 2011; Pilloni et al., 2011).

The T-RFLP data consisted of four data sets: GBA, GBB, GR and GV. These datasets were analyzed considering two periods: the first month, when the bacterial communities were monitored weekly (time points 0, 7, 14 21 and 28 days); and the four consecutive months, when the bacterial communities were monitored monthly (time points 60, 90, 120, 150). The relative abundances of the binned *Mnl*I fragments were used to monitor changes in the bacterial community along the oil biodegradation process. An ecological survey (Marzorati et al., 2008) was used to describe the bacterial community structure and dynamics of each dataset. Briefly, the range-weighted richness index (Rr) was estimated as the total number of peaks in the electropherogram. The dynamics of the community (Dy) was estimated by calculating the rate of change parameter (Δt) through moving-window analyses (MWA). First, a matrix of similarity was calculated based on the Pearson correlation coefficient. The percent change (percent change = 100 − percent similarity) was then calculated. The percent change value matrix was used to perform MWA by plotting the values between day x and day x − 7 days for the first month, and day x and day x − 30 days for the following months. The rate of change (Δt) was calculated as the average and standard deviation of the respective percent change values. In addition, moving-endpoint analyses (MEA) was performed, comparing the community profiles from different time points with the profile from the first sampling point as a reference fingerprint. The community organization (Co) was calculated as the percentage of the Gini coefficient (Wittebolle et al., 2009). To evaluate the most similar communities across time, a similarity matrix based on the Jaccard coefficient was computed considering the four datasets together and hierarchically clustered with the Ward's linkage method. All calculations were computed with the MASS and Vegan packages in the R statistical environment (version 3.0.1) (Oksanen et al., 2013; R Core Team, 2013). Co was computed as described in (Buckley et al., 2012).

## 3. Results

### 3.1. Samples and local characteristics

The sediments used in this experiment showed distinct properties between regions (Table 1). In GBA-GBB region, we found greater variability in elemental composition - OC ranging from 0.75 to 5.72% and TN 00:04 to 00:24%, while the GV-GR region was more homogeneous (OC 5.86 to 7:56% and TN 00:39 to 00:43%). The grain size distribution of GBA-GBB was mainly sandy and GV-GR estuary was typically mud (Silt + Clay > 86%). Another important differences between these regions are the salinity (GBA-GBB > 2 and GR-GR < 5) and the renewal time of the water mass, which for GBA-GBB are days and for GR-GV are hours.

### 3.2. Range-weighted richness (Rr)

This parameter translates the approximate carrying capacity for microbial diversity and ranged from 645 to 64 OTUs in the first time-interval and 5 to 46 OTUs in the second time-interval (Fig. 2). GR and GV had more OTUs than GBA and GBB at the beginning of the experiment. Upon petroleum exposure, all sites showed a dramatic decrease in OTUs, except GR which first showed an increase until day 7, followed by a

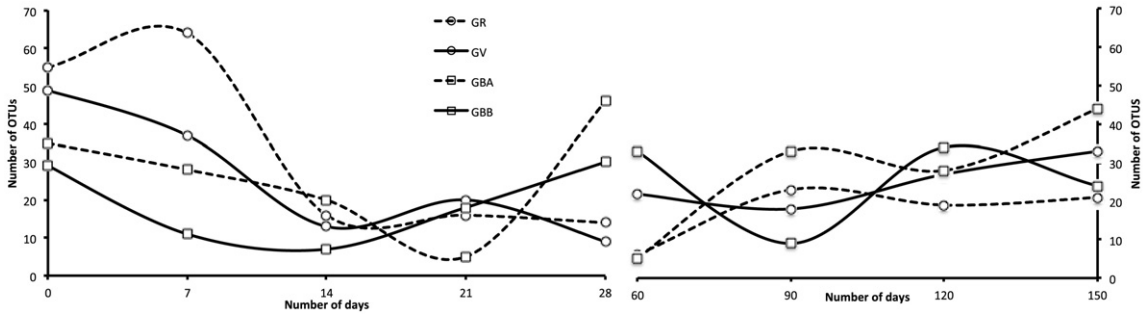


**Fig. 2.** Range-weighted richness (Rr), depicted as the number of OTUs, at the four sampling sites (GR, GV, GBA, and GBB) during the first and second time-intervals. The first time-interval is the weekly-based exposure to petroleum (days 0, 7, 14, 21, and 28) and the second time-interval is the monthly-based exposure to petroleum (days 60, 90, 120, and 150).

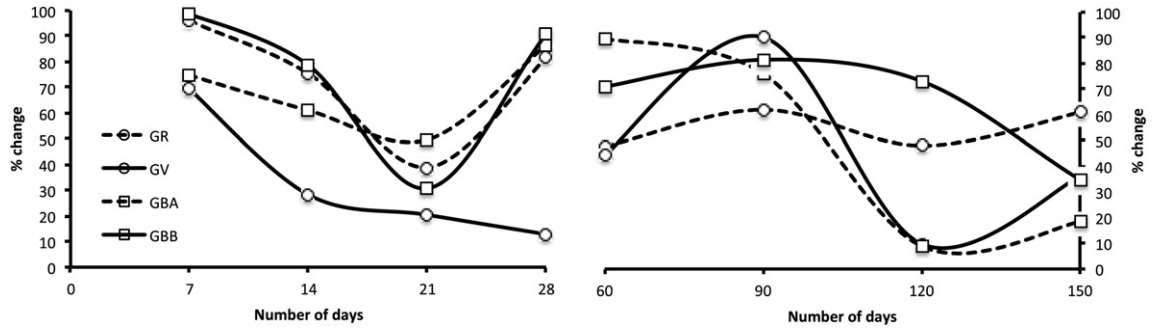


**Fig. 3.** The dynamics of the community (Dy) at the four sampling sites (GR, GV, GVA, GBB), during the first and second-time intervals, estimated by calculating the rate of change parameter (Δt) through moving-window analyses (MWA). The first time-interval represents weekly % of changes in the community in relation to the previous week. The second time-interval represents monthly % of changes in the community in relation to the previous month.

sharp decrease by day 14. In general, all sites lost OTUs by day 14; thereafter, there was a tendency of sudden increase in OTUs for the Guanabara sites, whereas the Gargaú sites stayed more stable. At GBA, this turning point appeared to be later at day 21. In the second time-interval, there is a fluctuation in the number of OTUs along the biodegradation process, and no common pattern is found among the four datasets. By the end of the experiment, GBA was the richest community and the only community richer than when compared to the beginning of the experiment, and GR was the least rich community.

### 3.3. Community dynamics (Dy)

Marked changes on bacterial community composition occur within the first week of heavy-oil exposure, reaching almost 100% in GR and GBB (Fig. 3). This trend was alleviated in the following weeks, and GR, GBA and GBB reached a minimum at day 21. For these communities, day 21 was a turning-point in community composition; for GV this turning-point occurred one week later, at day 28. During the second time-interval, the percent change values did not appear to have a common pattern among the communities (Fig. 3). These values fluctuate around 50% and 90%, but there was a drastic decrease from day 90 to day 120 for GV and GBA. By the end of the experiment, the least and most susceptible communities were GBA and GR, respectively. In general, the communities experienced more changes during the first time-interval than during the second time-interval (Table 2). For example, GBB changed on average 75% during the first time-interval, whereas GV changed on average only 33% during the same period. On the other hand, when the first time-point is taken as the reference for estimating the community change percent, it can be observed that the communities in each time-point of both time-intervals are completely different from the first time-point in GBA and GBB (Fig. 4). On average, GBA and GBB changed 92% and 99% and 97 and 96% in the first and second-time intervals, respectively (Table 2). Interestingly, GBA differed from GBB only during the first week, when it had a lower percent change value than GBB (Fig. 4). On the other hand, GR and GV changed on average less than GBA and GBB. GR and GV appeared to have a common pattern of community change with a striking difference from day 60 to day 90 (Fig. 4). By the end of the experiment, GR and GV was approximately 70% different from their respective first time-point.

**Table 2**
Average change in the community dynamics at the four sampling sites (GR, GV, GBA, GBB), according to moving-window analysis at the first-time interval (MWA1) and the second-time interval (MWA2), as well as according to moving-endpoint analysis at the first-time interval (MEA1) and the second-time interval (MEA2).

| | MWA 1 | MWA 2 | MEA 1 | MEA 2 |
|---|---|---|---|---|
| GR | 73 ± 24.31 | 55 ± 7.80 | 88 ± 10.47 | 67 ± 7.29 |
| GV | 33 ± 25.29 | 45 ± 33.52 | 70 ± 6.61 | 76 ± 8.80 |
| GBA | 68 ± 16.12 | 48 ± 40.53 | 92 ± 11.29 | 97 ± 1.45 |
| GBB | 75 ± 30.48 | 65 ± 20.68 | 99 ± 1.14 | 96 ± 2.12 |

### 3.4. Community organization (Co)

This parameter reflects the evenness of the community. Low Co values (0–40) are typical for a highly even community, while uneven communities have high Co values (70–100). The initial communities of GR and GV had a low organization (more even communities), whereas GBA and GBB had a medium organization (Fig. **5**). During the first week, the organization values of GR, GV and GBA increased, indicating an uneven community structure, especially for GR. After the third week, the organization of GV, GBA and GBB fluctuates around medium organization values, whereas GR stayed more stable, with a lower organization. By the end of the experiment, GV, GBA and GBB had a much uneven community than GR (Fig. **5**).

### 3.5. Hierarchical clustering

In order to compare the most similar communities, the four datasets were evaluated based on the Jaccard coefficient. Because of the complex dynamics, this coefficient was chosen, because it only takes into account the richness of the communities, whereas the Pearson coefficient is influenced by the abundance. Seven main clusters were observed (Fig. 6), which were divided into initial, intermediate and final communities. The initial communities (cluster 7) were grouped together with the second phase communities from GR (GR28-GR150) in cluster 6. The final communities from GBA, GBB and GV had each a distinct cluster (clusters 2, 3 and 5, respectively), and the final communities from GV were more similar to the initial communities. The intermediate clusters 1 and 4 included samples from days 7, 14, 21, 28 and 60, and is the only instance where samples from different locality are grouped together. The most distinct cluster (cluster 1) had samples from GBA and GBB and cluster 4 had samples from the 4 localities.

## 4. Discussion

The focus of this work was to test the reported hypothesis of a two-phase pattern of oil biodegradation (Kaplan & Kitts, 2004), by assessing the ecological aspects of mangrove bacterial communities under artificial heavy-oil exposure. To accomplish this, we used the Microbial Resource Management Framework (Rr, Dy and Co), and studied the responses of bacterial communities from two very different mangroves to artificial oil biodegradation. Guanabara Bay has a much higher salinity value than Gargaú and has been exposed to multiple oil spills, whereas in Gargaú there has never been an oil spill. Taking these two

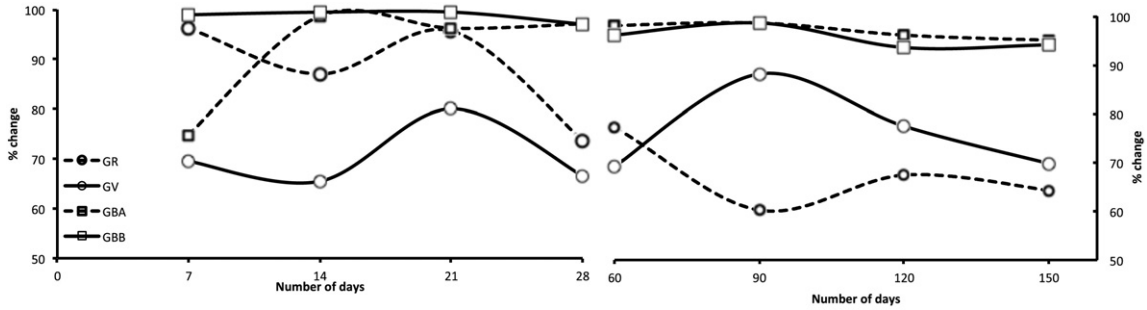


**Fig. 4.** The dynamics of the community (Dy) at the four sampling sites (GR, GV, GBA, GBB), during the first and second-time intervals, estimated by calculating the rate of change parameter (Δt) through moving-endpoint analyses (MEA). The first time-interval represents weekly % of changes in the community in relation to the first week. The second time-interval represents monthly % of changes in the community in relation to the first month.

factors together, our results indicate that oil exposure has a long-term effect in bacterial community structure, and that the bacterial assemblages are different as overall samples from Guanabara did not cluster together with samples from Gargaú. Oil contamination and the associated environmental changes have been shown as the predominant factor shaping the functional composition and structure of microbial communities (Lu et al., 2012).

Based on the hierarchical clustering, three major temporal changes can be observed in the communities along the biodegradation process: (1) initial communities at day 0; (2) intermediate communities from day 7 to day 60; and (3) final communities from day 90 to day 150. The initial communities clustered together with GR, from day 28 to 150. These are the communities with low organization, indicating that they have many rare OTUs in common. These communities clustered together with the final community of GV. During this period, GV increased its richness, slightly increased its evenness and had an abrupt low dynamics from day 90 to day 120. When taking the first day as a reference point (MEA), GV showed a tendency of decreasing in percent change from day 90 on. Altogether, these data suggest that GV is reestablishing its initial community. On the other hand, the final communities in Guanabara Bay cluster together, indicating final communities different from the initial communities, and different from each other. Strikingly, in the GBB cluster (cluster 3), day 60 appeared where day 90 is expected to be. The intermediate communities (clusters 1 and 4) had samples from different sampling sites clustered together. This may be an indication of a strong selection for hydrocarbonoclastic bacteria, presented in the four locations.

The composition of the developing bacterial community varies along the biodegradation steps, as well as among the four datasets. As showed in the hierarchical cluster, most samples did not cluster by geography, but by temporal shifts in the community. A visual inspection of the data clearly indicated different assemblages of samples from the four datasets in each time point. These different assemblages may be the result of microbial endemism, which has been shown over a range of environments (Nemergut et al., 2010). However, when considering abundance of OTUs, these different bacterial assemblages have some common most abundant OTUs, particularly OTU 249 and OTU 274.

Oil contamination had a significant effect on the composition of the communities, especially at the beginning of the experiment. Within seven days of exposure, GR and GBB communities changed almost 100% compared to their initial communities (Fig. 3a). GV and GBA also showed a change in their respective communities during this time, although to a lesser extent. These high percent values indicate the presence of highly dynamic communities (i.e. open communities). Nevertheless, during this same period, there was a decline in richness for all sites, except GR, indicating a loss of OTUs. Also, there was a shift in community organization during this time, especially for GR and GV, which went from low to high organization, indicating the dominance of few OTUs. Altogether, these data suggest a strong selection for hydrocarbon-degrading bacteria.

The dynamics of the communities along the experiment support the two-phase pattern of oil biodegradation (Kaplan & Kitts, 2004), with the breakpoint at 21 days for GR, GBA and GBB and at 28 days for GV. This two-phase pattern is characterized in the literature by a first phase of fast petroleum degradation with high abundance of few species, followed by a second phase of slower petroleum degradation with high richness of low abundant species. Our data suggest an overall tendency in richness decrease by day 21, although more marked by day 14. After day 21, these communities fluctuate between low (<10) and high (>30) richness values. Moreover, the organization of the communities after day 21 seems to reflect a marked different response between GR and the other communities.

This two-phase pattern of oil degradation has been related to the bioavailability of free total petroleum hydrocarbons (TPH) in the first

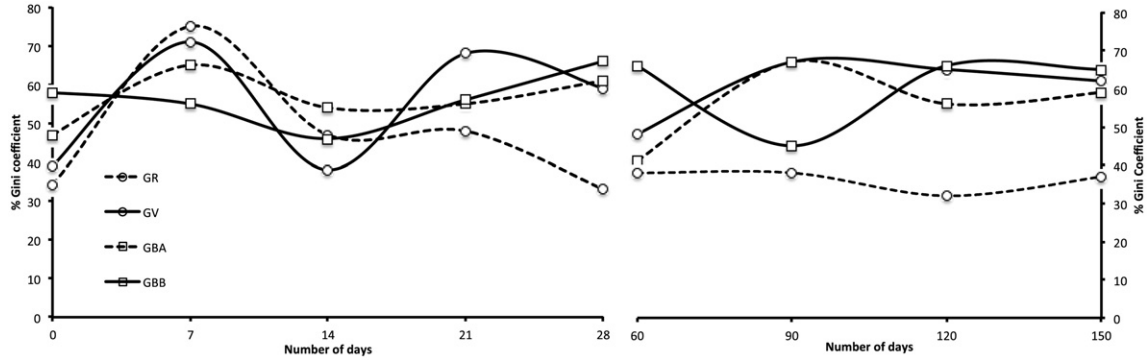


**Fig. 5.** The community organization (Co) at the four sampling sites (GR, GV, GBA, GBB) calculated as the percentage of the Gini coefficient, during the first and second time-intervals. The first time-interval is the weekly-based exposure to petroleum (days 0, 7, 14, 21, and 28), and the second time-interval is the monthly-based exposure to petroleum (days 60, 90, 120, and 150).

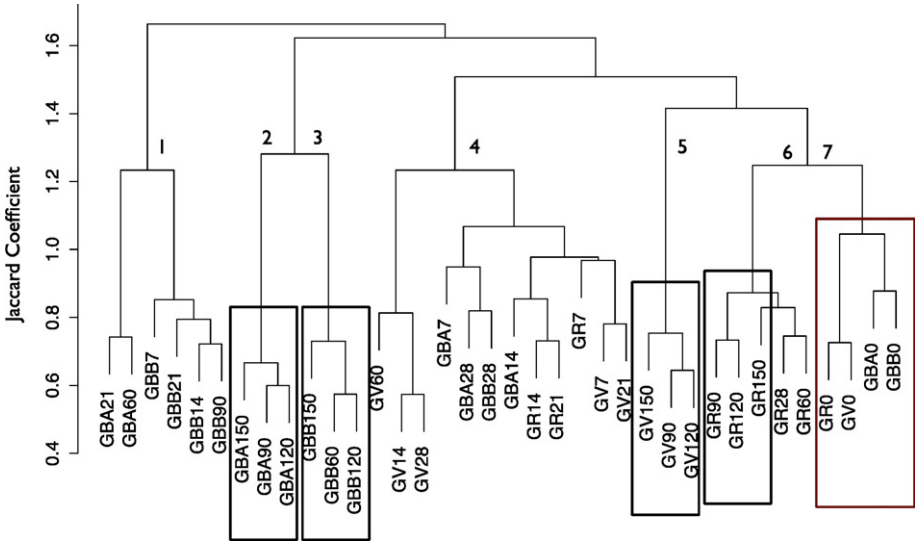


**Fig. 6.** Dendrogram of the four datasets (GBA, GBB, GR and GV for each time-interval considered - 0, 7, 14, 21, 28, 60, 90, 120, and 150 days), based on the Jaccard coefficient and using the Ward's linkage method. Samples are grouped in seven clusters, as indicated. The initial and final communities are indicated in red and black rectangles, respectively.

phase and with a slower desorption rate of soil-sequestered TPH in the second phase (Kaplan & Kitts, 2004). Nevertheless, it has been shown that the contamination levels did not affect this two-phase pattern (Admon et al., 2001). More recently, Sutton et al. (2012) showed that the presence of diesel contamination, rather than its concentration, dictated changes in community diversity, regardless of the different soil matrix type considered. In accordance to this, GBB also showed the two-phase pattern, even though this sampling site was sandy and leaching is expected to happen. It has been shown that soil structure is not static in space or time and that microbes alter this structure (Crawford et al., 2012). Therefore, another possible interpretation of the two-phase oil biodegradation pattern could be interspecies interactions that ought to happen in order to degrade complex petroleum derivatives.

The biophysical properties of soil are the product of both microbial genotypic and micro-environmental diversities (O'Donnell et al., 2007; Ruamps et al., 2011). The literature clearly shows that the fine fractions enrichment in soil or sediments (silt and clay) has a great capacity to retain different types of pollutants (in this case oil) and also natural organic matter; the organic matter also has different levels of reactivity and therefore it is important for the community microbial metabolism. So the interaction between abiotic factors and microbial community are fundamental and determine the kinetics of decomposition and mineralization of these pollutants.

This soil-microbe system is self-organizing as a consequence of the feedback between microbial activity and particle aggregation.

Heavy oil consists of a variety of chemically distinct hydrocarbons (Head et al., 2006), which requires a diverse range of microorganisms for its degradation (McGenity et al., 2011). Complementary effects through positive species interactions have been reported as a mechanism driving community dynamics in an experimental polyculture of crude oil degrading bacteria (Venail & Vives, 2012). In that experiment, the assemblages of mixed species with complementary metabolic systems were suggested to harbor a more complete machinery to better exploit the complex mixtures in crude oil. Interestingly, indigenous communities performed better than foreign ones, suggesting that in addition to adaptation to abiotic conditions, adaptation to the biotic environment of co-occurring species is also important for bacterial community dynamics.

Bacterial species interactions have also been experimentally demonstrated to drive the evolution of alternative resource use not observed in single-species communities (Lawrence et al., 2011). In this experiment, competition among the species resulted in character displacement and the evolution of some species to use the waste generated by other species. From a systems-biology perspective, it has also been suggested that a metabolic network is responsible for the biodegradation potential of a microbial community (Lorenzo, 2008; Pah et al., 2012; Pazos et al., 2003).

Gargaú was taken as the mangrove with no history of oil accidents and both sampling sites from this location had more OTUs and a lower community organization than the two sampling sites from Guanabara Bay. At the end of this experiment, oil exposure reduced the diversity in all sampling sites, except GBA. Perhaps, more importantly, community organization after day 21 differentiated GR from the other communities as the only community with low organization (i.e., high evenness). Initial community evenness has been related to maintaining functional stability and resilience of an ecosystem (Wittebolle et al., 2009). Surprisingly, GV did not follow this pattern, even though it also had low community evenness. Probably, the daily presence of oil contaminants from the fishing boats in the area might have an impact on the composition of this community.

Interspecies interactions can affect evolution and influence the ecosystem. Oil exposure had a drastic impact on the dynamic of the communities, when taking the initial community as the reference point (MEA values). During the oil incubation period, both sampling sites from Guanabara Bay showed change values close to 100% from the initial community, with the exception of GBA at day 7, suggesting that the initial community of GBA was already impacted by the presence of oil components. GR and GV also showed large changes in community composition related to the initial community, although to a lesser extent. At the end of the incubation period, GR and GV were approximately 30% similar to the initial community. We propose to designate such persisting community as the core bacterial community (CBC) in mangrove sediments under oil contamination, supporting the idea of community stability and resilience in samples from Gargaú, in contrast to Guanabara Bay, which is chronically polluted. Time to oil exposure is surely another important parameter to consider when evaluating community dynamic responses, as this may cause recurrent selection of hydrocarbonoclastic bacteria and, therefore, reduction in richness and increase in community organization, probably affecting the core microbiota and community stability and resilience thereof. Interestingly, GV had the smallest MEA values, except after day 60. This community increased in richness after day 90 and, at the same time, decreased in MEA values, suggesting an approximation to the initial community.

Also, this is seen in the hierarchical cluster, where the final communities of GV clustered together with the initial communities and the communities from GR (GR 28–150), indicating again the tendency of reestablishing the initial community.

In conclusion, the effect of oil exposure on the composition of the developing bacterial community is variable, time- and environmental-dependent. Our data corroborated the already reported two-phase pattern of oil biodegradation, although the original proposed explanation is questioned. This means that other plausible hypotheses of microbial interactions as the main drivers of this pattern need to be considered. The decreased richness associated with the high community organization at the beginning of the experiment indicates a strong selection for hydrocarbonoclastic bacteria soon after oil exposure. Different hydrocarbonoclastic bacteria may be selected at each sampling site at the beginning because of bacterial endemism, and this may reflect the different bacterial assemblages throughout the experiment. Species interactions along the experiment may explain the common two-phase pattern of community dynamics. Chronically polluted sites may be losing other functional groups as a result of recurrent selection for hydrocarbonoclastic bacteria, which affects ecosystem functioning. The cooperative behavior of microbes to self-construct a functional community is central to their success (McGenity et al., 2011), and community evenness is critical for the maintenance of functional stability and resilience of an ecosystem (Wittebolle et al., 2009). Although our data do not come from functional genes and caution need to be taken when interpreting community organization in relation to functional organization (Read et al., 2011), clearly there is a different response when comparing communities with or without oil contamination histories.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version doi:10.1016/j.marpolbul.2017.02.052. These data include the Google maps of the most important areas described in this article.

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