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Bacterial diversity patterns of the intertidal biofilm in urban beaches of Río de la Plata

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ABSTRACT

Intertidal benthic ecosystems in estuaries are productive sites where microbial processes play critical roles in nutrients mineralization, primary production and trophic web. In this groundwork study we analyzed the bacterial community of intertidal biofilms from Río de la Plata beaches with different anthropogenic impacts. Several environmental parameters were measured and bacterial assemblages were analyzed by 16S-rDNA pyrosequencing. The average OTU found per sample was 527.3 ± 122.5, showing similar richness and diversity among them. However, sites having the highest and lowest salinity displayed higher bacterial diversity. Assemblages from a site nearby an oil refinery, showing the lowest salinity and oxygen concentration, were clearly distinct from the rest. The weight of this splitting relied on OTUs belonging to *Thauera,* known by its ability to metabolize aromatic compounds. Our results suggest that intertidal bacterial assemblages would be structured by major estuarine variables such as salinity, and that anthropogenic-induced environmental parameters might also be relevant.

1. Introduction

In estuaries, the fine-grained and organic rich sediments act as sink for contaminants and hence their sediments are priority areas to identify potential bio-indicator species and biomarkers of pollution (Ducrotoy, 2010).

Since estuaries show high degree of variability in their physico-chemical characteristics, such as oxygen, temperature and salinity in the water column and bed sediment dynamics, these ecosystems have long been regarded as environmentally naturally stressed areas (Elliott and Whitfield, 2011). As a consequence, their biota is well adapted to cope with that stress and estuaries may be regarded as resilient while their ability to absorb stress without adverse effects has been referred as environmental homeostasis (Elliott and Quintino, 2007). Hence, these areas would be stressful only for marine or freshwater-adapted organisms, while for estuarine organisms the naturally occurring stress would be a positive state. It has been shown that natural stress in estuaries gives similar conditions to those found in anthropogenic stress, therefore the assessment of ecosystem structural features when looking for quality indicators makes the detection of the anthropogenic stress more difficult (Elliott and Quintino, 2007; García-Alonso et al. 2011). Sun et al. (2012) analyzed sediments from estuarine sites heavily modified and relatively unmodified and found shifts in both bacterial community composition and diversity by ARISA, a technique that allows analyzing bacterial communities to species level (Ramette, 2009). Same authors found these shifts to be strongly associated with metals concentration and claimed that were discernable from environmental variation inherent to complex estuarine environments. Thus, addressing the bacterial communities from estuarine sediments using high-resolution approaches, together to a thorough analysis of environmental variables could help to discriminate between assemblages corresponding to the natural estuarine variability and those shaped by anthropogenic impacts.

Although intertidal estuarine systems are dynamic, characterized by periodic fluctuations in several environmental parameters, they are spatially well-defined areas, becoming easy for monitoring studies. In this kind of zones, microbial processes play critical roles in the remineralization of nutrients and primary production. Many of the geochemical and biological processes mediated by microorganisms in intertidal systems occur within microbial biofilms, which form protective microenvironments that can structure a range of microbial processes (Decho, 2000) and exert a bottom up influence in the entire ecosystem.

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Río de la Plata is an important estuarine environment in the continent, located at 35°S on the Atlantic coast of South America. This system is a highly productive area, sustaining valuable fisheries of Uruguay, Argentina, and international fleets (Bisbal 1995; Martinez and Retta 2001; Acha et al., 2008). Specifically at Montevideo coastal area, exists one of the most contaminated harbours of the region (Danulat et al., 2002; Muniz et al., 2004). Gómez et al. (2009) found that microbenthic communities of Río de la Plata were governed by two gradients, the first one determined by anthropic factors and the second one to conductivity and turbidity. In the case of bacterioplankton communities, Alonso et al. (2010) showed that bacterial abundance and diversity patterns based on ARISA data were highest at the frontal zone, where turbid waters from Paraná and Uruguay rivers mix with the Atlantic Ocean (Nagy et al., 2008). In that work the authors also found a relatively high number of unique OTUs in the frontal zone, suggesting the existence of a bacterial assemblage specific of this zone of the estuary. Moreover, among the most abundant bacterial phyla they found that Bacteroidetes accounted for about one third of all bacteria at the frontal zone (Alonso et al., 2010). Thus, there are some clues about the existence of bacterial assemblages that are specific of the transitional, dynamic and changing environmental conditions found at estuaries, as well as the relevance of anthropogenic influence over the microbenthos species distribution. In spite of the mentioned knowledge, there is a total absence of information about intertidal prokaryotic communities of Río de la Plata.

Recent advances in sequencing strategies such pyrosequencing make possible a sensitive and accurate molecular detection of bacterial diversity, which happened to be one to two orders of magnitude greater than previous estimates. By using this kind of sequencing techniques it has been suggested that some members of microbial communities are always present but vary in population size depending on environmental conditions, such as those found at estuaries (Caporaso et al. 2012). Although there are numerous studies on the planktonic, epiphytic, and macroinvertebrate assemblages (Gómez and Bauer, 1998a,b, 2000; Rodrigues Capítulo et al., 2003; Licursi et al., 2006), there are few reports addressing the diversity and dynamics of intertidal microbial biofilms. In this study, we applied a high-throughput parallel tag sequencing (454-sequencing) to analyze the community composition of the intertidal biofilm from urban beaches of Río de la Plata estuary and its relationship to physicochemical factors showing high variability in the estuary, such as salinity, temperature, pH, dissolved oxygen (DO).

2. Methodology

2.1. Sampling site

Four sampling sites along the intertidal of the mid-estuary coastal areas of the Río de la Plata were selected. Montevideo Bay (S 34°52′22″ W 56°14′44″), inside a harbour where an oil refinery is located and receiving domestic and industrial discharges, described as a very polluted area (Danulat et al., 2002; Muniz et al., 2004; Venturini et al., 2012); Santa Catalina (S 34°53′36″, W 56°17′46″) with artisan fisheries and recreation activities and Pocitos (S 34°55′06″, W 56°08′49″) a urban beach with recreation activities. The fourth selected area was Penino (S 34°45′52″, W 56°25′27″), upstream to Montevideo and putative pristine (Fig. 1). Silt is the dominant fraction of sediment all around the area, with range values between 51% and 92% (Muniz et al., 2002). According to historic data, the assessment of the regional and overall ranking of eutrophic conditions determines that the system is moderately eutrophic (Nagy et al., 2002). Moreover, the inner part of Montevideo Bay is polluted by Cr, Pb and petroleum hydrocarbons (Venturini et al., 2004). Three replicates separated by 25 m of biofilm samples, 5 mm surface oxic sediment, were collected at low tide (Río de la Plata has a microtidal regimen and wind direction is the main factor influencing tides) with 50 ml sterile corers made up of plastic centrifuge tubes. All the samples were taken during the same day in may 2012 (Autumn), transported in cool boxes with ice and stored at −20 °C. The positions of sampling locations were recorded using a differential global positional system (DGPS).

Physicochemical parameters of interstitial water were measured *in situ* (temperature, conductivity, pH, and dissolved oxygen) using a multiparameter probe (Horiba). Since the assessed beaches are located in a highly populated area (Montevideo), or very close to it, total phosphorus (TP at 214.91 nm) in homogenised sediment (<2.0 mm) was measured in all samples as a proxy of urban impact (Howitt et al., 2014). Five hundred milligrams were digested in Teflon vials in a microwave oven (CEM modelo X-PRESS) with 9 mL de HNO_3, 4 mL de HF and 2 mL of HCl, heated until 180 °C with a ramp of 10 min and kept for 20 min at this temperature. Samples were heated until 180 °C with a ramp of 10 min and maintained for 20 min at this temperature. After cooled, samples were analysed by ICP-OES Varian 720 ES (based on U.S. EPA, 1996, method 3052).

2.2. DNA extraction and sequencing

DNA from sediment samples (1 g from each) was extracted using The ZR Soil Microbe DNA MiniPrep (Zymo Research). Purity and quantification of extracted DNA was assessed using a Nanodrop microvolume spectrophotometer (Thermo).

2.3. 454-sequencing

454 pyrosequencing was carried out at the UNC Microbiome Core Facility (University of North Carolina at Chapel Hill, NC). Initial amplification of the V1-V2 region of the bacterial 16S rDNA was performed on total DNA from samples. Master mixes for these reactions were prepared with Qiagen Hotstar Hi-Fidelity Polymerase Kit (Qiagen, Valencia CA), forward primer composed of the Roche Titanium Fusion Primer A (5′-CATCTCATCCCTGCGTGTCTCCGACTCAG-3′), a 10 bp Multiplex Identifier (MID) sequence (Roche, Indianapolis, IN) unique to each of the samples and the universal bacteria primer 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) (Edwards et al., 1989). The reverse primer was composed of the Roche Titanium Primer B (5′-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG-3′), the identical 10 bp MID sequence as the forward primer and the reverse bacteria primer 338R (5′-GCTGCCTCCCGTAGGAGT-3′) (Fierer et al., 2008), which span the V1–V2 hypervariable region of the bacterial 16S. Amplification of each sample was performed with following conditions: an initial denaturing step at 94 °C for 5 min, followed by a cycling of denaturing of 94 °C for 45 s, annealing at 50 °C for 30 s and a 1 min 30 s extension at 72 °C (35 cycles), a 10 min extension at 72 °C and a final hold at 4 °C. Each sample was gel purified individually using the Qiaquick Gel Extraction Kit (Qiagen, Valencia CA) (E-Gel Electrophoresis System (Life Technologies, Invitrogen division), and standardized prior to pooling. The 16S rDNA amplicons from the pooled sample were sequenced on a Roche 454 Genome Sequencer FLX Titanium instrument using the GS FLX Titanium XLR70 sequencing reagents and protocols. Initial data analysis and base pair calling were performed by Research Computing at the UNC (Chapel Hill, NC). The 16S rRNA gene amplicons were mostly treated using the RDP Pyrosequencing pipeline (http://pyro.cme.msu.edu/). Raw sequences obtained from each site were 3918 ± 571 for Penino, 3285 ± 1259 for Santa Catalina, 3223 ± 358 for Montevideo Bay and 4193 ± 892 for Pocitos. To yield high quality sequences, trimming of reads was performed using the RDP Pipeline initial process tool. The number of high quality sequences were 993 ± 245,

Fig. 1. Sampling sites along the northeast bank of the middle estuarine area of Río de la Plata. Urbanised area (Montevideo city) is depicted in grey. The oil refinery is shown in white.

702 ± 277, 982 ± 233 and 592 ± 81, for Penino, Santa Catalina, Montevideo Bay and Pocitos, respectively. Chimera sequences were removed using Decipher (Wright et al., 2011).

2.4. Diversity and community structure analyses

After trimming and removing sequences of poor quality, sequences were aligned and grouped into clusters sharing 97% similarity, which in this study were considered as operative taxonomic units (OTUs), using the RDP pipeline. Numbers of reads per OTU for each sample were normalized by sample size. The sequence with the minimum sum of the square of distances between sequences within a cluster was assigned as the representative sequence for that cluster or OTU (Cole et al., 2014). We found 149 sequences belonging to Bacillariophyta chloroplasts, which were removed from the database for further analyses. According to their relative abundance, we use the 0.1–1.0% range of OTUs relative abundance suggested by Pedrós-Alió (2006) and Fuhrman (2009) to define a 'rare' species, very rare when they were in a proportion $\leqslant$0.1% and abundant to those whose proportion was above 1% (Vergin et al., 2013). Alpha diversity was analyzed through rarefaction curves and two indices of diversity (Chao1 and Shannon) were calculated. Beta-diversity was addressed by non-metric multidimensional scaling (nm-MDS) of a Bray–Curtis similarity matrix of OTUs abundance data, and performed in PAST (Hammer et al., 2001). A 1-way multivariate analysis of similarity (ANOSIM) was used on the same matrix to test for community level differences between samples and sites (Ramette, 2007).

The Similarity percentages (SIMPER) routine in PAST was used to identify the OTUs responsible for particular aspects of the structure found in the Bray–Curtis similarity matrix, *i.e.* determining the OTUs responsible for the sample grouping observed in the nm-MDS plots.

2.5. Statistical analyses

All statistical analyses were performed using GraphPad Prism version 5.0 for Mac OS X, GraphPad Software (San Diego, CA, USA). Prior to analyses normality test were performed for each set of variables (Kolmogorov–Smirnov test); if normality was not obtainable, parametric tests were replaced by their non-parametric alternatives such as Mann–Whitney *U* test for paired comparisons (M–W) and Kruskal–Wallis ANOVAs for multiple comparisons (K–W). The differences between the relative abundance of each bacterial group between incubation times and treatments were analyzed using two-way ANOVAs. Post hoc comparisons between samples after ANOVA were performed using Bonferroni tests.

We used a principal components analysis (PCA) to summarize the environmental variation related to the different sites. We found that the first axis of the resulting PCA (PC 1) explained 70.76% of variance, and thus we used as a multivariate proxy to compare the environmental influence across sites on bacterial community. We used the scores from the first principal component (PC1) and regressed them against bacterial diversity (H′) and evenness (E). All variables were transformed to meet the assumptions of normality prior to the PCA and the calculations were performed in PAST (Hammer et al., 2001).

3. Results

3.1. Physicochemical environment

The physicochemical characteristics showed minor differences in pH, with lowest values corresponding to Montevideo Bay (7.65) and the highest for Penino (8.01) (Table 1). However salinity and dissolved oxygen clearly differ among areas. Montevideo Bay presented the lowest values with 1.5 and 2.47 mg L^{-1} for salinity and DO respectively. Interstitial water of Pocitos showed the highest value of salinity (25.1) (Table 1), while total phosphorus (TP) in Santa Catalina was significantly higher than in the other sites (ANOVA, $p < 0.0001$), although Montevideo Bay and Pocitos also showed high levels (Table 1). Penino was the site having the lowest concentration of this nutrient (ANOVA, $p < 0.0001$).

3.2. Characterization of biofilm bacterial community (Alpha diversity)

These analyses were performed using the number and frequency of sequences grouped into clusters that shared $\geqslant$97% similarity (OTUs). The average number of OTUs per sample was 527.3 ± 122.5, with 3107 different clusters for all the samples. Bacterial diversity addressed by rarefaction analysis and Shannon and Chao1 indexes showed that samples belonging to the sites with higher and lower salinities exhibited more diversity. Rarefaction curves suggest that at the phylogenetic depth employed ("species level") the sequencing effort was not large enough to capture the complete diversity of these communities, as the curves do not level off (the slope does not go to zero) with increasing sample size (Suppl. Fig. 1). The Chao1 index for clusters sharing 97% similarity

Table 1
Environmental parameters (averages) measured at each sampling site.

Site	Salinity	Temperature (°C)	OD (mg L^{-1})	pH	TP (μg g^{-1})
Penino	3.2	26.4	9.20	8.01	126.62
Montevideo Bay	1.5	28.8	2.47	7.65	284.29
Santa Catalina	8.5	21.0	8.83	7.95	707.83
Pocitos	25.1	28.0	9.10	7.97	466.63

calculated for each sample did not show significant differences (K–W, $P > 0.05$). The same conclusion is supported by other diversity indices, such as Shannon H′ (Table 2). Also, to examine how another aspect of diversity varied among groups, we calculated the evenness (E) for the 97% clusters. We found that in Montevideo Bay samples E was the highest, being this difference significant when related to Penino (unpaired *t*-test, $p \leqslant 0.001$) (Table 2).

3.3. Relationship between metrics of bacterial diversity and salinity

In order to visualize the influence of environmental parameters (temperature, DO and salinity) on community diversity, we plotted the Shannon H′ index obtained from each sample versus these parameters. The results showed that those samples having the extreme salinities in the gradient (Montevideo Bay and Pocitos) reached higher diversities (Fig. 2).

3.4. Across-site comparison (Beta-diversity analysis)

Differences among bacterial communities were firstly explored by comparing the frequency of each cluster (OTU) in each sample. The results showed that most of the defined OTUs had a frequency ⩽0.1% in all of the samples (Table 2), which according to Vergin et al. (2013) can be considered as "very rare". Interestingly, Penino, Santa Catalina and Pocitos samples have among 13% and 18% of the OTUs with a relative abundance between 0.1% and 1%, while in samples from Montevideo Bay the "rare" OTUs had the lowest observed abundance (3.91%). On the other hand, 20.14% of the OTUs from this site had frequencies ⩾1% and were considered as "abundant" OTUs (Table 2). Also, this splitting of clusters (OTUs) sharing ⩾97% similarity in three different categories allowed the clear discrimination between Montevideo Bay and the rest of the samples.

Bray–Curtis similarities between all samples using all OTUs were calculated and displayed in an nm-MDS plot, revealing that Montevideo Bay communities did not cluster together with the rest. However, analysis of similarity (ANOSIM) test of the microbial communities indicated no significant overall difference between communities ($p > 0.05$). In order to provide a better visualization of data, hierarchical clustering and nm-MDS based on the Bray–Curtis distance matrix was used (Fig. 3). Inside group similarities (between replicates from the same site) were ⩾0.4 in Montevideo Bay and Penino, while in Santa Catalina and Pocitos inside group similarities were not very different from those found between both sites, ranging from 0.2 to 0.4. Similarities <0.1 were found between Montevideo Bay and the other groups (Fig. 3). When we performed

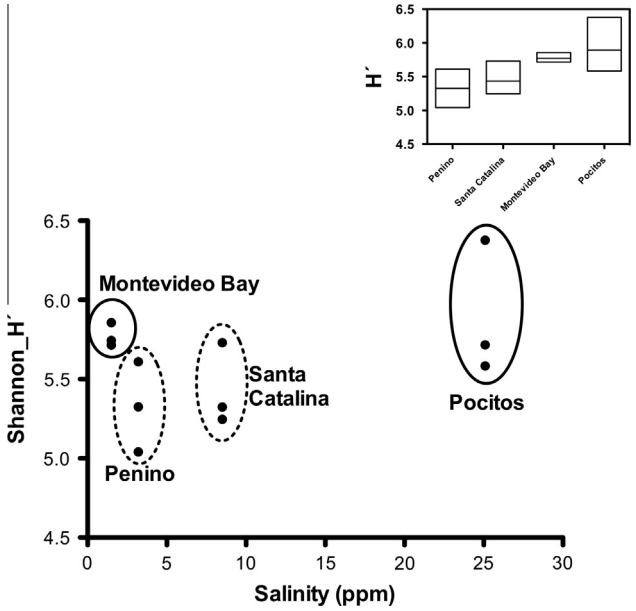

Fig. 2. Relationship between OTUs diversity (estimated as Shannon index H′) and salinity. Heavy line circles show those samples having the extreme salinities in the gradient, which coincided with the higher diversities. Dotted line circles show the intermediate salinities sites. Inset shows the H′ index of samples from each site, ordered according to the geographic gradient.

the analyses using the PC1 summarizing environmental variation through the sites and made regressions, no significant relationships to bacterial diversity estimated by H′ ($R^2 = 0.02$, $p = 0.64$) or E ($R^2 = 0.004$, $p = 0.8404$) were found.

Based on the nm-MDS ordination plot, where a clear, although no significant discrimination between Montevideo Bay and the rest of the sites could be detected, we performed a SIMPER analysis to know the weight of individual OTUs on this splitting. Therefore, after identification of the most relevant OTUs we checked their taxonomic identity given by the RDP pipeline. We found that four genera, *Thauera* (Betaproteobacteria), *Gaetbulibacter*, *Algoriphagus* and *Muriicola* (the three belonging to Bacteroidetes) contributed in 7% to group difference. Three of these genera, *Thauera*, *Gaetbulibacter* and *Algoriphagus* were present only in Montevideo Bay samples. In the case of *Thauera*, it comprised 4% of the whole community (average frequency of 0.040 ± 0.001).

3.5. Phylogenetic identification

In general, the phyla Actinobacteria, Bacteroidetes and Proteobacteria (Alpha, Beta, Gamma and Delta classes) accounted for 88.8%, 89.7%, 55.7% and 93.9% of the total community for Pocitos, Santa Catalina, Penino and Montevideo Bay, respectively (Fig. 4). Among the phylogenetic groups, significant differences in abundance were found between different samples, except for Actinobacteria that were the less abundant bacteria and did not change significantly between sampling sites. From Proteobacteria phylum,

Table 2
Rarity of OTUs (determined at ⩾ 97% similarity) found at each sampling site (categories according to Vergin et al. (2013)), and diversity calculated according to Chao1, Shannon (H′) and eveness (E).

Site	⩽0.1% (very rare)	0.1–1% (rare)	⩾1% (abundant)	Chao1 (± SD)	H′ (± SD)	E (± SD)
Penino	82.80%	16.10%	1.10%	1093.33 (194.03)	5.18 (0.24)	0.84 (0.02)
Montevideo Bay	75.95%	3.91%	20.14%	1380.71 (219.22)	5.69 (0.04)	0.91 (0.01)
Santa Catalina	80.97%	17.95%	1.08%	1217.16 (183.91)	5.42 (0.13)	0.88 (0.02)
Pocitos	85.81%	13.49%	0.71%	1374.71 (295.96)	5.70 (0.39)	0.88 (0.03)

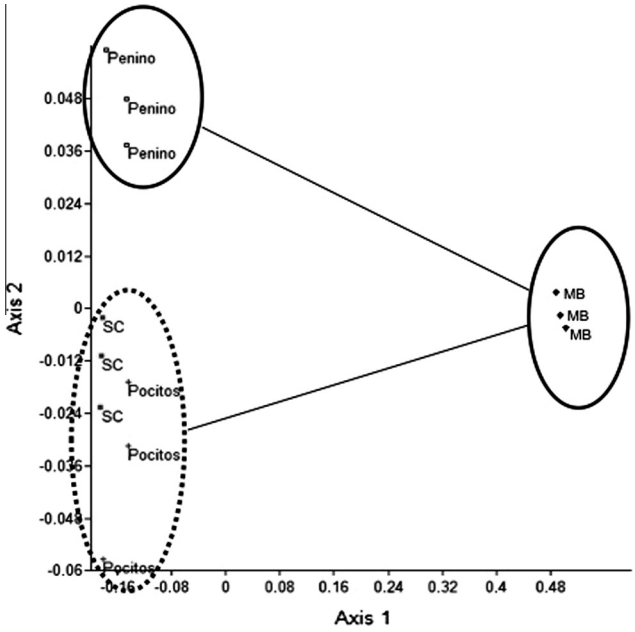

Fig. 3. Non-metric Multidimensional Scaling plot (nm-MDS) based on the OTUs found for each sample. For a better depiction, the nm-MDS plot was combined to the data obtained from hierarchical clustering based on Bray–Curtis similarities. The dashed circle indicates that similarities inside and between groups are $\geqslant 0.2 \leqslant 0.4$. Heavy line circles are depicted when the similarity inside a group was $\geqslant 0.4$. Straight lines show similarities between sites that were < 0.1. MB, Montevideo Bay, SC, Santa Catalina.

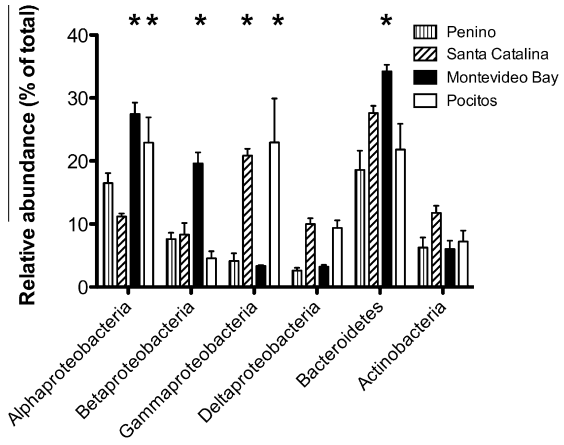

Fig. 4. Phylogenetic composition of bacterial communities at phylum and class levels. Asterisks indicate significant differences in the relative abundances of bacterial groups between sites (only those that were significantly higher than the rest are shown).

OTUs belonging to Alphaproteobacteria class were significantly higher in samples belonging to Montevideo Bay and Pocitos, the sites having the most contrasting salinities (ANOVA, $p < 0.05$; Fig. 4). However, alphaproteobacterial sequences from both sites were affiliated to different genera. In the case of Montevideo Bay the OTUs ranging 1–2% relative abundance were classified as *Roseobacter*, *Rhodobacter* and *Porphyrobacter*. On the other hand, alphaproteobacterial OTUs ranging 1–2% belonged to *Maritimibacter*, *Jannaschia* and *Seohaeicola* genera in Pocitos. Betaproteobacterial OTUs were significantly more abundant in Montevideo Bay samples, where reached abundances close to 20%, while in the rest of the samples dropped to 10% (ANOVA, $p < 0.05$; Fig. 4). When Gammaproteobacteria were analyzed, their relative abundances were strikingly higher in Santa Catalina and Pocitos (ANOVA, $p < 0.001$; Fig. 4). The phylum that exhibited high abundances ($\geqslant$15%) through all samples was Bacteroidetes, being those from Montevideo Bay significantly higher than in Pocitos and Penino (Fig. 4) (ANOVA, $p < 0.05$ and $p < 0.001$ respectively). In Montevideo Bay, Bacteroidetes OTUs assigned to *Gaetbulibacter* and *Algoriphagus* ranged abundances among 1% and 2.5%. In the case of Santa Catalina, where this phylum was also highly represented, all the representative sequences of most abundant OTUs belonged to *Muriicola* genus (between 1% and 8% relative abundance).

4. Discussion

The ability to characterise and discriminate closed areas in aquatic systems is a keystone tool for environmental impacts studies in ever changing conditions such as estuary. Thus, communities exposed to these stressors and with the ability to quickly respond to them, such as benthic microbes, are suitable targets for environmental impact studies. Among the techniques employed to assess the aquatic microbial diversity and community composition, deep sequencing techniques are increasingly being reported (Zinger et al. 2012). Therefore, microbial community assemblage analysis by 454-pyrosequencing of the 16S rRNA gene could be a distinctive approach to address the quality of aquatic ecosystems (Hu et al. 2014).

In the present work, all sediment samples, although belonging to a highly impacted zone (Burone et al., 2006; Muniz et al., 2002; Muniz et al., 2004; Venturini et al., 2004; García-Rodríguez et al., 2010), displayed a high bacterial diversity as demonstrated by the calculated Shannon diversity index (Table 2, Fig. 2). This result is in agreement with the concept that sediments are more phylogenetically diverse than any other environment type (Lozupone and Knight, 2007). When OTUs at 97% cut off where analysed, we found that among the environmental factors addressed, salinity seemed to have a role in the structure of sediment bacterial assemblages, since those sites having the lowest and highest salinities reached higher diversity (Fig. 3). However, as all sites showed quite similar diversities in spite of the environmental differences observed (specially in the cases of salinity and DO), no significant relationships between the environment and the calculated indexes (H′ and *E*) could be found when using PC1 scores. It is well known that salinity is one of the most relevant factors structuring estuarine bacterial communities (Bouvier and Del Giorgio 2002; Cottrell and Kirchman, 2003; Crump et al. 2004; Zhang et al., 2014). However, although salinity has been shown to shape the microbial communities in other coastal ecosystems, such as coastal lagoons and mangroves (Ikenaga et al., 2010; Alonso et al., 2013), the studies addressing its effect over intertidal bacterial communities are scarce. Comparison of bacterial diversity between sediment and water estuarine samples using 16S rDNA clone libraries, Feng et al. (2009) found that sediment harboured higher diversity. Similarly, Alonso et al. (2010) found that in Río de la Plata bacterioplankton were highly diverse and strongly structured by the environment, with a low similarity between planktonic and sediment bacterial assemblages. Further studies including a higher number of sites and comprising a wider salinity gradient should be performed in order to confirm if bacterial assemblages of intertidal biofilms are mostly structured by the main environmental variables of this estuary.

The results also showed that most of the defined OTUs had a frequency $\leqslant$0.1% in all of the samples, which, according to Vergin et al. (2013) could be defined as rare. This high abundance of rare taxa in most of the samples together to the finding that samples from the highly human-impacted site (Montevideo Bay) showed the highest frequency of abundant taxa ($\geqslant$1% relative abundance),

suggest the existence of environmental filters driving the community assembly. In fact, it has been described that Montevideo Bay, especially the inner zone where our samples were taken, is highly polluted with hydrocarbons derived from the oil refinery located nearby (Muniz et al., 2002, 2004; Venturini et al., 2004; García-Rodríguez et al., 2010). In this sense, TP concentrations found in this study are in the range of those described by the U.S. Environmental Protection Agency for urban areas, especially from storm water runoff (Howitt et al., 2014), confirming the highly urban and impacted nature of the study area. Moreover, previous works on foraminiferal fauna of this impacted area showed that richness of these animals was extremely low (Burone et al., 2006). Therefore, we hypothesized that intertidal biofilms harbour a myriad of rare OTUs, which are a bacterial seed bank adapted to and shaped by the estuarine changing conditions (Pedrós-Alió, 2006). When environmental conditions change owing to anthropogenic activities (such as wastewater or hydrocarbons spill) those taxa that can cope with the specific situation will grow and dominate the metabolism of the biofilm. In this regard, in Montevideo Bay samples, where the DO was significantly lower than in the rest of the sites, the more abundant OTUs belonged to *Thauera*, which are facultatively anaerobic Betaproteobacteria characterized for the ability of several species of the genus to metabolize aromatic compounds, under either aerobic or anaerobic conditions (Ramakrishnan, 2013; Liu et al., 2013). Due to this ability of using aromatic hydrocarbons either under anoxic conditions and because it contains unique pathways for the degradation of these aromatic compounds, *Thauera* has emerged as an important genus with anaerobic and facultative metabolism in environmental systems.

As it has been described elsewhere, each kind of aquatic ecosystem (freshwater, marine or transitional) has a typical bacterial assemblage (Glöckner et al., 1999; Lozupone and Knight 2007; Barberán and Casamayor 2010). Moreover, Alphaproteobacteria has been reported as the dominant group in the bacterioplankton assemblage of Río de la Plata (Alonso et al., 2010) and in coastal lagoons of the Atlantic Ocean (Piccini et al., 2006; Alonso et al., 2013). In our study, this group was equally represented in Pocitos and Montevideo Bay, suggesting that forces driving the bacterial assemblages of sediment are different from those acting over bacterioplankton communities. In the case of Bacteroidetes, a group that at the planktonic level was identified as the second most abundant group by Alonso et al. (2010), we found that members of this group were very abundant in all sampling sites, but were significantly higher in Montevideo Bay (Fig. 4). This group has been regarded as particle-attached, found at the turbidity maximum of estuaries (Bouvier and del Giorgio 2002) and in some cases their abundance was found to be constant along estuaries (Bouvier and del Giorgio, 2002; Crump et al., 2004; Kirchman et al., 2005; Kan et al., 2007). In all our samples, *Muriicola* sp., a seawater Flavobacteria found in coastal environments with a little known ecophysiology (Kahng et al., 2010), reached more than 2% of relative abundance, falling into the category of "abundant" OTUs. This finding implies that *Muriicola* would be ubiquitous in intertidal biofilms of the addressed area and suggests that Bacteroidetes would not be a good candidate as an indicator of anthropogenic impact.

In a previous study based on 16S rRNA gene clone libraries, Alonso et al. (2010) detected a dominance retrieval of gammaproteobacterial sequences in Río de la Plata sediment. In a similar way, Feng et al. (2009) reported that Gammaproteobacteria was the most abundant group in sediments from Changjiang estuary and from the coastal area of the China Sea. In our study, Gammaproteobacteria were highly abundant only in Santa Catalina and Pocitos samples. Deltaproteobacteria, although less abundant, exhibited a similar pattern and reached higher relative abundances where TP concentrations were also significantly higher than in the other sites (Fig. 4, Table 1). It has been demonstrated that *Deltaproteobacteria*, presumptively sulphate and sulphur/iron reducing, are strongly associated to chemical parameters and that sulphate-reducing bacteria (SRB) correlated positively with labile organic phosphorus in open sea sediments (Sinkko et al., 2011). Although in the present study sulphate concentration was not measured, the high abundance of Deltaproteobacteria in sites with high TP concentration suggests that SRB could be a relevant functional group in this estuarine biofilms. In fact, most of the genera identified inside this bacterial class belonged to SRB from Desulfobacteraceae family.

Beta-diversity analysis combining nm-MDS and Bray–Curtis based clustering discriminated Montevideo Bay samples from the rest (Fig. 3). Also, Pocitos and Santa Catalina samples showed to be highly similar among them and differentiated from Penino, probably explained by salinity. As it was mentioned above, OTUs belonging to Betaproteobacteria were significantly higher in Montevideo Bay samples. This site displayed the lowest DO concentration (ca. 30% saturation) and the highest temperature, is close to the oil refinery located at the innermost zone of the Montevideo harbour (Fig. 1) (Muniz et al., 2002, 2004; García-Rodríguez et al., 2010) and showed a high proportion of OTUs from *Thauera* genus. Thus, we propose that further studies focused on *Thauera* must be done in order to elucidate if would be suitable as bioindicator of oil-contaminated sediments.

Overall, the results of our study revealed that intertidal sediment bacterial assemblages seemed to be structured mostly by salinity, the main factor structuring communities of estuaries, and by environmental parameters that are directly linked to human activities, such as local oxygen availability and contaminants. It is assumed that higher biodiversity increase ecosystem capacity to resist and recover from perturbation, since maintains ecosystem functioning despite species loss and allows diversification of responses to perturbation. Moreover, community evenness has shown to favours the functioning of ecosystems under salinity stress (Wittebolle et al., 2009). The high bacterial diversity and evenness found in all samples from the present study suggest that biofilm communities of urban beaches of Río de la Plata are still resilient to the anthropogenic influence, so environmental homeostasis also acts at this biological level of organization.

5. Conclusions

In this work we assessed for the first time the composition of the prokaryotic bacterial community of intertidal biofilms in urban beaches of Río de la Plata. Our results, although did not cover the diversity exhaustively, showed that while the bacterial assemblage appeared to be mainly structured by salinity, anthropogenic induced environmental parameters might play a relevant role too. Dissolved oxygen seemed to be one of the factors probably explaining the differences in community structure, especially in the site strongly influenced by an oil refinery and domestic sewage (Montevideo Bay). At this site, sequences belonging to *Thauera* genus were identified, being this genus characterized by metabolizing aromatic compounds.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2014.08.039.

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