Bacterial diversity in deep-sea sediments under influence of asphalt seep at the São Paulo Plateau

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Abstract

Here we investigated the diversity of bacterial communities from deep-sea surface sediments under influence of asphalt seeps at the Sao Paulo Plateau using next-generation sequencing (NGS) method. Sampling was performed at North São Paulo Plateau using the human occupied vehicle Shinkai 6500 and her support vessel Yokosuka. The microbial diversity was studied at two surficial sediment layers (0-1 and 1-4 cm) of five samples collected in cores in water depths ranging from 2,456-2,728 m. Bacterial communities were studied through sequencing of 16S rRNA gene by Ion Torrent and clustered in operational taxonomic units. We observed high diversity of bacterial sediment communities as previously described by other studies. When we considered community composition, the most abundant classes were Alphaprotebacteria (33.2%), Acidimicrobiia (21.8%), Gammaproteobacteria (12.2%) and Deltaproteobacteria (8.9%). Most abundant OTUs at family level were from two uncultured bacteria from OM1 clade (19.5%) and Rhodospirillaceae (12.26%). The unexpected high abundance of Alphaproteobacteria and Acidimicrobiia in our deep-sea microbial communities may be related to the presence of asphalt seep at North São Paulo Plateau, since these bacterial classes have species capable of metabolizing hydrocarbon compounds.

Keywords: microorganisms, diversity, deep-sea sediment, São Paulo Plateau, asphalt seep

Introduction

Deep-sea ecosystems represent most of Earth surface. The seabed is composed of several types of habitat as hydrothermal vents, cold seeps, seafloor and subseafloor [1, 2]. Seafloor sediments are particularly interesting due to their geochemical characteristics, sedimentary dynamics and greater habitat stability that are important factors to structuring macro and microorganisms communities. Research on Microbial diversity in superficial sediment habitat has been intensified, in an effort to better understanding how spatial and temporal patterns are determined [3, 4].

Spatial distribution of microorganisms in deep-sea habitats has been studied in several locations, such as Arctic sediments in the Pacific Ocean [5], Siberian continental margin [6], eastern South Atlantic sediments near Angolan coast [7] and pockmarks from Southwestern Atlantic close to the Brazilian coast [8]. Although there are few studies in deep-sea habitats from South Atlantic Ocean, the knowledge of how, what and where microorganisms inhabit is incipient compared with similar environments from North Atlantic or other better studied deep-sea basins.

The Brazilian coast is known for the presence of huge oil fields under seafloor sediments [9, 10]. Campos and Santos basins are important oil-producing areas of Brazil, responsibly for more than 71% of the country's oil production [11]. Considering the existence of oil and gas reservoirs in these basins, it was expected that chemosynthetic ecosystems exist and a joint Japanese-Brazilian Iatá-Piúna cruise was conducted to investigate that hypothesis. This cooperative project integrated the Quest for the Limits of Life (QUELLE) 2013 carried out by JAMSTEC (Japan Agency for Marine-Earth Science and Technology). During the cruise that explored the deep seafloor of the North São Paulo Plateau in Espírito Santo Basin (2500-3600 m), asphalt seeps were found at a depth of 2,700 m colonized by non-chemosynthetic megafaunal organisms [12]. They also found that, in non-asphalt seeps areas, outcrops of mudstone were covered by black manganese oxide crusts and nodules were also present [12, 13, 14]. These two particular conditions of the study area may be important factors determining patterns of bacterial diversity.

A previous study carried out by the Iatá-Piúna consortium [13] using PCR-DGGE method found high and widespread dominance of Proteobacteria and Firmicutes at sediment samples, including asphalt seep area. The two predominant species were *Erythrobacter citreu*s strain VSW309 detected in hydrothermal vents and *Thalossospira xianhensis* strain MT02 a hydrocarbon-degrading marine bacterium. They also found that microbial community composition between sediment core depths was different.

Here we investigate the diversity of bacterial communities from deep-sea surface sediments under the influence of asphalt seeps at the Sao Paulo Plateau using next generation sequencing (NGS). Bacterial community assembly was accessed using 16S rRNA Ion Torrent PGM and by quantitative amplification (qPCR) with the aim of (1) describing bacterial taxonomic diversity and (2) estimating bacterial populations present in sediment depth layers.

Material and Methods

Description of sampling sites

Sediment samples were collected during 2nd leg of '*Iatá-piuna cruise*' expedition, a collaborative project between Brazil and Japan inserted in the QUELLE (Quest for Limit of Life) initiative from JAMSTEC (Japan Agency for Marine-Earth Science and Technology). Sediments samples were collected using the HOV '*Shinkai 6500*' and support vessel '*Yokosuka*' in Sao Paulo Plateau located off the coast of Espírito Santo and Rio de Janeiro states, composing the Campos and Espírito Santo basins.

The study area was separated in two regions, North São Paulo Plateau and South São Paulo Plateau (Figure 1). The northern region is located between coordinates 20°30' – 21°30' S and 39°30' – 38°30' W; and the southern region between coordinates 22°50' – 23°00' S and 39°10' – 38°30' W. A total of 14 sediment cores were sampled. Cores were subsampled on board at depth intervals of 0-1 cm, 1-4 cm, 4-7 cm, 7-10 cm and 10-13 cm. Each subsample was placed in sterile sample bags and stored at -20 °C. The top two layers (0-1 and 1-4 cm) of five sediment cores from North Sao Paulo Plateau were selected (Table 1). Samples N11, N12, and N13 were associated with asphalt seep and N06 and N14 were from background deep-sea.

DNA extraction

Bacterial community DNA was extracted from the top layers (0-1 and 1-4 cm) of five sediment cores from North Sao Paulo Plateau (Table 1) using PowerSoil® DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) following manufacturer's instructions with adaptations: sample consisted of 0.5 g of homogenized sediment and after mechanic cell lysis, a thermal shock step was added, heating samples at 55 °C for five minutes followed by 1 minute at -20 °C.

The integrity of extracted DNA was evaluated by electrophoresis in 1% agarose gel with TAE 1X (Tris 0.04M, glacial acetic acid 1M, EDTA 50 mM; in pH 8), visualized with SYBR®Safe (Invitrogen, Paisley, UK), and Lambda Hind III (Life Technologies, Carlbad, CA., EUA) used as molecular marker. DNA was quantified using NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, EUA) and Qubit® dsDNA HS (High Sensitivity) Assay (Life Technologies).

Ion Torrent PGM Sequencing

The bacterial 16S rRNA gene V3 and V4 variable regions were amplified with primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') [15]. PCR mixtures contained 0.5 µM of each primer, 0.7 U of Taq DNA Polymerase (Life Technologies, Carlbad, CA., EUA), 1X Buffer, 4 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.3 mg/mL BSA (Bovine Serum Albumin) and 4 ng of DNA template. Cycling conditions consisted of 5 min initial denaturation at 95 °C; 2 cycles of 1 min denaturation at 95 °C, 1 min annealing at 48 °C and 1 min extension at 72 °C; 2 cycles of 1 min at 95 °C, 1 min at 50 °C and 1 min at 72 °C; 2 cycles of 1 min at 95 °C, 1 min at 52 °C and 1 min at 72 °C; and 22 cycles of 1 min at 95 °C, 1 min at 54 °C and 1 min at 72 °C.

Amplicons libraries obtained were purified before emulsion step with Purelink PCR Purification Kit (Life Technologies, Carlbad, CA., EUA) and quantified using Qubit® dsDNA HS (High Sensitivity) Assay (Life Technologies). Emulsion PCR was carried out using Ion OneTouch 2™ Instrument, using the Ion PGM™ Template OT2 Reagents 400 Kit and enriched with OneTouch ES (Life Technologies). The sequencing of libraries was carried out in an Ion PGM™ System, using the Ion PGM Sequencing 400 Kit and deposited in two Ion 318 chip Kit v2 following the manufacturer's protocol (Life Technologies).

Quantitative PCR (qPCR)

Enumeration of bacterial populations was carried out by qPCR, performed in triplicates using SYBR Green I system detection (Invitrogen). Previous to qPCR, DNA was purified with the OneStep™ PCR Inhibitor Removal Kit (Zymo Research, USA) and diluted 1:5. The bacterial primers used were 27F 5'-AGAGTTTGATCMTGGCTCAG-3' and 518R 5'- GTATTACCGCGGCTGCTGG-3' [16]. Each reaction contained 12.5 µL of Platinum® Quantitative PCR SuperMix-UDG (Invitrogen), 0.2 µM of each primer, 0.5 µL of BSA (Bovine Serum Albumin), 5 µL of template DNA and ultra-pure water to complete 25 µL final volume. Amplification conditions to bacterial primers were: initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 1 min, annealing at 56 °C for 30 seconds and elongation at 72 °C for 30 seconds. The specificity of the reaction was verified against the denaturing curve with temperatures ranging from 72 °C to 96 °C. Data were analyzed using Applied Biosystems software, and values of cycle threshold (Ct), logarithmic correlation (R^2) between number of cycles and DNA quantity in samples and reaction efficiency were calculated. As positive controls of qPCR reactions, serial dilutions of 16S rRNA PCR product of *Escherichia coli* amplified with the primers 27F-1401R (Lane, 1991) were used. Thus, values of Ct

obtained in each reaction were utilized to determine the absolute quantity of DNA in samples. The cell number per gram of sediment was estimated dividing the 16S rRNA copy number by 3.5.

Bioinformatics

The first filter step was carried out using PGM software to remove low quality and polyclonal sequences. We performed bioinformatics analysis using the Brazilian Microbiome Project (BMP) pipeline [17]. BMP pipeline is a combination of VSEARCH [18] and QIIME [19] software. Using VSEARCH barcodes and primer sequences we removed from *fastq* file, sequences were filtered by length (fastq_trunclen 200) and quality (fastq_maxee 1.0), sorted by abundance and removed singletons. After that, OTUs were clustered and chimeras were removed. We assigned taxonomy using *uclust* method in QIIME (version 1.9.0) and SILVA 16S Database (version n132) as reference sequences [20]. The OTU table file was converted to BIOM and taxonomy metadata was added. Diversity indices of Chao1, Shannon and Simpson were calculated among samples.

Statistical analyses

The most abundant OTUs were filtered and a heat map was constructed considering taxonomic classification (Class and Order) and abundance using Ward's hierarchical clustering method (ward.d2) [21]. Cell number estimates between sediment layers were compared by paired T-test [22]. Moreover, we performed beta diversity analyses to compare similarities between samples through Principal Coordinates Analysis (PCoA) and using distance matrix of Bray-Curtis, Jaccard, Unweighted and Weighted Unifrac. The differences were tested using Permutational analysis of variance (PERMANOVA) [23]. All analyses were carried out using the statistical software R [24], *qiimer*, *ggplot2* [25], *phyloseq* [26] and *vegan* packages [27].

Results

We obtained 520,863 sequences and 5,229 OTUs clustered at 97% of similarity after quality control and bioinformatics analysis from 10 sediment samples using Ion Torrent PGM. The number of sequences varied among samples, ranging from 1,110 sequences in N12-1 to 119,010 in N13-1. All sequence data are listed in Table 2.

Alpha-diversity analysis showed that the number of observed species ($OTU_{0.03}$) ranged from 204 to 3,418, revealing a wide range of species inhabiting the Sao Paulo Plateau (Table 2). Samples with highest richness indices were N13-1 (3,418), N13-2 (3,142), and N14-1 (2,924). On the other hand, samples with lowest richness indices were N12-1 (204), N06-2 (321), and N11-4 (561). Similarly,

estimated richness by Chao1 index ranged from 307 to 3,668 species. However, in contrast to the higher variability observed in the number of OTUs and estimated richness, the Shannon and Simpson indices were more uniform among samples, ranging from 6.020 to 8.114, and 0.956 to 0.986, respectively (Table 2). The only exception was sample N12-1 that exhibited the lowest number of reads and, consequently, diversity indices and estimated richness values. We did not find significative differences of alpha-diversity between asphalt and non-asphalt seep areas.

In general, the community composition from all sediment samples was similar, with most sequences classified within the phyla Proteobacteria (55.79%, mean of all samples), Actinobacteria (24.20%), Chloroflexi (4.08%), Gemmatimonadetes (2.6%), and Acidobacteria (1.98%) (Figure 2). A similar trend was observed when sequences from the phyla Proteobacteria and Actinobacteria were analyzed at class level, with Alphaproteobacteria (33.2%), Acidimicrobiia (21.8%), Gammaproteobacteria (12.2%) and Deltaproteobacteria (8.9%) composing the sediment community (Figure 3).

The most abundant OTUs were an uncultured bacterium of the order Acidimicrobiales and family OM1 clade (Acidimicrobiia) (19.5%), followed by an uncultured bacterium of the order Rhodospirillales and family Rhodospirillaceae (Alphaproteobacteria) (12.26%), an uncultured bacterium of the order NB1-j (Deltaproteobacteria) (5.15%), and an uncultured bacterium of the order Rhizobiales and family OCS116 (Alphaproteobacteria) (4.61%). Among OTUs classified at genus level, *Jannaschia* (Rhodobacteraceae) (0.99%) was found in nine of ten samples.

Samples were not clustered by depth or asphalt seep presence/absence in heatmap (Figure 4) and PCoA analyses (Suppl. Figure 1). We did not identify significant correlation between community distance matrix used in the PCoA analysis and samples category (Suppl. Table 1). Heatmap analysis showed the clusterization of two sample groups. Further, OTUs were divided in three distinct groups, in which one group was related to Acidimicrobiales and Rhodospirillales orders, the two most abundant OTUs among samples, the second group composed by orders Rhizobiales, NB1-j, Rhodobacterales and Xanthomonadales, and a third group formed by less abundant orders.

Density of bacteria in sediment samples was evaluated by qPCR and ranged from 6.75×10^{2} to 4.88×10^{5} cells.g^{-1}. Some samples had low cell numbers such as N11-1 and N11-4 with 7.4×10^{3} and 6.75×10^{2} cells.g^{-1}, respectively. The sample with the highest density was N14.1 with 4.88×10^{5} cells.g^{-1}. No amplification occurred in sample N06-4 (Table 3). In the samples N11, N13 and N14, we observed a decrease in cell number with sediment depth, but this difference was not significant (Paired T-Test: $t= 1.09$, $p=0.35$, d.f=3).

Discussion

The discovery of asphalt seeps in North São Paulo Plateau was an important milestone in studies of hydrocarbon seep environments and their associated chemosynthetic communities. This asphalt seep is similar to asphalt systems found in Campeche Knolls of southern Gulf of Mexico [28] and Angola Margin [29]. However, Fujikura et al. (2017) analyzed the oil from the asphalt seep and their results indicated that this system was not capable of sustaining chemosynthetic communities. Nevertheless, our study was the first of investigate the diversity and composition of bacterial community using next generation sequencing in asphalt seep and non-asphalt seep.

Other studies developed in deep-sea surface sediments found similar values of observed species, Chao1 and Shannon indices [30, 31], indicating that these environments harbor highly diverse microbial communities, possibly due to their temporal stability, partitioning of resources and niche diversity, allowing the coexistence of distinct microbial metabolic traits [4, 32, 33]. The values of alpha diversity were not different between samples at the asphalt seep area and far from it. The low values found at samples N06-4, N11-4 and N12-1 are probably more related to the low number of reads produced in sequencing, that can directly influence the observed species and Chao1 estimator.

Beta-diversity analysis showed that the microbial communities distribution were not influenced by sediment depth or presence/absence of asphalt seep. Despite this, four of six samples from 0-1 cm layer had as the most abundant OTU an Acidimicrobiia from Acidimicrobiales order, while in the second layer 1-4 cm, the most abundant OTU in three of five samples was an Alphaproteobacteria from Rhodospirillales order (Figure 4). Jiang et al. (2018) observed that communities from surface sediments (0-4 cm) were more similar between them than communities from bottom sediments, independently whether samples were asphalt or non-asphalt seeps (16-20 cm). In our study, core sediments were sliced in two surface sediment samples (0-1 and 1-4 cm), which may explain the homogeneity between layers caused by dispersion or even by the mixture of sediments by deep-sea water currents [32, 34].

Proteobacteria and Actinobacteria comprised most of the taxonomic groups found in the samples, a pattern commonly observed in marine sediments throughout the world. However, at class level, we found a distinct bacterial community composition, dominated by Alphaproteobacteria (33.2%), Acidimicrobiia (21.8%), Gammaproteobacteria (12.2%) and Deltaproteobacteria (8.9%), in contrast with marine sediments from other regions of the globe, where the predominant taxa, from most to least abundant, are Gammaproteobacteria, Deltaproteobacteria, Planctomycetes, Actinobacteria and Acidobacteria [4, 7, 35].

The high abundance of Alphaproteobacteria and Acidimicrobiia in our samples may be explained by the presence of oil from the asphalt seep at São Paulo Plateau [12, 14]. Some Alphaproteobacteria

taxa are able to degrade hydrocarbon such as the Rhodobacteraceae family [36, 37] composed 5% of sequences present in our samples. Bacosa et al. (2018) found an increase in the relative abundance of Rhodobacteraceae in oil treatments and, using a metagenomic approach, they could also reconstruct seven genomes, one of them classified as Rhodobacteraceae and possessing several aromatic degradation genes. In a microcosm experiment carried out by Jung et al. (2010), an increase in the abundance of *Jannaschia* (Rhodobacteraceae) genus was found after an increase in oil concentration. In the present study, *Jannaschia* was found in both non-asphalt and asphalt seep samples. Other important taxon containing polycyclic aromatic hydrocarbon (PAH)-degrading bacteria is Rhodospirillaceae that was found in relatively high abundance in our samples (14.5%). *Thalassospira xianhensis* found by Jiang et al. (2018) belongs to Rhodospirillaceae, but we cannot confirm its presence using a short fragment of 16S rRNA gene.

Alphaproteobacteria contains several species which are highly abundant in superficial pelagic environments and have a broad spatial distribution. The most common example is *Pelagibacter ubique*, a ubiquitous Alphaproteobacteria present in all oceans that have important functions in biogeochemical cycles [43, 44]. However, some studies in deeper pelagic environments also found Alphaproteobacteria composing most of the microbial community [45, 46]. Therefore, this proximity between deep seawater and sediment surface may allow microbial community interchange, since both environments have similar chemical variables and suitable habitats for these microbial populations [47, 48].

Acidimicrobiia was highly abundant in North São Paulo Plateau sediments. The taxon Acidimicrobiia was recently created by updating taxonomic classification of Actinobacteria phylum to include the Acidimicrobidae subclass, assigned as Acidimicrobiia class [49]. Most of our sequences assigned to this phylum belonged to the order Acidimicrobiales, and the two most representative OTUs were classified in OM1 clade. This group was also found in deep-sea waters [45, 50], as an important component of deep-sea sediment core microbiome in several oceans [32]..

It is generally assumed that microbial cell densities in deep-sea sediment tend to decrease with increasing sediment depth [2]. In our study this tendency was not observed, with differences in bacterial densities not being significant. This can be explained by the low sediment core depth selected in our study (0-1 and 0-4 cm) and the microbial community interchange between deep seawater and sediment surface. In addition, when compared with similar habitats from the other oceans or deep-sea chemosynthetic habitats [52], lower cell numbers were found in our study, indicating that deep-sea sediment habitats in the North Sao Paulo Plateau are more oligotrophic, and sustain a low abundance microbial community.

Conclusions

Bacterial communities in the North Sao Paulo Plateau are diverse, despite their low abundance. The classes Alphaproteobacteria and Acidimicrobiia respond for most of the diversity found, differing from other communities from similar environments, in which Gammaproteobacteria are usually more abundant. We also found a high number of unclassified sequences and the most abundant of them was related to OM1 clade. This dominance of Alphaproteobacteria and Acidimicrobiia microorganisms may be likely related to the presence of asphalt seeps and/or interchange between deep seawater and sediment surface. However further studies are needed to answer that question.

Acknowledgements

We would like to thank Japan Agency for Marine-Earth Science and Technology (JAMSTEC), the Oceanographic Institute of the São Paulo University (IOUSP), the Brazilian Geological Survey (CPRM), Petróleo Brasileiro S.A. (Petrobras) and the Embassy of Japan in Brazil for assistance in this study. We would also like to thank Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) for financial support (Project number: 2013/09159-2) and CNPq for scholarship provided to A.O.S.L (Process 311010/2015-6); the operating team of the HOV Shinkai 6500 and the crew of the R/V Yokosuka for assistance with the survey; and all team of Laboratório de Ecologia Microbiana (LECOM) for productive discussions about our methods and results, and Kleber do Espirito-Santo Filho for help with maps.

Data

The nucleotide sequence data reported are available in the NCBI under BioProject PRJNA562874.

Authorship

The author LQ, RD, CN, PS, AL, YN, KF, HK and VP designed study, LQ, RD,DG,AS and VP performed research, LQ, AB, RD and DG analyzed data; and LQ, AB, RD, CN and VP wrote the paper.

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Tables

Table 1. Coordinates, depths and alphalt seep presence/absence of sediment samples from North São Paulo Plateau.

Dive No.	Sample	Core No.	Latitude (S)	Longitude (W)	Seafloor depth (m)	Asphalt seep area	Sediment Layer
1343	06	02	20°41'37.57"	38°38'11.86"	2728	No	N06-1cm
							N06-4cm
1345	11	05	20°43'8.05"	38°39'6.23"	2720	Yes	N11-1cm
							N11-4cm
1346	12	03	20°43'54.20"	38°39'43.76"	2651	Yes	N12-1cm
							N12-4cm
1346	13	04	20°43'54.20"	38°39'43.76"	2651	Yes	N13-1cm
							N13-4cm
1347	14	03	20°44'17.20"	38°40'7.61"	2456	No	N14-1cm
							N14-4cm

Table 2. Richness and alpha diversity data found in North region from São Paulo Plateau and obtained by Ion Torrent;

Samples	Reads	Observed species ($OTU_{0.03}$)	Chao1	Shannon	Simpson
N06-1cm	93300	2900	3145	7.983	0.982
N06-4cm	3425	321	440	6.953	0.983
N11-1cm	37861	945	1042	7.650	0.986
N11-4cm	6523	561	669	7.560	0.986
N12-1cm	1110	204	307	7.952	0.956
N12-4cm	64969	2128	2365	6.020	0.984
N13-1cm	119010	3418	3668	8.114	0.983
N13-4cm	87533	3142	3507	8.022	0.983
N14-1cm	80797	2924	3332	7.903	0.980
N14-4cm	26335	1835	2289	7.761	0.981

Table 3. Cell number of Bacteria 16S rRNA gene in samples of North São Paulo Plateau. SD: standard deviation.

	Samples					
Layers	N06	SD	N11	SD	N12	SD
1 cmbsf	6.65×10^{4}	3.98×10^{2}	7.4×10^{3}	4.71×10^{2}	8.68×10^{4}	8.15×10^{3}
4 cmbsf	-	-	6.75×10^{2}	9.87×10^{1}	9.17×10^{4}	6.09×10^{3}
Layers	N13	SD	N14	SD		
1 cmbsf	4.08×10^{4}	5.61×10^{3}	4.88×10^{5}	2.21×10^{4}		
4 cmbsf	7.58×10^{3}	7.6×10^{2}	9.66×10^{3}	2.58×10^{2}		

Obs.: Sample N06-4cm did not amplify.

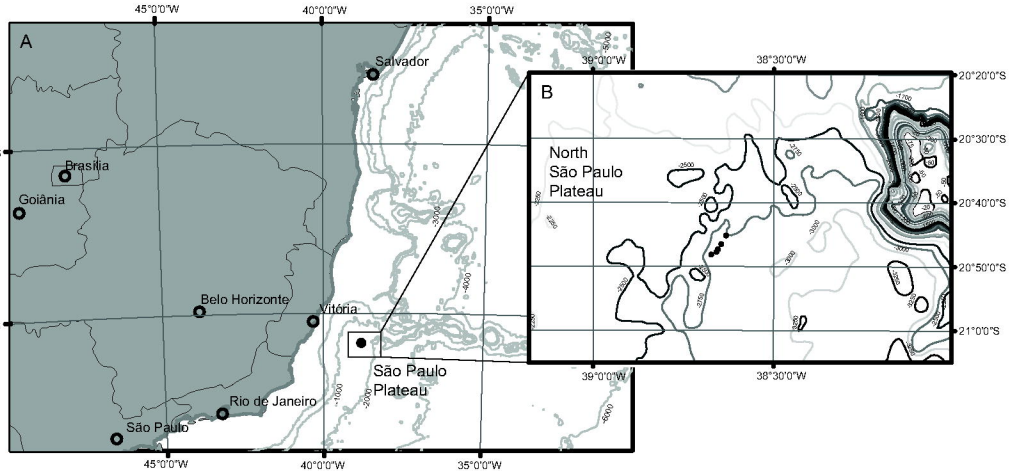
Figure Captions

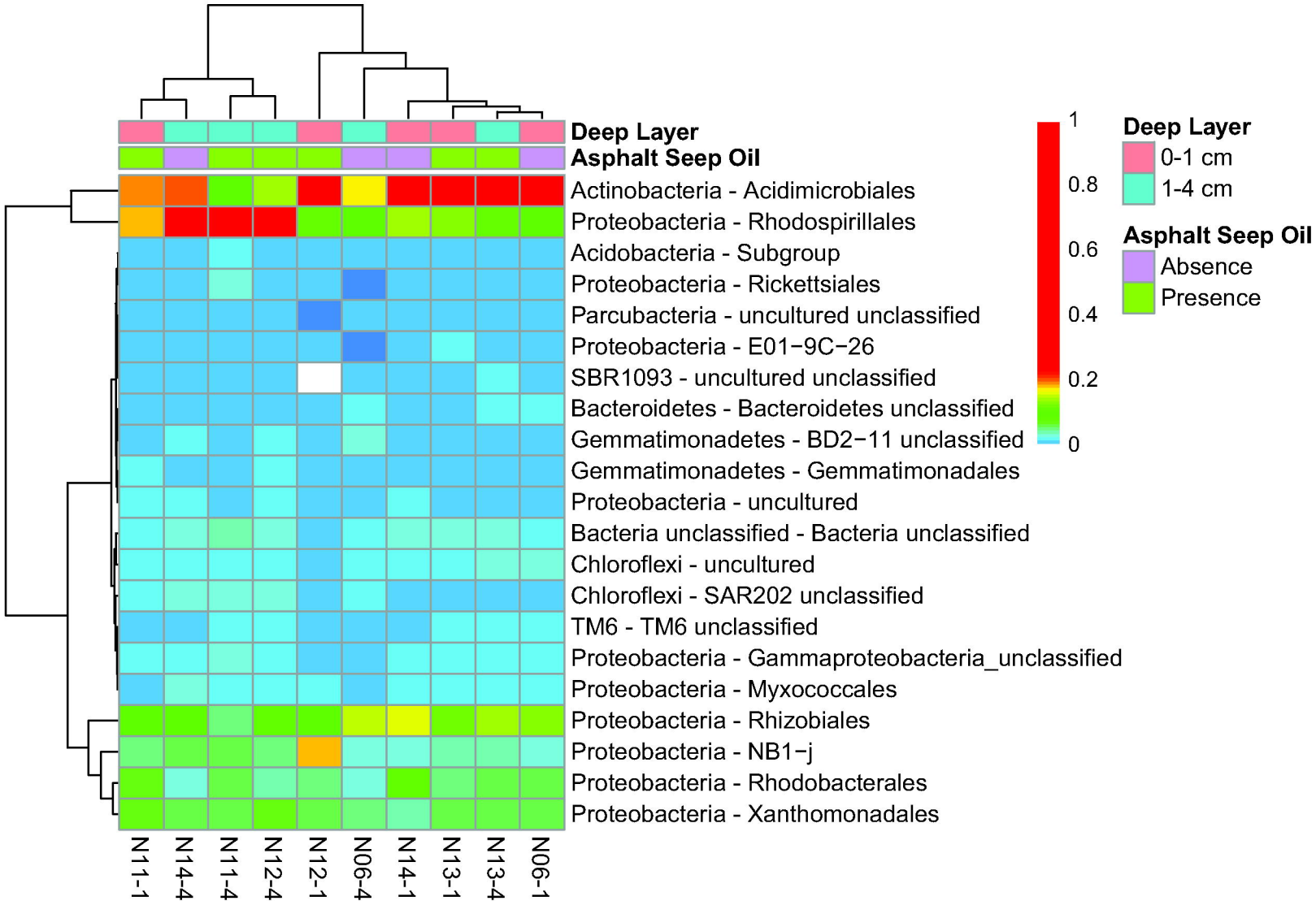
Figure 1. Map and location of samples in the São Paulo Plateau. (A) Location São Paulo Plateau and (B) distribution of superficial sediments samples in North São Paulo Plateau.

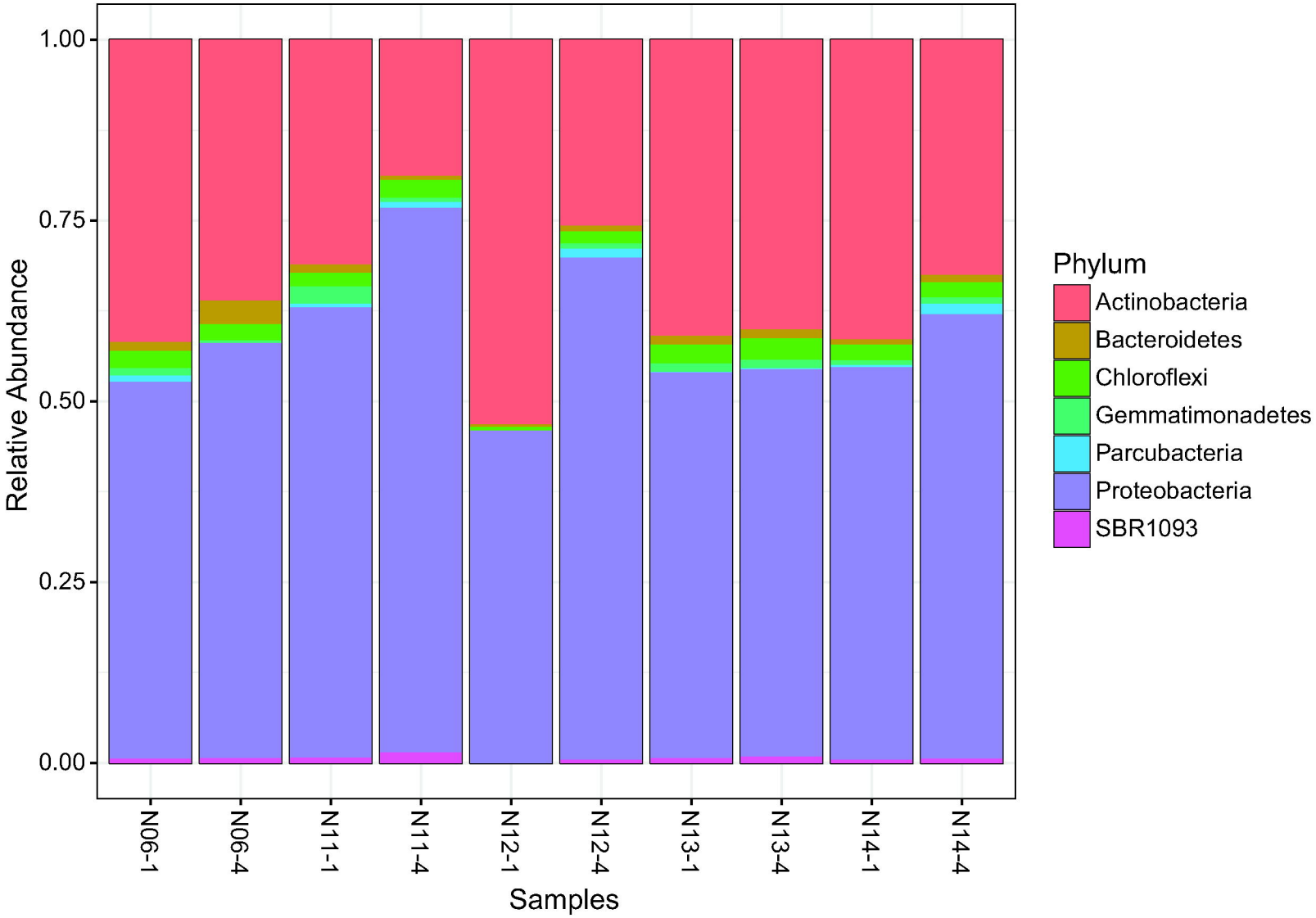
Figure 2. Heat map with abundant bacterial OTUs (21 OTUs) among the different depths and asphalt seep presence/absence in North São Paulo Plateau.

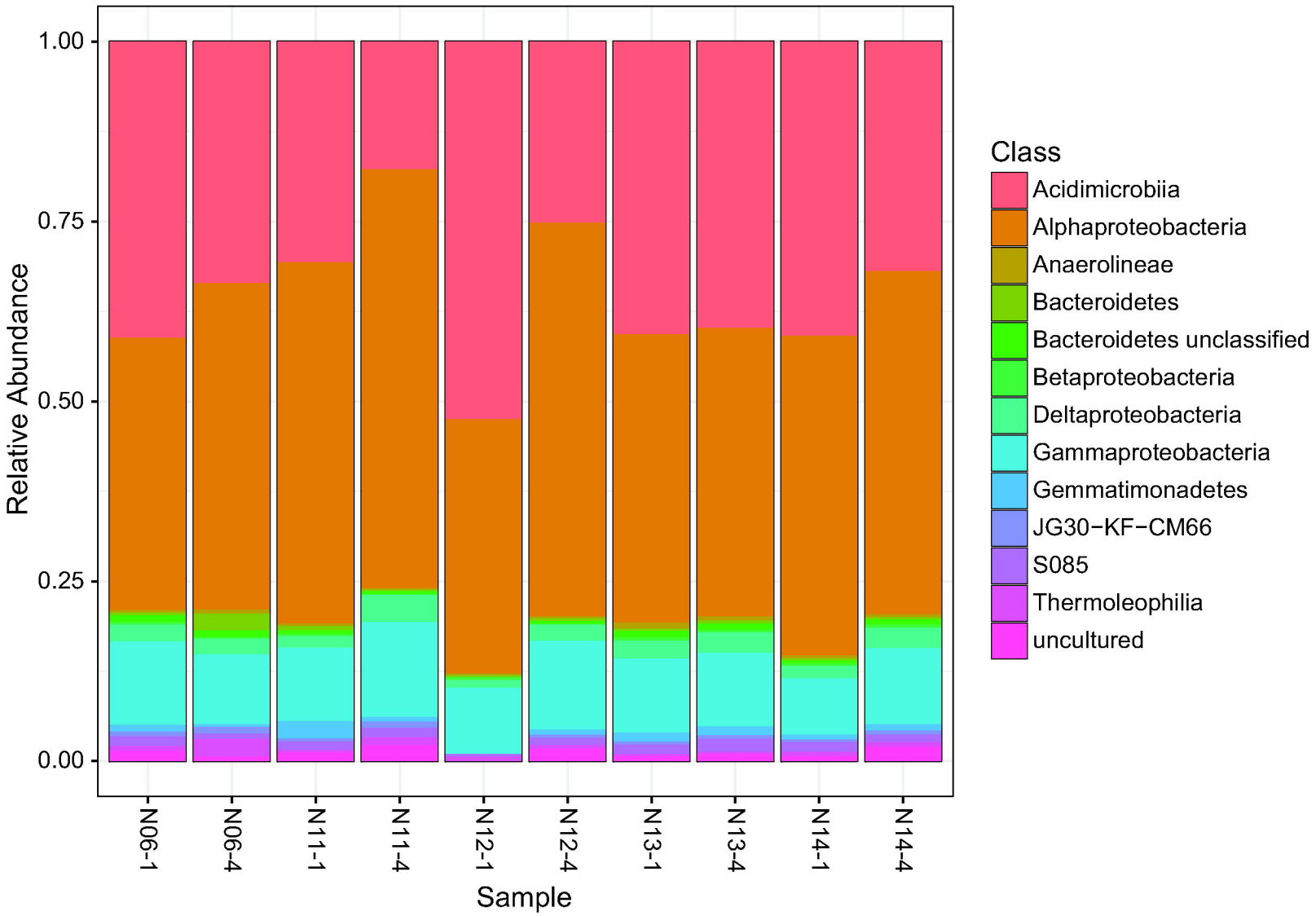
Figure 3. Relative abundance of the most abundant phyla (≈ 1%) found in North São Paulo Plateau.

Figure 4. Relative abundance of the most abundant classes (≈ 1%) found in North São Paulo Plateau.


A
45°0'0"W
40°0'0"W
35°0'0"W
15°0'0"S
20°0'0"S
Salvador
Brasília
Goiânia
Belo Horizonte
Vitória
São Paulo
Plateau
Rio de Janeiro
São Paulo
B
39°0'0"W
38°30'0"W
20°20'0"S
20°30'0"S
20°40'0"S
20°50'0"S
21°0'0"S
North
São Paulo
Plateau


Deep Layer
Asphalt Seep Oil
Actinobacteria - Acidimicrobiales
Proteobacteria - Rhodospirillales
Acidobacteria - Subgroup
Proteobacteria - Rickettsiales
Parcubacteria - uncultured unclassified
Proteobacteria - E01−9C−26
SBR1093 - uncultured unclassified
Bacteroidetes - Bacteroidetes unclassified
Gemmatimonadetes - BD2−11 unclassified
Gemmatimonadetes - Gemmatimonadales
Proteobacteria - uncultured
Bacteria unclassified - Bacteria unclassified
Chloroflexi - uncultured
Chloroflexi - SAR202 unclassified
TM6 - TM6 unclassified
Proteobacteria - Gammaproteobacteria_unclassified
Proteobacteria - Myxococcales
Proteobacteria - Rhizobiales
Proteobacteria - NB1−j
Proteobacteria - Rhodobacterales
Proteobacteria - Xanthomonadales
N11-1
N14-4
N11-4
N12-4
N12-1
N06-4
N14-1
N13-1
N13-4
N06-1
1
0.8
0.6
0.4
0.2
0
Deep Layer
0-1 cm
1-4 cm
Asphalt Seep Oil
Absence
Presence


1.00
0.75
0.50
0.25
0.00
Relative Abundance
N06-1
N06-4
N11-1
N11-4
N12-1
N12-4
N13-1
N13-4
N14-1
N14-4
Samples
Phylum
Actinobacteria
Bacteroidetes
Chloroflexi
Gemmatimonadetes
Parcubacteria
Proteobacteria
SBR1093


1.00
0.75
0.50
0.25
0.00
Relative Abundance
N06-1
N06-4
N11-1
N11-4
N12-1
N12-4
N13-1
N13-4
N14-1
N14-4
Sample
Class
Acidimicrobiia
Alphaproteobacteria
Anaerolineae
Bacteroidetes
Bacteroidetes unclassified
Betaproteobacteria
Deltaproteobacteria
Gammaproteobacteria
Gemmatimonadetes
JG30−KF−CM66
S085
Thermoleophilia
uncultured