FEMS Microbiology Letters, 2023, **370**, 1–8
DOI: 10.1093/femsle/fnad064
Advance access publication date: 3 July 2023
Research Letter – Environmental Microbiology and Microbial Ecology

The microbiome of the sponge *Aplysina caissara* in two sites with different levels of anthropogenic impact

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Editor: [Yu-Zhong Zhang]

Abstract

Despite the important roles that marine sponges play in ecosystem functioning and structuring, little is known about how the sponge holobiont responds to local anthropogenic impacts. Here we assess the influence of an impacted environment (Praia Preta) on the microbial community associated with the endemic sponge *Aplysina caissara* in comparison to a less-impacted area (Praia do Guaecá) from the coast of São Paulo state (Brazil, southwestern Atlantic coast). We hypothesized that the local anthropogenic impacts will change the microbiome of *A. caissara* and that the community assembly will be driven by a different process (i.e. deterministic versus stochastic) under distinct levels of impact. The microbiome at the amplicon sequence variants level was found to be statistically distinct between sponges from the different sites, and this was also seen for the microbial communities of the surrounding seawater and sediments. Microbial communities of *A. caissara* from both sites were found to be assembled by deterministic processes, even though the sites presented distinct anthropogenic impacts, showing a pivotal role of the sponge host in selecting its own microbiome. Overall, this study revealed that local anthropogenic impacts altered the microbiome of *A. caissara*; however, assembly processes are largely determined by the sponge host.

Keywords: porifera, barcoding, local pollution, community structure, deterministic and stochastic processes, southwestern atlantic coast

Introduction

Marine sponges (phylum Porifera) are key components of benthic communities, where they play essential roles in ecosystem functioning (Bell 2008). They also harbor complex bacterial, archaeal, fungal, and micro-eukaryotic microbiomes (Hardoim et al. 2021a, Thomas et al. 2016, De Mares et al. 2017, Moitinho-Silva et al. 2017, Nguyen and Thomas 2018, Nascimento-Silva et al. 2022). The symbiotic bacterial and archaeal communities have been shown to be stable over time (Hardoim and Costa 2014, Cárdenas et al. 2019), season (Erwin et al. 2015), geographical locations (Cárdenas et al. 2018), and depth (Steinert et al. 2016). Other studies evaluated how sponge microbiomes respond to various stressors, including temperature, acidification, eutrophication, sedimentation (Pita et al. 2018), carbonate chemistry (Morrow et al. 2015), nutrients (Simister et al. 2012, Luter et al. 2014), heavy metals (Tian et al. 2014, Gantt et al. 2017) and crude oil (Luter et al. 2019). In contrast, very little is known about how sponge-associated symbiont communities respond to general local anthropogenic impacts, such as pollutants.

The assembly of sponge microbiomes will most likely involve deterministic and/or stochastic processes (Zhou and Ning 2017). Deterministic processes presume that species traits, interspecies interactions (e.g. mutualism, predation, competition) and environmental factors (e.g. nutrients, temperature, salinity) shape the assembly of microbial communities (Vellend 2010, Zhou and Ning 2017). In the case of sponge-associated bacterial communities, this may be driven by host traits, microbe-microbe interactions and/or environmental conditions (Hardoim et al. 2021a, De Mares et al. 2017, Steinert et al. 2017, 2019). In addition, stochastic processes (e.g. immigration, spatio-temporal variation, historical contingency) have also been shown to influence microbial community assembly (Chen et al. 2017, Zhou and Ning 2017). Both processes are now recognized to occur concomitantly and show the specificity of the interaction with the host (Gravel et al. 2006, Chase and Myers 2011, Stegen et al. 2016, Zhou and Ning 2017). Microbial species likely have a differential ability to participate in or be influenced by deterministic and stochastic processes (Pandit et al. 2009, Székely and Langenheder 2014). However, whether pollutants have an impact on the balance of these processes in sponge microbiomes has not been studied.

The 700-km coast of São Paulo state (Southeast Brazil) is considered one of the benthic biodiversity hotspots of the Southwestern Atlantic (Soares et al. 2017) and marks the transition between tropical and warm temperate ecoregions (Spalding et al. 2007). Along this coastline, the region of São Sebastião hosts over 70 described sponge species (Custódio and Hajdu 2011) and could be

Received 17 March 2023; **revised** 26 June 2023; **accepted** 27 June 2023

considered a local hotspot of sponge biodiversity in Brazil. The São Sebastião Channel (SSC) divides the continental area of São Sebastião from the São Sebastião Island and contains the second biggest harbor (São Sebastião harbor) and the largest storage terminal of oil and gas in Brazil (the Terminal Aquaviário Almirante Barroso, TEBAR, da Silva and Bícego 2010, Amaral et al. 2016), which have operated since 1955 and 1967, respectively. Since their implementation, the region has faced several environmental impacts, such as landfills, dredging and oil spills (Amaral et al. 2016) and the continuous small-scale leakage of hydrocarbons from the pipelines that bring oil from the in-land facilities to TEBAR (Lamparelli and Ortiz 2007). In addition to this, the Araçá submarine sewage outfall discharges 44% of the urban wastewater of São Sebastião city in the area (CETESB 2020), imposing an additional anthropogenic pressure onto the local environment. Together, these factors directly affect the environmental complexity and biodiversity along the SSC (Amaral et al. 2016), resulting in steep, local gradients of anthropogenic impacts. This is further exemplified by the area of Praia Preta, which is close to the Araçá submarine sewage outfall, the São Sebastião harbor and the TEBAR (Hardoim et al. 2021b, Medeiros and Bícego 2004, Muniz et al. 2015). This location has been shown to have high concentrations of aliphatic hydrocarbons, petroleum biomarkers, fecal contaminations, and thermotolerant coliform bacteria in the sediment. This contrasts with the area of Praia do Guaecá, which has a much lower level of contaminants and can thus be considered a reference site in the studies of anthropogenic impacts (Hardoim et al. 2021b, Medeiros and Bícego 2004, da Silva and Bícego 2010, Muniz et al. 2015, Amaral et al. 2016, CETESB 2019, Birocchi et al. 2021). These two areas are therefore ideal to further our knowledge of the influence that local anthropogenic impacts have on sponges and the assembly of their microbiomes.

We collected and analysed the endemic sponge *Aplysina caissara* along with seawater and sediments from both sites. Our first hypothesis is that the local anthropogenic impacts change the sponge's microbiome. Furthermore, given the very specific interactions that bacteria have with sponges, we expect that the alterations in the microbiome are unique or distinct from the changes in the surrounding seawater or sediment. Our second hypothesis is that the interactions between microbes and the sponge result in more deterministic processes of microbiome assembly in comparison to seawater and sediment, and that the relative proportion of deterministic versus stochastic processes will vary with the local anthropogenic impacts.

Material and methods

Design and sampling

Samples were collected at Praia Preta (23°49′24.24″S–45°24′40.679″W) and at the Southern rock shores of Guaecá, hereafter called Praia do Guaecá (23°49′22.8″S–45°28′19.2″W) on the 13th of March 2019. These locations are around 6 km apart (Fig. 1). Sampling details were described in the Supplementary Material 1.

Microbial community analysis

Genomic DNA was extracted from 0.25 g of sponge choanosome samples using the DNeasy PowerSoil DNA isolation kit (QIAGEN, Germany) according to the manufacturer's protocol. Seawater samples (1 L) were filtered through 0.2-μm-pore-size nitrocellulose filters (Merck Millipore, USA) using a vacuum pump. After, the whole filters were cut into small pieces and directly used for DNA extraction. Sediment samples were mixed, sieved and aliquots of 0.25 g were used for DNA extraction.

Sponge barcoding

Identification and barcoding of sponges using the primer pair Diplo-cob-f1m and Diplo-cob-r1 (Lavrov et al. 2008) for a 364 bp fragment from the cytochrome b (*cob*) gene were performed as explained in detail in Hardoim et al. (2021c). Classical phylogenetic markers, as COI, ITS, 18S, and 28S rRNA either were not able to distinguish the *Aplysina* species or no amplification was obtained. Details on the phylogenetic analyses can be found in Supplementary Material 1.

16S rRNA gene sequencing and analyses

Briefly, the V4-region of the 16S rRNA gene of bacteria and archaea was amplified with the primer pair 515F-806R (Apprill et al. 2015, Parada et al. 2016). The reaction mixture and thermal cycle were performed as explained previously (Hardoim et al. 2021). The amplicons were subjected to Illumina sequencing using MiSeq platform.

The initial quality check of the sequences was performed with FastQC (Wingett and Andrews 2018). Sequence data were quality-filtered and trimmed using Trimmomatic version 0.36 (Bolger et al. 2014), truncating reads if the quality dropped below 25 in a sliding window of 4 bp. USEARCH version 11.0.667 (Edgar 2013) was used for further processing to merge and quality-filter sequencing reads, excluding reads with < 230 or > 300 nucleotides, in addition to reads with more than one ambiguous base or an expected error of >1. Filtered sequences were denoised and clustered into unique sequences (amplicon sequence variants, ASV) using the UNOISE3 algorithm (Edgar 2016a) implemented in USEARCH. Chimeric sequences were removed *de novo* during clustering and subsequently in reference mode using UCHIME2 (Edgar 2016b) with the Genome Taxonomy Database (GTDB, Parks et al. 2020). The ASVs were classified against GTDB using the BLCA algorithm (Gao et al. 2017). Sequences from mitochondria and chloroplasts were removed from the dataset based on the Greengenes 13_5 taxonomy (McDonald et al. 2012).

Ecological metrics and statistical analyses

The alpha-diversity measurements of Good's coverage (Good 1953), richness (Observed ASVs, CHAO, and ACE), diversity (Shannon—*H*' and inverse Simpson—D^2) and evenness (Pielou's evenness) were calculated using the package vegan v. 2.5–6 (Oksanen et al. 2019, R Core Team 2022). Vegan v. 2.5–6 was also used to perform an analysis of variance (ANOVA) for the alpha metrics (Oksanen et al. 2019, R Core Team 2022). A P value of ≤ 0.05 was considered statistically significant. The R package multcomp version 1.4–13 (Hothorn et al. 2016, R Core Team 2022) was applied to make multiple comparisons of means with Tukey contrasts.

The non-metric multidimensional scaling (nMDS) was used to visualize patterns of Bray–Curtis (BC) dissimilarities in community structure at the levels of ASV and class using the vegan package v. 2.5–6 (Oksanen et al. 2019, R Core Team 2022). Permutational multivariate analysis of variance (PERMANOVA) was used to test the significance of the differences across samples. Generalized linear models (GLM) were separately fitted to each ASV and class using the R package mvabund (Wang et al. 2021, R Core Team 2022) with a negative binomial distribution, given that a mean-variance relationship was observed. The resulting sum of likelihood ratio statistics and statistical significance was evaluated with ANOVA using mvabund (Wang et al. 2021, R Core Team 2022). Bubble

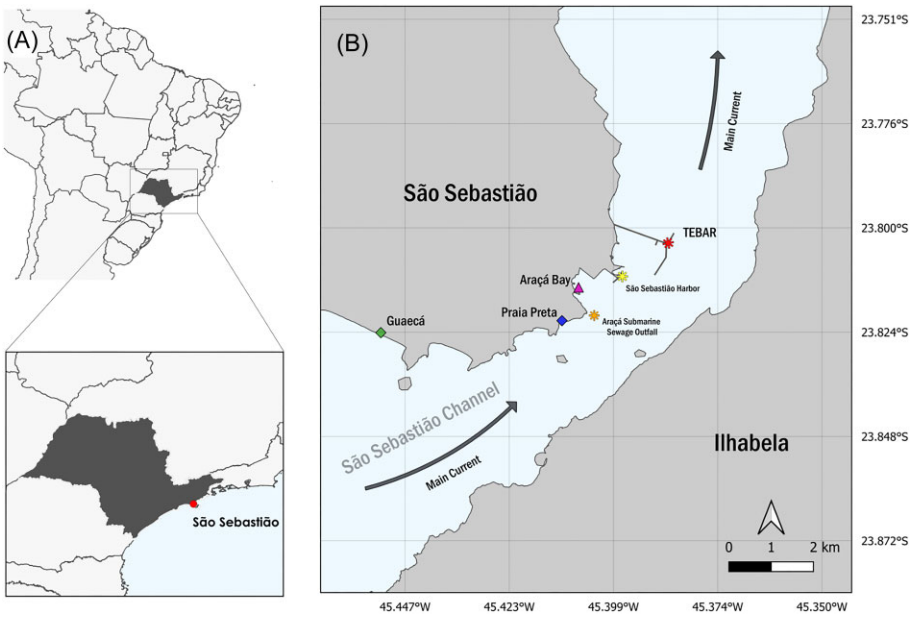

Figure 1. Map of the sampling sites and sources of pollution. Location of the sampling sites, the contaminated site of Praia Preta (blue diamond), less-impacted site of Praia do Guaecá (green diamond) on the São Sebastião Channel, Southeastern Brazil. Araçá Bay, one of the biodiversity hotspots in the region (magenta triangle). Sources of contamination: TEBAR (red asterisk), São Sebastião Harbor (yellow asterisk) and Araçá Submarine Sewage Outfall (orange asterisk).

plots were constructed for the most abundant classes, with relative abundance across all samples above 0.5%, using the R packages ggplot2 v3.3.2 and reshape2 v1.4.4 (Wickham. 2016, Wickham 2017, R Core Team 2022).

To test if the microbiomes of the sample types and sites were significantly different, the relative read abundances at the ASV level were evaluated. Analysis of compositions of microbiomes with bias correction (ANCOM-BC) was used to detect the differences in ASVs between the same sample type from different sites (Lin and Peddada 2020). ANCOM-BC evaluates the unknown sampling fractions and modifies the bias produced by their disparities among samples. The abundance data are modeled using a linear regression framework.

Community assembly analyses

The modified stochasticity ratio (MST) index was performed based on Bray–Curtis distance using the tNST (taxonomic normalized stochasticity ratio) function implemented in the R package NST (Ning et al. 2019, R Core Team 2022). The MST index is a particular transformation of NST, which ranges from 0% to 100%, where the former signifies no contribution of stochastic processes and the latter specifies that the community is driven by stochastic processes. The value of the MST index indicates a deterministic- or a stochastic-dominated community assembly when it is below or above 50%, respectively (Ning et al. 2019). Additionally, the Sloan neutral model was used to assess the potential contribution of stochastic processes to microbial community assembly in each sample type and site (Sloan et al. 2006, Burns et al. 2016). It predicts the correlation between the occurrence frequency of ASVs (the proportion of local communities in which each ASV was detected) and their abundances (the mean relative abundance across all local communities) (Sloan et al. 2006). In general, the most abundant taxa in the metacommunity are expected to be more widespread and be randomly sampled in any given sample, while the rare taxa are more likely to be lost in different local communities because of ecological drift. In the model, the estimated migration rate (m) evaluates the probability that a random loss of an individual in a local community would be replaced by a source community (i.e. more driven by stochastic processes) or by reproduction of a member of the local community (i.e. more driven by deterministic processes). The parameter R^2 specifies the overall fit to the neutral model, when its value is closer to 1, it indicates that the community was consistent with the neutral processes, while < 0 was considered unfit (Sloan et al. 2006). The 95% confidence interval of the model was calculated based on 1000 bootstrap replicates. Each treatment was used to predict the model using R scripts, as previously described (Burns et al. 2016). This analysis compares the fit of the neutral model (R^2) of a given sample type and site with the fit of random sampling (R^2.pois) of all sample types and sites. The model is considered fitted when R^2 is larger than R^2.pois.

Results

Aplysina caissara identification

Phylogenetic analysis of 364 bp-long sequences of the cytochrome b gene showed no intraspecific variations among the 10 individuals collected from both sites (Supplementary Figure 1). The proportion of nucleotide sites at which two sequences being compared are different (*P*-distance) between *A. caissara* from this study and those obtained from Recife (Northern Brazil) and Genbank was 0.82%. Phylogenetic reconstructions based on maximum likelihood and Bayesian inferences showed that *A. caissara* formed a robust cluster that is distinct from other *Aplysina* species.

Bacterial and archaeal alpha diversity

A total of 2 492 761 sequences for the V4-region of the 16S rRNA gene were obtained after quality control, removal of chimera, singletons, mitochondria and chloroplast, and assigned to 8339 amplicon sequence variants (ASVs, Supplementary Table 1). For the alpha diversity, the dataset was rarefied to 50 081 sequence reads per sample, resulting in a total of 1 502 430 sequences that were

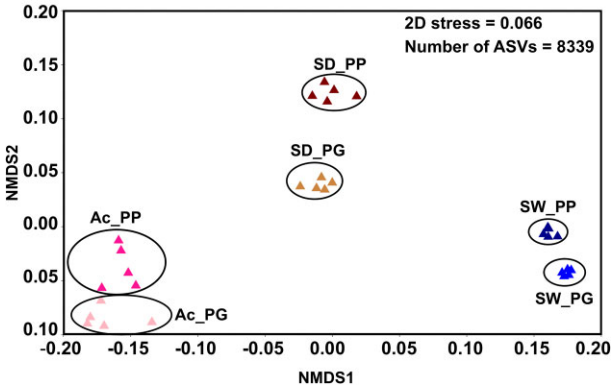

Figure 2. Microbial community structure at the ASV-level. Non-metric multidimensional scaling (nMDS) based on Bray–Curtis distances for ASV-level.

assigned to 8339 ASVs. The rarefaction curves demonstrated that the given sequencing effort captured most of the diversity for any given sample (Supplementary Figure 2), which was also supported by Good's coverage estimates of > 97.7% for all samples (Table 1).

The microbiome of sediment showed higher values for all alpha diversity metrics than the microbiome of *A. caissara* (Table 1). For the microbiome of *A. caissara*, the Shannon diversity and Pielou's evenness indices were significantly lower ($P < 0.05$) for the less-impacted site of Praia do Guaecá compared to the contaminated site of Praia Preta (Supplementary Table 2). In contrast, significantly higher richness and Shannon diversity indices ($P < 0.001$) were observed for the microbiome of sediments from Praia do Guaecá when compared to Praia Preta. All indices, except inverse Simpson, were significantly lower ($P < 0.001$) for the microbiome of seawater from Praia do Guaecá when compared to Praia Preta.

Comparison of community structure

The nMDS of the BC dissimilarity at the ASV level showed a clear separation between sample types as well as between sites (Fig. 2). These patterns for the factors sample types and sites were supported by permutational multivariate analysis of variance (PERMANOVA, $P < 0.001$, Supplementary Table 3a) and by GLM-based analysis ($P = 0.001$, Supplementary Table 3e), while there was no support for the interactions of these two factors (GLM, $P = 0.095$). Given these results, the differences in community structure were analyzed for each sample type separately (Supplementary Table 3b–d, f–h).

Analysis of compositions of microbiomes with bias correction (ANCOM-BC) between sites for each sample type was performed at the ASV-level. For the microbiome of *A. caissara*, 71 ASVs were significantly different (ANCOM-BC, $P < 0.05$, Supplementary Table 4) in relative read abundances between the contaminated site of Praia Preta and less-impacted site of Praia do Guaecá. From these enriched ASVs, 73.2% had a higher relative read abundance at Praia Preta and 26.8% at Praia do Guaecá. For the microbiome of seawater, 549 ASVs were significantly different (ANCOM-BC, $P < 0.05$) in relative read abundances between sites. A total of 60.3% of the ASVs had relative read abundances higher at Praia do Guaecá and 39.7% at Praia Preta. For the microbiome of sediments, 3202 ASVs showed distinct relative read abundances (ANCOM-BC, $P < 0.05$) between sites, of which 56% were higher at Praia Preta and 44% at Praia do Guaecá. Among these enriched ASVs, 48 and 36 were affiliated with genera known to contain members capable of degrading polycyclic aromatic hydrocarbons (PAHs) and linear alkylbenzenes (LABs), respectively (Supplementary Table 4).

The analysis of microbial community structure based on the BC dissimilarity of the 81 classes detected in the dataset was similar to that from ASV-based analyses for seawater and sediment samples, while replicates from *A. caissara* grouped together independently of the site (Supplementary Figure 3). This distinct pattern among sample types and the interaction between sample types and sites were statistically supported by PERMANOVA ($P < 0.001$) and GLM-based analysis ($P < 0.05$) as well as between sites (GLM, $P = 0.023$). For the microbiome of *A. caissara* from both sites, the classes with the highest relative read abundances were the *Gammaproteobacteria* and *Dehalococcoidia* (Supplementary Table 5, Fig. 3). The microbiome of seawater from both sites was dominated by reads assigned to the *Alphaproteobacteria* and *Cyanobacteriia* (Fig. 3). *Gammaproteobacteria* was the most abundant class in the microbiome of sediment from both sites, followed by *Cyanobacteria* in Praia Preta and *Nitrososphaeria* in Praia do Guaecá (Fig. 3).

Assemblage of microbial community

A MST analysis based on BC dissimilarity showed that the assembly of microbial communities of *A. caissara* was strongly driven by deterministic processes (MST < 4%, Fig. 4), independent of the investigated sites. The microbiome of seawater from Praia do Guaecá was also characterized by a stronger deterministic assembly processes when compared to Praia Preta (Fig. 4). In contrast, microbial community assembly in the sediment samples was largely dominated by stochastic process (MST > 79%).

The estimated immigration rate *m* of each community group revealed that the Sloan neutral model fitted well for the microbiome of *A. caissara* (Supplementary Table 6). The *m* values for the microbiome of *A. caissara* were smaller than those observed in the microbiome of the sediment, independent of the sites, which reinforces the importance of deterministic processes governing community assembly within the sponges.

Discussion

Microbial community structure

An increase in Shannon diversity and Pielou's evenness was observed in *A. caissara* at Praia Preta compared to Praia do Guaecá (Table 1, Supplementary Table 2). To the best of our knowledge, the vast majority of studies performed in those areas investigated PAHs and LABs in sediments (Hardoim et al. 2021b, da Silva and Bícego 2010, Muniz et al. 2015), which are biomarkers for petroleum and sewage contaminations, respectively, mainly due to the presence of the São Sebastião Harbor, TEBAR, and Araçá sewage outfall next to Praia Preta (Fig. 1). Whereas, Praia do Guaecá is considered a reference site, because it presented a much lower concentration of PAHs and LABs, with some of their constituents not being detected (Hardoim et al. 2021b, Medeiros and Bícego 2004, da Silva and Bícego 2010, Muniz et al. 2015, Amaral et al. 2016, CETESB 2019, Birocchi et al. 2021). The higher inputs of those pollutants in Praia Preta might have supported the increase in numbers of less-dominant microbial members that would be capable to degrade these compounds and/or the reduction in the abundance of members that are dominant in other less-impacted areas but lack this capacity.

The microbiomes of *A. caissara* and sediment from Praia Preta had more enriched ASVs than Praia do Guaecá, whereas the opposite occurred with the seawater. For the microbiomes of *A. caissara* and seawater from Praia Preta, four and five enriched ASVs,

Table 1. Ecological metrics values for richness, diversity, and evenness indices.

	Ac_PP	Ac_PG	SW_PP	SW_PG	SD_PP	SD_PG
Good´s coverage	99.86 ± 0.018	99.85 ± 0.009	99.20 ± 0.019	99.30 ± 0.013	97.78 ± 0.040	97.92 ± 0.026
Observed Richness	356 ± 9.94	360.8 ± 6.785	1193.8 ± 18.90	992.8 ± 17.72	5053.2 ± 50.72	5555.4 ± 22.04
CHAO	427.70 ± 23.51	443.3 ± 7.40	1713.50 ± 29.71	1457.90 ± 37.10	5821.74 ± 45.44	6203.73 ± 26.85
Ace	430.23 ± 24.33	440.6 ± 8.25	1664.12 ± 31.51	1445.14 ± 23.43	5778.25 ± 46.74	6135.08 ± 18.40
Shannon	4.29 ± 0.070	4.12 ± 0.02	4.26 ± 0.020	3.76 ± 0.033	7.23 ± 0.044	7.42 ± 0.015
Inverse Simpson	35.18 ± 6.94	20.7 ± 0.99	17.54 ± 0.54	9.62 ± 0.159	316.25 ± 18.40	299.26 ± 8.96
Pielou's evenness	0.73 ± 0.011	0.70 ± 0.003	0.60 ± 0.002	0.54 ± 0.003	0.85 ± 0.004	0.86 ± 0.0015

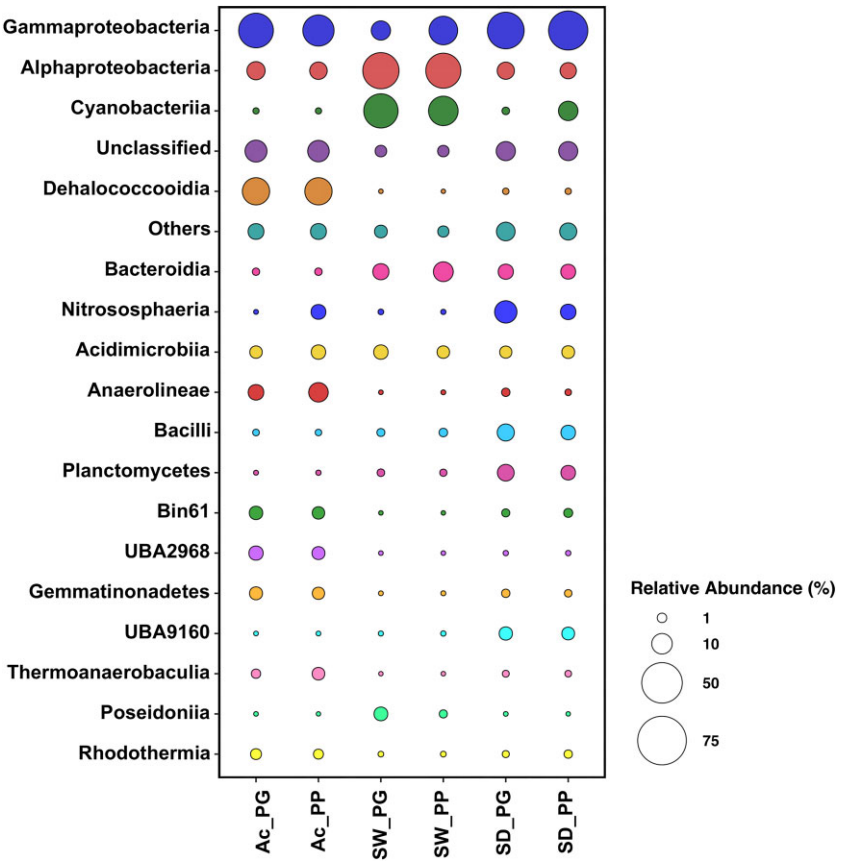

Figure 3. Microbial community composition. Bubble plot for the most relative read abundances classes. Ac: *Aplysina caissara*, SW: seawater, SD: sediment, PP: Praia Preta, PG: Praia do Guaecá.

respectively, were assigned to genera containing members that have been reported to be hydrocarbon-degraders in marine environment or possess the genes needed for PAHs degradation (Supplementary Table 4), whereas for the microbiomes of *A. caissara* and seawater from Praia do Guaecá, zero and three enriched ASVs, respectively, exhibited similar features (Dong et al. 2014, Brown et al. 2015, Yuan et al. 2015, Hazaimeh and Ahmed 2021, Xiao et al. 2021). For the sediments, there were 16 and 20 enriched ASVs from Praia Preta and Praia do Guaecá, respectively, encompassing members that have been described as hydrocarbon-degraders in the marine environment (Dong et al. 2014, Brown et al. 2015, Hidalgo et al. 2020, Hazaimeh and Ahmed 2021). It is still to be determined if those genera are indeed metabolizing PAHs *in situ*; however, these results indicate that the higher inputs of hydrocarbons in Praia Preta were sufficient to select for hydrocarbon degraders in the microbiomes of *A. caissara* and seawater.

Another source of pollution at Praia Preta was the Araçá submarine sewage outfall, the probable origin of the LABs used for the synthesis of alkylbenzene sulfonates (LAS), which are the most widely used anionic surfactants in detergents (Hardoim et al. 2021b, Scott and Malcolm 2000). The microbiomes of *A. caissara* and sediment from Praia Preta were enriched in two and 20 ASVs, respectively (Supplementary Table 4), assigned to the genera known to contain LAS degraders (Andrade et al. 2020, Kim et al. 2021), while no similar enrichment was observed in Praia do Guaecá. These genera are found in activated sludge used in domestic sewage or wastewater treatment plants, but the ability of their marine counterparts to degrade LAS in *A. caissara*, seawater and sediment still needs further investigation. Similar to PAHs, this indicates that the higher input of LAS in Praia Preta might have selected those members of the microbiome that would have the capacity to degrade these compounds. Overall, these results

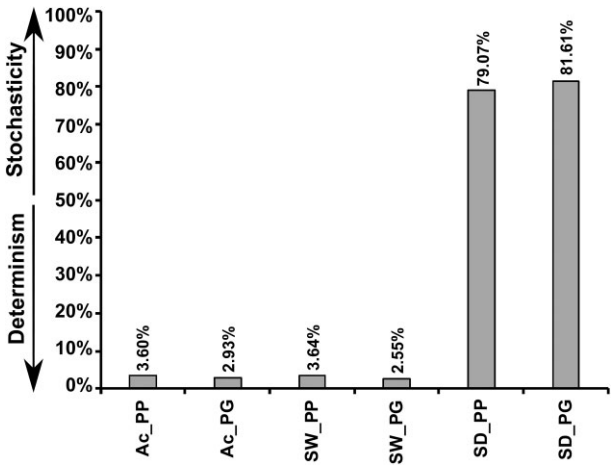

Figure 4. Microbial community assemblage. The modified stochasticity ratio (MST) of the microbial community. Ac: *Aplysina caissara*, SW: seawater, SD: sediment, PP: Praia Preta, PG: Praia do Guaecá.

together with the community structure at ASV- and class-levels (Fig. 2, Supplementary Figure S3), demonstrated that due to the specificity of the sponge microbiome, the alterations observed in the present study in *A. caissara* were distinct from those detected in seawater and sediments.

Assembly of microbial community

As hypothesized, a more deterministic and stochastic processes of microbiome assembly were observed in *A. caissara* and sediments, respectively. However, the seawater microbiome was mainly assembled by deterministic processes (Fig. 4). In contrast to our hypothesis, the local anthropogenic impacts did not influence the relative proportion of deterministic *vs*. stochastic processes in the assembly of the microbiome of *A. caissara*, seawater, and sediment.

Deterministic processes, such as host features, microbe-microbe interactions, and environmental conditions are thus most likely the main processes in the assembly of the microbiome of *A. caissara* as previously described (Hardoim et al. 2021a, De Mares et al. 2017, Steinert et al. 2017, 2019). The microbial communities of sediments collected at the bioturbation zones (10–15 cm below the seafloor) are mainly assembled by stochastic processes (Petro et al. 2017), which corroborated the results obtained here. The microbial communities of seawater contaminated with crude oil have been previously shown to be mainly assembled by deterministic processes (Nikolova et al. 2021) in agreement with what was seen in the present study.

Conclusions

The distinct inputs of toxic compounds affect the microbiome not only of the environment (i.e. seawater and sediment), which has been previously noted, but also of *A. caissara*. The structure of the host microbiome was modified, including members that could have the capacity to degrade toxic compounds, such as PAHs and LABs. As all *A. caissara* individuals are located relatively close to each other they most likely belong to the same population, and thus the host genetics and its derived traits are unlikely to explain any result observed in the present study. Interestingly, the assembly of microbiomes in *A. caissara* collected at both sites was mainly driven by deterministic assembly processes. The results obtained here provide the baseline to perform a controlled experiment to verify the effects of different concentrations of PHAs and LABs would have on the functional role and metabolism of the sponge microbiome. Together, it can provide a better understanding of how the sponge holobiont may adapt to pollution stressors.

Permits

Sampling was performed under the scientific collection permits A097B99 issued by Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado, 61460–2 issued by Sistema de Autorização e Informação sobre Biodiversidade do Instituto Chico Mendes de Conservação da Biodiversidade, both from the Ministério do Meio Ambiente and 260108-001.161/2013 issued by the Instituto Florestal, Secretaria do Meio Ambiente do Estado de São Paulo.

Author contributions

CH designed the experiment. GL-H and MRC identified the sponge species. CH collected and pre-processed the samples, prepared the samples for high-throughput sequencing. CH, PH and TT analyzed the data. CH, MRC, and TT wrote the manuscript. All authors revised the draft, approved the final manuscript version.

Acknowledgments

We would like to thank the CEBIMar/USP for allowing the use of the entire infrastructure and also the staff, especially Eduardo Honuma and Joseilto Medeiros de Oliveira for all the assistance with sampling and Emerson de Paula Candido for the bureaucratic organization. We are also very thankful to Ulisses Pinheiro from UFPE for providing *Aplysina* spp. samples. The authors also thank Gabriel Nascimento-Silva for the map. This is a contribution of NP-BioMar (Research Center for Marine Biodiversity—USP).

Supplementary data

Supplementary data are available at *FEMSLE Journal* online.

Conflict of interest: The authors declare no competing interests.

Funding

This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) through the Young Investigator project number No. 2016/17189-7 granted to CCPH. CCPH was also the recipient of a Young Investigator fellowship granted by FAPESP (No. 2017/10157-5). We thank Andressa C.M. Ramaglia who was the recipient of a Technical Training fellowship (TT-3) granted by FAPESP (No. 2018/12937-0) for her assistance in sampling and preprocessing of the samples.

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Received 17 March 2023; **revised** 26 June 2023; **accepted** 27 June 2023