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Coral microbial community dynamics in response to anthropogenic impacts near a major city in the central Red Sea

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ABSTRACT

Coral-associated bacteria play an increasingly recognized part in coral health. We investigated the effect of local anthropogenic impacts on coral microbial communities on reefs near Jeddah, the largest city on the Saudi Arabian coast of the central Red Sea. We analyzed the bacterial community structure of water and corals (*Pocillopora verrucosa* and *Acropora hemprichii*) at sites that were relatively unimpacted, exposed to sedimentation & local sewage, or in the discharge area of municipal wastewaters. Coral microbial communities were significantly different at impacted sites: in both corals the main symbiotic taxon decreased in abundance. In contrast, opportunistic bacterial families, such as e.g. Vibrionaceae and Rhodobacteraceae, were more abundant in corals at impacted sites. In conclusion, microbial community response revealed a measurable footprint of anthropogenic impacts to coral ecosystems close to Jeddah, even though the corals appeared visually healthy.

1. Introduction

Reef-building corals are metaorganisms living in symbiosis with dinoflagellates from the genus *Symbiodinium* and a plethora of other microbes, such as bacteria, archaea, fungi, and also viruses (Rosenberg et al., 2007). While *Symbiodinium* spp. are largely responsible for photoautotrophic energy production, other coral-associated microbes are shown to provide important contributions to coral holobiont functioning. They can facilitate the fixation of nitrogen in oligotrophic waters (Lema et al., 2012; Lesser et al., 2004) and play a role in many metabolic processes (Wegley et al., 2007), such as the cycling of sulfur compounds (González et al., 2003). Furthermore, it has been suggested that microbial communities may facilitate acclimatization of the coral holobiont to changes in the environment through rapid restructuring of the microbial community composition (Reshef et al., 2006), and studies indicate that an intact coral microbiome is essential to coral immunity and health (Krediet et al., 2013; Mao-Jones et al., 2010; Rosenberg et al., 2007).

Coral microbiomes are assumed to be host species specific (Sunagawa et al., 2010). However, this specificity is dependent on the health state of the coral; in diseased tissues different coral hosts display bacterial community compositions that are more similar in comparison to their healthy counterparts. This pattern has mostly been explored for visible coral disease states: in *Diploria strigosa*, *Montastraea cavernosa*, and *Orbicella annularis* from Curacao (Frias-Lopez et al., 2002) and in *Pavona duerdeni* and *Porites lutea* from Thailand (Roder et al., 2014a). Moreover, when compared across oceans, the disease microbiome of corals was found to be conserved across large geographic scales independent of host species (Roder et al., 2014b). Commonly, the development and progression of coral disease is associated with an increase in microbial diversity and the occurrence of opportunistic microbial taxa, such as e.g. Vibrionaceae and Rhodobacteraceae (Rosenberg and Ben-Haim, 2002), that can lead to changes in the function of the microbiome and the development of disease (Vega Thurber et al., 2009) and bleaching (Rosenberg and Falkovitz, 2004). On a global scale, outbreaks of coral bleaching and coral disease are amongst the most pervasive threats to coral reefs. In the Caribbean, for instance, White Plague Disease has driven Acroporid coral species to the brink of extinction within two decades (Miller et al., 2002) and in the Persian/Arabian Gulf, coral communities are projected to be on a trajectory of decline due to the combined impacts of bleaching and coral disease (Riegl et al., 2013).

Besides disease, local anthropogenic influences are also suspected to be drivers affecting the coral microbial community and coral health. For example, the experimental enrichment with inorganic nutrients of a

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coral reef habitat induced shifts in coral microbial communities of *Acropora hemprichii* (Jessen et al., 2013) and caused outbreaks of coral disease (Bruno et al., 2003; Vega Thurber et al., 2014). Further, damage from fishing gear causes wounds on coral colonies that can become infected and lead to higher rates of coral disease in fished reefs compared to protected areas (Lamb et al., 2015). Increased nutrients coupled with overfishing also has indirect effects on coral microbial communities, by promoting algal growth that may mediate microbe-induced coral mortality (Smith et al., 2006). Driven by burgeoning coastal populations, increasing amounts of sewage enter coastal ecosystems introducing high loads of inorganic nutrients, sediments, and organic compounds, which can have deleterious effects on coral reefs (Fabricius, 2005; Lamb et al., 2015; Wear and Thurber, 2015). Moreover, sewage also introduces many microbial taxa and recently a strong link between sewage-associated human pathogens and the development of coral disease in Caribbean corals has been made (Closek et al., 2014; Sutherland et al., 2010, 2011). Even in the absence of potential stressors or threats, prevailing environmental conditions are shown to align with coral-associated microbial communities. Roder et al. (2015) showed that highly structured microbiomes of *Ctenactis echinata* were encountered at sites where *C. echinata* was most common; these were sites characterized by open rocky substrates and clear water, indicating that microbiome composition reflects habitat adequacy.

Red Sea reefs are for the most part relatively unimpacted, but they border on coastal areas experiencing rapid population growth and increasing local anthropogenic influences. Evidence is accumulating that Red Sea reefs are changing as a result of these factors (Riegl et al., 2012), but to our knowledge, data on microbial infections of corals from the Red Sea is lacking and research exploring these coral reef habitats in their transition is urgently needed. The aim of this study was to investigate the effect of chronic anthropogenic pollution (i.e. sedimentation, sewage, nutrients) on microbial communities associated with seemingly healthy coral colonies of *Acropora hemprichii* and *Pocillopora verrucosa*, close to the metropolitan area of Jeddah, Saudi Arabia, in order to better understand the nature of anthropogenic impacts to Red Sea coral reefs.

2. Materials and methods

2.1. Sampling sites and anthropogenic impacts

The study sites were located in direct vicinity of the major city of Jeddah (current population 4 mio, annual 3% growth rate), Saudi Arabia, situated on the coast of the central Red Sea. Unlike many other parts of the Saudi Arabian Red Sea, the coastline around Jeddah has been extensively affected by development. For instance, there has been widespread infilling over the nearshore fringing reefs extending from the center of the city to 50 km northwards. Besides causing direct loss of coral communities and associated lagoonal habitat, at many points this has resulted in increased turbidity and sedimentation. Existing facilities for the treatment of wastewater often work over their recommended capacities and are insufficient. As a consequence there is extensive discharge of untreated or only partially-treated sewage into the oligotrophic coastal waters (Mudarris and Turki, 2006). Three large municipal wastewater outfalls release high volumes of effluent onto the coast and adjacent reefs; the outfalls at Al Shabab and Al Arbaeen respectively release approximately 35,000 $m^3\ d^{-1}$ and 68,000 $m^3\ d^{-1}$ of effluent within Jeddah Bay (El-Rayis and Moammar, 1998). At the third outfall at Al Kumrah, further to the south, approximately 300,000 $m^3\ d^{-1}$ of effluent are released (Basaham et al., 2009). In addition, numerous unapproved sewage outlets exist that discharge an estimated 99,000 $m^3\ d^{-1}$ of sewage near the shore over ca. 30 km of coastline extending north from the city center (Al-Farawati, 2010; Peña-García et al., 2014; Risk et al., 2009). In general, a series of previous studies have found a significant elevation in nutrient levels in the vicinity of these wastewater outfalls (e.g. Al-Farawati, 2010; El Sayed, 2002; Peña-García et al., 2014). Moreover, the local reefs are heavily fished, thus likely reducing the grazing pressure on benthic algae, which compete with corals for space and may affect their bacterial assemblages (Morrow et al., 2013). The combination of these impacts has resulted in declining hard coral cover on reefs in the area (DeVantier and Pilcher, 2000; Kotb, 2010; Pilcher and Alsuhaibany, 2000).

Given the above, six study sites were chosen to investigate influences of these prevalent anthropogenic impacts on coral-associated microbial communities (Fig. S1). The six sites had a similar geomorphology and provided paired replicates of three conditions. Two relatively unimpacted sites (henceforth referred to as sites A and B), one located at a patch Reef south of Ras Dha'l Lama at the entrance to Sharm Suleiman (site A: N 21°52′22.83″ E 38°58′01.61″) and the other at a fringing reef at Bohairat, off Al Zummrad to the northern end of the municipal area (site B: N 21°47′08.23″, E 39°02′28.56″) served as control sites. The second set of sites was located adjacent to the heavily-developed Jeddah Corniche, a major road running north along the shore from the city center, and the focus of ongoing coastal construction. These sites, which experience turbidity, sedimentation, and local sewage outfall were on the fringing reef south of Green Island (site C: N 21°36′54.53″, E 39°06′17.92″) and between Al Nawras and the Fakieh Aquarium (site D: N 21°34′34.21″, E 39°06′27.27″). The last two sites were located on reefs not far from Jeddah Port and the associated industrial zones at the southern end of the city (henceforth referred to as sites E and F). Site E was located on a patch reef off the center of Jeddah Bay close to the municipal wastewater outfalls of Al Shabab and Al Arbaeen (N 21°26′21.41″, E 39°06′28.49″) and site F was located south of Al Khumrah outfall (N 21°15′33.92″, E 39°06′42.37″). Both sites were within about 5 km of the three main discharge points from the city's main sewerage systems and treatment works, but proved sufficiently distant from the municipal wastewater discharge points to be only occasionally subject to elevated turbidity.

2.2. Benthic surveys

The line intercept transect (LIT) method (English et al., 1997; Loya, 1978) was used to assess differences between coral assemblages at the different sites, linked to differences in anthropogenic impacts (see above). At each site, a 30 m-long leaded line was laid along the reef following the 5 m depth contour. The substrate underlying the transect line was recorded cm by cm.

2.3. Coral and water sampling

At each of the six sites (i.e., A, B, C, D, E, F), three visually-healthy appearing colonies of the scleractinian corals *P. verrucosa* and *A. hemprichii* were sampled at 5 m depth by removing a branch. Sampled branches were placed in zip-lock bags under water and upon return to the boat were rinsed with filtered seawater (0.22 μm) and subsequently transferred into a N_2 dry shipper. Water samples were taken at the same depth as the coral samples, transported back to the shore in the dark in cooled boxes and 1 l of seawater was immediately filtered through 0.22 μm Durapore PVDF filters (Millipore, Billerica, MA, USA). The filters were subsequently frozen with the coral samples until transported to KAUST.

2.4. DNA extraction and sequencing

Coral fragments and PVDF filters were stored at −80°C. Prior to DNA extraction 1.5 ml AP1 extraction buffer (DNeasy Plant Mini Kit, Qiagen, Hilden, Germany) were applied on each coral sample while the frozen fragment was thawing. For each sample, coral tissue was blasted off the skeleton using airflow from a sterile pipet tip (1000 μl filter barrier tips, Neptune, USA) connected via a rubber hose to a bench top air pressure valve. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. For DNA

isolation from the water column, filters were thawed and re-frozen three times. Each filter (folded with filtered residue inside) was divided into three parts using sterile razorblades and each part was transferred into a separate 2 ml tube containing metal beads (Mobio, USA). 400 μl of AP1 buffer were added and the tubes were run in a tissue-lyser for 1 min at 30 Hz (TissueLyser II, Qiagen). DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. After extraction, the triplicate DNA isolations from each water filter were pooled and volume was reduced using a vacuum concentrator (CentriVap Complete, Labconco, USA). DNA concentrations of all samples were quantified on a NanoDrop 2000C spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

For amplicon sequencing on the Illumina MiSeq platform the variable region 5 and 6 of the 16S rRNA gene was amplified using the primers 784F [5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-AGGATTAGATACCCTGGTA '3] and 1061R [5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-CRRCACGAGCTGACGAC '3] (Andersson et al., 2008). Primers contained Illumina adapter overhangs (underlined above). PCRs were performed in triplicate (using 10–20 ng of DNA from each coral sample and 3–10 ng DNA from each water sample) with a Qiagen Multiplex PCR kit and a final primer concentration of 0.5 μM in a final reaction volume of 20 μl. The following PCR conditions were used: initial denaturing at 95 °C for 15 min, 27 cycles each at 95 °C for 30 s, 55°C for 90 s, and 72 °C for 30 s, followed by a final extension step at 72 °C for 10 min. The PCR products were visually assessed via 1% agarose gel electrophoreses (5 μl) and subsequently cleaned, indexed (Nextera XT indexing adapters), and cleaned again following the Illumina 16S manual for MiSeq. Amplicon PCRs for each sample were quantified on the BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) and by QuBit (Quant-IT dsDNA Broad Range Assay Kit, Invitrogen, Carlsbad, CA, USA) and pooled in equimolar ratios. Sequencing was performed at 8 pM with 10% phiX on the Illumina MiSeq, 2 × 300 bp paired-end v3 chemistry according to the manufacturer's specifications.

2.5. Data analysis

The software mothur (version 1.16.1; Schloss et al., 2009) was used for sequence editing and analyses. Briefly, reads were demultiplexed and sequences were quality trimmed, followed by a pre-clustering step (2 bp difference; Huse et al., 2010), removal of singletons, and alignment against the SILVA database (release 119; Pruesse et al., 2007). Chimeric sequences were removed using UCHIME (Edgar et al., 2011) and reads assigned to chloroplasts, mitochondria, archaea, and eukaryotes were excluded. Sequences were classified against Greengenes database (release gg_13_8_99; bootstrap = 60; McDonald et al., 2012) and subsampled to 39,450 reads per sample. Phylogenetically classified sequences were used to create bacterial community composition stack column plots at the family level using the means of relative abundances from replicated samples (n = 3). For further analyses, reads were clustered into Operational Taxonomic Units (OTUs), using a 97% similarity cutoff. Mothur was further used to obtain 'core' microbial OTUs (command: *get.coremicrobiome*) for *A. hemprichii*, *P. verrucosa*, and water samples.

For all subsequent analyses, the samples from the two replicated sites were pooled, i.e. site A and B were combined as 'unimpacted', site C and D as 'sedimentation & local sewage', site E and F as 'municipal wastewater'. Shared OTUs between the two coral species (*A. hemprichii* and *P. verrucosa*) under each of the three conditions (i.e. unimpacted, sedimentation & local sewage, and municipal wastewater) were obtained using Venn diagrams in mothur (command: *venn*). In addition, the statistical package indicspecies (Cáceres and Legendre, 2009) was employed to identify OTUs that were significantly associated with *A. hemprichii*, *P. verrucosa*, and water samples under the three conditions. The representative sequence of each indicator OTU was then BLASTed against GenBank nr database to identify previous occurrences of identical or highly similar bacteria. Alpha diversity measures (number of OTUs, Chao estimate of species richness, Shannon diversity, and Simpson diversity) were calculated separately for all corals and water under each condition, and differences between alpha diversity indices were assessed using one-way ANOVAs between conditions in each coral species and water (STATISTICA 10, StatSoft Inc.).

Multivariate analyses based on Bray-Curtis distances were conducted using PRIMER-E v6 (PERMANOVA+) software package (Clarke and Gorley, 2006). Non-Metric Multidimensional Scaling (nMDS) was used to visualize differences between 'water samples', 'coral species' (2 levels: *A. hemprichii*, *P. verrucosa*), and 'conditions' (3 levels: unimpacted, sedimentation & local sewage, and municipal wastewater). The dissimilarities between each coral species and the surrounding water for each condition were calculated using Similarity Percentage (SIMPER) analysis. Next, the differences in microbial communities between water and coral samples were tested in a one-factorial permutational MANOVA (PERMANOVA). Next, the differences in microbial communities in the water column were tested for the factor 'condition' in a one-factorial PERMANOVA. For the coral samples, a two-factorial PERMANOVA was run with the factors 'species', 'condition', and a possible interaction effect 'species * condition' (fully crossed). All multivariate tests were performed on log (x + 1) transformed data of OTU counts with PERMANOVA, using partial sum of squares and 9999 permutations under a reduced model. To test the contribution of rare bacterial members on the observed patterns, multivariate tests were repeated excluding all OTUs with less than 1000 sequence reads over all samples.

3. Results

In this study we investigated the influence of chronic anthropogenic impacts on microbial communities associated with two coral species, *A. hemprichii* and *P. verrucosa*, in the municipal area of Jeddah. Samples from sites subjected to two types of impacts, sedimentation & local sewage (sites C and D) and municipal wastewater outfall (sites E and F), were compared to samples from unimpacted sites (A and B). Besides assessment of the benthic community composition, we conducted amplicon sequencing of the 16S rRNA gene from 36 coral samples collected from these six sites (A–F), with three samples from each of two coral species (*A. hemprichii* and *P. verrucosa*), and a water sample per site, to characterize microbial community patterns. This yielded 13,527,693 sequence reads with a mean length of 309.8 bp. After quality filtering and exclusion of chimeras, 6,005,994 reads from 42,247 distinct sequences annotated to bacteria. Clustering of these sequences at the 97% similarity level resulted in 2,460 OTUs. All raw sequence data were deposited at NCBI Sequence Read Archive and can be accessed under BioProject accession number PRJNA287432.

3.1. Benthic community composition is distinct at sites under different anthropogenic impacts

Coral cover data from the LITs at each of the sites were compared as total substrate cover by hard coral and by soft coral genera (Fig. 1). Three contrasting assemblages were apparent: Site A differed from all the other sites in harboring the highest hard coral cover (40.3%) and also the lowest soft coral cover (1.8%). Although site A had the highest hard coral cover, fewer hard coral genera were recorded, reflecting dominance by *Acropora* spp. and *P. verrucosa*, and to a lesser extent by *Porites* spp. (Fig. 1A). Site B differed from site A by a reduced hard coral cover and higher coral genera diversity. The common feature of these two unimpacted sites was low cover of soft corals and almost complete lack of xeniids from the transect. In comparison, sites C and D (sedimentation & local sewage) showed intermediate levels of hard coral cover (16.9% and 25.1%, respectively), and possessed higher cover of soft corals (28.4% and 27.5%), that in places dominated the reef (personal observation). While xeniids (*Xenia* and *Heteroxenia* spp.) accounted for most of the soft coral at these two sites, there were also more other soft coral genera present than at any of the

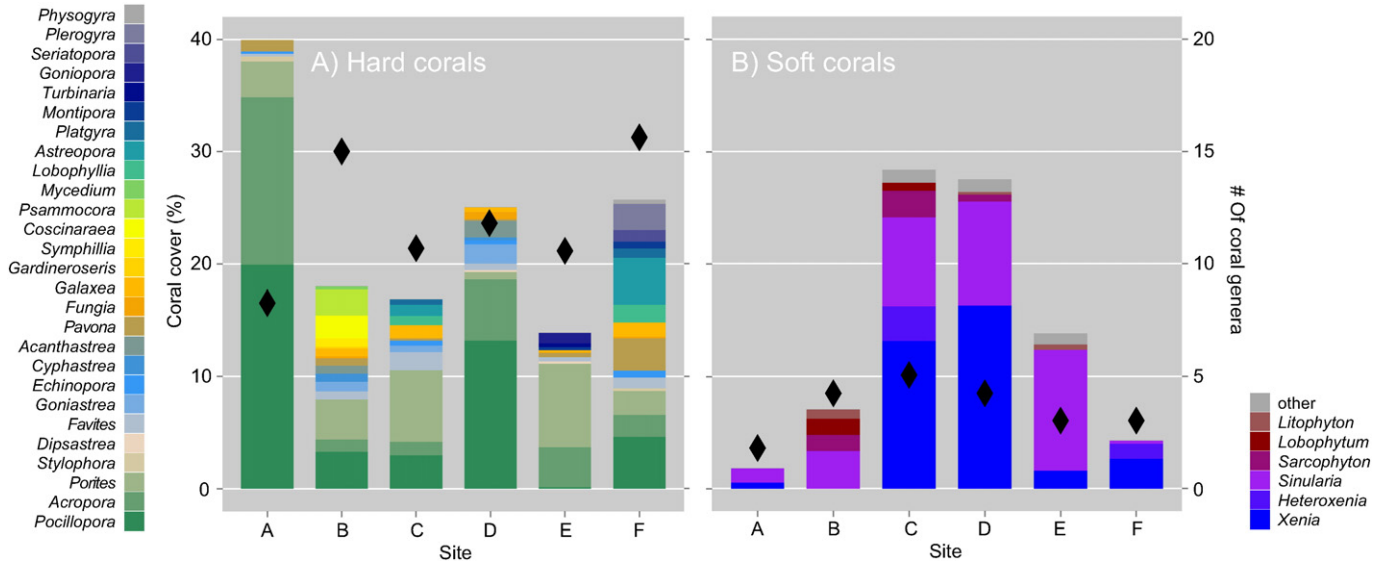

Fig. 1. Percent of (A) hard coral and (B) soft coral cover along 30-m transect lines at each of the study sites in the proximity of Jeddah, Saudi Arabia. Coral cover is given at the phylogenetic level of genus. Diamonds denote the number of coral genera recorded along each transect (second Y-axis).

other four stations (Fig. 1B). Finally, sites E and F (municipal wastewater) appeared similar to each other (Fig. 1); they had intermediate levels of hard coral cover similar to sites C and D, but exhibited lower substrate cover by xeniids and other soft corals than did sites C and D.

3.2. Microbial community composition of water reflects site differences

The microbial communities in the reef water entailed 991 OTUs. Importantly, replicated sites, i.e. unimpacted (A and B), sedimentation &

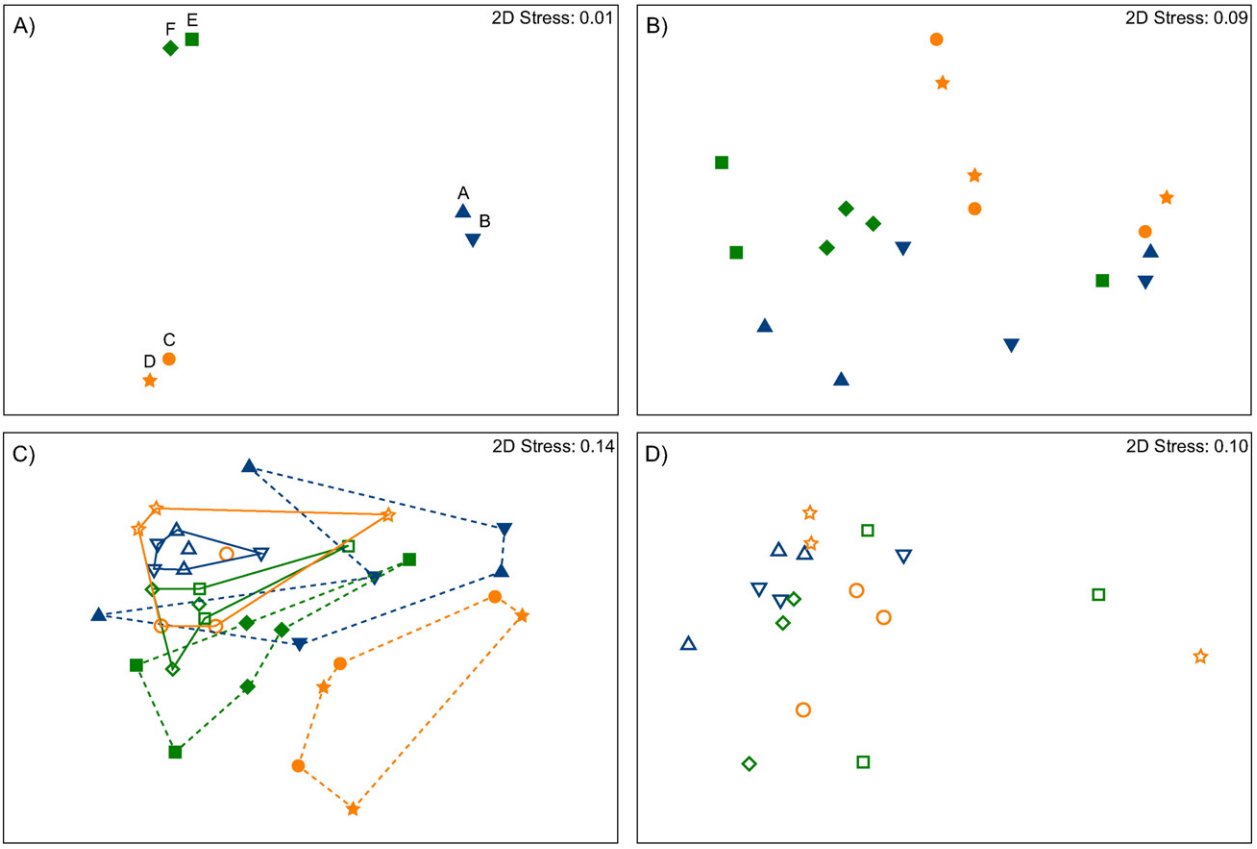

Fig. 2. Non-metric multidimensional scaling (nMDS) of bacterial communities from (A) seawater, (B) the hard coral *Acropora hemprichii* and (D) *Pocillopora verrucosa*, and (C) both coral species combined collected from six sites in the proximity of Jeddah, Saudi Arabia in the central Red Sea. Sites are indicated by the type of impact: unimpacted sites A (triangles facing up) and B (triangles facing down), sites C (circles) and D (stars) exposed to sedimentation & local sewage, sites E (squares) and F (diamonds) near the outfall of municipal wastewaters; in plot B–D) closed symbols = *A. hemprichii*, open symbols = *P. verrucosa*; in plot C) polygons mark out data points per impact and for *P. verrucosa* (solid) and *A. hemprichii* (dashed) separately. The plots are based on Bray Curtis distances of log (x + 1) transformed abundance data of bacterial OTUs. The stress values denote the goodness of fit.

local sewage (C and D), and municipal wastewater (E and F), displayed overlapping microbial profiles that were not significantly different from each other, but distinct from pairs of sites subjected to other impacts (Fig. 2A). Further, microbial communities in the water were significantly different from those in both coral species, with which 175 OTUs were shared (PERMANOVA, $F = 10.77$, $p < 0.001$, all pairwise comparisons $p < 0.0005$). Water samples from all locations were dominated by the uncultured actinobacterium OCS155, which made up between 34–41% of microbial abundance at all sites (Fig. 3). Flavobacteriaceae and Rhodobacteraceae increased in relative abundance at impacted sites compared to control sites (from 12 to 22% and from 3 to 7%, respectively), while Pelagibacteraceae decreased (from 17 to 5%).

On average, water samples contained 364 OTUs, which was more than twice that found in the coral samples (Table 1; *A. hemprichii*: 177, *P. verrucosa*: 117). There were no significant differences in alpha diversity between water samples from sites subject to different impacts (Table 1, ANOVA, $p > 0.05$). At the same time, microbial communities found in water and coral samples became more similar at the impacted sites (based on decreasing dissimilarity measure). Microbial communities of corals and water were least similar at unimpacted sites (dissimilarity to water: 93.9 and 91.6% for *A. hemprichii* and *P. verrucosa*, respectively). In comparison, similarity increased at the sites subjected to sedimentation & local sewage, despite the higher diversity at these sites, (dissimilarity to water: *A. hemprichii* 87.6%, *P. verrucosa* 90.4%) and those in the vicinity of the municipal wastewater outfalls (*A. hemprichii* 88.0%, *P. verrucosa* 86.6%).

3.3. Microbial community composition of corals in the central Red Sea

Combined over all samples, *A. hemprichii* contained roughly twice as many OTUs (1475) as *P. verrucosa* (712), of which 377 OTUs were shared, summing up to a total of 1810 coral-associated OTUs from 27 bacterial phyla. Both species were dominated by Gammaproteobacteria, which contributed an average of 71.54% and 81.23% of subsampled reads in *A. hemprichii* and *P. verrucosa*, respectively (Table S1). Some colonies of *A. hemprichii* also contained a large proportion of Alphaproteobacteria (median 6.79%, range 0.18–46.40% of reads). All colonies of *P. verrucosa* and some of *A. hemprichii* had high proportions of the bacterial family Endozoicomonaceae (Fig. 3, Table S2). *A. hemprichii* was further dominated by Pseudomonadaceae, which were less abundant in *P. verrucosa* (Fig. 3, Table S2). The two coral species shared three common OTUs: Otu0001 and Otu0002 from the family Endozoicomonaceae, and Otu0003 annotated as *Pseudomonas veronii* from the family Pseudomonadaceae (Table 2). Despite the occurrence of Otu0001 and Otu0002 in both corals, each of the species was dominated by only one of the two OTUs. Overall, both corals harbored distinct microbial communities (PERMANOVA, F 6.85, $p < 0.0005$).

3.4. Microbial community composition of corals under anthropogenic impact

The microbial community of *A. hemprichii* differed between unimpacted sites and the sites exposed to different anthropogenic impacts (Fig. 2B). For instance, Altermonadales, representing one of three major constituents of the *A. hemprichii* microbiome at unimpacted sites, were almost absent in corals from impacted sites (Fig. 3). There was the opposite change in the family Endozoicomonaceae; while they decreased in proportion at sites impacted by sedimentation & local sewage, they increased in proportion at the sites impacted by municipal wastewater (Fig. 3). Moreover, alpha diversity of microbial communities in *A. hemprichii* was significantly higher at the sites impacted by sedimentation & local sewage compared to all other sites. For instance, *A. hemprichii* samples from the sites impacted by sedimentation & local sewage had roughly twice as many OTUs (mean 264.3 OTUs per sample) as did those from the other sites (134.7 OTUs at unimpacted, 132.8 OTUs at municipal wastewater outfall). The same differences were apparent for the Chao estimator and the Shannon and Simpson diversity indices (Table 1).

Changes in the microbial community composition and bacterial species diversity and richness of *P. verrucosa* were less pronounced than those in *A. hemprichii* (Fig. 2C, D, Table 1) and apart from the microbial community composition at site E, all *P. verrucosa* colonies were dominated by the bacterial family Endozoicomonaceae. Nevertheless, there was a trend of a decreasing proportion in Endozoicomonaceae from

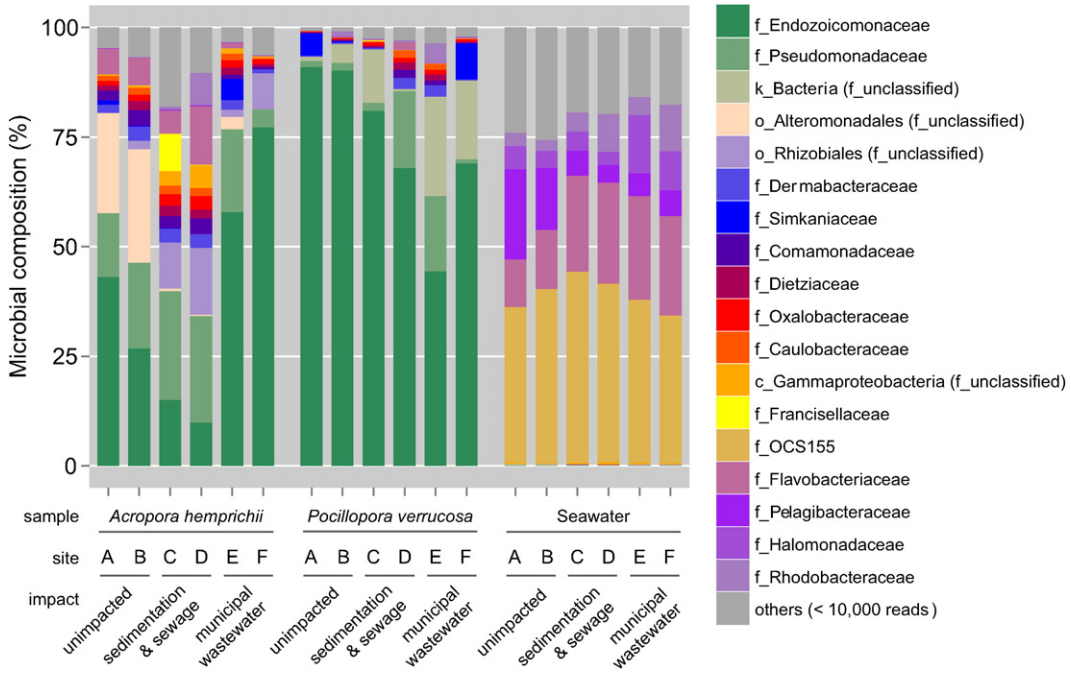

Fig. 3. Stack column plots showing the mean proportions of bacterial families contributing to the microbial assemblages of the coral species *Acropora hemprichii* and *Pocillopora verrucosa* collected from six sites exposed to varying anthropogenic impacts in the proximity of Jeddah, Saudi Arabia (n = 3 per species and site). A water sample was collected at each of the sites to examine the reef water assemblages (n = 1).

Table 1
Summary of alpha diversity indices (means ± SE) of microbial communities associated with the corals *Acropora hemprichii* and *Pocillopora verrucosa* and with water samples across different conditions (i.e., unimpacted, sedimentation & local sewage, municipal wastewater outfall) collected from sites in proximity to Jeddah, Saudi Arabia, central Red Sea. The sites are grouped by degree and type of anthropogenic impact.

Species	Condition	# of samples	OTU/sample	Chao	Shannon	Simpson	Inverse simpson
A. hemprichii	Unimpacted	6	134.7 (18.8)	162.2 (17.0)	1.66 (0.24)	0.37 (0.06)	3.18 (0.57)
	Sedimentation & local sewage	6	**264.3 (50.9)**	**293.6 (61.6)**	**2.57 (0.07)**	**0.18 (0.02)**	**5.91 (0.66)**
	Municipal wastewater outfall	6	132.8 (17.0)	164.3 (23.6)	1.44 (0.21)	0.40 (0.09)	2.85 (0.34)
P. verrucosa	Unimpacted	6	93.2 (7.3)	137.2 (7.2)	0.84 (0.08)	0.61 (0.05)	1.67 (0.12)
	Sedimentation & local sewage	6	126.5 (14.9)	164.6 (18.7)	0.90 (0.27)	0.67 (0.10)	1.77 (0.39)
	Municipal wastewater outfall	6	131.5 (17.0)	167.4 (25.9)	1.21 (0.18)	0.49 (0.07)	2.30 (0.37)
Water	Unimpacted	2	351.0 (16.0)	431.3 (3.2)	2.79 (0.01)	0.15 (0.00)	6.63 (0.01)
	Sedimentation & local sewage	2	398.5 (44.5)	586.5 (44.5)	2.84 (0.06)	0.14 (0.01)	6.97 (0.51)
	Municipal wastewater outfall	2	343.5 (29.5)	419.4 (68.9)	2.93 (0.05)	0.13 (0.01)	7.82 (0.58)

A. hemprichii-sedimentation & local sewage sites significantly different from *A. hemprichii* under all other conditions for all variables (ANOVA; $p < 0.05$).

unimpacted to impacted sites (Fig. 3). At all sites impacted by sedimentation & local sewage or by municipal wastewater, Endozoicomonaceae were reduced and unclassified bacteria and bacteria in the family Pseudomonadaceae prevailed instead (Fig. 3). This similarity in the response of *P. verrucosa*'s microbiome to both impacts was also reflected in the partial overlap of these samples in the MDS ordination (Fig. 2D).

Pooled over replicated sites, the microbial communities associated with the two coral species were significantly different between conditions (PERMANOVA, F 1.93, $p < 0.005$). Post-hoc tests revealed significant differences for all pairwise comparisons between the different conditions (all $p < 0.05$), but there was no significant interaction between the factors species and condition (PERMANOVA, $p > 0.05$). When less abundant microbes (OTUs < 1,000 reads) were excluded from the analysis, the differences between impacts were smaller and only significant between the unimpacted sites and those affected by sedimentation & local sewage (PERMANOVA, $p = 0.027$), indicating that the differences lie in less prominent members of the microbiome. Almost two thirds of the OTUs (Venn diagrams, 64.3% and 62.1% in *A. hemprichii* and *P. verrucosa*, respectively) were exclusively present at impacted sites, i.e. absent from corals at the unimpacted sites. For example, each colony of the two coral species at the unimpacted sites contained a mean 20 bacterial OTUs belonging to Alphaproteobacteria. In *P. verrucosa* this count increased by 50% to 30 OTUs at the sites impacted by sedimentation & local sewage and 34 OTUs at the sites close to the municipal wastewater outfall (Table S1). Colonies of *A. hemprichii* showed a comparable trend with a mean of 31 OTUs belonging to Alphaproteobacteria present in samples at the municipal wastewater outfall, and numbers of OTUs more than tripled to a mean of 64 OTUs belonging to Alphaproteobacteria at the sites impacted by sedimentation and local sewage (Table S1).

Microbial communities displayed coral species-specific patterns at the unimpacted sites (Fig. 2C), with both species sharing a lower proportion of OTUs (Venn diagrams, 17.73%) when compared to those at the impacted sites (Venn diagrams, 20.84%; C-F). Samples from both coral species at the municipal wastewater outfalls (E and F) clustered closely together (Fig. 2C, green data points) and shared a higher proportion of OTUs than at all other sites (Venn diagrams, 27.86%), while the proportion of shared OTUs at the sites impacted by sedimentation & local sewage (Venn diagrams, 17.73%; C and D) was comparable to that at the unimpacted sites. The response to sedimentation & local sewage was different between the species: *A. hemprichii* showed a strong bacterial community shift, also apparent in the alpha diversity metrics, whereas the response in *P. verrucosa* was not as distinct (Fig. 2B-D).

3.5. Bacteria indicative of anthropogenic impact on corals

To identify OTUs reflective of a given impact, we used indicspecies (Cáceres and Legendre, 2009) on OTU abundances over a range of conditions. We investigated the conditions (unimpacted, sedimentation & local sewage, municipal wastewater) separately for each coral species and combined over both species, and combinations of conditions (sedimentation & local sewage combined with municipal wastewater) within each species and over both species. Due to the design of the indicspecies analysis, the identified OTUs were exclusive for the specific host * condition combination tested. Hence, this analysis guided the identification of commonalities and uniqueness in the bacterial community response of two different coral hosts subjected to the same set of impacts. In *A. hemprichii* the prevalent order of Altermonadales decreased from 24.3% at unimpacted sites to below 1% at impacted sites (Fig. 3). Correspondingly, Otu0006 belonging to Alteromonadales was

Table 2
Common microbial OTUs of coral species (*Acropora hemprichii* and *Pocillopora verrucosa*) and water samples with taxonomic assignment of OTUs (bootstrap value given if <100). Only OTUs with at least 1% of reads in each sample were selected; common components of microbial communities of both coral species are marked in bold.

A. hemprichii		
14 of 18 samples	**Otu0003**	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Pseudomonadales; f_**Pseudomonadaceae**; g_*Pseudomonas*; s_*veronii* (83)
12 of 18	Otu0011	k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Dermabacteraceae (99); g_*Brachybacterium* (99); unclassified (99)
11 of 18	**Otu0002**	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Oceanospirillales; f_**Endozoicimonaceae**; unclassified
10 of 18	**Otu0001**	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Oceanospirillales; f_**Endozoicimonaceae**; unclassified
	Otu0007	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rhizobiales; unclassified; unclassified
P. verrucosa		
18 of 18 samples	**Otu0001**	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Oceanospirillales; f_**Endozoicimonaceae**; unclassified
13 of 18	**Otu0002**	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Oceanospirillales; f_**Endozoicimonaceae**; unclassified
12 of 18	**Otu0003**	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Pseudomonadales; f_**Pseudomonadaceae**; g_*Pseudomonas*; s_*veronii* (83)
8 of 18	Otu0008	k_Bacteria; unclassified
Water		
6 of 6 samples	Otu0004	k_Bacteria; p_Actinobacteria; c_Acidimicrobiia; o_Acidimicrobiales; f_OCS155; unclassified
	Otu0010	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rickettsiales; f_Pelagibacteraceae; unclassified
	Otu0013	k_Bacteria; p_Bacteroidetes; c_Flavobacteriia; o_Flavobacteriales; f_Flavobacteriaceae; unclassified
	Otu0014	k_Bacteria; p_Actinobacteria; c_Acidimicrobiia; o_Acidimicrobiales; f_OCS155; unclassified
	Otu0016	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rhodobacterales; f_Rhodobacteraceae; unclassified (95)

Table 3
Summary of significant OTUs/bacterial species associated with different coral species (*Acropora hemprichii* and *Pocillopora verruosa*) and impact combinations, based on indicspecies analysis (significance threshold $p < 0.05$). The association value indicates the strength of the association for the respective OTU with the tested sample group.

OTU	Mean read count (group)	Association value	*p*-Value	Lowest taxonomic level from Greengenes classification	Source, nearest relative [GenBank accession number]	Reference
Group: *A. hemprichii* (unimpacted)						
Otu0006	9998.2	0.968	0.002	Alteromonadales, J115 (Genus *Melitea*)	Gorgonia *Eunicella cavolini*, uncultured bacterium [JQ691580]	Bayer et al. (2013a)
Otu0136	9.2	0.809	0.001	Unclassified Oceanospirillales	Gorgonia *Eunicella cavolini*, uncultured bacterium [JQ691580]	Bayer et al. (2013a)
Otu430	3.5	0.648	0.035	Unclassified Bacteroidetes	Secondary effluent from wastewater treatment plant [GQ247293]	Herzberg et al. (2010)
Group: *A. hemprichii* (sedimentation & local sewage)						
Otu0181	4.8	0.816	0.002	Unclassified Rhodobacteraceae	White plague diseased coral tissue, uncultured bacterium [FJ203339]	Sunagawa et al. (2009)
Otu0117	57.2	0.783	0.008	Pseudoalteromonadaceae (Genus *Pseudoalteromonas*)	Deep sea polymetallic nodule, uncultured bacterium [JX227105]	Wu et al. (2013)
Otu0293	0.2	0.737	0.043	Unclassified Rhodospirillaceae	Intertidal soil from Arabian Sea, uncultured bacterium [JX240962]	Keshri et al. (in press)
Otu0083	105.7	0.725	0.026	Unclassified Deltaproteobacteria	Red Sea *Acropora humilis*, uncultured bacterium [KC668563]	Bayer et al. (2013b)
Otu0196	7.3	0.707	0.013	Unclassified Vibrionaceae	Marine sediments, *Photobacterium* sp. [JX134417]	Liu et al. (2011)
Otu0316	3.0	0.707	0.020	Unclassified Rhodobacteraceae	Marine biofilm, uncultured bacterium [HQ601615]	Witt et al. (2011)
Otu0320	2.8	0.707	0.013	Rickettsiales (Genus *Thalassiosira*)	South China Sea, uncultured bacterium [GU941045]	Unpublished
Otu0443	4.0	0.707	0.021	Desulfovibrionaceae (Genus *Desulfovibrio*)	Human gut, *Desulfovibrio desulfuricans* [KJ459867]	Unpublished
Otu0653	6.2	0.707	0.022	Oxalobacteraceae (Genus *Herminiimonas*)	Hydrocarbon-contaminated soil, uncultured bacterium [KP177362]	Unpublished
Otu0661	2.0	0.707	0.015	Unclassified Deltaproteobacteria	Salt marsh, uncultured bacterium [KF741529]	Darjany et al. (2014)
Otu0047	578.3	0.707	0.019	Unclassified Alphaproteobacteria	Biofilm, uncultured bacterium [EU917558]	Havemann and Foster (2008)
Otu0321	7.2	0.699	0.020	Unclassified Ucp1540	Deep sea sediments, uncultured bacterium [AB694518]	Hori et al. (2013)
Otu0270	4.5	0.694	0.018	Unclassified Sphingobacteriaceae	Hot spring, uncultured bacterium [FJ853446]	Kanokratana et al. (2004)
Otu0140	53.3	0.674	0.032	Unclassified Rhodobacteraceae	White patch syndrome infected tissue in *Porites* [KF179947]	Séré et al. (2013)
Otu0264	3.8	0.665	0.021	Rhodobacteraceae (Genus *Ruegeria*)	Marine sediments, Arabian Seas, uncultured bacterium [JQ258544]	Unpublished
Otu0481	5.0	0.645	0.048	Idiomarinaceae (Genus *Idiomarina*)	Salt marsh, *Idiomarina* sp. [FJ157171]	Unpublished
Otu0161	12.5	0.609	0.036	Unclassified Chitinophagaceae	Human skin, uncultured bacterium [HM341144]	Kong et al. (2012)
Otu0449	5.2	0.600	0.047	Unclassified Cytophagaceae	Deep coal seam groundwater, uncultured bacterium [AB294338]	Shimizu et al. (2007)
Group: *A. hemprichii* (municipal wastewater outfall)						
Otu0340	11.8	0.779	0.005	Unclassified Proteobacteria	Diseased *Orbicella faveolata*, uncultured bacterium [FJ425643]	Unpublished
Otu0176	54.3	0.707	0.014	Unclassified Endozoicomonaceae	Tissue of *Acropora* under sulfur enrichment, uncultured bacterium [FJ809497]	Raina et al. (2009)
Group: *A. hemprichii* (sedimentation & local sewage + municipal wastewater outfall)						
Otu0007	3525.9	0.932	0.002	Unclassified Rhizobiales	Sediments from a soda lake, *Cohaesibacter haloalkalitolerans* [NR108886]	Sultanpuram et al. (2013)
Otu0042	367.1	0.859	0.009	Unclassified Gammaproteobacteria	Deep sea sediments, uncultured bacterium [JX227676]	Wu et al. (2013)
Otu0057	71.6	0.764	0.017	Unclassified Francisellaceae	*Orbicella faveolata*, after 23 days in aquarium, uncultured bacterium [FJ202977]	Sunagawa et al. (2009)
Otu0147	7.6	0.701	0.026	Halomonadaceae (Genus *Halomonas*)	*Acropora palmata*, *Halomonas meridiana* [KP869065]	Unpublished
Otu0251	0.8	0.645	0.029	Unclassified Alphaproteobacteria	Ocean drilling core sample, uncultured bacterium [AB806475]	Unpublished
Group: *P. verrucosa* (unimpacted)						
Otu0081	9.3	0.868	0.001	Unclassified Endozoicomonaceae	*Pocillopora damicornis* in the Red Sea, uncultured bacterium [KC668791]	Bayer et al. (2013b)
Group: *P. verrucosa* (sedimentation & local sewage)						
Otu0392	1.0	0.707	0.018	Unclassified Endozoicomonaceae	*Stylophora pistillata* in the Red Sea, uncultured bacterium [KC669026]	Bayer et al. (2013b)
Otu0640	1.2	0.707	0.017	Unclassified Rickettsiales	Deep sea subarctic Pacific, uncultured bacterium [HQ674537]	Unpublished
Otu0501	7.8	0.700	0.037	Unclassified Phycisphaeraceae	Sediments of marine aquaculture farm, uncultured bacterium [KC631511]	Unpublished
Group: *P. verrucosa* (municipal wastewater outfall)						
Otu0308	15.0	0.739	0.012	Unclassified Alphaproteobacteria	*Mussismilia* spp., uncultured bacterium [JN106657]	Fernando et al. (2015)
Otu0058	5.0	0.674	0.047	Flavobacteriaceae (Genus *Gilvibacter*)	Coral reef water, uncultured bacterium [GU119204]	Sunagawa et al. (2010)

(continued on next page)

Table 3 (*continued*)

OTU	Mean read count (group)	Association value	*p*-Value	Lowest taxonomic level from Greengenes classification	Source, nearest relative [GenBank accession number]	Reference
Group: *P. verrucosa* (sedimentation & local sewage + municipal wastewater outfall)						
Otu0686	4.4	0.907	0.001	Amoebophilaceae (Genus SGUS912)	*Orbicella franski*, uncultured bacterium [GU118730]	Sunagawa et al. (2010)
Otu0082	151.8	0.906	0.001	Amoebophilaceae (Genus SGUS912)	*Orbicella franski*, uncultured bacterium [GU118730]	Sunagawa et al. (2010)
Otu0151	3.4	0.797	0.006	Amoebophilaceae (Genus SGUS912)	*Orbicella franski*, uncultured bacterium [GU118730]	Sunagawa et al. (2010)
Otu0262	2.0	0.645	0.049	Unclassified Bacteria	*Pocillopora damicornis* in the Red Sea, uncultured bacterium [KC668699]	Bayer et al. (2013b)
Group: *A. hemprichii* (sedimentation & local sewage) + *P. verrucosa* (sedimentation & local sewage)						
Otu0046	123.8	0.928	0.001	Rhodobacteraceae (Genus *Roseovarius*)	Crude oil-contaminated seawater, *Roseovarius nubinhibens* [KF146490]	Wang et al. (2014)
Otu0694	4.0	0.743	0.010	Sphingomonadaceae (Genus *Novosphingobium*)	Crude oil reservoir, uncultured bacterium [JQ088325]	Tang et al. (2012)
Group: *A. hemprichii* (municipal wastewater outfall) + *P. verrucosa* (municipal wastewater outfall)						
Otu0396	2.2	0.764	0.006	unclassified Saprospiraceae	*Mussismilia* spp., uncultured bacterium [JN106612]	Fernando et al. (2015)
Otu1089	1.9	0.680	0.009	Bacteriovoracaceae (Genus *Bacteriovorax*)	diseased *Orbicella faveolata*, uncultured bacterium [FJ403066]	unpublished
Group: *A. hemprichii* (sedimentation & local sewage + municipal wastewater outfall) + *P. verrucosa* (sedimentation & local sewage + municipal wastewater outfall)						
Otu0257	5.7	0.830	0.013	Balneolaceae (Genus *Balneola*)	Indian ocean seawater, uncultured bacterium [KJ752781]	Unpublished
Otu0412	3.2	0.785	0.016	Unclassified Alphaproteobacteria	Tissue of *Acropora* under sulfur enrichment, uncultured bacterium [FJ809311]	Raina et al. (2009)

the most abundant indicator OTU of *A. hemprichii* at unimpacted sites (Tables 3, S3). The same trend was apparent for the dominant family Endozoicomonaceae, the prevalence of which at unimpacted sites in *P. verrucosa* (average abundance = 90.6%) decreased by about 25% at the impacted sites (65.6%; Fig. 3). In *P. verrucosa*, the only indicator OTU at unimpacted sites was an unclassified bacterium, which also belonged to the family Endozoicomonaceae, but it was of comparably low abundance (Tables 3, S3).

The analysis of the effects of the impacts revealed distinct OTUs ($p < 0.05$) for different combinations of coral species and impacts. Corresponding to the increase in microbial richness and diversity in *A. hemprichii*, the sites affected by sedimentation & local sewage were characterized by the highest number of indicator OTUs. Of the 18 OTUs, four belonged to the family Rhodobacteraceae and of these one was classified to the genus *Ruegeria* (Otu0264). Five additional significant OTUs in that impact group were classified at the genus level: *Pseudoalteromonas* (Pseudoalteromonaceae), *Thalassiosira* (Rickettsiales), *Desulfovibrio* (Desulfovibrionaceae), *Herminiimonas* (Oxalobacteraceae), and *Idiomarina* (Idiomarinaceae; Table 3). Further bacterial families recorded were Vibrionaceae, Rhodospirillaceae, Ucp1540, Sphingobacteriaceae, Chitinophagaceae, and Cytophagaceae (Table 3). The corresponding samples of *P. verrucosa* from the sites impacted by sedimentation & local sewage were characterized by only three OTUs, which belonged to the Endozoicomonaceae (Otu0392), Phycisphaeraceae (Otu0501), and Rickettsiales (Otu0640). *A. hemprichii* collected at the sites of the municipal wastewater outfalls contained two indicator OTUs, of which one belonged to the family Endozoicomonaceae. Similarly, there were also two indicator OTUs identified for samples of *P. verrucosa* from the municipal wastewater outfall areas, one an unclassified Alphaproteobacterium and the other a bacterium from the genus *Gilvibacter* (Flavobacteriaceae).

In both comparisons (i.e., for each coral species analyzed over combined impacts and for both species combined analyzed over separated impacts) several additional OTUs were characteristic, representing a shared response to generally polluted environments within a host species or a response to an impact overlapping between hosts, respectively. For instance, *A. hemprichii* from all impacted sites had a relatively highly abundant indicator OTU belonging to the Rhizobiales (Otu0007), which was also present in some unimpacted samples but at lower abundances, and which was almost completely absent from *P. verrucosa* (Table S3). Furthermore, Otu0147 from the genus *Halomonas* (Halomonadaceae) was identified along with three other OTUs from *A. hemprichii*, while the corresponding group in *P. verrucosa* was characterized by four OTUs, three of which annotated to the genus *SGUS912* (Amoebophilaceae). The different impact groups combined over both species yielded two indicator OTUs for each impact analysis. For the sites impacted by sedimentation & local sewage, the OTUs common to both coral species belonged to the genera *Roseovarius* (Rhodobacteraceae) and *Novosphingobium* (Sphingomonadaceae), and for the sites near the municipal wastewater outfalls these were *Bacteriovorax* (Bacteriovoraceae) and an unclassified genus from the family Saprospiraceae (Table 3). Combined over all impacted sites and both species, Otu0257 *Balneola* (Baleolaceae) and an unclassified Alphaproteobacterium were significant indicators. At the control sites by comparison the two coral species had no indicator OTUs in common.

4. Discussion

4.1. Anthropogenic impacts and differences between study sites

The study of microbial communities over a range of chronic anthropogenic impacts along the Red Sea coast of Jeddah, Saudi Arabia, revealed an influence of prevailing environmental conditions on the microbial communities present in the water and associated with two coral species, *A. hemprichii* and *P. verrucosa*. Benthic data supported that site A, with relatively high hard coral cover and with low soft coral cover, was the least impacted. The benthic cover at the second control site (site B) was more similar to the impacted sites, which may be related to its location closer to the suburbs of Jeddah than site A, yet it was characterized by a lack of xeniid soft corals, distinguishing it from the more impacted sites. Sites C and D were the most impacted and dominated by soft corals, in particular xeniids that are known to opportunistically take over degraded reef habitats (Benayahu and Loya, 1985; Tilot et al., 2008). Sites E and F were under intermediate impact, being offshore of municipal sewage discharges. Similar consequences on reefs of such impacts, characteristic of rapidly developing tropical coasts, have been widely described (Fabricius, 2005; Schutte et al., 2010), including the deleterious effects of sewage discharge (Smith et al., 1981), sedimentation (Rogers, 1990), and reduced grazing pressure (Huse et al., 2010). In particular the reduced cover of hard corals on reefs subject to turbidity and sedimentation has been widely reported (Acevedo and Morelock, 1988; Rogers, 1990), with these reefs often also featuring high cover by soft corals, which can benefit from feeding on the introduced organic matter (van Katwijk et al., 1993).

Data on microbial communities from the water column at the six sites supported the structuring indicated by benthic cover. Microbes in reef-associated seawater can be affected by anthropogenic development, as has been demonstrated over a latitudinal transect along the Line Islands (Kelly et al., 2014) and a cross-shelf transect in the Great Barrier Reef (Alongi et al., 2015). The differences in water-associated microbes between impacted and unimpacted sites were present in this study, but relatively subtle given the generally similar structure of the microbial communities between sites. Overall, microbial communities in reef waters surrounding Jeddah were comparable to those collected in reefs 100 km to the north (Roder et al., 2015) and largely reflected common bacteria found in ocean surface communities (Rusch et al., 2007).

4.2. Coral microbial community composition at unimpacted sites

The coral species investigated were characterized by distinct microbial communities at unimpacted sites, a conclusion in agreement with previous findings of species specificity in microbial assemblages across many coral host taxa (e.g. Bayer et al., 2013b; Roder et al., 2014b; Sunagawa et al., 2010). For instance, the high abundance of bacteria belonging to the Alteromonadales exclusively in *A. hemprichii* was a distinct characteristic of the species' microbial community at unimpacted sites. The Endozoicomonaceae, a bacterial family most abundant in *P. verrucosa* in this study, have been suggested to be an integral part of pocilloporid coral microbiomes in the Red Sea (Bayer et al., 2013b), and Endozoicomonaceae were also abundant in *A. hemprichii*. It is important to note that the two coral species were associated with distinct Endozoicomonaceae OTUs that showed little overlap in their occurrence, thus supporting the notion of host-symbiont specificity that has recently been suggested for the *Endozoicomonas*-coral host relationship (Bayer et al., 2013b). Interestingly, the main OTU of Endozoicomonaceae in *A. hemprichii* was highly similar, but yet distinct (98% sequence identity) to an OTU recovered from the same coral host species collected about 100 km to the north (Jessen et al., 2013), indicating that geographic distance may play a role in fine-scale structuring of this association.

4.3. Coral microbial community composition under anthropogenic impact

The anthropogenic impacts close to Jeddah influenced the microbial assemblages of the corals *A. hemprichii* and *P. verrucosa*. In particular, host species specificity was decreased at the sites of municipal wastewater outfalls (sites E and F). Loss of species specificity and increase in similarity of coral microbial communities has previously been found in diseased corals (Frias-Lopez et al., 2002; Roder et al., 2014a). Commonalities in community shifts have also been reported in corals subject to different stressors, as in the experimental treatments of *Porites compressa* with increased temperature, inorganic nutrients, dissolved organic carbon, or reduced pH, in which the microbial communities of all experimental groups responded in a similar way (Vega Thurber et al., 2009). Also, seemingly healthy organisms have been found to follow this pattern under environmental stress, as demonstrated by clustering of host microbial communities of hard and soft corals by impact rather than by host species (Lee et al., 2012). It appears that negative impacts often disrupt the balance between the main microbial taxa associated with a coral, a tendency also observed in this study, as evidenced by a decrease in the dominant microbes belonging to the Endozoicomonaceae (*P. verrucosa*) and Altermonadales (*A. hemprichii*). In addition, the comparison of the microbial community response encompassing all OTUs with the analysis that only included those with more than 1,000 reads indicated that the observed differences were driven by changes in the less abundant microbial taxa. This is in line with the microbial stress-response in other coral species and further highlights the importance of the 'rare bacterial biosphere' (Pedrós-Alió, 2012; Sunagawa et al., 2010; Jessen et al., 2013).

Another indicator of a stressed coral holobiont may be the increase in microbial diversity and species richness. This pattern could be explained by a stress-induced destabilization of the host-microbial balance, leading to the uncontrolled emergence of opportunistic taxa that are otherwise suppressed (Rosenberg and Kushmaro, 2011). Generally, microbial communities were more diverse at impacted sites, but these differences were only significant for *A. hemprichii* at the sites exposed to sedimentation & local sewage (sites C and D). The microbial community of *A. hemprichii* showed a stronger response to pollution in all other metrics compared to the relatively stable/invariant community composition in *P. verrucosa*. Acroporids often belong to the first coral taxa to show signs of stress and they are known to be susceptible to sedimentation and terrestrial run-off (Fabricius, 2005; Nakajima et al., 2013). Similar to our observation, experimental exposure of *A. hemprichii* to simulated eutrophication and overfishing in the Red Sea has been found to increase microbial diversity over time, although the design of the study was lacking an experimental control (Jessen et al., 2013). On the one hand, increased microbial diversity may occur under comparably benign conditions, as deduced from the study on natural variability of the microbiome in the fungid coral *C. echinata* (Roder et al., 2015). On the other hand, increased microbial diversity also seems to be a typical characteristic accompanying coral disease, as illustrated e.g. in White Plague Diseased *Orbicella faveolata* (Sunagawa et al., 2009). In general, an instable or less robust microbial community, as observed in *A. hemprichii* during this study, may be a consequence of a coral's greater susceptibility to stress. At the same time, a largely invariant microbiome, as observed in *P. verrucosa* during this study, may in turn also represent a limiting factor of the coral holobiont to dynamically adjust its microbiome based on environmental conditions.

We investigated representative or indicative microbial taxa for different impacts by applying an indicator species analysis. The microbial communities of the corals at the impacted sites were characterized by a diverse set of bacterial indicator species, for many of which highly similar or identical bacteria have previously been reported from polluted environments and/or diseased corals (refer to Table S3 for sequence similarity). Five OTUs belonging to the bacterial family of Rhodobacteraceae marked the sites impacted by sedimentation & local sewage, some of the OTUs having previously been found in the tissues of diseased corals (Roder et al., 2014b; Séré et al., 2013; Sunagawa et al., 2009). Other coral disease-associated bacteria were also detected including Otu0196, a member of the Vibrionaceae, Otu0340, an unclassified Proteobacterium, and Otu1089 of the genus *Bacteriovorax* (Bacteriovoraceae), which is an opportunistic bacterium known to prey on Gram-negative bacteria (e.g. Crossman et al., 2013). It should be noted however that bacteria belonging to the Vibrionaceae are ubiquitous in marine waters and their exact role in coral microbial communities needs to be further addressed.

Another large group of OTUs in our indicator species analysis has previously been reported from polluted environments. This includes the genus *Herminiimonas* (Oxalobacteraceae), previously found in hydrocarbon-contaminated soil. A species from this genus (*H. arsenicoxydans*) is further known for its resistance to arsenic-contaminated industrial sludge (Muller et al., 2006). In addition, at the sites near the municipal wastewater outfalls, both corals shared a common indicator, Otu0396 from the Saprospiraceae, which has been found in the coral *Mussismilia* (Fernando et al., 2015), but was otherwise reported from an industrial wastewater treatment plant (Xia et al., 2008).

The presence of several abundant OTUs involved in nutrient cycling may potentially be related to the elevated nutrient levels at the impacted sites. These OTUs include two taxa potentially involved in nitrogen cycling (Lema et al., 2012), Otu0006 Rhizobiales and Otu0117 *Pseudoalteromonas* (Pseudoalteromonadaceae). At the sites subjected to sedimentation & local sewage, both corals shared two indicator OTUs in the genera *Roseovarius* (Rhodobacteraceae) and *Novosphingobium* (Sphingomonaceae), which have been reported in the context of crude oil-contaminated environments (Tang et al., 2012; Wang et al., 2014) and to play a role in the degradation of sulfur

compounds such as dimethylsulfoniopropionate (González et al., 2003). In addition, two OTUs were detected that have been associated with *Acropora* under sulfur enrichment (Raina et al., 2009). Another sulfur-reducing indicator Otu0443 *Desulfovibrio* (Desulfovibrionaceae) provides a connection to the presence of human sewage, given its previous isolation from the human gut. Furthermore, a second OTU belonging to Chitinophagaceae occurring on human skin was encountered exclusively at sites C and D (Kong et al., 2012), underscoring the anthropogenic influences at the sites.

Finally, it should be noted that the indicator analysis also yielded a plethora of benign taxa, previously associated with various coral species (e.g., Otu0057 Francisellaceae, Otu0083 unclassified Deltaproteobacteria, Otu0147 Halomonadaceae), or isolated from different portions of the marine environment, such as marine soil (Otu0042 unclassified Gammaproteobacteria, Otu0293 Rhodospirillaceae), biofilms (Otu0047 unclassified Alphaproteobacteria), or seawater (Otu0058 Flavobacteriaceae, Otu0257 Balneolaceae). In two cases, indicator OTUs from the Endozoicomonaceae (Otu0176 and Otu0392) were found to be associated with the impacted sites, suggesting that not all taxa from this bacterial family may be as beneficial to the coral microbiome as previously assumed (Mendoza et al., 2013) and warranting further investigation.

4.4. Acclimatization according to the probiotic hypothesis or deteriorating health states?

The shifts in microbial community structure between sites and impacts found in this study may be interpreted as acclimatization processes of the coral holobiont to the altered environmental conditions providing support to the probiotic hypothesis (Reshef et al., 2006). Alternatively, changes in associated microbial communities may represent impacted coral holobionts, in which the continuous exposure to stressors are not yet visually present (Rosenberg and Kushmaro, 2011). We argue that the changes we found under local anthropogenic impacts may represent coral microbial communities at a tipping point towards disease based on the following reasoning. Firstly, we observed loss of coral host specific microbial communities, i.e. higher similarity between corals at impacted sites. This is also known from diseased corals (Roder et al., 2014b) and corals exposed to various other stressors (Vega Thurber et al., 2009). Secondly, the increase in alpha diversity, in particular for *A. hemprichii* in response to sedimentation & local sewage, may indicate a stress-induced destabilization of the host-microbial balance, leading to emergence of opportunistic taxa (Rosenberg and Kushmaro, 2011). In congruence with the previous point, putative opportunistic taxa, such as Rhodobacteraceae and disease-associated bacteria, such as Vibrionaceae (Rosenberg and Ben-Haim, 2002) increased in abundance at the impacted sites. Together with the occurrence of various impact-specific OTUs in the corals previously recorded in wastewater and other polluted environments, this indicates a shift towards a microbial community with less favorable functions for the coral holobiont (Vega Thurber et al., 2009). Furthermore, the changes in the coral microbial communities observed at the impacted sites resemble those in healthy parts on diseased corals, displaying intermediate microbiomes (Closek et al., 2014). Lastly, our observations of changes in microbial community structure are corroborated by macro benthic data showing decreased hard coral cover at impacted sites.

5. Conclusions

In this study, we assessed the microbial community structure of two coral species and reef water at sites under chronic pollution close to the metropolitan area of Jeddah in order to understand the extent and nature of anthropogenic impacts to Red Sea coral reefs. Our data show that the anthropogenic environmental alterations left a measurable footprint on microbial assemblages, even though the corals from which the samples were collected appeared visually healthy. A concordant microbial community response to local anthropogenic impacts observed in multiple coral species may represent impacted holobionts, potentially at a tipping point towards disease. Our study furthermore highlights that microbial community typing of corals may serve as an early indicator of impact on coral reefs. As such, the presented results may therefore be interpreted as a warning for coral ecosystems surrounding Jeddah, implying the need to reduce these local anthropogenic impacts.

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Author contributions

RO and CRV conceived the study, RO and AP collected samples, MZ, AR, and AP conducted lab work, MZ, AR, and CRV analyzed data, and MZ wrote the manuscript with contributions from CRV, RO, and AR. CRV and KZ provided research materials and logistics. All authors read and approved the final manuscript.

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