diversity

MDPI

Article

An Update of Knowledge of the Bacterial Assemblages Associated with the Mexican Caribbean Corals *Acropora palmata, Orbicella faveolata,* and *Porites porites*

Joicye Hernández-Zulueta [1,2], Leopoldo Díaz-Pérez [1], Alex Echeverría-Vega [3], Gabriela Georgina Nava-Martínez [4], Miguel Ángel García-Salgado [4] and Fabián A. Rodríguez-Zaragoza [1,*]

1 Laboratorio de Ecología Molecular, Microbiología y Taxonomía, Departamento de Ecología, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Av. Ing. Ramón Padilla Sánchez, Zapopan CP 45200, Jalisco, Mexico; joicye.hernandez@academicos.udg.mx (J.H.-Z.); leopoldo.diaz@academicos.udg.mx (L.D.-P.)
2 Departamento de Biología Celular y Molecular, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Av. Ing. Ramón Padilla Sánchez, Zapopan CP 45200, Jalisco, Mexico
3 Centro de Investigación de Estudios Avanzados del Maule (CIEAM), Universidad Católica del Maule, Talca CP 3466706, Chile; aecheverria@ucm.cl
4 OCEANUS A.C. Calle Río Verde Lote 7 Mza. 235. Col. Proterritorio, Chetumal CP 77086, Quintana Roo, Mexico; gnavam01@gmail.com (G.G.N.-M.); info@oceanus.org.mx (M.Á.G.-S.)
* Correspondence: fabian.rzaragoza@academicos.udg.mx

Abstract: In this study, the bacterial microbiota associated with apparently healthy corals of *Acropora palmata*, *Orbicella faveolata*, and *Porites porites* and the surrounding seawater and sediment were evaluated via the MiSeq Illumina sequencing of the V4 region of 16S rRNA at three reef sites in the Mexican Caribbean. Bacterial assemblages associated with apparently healthy corals and sediments showed no significant differences between sites. The colonies of *A. palmata* showed a dominance of families Amoebophilaceae, Spirochaetaceae, Myxococcaceae, and Cyclobacteriaceae. Meanwhile, the colonies of *O. faveolata* and *P. porites* revealed a high prevalence of the Rhodobacteraceae and Kiloniellaceae families. The families Rhodobacteraceae, Cryomorphaceae, Cyanobiaceae, and Flavobacteriaceae were predominant in seawater samples, while Pirellulaceae, Nitrosococcaceae, and Woeseiaceae were predominant in sediments. Variations in *A. palmata* bacterial assemblages were correlated with salinity, sea surface temperature, and depth. These variables, along with nitrate, phosphate, and ammonium concentrations, were also correlated with changes in the bacterial composition of *P. porites*, seawater, and sediments. However, none of the environmental variables were related to the bacterial taxa of *O. faveolata*. Aerobic chemoheterotrophy and fermentation, followed by nitrate reduction and ureolysis, were the metabolic functions with the highest occurrence in the bacterial assemblages associated with all substrates.

Keywords: reef; 16S rRNA; bacteriome; microbial ecology; metabarcoding

Citation: Hernández-Zulueta, J.; Díaz-Pérez, L.; Echeverría-Vega, A.; Nava-Martínez, G.G.; García-Salgado, M.Á.; Rodríguez-Zaragoza, F.A. An Update of Knowledge of the Bacterial Assemblages Associated with the Mexican Caribbean Corals *Acropora palmata*, *Orbicella faveolata*, and *Porites porites*. *Diversity* **2023**, *15*, 964. https://doi.org/10.3390/d15090964

Academic Editors: Spiros Papakostas and Nikolaos Monokrousos

Received: 28 May 2023
Revised: 24 August 2023
Accepted: 24 August 2023
Published: 26 August 2023

1. Introduction

Coral reefs are considered among the most productive and biologically diverse ecosystems [1,2]. The structural units of these ecosystems are corals [3] and metaorganisms also referred to as holobionts [4,5]. Corals harbor a dynamic microbiota featuring a wide variety of taxa consisting of viruses and members of the Archaea, Eukarya, and Eubacteria domains [6,7]. Within this microbial assemblage are bacteria that can inhabit different niches within the coral, i.e., the mucopolysaccharide surface layer, skeleton, and tissues [8,9].

Bacterial assemblages have a large genetic and ecological pool contributing to corals' adaptive and evolutionary abilities [10]. These assemblages are involved in essential functions in host physiology and health such as protection against pathogens [6,11], protein

and nutrient supply (e.g., carbon, nitrogen, and sulfur) [7,12], and the synthesis of some essential vitamins [13].

Some investigations have reported that the taxonomic composition of the bacterial microbiota shows specificity towards coral genera or species, which influences their adaptation to environmental changes [6,14,15]. Morrow et al. [16] proposed that the diversity of bacterial assemblages associated with corals contributes to the resistance and resilience of these organisms to environmental changes such as thermal stress, nutrient limitations, pH changes, and ecosystem eutrophication [12]. This bacterial diversity is also influenced by geographic, seasonal, and physical factors of the coral habitats [7,17], which highlights the importance of understanding how the surrounding environment influences coral holobionts.

In recent decades, new-generation sequencing has deepened our knowledge of the non-culturable members in bacterial assemblages of corals [15,18]. Using these techniques, researchers have identified the bacterial taxa for the core microbiome of corals [19,20], indicating species specificity that likely provides functional benefits to the holobiont [21,22]. This type of sequencing is relevant for characterizing the bacterial assemblage structures of apparently healthy and diseased Caribbean corals [23].

Few studies, however, have characterized the bacterial microbiota associated with coral reefs in the Mexican Caribbean. Mckew et al. [24] found that the bacterial assemblages in the mucus of *Acropora* spp. and *Porites* spp. corals were more diverse than those found in Indonesia. The authors also observed that the corals showed specific bacterial assemblages different from that of the surrounding seawater. Conversely, Closek et al. [25] showed the specific composition and diversity of bacterial taxa in *Orbicella faveolata* coral colonies that were apparently healthy. Hernández-Zulueta et al. [23] reported that the bacterial assemblages associated with apparently healthy *Acropora palmata* and the surrounding seawater and sediments showed no significant differences between sampling sites (i.e., the Mexican Caribbean vs. the Gulf of Mexico).

Knowledge of the bacterial microbiota of apparently healthy corals and their surrounding environments is relevant because it allows researchers to identify possible changes in the structures of the microbial assemblages generated by environmental variation, which can trigger pathologies. Therefore, this study aimed to estimate the bacterial microbiota associated with apparently healthy corals of *A. palmata*, *O. faveolata*, and *Porites porites*, as well as the surrounding seawater and sediments, in three sites of the Mexican Caribbean. Bacterial assemblages associated with these substrates were characterized using MiSeq Illumina sequencing of the V4 region of 16S rRNA. In addition, we evaluated the potential relationship between the structure of the bacterial microbiota and environmental variables and described the putative metabolic functions of the most abundant taxa.

2. Materials and Methods

2.1. Area of Study and Fieldwork

Samples were collected during October 2021 at three sites in the Mexican Caribbean that are part of the northern sector of the Mesoamerican Reef System. (1) Chankanaab belongs to the Cozumel Reefs National Park (20°26′30.03″ N, 86°59′47.55″ W) and presents two-level reef development with a reef patch featuring high richness and total live coral cover; the dominant coral genera at this site are *Agaricia*, *Siderastrea*, *Porites*, and *Orbicella* [26–28]. This site experiences considerable tourism due to sport diving activities [27]. (2) Puerto Morelos (20°52′14.29″ N, 86°51′3.72″ W) corresponds to a protected natural area called Parque Nacional Arrecifes de Puerto Morelos. Reef development in this area is minor, discontinuous, and distributed as patches limited to shallow areas (<10 m), where the species *A. palmata*, *Siderastrea* spp., and *Pseudodiploria strigosa* present high coverage [29,30]. (3) Punta Maroma (20°42′50.96″ N, 86°58′38.60″ W) (Figure 1) is an unprotected reef with larger reef development than Puerto Morelos and a continuous, shallow (<10 m) barrier 5 km long, as well as high cover of the coral species *Acropora* spp., *Orbicella* spp., *Porites*

spp., and *Siderastrea* spp. This area's reef lagoon features a high proportion of sand, rubble, algae, and seagrasses [29,31].

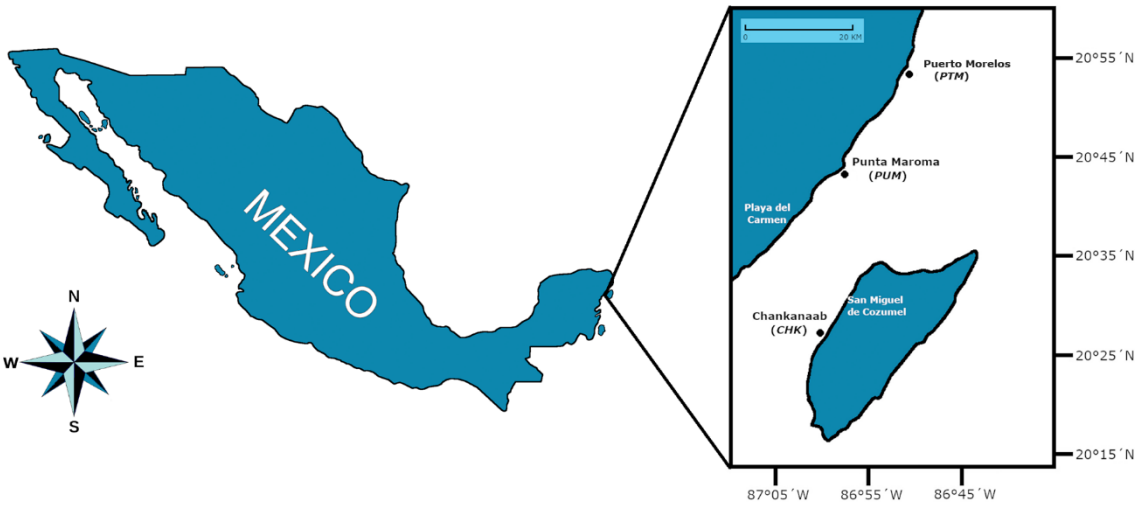

Figure 1. Study area and sampling sites in Mexican Caribbean: Chankanaab, Puerto Morelos, and Punta Maroma.

2.2. Sample Collection

At each site, three fragments (≈7 cm) of apparently healthy colonies of *A. palmata*, *O. faveolata*, and *P. porites* were collected through scuba diving according to Ainsworth et al. [19] and Bourne and Munn [32]. A minimum quantity of corals was sampled to limit the impact on these important reef builders.

Coral tissues were separated and stored according to the method described by Hernández-Zulueta et al. [15]. In addition, one liter of surrounding seawater was taken directly over the coral colony, and marine sediments adjacent to the colonies were collected and placed in sterile 50 mL plastic tubes. Seawater was filtered using Sterivex filtration units with a 0.22 µm pore size (Millipore, Billerica). All samples were preserved in anhydrous absolute ethyl alcohol (Baker's Reagent) and maintained at −20 °C until DNA extraction.

The following environmental parameters were determined in triplicate at each site: salinity (PSU), sea surface temperature (SST), sea depth, and pH. PSU was recorded with an ATC refractometer, SST and sea depth were measured with a ZOOP dive computer, and pH was measured with a Ketoket potentiometer. In addition, three seawater samples (150 mL) were collected for nutrient quantification ((nitrate (mg/L), nitrite (mg/L), ammonium (mg/L), and phosphate (mg/L) concentrations) at each site with an HACH DR900 portable colorimeter. The collected seawater was taken no more than 10 cm away from the substrates (i.e., corals, seawater, and sediments) to determine the environmental variables.

2.3. Sample Pretreatment and DNA Extraction

DNA extraction from coral tissue and sediment samples was performed with a Quick-DNA Fecal/Soil Microbe Kit (ZYMO Research) following the manufacturer's instructions. Seawater DNA was extracted from pre-cut filters with a MagMAX™ DNA Multi-Sample Ultra kit (ThermoFisher Scientific, Worcester, MA, USA) on a King Fisher Duo Prime kit. The DNA quality of each sample was evaluated on 1% agarose gel with SYBR safe staining. The concentration and purity of bacterial DNA were determined with a spectrophotometer (Jenway Genova Nano) at 280/260 nm. A total of 51 samples corresponding to the five substrates were processed in triplicate for each sample (i.e., *A. palmata*, *O. faveolata*, *P. porites*, seawater, and sediment) (n = 45) and the controls for DNA extraction methods (n = 6). Collection tubes from all samples were frozen until DNA sequencing was performed at the Molecular Research Laboratory (MR DNA, Shallowater, TX, USA).

2.4. PCR Amplification of the 16S rRNA Gene

The V4 region of the 16S rRNA gene was amplified via PCR primers 515f (GTGCCAGCMGCCGCGGTAA) and 806r (GGACTACHVGGGTWTCTAAT) as described by Caporaso et al. [33]. Amplification was performed using a Qiagen Kit HotStarTaq Plus Master Mix under the following conditions: initial denaturation at 94 °C for 3 min followed by 28 cycles, each set at 94 °C for 30 s, with 53 °C for 40 s and 72 °C for 1 min and a final elongation step at 72 °C for 5 min; lastly, incubation was performed at 4 °C after amplification.

2.5. Sequencing of the 16S rRNA Gene

After amplification, PCR products were analyzed on 2% agarose gel to determine the amplification success and relative band intensity. Samples were multiplexed with unique dual indices and pooled in equal proportions based on their molecular weight and DNA concentrations. The pooled samples were purified with calibrated Ampure XP beads. Then, the pooled and purified PCR product was used to prepare the Illumina DNA library. Sequencing was performed using MR DNA (www.mrdnalab.com, Shallowater, TX, USA, accessed on 21 April 2022) with MiSeq following the manufacturer's instructions.

2.6. Sequence Analysis and Taxonomic Identification Using the SILVA Database

Bioinformatic analyses were performed with QIIME 2 [34]. Raw sequence data were demultiplexed and quality filtered with the minimal quality median set to 30 using the q2-demux plugin. Sequences were independently cleaned, filtered, trimmed, dereplicated, merged, and had their chimeras removed using a modified Divisive Amplicon Denoising Algorithm 2 (DADA2 v. 1.20.0) pipeline [35]. Taxonomic classifications were assigned to amplicon sequence variants (ASVs) using the q2-feature-classifier [36] and classify–sklearn naïve Bayes taxonomy classifier against SILVA Database reference sequences (SILVA SSU 138 16.12.2019) [37]. To pass quality control, we eliminated singletons and rarefied the data in all sequences to a smaller sample size. Finally, the resulting ASV tables, taxonomy table, and metadata were analyzed using Primer-7 (Primer-e, Plymouth Marine Laboratory, Plymouth, UK) software [38,39]. All raw data were deposited in the SRA experiment database PRJNA836615.

2.7. Data Analysis

The model used in the present study considered apparently healthy samples of *A. palmata*, *O. faveolata*, *P. porites*, seawater, and sediment obtained at all sampling sites (i.e., Chankanaab, Puerto Morelos, and Punta Maroma).

Bacterial alpha diversity was assessed by estimating the number of ASVs (labeled as "ASV richness" (AR) in the present study) and Shannon diversity (H') in each sample of the analyzed substrates (i.e., *A. palmata*, *O. faveolata*, *P. porites*, seawater, and sediment). The variation in these community attributes (AR and H') was evaluated among sampling sites across analyzed substrates using a two-way experimental design with crossed factors:

$$Y = \mu + SU_i + SI_j + SU_i \times SI_j + \varepsilon_{ij} \quad (1)$$

where Y is the response variables (AR and H'), μ is the average of the variables analyzed, SU_i is the substrate factor (i.e., *A. palmata*, *O. faveolata*, *P. porites*, seawater, and sediment), SI_j is the sampling site factor (i.e., Chankanaab, Puerto Morelos, and Punta Maroma), and ε_{ij} is the cumulative error. The $SU_i \times SI_j$ term represents the interaction between the substrate and site factors. Both factors have a fixed effect (type I model). This experimental design was evaluated with a permutational multidimensional analysis of variance (PERMANOVA) constructed using a Euclidean distance matrix with data previously standardized to Z values.

Bacterial beta diversity analysis considered the variation in ASV composition and abundance, which was assessed with a second PERMANOVA constructed using the experi-

mental design described before, a Bray–Curtis distance matrix, and square root pretreated data. The factors that presented significant differences in the PERMANOVA global test were subjected to post hoc tests via pairwise comparisons. The statistical significance of the PERMANOVA global and post hoc tests was evaluated with 10,000 permutations of residuals under a reduced model and the type III sum of squares. In addition, NMDS ordination was used to visualize the bacterial ASV dissimilarities between the substrates and sites. This NMDS was performed based on the same data pretreatment, the same resemblance coefficient, and the results of the second PERMANOVA. Subsequently, the composition of the most dominant bacterial families was plotted in stacked bar charts.

Additionally, similarities in the composition and abundance of the most dominant bacterial families among the studied substrates were assessed with a shaded plot. In this shade plot, we constructed dendrograms based on two clustering strategies to analyze the similarity between substrates (Q-mode) and associated bacterial families (R-mode). The first dendrogram used the same data pretreatment and resemblance coefficient as the second PERMANOVA, while the second dendrogram used data standardized to relative abundance and Whittaker's association coefficient. In both cases, the average group linkage method and similarity profiling procedure (SIMPROF) were used for cluster identification based on the π test and 10,000 permutations.

Putative metabolic functions were identified with the database Functional Annotation of Prokaryotic Taxa (FAPROTAX v.1.2.4) [40]. This database includes information from 4600 taxa and assigns a putative metabolic function to each bacterial ASV based on the literature [40]. Putative functions not found on FAPROTAX were inferred from the literature by searching the family name and associated metabolic functions [40]. The relations between the bacterial families identified by the dominance analysis and their putative functions were analyzed using another shade plot. This shade plot was constructed using binary data on the incidence of the putative metabolic functions for each family or genus. For this process, we constructed a Sørensen similarity matrix and used the average group linkage method to create two dendrograms, one to associate putative functions (Q mode) and another to associate bacterial families (R mode). Cluster identification was performed following the SIMPROF (π) procedure, as described previously.

The relationships between the dominant bacterial families in each substrate and the environmental variables were assessed with the BIOENV procedure using Spearman correlation (ρ) and the best combinations of environmental variables that were statistically significant. The statistical significance of the BIOENV correlations was tested with 10,000 permutations. The BIOENV biological matrix was constructed based on the composition and abundance of the dominant bacterial families in each substrate, while the environmental matrix was composed of the variables sea surface temperature (SST), depth, pH, nitrate content (mg/L), nitrite content (mg/L), ammonium content (mg/L), and phosphate content (mg/L) (Table S1). The similarity and data pretreatment matrices for the biological matrix were the same as those for the second PERMANOVA, while standardization to the Z values and a Euclidean distance matrix were used for the environmental matrix. In addition, a principal coordinate analysis (PCO) was used to visualize the overall BIOENV results. Each PCO biplot was generated based on substrate type to analyze the relationship between the dominant bacterial families' composition and abundance and the environmental variables. Biplots were built with the same data pretreatment alongside the Bray–Curtis similarity and Euclidean distance matrices used in BIOENV. Environmental variables were also selected from the BIOENV outputs and projected as vectors using multiple correlation analysis. All analyses, i.e., the community metrics (AR and H'), PERMANOVA, NMDS, shade plots, SIMPROF, BIOENV, and PCO, were performed in the PRIMER v7 software [38,39].

3. Results

Illumina sequencing of the V4 region of the 16S ARNr yielded 1,537,905 high-quality sequences from the 51 samples, which included the 45 samples from five substrates (i.e.,

A. palmata, *O. faveolata*, *P. porites*, seawater, and sediment) and six negative controls. After removing ASVs from the negative controls, a total richness of 18,172 ASVs was obtained on all substrates. Bacterial ASVs were grouped into 70 phyla, 168 classes, 396 orders, 675 families, and 1308 genera. The highest number of ASVs was found in the sediments (8794), followed by *O. faveolata* (6649), *P. porites* (4252), and *A. palmata* (2189). The lowest ASV number was estimated in the seawater samples (1168) (Figure S1).

PERMANOVA results for community attributes (AR and H') showed significant differences in the interactions between substrate and site factors (Pseudo-F = 1.976, $p = 0.0412$), where the substrate was the most important factor, explaining 42.6% of the total variation (Table S2). Post hoc tests of the interactions by substrate factor showed significant differences between most substrates within each site (Table S3). The only exception to this rule was Chankanaab, where no differences were found between *O. faveolata* and seawater; sediments, *O. faveolate*, and *P. porites*; or *O. faveolata* and *P. porites*. In Puerto Morelos, the community attributes of *O. faveolata* bacteria were no different from those of the other substrates. In addition, no differences were observed between *P. porites* and seawater and *P. porites* and sediments. At Punta Maroma, the bacterial AR and H' of *O. faveolata* did not differ from the values in seawater, sediment, and *P. porites*; moreover, *P. porites* was no different from the sediment (Table S3). In contrast, post hoc tests of the factor interactions at the sampling site showed no significant differences in the substrates between the three sites, except in seawater at Chankanaab and Punta Maroma (Table S3).

The PERMANOVA found significant variation in the bacterial ASV composition and abundance in the interactions between the substrate and sampling site (Pseudo-F = 1.751, $p = 0.0001$). The substrate factor also explained the largest total variation in the model (30.6%) (Table S2). Post hoc tests of the interaction by substrate factor showed that, within each site, the bacterial assemblage of seawater was different from the assemblages in other substrates (Table S4). In Puerto Morelos, the bacterial taxa of *A. palmata* and sediments were also different (Table S4). Post hoc tests of interactions by sampling site showed significant differences only in the seawater bacterial assemblages between the three sites sampled (Table S4). NMDS ordination showed that most analyzed substrates had a considerable bacterial dissimilarity between themselves, particularly seawater, sediments, and *A. palmata*. However, *O. faveolata* and *P. porites* featured more similar bacterial assemblages (Figure 2).

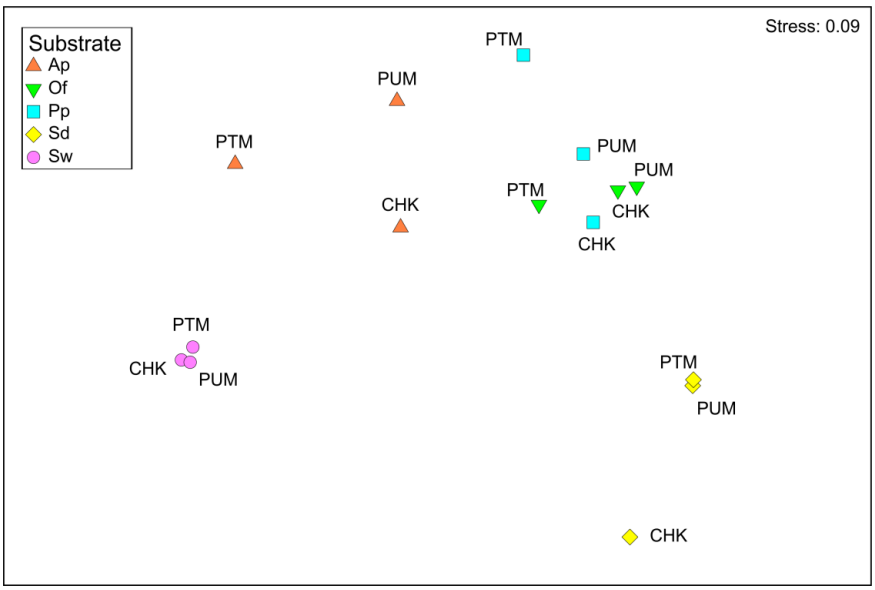

Figure 2. NMDS ordination shows the dissimilarity of the bacterial ASV's composition and abundance among the studied substrates across sampling sites. Codes: Ap is *A. palmata*; Of is *O. faveolata*; Pp is *P. porites*; Sw is seawater; Sd is sediments (Sd); CHK is Chankanaab; PTM is Puerto Morelos; PUM is Punta Maroma.

The families Rhodobacteraceae, Amoebophilaceae, Cryomorphaceae, Kiloniellaceae, Spirochaetaceae, Myxococcaceae, and Cyanobiaceae presented the highest absolute abundance (Figure 3a–c; Table S5). At Chankanaab, the Amoebophilaceae family showed high abundance in all three coral species (Figure 3a). In particular, the families Spirochaetaceae and Cyclobacteriaceae dominated in *A. palmata*, while Kiloniellaceae, Nitrosopumilaceae, and Rhodobacteraceae prevailed in *O. faveolata*. The coral *P. porites* also showed a high abundance of Kiloniellaceae and Rhodobacteraceae (Figure 3a). In the bacterial assemblage of Chankanaab seawater, the families Cryomorphaceae, Rhodobacteraceae, and Cyanobiaceae were found to be most dominant. Sediments from this site showed a high abundance of the Pirellulaceae family (Figure 3a; Table S5).

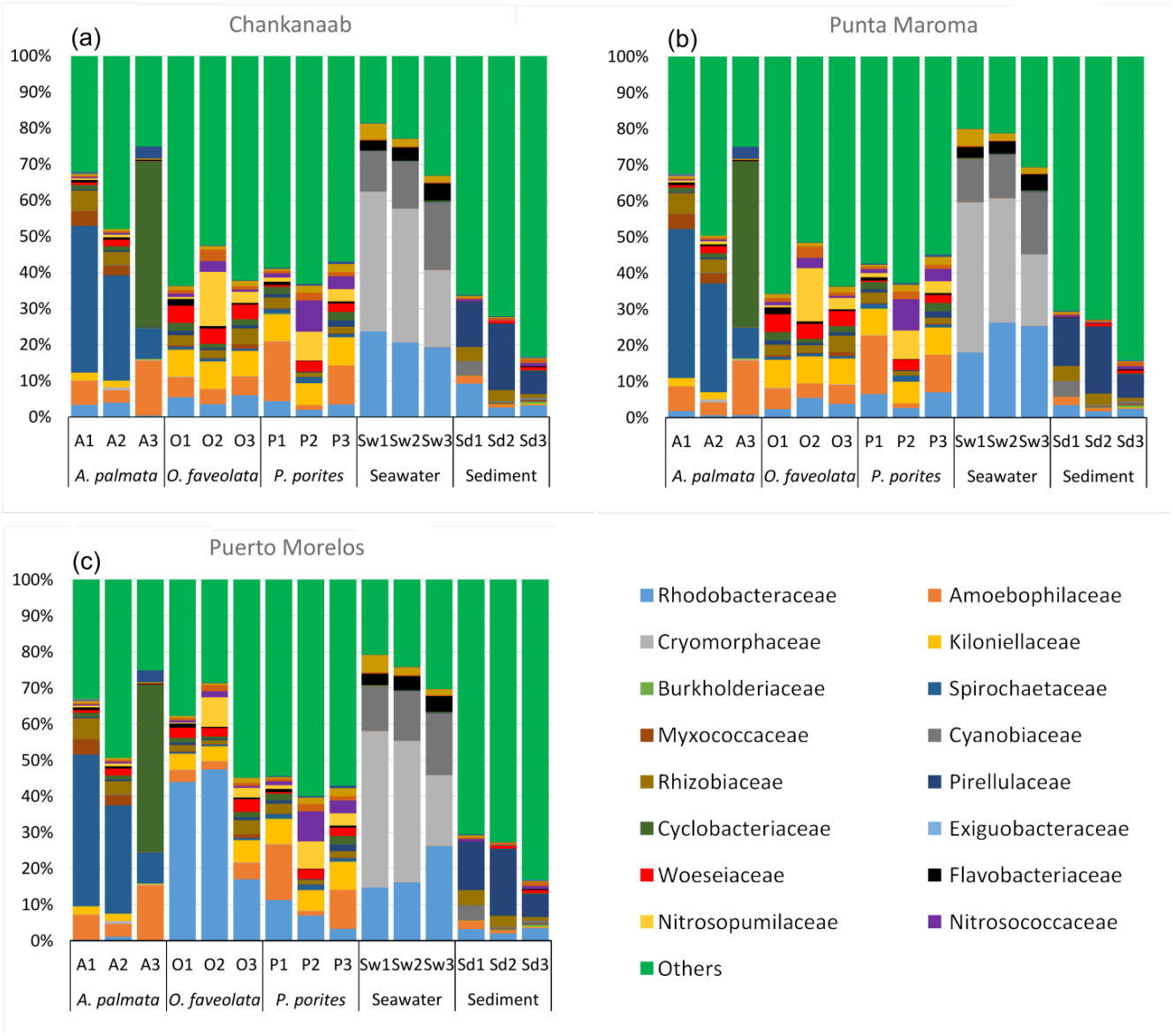

Figure 3. Stacked bar charts showing the composition and relative abundance of the main bacteria families by substrate and sampling site. The replicate codes comprise the substrate type (A is *A. palmata*; O is *O. faveolata*; P is *P. porites*; Sw is seawater; Sd is sediments) and their respective sample number.

At Punta Maroma, the Rhodobacteraceae family was dominant in *O. faveolata*, *P. porites*, and seawater (Figure 3b). A high abundance of the Amoebophilaceae family was observed in *A. palmata* and *O. faveolata*. Another family that prevailed in *A. palmata* was

Spirochaetaceae. In *O. faveolata* and *P. porites*, the families Kiloniellaceae and Nitrosopumilaceae dominated (Figure 3b). In seawater and sediments, the same families observed in Puerto Morelos dominated (Figure 3b; Table S5).

In Puerto Morelos, the Kiloniellaceae family presented high levels of abundance in corals and sediments (Figure 3c). Likewise, the Myxococcaceae and Amoebophilaceae families had a high prevalence in *A. palmata*. The corals *O. faveolata*, *P. porites*, and seawater presented large quantities of the Rhodobacteraceae family. The Rhizobiaceae family was also abundant in *P. porites*. The Cryomorphaceae, Cyanobiaceae, and Flavobacteriaceae families were dominant in seawater samples, while Pirellulaceae, Woeseiaceae, and Nitrosococcaceae were dominant in sediments (Figure 3c; Table S5).

The shade plots demonstrate that aerobic chemoheterotrophy (AeCh) (53%) and fermentation (Ferm) (32%) were the metabolic functions with the highest prevalence in the most dominant bacterial families among the five substrates studied (Figure 4a,b). The families with the highest number of putative functions were Rhodobacteraceae and Hyphomicrobiaceae with six; Xanthomonadaceae with five; Sandaracinaceae, Hyphomonadaceae, Desulfocapsaceae, and Gemmatimonadaceae with four; and Cryomorphaceae, Burkholderiaceae, Rhizobiaceae, Flavobacteriaceae, Vibrionaceae, Desulfosarcinaceae, Pseudomonadaceae, Sphingomonadaceae, and Oxalobacteraceae with three. The remaining 40 families had less than three functions (Figure 4b).

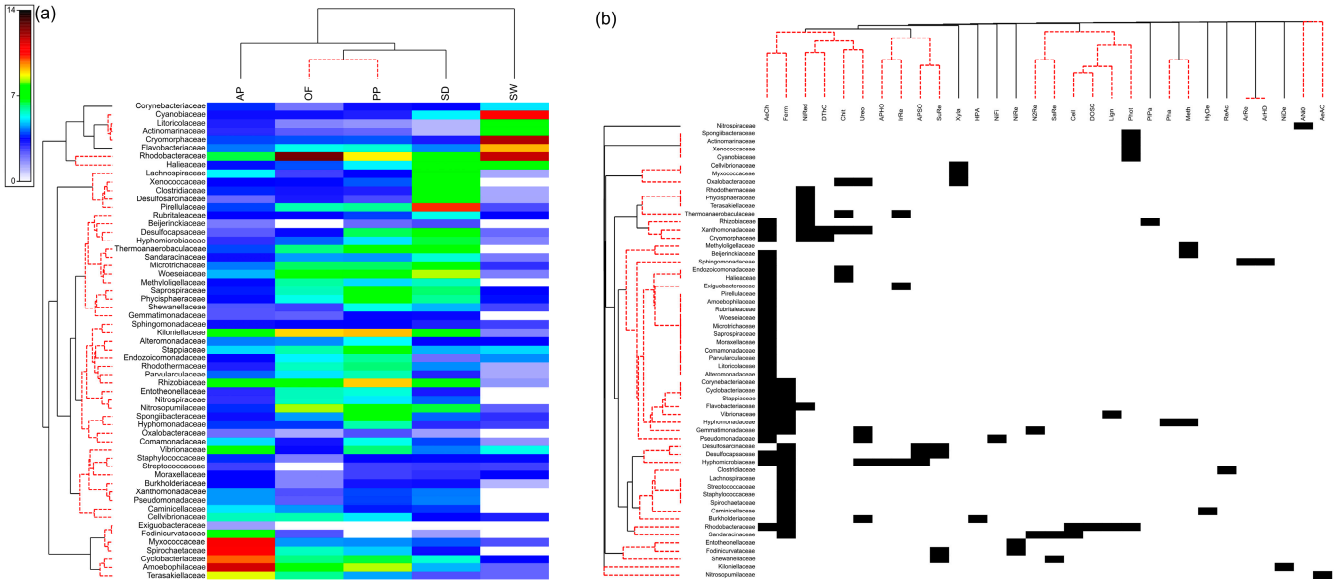

Figure 4. Shade plots show the dominant bacterial family of the substrates and their putative metabolic functions. (**a**) Dominant bacteria families of *A. palmata* (Ap), *O. faveolata* (Of), *P. porites* (Pp), seawater (Sw), and sediments (Sd). (**b**) Relationship between the dominant bacterial family and their putative metabolic functions. Codes: AeCh is aerobic chemoheterotrophy; Ferm is fermentation; NiRed is nitrate reduction; DThO is dark thiosulfate oxidation; Chit is chitinolysis; Ureo is ureolysis; IrRe is iron respiration; APSO is anoxygenic photoautotrophy S oxidizing; SuRe is sulfur respiration; Cell is cellulolysis; DOSC is dark oxidation of sulfur compounds; Meth is methylotrophy; Xyla is xylanolysis; HPA is human pathogens all; NiFi is nitrogen fixation; NiRe is nitrate respiration; N2Re is nitrogen respiration; SaRe is sulfate respiration; Lign is ligninolysis; Phot is photoheterotrophy; PlPa is plant pathogen; Pha is photoautotrophy; HyDe is hydrocarbon degradation; ReAc is reductive acetogenesis; ArRe is arsenate respiration; ArHD is aromatic hydrocarbon degradation; NiDe is nitrate denitrification; ANi0 is aerobic nitrite oxidation; AeAO is aerobic ammonia oxidation.

The Amoebophilaceae, Spirochaetaceae, and Myxococcaceae families were abundant in *A. palmata* coral (Figure 4a). The Amoebophilaceae family performed the metabolic function of aerobic chemoheterotrophy respiration (AeCh), Spirochaetaceae performed fermentation (Ferm), and Myxococccoccaceae carried out xylanolysis (Xyla) (Figure 4b).

Meanwhile, in *O. faveolata* and *P. porites* corals, the Rhodobacteraceae and Kiloniellaceae families predominated (Figure 4a). The Rhodobacteraceae family presented the following metabolic functions: AeCh, Ferm, cellulolysis (Cell), dark oxidation of sulfur compounds (DOSC), ligninolysis (Lign), and photoheterotrophy (Phot). Meanwhile, the Kiloniellaceae family carried out nitrate denitrification (NiDe). *P. porites* also showed a high abundance of the Rhizobiaceae family, in which AeCh, plant pathogens (PlPa), and nitrate reduction (NiRed) were reported (Figure 4a,b)

In seawater, the families Rhodobacteraceae, Cryomorphaceae, Cyanobiaceae, and Flavobacteriaceae were notably dominant (Figure 4a). Cryomorphaceae presented AeCh, NiRed, and dark thiosulfate oxidation (DThO). In addition, Cyanobiaceae performed Phot, and Flavobacteriaceae carried out AeCh, NiRed, and Ferm. In the sediments, Pirellulaceae, Nitrosococcaceae, and Woeseiaceae dominated. AeCh was observed in the Pirellulaceae and Woeseiaceae families, while aerobic nitrite oxidation (ANi0) was reported in Nitrosococcaceae (Figure 4a,b).

The BIOENV results showed the bacterial assemblages of *A. palmata* to be correlated with sea surface temperature (SST), salinity, and depth (Table S6). Combinations of these variables were also related to the bacterial composition of *P. porites*, seawater, and sediments, but only after including nitrate (NO_3^-), phosphate (PO_4), and ammonium (NH_4) (Table S6). pH and nitrite (NO_2^-) were only related to seawater and sediments. The bacterial taxa of *O. faveolata* were not correlated with any environmental variables (Table S6). The PCO biplots also showed that the subsets of environmental variables correlated with the dominant bacterial families differed among substrates. Salinity, depth, and SST were highly correlated in most substrates, particularly in *A. palmata* and *P. porites*. However, NO_3^- was highly correlated with *P. porites* and sediments, as was NO_2^- in seawater. These nutrients were mainly correlated with the Chankanaab site, while salinity and SST were correlated with Puerto Morelos (Figure 5).

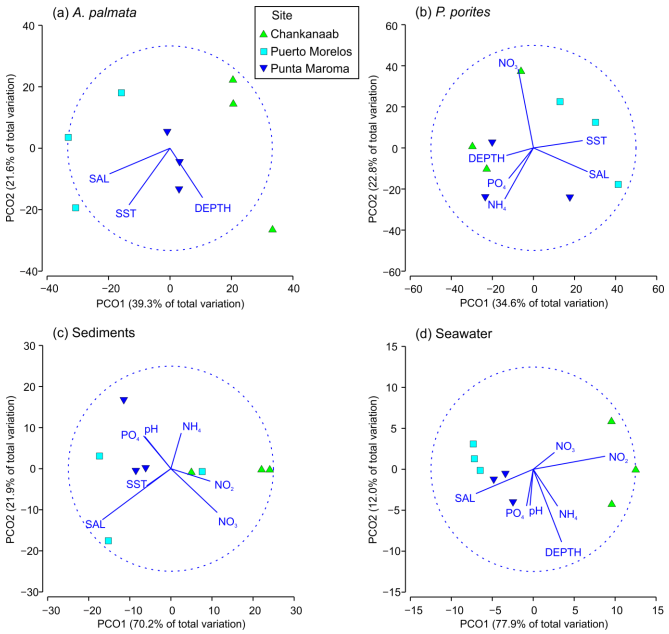

Figure 5. PCO biplots represent the relationship of dominant bacterial ASVs with different environmental variables by each studied substrate type across sampling sites. These biplots represent the BIOENV procedure's main results, and environmental variables are represented as vectors within a Pearson correlation circle. Codes: SST is sea surface temperature; SAL is salinity; NO_2 is nitrite; NO_3 is nitrate; NH_4 is ammonium; PO_4 is phosphate; pH is the potential of hydrogen. Note: The PCO biplot for *O. faveolata* was not performed because the BIOENV procedure did not find a significant correlation with environmental variables.

4. Discussion

In this study, the structures of bacterial assemblages associated with apparently healthy corals and sediments did not present spatial variation, but the seawater did show differences between sampling sites. Previous studies have reported that corals of the same species but from different sites present similar bacterial assemblages [4,14,15], suggesting that microbiota specificity ensures the holobiont's health, functionality, adaptability, and resilience to environmental changes [6,12]. The similarities between the coral and sediment microbiota suggest that such sediments are bacterial sources for holobionts. Some studies have proposed that sediments act as bacterial reservoirs in coral reefs [14,41]. Likewise, the bacterial assemblage in the sediments can be influenced by the coral mucus falling from the corals [42]. Some studies affirm that the corals may influence the surrounding sediments (in terms of granulometry, microbiome, meiobenthos composition, and organic matter) [43,44]. We suggest that sediment samplings at increasing distances from the coral would be necessary in the future to verify the hypothesis that sediments are the source of the coral microbiome. However, the lack of differentiation between most substrates is striking since it was previously reported that the absence of differences between substrates in coral reefs could indicate environmental disturbances [15]. Nonetheless, no signs of disturbance were observed in the sampling sites, and the measured physicochemical variables corresponded to those of oligotrophic marine ecosystems. In contrast, seawater's bacterial assemblages differed from those observed in corals. These results agree with those reported by Hernández-Zulueta et al. [23], de Voogd et al. [45], Zhang et al. [46], and Cleary et al. [47]. Therefore, these data confirm that corals correspond to different bacterial microbiota compared to the surrounding seawater. The family with the highest relative bacterial abundance in *O. faveolata*, *P. porites* corals, and the surrounding seawater was the Rhodobacteraceae family. Members of this family have been widely associated with tropical corals [48,49], suggesting that they establish mutualistic relationships with these organisms. However, these bacteria have been associated with poor reef health because they increase their abundance when corals are diseased and stressed [50–52]. Likewise, it was previously reported that several members of this family, such as *P. lutea*, are indicators of thermal stress [53].

The Rhizobiaceae family also presented a high abundance in the coral *P. porites*. Some members of this family are involved in the biological processes of nitrogen fixation and some plant diseases [54]. The high abundance of this family was also linked to diseased corals [55]. Conversely, the family Cryomorphaceae showed a high abundance in seawater samples. The dominance of this family, which belongs to the order Bacteroidetes, is consistent in coral reefs [56]. Moreover, microorganisms of this order have been used to induce microbialization in coral reefs [57].

The Amoebophilaceae family is highly abundant in *A. palmata* and *O. faveolata* corals. The genus *Candidatus amoebophilus* was reported to dominate the microbiome of Caribbean corals and is specifically associated with coral tissue, featuring a relative abundance of up to 70% among bacterial sequences [48,58]. However, the role of this microorganism in coral-associated bacterial assemblages is currently unknown.

A. palmata was strongly dominated by sequences related to the families Myxococcaceae and Spirochaetaceae. Some members of the Myxococcaceae family produce secondary metabolites that act as antimicrobials, antiparasitics, antivirals, cytotoxins, and anticoagulants [59]. Although the presence of this family has not been previously reported in corals, Myxococcaceae species could participate in holobiont defense. Moreover, members of the Spirochaetaceae family are characterized as free-living nonpathogenic anaerobic microorganisms capable of fixing and degrading organic carbon [60]. For example, the genus *Spirochaeta* is involved in nitrogen fixation [61]. Therefore, this family may be relevant for apparently healthy *A. palmata* corals by favoring carbon degradation and nitrogen fixation.

The family Cyanobiaceae presented a high abundance in the seawater samples. Members of this family (e.g., *Synechococcus*) are also considered key organisms for nitrogen fixation in coral reef ecosystems [62]. The Pirellula and Nitrosococcaceae families were

found to be prominent in sediments, and bacteria of the Pirellula family presented a wide range of physiological strategies, as some bacterial isolates are oligotrophic heterotrophic (micro) aerobic, and others are facultative fermenters or facultative NO_3^- reducers [63]. Conversely, members of the Nitrosococcaceae family from marine sediments are recognized as nitrifying bacteria because of their potential for ammonia oxidation in the nitrogen cycle [64]. Therefore, the presence of these families in marine sediments is relevant to the carbon and nitrogen cycles that develop in the reef ecosystem.

Analysis of putative metabolic functions of the most dominant bacteria showed many sequences assigned to aerobic chemoheterotrophy and fermentation. Aerobic chemoheterotrophy is performed by most microorganisms and is, therefore, classified as a broad ecosystemic function [65]. Ostria-Hernández et al. [22] reported that this metabolic function displayed the highest occurrence in the bacterial core microbiomes of corals from the Mexican Central Pacific. Aerobic chemoheterotrophy and fermentation are related to obtaining energy from the degradation of organic compounds. For example, some chemoheterotrophic bacteria can degrade dimethylsulfoniopropionate, a key compound in the marine sulfur cycle produced in large quantities in coral reefs [66]. Furthermore, bacterial fermentation allows corals to obtain energy from the degradation of amino acids in oligotrophic environments [67].

The bacterial assemblages of corals *A. palmata* and *P. porites*, seawater, and sediments were correlated with different environmental variables. The sea surface temperature (SST), for example, was correlated with the bacterial microbiota of *A. palmata, P. porites,* and sediments. An increase in this variable was reported to induce important changes in the structure and functionality of the bacterial assemblage [68] and in the occurrence of coral diseases generated by opportunistic pathogens [69]. Other environmental variables contributing to the variation in bacterial assemblages among substrates were salinity and concentrations of nitrite, nitrate, ammonium, and phosphate. Lee et al. [70] observed that salinity (SAL) and ammonium contribute to the composition of the bacterial microbiota of Red Sea corals. Furthermore, Hernández-Zulueta et al. [15] found that ammonium is one of the most significant environmental variables correlated with variation in bacterial OTU composition in coral tissues, seawater, and sediments from the Mexican Pacific. Our results are consistent with previous research indicating that marine environmental chemical components (i.e., the concentrations of nitrite, nitrate, ammonium, and phosphate) regulate the microbiota composition of corals and their surrounding habitats [71,72].

5. Conclusions

This study provides a foundation for monitoring the structure of coral bacterial assemblages in the Mexican Caribbean. Our results show that the coral's bacterial microbiota possess spatial stability, supporting the concept of coral–bacteria specificity. Furthermore, we found that the composition and abundance of the bacterial taxa of *A. palmata* and *P. porites*, seawater, and sediments were correlated with variables such as SAL, SST, nitrites, nitrates, ammonium, and phosphates. In terms of metabolic pathways, the most abundant bacterial families performed aerobic chemoheterotrophy and fermentation, which are vital functions for coral nutrition. This study could contribute to generating a baseline for microbial ecology in coral reefs in the Mexican Caribbean because a high incidence of diseases that affect the health of this ecosystem was reported in this region [23,25,52,73]. Further research on bacterial assemblages associated with coral ecosystems should be conducted to promote the maintenance, conservation, and restoration of this marine system in the Mexican Caribbean.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15090964/s1, Table S1: Average environmental variables at three sampling sites of the Mexican Caribbean; Figure S1: Venn's diagram showing number of amplicon sequence variants (ASVs) in each substrate; Table S2: Results of the two-way crossed PERMANOVA with replication of the community attributes (ASV richness [*AR*], Shannon diversity [*H'*, nats]), and the bacterial ASV composition and abundance among substrates and sampling sites; Table S3: Results

of the Post hoc tests of the two-way crossed PERMANOVA of the community attributes (ASV richness [AR] and Shannon diversity [H', nats]) of the interaction Substrate x Site, considering the substrate within and among sites; Table S4: Results of the Post hoc tests of the two-way crossed PERMANOVA of the bacterial ASV composition and abundance of the interaction Substrate x Site, considering the substrate within and among sites; Table S5: Dominant bacteria families of each substrate within each site; Table S6: BIO-ENV outputs with the best subsets of environmental variables correlated with the bacterial assemblage per substrate.

Author Contributions: All authors participated in the conception of the idea; J.H.-Z., L.D.-P. and F.A.R.-Z. designed the methodology; J.H.-Z., L.D.-P., A.E.-V., G.G.N.-M., M.Á.G.-S. and F.A.R.-Z. compiled the data; J.H.-Z. and F.A.R.-Z. analyzed the data; J.H.-Z., F.A.R.-Z. and A.E.-V. led the writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: Oceanus A.C. sponsored this work with support from Corporativo Ciudad Mayakoba (Puerto Morelos and Punta Maroma), Summit Foundation, and MARFUND (Cozumel). Also, financing from the Universidad de Guadalajara was provided as part of the following projects: (i) P3E2019 and P3E 2020 to F.A.R.-Z. and L.D.-P.; PRO-SNI2019 and PRO-SNI2020 to F.A.R.-Z., L.D.-P. and J.H.-Z.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Acknowledgments: We thank Edgar Salvador Samos Falcón, Rodrigo García Uribe, Melissa Llanes Lopez, and Gabriela Vargas (Oceanus A.C.) for helping us during fieldwork and the reviewers whose feedback has helped improve this paper. We also thank Rebeca Méndez-Hernández for assistance with the English language.

Conflicts of Interest: The authors declare they have no financial interests. Author Joicye Hernández Zulueta is on the board of Editors of Community Ecology.

References

1. Jackson, J.B.; Kirby, M.X.; Berger, W.H.; Bjorndal, K.A.; Botsford, L.W.; Bourque, B.J.; Bradbury, R.H.; Cooke, R.; Erlandson, J.; Estes, J.A.; et al. Historical overfishing and the recent collapse of coastal ecosystems. *Science* **2001**, *293*, 629–637. [CrossRef] [PubMed]
2. Woodhead, A.J.; Hicks, C.C.; Norström, A.V.; Williams, G.J.; Graham, N.A.J. Coral reef ecosystem services in the Anthropocene. *Funct. Ecol.* **2019**, *33*, 1023–1034. [CrossRef]
3. Carpenter, K.E.; Abrar, M.; Aeby, G.; Aronson, R.B.; Banks, S.; Bruckner, A.; Chiriboga, A.; Cortés, J.; Delbeek, J.C.; Devantier, L.; et al. One-third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science* **2008**, *321*, 560–563. [CrossRef]
4. Rohwer, F.; Breitbart, M.; Jara, J.; Azam, F.; Knowlton, N. Diversity of bacteria associated with the Caribbean coral *Montastraea franksi*. *Coral. Reefs* **2001**, *20*, 85–91.
5. Ziegler, M.; Grupstra, C.G.; Barreto, M.M.; Eaton, M.; BaOmar, J.; Zubier, K.; Al-Sofyani, A.; Turki, A.J.; Ormond, R. Coral bacterial community structure responds to environmental change in a host-specific manner. *Nat. Commun.* **2019**, *10*, 3092. [CrossRef]
6. Bourne, D.G.; Webster, N.S. Coral reef bacterial communities. In *The Prokaryotes*, 4th ed.; Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F., Eds.; Springer: Berlin/Heidelberg, Germany, 2013; pp. 163–187.
7. Vanwonterghem, I.; Webster, N.S. Coral reef microorganisms in a changing climate. *iScience* **2020**, *23*, 100972. [CrossRef]
8. Li, J.; Chen, Q.; Long, L.J.; Dong, J.D.; Yang, J.; Zhang, S. Bacterial dynamics within the mucus, tissue and skeleton of the coral *Porites lutea* during different seasons. *Sci. Rep.* **2014**, *4*, 7320. [CrossRef]
9. Bergman, J.L.; Shaw, T.; Egan, S.; Ainsworth, T.D. Assessing the coral microbiome at the scale of tissue-specific habitats within the coral meta-organism. *Front. Mar. Sci.* **2022**, *9*, 985496. [CrossRef]
10. van Oppen, M.; Medina, M. Coral evolutionary responses to microbial symbioses. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2020**, *375*, 20190591. [CrossRef]
11. Peixoto, R.S.; Rosado, P.M.; Leite, D.C.; Rosado, A.S.; Bourne, D.G. Beneficial Microorganisms for Corals (BMC): Proposed Mechanisms for Coral Health and Resilience. *Front. Microbiol.* **2017**, *8*, 341. [CrossRef] [PubMed]
12. Bourne, D.G.; Morrow, K.M.; Webster, N.S. Insights into the coral microbiome: Underpinning the health and resilience of reef ecosystems. *Annu. Rev. Microbiol.* **2016**, *70*, 317–340. [CrossRef]
13. Zhang, Y.; Yang, Q.; Ling, J.; Long, L.; Huang, H.; Yin, J.; Wu, M.; Tang, X.; Lin, X.; Zhang, Y.; et al. Shifting the microbiome of a coral holobiont and improving host physiology by inoculation with a potentially beneficial bacterial consortium. *BMC Microbiol.* **2021**, *21*, 130.

14. Carlos, C.; Torres, T.T.; Ottoboni, L. Bacterial communities and species-specific associations with the mucus of Brazilian coral species. *Sci. Rep.* **2013**, *3*, 1624. [CrossRef] [PubMed]
15. Hernández-Zulueta, J.; Araya, R.; Vargas-Ponce, O.; Díaz-Pérez, L.; Rodríguez-Troncoso, A.P.; Ceh, J.; Ríos-Jara, E.; Rodríguez-Zaragoza, F.A. First deep screening of bacterial assemblages associated with corals of the Tropical Eastern Pacific. *FEMS Microbiol. Ecol.* **2016**, *92*, fiw196. [CrossRef] [PubMed]
16. Morrow, K.M.; Muller, E.; Lesser, M.P. How Does the Coral Microbiome Cause, Respond to, or Modulate the Bleaching Process? In *Coral Bleaching. Ecological Studies*, 1st ed.; van Oppen, M., Lough, J., Eds.; Springer: Berlin/Heidelberg, Germany, 2018; Volume 233, pp. 153–188.
17. van Oppen, M.J.H.; Blackall, L.L. Coral microbiome dynamics, functions and design in a changing world. *Nat. Rev. Microbiol.* **2019**, *17*, 557–567. [CrossRef]
18. Voolstra, C.R.; Quigley, K.M.; Davies, S.W.; Parkinson, J.E.; Peixoto, R.S.; Aranda, M.; Baker, A.C.; Barno, A.R.; Barshis, D.J.; Benzoni, F.; et al. Consensus Guidelines for Advancing Coral Holobiont Genome and Specimen Voucher Deposition. *Front. Mar. Sci.* **2021**, *8*, 701784. [CrossRef]
19. Ainsworth, T.D.; Krause, L.; Bridge, T.; Torda, G.; Raina, J.B.; Zakrzewski, M.; Gates, R.D.; Padilla-Gamiño, J.L.; Spalding, H.L.; Smith, C.; et al. The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. *ISME J.* **2015**, *9*, 2261–2274. [CrossRef]
20. Hernandez-Agreda, A.; Gates, R.D.; Ainsworth, T.D. Defining the core microbiome in corals' microbial soup. *Trends Microbiol.* **2017**, *25*, 125–140. [CrossRef] [PubMed]
21. Hernandez-Agreda, A.; Leggat, W.; Bongaerts, P.; Herrera, C.; Ainsworth, T.D. Rethinking the coral microbiome: Simplicity exists within a diverse microbial biosphere. *mBio* **2018**, *9*, e00812-18. [CrossRef] [PubMed]
22. Ostria-Hernández, M.; Hernández-Zulueta, J.; Vargas-Ponce, O.; Díaz-Pérez, L.; Araya, R.; Rodríguez-Troncoso, A.P.; Ríos-Jara, E.; Rodríguez-Zaragoza, F.A. Core microbiome of corals *Pocillopora damicornis* and *Pocillopora verrucosa* in the northeastern tropical Pacific. *Mar. Ecol.* **2022**, *43*, e12729. [CrossRef]
23. Hernández-Zulueta, J.; Díaz-Pérez, L.; García-Maldonado, J.Q.; Nava-Martínez, G.G.; García-Salgado, M.A.; Rodríguez-Zaragoza, F.A. Bacterial assemblages associated with *Acropora palmata* affected by white band disease in the Mexican region of the Caribbean and Gulf of Mexico. *J. Sea Res.* **2022**, *185*, 102230. [CrossRef]
24. McKew, B.A.; Dumbrell, A.J.; Daud, S.D.; Hepburn, L.; Thorpe, E.; Mogensen, L.; Whitby, C. Characterization of geographically distinct bacterial communities associated with coral mucus produced by *Acropora* spp. and *Porites* spp. *Appl. Environ. Microbiol.* **2012**, *78*, 5229–5237. [CrossRef] [PubMed]
25. Closek, C.; Sunagawa, S.; DeSalvo, M.K.; Piceno, Y.M.; DeSantis, T.Z.; Brodie, E.L.; Weber, M.X.; Voolstra, C.R.; Andersen, G.L.; Medina, M. Coral transcriptome and bacterial community profiles reveal distinct Yellow Band Disease states in *Orbicella faveolata*. *ISME J.* **2014**, *8*, 2411–2422. [CrossRef] [PubMed]
26. Barranco, L.M.; Carriquiry, J.D.; Rodríguez-Zaragoza, F.A.; Cupul-Magaña, A.L.; Villaescusa, J.A.; Calderón -Aguilera, L.E. Spatiotemporal variations of live coral cover in the northern Mesoamerican Reef System, Yucatan Peninsula, Mexico. *Sci. Mar.* **2016**, *80*, 143–150.
27. Loreto-Viruel, R.M.; García-Beltrán, G.; Bezaury-Creel, J. Caracterización de los Arrecifes Coralinos de Isla Cozumel, Quintana Roo, México. *Amigos De Sian Ka'an Ser. Doc.* **2017**, *7*, 11–40.
28. Rioja-Nieto, R.; Álvarez-Filip, L. Coral reef systems of the Mexican Caribbean: Status, recent trends and conservation. *Mar. Pollut. Bull.* **2018**, *140*, 616–625. [CrossRef] [PubMed]
29. Rodríguez-Zaragoza, F.A.; Arias-González, J.E. Coral biodiversity and bio-construction in the northern sector of the mesoamerican reef system. *Front. Mar. Sci.* **2015**, *2*, 13. [CrossRef]
30. Caballero-Aragón, H.; Perera-Valderrama, S.; Cerdeira-Estrada, S.; Martell-Dubois, R.; Rosique-de la Cruz, L.; Álvarez-Filip, L.; Pérez-Cervantes, E.; Estrada-Saldívar, N.; Ressl, R. Puerto Morelos Coral Reefs, Their Current State and Classification by a Scoring System. *Diversity* **2020**, *12*, 272. [CrossRef]
31. Blanchon, P.; Richards, S.; Bernal, J.P.; Cerdeira-Estrada, S.; Ibarra, M.S.; Corona-Martínez, L.; Martell-Dubois, R. Retrograde Accretion of a Caribbean Fringing Reef Controlled by Hurricanes and Sea-level Rise. *Front. Earth Sci.* **2017**, *5*, 78. [CrossRef]
32. Bourne, D.G.; Munn, C.B. Diversity of bacteria associated with the coral *Pocillopora damicornis* from the Great Barrier Reef. *Environ. Microbiol.* **2005**, *7*, 1162–1174. [CrossRef]
33. Caporaso, J.; Lauber, C.; Walters, W.; Berg-Lyons, D.; Huntley, J.; Fierer, N.; Owens, S.; Betley, J.; Fraser, L.; Bauer, M.; et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* **2012**, *6*, 1621–1624. [CrossRef]
34. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [CrossRef]
35. Callahan, B.J.; Mcmurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.; Holmes, S.P. $DADA_2$: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [CrossRef]
36. Bokulich, N.A.; Kaehler, B.D.; Rideout, J.R.; Dillon, M.; Bolyen, E.; Knight, R.; Huttley, G.A.; Gregory Caporaso, J. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* **2018**, *6*, 90. [CrossRef] [PubMed]

37. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **2013**, *41*, D590–D596. [CrossRef] [PubMed]
38. Anderson, M.J.; Gorley, R.N.; Clarke, K.R. *Permanova for Primer Guide to Software and Statistical Methods*; PRIMER-E: Plymouth, UK, 2008; p. 214.
39. Clarke, K.R.; Gorley, R.N. *Primer v7: User Manual/Tutorial*; Primer-E Ltd.: Plymouth, UK, 2015; p. 192.
40. Louca, S.; Parfrey, L.W.; Doebeli, M. Decoupling function and taxonomy in the global ocean microbiome. *Science* **2016**, *353*, 1272–1277. [CrossRef]
41. Sweet, M.J.; Croquer, A.; Bythell, J.C. Bacterial assemblages differ between compartments within the coral holobiont. *Coral Reefs* **2011**, *30*, 39–52. [CrossRef]
42. Wild, C.; Huettel, M.; Klueter, A.; Kremb, S.G.; Rasheed, M.Y.; Jørgensen, B.B. Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. *Nature* **2004**, *428*, 66–70. [CrossRef]
43. Beltrán, Y.; Cerqueda-García, D.; Taş, N.; Thomé, P.E.; Iglesias-Prieto, R.; Falcón, L.I. Microbial composition of biofilms associated with lithifying rubble of *Acropora palmata* branches. *FEMS Microbiol. Ecol.* **2016**, *92*, fiv162. [CrossRef] [PubMed]
44. Sánchez-Quinto, A.; Falcón, L.I. Metagenome of *Acropora palmata* coral rubble: Potential metabolic pathways and diversity in the reef ecosystem. *PLoS ONE* **2019**, *14*, e0220117. [CrossRef]
45. de Voogd, N.J.; Cleary, D.F.; Polónia, A.R.; Gomes, N.C. Bacterial community composition and predicted functional ecology of sponges, sediment and seawater from the thousand islands reef complex, West Java, Indonesia. *FEMS Microbiol. Ecol.* **2015**, *91*, fiv019. [CrossRef] [PubMed]
46. Zhang, Y.Y.; Ling, J.; Yang, Q.S.; Wang, Y.S.; Sun, C.C.; Sun, H.Y.; Feng, J.B.; Jiang, Y.F.; Zhang, Y.Z.; Wu, M.L.; et al. The diversity of coral associated bacteria and the environmental factors affect their community variation. *Ecotoxicology* **2015**, *24*, 1467–1477. [CrossRef] [PubMed]
47. Cleary, D.F.R.; Polónia, A.R.M.; Becking, L.E.; de Voogd, N.J.; Purwanto; Gomes, H.; Gomes, N.C.M. Compositional analysis of bacterial communities in seawater, sediment, and sponges in the Misool coral reef system, Indones. *Mar. Biodivers.* **2018**, *48*, 1889–1901. [CrossRef]
48. Huggett, M.J.; Apprill, A. Coral microbiome database: Integration of sequences reveals high diversity and relatedness of coral-associated microbes. *Environ. Microbiol. Rep.* **2019**, *11*, 372–385. [CrossRef] [PubMed]
49. Luo, D.; Wang, X.; Feng, X.; Tian, M.; Wang, S.; Tang, S.L.; Ang, P., Jr.; Yan, A.; Luo, H. Population differentiation of Rhodobacteraceae along with coral compartments. *ISME J.* **2021**, *15*, 3286–3302. [CrossRef]
50. Roder, C.; Arif, C.; Daniels, C.; Weil, E.; Voolstra, C.R. Bacterial profiling of White Plague Disease across corals and oceans indicates a conserved and distinct disease microbiome. *Mol. Ecol.* **2014**, *23*, 965–974. [CrossRef]
51. Eaton, K.R.; Landsberg, J.H.; Kiryu, Y.; Peters, E.C.; Muller, E.M. Measuring Stony Coral Tissue Loss Disease induction and lesion progression within two intermediately susceptible species, *Montastraea cavernosa* and *Orbicella faveolata*. *Front. Mar. Sci.* **2021**, *8*, 717265. [CrossRef]
52. Alvarez-Filip, L.; González-Barrios, F.J.; Pérez-Cervantes, E.; Molina-Hernández, A.; Estrada-Saldívar, N. Stony coral tissue loss disease decimated Caribbean coral populations and reshaped reef functionality. *Commun. Biol.* **2022**, *5*, 440. [CrossRef]
53. Pootakham, W.; Mhuantong, W.; Yoocha, T.; Putchim, L.; Jomchai, N.; Sonthirod, C.; Naktang, C.; Kongkachana, W.; Tangphatsornruang, S. Heat-induced shift in coral microbiome reveals several members of the Rhodobacteraceae family as indicator species for thermal stress in *Porites lutea*. *Microbiologyopen* **2019**, *8*, e935. [CrossRef]
54. Alves, L.M.; Marcondes de Souza, J.A.; Varani, A.M.; de Macedo Lemos, E.G. The Family Rhizobiaceae. In *The Prokaryotes: Alphaproteobacteria and Betaproteobacteria*, 4th ed.; Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F., Eds.; Springer: Berlin/Heidelberg, Germany, 2014; pp. 419–437.
55. Rosales, S.M.; Huebner, L.K.; Clark, A.S.; McMinds, R.; Ruzicka, R.R.; Muller, E.M. Bacterial Metabolic Potential and Micro-Eukaryotes Enriched in Stony Coral Tissue Loss Disease Lesions. *Front. Mar. Sci.* **2022**, *8*, 776859. [CrossRef]
56. Frade, P.R.; Glasl, B.; Matthews, S.A.; Mellin, C.; Serrão, E.A.; Wolfe, K.; Mumby, P.J.; Webster, N.S.; Bourne, D.G. Spatial patterns of microbial communities across surface waters of the Great Barrier Reef. *Commun. Biol.* **2020**, *3*, 442. [CrossRef] [PubMed]
57. Glasl, B.; Robbins, S.; Frade, P.R.; Marangon, E.; Laffy, P.W.; Bourne, D.G.; Webster, N.S. Comparative genome-centric analysis reveals seasonal variation in the function of coral reef microbiomes. *ISME J.* **2020**, *14*, 1435–1450. [CrossRef] [PubMed]
58. Apprill, A.; Weber, L.; Santoro, A. Distinguishing between microbial habitats unravels ecological complexity in coral microbiomes. *mSystems* **2016**, *1*, e00143-16. [CrossRef]
59. Albataineh, H.; Stevens, D.C. Marine Myxobacteria: A few good halophiles. *Mar. Drugs* **2018**, *16*, 209. [CrossRef]
60. Baker, B.J.; Lazar, C.S.; Teske, A.P.; Dick, G.J. Genomic resolution of linkages in carbon, nitrogen, and sulfur cycling among widespread estuary sediment bacteria. *Microbiome* **2015**, *3*, 14. [CrossRef]
61. Lilburn, T.G.; Kim, K.S.; Ostrom, N.E.; Byzek, K.R.; Leadbetter, J.R.; Breznak, J.A. Nitrogen fixation by symbiotic and free-living spirochetes. *Sci. Rep.* **2015**, *292*, 2495–2498. [CrossRef]
62. Latysheva, N.; Junker, V.L.; Palmer, W.J.; Codd, G.A.; Barker, D. The evolution of nitrogen fixation in cyanobacteria. *Bioinformatics* **2012**, *28*, 603–606. [CrossRef]
63. Fuerst, J.; Sagulenko, E. Beyond the bacterium: Planctomycetes challenge our concepts of microbial structure and function. *Nat. Rev. Microbiol.* **2011**, *9*, 403–413. [CrossRef]

64. Marshall, A.J.; Longmore, A.; Phillips, L.; Tang, C.; Hayden, H.L.; Heidelberg, K.B.; Mele, P. Nitrogen cycling in coastal sediment microbial communities with seasonally variable benthic nutrient fluxes. *Aquat. Microb. Ecol.* **2021**, *86*, 1–19. [CrossRef]
65. Rivett, D.W.; Bell, T. Abundance determines the functional role of bacterial phylotypes in complex communities. *Nat. Microbiol.* **2018**, *3*, 767–772. [CrossRef]
66. Guibert, I.; Bourdreux, F.; Bonnard, I.; Pochon, X.; Dubousquet, V.; Raharivelomanana, P.; Berteaux-Lecellier, V.; Lecellier, G. Dimethylsulfoniopropionate concentration in coral reef invertebrates varies according to species assemblages. *Sci. Rep.* **2020**, *10*, 9922. [CrossRef] [PubMed]
67. Lima, L.F.O.; Alker, A.T.; Papudeshi, B.; Morris, M.M.; Edwards, R.A.; de Putron, S.J.; Dinsdale, E.A. Coral and seawater metagenomes reveal key microbial functions to coral health and ecosystem functioning shaped at reef scale. *Microb. Ecol.* **2023**, *86*, 392–407. [CrossRef] [PubMed]
68. Guo, A.; Li, J.; Wang, L.; Ju, H.; Li, Q.; Ren, L.; Zhang, S. Temperature affects antagonism among coral-associated bacteria. *Front. Mar. Sci.* **2022**, *9*, 840384. [CrossRef]
69. Van Woesik, R.; McCaffrey, K.R. Repeated thermal stress, shading, and directional selection in the Florida reef tract. *Front. Mar. Sci.* **2017**, *4*, 182. [CrossRef]
70. Lee, O.O.; Yang, J.; Bougouffa, S.; Wang, Y.; Batang, Z.; Tian, R.; Al-Suwailem, A.; Qian, P.Y. Spatial and species variations in bacterial communities associated with corals from the Red Sea as revealed by pyrosequencing. *Appl. Environ. Microbiol.* **2012**, *78*, 7173–7184. [CrossRef]
71. Jessen, C.; Villa Lizcano, J.F.; Bayer, T.; Roder, C.; Aranda, M.; Wild, C.; Voolstra, C.R. In-situ effects of eutrophication and overfishing on physiology and bacterial diversity of the Red Sea coral *Acropora hemprichii*. *PLoS ONE* **2013**, *8*, e62091. [CrossRef]
72. Rusch, A.; Gaidos, E. Nitrogen-cycling bacteria and archaea in the carbonate sediment of a coral reef. *Geobiology* **2013**, *11*, 472–484. [CrossRef]
73. Thome, P.E.; Rivera-Ortega, J.; Rodríguez-Villalobos, J.C.; Cerqueda-García, D.; Guzmán-Urieta, E.O.; García-Maldonado, J.Q.; Carabantes, N.; Jordán-Dahlgren, E. Local dynamics of a white syndrome outbreak and changes in the microbial community associated with colonies of the scleractinian brain coral *Pseudodiploria strigosa*. *PeerJ* **2021**, *9*, e10695. [CrossRef]