Exposure at low tide leads to different microbial abundance of intertidal coral *Acropora pulchra*

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Abstract

Corals thrive in symbiotic relationships with a variety of microorganisms, including endosymbiont algae. The interaction between coral and microbial associations has been extensively researched since it is thought to play a function in coral health. Temperature and light are two abiotic elements that contribute to coral life. Corals in reef flat environments frequently face variations in these two characteristics due to their proximity to shallow seas. This study aims to compare the microbial diversity and abundance associated with the coral *Acropora pulchra* on the reef flat under two conditions, namely corals that emerged to the surface at low tide (SF) and corals that submerged over time (SM) and to compare the microbial diversity of both with those found in its adjacent seawaters. Microbial analysis on 16S rRNA region V4 showed that the alpha diversity of coral microbial communities and seawaters was not significantly different. However, differences in abundance were noticed at the phylum and genus levels. With p-value < 0.05, PCoA analysis using the Bray-Curtis test showed that the coral microbial community was significantly different from the surrounding seawaters. This study indicates that, under different conditions, corals of the same species can be dominated by different microbial groups and confirms the uniqueness between coral microbes and its adjacent seawaters. The abundance of certain microbes is a host mechanism for survival.

Introduction

Coral reefs support the highest biodiversity in the oceans compared to other marine environments, and they are frequently referred to as oceanic tropical rainforests. Coral reef diversity can account for up to 5% of all known species on the planet and up to 35% of all known marine species[1]. Coral reefs offer ecosystem goods and services such as vital habitats for numerous species, fisheries services, coastal protection regulatory services, and cultural services such as tourism [2]. Meanwhile, coral reef ecosystems are under increasing threat of degradation and extinction as a result of bleaching and coral disease, as well as local environmental pressures such as water pollution, overfishing, and predation, as well as global pressures linked to rising seawater temperatures and acidification [3], [4].

Scleractinian hard corals are one of the active constructors of varied coral reef ecosystems [5]. The intimate connections between coral organisms called polyps and endosymbiont algae of the species *Symbiodinium*, usually referred to as zooxanthellae, resulted in the formation of this vast and complex reef structure. These corals live in symbiotic relationships with a variety of microbes, including algae, bacteria, archaea, fungi, protists, and viruses, among others [6]. The holobiont is a group of coral creatures and other microbes that dwell in close proximity to one another [7]. The symbiosis between the coral host and all of its microorganisms is so sophisticated that it is even thought of as a co-evolved microbiota [8].

The biological interactions between zooxanthellae-corals and their microbial communities have been extensively investigated and comprehended [9]–[11]. The coral holobiont's nourishment is met through a symbiotic relationship between coral microbes and algae *Symbiodinium* sp. The diazotrophic bacteria,

for instance, feed nitrogen to *Symbiodinium*, and the two have a nutrient-dependent interaction, whereas *Symbiodinium* releases carbon as a source of energy for coral hosts and their bacterial endosymbionts [12]. Microbial communities have a crucial role in coral health, as they participate in a variety of physiological processes, including nutrient cycling, pathogen defence and detoxification, and climate change adaptation [4], [13]. The stability and functional importance of microbial-coral relationships, on the other hand, remains largely unclear for other members of the microbial community [14].

This study investigated the bacterial community associated with coral *Acropora pulchra* on the reef flat. Corals that dominate the upper layers of the oceans are often exposed to fluctuating environmental conditions such as light intensity, temperature, pH, and nutrient availability. Due to these difficult living conditions, corals may be under pressure since elevate temperature couple with light exposure cause bleaching and even death of corals [15]. However, corals capable of surviving these conditions may experience unique microbial community changes as an important mechanism for physiological adaptation [16]. Furthermore, we also evaluated the differences in the microbial community of coral association with the microbial community in the seawaters around the reef flat. Due to currents and tides in the reef flat area, the planktonic microbial community alternates more often than that of coral endosymbiotic microbial community. This study provides a comparison of coral-associated microbial communities between corals that were exposed to air at low tide and coral that submerged over time and comparisons of both with microbial communities of the surrounding seawater.

Methods

Sample collection and physical measurement

The coral growth type of Acropora branching used in this study is *Acropora pulcra*, which is easy to locate in the structure of the reefs flat at the study site. The coral fragments were obtained on September 2020 from a reef flat on Madura's north coast (GPS coordinates: 6°53'04.7"S 113°05'18.7"E). The Hobo pendant MX2202 was fixed parallel to the SM coral site a week before sample collection. Water temperature and light intensity were measured at 10-minute intervals for a week, with a temperature precision of 0.5°C (Onset Computer Corporation, Canada).

Samples consisted of Submerged coral (SM), namely, coral tissue samples from *A. pulchra* that stayed below the superficies of seawater, Surface coral (SF), namely, samples from *A. pulchra* that emerged to the surface at low tide, and Seawater samples (SW) from surrounding coral reefs waters. An apparently healthy *A. pulchra* colony with a more than 10 cm diameter was selected as colony samples. The coral fragments were collected using sterilized forceps and taken from three colonies of *A. pulchra* for each condition according to the research purpose. Immediately, coral fragments were placed in a 30 ml dark bottle containing 20ml DESS (DMSO-Edta-salt solution). The bottles of coral samples were stored in a cooler box containing ice packs before being transported to local laboratory.

eDNA samples were obtained were taken from 1 liter of seawater near corals and vacuum-filtered via a cellulose membrane filter (pore size, 0.22 μm; diameter, 47 mm; MF millipore). The Millipore filter

membrane was stored in a 2 ml cryotube containing 1 ml of RNAlater preservation solution. All samples, including samples of corals and seawater microbes that have been collected, were transported to the Laboratory of Biodiversity and Marine Biosystematics, IPB University. Samples for molecular assay were stored on freezer at −20°C to maintain sample quality until extraction

DNA extraction

Prior to the DNA extraction, pre-treatment was carried out to obtain coral tissue samples. Modification of airbrushing using the water-pik method has been described by [17]. This method allows the coral tissue to entirely detach from the coral skeleton by spraying an amount of buffer from a pressurized air gun in a sturdy polyethylene bag [18]. The coral tissue was sprayed with filtered ice-cold 1X PBS to form a slurry in the bag then it was centrifuged to obtain a tissue pellet. The pellet was stored in a −20°C freezer before DNA extraction.

DNA from tissue samples was extracted with DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions with minor modification. Briefly, about 50 mg of the coral tissue were placed in 1.5 ml Eppendorf tubes. The amount of 180 ul buffer ATL and 20 ul Proteinase K buffer were added then were incubated at 56°C for 1 to 3 hours until the tissue loose. DNA extractions were then conducted according to the manufacturer's instructions. For seawater samples, membrane filters were thawed and placed in 1.5 ml Eppendorf tubes. The amount of 270 ul ATL buffer and 30 ul Proteinase K buffer was added, and the tube was incubated at 56°C for an hour. DNA extraction was then proceeded according to kit’s protocols. DNA was eluted in only 150 ul AE buffer to increase DNA quality in the last step.

The genomic quality of DNA was examined by electrophoresis in 1.0% agarose gel (110 V, for 20 min). The 16S rRNA region V4 sequence was amplified using universal bacterial primers 515F forward: (5'-GTG CCA GCM GCC GCG GTA A-3') and reverse 806Rb: (5'-GGA CTA CHV GGG TWT CTA AT-3') [3], [13]. All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). The PCR products were quantified and checked for quality by adding same mix volume of 1X loading buffer (contained SYB green) and electrophoresis in 2.0% agarose gel. Samples with the appearance of a bright band between 400–450 bp were chosen for further steps.

The PCR products that have been examined are collected with the same concentration. The mixture was further purified with the Qiagen Gel Extraction Kit (Qiagen, Germany). The libraries were processed with the NEBNext® UltraTM DNA Library Prep Kit for Illumina and quantified with a Qubit 2.0 Fluorometer (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA) and Q-PCR, then sequenced with the Illumina HiSeq2500 platform (San Diego, CA, USA).

Data analysis and bioinformatics

The temperature and light intensity recordings were processed in Excel, while the sequencing data were processed using the QIIME2 software [19]. QIIME2 software is used to combine, filter the quality of

readings, clean from noise and chimeras, namely "artifactual" amplicons (by-products) that are formed during the PCR process, clustered them into OTU (Operational Taxonomical Units) and classify readings from raw sequences in the file .fastq format from the NGS machine. The determination of OTU was carried out at the similarity level of 97% (species level) and the assignment of OTU using SILVA v138 reference data [20] with a confidence level of 0.8~1. Software R version 4.0.5 was used to calculate the alpha diversity index including the *Chao1* and ACE indices to estimate the total OTU in each sample (richness), the Shannon-Wiener index (*H'*) for species diversity analysis, and the Simpson index (*D*) was used to determine species dominance. R software is also used to visualize microbial composition using the ggplot2 package [21], create a cluster heatmap using the pheatmap version 1.0.12 package [22], create a proportional Venn diagram with the Venneuler package [23] and plot Principal Coordinate of Analysis (PCoA) using the phyloseq package.

For statistical analysis, Wilcoxon's test was used and observed at p-value<0.05 or 5% significance level to compare in pairs the diversity in each group of microbial samples. PCoA plots were made based on the Bray-Curtis distance using the PERMANOVA test to describe the similarity in the microbial community structure of each sample group.

Result

Seawater temperature and light intensity measurements

The seawater temperature warmed up during midday with a difference of ~2°C and cooled down with a difference of ~1.5°C at night. The highest temperature recorded was 33.64°C during the heating time during the day (13:50), and the lowest was 26.16° C at night (19:50), with an overall fluctuation of ~7.5°C. The largest daily temperature fluctuations occurred at low tide on September 14, 2020 with a range of ~6.6°C (33.64°C–27.01°C), while the smallest daily fluctuations occurred during the first day of high tide on September 10, 2020 with range of 4.14°C (31.60°C–27.47°C).

The light intensity can describe how much sunlight penetrates the water column. During the recording period, which took place in the summer, the light intensity was high because the sky was generally clear with relatively shallow waters, besides the low tide occurred from the afternoon until the evening. Coral reefs can be exposed for about four hours when the deepest low tide occurs. The highest light intensity occurred on September 11, 2020 at 13:40, reaching 22,451 Lux while the lowest was recorded on September 16, 2020 at 15:20 at 7,577 Lux. The light intensity on the first four days of recording showed a high level, but it decreased in intensity on the next three days. During the first few days of recording, exposure only occurred at sunset, so the emerged corals were not exposed to solar radiation. The water receded earlier in the next few days; thus, the corals were exposed to afternoon solar radiation.

Alpha and beta diversity of microbial community

Microbes from the 16S rRNA region hypervariable V4 gene obtained from coral tissue samples of *A. pulchra* and the seawaters around the coral were sequenced and have been successfully represented

into 9,309 OTUs from a total of 1,240,011 reads after going through the quality filtering step, and OTU clustering at a similarity level of 97%. The length reads of the sequence ranged from 226–253 bp. The number of OTUs found in the seawater samples was more (7,746 OTUs) than the OTUs found in the coral samples of *A. pulchra* (7,331 OTUs). The Wilcoxon significance test showed $p>0.05$, which means there is no significant difference between each sample group.

Median calculation of the estimated *Chao1* richness in the SW, SM and SF sample groups, respectively, was 6,151; 4,446; and 4,933; while the ACE median estimation were 6,180; 4,516; and 5.025; respectively. Both estimates show that the higher the value, the higher the species richness. The Shannon index in both groups of coral samples had similar values (SM 3.17; SF 3.05) but differed from SW (6.82). Simpson's diversity index shows the same thing, both groups of coral samples have a similar value (0.69) while SW has a higher value (0.99).

The results of the determination of the OTU taxonomy based on SILVA reference data obtained 44 phyla, three of which came from the Archaea group. Proteobacteria was the dominant phylum (0.45–0.33) in both coral samples and seawater samples. The dominance was represented by the Alphaproteobacteria, Deltaproteobacteria and Gammaproteobacteria classes, which were found to be more abundant in the coral microbial community (Gammaproteobacteria: 0.37–0.33) than seawater (Gammaproteobacteria: 0.17). Phylum Thaumarchaeota is an Archaea found abundantly in seawater samples but not in coral samples. Class members of this phylum in the sample are only found from the class Nitrososphaeria. Another phylum also abundant in seawater samples and classified as Archaea is Euryarchaeota, obtained from 27 OTUs. The predominance of the phylum Acidobacteria is represented by the classes Acidobacteriia and Holophagae. Presented in Figure 3, the microbial composition of each sample group shows that the seawater community is more evenly distributed than the coral sample group.

The venn diagram shows the distribution of OTUs in each sample group. Both SM and SF coral samples shared 501 OTUs (5.3%), had 417 OTUs and 645 unique OTUs, respectively. There are 1,336 OTUs (14.3%) which are both owned by Surface samples and Seawater samples, while the same OTUs owned by Submerged samples and Seawater samples are only 649 (6.9%). The number of OTUs found not only in coral samples but also in seawater was 3,783. Meanwhile, the OTUs that were only found in seawater samples were 1,978. The total detected OTUs in Seawater, Surface, and Submerged Water samples were 7,746; 6,265; and 5,350; respectively. Seawater samples have the microbial community with the highest number of unique OTUs (Fig. 2).

The PCoA plot shows the clustering pattern between the microbial communities found in the sample group with the percentage of variation on axis 1 of 57.9% and axis 2 of 25.4%. The submerged and surface coral sample groups are in close distribution areas (clustered), but both are separated from the seawater sample group. Based on permutational multivariate analysis of variance (PERMANOVA), the microbial community between coral samples and the surrounding seawaters was significantly different with a p-value < 0.05 (Fig. 4).

The most abundant genus of microbes

The heatmap clusters of the 40 most abundant genera in all samples are presented in Fig 5. The heatmap depicts the genera in the coral samples forming a separate cluster with the genus belonging to the seawater sample. The displayed genus comes from the eight most abundant phyla, namely Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Nitrospirae, Proteobacteria, Thaumarchaeota, and Verrucomicrobia. The dendogram on the left shows the genus in each sample grouped by abundance with values shown on the colour gradient according to the label. Columns are grouped by Bray-Curtis distance, while rows are grouped by Euclidean distance from the abundance profile of each genus.

The genera clustered in the coral samples mostly came from the phylum Proteobacteria, while the genera from the SW were more diverse from the phyla Proteobacteria, Actinobacteria and Acidobacteria. The genus *Methyloversatilis* is abundant in SM samples with only one representative OTU. The genus *Endozoicomonas* (7 OTUs in the dataset) and *Ralstonia* (2 OTUs in the dataset) were dominant in the SF samples. The most abundant genera in SW were *Acidibacter, Gaiella, Streptomyces, and Sphingomonas*, which are Proteobacteria and Actinobacteria phyla members. The genera *Cupriavidus, Acinebacteria, Mesorhizobium* and *Caulobacter*, which were found to be abundant in the coral sample group, were found to be small in seawater samples, while the microbial group from *unidentified_Cyanobacteria* was abundant in the SW and on SM only. OTU_7639 is listed in the genus-level on cluster heatmap since even if only one representative OTU, it has a striking proportion of both coral samples group with a percentage of more than 40%.

Discussion

The different dominance microbial groups between seawater and coral

The results of paired Wilcoxon test showed that seawater samples' diversity was not significantly different ($p>0.05$) with the two coral samples carried out in pairs. Another study also showed similar results that the bacterial community associated with corals *Acropora millepora*, *Galaxea fascicularis* and *Porites lutea* was not significantly different from the bacterial community in the surrounding waters despite the high community diversity [24]. This is presumably because there are many minor OTUs, only found in one sample with a small number of readings.

The results of the taxonomic assignment to the SILVA reference showed, from all samples, the predominant microbial class in samples from the phylum Proteobacteria was Alphaproteobacteria, Deltaproteobacteria and Gammaproteobacteria. All three are considered to be the major bacterial classes of the dominant coral microbial resident community group across coral species, geographic areas and at various depths [14]. In contrast to the aquatic microbial community, the Cyanobacteria phylum was found to be more abundant in seawater samples than the two groups of coral samples. This phylum has the highest diversity in shallow water where they can form stretches in the intertidal and infralittoral zones [25]. As aquatic bacteria, the abundance of cyanobacteria found on corals can cause black band disease (BBD) due to the formation of a layer covering the coral skeleton [26]. Furthermore, phylum

Thaumarchaeota and Euryarchaeota which are Archaea were also found abundantly in seawater samples but not in coral samples. The diversity of seawater microbes is higher than the microbes that live in corals tissue, as indicated by the Shannon index of 6.82 and the number of OTUs owned by 7,746. The aquatic environment is more volatile due to the influence of tides and changes in physical and chemical properties that encourage microbial diversity.

The predominance of the phylum Acidobacteria is represented by the classes Acidobacteriia and Holophagae, which are well annotated at the class level, but most of the sequences from this phylum are annotated in unidentified or unclassified Acidobacteria. At least 2% reads of seawater samples have not been annotated at a level below kingdom, while on coral samples has not about 43% to do so. The large percentages aforementioned are owned by 1 OTU (OTU 7639), which is only annotated on Kingdom Bacteria which indicates that the base sequence of this bacterium is unique or has not been cultured. Taxonomic identification in microbial metabarcoding studies generally facilitated by using reference databases such as Silva [20] or Greengenes [27]. However, these references include sequences from biomedical-based studies and are often difficult to use to identify sequences derived from marine biota [28]. Database enrichment, especially related to coral microbes, needs to be improved considering that this research topic is still in great demand. On the other hand, considering that the primers used targeting the V4 region of the 16S rRNA gene, where this region is the lowest mutation rate or most conservative, the sequences obtained will have poor resolution in identification at lower taxa levels (below of class). On the other hand, regions V2 and V8 are the areas that mutate the fastest so that they are able to distinguish genera in one family or even species in one genus [29]. Metabarcoding analysis using the V2 or V8 regions of the 16S rRNA gene will provide an overview of community diversity in lower taxa that is useful for further studies.

The microbial diversity of the seawater samples compared to the coral samples depicted in the PCoA ordinate plot shows the two samples representing seawater apart from the coral samples. In contrast, coral microbial diversity did not differ between SM and SF because it was scattered in clusters between coral samples. These results were corroborated by the PERMANOVA test which was carried out between types of samples with a value of $p<0.05$. The diversity of microbial species seems to be relatively the same within one species and is more influenced by other factors such as differences in season, location, type and environmental stress [30], regarding different species, time and location and related to different environmental pressures [3].

Microbial communities at the genus level, namely *Cupriavidus*, *Acinetobacter*, *Mesorhizobium* and *Caulobacter* were found to be abundant in coral samples but few in aquatic samples (Figure 5). This indicates that this microbial group is generally associated with coral *A. pulchra*. The abundant genus in seawater samples is associated with roles related to natural biochemical processes such as Acidibacter as a sulfur oxidizing agent and Fe(III) iron [31], Gaiella from the only strain studied that plays a role in the assimilation of carbohydrates, organic acids , and amino acids [32], and Streptomyces produce various bioactive compounds and enzymes so that they are potential sources of antibiotics [33].

The aquatic microbial community is more diverse, while the coral microbial community is dynamic due to different environmental factors and different habitats where the colony lives [16]. However, interactions between coral resident microbes and waters near corals can occur by influencing the selection of associated microbes on corals. At least 3,783 OTUs (Fig. 2) were found in coral and aquatic samples, indicating that microbial members of corals could be recruited in the environment. This group of microbes may be bacteria that facilitate the exchange of coral metabolites into the surrounding microbial food web so that biogeochemical cycles between corals and surrounding waters can occur [34].

Environmental factors affecting coral microbial abundance

The temperature records demonstrate a diurnal tidal type, which is defined by the presence of one high tide and one low tide in a half-day (12-hour) period. In the intertidal plains, a diurnal temperature change of roughly 2–4°C during a sunny day is considered usual [35]. As indicated in the study, environmental parameters in shallow water areas encounter fluctuations in temperature, oxygen concentration, pH, salinity, and nutrient content during the tidal period [36]. During the day, photosynthesis can raise the concentrations of oxygen and pH while decreasing the concentrations of nutrients. On the other hand, due to increased biota respiration in the waters at night, oxygen concentration and pH decreased. Of particular, the physical and chemical characteristics of the waters have such an impact on the presence of associated biota in the area.

The highest temperature recorded was 33.64°C during daytime heating, and the average heating in a week was 31.37°C. Even though the temperature at the study site was outside the optimum temperature for coral growth (27°C), the corals were still able to survive and adapt well. A study stated that an increase in sea surface temperature with a difference of 2°C from the average monthly temperature could significantly reduce corals' calcification process and linear elongation [37]. Another study showed an increase in temperature of 4°C with exposure to light can cause death and loss of color on the 22nd day of treatment [15]. With the increasing sea surface temperature, corals adapt to survive in conditions outside of their optimum temperature. Subsequent studies suggested that adaptation may occur in corals located in intertidal areas and exposed during tidal periods with temporary and sporadic changes in microbial communities [38].

When the water temperature warms up, coral will respire more actively and increase their metabolic rate such their production of waste (containing nitrogenous waste) also increases. The genera that were abundant in the SF were a group of bacteria that supported the enhancement of this function, for example *Endozoicomonas* plays a role in the carbohydrate cycle and provides protein for its host [39], [40], *Stenotrophomonas* plays a role in the sulfur cycle [41] along with *Herbaspirillum* both are nitrogen-fixing bacteria [42] and *Ralstonia* has denitrifying ability [43]. In addition, the effect of light on corals is associated with primary production that occurs during the day, promoting metabolism that affects pH and oxygen concentration in the water. Some coral microbes utilize light to regulate the production of metabolites that function specifically to combat opportunistic pathogens [44].

The genera *Methyloversatilis*, *Methylobacillus, Flavobacterium, Sphingopyxis, Novosphingobium,* and *Brevundimonas* were the most abundant in the SM. *Methyloversatilis* and *Methylobacillus* are a group of methylotrophic bacteria that have the ability to utilize single-carbon compounds (such as methane and methanol) as carbon and energy sources [45]. The abundance of methylotrophic bacteria in SM may be influenced by the availability of C_1 compounds in the water column and the ability of these bacteria to denitrify [46]. *Flavobacterium* includes opportunistic bacteria and often causes disease in fish. Although this bacterium is found everywhere, including in soil and aquatic habitats, it is sensitive to high temperatures and grows well in the temperature range of 30–35°C [47]. *Novosphingobium* and *Sphingopyxis* of the same family (Sphingomonadaceae) have been associated with their adaptability to nutrient deficient (oligotrophic) conditions and their role in the degradation of polyaromatic compounds [48]. From these characteristics, the abundance of the two genera can be an indication of polluted environmental conditions [49].

Microbial diversity in the two coral conditions in the study did not differ significantly, but differences in microbial types could be detected from the unique OTUs in both coral samples, where there were 645 OTUs in Surface samples and 417 OTUs in Submerged samples. Corals acquire their symbionts in two ways, namely horizontally, which means that new symbionts are taken from the environment by each generation of hosts, and vertically by passing through the female lineage [50]. The difference in species in the two samples could occur because the corals obtained their symbionts vertically and horizontally. Meanwhile, differences in the abundance of coral microbial communities can be influenced by environmental conditions (such as temperature, light and the presence of nutrients) and host preferences for survival in certain conditions. Therefore, the addition of an analysis of other environmental factors in future research will provide a more comprehensive understanding of the research results.

Conclusion

The alpha diversity of both coral samples and water samples was not significantly different and was dominated by Proteobacteria. However, the dominance on microbial community between coral and seawater was noticable different at the phylum level. Differences in dominance were also shown at the genus level, where corals exposed to air at low tide were dominated by members of the genera Endozoicomonas, Herbaspirillum, Stenotropomonas, Pseudomonas and Ralstonia, while corals that were always submerged in the water column were dominated by members of Methyloversatilis, Methylobacillus, Flavobacterium, Sphingopyxis, Novosphingobium, and Brevundimonas. The results of this study can provide an indication that differences in the abundance of coral microbial groups can be influenced by environmental conditions.

Declarations

Data Availability The datasets generated from this study are available from the corresponding author on a request.

Code Availability Not applicable.

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Author Contributions DNA, RR, NPZ and HM designed the study and revised the manuscript. DNA carried out the experiments and analyzed the data while RR supervised the experiments. All the authors contributed to the interpretation of results and made a signifcant contribution until approval of the final manusicript.

Ethics Declarations

Conflict of interest The authors declare no conflict of interest.

Consent for Publication Not applicable

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Tables

Tabel 1 Diversity estimation index of *Acropora pulchra* coral-association bacteria.

Sample Group	Indices*			
	Chao1	ACE	Shannon	Simpson
SW	6151.18	6180.14	6.82	0.99
SM	4446.02	4516.06	3.17	0.69
SF	4933.25	5025.46	3.05	0.69

* Diversity indice (*Chao1*, ACE, Shannon, and Simpson) values were presented in the median calculation

Figures

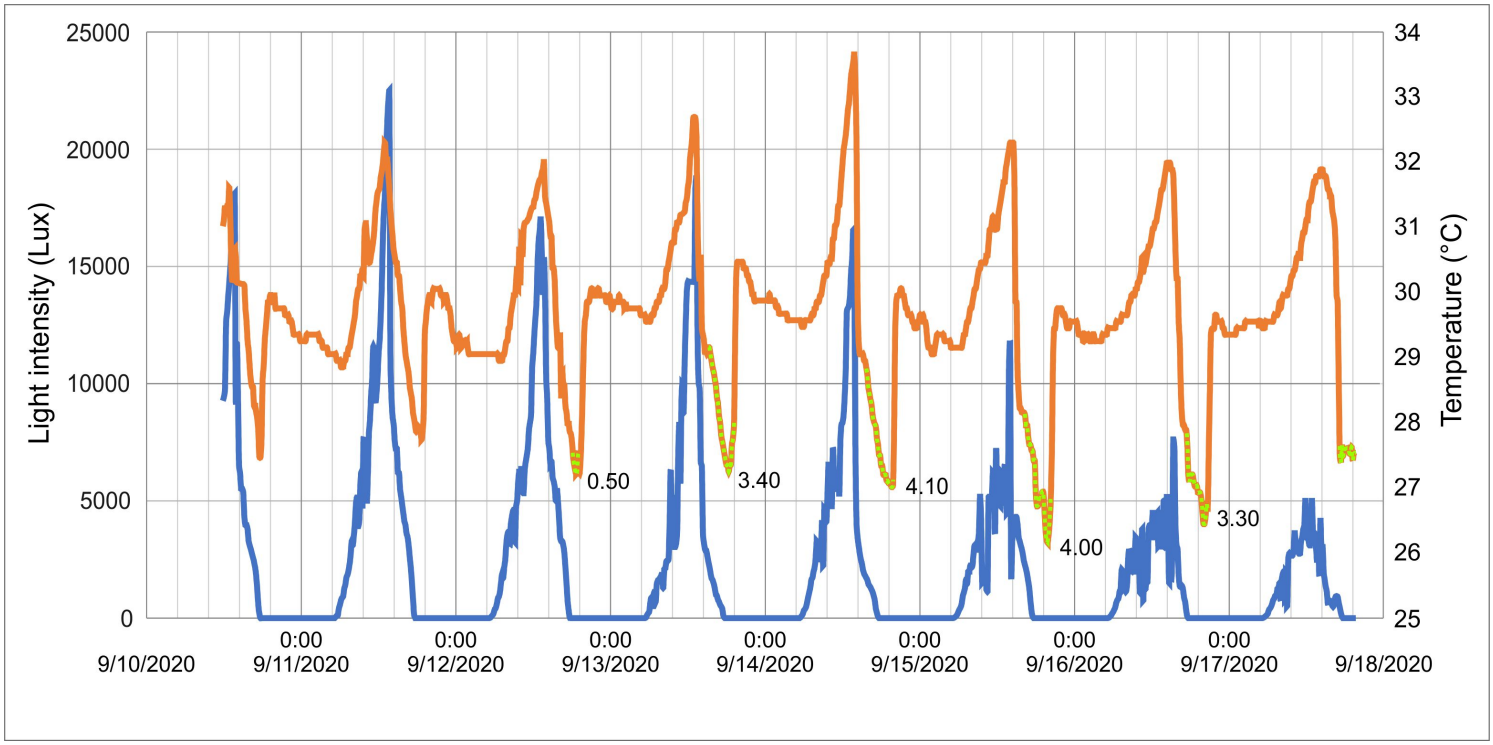

Figure 1

Temperature records (orange line) and light intensity records (blue line) from September 10 to September 17, 2020. The green dashed lines show the time from when the coral first appeared on the surface till they were re-submerged. The number represents the length of time that the low tide lasts (00.50 means 0 hours 50 minutes).

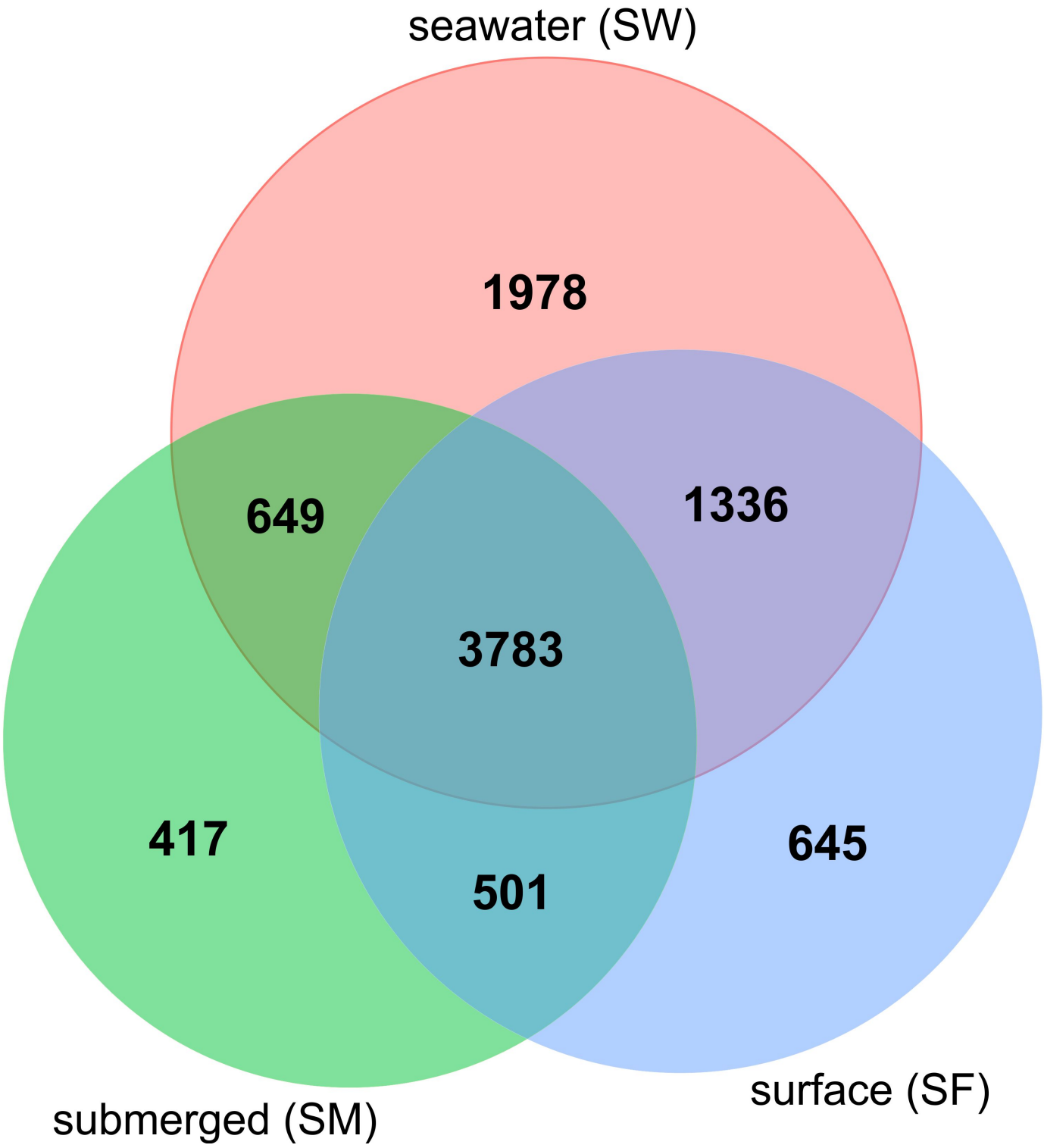

Figure 2

Venn diagram showing the number distribution of OTUs in each sample group between submerged (SM), surface (SF) and Seawater (SW).

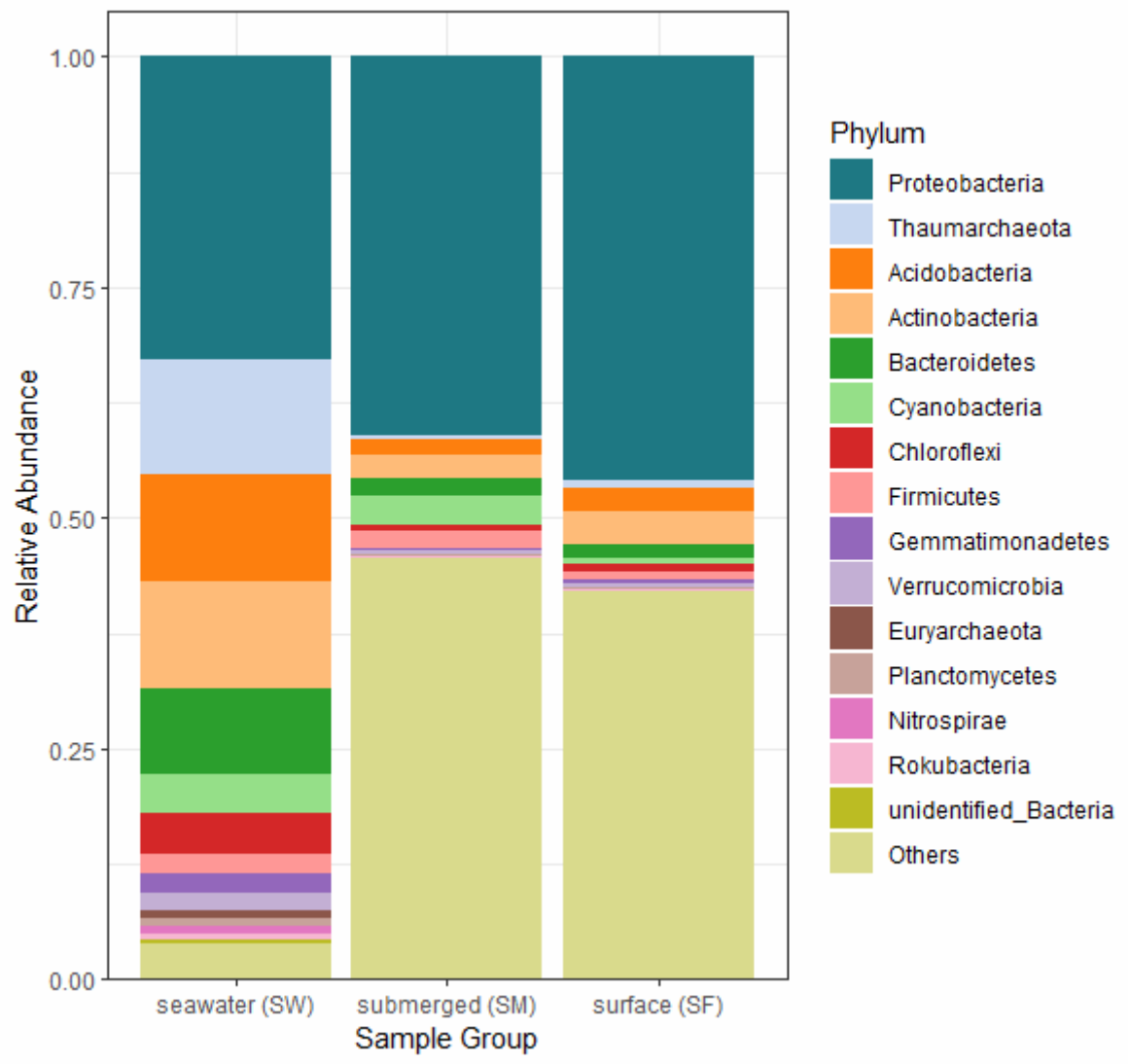

Figure 3

The relative abundance of microbial communities at phylum level of the *Acropora pulchra* association with a group sample from seawater, surface and submerged.

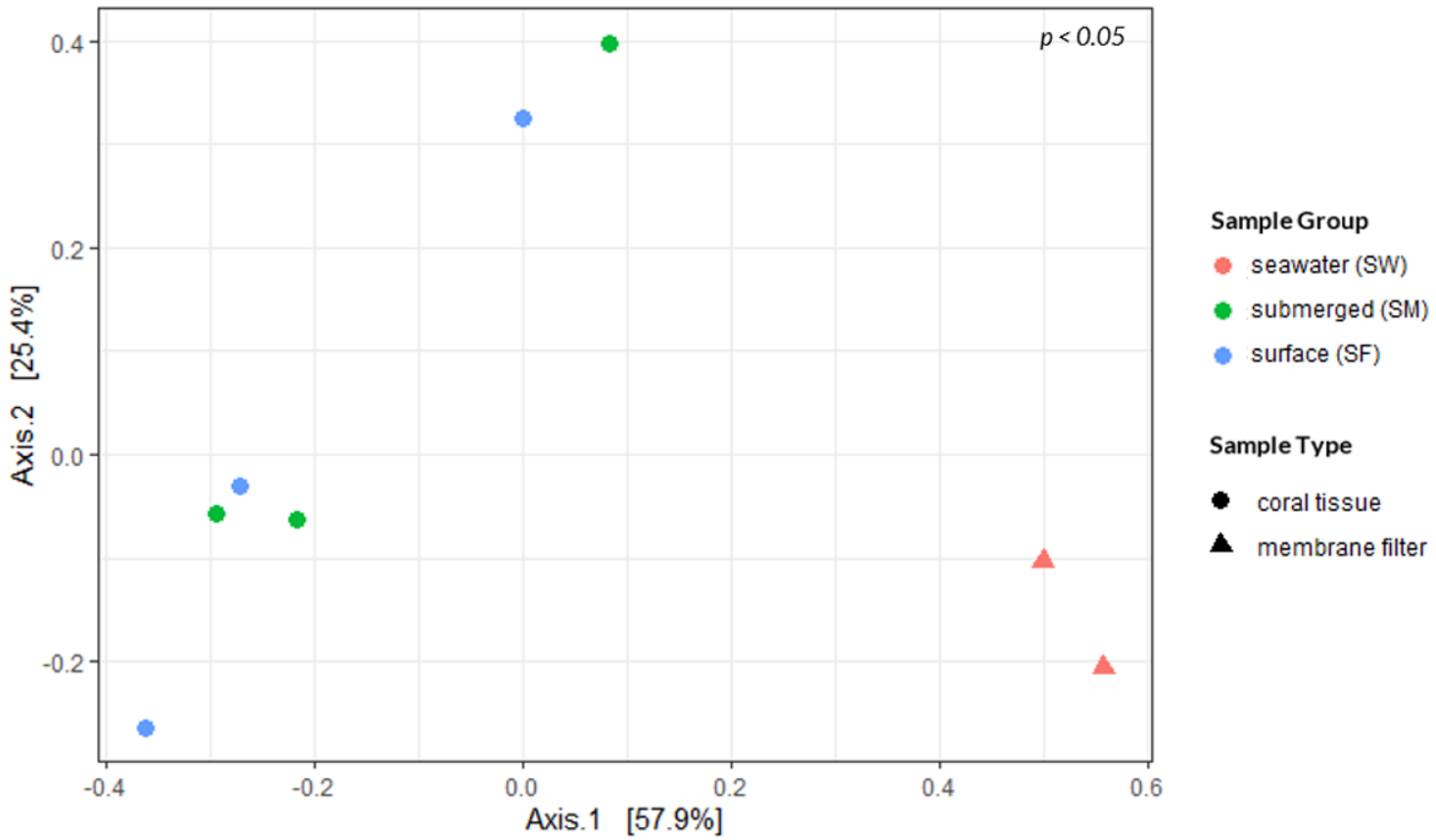

Figure 4

Principal coordinate of analysis based on the Bray-Curtis dissimilarity matrix of the association microbial community *Acropora pulchra* and the waters adjacent coral.

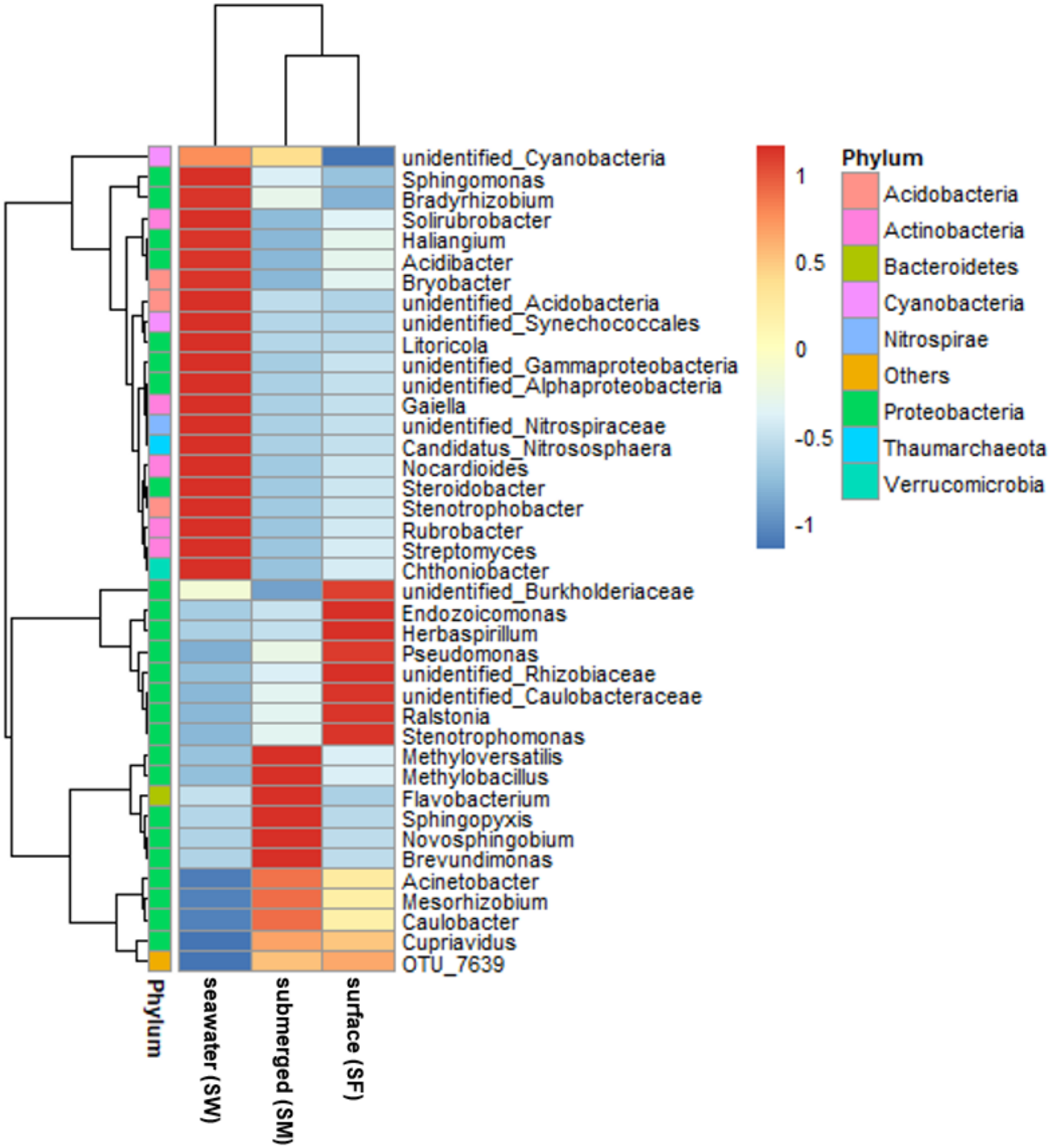

Figure 5

The cluster heatmap of the 40 most abundant genera that were successfully annotated at the genus level is displayed based on the sample group.