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2022

Understanding the Environmental Implications of the Microbiome of Canals in Bangkok, Thailand

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Understanding the Environmental Implications of the Microbiome of Canals in Bangkok,

Thailand

A Thesis Presented

by

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To the Keck Science Department

of

Claremont McKenna, Scripps, and Pitzer Colleges

In Partial Fulfillment of

The Degree of Bachelor of Arts

Senior Thesis in Organismal Biology

[Submission Date]

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Abstract

The canals of Bangkok, Thailand hold significant economic and social value, yet the increasing urbanization of the city has resulted in increased water pollution. Agricultural runoff and urban waste contribute to the degradation of the water quality, which has impaired its safe usage by the people of the city. This study focused on analyzing the microbiome of the water in the canals in correlation with the surrounding environment, both in and out of the water. Ten sites along the Bangkok canals were analyzed. DNA was isolated for the sequencing of the 16s rRNA gene to determine the microbial diversity of the area and its relation to the water quality. Sites 8, 9, and 10, which were located further inland, were found to consist of a different microbiome compared to sites 1 through 7. The conductivity, total dissolved solids, and salinity were also different between the two groups. Beta diversity analysis suggests that location along the canal likely plays a strong role in determining the microbiome of the water. Understanding the interaction of the water chemistry with the microbiology of the canals allows for local communities to better enact measures to improve the water quality.

Intro

Water is a vital resource for societal development, contributing to socio-economic growth, production of food and energy, and maintenance of the general health of the ecosystem and the people within them. The quality of water is heavily tied to public health, with poor quality water potentially leading to various waterborne diseases. Contaminated water is one of the leading causes of child mortality, especially in developing countries, where it is tied to inadequate water supply or sanitation (Nations, n.d.). Diarrhea, which can occur as a result of pathogens transmitted in the water, are responsible for nearly one million deaths each year (Levallois & Villanueva, 2019). Cholera, a diarrheal infection, affects an estimated 1.3 to 4 million individuals worldwide each year, with around 21,000 to 143,000 deaths (*General Information | Cholera | CDC*, 2022). In addition to bacterial infections, another problem tied to water quality in industrialized countries is chemical pollution. Exposure to chemicals in the

water can lead to the development of a wide range of diseases, including cancer and cardiovascular disease (Levallois & Villanueva, 2019). Yet despite the serious health ramifications of not having access to good-quality drinking water, it is estimated by the World Health Organization (WHO) that 10% of the world's population does not have access to clean drinking water; around 738,700,000 individuals do not have access to something that should be prioritized as a basic human right as recognized by the UN in 2010.

The focus of this study was to understand the microbial population of the water in the canals of Bangkok and analyze their relation to the water chemistry and local environment. The Chao Phraya River, which flows through Bangkok and into the Gulf of Thailand, is the major body of water that runs alongside the canals from which the water samples analyzed in this study were collected. It is the second longest river in Southeast Asia and is the largest in Thailand (Hungspreugs et al., 1989). The river basin where it is located holds considerable importance in agriculture and industry in Southeast Asia (Chaiwongsaen et al., 2019). Water quality in the river is heavily affected by upstream factors, due to the fact that multiple tributaries, including the Ping, Wang, Yom, and Nan Rivers flow into the Chao Phraya River (Simachaya, n.d.). About 13 million people rely on the river in their daily lives, using it for various purposes including consumption and irrigation. Additionally, canals and rivers serve as an avenue of transportation for boats and a site of recreation, as well as a site for river markets and festivals.

The rapid urbanization of Bangkok has resulted in an increase in population as well as industrial and agricultural activity, which has stressed the environment by increasing the amount and rate by which the water is polluted (Hungspreugs et al., 1989). The water in the canals of Bangkok, Thailand are subject to various forms of pollution, including industrial sewage, agricultural runoff and domestic waste, which can all be tied to the urbanization of the city. The

majority of pollution in the water is of domestic origin in the form of faecal bacteria and organic waste. While water treatment plants do exist, their range is limited and lack the capacity to treat all of the water that flows through the river (Simachaya, n.d.).

One major way in which humans influence water quality is through agricultural land. Peri-urban (located in areas that are directly adjacent to a city or urban area) aquaculture farms in Bangkok are closely linked to the quality of water in local canals. Not only does the water in the canal influence the quality of water being utilized for the purposes of aquaculture, but the aquaculture itself also influences the quality of water in the canals. A previous study found that the water collected from local canals used in the farms contained various types of micropollutants, including "faecal coliforms, *Bacteriodes*, *Prevotella*, human *E. coli*, Tetracycline resistant genes, and nitrogen" (Mrozik et al., 2019), which have been linked to algal blooms through eutrophication. Eutrophication happens when a body of water becomes enriched with excessive nutrients, occurring in the aquaculture ponds, even without the addition of fertilizer. In addition to eutrophication related concerns, excessive levels of nitrogen have also been tied to an elevated risk of developing cancer and producing birth defects (Schaider et al., 2019). The application of nitrogen and phosphorus, which are key nutrients in plant growth, are heavily utilized in the farming process. Urbanization and forest encroachment, which decreases infiltration of water into soil, result in increased surface runoff; this leads to key nutrients entering waterways and causing eutrophication (Chotpantarat & Boonkaewwan, 2018). Aquaculture can also influence water in the canals, where an increase in salinity and the herbicide diuron, used to control weed and algal blooms, has been observed in water near aquaculture farms (Mrozik et al., 2019).

What defines the microbiome of different locations in the canals of Bangkok is important for better understanding the types of bacteria affecting people using the water and the human influence of their presence. Understanding the factors influencing differences in water quality based on location and how the water quality affects the microbiology of the water helps to create a more holistic view about the canal water. While there are temporary solutions to poor water quality in the canals, such as utilizing tap water for agriculture, they do not solve the underlying issue that will continue to fester and potentially build into an even more severe issue. By better understanding the microbiome of the water and how the local water chemistry influences it, it becomes possible to pinpoint possible sources affecting the water, allowing for these sources to be targeted to improve the overall water quality.

Methods

Sample Collection

This study was conducted as a collaborative effort with Professor Kanjanee Budthimedhee from the King Mongkut's University of Technology Thonburi (KMUTT) and her students to study canal water quality, specifically the potential correlation between the canal microbiome and water chemistry on the local environment. Water samples were collected at 23 sites along the canals of Bangkok, Thailand, specifically in the region to the west of the Chao Phraya River, although only 10 sites were analyzed for their microbiome. The specific site locations were selected because of the interest of the collaborators in Thailand in studying this area of the canal for its proximity to SAFETist Farm, which has ties KMUTT. The distance between sites was not always equal due to the difficulty of collecting water samples at certain locations along the canals. The microbiome samples were collected through a Fisherbrand™ polyethersulfone syringe filter using a 50 mL BD syringe. ZymoBIOMICS™ DNA/RNA shield

was used as a stabilization solution to preserve and prevent the degradation of the genetic material in the samples.

DNA Prep for Sequencing

To analyze the microbiology of water from the canals in Bangkok, Thailand, we sequenced the 16s rRNA gene. This gene was analyzed because it is highly conserved and evolves slowly, remaining relatively unchanged through evolution. This allows for species-specific identification to analyze the present microbiome. The gene consists of constant and variable regions. The constant regions are the same across species and can therefore be used to analyze phylogenetic relationships between species, while the variable regions differ across species and can be used for the identification of specific species. The V3/V4 regions, two variable regions of the 16s rRNA gene, were analyzed to identify the specific bacteria present in the samples used in this study. The DNA of the samples was isolated using the ZymoBIOMICS™ DNA Miniprep Kit. To reduce potential damage to the filter papers used to collect the water, Lysis solution was used to backwash the samples into Lysis tubes, where the cells in the samples were then lysed using a MP Fastprep-24. The final DNA samples were eluted using ZymoBIOMICS™ DNase/RNase Free Water. The quantity of genetic material present after DNA isolation was analyzed using a Nanodrop, a spectrophotometer, to find the concentration of double-stranded DNA. A PCR using 27 and 1497R primer was run on the 16s rRNA gene (variable region) to check that it can be detected in the samples. It was found that the 16s rRNA gene was visible and present in all samples.

Sequence Analysis

Samples 1 through 10, from the 23 original samples, were sent to the sequencing facility, SeqCenter, for analysis of the genetic material to determine the microbiome of the samples. The

V3/V4 regions of the 16s gene were targeted and the sequenced samples underwent quality control using bcl-convert, which converts binary base call files (raw data files generated from Illumina Sequencers) to FASTQ files. FASTQ files store raw sequence data and quality scores, which can then be used for further data analysis. Qiime2's dada2 plugin was used to denoise the sequences, which removed unexplained variability from the total genetic material, and assign them SILVA taxonomy labels.

Data Analysis

The microbial composition data obtained from the sequencing facility was analyzed using Microbiome Analyst, a web-based platform used for the analysis of microbial data. The Marker Data Profiling module of the platform, which analyzes marker-gene survey data, was used to conduct statistical analysis and visualization of the microbial composition data in correlation with the water chemistry information at each site. Both the relative frequency of microbes in the samples and the exact counts, where the frequencies determined by the sequencing facility were multiplied by the total number of reads that were denoised, were used for the analysis. The water chemistry information, which included pH, salinity, conductivity, total dissolved solids, dissolved oxygen, and the water quality index (WQI), was collected independent of this study, so not all of the microbial sample locations had a corresponding water chemistry location. Sample locations 4, 7, and 9 did not have a corresponding water chemistry location, so the nearest point with data was used as a proxy. Due to limited direct access to the local environment, Google Maps was used to study the land surrounding each site for potential contributing factors to the site microbiome.

The table including the composition of microbes at each sample site and water chemistry data were uploaded on MicrobiomeAnalyst for Marker Data Profiling. The water chemistry data

was sorted into bins for comparison, with at least two samples present in each group. None of the sample counts were filtered out before analysis. The data was scaled through total sum scaling, which divides the count of reads with the same operational taxonomic unit from a sample by the total number of reads per sample. No other data normalization steps were performed.

The two primary types of analysis run on the data included the comparison of the bacterial composition of the ten most common genera between sites and the beta-diversity analysis of each bacterial sample population against water chemistry factors, which compares the species diversity between different communities. The analysis was conducted on the genus level to identify the specific types of bacteria in the area without being too specific for the bacteria to be uncultured species.

The Chao1 index, which accounts for unobserved species based on low species richness, was used to analyze the alpha diversity of the samples, with an ANOVA run to compare the microbial population to the water chemistry to test correlation. For beta diversity, the data was ordained (differences between samples of multivariate data analyzed and presented based on fewer dimensions) using Principal Coordinates Analysis (PCoA), with the Bray-Curtis Index used to measure the dissimilarity between different sites. In this process, the data is manipulated in a manner that allows for it to be visualized to show similarities between samples, the closer the points are to each other, the more similar the microbial communities are. A Permutational ANOVA (PERMANOVA) was run to test the statistical significance of the beta-diversity groupings. The PERMANOVA tested if the centroids (center) of each cluster, as defined by a specific feature of water quality, differed significantly from each other in ordination. Significance would indicate that the factor of water quality used for grouping likely has an effect on the observed differences observed in the microbiomes.

Results

Water Quality Trend

From the 23 total water samples collected, the water from sites 1 through 10 were

analyzed for their microbiome. The water collection site numbers correlate with increasing

proximity to the coast, with site 1 being closest to the coast and site 10 being the furthest inland.

The pH, conductivity, salt concentration, total dissolved solids, temperature, dissolved oxygen,

temperature, and water quality index were included for the comparison between water quality

and microbiome.

Of these factors, it was found that conductivity, salt concentration, and total dissolved

solids were significantly different between sites 1 through 7 and sites 8 through 10 (Fig. 1; Table

1). These aspects are positively correlated with each other; as salt concentration increases, total

dissolved solids and conductivity (dissolved salt conducts electrical current) increases, with a

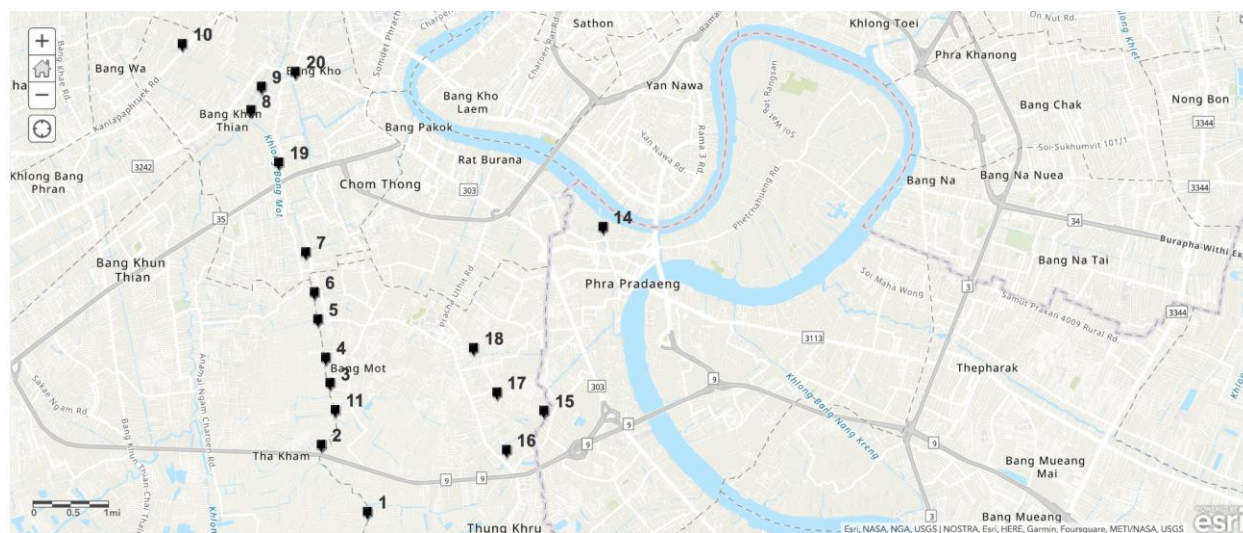positive correlation also existing between conductivity and total dissolved solids.

Figure 1: Map of the region in Bangkok where the samples were collected, with sites numbered 1

through 10 being analyzed (ArcGIS).

Sample Site	Canal (Khlong)	pH	Conductivity	Salt	Total Dissolved Solids	Dissolved Oxygen	Temperature	Water Quality Index
1	Khlong Rang Maenam	7.37	3059.29	1.55	1537.14	8.24	28.74	31
2	Khlong Ratchaphruek	7.15	3299.03	1.8	1780.57	7.1	28.51	30
3	Khlong Bang Mot	7.12	2483.29	1.27	1257.71	6	28.6	30
4	Khlong Bang Mot (1)	7.16	2478.43	1.41	1385.29	6.63	29.26	30
5	Khlong Bang Mot (2)	7.08	2232.89	1.29	1294.14	5.36	28.29	30
6	Khlong Bang Mot (3)	7.14	2361.29	1.18	1195.29	6.57	28.47	30
7	Khlong Bang Mot (4)	7.1	2047.43	1.04	1050.43	6.43	28.44	30
8	Khlong Sanam Chai	6.97	555.57	0.28	281.86	5.13	29	35
9	Khlong Sanam Chai (1)	6.96	546	0.27	275.71	4.79	29.54	35
10	Khlong Bang Prathum	7.01	557	0.28	281.71	4.89	29.5	35

Table 1: Location and water chemistry of sites selected for analysis

Microbiome Trend

While the data provided by SeqCenter was the proportion of each bacteria found in the samples, the analysis of the microbiome was performed with the actual counts to account for the possibility of locations having different total population sizes from which the proportions are drawn from. To calculate the total count of bacteria in the samples, the proportion was multiplied by the total reads denoised.

Between the ten sites, a more similar microbial composition within the samples collected at sites 1 through 7 and within those collected at sites 8 through 10 compared to between the two groupings was observed (figure 1). This pattern was consistent between analyses conducted using the relative frequency and using the actual count of microbes in each sample. It was determined that site 7 had the greatest amount of bacteria, while the other locations had a similar total bacterial count. There were three bacteria genera where the concentration differed between sites, which included cyanobium pcc6307, CL500 29 marine group, and c39. Sites 8, 9, and 10 were shown to have had less cyanobium pcc6307, less CL500 29 marine group, and more c39 (figure 1) than sites 1 through 7.
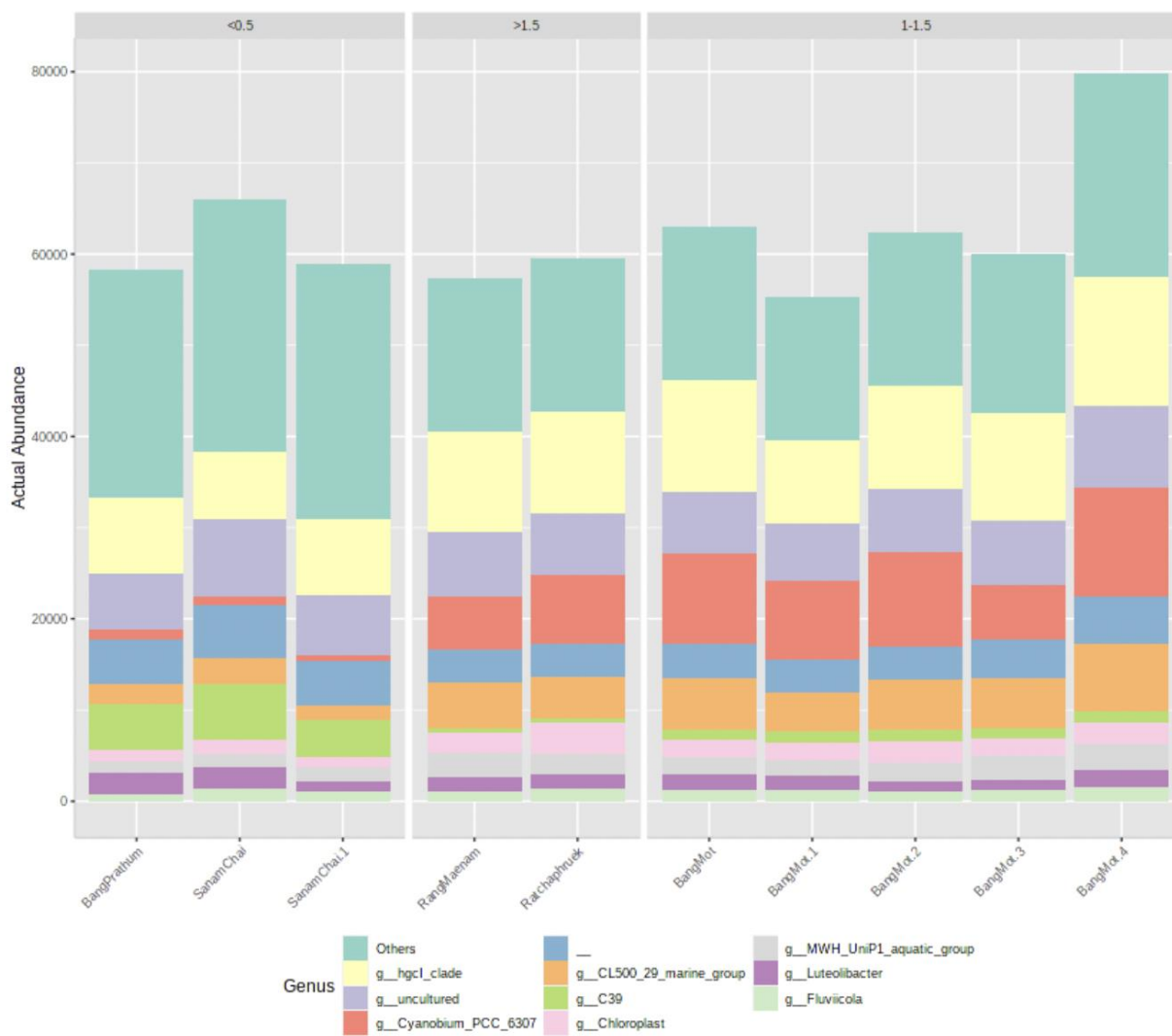
Figure 2: Total count of the ten most common bacteria genus at each site, with less common genus classified under 'others', sorted by salinity level (<0.5 ppt, >1.5 ppt, 1-1.5 ppt).

Cyanobium pcc6307

Cyanobium pcc6307 is a type of cyanobacteria, a bacteria that produces oxygen through photosynthesis (Shih et al., 2013). More specifically, cyanobium is a type of picocyanobacteria, a small cyanobacteria ranging from 0.2–2.0 µm that is recognized for their importance in the food web, serving as a primary food source for many planktonic organisms due to their small size, and

as a primary producer (Śliwińska-Wilczewska et al., 2018). Algal blooms can occur when conditions in the water are such that their growth rate increases. One factor that influences cyanobacteria growth is the presence of nutrients like nitrogen and phosphorus, which increase algal growth. These nutrients are common in an agricultural setting, where they can be present in manure and fertilizer, and can end up in nearby waterways through runoff. The presence of Cyanobium pcc6307 in the water could be influenced by agricultural activity, with more farmland being present near the sites that the bacteria was more common in. This bacteria stands out in that it is typically found in freshwater, yet in this instance was found to be more prevalent in areas of higher salinity.

Cl500 29 marine group

Cl500 29 marine group is a type of actinobacteria, a gram-positive bacteria with high guanine and cytosine content. Of the naturally derived antibiotics in clinical use, around two-thirds of them are produced by actinobacteria (Barka et al., 2015). While it is primarily found in marine environments, it has also been reported in bodies of freshwater (Papale et al., 2020).

C39

C39 is a type of proteobacteria. It has primarily been found in bodies of water that are low in salinity, which matches its higher prevalence in sites lower in salinity. The prevalence of C39 in a population is positively correlated with pH and negatively correlated with total dissolved solids (Zhu et al., 2022). While the results do not match the expected trend for pH, they do match for total dissolved solid, which could be related to how significantly the values of pH and total dissolved solids differ between sites.

It was observed that water chemistry features that differed significantly between the two groups of sites were the salinity (figure 2), total dissolved solids, and conductivity; these factors are connected in how they can influence and be influenced by one another. Sites 1 through 7 have higher salinity, total dissolved solids, and conductivity than sites 8 through 10.

There was shown to be a significant effect of salt, conductivity, TDS, DO, temperature and the WQI on the beta diversity between sites. The results of the PERMANOVA were the same for salt, conductivity, and total dissolved solids (f-value: 21.001, R^2: 0.85715, p: 0.001; fig. 2, 4, 5). There is overlap in the grouping for temperature, but the p-value shows that the overlap is not significant enough to affect the strength of temperatures effect on the microbiome (f-value: 9.9498, R^2: 0.55431, p: 0.009; fig. 7). pH was the only factor of water chemistry not found to have a significant effect on the microbiome (f-value: 0.9355, R^2: 0.1047, p: 0.307; fig. 3).
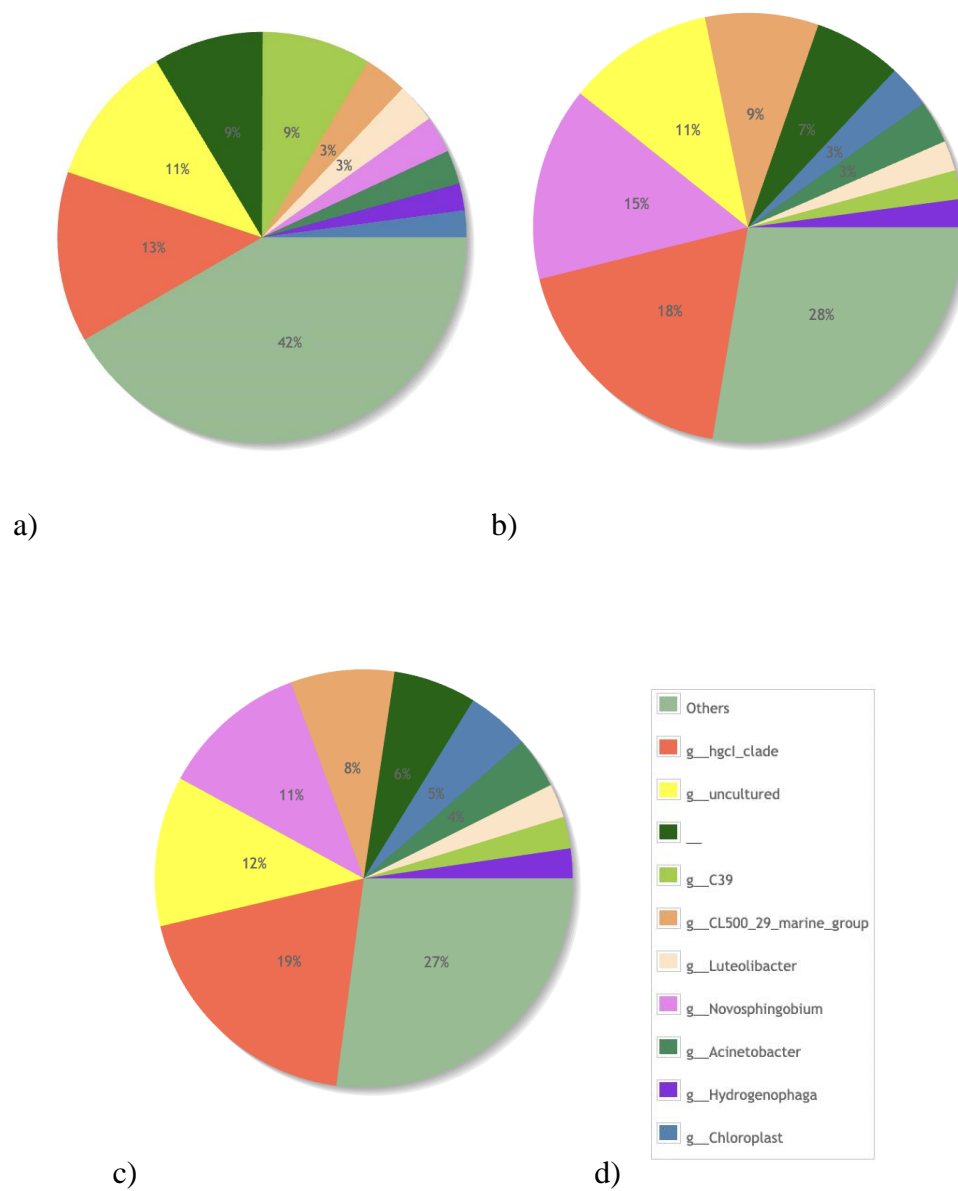
Figure 3: Pie chart of the relative abundance of the 10 most common genus when merged into groups based on salinity level (ppt) a) <0.5, b) 1-1.5, c) >1.5, d) genus legend.
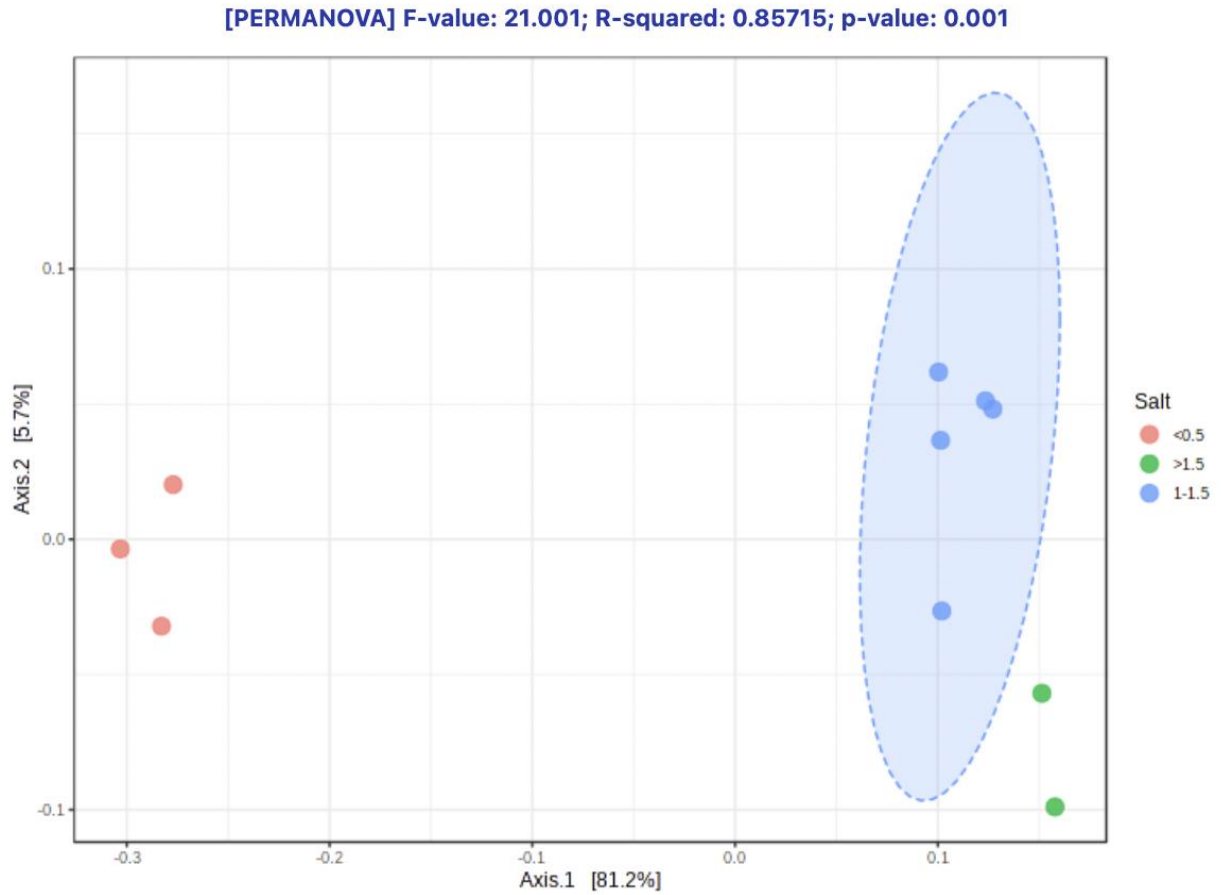
Figure 4: Beta diversity between sites, as measured using PERMANOVA, with grouping based on salinity.
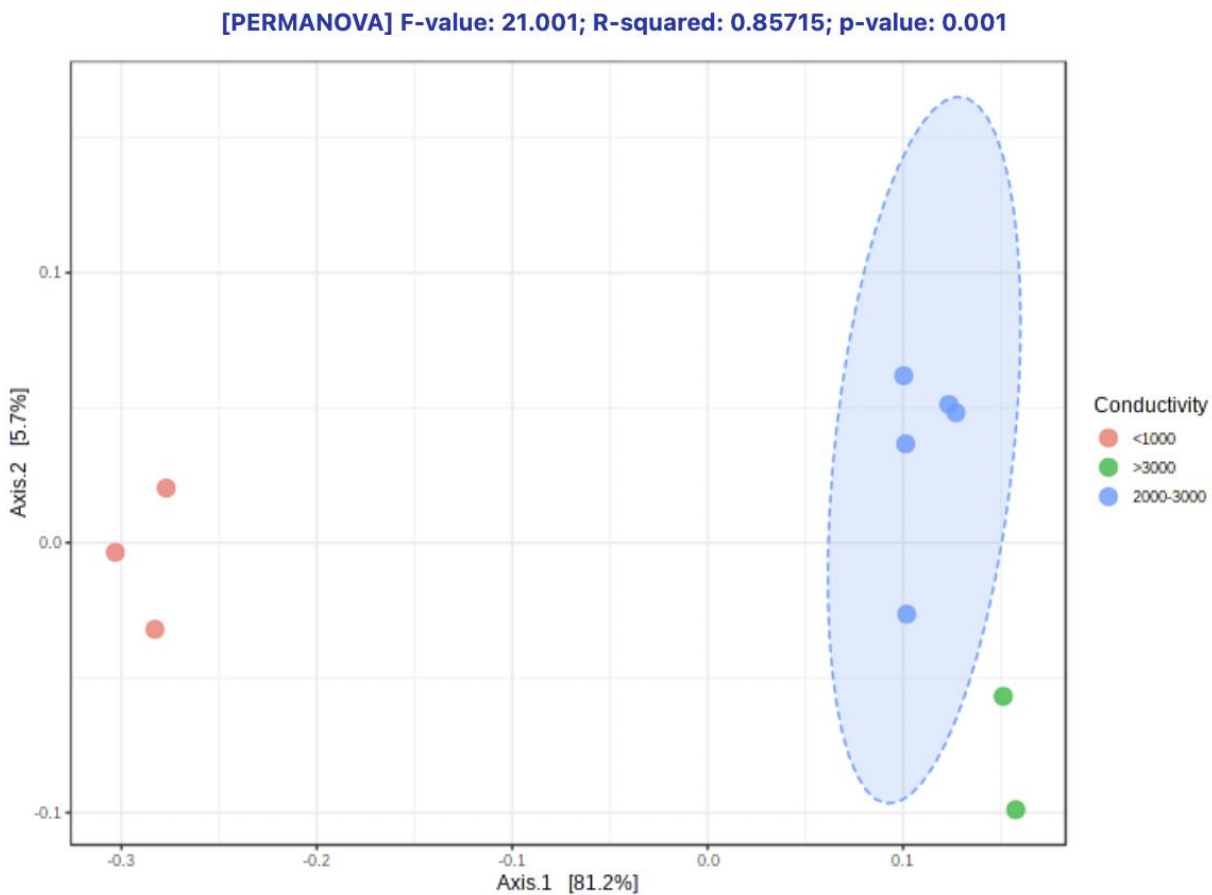
Figure 5: Beta diversity between sites, as measured using PERMANOVA, with grouping based on conductivity.
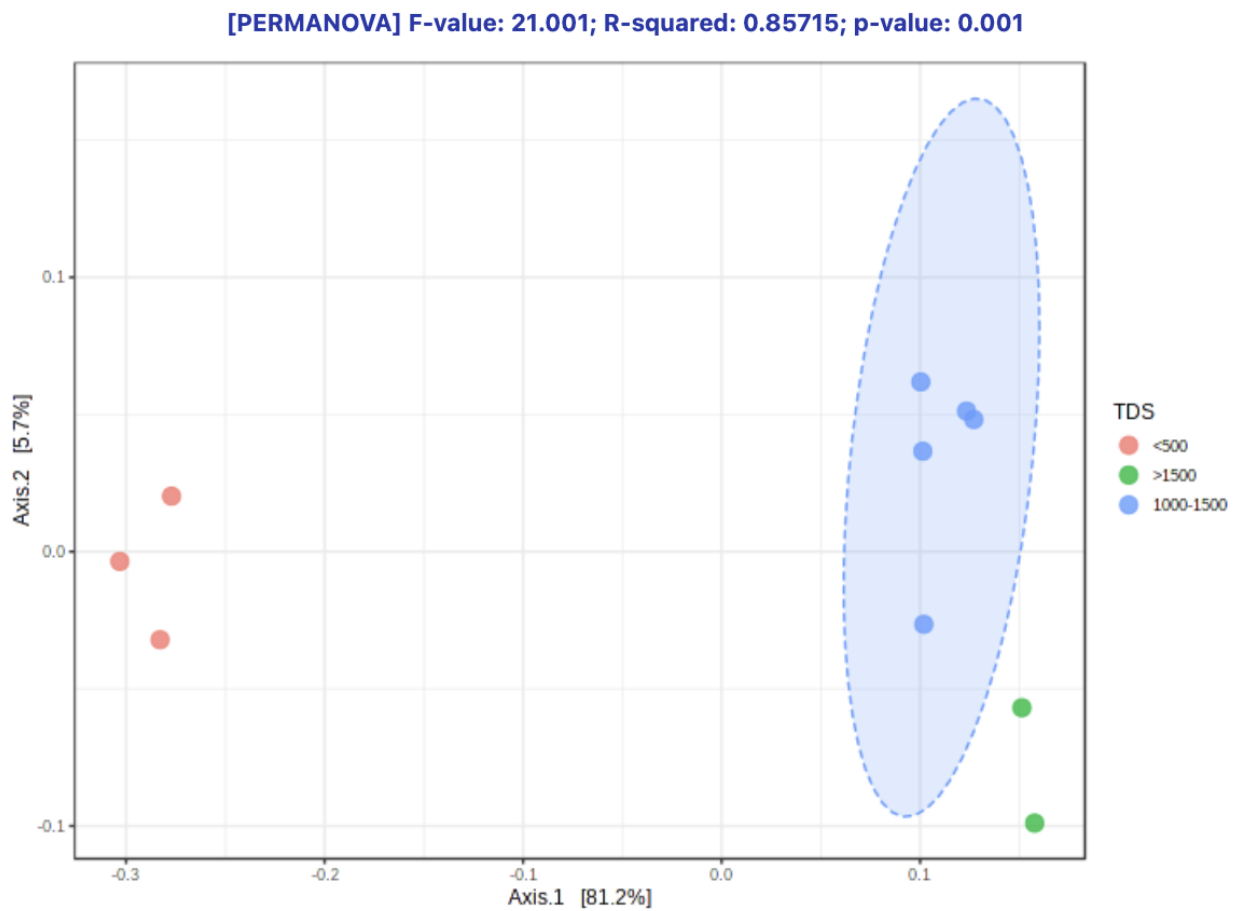
Figure 6: Beta diversity between sites, as measured using PERMANOVA, with grouping based on total dissolved solids (TDS).

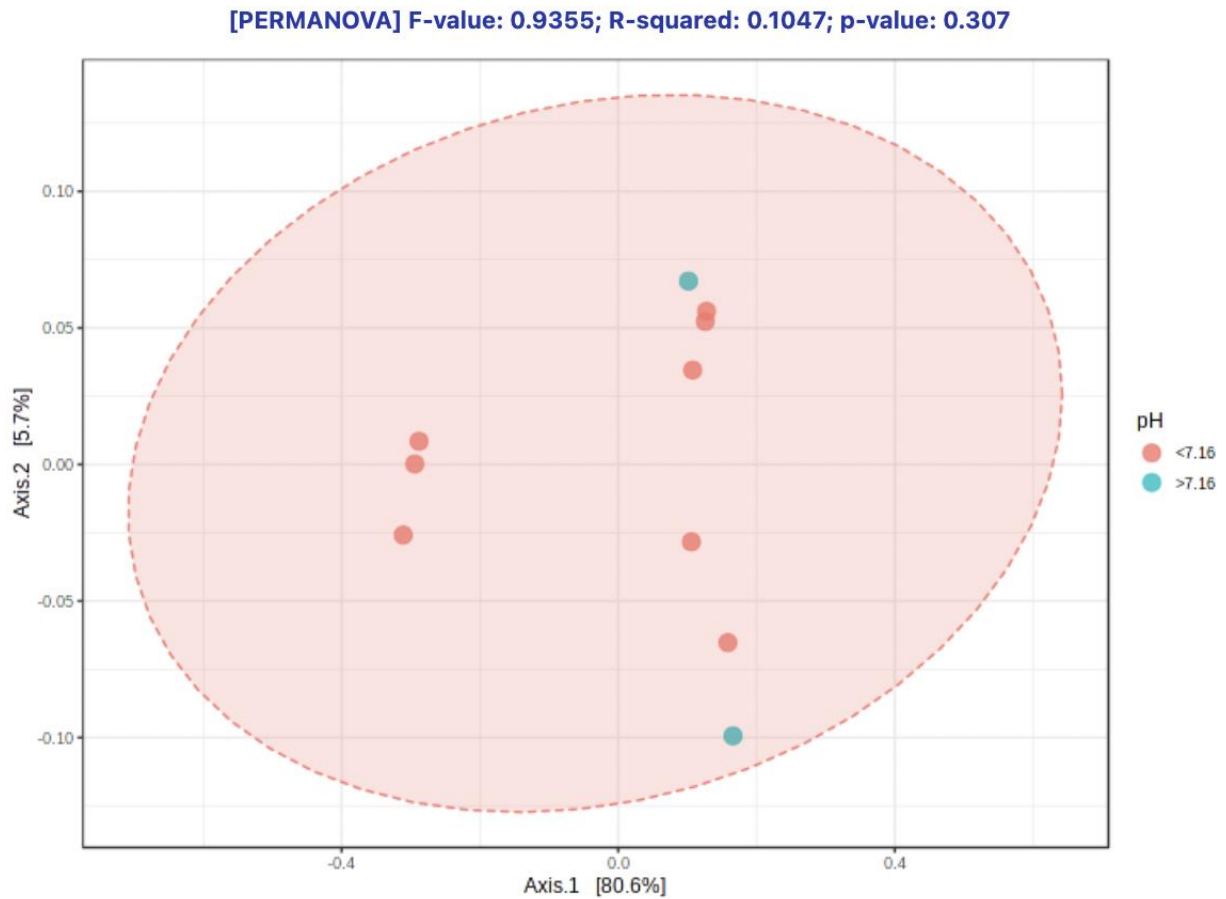**[PERMANOVA] F-value: 0.9355; R-squared: 0.1047; p-value: 0.307**

Figure 7: Beta diversity between sites, as measured using PERMANOVA, with grouping based on pH.
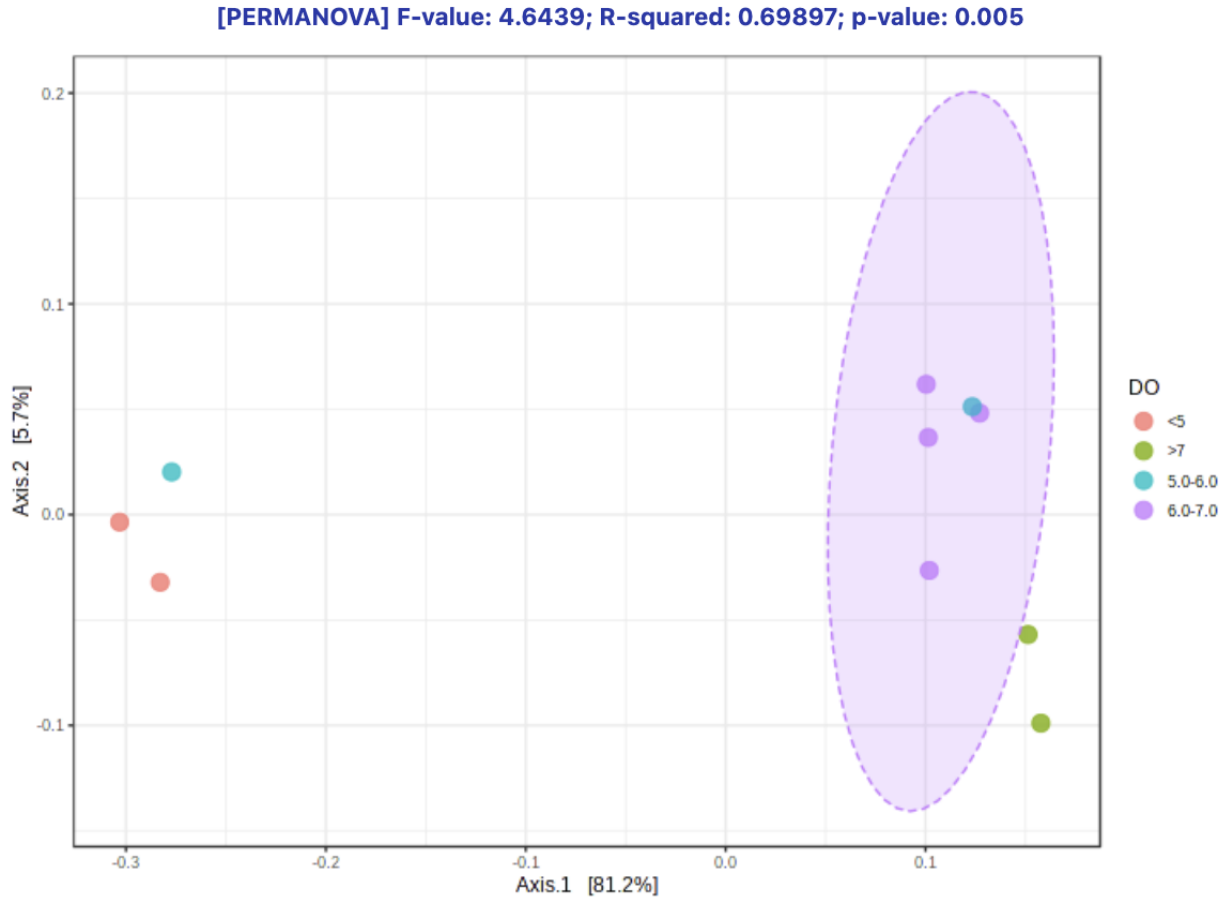
Figure 8: Beta diversity between sites, as measured using PERMANOVA, with grouping based on dissolved oxygen (DO).
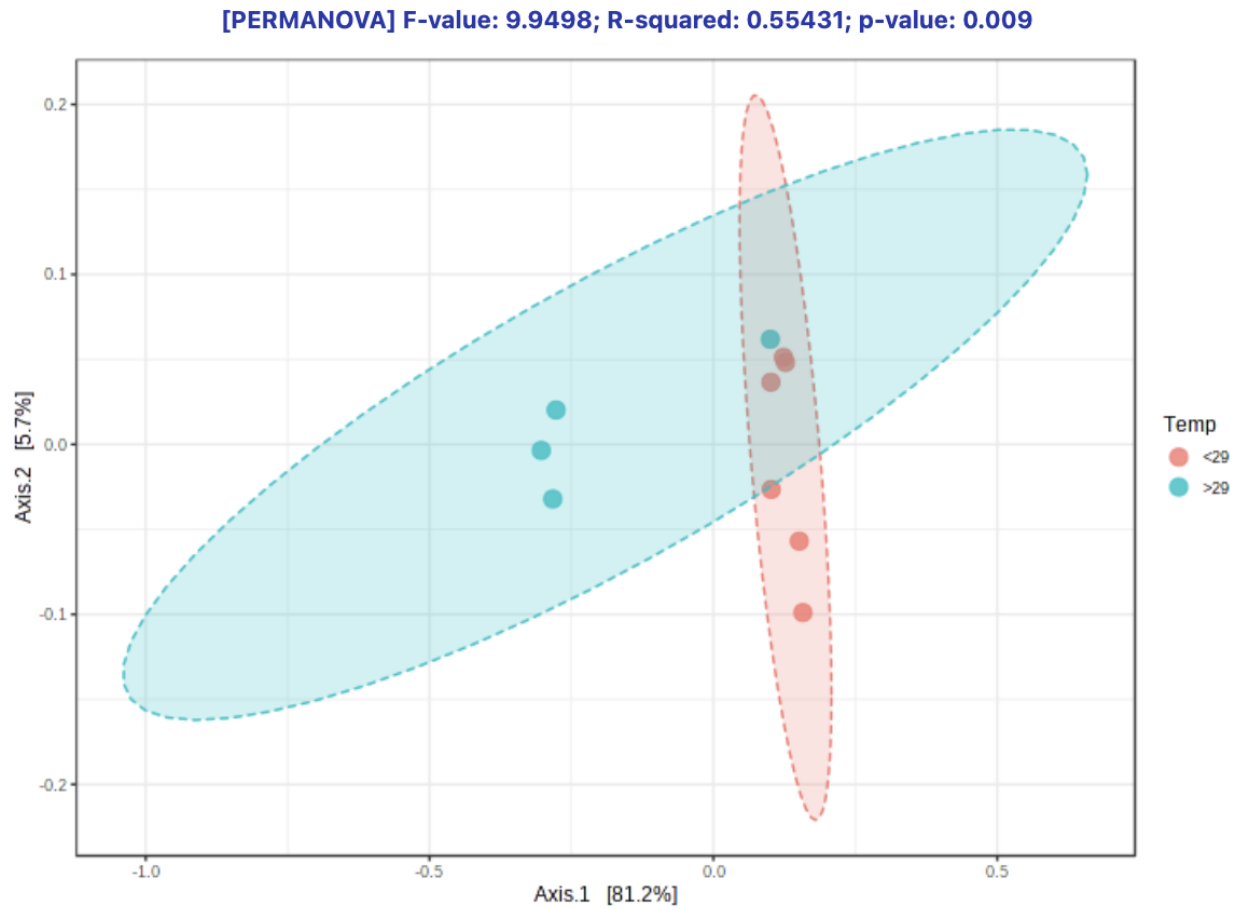
Figure 9: Beta diversity between sites, as measured using PERMANOVA, with grouping based on temperature.
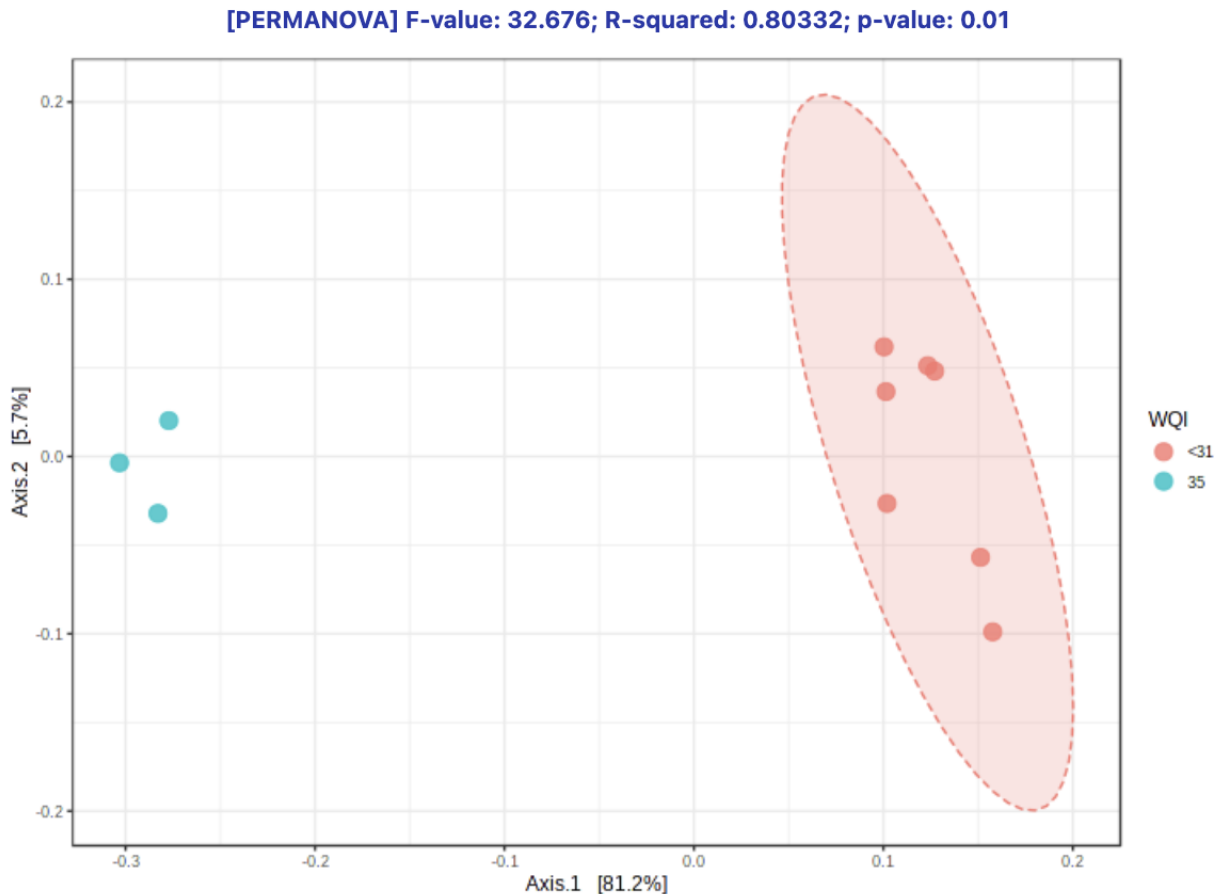
[PERMANOVA] F-value: 32.676; R-squared: 0.80332; p-value: 0.01

Figure 10: Beta diversity between sites, as measured using PERMANOVA, with grouping based on the water quality index.

Discussion

The trend observed in the values for factors of water chemistry in relation to each other match what was expected. As salinity (Fig. 4) increased, conductivity (Fig. 5) and total dissolved solids (Fig. 6) increased as well, which matches the expected correlation between these features of water quality. The pattern observed for dissolved oxygen matched the expected pattern based on the temperature of the water, with higher levels of dissolved oxygen (Fig. 8) being observed at sites colder in temperature (Fig. 9), as cold water can hold onto more dissolved oxygen than warm water. The results for the WQI (Fig. 10) match the expected pattern based on TDS (Fig. 6),

with regions lower on the water quality index having higher amounts of total dissolved solid, which could encompass solids that negatively impact organisms in the surrounding water.

While a significant correlation was observed between salt, conductivity, TDS, DO, temperature and the WQI and the microbiome beta diversity between sites, external factors likely also affected the type of bacteria found at each site. Overlap in data points occurred when grouped by dissolved oxygen, where sites with dissimilar levels of dissolved oxygen were located close together on the PCoA plot even if their measure level of dissolved oxygen differed. However, the points from similar sites are grouped closely together on the plot. This suggests that water quality alone cannot explain variation between sites and that location-dependent environmental factors may also play a role. More information needs to be collected on the features of the land surrounding the sample sites to better understand its potential effect on the microbial composition of the water.

A potential contributing factor to this observed microbiome pattern based on location is the use of the surrounding area for agriculture. One bacteria genus that differs between sites is cyanobium pcc6307, a type of cyanobacteria. Cyanobacteria is tied to algal blooms through the increase of nutrients in the water, such as the nitrogen and phosphorus that can come from manure or fertilizer used in agriculture. Based on google map images, it appears that the region surrounding sites 1 through 7, where cyanobium pcc6307 was more common, consists primarily of farmland. This contrasts with the region surrounding sites 8 through 10, which appear to have a more urbanized landscape.

While there appeared to be a correlation between the microbiome of the water and some factors of water chemistry, the surrounding location of the sampling site likely had a stronger role in controlling the types of bacteria found in the water. The PCoA plot for dissolved oxygen

(Fig. 8) showed sites differing in level of dissolved oxygen instead being grouped close to sites similar in location, suggesting a potentially stronger correlation between location and differences in microbiome than between dissolved oxygen and differences in microbiome. The correlation found to exist between salinity, total dissolved solids, and conductivity, the three factors that were the most significantly different between sites with significantly different microbiomes, were likely the result of changing levels of salinity based on the location of the site in relation to the Gulf of Thailand, with sites closer to the coast being higher in salinity and sites closer inland being lower in salinity. As salt decreases further inland, there is a corresponding decrease in the amount of total dissolved solids and the conductivity of the water. Further study into the features of the land surrounding the canals would be helpful in determining how exactly the local environment might be affecting the microbiome.

Another relationship of note was that while there appeared to be a significant correlation between the water quality index and the microbiome, the water quality indices all fell into the range considered by the EPA to be of poor quality (US EPA, 2013). The water quality index of the sample sites does not cover a wide enough quality range to truly account for how varying levels might affect the microbiome. Thus, more testing is required at sites that are considered to have a good water quality index to make more definitive conclusions about the relationship between these factors and the microbiome.

This lack of diversity in data points can also be observed in other measures of water quality. The non-significant relation between pH and the microbiome may also be the result of the lack of variability in the pH range at the sites tested, with all of the sites being neutral (around 7). Additionally, while temperature was found to have a significant effect on the microbiome, the overlap in data points on the PCoA plot may have been influenced by similarity

in temperature value between sites. Due to the small range of values for pH and temperature, testing more sites with a wider range of values would be helpful in determining their effect on the microbiome.

Since the water analyzed in this study only comes from one collection period, there is insufficient microbial data to determine potential temporal changes in the microbiome or if the results are significant in terms of the overall microbiology of the canals. It would not be possible to discern from the current data if there are changes to the microbiome over time and if the observations made represent a temporary occurrence. With more data gathered over time, it would be possible to observe temporal patterns in the microbiome and have more information that can be compared with the water chemistry and local land usage. Additionally, data collected over time could also be helpful in determining if and how climate change affects the microbiome or water chemistry of the sites. This would be helpful in understanding the flexibility required of any future policies regarding water quality to properly account for changes over time.

Future steps for the project include more social outreach with the local community to build and maintain a data source with information about the overall quality of water in the canals for people to access. One project currently being developed to make the information more accessible to the public is an ArcGIS map, which will map out the location of each site, accompanied by information about the water chemistry and microbiome present (figure 9). The map was made in collaboration with water chemistry information collected by Professor Kanjanee Budthimedhee from the King Mongkut's University of Technology Thonburi and her students. Another potential outreach project is to create opportunities for community-based data collection. The focus of these future steps is to increase access to information about the water so

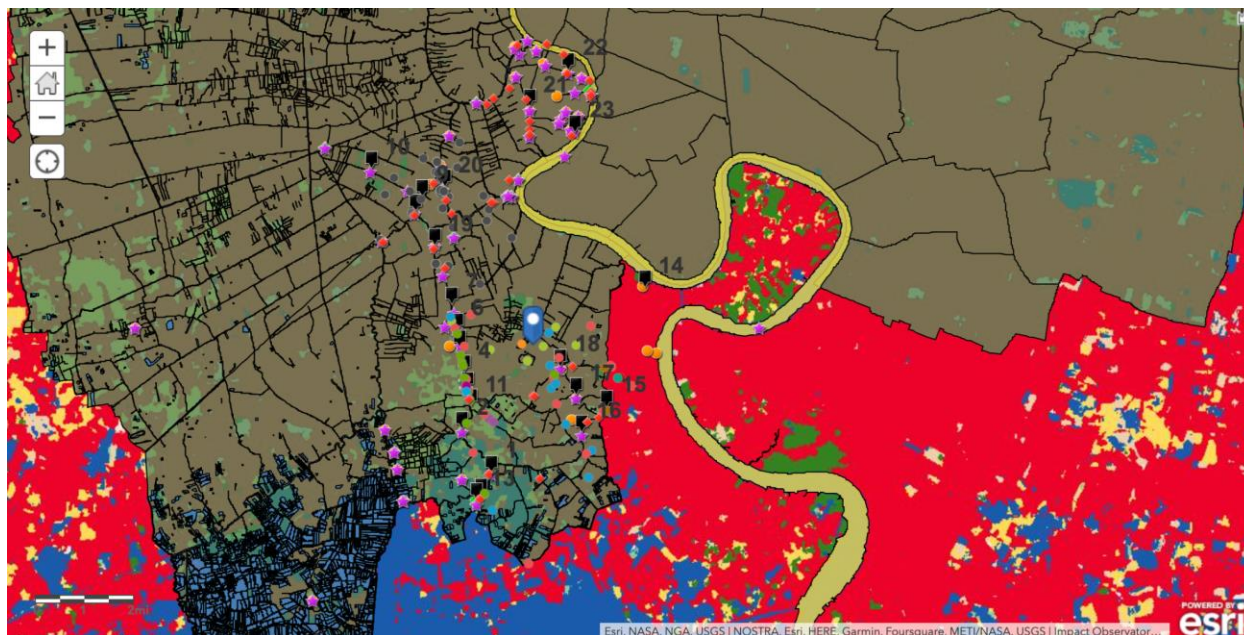that the local community can use it to determine policies for improving its quality.

Figure 11: ArcGIS Map of the sample sites: https://arcg.is/0WS8v80

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