Here's the Dirt: Sedimentation Effects on Coral Microbiomes

By
Justin Berg

A thesis submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

IN

BIOLOGY

UNIVERSITY OF GUAM

May 13, 2022

Abstract

In southern Guam, rainfall and resulting river discharge leads to large sediment deposits into several bays, including Fouha Bay. These sediments originate from lateritic clay soils, exposed due to arson, development and poor land management. In the late 1980's, Fouha Bay experienced high coral mortality due to sediment runoff following land clearing and road construction. The effects of road construction and subsequent anthropogenic influence have affected coral cover, composition, and diversity in Fouha Bay and adjacent bays. Following storms, coral is exposed to lowered salinity, reduced light-levels due to suspended particles, and tissue necrosis due to sediment settling on coral. While these factors can affect coral health and in turn shape coral community composition, effects on the microbial communities associated with corals living in these habitats are not fully understood. *Porites lobata* dominates Fouha Bay at the inner and outer sites that differ in sedimentation severity. Over the course of 8-month study, covering the wet and dry seasons, 8 coral samples from the inner and outer bay sites were collected monthly for DNA extraction and 16S rRNA metabarcoding of bacterial microbiomes. Two hypotheses were tested: 1) The coral-associated prokaryotic diversity of *P. lobata* will not be stable throughout the seasons and across the sites in Fouha Bay and 2) the coral associated prokaryotic metabolic functions of *P. lobata* will not shift between sites and seasons in Fouha Bay. A more stable coral microbiome was observed in the outer bay site due to year-round low levels of sedimentation while the largest microbial shift was observed in the inner bay site during the wet season. Key metabolic pathway shifts were observed at the inner site during the wet season driven by increased sedimentation and nutrient availability. A better understanding of the seasonal dynamics of *P. lobata's* microbiome and metabolic functions will provide increased understanding of effects of river runoff and sedimentation on the coral holobiont.

Key words: coral microbiome, microbial biodiversity, sedimentation

Table of Contents

Tables

Figures

Background

Anthropogenic Effects on Small Islands

Increased eutrophication and sedimentation are affecting coastal coral reefs worldwide (Burke et al., 2011; Golbuu et al., 2008; Howarth et al., 2011). Soil erosion and nearshore sedimentation are one of the primary threats to marine ecosystems for islands across the Pacific, such as Guam (Abraham et al., 2004; Richmond, 1993). Guam experiences large tropical storms that can lead to high erosion rates and sediment loading onto nearshore reefs (Minton, 2005). In southern Guam, there are 14 watersheds that run along the mountain ridges on the western coast. Of the 100 named rivers and streams on Guam, 46 drain directly into the Pacific Ocean and onto the coral reefs surrounding the island (Figure 1A). River and stream runoff discharge not only fresh water, but erodible solids such as soil, pesticides, and debris. Observed changes in coral cover and composition from inshore to offshore coral reef systems are partially attributable to differences in sedimentation (DeVantier et al,. 2006; Golbuu et al., 2008; Sweatmanet al., 2011). Corals that are better adapted or acclimated to sedimentation are usually more abundant in reefs exposed to higher sediment loads (Done, 1982; Sweatman et al., 2007). Coastal coral reefs have adapted to natural erosion processes. However, increased sedimentation due to human activities leads to declines in community diversity and species abundance near river mouths (Macdonald et al., 1997; Ramos-Scharrón & MacDonald, 2005; Rongo, 2004).

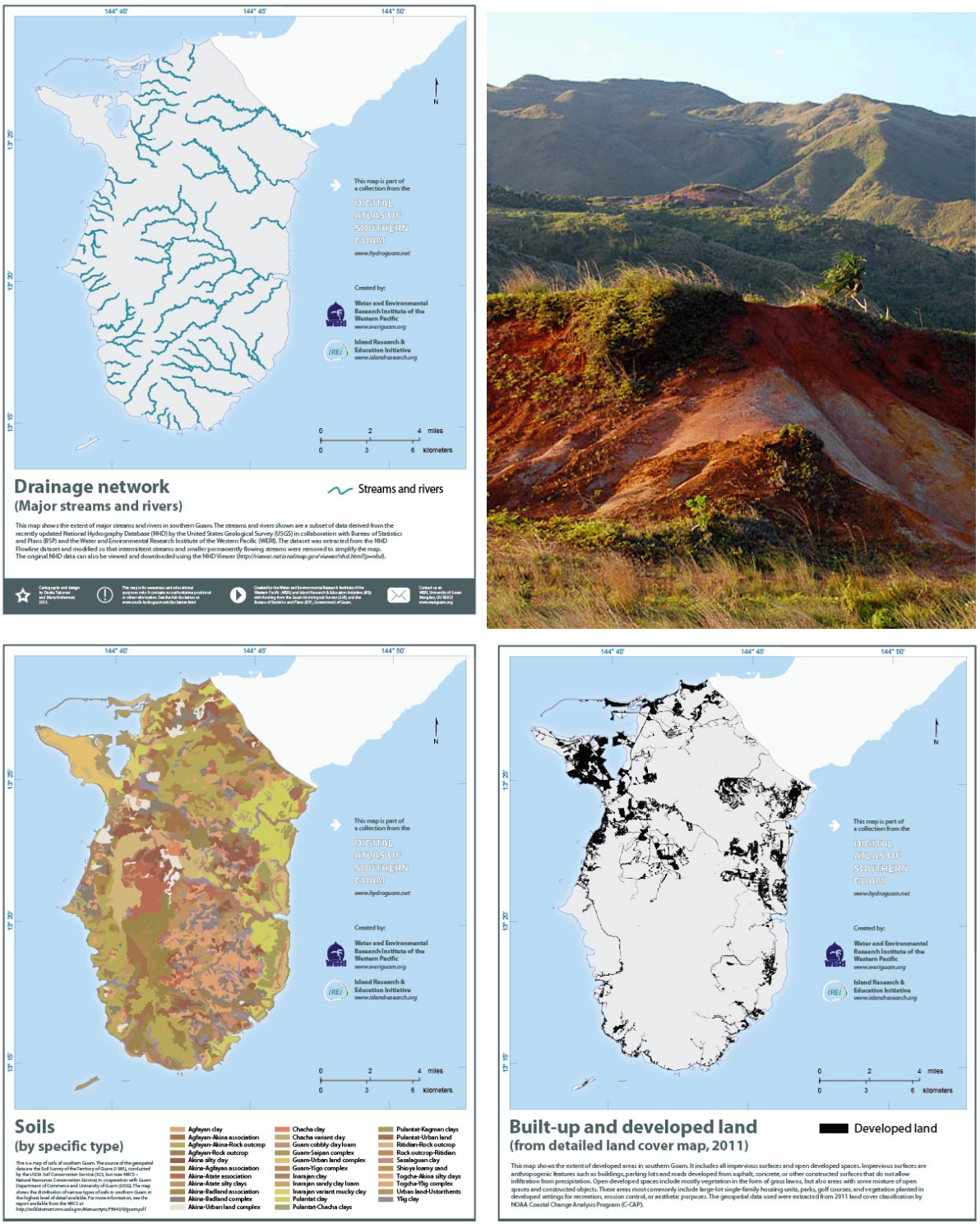


Figure 1 A-D. (A) Map of southern Guam depicting the drainage network of major rivers and streams. (B) The highly erodible badlands of southern Guam. (C) Map of southern Guam depicting soil types from the Soil Survey of the Territory of Guam in 1985. (D) Map of southern Guam depicting anthropogenic features (black) and natural land (gray) in 2011. (Digital Atlas of Southern Guam)

One of Guam's largest impacts on nearshore coral reefs is arson-derived fires. Prior to human arrival on Guam, wildland fires were none-existent due to Guam's naturally high humidity and rare lightning storms (Minton, 2006). Now, almost all fires on Guam are intentionally set by humans. Farmers, homeowners, and hunters light fires to clear vegetation, burn trash, and clear land to hunt pig and deer (Minton, 2006). Fires have led to a replacement of forests by savannah (Athens and Ward, 2004) and highly exposed clay areas known as Badlands (Figure 1B). Young native trees are highly susceptible to fires, which allows fast-growing, non-native savannah grasslands to replace forests. In 1985, the Soil Survey of the Territory of Guam was conducted by the USDA Soil Conservation Service, Guam Department of Commerce, and the University of Guam (Figure 1C) to map the soil types across southern Guam.

Badlands and burned savannah have the highest erosion rates with 35% topsoil loss (Minton, 2006). Timing of burning events can affect erosion rates. Areas burned earlier in the dry season erode less than areas burned at the start of the wet season (Minton, 2006). The anthropogenically driven shift from forests to grasslands not only affects terrestrial ecosystems, but increased topsoil deposits into watersheds affect downstream coral reefs and can lead to mass coral mortality. Without anthropogenic influences, natural levels of suspended sediments on reefs are usually less than 5 mg/l and rarely exceed 40 mg/l (Kleypas, 1996; Larcombe et al., 1995), but on reefs adjacent to degraded watersheds, suspended sediments can reach 1,000 mg/l during periods of heavy rains (Rongo, 2004; Wolanski et al., 2003).

Other territories of the United States face similar sedimentation impacts, notably the US Virgin Islands. Over the past few decades, development increased on the island of Saint John due to increased tourism and part-time inhabitants (MacDonald et al., 2001). From 1990 to 2000, the Coral Bay area of St. John saw a population increase of 80% (U.S. Census Bureau, 2000) that gave rise to increased infrastructure, including new roads, residential and commercial construction (Brooks et al.,

2007). Reduction of natural vegetation and additional unpaved roads greatly increased erosion in the US Virgin Islands (Macdonald et al., 1997; Ramos-Scharrón and MacDonald, 2005) linked upslope sediment production with increased sediment impacts in the downstream marine environment (Nemeth and Sladek Nowlis, 2001).

Similar to the US Virgin Islands, Guam's population has doubled in the last 50 years (Guam Demographic Profile, 2019) leading to increased infrastructure and construction. While southern Guam remains less populated than the north (Figure 1D), construction and other human activities result in increased runoff and suspended sediment discharge into surrounding rivers and bays. In Fouha Bay, southern Guam, a large coral mortality event took place between 1988 and 1990 due to sediment runoff following land clearing and road construction (Richmond, 1993). The effects of this mortality event can still be seen today with lower coral diversity and abundance compared to baseline surveys (Randall and Birkeland, 1978).

Sedimentation

Terrestrial runoff or wave resuspension of deposits can expose corals to nutrient-rich sediment that can bleach or kill exposed tissues (Weber et al., 2006). Erosion and sediment runoff into coastal waters are natural processes, but anthropogenic effects have rapidly increased runoff (Rawlins et al. 1998). Areas exposed to coastal development are common zones with increased sedimentation, elevated in rich organic matter, that cover reef organisms after flood plumes and resuspension events (Nemeth and Sladek Nowlis, 2001). Across southern Guam, rainfall and resulting river discharge led to large sediment deposits into coastal habitats.

High suspended sediment concentrations can smother corals leading to mortality (Loya, 1976), decrease larval settlement by reducing available substrate, increase the energetic costs of corals to remove sediment (Stafford-Smith and Ormond, 1992), and reduce available energy for calcification (Bak, 1978; Walker and Ormond, 1982) and reproduction. These shifts in energetic

costs can promote tissue infection (Bruno et al., 2003; Fabricius, 2005; Nugues & Roberts, 2003) and leave corals exposed to pathogens and susceptible to disease. During acute sediment stress events, such as high river runoff during tropical storms, corals can experience lower light conditions that reduce photosynthesis while increasing energy expenditure leading to mortality (Erftemeijer et al., 2012).

Increased turbidity and sedimentation can create a tissue barrier that prevents gas exchange and removal of metabolic waste products (Stafford-Smith and Ormond, 1992). The organic matter in sediment can lead to microbially induced anoxia and reduced pH, which can cause coral mortality within as little as 15-48 hours (Miriam Weber et al., 2012). Sediment and silt enriched with organic materials have been shown to kill juvenile coral within hours to days while silt without enrichment was removed by the organism (Fabricius et al., 2003; Fabricius and Wolanski, 2000). Coral's sediment tolerance can be attributed to its efficiency in sediment removal and a timely shift from photo-autotrophy to heterotrophy (Anthony, 2000; Anthony and Fabricius, 2000). Corals that can acclimate to lower light conditions and quickly remove sediment may be more suited for reefs inundated with resuspended sediment. Nutrients in rivers from fertilizers, sewage, and eroding soils have increased globally compared to preindustrial times, affecting about 25% of coral reefs around the world (Burke et al., 2011). Sediment contains harmful substances such as pesticides and nutrients (Peters et al., 1997; Richmond, 1993), further exacerbating the impact of sedimentation. These nutrients and pollutants can attach to sediment particles and rapidly stimulate algal growth, creating a coral-to-algae phase shift (Minton, 2006).

The Coral Microbiome

A coral colony represents a complex holobiont comprised of the host coral and a microbial community including endosymbiotic algae and endolithic bacteria and archaea, viruses, and other protists (Bourne and Munn, 2005; Koren and Rosenberg, 2006; Rohwer et al., 2002; Sunagawa et al.,

2010). These microorganisms and their genetic material represent the coral microbiome and facilitate metabolic adaptations of the coral holobiont to local environmental conditions (Ainsworth et al., 2015; Kelly et al., 2014). The core microbiome is largely stable and associated with host microbiome interactions that are less sensitive to their environmental surroundings (Hester et al., 2016). However, there can be shifts during adaptation to environmental change (McFall-Ngai et al., 2013; Santos et al., 2014). Some coral-associated prokaryotes have been connected with beneficial functions in the coral holobiont (Grottoli et al., 2018) while others have shown deleterious effects on coral health and physiology (Peixoto et al., 2017).

In locations with reduced coral cover and increased fleshy algae, the coral microbial community can show a higher abundance of microbial pathogens (Dinsdale et al., 2008). Increased abundance of pathogens is positively correlated with increased prevalence of coral diseases (Sandin et al., 2008), highlighting that coral-associated prokaryotic communities may be linked to coral health. A "healthy", or beneficial (Peixoto et al., 2017) microbiome may guard coral from pathogens under stressful environmental conditions (Sweet and Bulling, 2017) and protect from further harmful processes (Webster and Reusch, 2017). Corals with a more diverse and stable microbiome tend to have higher resilience (Grottoli et al., 2018), a phenomenon linked to the insurance hypothesis. The insurance hypothesis posits that microbial diversity stabilizes microbial community function (Yachi and Loreau, 1999). Thus, the loss of diversity can lower microbial community function. However, sewage and sedimentation may bring microorganisms onto the reef microbial community which may destabilize microbiome function (Ziegler et al., 2016). Studies on the effects of sedimentation on corals have typically shown destabilization and decreased coral health (Fabricius, 2005; Goreau and Yonge, 1968; Rogers, 1990).

The Core Microbiome: Identity or function?

The *core microbiome* is composed of host species-specific members that are common among two or more communities (Hamady and Knight, 2009; Hernandez-Agreda et al., 2016; Sweet and Bulling, 2017; Turnbaugh et al., 2007). Identifying the Operational Taxonomic Units (OTUs) in the core microbiome is imperative due to their ability to remain relatively stable during environmental changes, continuing to provide key metabolic functions (Chu and Vollmer, 2016; Ainsworth et al., 2015; Shade and Handelsman, 2012; Shafquat et al. 2014). Central metabolic pathways associated with the core microbiome can aid in the understanding of how host-microbiome interactions are established and maintained (Ainsworth et al., 2015; Shafquat et al., 2014). Coral-associated prokaryotes can share core genes required to aid in the host's metabolic functions and other metabolic genes that can aid in host fitness. The same specialized metabolic genes and pathways can occur in different coral-associated prokaryotes under similar environmental conditions, as such, different groups may provide the same core functional redundancy (Martiny et al., 2006; Martiny et al., 2009). The functions of the core coral microbiome may be more important than taxonomic composition due to the wide range of functions across complex microbial communities (Burke et al., 2011). While taxonomic composition may shift across environmental conditions such as sedimentation gradients, microbiome function may be conserved across taxonomic groups.

Along with core coral microbiome, there is a variable *dynamic* or accessory microbiome, that changes with seasonality and habitat (Hester et al., 2016). The dynamic microbiome has the ability to adapt or evolve when facing environmental changes through microbiome-mediated transgenerational acclimatization (MMTA) (Webster and Reusch, 2017). This transient microbiome can be associated with strategic changes to quickly acclimate the holobiont to changes in the environment. Understanding the dynamic coral microbiome has implications for coral restoration and resilience due to identification of corals capable of adaptation to environmental conditions (Singh et al., 2013; Ziegler et al., 2017).

Environmental Effects on the Coral Microbiome

Coral reefs and their associated microbial communities are facing long-term, global disturbances such as rising temperatures. However, additional increases in chronic anthropogenic impacts are rapidly degrading reef conditions and shifting coral microbiomes (De'Ath et al., 2012; Pandolfi et al., 2003). Variations in the coral microbiome can be partially attributed to seasonal variability (Angly et al., 2016; Kimes et al., 2013; Meyer et al., 2014). Guam experiences a dry and wet season with increased rainfall and river discharge during the wet season. Increased river drainage from rain events directly relate to increased sedimentation and concentrations of pollutants (Angly et al., 2016; Brodie et al., 2010; Moreno-Madriñán et al., 2015). During high rainfall events, bacteria that are common among soil and plant root microbiomes can be found in near-shore marine waters (Angel et al., 2016), indicating runoff from nearby eroded areas.

Environmental stressors can directly or indirectly shift microbial communities, potentially leading to disease (Harvell et al., 2007; Mouchka et al., 2010), but the existing coral microbiome may play a role in limiting establishment of pathogenic microbes. Corals have the ability to acquire new symbionts to mitigate adverse environmental conditions that have led to the development of the coral probiotic hypothesis (Reshef et al., 2006; Rosenberg et al., 2007) . The hypothesis suggests that corals can shift their holobiont by selecting microbes to promote growth and persistence of the coral host under harsh environmental conditions (Rosenberg et al., 2007). One prominent bacterial genus in healthy corals is *Endozoicomonas* diseased or compromised corals often characterized by lower abundances of *Endozoicomonas* (Bayer et al., 2013; Glasl et al., 2016; Meyer et al., 2014; Morrow et al., 2017; Neave et al., 2016; Vezzulli et al., 2013). *Endozoicomonas* belongs to the family Endozoicomonadaceae in the order Oceanospirallales, a group of heterotrophic aerobic marine bacteria (Peixoto et al., 2017). Though not fully understood, *Endozoicomonas* is a diverse and flexible symbiotic group (Neave et al., 2017) that exists worldwide across several marine hosts. Some strains

of *Endozoicomonas* have been shown to produce antimicrobial compounds (Ritchie, 2006; Rua et al., 2014) that could act as a biological control for coral pathogens and prevent possible disease. A long-term study found a positive correlation between Proteobacteria and Actinobacteria abundances in the coral microbiome when Actinobacteria abundance was low, opportunistic Proteobacteria were more common suggesting that antibiotic producing Actinobacteria suppress opportunistic bacteria (Zaneveld et al., 2016).

The coral genus *Porites* has been a common study species for coral microbiome research across the globe (Glasl et al., 2016; Hadaidi et al., 2017; Meyer et al., 2014; Ziegler et al., 2019). *Porite*s species have been shown to act as generalists with high variation in their microbial communities compared to the coral genus *Acropora* (Dunphy et al., 2019). In Fouha Bay, *P. lobata* is the dominant structure-building species that can persist near the mouth of the river and continuing to grow along a sediment gradient (Rongo 2004). The coral microbiome can show plasticity, adapting to habitat differences and available nutrients (Kelly et al., 2014). Corals can also provide specific host-derived nutrients that allow for bacterial colonization (Littman et al., 2010) that can promote coral growth through beneficial bacteria. My work aimed to elucidate these interactions in *P. lobata* across the strong environmental gradient of Fouha Bay.

Objectives

The goal of this study was to examine the effects of sedimentation on the coral microbiome of *Porites lobata.*

Hypotheses

H_{01}: The coral-associated prokaryotic diversity of *P. lobata* will not be stable throughout the seasons and across the sites in Fouha Bay.

H_{A1}: The coral-associated prokaryotic diversity of *P. lobata* will be stable throughout the seasons and across the sites in Fouha Bay.

H_{02}: The coral-associated prokaryotic metabolic functions of *P. lobata* will not shift between sites and seasons in Fouha Bay.

H_{A2}: The coral-associated prokaryotic metabolic functions of *P. lobata* will shift between sites and seasons in Fouha Bay.

Materials and Methods

Sampling Sites

Coral samples were collected at two sites in Fouha Bay from September 2019 to May 2020 that represent the extreme ends of a sedimentation gradient (Table 1 and Figure 2). The inner site (13.306, 144.657) was located closer to the mouth of the river than the outer site (13.305, 144.657). The inner and outer bay sites were chosen due to availability of past environmental data, which showcase stark differences in sedimentation, accessibility from land and boat, and the typical yearly transport of river discharge into Fouha Bay from the La Sa Fua River (Randall and Birkeland 1978, Rongo 2004). The 400-meter-wide bay has a coral reef bisected by a channel that starts at the mouth of the La Sa Fua River. The channel's depth ranges from less than one meter near the river-mouth to 11 m depth at the mouth of the bay. Poor land management in the late 1980's during nearby road construction led to high sediment runoff into the bay that buried and killed many corals (Richmond). This led to a shift in coral reef community composition. Prior to road construction, there were 155 coral species in Fouha Bay (Randall and Birkeland 1978). Two decades later, there were 92 recorded species in Fouha Bay (Rongo 2004). Fouha Bay's high sedimentation rate stems from human induced fires, increased construction in undisturbed areas, and shifts from natural forests to invasive savannah grassland and badlands due to arson. The scorched earth exposes steep slopes with highly erodible lateritic soils (Rongo 2004). Between the wet and dry season, the La Sa

Fua watershed has an average annual rainfall of 2.5 m (United States Geological Survey). The dry season usually occurs from December to June and the wet season usually lasts from July to November with August to October as the wettest months.

Table 1. Coral sample collection dates during the wet season (September 9 – October 22) and dry season (December 7 – May 10) with number of corals collected from each site that yielded sequenced data.

Coral Sample Collection Dates	Inner Coral Collected with Sequence Data	Outer Coral Collected with Sequence Data
September 9	7	7
September 24	8	7
October 22	8	7
December 7	7	6
December 27	6	6
February 5	8	7
February 26	7	7
May 10	6	8

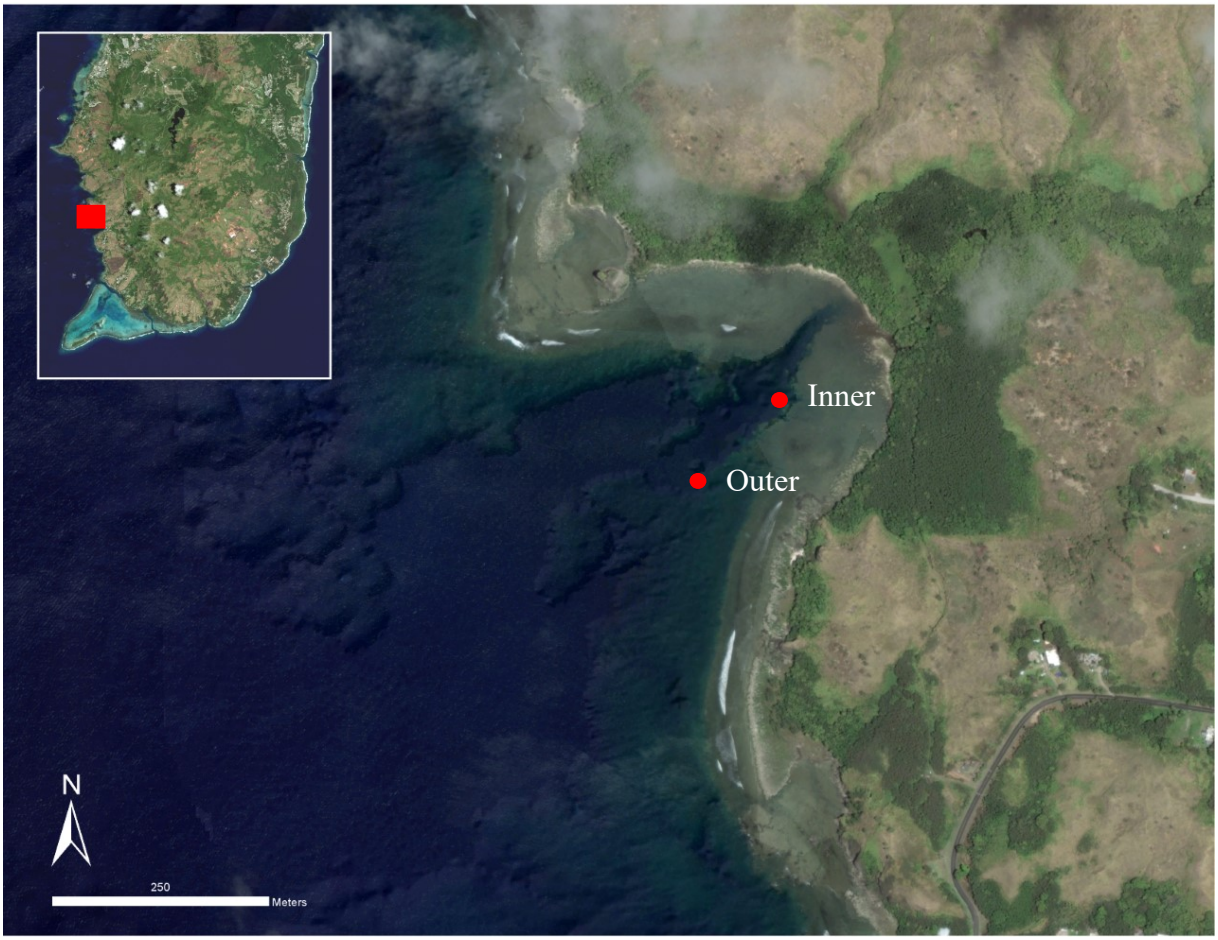


Figure 2. Inner (144°39'25.52E, 13°18'22.09N) and Outer (144°39'25.39E, 13°18'19.62N) Bay sites in Fouha Bay, Guam (Satellite imagery copyright 2014 DigitalGlobe Inc.; 2001 SHOALS Lidar bathymetry data provided by the U.S. Army Engineer Joint Airborne Lidar Bathymetry Technical Center of Expertise).

Sample collection

In Fouha Bay, *Porites lobata* is one of two species alongside *Leptastrea purpurea* that can survive the harshest conditions from river discharge such as high sedimentation rates, altered pH from freshwater runoff, and reduced salinity. Eight *P. lobata* specimens were collected at the Inner and Outer sites across the wet and dry seasons. Samples were collected across a 3-month period during the wet season and a 5-month period during the dry season. Each colony was marked with a numbered tag in nearby substrate to ensure replicate sampling of the same coral colonies through time. The parent colonies were located between 2-5 m depth at each site. A YSI 6-Series

Multiparameter Water Quality Sonde (Yellow Springs, Ohio) was deployed at each site to measure conductivity and temperature. Local watershed drainage and rainfall data were obtained from the U.S. Geological Survey (2019-2020) to analyze shifts in precipitation between the wet and dry season. Over the course of the 8-month study, 126 coral tissue samples were collected. The coral samples were collected from the center of the colonies with a hammer and chisel, placed individually in a pre-labeled whirley pack, and immediately frozen in liquid nitrogen. Upon return to the lab, samples were stored at -80°C. Each coral sample was grouped by month and location, ultimately categorized by site and season (Inner Wet, Inner Dry, Outer Wet, and Outer Dry).

Bacterial 16S metabarcoding

16S rRNA was extracted from tissue samples of 126 *P. lobata* using a DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) in conjunction with a QIAcube Connect (Qiagen, Hilden, Germany) following the manufacturer's protocol. The pure DNA extract was quantified with a Quibit (Qubit, Carlsbad, CA). DNA samples were sent to CD Genomics (Long Island, NY) for PCR amplification and for Illumina NovaSeq 6000 Sequencing (Illumina, San Diego, CA). The V4 hypervariable region of 16S ribosomal DNA was targeted for PCR with a 515F and 806R universal bacterial primers. Paired-end reads were assigned using samples' unique barcodes and the primer and barcode sequences were removed. Paired-end reads were merged using Fast Length Adjustment of Short reads (FLASH)(Magoč and Salzberg, 2011). This tool was designed to merge paired-end reads. These assembled raw tags were quality filtered retaining sequences with a Phred score of 20 to obtain high-quality clean tags using the QIIME V1.9.1 quality control protocol (Caporaso et al., 2012). Chimeras were detected using the UCHIME algorithm (Edgar et al., 2011) with the tags being compared to the reference database SILVA (https://www.arb-silva.de/). The final tags for analysis were obtained once the detected chimeras were removed.

Only sequences between 252 and 256 base pairs length were retained. Sequences were dereplicated using VSEARCH (Rognes et al., 2016) to improve downstream processing speeds. Operational Taxonomic Units (OTUs) were assigned at 99% similarity using de novo clustering (Westcott and Schloss, 2015). Chimeric sequences were identified and removed from the clustered sequences using VSEARCH (Rognes et al., 2016). The clustered sequences were rarefied at 60,000 sequences based off a rarefaction curve to account for variation across samples. The taxonomy was assigned in QIIME using the RDP classifier (Wang, 2007) against the GreenGenes Database (DeSantis et al., 2006). Chloroplast, mitochondria, and taxa only present in one sample (singletons) were removed from the OTU table. A multiple sequence alignment was performed on these sequence sets using Mafft (Katoh et al., 2002). The alignment was masked, or filtered, to remove highly variable positions that could add noise to a downstream phylogenetic inference. The unrooted phylogenetic tree was created using the Fasttree program (Price MN, Dehal PS, and Arkin AP, 2009) and the tree rooted created midpoint rooting (Kinene et al., 2016).

Bacterial diversity and function

The non-normalized OTU abundance matrix of the microbial community was used to calculate the microbial diversity indices for each of the 112 coral samples that remained after quality filtering. The OTUs were visualized in a taxa barplot at the phylum and genera levels in QIIME 2.0 (Bolyen et al., 2019). Taxonomic heat trees were created to show differences in taxonomic abundances across sites and seasons. Core microbial taxa that were present across 95% of samples were analyzed in QIIME across the site, season, site/season, and specific months. These core microbes were visualized as Venn Diagrams in R (Chen and Boutros, 2011). Alpha diversity was inferred using 7 unique analyses (Figure 3). However, the bacterial communities were analyzed using the Shannon Diversity Index for richness and evenness across site and season combinations. The

Shannon diversity scores were used in an ANOVA to test for differences amongst site/season combinations. An additional Tukey's Honest Significant Difference Test was used to test if there were significant differences among the site/season groups. The bacterial communities were analyzed using the weighted and unweighted unifrac distances to compare relative relatedness while incorporating their phylogenetic distances. Unweighted unifrac distances were used for further analysis, as this metric considered presence and absence of taxonomic groups without the abundance being a factor as the dominance of specific taxa would skew the data. The unweighted unifrac distances and Shannon diversity indices were used to create a Principal Coordinate Analysis (PCoA) for visualization for the site/seasonal groups. A three-dimensional Emperor Plot was created in QIIME 2.0 with an additional Biplot created in QIIME 2.0 highlighting the 5 most influential taxa across all site/season groups. An additional Emperor plot and Biplot were created to visualize the inner site, wet season group against the other combined groups according to the Tukey Honest Significant Difference Test.

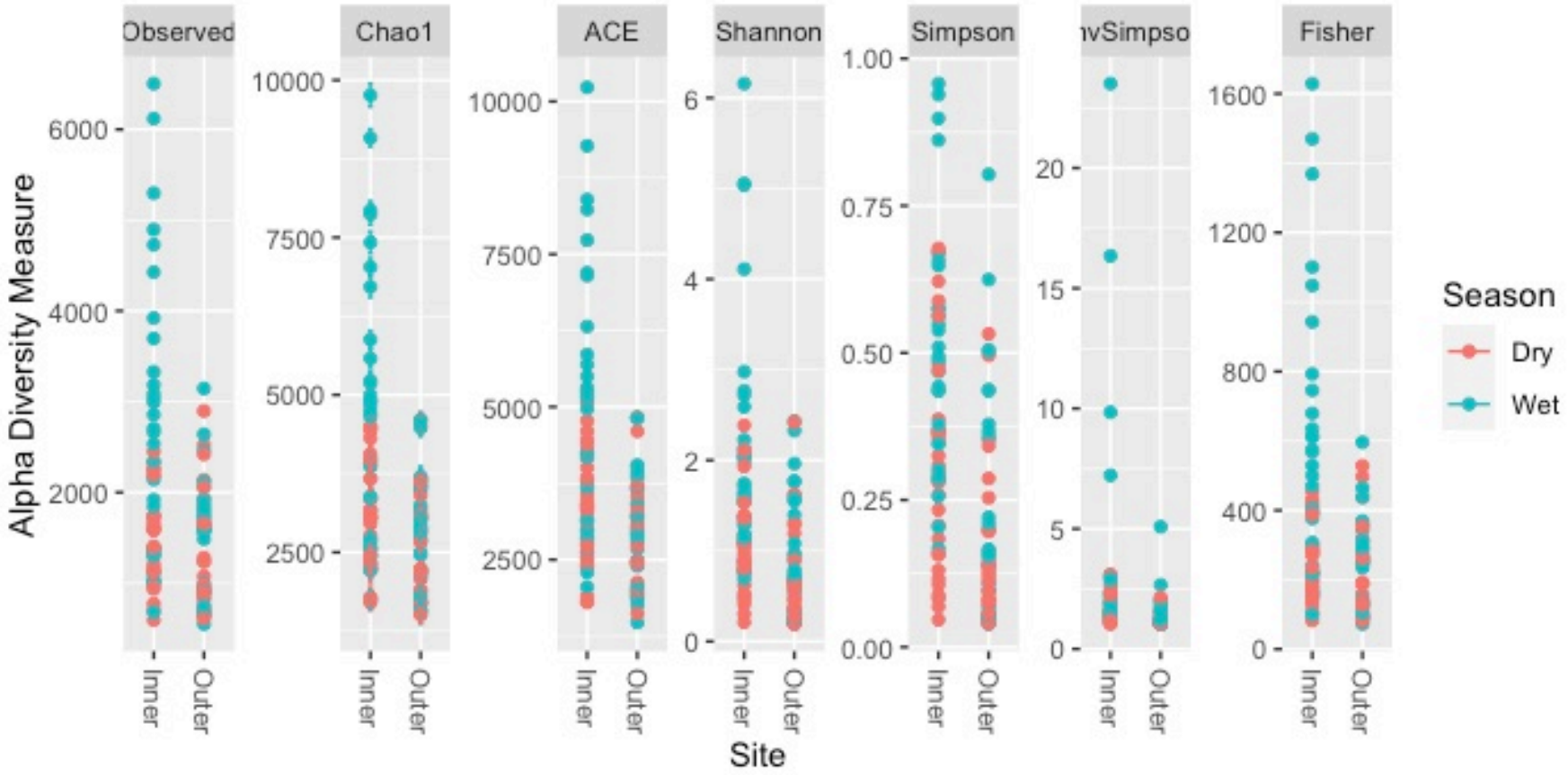


Figure 3. Coral microbiome alpha diversity indices for the inner and outer sites during the dry (red) and wet (blue) season.

Multivariate analyses tested the statistical difference of the microbial community structure in relation to site and seasonal. A Permutation Analysis of Variance (PERMANOVA) was conducted in QIIME with the default permutation settings (999) using the unweighted unifrac distance matrix. Analysis of Similarity (ANOSIM) and Adonis tests (Simpson et al., 2022) were used to evaluate the influence that site, season, and site/season have on the bacterial communities (Table 2 and Table 3).

Table 2. PERMANOVA results for Analysis of Similarity (ANOSIM) across site/seasons.

Method Name	**ANOSIM**
Test statistic name	R
Sample size	112
Number of groups	4
Test statistic	0.429949
P-value	0.001
Number of permutations	999

Table 3: PERMANOVA results for Adonis Test with Season, Site, and Sit / Season.

Adonis Test Results		
	R^2	Pr(>F)
Season	0.034359	0.001
Site	0.057026	0.001
Site / Season	0.130934	0.001

PICRUSTV2 (Caicedo et al., 2020), was used to assess metabolic functions between the Inner Wet group and the combined grouping of Inner Dry, Outer Wet, and Outer Dry to test if specific functions were conserved despite changing microbial diversity. The EC number database was used to predict the copy numbers of gene families that the relative abundance of OTUs were weighted with. These weighted EC abundancies were used to infer MetaCyc pathway abundances. The R package aldex (Gloor et al., 2020) was then used to create a general linear model (GLM) for pathway enrichment comparisons across the site/season groups. The inferred MetaCyc pathway were tested for statistical significance ($P<0.05$) and for their effect sizes. The effect sizes were then transformed by 1.42 (Fernandes et al., 2018) to convert to a more conservative Cohen's D score

with a large effect size considered 0.8. Significant metabolic functions that were overrepresented or underrepresented (P-value of <0.05) and had an effect size >1.12 and <-1.12.

Results

Environmental Data

Temperature ranged from ~31.5°C to ~26°C and were consistent between sites. However, there was a ~2°C drop from the wet season ending in November to the dry season starting in December. The conductivity ranged from ~55 to ~40 mS/cm with a small shift from the wet to the dry season. During this time, monthly river discharge (m^3/s) and monthly river gauge (m) height at the La Sa Fua River (U.S. Geological Survey) that showed consistent shifts from the wet season into the dry season (Figure 4).

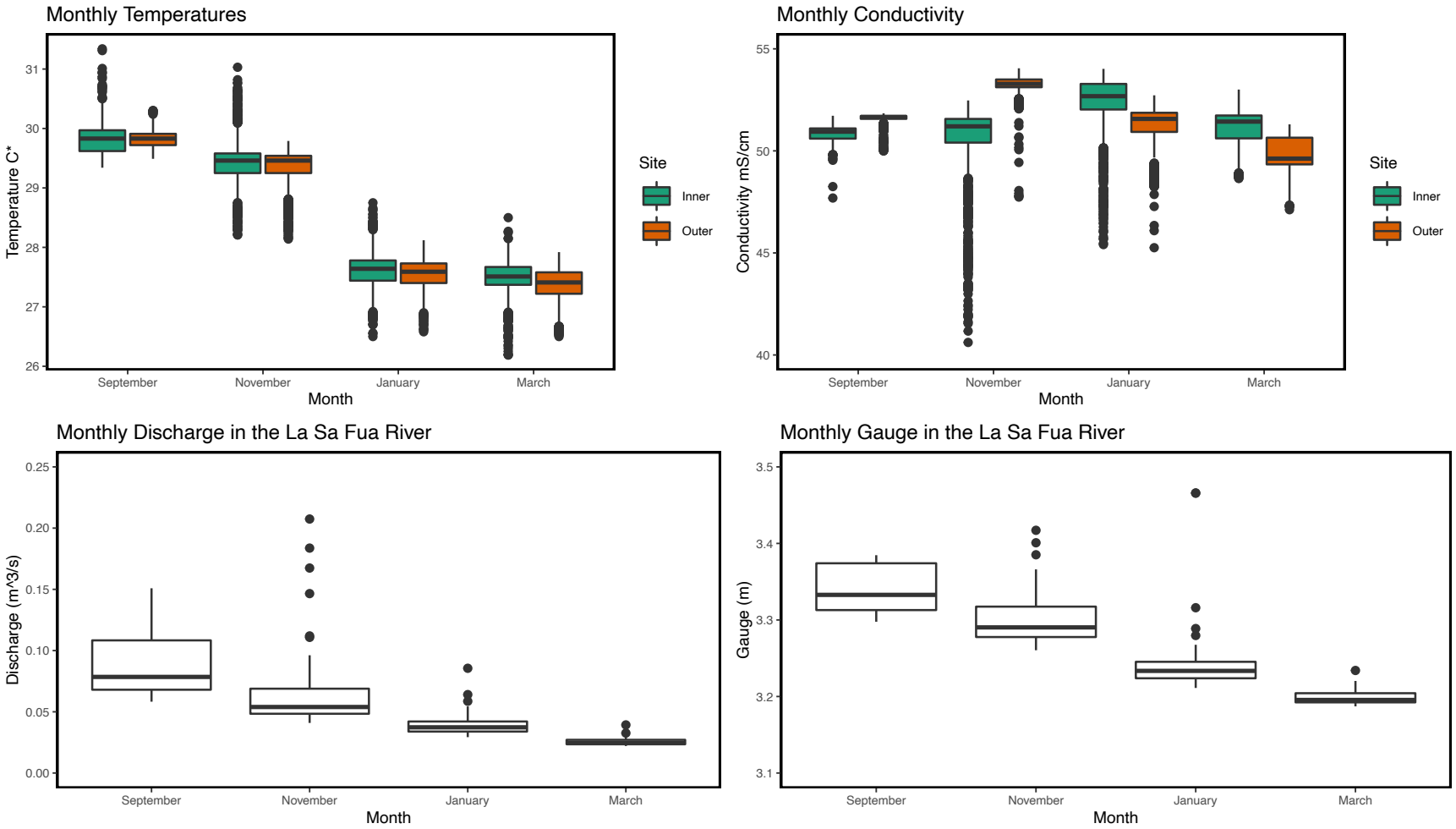


Figure 4. Environmental Data in Fouha Bay from the YSI 6-Series Multiparameter Water Quality Sonde and U.S. Geological Survey.

Bacterial Community Structure and Distribution across coral samples

A total of 126 coral samples yielded an average of 71,00 sequences. 3 outliers were filtered from the analysis that were not a sequence length of 252 to 256 base pairs. Based off the rarefaction curve at 60,000 reads, 11 samples were removed from further analysis due to low sequence yield. The coral samples yielded 113,698 OTUs. After filtering the samples and sequence reads, there were 112 total samples used to represent the Wet and Dry season at the Inner and Outer Bay Sites.

Bacterial Community Diversity

Across the 68 unique phyla in this study, each sample was largely dominated by the phylum Proteobacteria (93.3%) (Figure 5). Three groups allotted for 4.5% of non-rare taxa (above 1%), comprising of Firmicutes (1.7%), Cyanobacteria (1.4%), and Bacteroidetes (1.4%). At the genus level, the microbial communities showed to diversification across site/seasons (Figure 6). Across the 1646 genera across all samples, an unknown genus of the family Endozoicomonadaceae dominated comprising an average of 81.0%. The other dominant genus was *Herbaspirillium* (5.2%) with all other genera remaining rare with less that 1% overall abundance. The Inner Wet group showed the highest taxonomic diversity while the Outer Dry group showed the lowest taxonomic diversity (Figure 7).

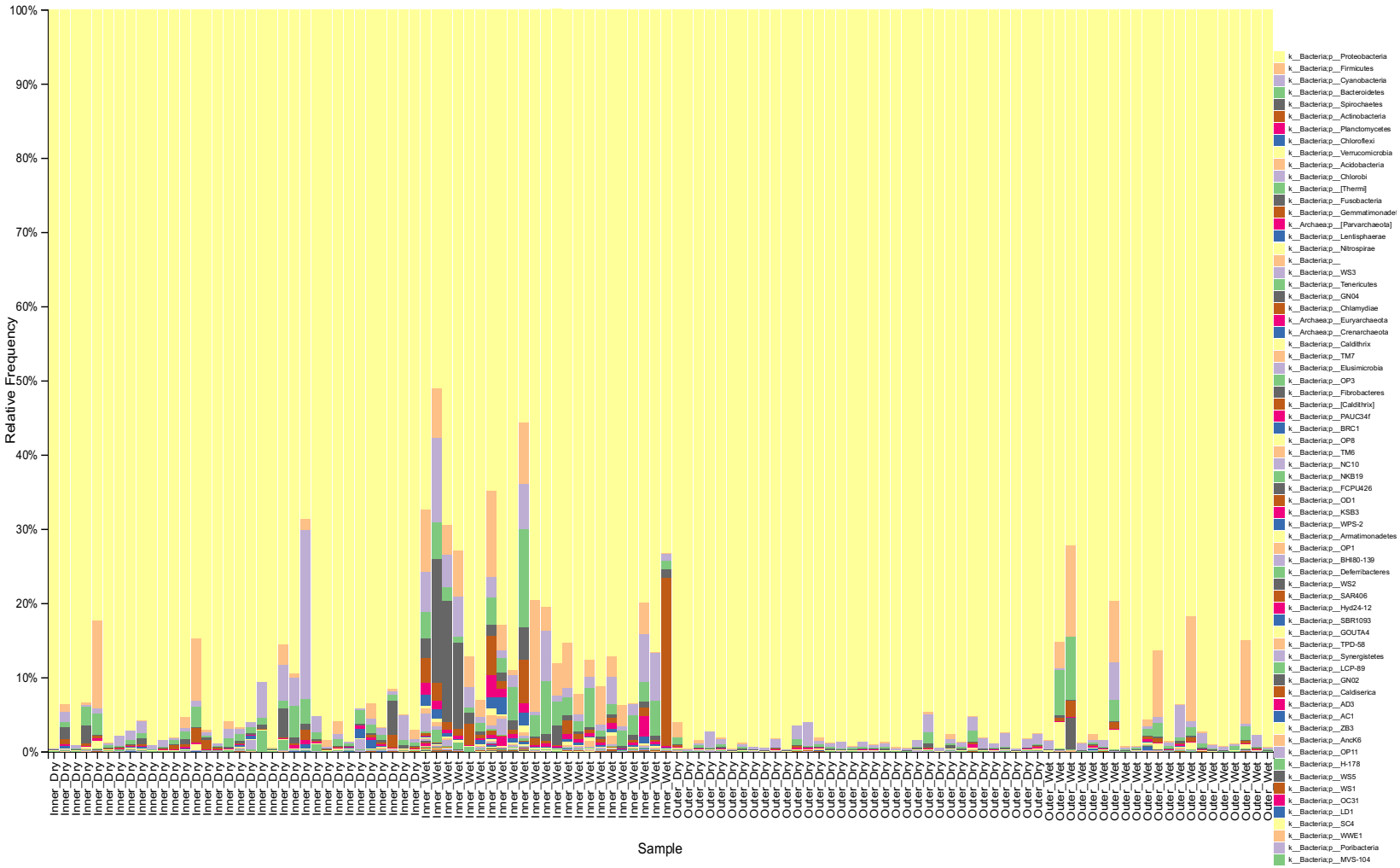


Figure 5. Taxonomic relative frequency percent at the phyla level across each site/season group (Inner Dry, Inner Wet, Outer Dry, Outer Wet) with the dominant phyla *Proteobacteria.*

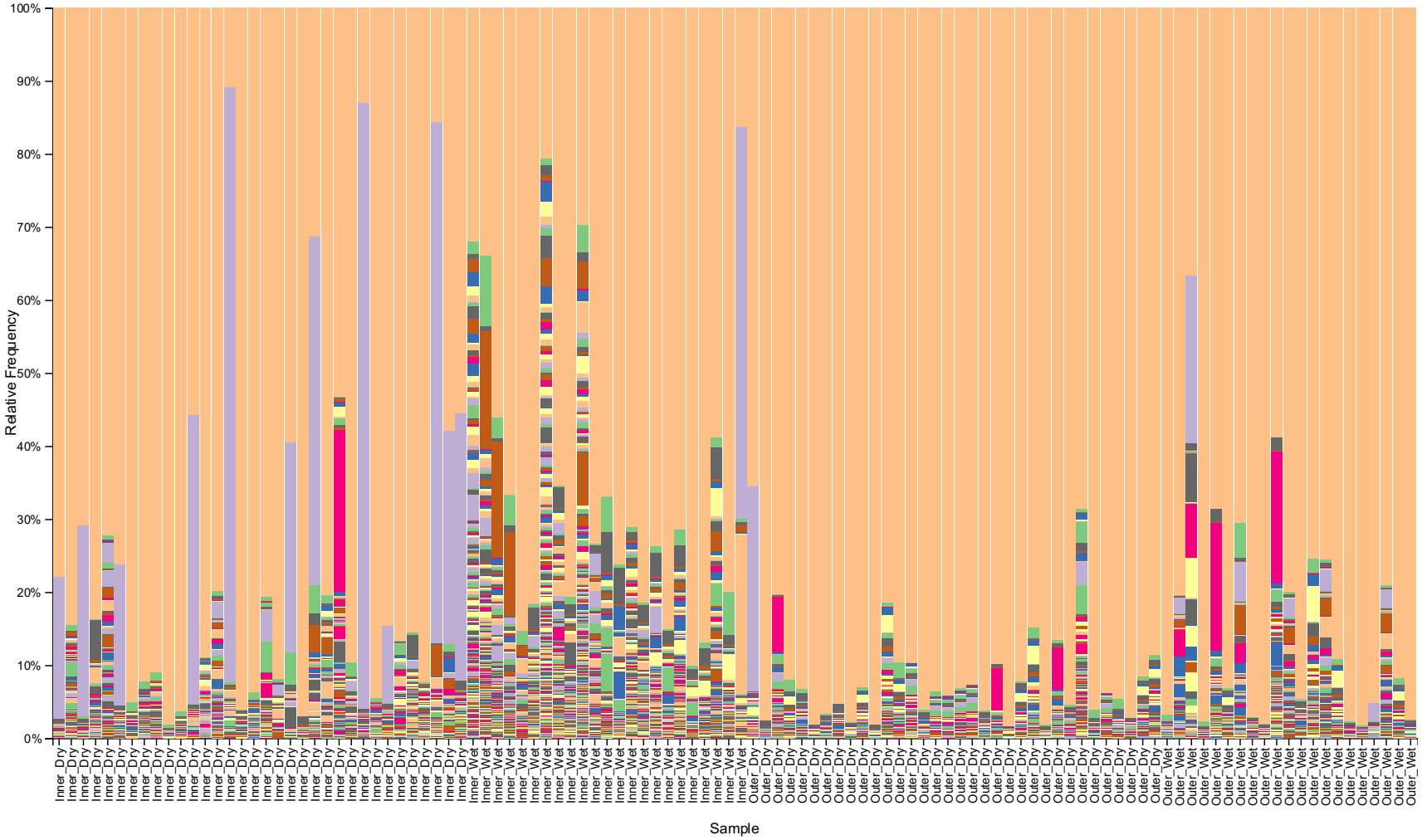


Figure 6. Taxonomic relative frequency percent at the genus level across each site/season group (Inner Dry, Inner Wet, Outer Dry, Outer Wet) with the dominant unknown genus from *Endozoicomonadaceae*.

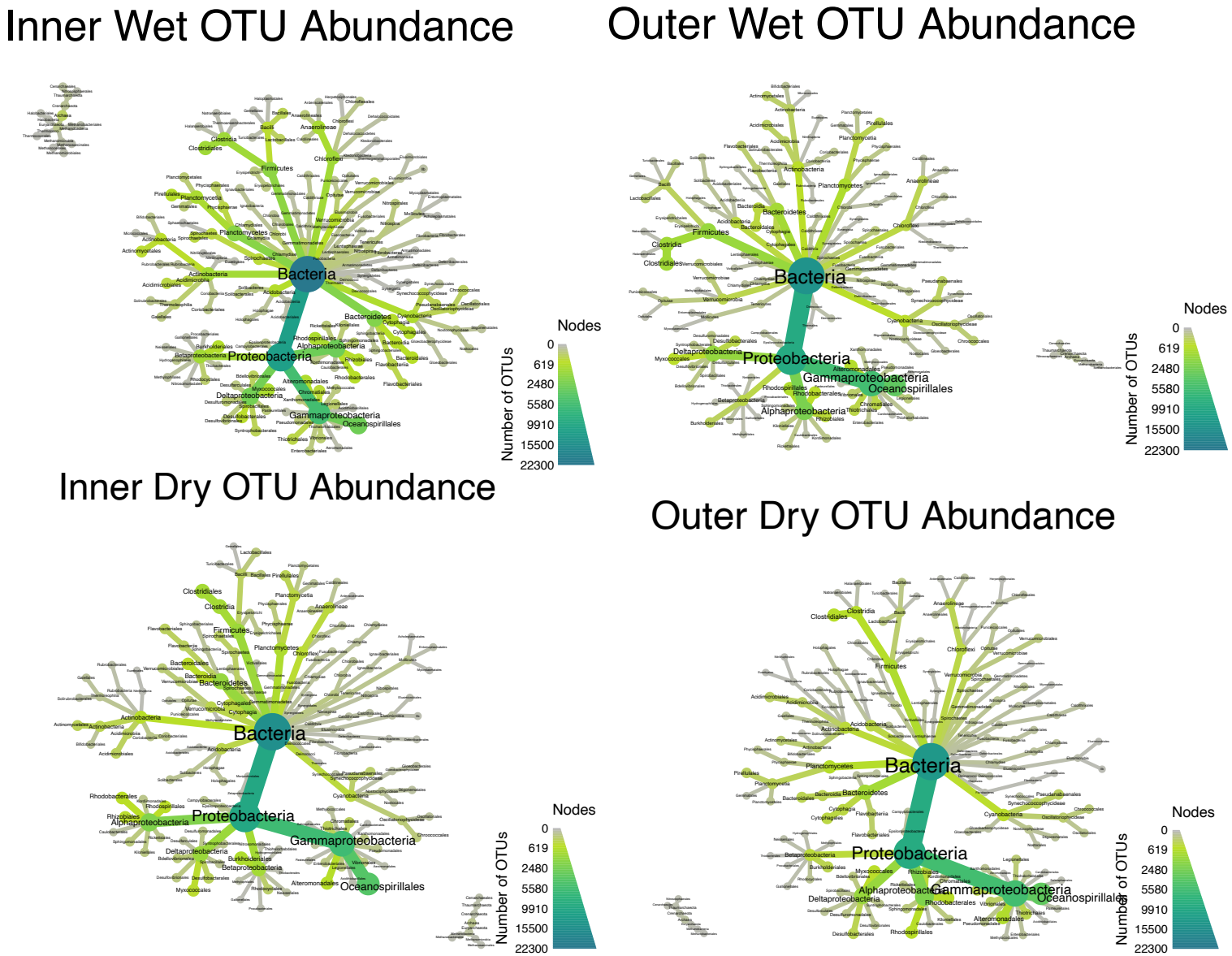


Figure 7. Heat tree based on taxonomic abundance for the Inner Wet, Outer Wet, Inner Dry, and Outer Dry groups.

Taxa that existed in 95% of a given microbiome were considered core microbes. Season and site showed no difference in their core microbiome (Figure 8), but the site/season groups and the Inner Wet group differed with 13 unique core microbes. The individual months in the Inner Wet group showed an increase in core microbes through time with the last time point of the dry season, October 22, consisting of 105 unique core microbes (Figure 8).

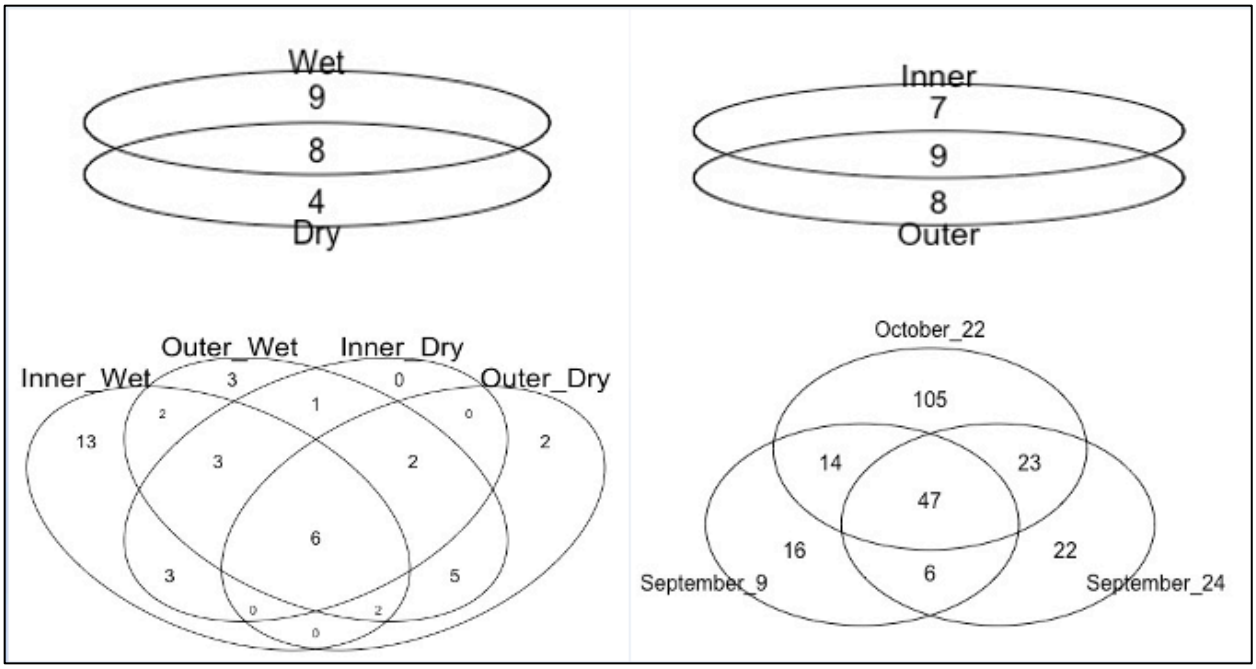


Figure 8. Core microbial taxa with comparisons between site, season, site and season, and the months of the Inner Wet Season.

Microbiome diversity at the Inner and Outer Sites follows seasonality (Figure 9). The Inner Wet group shows the highest diversity with a sharp decline entering the dry season (Figure 9). The Outer sites tend to display consistently low diversity across seasons (Figure 9). The Inner Wet groups high relative diversity is driven by the decrease in Proteobacteria (84.3%) and an increase in other groups such as Firmicutes (3.6%), Cyanobacteria (2.6%), Bacteroidetes (2.5%), and other rare taxa. At the genus level, a parallel increase of relative diversity with a decrease in the unknown genus of the family Endozoicomonadaceae (65.3%) and an increase in *Herbaspirillium* (8.4%) and *Chroocaccales* (1.7%) was observed. While the Inner Wet group was still dominated by Proteobacteria

and unknown genus of the family Endozoicomonadaceae at the phylum and genus level, there was a stark increase in rarer taxa that would revert to a lower relative diversity at the Inner Dry group and the Outer site during both seasons. An ANOVA of Shannon Diversity scores showed that the site/season groups were significantly different (P = 9.87e-09). A Tukey's Honest Significant Difference Test showed that the Inner Wet group was significantly different from the Inner Dry, Outer Wet, and Outer Dry groups.

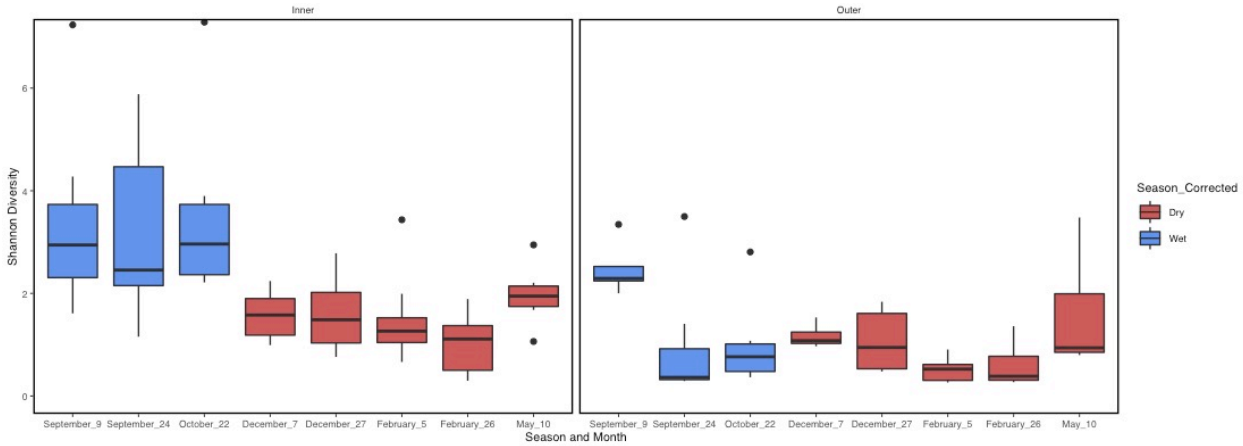


Figure 9. Shannon Diversity of the Inner and Outer site through the Wet (Blue) and the Dry Season (Red).

In the PCoA, the Inner Wet group formed a cluster sharing the most overlap with the Inner Dry group while Outer Dry group and Outer Wet groups formed a separate cluster (Figure 10). The three-dimensional Emperor Plot (Axis 1=12.52%, Axis 2= 5.565%, Axis 3= 4.223%) highlighted the separate clustering of the Inner Wet group away from the other individual site/season groups (Figure 11 A). The second Emperor Plot highlighted the isolated Inner Wet group against the other clustered groups dictated by the Tukey's Honest Significant Difference test (Figure 12A). The Biplot showcased the site/season groups overlayed with 5 most influential taxa indicated with arrows: Oceanospirallales Endozoicimonaceae, Oxalobacteraceae *Herbaspirillum*, Spirochaetaceae Spirochaeta, *Photbacterium damselae*, and Nostocales Nostocaceae (Figure 11B and 12B). It is worth noting that the unknown Endozoicomonadaceae (81.0%) and *Herbaspirillium* (5.2%) were the two

most abundant and influential taxa that separate the Inner Dry, Outer Wet, and Outer Dry groups away from the Inner Wet Group.

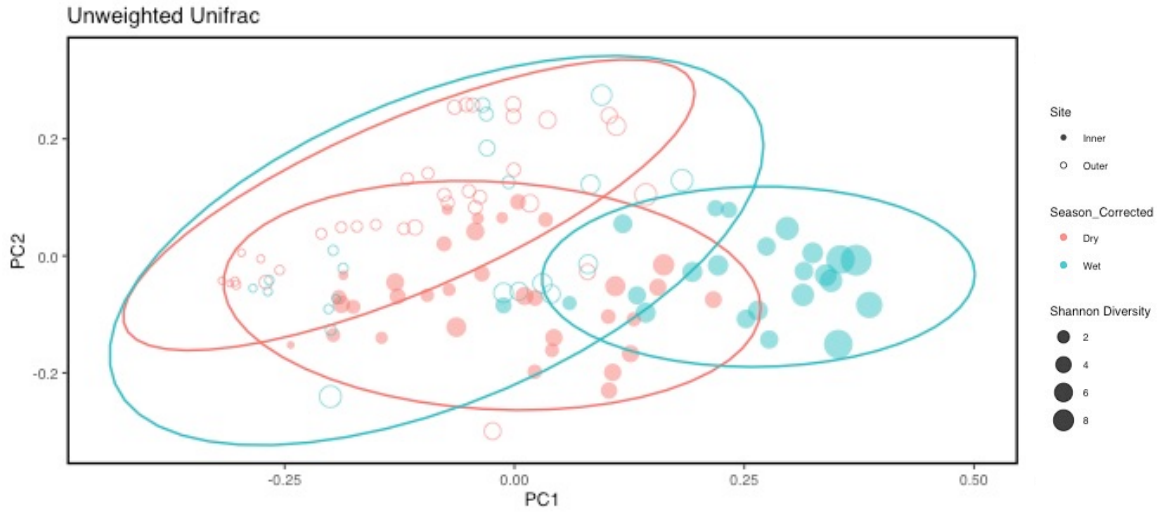


Figure 10. Each circle represents a coral sample from the study based on the presence/absence of bacterial taxa groups and their phylogenic distances. The seasons are represented by color (red and blue), the filled in and outlined circles represent site location, and the size of circle represents the Shannon diversity score.

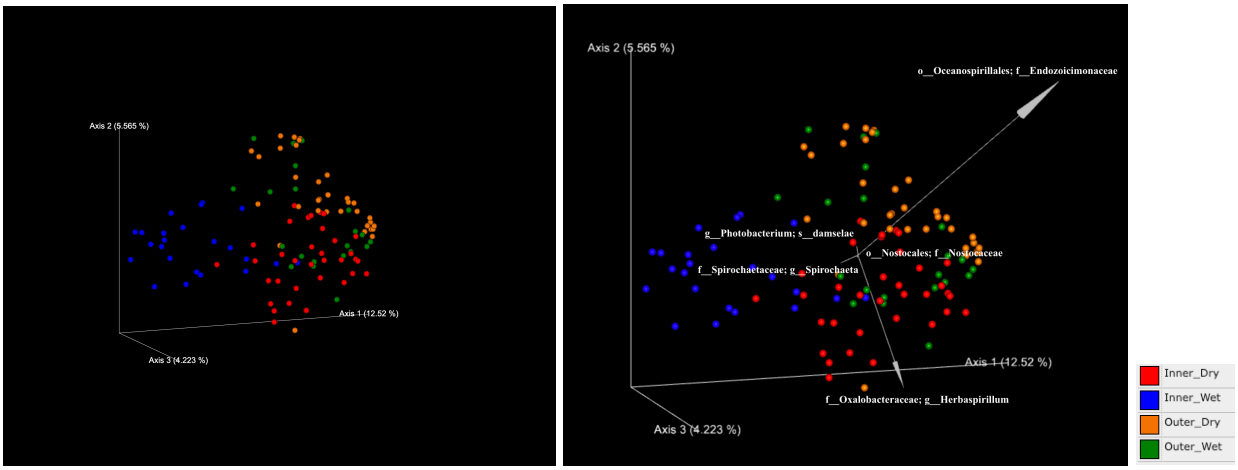


Figure 11 A-B. Three-dimensional Emperor plot of unweighted unifrac distances and Biplot of unweighted unifrac distances with 5 most influential taxa across site/seasons (Axis 1=12.52%, Axis 2= 5.565%, Axis 3= 4.223%).

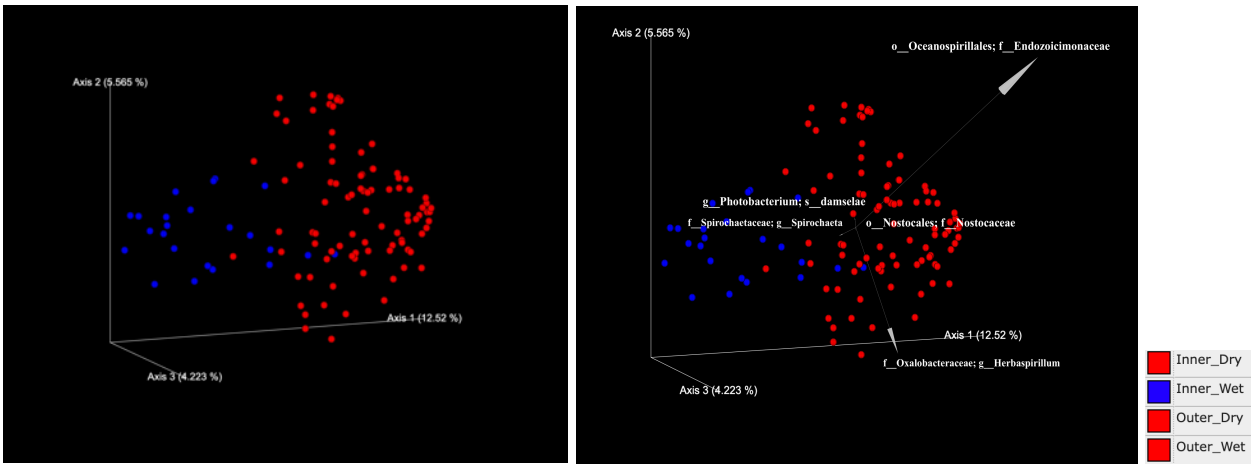


Figure 12 A-B. Three-dimensional Emperor plot of unweighted unifrac distances and Biplot of unweighted unifrac distances with 5 most influential taxa of the Inner Wet and other combined groups (Axis 1=12.52%, Axis 2= 5.565%, Axis 3= 4.223%).

The beta diversity of the site/season groups were tested via a PERMANOVA showing a significant difference across 999 permutations (Pseudo-F= 5.42, P-value=0.001). Further beta diversity tests showed a significant difference through the Analysis of Similarity (ANOSIM) and Adonis test. The ANOSIM showed the Inner Wet group's distance within itself was significantly lower than compared to distances to the other groups across 999 permutations (P=0.001, R=0.43) (Figure 13). An R value of 0.43 indicated a difference between groups while still containing some overlap. An Adonis test showed which factor was most influential in driving overall microbial diversity of a given group. While site, season, and site/season were all significant (P=0.001), the season alone had the least influence (R^2=0.034) and the site alone also had lower influence (R^2=0.057). The site/season combination showed the largest influence (R^2=0.13) likely driven by the Inner Wet group.

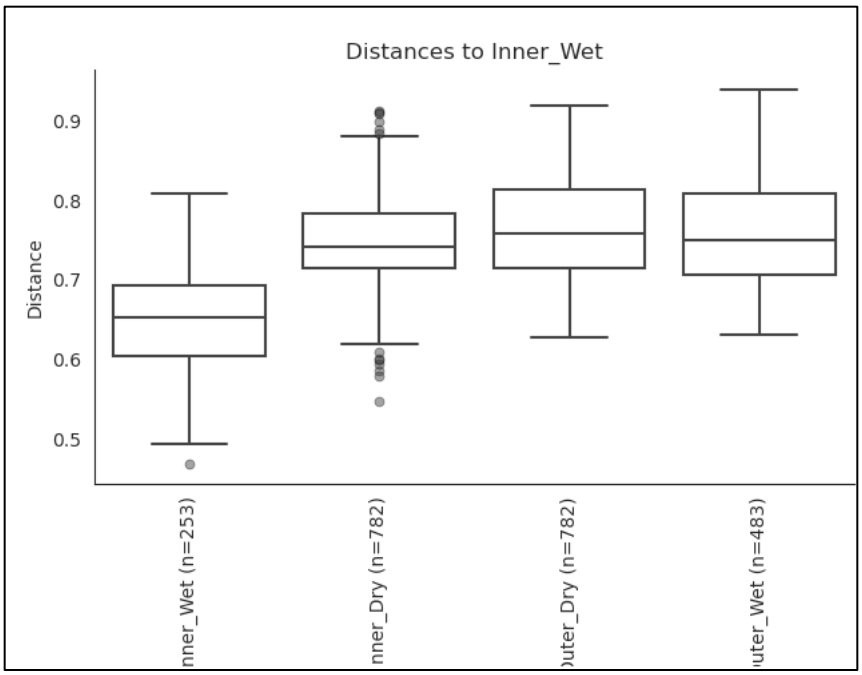


Figure 13. Analysis of Similarity (ANOSIM) visualizing distances between the Inner Wet Season against Inner Dry Season, Outer Dry Season, and the Outer Wet Season.

Microbiome Functional Profile

The bacterial functional metabolic pathways for each sample were inferred using PICRUSTV2 to identify the functional metabolic pathways that were overrepresented or underrepresented. The Inner Wet group was compared against a combined group of all others based on the Tukey Host Significant Difference Test. There were 175 significant functional pathways ($P<0.05$) with an effect size >1 (overrepresented) and <-1 (underrepresented) (Supplemental Figure 1). After transforming effect sizes by 1.42 to the more widely accepted Cohen's D, there were 26 functional metabolic pathways considered significant ($P<0.05$) with a large effect size (>0.8) (Table 4 and Figure 14). 5 overrepresented pathways were related to the TCA cycle IV, denitrification, and sulfate pathways. Of the 21 underrepresented pathways, the pathways were largely related to fermentation, carbohydrate synthesis, and amino acid pathways (Table 4).

Table 4. List of 26 significant metabolic functional pathways and their effect size.

Pathway	E**ffect** size (D)
TCA cycle IV (2-oxoglutarate decarboxylase)	1.35
DENITRIFICATION-PWY	1.21
thiamine diphosphate salvage II	1.13
SULFATE-CYS-PWY	1.13
UDPNAGSYN-PWY	1.13
ARGORNPROST-PWY	-1.13
hexitol fermentation to lactate, formate, ethanol and acetate	-1.15
superpathway of N-acetylneuraminate degradation	-1.17
mevalonate pathway I (eukaryotes and bacteria)	-1.18
FOLSYN-PWY	-1.19
chondroitin sulfate degradation I (bacterial)	-1.20
mycothiol biosynthesis	-1.20
superpathway of geranylgeranyldiphosphate biosynthesis I (via mevalonate)	-1.22
4-deoxy-L-threo-hex-4-enopyranuronate degradation	-1.23
glycerol degradation to butanol	-1.24
RHAMCAT-PWY	-1.30
2-nitrobenzoate degradation I	-1.31
2-amino-3-carboxymuconate semialdehyde degradation to 2-hydroxypentadienoate	-1.33
pyruvate fermentation to acetone	-1.33
L-tryptophan degradation to 2-amino-3-carboxymuconate semialdehyde	-1.34
LEU-DEG2-PWY	-1.34
reductive TCA cycle I	-1.36
mono-trans, poly-cis decaprenyl phosphate biosynthesis	-1.36
GALACT-GLUCUROCAT-PWY	-1.40
L-tryptophan degradation IX	-1.44
methyl ketone biosynthesis (engineered)	-1.47

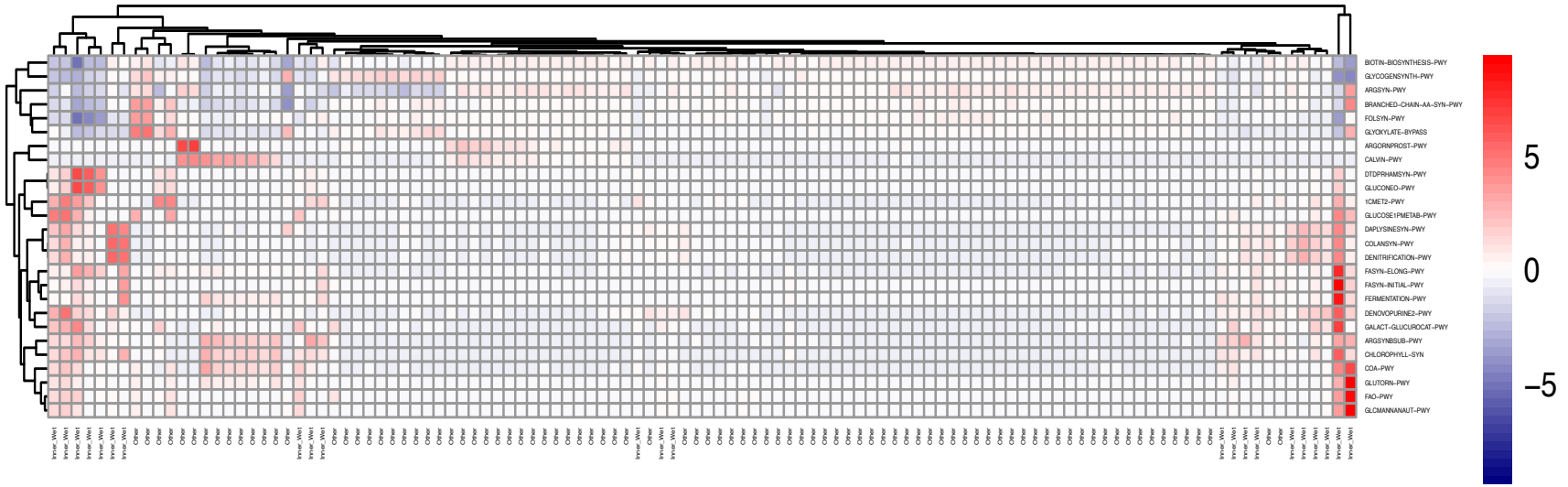


Figure 14. Z scores for the overrepresented or underrepresented functional metabolic pathways with large effect size (D) post transformation in coral samples ($p<0.05$).

Discussion

Environmental Differences in Fouha Bay

From September of 2019 to May of 2020, Fouha Bay showed stark differences in environmental parameters and microbial diversity between the wet and dry season. The initial intention of the project was to sample Inner and Outer sites in Fouha Bay for two four-month periods that transitioned from the wet to the dry season. However, the wet season ended earlier than anticipated nearing the end of November 2019 that ultimately shifted to a 3-month period during the wet season and a 5-month period during the dry season (Figure 4). It is worth noting that there were two typhoons, Typhoon Francisco and Typhoon Krosa, that originated near the Marianas at the beginning of August 2019 and were severe enough to delay the start of this project. While increased sedimentation and runoff typically dissipate before reaching the Outer site, these large tropical disturbances largely smothered the bay in sediment and brackish water that could have influenced the microbial diversity at the first time point in the study.

Microbiome Diversity

A previous study of *P. lobata* microbiomes along the Fouha Bay sediment gradient during one time point in the dry season showed no significant difference in microbiome along the gradient (Fifer et al., 2022). When compared, the relative microbial diversity among site and seasonal groups along the same gradient in this study were significantly different between the site/season groups. The Inner Wet group had the highest diversity and microbial instability of taxonomic groups (Figure 7) attributed to the Inner site's proximity to the river with increased river runoff, sedimentation, and available nutrients during the wet season. The Outer Dry group likely showed the highest microbial stability due to environmental stability between wet and dry seasons as well as the distance from the mouth of the La Sa Fua River that allowed for freshwater and sediment dissipation. The core

microbiome was influenced most at the site/season level, specifically the Inner Wet group. This group of rarer taxa unique to the Inner Wet group also increased through time within this site/season from 16 to 105. This shift was likely driven by chronic nutrient and sediment availability that increased influx of microbes, with coral microbiomes shifting to facilitate persistence of the host under harsh environmental conditions (Rosenberg et al., 2007). The correlation between the seasonal environmental shift and the drastic drop in core microbes unique to the Inner Dry group further indicates that the severe wet season conditions could drive the increase in core microbes. The harsh conditions experienced at the Inner Wet site may have allowed rarer taxa to outcompete Endozoicomonadaceae that decreased from 81.0% relative abundance across all samples to 65.3% in the Inner Wet group. Alternatively, the absolute number of bacteria in the microbiome may have increased.

While the microbiome of *P. lobata* colonies were largely dominated by a single bacteria phylum, Proteobacteria (93.3%) (Figure 5), an unknown genus of the family Endozoicomonadaceae (81%), and the genus *Herbaspirillium* (5.2%) (Figure 6), each site/season were characterized by unique prokaryotes. The Inner Dry group was characterized by Endozoicomonadaceae, thought to contribute to coral health (Neave et al., 2016) through production of antimicrobial compounds (Ritchie, 2006; Rua et al., 2014), and a stark increase in *Herbaspirillium*, a common river bacterium found in soil and plant microbiomes (Angel et al., 2016). The Inner Dry group's decreased exposure to rain events and reduced flushing of sediment load likely led to an accumulation of *Herbaspirillium* close to the mouth of the La Sa Fua River. The Inner Wet group was characterized by Endozoicomonadaceae and showed a significant increase in rarer taxa likely a result of the harsh environmental conditions *and* proximity to the river. Despite dominance by Endozoicomonadaceae, the Outer Dry group juxtaposed the Inner Wet group with the highest stability and lowest diversity of bacterial taxa. This can be explained by opposing sites and seasons with Inner Wet having the

harshest conditions near the river mouth and the Outer Dry having the least harsh environmental variation at the outer site. The Outer Wet group was dominated by Endozoicomonadaceae and an increase in Firmicutes that are largely seen in soils and sedimentation. This increase is likely driven by chronic dispersed sediment and nutrients affecting the inner and outer bay in the wet season. When comparing the Inner Wet group to the other groups, Endozoicomonadaceae and *Herbaspirillium* influenced the clustering of the Inner Dry, Outer Wet, and Outer Dry groups separate from the Inner Wet group (Figure 11A and 12B).

The site/seasonal groups' beta diversity was significantly different from the Inner Wet group (Figure 10) (Table 2). The Unweighted Unifrac distance matrix showed significance that site and season each played on the bacterial diversity and the effect that site/season played in conjunction. While the *post hoc* test indicated that the Inner Wet group's diversity was higher compared to the other groups' diversity, the outer sites highlighted the effect of seasonality. The Outer Dry group was encompassed within the Outer Wet group, suggesting that the wet season added diversity rather than having a compositional shift (Figure 10). The wet season is characterized by a compositional shift of the microbiome for inner and outer sites, as they are entirely separated (Figure 10). Importantly, the Inner Wet group showed the highest diversity during the harsh conditions of the wet season with likely highest nutrients. This increase in diversity is surprising considering that species of *Porites* are usually thought of as microbiome regulators (Li et al., 2014).

Microbiome Function

Underrepresented metabolic pathways in the Inner Wet group outnumbered overrepresented pathways when compared to the other groups (Table 4). The overrepresented functional pathways that were statistically significant and had a large effect size were related to the reverse tricarboxylic acid (TCA) cycle, denitrification, and sulfate assimilation and cysteine

biosynthesis. These enriched functional pathways were likely associated with the increase in sedimentation and available nutrients through the carbon, nitrogen, and sulfur cycles. The overrepresentation of the TCA cycle can be attributed to increased carbon fixation, as CO_2 and the photosynthate translocate to the host (Fiore et al., 2020), often seen in Cyanobacteria, which were identified in the microbiome. With an increase in nutrients in river runoff, specifically nitrogen, the denitrification pathway may have been overrepresented to compensate for a shift in the N:P ratio. Denitrification may have also been overrepresented due to smothering of the corals with sediment, creating areas for anaerobic bacterial metabolism and nitrogen related byproducts (Shashar et al., 1993). The sulfate assimilation and cysteine biosynthesis pathway may have been upregulated, as the sulfur-containing amino acid, cysteine, plays a key role in the synthesis of antioxidants and vitamins (Saito 2004). *Acropora* is believed to not possess an essential enzyme for cysteine biosynthesis and ultimately relies on its symbionts for survival during harsh environmental conditions (Shinzato et al., 2011). By contrast, *Porites* can synthesize their own cysteine *and* rely on its symbionts during harsher conditions (Shinzato et al., 2014).Cysteine stabilizes proteins and is likely vital for mitigating the impacts of environmental disturbances in corals. The likely increase in sulfur and sulfate at the Inner site in Fouha Bay may have come from fires in the hills above the La Sa Fua River. Ash and organic matter may be swept downstream altering the sulfur levels in Fouha Bay.

While the insurance hypothesis states microbial diversity stabilizes microbial community function (Grottoli et al., 2018), increased sedimentation and nutrient loads can destbilize microbial communities through influx of additional microbial diversity (Ziegler et al., 2016). The Inner Wet group shows a stark increase in prokaryotic diversity with significantly more underrepresented metabolic pathways than the overrepresented pathways, indicating microbial function instability. This microbial functional instability in the Inner Wet group can be attributed to the harsh environmental conditions and proximity to the river. When compared to the 5 overrepresented

functional pathways, the Inner Wet group had 21 underrepresented functional pathways. The pathways were largely related to fermentation, carbohydrate synthesis, and amino acid pathways attributable to *Endozoicomonas* that may aid in overall coral health, growth, and sexual reproduction (Bayer et al., 2013; Neave et al. 2016, 2017) under dry season conditions and become disrupted during harsh environmental conditions as seen in 2019. A reduction in fermentation-related pathways and increase in denitrification suggests a shift from aerobic to anaerobic bacterial metabolism, consistent with increased sediment and nutrient loads that likely reduce available oxygen. As the Inner site is inundated with river runoff, key microbial functions may become underrepresented, but function at sufficient levels to allow for coral holobiont persistence. While the coral may not be able to survive in this state indefinitely, the change of season with the emergence of the dry season may allow for a return to homeostasis.

Conclusions

In this study, the coral microbiome of *Porites lobata* was assessed for shifts in prokaryotic diversity across the wet and dry season at an inner and outer site in Fouha Bay. The data showed that site and season had the highest influence on the coral microbiome diversity in Fouha Bay. Site and season led to shifts in key metabolic functions of the microbiome at the inner site during the wet season. Following the wet season, microbiome diversity and function returned to a normal state. This study highlights the anthropogenic impacts on watersheds and the resulting shifts od coral microbiome communities. Interestingly, shifts in microbiome diversity and function were observed in P. lobata, a coral generally considered to tightly regulate its microbiome even when impacted by environmental changes.

References

Abraham, T., et al. "Status of the Coral Reefs in Micronesia and American Samoa." *Status of Coral Reefs of the World*, 2004.

Angel, Roey, et al. "The Root-Associated Microbial Community of the World's Highest Growing Vascular Plants." *Microbial Ecology*, 2016, doi:10.1007/s00248-016-0779-8.

Angly, Florent E., et al. "Marine Microbial Communities of the Great Barrier Reef Lagoon Are Influenced by Riverine Floodwaters and Seasonal Weather Events." *PeerJ*, 2016, doi:10.7717/peerj.1511.

Anthony, K. R. N. "Enhanced Particle-Feeding Capacity of Corals on Turbid Reefs (Great Barrier Reef, Australia)." *Coral Reefs*, 2000, doi:10.1007/s003380050227.

Anthony, Kenneth R. N., and Katharina E. Fabricius. "Shifting Roles of Heterotrophy and Autotrophy in Coral Energetics under Varying Turbidity." *Journal of Experimental Marine Biology and Ecology*, 2000, doi:10.1016/S0022-0981(00)00237-9.

Athens, J. Stephen, and Jerome V. Ward. "Holocene Vegetation, Savanna Origins and Human Settlement of Guam." *Pacific Odyssey: Archaeology and Anthropology in the Western Pacific. Papers in Honor of Jim Specht.*, 2004.

Bak, Rolf P. M. "Lethal and Sublethal Effects of Dredging on Reef Corals." *Marine Pollution Bulletin*, 1978, doi:10.1016/0025-326X(78)90275-8.

Bayer, Till, et al. "The Microbiome of the Red Sea Coral Stylophora Pistillata Is Dominated by Tissue-Associated Endozoicomonas Bacteria." *Applied and Environmental Microbiology*, 2013, doi:10.1128/AEM.00695-13.

Bolyen, Evan, et al. "Reproducible, Interactive, Scalable and Extensible Microbiome Data Science Using QIIME 2." *Nature Biotechnology*, vol. 37, no. 8, 2019, pp. 852–57, doi:10.1038/s41587-019-0209-9.

Bourne, David G., and Colin B. Munn. "Diversity of Bacteria Associated with the Coral Pocillopora Damicornis from the Great Barrier Reef." *Environmental Microbiology*, 2005, doi:10.1111/j.1462-2920.2005.00793.x.

Brodie, Jon, et al. "Dispersal of Suspended Sediments and Nutrients in the Great Barrier Reef Lagoon during River-Discharge Events: Conclusions from Satellite Remote Sensing and Concurrent Flood-Plume Sampling." *Marine and Freshwater Research*, 2010, doi:10.1071/MF08030.

Brooks, Gregg R., et al. "Sedimentary Development of Coral Bay, St. John, USVI: A Shift from Natural to Anthropogenic Influences." *Caribbean Journal of Science*, 2007, doi:10.18475/cjos.v43i2.a8.

Bruno, John F., et al. "Nutrient Enrichment Can Increase the Severity of Coral Diseases." *Ecology Letters*, 2003, doi:10.1046/j.1461-0248.2003.00544.x.

Burke, Lauretta, et al. "Reefs at Risk Revisited." *Reefs at Risk Revisited*, 2011, doi:10.1016/0022-0981(79)90136-9.

Caicedo, H. Hugo, et al. "Overcoming Barriers to Early Disease Intervention." *Nature Biotechnology*, vol. 38, no. 6, 2020, pp. 669–73, doi:10.1038/s41587-020-0550-z.

Caporaso, J. Gregory, et al. "Ultra-High-Throughput Microbial Community Analysis on the Illumina HiSeq and MiSeq Platforms." *ISME Journal*, 2012, doi:10.1038/ismej.2012.8.

Chen, Hanbo, and Paul C. Boutros. *VennDiagram: A Package for the Generation of Highly-Customizable Venn and Euler Diagrams in R*. 2011, doi:10.1186/1471-2105-12-35.

Chu, Nathaniel D., and Steven V. Vollmer. "Caribbean Corals House Shared and Host-Specific Microbial Symbionts over Time and Space." *Environmental Microbiology Reports*, 2016, doi:10.1111/1758-2229.12412.

"Coral Disease, Environmental Drivers, and the Balance between Coral and Microbial Associates." *Oceanography*, 2007, doi:10.5670/oceanog.2007.91.

D Ainsworth, Tracy, et al. "The Coral Core Microbiome Identifies Rare Bacterial Taxa as Ubiquitous Endosymbionts." *The Isme Journal*, vol. 9, The Author(s), Apr. 2015, p. 2261, https://doi.org/10.1038/ismej.2015.39.

De'Ath, Glenn, et al. "The 27-Year Decline of Coral Cover on the Great Barrier Reef and Its Causes." *Proceedings of the National Academy of Sciences of the United States of America*, 2012, doi:10.1073/pnas.1208909109.

DeSantis, T. Z., et al. "Greengenes, a Chimera-Checked 16S RRNA Gene Database and Workbench Compatible with ARB." *Applied and Environmental Microbiology*, vol. 72, no. 7, 2006, pp. 5069–72, doi:10.1128/AEM.03006-05.

DeVantier, L. M., et al. "Species Richness and Community Structure of Reef-Building Corals on the Nearshore Great Barrier Reef." *Coral Reefs*, 2006, doi:10.1007/s00338-006-0115-8.

Dinsdale, Elizabeth A., et al. "Microbial Ecology of Four Coral Atolls in the Northern Line Islands." *PLoS ONE*, 2008, doi:10.1371/journal.pone.0001584.

Done, T. J. "Patterns in the Distribution of Coral Communities across the Central Great Barrier Reef." *Coral Reefs*, 1982, doi:10.1007/BF00301691.

Dunphy, Courtney M., et al. "Structure and Stability of the Coral Microbiome in Space and Time." *Scientific Reports*, 2019, doi:10.1038/s41598-019-43268-6.

Edgar, Robert C., et al. *UCHIME Improves Sensitivity and Speed of Chimera Detection*. Vol. 27, no. 16, 2011, pp. 2194–200, doi:10.1093/bioinformatics/btr381.

Erftemeijer, Paul L. A., et al. "Environmental Impacts of Dredging and Other Sediment Disturbances on Corals: A Review." *Marine Pollution Bulletin*, 2012, doi:10.1016/j.marpolbul.2012.05.008.

Fabricius, K. E., et al. "Effects of Transparent Exopolymer Particles and Muddy Terrigenous Sediments on the Survival of Hard Coral Recruits." *Estuarine, Coastal and Shelf Science*, 2003, doi:10.1016/S0272-7714(02)00400-6.

Fabricius, K. E., and E. Wolanski. "Rapid Smothering of Coral Reef Organisms by Muddy Marine Snow." *Estuarine, Coastal and Shelf Science*, 2000, doi:10.1006/ecss.1999.0538.

Fabricius, Katharina E. "Effects of Terrestrial Runoff on the Ecology of Corals and Coral Reefs: Review and Synthesis." *Marine Pollution Bulletin*, 2005, doi:10.1016/j.marpolbul.2004.11.028.

Fernandes, Andrew D., et al. *A Reproducible Effect Size Is More Useful than an Irreproducible Hypothesis Test to Analyze High Throughput Sequencing Datasets*. 2018, pp. 1–11, http://arxiv.org/abs/1809.02623.

Fifer, James E., et al. "Microbiome Structuring Within a Coral Colony and Along a Sedimentation Gradient." *Frontiers in Marine Science*, vol. 8, no. January, 2022, doi:10.3389/fmars.2021.805202.

Fiore, Cara L., et al. "Trait-Based Comparison of Coral and Sponge Microbiomes." *Scientific Reports*, vol. 10, no. 1, Springer US, 2020, pp. 1–17, doi:10.1038/s41598-020-59320-9.

Glasl, Bettina, et al. "The Microbiome of Coral Surface Mucus Has a Key Role in Mediating Holobiont Health and Survival upon Disturbance." *ISME Journal*, 2016, doi:10.1038/ismej.2016.9.

Golbuu, Yimnang, et al. "Gradients in Coral Reef Communities Exposed to Muddy River Discharge in Pohnpei, Micronesia." *Estuarine, Coastal and Shelf Science*, 2008, doi:10.1016/j.ecss.2007.06.005.

Goreau, T. F., and C. M. Yonge. "Coral Community on Muddy Sand." *Nature*, 1968, doi:10.1038/217421a0.

Grottoli, Andréa G., et al. "Coral Physiology and Microbiome Dynamics under Combined Warming and Ocean Acidification." *PLoS ONE*, 2018, doi:10.1371/journal.pone.0191156.

Hadaidi, Ghaida, et al. "Stable Mucus-Associated Bacterial Communities in Bleached and Healthy

Corals of Porites Lobata from the Arabian Seas." *Scientific Reports*, 2017, doi:10.1038/srep45362.
Hamady, Micah, and Rob Knight. "Microbial Community Profiling for Human Microbiome Projects: Tools, Techniques, and Challenges." *Genome Research*, 2009, doi:10.1101/gr.085464.108.
Hernandez-Agreda, Alejandra, et al. "The Microbial Signature Provides Insight into the Mechanistic Basis of Coral Success across Reef Habitats." *MBio*, 2016, doi:10.1128/mBio.00560-16.
Hester, Eric R., et al. "Stable and Sporadic Symbiotic Communities of Coral and Algal Holobionts." *ISME Journal*, 2016, doi:10.1038/ismej.2015.190.
Howarth, Robert, et al. "Coupled Biogeochemical Cycles: Eutrophication and Hypoxia in Temperate Estuaries and Coastal Marine Ecosystems." *Frontiers in Ecology and the Environment*, 2011, doi:10.1890/100008.
Katoh, Kazutaka, et al. "MAFFT: A Novel Method for Rapid Multiple Sequence Alignment Based on Fast Fourier Transform." *Nucleic Acids Research*, vol. 30, no. 14, 2002, pp. 3059–66, doi:10.1093/nar/gkf436.
Kelly, Linda W., et al. "Local Genomic Adaptation of Coral Reef-Associated Microbiomes to Gradients of Natural Variability and Anthropogenic Stressors." *Proceedings of the National Academy of Sciences of the United States of America*, 2014, doi:10.1073/pnas.1403319111.
Kimes, Nikole E., et al. "The Montastraea Faveolata Microbiome: Ecological and Temporal Influences on a Caribbean Reef-Building Coral in Decline." *Environmental Microbiology*, 2013, doi:10.1111/1462-2920.12130.
Kinene, T., et al. "Rooting Trees, Methods For." *Encyclopedia of Evolutionary Biology*, no. January, 2016, pp. 489–93, doi:10.1016/B978-0-12-800049-6.00215-8.
Kleypas, J. A. "Coral Reef Development under Naturally Turbid Conditions: Fringing Reefs near Broad Sound, Australia." *Coral Reefs*, 1996, doi:10.1007/BF01145886.
Koren, Omry, and Eugene Rosenberg. "Bacteria Associated with Mucus and Tissues of the Coral Oculina Patagonica in Summer and Winter." *Applied and Environmental Microbiology*, 2006, doi:10.1128/AEM.00554-06.
Larcombe, P., et al. "Factors Controlling Suspended Sediment on Inner-Shelf Coral Reefs, Townsville, Australia." *Coral Reefs*, 1995, doi:10.1007/BF00367235.
Li, Jie, et al. *Bacterial Dynamics within the Mucus, Tissue and Skeleton of the Coral Porites Lutea during Different Seasons*. doi:10.1038/srep07320.
Littman, Raechel A., et al. "Responses of Coral-Associated Bacterial Communities to Heat Stress Differ with Symbiodinium Type on the Same Coral Host." *Molecular Ecology*, 2010, doi:10.1111/j.1365-294X.2010.04620.x.
Loya, Y. "Effects of Water Turbidity and Sedimentation." *Bulletin of Marine Science,* 1976.
Macdonald, Lee H., et al. "Paradise Threatened: Land Use and Erosion on St. John, US Virgin Islands." *Environmental Management*, 1997, doi:10.1007/s002679900072.
MacDonald, Lee H., et al. "Runoff and Road Erosion at the Plot and Road Segment Scales, St John, US Virgin Islands." *Earth Surface Processes and Landforms*, 2001, doi:10.1002/1096-9837(200103)26:3<251::AID-ESP173>3.0.CO;2-X.
Magoč, Tanja, and Steven L. Salzberg. "FLASH: Fast Length Adjustment of Short Reads to Improve Genome Assemblies." *Bioinformatics*, vol. 27, no. 21, Nov. 2011, pp. 2957–63, doi:10.1093/BIOINFORMATICS/BTR507.
Martiny, Adam C., Maureen L. Coleman, et al. "Phosphate Acquisition Genes in Prochlorococcus Ecotypes: Evidence for Genome-Wide Adaptation." *Proceedings of the National Academy of Sciences of the United States of America*, 2006, doi:10.1073/pnas.0601301103.
Martiny, Adam C., Satish Kathuria, et al. "Widespread Metabolic Potential for Nitrite and Nitrate Assimilation among Prochlorococcus Ecotypes." *Proceedings of the National Academy of Sciences of*

the United States of America, 2009, doi:10.1073/pnas.0902532106.
McFall-Ngai, Margaret, et al. "Animals in a Bacterial World, a New Imperative for the Life Sciences." *Proceedings of the National Academy of Sciences of the United States of America*, 2013, doi:10.1073/pnas.1218525110.
Meyer, Julie L., et al. "Community Shifts in the Surface Microbiomes of the Coral Porites Astreoides with Unusual Lesions." *PLoS ONE*, 2014, doi:10.1371/journal.pone.0100316.
Minton, Dwayne. *Fire, Erosion, and Sedimentation in the Asan-Piti Watershed and War in the Pacific NHP, Guam*. Jan. 2006.
Moreno-Madriñán, Max J., et al. "Using Remote Sensing to Monitor the Influence of River Discharge on Watershed Outlets and Adjacent Coral Reefs: Magdalena River and Rosario Islands, Colombia." *International Journal of Applied Earth Observation and Geoinformation*, 2015, doi:10.1016/j.jag.2015.01.008.
Morrow, Kathleen M., et al. "Allelochemicals Produced by Brown Macroalgae of the Lobophora Genus Are Active against Coral Larvae and Associated Bacteria, Supporting Pathogenic Shifts to Vibrio Dominance." *Applied and Environmental Microbiology*, 2017, doi:10.1128/AEM.02391-16.
Mouchka, Morgan E., et al. "Coral-Associated Bacterial Assemblages: Current Knowledge and the Potential for Climate-Driven Impacts." *Integrative and Comparative Biology*, 2010, doi:10.1093/icb/icq061.
Neave, Matthew J., Rita Rachmawati, et al. "Differential Specificity between Closely Related Corals and Abundant Endozoicomonas Endosymbionts across Global Scales." *ISME Journal*, 2017, doi:10.1038/ismej.2016.95.
Neave, Matthew J., Amy Apprill, et al. "Diversity and Function of Prevalent Symbiotic Marine Bacteria in the Genus Endozoicomonas." *Applied Microbiology and Biotechnology*, 2016, doi:10.1007/s00253-016-7777-0.
Nemeth, Richard S., and J. Sladek Nowlis. "Monitoring the Effects of Land Development on the Near-Shore Reef Environment of St. Thomas, USVI." *Bulletin of Marine Science*, 2001.
Nugues, M. M., and C. M. Roberts. "Coral Mortality and Interaction with Algae in Relation to Sedimentation." *Coral Reefs*, 2003, doi:10.1007/s00338-003-0338-x.
Pandolfi, John M., et al. "Global Trajectories of the Long-Term Decline of Coral Reef Ecosystems." *Science*, 2003, doi:10.1126/science.1085706.
Peixoto, Raquel S., et al. "Beneficial Microorganisms for Corals (BMC): Proposed Mechanisms for Coral Health and Resilience." *Frontiers in Microbiology*, 2017, doi:10.3389/fmicb.2017.00341.
Peters, Esther C., et al. "Ecotoxicology of Tropical Marine Ecosystems." *Environmental Toxicology and Chemistry*, 1997, doi:10.1897/1551-5028(1997)016<0012:EOTME>2.3.CO;2.
Ramos-Scharrón, Carlos E., and Lee H. MacDonald. "Measurement and Prediction of Sediment Production from Unpaved Roads, St John, US Virgin Islands." *Earth Surface Processes and Landforms*, 2005, doi:10.1002/esp.1201.
Rawlins, B. G., et al. "Review of Agricultural Pollution in the Caribbean with Particular Emphasis on Small Island Developing States." *Marine Pollution Bulletin*, 1998, doi:10.1016/S0025-326X(98)00054-X.
Reshef, Leah, et al. "The Coral Probiotic Hypothesis." *Environmental Microbiology*, 2006, doi:10.1111/j.1462-2920.2006.01148.x.
Richmond, Robert H. "Coral Reefs: Present Problems and Future Concerns Resulting from Anthropogenic Disturbance." *Integrative and Comparative Biology*, 1993, doi:10.1093/icb/33.6.524.
Ritchie, Kim B. "Regulation of Microbial Populations by Coral Surface Mucus and Mucus-Associated Bacteria." *Marine Ecology Progress Series*, 2006, doi:10.3354/meps322001.
Rogers, CS. "Responses of Coral Reefs and Reef Organisms to Sedimentation." *Marine Ecology*

Progress Series, 1990, doi:10.3354/meps062185.
Rognes, Torbjørn, et al. "VSEARCH: A Versatile Open Source Tool for Metagenomics." *PeerJ*, vol. 2016, no. 10, 2016, pp. 1–22, doi:10.7717/peerj.2584.
Rohwer, Forest, et al. "Diversity and Distribution of Coral-Associated Bacteria." *Marine Ecology Progress Series*, 2002, doi:10.3354/meps243001.
Rosenberg, Eugene, et al. "The Role of Microorganisms in Coral Health, Disease and Evolution." *Nature Reviews Microbiology*, 2007, doi:10.1038/nrmicro1635.
Rua, Cintia P. J., et al. "Diversity and Antimicrobial Potential of Culturable Heterotrophic Bacteria Associated with the Endemic Marine Sponge Arenosclera Brasiliensis." *PeerJ*, 2014, doi:10.7717/peerj.419.
Saito, Kazuki. "Sulfur Assimilatory Metabolism. The Long and Smelling Road." *Plant Physiology*, vol. 136, no. 1, Sept. 2004, pp. 2443–50, doi:10.1104/pp.104.046755.
Sandin, Stuart A., et al. "Baselines and Degradation of Coral Reefs in the Northern Line Islands." *PLoS ONE*, 2008, doi:10.1371/journal.pone.0001548.
Santos, Henrique F., et al. "Climate Change Affects Key Nitrogen-Fixing Bacterial Populations on Coral Reefs." *ISME Journal*, 2014, doi:10.1038/ismej.2014.70.
Shade, Ashley, and Jo Handelsman. "Beyond the Venn Diagram: The Hunt for a Core Microbiome." *Environmental Microbiology*, 2012, doi:10.1111/j.1462-2920.2011.02585.x.
Shafquat, Afrah, et al. "Functional and Phylogenetic Assembly of Microbial Communities in the Human Microbiome." *Trends in Microbiology*, 2014, doi:10.1016/j.tim.2014.01.011.
Shashar, N., et al. "Extreme Diel Fluctuations of Oxygen in Diffusive Boundary Layers Surrounding Stony Corals." *Biological Bulletin*, vol. 185, no. 3, 1993, pp. 455–61, doi:10.2307/1542485.
Shinzato, Chuya, Mayuri Inoue, et al. "A Snapshot of a Coral 'Holobiont': A Transcriptome Assembly of the Scleractinian Coral, Porites, Captures a Wide Variety of Genes from Both the Host and Symbiotic Zooxanthellae." *PLoS ONE*, vol. 9, no. 1, Public Library of Science, Jan. 2014, doi:10.1371/JOURNAL.PONE.0085182.
Shinzato, Chuya, Eiichi Shoguchi, et al. "Using the Acropora Digitifera Genome to Understand Coral Responses to Environmental Change." *Nature*, 2011, doi:10.1038/nature10249.
Simpson, Gavin L., et al. *Package 'Vegan' R Topics Documented:* 2022.
Singh, Yadvir, et al. "Emerging Importance of Holobionts in Evolution and in Probiotics." *Gut Pathogens*, 2013, doi:10.1186/1757-4749-5-12.
Stafford-Smith, M. G., and R. F. G. Ormond. "Sediment-Rejection Mechanisms of 42 Species of Australian Scleractinian Corals." *Marine and Freshwater Research*, 1992, doi:10.1071/MF9920683.
Sunagawa, Shinichi, et al. "Threatened Corals Provide Underexplored Microbial Habitats." *PLoS ONE*, 2010, doi:10.1371/journal.pone.0009554.
Sweatman, H., et al. "Assessing Loss of Coral Cover on Australia's Great Barrier Reef over Two Decades, with Implications for Longer-Term Trends." *Coral Reefs*, 2011, doi:10.1007/s00338-010-0715-1.
Sweatman, H, et al. "Status of Near-Shore Reefs of the Great Barrier Reef 2004." *Marine and Tropical Sciences Research Facility Research Report Series*, 2007.
Sweet, Michael J., and Mark T. Bulling. "On the Importance of the Microbiome and Pathobiome in Coral Health and Disease." *Frontiers in Marine Science*, 2017, doi:10.3389/fmars.2017.00009.
Turnbaugh, Peter J., et al. "The Human Microbiome Project." *Nature*, 2007, doi:10.1038/nature06244.
Vezzulli, Luigi, et al. "16SrDNA Pyrosequencing of the Mediterranean Gorgonian Paramuricea Clavata Reveals a Link among Alterations in Bacterial Holobiont Members, Anthropogenic Influence and Disease Outbreaks." *PLoS ONE*, 2013, doi:10.1371/journal.pone.0067745.

Walker, D. I., and R. F. G. Ormond. "Coral Death from Sewage and Phosphate Pollution at Aqaba, Red Sea." *Marine Pollution Bulletin*, 1982, doi:10.1016/0025-326X(82)90492-1.

Weber, M., et al. "Sedimentation Stress in a Scleractinian Coral Exposed to Terrestrial and Marine Sediments with Contrasting Physical, Organic and Geochemical Properties." *Journal of Experimental Marine Biology and Ecology*, 2006, doi:10.1016/j.jembe.2006.04.007.

Weber, Miriam, et al. "Mechanisms of Damage to Corals Exposed to Sedimentation." *Proceedings of the National Academy of Sciences*, May 2012, p. 201100715, doi:10.1073/pnas.1100715109.

Webster, Nicole S., and Thorsten B. H. Reusch. "Microbial Contributions to the Persistence of Coral Reefs." *ISME Journal*, 2017, doi:10.1038/ismej.2017.66.

Westcott, Sarah L., and Patrick D. Schloss. "De Novo Clustering Methods Outperform Reference-Based Methods for Assigning 16S RRNA Gene Sequences to Operational Taxonomic Units." *PeerJ*, vol. 2015, no. 12, 2015, doi:10.7717/peerj.1487.

Wolanski, Eric, et al. "Water and Fine Sediment Dynamics in Transient River Plumes in a Small, Reef-Fringed Bay, Guam." *Estuarine, Coastal and Shelf Science*, 2003, doi:10.1016/S0272-7714(02)00321-9.

Yachi, Shigeo, and Michel Loreau. "Biodiversity and Ecosystem Productivity in a Fluctuating Environment: The Insurance Hypothesis." *Proceedings of the National Academy of Sciences of the United States of America*, 1999, doi:10.1073/pnas.96.4.1463.

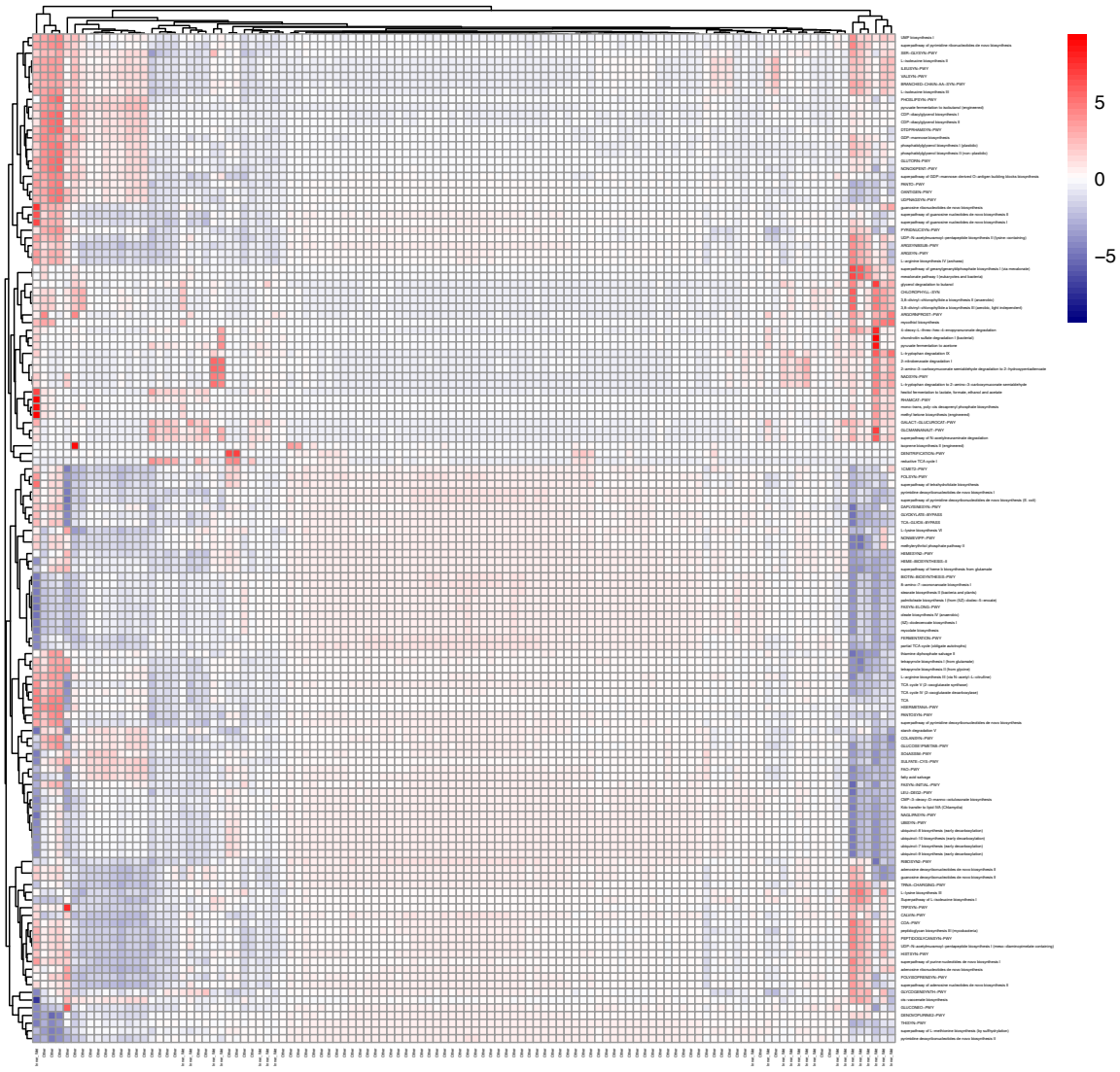
Zaneveld, Jesse R., et al. "Overfishing and Nutrient Pollution Interact with Temperature to Disrupt Coral Reefs down to Microbial Scales." *Nature Communications*, 2016, doi:10.1038/ncomms11833.

Ziegler, Maren, Francois O. Seneca, et al. "Bacterial Community Dynamics Are Linked to Patterns of Coral Heat Tolerance." *Nature Communications*, 2017, doi:10.1038/ncomms14213.

Ziegler, Maren, Carsten G. B. Grupstra, et al. "Coral Bacterial Community Structure Responds to Environmental Change in a Host-Specific Manner." *Nature Communications*, 2019, doi:10.1038/s41467-019-10969-5.

Ziegler, Maren, Anna Roik, et al. "Coral Microbial Community Dynamics in Response to Anthropogenic Impacts near a Major City in the Central Red Sea." *Marine Pollution Bulletin*, 2016, doi:10.1016/j.marpolbul.2015.12.045.

Supplemental Figure



Supplemental Figure 1. Z scores for the overrepresented or underrepresented functional metabolic pathways with large effect size in coral samples prior to Cohen transformation ($p<0.05$).