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Differential Responses of Coral-Associated Microbiomes to Elevated Temperatures Across the Indonesian Archipelago at Species, Local, and Regional Scales

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Differential Responses of Coral-Associated Microbiomes to Elevated Temperatures Across the Indonesian Archipelago at Species, Local, and Regional Scales

A dissertation submitted in partial satisfaction of the requirement for the degree Doctor of Philosophy in Biology

by

Rita Rachmawati

2018

ABSTRACT OF THE DISSERTATION

Differential Responses of Coral-Associated Microbiomes

to Elevated Temperatures Across the Indonesian Archipelago

at Species, Local, and Regional Scales

by

Rita Rachmawati

Doctor of Philosophy in Biology

University of California, Los Angeles, 2018

Professor Paul Henry Barber, Chair

Coral reefs are among the world's most biodiverse and valuable ecosystems, but they are in global decline. One major concern for the future of reefs is mass coral bleaching, a phenomenon where corals turn white after expelling their endosymbiotic algae (*Symbiodinium*) during periods of elevated sea surface temperatures. Unfortunately, the frequency and severity of mass coral bleaching is increasing as climate change results in global sea surface temperature rise. Extensive research has examined how *Symbiodinium* variation influences bleaching susceptibility, yet *Symbiodinium* is only one part of a complex coral holobiont that also include bacteria and archaea.

This dissertation investigates variation in coral microbiomes in the world's largest and most diverse coral reef ecosystem, the Indonesian Archipelago. Using a common garden approach, Chapter 1 investigates how the microbiome of *Porites lobata* varies with water temperature among two nearby reef ecosystems that experience different levels of coral bleaching—Amed and Pemuteran, Bali. Results reveal significant local variation in microbiomes, but no consistent changes in microbiomes with temperature. Chapter 2 examines how the coral microbiomes of three species of coral with different bleaching susceptibility (*Acropora millepora, Pocillopora verrucosa,* and *Porites lobata*) respond to elevated water temperatures in a single location, Manokwari, West Papua. Results show significant differences in microbiomes in all three species, indicating species-specific relationships between coral host and microbial symbionts. However, while microbiomes changed with elevated temperature, there were no consistent patterns by species or by propensity to bleach. Lastly, chapter 3 examines natural variation in microbiomes of these same three species of coral across 5000 km of the Indonesian Archipelago, including reefs with historically low, moderate, and high bleaching susceptibility. Results varied by species: microbiomes of *Acropora millepora* showed no significant geographic variation; microbiomes of *Pocillopora verrucosa* were significantly different among most locations, but not among regions; and microbiomes of *Porites lobata* showed significant variation based on regional variation in bleaching susceptibility, but not among individual localities. Combined, these studies show that coral microbiomes are complex, varying by species, reef, and geographic region, providing novel insights into the nature and specific relationship of microbiomes to coral host and their environment

The dissertation of Rita Rachmawati is approved.

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Willette, D.A., S.E. Simmonds, S.H. Cheng, S. Esteves, T.L. Kane, H. Nuetzel, N. Pilaud, **R. Rachmawati**, and P.H. Barber. 2017. Using DNA barcoding to track seafood mislabeling in Los Angeles restaurants. *Conservation Biology* **31**:1076-1085

Simmonds, S.E., V. Chou, S. Cheng, **R. Rachmawati**, H. Calumpong, IG.N. Mahardika, and P.H. Barber. 2018. Evidence of ecological divergence from coral-eating snails (Genus *Coralliophila*) in the Coral Triangle. *Journal of Biogeography. in revision*.

Presentations

Oral presentations

Rachmawati, R. Apprill, A. and Paul H. Barber. Variations in Coral Microbiomes among Indonesian Reefs with Different Levels of Bleaching. The 13th International Coral Reefs Symposium. Honolulu, Hawaii, June 2016

Rachmawati, R. Apprill, A. and Paul H. Barber. Coral Symbionts and the Host Susceptibility to Bleaching. Symbiosis Workshop 2015. Yosemite, CA, May 2015

Rachmawati, R. and Paul H. Barber. Differential Susceptibility to Bleaching among Coral Types and Regions in Indonesian Waters. The 1st Pan-Pacific Advanced Studies Institute (PacASI) Workshop on Genomic Applications to Marine Science and Resource Management in South East Asia. Dumaguete, Philippines, July 2012.

Rachmawati, R. Climate Change Impacts on Coral Reef Ecosystems in Indonesian Waters. Indonesian Studies Conference 2012: Indonesia in Global and Transnational Perspective. Los Angeles, CA, April 2012

Rachmawati, R. Global Warming Impact on Coral-zooxanthellae Symbiosis. Coral Reef National Conference. Jakarta, Indonesia, 2008

Rachmawati, R. Paleoclimate Study Using Coral as a Proxy, Ankara, Turkey, 2006

Rachmawati, R. Fisheries in Indonesia. The University of the Sea. Marion Dufresne, Australian waters, 2005

Rachmawati, R., K. Soewardi, D.G. Bengen, R. Affandi. Principal Component Analysis as Determination Method of Fish Population Based on Morphometrical Data. National Seminar of PERSADA (Japan Student Alumni) VIII. Bogor, Indonesia, 2001.

Rachmawati, R., K. Soewardi, R. Affandi, D.G. Bengen. Potential Growth Estimation of Giant Gouramy (*Osphronemus goramy*, Lacepede) Using RNA/DNA Ratio. The JSPS –DGHE International Symposium on Fisheries Science in Tropical Area. Bogor, Indonesia, 2000.

Poster presentations

Rachmawati, R. A. Apprill, and Paul H. Barber. Variation of symbiotic microbes between healthy and bleached corals. 19th Annual Biology Research Symposium Los Angeles, CA, May 2016

Rachmawati, R. and Paul H. Barber. Temperature tolerance of corals from Indonesian reefs with different bleaching susceptibility. 18th Annual Biology Research Symposium Los Angeles, CA, May 2015

Rachmawati, R. and Paul H. Barber. Temperature tolerant range of corals of different susceptibility reefs in Indonesia. Symbiosis Workshop 2014, Yosemite, CA, May 2014.

Rachmawati, R. and Paul H. Barber. Variation in bleaching susceptibility of Indonesian corals. Symbiosis Workshop 2013, Yosemite, CA, May 2013.

Rachmawati, R. and Paul H. Barber. Differential bleaching responses of Indonesian corals. Symbiosis Workshop 2012, Yosemite, CA, May 2012.

Rachmawati, R. Adaptation strategy of coral to prevent more severe destruction under elevated temperatures. International Symposium on Ocean Science, Technology and Policy, the World Ocean Conference (WOC), Manado, Indonesia, 2009.

Rachmawati, R. How can coral absorb atmospheric carbon?. International Symposium on Ocean Science, Technology and Policy, the World Ocean Conference (WOC), Manado, Indonesia, 2009.

Rachmawati, R. Climate change impact on coral reef ecosystem, Ankara, Turkey, 2006.

Rachmawati, R. & S. Horiike. The box corer. Marion Dufresne, Australian waters, 2005

INTRODUCTION

Coral reefs are among the world's most biodiverse (Ray 1988) and valuable ecosystems, providing an estimated $9.9 trillion USD per year in economic goods and services worldwide (Costanza et al. 2014). More than 500 million people depend upon coral reefs for food and livelihoods (Wilkinson and Salvat 2012), and in 23 countries, reefs provide an excess of 15% of local GDP (Burke et al. 2011).

Despite these tremendous benefits, reef ecosystems are in global decline. Burke et al. (2011) indicate that 75% of global reefs are threatened by local activities and global climate. Of these threats, global climate is a growing concern because of ocean acidification (Hoegh-Guldberg et al. 2017) as well as coral bleaching (Hoegh-Guldberg and Ridgway 2016). Coral bleaching occurs with the symbiosis between a coral animal and photosynthetic algae, *Symbiodinium,* breaks down (Lough and van Oppen 2009) As coral expel these endosymbiotes, the coral loses its color and 90% of it's nutrition, both of which are provided by the algae (Gordon and Leggat 2010). Given current climate trends, coral bleaching is predicted to get worse, threatening the future of these ecosystems (Hughes et al 2017).

Indonesia is home to nearly 20% of the world's coral reefs, the most of any nation (UN 2010). Indonesia is also the heart of the Coral Triangle, the worlds largest and most biologically diverse marine ecosystem (Roberts et al. 2002, Veron et al 2011) and about 40% of it's 240 million inhabitants depend upon these ecosystems for food and livelihoods. Unfortunately, Indonesian reefs are not immune to the impacts of coral bleaching resulting from rising global temperatures. However, reefs in Indonesia experience different levels of bleaching, with Western Indonesia experiencing the most severe bleaching with high mortality (Guest et al. 2012), while

Eastern Indonesia experiencing mild bleaching with mostly fully recovered (Setiasih and Wilson 2010), providing potential insights into characteristics that allow corals to withstand elevated temperatures without bleaching. While the role of *Symbiodinium* to coral host sensitivity level to bleaching has been well studied (Berkelmans and van Oppen 2006; Bellantuono et al. 2011; Keshavmurthy et al. 2014), less is known about how coral-associated microbiome vary heat stress, and how that variation might influence bleaching susceptibility. To help address this knowledge gap, I employed common garden temperature experiments on multiple coral species from six localities along Indonesian archipelago. Chapter 1 investigates how the microbiomes of a single species of coral varies with water temperature among two nearby reef ecosystems that experience different levels of coral bleaching. Chapter 2 examines the coral microbiomes of multiple species of coral respond to elevated water temperatures in a single location. Finally, chapter 3 examines the microbiomes of multiple species of coral across 5000 km of the Indonesian Archipelago, to test for local, regional, and species-specific differences in coral microbiomes that may explain regional variation in bleaching susceptibility. Combined, these studies provide novel insights into the nature and specific relationship of microbiomes to coral host and their environment.

Chapter 1

Chapter 1 examine how microbiomes from corals in reefs that experience different levels of bleaching might respond differently to heat stress, potentially impacting their sensitivity to bleaching. To test this, I conducted a common garden temperature experiment using *Porites lobata* from two reefs from Bali, Indonesia that with a history of severe (Pemuteran) and moderate (Amed) bleaching. Replicate fragments of multiple coral colonies from each location

were exposed to temperatures ranging from 30 °C (normal field conditions) to 36 °C, and then their microbial communities were documented by sequencing the V4 region of SSU rRNA genes. Results showed that bacterial and archaeal communities associated with *P. lobata* from the two locality were significantly different in composition, showing that coral micriobiomes can vary on local scales. Although microbiomes changed across temperature, there was substantial overlap in these communities. Moreover, even though microbial communities in corals from both locations changed, there was no consistent response to increasing temperature. Of 11 microbial taxa that increased in abundance with increasing temperature, only 1 increased in corals from both locations. Similarly, of 10 microbial taxa that decreased in abundance with decreasing temperature, only 1 decreased in corals from both locations. Further research is required to determine whether the microbes that changed similarly in both locations play a role in helping corals adapt to elevated temperatures, and whether the differential responses of their microbiomes play a role in the local variation in bleaching susceptibility.

Chapter 2

Different species of coral respond differently to environmental stress, with some taxa being more sensitive and more prone to bleach than others. While this variation could be a function of the coral animal, other components of the coral holobiont, such as microbiomes which are known to have strong impact on coral host condition, could influence how corals respond to environmental thermal stress. To examine how microbiomes change in response to thermal stress in coral taxa that sensitive and resistant to coral bleaching, I examined the microbiomes of *Acropora millepora*, *Pocillopora verrucosa*, and *Porites lobata* in Manokwari, West Papua. Replicate coral fragments were exposed to normal (30 °C) and elevated (32 °C, 34

°C, and 36 °C) water temperatures, resulting in obvious bleaching in *A. millepora* and *P. verrucosa,* but not *P. lobata*. Coral microbiomes (bacteria and archaea) were then assessed by sequencing the V4 region of SSU rRNA genes. Results revealed unique microbial community profiles in all three taxa. Proteobacteria was the predominant taxon and these, along Bacteroidetes and Firmicutes which were also abundant, were present in all three species. Despite these similarities, each coral species harbored one unique phylum in relatively high frequency. At the genus level, *Endozoicomonas* was dominant in *A. millepora* and *P. verrucosa*, whereas *Pseudoalteromonas* and Rhodobacteraceae bacteria were the most abundant in *P. lobata*. Microbial communities associated with these three coral species responded differently to increasing water temperature. In *A. millepora, Endozoicomonas* decreased in frequency with increasing temperature, almost disappearing at the highest temperature while *Vibrio* became the dominant taxon. In contrast, frequency of *Endozoicomonas* increased with increasing temperature in *P. verrucosa*, but then nearly disappeared at 36 °C where Rhodobacteraceae became the dominant taxon. There was no strong pattern among temperature treatments in *P. lobata* microbiomes due to high intra-species variation. Results clearly demonstrate that different species of coral from the same reef can have unique microbiomes, demonstrating a degree of specificity in the relationship between corals and their associated microbes. However, although two of three species clearly bleached, while the other showed no visual signs of color loss, there were no clear patterns of coral microbiome changes associated with these changes, other than potentially-pathogenic bacteria replacing dominant microbial taxa seen at lower temperatures. As such, it remains unclear what role microbiome changes may play in species specific variation in bleaching susceptibility in corals.

Chapter 3

Historically, different reefs in Indonesian waters have exhibited different levels of coral bleaching, with severe bleaching in Western Indonesia, and limited bleaching in Eastern Indonesia. To test whether coral microbiomes may contribute to this regional variation in bleaching susceptibility, I sequenced the V4 region of SSU rRNA genes of *Pocillopora verrucosa* and *Porites lobata* from six locations in Indonesian archipelago that experienced very different levels of bleaching in 2010. In addition, *A. millepora* was sampled from two locations. Results show significantly different microbial communities in each of the three species, although each had a unique geographical pattern. There were no significant differences in the microbiome of *A. millepora,* despite representing two populations with very different bleaching histories. In *P. verrucosa,* there were significant differences in the microbiomes among most of the populations, demonstrating a strong signal of local differentiation, although there was no signal of microbiome variation associated with regional variation in bleaching history. In constrast, *P. lobata* exhibited limited local differentiation, but microbiomes showed significant differences among populations with a history of severe and moderate bleaching, and populations with a history of limited or no bleaching. These results show a complex pattern of microbiome variation, demonstrating species-specific, local, and regional variation. Although the regional variation corresponds with history of bleaching susceptibility, further work will be required to determine whether these microbiomes contribute to this pattern, or are a result of regional variation in other biological or environmental variables.

REFERENCES

Bellantuono, A.J., O. Hoegh-Guldberg, and M. Rodriguez-Lanetty. 2012. Resistance to thermal stress in corals without changes in symbiont composition. *Proc Biol Sci* **279**: 1100–1107.

Berkelmans, R., and M.J.H. van Oppen. 2006. The role of zooxanthellae in the thermal tolerance of corals: a ‘‘nugget of hope’’ for coral reefs in an era of climate change. *Proceedings of the Royal Society B* **273**:2305–2312.

Burke, L., K. Reytar, M. Spalding, and A. Perry. 2011. Reefs at Risk Revisited. Washington, D.C., World Resources Institute (WRI), The Nature Conservancy, WorldFish Center, International Coral Reef Action Network, UNEP World Conservation Monitoring Centre and Global Coral Reef Monitoring Network.

Costanza, R., R. de Groot, P. Sutton, S. van der Ploeg, S.J. Anderson, I. Kubiszewski, S. Farber, and R.K. Turner. 2014. Changes in the global value of ecosystem services. *Global Environmental Change* **26**: 152-158.

Hughes et al. 2017 *Science* 05 Jan 2018: Vol. 359, Issue 6371, pp. 80-83 DOI: 10.1126/science.aan8048

Keshavmurthy et al. (2014), Can resistant coral-Symbiodinium associations enable coral communities to surviveclimate change? A study of a site exposed to long-term hot water input. *PeerJ* **2**:e327.

Lough, J.M. and M.J.H. van Oppen. Introduction: Coral Bleaching Patterns Processes, Causes and Consequences. 1–5 *In* Ecological Studies. Springer Berlin Heidelberg, 2009.

Ray, G.C. 1988. Ecological diversity in coastal zones and oceans. Pp. 36-50. *In:* Wilson, E.O (ed.). Biodiversity. National Academy Press, Washington, D.C., U.S.A.

Roberts, C.M., et al. 2002. Marine Biodiversity Hotspots and Conservation Priorities for Tropical Reefs. Science, 295(5558): 1280-1284.

Setiasih, N. and J. Wilson. 2010. A regional overview on the 2010 coral bleaching event in Southeast Asia: Indonesia. *in* Status of coral reefs in East Asian seas region: 2010. The Global Coral Reef Monitoring Network. Tokyo, Japan.

United Nations Environment Programme. (2001). World Atlas of Coral Reefs. Retrieved December, 2010.

Veron J.E.N. et al. (2011) The Coral Triangle. *In*: Dubinsky Z., Stambler N. (eds) Coral Reefs: An Ecosystem in Transition. Springer, Dordrecht

Wilkinson C. and B. Salvat. 2012. Coastal resource degradation in the tropics: Does the tragedy of the commons apply for coral reefs, mangrove forests and seagrass beds? *Marine Pollution Bulletin 64(6): 1096-1105.*

Gordon B.R. and W. Leggat. 2010. Symbiodinium—Invertebrate Symbioses and the Role of Metabolomics Mar. Drugs, 8(10), 2546-2568; doi:10.3390/md8102546

Guest, J.R., A.H. Baird, J.A. Maynard, E. Muttaqin, A.J. Edwards, et al. 2012. Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. *PLOS ONE* **7**: e33353. pmid:22428027

Hoegh-Guldberg, O., and Ridgway, T. 2016. Coral bleaching comes to the great barrier reef as record-breaking global temperatures continue. *The Conversation*, March 21, 2016 theconversat AJ ion.com.

Hoegh-Guldberg, O., Poloczanska, E.S., Skirving, W., Dove, S. 2017. Coral Reef Ecosystems under Climate Change and Ocean Acidification. Frontiers in Marine Science 4: 158.

CHAPTER 1

Differential response of *Porites lobata* microbiomes to thermal stress in two nearby reef systems

Introduction

Coral reefs are the most diverse marine ecosystems in the world (Reaka-Kudla 2001) and provide essential ecosystem services to human societies (Cesar et al. 2003), such as fisheries, coastal protection, tourism, and pharmacological products (Bruckner 2002), among others. Unfortunately, these valuable marine ecosystems are increasingly threatened and degraded worldwide (Osborne et al. 2017). While many of the threats to coral reefs are local (e.g. overfishing, sedimentation, land reclamation, etc.; Burke et al. 2012) others are global in nature. Of particular concern is rising global sea surface temperature (SST) and the associated increase in frequency and severity of coral bleaching (Bruno & Valdivia 2016; De'ath et al. 2017).

Coral bleaching is a process by which corals expel their endosymbiotic zooxanthellae, resulting in the loss of color and autotrophic abilities within corals (Lough and van Oppen 2009). Coral bleaching is believed to be an adaptive response to temperature stress, allowing corals to exchange *Symbiodinium* strains adapted to average temperature regimes in favor of strains adapted to elevated temperatures (Baker 2001; Mieog 2009). While individual corals and reef ecosystems have varying sensitivities to bleaching, extended anomalies of 2 °C above the maximum of the monthly mean SST can lead to bleaching (Kayanne 2017).

Historically, coral bleaching has been infrequent and local in scale. However, increasing SST associated with climate change is resulting in "mass coral bleaching" events—dramatic regional-scale events where a high proportion of corals severely bleach (Berkelmans et al. 2004).

Unfortunately, current climate trends are resulting in larger mass bleaching events that occur more frequently (Hughes et al. 2017; Kayanne 2017). With limited time to recover between bleaching events, coral survival rates from bleaching are decreasing (Pandolfi 2014; Graham et al. 2015; Ainsworth et al. 2016; Hughes et al. 2017), resulting in mass mortality. For example, in 2016, the Great Barrier Reef sustained successive mass bleaching events where 85% of surveyed reefs where bleached, compared to 56% in 2002 and 43% in 1998 (Hughes et al. 2017). Such high coral mortality can occur from either extremely high temperatures or prolonged periods of elevated temperature (Glynn 1984; Oliver et al. 2009; Eakin et al. 2016; Perry and Morgan 2017) compromising the ecological health and sustainability of coral reef and the socio-economic benefits we derive from them (Westmacott et al. 2000).

While bleaching now impacts virtually all coral reef ecosystems globally, not all coral species are equally susceptible to bleaching. For example, massive corals in the genus *Porites* tend to be less sensitive to temperature stress compared to species in *Acropora* and *Pocillopora* (Marshall & Bair 2000; Loya et al. 2001; McClanahan et al. 2005). As such, these taxa may have higher survival rates during future elevated ocean temperature scenarios (Cacciapaglia & van Woesik 2015). Moreover, certain *Porites* taxa (e.g. *P. lobata*) have extensive geographic distributions, suggesting that they are more capable of surviving varied environmental conditions than endemic and range-restricted corals (Burman et al. 2012).

Corals are a complex association of the coral animal, symbiotic algae, and associated microbial communities (Rohwer et al. 2002), and as such, their ability to survive temperature stress is a function of the entire coral holobiont. There has been significant effort in trying to understand the role of *Symbiodinium* in regional and taxonomic variation in bleaching susceptibility (e.g. Iglesias-Prieto et al. 1992; Buddemeier and Fautin 1993; Rowan and

Knowlton 1995; Kinzie III et al. 2001; Baker et al. 2004; Abrego et al. 2008; Sampayo et al. 2008; Leggat et al. 2011). Briefly, *Symbiodinium* clades vary in their thermal tolerance (Rowan et al. 1997), with some clades being more sensitive to temperature stress (e.g. clades A and C) while others (e.g. clade D) are more tolerant to high temperatures (Baker 2003). As such, a higher percentage of clade D in the *Symbiodinium* community increases the probability of the coral holobiont surviving periods of increased temperatures (Berkelmans & van Open 2006; Stat & Gates 2010). However, while our understanding of the role of *Symbiodinium* in coral bleaching variation continues to increase, comparatively less attention has been paid to the microbial components of the holobiont.

Research shows that, like *Symbiodinium* communities, the composition of microbial communities can also change during bleaching events (Ritchie et al. 1994; Bourne 2008). For example, healthy and bleached colonies of *Pocillopora verrucosa* and *Acropora* spp. harbor different bacterial communities (Jindal et al.1995; Ainsworth and Hoegh-Guldberg 2009). In addition to community shifts, increasing temperature can result in coral-associated microbial communities shifting from autotrophy to heterotrophy, reducing the health of coral hosts (Littman et al. 2011). Community shifts can change coral microbiome composition, so bleaching can result in an increase of pathogenic-related species (Littman et al. 2011) and susceptibility to opportunistic infections from *Vibrio* (Banin et al. 2001). For example, while both bleached and non-bleached corals may host pathogenic bacterial taxa, such as *Vibrio* and Acidobacteria, bleached corals generally host higher densities (Mouchka et al. 2010, Ritchie, 1994). Moreover, the abundance of pathogenic *Vibrio* increases significantly in coral colonies with increasing water temperature (Ben-Haim et al. 2003), suggesting that *Vibrio* infections in coral reefs environments may be climate-linked (Vezzulli et al. 2010). As a result, *Vibrio* can dominate the

coral-associated microbial community during bleaching events (Bourne 2008), potentially negatively impacting coral health.

While coral microbiomes are impacted by bleaching and temperature, these communities can also vary across geography. For example, microbial communities of *Pachyseris speciosa* differ across the Great Barrier Reef (Hernandez-Agreda et al. 2016). Similarly, Neave et al. (2017) showed that multi-species of Pociloporidae had pronounced differences among individual species sampled from different geographic locations. Combined, these results show that both geography and taxonomy impact coral microbiomes (Neave et al. 2017). Thus, if coral microbiomes play a role in coral bleaching, it is possible that geographic variation in microbiomes could result in regional variation in coral bleaching.

Indonesia is home to the world's most diverse coral reef ecosystems (Roberts et al. 2002; Veron et al. 2011). Interestingly, these reefs experience significant geographic variation in coral bleaching. The waters of Eastern Indonesia (e.g. West Papua) experienced relatively low levels of bleaching. For example, in 2010, no bleaching was reported in the waters near Manokwari and Cendrawasih Bay (West Papua), and very mild bleaching (~5%) occurred in Raja Ampat (Setiasih and Wilson 2010). In contrast, during the same period, moderate bleaching (40%-60%) was reported in Bali (Setiasih and Wilson, 2010) and severe mass bleaching events (up to 80%) occurred in the islands of Western Indonesia (Rudi et al. 2012), despite having similar period per year experiencing warm water as Eastern Indonesia during 2007-2016 (Kusuma et al. 2017).

In this study, we examine coral-associated microbial variation between two coral reef ecosystems on the island of Bali, Indonesia, that exhibit different bleaching susceptibility. Implementing a common garden approach, we experimentally increased water temperatures on *P. lobata* from both reefs and sequenced bacterial and archaeal small subunit (SSU) ribosomal

RNA (rRNA) genes to determine how coral microbiome communities respond to thermal stress, and if there are community shifting, whether the shifts correspond to geographic variation in bleaching susceptibility.

Materials and Methods

Site description and sampling

We sampled *Porites lobata* from two locations on the island of Bali (Figure 1.1), Indonesia that experienced different levels of bleaching in 2009-2010 despite being separated by only 133 km of coastline. Pemuteran is located on the north coast of Bali facing the Java Sea. Amed is located on the east coast of Bali, adjacent to the Lombok Strait. Setiasih and Wilson (2010) reported that in 2009, 60% of corals in Pemuteran bleached, while 40% of corals in Amed bleached, suggesting that the latter was more resistance to bleaching.

At each location, we collected three 15-25 cm colonies that were about 5 m apart at 6-8 m depth using SCUBA. To ensure that results were not impacted by coral health, we only chose healthy colonies that showed no obvious evidence of disease, attached predators, or boring organisms. These coral colonies were then transported to the field experiment site in shaded, aerated seawater tanks to limit coral stress.

Temperature experiment

To control for regional environmental variation, we established a series of "common garden" saltwater tanks equipped with protein skimmers to maintain water quality and aerate the water, and automatic heaters to maintain constant temperature. We then added field-collected

coral colonies to these experimental treatment tanks where we set water temperature at 30 °C to mimic existing conditions on the reef. To maintain water quality during holding and acclimation periods, we changed ~10% of the water daily with freshly collected seawater. However, we did not add or change seawater during the 12 h of treatment to ensure seawater changes did not impact the results.

After a one-day acclimation period to allow the coral colonies to recover from transportation and handling stress, we used a hammer and chisel to carefully generate five replicate fragments from each coral. We then placed the resulting replicate coral fragments into one of five experimental common garden treatment tanks, such that each experimental treatment tank contained one fragment from each of the six field collected coral colonies. We then allowed coral fragments an additional day to recover from the fragmentation handling stress before increasing temperatures for bleaching experiment. To achieve the targeted temperatures in a biologically realistic way, we increased temperatures gradually at a rate not exceeding 1 °C in 12 h.

To expose corals to a range of thermal stress, we used five temperature treatments: 30 °C as control or "normal" temperature based on local reef SST, 32 °C to represent elevated SST and upper temperature stress tolerance (1–3 °C above mean long-term annual maximum temperatures, Marshall and Baird, 2000; Glynn et al. 2001; Podesta and Glynn 2001; Wellington et al. 2001), 34 °C to represent extreme temperature during strong El Niño (4 °C above normal temperature, Barber and Chavez 1983), and 35 °C and 36 °C to represent further temperature extremes. Coral fragments were kept at target temperatures for 12 h to allow microbial communities to respond to the changing conditions.

To document changes resulting from thermal stress, we took pictures of all experimental fragments at the end of the treatment period. Following the conclusion of the temperature

challenges, we collected small piece (~2 cm) of samples from each fragment and preserved each of replicates in 95% ethanol and stored them at -20 °C for subsequent genetic analysis.

DNA Extraction

We extracted DNA from all 30 samples (5 fragments of 3 coral colonies collected from each of 2 field sites) for SSU rRNA gene-based microbial community profiling. To remove coral tissue from the skeleton prior to DNA extraction, we drained the ethanol from each sample and then used an airbrush and cold sterile 1X phosphate-buffered saline (PBS). We centrifuged the resulting tissue slurry at 5000 rpm for 20 min at 4 °C to pellet the coral tissue and associated microbial cells, and stored the tissue pellet at -20 °C until extraction. We extracted < 50 mg of each tissue pellet using a MoBio DNA Ultra Clean kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's recommendations with additional heating at 56 °C for 30 min with 400 rpm shaking before vortexing them for 15 min. DNA extractions were stored in -20 °C.

SSU rRNA gene amplifications and sequencing

We characterized variation in bacteria and archaea communities among experimental colonies by sequencing the hypervariable 4 region of the SSU rRNA gene. We amplified individual coral DNA extractions using forward primer set 515F: GTGCCAGCMGCCGCGGTAA and reverse primer set 806RB: GGACTACNVGGGTWTCTAAT (Caporaso et al. 2012; Apprill et al. 2015). These PCR reactions were dual indexed (Kozich et al. 2013) using an Illumina (San Diego, CA, USA) adapter, 8 bp index sequence, 10 bp pad sequence, and 2 bp linker sequence. We amplified each sample in triplicate 20-µl reactions containing 1 to 3 µl of template DNA, based on DNA

extraction concentration. Each PCR reaction contained 1 U of GoTaq® Flexi DNA Polymerase (Promega, Madison, USA), 5X Colorless GoTaq® Flexi Buffer, 2 mM MgCl2, 160 µM dNTP mix (Promega, Madison, USA), and 160 nM of each barcoded primer. Thermocycling parameters were: an initial denaturation for 2 min at 95 °C followed by an amplification cycle of 20 s at 95 °C, 15 s at 55 °C and 5 min at 72 °C and a final extension for 10 min at 72 °C. The PCR was run at Bio-Rad C1000 Touch™ Thermal Cycler (< 5 °C/s of ramp rate, Bio-Rad Laboratories, Hercules, CA, USA), and PCR cycles varied from 20-40 cycles to achieve consistent amplification strength across all samples.

To visualize PCR products, we electrophoresed each sample in a 1% agarose 1X TBE buffer gel run for 60 min at 110 V. We then photodocumented the resulting gel by staining with SYBR Safe (Invitrogen, Carlsbad, CA, USA) and illuminating with an UltraBright LED Transilluminator, 470nm (LB-16) (Maestrogen, Nevada, USA). Successful PCRs were indicated by the presence of a distinct, thin band around 292 bp in length using the Hyperladder 50 bp DNA ladder (5 ng µl−1) (Bioline USA Inc., Taunton, MA, USA) as reference. We excised these bands and then purified them using the Qiagen MinElute gel extraction kit (Qiagen, Hilden, Germany). We eluted the purified PCR product in 10 µl EB buffer and then quantified DNA concentrations using a Qubit 2.0 Fluorometer with the dsDNA High Sensitivity Assay (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA).

To achieve similar numbers of sequences from each coral fragment, we pooled approximately 2 ng of each purified amplicon and control amplicons in one library. In addition to the experimental samples, we used Microbial Mock Community B (Staggered, Low Concentration, v5.2L, for 16S rRNA Gene Sequencing, HM-783D obtained through BEI Resources, NIAID, NIH as part of the Human Microbiome Project) as a sequencing control. As a

negative control, we included three negative PCR controls from 30, 35 and 40 cycle PCR reactions. As with sample DNA, each control was uniquely barcoded and included in the library. These libraries were then sequenced using 250 bp paired-end sequencing on an Illumina MiSeq (San Diego, CA, USA).

Bioinformatics

To analyze the resulting microbial community DNA sequences, we used Mothur v1.34.4 (Schloss et al. 2009). First, we removed reads that were longer than 255 bp or that had ambiguous positions using the *screen.seqs* command. We then classified the resulting contigs against the SILVA database v119 (Pruesse et al. 2007) using the *classify.seqs* command with the 'knn' method. To exclude DNA sequences that are not from Bacteria or Archaea, we removed all sequences classified as chloroplast, eukaryota, mitochondria, and 'unknown' using the *remove.lineage* command, and then we detected and removed chimeras using the *chimera.uchime* and *remove.seqs* commands, respectively. Next, we used *remove.groups* command to remove all other groups, such as sequencing controls, that were not included in the next analyses or used *get.groups* to pick a certain dataset for further analysis. Finally, prior to further data analyses, we subsampled the resulting sequences close to the lowest sequence number using *sub.sample* command to reveal most of the diversity in the analyzed group.

Statistical analyses

To cluster the resulting sequence reads into groups based on similarity, we employed Minimum Entropy Decomposition (MED; Eren et al. 2014) using least sequence difference. To do this, we first converted fasta and group files with Mothur format into a MED-compatible

formats using "headersMED.py" command in Python (Neave et al. 2017). We ran MED using default settings and a minimum substantive abundance criterion (*M*) of total sequences/10,000 as recommended by Eren et al. (2014). MED software was then used to generate Nonmetric Multidimensional Scaling (NMDS) plots based on similarity indices). We ran an MED analysis using 16,700 sequence subsample and $M = 50$ to examine the difference of microbial communities of samples from Amed and Pemuteran. To determine in more detail whether the cluster was more based on temperature or colonies, we generated MED plots based on temperature treatments for each location, with additional label on each colony, using 16,700 and 19,100 sequences and $M = 25$ and 28 for Amed and Pemuteran, respectively. To determine whether the resulting clusters were significantly different, we employed the analysis of similarity algorithm (ANOSIM) in Primer-E (Clarke and Gorley, 2015). ANOSIM compares the distance ranks between groups to ones within groups to test the similarity level of two or more groups of nonparametric data (Clarke, 1993).

Diversity of Microbial Communities

To examine overall differences in microbial diversity, we analyzed a variety of diversity indices from abundance data comparison of DNA sequences to the SILVA database v119 (Pruesse et al. 2007). Chao-1 richness estimates the total number of OTUs, Simpson diversity estimate (1-D) examines the dominance of high abundant OTUs, Shannon diversity estimate integrates the richness and evenness of microbial community, and Beta Diversity-Whittaker compares the diversity and composition between two samples). In all cases, we evaluated significance using Kruskal-Wallis and Mann-Whitney tests as implemented in the PAST statistics software package (v3.14, Hammer et al. 2001). We also used this software to run

multivariate statistical tests (permutational multivariate analysis of variance (PERMANOVA) and ANOSIM), and Similarity Percentages (SIMPER) to calculate contribution of every variable to separate groups.

To further explore different patterns of microbial diversity, we generated bar plots of relative abundance of major microbial groups at phylum and genus levels based on the abundance of sequences of these groups in each sample. For each bar plot, we included the 5 most abundant microbial phyla, and 10 most abundant microbial genera from Amed and Pemuteran. To visualize changes in these communities with increasing temperature, we plotted the relative frequency of these groups across temperature treatments and then plotted major microbial groups that increased, decreased, or fluctuated across temperature treatments.

Results

Fragments of *P. lobata* exhibited no clear visual signs of color change after 12 hours in any of the temperature treatments (Figure 1.2). After trimming and removing non-targeted sequences, a total of 1,247,560 high-quality sequences were recovered, resulting in an average number of sequences per sample of 42,702 ± 19,125 (± SD) for Amed and 40,468 ± 15,489 (± SD) for Pemuteran. Employing a 97% sequence similarity threshold, these sequences clustered into a combined total of 651 microbial operational taxonomic units (OTUs), 564 that were found in Amed and 532 in Pemuteran. Comparison of these sequences to the SILVA database v119 (Pruesse et al. 2007) revealed that 50% of the OTUs are undescribed at the genus level.

Amed and Pemuteran have different profiles of *P. lobata*-associated microbiome

NMDS plot on all data using Minimum Entropy Decomposition (MED) revealed that microbial communities from Amed and Pemuteran form significantly different clusters (Figure 1.3) based on the analysis of similarity (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA) ($P < 0.001$). Although ~6% more OTUs were recovered in Amed than Pemuteran, the average number of OTUs per sample and the Chao-1 per sample were not significantly different between these two locations (Figure 1.4A; $P > 0.05$, Kruskal-Wallis). Moreover, Simpson, Shannon, and Beta diversity indices also were not significantly different across samples (Figure 1.4B, C, D; $P > 0.05$, Kruskal-Wallis).

The three most abundant microbial phyla were the same at both locations. Proteobacteria was the most predominant phylum, representing > 60% of all microbial sequences, followed by Bacteroidetes and Firmicutes (Figure 1.5A). The main difference between locations was that Lentisphaerae was associated with Amed, whereas phylum Planctomycetes was associated with Pemuteran.

At the class level, both locations had roughly equal Gammaproteobacteria abundance, but Pemuteran had roughly double Alphaproteobacteria and Flavobacteriia (data not shown). In contrast, Amed had roughly double Deltaproteobacteria, Clostridia (Firmicutes), undescribed Bacteria, Bacteroidia (Bacteroidetes), and Epsilonproteobacteria. Oligosphaeria (Lentisphaerae), was associated with Amed, whereas Sphingobacteria (Bacteroidetes) and Planctomycetacia (Planctomycetes) were more associated with Pemuteran.

At the family level, Rhodobacteraceae was dominant in Pemuteran, representing 25% of all sequences, which was more than four-fold greater than the second most abundant family, Colwelliaceae (Gammaproteobacteria, data not shown). Other abundant taxa in Pemuteran

include Flavobacteraceae (Bacteroidetes) and Saprospiraceae (Bacteroidetes). While Rhodobacteraceae was also most abundant in Amed, it was far less abundant than Pemuteran, and less than double of the second most abundant family in Amed, undescribed taxa in phylum Bacteroidetes. Other abundant taxa in family level include undescribed taxa in Bacteroidetes, Desulfovibrionaceae (Deltaproteobacteria), Vibrionaceae (Gammaproteobacteria), Family XII (Firmicutes), and undescribed taxa in class Oligosphaeria (Lentisphaerae).

SIMPER showed that the top 15 OTUs accounted for nearly 60% of the difference in microbial profiles difference between Amed and Pemuteran. Experimental colonies from Pemuteran had higher abundances of undescribed Rhodobacteraceae (Alphaproteobacteria), Colwelliaceae (Gammaproteobacteria), Flavobacteriaceae (Bacteroidetes) and Saprospiraceae (Bacteroidetes). Whereas colonies from Amed had more undescribed Bacteroidetes, Marinilabiaceae (Bacteroidetes), as well as *Desulfovibrio* (Deltaproteobacteria) and *Fusibacter* (Firmicutes) (Figure 1.5B). In addition, undescribed Oligosphaeria (Lentisphaerae) had a high association with *P. lobata* in Amed.

Colony-based microbial profile of *P. lobata* colonies

In addition to variation between locations, microbiomes also showed variation among colonies within location. In Amed, despite significant difference of microbial communities between lower (30 °C, 32 °C) and higher (34 °C, 35 °C, 36 °C) temperature treatments (Figure 1.6A, $P < 0.05$ ANOSIM and PERMANOVA), microbial profiles clustered more based on colony than temperature ($P < 0.005$ ANOSIM and PERMANOVA). The pairwise test showed that the microbial profiles of colonies 1 and 3 from Amed were significantly different ($P < 0.005$) from colony 2. While, colony-based clustering was not as pronounced in samples from

Pemuteran (Figure 1.6B), significant clustering by colony was also observed *(P* < <0.05 ANOSIM and PERMANOVA). In the Pemuteran community, microbial profiles of colonies 2 and 3 were significantly different ($P < 0.05$) from colony 1.

Microbial community changes with temperature

In *Porites lobata* from Amed, the first five most abundant phyla, Protobacteria, Bacteroidetes, Firmicutes, Lentisphaerae, and unclassified phyla, were present in all treatments. In the extreme temperature treatment (36 °C) there was also Spirochaetae, which replaced an undescribed phylum of bacteria as the fifth most abundant phylum (Figure 1.7A). In general, the dominant taxa at the genus level of Amed samples remained the dominant taxa in all temperature treatments (Figure 1.7B). In Pemuteran, the four most abundant phyla were the same in Amed, except that Planctomycetes replaced Lentisphaerae, and they appeared in all temperatures (Figure 1.8A). More specifically, Proteobacteria was highly dominant at similar abundances (67−76%) in all temperatures. Although ANOSIM based on MED OTUs showed no difference between microbial from both location with increasing temperatures, variation of microbial genera among temperatures in Pemuteran was more complex (Figure 1.8B).

At the genus level, three basic patterns of change were observed. There were numerous genera that increased in frequency with increasing temperature. Although some taxa (e.g. *Arcobacter*) increased in both Amed and Pemuteran, responses of individual genera were largely unique to each location. For example, in Amed, *Endozoicomonas* had about triple abundance at extreme temperatures than at lower temperatures (Figure 1.8A). In contrast, in Pemuteran, *Desulfovibrio* and undescribed Desulfobacteracea increased more than three times at high temperatures (Figure 1.8B).

While some taxa increased in frequency with increasing temperature, others decreased. Of particular note are taxa like *Endozoicomonas* that had opposite responses to temperature in the two locations, substantially increasing in abundance in Amed (Figure 1.9A), while decreasing dramatically in Pemuteran (Figure 1.9B). Some genera (e.g. undescribed Rhodobacteraceae) tended to decrease with increasing temperatures in both locations.

Lastly, other taxa fluctuated across temperatures (Figure 1.10). For example, *Vibrio* and *Marinifilum* were two genera with similar responses in both locations; they both initially increased in abundance with increasing temperature but then decreased to levels closer to original abundances with further temperature increase. However, other taxa (e.g. *Desulfovibrio*, *Shimia*) showed no clear patterns.

Discussion

Despite close geographic proximity, *P. lobata* microbiomes from Pemuteran and Amed were significantly different, in composition and response to thermal stress, exceeding previously reported differences in geography and bleached/health *P. lobata* (Hadaidi et al. 2017). However, large changes in relative abundance of dominant microbial taxa were not associated with significant changes of measures of microbial diversity, suggesting that significant differences in microbiomes must result from shifts in overall community composition rather than loss of overall microbial diversity. As such, diversity indices may be insufficient to document changes in coral microbial communities, as changes in overall community composition could result in functional changes of microbiomes (Dinsdale et al. 2008).

Previous studies show that reefs from Pemuteran are more prone to bleach than reefs from Amed (Setiasih and Wilson, 2010). The significant differences in coral microbiomes in

Amed and Pemuteran combined with recent work (Ziegler et al. 2017) directly implicating coral microbiomes with heat stress tolerance suggest that local variation in microbial communities could contribute to observed local variation in bleaching susceptibility, although future experiments will be required to directly test this hypothesis.

Local variation in *Porites lobata* microbiomes

That Proteobacteria dominated the coral microbiome from both study sites was not surprising as this phylum is a common result in other coral microbial studies (Rohwer et al. 2002, Littman et al. 2009, Sunagawa et al. 2010, Blackall et al. 2015). Within the Proteobacteria, both locations had similar abundance of Gammaproteobacteria, a microbial group that includes highly abundance bacteria, *Endozoicomonas,* which can represent up to 90% of the coral microbiome (Bayer et al. 2013a, 2013b; Neave et al. 2017). However, *P. lobata* from Pemuteran had nearly twice the abundance of Alphaproteobacteria. In Caribbean corals, increase in Alphaproteobacteria was associated with white plague disease (Cárdenas et al. 2012) as was decrease in *Endozoicomonas* (Meyer et al. 2014), suggesting that corals from Pemuteran may be less healthy than those from Amed, which could explain the higher propensity of Pemuteran corals to bleaching.

Important family levels differences were also observed between these two regions that could also potentially impact their response to thermal stress. Although Desulfovibrionaceae were observed in *P. lobata* in both locations, this was the 3rd most abundant microbial group in Amed coral microbiomes, but not among the 10 most abundant groups in Pemuteran (data not shown). Most strains in Desulfovibrionaceae are sulfate-reducing and anaerobic bacteria (Kuever 2014). In addition, they are mostly mesophiles, which has upper temperature range of 35-42 °C

(Canganella and Wiegel 2011). If these microbes play a critical functional role in coral health, elevated thermal tolerance could potentially help coral hosts to cope with thermal stress. Indeed, this group had higher frequency in *P. lobata* from Amed, which have greater tolerance of elevated temperatures. Interestingly, Rhodospirillaceae, which was associated with high heat tolerance in *Acropora* (Ziegler et al. 2017), was not among the common microbial taxa in *P. lobata* in Bali, despite being present in *P. lobata* from the Red Sea, including colonies that had bleached (Hadaidi et al 2017). This result suggest that there might be functional redundancy in coral microbiomes across global oceans.

Although Colwelliaceae was the second most abundant microbial group in Pemuteran, it was only the 15th most abundant in Amed. Colwelliaceae has genes that can cope with reactive oxygen species (ROS, Methé et al. 2005) produced by *Symbiodinium* during photooxidative stress (Gardner et al. 2017; Parrin and Blackstone 2017; Wang et al. 2017), such as during conditions that promote bleaching. As such, high frequency of these microbes in Pemuteran could be adaptive. However, other Colwelliaceae are associated with diseased corals (Thompson et al. 2006) and could signal unhealthy conditions on these reefs. Unfortunately, the Colwelliaceae in this study are undescribed. However, given that abundance of these Colwelliaceae bacteria didn't change much in *P. lobata* across all temperature treatments suggests that this undescribed group may not be impacted by thermal stress.

Previous studies showed that coral-associated microbiomes differed more across geographic locations than among coral species within a given reef system (Littman et al. 2009, 2011), including subtle differences among *P. lobata* from reefs in the Red Sea (Hadaidi et al 2017). Similarly, our results show that the abundance and composition of microbial communities of *P. lobata* differed in between two coral reef systems on the island of Bali, including major

differences in the most abundant taxa in the microbiome. While this result could indicate that local environments play a major role in shaping the *P. lobata* microbiome, location specific variation persisted even when corals from the two localities were placed in a common garden. In fact, coral fragments from the same reef were more similar even when placed in different tanks, than coral fragments from different localities placed in the same tank and showed strong colony-specific associations. The stability of these local differences in a common garden suggests that environmental variation cannot be the only cause of local differences in *P. lobata* microbiomes —coral hosts may also mediate differential microbial associations in these two reef ecosystems. Alternatively, these results could occur if there was insufficient time for microbiomes to change. Indeed, Ziegler et al. (2017) showed that it took 17 months for the microbiome of corals transplanted from one environment to match the coral microbiomes native to that environment. However, the rapid changes seen with changing temperature, below, suggests the capacity for rapid microbiome shifts in *P. lobata*.

Interestingly, despite significant differences in *P. lobata* microbial profiles from Amed and Pemuteran, there was no significant difference in standard biodiversity measures, such as species richness, evenness, etc., a result that matches previous results by Tracy et al. (2015). This result indicates that temperature induced changes in microbiomes (below) must result from shifts in abundance of microbial taxa, rather increase or decrease of microbial diversity.

Reef specific microbiome response to thermal stress

Microbiomes are sensitive to disturbance (Allison and Martiny 2008) and can change in corals associated with thermal stress (Ziegler et al. 2017; Hadaidi et al. 2017). As such, it is not surprising that the microbiomes of *P. lobata* from two reefs in Bali changed with increasing

temperature. What is surprising is the lack of similarity in their response to increasing temperature in a common garden. Of 11 microbial genera with large increases in abundance, only one—*Arcobacter*—increased in both locations. All other taxa were unique to a single location. Some *Arcobacter* are pathogenic and associated with coral diseases (Rice et al. 1999; Vandenberg et al. 2004). Therefore, increasing abundance with increasing temperature could indicate declining coral health. However, *Arcobacter* can also be nitrogen fixers (McClung et al. 1983; Wirsen et al. 2002), so their increase could be associated with changes in holobiont metabolic processes.

Similarly, of 10 taxa that decreased in abundance, only one—undescribed Rhodobacteracea—declined in both locations. Given that this taxon is undescribed, it is unknown whether its decline indicates any important physiological shifts. However, decline of *Endozoicomonas,* as observed in Pemuteran, is associated with coral disease in the Caribbean (Meyer et al. 2014). As such, this change could hint at declining coral health in Pemuteran with increased temperature.

Yilmaz et al. (2016) report similarly idiosyncratic responses of dominant microbial taxa to changing temperature. This pattern may result from many of the microbial taxa in this study (e.g. Proteobacteria, Bacteroidetes) being large, with their members having very diverse activities and roles. Some taxa like Bacteroidetes can also adapt quickly to changing environments through genetic rearrangements, gene duplications and lateral gene transfers (Thomas et al. 2011) so this taxon could play different roles in their hosts. What is unusual, however, is that groups like *Endozoicomonas* decrease by 2-fold in Pemuteran while increasing more than 4-fold in Amed in response to the same temperature increases. Given that *Endozoicomonas* is closely associated with healthy corals (Meyer et al. 2014),this result provides

circumstantial evidence that this taxon could contribute to the different bleaching dynamics reported in the two locations by Setiasih and Wilson (2010).

Dynamics of *Vibrio*

The dramatic increase in abundance of Vibrionaceae with increasing temperature in *P. lobata* from Pemuteran was not unexpected as Vibrionaceae is commonly associated with bleached (Bourne et al. 2008; Vega Thurber et al. 2009) and diseased (Kushmaro et al. 2001; Ben-Haim et al. 2003) corals. In particular, *Vibrio shiloi* is virulent at warm temperatures (25–30°C) where it produces toxic compounds that disrupt the photosynthetic activity of *Symbiodinium* and increase the chances of the coral host bleaching (Rosenberg et al. 2009); however, it lacks virulence at lower temperatures. Similarly, *V. coralliilyticus* was only found in bleached *Pocillopora damicornis* (Rosenberg et al. 2009) in warm (24–29 °C) water only (Ben-Haim et al. 2003). As such, it is likely that the increase in *Vibrio* seen in Pemuteran is a direct response to temperature and associated coral stress.

Although the observed increase of *Vibrio* with increasing temperature in Pemuteran was expected, in Amed, *Vibrio* abundance was relatively high across all temperatures, peaking in abundance at 34 °C, where it became one of the most dominant microbial groups. While seemingly paradoxical, *Vibrio* can occur in high abundances on healthy corals (Koren and Rosenberg 2008; Littman et al. 2011; Kellogg et al. 2013). In fact, some *Vibrio* produce compounds that can suppress the growth of pathogenic bacteria, including other *Vibrio* species (Rypien et al. 2010). Thus, the conflicting responses of *Vibrio* in *P. lobata* from Pemuteran and Amed could result from the presence of different strains in their respective microbiomes.

Furthermore, while the *Vibrio* abundance in our study increased with temperature, at extreme temperatures (35 °C and 36 °C) their abundances dropped again to initial abundances. Stressed corals have mechanisms to promote coral antibacterial activity (CAA), where they release antibacterial compounds to combat etiological agents, such as *V. coralliilyticus* (Geffen et al. 2009). One of these antibacterial compounds is tropodithietic acid (TDA), produced by a *Pseudovibrio* strain, which is able to suppress the growth of virulent *V. coralliilyticus* and *V. owensii* (Raina et al. 2016). Thus, differential responses among the two locations could indicate different CAA, although this seems unlikely within a single species.

High percentage of undescribed taxa

Our understanding of coral-associated microbial diversity lags behind terrestrial environments and their associated fauna. As such, it is common that marine microbiome studies reveal a substantial number of unknown taxa (Yilmaz et al. 2016). Our study also revealed large amount of undescribed microbial taxa. After the five most abundant phyla, all of the remaining phyla with abundance >4% were unclassified or undescribed. Among the three most abundant classes under the Bacteroidetes phylum (data not shown), the most abundant one was undescribed. In addition, the dominant genus in Pemuteran and the most abundant genus in Amed were the undescribed taxa from the Rhodobacteraceae family and Bacteroidetes phylum, respectively. All highly abundant genera in the Bacteroidetes phylum are undescribed. The total undescribed OTUs in Amed and Pemuteran are 52% and 50%, which comprise 58% and 70% of respective sequences. These are much higher percentages than found in studies on other coral species, such as *Orbicella faveolata* (16.9%) and *Gorgonia ventalina* (35.5%) (Tracy et al. 2015). This result likely stems from fewer reference databases from marine habitats (Werner et

al. 2012) and highly novel bacteria in corals (Koren and Rosenberg 2006; Sunagawa et al. 2010; Chen et al. 2011). In addition, a study by Tracy et al. (2015) found that the proportion of unclassified OTUs in the communities consistently increased during and after disturbance events, so the high proportion of unclassified microbes in this study could also result from the high water temperatures. However, Indonesia is the most biologically diverse marine ecosystem on the planet (Roberts et al. 2002; Veron et al. 2011), and among the least studied (Fisher et al. 2010). As such, it may be that this region harbors a high degree of unknown microbial diversity as has been reported for other marine taxa (Barber and Boyce 2006), an hypothesis that merits further exploration.

FIGURES

Figure 1.1. Study sites: Pemuteran and Amed (Bali, Indonesia)

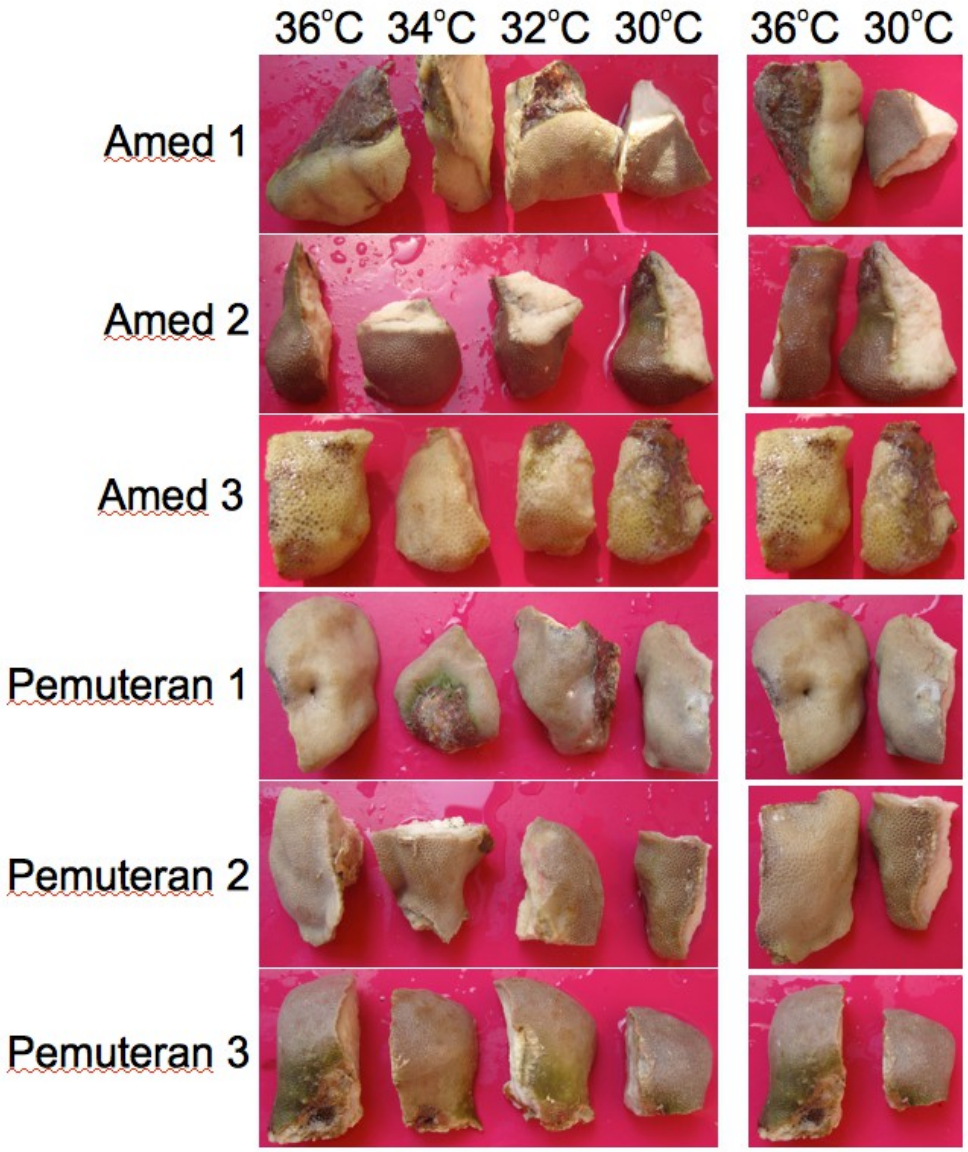


Figure 1.2. Representative photographs of *Porites lobata* corals taken following the temperature experiment, from reefs in Amed and Pemuteran, Indonesia. Left: fragments treated at 30 °C, 32 °C, 34 °C, 36 °C. Right: direct comparison of fragments at 30 °C and 36 °C.

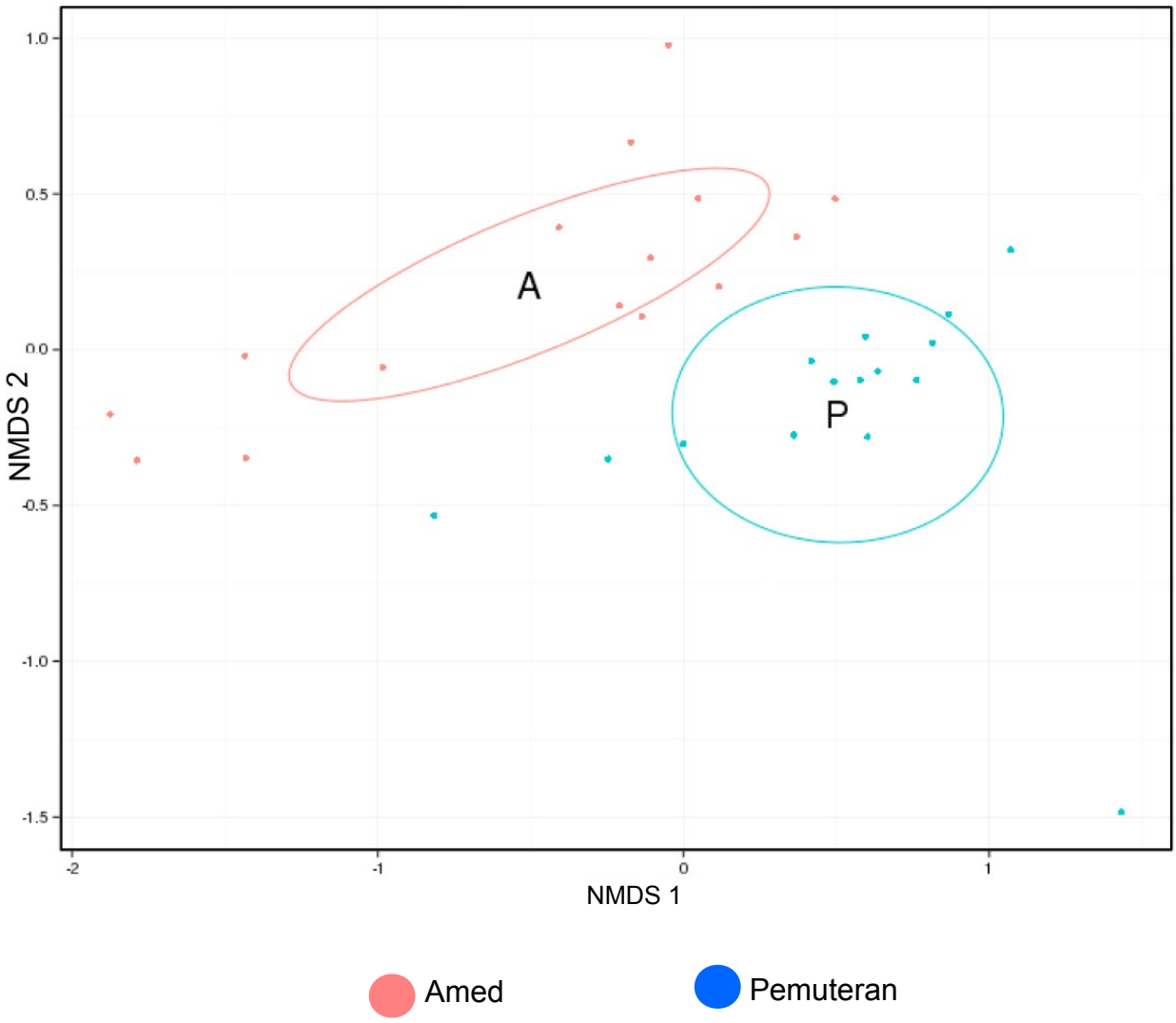
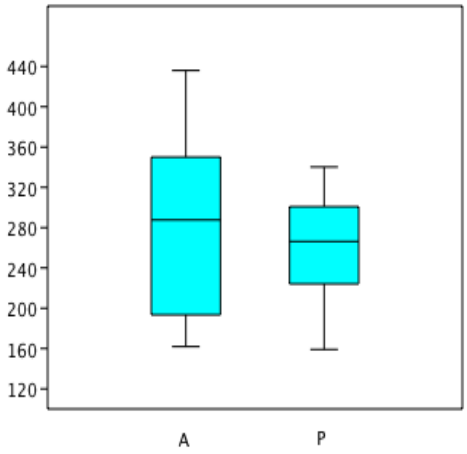
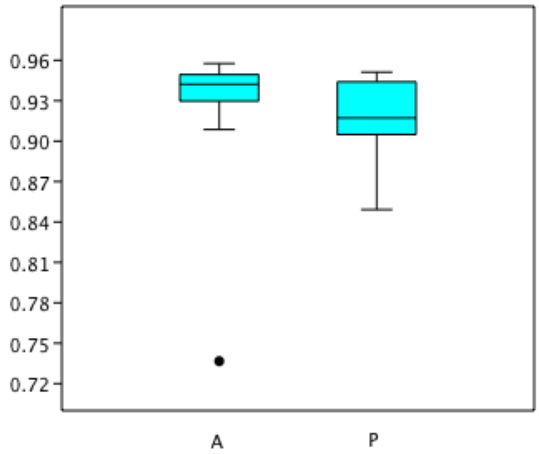


Figure 1.3. Nonmetric multidimensional scaling (NMDS) analysis of SSU rRNA gene sequence data, from common garden experiment on *Porites lobata* from 2 locations with different levels of sensitivity to bleaching in Bali (Central Indonesia). The sequences were grouped into minimum entropy decomposition (MED) nodes and compared using Jaccard similarity index. P = Pemuteran (experienced more severe bleaching), A = Amed (experienced less severe bleaching).

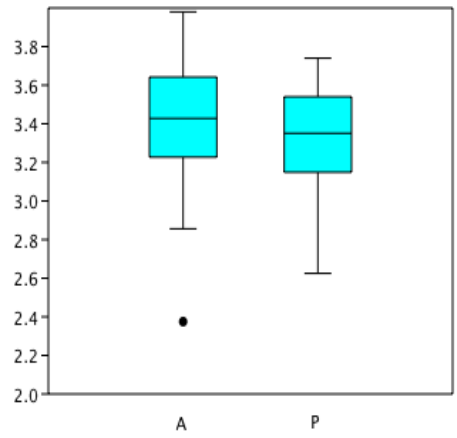
A. Chao-1



B. Simpson



C. Shannon



D. Beta Diversity

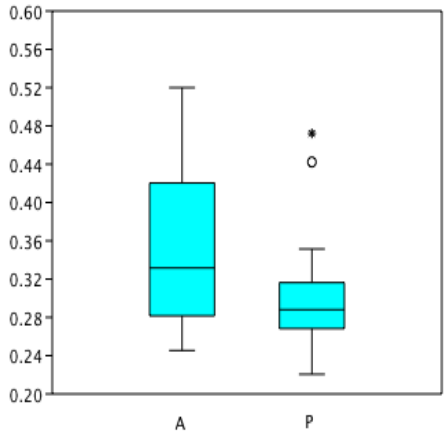
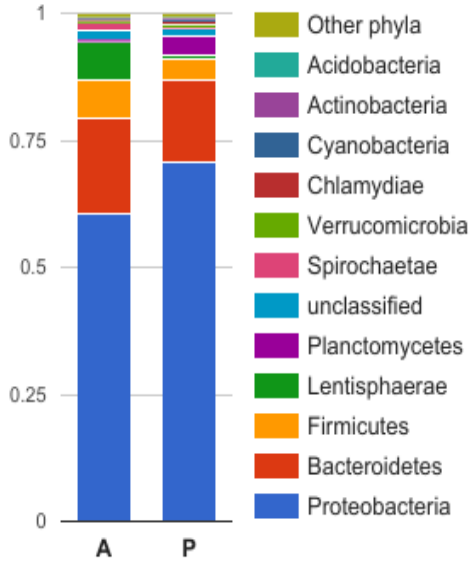


Figure 1.4. Box plots of **A.** Chao-1 richness estimate, **B.** Simpson diversity estimate (1-D), **C.** Shannon diversity estimate, and **D.** Beta diversity (Whittaker) for all temperature treatments comparing Amed and Pemuteran. A = Amed (lower sensitivity to bleaching), P = Pemuteran (higher sensitivity to bleaching). Significance tests were based on Mann-Whitney. All test results are not significant ($P > 0.05$). Dots, circle, and asterisk are outliers.

A. Phylum



B. Genus

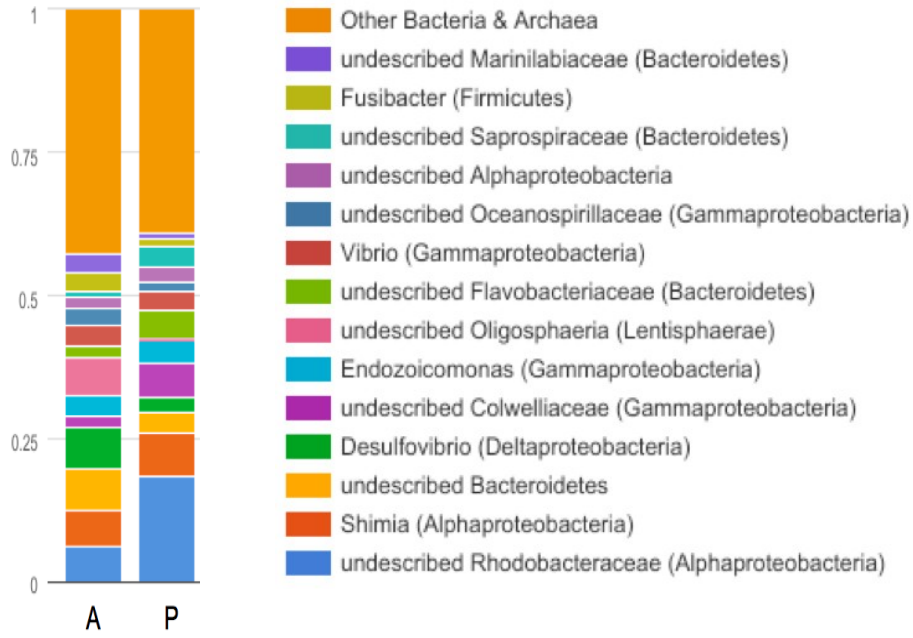
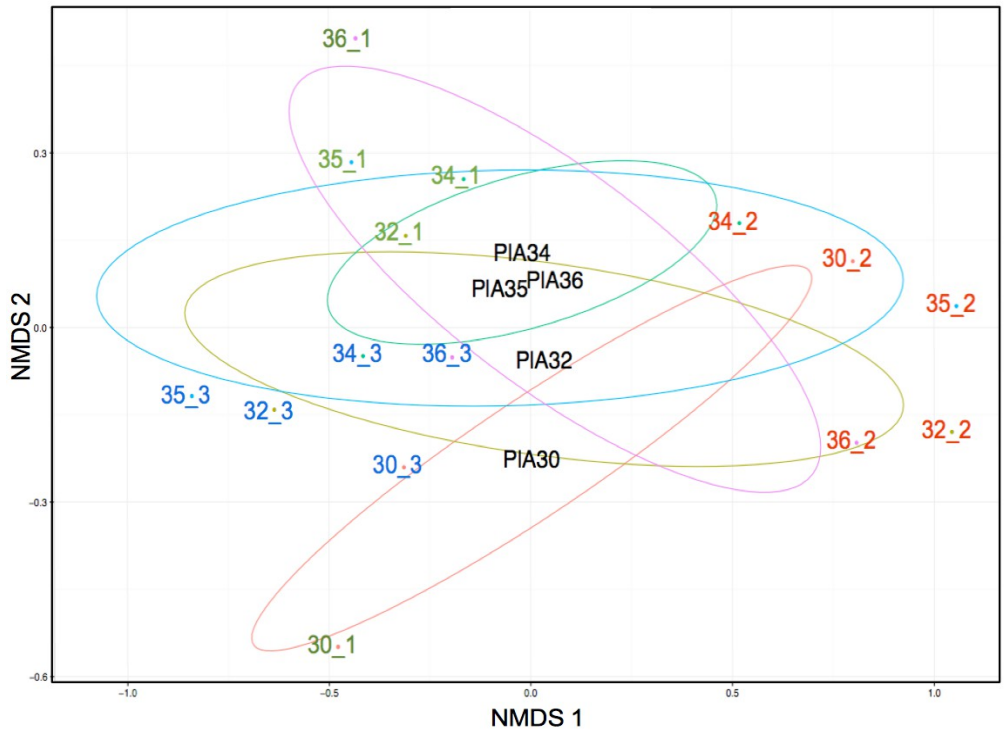


Figure 1.5. Distribution of combined the 10 most abundant *P. lobata*-associated microbial taxa from common garden experiment. A=Amed, P=Pemuteran. **A**. Phylum level, **B**. Genus level.

A. Amed



B. Pemuteran

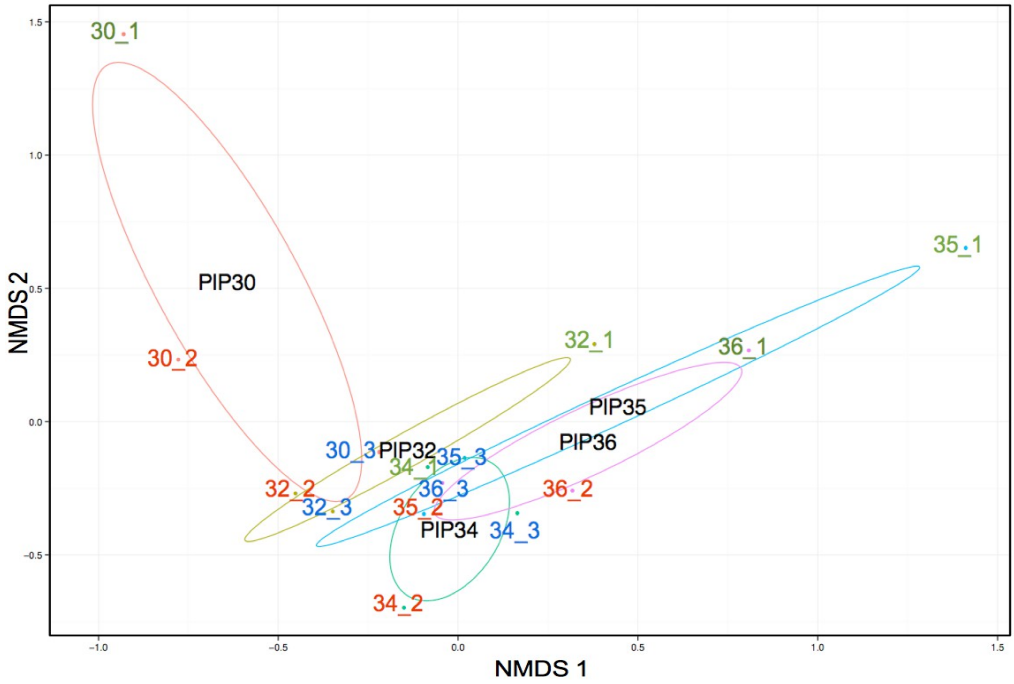
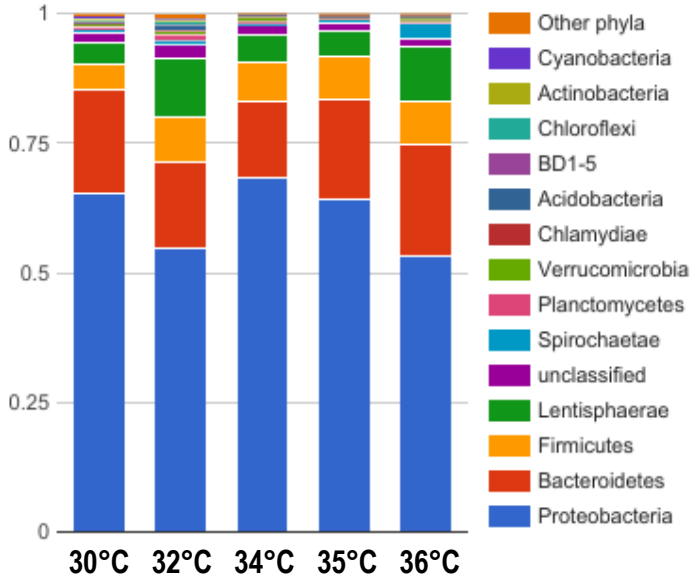


Figure 1.6. NDMS plots of microbial profiles variation of each experimental fragment. **A**. Amed. **B**. Pemuteran. Colored numbers=colonies (n=3; green=colony 1, red=colony 2, blue=colony 3). Dots and colored ovals=temperature treatments; PlP30 = 30°C, PlP32 = 32°C, PlP34 = 34°C, PlP35 = 35°C, PlP36 = 36°C

AMED

A. Phylum



B. Genus

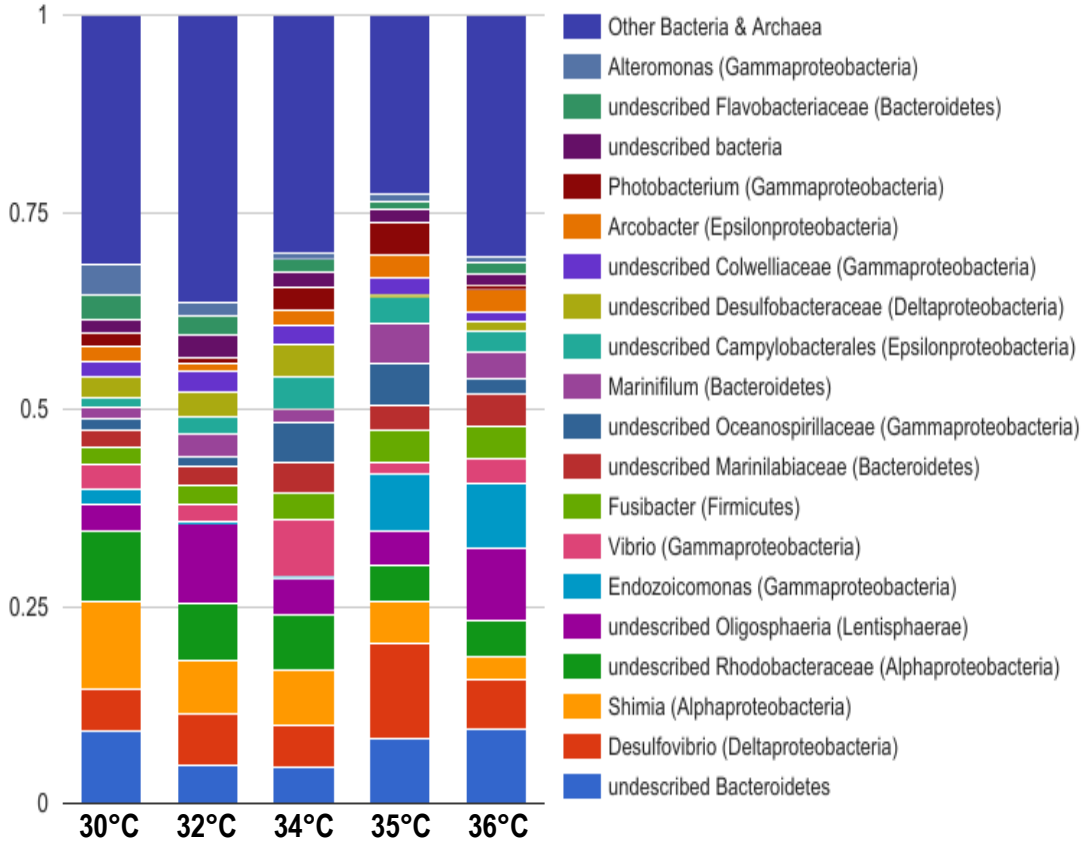
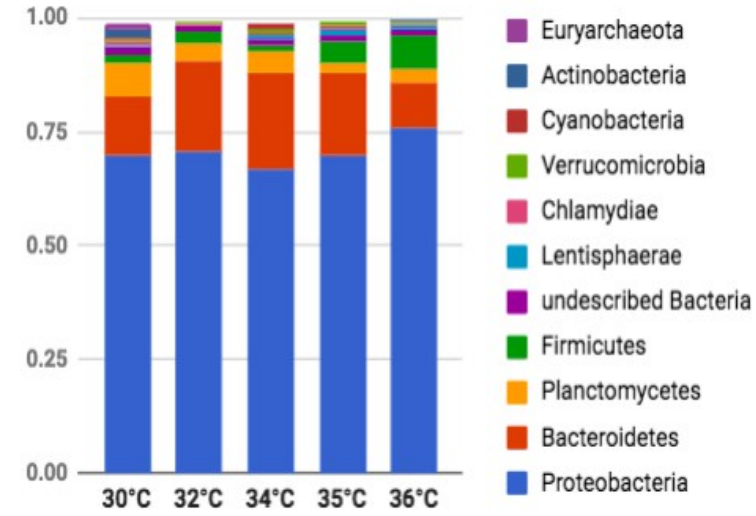


Figure 1.7. Compilation of **A.** 5 most abundant phyla, and **B.** 10 most abundant genera, of *P. lobata*-associated microbial taxa of all five temperature treatments from Amed.

PEMUTERAN

A. Phylum



B. Genus

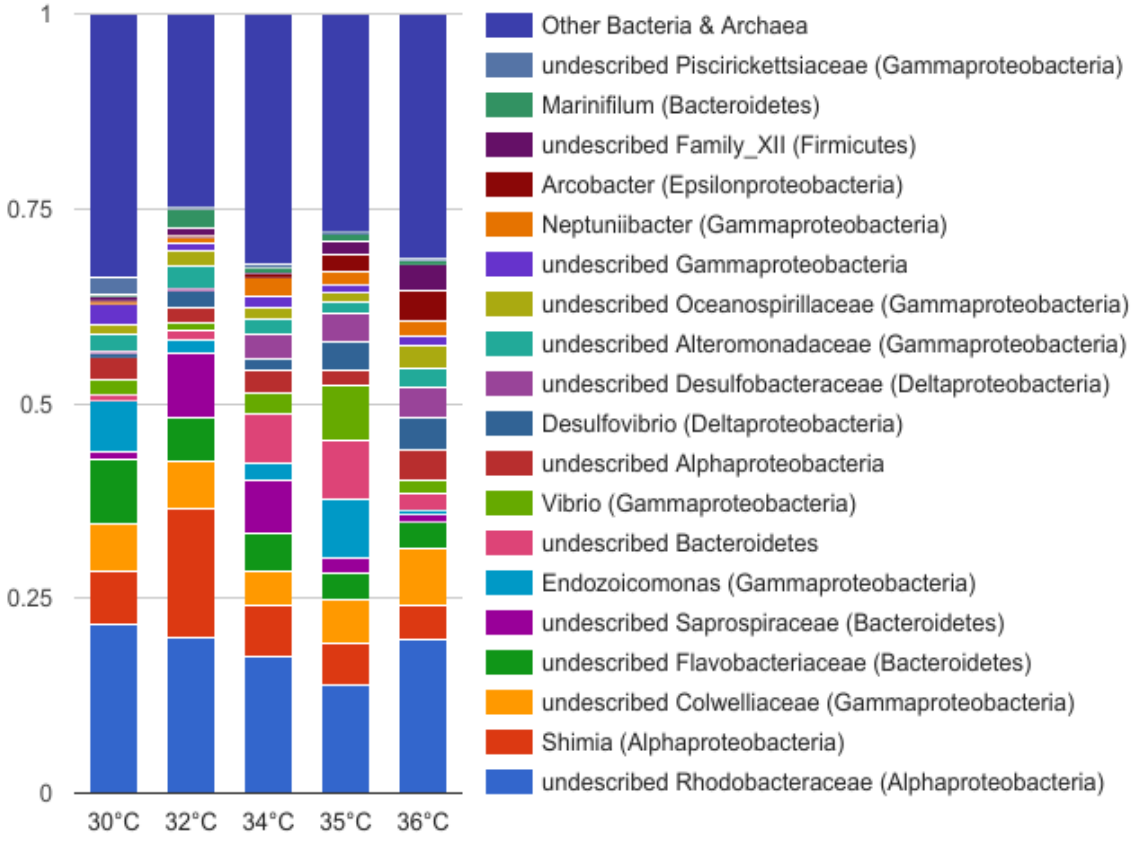
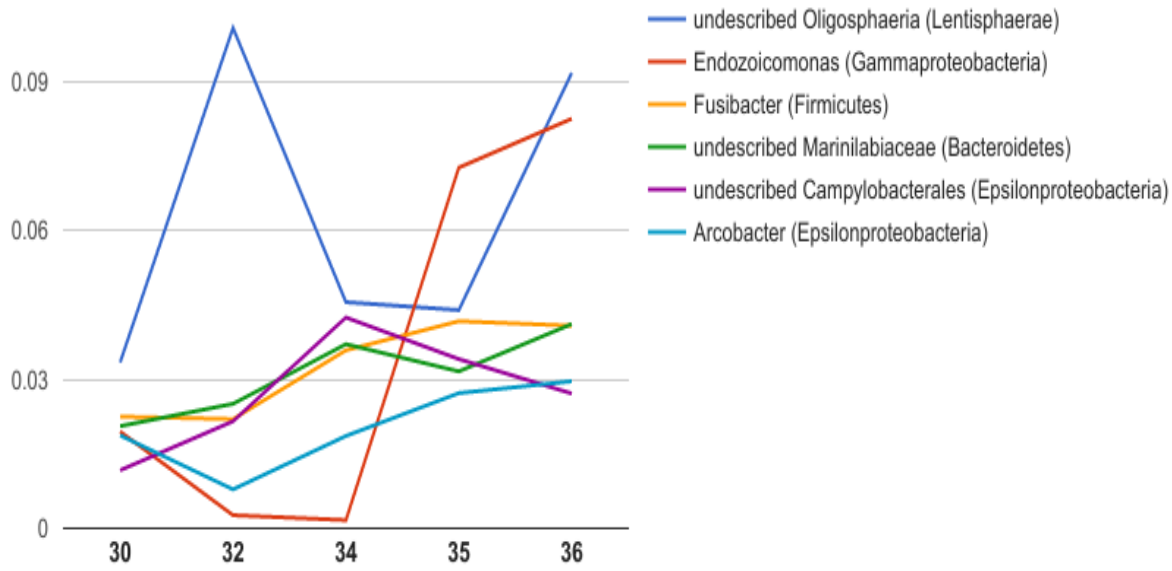


Figure 1.8. Compilation of **A.** 5 most abundant phyla, and **B.** 10 most abundant genera, of *P. lobata*-associated microbial taxa of all five temperature treatments from Pemuteran.

A. Amed -Increasing



B. Pemuteran - Increasing

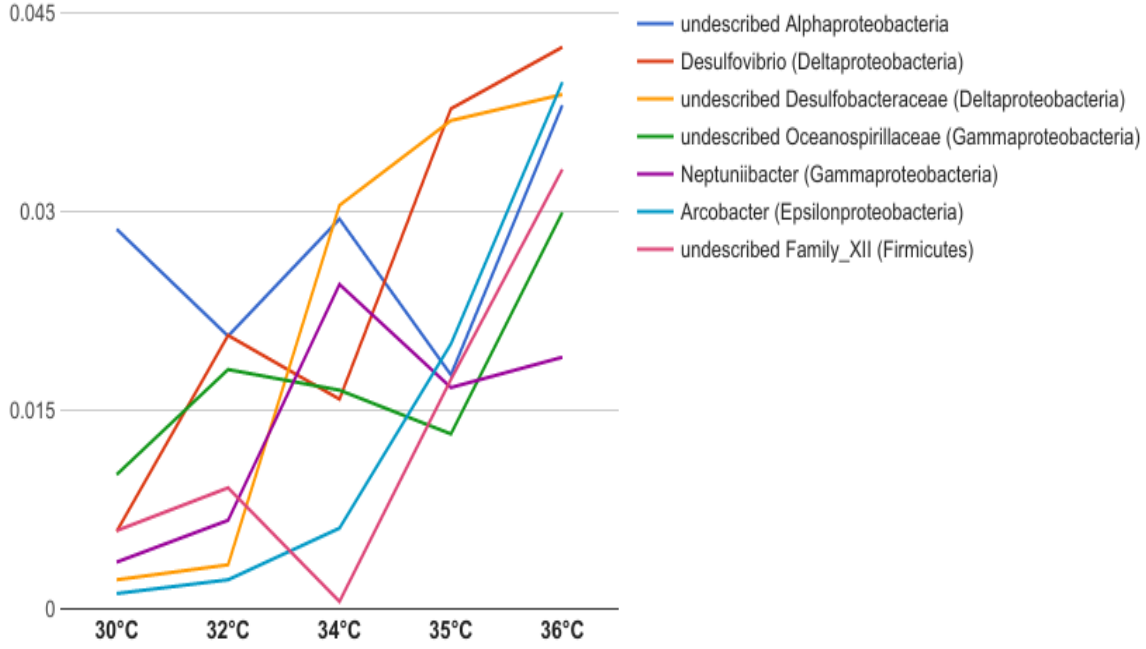
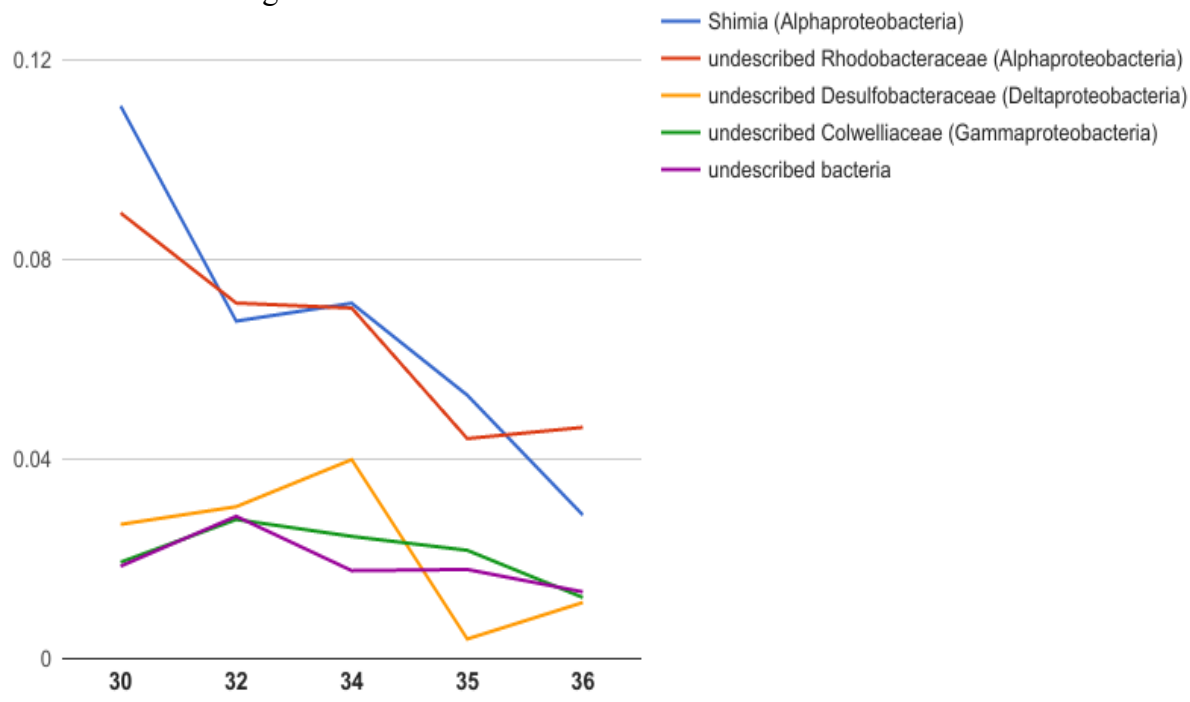


Figure 1.9. *Porites lobata*-associated microbial genera that had relative abundances increased with increasing temperature. **A.** in Amed, **B.** in Pemuteran. Y-axis is relative abundance.

A. Amed - Decreasing



B. Pemuteran - Decreasing

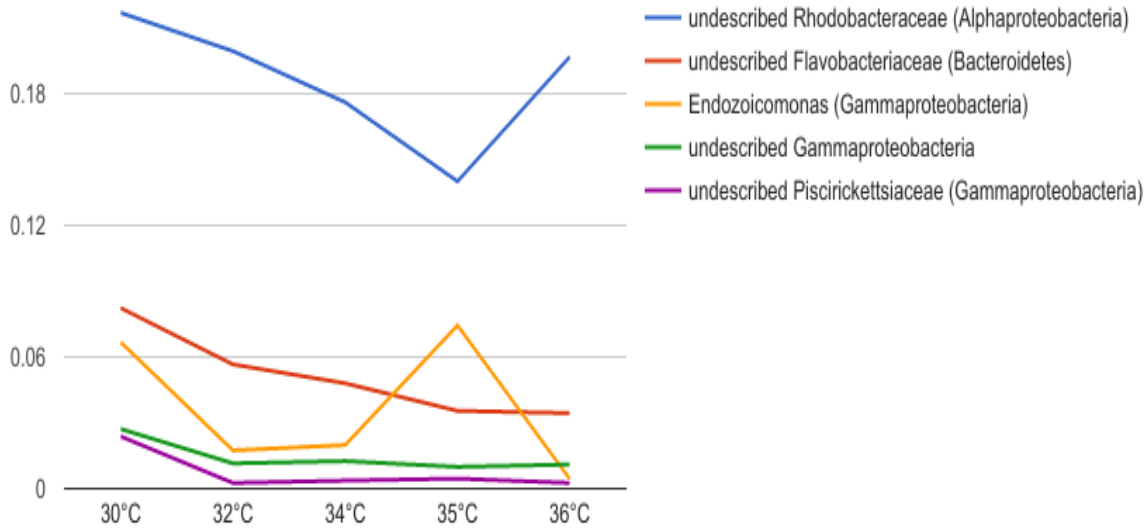
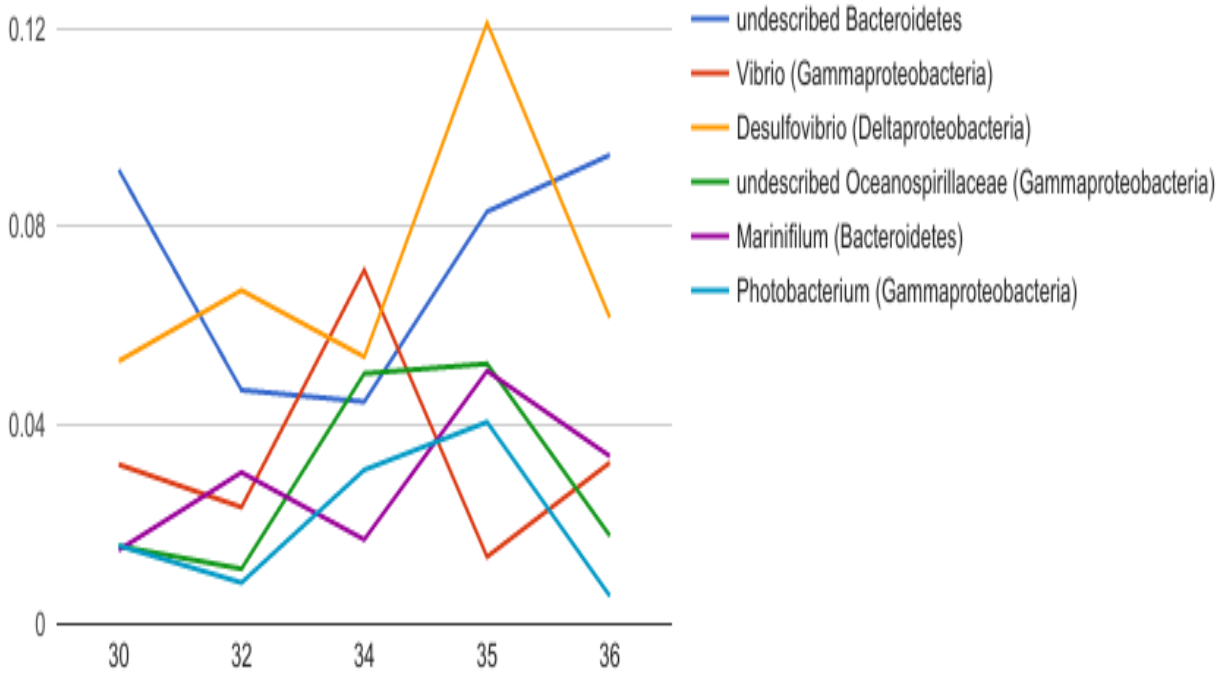


Figure 1.10. *Porites lobata*-associated microbial genera that had relative abundances decreased with increasing temperature. **A.** in Amed, **B.** in Pemuteran. Y-axis is relative abundance.

A. Amed - Fluctuated



B. Pemuteran- Fluctuated

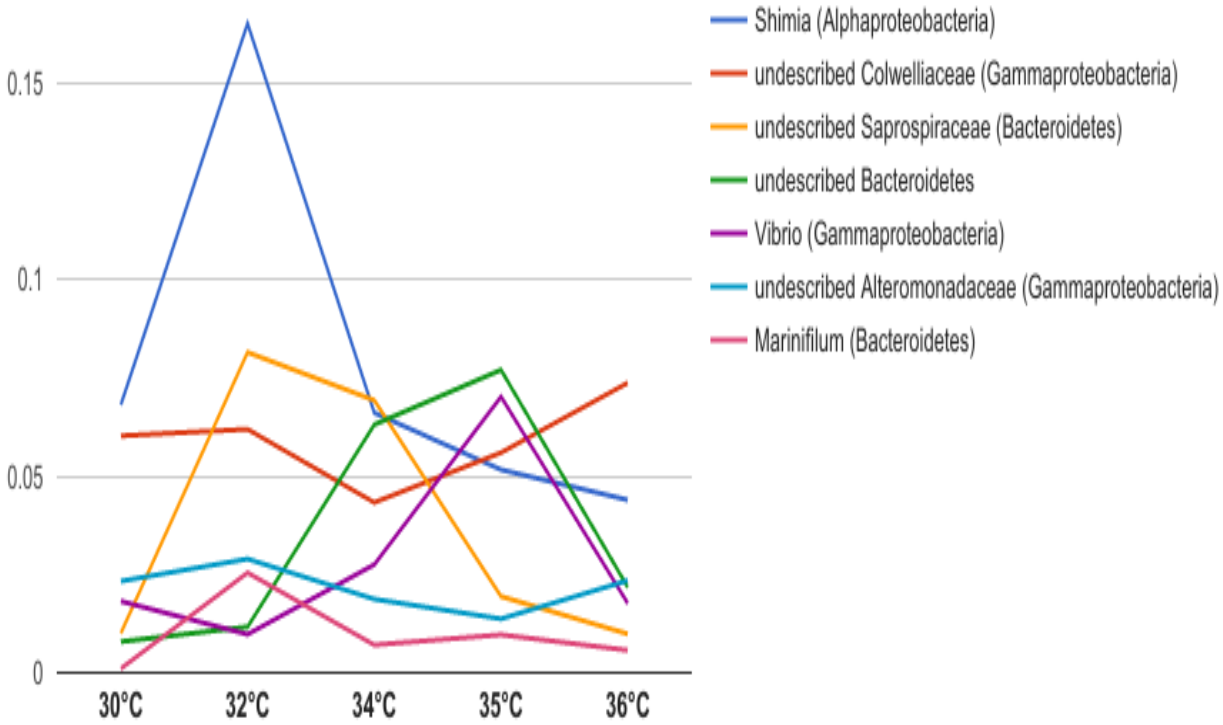


Figure 1.11. *Porites lobata*-associated microbial genera that had fluctuated relative abundances with increasing temperature. **A.** in Amed, **B.** in Pemuteran. Y-axis is relative abundance.

REFERENCES

Abrego, D., K.E. Ulstrup, B.L. Willis, and M.J.H. van Oppen. 2008. Species specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proc. R. Soc. Lond. Ser. B-Biol. Sci*. **275**:2273–2282.

Ainsworth, T.D. et al. 2016. Climate change disables coral bleaching protection on the Great Barrier Reef. *Science* **352**: 338–342.

Ainsworth, T.D. and O. Hoegh-Guldberg. 2009. Bacterial communities closely associated with coral tissues vary under experimental and natural reef conditions and thermal stress. *Aquat Biol* **4**: 289–296.

Allison, S.D. and J.B.H. Martiny. 2008. Resistance, resilience, and redundancy in microbial communities. *Proc Natl Acad Sci U S A* **105:** 11512–11519.

Apprill, A., S.P. McNally, C.R. Parsons, and L. Weber. 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat Microb Ecol* **75**: 129–137.

Baker, A.C. 2001. Reef corals bleach to survive change. *Nature* **411**: 765 - 766.

Baker, A.C. 2003. Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of Symbiodinium. *Annu. Rev. Ecol. Evol. Syst.* **34**: 661.

Baker, A.C., C.J. Starger, T.R. McClanahan, and P.W. Glynn. 2004. Corals' adaptive response to climate change. Shifting to new algal symbionts may safeguard devastated reefs from extinction. *Nature*, **430**: 741.

Banin, A., T. Israely, M. Fine, Y. Loya, and E. Rosenberg. 2001. Role of endosymbiotic zooxanthellae and coral mucus in the adhesion of the coral-bleaching pathogen *Vibrio shiloi* to its host. F*EMS Microbiology Letters* **199**: 33-37.

Barber, P. & S.L. Boyce. 2006. Estimating diversity of Indo-Pacific coral reef stomatopods through DNA barcoding of stomatopod larvae. *Proc. R. Soc. B.* **273:** 2053-2061.

Barber, R.T. and F.P. Chavez. 1983. Biological consequences of El Niño. *Science* **222(4629)**: 1203-1210.

Bayer, T., C. Arif , C. Ferrier-Pagès, D. Zoccola, M. Aranda, and C.R. Voolstra. 2013a. Bacteria of the genus *Endozoicomonas* dominate the microbiome of the Mediterranean gorgonian coral *Eunicella cavolini*. *Mar Ecol Prog Ser* **479**: 75–84.

Ben-Haim, Y., F.L. Thompson, C.C. Thompson, M.C. Cnockaert, B. Hoste, J. Swings, and E. Rosenberg. 2003. *Vibrio coralliilyticus* sp. nov., a temperature-dependent pathogen of the coral *Pocillopora damicornis*. *Int J Syst Evol Microbiol* **53**: 309–315.

Berkelmans, R. and M.J.H. van Oppen. 2006. The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proc. R. Soc. B.* **273:** 2305–2312.

Berkelmans, R., G. De'ath, S. Kininmonth, and W. Skirving. 2004. A comparison of the 1998 and 2002 coral bleaching events on the Great Barrier Reef: spatial correlation and predictions. *Coral Reefs* **23**:74–83.

Blackall, L.L., B. Wilson, and M.J.H. van Oppen. 2015. Coral-the world's most diverse symbiotic ecosystem. *Mol. Ecol.* **24**, 5330–5347.

Bourne, D., Y. Iida, S. Uthicke, and S. Smith-Keune. 2008. Changes in coral-associated microbial communities during a bleaching event. *ISME J.* **2112**, 350–363.

Bruckner, A.W. 2002. Life-saving products from coral reefs. *Issues in Science and Technology* **18**, no. 3 (Spring).

Bruno, J.F. and A. Valdivia. 2016. Coral reef degradation is not correlated with local human population density. *Scientific Reports 6 Springer Nature.*

Buddemeier, R.W. and D.G. Fautin. 1993. Coral bleaching as an adaptive mechanism. *Bioscience,* **43**:320–326.

Burke, L., K. Reytar, M. Spalding, and A. Perry. 2012. Reefs at Risk Revisited in the Coral Triangle. Washington, DC: World Resources Institute. http://www.wri.org/reefs. 86p.

Burman, S.G., R.B. Aronson, and R. van Woesik. 2012. Biotic homogenization of coral assemblages along the Florida reef tract. *Mar Ecol Prog Ser* **467**: 89–96.

Cacciapaglia, C. and R. van Woesik. 2015. Reef-coral refugia in a rapidly changing ocean. *Global Change Biology* **21:** 2272–2282.

Canganella, F. and J. Wiegel. 2011. Extremophiles: from abyssal to terrestrial ecosystems and possibly beyond. *Naturwissenschaften* **98**: 253.

Caporaso,, J.G., C.L. Lauber, W.A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer et al. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* **6**: 1621–1624.

Cárdenas, A., L.M. Rodriguez-R, V. Pizarro, L.F. Cadavid and C. Arévalo-Ferro. 2012. Shifts in bacterial communities of two caribbean reef-building coral species affected by white plague disease. *The ISME Journal* **6**: 502–512.

Cesar, H., L. Burke, and L. Pet-Soede. 2003. The Economics of Worldwide Coral Reef Degradation. Arnhem, the Netherlands: Cesar Environmental Economics Consulting.

Chen, C., C. Tseng, C.A.Chen, and S. Tang. 2011. The dynamics of microbial partnerships in the coral *Isopora palifera*. *ISME J* **5**: 728–740.

Clarke, K.R. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18**: 117-143.

Clarke, K.R. and R.N. Gorley. 2015. PRIMER v7: User Manual/Tutorial. PRIMER-E, Plymouth, 296pp.

De'ath, G., K.E. Fabricius, H. Sweatman, and M. Puotinen. 2012. The 27-year decline of coral cover on the Great Barrier Reef and its causes. *Proc Natl Acad Sci* **109**, 17995–17999.

Dinsdale, E.A., R.A. Edwards, D. Hall, F. Angly, M. Breitbart, J.M. Brulc, et al. 2008. Functional metagenomic profiling of nine biomes. *Nature* **452**: 629–632.

Eakin, C.M. et al. 2016. Global coral bleaching 2014–2017. *Reef Currents* **31**: 1.

Eren, A.M., H.G. Morrison, P.J. Lescault, J. Reveillaud, J.H. Vineis, and M.L. Sogin. 2014. Minimum entropy decomposition: unsupervised oligotyping for sensitive partitioning of high- throughput marker gene sequences. *ISME J* **9**: 968–979.

Fisher, R., R.A. O'Leary, S. Low-Choy, K. Mengersen, N. Knowlton, R.E. Brainard, et al. 2015. Species richness on coral reefs and the pursuit of convergent global estimates. *Curr. Biol.* **25**, 500–505.

Gardner, S.G., J-B. Raina, P.J. Ralph, K. Petrou. 2017. Reactive oxygen species (ROS) and dimethylated sulphur compounds in coral explants under acute thermal stress. *Journal of Experimental Biology* **220**: 1787-1791.

Geffen, Y., E.Z. Ron and E. Rosenberg. 2009. Regulation of release of antibacterials from stressed scleractinian corals. *FEMS Microbiol Lett* **295**: 103–109.

Glynn, P. 1984. Widespread Coral Mortality and the 1982–83 El Niño Warming Event. *Environmental Conservation* **11(2)**: 133-146.

Glynn, P.W., J.L. Mate, A.C. Baker, and M.O. Calderon. 2001. Coral bleaching and mortality in Panama and Ecuador during the 1997–98 El Niño–Southern Oscillation event: spatial/temporal patterns and comparisons with the 1982– 1983 event. *Bulletin of Marine Science* **69**:79–109.

Graham, N.A.J., S. Jennings, M.A. MacNeil, D. Mouillot, and S.K. Wilson. 2015. Predicting climate-driven regime shifts versus rebound potential in coral reefs. *Nat. Res. Let.* doi:10.1038/nature14140.

Hadaidi, G., T. Rothig, L.K. Yum, M. Ziegler, C. Arif, C. Roder, et al. 2017. Stable mucus-associated bacterial communities in bleached and healthy corals of *Porites lobata* from the Arabian Seas. *Sci Rep* **7**: 45362.

Hernandez-Agreda, A., W. Leggat, P. Bongaerts, and Ainsworth, T. D. (2016). The microbial signature provides insight into the mechanistic basis of coral success across reef habitats. MBio 7, e00560–16. doi: 10.1128/mBio.00560-16

Hughes, T.P., J.T. Kerry, M. Álvarez-Noriega, J.G. Álvarez-Romero, K.D. Anderson, et al. 2017. Global warming and recurrent mass bleaching of corals. Nature, 543: 373-378.

Iglesias-Prieto, R., J.L. Matta, W.A. Robins, and R.K. Trench. 1992. Photosynthetic response to elevated temperature in the symbiotic dinoflagellate *Symbiodinium microadriaticum* in culture (bleaching/coral reefs). *Proc. Natl. Acad. Sci*., **89**: 10302-10305.

Jindal, A., K.B. Ritchie, R.L. Hayes, T.J. Goreau, and G.W. Smith. 1995. Bacterial ecology of selected corals following the 1994 south central pacific bleaching event. 26-34.

Kayanne, H. 2016. Validation of degree heating weeks as a coral bleaching index in the northwestern Pacific. *Coral Reefs* **36**: 63–70.

Kellogg, C.A., Y.M., Piceno, L.M., Tom, T.Z., DeSantis, M.A., Gray, D.G., Zawada, and G.L. Andersen. 2013. Comparing bacterial community composition between healthy and White Plague-like disease states in *Orbicella annularis* using PhyloChip G3 microarrays. *PLoS ONE* **8**: e79801.

Kinzie, III, R.A., M. Takayama, S.R. Santos, and M.A. Coffroth. 2001. The Adaptive Bleaching Hypothesis: Experimental Tests of Critical Assumptions. *Biol. Bull.* **200**: 51-58.

Koren, O. and E. Rosenberg. 2006. Bacteria associated with mucus and tissues of the coral *Oculina patagonica* in summer and winter. *Appl Environ Microbiol* **72**: 5254– 5259.

Koren, O. & E. Rosenberg. 2008. Bacteria associated with the bleached and cave coral *Oculina patagonica*. *Microb Ecol,* **55**: 523.

Kozich, J.J., S.L. Westcott, N.T. Baxter, N.T. Highlander, and P.D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl Environ Microbiol* **79**: 5112–5120.

Kuever J. 2014. The Family Desulfobacteraceae. *In*: Rosenberg E., E.F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson (eds) The Prokaryotes. Springer, Berlin, Heidelberg.

Kushmaro, A., E., Banin, Y., Loya, E., Stackebrandt, and E. Rosenberg. 2001. *Vibrio shiloi* sp. nov., the causative agent of bleaching of the coral *Oculina patagonica*. *Int J Syst Evol Microbiol* **51**: 1383–1388.

Kusuma, D.W. et al. 2017. *IOP Conf. Ser.: Earth Environ. Sci.* **98** 012038.

Leggat, W., F. Seneca, K. Wasmund, L. Ukani, D. Yellowlees, et al. 2011. Differential responses of the coral host and their algal symbiont to thermal stress. *PLoS ONE* **6(10)**: e26687.

Littman, R., B.L., Willis, and D.G. Bourne. 2011. Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. *Environ Micro- biol Rep* **3**: 651–660.

Littman, R., B.L., Willis, C. Pfeffer, and D.G. Bourne. 2009. Diversities of coral-associated bacteria differ with location, but not species, for three acroporid corals on the Great Barrier Reef. *FEMS microbiology ecology* **68(2)**: 152-163.

Lough, J.M. and M.J.H. van Oppen. 2009.Introduction: Coral Bleaching Patterns Processes, Causes and Consequences. 1–5 In Ecological Studies. Springer Berlin Heidelberg.

Loya, Y., K., Saka, K., Yamazato, Y., Nakano, H., Sambali, and R. van Woesik. 2001. Coral bleaching: the winners and the losers. *Ecol Lett* **4**: 122–131.

Marshall, P.A. and A.H. Baird. 2000. Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. *Coral Reefs* **19**: 155–163.

McClanahan, T.R., J. Maina, P.R. Moothien, et al. 2005. Effects of geography, taxa, water flow, and temperature variation on coral bleaching intensity in Mauritius. *Marine Ecology Progress Series,* **298:** 131–142.

McClung, C.R., D.G. Patriquin, and R.E. Davis. 1983. *Campylobacter nitrofigilis* sp. nov., a nitrogen-fixing bacterium associated with roots of *Spartina alterniflora* Loisel. *Int J Syst Bacteriol* **33**: 605–612.

Methé, B.A., K.E. Nelson, J.W. Deming, B. Momen, E. Melamud, et al. 2005. The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses. *Proc Natl Acad Sci USA* **102**:10913–10918.

Meyer, J.L., V.J. Paul, and M. Teplitski. 2014. Community shifts in the surface microbiomes of the coral *Porites astreoides* with unusual lesions. *PLoS ONE* **9(6)**: e100316.

Mieog, J.C. 2009. Flexibility of the coral-algal symbiosis in the face of climate change: investigating the adaptive bleaching hypothesis. Thesis dissertation. The University of Groningen.

Mouchka, M.E., I. Hewson, and C.D. Harvell. 2010. Coral-associated bacterial assemblages: current knowledge and the potential for climate-driven impacts. *Integr. Comp. Biol*. **50:** 662–674.

Neave, M. J., R. Rachmawati, L. Xun, C. T. Michell, D. G. Bourne, A. Apprill, and C.R. Voolstra. 2017. Differential specificity between closely related corals and abundant *Endozoicomonas* endosymbionts across global scales. *ISME J.* **11**: 186–200.

Oliver, J.K., R. Berkelmans, and C. M. Eakin. 2009. *in* van Oppen, M.J.H. and J. M. Lough (eds). Ecological Studies: Analysis and Synthesis, 21–39.

Osborne, K., A.A. Thompson, A.J. Cheal, et al. 2017. Delayed coral recovery in a warming ocean. *Glob Change Biol.* **23**:3869–3881.

Pandolfi, J.M. 2015. Ecology: Deep and complex ways to survive bleaching. *Nature* **518**: 43–44.

Parrin, A. and N. Blackstone. 2017. The role of light and photo-oxidative stress in coral bleaching. *FASEB Journal* **31** 889.11.

Perry, C.T. and K.M. Morgan. 2017. Bleaching drives collapse in reef carbonate budgets and reef growth potential on southern Maldives reefs. *Sci Rep* **7**:40581.

Podesta, G.P. and P.W. Glynn. 2001. The 1997–98 El Niño event in Panama and Galapagos: an update of thermal stress indices relative to coral bleaching. *Bulletin of Marine Science* **69**:43–59.

Pruesse, E., C. Quast, K. Knittel, B. Fuchs, W. Ludwig, J. Peplies, et al. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research* **35**: 7188-7196.

Raina, J., D. Tapiolas, C. Motti, S. Foret, and T. Seemann. 2016. Isolation of an antimicrobial compound produced by bacteria associated with reef-building corals. *PeerJ.* **4**:e2275.

Reaka-Kudla, M.L. 2001. Known and unknown biodiversity risk of extinction and conservation strategy in the sea. 19–33 *In* Waters in Peril. Springer US.

Rice, E.W., M.R. Rodgers, I.V. Wesley, C.H. Johnson, and S.A. Tanner. 1999. Isolation of *Arcobacter butzleri* from ground water. *Letters in Applied Microbiology* **28**: 31–35.

Ritchie, K.B., J.H. Dennis, T. McGrath, and G.W. Smith. 1994. Bacteria associated with bleached and non-bleached areas of *Montastrea annularis*. *In* Kass, L. (ed.) Proc 5th Symp Nat Hist Bahamas. San Salvador, Bahamas: Bahamian Field Station, pp. 75– 80.

Roberts, C.M., et al. 2002. Marine biodiversity hotspots and conservation priorities for tropical reefs. *Science*, **295(5558)**: 1280-1284.

Rohwer, F., V. Seguritan, F. Azam, and N. Knowlton. 2002. Diversity and distribution of coral-associated bacteria. *Mar Ecol Prog Ser* **243**: 1–10.

Rosenberg, E., A. Kushmaro, E. Kramarsky-Winter, E. Banin and L. Yossi. 2009. The role of microorganisms in coral bleaching. *The ISME Journal* **3**: 139–146.

Rosenberg, E., O. Koren, L. Reshef, R. Efrony and I. Zilber-Rosenberg. 2007. The role of microorganisms in coral health, disease and evolution. *Nat Rev Microbiol* **5**: 355-362.

Rowan, R. and N. Knowlton. 1995. Intraspecific diversity and ecological zonation in coral–algal symbioses. *Proc. Natl. Acad. Sci*. **92,** 2850–2853.

Rowan, R., N. Knowlton, A. Baker, and J. Jara. 1997. Landscape ecology of algal symbionts creates variation in episodes of bleaching. *Nature* **388**: 265-269.

Rudi, E., T. Iskandar, N. Fadli, and H. Hidayati. 2012. Impact of mass coral bleaching on fish reef community and fishermen catches at Sabang, Aceh Province, Indonesia. AACL Bioflux 5(5): 309-320.

Rypien, K.L., J. R. Ward, and F. Azam. 2010. Antagonistic interactions among coral-associated bacteria. *Environ. Microbiol.* **12**, 28–39.

Sampayo, E.M., T. Ridgway, P. Bongaerts, and O. Hoegh-Guldberg. 2008. Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. *PNAS*, **105, 30:** 10444-10449.

Schloss, P.D., S.L. Westcott, T. Ryabin, J.R. Hall, M. Hartmann, E.B. Hollister, et al. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75**: 7537–7541.

Setiasih, N. and J. Wilson. 2010. A regional overview on the 2010 coral bleaching event in Southeast Asia: Indonesia. *in* Status of coral reefs in East Asian seas region: 2010. The Global Coral Reef Monitoring Network. Tokyo, Japan.

Stat, M. and R.D. Gates. 2011. Clade D *Symbiodinium* in scleractinian corals: a "nugget" of hope, a selfish opportunist, an ominous sign, or all of the above? *J. Mar. Biol.* **2011**: 1–9. doi: 10.1155/2011/730715

Sunagawa, S., C.M. Woodley, and C.M. Medina. 2010. Threatened corals provide underexplored microbial habitats. *PLoS ONE* **5**: e9554.

Thomas, F., J-H. Hehemann, E. Rebuffet, M. Czjzek, and G. Michel. 2011. Environmental and gut Bacteroidetes: the food connection. *Frontiers in Microbiology* **2**:93.

Thompson, F.L., Y. Barash, T. Sawabe, G. Sharon, J Swings, and E. Rosenberg. 2006. *Thalassomonas loyana* sp. nov., a causative agent of the white plague-like disease of corals on the Eilat coral reef. *Int J Syst Evol Microbiol* **56**:365–368.

Tracy, A.M., O. Koren, N. Douglas, E. Weil, and C.D. Harvell. 2015. Persistent shifts in Caribbean coral microbiota are linked to the 2010 warm thermal anomaly. *Environmental Microbiology Reports* **7**: 471–479.

Vandenberg, O., A. Dediste, K. Houf, S. Ibekwem, H. Souayah, S. Cadranel, N. Douat, G. Zissis, J.P. Butzler, and P. Vandamme. 2004. *Arcobacter* species in humans. *Emerging Infectious Diseases* **10(10)**:1863-1867.

Vega Thurber, R., D. Willner-Hall, B. Rodriguez-Mueller, C. Desnues, R.A. Edwards, F. Angly, et al. 2009. Metagenomic analysis of stressed coral holobionts. *Environ. Microbiol.* **11**: 2148–2163.

Veron, J.E.N. et al. 2011. The Coral Triangle. *In*: Dubinsky Z. and N. Stambler (eds). Coral Reefs: An Ecosystem in Transition. Springer, Dordrecht.

Vezzulli, L., M. Previati, C. Pruzzo, A. Marchese, D.G. Bourne, et al. 2010. *Vibrio* infections triggering mass mortality events in a warming Mediterranean Sea. *Environ Microbiol* **12**: 2007–2019.

Wang, J., S. Keshavmurthy, T. Chu, and CA. Chen. 2017. Diverse responses of *Symbiodinium* types to menthol and DCMU treatment. *PeerJ* **5**:e3843.

Wellington, G.M., P.W. Glynn, A.E. Strong, S.A. Navarette, E. Wieters, and D. Hubbard. 2001. EOS *Trans Am Geophys Union*, **82**:1–6.

Werner, J., O. Koren, P. Hugenholtz, T.Z. DeSantis, W.A. Walters, J.G. Caporaso, et al. 2012. Impact of training sets on classification of high-throughput bacterial 16s rRNA gene surveys. *ISME J* **6**: 94–103.

Westmacott, S., H.S.J. Cesar, L. Pet-Soede and O.Lindén. 2000. Coral bleaching in the Indian Ocean: socio-economic assessment of effects. *in* Cesar H.S.J. Collected essays on the economics of coral reefs. CORDIO. Kalmar, Sweden.

Wirsen, C.O., S.M. Sievert, C.M. Cavanaugh, S.J. Molyneaux, A. Ahmad, L.T. Taylo, E.F. DeLong and C.D. Taylor. 2002. Characterization of an autotrophic sulfide-oxidizing marine *Arcobacter* sp. that produces filamentous sulfur. *Appl Environ Microbiol* **68**: 316–332.

Yilmaz, P., P. Yarza, J.Z. Rapp, and F.O. Glöckner. 2016. Expanding the world of marine bacterial and archaeal clades. *Frontiers in Microbiology* **6.**

Ziegler, M., F.O. Seneca, L.K. Yum, S.R. Palumbi, and C.R. Voolstra. 2017. Bacterial community dynamics are linked to patterns of coral heat tolerance. *Nat Commun* **8**: 14213.

CHAPTER 2

Differential response of coral microbiomes to thermal stress

Introduction

Coral reefs are the most diverse marine ecosystems on the planet (Reaka-Kudla 2001), providing a variety of ecological services, including biological support for coastal and offshore ecosystems, biogeochemical cycling, climate records, and social and cultural services for coastal communities. Despite their importance, coral reefs are highly threatened worldwide (Cesar et al. 2003; Osborne et al. 2017). In Indonesia, a country that is home to > 19% of the world's coral reefs (Roberts et al. 2002; Veron et al. 2011), overfishing, pollution, and coastal development are having major negative impacts on coral reef ecosystems. Currently more than 85% of Indonesian reefs are at risk (Burke et al. 2002) decreasing their environmental and economic productivity (Jokiel 2004; Stambler and Dubinsky 2004).

In Indonesia and worldwide, indirect environmental threats related to climate change are a growing concern (Baker et al. 2008; Carpenter et al. 2008; Ainsworth et al. 2016). In particular, elevated global temperatures are resulting in mass coral bleaching (Kayanne 2017), contributing to significant declines of coral reefs (Ainsworth et al. 2016). More disturbing is that the frequency of mass bleaching events has increased. While only three coral bleaching events were recorded in the previous century, in the last three decades alone, there have been dozens of reports of coral bleaching and the frequency and severity of these bleaching events are increasing (Donner et al. 2017; Hughes et al. 2017). Moreover, the geographic area impacted by coral bleaching leading to mass mortality is also increasing (Hughes et al. 2017).

Models of climate change indicate that atmospheric temperatures will continue to rise for the foreseeable future (Eakin et al. 2016; Donner et al. 2017) with associated sea surface temperature (SST) increases across much of the world's oceans (Hoegh-Guldberg et al. 2014). Of particular concern, the highest rate of increasing SST (0.13 °C per decade) is occurring in the Coral Triangle and Southeast Asia (Hoegh-Guldberg et al. 2014), impacting the world's largest and most diverse coral reef ecosystem (Roberts et al. 2002, Bellwood and Meyer 2009; Veron et al. 2011) on which up to 100 million depend upon for jobs and food (Cesar et al. 2003). In the face of such dramatic environmental changes, effective conservation strategies for coral reefs must focus on better understanding the process of coral bleaching and the causes of natural variation in resistance to bleaching. Such information may help support the design of strategic networks of marine protected areas designed to maximize conservation of coral reef biodiversity (West and Salm 2003).

While no reefs are immune to coral bleaching, some are more sensitive than others (Obura 2005), with coral endosymbionts (*Symbiodinium* spp.) playing an important role in thermal sensitivity. In particular, clade D *Symbiodinium* are less sensitive to heat stress and are most likely to survive during extreme seawater temperature events (Baker et al. 2004). However, in addition to *Symbiodinium*, the coral holobiont includes a host other diverse microorganisms, including bacteria and archaea (Rohwer et al. 2002). These microbial communities can be sensitive to environmental change, including increasing ocean temperature (Ritchie et al. 1994; Bourne 2008). In particular, these microbial communities can change during elevated SST that lead to bleaching, resulting in different microbiome composition (Ritchie 1994; Jindal et al.1995; Mouchka et al. 2010).

Although no corals are completely immune to bleaching, different species or families of corals also respond differently to heat stress. For example, corals from the genera *Acropora* and *Pocillopora* can be more sensitive to heat stress and bleaching than some massive *Porites* corals (Marshall and Bair 2000; Loya et al. 2001; McClanahan et al. 2004; Guest et al. 2012). In fact, the large geographic range of *Porites* corals illustrates their high capacity to thrive in different environments and fluctuations in climate (Burman et al. 2012). Such taxon-specific sensitivities will likely translate into varied survivorship as the Earth's temperature continues to increase (Logan et al. 2014; Cacciapaglia and van Woesik 2015; Hughes et al. 2017), reshaping contemporary reef ecosystems, the biodiversity they support, and ecosystem services that they provide.

Varied responses of coral taxa to thermal stress leading to bleaching could be an intrinsic property of the coral host. However, increasingly research shows that coral microbiomes can be specific to genera or species of coral (Littman et al. 2009; Neave et al. 2017). For example, *Pocillopora verrucosa* had different microbial compositions from *Stylophora pistillata,*which is from the same family Pocilloporidae, and its compositions were relatively consistent across diverse sampling locations worldwide (Neave et al. 2017). Similarly, despite being part of the same genus, *Acropora millepora, A. tenuis,* and *A. valida* living on the same reef had unique, species-specific microbial communities, although this host species-associated difference was not as strong as locality difference (Littman et al. 2009). Taxon-specific variation in coral microbiomes raises the possibility that these microbes could also contribute to the response of individual coral taxa to thermal stress, potentially contributing to taxon specific response to thermal stress.

Indonesia is home to 39,500 km^2 of reefs (Burke et al. 2012) with the highest diversity of coral species in the world (Roberts et al. 2002; Veron et al. 2011). Located at the convergence of the Indian and Pacific Oceans with reefs spanning 18 degrees latitude and 46 degrees longitude, Indonesia is an ideal place to examine variation in bleaching sensitivity among regions and coral species. Of particular interest are the reefs of Eastern Indonesia that have historically only experienced mild levels of bleaching. For example, during 2010 global mass bleaching event West Papua had no bleaching in Manokwari and Cendrawasih Bay and only mild bleaching observed in Raja Ampat (Setiasih and Wilson 2010) as compared to Aceh (western tip of Sumatera) that experienced 80% of bleaching (Guest et al. 2012; Rudi et al. 2012), while both regions experienced similar duration of warm water in 2007-2016 period (Kusuma et al. 2017).

In this study, we examine variation in sensitivity to coral bleaching in three species of coral from different families (*Acropora millepora*, *Pocillopora verrucosa*, *Porites lobata*) from Manokwari (West Papua). Then, we explore their associated variation in microbial communities to examine possible relationships with their capacity to cope temperature stress.

Materials and Methods

Temperature experiments

To assess relative sensitivity among coral taxon to bleaching associated with high temperature and examine the concomitant response of coral-associated microbial communities, we collected coral fragments from Manokwari Bay, West Papua, Indonesia (Figure 2.1), a massively biodiverse region of Indonesia with no recorded mass bleaching events. To represent a diversity of coral morphologies, we focused on three different families: *Acropora millepora*, a

fine branching coral from family Acroporidae, *Pocillopora verrucosa*, a massive branching coral from family Pocilloporidae, and *Porites lobata*, a massive coral from family Poritidae.

We collected 3 small colonies, which were at least 5 m apart, for each species at about 2–4 m depth using SCUBA, selecting healthy colonies with no obvious signs of bleaching, disease, boring organisms, or coralivorous predators. To avoid possible cross contamination of microbiomes, we placed each colony collected into a different bag to prevent physical contact during transport. We then transported all colonies to the field station in separate aerated containers filled with local seawater and shaded the containers to reduce coral stress from direct sunlight.

At the field station, we transferred all colonies to experimental tanks filled with seawater from the same reef where the corals were collected. We used automatic heaters to maintain constant temperature, and monitored water temperature with a thermometer. We also equipped each tank with a protein skimming system that worked continuously to reduce and neutralize particulates in the tank as well as to oxygenate the water.

Prior to beginning temperature treatments, we equilibrated all tank temperatures to 30 °C to mimic the sea surface temperature on the reef from which we collected the corals, and this temperature served as our baseline as control. We allowed all colonies one day to acclimate to the experimental tanks and to recover from transportation and handling stress. We then fragmented each coral colony into four fragments and placed them into the experimental tanks such that each experimental tank became a common garden environment containing nine coral fragments, representing three different colonies of three coral species. Following colony fragmentation, we allowed an additional day to let corals fragments recover from handling and fragmenting stress.

Once corals acclimated, we gradually increased water temperatures to represent different scenarios of elevated sea surface temperature (SST). The first treatment was 32 °C, representing 1–3 °C above mean long-term annual maximum temperatures, which is the upper thermal tolerance of most corals (Marshall and Baird 2000; Glynn et al. 2001; Podesta and Glynn 2001; Wellington et al. 2001) and near-term forecast of the Intergovernmental Panel on Climate Change (IPPC) projected SST**.** The second treatment was 34 °C, a temperature that leads to bleaching in corals, even over short periods of exposure (Barber and Chavez 1983). The last treatment, 36 °C, represented a temperature extreme designed to ensure bleaching in all experimental corals. We increased temperatures gradually (not exceeding 1 °C in 12 h, Barron et al. 2010) to avoid additional stress from abrupt temperature change and to let corals acclimate to gradual temperature change as they experience in nature. During acclimation and increasing temperatures, we replaced 10% of the water in each tank with fresh seawater from the original reef each day to maintain water quality while avoiding shock from completely changing experimental seawater. However, to avoid having seawater changes impact results, we did not change or add seawater during the 12 h of temperature treatment.

To document changes in the coral microbiomes, we collected a small sample from each coral fragment prior to increasing water temperatures to serve as a baseline, and preserved these fragments in 95% ethanol. We then collected additional samples from each fragment 12 hours after reaching the maximum water treatment temperature (e.g. 32 °C, 34 °C, or 36 °C), applying the same preservation method. We stored all samples at -20 °C.

DNA extraction

We did extractions on two experimental colonies of each treatment and each coral species. We extracted coral DNA by first draining all ethanol, and then airbrushing each sample using cold sterile 1X phosphate-buffered saline (PBS), allowing us to remove most coral tissue and associated microbial cells from the skeleton. We centrifuged the resulting tissue slurry at 5000 rpm for 20 min at 4 °C to create a tissue pellet. We used less than 50 mg of each tissue pellet to extract the DNA using a MoBio DNA Ultra Clean kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's recommendations, with the modification of an additional 30 min incubation period at 56 °C with 400 rpm shaking followed by a 15 min vortexing step to help further break up coral tissues prior to extraction. All DNA extractions were stored at -20 °C.

SSU rRNA gene amplifications and sequencing

To determine the composition of, and variation among, bacterial & archaeal communities of all experiment coral colonies, we amplified 253 bp of the hypervariable region 4 (V4) of their small subunit (SSU) ribosomal RNA (rRNA) genes using primers 515F: GTGCCAGCMGCCGCGGTAA and 806RB: GGACTACNVGGGTWTCTAAT (Caporaso et al. 2012; Apprill et al. 2015). Then, following Kozich et al. (2013) we dual indexed all PCR reactions by added primers with an Illumina (San Diego, CA, USA) adapter, 8 bp index sequence, 10 bp pad sequence, and 2 bp linker. We amplified each sample in 20-µl PCR reactions containing 1 to 3 µl of template DNA, depending on DNA extraction concentration, 1 unit of GoTaq® Flexi DNA Polymerase (Promega, Madison, USA), 5X Colorless GoTaq® Flexi Buffer, 2 mM MgCl2, 160 µM dNTP mix (Promega, Madison, USA), and 160 nM of each barcoded primer, and adjusted volume of sterile molecular grade water. We amplified PCR

reactions in triplicate in a Bio-Rad C1000 Touch™ Thermal Cycler (< 5 °C/s of ramp rate, Bio-Rad Laboratories, Hercules, CA, USA) using an initial denaturation for 2 min at 95 °C followed by 30 cycles of 20 s at 95 °C, 15 s at 55 °C and 5 min at 72 °C and a final extension for 10 min at 72 °C.

To visualize the quality of the resulting PCR products, we electrophoresed them in a 1% agarose gel with 1X TBE buffer for 60 min at 110 V with the 50bp Hyperladder (5 ng μl−1) (Bioline USA Inc., Taunton, MA, USA) as reference. To visualize the electrophoresis results, we stained gels with SYBR Safe (Invitrogen, Carlsbad, CA, USA) illuminated with an UltraBright LED Transilluminator, 470nm (LB-16) (Maestrogen, Nevada, USA). In cases of weak PCR, we rerun the PCR with more cycles (35 or 40); similarly, if cases of too strong PCR, we reduced the number of cycles (e.g. 25 cycles). This process allowed us to obtain consistent amplification across all fragments. We recovered and purified PCR products from agarose gel slices using the Qiagen MinElute gel extraction kit (Qiagen, Hilden, Germany) and eluted them in 10 μl EB buffer. We then quantified DNA concentrations of purified DNA using a Qubit 2.0 Fluorometer with the dsDNA High Sensitivity Assay (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA) before pooling them into a library.

To achieve similar sequence depth across all samples, we pooled approximately 2 ng of each purified sample DNA along with all controls. For positive control, we used Microbial Mock Community B (Staggered, Low Concentration, v5.2L, for 16S rRNA Gene Sequencing, HM-783D obtained through BEI Resources, NIAID, NIH as part of the Human Microbiome Project). In addition, we included three negative PCR controls from 30, 35 and 40 cycles PCR reactions as negative controls. We then sequenced these libraries using 250bp paired-end sequencing on an Illumina MiSeq (San Diego, CA, USA).

Bioinformatics and data analyses

To determine the composition and abundance of coral-associated microbiomes from Illumina sequence results, we used Mothur v1.34.4 (Schloss et al. 2009). Briefly, we used *make.contigs* to combine the overlapping segments of sequenced amplicons, the used *screen.seqs* to remove contigs > 255 bp or contigs with ambiguous positions. We then classified the resulting contigs against the SILVA database v119 (Pruesse et al. 2007) using the *classify.seqs* command with the 'knn' method, using the *remove.lineage* command to exclude DNA sequences that were not bacteria or archaea (e.g. chloroplast, eukaryota, mitochondria, and 'unknown'). Next, we used *chimera.uchime* and *remove.seqs* to detect and remove chimeras, respectively. We then used *remove.groups* and *get.groups* to remove all other groups, such as positive and negative controls that were not included in the next analyses, and to isolate certain parts of the dataset for subsequent analysis, respectively. Finally, we used *sub.sample* to subsample 8,000 sequences, standardizing the number of sequences from each experimental coral sample for downstream analyses.

To examine the composition of coral microbial communities, we used Minimum Entropy Decomposition (MED; Eren et al. 2014) to cluster the resulting datasets based on the least sequence difference. First, we used "headersMED.py" command in Python (Neave et al. 2017) to convert fasta and group files into MED-compatible formats. We then used MED to generate Nonmetric Multidimensional Scaling (NMDS) plots based on the ranks of Jaccard distance. For MED analyses, we used all samples that generated more than 8,000 high-quality sequences, regardless the temperature treatments applied, to represent a combined microbiome signature across all treatments.

To determine whether the Jaccard distance-based NMDS clusters were significantly different, we used the analysis of similarity algorithm (ANOSIM), based on Jaccard distance with 9,999 permutations. ANOSIM is a non-parametric analysis that compares the distance ranks between groups to ones within groups to test the similarity level of two or more groups (Clarke 1993). We used Primer-E (Clarke and Gorley, 2015) to run ANOSIM analysis using MATRIX-PERCENT data from the MED analyses. In addition, we also analyzed diversity indices, such as Chao-1 richness estimate to estimate the total number of OTUs in each sample, evenness (e^H'/S, S=number of OTUs) to examine variation in the abundance of OTUs, Shannon diversity estimate (H') to estimate taxonomic richness based on relative abundance and evenness of OTUs, Simpson diversity estimate (1-D, D=dominance) to estimate the dominance of OTUs in the microbial community, and Whittaker beta diversity to examine the variation in community diversity among samples. We then used Kruskal-Wallis and Mann-Whitney tests as employed in the PAST statistical software package (v3.14, Hammer et al. 2001) to determine statistical significance of diversity measures. To compare diversity of major microbial groups across taxa, we combined data from all treatments. However, to examine species-specific differences in response to thermal stress, we generated species specific bar plots across all temperature treatments. Lastly, we examined differences in the distribution of major OTUs among groups by calculating their contribution to the difference using similarity percentages (SIMPER, Bray-Curtis distance) run in PAST (v3.14, Hammer et al. 2001).

Results

Visually, *Acropora millepora*, *Pocillopora verrucosa* and *Porites lobata* showed different responses to elevated water temperatures in a common garden. After only 12 hours,

both *A. millepora* and *P. verrucosa* started to pale at 34 °C and lost most of their color at 36 °C. In contrast, *P. lobata* did not show visual paling or obvious loss of color in any of the temperature treatments (Figure 2.2).

Deep-sequencing on the hypervariable region 4 (V4) of the SSU rRNA genes revealed 1,060,424 high-quality sequences. After trimming and removing non-targeted contigs average number of sequences per sample were 17,107 ± 9,103 (SD) for *A. millepora*, 23,601 ± 19,916 (SD) for *P. verrucosa* and 25,568 ± 22,646 (SD) for *P. lobata*. Comparison of sequences to the SILVA database v119 (Pruesse et al. 2007) applying a 97% similarity threshold generated a total of 656 microbial operational taxonomic units (OTUs), with *A. millepora, P. verrucosa* and *P. lobata* samples harbored a total of 456, 441, and 475 OTUs, respectively.

Based on Chao-1 biodiversity indices, *A. millepora* had significantly higher estimated total OTUs per coral fragment than *P. verrucosa* and *P. lobata* ($P < 0.05$, Kruskal-Wallis and Mann-Whitney pairwise tests), whereas the latter two were not significantly different from each other (Figure 2.3A). Evenness was not significantly different between *A. millepora* and *P. lobata*, but *P. verrucosa* had significantly less evenness, indicating the presence of more predominant OTUs, compared to *A. millepora* and *P. lobata* ($P < 0.01$, Figure 2.3B). Furthermore, all Mann-Whitney pairwise comparisons among these three coral species for Shannon and Simpsons biodiversity indices and beta diversity were significantly different ($P < 0.01$, $P < 0.01$, $P < 0.005$, respectively, Figure 2.3C, D, E).

Species-specific variation in microbiome composition,was further supported by MED-based NMDS plots (Figure 2.4) showing clear differences in coral host microbiomes that were significantly different (ANOSIM, $P < 0.001$). *Acropora millepora* had the most tightly clustered bacterial profiles among experimental fragments, while *P. lobata* had the most dispersed.

Species-specific patterns

Proteobacteria dominated the microbiomes of all three coral taxa (68–74%) followed by Bacteroidetes and Firmicutes (Figure 2.5A). In addition to these three common phyla, each coral species had a unique association with one additional abundant phylum. For example, Spirochaetae was strongly associated with *P. verrucosa,* which, combined with the other three dominant microbial taxa, comprised > 99% of all bacteria and archaea in the *P. verrucosa* microbiome; the remaining 24 phyla comprising less than 1%. For *A. millepora,* the three dominant microbial taxa plus Cyanobacteria comprised almost 90% of their microbiome, while the remaining ~10% came from 25 other phyla. For *P. lobata,* the three dominant microbial taxa plus Actinobacteria comprised 94.5% of the microbiome,whereas the remaining 5.5% came from 24 other phyla.

While more than 87% of the sequences from the three coral species belonged to microbes in only three phyla, the composition of microbiomes were very different at the genus level. In *P. verrucosa, Endozoiconomas* was dominant, representing more than 60% of the samples. In contrast, *Endozoicomonas* less common in *A. millepora* and very rare in *P. lobata*. Instead, *P. lobata* had high levels of *Pseudoalteromonas* and an undescribed *Rhodobacteraceae*. *Vibrio* was the only genus with similar relative abundances across all three coral species (~2%).

The most abundant genus in the *A. millepora* microbiome was *Endozoicomonas* (Gammaproteobacteria), representing > 17.5% of total abundance (Figure 2.5B). The next most abundant were undescribed Rhodobacteraceae (7.7%, Alphaproteobacteria) and *Pseudomonas* (6%, Gammaproteobacteria), the latter which was only recovered in *A. millepora* microbiome. All of the remaining ten most abundant genera had less than 4% abundances, including undescribed taxa of family SAR86 clade (Gammaproteobacteria), *Prochlorococcus* (Cyanobacteria),

undescribed taxa of Family I from Subsection I (Cyanobacteria), and undescribed taxa of order SAR11 clade (Alphaproteobacteria) that were unique to this coral host species.

Within *P. verrucosa, Endozoicomonas* represented 62% of all sequences (Figure 2.5B). followed by *Fusibacter* (5.1%, Firmicutes) and undescribed Bacteroidetes (4.6 %), the former being found largely only within the *P. verrucosa* microbiome. Other common genera in *P. verrucos,* included *Desulfovibrio* (Deltaproteobacteria) and undescribed Spirochaetaceae with 3–4% relative abundances, followed by three genera with ~1–2% relative abundance, including undescribed Clostridiales (Firmicutes), *Spirochaeta*, and undescribed Flammeovirgaceae (Bacteroidetes) that were unique to the *P. verrucosa* microbiome.

In contrast to the other taxa, *Endozoicomonas* was rare in *P. lobata.* Instead*,* the most abundant genus was *Pseudoalteromonas* (14.3%, Gammaproteobacteria), a taxon not found in significant abundance in two other coral species. The second most abundant genus was undescribed Rhodobacteraceae (12.7%, Figure 2.5B). Three additional taxa, *Cobetia* (6.1%, Gammaproteobacteria), undescribed Vibrionaceae (5.3%, Gammaproteobacteria), and undescribed Alteromonadaceae (4.0%, Gammaproteobacteria), were abundant members of the *P. lobata* microbiome, but rarely observed in *P. verrucosa* or *A. millepora*. Other common, but low abundance taxa within the *P. lobata* microbiome were *Cloacibacterium* (Bacteroidetes), followed by *Phyllobacterium* (Alphaproteobacteria), undescribed Alphaproteobacteria, and undescribed Saprospiraceae (Bacteroidetes).

Microbiome response to thermal stress

Despite having the least variation, the microbiome of *A. millepora* changed dramatically across temperature (ANOSIM, $P < 0.05$). While many of major taxa were present at 30 °C to 34

°C treatments, there was a clear decrease in *Endozoicomonas,* which decreased by more than 50% as fragments paled with increasing temperature (Figure 2.6). There were also major decreases in *Hydrogenophilus* (Betaproteobacteria) and *Bacillus* (Firmicutes), which were the next most abundant genera after *Endozoicomonas* at 30 °C. In contrast, microbiome profiles show increases in *Pseudomonas,* an undescribed Rhodobacteraceae, undescribed taxa of family SAR86 clade (Gammaproteobacteria), and undescribed taxa of order SAR11 clade (Alphaproteobacteria) (Figure 2.6).

The largest change in the *A. millepora* microbiome occurred in the 36 °C treated fragments, where *Vibrio* became the dominant taxa (~40%) despite being virtually absent at lower temperatures (Figure 2.6). *Psychrobacter* (Gammaproteobacteria) also increased greatly at the highest temperatures as did *Neptuniibacter* (Gammaproteobacteria), which was found in the highest temperature treatment despite being absent in lower temperature treatments samples.

Despite obvious shifts in frequencies, *P. verrucosa* microbiomes did not change significantly across temperature (ANOSIM, $P > 0.05$, Figure 2.7). An undescribed Bacteriodetes, *Vibrio*, *Shimia* (Alphaproteobacteria), and undescribed Alteromonadaceae virtually disappeared in the 32 °C treatment and remained very low at (34 °C) when *P. verrucosa* fragments visually paled. In addition, *Cupriavidus (Betaproteobacteria)* was only present in the 34 °C treatment.

The biggest changes were seen in *Endozoicomonas,* which increased from about 50% to more than 92% of the *P. verrucosa* microbiome sequences at 34 °C, when fragments visually paled, and then to less than 2.5% at 36 °C when samples appeared loosing color (Figure 2.7). As *Endozoicomonas* decreased, undescribed Rhodobacteraceae became the dominant OTU, comprising 17.5% of all sequences) with additional major increases in undescribed

Flammeovirgaceae and undescribed *Bacteroidetes* with contribution 11.7% and 7.3%, respectively. *Vibrio* was also abundant in 36 °C treated fragments (~7.2% of sequences) despite having low abundance at 32 °C and 34 °C. In addition, there were many taxa that were only observed in 36 °C fragments, including *Alteromonas*, undescribed Colwelliaceae (Gammaproteobacteria), *Thalassolituus (Gammaproteobacteria)*, undescribed taxa of order OCS116_clade (Alphaproteobacteria), *Clostridium_sensu_stricto*, and undescribed Pseudoalteromonadaceae.

Unlike the other two coral species, *P. lobata* fragments did not show any visual color change or loss of color even at the highest temperature treatment (36 °C), but their microbiomes changed greatly with increasing temperature (Figure 2.8). Unlike the previous two coral taxa, however, there were no clear taxonomic patterns and no significant differences among microbiomes at the different temperature treatments (ANOSIM $P > 0.05$). *Staphylococcus* (Firmicutes), *Cobetia,* and undescribed Bacillaceae (Firmicutes) were common at 30 °C but virtually absent at higher temperatures. *Pseudoalteromonas* was rare at 30 °C and 36 °C, but common at 32 °C and 34 °C. Unlike the other coral taxa, *Vibrio* was present at modest levels at all temperatures and did not increase dramatically at 36 °C. Instead, *Phyllobacterium* and *Arcobacter* (Epsilonproteobacteria) were the only taxa to increase substantially at 36 °C, but not at lower temperatures.

Discussion

Although colonies of the three coral taxa came from a single reef that historically has low susceptibility to bleaching (Markus Krey, personal communication), their different responses to common garden thermal stress experiments indicate that corals in this region are not immune to

thermal stress. The three coral species texted varied in response to thermal stress. Of particular concern was the sensitivity of the branching corals, *A. millepora* and *P. verrucosa* that visibly bleached quickly at elevated temperatures. Given that much of Eastern Indonesia's coral diversity are branching corals—there are 140 species of *Acropora* alone in West Papua (Veron 2002)—rising sea surface temperatures may disproportionally impact these species, radically transform the diversity of these reef ecosystems (Pratchett et al. 2011; Rudi et al. 2012).

Despite being held in a common garden where microbiomes could be shaped by the similar environment (Apprill et al. 2009, 2012), SSU rRNA genes sequencing data showed that each coral species had a unique microbiome profile, similar to previous studies (e.g. Neave et al. 2017). Species specific microbial associations suggest that coral taxa have specific anatomical and physiological characteristics that provide different niches for microorganism (Ainsworth et al. 2015) and/or that microbes provided unique functions to support host corals (Dinsdale et al. 2008).

Not only were the microbiomes of these three coral taxa distinct, they also responded very differently to the same thermal challenges. Combined, our results highlight that microbiome changes are a function of coral host and environment, rather than environment alone. Given that microbes play important roles in coral physiology (see Bourne et al. 2016 for review), the observed microbiome changes could be specific responses for rapid adaptation to periods of elevated water temperatures.

Varied responses of corals to thermal stress

Eastern Indonesian reefs are less susceptible to bleaching than other Indonesian reef ecosystems (Setiasih and Wilson 2010). However, corals from this reef are not immune to

thermal stress, with *A. millepora* and *P. verrucosa* showing clear signs of bleaching, but only at temperatures (36 °C) well beyond the worse scenario of long-term projected increased SST in this region (Collins et al. 2014). These two corals were more sensitive to thermal stress than *P. lobata,* consistent with previous studies (Marshall & Bair 2000; Loya et al. 2001; McClanahan et al. 2005) that report massive corals like *P. lobata* were more tolerant of thermal stress than branching corals.

Multiple factors could promote greater resistance of *Porites* to bleaching under thermal stress. Hoegh-Guldberg and Salvat (1995) proposed that *Porites* may harbor *Symbiodinium* with better photoprotection, while others suggest these corals are more efficient at remove toxins that potentially damage coral tissues (Loya et al. 2001; Nakamura and van Woesik 2001). Whatever the cause, this physiological flexibility could allow *P. lobata* to survive in wider range of environments (Burman et al. 2012). As such, *P. lobata* is predicted to have higher survivorship rates under future elevated global temperature scenarios (Cacciapaglia and van Woesik 2015).

Common microbial phyla

Echoing previous results (Rohwer et al. 2002; Littman et al. 2009; Sunagawa et al. 2010; Blackall et al. 2015), Proteobacteria dominated all coral microbiomes in this study, indicating that Proteobacteria are an important part of the microbiome of corals. Proteobacteria is the largest phylum of bacteria with more than 500 genera and 2000 species, and contains taxa with diverse functions and habitats (Karel et al. 2006), supporting holobiont function in a variety of niches.

Although not as abundant as Proteobacteria, Bacteroidetes and Firmicutes were also found in high abundance in all the coral species, suggesting they are part of the major coral

microbiome as well. Bacteroidetes and Firmicutes are common gut microbes, comprising 98% of mammal gut microbiota (Ley et al. 2006) and likely come from the digestive tissues of the whole polyps extracted in this study. In people, bacteria from these phyla can change from commensal to pathogenic (Thomas et al. 2012), increasing in abundance in people with gut metabolic disorders (Ismail et al. 2012). Interestingly, undescribed Flammeovirgaceae (Bacteroidetes) and undescribed Bacteroidetes are only seen in high abundance in the 36 °C *P. verrucosa* samples, suggesting either a major change in the gut microbiome of these corals, or the rapid increase in opportunistic pathogenic types.

Species-Specific Patterns

While the three most abundant phyla were common to all three coral taxa, the fourth most abundant phylum was unique to each coral species. For example, *A. millepora* was uniquely associated with Cyanobacteria, especially *Prochlorococcus* and undescribed Family I of Subsection I. Cyanobacteria are photoautotrophs (Soo et al. 2017) and can play an important role providing nitrogen-based food for the host (Lesser et al. 2004, 2007). On the surface, the presence of Cyanobacteria in *A. millepora*—a shallow water, high light coral species—is paradoxical because Cyanobacteria play an important role in nitrogen fixation, providing nutrition to *Symbiodinium* in low light environments (Lesser et al. 2007). However, *Prochlorococcus*—the dominant Cyanobacteria in the *A. millepora* microbiome—can also thrive in shallower water with high sunlight environments (Partensky et al. 1999). Given that *Symbiodinium* can experience photoinhibition (Henigge et al. 2011) and shallow water corals are more susceptible to bleaching than deeper corals (Marshall and Baird 2000) due to increase in solar irradiance and oxidative stress (Richier et al. 2008), it is possible that Cyanobacteria play

an important role in maximizing *Symbiodinium* and coral health in shallow water corals like *A. millepora.*

In contrast to *A. millepora*, *P. verrucosa* had a strong association with Spirochaetae that can dominate the microbiome of deep sea corals (Lawler et al. 2016) and be a significant part of tropical coral microbiomes as well (Casas et al. 2004; Kimes et al. 2010, 2013; Closek et al. 2014). *Spirochaeta* are diseases agents in humans and animals (Schwan 1995; Villegas et al. 2004; McBride et al. 2005; Miklossy 2011) and are associated with diseased corals as well (Casas et al. 2004; Closek et al. 2014). However, Closek et al. (2014) found highest abundances in healthy corals, with decreasing abundance with disease progression. Both free living and symbiotic Spirochaetae can be nitrogen fixers (Lilburn et al. 2015), including in scleractinian corals (Kimes et al. 2010). Spirochaetae are also found in diverse (Leschine et al. 2006) and extreme (Hoover et al. 2003) environments and employ a diversity of metabolic pathways (Baker et al. 2015). Given the above, Spirochaetae are likely an important part of the *P. verrucosa* microbiome, and the wide range distribution of *P. verrucosa* (Glynn and Ault 2000; Neave et al. 2017) may be facilitated by the metabolic flexibility of these microbes.

Actinobacteria, a major component of some coral microbiomes (Lampert et al. 2006), was uniquely associated with *P. lobata.* Actinobacteria are reported in a diversity of *Porites* (Wegley et al. 2007, Nithyanand & Pandian 2009), including *P. lobata* in Hawaii both unbleached and bleached colonies in the Red Sea (Haidadi et al. 2017). Like Spirochaetae, which were associated with *P. verrucosa*, many Actinobacteria can be nitrogen fixers. Moreover, they produce secondary metabolites that have potentially beneficial anti-microbial properties. It is notable that known pathogenic microbes like *Vibrio* did not increase in *P. lobata* with increasing temperature, like they did in *A. millepora* or *P. verrucosa*, species that didn't not harbor

significant amounts of Actinobacteria.

Microbial communities changing with increasing temperatures

Although there were broad scale commonalities—and species-specific differences—in the microbial taxa observed in the three coral taxa in this study, each of these microbiomes responded differently to thermal stress. For instance, in *A. millepora, Endozoicomonas* abundance was reduced by half in pale fragments and another half in bleached fragments, supporting the notion that *Endozoicomonas* is associated with healthy corals (Neave et al. 2017) and that it can be outcompeted by opportunist bacteria in stressed environments.

In particular, two potential pathogens, *Pseudomonas* and *Vibrio,* increased in *A. millepora* with increasing temperature. Despite being a common coral pathogen (Banin et al. 2000; Ben-Haim et al. 2003; Sussman et al. 2008; Kesarcodi-Watson et al. 2009) healthy corals can also have high abundances of *Vibrio* (Koren and Rosenberg 2008; Littman et al. 2011; Kellogg et al. 2013) which can have anti microbial properties (Rypien et al. 2010). Similarly *Pseudomonas* from soft corals can also have anti-microbial activity (Radjasa et al. 2007). Thus, will increases in taxa like *Vibrio* are common in thermally stressed corals (Banin et al. 2001; Kushmaro et al. 2001; Rosenberg & Falkovitz 2004), it is unclear whether these changes indicate an opportunistic infection associated with declining coral health at increased temperatures, or a facultative response to ward off such infections.

In contrast to *A. millepora,* relative abundance of *Endozoicomonas* increased sharply in *P. verrucosa* with increasing temperature, before disappearing at 36 °C fragments where it was replaced by a pathogenic-like bacteria, but not *Vibrio*. While *Endozoicomonas* is the dominant genus in *P. verrucosa* (Neave et al. 2017), it is surprising that abundance increased with elevated

water temperatures that left coral fragments pale. While this response could result if other members of the *P. verrucosa* microbiome were more negatively impacted by elevated temperatures than *Endozoicomonas,* the fact that *Endozoicomonas* decreased with increasing temperature in *A millepora* suggests that it is negative impacted by elevated temperatures. Either way, this result shows that responses of the coral holobiont to thermal stress are complex.

Fusibacter was another taxon associated with *P. verrucosa*. Found in a wide range of corals (Glynn and Ault 2000; Neave et al. 2017), this taxon is tolerant of variation in salinity (Ravot et al. 1999; Hania et al. 2012; Fadhlaoui et al. 2015), temperatures, and pHs (Hania et al. 2012; Fadhlaoui et al. 2015), so could help corals adapt to change environments. However, *Desulfovibrio* and undescribed Rhodobacteraceae were associated with thermally stressed *P. verrucosa*, but were found in fragments below 36 °C only. Some species of *Desulfovibrio* are suspected in generating coral diseases such as Black Band Disease (Frias-Lopez et al. 2004; Richardson et al. 2009; Miller and Richardson 2011; Sato et al. 2011; Meyer et al. 2016) and Rhodobacteraceae can be associated with White Plague Disease (WPD) (Sunagawa et al. 2009; Cárdenas et al. 2012), including *Porites* (Roder et al. 2014). Similarly, many *Vibrio* and *Alteromonas,* taxa found in high abundance in bleached *P. verrucosa,* are often pathogens (Banin et al. 2000; Ben-Haim et al. 2003; Thompson et al. 2004; Miller and Richardson 2011), although occasionally these species can produce compounds that inhibit growth of other bacteria (Rypien et al. 2010). Combined, these results suggest that opportunistic pathogens increased in *P. verrucosa* with increasing temperature.

Although *Endozoicomonas* showed clear patterns in *A. millepora* and *P. verrucosa*, it was not found in *P. lobata.* Although both healthy and bleached *P. lobata* from the Red Sea lacked *Endozoicomonas* (Hadaidi et al. 2017), *Endozoicomonas* was found in experimental fragments of

P. lobata from two locations in Bali (Rachmawati et al. Chapter 1) as well as samples of wild *P. lobata* from the same reefs used in this study (Rachmawati et al. Chapter 3). Results showed that *P. lobata* exhibited the widest range of microbial profiles of all three coral taxa. Given this variation and the small sample sizes used in this study, and in Hadaidi et al. (2017), it is possible that natural variation impacts these results, and that the three colonies used for the experiment randomly did not harbor *Endozoicomonas* in significant amounts.

There are, however, additional explanations for the high variability, and apparently random response of *P. lobata* fragments to elevated temperature. *Porites lobata* is a species complex (Iliana Baums, personal communication). Given that our field site was in the center of global coral diversity (Veron et al. 2011), it is possible that corals sampled represent unique lineages with different microbial associations. In addition, *P. lobata* has high phenotypic plasticity (Barshis et al. 2010) that might affect their high variation in maintaining symbiotic communities. Further studies will be required to test these possibilities.

Interestingly, unlike *A. millepora* and *P. verrucosa* that had high relative abundance of pathogenic microbes like *Vibrio* in 36 °C fragments, relative abundance of *Vibrio* was relatively low and constant in *P. lobata.* It is possible that this is because *P. lobata* fragments never because sufficiently stressed to allow the increase of opportunities pathogens. However, another possibility is that *P. lobata* was the only coral in this study with high relative abundance of *Pseudoalteromonas*, which was the most abundant taxon at 32 °C and 34 °C. Many *Pseudoalteromonas* produce inhibitors that can limit the growth of pathogenic microbiota (Holmstrom and Kjelleberg 1999), potentially explaining the relatively low abundances of *Vibrio.* However, undescribed Vibrionaceae and undescribed Alteromonadaceae also had relatively high abundances in *P. lobata* fragments during the different temperature experiments.

Most Vibrionaceae and Alteromonadaceae are pathogenic and opportunistic, but some of them can inhibit or reduce other opportunist microbes (Rypien et al. 2010). As such, it is unclear how to interpret the varied responses of *P. lobata* to elevated temperatures.

With *A. millepora* and *P. verrucosa,* the increase of potentially pathogenic microbes and loss of core beneficial microbes, like *Endozoicomonas,* are clearly associated with high temperature and bleaching, suggesting some relationship (either cause or effect). However, the highly variable responses of *P. lobata* microbiomes suggest that the dominant microbial community may not play a significant role in response to thermal stress and bleaching. Indeed, Hadaidi et al. (2017) found the dominant taxa in bleached and unbleached *P. lobata* microbiomes to be nearly identical. More detailed studies will be required to understand the high variation in *P. lobata* microbiome response to thermal stress, and whether changes in coral microbiomes associated with thermal stress may help protect corals from bleaching, or promote it.

Diversity and Resilience

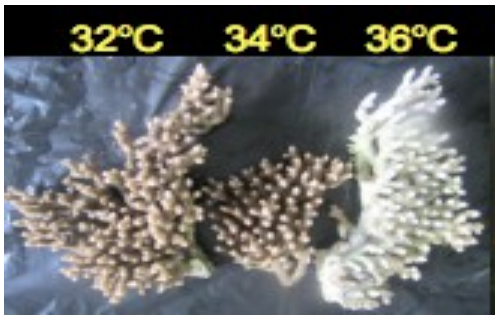
On ecosystem scales, biodiversity promotes resilience (Hughes et al. 2015, Oliver et al. 2015. Similarly, *Symbiodinium* community diversity promotes resilience of corals to environmental stress or change (Baker 2003). However, it is unclear whether this is true at the microbial level. While there were significant differences in the various diversity metrics among the three species, in many cases *A. millepora* had similar (Simpson, Shannon) or higher (Chao-1) diversity metrics than *P. lobata,* even though the former was much more sensitive to temperature stress than the latter. However, *P. lobata* had the highest Beta diversity, indicating high microbiome variation among samples. This result could suggest that *P. lobata* have broader microbial associations, and could help it cope better with environmental stresses, consistent with

empirical data (Marshall and Baird 2000; Loya et al. 2001; McClanahan et al. 2004).

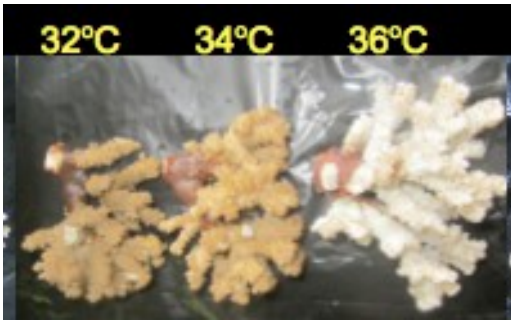
FIGURES

Figure 2.1. Study site: Manokwari (West Papua, Indonesia)

Acropora millepora



Pocillopora verrucosa



Porites lobata

Figure 2.2. Visual morphology after 12 h exposed to different elevated temperatures for *Acropora millepora* (top), *Pocillopora verrucosa* (middle) and *Porites lobata* (bottom). All samples are from reefs in Manokwari (West Papua, East Indonesia) that are reported to be non-sensitive to bleaching.

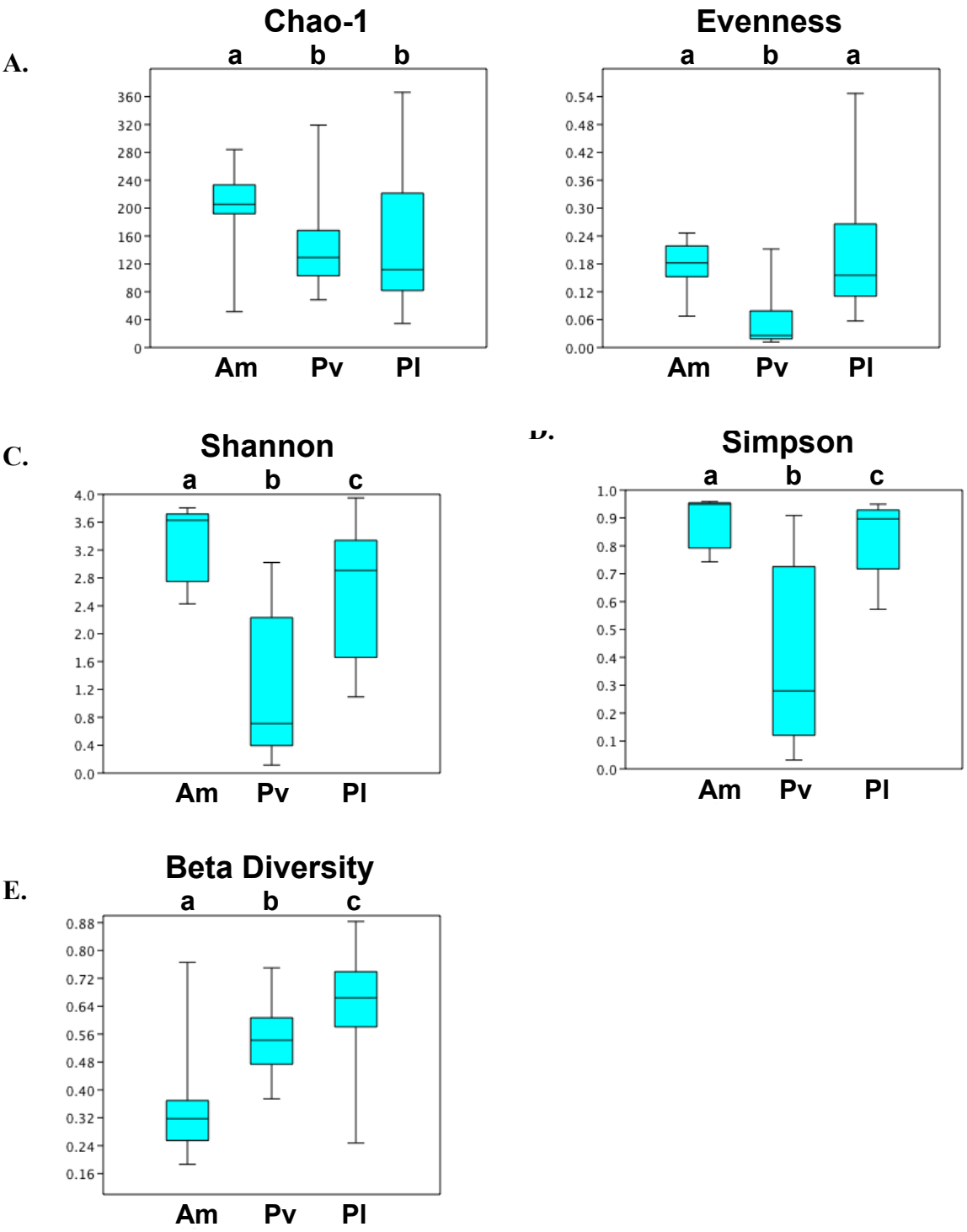


Figure 2.3. Box plots of alpha and beta diversity indices. Kruskal-Wallis tests and Mann-Whitney pairwise determine the differential distances, marked by letters. **A.** Chao-1 estimation ($p<0.05$), **B.** Evenness ($p<0.01$), **C.** Shannon diversity index ($p<0.01$), **D.** Simpson diversity index ($p<0.01$), **E.** Beta diversity ($p<0.0001$). Am = *Acropora millepora*, Pv = *Pocillopora verrucosa*, Pl = *Porites lobata.*

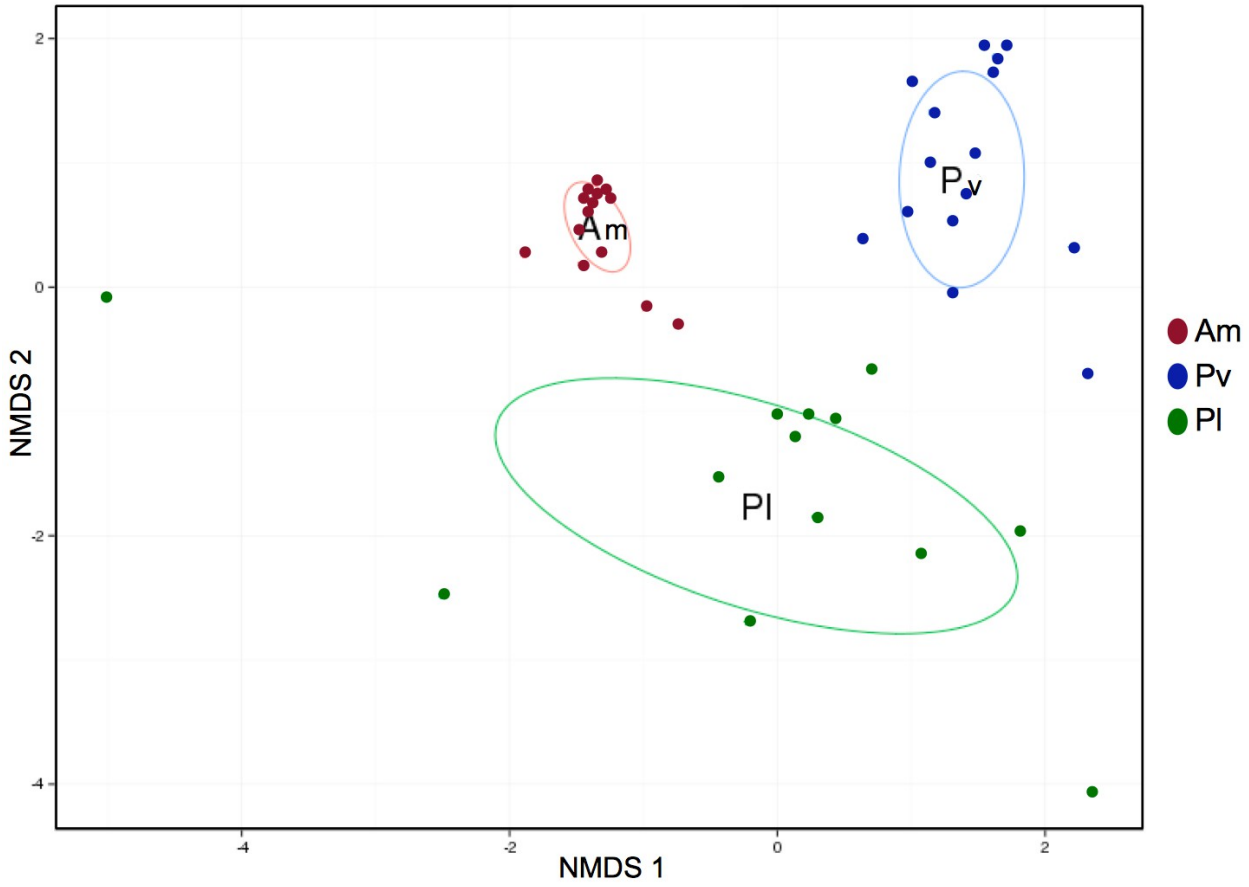


Figure 2.4. Nonmetric Multidimensional Scaling (NMDS) plot of microbial profile of three coral species in Manokwari (West Papua, Eastern Indonesia). Am = *Acropora millepora*, Pv = *Pocillopora verrucosa*, Pl = *Porites lobata.*

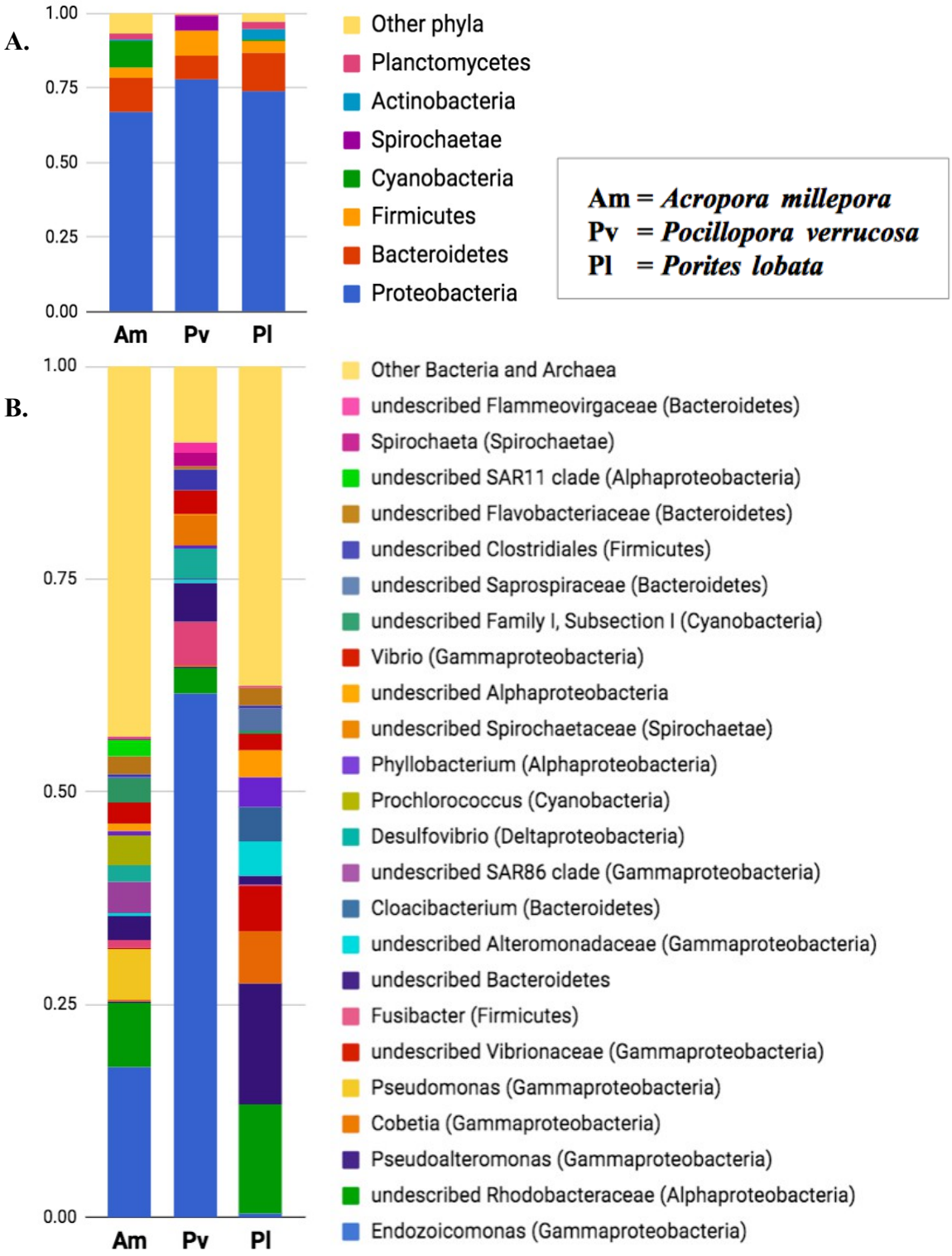


Figure 2.5. Bar plots of microbial profile of three coral species from common garden temperature experiment. **A.** Phylum, compilation of 5 most abundant phyla of each coral species, **B.** Genus, compilation of 10 most abundant genera of each coral species.

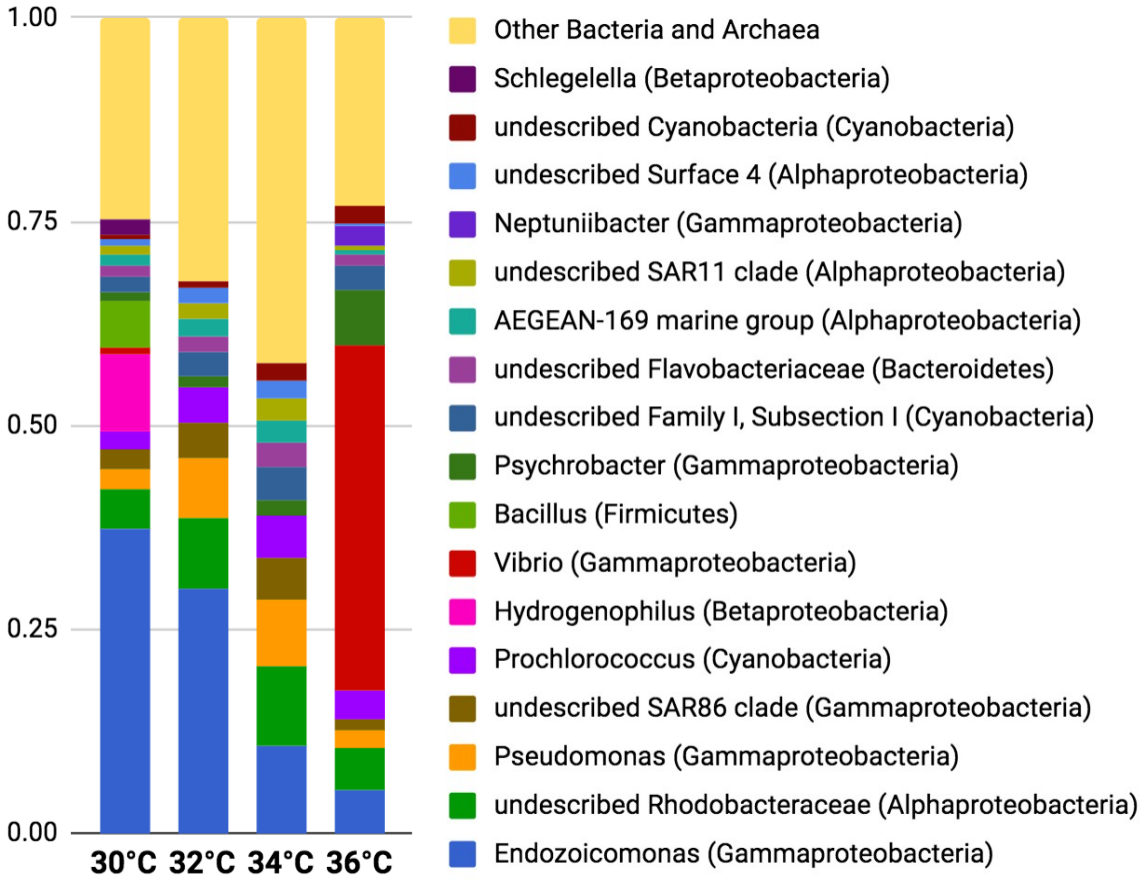


Figure 2.6. Bar plot of *Acropora millepora*-associated microbial profile (genus level) after 12 h temperature treatments in Manokwari (West Papua, Eastern Indonesia). Compilation of 10 most abundant taxa of each temperature treatment.

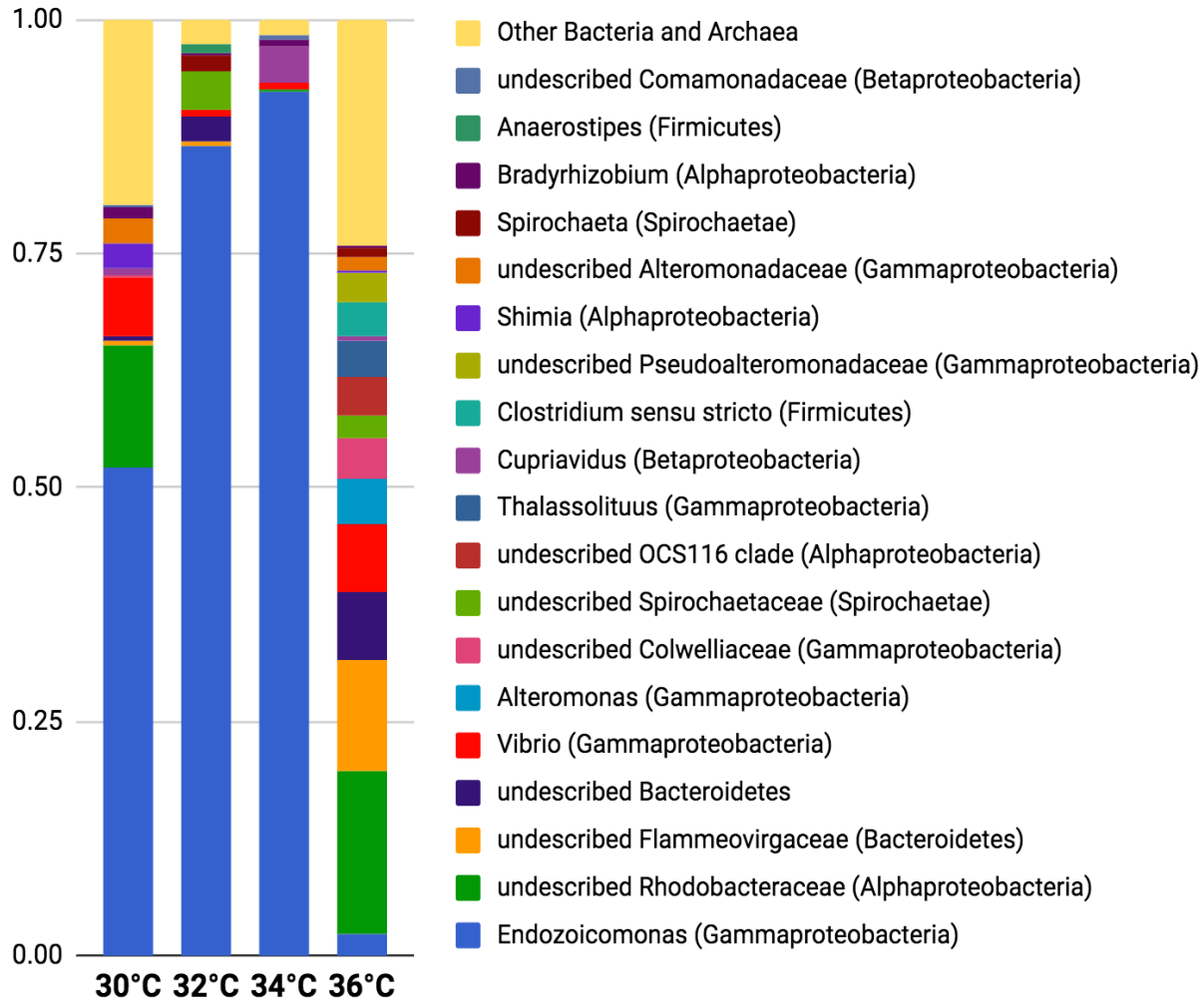


Figure 2.7. Bar plot of *Pocillopora verrucosa*-associated microbial profile (genus level) after 12 h temperature treatments in Manokwari (West Papua, Eastern Indonesia). Compilation of 5 most abundant taxa of each temperature treatment plus the next 5 most abundant of 36 °C.

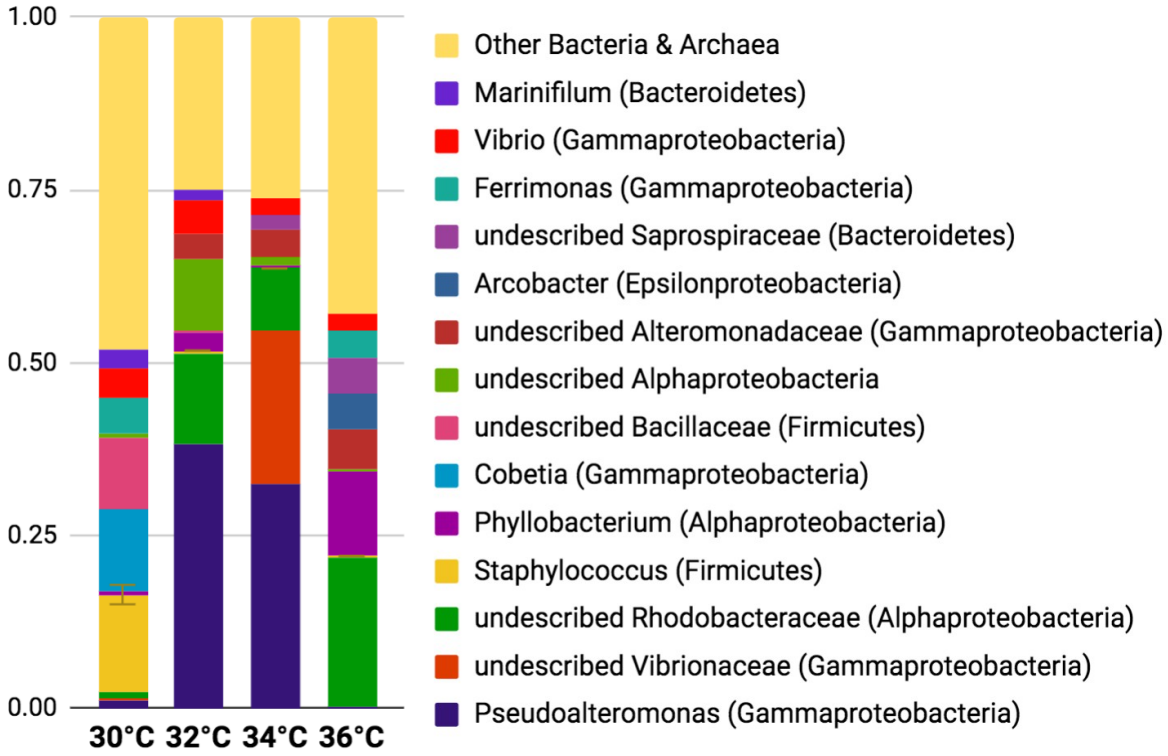


Figure 2.8. Bar plot of *Porites lobata*-associated microbial profile (genus level) after 12 h temperature treatments in Manokwari (West Papua, Eastern Indonesia). Compilation of 4 most abundant taxa of each temperature treatment.

REFERENCES

Ainsworth, T., L. Krause, T. Bridge, G. Torda, J-B. Raina, M. Zakrzewski, et al. 2015. The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. *ISME J*. **9:** 2261–2274.

Ainsworth, T.D., S.F. Heron, J.C. Ortiz, P.J. Mumby, A. Grech, et al. 2016. Climate change disables coral bleaching protection on the Great Barrier Reef. *Science* **352**: 338–342.

Apprill, A., H.Q. Marlow, M.Q. Martindale, and M.S. Rappé. 2009. The onset of microbial associations in the coral *Pocillopora meandrina*. *ISME J* **3**: 685–699.

Apprill, A., H.Q. Marlow, M.Q. Martindale, and M.S. Rappé. 2012. Specificity of associations between bacteria and the coral Pocillopora meandrina during early development. *Appl Environ Microbiol* **78**: 7467–7475.

Apprill, A., S.P. McNally, C.R. Parsons, and L. Weber. 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat Microb Ecol* **75**: 129–137.

Baker, A.C. 2003. Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of Symbiodinium. *Annu. Rev. Ecol. Evol. Syst.* **34**: 661.

Baker, A.C., C.J. Starger, T.R. McClanahan, and P.W. Glynn. 2004. Corals' adaptive response to climate change. Shifting to new algal symbionts may safeguard devastated reefs from extinction. *Nature*, **430**: 741.

Baker, A.C., P.W. Glynn, and B. Riegl. 2008. Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. *Estuar Coast Shelf Sci,* **80**:435–471.

Banin, A., T. Israely, M. Fine, Y. Loya, and E. Rosenberg. 2001. Role of endosymbiotic zooxanthellae and coral mucus in the adhesion of the coral-bleaching pathogen *Vibrio shiloi* to its host. F*EMS Microbiology Letters* **199**: 33-37.

Barber, R.T. and F.P. Chavez. 1983. Biological consequences of El Niño. *Science* **222(4629)**: 1203-1210.

Barshis, D. J., Stillman, J. H., Gates, R. D., Toonen, R. J., Smith, L. W. And Birkeland, C. 2010, Protein expression and genetic structure of the coral *Porites lobata* in an environmentally extreme Samoan back reef: does host genotype limit phenotypic plasticity?. *Molecular Ecology,* **19**: 1705–1720.

Bellwood, D. R. and Meyer, C. P. 2009, Searching for heat in a marine biodiversity hotspot. *Journal of Biogeography*, **36:** 569–576.

Ben-Haim, Y., F.L. Thompson, C.C. Thompson, M.C. Cnockaert, B. Hoste, J. Swings, and E. Rosenberg. 2003. *Vibrio coralliilyticus* sp. nov., a temperature-dependent pathogen of the coral *Pocillopora damicornis*. *Int J Syst Evol Microbiol* **53**: 309–315.

Blackall, L.L., B. Wilson, and M.J.H. van Oppen. 2015. Coral-the world's most diverse symbiotic ecosystem. *Mol. Ecol.* **24**, 5330–5347.

Bourne, D.G., Morrow, K.M., and Webster, N.S. (2016) Insights into the coral microbiome: Underpinning the health and resilience of reef ecosystems. *Annu Rev Microbiol* **70**: 317–340.

Burke, L., E. Selig, and M. Spalding. 2002. Reefs at Risk in Southeast Asia. Washington, DC: World Resources Institute.

Burke, L., K. Reytar, M. Spalding, and A. Perry. 2012. Reefs at Risk Revisited in the Coral Triangle. Washington, DC: World Resources Institute. http://www.wri.org/reefs. 86p.

Burman, S.G., R.B. Aronson, and R. van Woesik. 2012. Biotic homogenization of coral assemblages along the Florida reef tract. *Mar Ecol Prog Ser* **467**: 89–96.

Cacciapaglia, C. and R. van Woesik. 2015. Reef-coral refugia in a rapidly changing ocean. *Global Change Biology* **21:** 2272–2282.

Caporaso,, J.G., C.L. Lauber, W.A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer et al. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* **6**: 1621–1624.

Cárdenas, A., L.M. Rodriguez-R, V. Pizarro, L.F. Cadavid and C. Arévalo-Ferro. 2012. Shifts in bacterial communities of two caribbean reef-building coral species affected by white plague disease. *The ISME Journal* **6**: 502–512.

Carpenter, K.E., et al. 2008. One-third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science* **321**:560–563.

Casas, V., Kline, D.I., Wegley, L., Yu, Y., Breitbart, M., and Rohwer, F. 2004. Widespread association of Rickettsiales-like bacterium with reef-building corals. *Environ Microbiol* **6**: 1137–1148.

Cesar, H., L. Burke, and L. Pet-Soede. 2003. The Economics of Worldwide Coral Reef Degradation. Arnhem, the Netherlands: Cesar Environmental Economics Consulting.

Clarke, K.R. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18**: 117-143.

Clarke, K.R. and R.N. Gorley. 2015. PRIMER v7: User Manual/Tutorial. PRIMER-E, Plymouth, 296pp.

Dinsdale, E.A., R.A. Edwards, D. Hall, F. Angly, M. Breitbart, J.M. Brulc, et al. 2008. Functional metagenomic profiling of nine biomes. *Nature* **452**: 629–632.

Eakin, C.M. et al. 2016. Global coral bleaching 2014–2017. *Reef Currents* **31**: 1.

Donner SD, Rickbeil GJM, Heron SF (2017) A new, high-resolution global mass coral bleaching database. *PLoS ONE* **12(4):** e0175490. https://doi.org/10.1371/journal.pone.0175490

Glynn, P.W., J.L. Mate, A.C. Baker, and M.O. Calderon. 2001. Coral bleaching and mortality in Panama and Ecuador during the 1997–98 El Niño–Southern Oscillation event: spatial/temporal patterns and comparisons with the 1982– 1983 event. *Bulletin of Marine Science* **69**:79–109.

Guest JR, Baird AH, Maynard JA, Muttaqin E, Edwards AJ et al. (2012) Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. *PLOS ONE* **7:** e33353.

Hadaidi, G., T. Rothig, L.K. Yum, M. Ziegler, C. Arif, C. Roder, et al. 2017. Stable mucus-associated bacterial communities in bleached and healthy corals of *Porites lobata* from the Arabian Seas. *Sci Rep* **7**: 45362.

Hammer, Ø., Harper, D.A.T., Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4(1)**: 9pp.

Hennige, S.J., McGinley, M.P., Grottoli, A.G. et al. 2011. Photoinhibition of Symbiodinium spp. within the reef corals *Montastraea faveolata* and *Porites astreoides*: implications for coral bleaching*Mar Biol* **158**: 2515. https://doi.org/10.1007/s00227-011-1752-1

Hoover, R., Pikuta, E., Bej, A., Marsic, D., Whitman, W., Tang, J., Krader, P. 2003. *Spirochaeta americana* sp. nov., a new haloalkaliphilic, obligately anaerobic spirochaete isolated from soda Mono Lake in California. *International Journal of Systematic and Evolutionary Microbiology. **53:** 815-821.*

Hughes, T.P., J.T. Kerry, M. Álvarez-Noriega, J.G. Álvarez-Romero, K.D. Anderson, et al. 2017. Global warming and recurrent mass bleaching of corals. *Nature,* **543**: 373-378.

Jindal, A., K.B. Ritchie, R.L. Hayes, T.J. Goreau, and G.W. Smith. 1995. Bacterial ecology of selected corals following the 1994 south central pacific bleaching event. 26-34.

Jokiel, P. L. 2004. Temperature stress and coral bleaching. p 401–425 *in* E. Rosenberg and Y. Loya, editors. Coral health and disease. Springer, Berlin, Germany.

Kayanne, H. 2017. Validation of degree heating weeks as a coral bleaching index in the northwestern Pacific. *Coral Reefs* **36**: 63–70.

Kellogg, C.A., Y.M., Piceno, L.M., Tom, T.Z., DeSantis, M.A., Gray, D.G., Zawada, and G.L. Andersen. 2013. Comparing bacterial community composition between healthy and White Plague-like disease states in *Orbicella annularis* using PhyloChip G3 microarrays. *PLoS ONE* **8**: e79801.

Kimes NE, Van Nostrand JD, Weil E, Zhou J, Morris PJ. 2010. Microbial functional structure of Montastraea faveolata, an important Caribbean reef-building coral, differs between healthy and yellow-band diseased colonies. *Environ Microbiol* **12:** 541–556.

Kimes, N.E., Johnson, W.R., Torralba, M., Nelson, K.E., Weil, E., and Morris, P.J. 2013. The Montastraea faveolata microbiome: ecological and temporal influences on a Car- ibbean reef-building coral in decline. *Environ Microbiol* **15**: 2082–2094.

Koren, O. & E. Rosenberg. 2008. Bacteria associated with the bleached and cave coral *Oculina patagonica*. *Microb Ecol,* **55**: 523.

Kozich, J.J., S.L. Westcott, N.T. Baxter, N.T. Highlander, and P.D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl Environ Microbiol* **79**: 5112–5120.

Kuever J. 2014. The Family Desulfobacteraceae. *In*: Rosenberg E., E.F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson (eds) The Prokaryotes. Springer, Berlin, Heidelberg.

Kushmaro, A., E., Banin, Y., Loya, E., Stackebrandt, and E. Rosenberg. 2001. *Vibrio shiloi* sp. nov., the causative agent of bleaching of the coral *Oculina patagonica*. *Int J Syst Evol Microbiol* **51**: 1383–1388.

Kusuma, D.W. et al. 2017. *IOP Conf. Ser.: Earth Environ. Sci.* **98** 012038.

Lampert, Y., Kelman, D., Dubinsky, Z., Nitzan, Y., and Hill, R. T. (2006). Diversity of culturable bacteria in the mucus of the Red Sea coral *Fungia scutaria. FEMS Microbiol. Rev*. **58**, 99–108. doi: 10.1111/j.1574-6941.2006. 00136.x

Leschine, S., Paster, B., Canale-Parola, E. 2006. Free-living saccharolytic spirochetes: The genus Spirochaeta. Prokaryotes. **7**. p. 195-210.

Lesser MP, Mazel CH, Gorbunov MY, Falkowski PG. 2004. Discovery of symbiotic nitrogen-fixing cyano- bacteria in corals. *Science* **305**: 997–1000.

Lesser, M. P., Bythell, J. C., Gates, R. D., Johnstone, R. W., and Hoegh-Guldberg, O. 2007. Are infectious diseases really killing corals? Alternative interpretations of the experimental

and ecological data. *J. Exp. Mar. Bio. Ecol*. **346:** 36–44. doi: 10.1016/j.jembe.2007.02.015

Littman, R., B.L., Willis, and D.G. Bourne. 2011. Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. *Environ Micro-biol Rep* **3**: 651–660.

Littman, R., B.L., Willis, C. Pfeffer, and D.G. Bourne. 2009. Diversities of coral-associated bacteria differ with location, but not species, for three acroporid corals on the Great Barrier Reef. *FEMS microbiology ecology* **68(2)**: 152-163.

Logan CA, Dunne JP, Eakin CM, Donner SD. 2014. Incorporating adaptive responses into future projections of coral bleaching. *Glob Change Biol.* **20(1)**:125–39.

Loya, Y., K., Saka, K., Yamazato, Y., Nakano, H., Sambali, and R. van Woesik. 2001. Coral bleaching: the winners and the losers. *Ecol Lett* **4**: 122–131.

Marshall, P.A. and A.H. Baird. 2000. Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. *Coral Reefs* **19**: 155–163.

McBride A, Athanazio D, Reis M, Ko A (005. Leptospirosis. Current Opinion in Infectious Diseases. **18 (5)**: 376–86. PMID 16148523. doi:10.1097/01.qco.0000178824.05715.2c.

McClanahan TR, McLaughlin SM, Davy JE et al. 2004 Observations of a new source of coral mortality along the Kenyan coast. *Hydrobiologia*, **530/531:** 469–479.

McClanahan, T.R., J. Maina, P.R. Moothien, et al. 2005. Effects of geography, taxa, water flow, and temperature variation on coral bleaching intensity in Mauritius. *Marine Ecology Progress Series,* **298:** 131–142.

Meyer, J. L., Rodgers, J. M., Dillard, B. A., Paul, V. J., & Teplitski, M. 2016. Epimicrobiota Associated with the Decay and Recovery of Orbicella Corals Exhibiting Dark Spot Syndrome. *Frontiers in Microbiology*, **7:** 893. http://doi.org/10.3389/fmicb.2016.00893

Miklossy, J. 2011. Alzheimer's disease - a neurospirochetosis. Analysis of the evidence following Koch's and Hill's criteria. *Journal of Neuroinflammation*. **8**: 90. ISSN 1742-2094. PMC 3171359 . PMID 21816039. doi:10.1186/1742-2094-8-90.

Mouchka, M.E., I. Hewson, and C.D. Harvell. 2010. Coral-associated bacterial assemblages: current knowledge and the potential for climate-driven impacts. *Integr. Comp. Biol*. **50:** 662–674.

Nakamura, T., and R. van Woesik. 2001. Differential survival of corals during the 1998 bleaching event is partially explained by water-flow rates and passive diffusion. *Marine Ecology Progress Series* **212**:301–304.

Neave, M. J., R. Rachmawati, L. Xun, C. T. Michell, D. G. Bourne, A. Apprill, and C.R. Voolstra. 2017. Differential specificity between closely related corals and abundant *Endozoicomonas* endosymbionts across global scales. *ISME J.* **11**: 186–200.

Nithyanand, P. and S.K. Pandian. 2009. Phylogenetic characterization of culturable bacterial diversity associated with the mucus and tissue of the coral Acropora digitifera from the Gulf of Mannar. *FEMS Microbiology Ecology,* **69**: 384–394. doi:10.1111/j.1574-6941.2009.00723.x

Obura, D. O. 2005. Resilience and climate change: lessons from coral reefs and bleaching in Western Indian Ocean. *Estuarine Coastal and Shelf Science* **63**:353–372.

Osborne, K., A.A. Thompson, A.J. Cheal, et al. 2017. Delayed coral recovery in a warming ocean. *Glob Change Biol*. **23**:3869–3881.

Podesta, G.P. and P.W. Glynn. 2001. The 1997–98 El Niño event in Panama and Galapagos: an update of thermal stress indices relative to coral bleaching. *Bulletin of Marine Science* **69**:43–59.

Pratchett et al. 2011. Changes in biodiversity and functioning of reef fish assemblages following coral bleaching and coral loss. *Diversity* **3(3**): 424-452.

Pruesse, E., C. Quast, K. Knittel, B. Fuchs, W. Ludwig, J. Peplies, et al. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research* **35**: 7188-7196.

Radjasa et al. 2007. Antibacterial activity of marine bacterium *Pseudomonas* sp. associated with soft coral *Sinularia polydactyla* against *Streptococcus equi* subsp. zooepidemicus. *International Journal of Pharmacology* **3 (2**) : 170-174

Reaka-Kudla, M.L. 2001. Known and unknown biodiversity risk of extinction and conservation strategy in the sea. 19–33 *In* Waters in Peril. Springer US.

Richier, S., J-M. Cottalorda, M.M.M. Guillaume, C. Fernandez, D. Allemanda, and P. Furla. 2007. Depth-dependant response to light of the reef building coral, *Pocillopora verrucosa*: Implication of oxidative stress. https://doi.org/10.1016/j.jembe.2007.12.026

Ritchie, K.B., J.H. Dennis, T. McGrath, and G.W. Smith. 1994. Bacteria associated with bleached and non-bleached areas of *Montastrea annularis*. *In* Kass, L. (ed.) Proc 5th Symp Nat Hist Bahamas. San Salvador, Bahamas: Bahamian Field Station, pp. 75– 80.

Roberts, C.M., et al. 2002. Marine biodiversity hotspots and conservation priorities for tropical reefs. *Science*, **295(5558)**: 1280-1284.

Roder, C., C. Arif, C. Daniels, E. Weil, and C.R. Voolstra. 2014. Bacterial profiling of White Plague Disease across corals and oceans indicates a conserved and distinct disease microbiome. *Mol Ecol* **23:** 965–974.

Rohwer, F., V. Seguritan, F. Azam, and N. Knowlton. 2002. Diversity and distribution of coral-associated bacteria. *Mar Ecol Prog Ser* **243**: 1–10.

Rosenberg E, Falkovitz L. 2004. The Vibrio shiloi/Oculina patagonica model system of coral bleaching. *Ann Rev Microbiol* **58**: 143–159.

Rudi, E., T. Iskandar, N. Fadli, and H. Hidayati. 2012. Impact of mass coral bleaching on fish reef community and fishermen catches at Sabang, Aceh Province, Indonesia. *AACL Bioflux* **5(5**): 309-320.

Rypien, K.L., J. R. Ward, and F. Azam. 2010. Antagonistic interactions among coral-associated bacteria. *Environ. Microbiol.* **12**, 28–39.

Sato, Y., Willis, B.L. and Bourne, D.G. 2013. Pyrose- quencing-based profiling of archaeal and bacterial 16S rRNA genes identifies a novel archaeon associated with black band disease in corals. *Environ Microbiol* **15:** 2994–3007.

Schloss, P.D., S.L. Westcott, T. Ryabin, J.R. Hall, M. Hartmann, E.B. Hollister, et al. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75**: 7537–7541.

Schwan T 1996. Ticks and Borrelia: model systems for investigating pathogen-arthropod interactions. *Infect Agents Dis.* **5 (3)**: 167–81. PMID 8805079.

Setiasih, N. and J. Wilson. 2010. A regional overview on the 2010 coral bleaching event in Southeast Asia: Indonesia. *in* Status of coral reefs in East Asian seas region: 2010. The Global Coral Reef Monitoring Network. Tokyo, Japan.

Soo Rochelle M. et al, 2017 On the origins of oxygenic photosynthesis and aerobic respiration in Cyanobacteria, *Science*. DOI: 10.1126/science.aal3794

Stambler, N. and Z. Dubinsky. 2004. Stress effects on metabolism and photosynthesis of hermatypic corals. Coral Health and Disease, Eds. Rosenberg, E. and Y. Loya. Springer, 195.

Sunagawa, S., T.Z. DeSantis, Y.M. Piceno, E.L. Brodie, M.K. DeSalvo, C.R. Voolstra, et al. 2009. Bacterial diversity and White Plague Disease-associated community changes in the Caribbean coral *Montastraea faveolata. ISME J* **3**: 512–521.

Sunagawa, S., C.M. Woodley, and C.M. Medina. 2010. Threatened corals provide underexplored microbial habitats. *PLoS ONE* **5**: e9554.

Veron, J.E.N. 2002. *in* McKenna, S.A., G.R. Allen, and S. Suryadi (eds). 2002. A Marine Rapid Assessment of the Raja Ampat Islands, Papua Province, Indonesia. RAP Bulletin of Biological Assessment 22. Conservation International, Washington, DC.

Veron, J.E.N. et al. 2011. The Coral Triangle. *In:* Dubinsky Z. and N. Stambler (eds). Coral Reefs: An Ecosystem in Transition. Springer, Dordrecht.

Villegas I, A., Borobio Aguilar E, Beloqui Perez R, de Llano Varela P, Oquiñena Legaz S, Martínez-Peñuela Virseda JM 2004. Colonic spirochetes: an infrequent cause of adult diarrhea. Gastroenterol Hepatol (in Spanish). 27 (1): 21–3. PMID 14718105.

Wegley, L., Edwards, R., Rodriguez-Brito, B., Liu, H., and Rohwer, F. (2007). Metagenomic analysis of the microbial community associated with the coral *Porites astreoides. Environ. Microbiol.* **9**, 2707–2719. doi: 10.1111/j.1462- 2920.2007.01383.x

West, J.D. and R.V. Salm. 2003. Resistance and Resilience to Coral Bleaching: Implications for Coral Reef Conservation and Management. *Conservation Biology*, **17, 4**: 956–967.

CHAPTER 3

Variation of multiple scleractinian coral microbiomes across the Indonesian Archipelago

Introduction

Coral reef ecosystems occupy a mere 0.012% of the ocean floor (Roberts et al. 2002). However, they are the world's most biologically diverse marine habitats (Reaka-Kudla 2001), and are home to at least one quarter of all marine species and thirty-two of the thirty-three animal phyla (Spalding et al. 2001). Unfortunately, global warming caused by increasing atmospheric CO_2 levels (Eakin et al. 2016; Donner et al. 2017) is resulting in mass coral bleaching and ocean acidification that significantly threaten the long-term persistence of coral reefs worldwide (Hoegh-Guldberg 2007). These ecosystem changes will significantly reduce the biodiversity of shallow reef coral communities and lead to future extinctions (Carpenter et al. 2008).

Scientists first observed major coral bleaching events resulting in worldwide mass mortality of coral colonies in the 1970s and 80s, when atmospheric CO_2 levels exceeded 320 ppm, leading to rising sea surface temperature (Hoegh-Guldberg 1999). Since then, the Earth's temperature has continued to increase significantly (Kayanne 2017) as has the frequency and severity of global mass bleaching events (Donner et al. 2017; Hughes et al. 2017). For example, in 2016, the Great Barrier Reef, one of the world's largest coral reef ecosystems, suffered successive bleaching events impacting 75-85% of corals (Hughes et al. 2017).

Models based on current climate trends predict that large-scale severe bleaching events will occur annually by 2043 (van Hooidonk et al. 2016). Given that CO^2 levels and temperatures are predicted to rise under even the most optimistic IPCC scenarios (Eakin et al. 2016; Donner et

al. 2017), it is essential to find effective conservation strategies to help coral reefs survive the effects of climate change in the near future. Towards this end, some are advocating for genetically engineering *Symbiodinium* (e.g. Levin et al. 2017) to increase coral resistance to bleaching. This focus is based on the fact that it is the breakdown of the coral-*Symbiodinium* symbiosis that results in coral bleaching (Lough and van Oppen 2009) and *Symbiodinium* vary in their response to thermal stress (Rowan et al. 1997; Baker 2003, Baker et al. 2004). However, another strategy is to protect reef ecosystems with natural resistance to bleaching. Understanding why these corals resist bleaching can provide information to supports the strategic networks of marine protected areas designed to maintain coral reef biodiversity (West and Salm 2003).

While mass bleaching is a global concern, it is particularly concerning in countries like Indonesia. Indonesian reefs are the world's most diverse and are home to nearly 20% of global reef habitat (Roberts et al. 2002; Veron et al. 2011). These reefs support the nutritional needs of over 100 million people and are a major economic resource (Westmacott et al. 2000). However, these ecosystems have already suffered significant declines and over 90% of the remaining reefs are threatened or severely threatened (Burke et al. 2012).

Mass coral bleaching was first noted in Indonesia during the 1982-/1983 El Niño Southern Oscillation (ENSO); sea surface temperatures (SST) exceeded 33 °C for six months (2–3 °C above mean SST), resulting in up to 90% coral mortality in the Seribu Islands, especially in corls from genera *Acropora* and *Pocillopora* (Brown and Suharsono 1990). However, during the same time period, no bleaching was observed in Komodo National Park (West and Salm 2003). Similarly, during the 1998 ENSO, mass coral bleaching impacted 50% of corals in Karimunjawa National Park, Java (Habibi 2007). In 2010, another periods of mass coral bleaching hit Indonesia, with Aceh at the tip of Western Sumatra experienced up to 90% (Guest et al. 2012),

yet reefs in Eastern Indonesia, particularly Western Papua, showed minimal bleaching (Setiasih and Wilson 2010). These patterns suggest regional variation in bleaching susceptibility of corals across Indonesia with Western Indonesia being highly susceptible and Eastern Indonesia being the most resistant, with intermediate responses in between. Further evidence for bleaching resistance in Eastern Indonesia comes from SST data showing that from 2007-2016, the average SST in Eastern Indonesia were warmer for longer than Western Indonesia waters (Kusuma et al. 2017), which would predict that Eastern Indonesia reefs would bleach more.

Coral bleaching occurs when the mutualistic relationship between a coral host and its endosymbiotic algae, *Symbiodinium,* breaks down (Lough and van Oppen 2009), a response that may be adaptive, allowing corals to change one *Symbiodinium* community for a physiologically more appropriate one under changing environmental conditions ("the Adaptive Bleaching Hypothesis" Buddemeier and Fautin 1993; Kinzie III et al. 2001). As such, a lot of research has focused on understanding the relationship between coral hosts and their endosymbiotic algae (e.g. Karako et al. 2002; Coffroth et al. 2006) and how *Symbiodinium* could result in variation in bleaching susceptibility (Rowan et al. 1997; Baker 2003, Baker et al. 2004).

However, *Symbiodinium* is only one part of the coral holobiont. It also includes other microbiota, such as bacteria, archaea, viruses, fungi, and protozoa (Rohwer et al. 2002; Wegley et al. 2007), and we are only beginning to understand the roles of these communities (see Hernandez-Agred 2017 for review). However, microbiomes almost certainly impact coral health and response to thermal stress. For example, nitrogen-fixing bacteria in corals support significant physiological and ecological benefits to host mutualistic bacteria (Williams et al. 1987), and reduction of these bacteria could reduce nitrogen available for algal photosynthesis, potentially impacting the coral-*Symbiodinium* symbiosis (Rädecker et al. 2015). Additionally, some bacteria

behave differently in different environmental conditions where some mutualists can turn into opportunistic pathogens (French-Constant et al. 2003).

In general, the coral holobiont is shaped by the environment and the coral host (Kelly et al. 2014), and certain types of bacteria are indicators of coral host health (Rohwer et al. 2001; Speck and Donachie 2012; Rodriguez-Lanetty et al. 2013; Bayer et al. 2013; Jessen et al. 2013; Ainsworth et al. 2015). However, similar to the Adaptive Bleaching Hypothesis, the Coral Probiotic Hypothesis suggests that coral microbiomes are dynamic, allowing the coral holobiont to rapidly adapt to short (days to weeks) or long term (many years) environmental changes via mutation and selection (Reshef et al. 2006). This view is supported by recent studies that show a stable core microbiome, a microbiome association that fills functional niches, along with additional microbial variation (Hernandez-Agreda et al. 2016).

Studies on coral microbiomes show that microbiomes may have species-specific patterns. For example, *Stylophora pistillata* and *Pocillopora verrucosa* have different microbial profiles, where the latter ones are consistent across global reefs (Neave et al. 2017). Even different colors of species like *Montipora capitata* harbored distinct bacterial profiles (Shore-Maggio et al. 2015). In Indonesia, *Acropora millepora, Pocillopora verrucosa,* and *Porites lobata*, had unique microbiomes, and responded differently to increasing water temperature (Rachmawati et al. Chapter 2).

In addition to species-specific differences, microbial profiles of individual coral taxa can vary among locations (Litman et al. 2009; Neave et al. 2017), including reefs that are very close in proximity (Hadaidi et al. 2016, Rachmawati et al. Chapter 1). While such variation could be stochastic, recent studies indicate that it is likely functional with different microbial groups filling specific niches in different habitats (e.g. Hernandez-Agreda et al. 2016) and influencing

susceptibility to bleaching (Ziegler et al. 2017), including the initiation of bleaching (Kushmaro et al. 1997, 2001).

Given the above, it is possible that regional variation in bleaching observed in the Indonesian Archipelago could be influenced by coral microbial communities, with certain microbial taxa associated with bleaching resistant reefs in Eastern Indonesian, and others associated with reefs in Central and Western Indonesia that are more prone to bleaching. A non-mutually exclusive alternative is that pathogenic bacteria may be less common in Eastern Indonesia and more common in Central and Western Indonesia. To test these hypotheses, we compared the microbial profiles of *Acropora millepora, Pocillopora verrucosa,* and *Porites lobata,* from Indonesian reefs with different susceptibility levels to bleaching.

Methods

Study Sites and Sampling

We collected three coral species, *Acropora millepora, Pocillopora verrucosa,* and *Porites lobata* from three regions spanning the Indonesia Archipelago (Figure 3.1) that experienced different levels of coral bleaching. Weh Island in Aceh-Western Sumatra is a region of Western Indonesia that experienced the most severe coral bleaching in 2010 (Guest et al. 2012; Rudi et al. 2012) based on percentage corals bleached. In contrast, Raja Ampat and Manokwari in West Papua is a region of Eastern Indonesia that experienced minimal bleaching (Setiasih and Wilson 2010; Markus Krey, personal communication). In the middle, the reefs of Bali (Amed and Pemuteran) and Wakatobi (Southeast Sulawesi) have intermediate levels of bleaching (Setiasih and Wilson 2010).

We collected up to ten samples of each coral per reef. We used SCUBA to collect *P. verrucosa* and *P. lobata* at 6-8m depth in 3 reefs of each six locations. We collected *A. millepora* at < 5 m depth from 2 reefs in Amed and 3 reefs in Manokwari. We collected *P. lobata* using a chisel and hammer, and a bone cutter to collect *P. verrucosa.* To maximize our probability of sampling distinct genes and not clones, we sampled colonies that were at least 5 m apart, and put samples into separate plastic bags underwater to avoid cross contamination. We then preserved samples in 95% ethanol and stored them at -20 °C.

DNA Extraction

We extracted three to five samples of each species per reef by airbrushing with cold sterile 1X PBS (phosphate buffered saline). We then centrifuged tissue slurries at 4 °C for 20 min at 5000 rpm to pellet the coral tissues and stored the pellet at -20 °C before extraction. To lyse coral tissue, we heated ~ 50 mg of pelleted tissue at 56 °C for 30 min while shaking at 400 rpm, followed by 15 min of vortexing at high speed. To extract DNA, we used the DNA Ultra Clean kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's recommendations, and then eluted DNA into 50 µL of elution buffer. We estimated the relative dsDNA concentration and quality using BioDrop µLite (Isogen Life Science, De Meern, the Netherlands) to predict the optimum number of PCR cycles to use for amplification.

Library Preparation

To target the hypervariable V4 region of bacterial and archaeal small subunit (SSU) ribosomal RNA (rRNA) genes, we performed triplicate PCR in 20 µl reactions containing 1–3 µl DNA extraction with the 16S primer set 515F: GTGCCAGCMGCCGCGGTAA and 806RB:

GGACTACNVGGGTWTCTAAT (Caporaso et al. 2012; Apprill et al. 2015) combined with an Illumina adapter, 8 bp index sequence, 10 bp pad sequence, and 2 bp linker sequence for the dual-index sequencing strategy (Kozich et al. 2013). We used 1 µl of GoTaq® Flexi DNA Polymerase (Promega) in 20 µl 5X Colorless GoTaq® Flexi Buffer mixed with 250 nM MgCl2, 200 µM dNTP mix (Promega, Madison, USA) for 5 PCR reactions. In each PRC reaction, we used unique combination of 160 nM of each barcoded primer. We conducted PCR in a BioRad C1000 Touch™ Thermal Cycler (< 5 °C/s ramp rate) with the following parameters: 2 min at 95 °C for the initial denaturation followed by cycle iterations of 20 s at 95 °C, 15 s at 55 °C and 5 min at 72°C and a final extension for 10 min at 72°C. To increase the consistency of amplification across samples, each sample used its optimum iterated cycle among 20, 25, 30, 35, and 40 cycles, that was determined at the next step.

To verify successful PCR, we electrophoresed PCR products at 110 V for 60 min on a 1% agarose gel using 1X TBE buffer, and then SYBR Safe (Invitrogen, Carlsbad, CA, USA) stained DNA was visualized on an UltraBright LED Transilluminator, 470nm (LB-16) (Maestrogen, Nevada, USA). The optimum cycle used for each sample was determined based on the presence of a clear thin targeted band on the gel. We excised the targeted band, then purified gel slices with Qiagen MinElute Gel Extraction Kit (Qiagen, Hilden, Germany), eluted the recovered DNA in 10 µl EB buffer, and then quantified the purified DNA using a Qubit 2.0 dsDNA HS assay (Invitrogen, New York, USA).

For DNA sequencing, we added 2 ng of purified DNA from each sample to a pooled library. The library was then sequenced in two separate 250 bp paired-end Illumina MiSeq runs. As controls, a positive control of a mock community and three negative controls from 30, 35 and 40 cycle PCR reactions were also uniquely barcoded and included in both runs. The positive

control used was Microbial Mock Community B (Staggered, Low Concentration), v5.2L, for 16S rRNA Gene Sequencing, HM-783D obtained through BEI Resources, NIAID, NIH as part of the Human Microbiome Project.

Bioinformatics and statistics

We analyzed the sequence data using Mothur v1.34.4 (Schloss et al. 2009). We started by combining all reads from both runs using *merge.files*. We used *make.contigs* command to combine the reads. Contigs with length more than 255 bp or that had ambiguous positions were removed using *screen.seqs* command. Next, we used *classify.seqs* command with the 'knn' method to classify the contigs against the SILVA database v119 (Pruesse et al. 2007). We grouped taxa into operational taxonomic units (OTUs) based on a 97% sequence similarity threshold, which were then identified to the lowest taxonomic level using the SILVA database after removing all unwanted reads belonging to chloroplasts, eukaryota, mitochondria, and unknown using *remove.lineage* command. We also removed chimeras using *remove.seqs* command after using the *chimera.uchime* command to identify them. Finally, we removed all sequencing controls, and other groups that were not included in the next analyses using *remove.groups* command, and then subsampled 8000 sequences using *sub.sample* command to yield equal sequencing coverage across all samples. To generate different datasets, we picked certain samples using *get.groups*.

To explore similarity and differences of microbial communities, we employed clustering algorithms based on the least difference of sequence, using Minimum Entropy Decomposition (MED, Eren et al. 2014). We used "headersMED.py" command in Python to convert the fasta and group files with Mothur format into a MED-compatible format (Neave et al. 2017). MED

was then run on the sequences using default settings and a minimum substantive abundance criterion (*M*) of total sequences/10,000 (Eren et al. 2014). We used MED with *M* of 66 for comparing microbiome in six locations and *M* of 31 when comparing *A. millepora* between Amed and Manokwari to generate Nonmetric Multidimensional Scaling (NMDS) plots based on Jaccard distance. The distribution in NMDS plots used new sequences groups, with >99% similarity, generated by each MED analysis. We refer to these data when using term 'MED OTUs' in further analyses. To evaluate whether groupings identified in NMDS plots were significantly different, we used an analysis of similarity algorithm (ANOSIM) based on Jaccard distance with 9,999 permutations. We also calculated the contribution of every OTU to separate groups using similarity percentages (SIMPER) analysis based on Bray-Curtis distance.

To explore differences in overall microbial diversity among coral taxa and geographic regions, we calculated multiple diversity indices on OTU data, including Chao-1 richness estimate, Simpson diversity estimate (1-D), Shannon diversity estimate, and Evenness, and then determined significance using a Mann-Whitney test. For all diversity indices and associated statistical analyses including ANOSIM and SIMPER, we used the PAST statistics software package (v3.14, Hammer et al. 2001).

Results

Microbiome variation between hosts

NMDS plots of the SSU rRNA genes sequences pooled from Amed and Manokwari showed that microbial communities tended to cluster by species (Figure 3.2), with *P. verrucosa* having the least variation and *P. lobata* having the most. Analysis of similarity (ANOSIM) based

on MED OTUs indicated that the microbial profiles of these three coral species were different from each other ($P < 0.05$).

All coral species harbored high amounts of Proteobacteria, with this phyla comprising > 90% of the microbial taxa in *A. millepora*, while *P. verrucosa* and *P. lobata* had about 77% and 60%, respectively (Figure 3.3A). The second most abundant phylum, Firmicutes, was also present in all coral species but in much lower proportion; *P. lobata* had the highest proportion and *A. millepora* the lowest. Bacteroidetes was the third most abundant phylum in *P. verrucosa* and *P. lobata,* with no large differences in relative abundance, but was only the fourth most abundant phylum in *A. millepora*; a group of all unclassified bacteria in phylum level was the third most abundant group. While *P. lobata* harbored the least Proteobacteria, it had a relatively high proportion of other phyla, including Lentisphaerae, Cyanobacteria, and Fusobacteria.

Endozoicomonas was the most common genus across all coral hosts, although it was not the most abundant taxa in each coral species. Relative abundance of *Endozoicomonas* varied greatly with *A. millepora* having almost twice that of *P. verrucosa*, which was nearly double *P. lobata* (Figure 3.3B). In *A. millepora, Endozoicomonas* had almost equal frequency to *Cupriavidus*, the most abundant taxa in this species. In contrast, *Vibrio* (Gammaproteobacteria) was the most abundant taxa in both *P. verrucosa* and *P. lobata.* While present in both *P verrucosa* and *P. lobata,* the former had higher frequencies (6–8%) of undescribed Rhodobacteraceae (Alphaproteobacteria), undescribed Family XII (Firmicutes), and *Ferrimonas* (Gammaproteobacteria). Similarly, both corals had undescribed Clostridiaceae 1 (Firmicutes), but the frequency (3.6%) was higher in *P. lobata*.

SIMPER analysis showed that five microbial taxa accounted for 50% of the variation among coral microbiomes (Table 3.2). In pair-wise comparisons, three genera (*Endozoicomonas,*

Vibrio, and *Cupriavidus*) were responsible for about half of the variation between *A. millepora* and *P. verrucosa*, four genera (*Endozoicomonas, Vibrio, Cupriavidus,* and undescribed Oligosphaeria) explained half of the variation between *A. millepora* and *P. lobata*, and six taxa (*Endozoicomonas, Vibrio*, undescribed Rhodobacteraceae, undescribed Family XII (Firmicutes), *Ferrimonas*, and *Photobacterium*) explained almost 50% of the difference between *P. verrucosa* and *P. lobata*.

Microbiome variation across geography

In *A. millepora,* microbial profiles were very different across the two sampled localities. While Proteobacteria was the most predominant phylum associated with *A. millepora* in both Amed and Manokwari, in the latter they were extremely dominant, comprising more than 95% of the microbial community (Figure 3.4A). In contrast, Amed had fewer Proteobacteria, and Firmicutes represented 17% of sequences, with Actinobacteria and Bacteroidetes each comprising about 3%. At the genus level, populations from Manokwari had much higher levels of *Cupriavidus* and *Vibrio,* while these were in relatively low abundance in Amed (Figure 3.4B). In contrast, *Streptococcus, Pseudomonas* and *Phyllobacterium* were relatively common in Amed and rare in Manokwari. Despite these large frequency difference in common microbial taxa, NMDS plots (Figure 3.5) and ANOSIM showed these two locations were not significantly different ($P > 0.05$).

For *P. verrucosa,* Proteobacteria also dominated the microbiomes at all locations. While Firmicutes and Bacteroidetes were found in all locations, abundances varied greatly. In general Firmicutes had high relative abundance except for Raja Ampat where it had < 4% relative abundance, and Manokwari where it had only 1% (Figure 3.6A). Substantial variation was also seen in microbial genera. *Endozoicomonas* varied from nearly 50% of the sequences in

Manokwari to less than 3% in Aceh. Similarly, taxa like *Vibrio* varied from over 35% in Aceh to less than 5% in Raja Ampat (Figure 3.6B). Although NMDS plots showed a lot of overlap among the microbiomes of *P. verrucosa* across Indonesia, (Figure 3.7), most of the locations were different from each other (ANOSIM, $P < 0.005$), except for two pair-wise comparisons: Manokwari-Aceh and Manokwari-Pemuteran. The microbial communities of Amed and Pemuteran were the most different ($P < 0.0001$) despite both being reefs from Bali.

Similarly, Proteobacteria dominated the microbiomes of *Porites lobata* across all locations (Figure 3.8A). While Firmicutes and Bacteroidetes were also abundant, frequencies varied from 22% and 9%, respectively, in Amed to virtually absent in Manokwari. There was similarly large variation in abundance at the genus level, with *Vibrio* comprising over 50% of the microbiome in Pemuteran, to < 10% in Wakatobi (Figure 3.8B). There were similarly large fluctuations in other common taxa, such as *Endozoicomonas, Cobetia, Pseudomonas, Incertae-Sedis,* and *Cupriavidus.* Despite this variation, there was substantial overlap in the NMDS plot (Figure 3.9), and only four pairs of locations were statistically different; Wakatobi was significantly different from Pemuteran, Amed, and Raja Ampat ($P < 0.05$), and Manokwari and Aceh were also significantly different ($P < 0.005$).

Interestingly, bar plots based on MED shows that while microbiomes may be similar at the phylum or genus level across geography, there is very little similarity in the actual taxa when a 99% sequence similarly threshold is employed. For example, while both locations of *A. millepora* harbor substantial *Endozoicomonas* (Figure 3.4B), these are not the same *Endozoicomonas* OTUs*,* with Amed having about 25% of *Endozoicomonas-3,* while this group was not abundant in Manokwari (Figure 3.10). Similarly, in *P. verrucosa, Vibrio-1* was present in all populations except Raja Ampat (Figure 3.11), even though *Vibrio* was a genus present in

Raja Ampat (Figure 3.6B). Likewise, in *P. lobata, Vibrio-1* was observed in all populations, but it was very rare in Wakatobi and Manokwari (Figure 3.12). Instead, Wakatobi had high levels of *Vibrio-2.* Other locations like Amed have high levels of *Vibrio-3*, which was not part of abundant groups in all other populations.

Microbial variation and bleaching sensitivity

When populations are grouped by bleaching susceptibility level with Aceh and Pemuteran representing "high sensitivity" and Amed, Wakatobi, Raja Ampat, and Manokwari representing "low to moderate sensitivity", NMDS plots show different distributions (Figure 3.13). For *P. verrucosa,* high bleaching regions clustered tightly together in the center of the distribution of low bleaching sites (Figure 3.13A), although these distributions were not statistically different. In contrast, *P. lobata*-associated microbial were statistically different between two groups ($P < 0.01$) despite partially overlapping (Figure 3.13B).

Discussion

Results of this study show a complex pattern of microbiome differentiation. Microbiomes were significantly different across all three coral species, mirroring other studies (e.g. Neave et al. 2017) showing species specific differences in coral microbiomes, including how the microbiomes of different coral taxa respond to thermal stress (Rachmawati et al, Chapter 2). Moreover, even when corals shared similar taxa (e.g. *Endozoicomonas* or *Vibrio*), the exact OTUs varied among the three species, indicating fine scale variation in coral microbiomes.

Although *Symbiodinium* type plays an important role in coral adaptation to elevated sea surface temperatures (Baker et al. 2004), results of microbiomes across regions of Indonesia with

different histories of coral bleaching were mixed. One species, *P. lobata*, had significant differences in microbiomes among regions where severe bleaching is common and regions where it is rare suggests that microbiomes could also play a role, as reported by recent studies of coral microbiomes and thermal tolerance (Hadaidi et al. 2017; Ziegler et al. 2017). However, *P. lobata* was the only taxon in this study to display this regional microbiome variation.

There were also mixed results in tests of microbiome differentiation across localities. Microbiomes of *P. verrucosa* showed pronounced variation among reef ecosystems spanning the Indonesian Archipelago, supporting results of other studies that report geographic variation in microbiomes (Litman et al. 2009; Hadaidi et al 2016), including Indonesian reefs in very close in proximity (Rachmawati et al. Chapter 1). However, there was limited support for local differentiation of *P. lobata* microbiomes, and no differentiation in *A. millepora.* This result indicates that coral microbiomes must respond differently to local environmental conditions.

Microbiome variation among species

Results showed Proteobacteria was the most dominant phyla across all taxa. This is a common result seen across coral microbiome studies (Rohwer et al. 2002; Littman et al. 2009; Sunagawa et al. 2010; Blackall et al. 2015; Neave et al. 2017), likely because this phylum is very diverse (Gupta 2000) and contains taxa with diverse ecological roles, including mutualistic microbes, such as *Endozoicomonas* (Bayer et al. 2013a, 2013b; Neave et al. 2017), as well as pathogenic taxa like *Vibrio* (Bourne et al. 2008; Vega Thurber et al. 2009). *Endozoicomonas* is a beneficial coral symbiont that is a common component of the coral microbiome (Bayer et al. 2013; Neave et al. 2017), although some studies found this genus might also related to marine organism diseases (Katharios et al. 2015; Ziegler et al. 2016). In contrast, *Vibrio* are commonly

pathogenic (Banin et al. 2000; Kushmaro et al. 2001; Ben-Haim and Rosenberg 2002; Ben-Haim et al. 2003; Luna et al. 2010; Wilson et al. 2012), although some studies report *Vibrio* in healthy corals (de Castro et al. 2010; Kvennefors et al. 2010; Ainsworth et al. 2015; Kemp et al. 2017). The presence of these microbial groups across three divergent coral species show their importance in the microbial communities of coral reefs. Interestingly, while *Endozoicomonas* and *Vibrio* were two of the most abundant taxa overall, *Cupriavidus* was the most abundant taxon in *A*. *millepora*. In plants, *Cupriavidus* are diazotrophic, fixing nitrogen for photosynthesis (Florentino et al. 2010), and thus could aid photosynthesis in *Symbiodinium*. However, *Cupriavidus* is not reported from *A. millepora* from the Great Barrier Reef (Lema et al. 2012). Combined with strong regional variation of *Cupriavidus* in *A. millepora* across Indonesia, it remains unclear how important *Cupriavidus* is to the health of coral hosts.

Despite high relative abundance, Proteobacteria abundance varied greatly across all taxa, comprising ~90% of the *A. millepora* microbiome, compared to <80% in *P. verrucosa* and about 60% in *P. lobata.* In contrast, Bacteroidetes and Fimicutes were relatively common (~20-25% relative abundance) in *P. verrucosa* and *P. lobata* while being more rare in *A. millepora.* High levels of Bacteoidetes in *P. verrucosa* and *P. lobata* is not surprising given that these taxa are reported in previous studies (Neave et al. 2017; Hadaidi et al 2017). However, while Firmicutes is reported from other *Porites* (Wegley et al. 2017)*,* including *P. lobata* (Richie et al. 2005), Firmicutes is rare in *P. verrucosa* across it's global range (Neave et al. 2017). Some Firmicutes produce antimicrobial products in corals that could combat pathogenic bacteria (Umagowsalya et al. 2015). However, others, such as *Clostridium*, *Fusibacter*, and many undescribed Firmicutes, are potentially pathogenic as they were found at black band disease (BBD) coral surface in low occurrence (Miller and Richardson 2011). In either case, the presence of Firmicutes in all three

taxa could be indicative of compromised health of these corals, a result that would be consistent with studies showing that many Indonesian coral reefs are severely stressed (Burke et al. 2012).

Regional variation in coral microbiomes

Reefs surveyed in this study include reefs from Western Sumatra that experience severe bleaching (Guest et al. 2012), reefs in Eastern Indonesia that only experience mild bleaching (Setiasih and Wilson 2010) and reefs in the middle with intermediate levels of bleaching (Setiasih and Wilson 2010). Results from NMDS distribution and ANOSIM showed significant differences in the microbial communities from *P. lobata* in bleaching resistant areas and areas more susceptible to bleaching, but not in *P. verrucosa*. Despite significant differences, there was no single taxon or group of taxa that was associated with these regional differences. This pattern could result if taxonomic resolution is insufficient to distinguish the key members of the microbial communities associated with bleaching susceptibility. Alternatively, this pattern could result if it's the interaction among components of the microbial community (or components thereof), rather than individual taxa, that result in regional differences. Either way, the regional differences seen in *P. lobata* are only associations—they do not imply causality—and there are several reasons why regional variation in bleaching could be observed.

The first potential driver of this pattern is that there could be significant differences in regional water temperatures. If the waters of Western Indonesia are warmer than Eastern Indonesia, eastern reefs could be more impacted by subsequent SST rise, resulting in greater propensity to bleach. In fact, however, the waters of Eastern Indonesia were warmer, and warmer for longer than Western Indonesia from 2007-2016 (Kusuma et al. 2017). As such, these reefs are closer to temperatures that induce coral bleaching so should be more likely—not less likely—

to bleach. Given that less bleaching is seen in Eastern Indonesia where SST would predict more bleaching, regional SST temperature differences cannot directly account for regional variation in bleaching.

Regional variation in SST, however, could influence regional variation in bleaching susceptibility by shaping the composition of the coral holobiont, such as *Symbiodinium* and other microbial components of the microbiome. Previous work on *Tridacna* (giant clams that harbor the same *Symbiodinium* strains as corals) show that populations in Eastern Indonesia that experience higher temperatures have higher frequencies of thermally tolerant clade D haplotypes (DeBoer et al. 2012). If higher SST also results in selection for more thermally tolerant *Symbiodinium* in Eastern Indonesian corals, the resulting increase in clade D symbionts could increase resistance to bleaching. Similarly, if higher SSTs also selected for microbes associated with higher thermotolerance (Tse-Dinh et al. 1997), these changes could make corals in Eastern Indonesia "preadapted" to surviving additional SST increases. This hypothesis could be tested through reciprocal transplants, such as those performed by Ziegler et al. (2017) that demonstrated adaptation of microbial communities to warmer microhabitats.

Local variation in coral microbiomes

In addition to differences of *P. lobata* microbiomes associated with regional variation in bleaching sensitivity, there were also significant differences between the microbiomes of *P. verrucosa* from the 6 reefs tested. Previous studies in corals have shown geographic variation in microbiomes (Hadaidi et al. 2017), including reefs in Indonesia separated by short geographic distances (Rachmawati et al. Chapter 1). Interestingly, minimal differentiation among populations was observed among microbiomes in *P. lobata* and none was observed in *A.*

millepora. Microbiomes are shaped by environmental differences (Roder et al. 2015). Given the complexity of the waters of the Indonesian Archipelago, it would not be surprising that these 6 populations spanning 5000 experience different environmental conditions (do you have refs for this?). However, it is unclear why these differences would have a profound impact on *P. verrucosa,* but not the microbiomes of the other taxa. The only explanation is that coral microbiomes are shaped by both the environment and coral host, as indicated by Kelly et al. (2014).

High abundance of potential pathogens

Microbes like *Vibrio* are commonly associated with stressed or diseased corals (Banin et al. 2000; Ben-Haim et al. 2003; Thompson et al. 2004). However, *Vibrio* accounted for more than 25% of the microbiomes of *P. verrucosa* and *P. lobata,* including regions of Eastern Indonesia like Raja Ampat and Manokwari, that are among the healthiest and most diverse reefs in Indonesia. Previous studies report very few *Vibrio* in healthy coral microbiomes (Hadaidi et al. 2017) although others report abundances of up to 35% (Ceh et al. 2011). Similarly, other taxa common in our microbiomes include *Clostridium, Fusibacter*, and some undescribed Firmicutes, that can be pathogenic and associated with black band disease (BBD) (Miller and Richardson 2011, Meyer et al. 2016).

The reefs of Eastern Indonesian (Raja Ampat, Manokwari) are among the healthiest and most diverse reefs in Indonesia (Veron 2002; Veron et al. 2011). The high abundance of potentially pathogenic microbes in this region may be normal, healthy corals may be able to sustain high pathogen loads. Alternatively, many of these taxa have both beneficial and pathogenic forms (Ainsworth et al. 2015) so the abundance of pathogens may be low. However,

a more concerning possibility is that the high abundance of these taxa indicate that Indonesian reefs are increasingly stressed, and that these results represent a microbial canary in a coalmine. Given the biological and economic importance of these ecosystems, future research should explore these unusual patterns.

FIGURES

S ● Aceh (western Sumatra)
P ● Pemuteran (northwest Bali)
A ● Amed (eastern Bali)
W ● Wakatobi (Southeast Sulawesi)
R ● Raja Ampat (western West Papua)
M ● Manokwari (eastern West Papua)

Figure 3.1. Study sites comprise 6 locations represent regions with different severity levels of bleaching experiences with trends of decreasing levels from west to east.

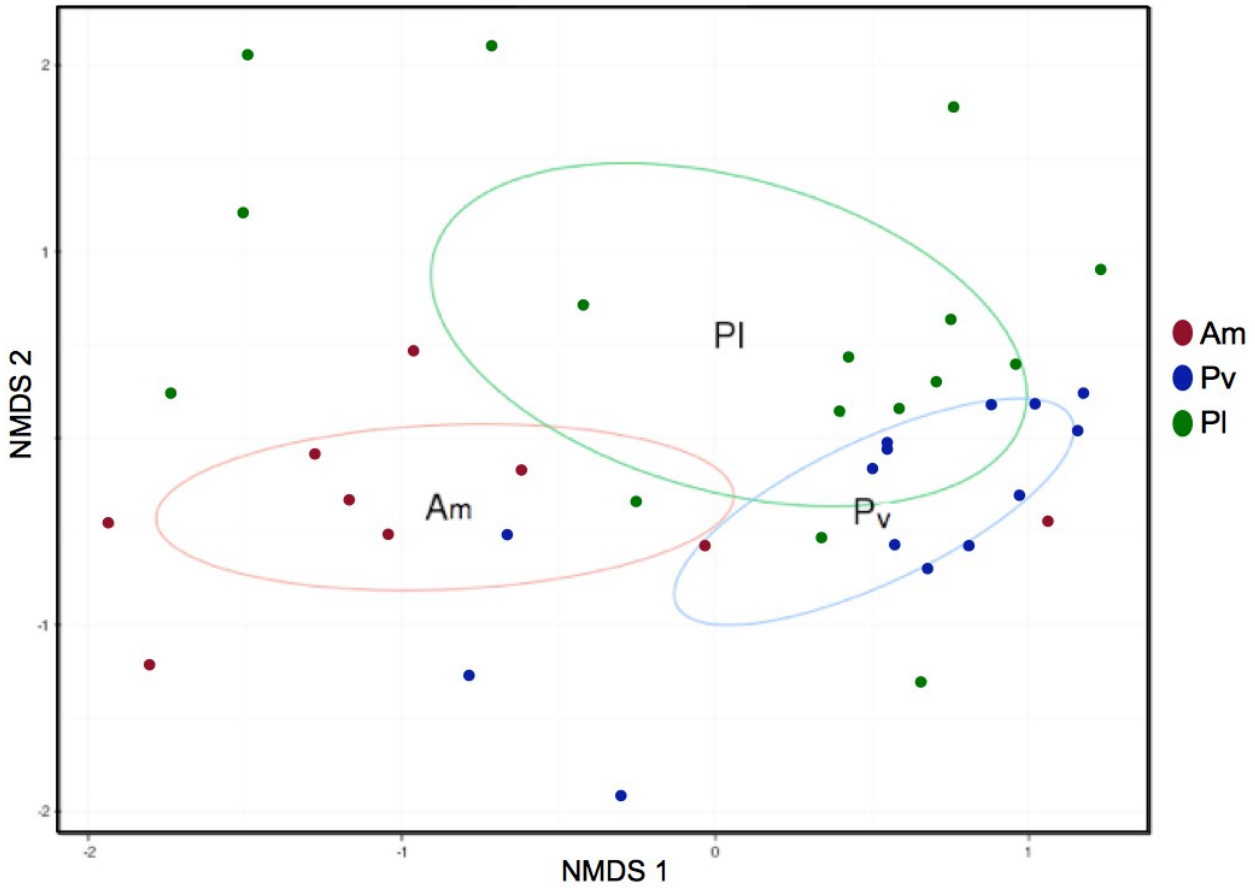
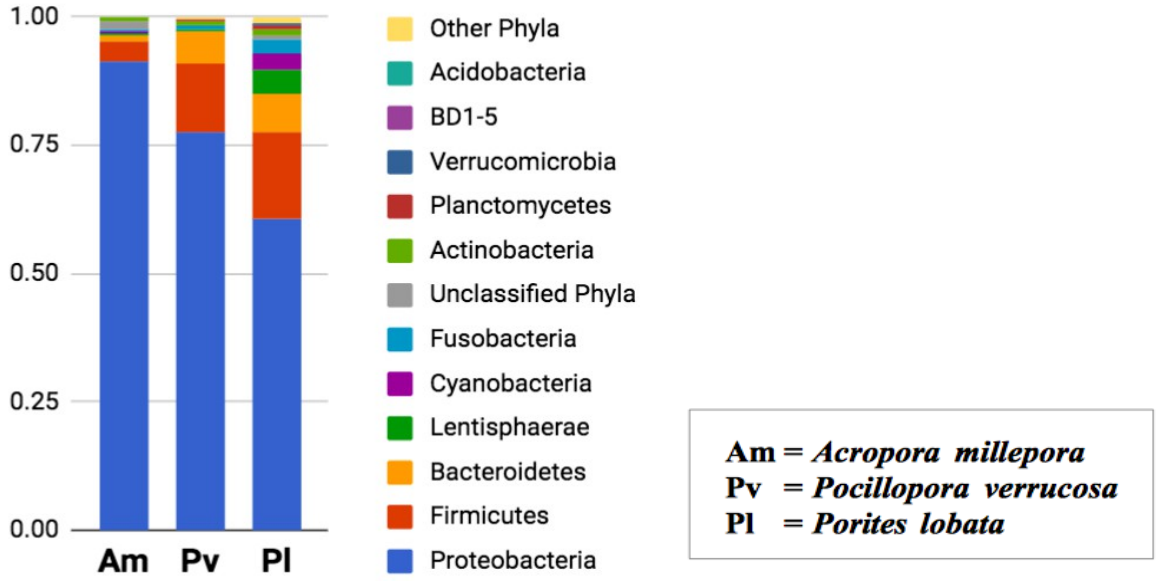


Figure 3.2. Nonmetric multidimensional scaling (NMDS) analysis of SSU rRNA gene sequence data of three coral species, *Acropora millepora* (Am), *Pocillopora verrucosa* (Pv), and *Porites lobata* (Pl). We combined Amed and Manokwari coral-associated microbial profiles to compare among these three coral species. The sequences were grouped into minimum entropy decomposition (MED) nodes and compared using Jaccard similarity index.

A. Phylum



B. Genus

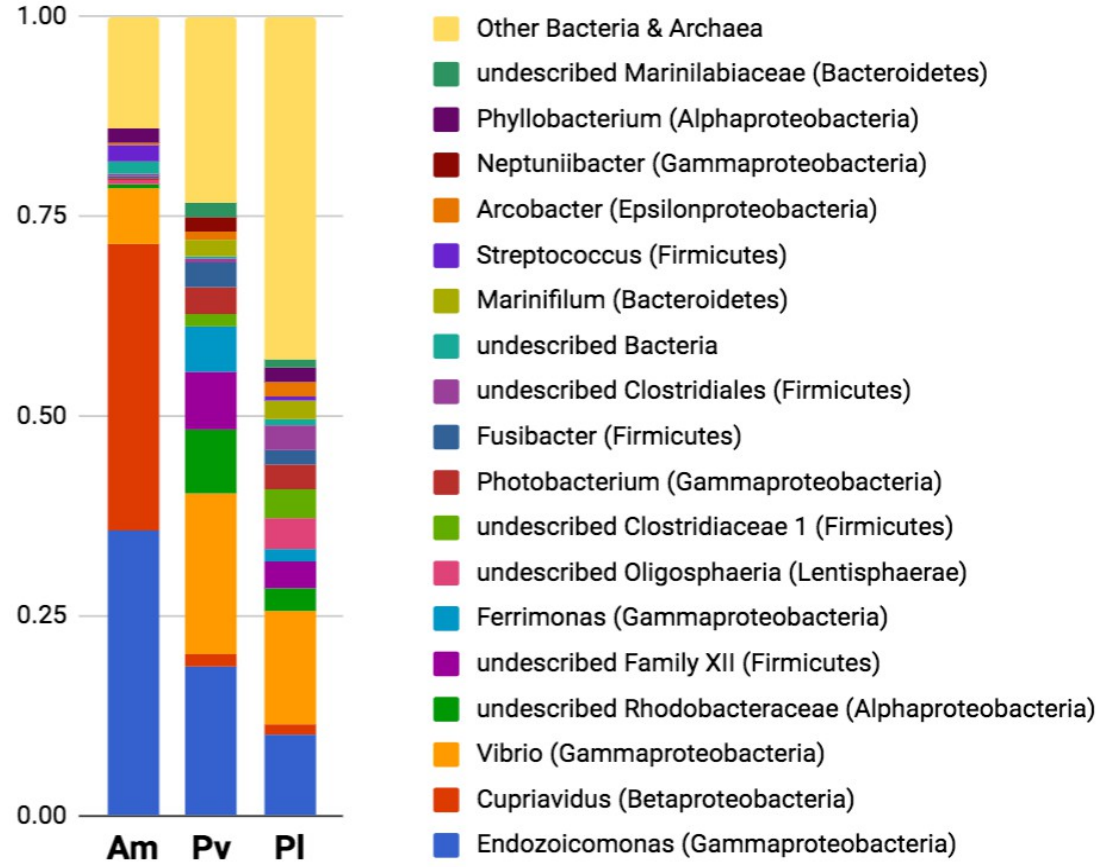
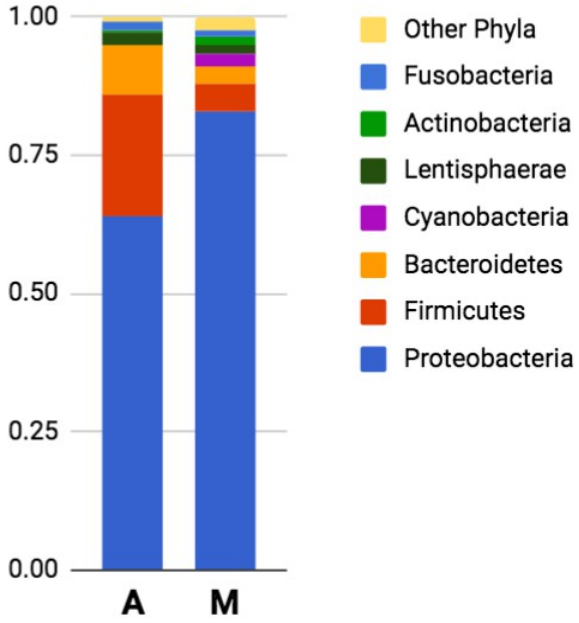


Figure 3.3. Combined Amed and Manokwari coral-associated microbial profiles of three coral species. **A.** Bar plot of distribution of the combined 5 most abundant microbial phyla, **B.** Bar plot of distribution of the combined 10 most abundant microbial genera.

A. Phylum



B. Genus

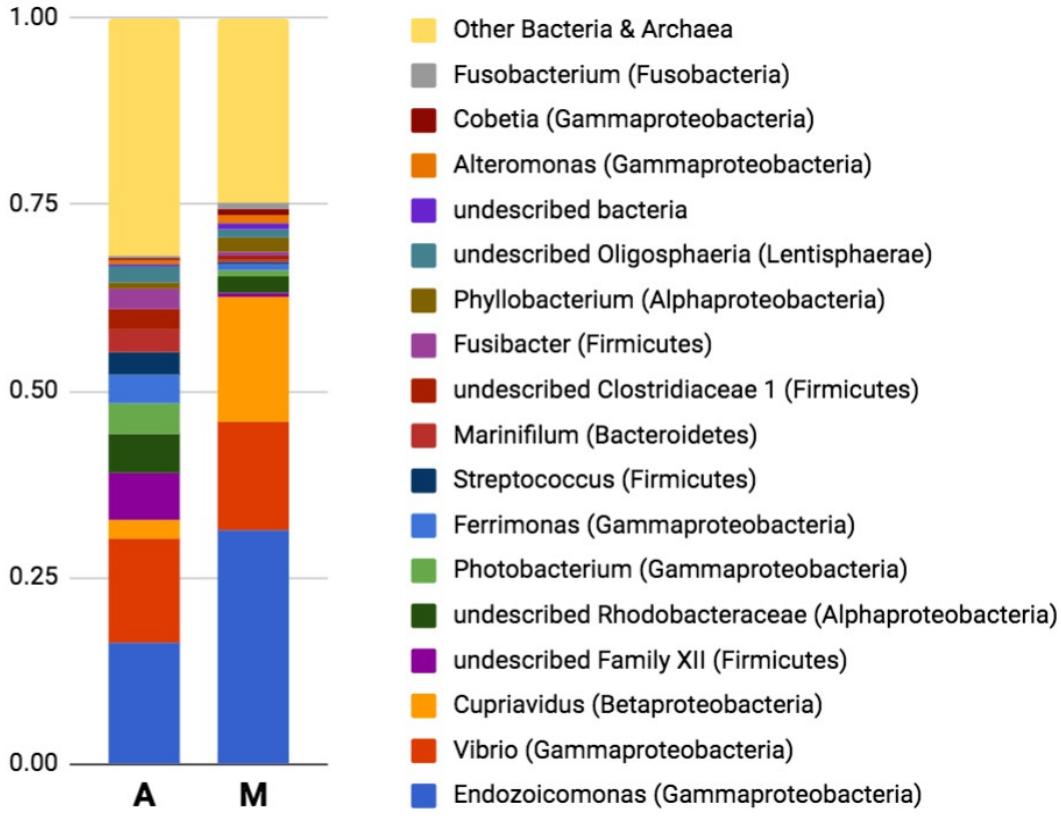


Figure 3.4. Comparison of ***Acropora millepora***-associated microbial profiles between Amed (A) and Manokwari (M). **A.** Bar plot of distribution of the combined 5 most abundant microbial phyla, **B.** Bar plot of distribution of the combined 10 most abundant microbial genera.

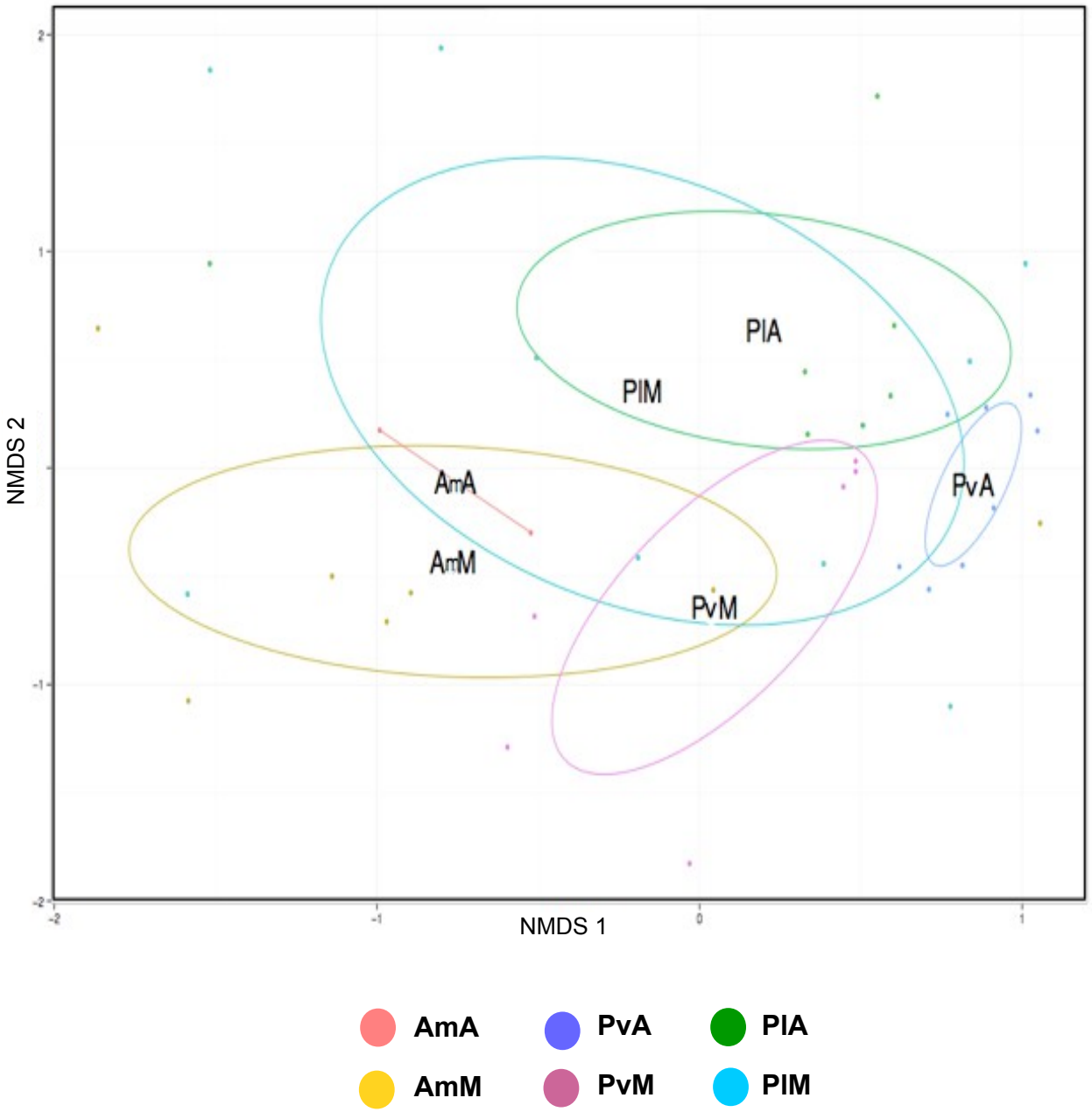
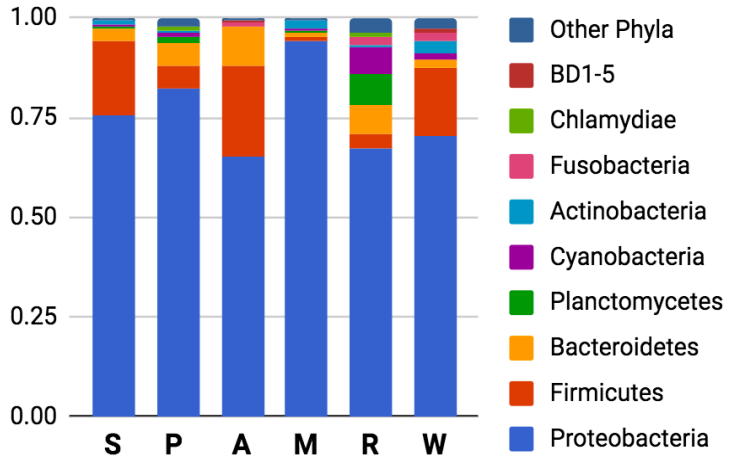


Figure 3.5. Nonmetric multidimensional scaling (NMDS) analysis of SSU rRNA gene sequence associated with ***Acropora millepora*** (Am) from two locations, Amed (A) and Manokwari (M), showing their variation of community profiles compared to the other two coral host species, *Pocillopora verrucosa* (Pv) and *Porites lobata* (Pl), in the same locations. The sequences were grouped into minimum entropy decomposition (MED) nodes and compared using Jaccard similarity index.

A. Phylum



B. Genus

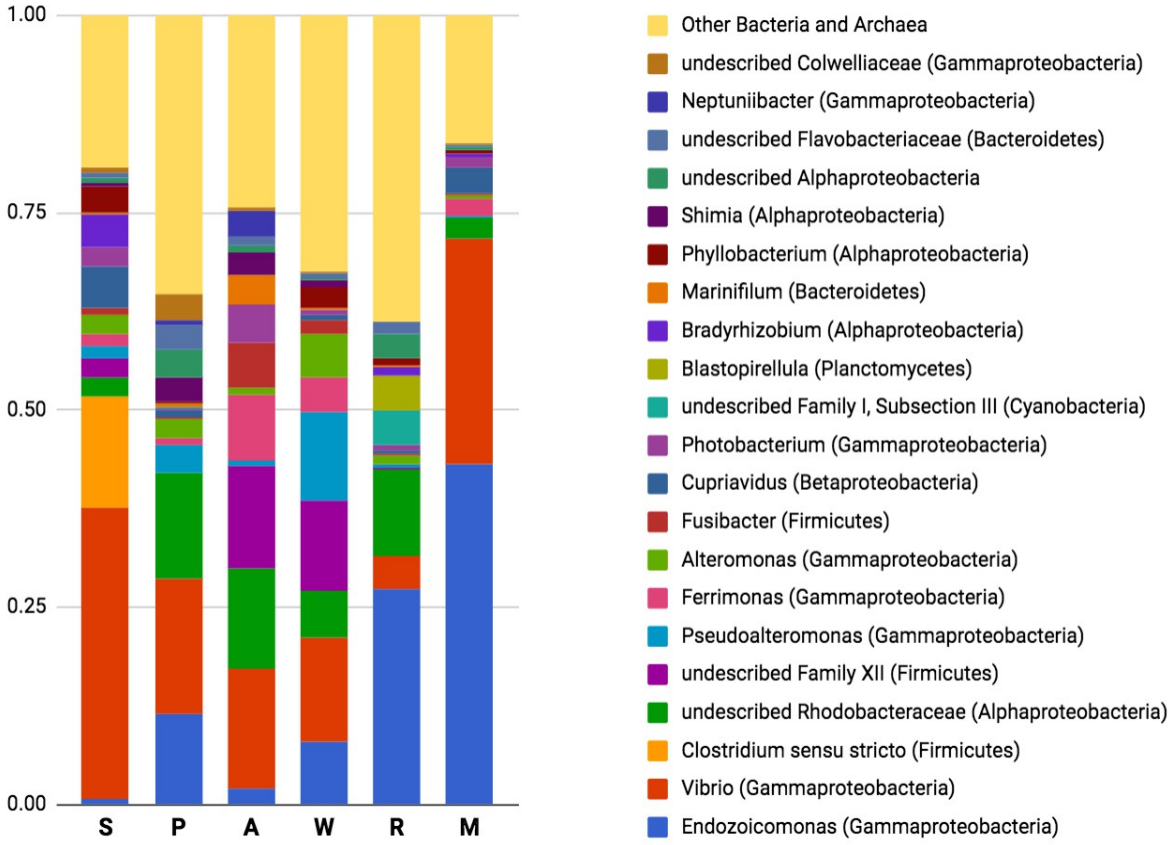


Figure 3.6. Bar plots of ***Pocillopora verrucosa***-associated microbial profiles from six locations. From west (more sensitive to bleaching) to east (more resistant to bleaching): S = Weh Island (Aceh Nanggroe Darussalam, West Indonesia), P = Pemuteran (Bali, Central Indonesia), A = Amed (Bali, Central Indonesia), W = Wakatobi (Southeast Sulawesi, Central Indonesia), R = Raja Ampat (West Papua, Eastern Indonesia), and M = Manokwari (West Papua, Eastern Indonesia). **A.** Compilation of 5 most abundant microbial phyla, **B.** Compilation of 10 most abundant microbial genera.

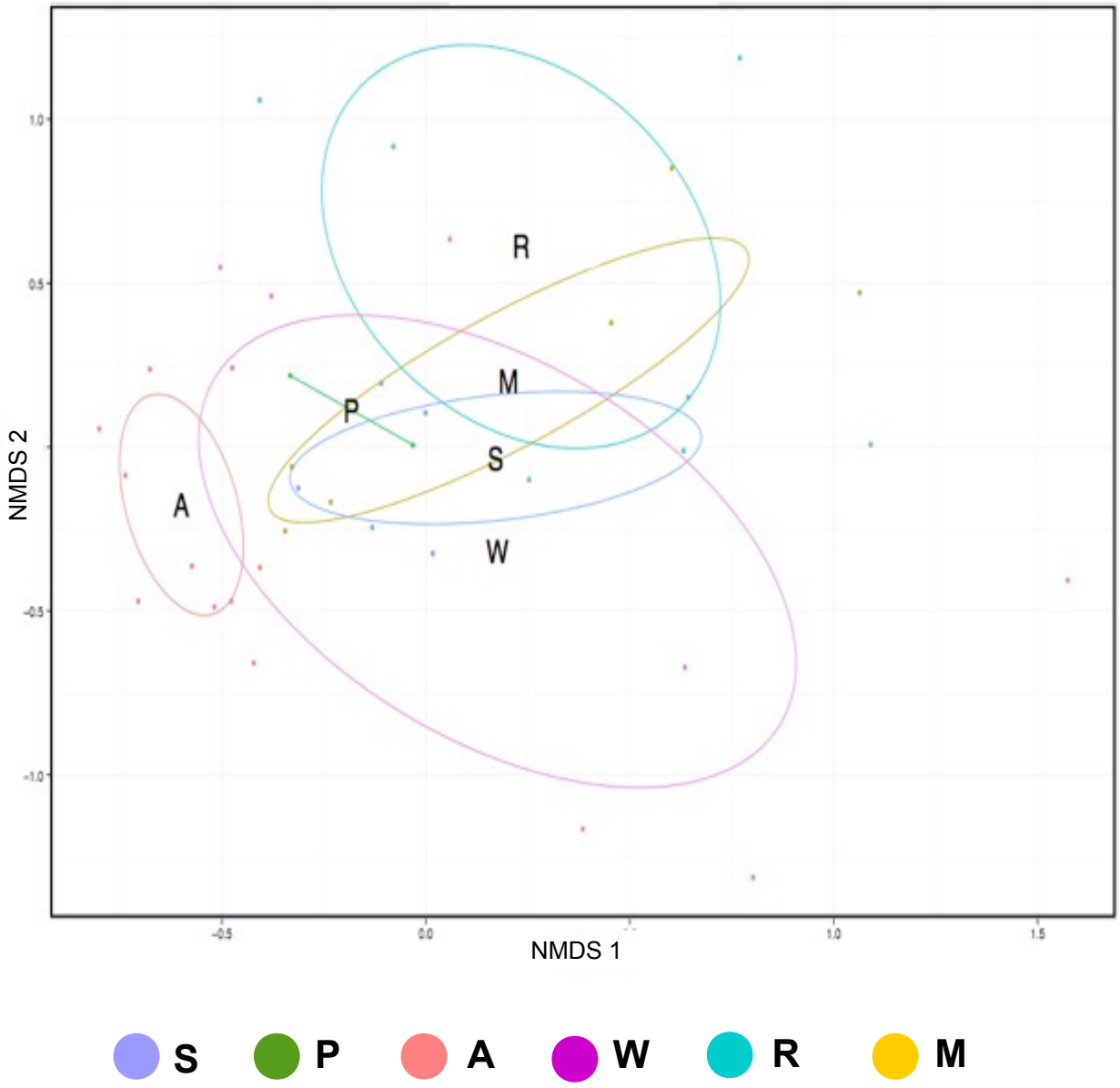
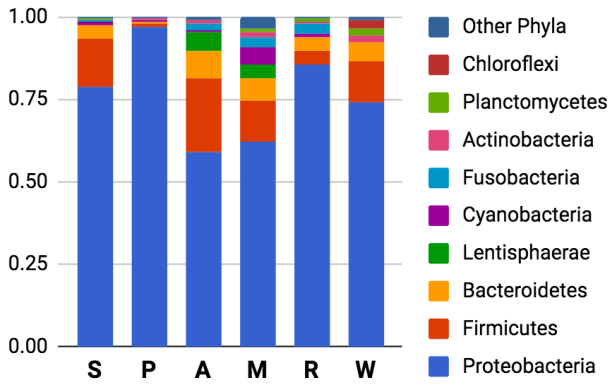


Figure 3.7. Nonmetric multidimensional scaling (NMDS) analysis of SSU rRNA gene sequence associated with ***Pocillopora verrucosa*** from six locations. From west (more sensitive to bleaching) to east (more resistant to bleaching): S = Weh Island (Aceh Nanggroe Darussalam, West Indonesia), P = Pemuteran (Bali, Central Indonesia), A = Amed (Bali, Central Indonesia), W = Wakatobi (Southeast Sulawesi, Central Indonesia), R = Raja Ampat (West Papua, Eastern Indonesia), and M = Manokwari (West Papua, Eastern Indonesia). The sequences were grouped into minimum entropy decomposition (MED) nodes and compared using Jaccard similarity index.

A. Phylum



B. Genus

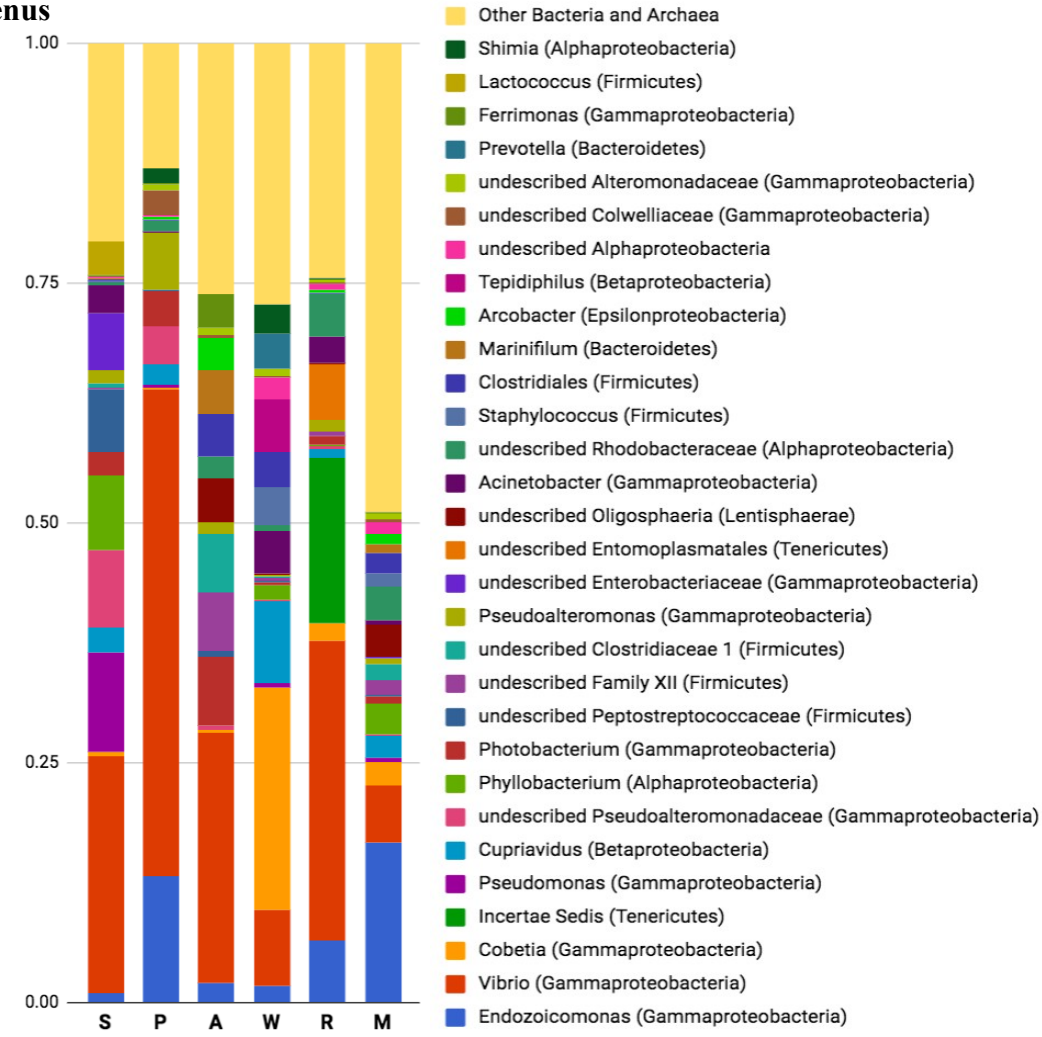


Figure 3.8. Bar plots of ***Porites lobata***-associated microbial profiles from six locations. From west to east are more resistant to bleaching: S = Weh Island (Aceh Nanggroe Darussalam, West Indonesia), P = Pemuteran (Bali, Central Indonesia), A = Amed (Bali, Central Indonesia), W = Wakatobi (Southeast Sulawesi, Central Indonesia), R = Raja Ampat (West Papua, Eastern Indonesia), and M = Manokwari (West Papua, Eastern Indonesia). **A.** Compilation of 5 most abundant microbial phyla, **B.** Compilation of 10 most abundant microbial genera.

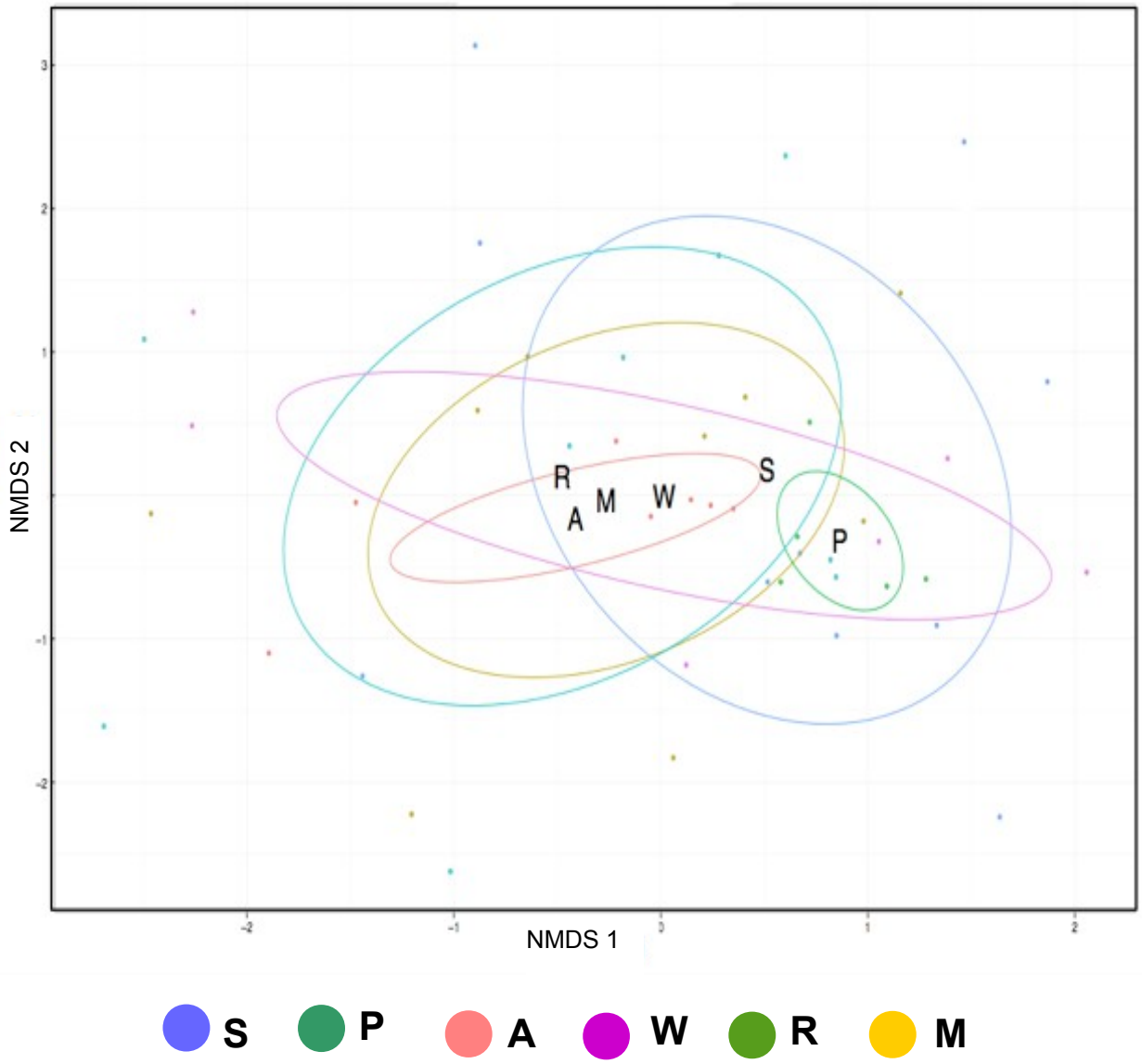


Figure 3.9. Nonmetric multidimensional scaling (NMDS) analysis of SSU rRNA gene sequence associated with ***Porites lobata*** from six locations. From west (more sensitive to bleaching) to east (more resistant to bleaching): S = Weh Island (Aceh Nanggroe Darussalam, West Indonesia), P = Pemuteran (Bali, Central Indonesia), A = Amed (Bali, Central Indonesia), W = Wakatobi (Southeast Sulawesi, Central Indonesia), R = Raja Ampat (West Papua, Eastern Indonesia), and M = Manokwari (West Papua, Eastern Indonesia). The sequences were grouped into minimum entropy decomposition (MED) nodes and compared using Jaccard similarity index.

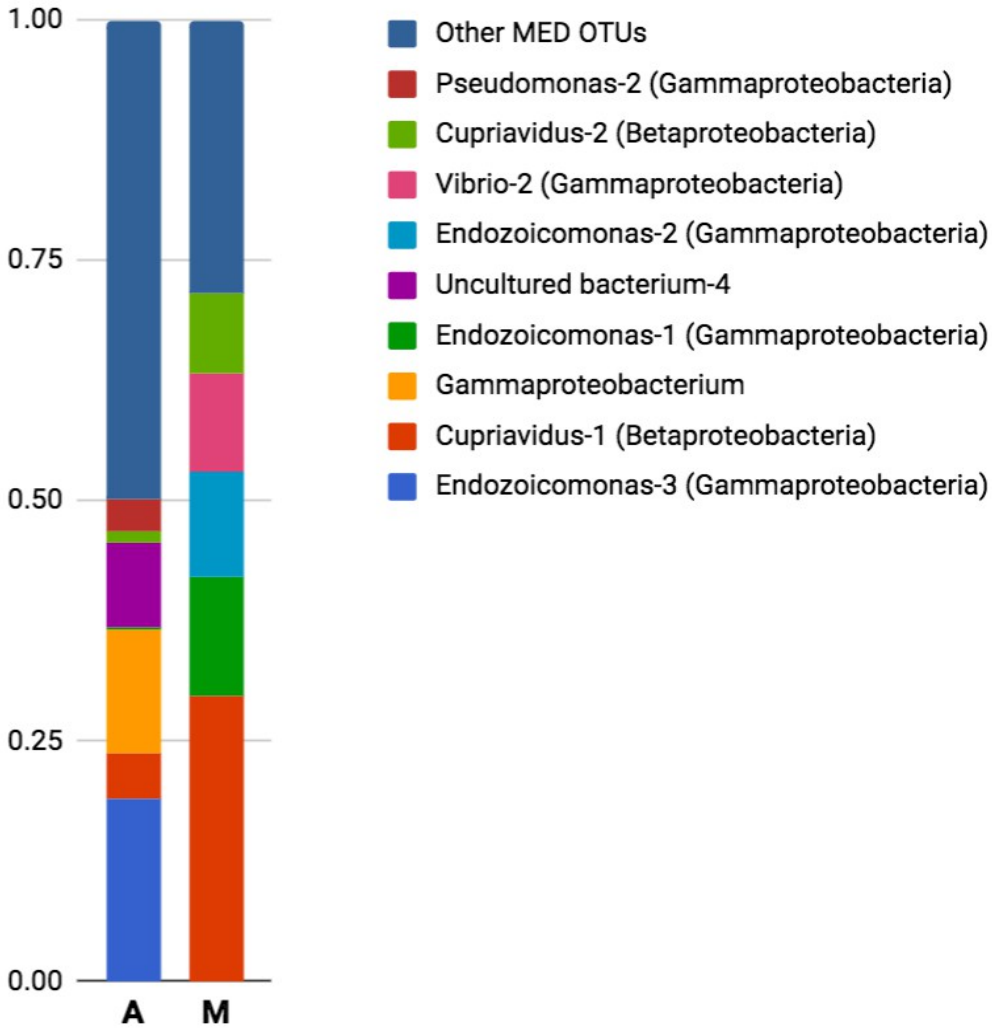


Figure 3.10. Bar plot of compilation 5 most abundant MED OTUs of *Acropora millepora* in Amed (A) and Manokwari (M).

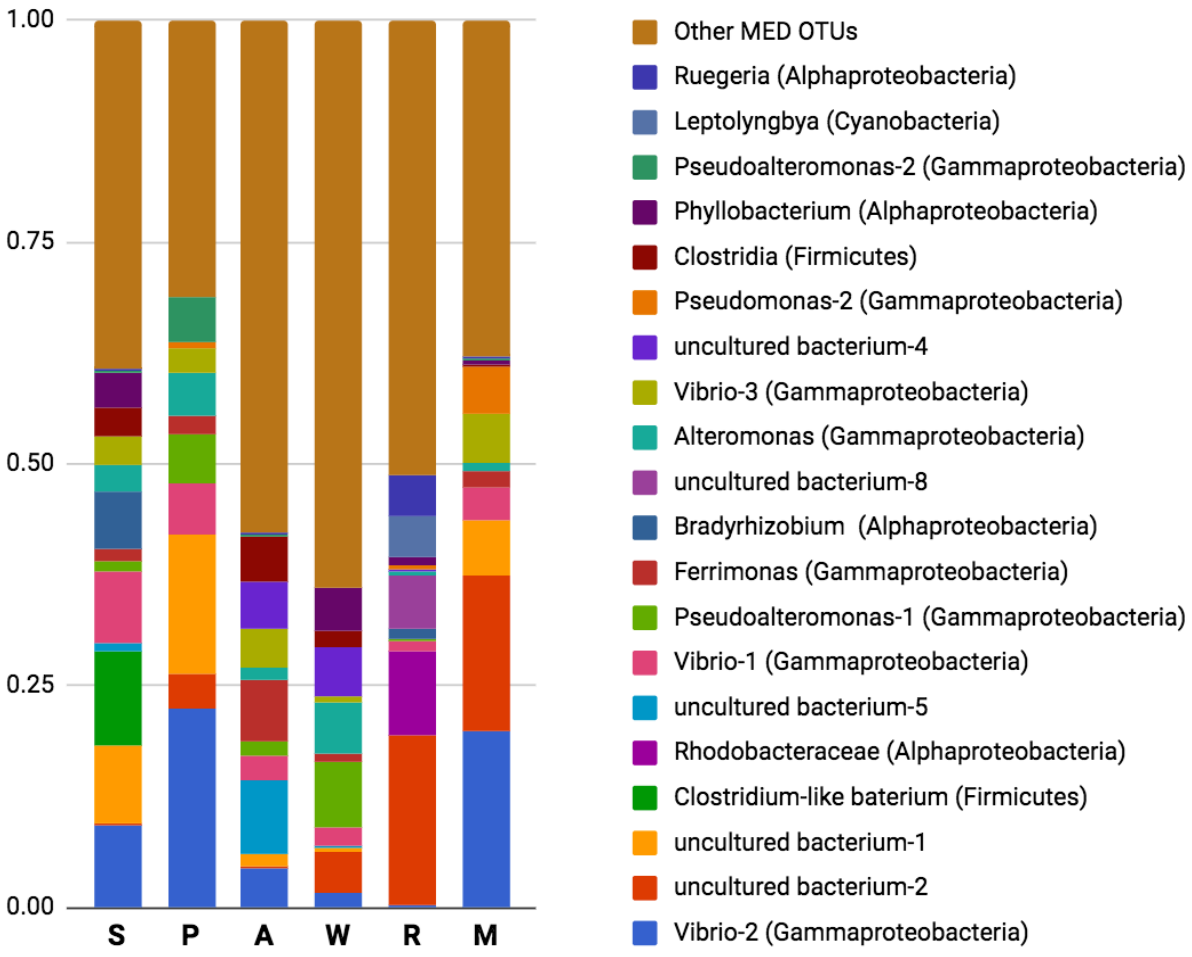


Figure 3.11. Bar plot of compilation 5 most abundant MED OTUs of microbial associated with ***Pocillopora verrucosa*** in each of six locations. From west (more sensitive to bleaching) to east (more resistant to bleaching): S = Weh Island (Aceh Nanggroe Darussalam, West Indonesia), P = Pemuteran (Bali, Central Indonesia), A = Amed (Bali, Central Indonesia), W = Wakatobi (Southeast Sulawesi, Central Indonesia), R = Rajaampat (West Papua, Eastern Indonesia), and M = Manokwari (West Papua, Eastern Indonesia). Each MED OTU here are the same as ones in Figure 3.12.

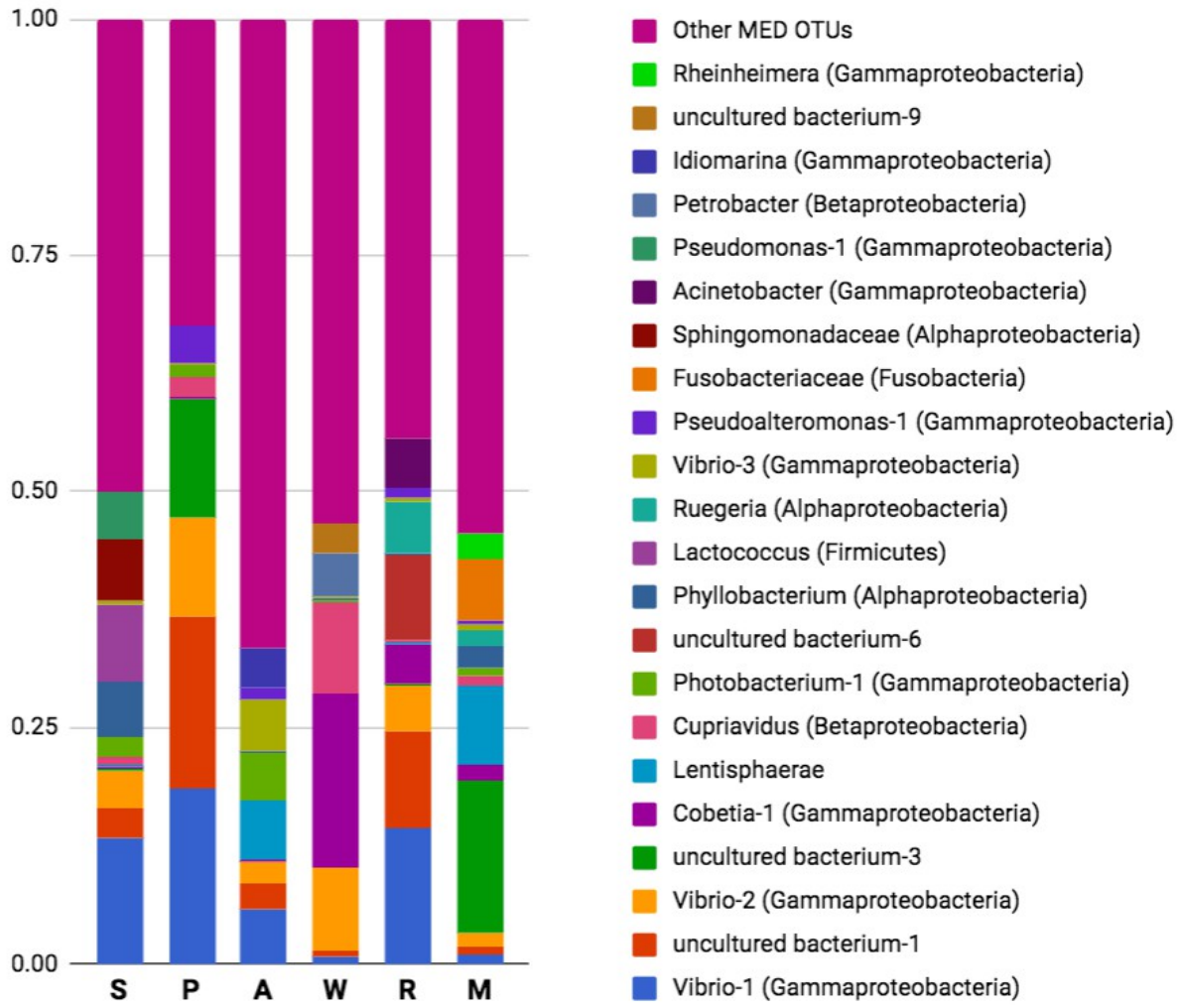


Figure 3.12. Bar plot of compilation 5 most abundant MED OTUs of microbial associated with ***Porites lobata*** in each of six locations. From west (more sensitive to bleaching) to east (more resistant to bleaching): S = Weh Island (Aceh Nanggroe Darussalam, West Indonesia), P = Pemuteran (Bali, Central Indonesia), A = Amed (Bali, Central Indonesia), W = Wakatobi (Southeast Sulawesi, Central Indonesia), R = Rajaampat (West Papua, Eastern Indonesia), and M = Manokwari (West Papua, Eastern Indonesia). Each MED OTU here are the same as ones in Figure 3.11.

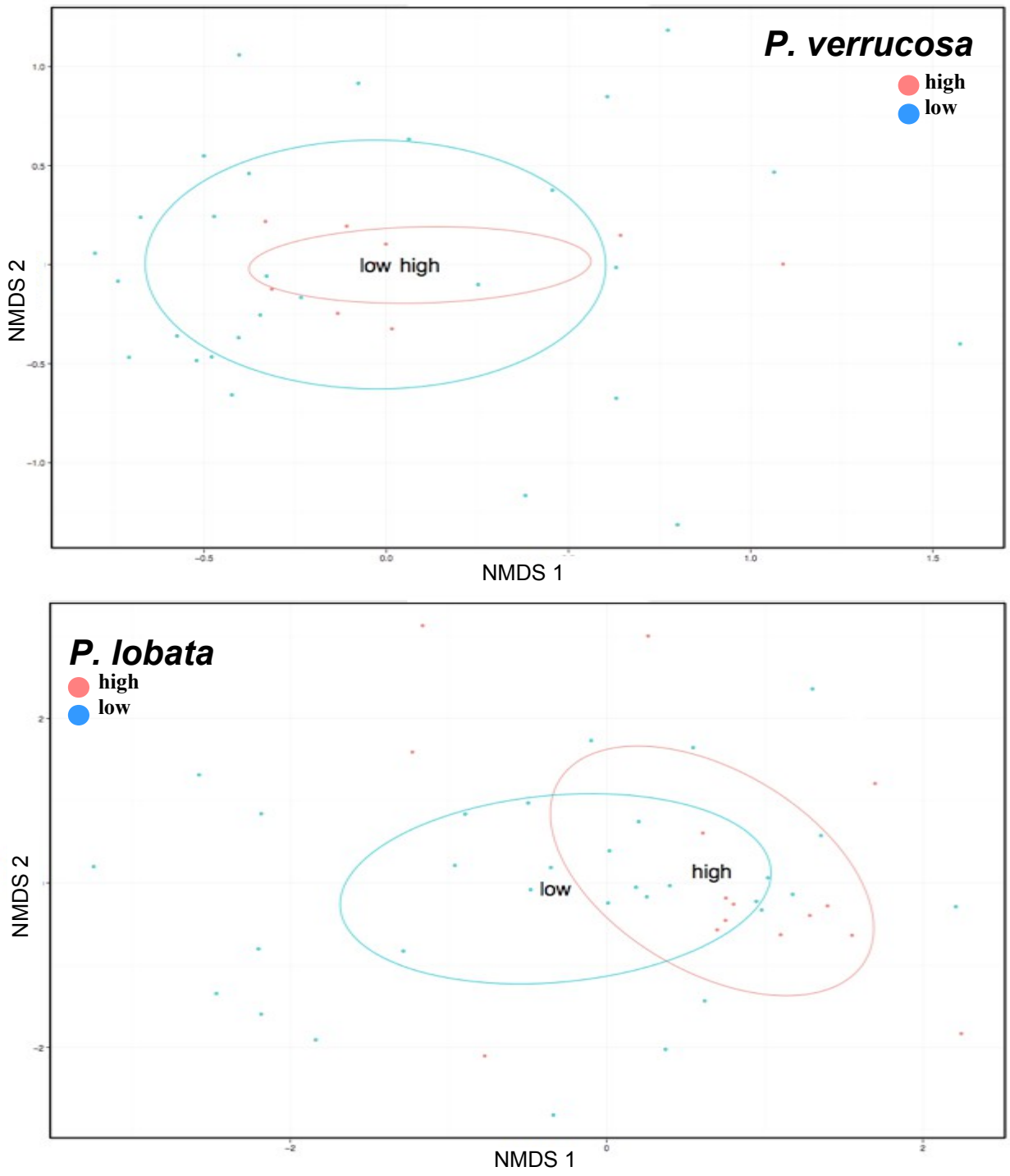


Figure 3.13. NMDS (Jaccard distance) plots of coral symbiotic microbiome in two reefs groups that experienced moderate to severe bleaching (S = Weh Island (Aceh Nanggroe Darussalam, West Indonesia) and P = Pemuteran (Bali, Central Indonesia), high) and mild to moderate bleaching (A = Amed (Bali, Central Indonesia), W = Wakatobi (Southeast Sulawesi, Central Indonesia), R = Rajaampat and M = Manokwari (West Papua, Eastern Indonesia), low). **Top:** associated with *Pocillopora verrucosa.* **Bottom:** associated with *Porites lobata.*

REFERENCES

Ainsworth, T., L. Krause, T. Bridge, G. Torda, J-B. Raina, M. Zakrzewski, et al. 2015. The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. *ISME J*. **9:** 2261–2274.

Apprill, A., S.P. McNally, C.R. Parsons, and L. Weber. 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat Microb Ecol* **75**: 129–137.

Baker, A.C. 2003. Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of Symbiodinium. *Annu. Rev. Ecol. Evol. Syst.* **34**: 661.

Baker, A.C., C.J. Starger, T.R. McClanahan, and P.W. Glynn. 2004. Corals' adaptive response to climate change. Shifting to new algal symbionts may safeguard devastated reefs from extinction. *Nature*, **430**: 741.

Banin, A., T. Israely, M. Fine, Y. Loya, and E. Rosenberg. 2001. Role of endosymbiotic zooxanthellae and coral mucus in the adhesion of the coral-bleaching pathogen *Vibrio shiloi* to its host. F*EMS Microbiology Letters* **199**: 33-37.

Bayer, T., C. Arif , C. Ferrier-Pagès, D. Zoccola, M. Aranda, and C.R. Voolstra. 2013a. Bacteria of the genus *Endozoicomonas* dominate the microbiome of the Mediterranean gorgonian coral *Eunicella cavolini*. *Mar Ecol Prog Ser* **479**: 75–84.

Bayer T, Neave MJ, Alsheikh-Hussain A, Aranda M, Yum LK, Mincer T et al. 2013b. The microbiome of the Red Sea coral *Stylophora pistillata* is dominated by tissue-associated *Endozoicomonas* bacteria. *Appl Environ Microbiol* **79**: 4759–4762.

Ben-Haim, Y., F.L. Thompson, C.C. Thompson, M.C. Cnockaert, B. Hoste, J. Swings, and E. Rosenberg. 2003. *Vibrio coralliilyticus* sp. nov., a temperature-dependent pathogen of the coral *Pocillopora damicornis*. *Int J Syst Evol Microbiol* **53**: 309–315.

Blackall, L.L., B. Wilson, and M.J.H. van Oppen. 2015. Coral-the world's most diverse symbiotic ecosystem. *Mol. Ecol.* **24**, 5330–5347.

Bourne, D., Y. Iida, S. Uthicke, and S. Smith-Keune. 2008. Changes in coral-associated microbial communities during a bleaching event. *ISME J.* **2112**, 350–363.

Brown, B.E. and Suharsono. 1990. Damage and recovery of coral reefs affected by El Nino related seawater warming in the Thousand Islands, Indonesia. *Coral Reefs*, **8**:163-170

Buddemeier, R.W. and D.G. Fautin. 1993. Coral bleaching as an adaptive mechanism. *Bioscience,* **43**:320–326.

Burke, L., K. Reytar, M. Spalding, and A. Perry. 2012. Reefs at Risk Revisited in the Coral Triangle. Washington, DC: World Resources Institute. http://www.wri.org/reefs. 86p.

Caporaso,, J.G., C.L. Lauber, W.A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer et al. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* **6**: 1621–1624.

Carpenter, K.E., et al. 2008. One-third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science* **321**:560–563.

DeBoer, T.S., A.C. Baker, M.V. Erdmann, Ambariyanto, P.R. Jones, and P.H. Barber. 2012. Patterns of Symbiodinium distribution in three giant clam species across the biodiverse Bird's Head region of Indonesia. *Marine Ecology Progress Series,* **444**: 117-132.

Donner SD, Rickbeil GJM, Heron SF (2017) A new, high-resolution global mass coral bleaching database. *PLoS ONE* **12(4):** e0175490. https://doi.org/10.1371/journal.pone.0175490

Eakin, C.M. et al. 2016. Global coral bleaching 2014–2017. *Reef Currents* **31**: 1.

Eren, A.M., H.G. Morrison, P.J. Lescault, J. Reveillaud, J.H. Vineis, and M.L. Sogin. 2014. Minimum entropy decomposition: unsupervised oligotyping for sensitive partitioning of high- throughput marker gene sequences. *ISME J* **9**: 968–979.

Guest JR, Baird AH, Maynard JA, Muttaqin E, Edwards AJ et al. (2012) Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. *PLOS ONE* **7:** e33353.

Habibi, A. 2007. Coral Bleaching in Karimunjawa National Park. Taka Foundation.

Hadaidi, G., T. Rothig, L.K. Yum, M. Ziegler, C. Arif, C. Roder, et al. 2017. Stable mucus-associated bacterial communities in bleached and healthy corals of *Porites lobata* from the Arabian Seas. *Sci Rep* **7**: 45362.

Hammer, Ø., Harper, D.A.T., Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4(1)**: 9pp.

Hernandez-Agreda, A., W. Leggat, P. Bongaerts, and Ainsworth, T. D. 2016. The microbial signature provides insight into the mechanistic basis of coral success across reef habitats. *MBio* **7**, e00560–16. doi: 10.1128/mBio.00560-16

Hoegh-Guldberg, O., 1999. Climate Change, coral bleaching and the future of the world's coral reefs**.** *Mar. Fresh. Res.,* **50***,* 839.

Hoegh-Guldberg, O., P.J. Mumby, A.J. Hooten, R.S. Steneck, P. Greenfield, E. Gomez, C.D. Harvell, P.F. Sale, A.J. Edwards, K. Caldeira, N. Knowlton, C.M. Eakin, R. Iglesias-

Prieto, N. Muthiga, R.H. Bradbury, A. Dubi, M.E. Hatziolos. 2007. Coral Reefs Under Rapid Climate Change and Ocean Acidification. *Science*, **318**, 5857, 1737-1742.

Hughes, T.P., J.T. Kerry, M. Álvarez-Noriega, J.G. Álvarez-Romero, K.D. Anderson, et al. 2017. Global warming and recurrent mass bleaching of corals. *Nature,* **543**: 373-378.

Kayanne, H. 2017. Validation of degree heating weeks as a coral bleaching index in the northwestern Pacific. *Coral Reefs* **36**: 63–70.

Kinzie, III, R.A., M. Takayama, S.R. Santos, and M.A. Coffroth. 2001. The Adaptive Bleaching Hypothesis: Experimental Tests of Critical Assumptions. *Biol. Bull.* **200**: 51-58.

Kozich, J.J., S.L. Westcott, N.T. Baxter, N.T. Highlander, and P.D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl Environ Microbiol* **79**: 5112–5120.

Kushmaro, A., E., Banin, Y., Loya, E., Stackebrandt, and E. Rosenberg. 2001. *Vibrio shiloi* sp. nov., the causative agent of bleaching of the coral *Oculina patagonica*. *Int J Syst Evol Microbiol* **51**: 1383–1388.

Kusuma, D.W. et al. 2017. *IOP Conf. Ser.: Earth Environ. Sci.* **98** 012038.

Lema, K. A., Willis, B. L., and Bourne, D. G. 2012. Corals form characteristic associations with symbiotic nitrogen-fixing bacteria. *Appl. Environ. Microbiol.* **78**: 3136–3144.

Littman, R., B.L., Willis, C. Pfeffer, and D.G. Bourne. 2009. Diversities of coral-associated bacteria differ with location, but not species, for three acroporid corals on the Great Barrier Reef. *FEMS microbiology ecology* **68(2)**: 152-163.

Lough, J.M. and M.J.H. van Oppen. 2009.Introduction: Coral Bleaching Patterns Processes, Causes and Consequences. 1–5 In Ecological Studies. Springer Berlin Heidelberg.

Meyer, J.L., V.J. Paul, and M. Teplitski. 2014. Community shifts in the surface microbiomes of the coral *Porites astreoides* with unusual lesions. *PLoS ONE* **9(6)**: e100316.

Neave, M. J., R. Rachmawati, L. Xun, C. T. Michell, D. G. Bourne, A. Apprill, and C.R. Voolstra. 2017. Differential specificity between closely related corals and abundant *Endozoicomonas* endosymbionts across global scales. *ISME J.* **11**: 186–200.

Pruesse, E., C. Quast, K. Knittel, B. Fuchs, W. Ludwig, J. Peplies, et al. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research* **35**: 7188-7196.

Reaka-Kudla, M.L. 2001. Known and unknown biodiversity risk of extinction and conservation strategy in the sea. 19–33 *In* Waters in Peril. Springer US.

Roberts, C.M., et al. 2002. Marine biodiversity hotspots and conservation priorities for tropical reefs. *Science*, **295(5558)**: 1280-1284.

Roder C, Arif C, Daniels C, Weil E, Voolstra CR. 2014. Bacterial profiling of White Plague Disease across corals and oceans indicates a conserved and distinct disease microbiome. *Mol Ecol* **23:** 965–974.

Rohwer, F., V. Seguritan, F. Azam, and N. Knowlton. 2002. Diversity and distribution of coral-associated bacteria. *Mar Ecol Prog Ser* **243**: 1–10.

Rowan, R., N. Knowlton, A. Baker, and J. Jara. 1997. Landscape ecology of algal symbionts creates variation in episodes of bleaching. *Nature* **388**: 265-269.

Rudi, E., T. Iskandar, N. Fadli, and H. Hidayati. 2012. Impact of mass coral bleaching on fish reef community and fishermen catches at Sabang, Aceh Province, Indonesia. *AACL Bioflux* **5(5)**: 309-320.

Schloss, P.D., S.L. Westcott, T. Ryabin, J.R. Hall, M. Hartmann, E.B. Hollister, et al. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75**: 7537–7541.

Setiasih, N. and J. Wilson. 2010. A regional overview on the 2010 coral bleaching event in Southeast Asia: Indonesia. *in* Status of coral reefs in East Asian seas region: 2010. The Global Coral Reef Monitoring Network. Tokyo, Japan.

Sunagawa, S., C.M. Woodley, and C.M. Medina. 2010. Threatened corals provide underexplored microbial habitats. *PLoS ONE* **5**: e9554.

Thompson, F.L., Y. Barash, T. Sawabe, G. Sharon, J Swings, and E. Rosenberg. 2006. *Thalassomonas loyana* sp. nov., a causative agent of the white plague-like disease of corals on the Eilat coral reef. *Int J Syst Evol Microbiol* **56**:365–368.

Vega Thurber, R., D. Willner-Hall, B. Rodriguez-Mueller, C. Desnues, R.A. Edwards, F. Angly, et al. 2009. Metagenomic analysis of stressed coral holobionts. *Environ. Microbiol.* **11**: 2148–2163.

Veron, J.E.N. 2002. *in* McKenna, S.A., G.R. Allen, and S. Suryadi (eds). 2002. A Marine Rapid Assessment of the Raja Ampat Islands, Papua Province, Indonesia. RAP Bulletin of Biological Assessment 22. Conservation International, Washington, DC.

Veron, J.E.N. et al. 2011. The Coral Triangle. *In*: Dubinsky Z. and N. Stambler (eds). Coral Reefs: An Ecosystem in Transition. Springer, Dordrecht.

Wegley, L., Edwards, R., Rodriguez-Brito, B., Liu, H., and Rohwer, F. (2007). Metagenomic analysis of the microbial community associated with the coral *Porites astreoides. Environ. Microbiol.* **9**, 2707–2719. doi: 10.1111/j.1462- 2920.2007.01383.x

West, J.D. and R.V. Salm. 2003. Resistance and Resilience to Coral Bleaching: Implications for Coral Reef Conservation and Management. *Conservation Biology*, **17, 4**: 956–967.

Westmacott, S., H.S.J. Cesar, L. Pet-Soede and O.Lindén. 2000. Coral bleaching in the Indian Ocean: socio-economic assessment of effects. *in* Cesar H.S.J. Collected essays on the economics of coral reefs. CORDIO. Kalmar, Sweden.

Ziegler, M., F.O. Seneca, L.K. Yum, S.R. Palumbi, and C.R. Voolstra. 2017. Bacterial community dynamics are linked to patterns of coral heat tolerance. *Nat Commun* **8**: 14213.