Deciphering the bacterial microworld in corals: structure, variability and persistence

Thesis submitted by

Lic in Biology Alejandra Isabel Hernández Agreda

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College of Public Health, Medical and Veterinary Sciences

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Thesis committee:

- ***Dr. Tracy D. Ainsworth*** – Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University and School of Biological, Earth and Environmental Science, University of New South Wales
- ***Associated Professor William Leggat*** - Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University; The College of Public

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Abstract

Decades of research have defined the coral meta-organism as a complex microbial system due to the diversity, abundance and variability of the associated microorganisms. Bacteria are the most studied group of coral-associated prokaryotes, and they are located within the mucus, skeleton, tissues and cellular spaces. Abiotic factors including light irradiance, current, pH, and oxygen generate distinct micro-niches that differ between coral species, depths, reefs, and bioregions. This variety in microhabitats leads to enormous configurations of hundred of thousand bacteria, from tens of thousand phylotypes, associated with each coral species. The variability, diversity and richness of these bacterial communities have undermined the capacity to identify bacterial phylotypes in symbiosis with corals, to describe their functional roles and to establish the characteristics of a healthy bacterial community in corals.

Herein, I dissect the variability, diversity and richness of bacterial communities in healthy corals. I aim to (1) define the characteristics of a healthy coral microbiome, (2) evaluate the presence of universal bacterial symbionts in coral-associated bacterial assemblages, and (3) identify factors generating variability in assessments of the coral-associated bacterial communities. In doing so, I developed a conceptual framework that extends on the core microbiome concept, attempting to structure and understand the high diversity observed in coral-associated microbial communities.

Initially, I compared sample preservation and preparation methodologies with samples from the corals *Goniastrea edwardsi* and *Isopora palifera* collected from Heron Island (Southern Great Barrier Reef, Australia). I showed that preservation in DMSO and 4% paraformaldehyde solution generate comparable composition results to traditional snap freezing in liquid nitrogen for generating 16S microbiome datasets. Furthermore, I showed that homogenization with beat

beating is the most reliable, reproducible and practical method for rapid sample preparation. I further evaluated the bacterial communities associated with the coral polyp and coenosarcs from the widely distributed coral *Pocillopora damicornis*. Although overall bacterial communities appeared similar between microhabitats, differences were evident when comparing diversity, dispersion and core, low-abundance bacteria. These results highlight the importance of considering rare bacteria in the coral microbiome, and the efficiency of core microbiome concept in detecting fine-scale differences.

To address variability across broad geographic and ecological scales, I identified and quantified the bacterial community of three depth generalist corals, *Pachyseris speciosa*, *Mycedium elephantotus* and *Acropora aculeus*, at distinct depth intervals (10, 20, 40, and 60-80 m), across a broad latitudinal range in two distinct bioregions (the Great Barrier Reef and the western Coral Sea). I demonstrated that bacterial communities are comparable in richness, diversity and taxonomic structure. In the three coral species, the response of bacterial communities structure is reflective of differences in reef location and bioregion. I further identified ubiquitous bacterial phylotypes (core microbiome) for each species, and determined bacteria consistently associated with both shallow and mesophotic reefs. Coupling the core microbiome framework with an analysis of beta-diversity, taxonomic breadth, taxonomic redundancy and functional prediction on the databases of the three species, I further identified and quantified the variability associated to species, bioregions, reefs and individuals. I demonstrated that bacterial communities in corals show taxonomical, and potentially functional, redundancy in both the resident community and the core microbiome.

Based on these results, I propose a conceptual framework defining bacterial communities in healthy corals as three layers: an *environmentally responsive community* (thousand of phylotypes, transient and variable), an *individual microbiome* (~500-600 OTUs, variable

between reefs but with consistent taxonomy and function) and a *core microbiome* (few bacteria likely to be in symbiosis showing functional redundancy). This conceptual framework provides structure to the observed high levels of diversity and indicates that bacterial communities in corals are not as complex as previously considered. Given the ongoing degradation of reef environments and the increasing frequency and severity of anthropogenic stressors, future research should be directed towards identifying direct links of microbial contributions to coral resistance and resilience, including an understanding of their individual roles, functional redundancy, and their localization within coral niches.

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Publications produced during my PhD candidature

Peer reviewed papers:

- **Hernandez-Agreda A**, Gates RD, Ainsworth TD. 2017. Defining the core microbiome in corals' microbial soup. *Trends in Microbiology*, 25 (2). pp. 125-140.

- **Hernandez-Agreda A**, Leggat W, Bongaerts P, Ainsworth TD. 2016. The microbial signature provides insight into the mechanistic basis of coral success across reef habitats. *mBio*, 7 (4). pp. 1-10.

- **Hernandez-Agreda A**, Leggat W, Bongaerts P, Herrera C, Ainsworth TD. Rethinking the coral microbiome. Simplicity exits within a diverse microbial biosphere. Accepted - *mBio.*

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- *13th International Coral Reef Symposium.* June 2016. Honolulu, Hawaii. **Hernández A**, AI; P Bongaerts; W Leggat; TD Ainsworth. Exploring coralassociated bacteria over an extreme depth gradient: assessing the presence of ubiquitous symbionts.

- *ASLO 2017 Aquatic Sciences Meeting*. February 2017. Honolulu, Hawaii. **Hernández A**, AI; P Bongaerts; W Leggat; TD Ainsworth. Persistence and functional importance: identifying bacterial likely to be promotors of corals' success.

- *Coral Reef Futures Symposium* 2017. June 2017. Canberra, Australia. **Hernández A**, AI; P Bongaerts; W Leggat; TD Ainsworth. Defining microbiome in coral's microbial soup.

- *Origins and Function of the Animal Metaorganism.* March 2018. **Hernández A**, AI; W Leggat; P Bongaerts; C Herrera; TD Ainsworth. Rethinking the coral microbiome: simplicity in a diverse microbial biosphere.

Science communication pieces:

- **Hernandez-Agreda A**, McMahon R, Ainsworth TD, Martin J. What we have in common with corals and their unexplored microbial world, via The Conversation (September 12th, 2016).

Chapter 1: General Introduction

The coral microbiome

Microbial communities in corals represent a complex study system due to the high diversity and variability encountered within each coral host species and individual. Here I review the knowledge on coral-associated microbial communities and identify factors that influence this diversity and variability. This section provides an introduction to the topics explored in this thesis, followed by a more in-depth review and meta-analysis in Chapter 2 ('Defining healthy microbiome: a meta-analysis').

As a holobiont or meta-organism, corals are inhabited by diverse microbial communities including microalgae, bacteria, fungi, Archaea and viruses (Blackall, Wilson et al. 2015). Biological interactions between these microbes and the coral host are not yet fully understood, and just recently, we have started to comprehend the dimensions in richness, diversity and abundance of those communities. Symbiosis with the photosynthetic endosymbionts of the genus *Symbiodinium* is, so far, the most studied and better understood biological interaction between coral and any member of the microbial community (Davy, Allemand et al. 2012). The coral rely on the symbiotic relationship with these dinoflagellates for up to 95% of their nutrient uptake (Muscatine, McCloskey et al. 1981, Muscatine, Falkowski et al. 1984), and where this endosymbiosis is disrupted for long periods of stress, the coral can perish (Glynn 1984, Baird and Marshall 2002, Eakin, Morgan et al. 2010). However, the remaining members of the microbial community, the stability, and functional contribution have remained mostly unknown despite decades of coral reef research.

In particular for bacteria, one of the factors limiting our understanding of the host-microbe interaction is the variability that is evident in community dynamics (Rohwer, Seguritan et al. 2002, Bourne and Munn 2005). Coral-associated bacterial communities are highly variable, diverse and abundant; and differentiating stable, functionally significant interactions within

these communities is challenging. Community variability occurs at different spatial and temporal scales, ranging from the microbial niche within a coral colony (centimeters, (Rohwer, Seguritan et al. 2002)) to different biogeographical regions (thousand of kilometers, i.e. genus *Porites* (Wegley, Edwards et al. 2007, Li, Chen et al. 2013, Zhang, Ling et al. 2015)). Due to the broad physiological and metabolic characteristics of the prokaryotic phylum Bacteria, these microorganisms can inhabit virtually any environment on earth (Kim and Gadd 2008). Thus, corals represent a varied ecosystem for bacterial communities, which have been found inhabiting the surface mucus layer (Carlos, Torres et al. 2013, Glasl, Herndl et al. 2016), coral tissue (Ainsworth, Fine et al. 2006, van de Water, Ainsworth et al. 2015, Neave, Rachmawati et al. 2016) and the skeleton (Yang, Lee et al. 2016). These three microhabitats represent distinct microniches for bacteria, and consequently, the communities associated to each are likely to differ in richness, composition and structure (Sweet, Croquer et al. 2011, Apprill, Weber et al. 2016). While the exact ecological and biological factors influencing microbial community structure have not yet been determined, reef environment is likely to have an important role in generating variability on bacterial communities (Lee, Yang et al. 2012, Morrow, Moss et al. 2012, Rodriguez-Lanetty, Granados-Cifuentes et al. 2013). Light intensity, temperature, turbidity, pH or any environmental disturbance resulting in stress response in the coral can potentially impact the community structure in bacteria (Thurber, Willner-Hall et al. 2009, Littman, Willis et al. 2011, Grottoli, Dalcin Martins et al. 2018). As evident by recent findings that distinct bacterial communities exist between organisms of the same species located in different reefs (Morrow, Moss et al. 2012) and on the same reef but at different depths (Glasl, Bongaerts et al. 2017). Furthermore, the vast majority of literature on coral-associated bacteria has arisen from shallow reefs (Olson and Kellogg 2010), and such our understanding of depth-related community variability is limited. Recent advances in diving and remotely operated underwater technology have accelerated research into the microbial

communities of mesophotic (i.e. >30 m depth) and deep-sea corals, allowing the identification of similarities and discrepancies with shallow coral-associated microbial communities (Ainsworth, Krause et al. 2015, Meistertzheim, Lartaud et al. 2016, Glasl, Bongaerts et al. 2017, Gonzalez-Zapata, Bongaerts et al. 2018). Biological processes as diseases, reproduction and competition for space are some of the factors that can also generate structural changes in bacterial communities in corals (Barott, Rodriguez-Mueller et al. 2012, Cardenas, Rodriguez et al. 2012, Ceh, Raina et al. 2012).

Bacterial communities on corals are defined as highly diverse (Blackall, Wilson et al. 2015, Bourne, Morrow et al. 2016). This diversity is broadly described in literature reports of coral species harboring more than a hundred thousand individual bacteria belonging to over 30 thousand distinct phylotypes (Ainsworth, Krause et al. 2015, Zhang, Ling et al. 2015, Meyer, Rodgers et al. 2016), many of them not fully taxonomically identified or described. Thus, the variability, abundance and diversity of bacterial communities in corals have impaired our capacity of

i) identifying bacterial phylotypes potentially in stable symbiosis with corals,

ii) describing the functional roles that these symbiotic bacterial phylotypes may be playing to support coral wellbeing, and

iii) defining the characteristics of a healthy state of a bacterial community.

Answering these questions is crucial to identify the mechanisms through which bacteria, as physiologically and metabolically versatile organisms, may contribute to coral resilience and recovery from disturbances. Identifying those mechanisms is crucial as disturbances to coral reefs are increasing in frequency and magnitude (e.g. bleaching, (Hughes, Kerry et al. 2017, Hughes, Anderson et al. 2018)), and the associated decline ultimately impacts the livelihood

of human populations depending on reefs. To better understand the coral bacterial community structure, function and symbioses, we first need to identify the attributes of a normal and healthy bacterial community. Through accurately characterizing the normal and healthy status, we can define disturbed and dysbiotic states and identify holobiont states associated with a lack of, or low, performance in delivering goods and services to the host. Ultimately, this knowledge may provide avenues to enhance resilience and accelerate coral recovery (e.g. through the active manipulation of coral microbiota).

Thesis objectives

The goal of this thesis was to advance the understanding the healthy coral microbiome by evaluating spatial patterns in the composition and structure of bacterial communities in corals, identifying their relevant scales of variation and proposing potential processes driving them. To achieve this goal, I establish the following objectives:

- Objective 1: Define the characteristics of a healthy coral microbiome.
- Objective 2: Evaluate the presence of universal bacterial symbionts in coral-associated bacterial assemblages.
- Objective 3: Identify and quantify natural and artificial factors generating variability in coral-associated bacterial communities.

Objective 1: Define the characteristics of a healthy coral microbiome.

Due to the extreme variability, diversity and richness of microbes observed in association with corals, the definition and characteristics of a healthy microbiome have not yet been fully established. Firstly, I will approach this question by undertaking a meta-analysis of the published literature on the coral microbiome (Chapter 2). Through this meta-analysis, I

determine the variability of the coral microbiome to give a current status of the definition of the healthy microbiome. Besides, this exercise exposes critical gaps in our knowledge, and therefore an ecological framework is proposed to investigate the complex microbial systems in corals. Following this, in Chapter 5, I will empirically determine the common attributes of a healthy microbiome among coral species. Finally, a formal definition of the healthy coral microbiome is proposed in Chapter 7 together with an integrative discussion of all the chapters of this thesis.

Objective 2: Evaluate the presence of universal bacterial symbiont in coral-associated bacterial assemblages.

The core microbiome concept is one of the frameworks that has facilitated the understanding of other complex microbial systems. In Chapter 2 this concept is extensively reviewed, and considerations on its application in corals are proposed. Its applicability is then empirically tested in Chapters 4, 5, 6. Discussion in regards to its applicability and utility in advance of understanding healthy coral microbiome are discussed in Chapter 7.

Objective 3: Identify and quantify natural and artificial factors generating variability in coral-associated bacterial communities.

Factors driving the variability of coral-associated bacterial communities are reviewed as part of the literature review (Chapter 2). However, in the meta-analysis the definition of healthy and its dissimilarities with dysbiotic/disturbed states of the coral microbiome are resultant from studies with different sampling and manipulation (laboratory and bioinformatics) methods. Therefore, the natural factors generating variability in coral-associated bacteria are identified across species, depth, reefs and bioregions in Chapter 4 and 5, and within individuals in Chapter 6. Artificial factors that can impact the perception of bacterial communities in corals are

evaluated in Chapter 3, with the evaluation of preservation and homogenization methods on the composition and structure of bacterial communities in corals.

Thesis outline

This thesis consists of seven chapters written in publication-format, intended for publication in peer-reviewed journals (*see* Figure 1-1 for an outline). Chapters for publication (Chapters 2 – 7) have shared authorship with three members of my committee Tracy Ainsworth (Chapters 2 – 7), William Leggat (Chapters 3 – 6) and Pim Bongaerts (Chapters 4 and 5). Tracy Ainsworth and William Leggat contributed to the development of the questions and design of the samplings, funding, training on laboratory and bioinformatics tools, analyses and interpretation of results and preparation of manuscripts for submission. Pim Bongaerts contributed to the development of the questions and design of the samplings and preparation of manuscripts for submission. Also, two chapters are co-authored with two collaborators; Ruth D. Gates contributed to the conceptualization and development of Chapter 2 and Cesar Herrera Acosta in the data analysis of Chapter 5. Data produced in this thesis have been made publically available in the National Centre for Biotechnology Information (NCBI), with project identification in the relevant chapters. Figures and tables illustrating the results are showed where relevant along the thesis. Supplementary material, principally statistical analyses, supporting results are identified and listed in the appendices. I have created all the listed figures and tables and carried out the statistical analyses unless otherwise specified.

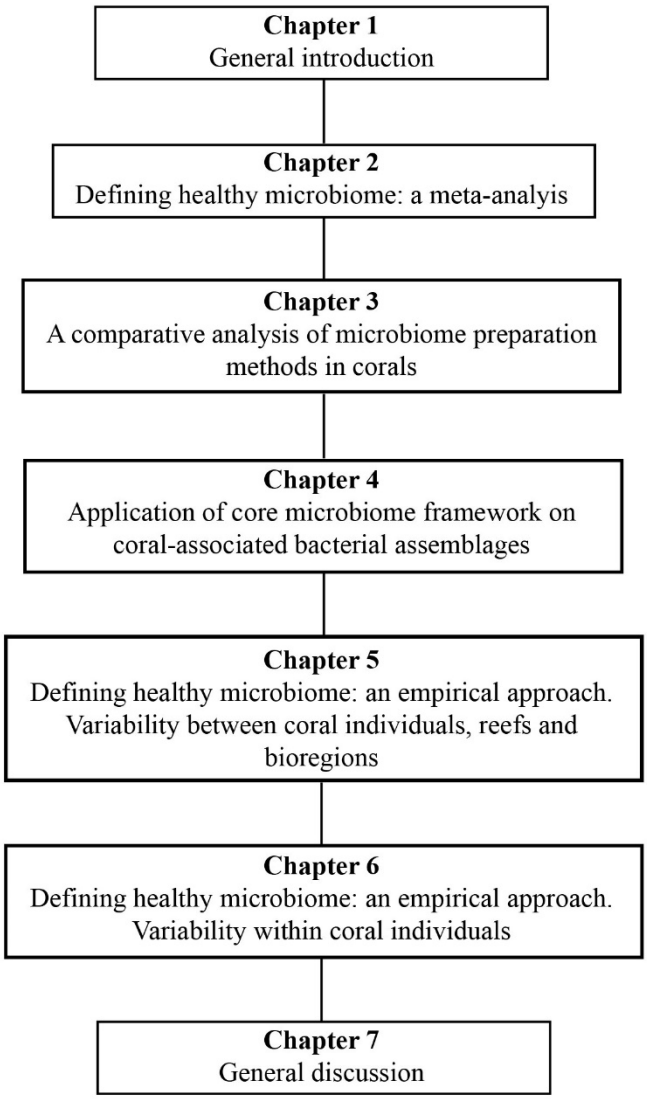


Figure 1-1: Diagram of thesis structure.

Chapter 1 (this chapter) represents the general thesis introduction, providing an initial introduction to microbial communities in corals and outlining thesis objectives.

Chapter 2 reviews the literature through a meta-analysis to describe the current definition of healthy state in bacterial communities and propose concepts may contribute to developing the concept of the healthy microbiome in corals. This chapter is published in *Trends in Microbiology* (Hernandez-Agreda, Gates et al. 2017). I conducted the literature review and wrote the chapter, and Tracy Ainsworth and Ruth D. Gates contributed to the development of proposals to impulse the research field and in the editing of the manuscript.

Chapter 3 evaluates the effect of preparation methods on the attributes of bacterial datasets and the perception of bacterial communities in healthy corals. This chapter is under review in *Frontiers in Microbiology*. I collected the coral specimens, conducted laboratory, bioinformatics and statistical analysis and wrote the paper, Tracy Ainsworth and William Leggat assisted in the editing of the manuscript.

Chapter 4 characterizes bacterial community on a healthy coral along of different spatial scales and in a depth gradient to identify relevant scales on the variability of bacterial communities. This chapter also tests the concept of core microbiome developed in Chapter 2. This chapter is published in *Mbio* (Hernandez-Agreda, Leggat et al. 2016). Pim Bongaerts collected coral specimens (as part of the XL Catlin Seaview Survey project) and assisted in the editing of the manuscript. I conducted laboratory, bioinformatics and statistical analysis and wrote the paper, Tracy Ainsworth and William Leggat assisted in the interpretation of the results and the editing of the manuscript.

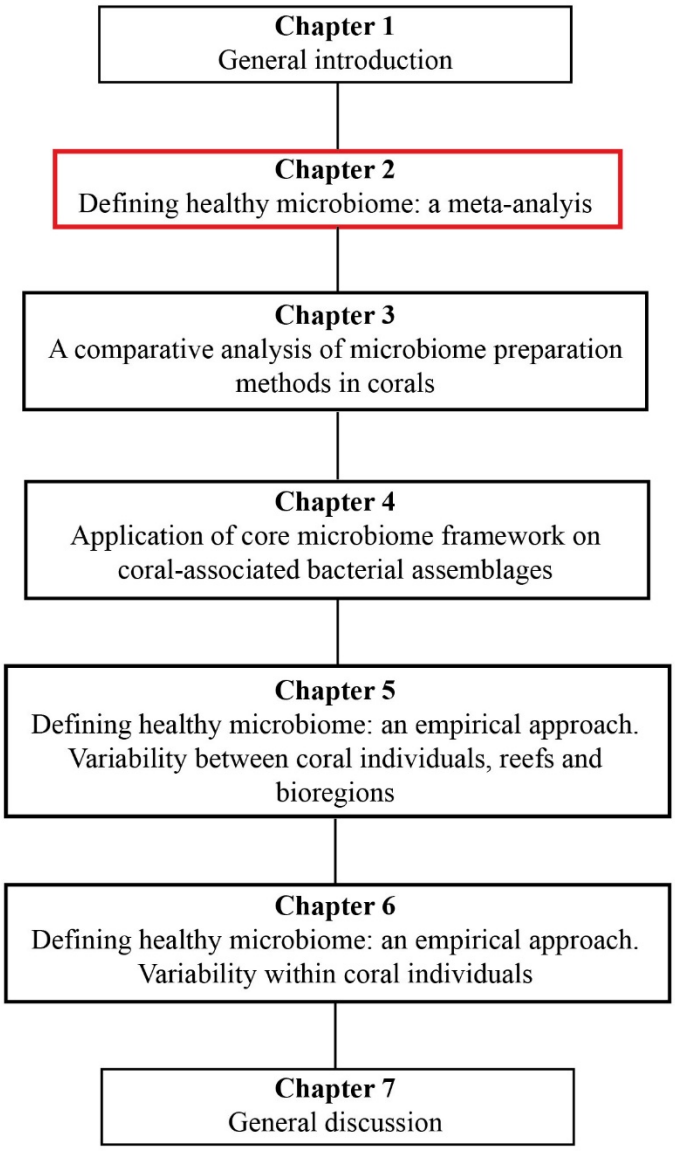
Chapter 5 tests some of the assumptions made in the literature about healthy bacterial communities in corals. Bacterial communities across coral species are analyzed in depth, using

ecological approaches proposed in Chapter 2, to identify common characteristics among coral individuals and establish attributes of bacterial communities in healthy corals. This chapter has been accepted in *mBio*. Pim Bongaerts collected coral specimens (as part of the XL Catlin Seaview Survey project) and assisted in the editing of the manuscript. I conducted laboratory, bioinformatics and statistical analysis and wrote the paper, Cesar Herrera contributed with the analysis of beta-diversity, Tracy Ainsworth and William Leggat assisted in the interpretation of the results and the editing of the manuscript.

Chapter 6 evaluates the variability of bacterial communities within microhabitats of corals. This chapter has been submitted to *Applied and Environmental Microbiology*. I collected the coral specimens, conducted laboratory and statistical analysis and wrote the paper, William Leggat ran bioinformatics analyses and assisted in the editing of the manuscript, and Tracy Ainsworth assisted in the editing of the manuscript.

Chapter 7 summarized the previous chapters, discusses the concept and characteristics of health on bacterial communities in corals and proposes priority research areas to progress in the understanding of microbes' roles in coral wellbeing. This chapter is under review in *FEMS Microbiology Letters*. I wrote the manuscript, Tracy Ainsworth contributed to the development of the approach for this chapter and its editing, and William Leggat and Pim Bongaerts assisted with the editing of the manuscript.

Chapter 2: Defining the Core Microbiome in Corals' Microbial Soup



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The coral microbiome symbioses and functional contributions of coral-associated bacteria

Multi-organism partnerships are widespread in nature and can form the basis of organism and ecosystem (*see* Glossary) success in space and time (Herre, Knowlton et al. 1999, Leigh 2010, Bordenstein and Theis 2015). Many of these symbioses have been studied in detail, and the benefits provided by the symbiont to the host have been well documented (McFall-Ngai 2008, Relman 2008). However, there are other systems in which symbiosis, particularly bacterial symbiosis, are hypothesized as an underlying mechanism of the host success, but the exact nature of the symbiosis has not, or can not yet be, determined. Corals and coral reefs are one such ecosystem where specific bacteria, and bacterial communities, are hypothesized as crucially important in both organism function and ecosystem dynamics (Graham, Ainsworth et al. 2011, Blackall, Wilson et al. 2015). However, the contributions made by specific bacterial symbionts have not yet been accurately deciphered.

The coral **microbiome** is one of the most complex microbial biospheres studied to date (Blackall, Wilson et al. 2015). Corals host thousands of bacterial **phylotypes**, in species-specific associations across broad geographical and temporal scales that have been hypothesized as functionally significant (Ritchie and Smith 1997, Rohwer, Breitbart et al. 2001, Rohwer, Seguritan et al. 2002, Morrow, Moss et al. 2012). The coral microbiome, its **composition**, spatial-temporal variability, and response to environmental change have been studied to date in over 25 coral species, from reef locations around the world, and from corals in both healthy and diseased states (Frias-Lopez, Zerkle et al. 2002, Rohwer, Seguritan et al. 2002, Bourne and Munn 2005, Ritchie 2006, Bourne, Iida et al. 2008, Thurber, Willner-Hall et al. 2009, Barott, Rodriguez-Brito et al. 2011, Ceh, van Keulen et al. 2011, Littman, Willis et al. 2011, Cardenas, Rodriguez et al. 2012, Ceh, Raina et al. 2012, Thurber, Burkepile et al.

2012, Wilson, Aeby et al. 2012) (Supplementary Table B-1). As in other systems, advances in molecular technologies have unveiled the richness and composition of bacterial communities of the coral host, and high throughput sequencing is now universally applied to evaluate bacterial diversity (Claesson, Wang et al. 2010, Wu, Lewis et al. 2010). This methodology provides a representation of the bacterial **community**, identifies **rare** and less abundant species and provides insight into the conservation of bacterial phylotypes within **microhabitats** and between individual hosts (Pedros-Alio 2006, Sogin, Morrison et al. 2006, Sunagawa, Woodley et al. 2010). An average of 995 distinct bacterial **operational taxonomic units (OTUs)** and 22,520 sequences were identified from each coral species when sequencing technologies were first applied to the coral microbiome (Supplementary Table B-1). These estimates of microbiome complexity have recently increased substantially as higher sequencing coverage has been obtained. Now upwards of 100,000 bacterial OTUs and millions of reads are reported from each coral species (Zhang, Ling et al. 2015, Hernandez-Agreda, Leggat et al. 2016, Meyer, Rodgers et al. 2016). In general, the dominant associations that have been identified in these studies are assumed as the healthy symbiotic state of the coral-associated bacterial communities (Littman, Willis et al. 2011, Cardenas, Rodriguez et al. 2012, Croquer, Bastidas et al. 2013, Lee, Davy et al. 2015).

Gamma- and Alphaproteobacteria dominate the bacterial communities of corals, other highly abundant bacteria include members of the phyla Bacteriodetes, Firmicutes, Actinobacteria and Cyanobacteria. *Endozoicomonas* are generally the highest **abundance** genera in the coral microbiome (Blackall, Wilson et al. 2015) (*see* citations in Supplementary Table B-1). The consistency of dominant associations between individuals, within and between coral species, across spatio-temporal scales, and depth gradients, have only recently been investigated. In fact, one of the most significant findings that have arisen from the recent deep sequencing of the coral microbiome is the substantial variability that is evident between individuals, species,

and reef habitats. The most revealing of which is the variability that occurs between individuals (Ainsworth, Krause et al. 2015, Hester, Barott et al. 2015, Hernandez-Agreda, Leggat et al. 2016). For example, studies investigating ubiquitous associations across individuals find that over 60% of the identified bacterial OTUs of the coral microbiome are present in less than 10% of individuals studied, many of which include some of the most highly abundant bacteria within a single individual (Ainsworth, Krause et al. 2015, Hernandez-Agreda, Leggat et al. 2016). There is also substantial variation in the occurrence (and persistence) of some of the most abundant members of the microbiome. For example *Endozoicomonas* are the most abundant group in the coral microbiome but are also highly variable within and between coral species (Bayer, Neave et al. 2013, Meyer, Paul et al. 2014, Morrow, Bourne et al. 2014) (*see* review (Blackall, Wilson et al. 2015)). Williams, Brown et al. (2015) have proposed that the variability between individual seen in corals be correlated to the age of the coral colony. Thus, like other systems studied, the coral microbiome is likely to be highly variable until the colony reaches adulthood, at which time the microbiome stabilizes as the adult microbial signature (Williams, Brown et al. 2015). Studies of coral reproduction have also revealed that bacterial colonization in corals can occur principally by horizontal uptake (Apprill, Marlow et al. 2009, Littman, Willis et al. 2009, Lema, Bourne et al. 2014) and occasionally by vertical transmission (Sharp, Distel et al. 2012). While Neave, Rachmawati et al. (2016) suggest that substantial variation in microbiome structure, including *Endozoicomonas,* could be linked to reproductive strategies of the coral host (i.e. brooding and broadcast spawning corals). The seasonal variability in the microbiome indicates that abiotic factors strongly influenced these associations, and correlations with carbon availability suggest a strong influence of the by-products of photosynthesis from the corals' symbiotic eukaryotes (*Symbiodinium* spp.) on **community structure** (Carlos, Torres et al. 2013, Kimes, Johnson et al. 2013, Lema, Willis et al. 2014, Li, Chen et al. 2014, Williams, Brown et al. 2015). There are in fact many factors of the coral

animal and the coral reef **habitat** that are likely to influence microbial diversity, abundance, occurrence and persistence (Figure 2-1). The bacterial community of corals is responsive to environmental variables, biological events and also to factors that result in stress response in the host (Figure 2-2, Supplementary Table B-1) (*see* review (Schwartzman and Ruby 2016)). Stress in corals manifests in response to variations in sea surface temperature, salinity, nutrients, pH, loading of dissolved organic carbon, disease and competition for space. All of these factors also have the potential to impact bacterial groups, OTU abundance, and may also result in the disappearance, or appearance, of some bacteria. Biotic and abiotic factors also influence activation and expression of genes related to virulence and secondary metabolites in the metagenome (Dinsdale, Edwards et al. 2008, Thurber, Willner-Hall et al. 2009).

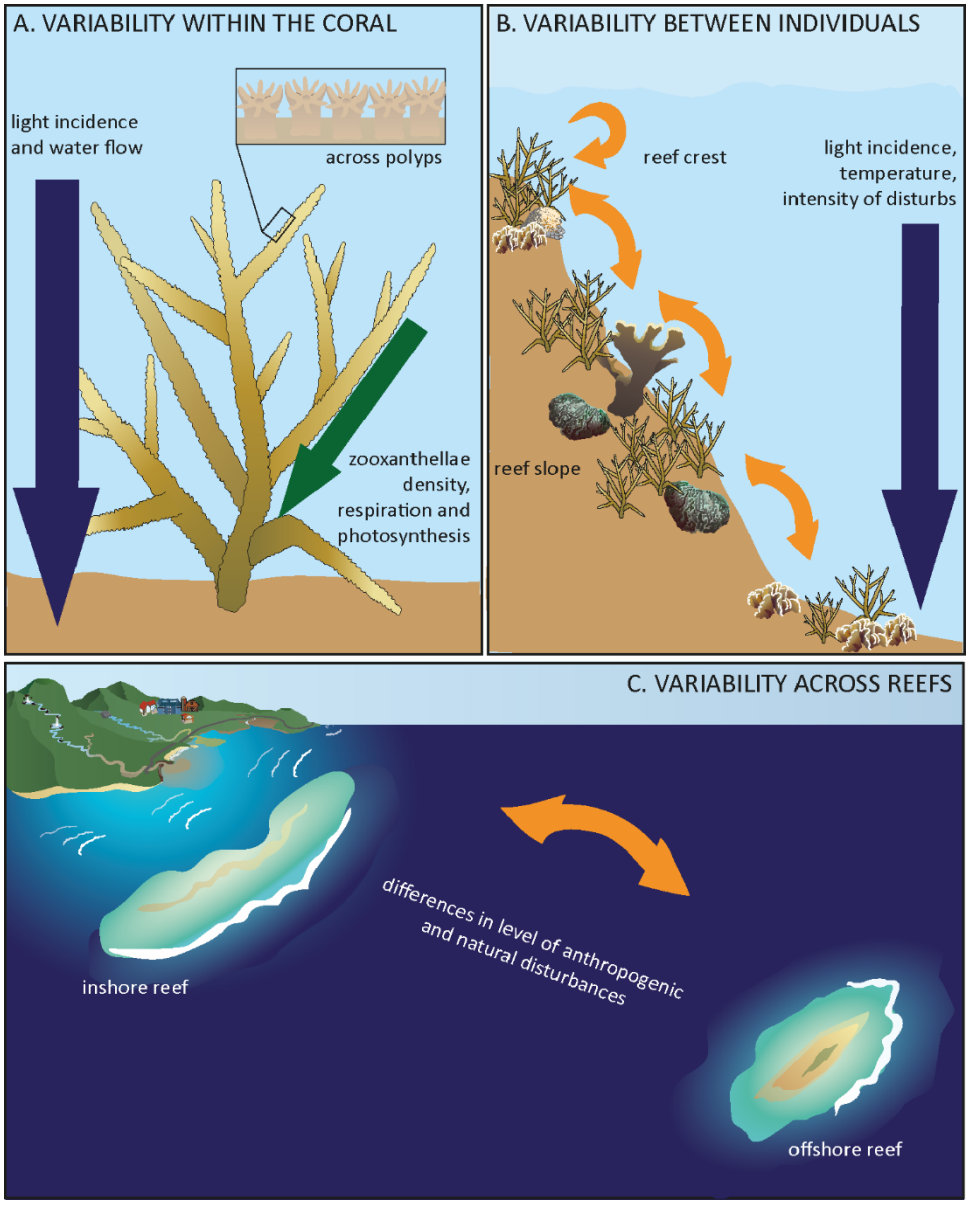


Figure 2-1: Complexity of coral host habitat and coral reef environment. Environmental and biological factors can generate different habitats conditions A) within coral colonies, B) between coral colonies and C) across reefs. This high variability at different spatial scales converts corals in a complex host, where adequate hypothesis, robust replication and the development of microbial ecology concepts considering this complexity become essential to define and understand bacterial symbioses.

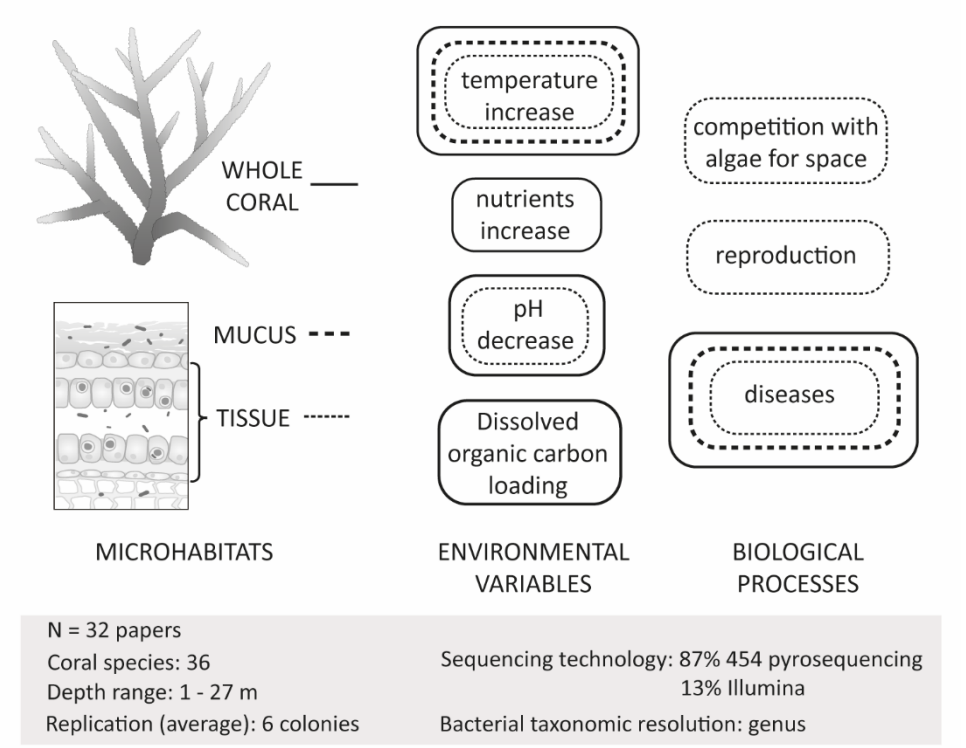


Figure 2-2: Bacterial community in corals is responsive to environmental factors and biological events. Meta-analysis results evidence that in shallow corals bacterial community composition and abundance within different microhabitats shifts in response to changes in environmental factors and response to biological processes (*see* details in Supplementary Table B-1).

Bacterial communities are proposed to have important critical contributions to the health, nutrition and nutrient cycling in the coral host (Ritchie 2006, Lesser, Falcon et al. 2007, Raina, Tapiolas et al. 2009, Lema, Willis et al. 2012, Peixoto, Rosado et al. 2017). Bacteria in the coral's surface mucus layer are hypothesized as important in the provision of antibiotic activity and likely provide population control, preventing pathogen colonization and invasion (Ritchie 2006). **Diazotrophic bacteria** have also been consistently found within bacteria community of the coral tissue, skeleton and mucus (within the family Cyanobacterium and order Rhizobiales) (Shashar, Cohen et al. 1994, Rohwer, Seguritan et al. 2002, Lesser, Mazel et al. 2004, Lesser, Falcon et al. 2007, Lema, Willis et al. 2012). The ubiquitous nature of diazotrophic bacteria in newly released larvae and juvenile corals (Lema, Bourne et al. 2014) and at different seasons and locations (Lema, Willis et al. 2014) indicates the potential

importance of nitrogen-fixing bacteria in the coral **holobiont** at all life history stages. The degradation of the organic compound dimethylsulfoniopropionate (DMSP) and its products dimethylsulfide (DMS) and acrylic acid are also carried out by bacteria (Curson, Rogers et al. 2008). Genera of coral-associated bacteria *Spongiobacter*, *Pseudomonas*, *Roseobacter*, and *Vibrio* spp. can metabolize DMSP, DMS and acrylic acid (Raina, Tapiolas et al. 2009), and provide strong evidence for the role of coral-associated bacteria in sulfur cycling. However, differentiating the functionally important bacteria associated with corals has been limited due to the complexity of both the coral host habitat and the coral reef environment (Figure 2-1). The microhabitat association of only a few coral-associated bacteria has been determined. Bacterial aggregations, dominated by members of γ-proteobacteria, have been found within coral epidermal cell layer (Ainsworth, Fine et al. 2006, van de Water, Ainsworth et al. 2015), and a higher abundance of aggregates has been found in corals held in captive conditions (Ainsworth and Hoegh-Guldberg 2009). *Endozoicomonas* aggregations have also been localized within both of the coral cell layers (Neave, Rachmawati et al. 2016) and members of the phylum *Actinobacteria* and *Ralstonia sp.* have also been found within the cellular space of coral that is inhabited by the endosymbiotic dinoflagellate (peri-algal space) (Ainsworth, Krause et al. 2015). Members of the Alpha-, Gammaproteobacteria, Cyanobacteria, Flavobacteria, and Firmicutes have been isolated from the coral gastric cavity (Agostini, Suzuki et al. 2012). Localization of specific bacteria within cell layers and habitats may suggest that some of these associations play an essential role in the coral nutrient uptake.

Research into the coral microbiome is however still in its infancy. Research has yet to determine the stability of the vast majority of bacterial associations of corals and the influence of factors including feeding strategy (autotrophy and heterotrophy), feeding time, growth stage, immune status, and patterns of microbial succession remain unresolved. The complexity of the reef habitat, the coral organism, and a diversity of life history traits are all likely to influence

the microbiome and need to be considered when differentiating microbial states as symbiotic, **mutualistic**, **commensal**, parasitic, pathogenic, or dysbiotic. Overcoming the constraints that are inherent in highly complex environmental systems, such as coral reefs, and differentiating potential symbioses will, therefore, be reliant on utilizing the theoretical and technical advances made in more extensively studied model systems. In this review, I examine how the core microbiome concept in conjunction with community ecology principals is a framework that can be applied to facilitate identifying potentially important microbes in corals.

The coral microbiome: bacterial habitats

In corals, bacteria have been reported inhabiting three unique microbial habitats (Figure 2-3); the surface mucus layer, the symbiosome space within the coral tissue, and the exposed skeleton (Bourne and Munn 2005, Koren and Rosenberg 2006, Ritchie 2006, Sweet, Croquer et al. 2011).

The surface mucus layer (SML, Figure 2-3B1) is an interface zone between the surrounding seawater and the coral epithelium and plays an essential role in heterotrophic feeding, sediment clearing, defense against pathogens, and protection during environmental stresses. The microbial community within this zone is likely to be a highly structured biofilm, exposed to substantial biotic and abiotic variation. Host mucus secretion and the microbial biofilm present in this layer are released to seawater and drive microbial turnover, biofilm succession and composition of the coral mucus-associated community (Bythell and Wild 2011). The nutrient composition, oxygen levels, pH and rate of production of the surface mucus layer vary across the day, between coral species, depth and as a response to environmental changes (e.g. sedimentation, radiation). All these characteristics result in a highly variable microbial habitat within which the bacterial community is likely also to be extremely dynamic (Sweet, Croquer et al. 2011). In similar, highly variable environments of benthic microbial mats fluctuations in

environmental conditions generate a physicochemical gradient of various micro-niches available for colonization (Bolhuis, Cretoiu et al. 2014). However, it is still poorly understood how the changes in mucus chemistry, mucus release, and colonization during regular sedimentation on corals regulate microbial population dynamics on the surface microbial biofilm.

The term coral tissue has been used to group the tissues layers of the coral, namely the epithelium, mesoglea, gastroderm and calicoblastic epithelium (Figure 2-3B). The algal endosymbiont *Symbiodinium* sp. resides within the gastrodermal layer, within a single gastrodermal cell where each algal cell is surrounded by the host-derived symbiosome membrane (Yellowlees, Rees et al. 2008). The pocket created by this membrane within the coral cell is referred to as the peri-algal space, and is an interface zone in which nutrient and metabolite transfer between the algal symbionts and the host cell occurs (Kazandjian, Shepherd et al. 2008). Difficulties in isolating this space have however interfered with detailed characterization of the specific conditions that are unique to this microhabitat. Using fluorescence *in situ* hybridization, bacteria from genus *Propionibacterium sp*. and *Ralstonia sp.* have been recently reported as potential universal coral symbionts inhabiting the cellular space of coral dinoflagellate in a high relative abundance (Ainsworth, Krause et al. 2015). Gammaproteobacteria and specifically *Endozoicomonas* have also been localized to the both the epidermal (outer) and gastrodermal (inner) tissue layers (Ainsworth, Fine et al. 2006, van de Water, Ainsworth et al. 2015, Neave, Rachmawati et al. 2016). Their localization suggests bacteria are likely to play an essential role in corals or coral nutrient uptake, but also raises questions such as, how bacteria can evade the coral immune systems and inhabit this space, what is the role of the dinoflagellate in the association, and how stable is this relationship.

The coral skeleton (Figure 2-3B2) is a microbial habitat that is also exposed to the surrounding seawater but unlike the SML is protected from wave and water action, sedimentation, and light penetration (due to the overlaying algal rich coral tissues), and as a result this region is environmentally stable (Shashar, Banaszak et al. 1997). Research in the microbial community in this zone (the **endolithic** community) has focused on documenting the micro-algae and fungi presence (Le Campion-Alsumard, Golubic et al. 1995, Le Campion-Alsumard, Golubic et al. 1995, Fine and Loya 2002, Rädecker, Pogoreutz et al. 2015). The microalgae within the endolithic layer are proposed to provide alternative energy supply to the coral host and the community rapidly blooms following changes to the overlaying coral tissues that allow increase light to penetrate into the skeleton (Rädecker, Pogoreutz et al. 2015). The endolithic layer hosts an abundant bacterial community, which like the algal community blooms following changes to the overlying coral tissues (Rosenberg, Koren et al. 2007, Rädecker, Pogoreutz et al. 2015).

The microbial habitats of the coral tissue and skeleton structure, therefore, provide distinct and dynamic environments within which bacterial communities can form, and in doing so potentially both provide benefit and a source of disease, to the coral host.

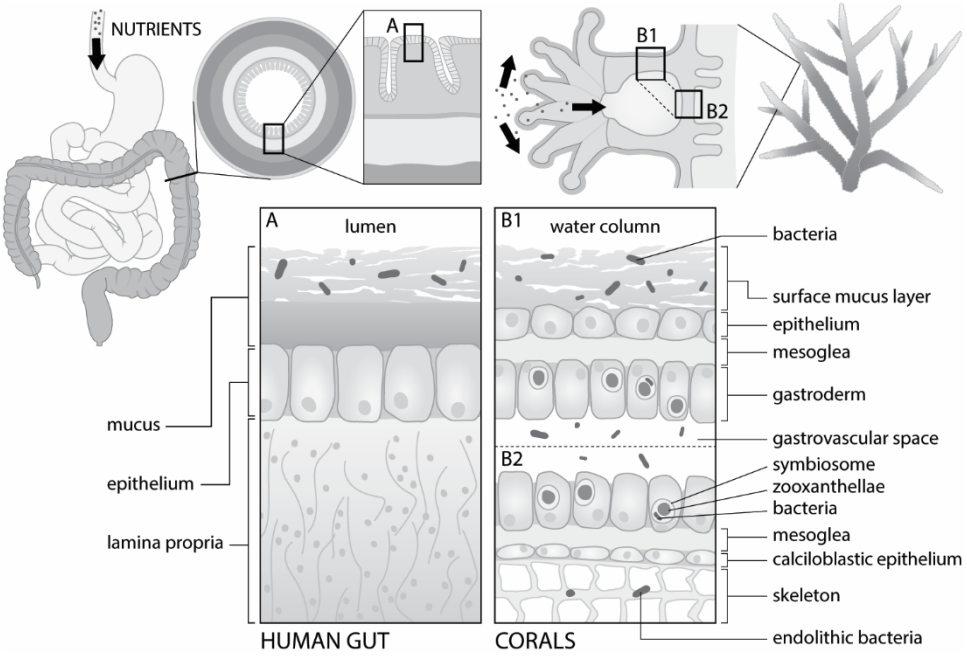


Figure 2-3: Microhabitat similarities between the human gut and corals. Axial view of microhabitats in A) human large intestine, and B) corals [tissue of walls (B1) and base (B2) of polyps]. The human gut and corals have structural similarities in their microhabitats distribution and characteristics. Knowledge about symbiosis and mechanisms driving microbial colonization and dynamics have significantly advanced in humans; and based on this similarity between systems, corals can follow some of those advances done in the human gut system to accelerate the understanding of coral-bacterial symbiosis.

Human gut and corals: similar systems, different drivers

The similarities between corals' and humans' gut microbial systems open a path to understanding corals' microbial symbiosis based on the progress of humans' gut as a model system. Composition and abundance of the bacterial community in both, human gut and corals, vary longitudinal and axially (Ainsworth, Thurber et al. 2010, Belizario and Napolitano 2015), although the factors promoting those variations are different. For example in the human gut, pH changes from the esophagus (pH <4.0) through the stomach (pH 2) to the small intestine (pH 5-7), representing a variety of habitats with different bacterial composition (Jandhyala, Talukdar et al. 2015). Likewise, due to the structural complexity of diverse morphologies of corals (branching, plating and laminar and massive/encrusting), factors as temperature, light, water flow and zooxanthellae density vary significantly within colonies, generating many

microhabitats and harboring different microbial communities (Figure 2-1A,B) (Ainsworth, Thurber et al. 2010). In an axial vision, the tissues in both human gut and corals are structurally similar: a mucus layer as an external barrier protecting epithelial cells (Figure 2-3) (Ritchie 2006, Bythell and Wild 2011, Tuddenham and Sears 2015). However, in the human colon, mucus has two homogeneous layers: an external and dynamic one, low adherent with bacteria living on it; and an internal one, close to the epithelium, denser and devoid of bacteria (Tuddenham and Sears 2015) (Figure 2-3A). Corals also possess a homogeneous mucus layer across colony surface; nonetheless, it is inhabited by a highly abundant bacteria community (Ritchie 2006, Bythell and Wild 2011) (Figure 2-3B1). Unlike these similarities, corals have many differences with the human gut system; the principal is that corals are an open system, where the host and the microbial community are vulnerable and responsive to changes in host environment (e.g. temperature, pH, salinity, between others) and biological processes (e.g. reproduction and competition). Whereas human gut is a closed system, where changes in microbial community occur in response to diet or antibiotics (Backhed, Fraser et al. 2012).

The core microbiome framework

The core microbiome framework aims to identify potentially crucial microbes within microbial communities based on the persistence of the microbe within the host, and within a niche across spatio-temporal boundaries. Research into the identity and functional contribution of core microbes, and a core microbiome was first applied to understanding the bacterial communities that are associated with humans (Fierer, Hamady et al. 2008, Turnbaugh and Gordon 2009, Turnbaugh, Hamady et al. 2009, Qin, Li et al. 2010). The Human Microbiome Project (HMP) aimed at understanding the role of microbes in human health, and the factors influencing microbial distribution, ecology and evolution. This research explored the bacterial community

of the human gut, unveiling both persistent bacterial functional roles and a highly variable bacteria community (Turnbaugh, Ley et al. 2007, Turnbaugh, Hamady et al. 2009). The core microbiome was identified as group of consistent functional microbial genes (Turnbaugh and Gordon 2009, Turnbaugh, Hamady et al. 2009), and both the core and variable bacterial community members were found to be influenced by the diet, genotype and developmental stage (Turnbaugh, Hamady et al. 2009, Spor, Koren et al. 2011, Tims, Zoetendal et al. 2011). One example of a bacterium persistently found across individuals is *Faecalibacterium prausnitzii* (Qin, Li et al. 2010). This bacterium contributes to human health through the production of butyrate, a short-chain fatty acid that acts in a regulatory role in colon walls, as a defense barrier enhancer, an intestinal motility modulator and provides anti-inflammatory action (Canani, Costanzo et al. 2011, Miquel, Martín et al. 2013, Miquel, Leclerc et al. 2015, Quevrain, Maubert et al. 2016).

Researchers have since adopted the core microbiome concept across microbial systems (Table 2-1). Differentiating the core microbiome from the broader microbial community has been applied to understanding the microbial role in host organisms, including plants and sponges, and in ecosystems including soils, beaches and oceans (Table 2-1) (Turnbaugh, Hamady et al. 2009, Lundberg, Lebeis et al. 2012, Schmitt, Tsai et al. 2012, Newton, Huse et al. 2013, Vik, Logares et al. 2013, Kembel, O'Connor et al. 2014, Shade, Jones et al. 2014, Staley, Gould et al. 2014). As such a core microbiome is defined as the group of microbes commonly found within a host's microbiome, using persistence of the association as the criterion to select microbes potentially providing a critical function within the habitat in which they are found.

Table 2-1: Comparison of the Application of the Core Microbiome Approach

Subject/ Species/Habitat	N. of total OTUs	N. of OTUs in core microbiome	Core taxonomic level	% cut-off[a]	Justification	Sequencing platform	Core phylotypes with functional role (examples)	Reference
Human								
Gut	-	a set of microbial functional genes	-	100	Core defined as shared microbial functional genes	454	-	(Turnbaugh, Hamady et al. 2009)
Gut	3.3 million microbial genes, ≈ 1,000 bacteria species	75, 57 and 18	Species, strains	50, 90 and 100	Arbitrary	Illumina	*F. prausnitzii* (Canani, Costanzo et al. 2011, Miquel, Martín et al. 2013, Miquel, Leclerc et al. 2015, Quevrain, Maubert et al. 2016), *Bacteroides uniformis* (Renouf and Hendrich 2011, Li, Li et al. 2014), *Ruminococcus bromii* (Ze, Duncan et al. 2012), *Bacteroides thetaiotaomicron* (Cameron, Maynard et al. 2012, Varyukhina, Freitas et al. 2012)	(Qin, Li et al. 2010)
Hands	4,742	5	Genus	100		454		(Fierer, Hamady et al. 2008)
Plants								
Arabidopsis thaliana[b]	18,783[c]	97	Family	-	Based on OTU abundances analysis, GLMM	454		(Lundberg, Lebeis et al. 2012)

Subject/ Species/Habitat	N. of total OTUs	N. of OTUs in core microbiome	Core taxonomic level	% cut-off[a]	Justification	Sequencing platform	Core phylotypes with functional role (examples)	Reference
A. thaliana and three relatived species[b]	88,731	9	Genus	-	Based on OTU abundances, as the intersection of enriched bacteria determined by three statistical analyses	454		(Schlaeppi, Dombrowski et al. 2014)
57 tree species, leaves	7,293	104	Family or Genus	95	Arbitrary	Illumina		(Kembel, O'Connor et al. 2014)
Sponges								
32 sponges species	2,567	3	Phylum or Class	70	Arbitrary, based on presence in the majority of sponges species	454		(Schmitt, Tsai et al. 2012)
Corals								
Acropora granulosa	1,508	7 universal 149	Kingdom to Genus	30	Lowest percentage at which core OTU abundance is stable across core microbiomes	454		(Ainsworth, Krause et al. 2015)
Leptoseris spp.	1,424	204						(Ainsworth, Krause et al. 2015)
Montipora capitata	1,433	350						(Ainsworth, Krause et al. 2015)
Pachyseris speciosa	173,690	8	Kingdom to Genus	80	Arbitrary, core phylotypes were present in all spatial scales and depth considered	Illumina		(Hernandez-Agreda, Leggat et al. 2016)

Subject/ Species/Habitat	N. of total OTUs	N. of OTUs in core microbiome	Core taxonomic level	% cut-off[a]	Justification	Sequencing platform	Core phylotypes with functional role (examples)	Reference
Stylophora pistillata	560[c,d,e]	1	Genus (*Endozoicomonas*)	79	This study was focused in evaluating *Endozoicomonas* prevalence across different geographic regions.	Illumina		(Neave, Rachmawati et al. 2016)
Pocillopora verrucosa	655 [c,d,e]	1		85				(Neave, Rachmawati et al. 2016)
Antillogorgia elisabethae	502 and 281[c,e,f]	27 and 48[f,g]	Kingdom to Genus	50	Arbitrary	454		(Robertson, Haltli et al. 2016)
Anthothela grandiflora	55 [c,e]	1	Genus	100		454		(Lawler, Kellogg et al. 2016)
Anthothela sp.	110 [c,e]	7	Phylum to Genus					(Lawler, Kellogg et al. 2016)
Corallium rubrum	250 [c,e]	12	Phylum to Family	100		Illumina		(van de Water, Melkonian et al. 2016)
Acropora cervicornis	87,668[c] (all coral species together)	6 universal 12	Phylum to Genus	50	Arbitrary	Illumina		(Chu and Vollmer 2016)
Acropora palmata		15						(Chu and Vollmer 2016)
Diploria labyrinthiformis		11						(Chu and Vollmer 2016)

Subject/ Species/Habitat	N. of total OTUs	N. of OTUs in core microbiome	Core taxonomic level	% cut-off[a]	Justification	Sequencing platform	Core phylotypes with functional role (examples)	Reference
Diploria strigosa		11						(Chu and Vollmer 2016)
Porites astreoides		13						(Chu and Vollmer 2016)
Porites furcata		14						(Chu and Vollmer 2016)
Beaches								
Sand	23,670	26	Family, Genus	75	Arbitrary	454		(Newton, Huse et al. 2013)
Water	19,411	62						(Newton, Huse et al. 2013)

[a] Minimal percentage of individuals (microbiomes) where bacteria or microbial genes have to be present to be considered as part of the core microbiome.
[b] Root system.
[c] Rarefied or subsampled data.
[d] Based on the average of the three methods applied to assign OTUs.
[e] Average.
[f] Based on the amplified region (V1/V2 and V4).
[g] OTUs present in water column in higher abundance that in the octocoral were not considered in core microbiome.

The coral core microbiome

The core microbiome framework is still a young concept for corals. The core microbiome has been explored in 16 coral species, across ocean depths and geographically separated locations. Core annotations have ranged from 30% of persistence with studies using the 454-sequencing platform, to 100% using the Illumina sequencing platform (Table 2-1). Ainsworth, Krause et al. (2015) used a 30% core described between 149 to 350 phylotypes per coral species, and 7 shared phylotypes among coral species. Hernandez-Agreda, Leggat et al. (2016) identified 8 persistent phylotypes at different spatial scales and depth gradients using core 80% (Illumina). van de Water, Melkonian et al. (2016) found 12 bacterial species using a 100% core in 23 individuals (Illumina) collected from five disparate reef sites over a 3-month period. The core microbiome has also been investigated in one octocoral host (454 platform) finding 27 OTUs at 50% persistence across 17 individuals (Robertson, Haltli et al. 2016). In the cold-water octocoral *Anthothela grandiflora* only one OTU was evident in a 100% core microbiome in 12 individuals (454 platform), further study at the genus level found between 7 OTUs in the core microbiome (Lawler, Kellogg et al. 2016). Chu and Vollmer (2016) undertook a spatio-temporal study of the core microbiome in 100 tagged individuals of six coral species (collected three times during one year period from four reef habitats) identifying between 11 and 15 members of a 50% core microbiome (Illumina). These studies identify several crucial factors that need to be considered when annotating core microbiomes, using core microbiome analysis for identifying potential symbioses and comparing core annotations between studies. Confounding factors include the criteria used for clustering of samples (i.e. the host taxonomic level, reef site, reef depth, and time and season of sampling) and the bacterial taxonomic level used for annotations (Table 2-1). Robertson, Haltli et al. (2016) also show that the targeted region of the 16S rRNA gene, finding that targeting V1/V2 and V4 regions yielded differences

in the number of OTUs annotated as core associations (Table 2-1). Furthermore Ainsworth, Krause et al. (2015) show that a whole colony community approach (i.e. homogenized intact host samples) does not reflect bacterial associations with the corals' microhabitats (polyp tissue, skeleton and gastrodermis). This study showed that the core microbiome within in each microhabitat differs despite phylotypes being common across coral species.

The criteria that have been used to date to define the persistence of bacterial species within a coral population (core microbiome) have been arbitrary. However attributing significance to members of the microbiome solely through high or low relative abundance within a given study while ignoring persistence, is also an arbitrary process that can be heavily impacted by technical and biological factors (Supplementary Table B-1). Doing so overlooks key information about the microbiome that exists within the data. Hernandez-Agreda, Leggat et al. (2016) suggest that the coral microbiome should be conceptualized as three main components; (i) a ubiquitous core microbiome; (ii) a dynamic site and/or species-specific community; and (iii) a highly variable community reflective of the biotic and abiotic fluctuations. For each of the coral systems that have been investigated to date for the presence of a core microbiome, features such as reef location, reef habitat and patchiness of microbial habitat within the host morphology have been overlooked. In differentiating core microbiome and potential symbioses within highly dynamic and complex systems, such as corals, it is necessary to account for the unique challenges of those systems. The core microbiome framework provides a means by which to identify and analyze potentially important bacteria and bacterial groups (also defined as Beneficial Microorganisms for corals, BMC (Peixoto, Rosado et al. 2017)) within the coral microbial soup, based on their ubiquitous association within a coral group, reef habitat, coral species or within a microbial niche.

Technical considerations and theoretical frameworks for applying core microbiome framework.

The criteria used to define and annotate a core microbiome across different systems have traditionally been arbitrary, and there has not been a consensus reached in any of the organisms in which the concept has been applied (Table 2-1). Both technical considerations and the theoretical framework need to be considered in developing and applying the core microbiome framework and comparing core microbiomes between studies. These factors include the study design, target habitat and sample size, sequencing approach, replication and effort, and the analysis tool applied. Addressing these knowledge gaps will be a crucial step in reliably applying mechanistic models and interpreting patterns that are observed in nature.

Technical considerations

Within the application of a community framework, there are several levels of information that are commonly reported in the study of microbiomes and should be considered in determining criteria for the core microbiome and when comparing studies (Hamady and Knight 2009). These factors include:

(i) Replication (number of samples),

(ii) Sampling effort (sequencing and sequences analyses),

(iii) Community membership, and

(iv) Study design.

(i) Replication: A crucial factor in defining the core microbiome is the number and quality of the replicates. Replicates need to be random and independent (Hurlbert 1984); for example, to

be considered individuals and different biological units coral colonies should be similarly sized, disparate, and randomly selected for similar reef environments. Samples from colonies should also be collected at similar locations within the coral colony, targeting the same macro and micro colony structures (Figure 2-1). In environmental scenarios, standardizing the number of samples required can also be challenging due to logistical considerations such as remoteness, field time, collection logistics and equipment needs. However, both a priori and post hoc power analysis tests are alternative strategies for investigating adequate replication in these scenarios (Faul, Erdfelder et al. 2007, Johnson, Barry et al. 2015). For example, post hoc power analyses are informative about experimental design where no statistical differences have been detected, and a small number of replicates have been used (probability of a false negative, type II error, e.g. (Montilla, Ramos et al. 2016)); whereas a priori power analysis are useful in defining the sample size (number of replicates, sampling effort) necessary to detect an effect or statistical difference (e.g. (Harasti, Malcolm et al. 2015)).

(ii) Sampling effort: In microbial ecology, alpha diversity (number of taxa per samples) and beta diversity (turnover of taxa between samples expressed as pairwise sample dissimilarity) indexes are widely used to represent and analyze community richness (with the assumption that OTUs are reflective of taxon (Mihaljevic 2012, Shade and Handelsman 2012)). However, the weaknesses of alpha and beta diversity measures identified in macro-ecology are also applicable to micro-ecology (e.g. (Tóthmérész 1995, Koleff, Gaston et al. 2003, Legendre, Borcard et al. 2005, Bennett and Gilbert 2016)) and apply to the core microbiome framework. The sensitivity of sample size and resolution is one of the most relevant of these. Factors such as marker, primer and sequencing region chosen, sequencing depth selected (i.e. number of sequences per sample relates to sampling effort), alignment and clustering algorithms used to assign OTUs (e.g. nearest vs. furthest neighbor) (Hamady and Knight 2009, Shade and Handelsman 2012, McMurdie and Holmes 2014, Schlaeppi, Dombrowski et al. 2014, Lynch

and Neufeld 2015) affect the number of OTUs generated in sequencing-based estimates of community composition. Sequencing technologies have also rapidly improved the depth of sampling (effort) within micro-ecological studies, and as a result, the characterization of bacterial communities and identification of previously undetectable bacteria continues to expand (Shendure and Ji 2008, Luo, Tsementzi et al. 2012). For example, deeper sequencing has allowed for the detection of low abundance bacteria, rare bacteria, and bacteria previously thought to have limited range. For example, bacteria reported as "sponge-specific" (Taylor, Radax et al. 2007, Schmitt, Tsai et al. 2012) are now also detected in low abundance within the water column (Taylor, Tsai et al. 2013), giving insight into the potential source of bacterial colonization, bacterial mobility and sources/sinks of symbiotic bacteria. These advances in sampling effort within samples, habitats, host systems and ecosystems are rapidly changing our understanding of bacterial persistence and associations in nature. In addition, the Abundance-Ubiquity (AU) Test determines the probability that a bacterial phylotype is present but not sampled within a dataset; the authors show that for some, but not all, bacteria there is a strong correlation between abundance and occurrence (Hester, Barott et al. 2015).

(iii) Community membership: Analyses of membership (sample occurrence frequency and composition) are based on presence/absence data and are used to detect taxa present in one or more microbiomes (Shade and Handelsman 2012). However, a reliance on membership analysis presents two clear challenges. Firstly, membership analysis does not consider bacterial abundances, and secondly, the criteria to define persistent bacteria can be arbitrary within a biological and ecological context (Shade and Handelsman 2012) (Table 2-1). Persistence refers to the percentage of samples where a phylotype is present (i.e. considered constantly found). A threshold for persistence can be either fixed to a determined (abrupt) change in species richness across samples, or arbitrary, depending on the authors' definition of evidence for persistence (e.g. >50% of samples). Persistence can also be defined (and influenced) by

boundaries within a biologically or ecologically relevant time scale; within bio-geographical regions; and within the microbial topographical landscape (Shade and Handelsman 2012). This impact of both space and time on persistence, and as such the designation of microbes as members of core microbiome, has not been well explored in any systems studied to date. For example studies of the human gut microbiota over a 2.5 to 5 year time period have revealed dynamic states of health and variations in both composition and richness related to niche microbial diversity (Koenig, Spor et al. 2011, Spor, Koren et al. 2011).

(iv) Study design: There are several important reasons why a consensus has not been reached in any system but an essential limiting factor is study design. It is crucial to consider the number of replicates of the lower level of the sampling design in conjunction with the sequencing method applied as a starting point in selecting the core percentage. For example, in a mensurative study evaluating bacterial community associated to X organism variability across spatial scales, a hierarchal design (with two regions and five locations per region) and a replication of 10 organisms per location would result in 100 total individuals sampled. Thus for an OTU being excluded in a complete location (the lower level of this design) it has to be absent in 10 organisms, which means the OTU must be present in at least 90% of the samples. Furthermore, different deep sequencing methods impact the detection number of bacterial phylotypes (Shendure and Ji 2008, Luo, Tsementzi et al. 2012). Hence, it is expected that results of deeper sequencing (higher effort) will give the most accurate representation of bacterial communities (or at least closer to real bacterial composition) (Luo, Tsementzi et al. 2012), which should be considered when assessing bacterial occurrence and core assignation. For example, it is likely to be accurate to define a core microbiome around 90% from a study using an Illumina sequencing platform, whereas when sequencing with a 454 sequencing platform, a more conservative percentage (e.g. 50% to 80%) may be necessary in defining core

microbiome to avoid the impact of sampling effort particularly where there is low replication within the lower level of the study design.

Theoretical framework

Theoretical approaches that are common to macro-ecology and community theory can inform complex microbial community ecology, the search for potential symbioses, and the application of the core microbiome concept (Mihaljevic 2012). Two approaches are used to investigate ecological patterns, a mechanism-based approach and a pattern-based approach. A mechanism-based approach aims to understand the distribution in local habitats of a regional pool of species. Whereas a pattern-based approach examines the structure of natural communities and how environmental gradients generate these structures at different spatial scales (*see* recent reviews in (Logue, Mouquet et al. 2011, Mihaljevic 2012)). Each of these approaches can provide information into micro-ecological patterns and also produce very different insights into microbial symbioses. For example, under a mechanism-based approach, the bacterial community associated with a whole coral colony can be considered as a regional pool and coral compartments as local habitats. By exploring changes in this microbial pool within the different coral compartments (local habitats), the processes structuring compartmentalization of coral bacterial communities can be deciphered. Under a pattern-based approach, for example exploring coral microbial communities within environmental gradients (e.g. depth generalist corals and depth specialist corals) and at different temporal and spatial scales, the response of the microbial structure and specific members of the microbiome, to biotic and abiotic factors can be deciphered. Within this framework, meta-community theory is of particular relevance to the study of microbial community patterns and the identification of persistent associations in the microbiome.

A meta-community approach aims to provide a structure from which to evaluate patterns of diversity and distribution and identify the mechanisms that generate these patterns (e.g. dispersal, local diversification, environmental selection, and ecological drift) (Costello, Stagaman et al. 2012). For example, a meta-community is described as a group of multiple local communities (patches) connected by the dispersal of interacting species within the communities (Logue, Mouquet et al. 2011). In applying this to microbial systems in general, and the study of coral bacterial symbioses, both the host and the unique microbial habitats within the host coral, can be considered patches within the community. Microbial dispersal can occur both between individual hosts and between the microbial habitats. In this sense, the coral host represents a multitude of potentially dynamic microbial niches (Ainsworth, Thurber et al. 2010, Sweet, Croquer et al. 2011) (Figure 2-1, Figure 2-3B).

Microorganism's and microbial communities develop within the microscale (microns), and the microbial habitat in which they reside is influenced not only by the abiotic conditions but also by interactions between microbes and the coral. The application of community frameworks and core microbiome analysis are, for now, limited due to insufficient information on the processes that contribute to, and influence, microbial distributions within, and between, the coral host. Determining the microbial habitat and niche utilization within corals will require detailed information on the discrete or continuous micro-scale environment (Konopka 2009). For example in other systems, it has been shown diversification and environmental selection within micro-scale habitats of the host, and the host can influence the physicochemical conditions of the habitat, for example, carbon sources (Turnbaugh, Ley et al. 2006, Costello, Stagaman et al. 2012). The host immune system also functions as an environmental filter limiting the niches available for microbial colonization (Costello, Stagaman et al. 2012). The plant microbiome is one key example of microhabitat partitioning and the influence of the host on the microbial meta-community symbiosis plants. Plants modify the space-surrounding root through the

secretion of a wide range of compounds (niche differentiation mediated by the host) that attract bacteria within the soil. Then, using a second filter (predominantly the innate immune system) the plant selects specific bacteria present in the space-surrounding root that are can colonize the root (Bulgarelli, Schlaeppi et al. 2013, Schlaeppi, Dombrowski et al. 2014). The human body is another example whereby structural and functional aspects of spaces such as the skin, mouth, esophagus and gastrointestinal tract, provide diverse habitats for distinct communities of microbes (Spor, Koren et al. 2011). In the human gut, diet composition (environmental factor) modifies the microbial gut environment favoring some bacteria over others (Turnbaugh, Hamady et al. 2009, Spor, Koren et al. 2011). The human immune system also restricts the niches that are available to colonize the gut (Costello, Stagaman et al. 2012) producing an impenetrable mucus (Johansson, Phillipson et al. 2008), generating antibodies (Duerkop, Vaishnava et al. 2009), and stimulating innate and adaptive local responses (Macpherson, Geuking et al. 2012). The coral host is also a complex organism within a dynamic abiotic and biotic environment. Accounting for both environmental and host influences over the microbial habitat, the community within that habitat, and how the community interacts with the host is, therefore, key to overcoming many difficulties in understanding the functional role of the coral microbiome.

Community profiling in conjunction with multi-omics to decipher functionally significant symbioses.

The core microbiome framework is one of many tools that are informative for deciphering symbioses in complex microbial communities. In many systems, multi-omic approaches have been the necessary next step to fully understand the functional significance of the microbiome (Franzosa, Hsu et al. 2015). Multi-omic methods integrate taxonomic profiling, with functional profiling [transcriptomic (RNA), proteomics (proteins), and metabolomics (metabolite data)]

to characterize the functional contribution of microbes and microbial communities to the host (Franzosa, Hsu et al. 2015, Gilbert, Quinn et al. 2016). However, taxonomic profiling that accounts for the complexity of the system is a necessary initial step in understanding community variability and detecting changes in the composition of microbial communities in response to a disturbance. From the initial taxonomic profiling it is possible to derive questions related to functional community change when the stability is lost (e.g. in humans: diseases, change of diet, use of antibiotics). By integrating taxonomic and functional profiles, and host responses, it is possible to gain greater insight into mechanisms through which bacteria develop, and define a functional role to the host and determine how this role can change in response to disturbance. Furthermore, research into the human microbiome has revealed that the most common restriction to metagenomics is the taxonomic resolution, finding that some of the most functionally important microbial changes occur at the level of strain (e.g. highly pathogenic *Escherichia coli* strains (Karch, Tarr et al. 2005)). Currently, human microbiome research is moving towards strain-level profiling and gene content techniques such as single-cell sequencing and whole-metagenome shotgun (WMS) to identify variation across microbial genomes (Franzosa, Hsu et al. 2015). However, to date, human microbiome research is the only system that has attempted to resolve strain level of taxonomy (Table 2-1). Applying this level of taxonomic resolution and multi-omic approaches to coral systems would require a sound understanding of the microbial community dynamics.

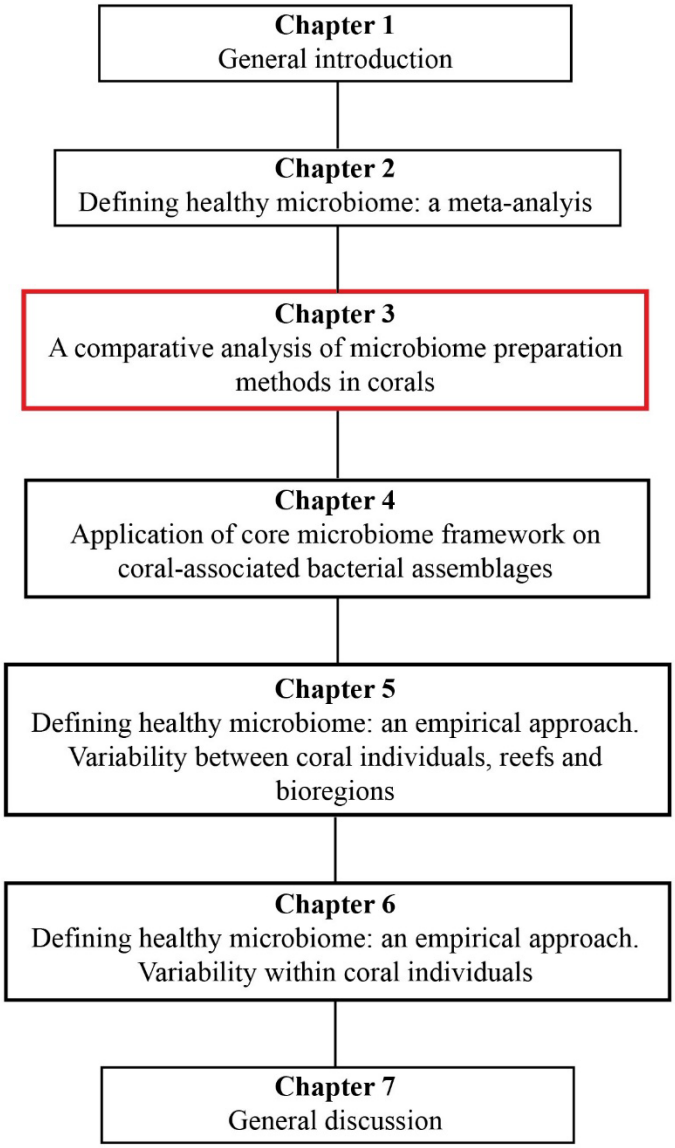
Meta-analysis on the current literature reveals that coral microbial ecology has recently stalled both at the level of taxonomic profiling and at understanding functional the role (Supplementary Table B-1) (Thurber, Willner-Hall et al. 2009, Littman, Willis et al. 2011, Barott, Rodriguez-Mueller et al. 2012). To date, taxonomic resolution in coral microbiome studies is determined at the genus level, which is a comparatively low taxonomic resolution and is the result of taxonomy assignment reliant of multi-host databases and the lower sampling

effort in such non-model systems. Moreover, unlike human research, coral microbial ecology has been hampered in space and time in regard to replication. Meta-analysis of the coral literature shows that to date less than 33 colonies (from different sites and time points but considered as replicate individuals) have been included in each study, and on average only 6 colonies (individuals) are used per study (Supplementary Table B-1). Also, most studies investigating disturbance and stress responses of the coral microbiome compare only two temporal states (before and during, or immediately after the stimuli) or impacted versus not impacted (i.e. diseased or bleached). Long-term studies addressing colonization and seasonal dynamics have studied corals for only up to one year (Chen, Tseng et al. 2011, Carlos, Torres et al. 2013, Lema, Bourne et al. 2014, Lema, Willis et al. 2014, Li, Chen et al. 2014, Angly, Heath et al. 2016) which limits conclusions on bacterial succession in corals' lifetime. Utilizing the theoretical and technical advances made in human system can dramatically accelerate our understanding of the coral microbiome (Figure 2-3). Moving beyond taxonomic profiling, to a more integrated vision of microbial-host relationship will be challenging and require the standardization of methods to validate results across coral species and geographical regions.

Concluding Remarks

Corals harbor a complex microbial biosphere that is still poorly understood. The complexity of the system and technological and methodological challenges have hampered progress in understanding the mechanisms mediating microbial colonization and symbiosis in microhabitats and ultimately the identification of bacterial contribution to coral health and resilience. Corals, as complex systems, require an integrated vision of microbial dynamics and host responses to overcome the current knowledge gaps and address these critical questions.

Chapter 3: A comparative analysis of microbial DNA preparation methods for use with massive and branching coral growth forms



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Introduction

Sequencing of the gene 16s rRNA is now by far the most common technique used to study the microbiome (Shokralla, Spall et al. 2012, D'Amore, Ijaz et al. 2016, Lear, Dickie et al. 2018). The reliability of this method is directly related to the accuracy and precision of capturing entire communities of highly diverse, abundant and uncultivable microbes (Rajendhran and Gunasekaran 2011, D'Amore, Ijaz et al. 2016, Thompson, Sanders et al. 2017). A number of steps are required to undertake this process, starting with the initial sampling protocol, through to the analysis (Lear, Dickie et al. 2018). Throughout the process of generating a microbiome dataset, the protocol that is used can impact many attributes of the microbial dataset, and consequently, our understanding of the microbial community. Methods that can influence the final dataset may be related to the initial preservation of samples (Vlčková, Mrázek et al. 2012, Gray, Pratte et al. 2013, Rocha, Coelho et al. 2014), DNA extraction and amplification (Pinto and Raskin 2012, Soergel, Dey et al. 2012, Ghyselinck, Pfeiffer et al. 2013)], as well as a number of metrics related to downstream sequence analysis (McMurdie and Holmes 2014). The current study aims to evaluate the influence of sample preservation methods and DNA extraction protocols on the microbiome datasets of coral.

As preservation methods, three reagents are commonly employed in marine research efforts to identify and characterize the microbiome. Each of these preservation methods has been developed to overcome various limitations of working in remote field sites, where access to fully equipped laboratories is limited (Nagy 2010). For example, salt saturated dimethyl sulfoxide (DMSO) – EDTA (Seutin, White et al. 1991) is one of the most widely used preservation methods in marine sampling protocols as it can be transported long distances and remains stable over long time periods (Dawson, Raskoff et al. 1998). Snap freezing has also become widely used as the sample is preserved immediately upon collection with the handling

of the sample required and minimal exposure of the sample to preservation artifacts (Fouhy, Deane et al. 2015, Vandeputte, Tito et al. 2017). However, this method has been limited by the capacity to transport and store liquid nitrogen or dry ice in remote areas. Finally, due to advances in DNA extraction protocols, fixation with formaldehyde (PFA) based solutions (for example 4% PFA) has recently become more widely used to preserve DNA, and has been applied to microbiome studies in plankton, humans, plants, sponges, and corals (Dinsdale, Pantos et al. 2008, Tang, Hong et al. 2011, Lundberg, Lebeis et al. 2012, Raina, Tapiolas et al. 2013, Adam, Klawonn et al. 2016, Bruder, Dorkes et al. 2016, Neave, Rachmawati et al. 2016, Guerrero-Feijóo, Sintes et al. 2017). Like DMSO, sample preservation in paraformaldehyde provides an easily transportable and widely applicable preservation system but has not yet been widely taken up in environmental microbiome studies. The process of generating a microbiome dataset also requires homogenization of the preserved sample before employing DNA extraction protocols (Elbrecht and Leese 2015, Lear, Dickie et al. 2018). To date, the homogenization processes used in studying the coral holobiont microbiome have varied between studies. In general, some form of the crushing of the entire coral sample is employed, crushing the hard coral skeleton and overlaying tissues involves either the use of a mortar and pestle or a French press while the sample is held in liquid nitrogen to prevent DNA degradation (*see* meta-analysis in Chapter 2, (Ng, Chan et al. 2015, Samodha, Wang et al. 2015, Shore-Maggio, Runyon et al. 2015, Zhang, Ling et al. 2015)). Sample lysis and DNA extraction are then applied to a sub-sample of the generated homogenate, for example using approximately 20 mg of homogenate samples in cell lysis buffer before DNA extraction (e.g. Ainsworth, Krause et al. (2015)). Homogenization through bead beating of a small sub-sample has also been applied to extraction protocols without the use of before crushing (e.g. Weber, DeForce et al. (2017)). The bead beating method combines physical force applied on spheres with cell lysis before DNA extraction (Lear, Dickie et al. 2018). This method utilizes a smaller sample

(for example in coral studies ~1-2 cm of the entire coral branch) and uses the beads to strip the overlaying tissues from the coral skeleton during the chemical cell lysis. This approach provides a quicker and more cost-effective means of sample preparation, but results are less of the coral skeleton been broken down and therefore may alter the resulting dataset due to less of the endolithic microbiome (microbes contained within the skeleton) being released. There are many advantages and disadvantages to different sample preservation and preparation methods that have been employed in coral, and marine microbiome studies including transport, handling time, handling effort, total cost, and applicability in remote field locations. Despite comparison of DNA extraction kits and homogenization methodologies (Weber, DeForce et al. 2017) very few studies have directly compared preservation and processing methods to determine their impact on the resulting datasets (e.g. Gray, Pratte et al. (2013)). However, there are studies comparing the microbiome datasets generated from multiple studies (Mouchka, Hewson et al. 2010, Miller and Richardson 2011).

Assessing preservation and homogenization methods can provide insights into protocols best suited for use in remote locations and assist in standardising approaches across different studies undertaken worldwide. Standardized protocols are particularly relevant for microbiome studies on coral reefs. The worldwide degradation of coral reef ecosystems is driving more and more studies to be undertaken on the coral microbiome. Studies are aiming to define the characteristics of microbial communities of healthy organisms and also dysbiotic and unhealthy coral reef ecosystems (Ainsworth and Gates 2016, Bourne, Morrow et al. 2016). Coral reef ecosystems are often remote and located offshore, and sampling undertake in these areas often represents a compromise in the number of samples taken and the quality of the preservation method. These logistical constraints are acknowledged as potential influencing factors on the microbiome datasets that are generated and consequently, on our perception of the attributes of microbial communities. The current study aims to evaluate the influence of sample preservation

methods and two DNA extraction protocols on the microbiome datasets generated from two coral species.

Materials and Methods

Coral collection and preservation

On January 2015, fragments of corals *Goniastrea edwardsi* (*n*=25, <3 cm diameter) and *Isopora palifera* (*n*=25, <5 cm long) were collected in the reef flat in Coral Gardens reef adjacent to Heron Island Research Station, Australia (23°26.5248'S, 151°54.754'E). For each species five coral fragments were collected from five colonies separated by >3 m, using a hammer and chisel (Figure 3-1). The samples were held in seawater and after collection; fragments were immediately transported to the adjacent laboratory for preservation. For each colony, samples were preserved using three reagents: two samples were snapped frozen in liquid nitrogen and stored at – 80°C, two samples were preserved in salt-saturated 20% dimethyl sulfoxide (DMSO) – 0.5 M EDTA and stored at 4°C, and one sample was fixed in 4% Paraformaldehyde solution (PFA) and stored at 4°C. After 14h samples fixed in PFA were rinsed and stored in sterile 3x phosphate buffered saline at 4°C. 4% Paraformaldehyde solution and 3x phosphate buffered saline were prepared using DNA/RNA-free water on the same day of coral collection. Fragments were shipped to James Cook University, Townsville, Australia. Until their processing, fragments preserved in PFA and DMSO were stored at 4°C, and snap frozen fragments at – 80°C. Coral collections were collected under permits supplied by the Great Barrier Reef Marine National Park Authority (Townsville, Australia, G15/37488.1).

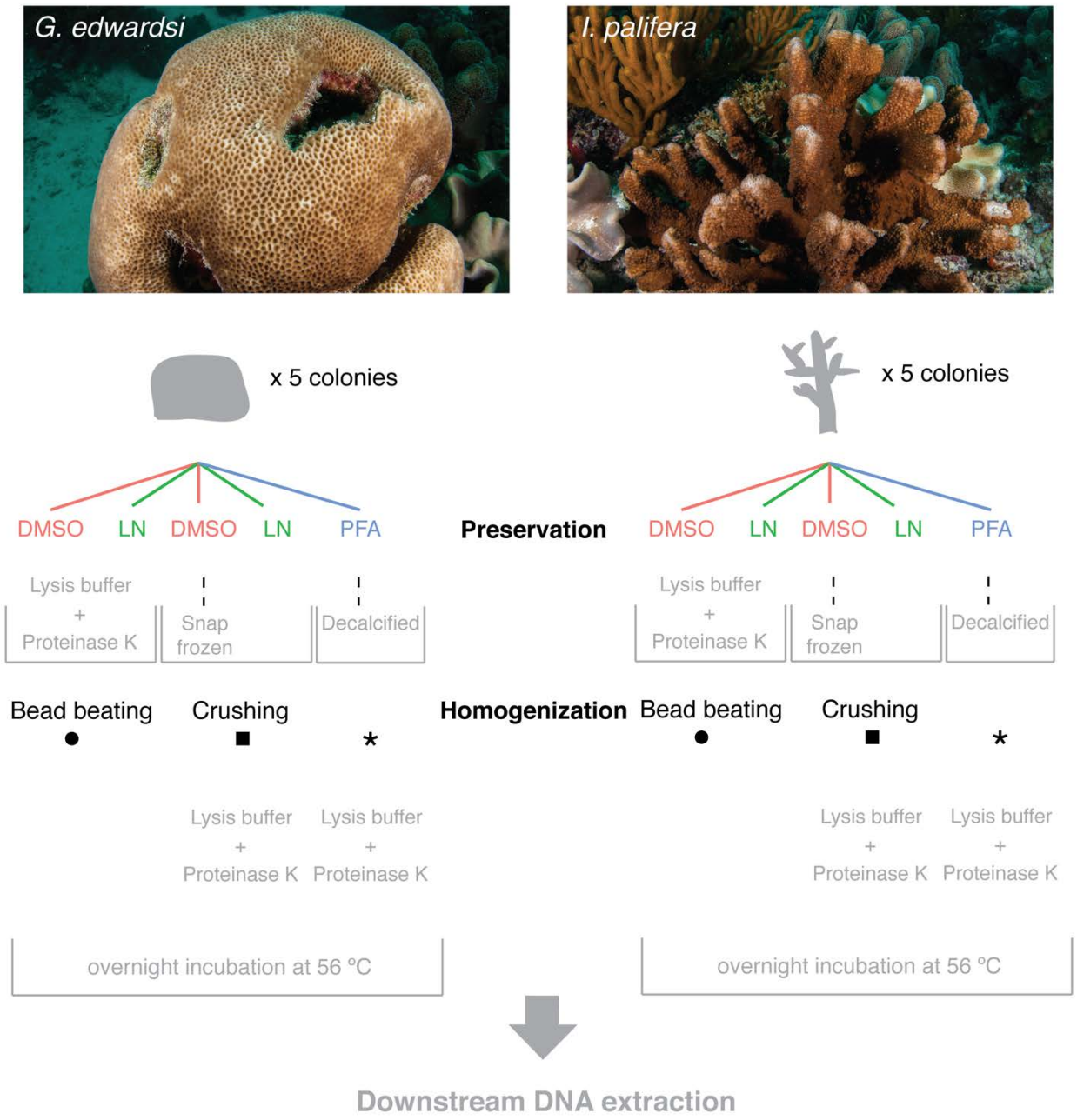


Figure 3-1: Flow diagram of experimental design. LN: liquid nitrogen, DMSO: salt saturated dimethyl sulfoxide (DMSO) – EDTA, PFA: Paraformaldehyde. Photos by Ed Roberts.

Sample homogenization and decalcification

For a mixed combination preservation reagent x homogenization method, samples preserved in liquid nitrogen and DMSO were homogenized using two methods, bead beating and crushing. To standardize the sample size, a subsample of 0.173 (±0.04) g of coral, including tissue and skeleton, were used for both methods. Homogenization under liquid nitrogen is necessary to ensure a uniform homogenization across the entire sample, thus per colony samples preserved in DMSO were snap frozen before crushing. As such one sample preserved in liquid nitrogen and one in DMSO (after snap frozen) were crushed in liquid nitrogen applying up to 40 psi of pressure with a French press, followed by manual homogenization to a fine powder using mortar and pestle on dry ice (Figure 3-1). The resulting powder was used for subsequent steps in cell lysis and DNA extraction. All instruments were sterilized before use with each sample. The resulting homogenate was used in the DNA extraction outlined below (*see DNA extraction, amplification and sequencing protoco*l).

For homogenization using bead beating, the same amount of coral tissue/skeleton from each sample preserved in DMSO and liquid nitrogen were individually placed into 2 ml tubes with 1.0 mm silica spheres for immediate lysis and DNA extraction. To each tube was added 360 µl of lysis buffer (QIAmp® DNA Mini Extraction kit, Qiagen) and 40 µl of Proteinase K. A FastPrep-24^{TM} 5G (MP) homogenizer was used to run three rounds of 20 seconds each to homogenize the sample.

For each sample preserved in PFA and stored in PBS, the entire coral sample was decalcified with repetitive washes of DNA/RNA-free 20% EDTA at 4°C over a 2-week period. After decalcification of the entire coral sample, 0.04 (±0.004) g of the resulting coral tissue was used from each colony for successive steps in DNA extraction.

DNA extraction, amplification and sequencing protocol

As coral samples, tissue from the decalcified PFA fixed, and the powder from crushed samples were placed in 1.5 ml tubes and 360 µl of lysis buffer (QIAmp® DNA Mini Extraction kit, QIAGEN) and 40 µl of Proteinase K.

Together with homogenized samples from the bead beating method, all samples were incubated overnight at 56°C and posteriorly purified using a silica-membrane-based nucleic acid technique as manufacture's protocol (QIAmp® DNA Mini Extraction kit, QIAGEN). Extracted DNA concentration and purity were quantified using Qubit Fluorometer and Qubit® dsDNA High-sensitivity Assay Kit (Life Technologies, NSW, Australia). Extracted DNA was stored at -20°C prior to PCR amplification and sequencing. DNA extraction and sequencing were performed on negative controls as well. DNA extraction, amplification and sequencing were performed on negative controls (no sample template) as well.

Genomic template primers 27F/519R (v1-v3 region) and barcode on the forward primer were used in a 30-cycle PCR using HotStarTaq plus master mix kit (QIAGEN, USA) to amplify bacterial 16S rRNA gene amplicons. PCRs were run under following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds, and 72°C for 1 min, a final elongation at 72°C for 5 minutes. Based on molecular weight and DNA concentration, amplicon products from different samples were pooled and purified using calibrated Ampured XP beads. DNA libraries were prepared with purified and pooled samples following Illumina TruSeq DNA library preparation protocol. Sequencing was performed at MR DNA (Shallowater, Texas, USA) on a MiSeq platform under manufactures' protocol. 16s rRNA raw sequences are available in the National Center for Biotechnology Information (NCBI) Short Read Archive (SRA) under the project number PRJNA432131.

Sequence analysis

Sequence data were processed using the open-source software Quantitative Insights Into Microbial Ecology (QIIME, version 1.9) (Caporaso, Kuczynski et al. 2010). Barcodes, ambiguous base calls, homopolymer runs exceeding 8 bp or sequences below 200 bp were removed from raw sequence data. Chimeras sequences were removed using Usearch61 (Edgar, Haas et al. 2011). 97% cluster similarity was used to define Operational Taxonomic Units (OTUs), and RDP classifier (Wang, Garrity et al. 2007) was used against a curated Greengenes database (v. 13_8) (DeSantis, Hugenholtz et al. 2006) to assign taxonomy to OTUs. Chloroplast, mitochondria, unidentified and unassigned OTUs were removed from resulting OTU tables.

Statistical analyses

Differences between preservation and homogenization methods were analyzed using PRIMER v7 and PERMANOVA+ (Anderson, Gorley et al. 2008). The overall performance of each methodology was also assessed through the comparison of the number of sequences and number of OTUs. Bacterial assemblage structure was evaluated as structure and composition. As part of a comprehensive evaluation of the different preservation and homogenization methods, singletons and low read OTUs were kept in data analysis. For the analysis of relative abundance, a fourth root transformation and standardization by sample by total was applied to the OTU table. The OTU table was also converted to presence/absence to evaluate bacterial composition. Differences between methodologies were evaluated with a design considering both *Preservation* and *Homogenization* as fixed factors with two levels each (DMSO and liquid nitrogen; and bead beating and crushing, respectively). Individual comparisons between decalcified PFA fixed samples and other samples under preservation-homogenization combinations were assessed with a design considering the combination preservation-

homogenization as *Treatments*, a fixed factor with 5 levels (DMSO-BB, DMSO-Cr, LN-BB, LN-Cr and PFA-decalcified).

Differences between preservation and homogenization methods and treatments were identified by permutational multivariate analysis of variance (PERMANOVA) on Euclidian distances (number of sequences and OTUs), Bray-Curtis (BC) and Sorensen dissimilarity matrices (relative abundance and presence/absence data, respectively). PERMANOVA analyses were run under the following parameters: Type III (partial) sums of squares, fixed effects sum to zero for mixed terms, number of permutations 9,999 and as permutation method, permutation of residuals under a reduced model for the assessment of differences between preservation and homogenization methods, and unrestricted permutation of raw data for analysis of differences between treatments. Adjusted Bonferroni *p-value* was used to determine significant differences between PFA fixed samples and other samples under preservation-homogenization combinations. Coral species data were analyzed separately since differences between them were detected (Supplementary Table C-5, C-6 and Supplementary Figure C-1). Two-dimensional nonmetric dimensional scaling (nMDS) plots (Clarke 1993) are presented to illustrate PERMANOVA results.

The OTUs present across samples of a treatment (core 100% per treatment) were determined using the command *compute_core_microbiome.py* in QIIME. Venn diagrams were generated using Venn diagram software (Bioinformatics and Evolutionary Genomics, http://bioinformatics.psb.ugent.be/webtools/Venn/). Graphs were produced using 'ggplot2' package (Wickham 2016) in R (Team 2013).

Results

Number of sequences and Operational Taxonomic Units (OTU)

The number of sequences and the number of OTUs generated was highly variable within all the replicates and between the treatments, and negative controls did not amplify and did not generate sequences (Figure 3-2, Table 3-1). High variability in the number of sequences and OTUs is evident in the preservation-homogenization treatment of *I. palifera* samples *(*Figure 3-2, B and D) where overall patterns observed in the number of sequences per sample are also observed in the number of OTUs. In *G. edwardsi*, the efficiency of preservation interacts with homogenization method used (Figure 3-2, A and C) and for the homogenization method bead beating both the number of sequences and OTUs are higher with DMSO than in those samples preserved in liquid nitrogen, but the opposite occurs with the crushing homogenization method. As such, there is no significant difference detected between preservation or homogenization methods (Supplementary Table C-1, C-2). On average all of the preservation methods resulted in between 42 and 47 thousand sequences, notably the combination of DMSO-crushing resulted in on average only 22 thousand sequences (Table 3-1). On average, the number of OTUs generated was between 1,351-2,528. Notably, the PFA–decalcification method retrieved comparable results to the other methods for both the number of sequences and the number of generated OTUs.

Similarly, in *I. palifera* there were no statistical differences detected between the preservation methods (Figure 3-2, B and D, Supplementary Table C-3, C-4) and the number of sequences and OTUs were highest in PFA – decalcification method. On average the lowest number of sequences were retrieved from the crushing protocol (28-29 thousands of sequences), followed by for both preservation methods when bead beading (37-42 thousands of sequences), and PFA

with the highest value, doubling and triplicating the value observed with other methods (92 thousand of sequences, Table 3-1).

Table 3-1: Number of sequences and OTUs per treatment. Counts are estimated on raw data after filtering out chloroplast, none and unassigned OTUs.

Coral species	Method		N. samples	N. sequences (total)	N. sequences (av. by samples)	N. OTUs (total)	N. OTUs (av. by samples)
	Preservation	**Homogenization**					
G. edwardsi	DMSO	Bead beating	5	235,026	47,005	10,673	2,135
	DMSO	Crushing	5	110,638	22,128	7,654	1,531
	Liquid nitrogen	Bead beating	5	212,461	42,492	10,588	2,118
	Liquid nitrogen	Crushing	5	211,476	42,295	12,640	2,528
	PFA	Decalcified	4	190,770	47,693	5,403	1,351
I. palifera	DMSO	Bead beating	5	186,431	37,286	5,356	1,071
	DMSO	Crushing	5	140,986	28,197	5,910	1,182
	Liquid nitrogen	Bead beating	5	211,421	42,284	8,703	1,741
	Liquid nitrogen	Crushing	5	146,383	29,277	4,448	890
	PFA	Decalcified	5	476,060	95,212	9,679	1,936

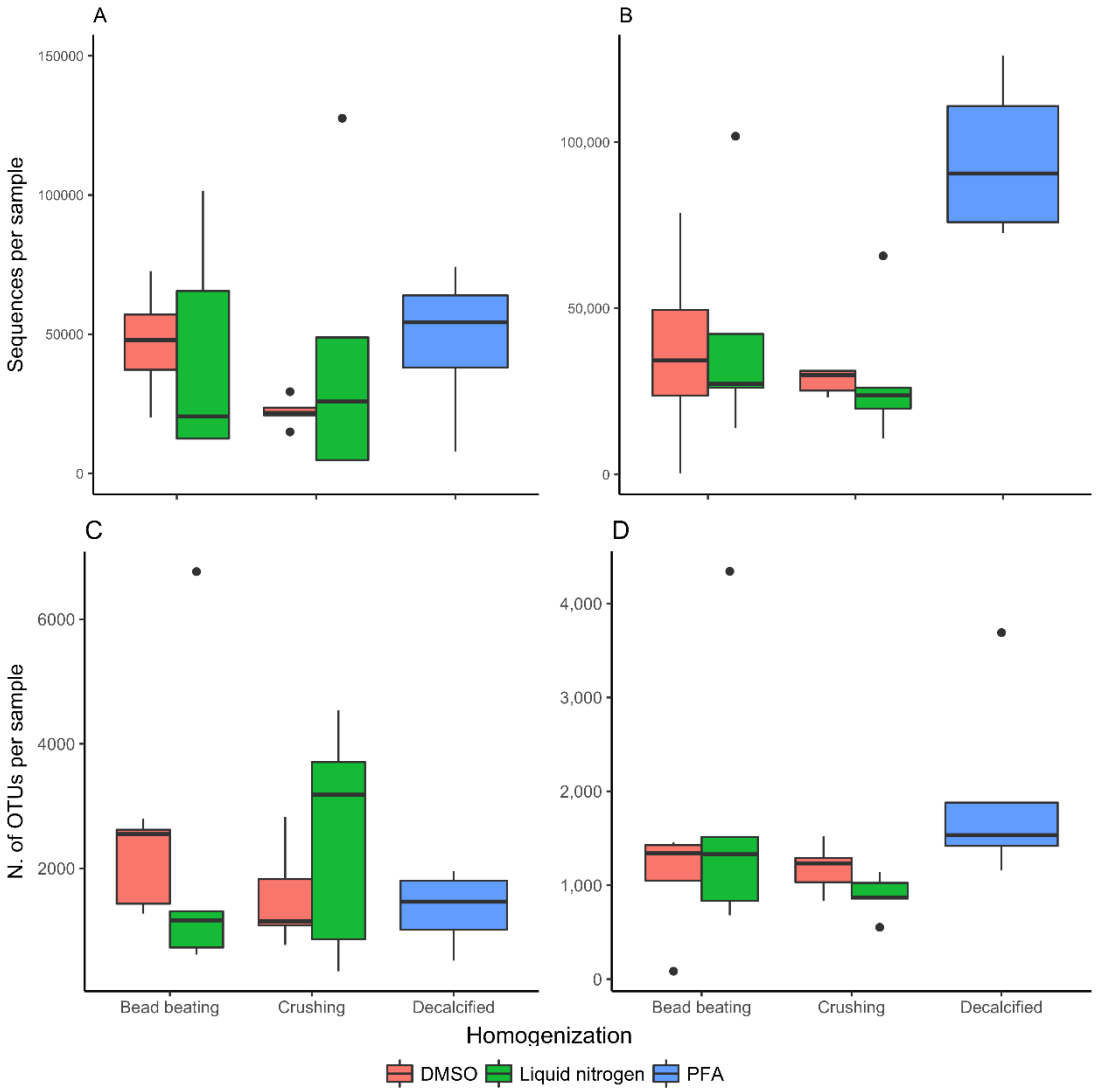


Figure 3-2: Number of sequences and OTUs per sample for *G. edwardsi* (A, C) and *I. palifera* (B, D). Boxplots are based on raw data after filtering out chloroplast, non-identified and unassigned sequences.

Community composition and structure

An analysis of the community structure indicated differences in composition and structure between both coral species (Supplementary Figure C-1, Supplementary Table C-5, C-6). Exploring the coral species separately, I found there are no significant differences for either composition or structure of the community retrieved from preservation with DMSO and liquid nitrogen and homogenization using bead beating and crushing methods. In the massive coral *G. edwardsi* bacterial community, only 7% of the variation resulted from preservation methods (Figure 3-3A, Supplementary Figure C-2A, Supplementary Table C-7, C-8). I also found there are no evident differences between PFA-decalcification bacterial community composition and structure and the community structure of other methods (Supplementary Table C-9, C-10). Similarly, for *I. palifera*, no differences were detected between DMSO and liquid nitrogen preservation and bead beating and crushing homogenization, and 13% and 9% of variation are assigned respectively (Figure 3-3B, Supplementary Figure C-2B, Supplementary Table C-11, C-12). Contrary to the observed in *G. edwardsi*, bacterial community composition and structure of PFA-decalcified individuals in *I. palifera* are different to the community in individuals preserved with DMSO, regardless the homogenization method (DMSO - Bead beating and crushing in Figure 3-3B, Supplementary Figure C-2B, Supplementary Table C-13, C-14).

Figure 3-3: Bacterial communities are similar regardless the preservation and homogenization method used in *G. edwardsi* (A), in *I. palifera* (B) bacterial assemblages treated with PFA-decalcified differ from the other methods. Non-metric MDS based on Bray-Curtis dissimilarity of relative abundance data (fourth root-transformed). Green: Liquid nitrogen, red: DMSO, circles: bead beating, squares: crushing. Colonies indicated with numbers. For presence/absence equivalent results *see* Supplementary Figure C-2 and Supplementary Table C-8, C-10, C-12, C-14.

Rare, common and core microbiome

I found that the highly persistent, and highly abundant, bacterial phylotypes were captured by all the preparation protocols used in the current study (black dots in Figure 3-4). Interestingly in each methodology, a specific group of bacteria only occurred in between one to three individuals and low abundance (bright colored dots in Figure 3-4). Differences between the preservation and homogenization methods occur in a fraction of the community that is rare; low abundant and low persistent bacteria.

Dissecting the number of OTUs by their percentage of persistence demonstrated similar performance between the methods assessed (Supplementary Table C-15, Supplementary Figure C-3, C-4). Across the methodologies, singletons represented 60% of the total of phylotypes, and while increasing the persistence, the number of OTUs decrease within the same order of magnitude. Each methodology captured differently bacterial community (Figure 3-5); in the sense that phylotypes resulting more persistent (core 100%) and more dominant OTUs (dominant phylotypes based on the cut-off, and top 10 dominant phylotypes) differed between methodologies (Supplementary Table C-16, C-17). However, some taxa were consistently detected in all the methodologies with the same dominance or occurrence. For example, for both *G. edwardsi* and *I. palifera* core 100%, OTUs from Family Endozoicimonaceae (except DSMO-BB in *I. palifera*) and genera, *Diaphorobacter* and *Propionibacterium* were detected in all the methodologies employed. OTUs from the Order Kiloniellales, Families Endozoicimonaceae, Flammeovirgaceae, Phyllobacteriaceae, Rhodobacteraceae and genera *Corynebacterium*, *Diaphorobacter*, SGUS912, *Propionibacterium*, and *Pseudomonas* were dominant across methodologies for *G. edwardsi* bacterial community. In *I. palifera*, OTUs from the Family Aerococcaceae were consistently found as dominant in the bacterial community (Supplementary Table C-16, C-17).

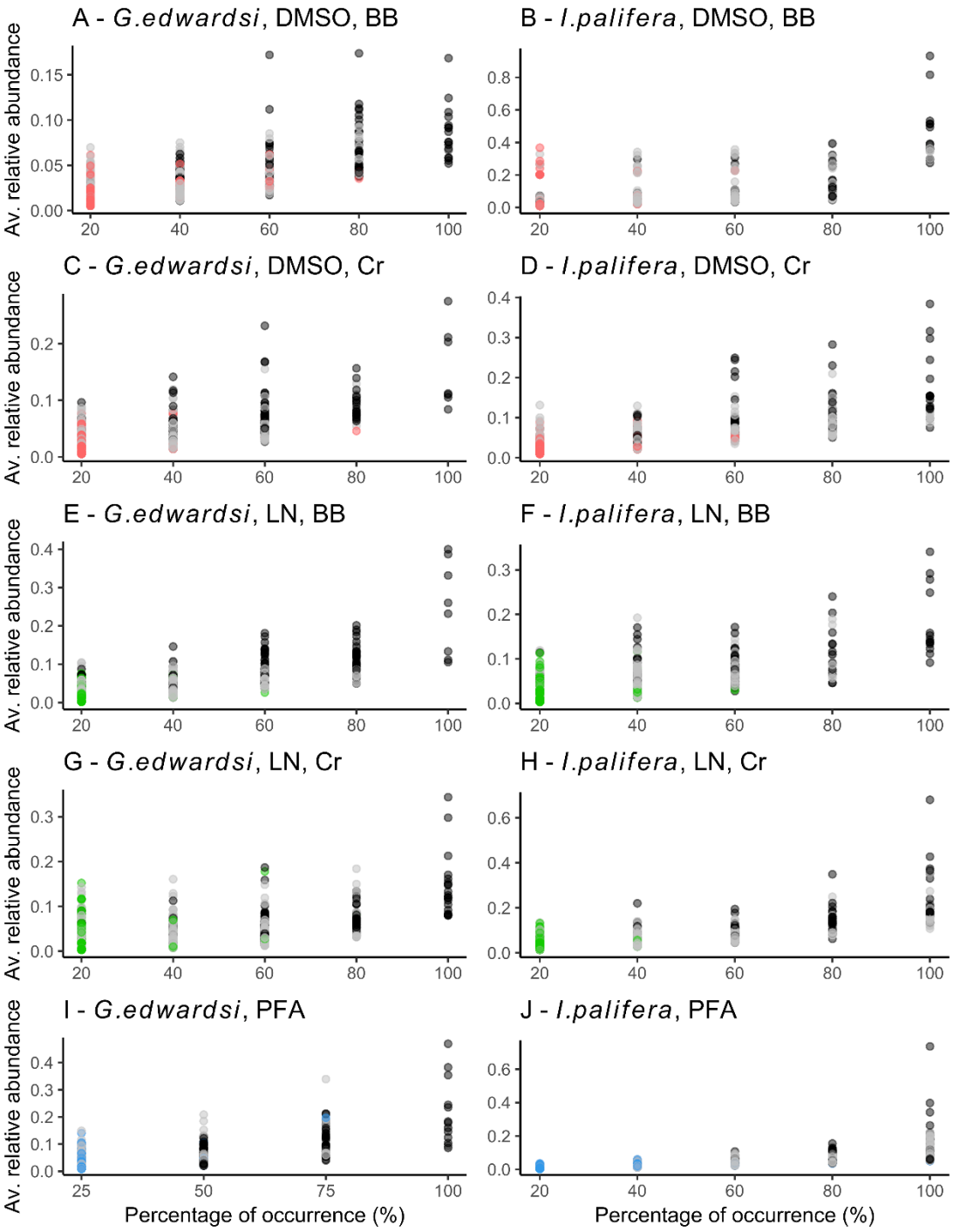


Figure 3-4: Common/shared and specific phylotypes in bacterial assemblages sampled by different preservation and homogenization methods. Graphs of average of relative abundance vs. percentage of occurrence across methodologies revealed that specific bacterial phylotypes for each method are rare, low occurrence and low abundance (red, green and blue dots across figures). Highly persistent, and mostly dominant OTUs, are common/shared OTUs across methodologies (grey and black dots). Red, green and blue dots: OTUs present uniquely in the assemblage sampled by the referred combination method; grey dots: OTUs present in between two to four of the methods used; black dots: OTUs present in all the methods used. Left side: *G. edwardsi*, right side: *I. palifera*. Green: Liquid nitrogen, red: DMSO, blue: PFA. LN: liquid nitrogen, BB: bead beating, Cr: crushing.

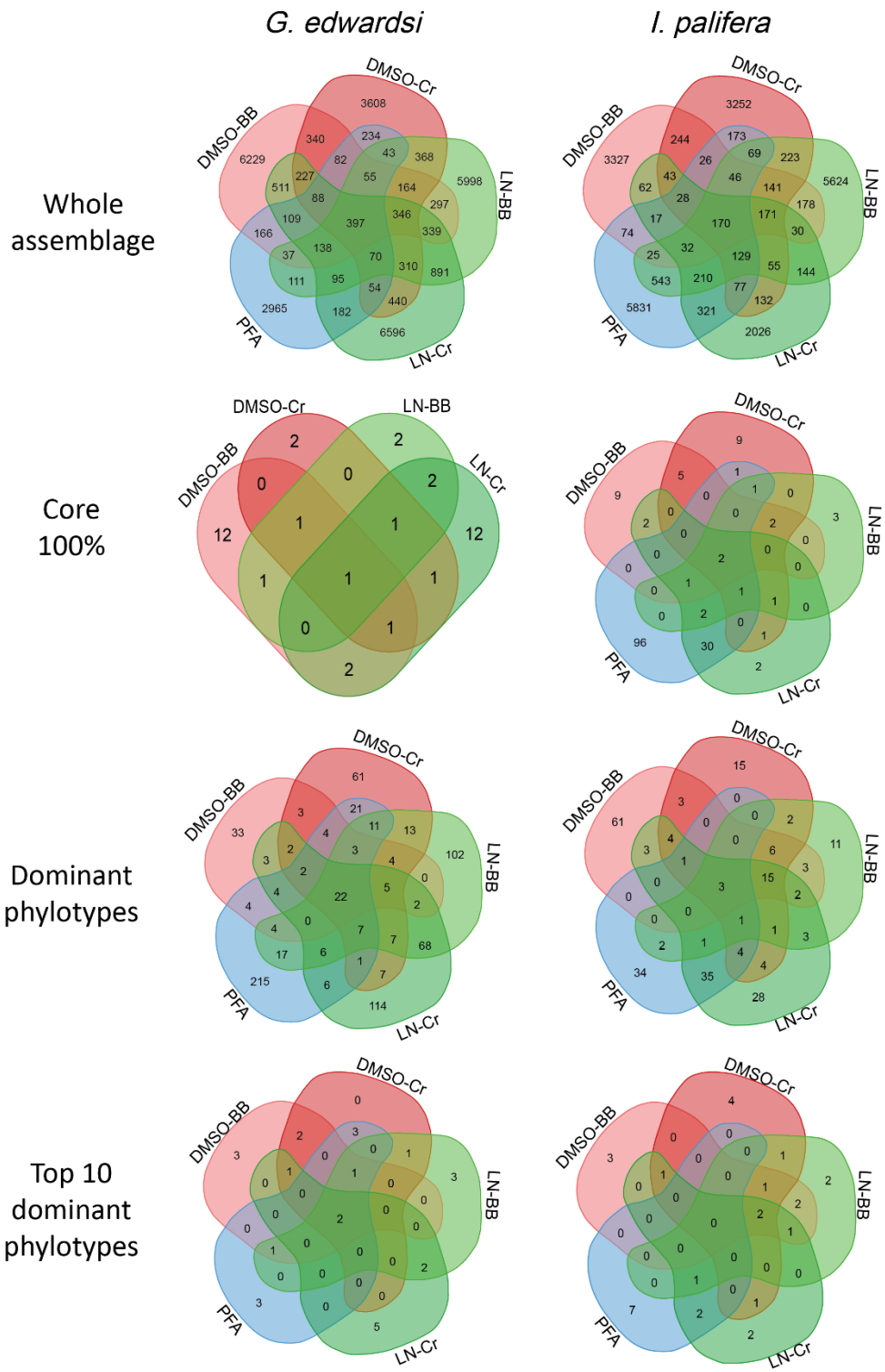


Figure 3-5: Common/shared phylotypes variate in persistence among preservation and homogenization methods. Venn diagrams for the whole bacterial community, core 100%, dominant phylotypes (cut-off relative abundance ≥ 0.05 for *G. edwardsi* and 0.1 *for I. palifera*) and top 10 dominant phylotypes. Venn diagrams reflect approximately 39% and 45% of OTUs of the whole bacterial assemblages in *G. edwardsi* and *I. palifera* are shared among methodologies. However, shared bacterial phylotypes are not consistently highly persistent, dominant or among the top 10 dominant OTUs across methodologies. Conversely, 'importance' of bacterial phylotype, expressed as persistence

or relative abundance, varies among preservation and homogenization methods (Supplementary Figure C-3, C-4 and Supplementary Table C-15). For OTUs taxonomic identification in core 100%, dominant phylotypes and top 10 dominant phylotypes *see* Supplementary Table C-16, C-17.

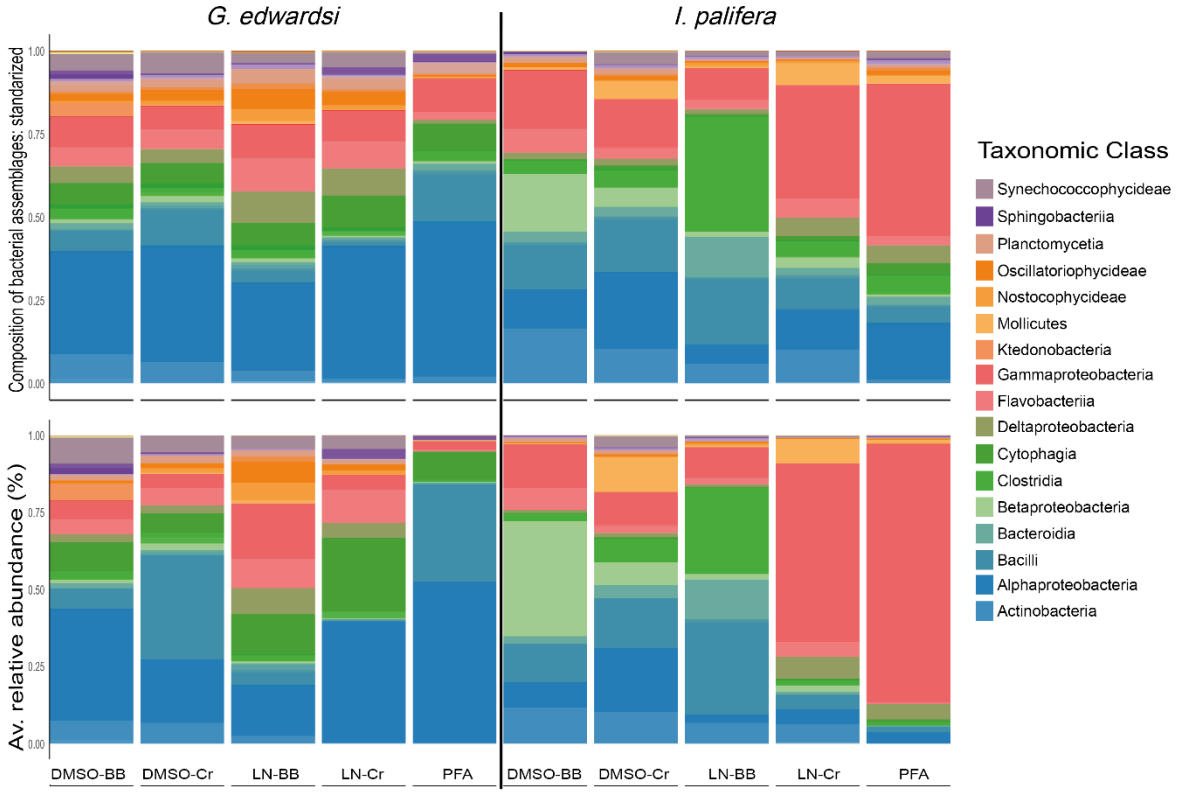


Figure 3-6: Variation of taxonomic composition and structure among preservation and homogenization methods. Taxonomical composition is consistent among methodologies for *G. edwardsi* (top left), however, taxonomical structure is distorted when is evaluated considering relative abundance (bottom left). For *I. palifera*, taxonomic composition is not consistent across methodologies, with major discrepancies in classes Gammaproteobacteria, Clostridia, Betaproteobacteria, Bacilli, Alphaproteobacteria and Actinobacteria (top right). Discrepancies are enhanced when relative abundance is considered (bottom right). For results by colony *see* Supplementary Figure C-5. Major taxonomic classes are presented in the legend, for complete legend *see* Supplementary Figure C-6.

Taxonomical composition and structure

The taxonomical composition, structure and diversity were similar across methodologies for both species (Figure 3-6, Supplementary Table C-18); however, for *I. palifera* some of the classes were overrepresented. Consistently high numbers of bacterial phylotypes belonging to classes Alphaproteobacteria, Cytophagia, Flavobacteriia and Gammaproteobacteria, were evident in *G. edwardsi*. However, small differences occurred in low occurrence classes as

Actinobacteria, Sphingobacteriia and Synechococcophycideae. For *I. palifera*, bacterial classes with a higher number of OTUs were less evident across methodologies. For colonies preserved in DMSO, classes with the higher number of bacterial phylotypes were Gammaproteobacteria and Bacilli, but differences were raised between homogenization methods for classes Alpha-, Betaproteobacteria and Actinobacteria. High similarity was evident between liquid nitrogen-crushing, and PFA treated colonies, where Gammaproteobacteria and Alphaproteobacteria were the groups with the higher number of OTUs, whereas LN-BB had an overall distinct taxonomic representation with Clostridia as the class with the higher percentage in composition. As expected, variability between colonies was evident. However, representation of taxonomical structure per colony is similar across methodologies (Supplementary Figure C-5 top).

The taxonomical structure observed in *G. edwardsi* was less evident in most of the treatments when evaluating relative abundance of same classes (Figure 3-6 and Supplementary Figure C-5 bottom), and differences between methods observed in *I. palifera* are enhanced. In relative abundance, the dominance of the classes Alphaproteobacteria, Cytophagia and Gammaproteobacteria were still evident in *G. edwardsi* individuals homogenized using bead beating, regardless the preserving method. Increases in the dominance of Bacilli and Cytophagia were evident when homogenizing with the crushing method, regardless of the preserving method. PFA taxonomical structure is dominated by Alphaproteobacteria, Bacilli and Cytophagia, with the representation of Gammaproteobacteria was smaller. For *I. palifera*, dominant groups in taxonomic composition had the higher percentages of relative abundance. Alpha-, Betaproteobacteria, Bacilli and Clostridia dominated DMSO-BB, DMSO-Cr, and LN BB, however, Gammaproteobacteria still appeared as the second more dominant class. LN-Cr and PFA showed a similar community but very different from the other methodologies, with the dominance of Gammaproteobacteria, and other groups with lower relative abundance. The

contrast between the taxonomic structure and the relative abundance evidenced incongruences observed when comparing 10% and 10 top more abundant OTUs between different methodologies (Figure 3-5).

Discussion

Here I show that sample preservation and processing methodologies generate coral microbiome databases similar in composition, but with structural discrepancies. I find that there is substantial variability in the microbiome between colonies, regardless of the preparation method utilised and this within individual variability is greater than variability resulting from the preparation method employed. No statistical differences are detected in the number of sequences, OTUs or community composition and structure of the microbiome. Similarly, across methodologies, the same taxonomical classes were retrieved, and there are groups of highly occurrent and dominant phylotypes consistently detected. However, there are some evident differences in the percentages of representation of the phylotypes across methodologies. Rare – low abundance bacterial phylotypes represent a high percentage of the assemblage and are specific per preservation-homogenization method. As a result, groups of phylotypes rare – low abundance, dominant and persistent vary between methodologies. Taken together these results indicate that each methodology is sensitive to specific groups of bacteria. Variations in relative abundance and persistence of shared bacterial phylotypes across methods indicate that both parameters should be considered in conjunction in studies aiming to determine the complexity of bacterial communities and to select phylotypes of interest (i.e. ubiquitous bacterial phylotypes).

If the objective is to evaluate the microbiome composition, the two most widely utilised preservation protocols, DMSO and liquid nitrogen, coupled with homogenization through either bead beating and crushing methods are directly comparable. The microbiome dataset

generated through paraformaldehyde preservation methods is similar to that of other methods depending on the coral growth form or species. I show that preservation with paraformaldehyde is directly comparable with the other preservation-homogenization methods for *G. edwardsi* bacterial assemblages. For example, in *G. edwardsi* paraformaldehyde fixation generates a bacterial assemblage with attributes similar to the assemblages retrieved from other methodologies. The number of sequences and OTUs are in the same order of magnitude, and community structure is comparable. For *I. palifera* bacterial assemblages, PFA treated colonies are comparable to those preserved with liquid nitrogen, but bacterial assemblages retrieved from individuals of *I. palifera* preserved with PFA are different to that of those preserved in DMSO, the number of sequences and OTUs are in fact higher in PFA preserved samples than the other methodologies. Also, the community structure of PFA preserved individuals seems to be more similar across the coral colonies, with less variation between individuals. In *I. palifera* I also show that the taxonomical structure of individuals preserved with PFA is similar to those preserved in liquid nitrogen and crushed, and PFA shows similar results in relative abundance vs. percentage of occurrence. Thus, highly persistent and abundant bacterial phylotypes (see top 10 dominant phylotypes, Supplementary Table C-17) are also present in PFA detected bacterial assemblage, and as observed in assemblages treated with other methods, specific OTUs for this method of preservation are present as rare members of the assemblage.

Selecting preservation and homogenization methodology can be influenced by the logistics of the sampling effort without greatly impacting the composition of the microbiome dataset generated. The methods explored in the current study present diverse advantages concerning safety, practicality, reproducibility and risk of cross-contamination that must be considered when selecting preservation and homogenization methods (Nagy 2010). For example, DMSO requires handling of dangerous chemicals and training in the preparation of the reagents, which can be a limiting factor for monitoring programs using sampling protocols conducted in

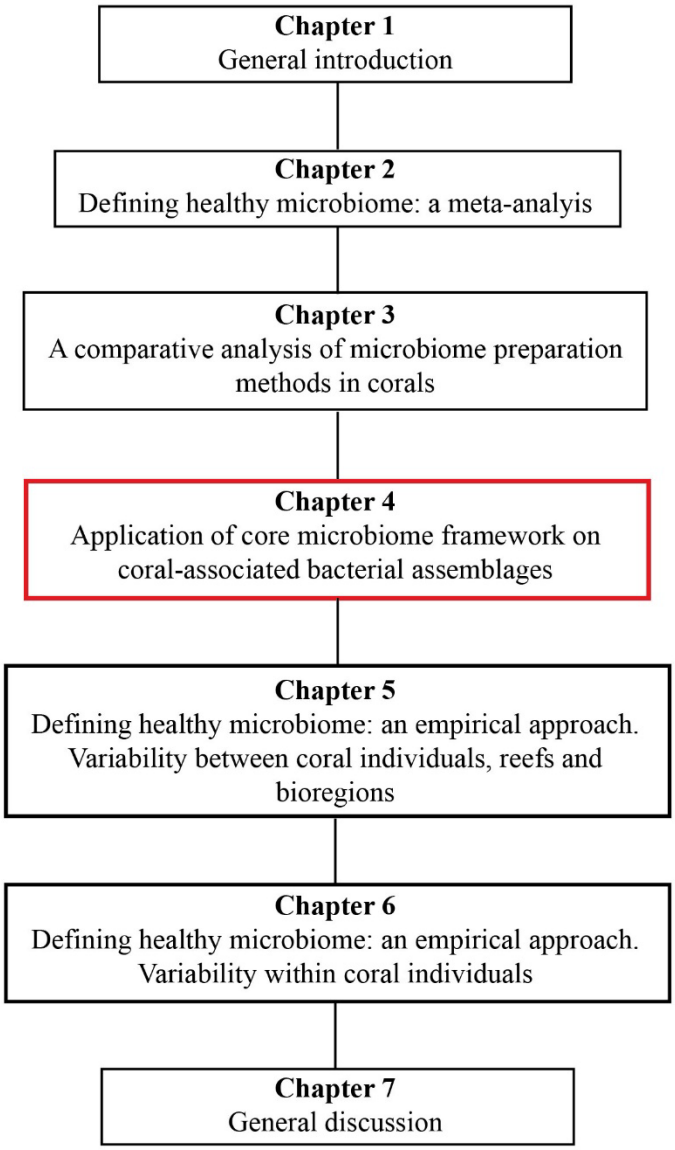
association with volunteer groups, but it is a stable preservative in the long term, and no refilling of handling is required after sample collection. Preservation with DMSO can be done in the field at room temperature, and once in the final destination, samples can be allocated at -20°C to avoid DMSO evaporation. Therefore, sample refrigeration is not necessary for the short-term when preserving with DMSO. Preservation in paraformaldehyde is a similarly fast and easy method in the field but requires further handling after sample collection for storage of the sample in Phosphate buffered saline (non-hazardous) to avoid over-fixation of the sample. PFA is hazardous, and handling requires training and safety equipment, with similar limitation for untrained personnel. The advantage of PFA over the other methods is that it is ideal to preserve tissue structure and allows more detailed assessment of the health and condition of the samples collected, allowing for histological analysis to be conducted, the identification of bacteria niches through, fluorescence *in situ* hybridization (FISH, e.g. Bythell, Barer et al. (2002), Ainsworth, Fine et al. (2006), Apprill, Marlow et al. (2009), Apprill, Marlow et al. (2012), Ainsworth, Krause et al. (2015)) and other DNA based studies to be carried out with minimal chemical contamination. Liquid nitrogen, however, is currently the most common method of preservation for analysis of both DNA and RNA. But access to liquid nitrogen and -80°C freezers are limited in remote areas, and the transport of liquid nitrogen is prohibited in planes and boats. Preservation with liquid nitrogen also presents a disadvantage for the shipment of samples in specialist dewars, which is time sensitive and expensive (Nagy 2010). Logistical considerations such as these are likely to impact the preferred method of preservation for any given study of the microbiome in coral and other marine organism samples. However, I show that direct comparisons of the composition in databases generated with the preparation methods are likely to be a reliable and accurate insight into the microbiome.

In selecting appropriate methodologies for generating microbiome datasets, it is essential to consider that there are steps in the DNA extraction process that can potentially produce cross-contamination and the homogenization protocol used is one of critical consideration. In this study, negative controls (no template) were used along the DNA extraction, amplification and sequencing, under the same conditions as other samples. These controls did not produce sequences; herein it is not possible to estimate which method is effectively more susceptible to crossed-contamination. However, based on the characteristics of the methodologies, I argue that the bead beating is less susceptible. Bead beating is highly reproducible and practical as the homogenization is carried out by a programmed machine and 24 samples can be homogenized at a time. Therefore the risk of cross-contamination between samples is low because there is little overlap in the handling of the samples (Lear, Dickie et al. 2018). Crushing samples with a French press or mortar/pestle is however widely employed homogenization method in the study coral holobiont microbiome degradation (*see* meta-analysis in Chapter 2, (Ng, Chan et al. 2015, Samodha, Wang et al. 2015, Shore-Maggio, Runyon et al. 2015, Zhang, Ling et al. 2015)). However, reproducibility is questionable since the homogenization with mortar and pestle is manual, and variable between samples and the number of samples to homogenise is dependent on the capacity to clean and sterilise the instruments and keep them frozen during the process to prevent the sample becoming mucus bound at room temperature. As such, the risk of cross-contamination is high, because the material is in contact with laboratory instruments and open to the environment while homogenization with mortar and pestle is carried out. Thus, I recommend that future studies apply a bead-beating approach to sample preparation rather than sample crushing.

In conclusion, these results indicate that comparisons of 16s rRNA databases across different preservation and homogenization methods should be restricted to overall microbiome composition and diversity of OTUs. Important variations were observed in criteria based on

the occurrence and relative abundance of phylotypes, herein comparisons relying in these attributes should be avoided across different preservation and preparation methodologies. Regardless of the methodology employed, the variability among coral colonies, as shown in the current study, raises the importance of adequate colony replication (Gray, Pratte et al. (2013) and this study). These results demonstrate the importance of replication when assessing the relative abundance and persistence of dominant or key bacteria. As it has been explored in the literature 16s rRNA amplicon sequencing has, as any sampling method, caveats and bias (Hamady and Knight 2009), and focusing in one attribute of the community limit the overall picture of the bacterial community. The literature offers many alternatives that vary in the degree of importance to the abundance and persistence of bacterial phylotypes; e.g. Abundance-Ubiquity test (Hester, Barott et al. 2015), Core microbiome (Ainsworth, Krause et al. 2015, Hernandez-Agreda, Gates et al. 2017) and Indicator species (De Cáceres and Legendre 2009). If the purpose of the study is to identify key bacterial species, the use of 16s rRNA amplicon and the exploration of relative abundance as well as the persistence of individual bacteria will contribute to select small groups bacterial phylotypes over which hypothesis can be raised (e.g. out of 100,000s bacterial phylotypes, select a group 20-100 bacterial OTUs for further exploration). The determination of the importance of those bacteria and the characteristics of the potential symbiosis with the host will depend on further analysis of the niche occupation, metabolic and physiological characteristics and the determination of a real symbiosis.

Chapter 4: The microbial signature provides insight into the mechanistic basis of coral success across reef habitats



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Introduction

Identifying specific bacteria that provide critical functional contributions to a host organism (and the ecosystem it is part of) requires an understanding not only of the bacterial population but of the persistence and stability in time and space of both the functional microbial niches and the bacteria that utilise them. Determining crucial functional bacteria is a challenging task given that bacterial communities tend to be both highly diverse and highly variable, and functional niches can be extremely difficult to identify in highly complex communities. Differentiating the bacterial associations with corals is an example of this challenge. As in all other natural systems, bacterial communities associated with corals are proposed to have important critical contributions to their health (Castillo, Lodeiros et al. 2001, Ritchie 2006), nutrition (Lesser, Falcon et al. 2007, Lema, Willis et al. 2012) and nutrient cycling (Raina, Tapiolas et al. 2009, Raina, Dinsdale et al. 2010). However, the microbiome associated with corals is one of the most complex and diverse, which has been studied to date (Blackall, Wilson et al. 2015). Corals harbour thousands of bacterial phylotypes and the communities they form vary structurally (composition and abundance) between coral species across geographical, spatial and temporal scales (Mouchka, Hewson et al. 2010, Garren and Azam 2012, Sharp and Ritchie 2012).

The structure of bacterial communities in corals has been shown to be highly variable and to respond to many biotic and abiotic factors (Mouchka, Hewson et al. 2010, Garren and Azam 2012, Sharp and Ritchie 2012). Biological events, such as algal competition, reproduction and diseases, as well as changes in environmental variables including temperature, pH, nutrients and dissolved organic carbon, generate shifts in the composition, richness and abundance of coral-associated bacteria (Thurber, Willner-Hall et al. 2009, Littman, Willis et al. 2011, Ceh, Raina et al. 2012, Thurber, Burkepile et al. 2012, Croquer, Bastidas et al. 2013, Morrow,

Bourne et al. 2014). Moreover, the responses of the bacterial community (and community members) differ between host coral species, as well as between stimuli (Sunagawa, Woodley et al. 2010). Thus, while there is evidence that the coral-associated bacterial communities change in response to disturbances, there is substantial confusion about the impact of underlying natural variability in patterns of coral-associated bacteria. For example, the coral endosymbiosis with the dinoflagellate *Symbiodinium* generates patchy microhabitats with different environmental conditions within an individual host (Ainsworth, Thurber et al. 2010). Bacterial communities differ along the host colony and between niche compartments, such as the surface mucus, the symbiosome (host-derived membrane enclosing *Symbiodinium* (Yellowlees, Rees et al. 2008)) and the skeleton (Sweet, Croquer et al. 2011, Ainsworth, Krause et al. 2015). Therefore, despite over a decade of research documenting coral-associated bacteria, the identities of specific bacteria playing essential roles in corals and their responses to biotic and abiotic variables, remain poorly characterized. A core microbiome approach, focused on the identification of ubiquitous bacteria rather than highly abundant bacteria, has been suggested as an alternative for differentiating stable and functionally significant coral-bacteria interactions, overcoming the complexity of bacterial communities, and functionally differentiating bacterial symbioses (Ainsworth, Krause et al. 2015, Hester, Barott et al. 2015).

The high degree of variability of the bacterial communities, the complexity of the coral host habitat and the coral reef environment, and the difficulties in identification of functionally important bacteria in corals, have together contributed to substantial uncertainty regarding the identity, role, and significance of bacterial symbioses on corals. Addressing this uncertainty requires a comprehensive analysis of the diversity, commonality and rarity of bacterial phylotypes on coral hosts. To do so, sample sizes (number of individual hosts investigated) need to be greatly increased, as does the diversity of reef habitats for the same host species. The environmental-generalist coral *Pachyseris speciosa* (Figure 4-1) is one coral species that

is found in most reef environments of the Great Barrier Reef and the Coral Sea (Veron 2000, Bongaerts, Bridge et al. 2011), and as such, represents an ideal model to test the bacteria-persistence hypothesis (i.e. presence of ubiquitous bacteria within hosts across diverse habitats). The Great Barrier Reef (GBR) represents the largest coral reef ecosystem in the world, extending over 2,300 km (14 degrees of latitude) and encompassing a surface area of 348,000 km^2. The adjacent Coral Sea Commonwealth Marine Reserve (CSCMR) (989,842 km^2) is located east of the GBR and represents a vast region (989,842 km^2) containing numerous coral atolls that, to date, have remained mostly unstudied ("Coral Sea Commonwealth Marine Reserve - Overview", 2016). Host-microbiome interactions and/or symbioses are potential mechanisms by which environmental-generalist coral species can successfully occupy a broad range of reef habitats. Here, I characterize the bacterial community of *P. speciosa* from reefs across the GBR and the Coral Sea.

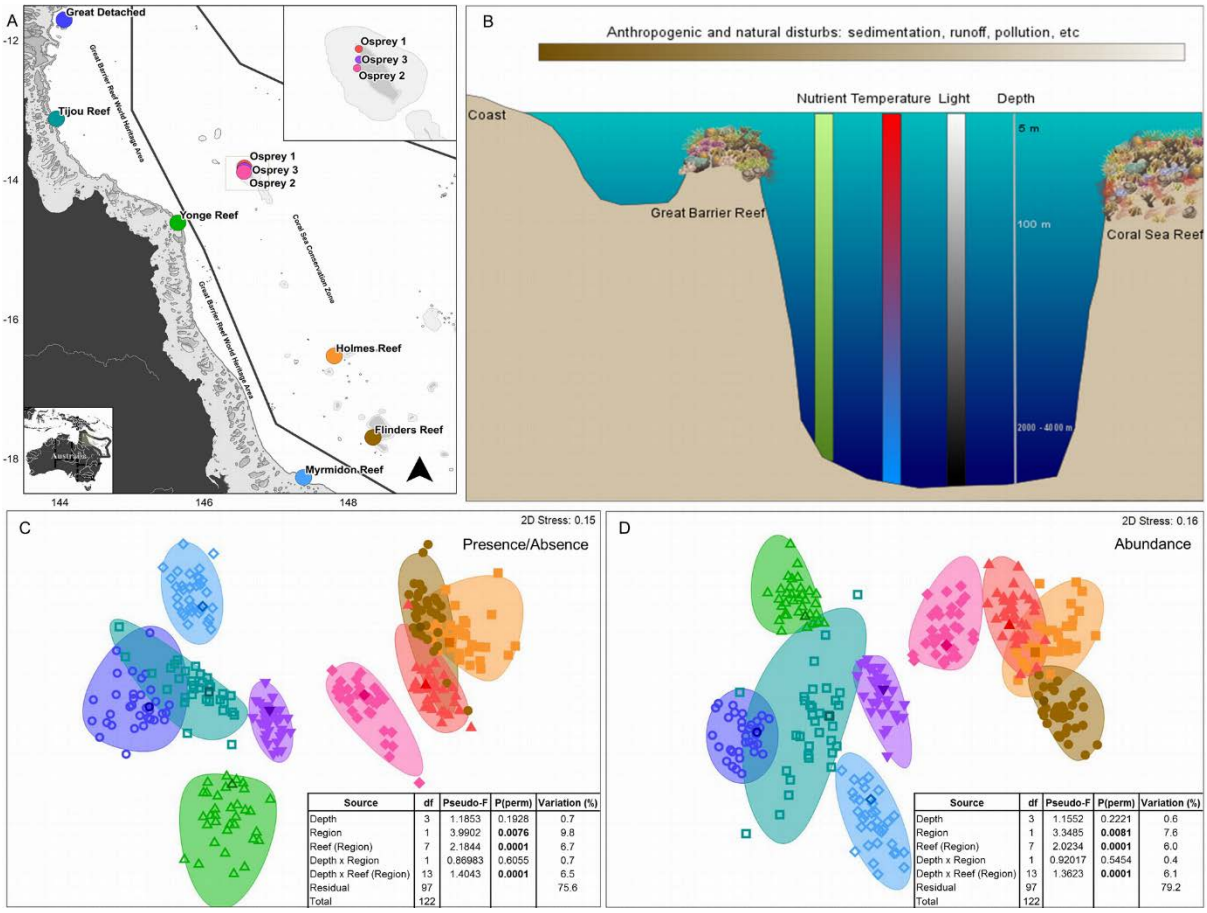
Figure 4-1: Host *Pachyseris speciosa*, a depth-generalist coral.

Materials and Methods

Experimental design and samples preservation

Fragments of the plating coral *P. speciosa* (Figure 4-1, *n*=123) were collected from reefs of the Great Barrier Reef and the Coral Sea (Figure 4-2A, Supplementary Table D-1) during Catlin Seaview Survey expeditions. Corals were sampled on SCUBA at shallow and intermediate depths (~10 m, ~20 m and ~40 m) during September - December 2012, whereas deep coral samples (60-80 m) were collected using a remotely operated vehicle (ROV) in November 2013. Here I utilised a nested hierarchical design considering the following three factors: a) Depth (fixed factor), four levels: 10 m (± 3 m), 20 m (± 2 m), 40 m (± 3 m), 60 to 80 m; b) Region (fixed factor, Figure 4-2A), with two levels - Great Barrier Reef (GBR), Coral Sea (CS); and c) Reefs (random nested in Region, Figure 4-2A), with nine levels - Great Detached, Tijou Reef, Yonge Reef and Myrmidon Reef in GBR and Osprey 1 (Dutch Towers), Osprey 2 (Halfway Wall), Osprey 3 (Bigeye Ledge), Holmes Reef and Flinders Reef in CS. Lower mesophotic depths (60 to 80 m) were only sampled in the Coral Sea, whereas the intermediate depth of 20 m was only sampled at Osprey 1 to 3. Four or five coral fragments were collected per depth in each reef.

Coral fragments (~3 cm^2) were preserved in salt-saturated 20% dimethyl sulfoxide (DMSO) - 0.5M EDTA and stored at -20 °C. Sample collection was under permits supplied by the Great Barrier Reef Marine National Park Authority (Townsville, Australia) and Commonwealth Marine Reserves, Department of the Environment (Hobart, Australia).



Source	df	Pseudo-F	P(perm)	Variation (%)
Depth	3	1.1853	0.1928	0.7
Region	1	3.9802	**0.0076**	9.8
Reef (Region)	7	2.1844	**0.0001**	6.7
Depth x Region	1	0.86983	0.6055	0.7
Depth x Reef (Region)	13	1.4043	**0.0001**	6.5
Residual	97			75.6
Total	122			

Source	df	Pseudo-F	P(perm)	Variation (%)
Depth	3	1.1552	0.2221	0.6
Region	1	3.3485	**0.0081**	7.6
Reef (Region)	7	2.0234	**0.0001**	6.0
Depth x Region	1	0.92017	0.5454	0.4
Depth x Reef (Region)	13	1.3623	**0.0001**	6.1
Residual	97			79.2
Total	122			

Figure 4-2: Biographic differences in coral-associated bacteria (holobiont) structure of *P. speciosa*. A) Study sites in the Great Barrier Reef (GBR, left) and Coral Sea (CS, right). B) Environmental factors changing in depth and with closer proximity to the coast. C) Differences in bacterial composition between GBR and CS and between reefs (nMDS, Bray-Curtis; inset PERMANOVA table). D) Differences in bacterial abundance between GBR and CS and between reefs (nMDS, Bray-Curtis, data: fourth root transformed, sample standardized by total; inset PERMANOVA table). Factors: i) Region: Great Barrier Reef (GBR) and Coral Sea (CS); ii) Reef(Region): Great Detached (GBR), Tijou Reef (GBR), Yonge Reef (GBR), Myrmidon Reef (GBR), Osprey 1 (CS), Osprey 2 (CS), Osprey 3 (CS), Holmes Reef (CS) and Flinders Reef (CS); iii) Depth: 10 m, 20 m, 40 m, 60-80 m. Variation (%) refers to components of variation.

DNA extraction and sequencing

DNA was extracted from approximately 1.4 gr (± 0.2 gr) of each coral fragment using a modified protocol from MOBIO PowerPlant Pro DNA Isolation Kit (MoBio, Carlsbad, CA, USA: Cat. No. 13400-50). As described by Sunagawa, Woodley et al. (2010), the modification of the MoBio protocol consisted of digesting samples in Proteinase K (final concentration, ≈0.8 mg mL^{-1}; Invitrogen) at 65 °C for 30 min after homogenization. The purity and quantity of

bacterial DNA were determined using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, USA) and PCRs. Samples were held at -20 °C before PCR amplification.

To determine the composition of the bacterial assemblage and the relative abundances of its members, bacterial 16S rRNA gene amplicons were amplified from genomic template primers 27F/519R in a single-step 30-cycle PCR (HotStarTaq plus master mix kit; Qiagen, USA). PCRs were conducted under the following conditions: 94°C for 3 min, followed by 28 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 1 min, followed by a final elongation step at 72°C for 5 min. After the amplification, to check the success of amplification and the relative intensities of the bands, amplicon products were checked in 2% agarose gel and based on molecular weight, and DNA concentrations, amplicon products from different samples were pooled in equal proportions. Pooled samples were purified utilizing calibrated Ampure XP beads and sequenced using Illumina TruSeq DNA library preparation protocol (MR DNA, Shallowater, TX, USA). Sequences have been submitted to the National Center for Biotechnology Information (NCBI) Short Read Archive (SRA) under the project number PRJNA328211.

Sequence analysis

Sequence data were analysed using Quantitative Insights Into Microbial Ecology [QIIME] (Caporaso, Kuczynski et al. 2010). Barcodes, primers and short sequences (<200 bp) were removed, and sequences with ambiguous base calls and with homopolymer runs exceeding 8 bp were discarded. The sequences were denoised and chimeras removed. Operational Taxonomic Units (OTUs) were defined with clustering at 97% similarity. Taxonomy was assigned to OTUs in QIIME using RDP classifier (Wang, Garrity et al. 2007) against a curated Greengenes database (DeSantis, Hugenholtz et al. 2006). Chloroplast and unidentified OTUs were excluded from the OTU table.

Statistical analysis and Core microbiome

Statistical analysis and data mining was conducted using PRIMER v7 + PERMANOVA (Anderson 2001). As our hypothesis was focused on the determination of highly persistent bacteria in corals, bacteria present in less than 5% of the samples (≤6 samples) were excluded from the analyses, as they were considered rare bacteria in the coral-associated assemblage (the whole assemblage is evaluated in Chapter 5). This filter reduced the number of phylotypes from 173,690 to 4,446 OTUs. Normalized relative abundance was obtained using a fourth root transformation and standardization by sample by the total. The matrix of abundances was converted to presence/absence to analyse the composition of the bacterial assemblage. For both matrices, significant differences in the bacterial assemblages were identified by permutational multivariate analysis of variance (PERMANOVA) using Bray-Curtis distances and explanatory variables as listed above in *Experimental design and samples preservation*. Observed patterns (significant differences at any level) were evaluated with a pairwise comparison. Statistical significance of the F test was assessed with 9,999 permutations. To visualise PERMANOVA results, nonmetric dimensional scaling (nMDS) plots using 95% bootstrap regions and averages for the factor Reef were generated from Bray-Curtis similarity matrices of relative abundance and presence/absence data.

The core microbiome of all the data and for each of the factors considered in the experimental design and their combinations were identified using QIIME. Phylotypes consistently present in >80% of the samples were considered highly persistent bacteria, a conservative representation of the core microbiome, selected based on previous research on core microbiome annotations (Ainsworth, Krause et al. 2015). Phylotypes present in 50-79% of the samples were considered persistent bacteria, whereas OTUs not consistently present in at least 50% of the samples were taken as natural variability across colonies.

A dendrogram was constructed using the Interactive Tree of Life software (http://itol.embl.de) (Letunic and Bork 2007, Letunic and Bork 2011) from a phylogenetic tree produced in QIIME. Venn diagrams were generated from 50% and 80% core microbiome data and visualized using Venn diagram software (Bioinformatics and Evolutionary Genomics, http://bioinformatics.psb.ugent.be/webtools/Venn/).

Meta-analysis

Sequences of the phylotypes that were part of the 50% and 80% core microbiome were searched against the nucleotide database of the National Center for Biotechnology Information (NCBI) using Basic Local Alignment Search Tool (BLAST) algorithm. Moreover, to determine whether the 50% and 80% core microbiome phylotypes have been reported as part of specific coral microhabitats, the same sequences were compared with the *Acropora granulosa* nucleotide database (Ainsworth, Krause et al. 2015) using a BLAST search. Based on the nucleotide database, sequences with ≥97% identity were classified in four categories and their combinations: a) not reported in corals, b) symbiotic, c) endosymbiotic, and d) holobiont (in other corals). The category *symbiotic* represent the bacteria reported in coral tissue, composed by endosymbiotic and episymbiotic tissue regions, and *endosymbiotic* correspond to coral endodermal cells, excluding skeleton and mucus (Ainsworth, Krause et al. 2015). *Holobiont* constitutes bacteria reported in other coral species as part of the whole bacterial assemblage. Sequences annotated as chloroplast were not considered in the analyses.

Map of sites

The map of sites was produced with the software QGIS using the Group Layer 'GBRMPA features', data courtesy of the Great Barrier Reef Marine Park Authority (GBRMPA), Copyright Commonwealth of Australia (2007). Bathymetry was obtained from “Great Barrier Reef and Coral Sea Bathymetry” dataset (Beaman 2010), available in www.deepreef.org.

Results and Discussion

I propose that the coral holobiont of the environmental-generalist coral *P. speciosa* should be considered as three functionally different fractions, as follows: a ubiquitous core microbiome consisting of a small group of bacteria that are persistent across spatial scales and along depth gradients and are likely to be symbiotic. Second, a spatially and/or regionally explicit core microbiome that is composed of bacteria found consistently in individuals within specific environmental regimes and that likely aid coral success within the environment. Third, a highly variable bacterial community that is responsive to processes occurring at both large (hundreds of kilometres; for example, reef regions) and small (meters; for example, depth ranges) spatial scales.

The bacterial community of P. speciosa

An Operational Taxonomic Unit (OTU) database containing 4,176,251 high quality reads and comprising 173,690 OTUs was generated for all corals (n=123) sampled within the study. OTUs with a percentage of occurrence <5% were excluded (i.e., those found in less than 6 of the 123 coral samples) as these were considered to be transient members, which reduced the number of OTUs to 4,446 phylotypes. No clustering was observed by collection dates (Supplementary Table D-1, Figure D-1).

I find that the bacterial community structures are different between regions, reefs, and in some reef locations, between depths (Figure 4-2A, 4-2C and 4-2D, Supplementary Tables D-2 to D-7). Biotic and abiotic processes occurring at those regional scales (factor Regions, scale 10-100 km) are likely to substantially influence coral holobiont bacterial communities, both regarding composition and abundance. The Coral Sea reefs are in oceanic waters, where variables like flow rate, mixing and tidal currents, temperature and concentration of nutrients are vastly different than in the reefs of the Great Barrier Reef lagoon (Figure 4-2B).

Bacterioplankton, biofilms, and coral holobiont bacterial communities have previously been shown to be responsive to alterations in water quality (Thurber, Willner-Hall et al. 2009, Morrow, Moss et al. 2012, Lema, Willis et al. 2014). Moreover, environmental variables like nutrient concentration, temperature, and light vary significantly across a reef depth gradient (Figure 4-2B). Differences in water quality and oceanography between the reefs from the GBR and the CS could be affecting the structure of coral-associated bacteria. However, there is currently a substantial lack of information about how bacterial communities in corals change in relation to reef depth since 1) variation in these factors are site specific (evidenced in our results), 2) these factors have been evaluated in isolation; and 3) bacterial communities in corals have been studied principally at shallow depth (0-30 m), and only sparsely studied in mesophotic reef zones (depths from 30m to 200 m) (Bruck, Bruck et al. 2007, Lesser, Falcon et al. 2007, Santiago-Vazquez, Bruck et al. 2007). These findings here support previous studies that have indicated that coral-associated bacteria communities (holobionts) are highly responsive to environmental conditions that can change over distances ranging from meters (as in depth gradient) to hundreds of kilometres (between reefs and regions).

Persistent bacteria: a core microbiome

These results provide the most comprehensive evidence to date for the presence of a small group of bacteria that are ubiquitously associated with corals regardless of abiotic environmental factors. Despite the high diversity and variability of coral-associated bacteria found across spatial scales, I found that of the 173,690 bacterial phylotypes recovered from *P. speciosa* only 9 bacterial phylotypes are present in over 90% of coral individuals (red labels Figure 4-3), and only 97 phylotypes are found in over 50% of individual coral colonies. Traditionally, coral-associated bacteria have been analysed by focusing on highly abundant bacteria, regardless of their occurrence across individual corals. However, abundance measures

are known to be biased by the method of sample preparation, sample handling and data generation (Hamady and Knight 2009, Shade and Handelsman 2012, Lynch and Neufeld 2015). These results clearly show that studies focusing on the importance of highly abundant bacteria overlook the frequently occurring bacteria that are generally in relatively low abundance and/or rare in the whole colony (or holobiont) community analyses (inset Figure 4-3). The meta-analysis (Nucleotide BLAST of the National Center for Biotechnology [NCBI] database) reveals that of the 97 bacterial phylotypes consistently present in over 50% samples, 49 have been previously reported in specific coral microhabitats (symbiont and endosymbiont) and/or as part of coral microbiota (Supplementary Table D-9). These results highlight the importance of considering persistence instead of abundance to define potentially functional important bacteria in association with reef-building corals. I also find that only two bacterial phylotypes are both highly persistent (ubiquitous) and highly abundant within community analyses (OTUs 306 and 25296, Figure 4-3). These two bacteria are both novel reports for within the coral microbiome. The novel identification of two highly abundant ubiquitous bacteria is likely the result of two factors: the large sampling design of the current study (*n*=123 corals, the largest undertaken to date) and the application of Illumina sequencing technology, allowing a great depth of sequencing within the coral microbiome. This result highlights that the application of sampling designs with greater depth of coverage is likely to be crucial in the identification of potential symbiotic bacteria within high diversity (>170,000) coral community analyses.

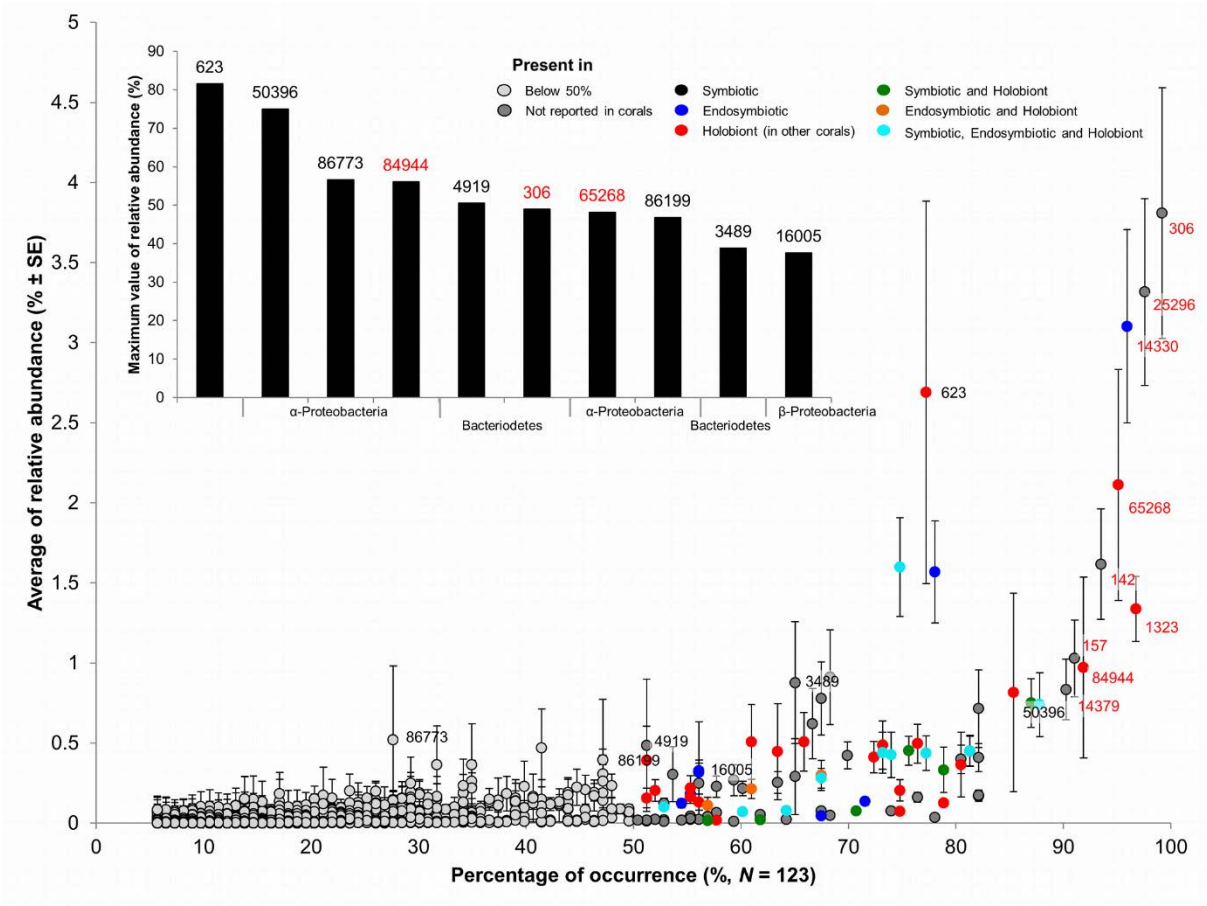


Figure 4-3: Comparison average of relative abundance and percentage of occurrence. Each point represents an Operational Taxonomic Unit (OTU). Point colours indicate microhabitats were these OTU have been previously reported (NCBI, identities ≥97%). Labels display OTUs numbers; in red, the top nine OTUs with highest percentage of occurrence. *See* Supplementary Table D-8 for complete taxonomic identification. Bar chart shows the top ten OTUs with highest maximum values of relative abundance and their taxonomic classification.

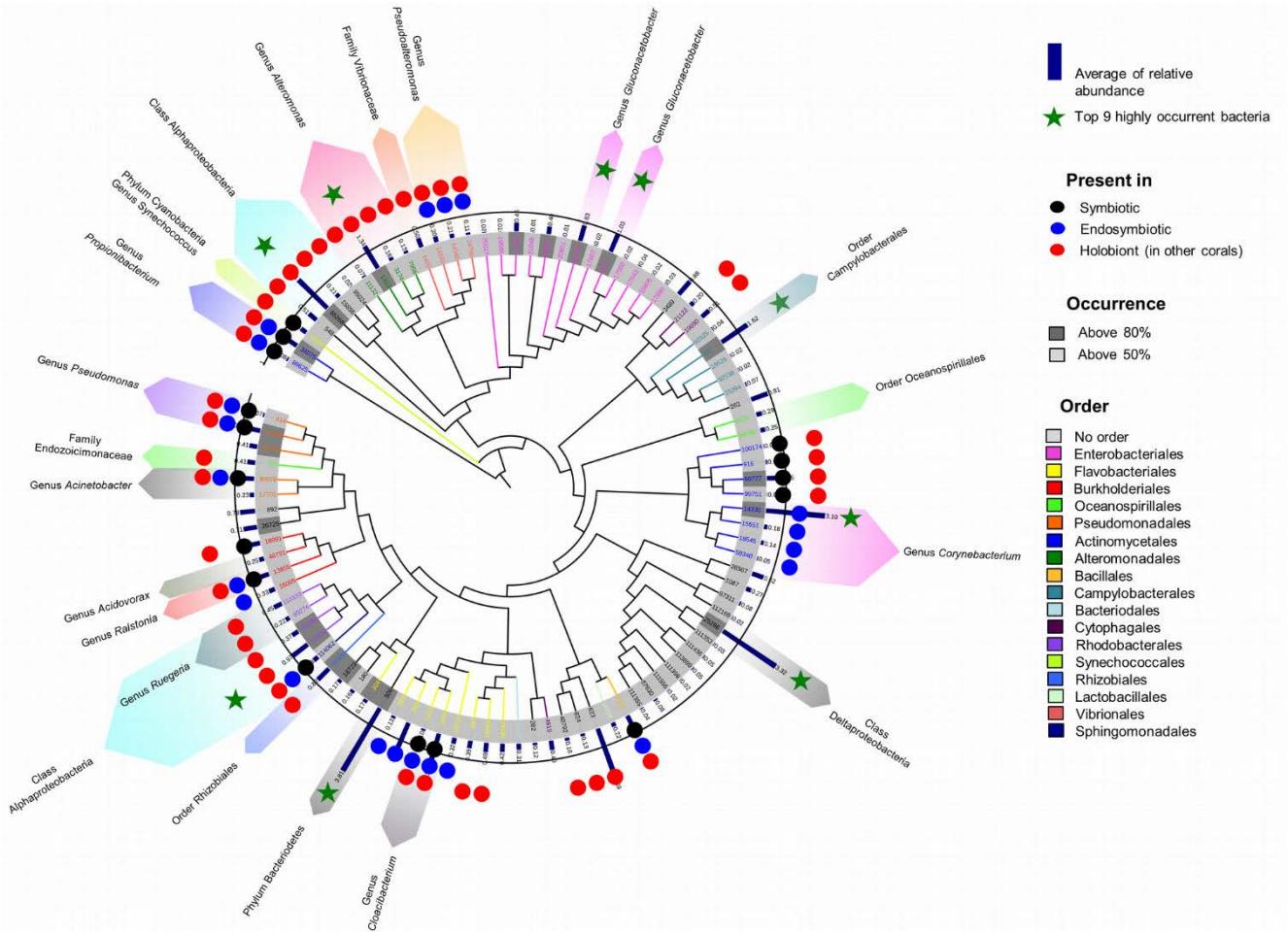


Figure 4-4: Dendrogram (Tree of life) of 97 bacteria with high percentage of occurrence (≥50%). External blue bars represent average of relative abundance; circles, the microhabitats were OTUs have been previously reported (NCBI, identities ≥97%); and intensity of grey shades, the range of percentage of occurrence. Colours of the leaf indicate the taxonomic Order. The top nine persistent bacteria are identified with a green star. With colour rows, other bacteria groups recognized as important bacteria in corals. *See* Supplementary Table D-8 for complete taxonomic identification and relevant citations.

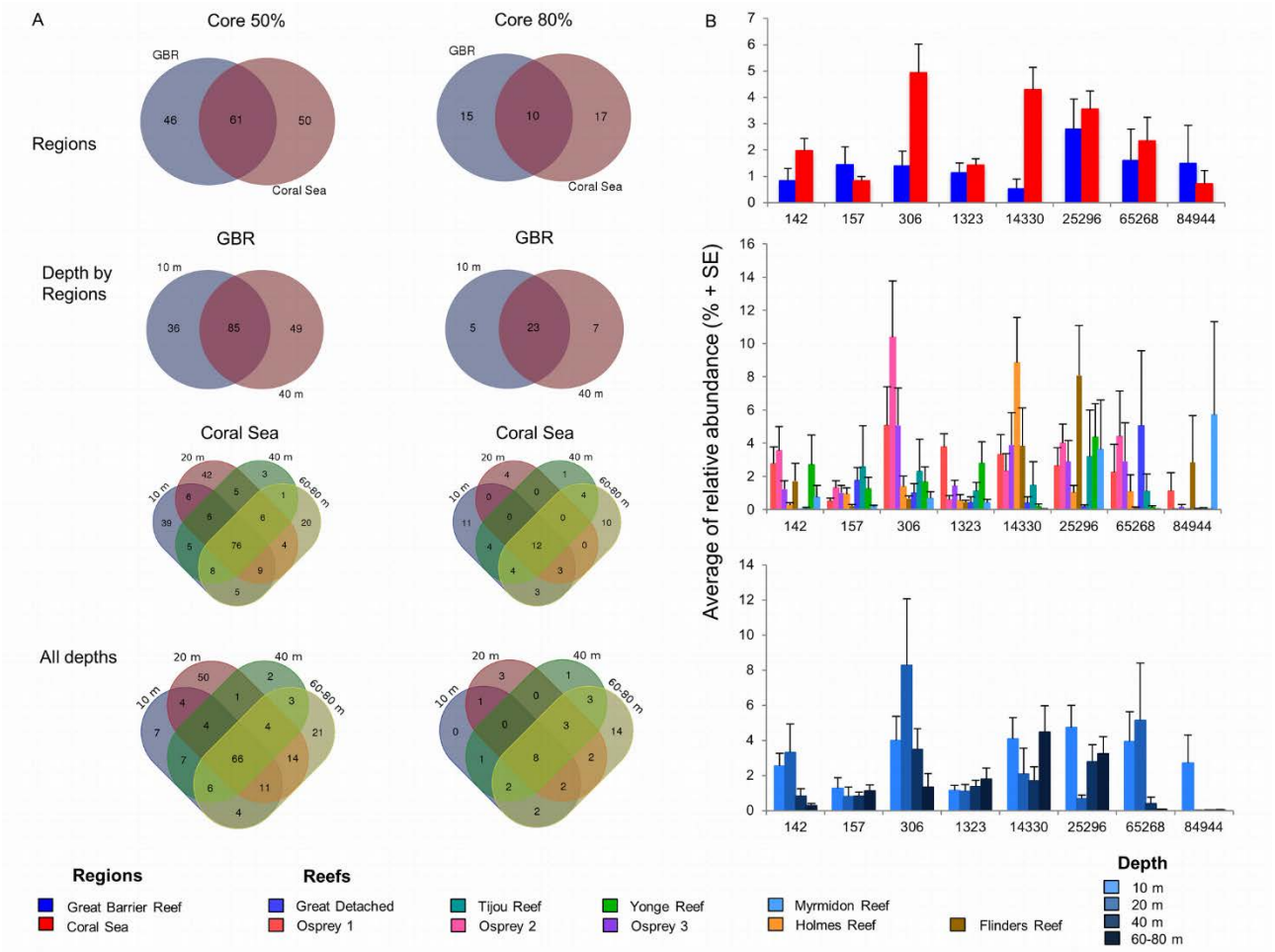


Figure 4-5: Presence of the eight highly persistent bacteria in coral core microbiome. A) Venn diagram of Regions, Depth by Regions and All depths using 50% and 80% as percentage of occurrence to define coral core microbiome. Numbers inside the area represent number of OTUs part of the core microbiome; in the intersection, number of OTUs in common. B) Average of relative abundance of the eight highly persistent bacteria between Regions, Reefs and along depth gradients. *See* Supplementary Table D-8 for complete taxonomic identification.

In the current study, bacterial phylotypes persistently found in over 50% of the samples (designated the core microbiome in *P. speciosa*) predominantly belong to the phyla *Proteobacteria* (61.9%), *Actinobacteria* (10.3%), *Bacteriodetes* (17.5%), *Cyanobacteria* (1%) and *Firmicutes* (2.1%). In the phylum Proteobacteria, 53.3% of the core OTUs are class Gammaproteobacteria, whereas Alpha- (16.7%), Beta- (6.7%), Delta- (13.3%) and Epsilonproteobacteria (8.3%) have lower percentages of representation (Supplementary Tables D-8, D-9). From nine highly persistent bacterial phylotypes (defined as core due to their presence in ≥90% of all coral colonies, Figure 4-4), four were identified as belonging to genera *Corynebacterium*, *Alteromonas* and *Gluconacetobacter*; whereas the rest were assigned to higher taxonomic levels. The phylotypes with the highest levels of occurrence, OTUs 306 and 25296, were identified as phylum *Bacteriodetes* and class *Deltaproteobacteria*, respectively (Figure 4-4) and are novel reports for corals (Figure 4-3). Similarly, OTUs 142, 84944 and 65268 were assigned to orders *Campylobacterales* and *Rhodobacteraceae* and class *Alphaproteobacteria*, respectively; the last two found as part of the holobiont bacteria community in previous studies (Reis, Araujo et al. 2009, Séré, Tortosa et al. 2013), whereas OTU 142 is also a novel report in corals.

Eight of the nine highly persistent and ubiquitous bacteria are present in both reef regions (the Coral Sea and GBR) and all depths (10-80 m), but the abundance of each phylotype was found to vary between regions and reefs and along the depth gradient (Figure 4-5A and B, Supplementary Table D-9). Three of the core bacteria have previously been shown to contribute to defence against pathogens and to nutrient intake in other organisms. Members of the genus *Corynebacterium* can uptake and metabolise urea (Siewe, Weil et al. 1998) as a nitrogen source and synthetize pyrazine (Dickschat, Wickel et al. 2010), a precursor of antibiotic, antitumor and diuretic substances in humans. Members of the genus *Alteromonas* have been reported as part of coral mucus and skeleton bacterial communities and can

metabolize dimethylsulfide (DMS) (Raina, Tapiolas et al. 2009), an essential organic compound in the cycling of sulphur, and to incorporate and translocate nitrogen into zooxanthellae in coral larvae (Ceh, Kilburn et al. 2013). Moreover, members of the genus *Alteromonas* can produce isatin, an antibiotic (Majik, Rodrigues et al. 2014) and antifungal (Gil-Turnes, Hay et al. 1989) compound in marine organisms. Bacteria of the genus *Gluconacetobacter* are diazotrophic and colonize intracellular spaces and vascular tissues in sugarcane and rice plants (Carvalho, Balsemao-Pires et al. 2014). As well as contributing to nitrogen fixation, *Gluconacetobacter* bacteria produce plant growth hormones, improving nutrient acquisition (Carvalho, Balsemao-Pires et al. 2014) and stimulating plant defence response (Arencibia, Vinagre et al. 2006). In corals, this genus has been reported in *Montipora* corals as part of the diazotrophic bacterial community (Olson, Ainsworth et al. 2009); however, these particular phylotypes are novel in the literature on corals. The ubiquity of these eight core bacteria in corals across such vast geographic (several degrees of latitude and two distinct regions) and environmental gradients (10-80 m depth gradient) suggests a highly stable symbiosis between corals and these bacterial phylotypes. The identification of potential key bacterial symbioses thus enables us to differentiate important bacteria and gives rise to hypotheses about how and when symbioses occur, and how critical functional roles are accomplished. Similarly, determining explicitly conserved interactions across individual corals from different regions and depths can allow us to determine potential interactions that aid coral success under vastly different environmental regimes. However, differences in the annotation of core or ubiquitous bacteria between studies are likely, due to several factors, including the type of host species, reef locations, depth of sequencing undertaken, degree of host replication, and methodology or criteria used for determining occurrence across samples (Garcia, Croquer et al. 2004, Ainsworth, Krause et al. 2015, Blackall, Wilson et al. 2015, Hester, Barott et al. 2015, Lynch and Neufeld 2015).

Spatially variability in core microbiomes

I therefore also further identify the potential for spatially explicit core microbiomes in *P. speciosa*: the annotations of core bacteria were analysed independently for both presence in 50% of the samples, and in 80% of the samples from each region, depth and depth by region. The GBR and CS corals were found to have different core microbiome communities (Figure 4-5). However, there is a group of bacteria that are common, and therefore independent of the environmental variation between regions. Similar outcomes were observed for depth gradient per region and at all depths. For example, the 80% core microbiome for each of the ocean depths (i.e., determined separately between 10 m and 80 m) is constituted principally of the eight highly persistent (core) bacteria, thereby providing evidence for a symbiosis that can adjust to environmental conditions. An additional 14 phylotypes are also evident in 80% of samples from the 60-80 m depth range, suggesting that there are habitat partitioning and ecological diversification in core bacterial associations of corals related to reef depth. Niche differentiation and higher genotypic diversity at mesophotic depths have also been observed for coral hosts and their photosymbiotic partners, the *Symbiodinium* dinoflagellates, and this could indicate a bacterial community adaption, or facilitation, to deep environmental conditions (e.g. light, temperature and nutrient availability) (Frade, De Jongh et al. 2008, Bongaerts, Riginos et al. 2010, Bongaerts, Frade et al. 2013, Bongaerts, Frade et al. 2015).

Three functionally different fractions of the coral holobiont

These data support the hypothesis that three functionally distinct bacterial fractions represented *P. speciosa* microbiome. The symbiotic fraction is represented by a group of ubiquitous core bacteria that are likely to be highly conserved in corals. These eight ubiquitous bacteria are from the classes Actinobacteria, Alpha-, Delta-, Epsilon- and Gammaproteobacteria and the phylum Bacteriodetes. High specificity in host-microbe interactions and symbioses has been

observed in other natural systems, as it is the case of squid-vibrio symbiosis. During the embryogenesis, bobtail squid, *Euprymna scolopes,* develops appendages covered by cilia through which *Vibrio fisheri* colonization occurs. *E. scolopes* squid do not establish symbiosis with any other bacteria, and the cilium appendages are lost once the symbiosis is established, making this one of the most specific bacterial symbiosis studied to date (Nyholm and McFall-Ngai 2004). The contribution and mechanisms of selection for highly specific, core interactions also need to be investigated in coral, since by doing so, we can have greater insight into the capacity for bacterial symbioses to provide ecological advantages to coral.

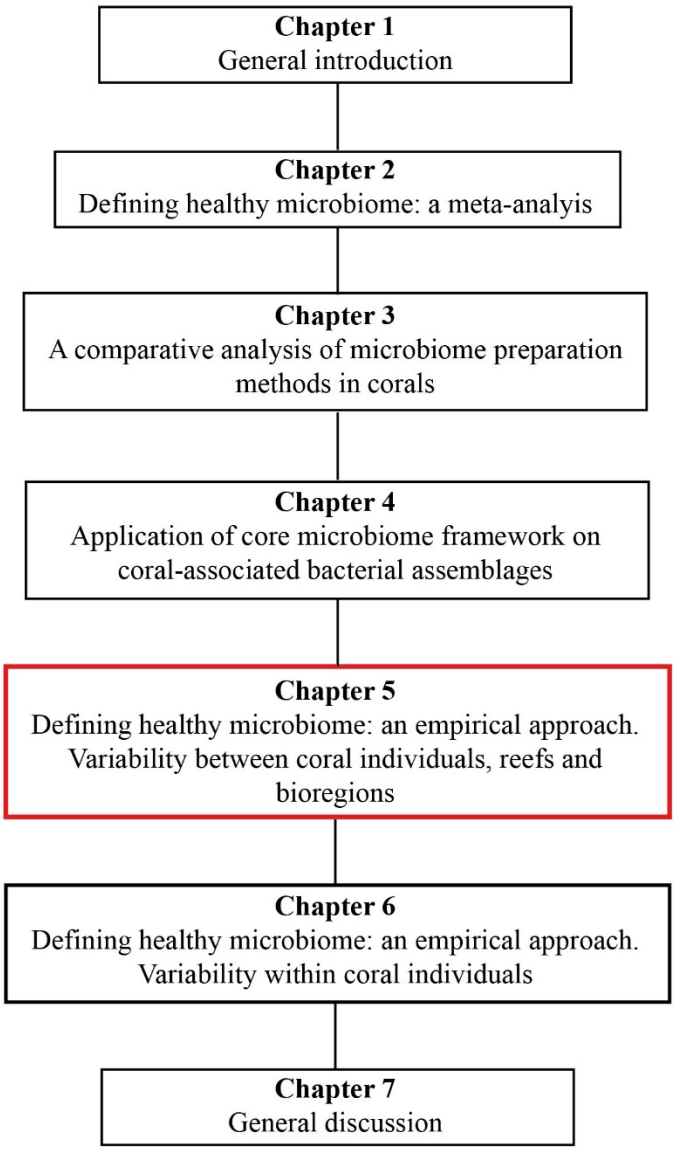
I also provide evidence for a functional niche fraction of the coral microbiome. Bacteria that are persistent in specific environmental regimes that are likely to contribute to coral success in particular habitats are characteristic of this functional niche. For example, in *P. speciosa,* this niche fraction is filled by the 14 phylotypes persistently present in the 60-80 m depth range, composed by classes *Actinobacteria*, *Bacilli*, *Flavobacteriia*, *Synechococcophycideae*, *Alpha-*, *Beta-*, *Delta-* and *Gammaproteobacteria*. Many similar examples of functional niches have been presented for plants, where bacteria present in the rhizosphere (habitat directly surrounding the root) assist the plant in overcoming abiotic stresses like drought, high and low temperatures, salinity, flooding, heavy metals, organic pollutants and nutrient deficiency (*see* review in Selvakumar, Panneerselvam et al. (2012)). Soil and host type, as well as developmental stage, are factors that influence the rhizospheric microbiome (Bulgarelli, Rott et al. 2012, Lundberg, Lebeis et al. 2012, Chaparro, Badri et al. 2013).

P. speciosa also hosts numerous bacteria (hundreds of thousands) whose occurrence and abundance are highly variable, and these are likely to be highly responsive to biotic and abiotic processes occurring at diverse spatial scales. This fraction of the bacterial community could principally inhabit coral mucus, which is high in nutrients and has a fast turnover and rapidly

changing abiotic conditions. The surface mucus environment, being the most external coral microhabitat, is exposed to and directly affected by changes in the marine environment, including nutrient fluctuations in the water column, water flow, sedimentation (Brown and Bythell 2005, Sweet, Croquer et al. 2011, Morrow, Moss et al. 2012, Li, Chen et al. 2014). These micro- and macro- scale conditions likely create a fluctuating biotic and abiotic environment that attracts and supports a large diversity of bacteria that can colonise microniches, form biofilms, and utilise nutrients.

The occurrence of eight bacteria within over 100 individual *P. speciosa* colonies collected from 9 geographically distinct coral reefs and at depths of 10m to 80 m provides substantial evidence for the existence of a coral core microbiome and stable bacterial symbioses in corals. To understand the long-term stability of coral bacterial symbiosis, as well as its universality, it is now crucial to test the hypothesis of core microbiome ubiquity across coral species and temporal scales. The functional role of the core microbiome will be affected mainly by the host's environment and the conditions in the microhabitat within the host where these bacteria exist; therefore, it is crucial to determine precisely where in the coral host these bacteria reside. Meta-analysis suggests highly likely these bacteria are found in close association with the coral tissues (Supplementary Table D-9). Results in this chapter provide the first evidence of higher diversity in the bacterial communities and core microbial associations of corals existing in the mesophotic zone of reefs. Furthermore, the high bacterial diversity in corals collected from deeper reefs suggests that there are functional niches in which corals can adapt their microbial associations to suit the environmental conditions and utilise available nutrients. In vulnerable ecosystems, like coral reefs, the evaluation of hosts and their symbioses in time and space is fundamental to understanding how these organisms and the ecosystems they support will be impacted by climate change and to what extent they will be able to overcome it.

Chapter 5: Rethinking the coral microbiome. Simplicity exists within a diverse microbial biosphere



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Introduction

Deciphering the functional contribution of symbiotic bacteria to host health is imperative to determine the mechanistic basis of coral health, survival, and resilience in a rapidly changing environment. However, to date, the challenge remains to understand which of the thousands of bacteria that are in association with a particular coral host species (*species-specific microbiome*) have a significant contribution to the well-being of individual corals in their natural habitat (Knowlton and Rohwer 2003, Ainsworth and Gates 2016, Bourne, Morrow et al. 2016). Accurately documenting the taxonomic structure of the coral microbiome has been crucial to this aim and over the past decade, numerous studies have aimed to define the characteristics of the healthy coral microbiome (Bourne, Morrow et al. 2016, Hernandez-Agreda, Gates et al. 2017). Importantly though it is the interactions of the individual with its' microbiome (*individual microbiome*) that impact the coral health. Distinguishing the individual's microbiome from that of species-specific microbial consortia is therefore critical to identify the symbiotic microbial roles. Here I argue that the diversity, structure and potential function of the coral microbiome are much lower and less complex than has previously been reported. I show that over 96% of the microbiome associated with a coral species (as a whole) are in fact not found in association with the majority of coral individuals. I, therefore, propose that the coral microbiome can be conceptualised into 3 distinct layers (Figure 5-1): (1) the *environmentally responsive community*, which is predominantly a transient community encompassing thousands of distinct bacterial phylotypes, of which very few are consistently associated with a single host individual, (2) the *resident community* consisting of phylotypes principally from three critical bacterial classes; and (3) the *core microbiome* consisting of a few ubiquitous, potentially symbiotic, bacterial phylotypes.

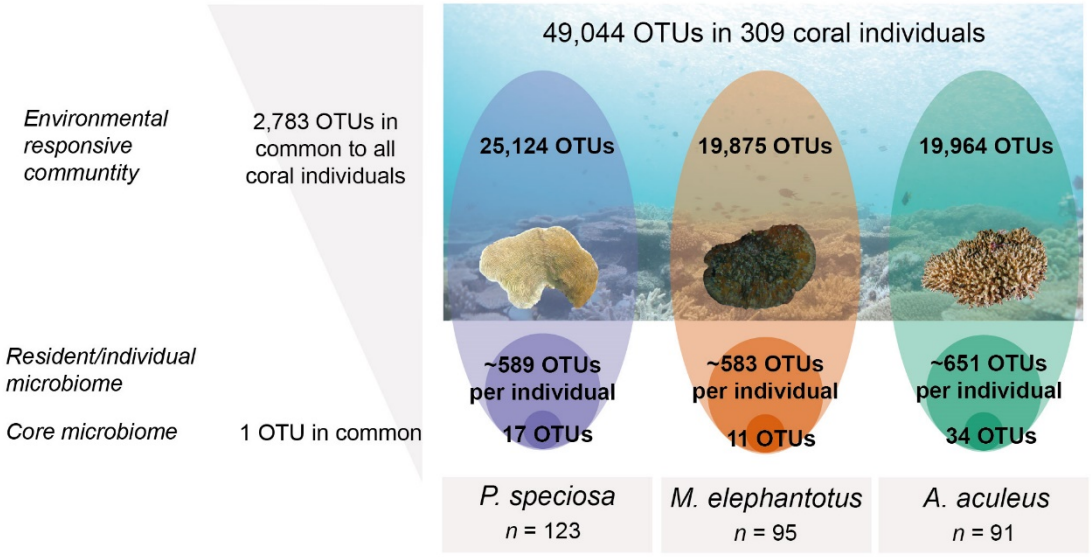


Figure 5-1: Coral microbiome conceptualised into three distinct layers. I propose to understand microbiome of each coral individual as three distinct groups of bacteria with different levels of impact from the environment and the host. An *environmentally responsive community* with thousands of bacterial phylotypes, transient and highly variable across coral individuals; *individual microbiome*, ~500-600 OTUs variant among reefs at the level of OTUs but consistently belonging to three major taxonomical classes; and *the core microbiome*, few bacterial phylotypes, potentially symbiotic. Taxonomical and functional redundancy is potentially occurring in the *resident community* and *core microbiome*. Reef picture: Alexander J Fordyce.

Across ecological systems, the concept of taxonomical and functional redundancy has been employed to characterise and conceptualise healthy and disturbed ecosystem states (Bellwood, Hughes et al. 2004, Moretti, de Bello et al. 2009, Carmona, Azcarate et al. 2012, Lozupone, Stombaugh et al. 2012). Here I investigate the microbiome of 309 individuals for three highly abundant and widespread Indo-Pacific species (DeVantier and Turak 2017), the plating corals *Mycedium elephantotus* and *Pachyseris speciosa* and the branching coral *Acropora aculeus.*

Materials and Methods

Coral collection and preservation

Fragments of corals *Mycedium elephantotus* (*n*=95) and *Acropora aculeus* (*n*=91) were collected from 10 reefs from northern reefs of the Great Barrier Reef and the Coral Sea during

Caitlin Seaview Survey expeditions. Coral specimens were collected at shallow and intermediate depth (10 to 40 m) between September and December 2012, and at mesophotic depth (60 to 80 m) in November 2013 (Supplementary Table E-1). Temporal clustering was not observed in the samples (Figure 5-2, Supplementary Figure E-1). Fragments of corals *Mycedium elephantotus* (*n*=95) and *Acropora aculeus* (*n*=91) were collected from 10 reefs from northern reefs of the Great Barrier Reef and the Coral Sea between September and December 2012, in November 2013, and in November-December 2014 (Caitlin Seaview Survey). A nested hierarchical design was used for collection and data analysis: (i) Coral species; (ii) Region [fixed factor, two levels: Great Barrier Reef (GBR) and the Coral Sea (CS)]; (iii) Reefs [random factor nested in Region, ten levels: for the GBR: Great Detached, Tijou Reef, Day Reef, Yonge Reef and Myrmidon Reef; for the CS: Flinders Reef, Holmes Reef, and in Osprey Reefs: Dutch Towers, Halfway Wall (also known as Nautilus Wall), Bigeye Ledge]; and (iv) Depth [fixed factor, four levels: 10m (±3 m), 20 m (± 2 m), 40 m (±3 m), and 60 to 80 m]. Based on the bathymetric distribution of the coral species (Englebert, Bongaerts et al. 2017) between 3 and 8 individuals were collected at each depth using SCUBA and a Remotely Operated Vehicle (ROV), and samples were preserved in salt-saturated 20% dimethyl sulfoxide (DMSO) with 0.5 EDTA (at – 20 °C). Coral specimens were collected under permits supplied by the Great Barrier Reef Marine National Park Authority (Townsville, Australia) and Commonwealth Marine Reserves, Department of the Environment (Hobart, Australia). For coordinates and site information of reef localities *see* Hernandez-Agreda, Leggat et al. (2016) and Englebert, Bongaerts et al. (2017).

DNA extraction, amplification and sequencing

DNA extraction was performed using 0.4 (±0.2) g of each coral fragment using the modified protocol of MoBio PowerPlant pro DNA isolation kit (catalog no. 13400-50; MoBio, Carlsbad,

CA) described by Sunagawa, Woodley et al. (2010) and DNA concentration was estimated in a Qubit Fluorometer using the Qubit dsDNA HS Assay Kit (Thermo Scientific, Wilmington, DE). Amplified DNA was stored at -20°C before PCR amplification. Genomic template primers 27F/519R were used to amplify bacterial 16S rRNA gene amplicons for examining bacterial assemblage structure. Gene amplicons were amplified in a single-step, 30-cycle PCR (HotStarTaq plus master mix kit; Qiagen, United States). The conditions for PCR as follows: 94°C for 3 min, followed by 28 cycles of 94°C for 30s, 53°C for 40s, and 72°C for 1 min, to finish with a final elongation step at 72°C for 5 min. PCR products were checked in 2% agarose gel and pooled in equal proportions based on molecular weight and DNA concentrations. Calibrated ampured XP beads were used to purify pooled individuals. DNA library preparation and Illumina TruSeq DNA sequencing were performed under MR DNA protocols (MR DNA; Shallowater, Texas, USA) 300bp paired-end MiSeq.

Sequence analysis

To establish common phylotypes among coral species *M. elephantotus* and *A. aculeus* data (newly generated here) were jointly analysed with *P. speciosa* sequence data (previously analysed in Chapter 4 (Hernandez-Agreda, Leggat et al. 2016)). Sequence data analysis was performed using the open-source software Quantitative Insights Into Microbial Ecology (QIIME, version 1.9) (Caporaso, Kuczynski et al. 2010). Sequences with ambiguous base calls, with homopolymer runs exceeding 8 bp or below 200 bp were discarded. Barcodes, primers and chimeras were removed from sequences prior analysis (Usearch61 (Edgar, Haas et al. 2011) for chimera removal). Operational Taxonomic Units (OTUs) were defined and taxonomically identified with 97% cluster similarity using RDP classifier and Greengenes database (version 13_8 (DeSantis, Hugenholtz et al. 2006)).

Statistical analysis, taxonomical redundancy and core microbiome

Data mining, statistical and taxonomical redundancy analyses were performed in PRIMER v7 and PERMANOVA+(Anderson, Gorley et al. 2008). Composition and structure was analysed by composition and structure. A fourth root transformation and standardization by individual by total was applied to raw abundance OTU tables to normalize to relative abundance. Fourth root transformation was selected to balance the contribution of rare and highly abundant bacteria (Clarke, Gorley et al. 2014). Raw abundance OTU tables were also converted to presence/absence data to analyse bacterial composition. For both, relative abundance and composition data, significant differences in the factors of the design were identified by permutational multivariate analysis of variance (PERMANOVA) using 9,999 permutations, Bray-Curtis and Weighted Unifrac distances (on relative abundance data), and Sorensen and Unweighted Unifrac distances (on composition data). Coral species data were wrangled and analysed separately after detection of structural differences between them (i.e. excluding the factor *coral species* from statistical analysis). Pairwise comparisons were used to further exploration of significant differences at any factor. PERMANOVA results were visualized in a non-metric dimensional scaling (nMDS)(Clarke 1993) plots using 95% bootstrap reefs or centroids (identified in figure legends).

A stepwise selection of species (BVSTEP routine) was used to create subset matrices of selected OTUs reflecting the abundance pattern observed in the normalized relative abundance matrix (Somerfield and Clarke 1995, Clarke and Warwick 1998, Clarke, Somerfield et al. 2006). Using Bray-Curtis distance matrices, Spearman rank as correlation method and Rho (ρ) >0.95and Delta Rho 0.001 as stop criteria, BVSTEP was run for each reef per coral species. Taxonomical and functional redundancy was evaluated on resulting subset matrices and the core microbiome for each coral species.

Phylotypes consistently present in ≥ 80% of the individuals were considered highly persistent core microbiome (Ainsworth, Krause et al. 2015). Core 80% was identified for (i) each coral species, (ii) each reef per coral species; and (iii) each depth per reef per coral species using the command *compute_core_microbiome.py* in QIIME. Core 80% matrices were created as a subset matrices selecting Core 80% OTUs from the normalized relative abundance matrix. Taxonomical redundancy was evaluated using the indices average [Δ+] (Clarke and Warwick 1998) and variation [Λ+] (Clarke and Warwick 2001) of taxonomical distinctness. Venn diagrams for Core 80% and the whole bacterial community were generated using Venn diagram software (Bioinformatics and Evolutionary Genomics, http://bioinformatics.psb.ugent.be/webtools/Venn/).

Predicted functional profiling based on bacterial taxonomy

The Galaxy web version of Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt (Langille, Zaneveld et al. 2013)) was used to produce a prediction of the metagenomic functional content of the subset matrices of representative phylotypes and the Core 80% matrices. Each matrix was normalized by copy number using Greengenes database (v. 13_8 (DeSantis, Hugenholtz et al. 2006)) as reference and metagenome prediction was produced KEGG Orthology groups (KOs) were summarized at 3 different levels of KEGG-Pathway. The predicted functional profiling of representative OTUs in *P. speciosa* individuals from Myrmidon reef was not estimated since the OTUs were not present in Greengenes database. Differences in KEGG Pathways were assessed by as indicated previously (*see* '*Statistical analysis, taxonomical redundancy and core microbiome*'). Graphs were produced with '*ggplot2*' (Wickham 2016) as implemented in R (Team 2013).

Beta-diversity (turnover)

Beta-diversity was analyzed using packages *betapart* (Baselga 2010, Baselga and Orme 2012, Baselga, Orme et al. 2012) and *geosphere* in R (Team 2013). Pair-wise turnover was computed per each presence-absence OTU tables using Sorensen dissimilarity index. Distance matrixes (one per coral species) among pairs of samples were calculated using their geographic locations and the Vincenty ellipsoid method. Due to the impossibility to assign a geographic location to specific coral colonies, I assigned a random location to each colony sampled in each reef (3-33 meters from the reef coordinate). The relationship between the fraction of species shared (i.e. turnover) and distance for each pair of samples per coral species was explored in plots produced with '*ggplot2*' (Wickham 2016) in R (Team 2013).

Results and Discussion

The diversity and species-specific patterns of the microbiome observed for the three species are consistent with that which has been reported to date (Blackall, Wilson et al. 2015), in that collectively, corals host highly diverse bacterial interactions responsive to the hosts' reef environment (Figure 5-2). Over 79,000 distinct Operational Taxonomic Units (OTUs) are generated from 17 million sequences within the collective dataset (Supplementary Table E-2), which result in 49,000 distinct OTUs (from ~6 million sequences) after filtering chloroplasts and unidentified/unassigned sequences. From those OTUs, about half are species-specific, and half are shared between the 3 coral species (Figure 5-3B, Supplementary Table E-2), with each coral species hosting on average ~20-25k OTUs, (*M. elephantotus* 19,964 OTUs, *P. speciosa* 25,124 OTUs, and *A. aculeus* 19,875 OTUs). Importantly, I find that each individual coral hosts' very few of these species-specific bacterial phylotypes. For example, *P. speciosa* individuals host on average only 589 ± 39 OTUs (*n*=123 coral individuals), similarly, on average, *M. elephantotus* individuals host 583 ± 62 OTUs (*n*=95 corals) and for *A. aculeus* 651

± 41 OTUs (*n*=91 corals). As such, regardless of the coral species, the reef site in which they reside, or other environmental variables (such as reef depth, nutrient availability and light availability) an individual coral colony will harbour only 2-3% of the total number of bacteria that are found in association within the species (*species-specific microbiome*). I also find that the bacterial associations of individual corals are overwhelming constrained to 2-3 dominant bacterial classes (Figure 5-3A,C). These findings are consistent with the characteristics of the microbiome of holobiont models across the phyla that have been studied to date, including hydra (Augustin, Fraune et al. 2012, Bosch 2012), nematode *Caenorhabditis elegans* (Zhang, Berg et al. 2017), plants (Bulgarelli, Schlaeppi et al. 2013, Edwards, Johnson et al. 2015) and also humans (Huttenhower, Gevers et al. 2012, Lozupone, Stombaugh et al. 2012), in that the host microbiome is highly structured, habitat specific and functionally redundant. As such, it contrasts with previous studies hypothesising the coral microbiome as an exceptionally diverse microbial biosphere compared to other organisms. In that corals are widely documented, as found in the current study, as uniquely hosting thousands of bacteria, in species-specific interactions. However, these high numbers of species-specific interactions are due to a highly transient microbiome (Figure 5-2), likely reflective of the highly dynamic symbiotic state and open interaction with the surrounding environment. I further suggest that the substantial taxonomic redundancy within an individual corals' microbiome reflects functional redundancy within waste production, utilisation and nutrient cycling, of the highly efficient photoendosymbiotic host system.

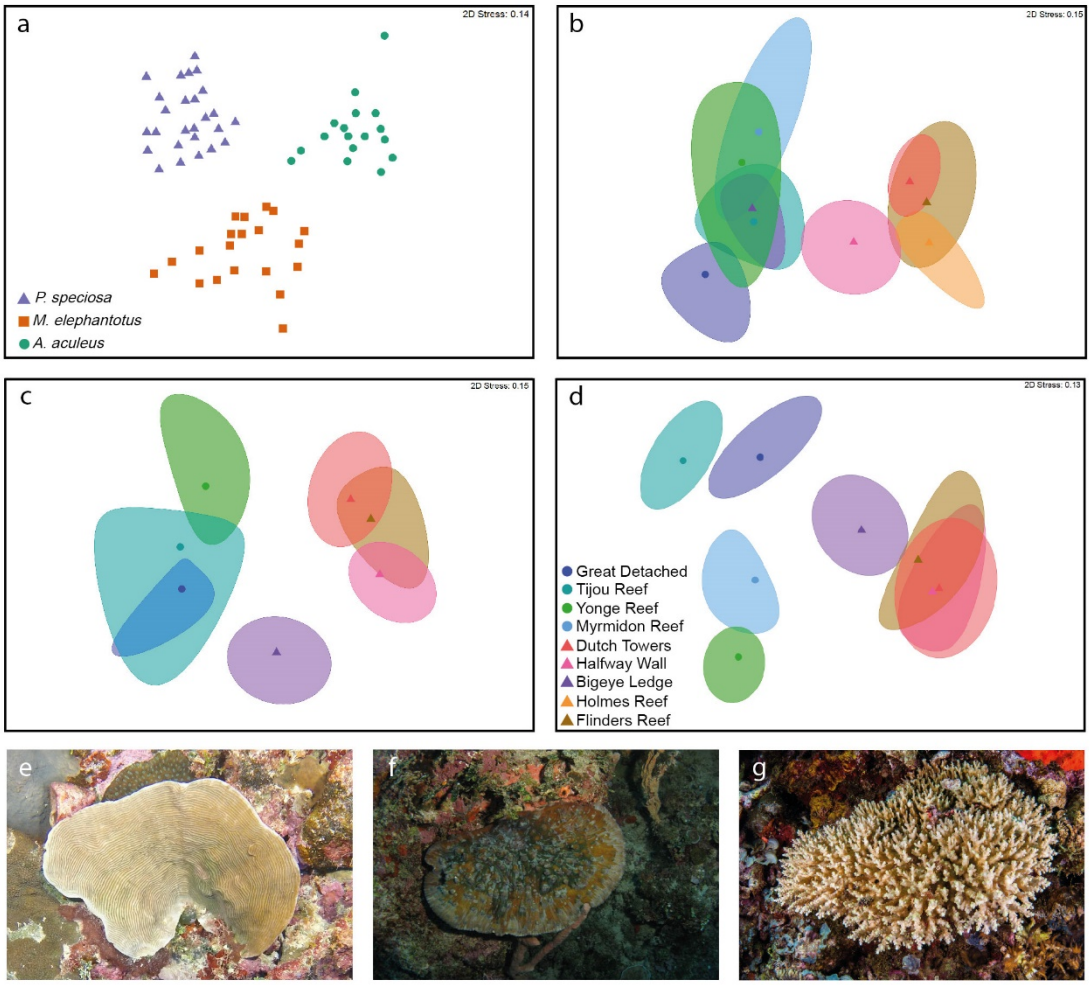


Figure 5-2: Bacterial communities structurally differ spatially and between coral species. Non-metric MDS based on relative abundance to illustrate differences between coral species (a; PERMANOVA, $p<0.01$, Supplementary Table E-4) and between reefs for *P. speciosa* (b), *M. elephantotus* (c; excluding Myrmidon reef), and *A. aculeus* (d; excluding Holmes reef). NMDS based on Bray-Curtis dissimilarity of fourth root-transformed data. (a) Centroids, (b-d) bootstrap area and average for reefs. Circles denote GBR reefs, triangles CS reefs. For presence/absence equivalent results *see* Supplementary Figure E-2. *A. aculeus* photo: Ed. Roberts.

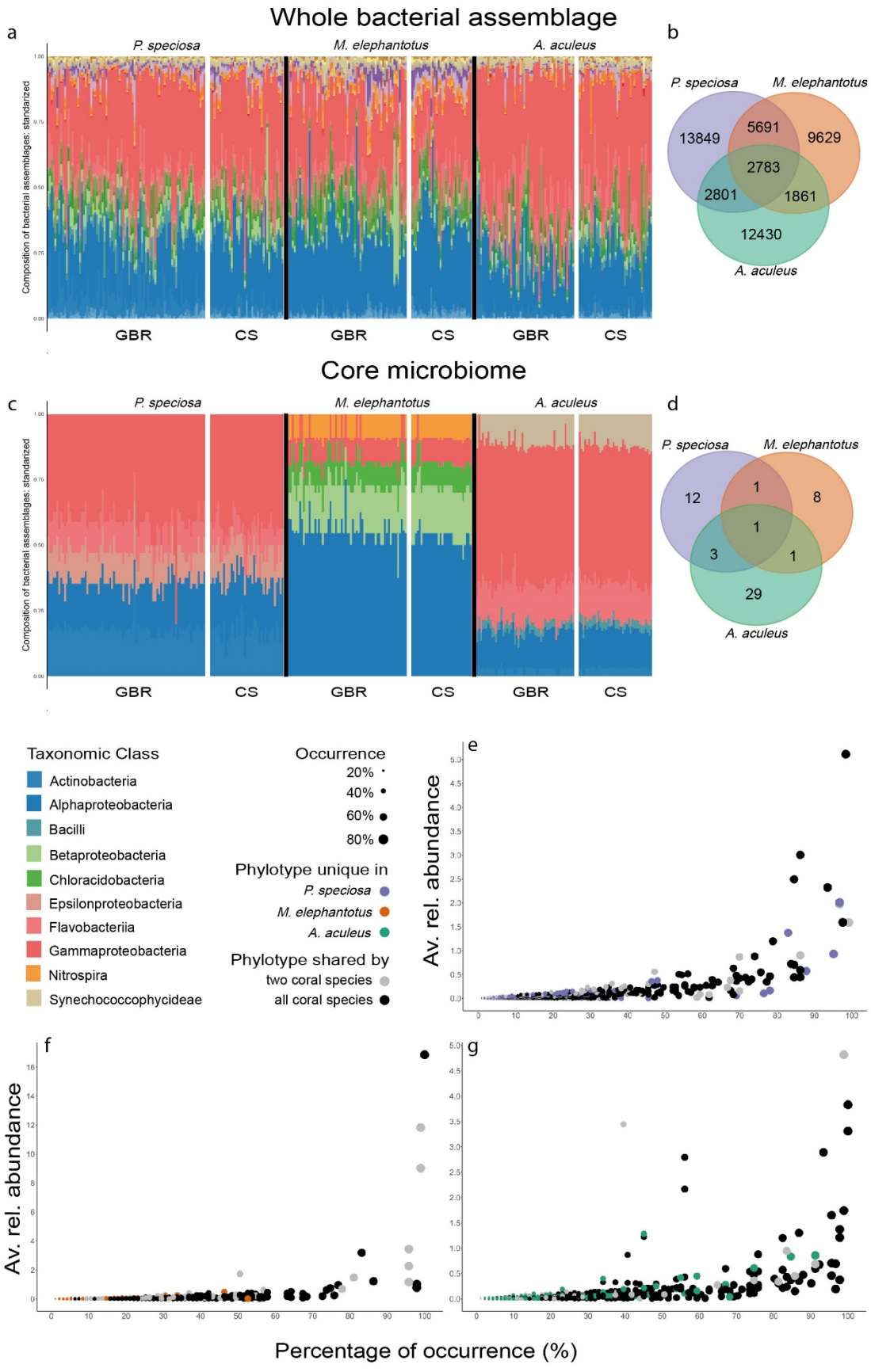

Whole bacterial assemblage
a
P. speciosa
M. elephantotus
A. aculeus
Composition of bacterial assemblages: standarized
GBR
CS
b
P. speciosa
M. elephantotus
A. aculeus
13849
5691
9629
2783
2801
1861
12430
Core microbiome
c
d
12
1
8
1
3
1
29
Taxonomic Class
Actinobacteria
Alphaproteobacteria
Bacilli
Betaproteobacteria
Chloracidobacteria
Epsilonproteobacteria
Flavobacteriia
Gammaproteobacteria
Nitrospira
Synechococcophycideae
Occurrence
20%
40%
60%
80%
Phylotype unique in
P. speciosa
M. elephantotus
A. aculeus
Phylotype shared by
two coral species
all coral species
e
f
g
Av. rel. abundance
Percentage of occurrence (%)

Figure 5-3: Coral microbiome is composed by common and species-specific phylotypes in a taxonomical stable structure across individuals. Taxonomic structure of coral-associated bacteria within individual coral hosts (a) is reflected by that of the core microbiome (c), Alpha- and Gammaproteobacteria dominate bacterial assemblage composition despite the variability across spatial scales (Figure 5-2); bacterial classes are structurally stable across individuals. Common bacterial phylotypes of the three commonly occurring coral species *(species-specific microbiome* and *resident microbiome* (b)) and core microbiome of individual *P. speciosa*, *M. elephantotus,* and *A.aculeus* (d). Graphs of average of relative abundance vs. percentage of occurrence across coral individuals for *P. speciosa* (e), *M. elephantotus* (f) and *A. aculeus* (g) reveal that highly persistent (>80%) OTUs rarely are species-specific (Note the difference in scale in relative abundance). Bacterial assemblages' composition: number of OTUs belonging to the taxonomic level Class, standardized by individual by total. For legend in extend *see* Supplementary Figure E-12.

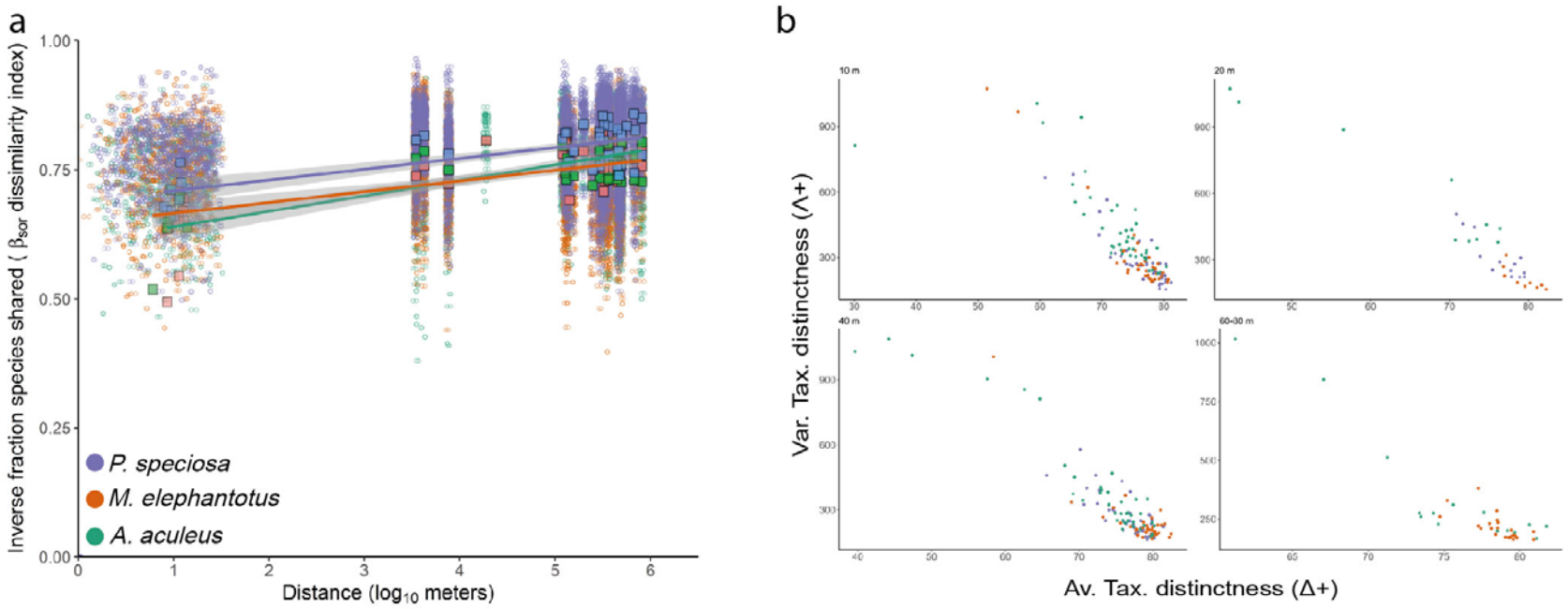


Figure 5-4: Taxonomical structure evidenced in beta-diversity (turnover, (a)) and taxonomic breadth (b). A minimal increase on dissimilarity (i.e. inverse of species shared) is observed between pair of individuals across spatial scales (a). Depending on the spatial scales composition of coral microbiome is conserved in 50-23% (same reef) and 27-18% (distinct reefs). Pattern of high taxonomic relatedness-low taxonomic evenness in the three species regardless the depth supports the taxonomic structure observed in coral microbiome (b). Together these results suggest that a fraction of the coral microbiome is conserved and taxonomic structured, regardless the reef environment.

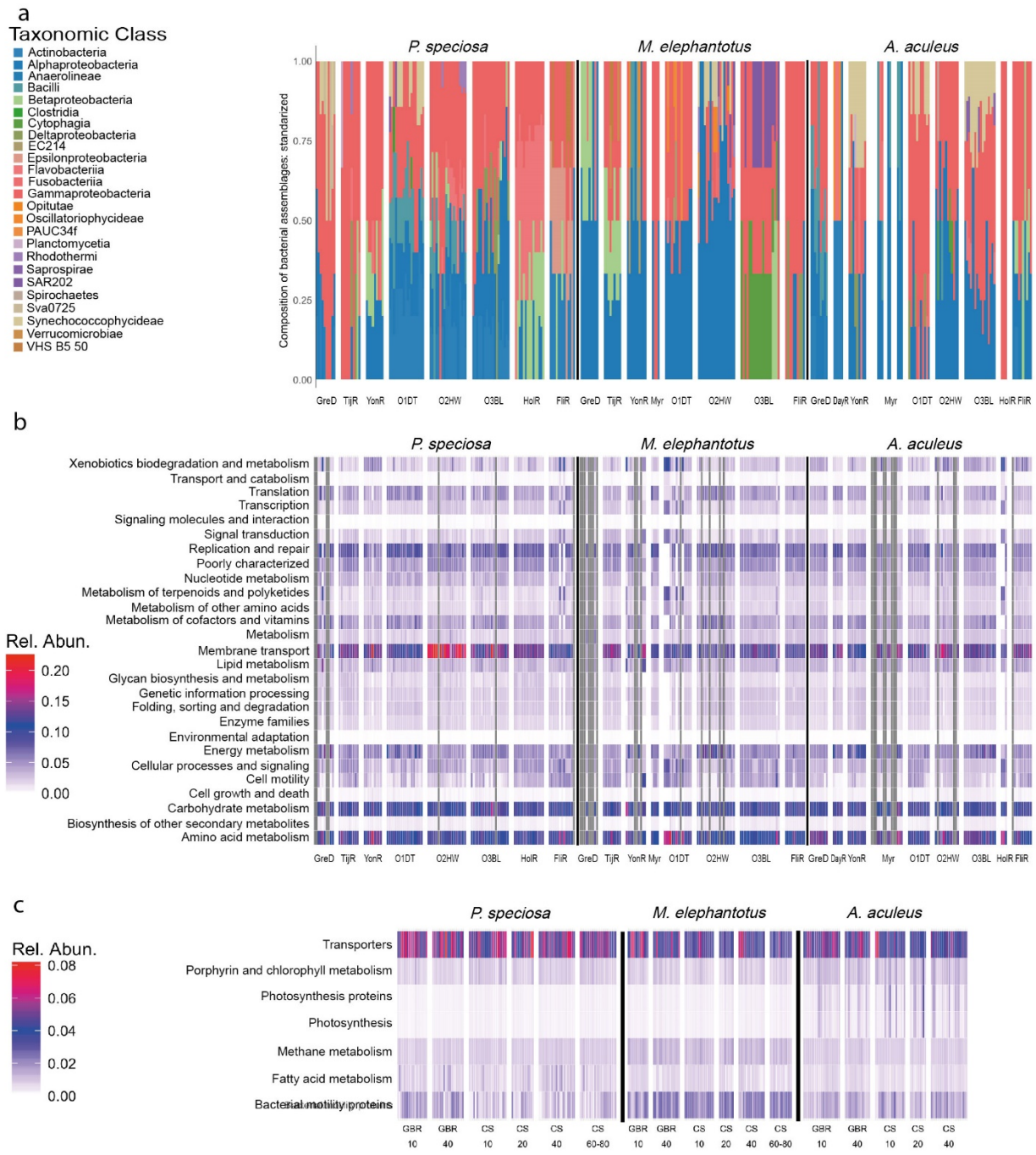


Figure 5-5: Different bacterial taxonomy structure on representative and highly persistent OTUs (core microbiome) encodes similar functional capabilities. For each coral species, representative OTUs (a) and core microbiome have distinct taxonomical structure (Figure 5-3C), but equivalent prediction on functional content (B and C). Functional prediction content generated from relative abundance of KEGG KO genes, normalized, and standardized by individual by total.

The composition of bacterial assemblages observed in individual corals, based on the number of OTUs per bacterial Class, reveals that Alpha- and Gammaproteobacteria consistently are the dominant taxonomic classes across coral species, geographic regions and depth (Figure 5-3). Here I report that the numerical dominance of phylotypes within two bacterial classes, reflected in taxonomic structure, is similar to that which has been reported in the numerical abundance of phylotypes within the Alpha- and Gammaproteobacteria (Blackall, Wilson et al. 2015, Bourne, Morrow et al. 2016, Hernandez-Agreda, Gates et al. 2017). Thus, I show evident taxonomical redundancy is evident within the individual corals' microbial assemblage. This pattern of constrained diversity is even more evident when evaluating beta-diversity and the indexes of complexity to assess the microbiome.

Variations in composition of bacterial assemblages (beta-diversity, turnover) (Baselga 2010) show that the bacterial distance–decay relationship for the three corals species is constant across spatial scales, which is a unique feature of the coral microbiome compared to other ecological systems (Condit, Pitman et al. 2002, Green, Holmes et al. 2004, Bates, Clemente et al. 2013) (Figure 5-4A). This indicates that regardless of community drivers related to reef environment, the composition of the coral microbiome system is structured at the individual level. The structure and complexity of the assemblage, expressed as taxonomic relatedness (average of taxonomic distinctness, Δ^+) (Clarke and Warwick 1998) and taxonomic evenness (variation of taxonomic distinctness, Λ^+) (Clarke and Warwick 2001), provides a measure of the taxonomic spread of communities (Gibson, Barnes et al. 2001) which has been applied in macroecology (Thrush, Hewitt et al. 2003, McClanahan, Ateweberhan et al. 2007, Alahuhta, Toivanen et al. 2017) and more recently utilised in microbial ecology (Moss, Nocker et al. 2006, Xu, Jiang et al. 2011). These measurements allow us to assess biodiversity changes on spatial and temporal scales and the response to disturbances. By utilising this approach in the current study, I find that the taxonomic relatedness is inversely proportional to the taxonomic

evenness, thus indicating that the *individual microbiome* with high taxonomical complexity (high average, Δ^+) are more even (low variance, Λ^+), and vice-versa. This pattern is consistent across all individuals of the three coral species studied, at all depths evaluated and across all reef locations (Figure 5-4B). Interestingly I further demonstrate that *M. elephantotus* and *P. speciosa* showed a constrained range in both average and variation of complexity beyond 20 m in reef depth. In contrast, *A. aculeus* exhibits a broad response ranging across all reef depths. These results suggest that coral growth form, branching versus plating and/or massive forms, has a substantial influence over the complexity and variation of the coral microbiome, particularly across reef depths.

A key feature of the *individual microbiome* in all three corals species studied here is the characteristically small group of highly persistent OTUs (*core microbiome*) (Figure 5-3C-G). Each coral species' core microbiome compromises phylotypes ranging from rare to highly dominant across reef habitats. Also, only very few bacterial phylotypes were shared between the three coral species. I also find similarities in the taxonomical structure of the core bacteria across species and individuals (core microbiome herein defined at occurrence >80% individuals within the study, Figure 5-3C,D) and find that in each coral species the core microbiome was equivalent in taxonomical complexity and functional capabilities (Figure 5-5C, Supplementary Figure E-9-11). The core microbiome of both *P. speciosa* and *A. aculeus* was dominated by Gammaproteobacteria phylotypes and included several phylotypes with relative abundance between 0.43 - 5.11% and 0.15 - 4.82%, respectively (Figure 5-3, e.g. Supplementary Table E-3). Overall the core microbiome of *P. speciosa* and *A. aculeus* showed similar taxonomical structure (Figure 5-3C) as observed in the bacterial assemblage of the species as a whole (Figure 5-3A), with high numbers of phylotypes from Alpha- and Gammaproteobacteria. Interestingly, the core microbiome of the coral *M. elephantotus* was unique compared to the other two depth generalist corals. Most notably, I find that the

Alphaproteobacteria are the dominant grouping within the coral microbiome (Figure 5-3A), followed by Betaproteobacteria. Furthermore, in *M. elephantotus* the most dominant 11 phylotypes accounted for relative abundance between 0.78 - 16.85% (>80% occurrence in Figure 5-3F, Supplementary Table E-3). Moreover, *M. elephantotus* hosts 3 phylotypes with a high relative abundance (> 9%) which together account for 37.7% of the relative abundance. These phylotypes belong to the classes Alphaproteobacteria, Betaproteobacteria, and class Chloracidobacteria. The three coral species have common and distinct biomechanical, morphological, reproductive and ecological traits. They are all of colonial morphology attached to the sediment, broadcast spawners, hermaphrodite and coincide in their *Symbiodinium* clade association (C3, C3h in common) (Madin, Anderson et al. 2016). However, they also have distinctive traits that may potentially influence microbial associations. *M. elephantotus* belongs to a robust major clade with encrusting and laminar growth form that have large polyps (7.5-10 mm). *P. speciosa* and *A. aculeus* belong to complex clades, show small polyp size (0.8-1 mm and 2.4-4.6 mm, respectively) and are variable in growth forms (corymbose and laminar, respectively) (Madin, Anderson et al. 2016). Whilst the relationship between some of these traits and the coral microbial community have been explored (morphology (Sunagawa, Woodley et al. 2010, Liang, Yu et al. 2017), phylogeny (Sunagawa, Woodley et al. 2010), mode larval development (Apprill, Marlow et al. 2009, Sharp, Distel et al. 2012, Lema, Bourne et al. 2014, Leite, Leão et al. 2017) , zoonxanthellae clade (Pantos, Bongaerts et al. 2015)), to date no direct or conclusive relationship has been draw.

In the current study, only one phylotype, a member from the family Alteromonadaceae (OTU 806717) was found to be highly persistent in the three depth generalist coral species across the entire 10-80 m depth range (Figure 5-3D). This bacterial phylotype was not only highly persistent in that it was present in 98.4% of individuals, but I find it is also in high relative abundance within both *A. aculeus* and *P. speciosa* individuals. This study is the first report of

a persistent bacterial phylotype within this group across coral species and a broad range of geographical and depth reef locations. However, genera from the family Alteromonadaceae have been previously reported occurring in the core microbiome of other coral species (Leite, Salles et al. 2018) and coral early life stages (Sharp, Distel et al. 2012, Ceh, van Keulen et al. 2013) . The persistence of this specific taxon could indicate that it plays a critical functional role, not played by any other taxa or played more efficiently by this taxon (as suggested by Leite, Salles et al. (2018)). Members of this group have been suggested as symbiotic in other marine habitats and are reported as important in chemical defences against pathogenesis (Gil-Turnes, Hay et al. 1989, Ashton, Rosado et al. 2003). The potential role of members of the Alteromonadaceae family, in association with corals, has yet to be explored.

Given that the reef environment varies across the depth gradient and abiotic factors within the reef impact nutrient acquisition and cycling within the coral host (Einbinder, Mass et al. 2009, Lesser, Slattery et al. 2010), these are also likely to be a driver of microbiome structure. Therefore here I also aimed to determine if bacterial associations were reflective of within, reef locations (depth of sampling) from which the corals were collected. Here I identified bacteria present within all host corals at depth for each reef location. All three coral species showed persistent bacterial phylotypes across the sampled depths (intersection of the Venn diagrams, Supplementary Figure E-3). However, I find at all of the depths, the *A. aculeus* microbiome harbours the highest diversity and highest number of phylotypes than that of the other two coral species. Interestingly the family Endozoicomonaceae was present in the core microbiome of all sampled depths for *A. aculeus*, but only occasionally present in *M. elephantotus* corals where it was found in some individuals collected at 10 m (Tijou Reef) and 40 m (Flinders Reef) and *P. speciosa* at 10 m (Myrmidon Reef, Dutch Towers), 40 m (Bigeye Ledge) 60-80 m (Halfway Wall). Similarly, phylotypes from the Phylum Cyanobacteria, including the genera *Synechococcus* and *Prochlorococcus*, were present in all individuals of the coral *A. aculeus* at

all depths and reefs, but only occasionally in *M. elephantotus* (10 m at O1DT and O3BL, 20 m at O2HW, 40 m GreD and TijR, and 60-80 m at O1DT) and *P. speciosa* (all depth at TijR and YonR, 10 m at O1DT and O3BL, 40 m at MyrR, and 60-80 m at O2HW, O3BL and FliR). This clear structuring of the coral microbiome was evident across the biogeographical and depth ranges of the current study, highlighting that redundancy is a consistent feature of the coral microbiome regardless of reef region, reef site and reef depth (Supplementary Figure E-3-6).

Significantly, analyses over the different spatial scales and depth gradients of the 309 corals analysed in the current study showed that the number of sequences, phylotypes and taxonomic structure of depth generalist corals *P. speciosa*, *M. elephantotus* and *A. aculeus* were comparable (Supplementary Figure E-4-8, Supplementary Table E-4). While I find differences in the microbiome between geographic regions of the three coral species (GBR vs. CS; PERMANOVA, $p<0.05$), differences between reef localities (within region; PERMANOVA, $p<0.05$, Supplementary Table E-5-8) and depths were not consistent across species (a posteriori analysis, Figure 5-2, Supplementary Table E-9-12). For example, I find that at Halfway wall, Bigeye Ledge (Osprey), and Flinders of the Coral Sea, the bacterial assemblages within these reef localities showed similar assemblage structure at 10, 20, but unique assemblages at 40 m and 60-80. Likely reflective of changes associated with mesophotic conditions (in this case 40 m to deeper), these results suggest substantial variation in the within an individual, and within the environment, drivers (such as light penetration, shading, nutrient availability, and water flow). This is highly likely within the diverse habitats of any coral reef environment whereby corals may be exposed to greater upwelling, flow or shading dependant on local reef factors, all of which are known to affect the physiology of the coral, its nutrient cycling and its reliance of heterotrophic and autotrophic feeding (Einbinder, Mass et al. 2009, Kahng, Garcia-Sais et

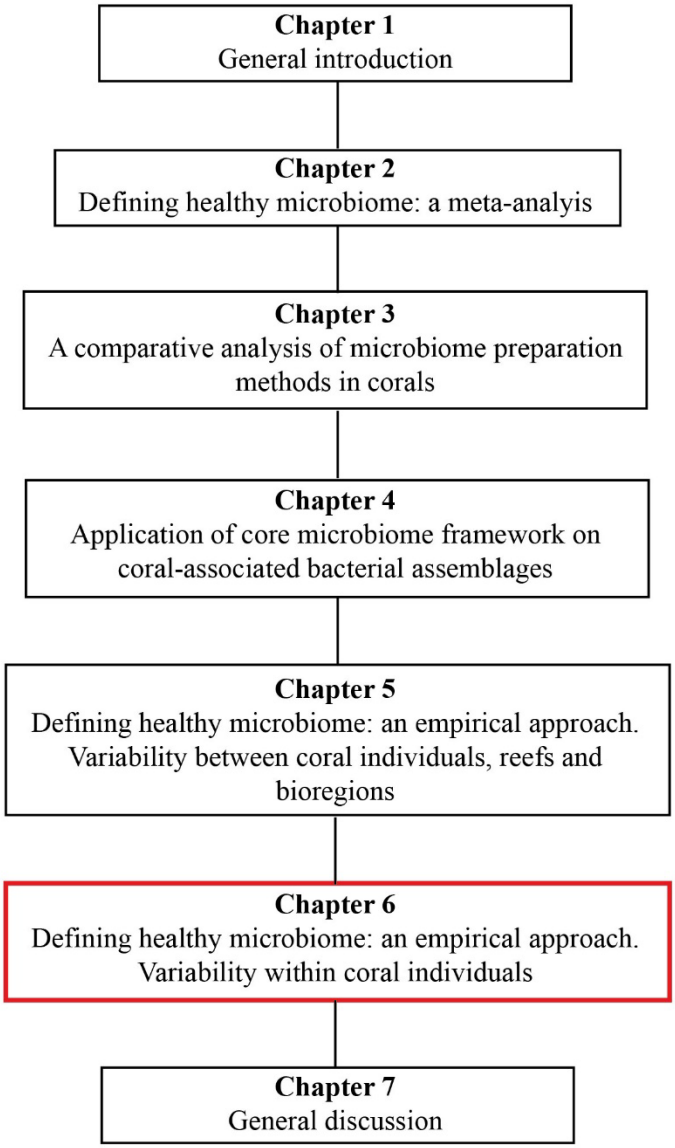
al. 2010, Lesser, Slattery et al. 2010, Bongaerts, Frade et al. 2015, Englebert, Bongaerts et al. 2017).

Finally, representative phylotypes from the transient community in each reef for each coral species were selected as groups of OTUs with a multivariate pattern reflective of that observed in the *species-specific microbiome* (BVSTEP algorithm, ρ >0.95). I have then evaluated taxonomic relatedness and conducted functional prediction analysis within each individual coral. I find that the taxonomic redundancy is reflected in the functional predictions for the coral microbiome (Figure 5-5A,B). I also find that the core microbiome and representative phylotypes per reef were equivalent in taxonomical complexity (Δ^+ and Λ^+, Figure 5-3C and Figure 5-5A, Supplementary Table E-13) and functional predictions (Figure 5-5B,C and Supplementary Figure E-9-11). Equivalent taxonomic complexity and functional predictions further demonstrate, that regardless of the composition of the assemblages per reef, they are taxonomically, and likely, functionally, redundant within the coral host. For example, transporters, porphyrin and chlorophyll metabolism, photosynthesis proteins, photosynthesis, methane metabolism, fatty acid metabolism and bacterial motility proteins are consistently enriched within the coral-associated bacteria assemblage of individual corals across reef habitats. This is likely to indicate that within the nutritionally dynamic coral host, there are relatively few niche microhabitats available and that those niches are highly consistent across species and reef environments.

In conclusion, I find that the observed simplicity and structure of the corals' diverse microbial biosphere, in which there is substantial taxonomic and functional redundancy, is consistent across coral species, bioregions and reef depths, suggesting that within diverse microbial biospsheres such as the coral, simplicity exists with the meta-organism. For the individual coral meta-organisms' microbiome, this simplicity is on average, 605 bacterial phylotypes, and is

likely to be reflective with the gut (Ainsworth, Fine et al. 2006) and its digestive process, within the mucus rich habitats of the surface mucus layer (Ritchie 2006) and skeleton (Yang, Lee et al. 2016), involved in nutrient cycling and waste utilisation and which is open to the reef environment. Conceptualising the coral microbiome as a system of transient, resident and core microbiomes, is likely going to result in deciphering the microbial contribution to corals' symbiotic and dysbiotic states more achievable.

Chapter 6: Diversity, variability and rare bacterial associations differ between the microhabitats of the coral host



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Introduction

The microbial communities of corals have been described as highly complex and highly variable (Blackall, Wilson et al. 2015). In the last two decades, the primary focus of research has been to dissect this variability across various spatial scales, including bioregions, reefs, and across species bounds (i.e. Li, Chen et al. (2013), Samodha, Wang et al. (2015), Neave, Rachmawati et al. (2017); see review in Chapter 2). Corals offer a variety of niche microbial habitats, which are hypothesized to be inhabited by different microbial communities. Within-colony, variability has also been identified as a potential driver of the disparity observed within larger-scale studies of the coral microbiome (Ainsworth, Thurber et al. 2010). Within a colony, microbial community variability has the potential to occur at different scales. For example, compared to the base of the coral branches, branch tips experience differences in water flow, light penetration and irradiance. Coral bases are also shown to have higher symbiont densities than the tip, potentially generating physio-chemical gradients that alter the microbial environment (Rohwer, Seguritan et al. 2002, Ainsworth, Thurber et al. 2010). The coral surface mucus layer, host tissues and the coral skeleton are also areas where distinct physiological processes occur and exposure to the reef environment differs significantly, resulting in structurally distinct bacterial communities (Figure 6-1) (Shashar, Banaszak et al. 1997, Brown and Bythell 2005, Sweet, Croquer et al. 2011, Rädecker, Pogoreutz et al. 2015, Apprill, Weber et al. 2016, Glasl, Herndl et al. 2016, Putnam, Barott et al. 2017). Within the tissue, interchange of nutrients in different coral cell types (epithelium, mesoglea, gastroderm and calicoblastic epithelium) also has the potential to drive dissimilarities in the microbiome (Ainsworth, Fine et al. 2006, Rädecker, Pogoreutz et al. 2015, van de Water, Ainsworth et al. 2015, Neave, Rachmawati et al. 2017). For example, the endosymbiotic microalgae *Symbiodinium* is enclosed in a host-derived membrane within the gastrodermal layer, which makes this environment highly restricted to the physiological dynamics of the zooxanthellae (Kazandjian,

Shepherd et al. 2008, Yellowlees, Rees et al. 2008). Only a few bacteria from the genera *Propionibacterium* and *Ralstonia* have been detected inhabiting this space (Ainsworth, Krause et al. 2015) and hypotheses have been raised in regards to the potential functional role played by these bacteria.

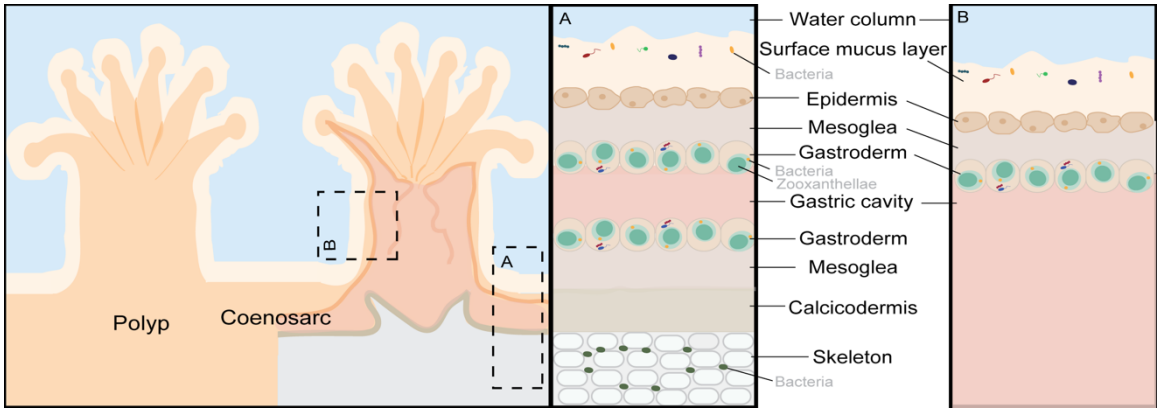


Figure 6-1: Coral microhabitats Tissue layers in A) coenosarcs and B) polyps.

As corals colonies are comprised of millions of interconnected, individual polyps (Figure 6-1) (Putnam, Barott et al. 2017). Polyps' walls are connected to coenosarc tissue, which is a continual tissue layer connecting the coral skeleton and polyps in a continuous sheet of colonial tissue (Galloway, Work et al. 2006, Ruppert, Fox et al. 2009). The coenosarc tissue is comprised of the corals two tissue layers; the epidermis and gastrodermis, which contains the endosymbiotic zooxanthellae *Symbiodinium*; and a connective extracellular matrix, mesoglea (Galloway, Work et al. 2006, Tambutté, Allemand et al. 2007). However, physical and functional differences between polyps and coenosarcs also mean they are likely to represent distinct micro-habitats, providing niche environments in which microbial communities associate. For example, oxygen saturation and light irradiance differ between polyps and coenosarcs (Wangpraseurt, Larkum et al. 2012, Wangpraseurt, Pernice et al. 2015). Ulstrup, Ralph et al. (2006) demonstrated that in branching coral *Pocillopora damicornis*, oxygen saturation varies between the polyp and coenosarc, light absorption is higher in polyps, and in both shade- and sun-adapted colonies, the maxima of relative electron transport rates in the

coenosarc are double than that of the polyp tissues. Wangpraseurt, Larkum et al. (2012) also provided evidence for consistently higher irradiance in polyp over coenosarc tissues in four coral species. Furthermore, the coenosarcs (or coenenchyme) are open spaces in direct contact with the water column, whereas coral polyps include the gastric cavity and mouth surrounded by tentacles, with thicker tissue and a higher density of endosymbionts (Galloway, Work et al. 2006, Tambutté, Allemand et al. 2007). Heterotrophic feeding within the coral polyp (Houlbreque and Ferrier-Pages 2009) provides up to 30% of the total carbon uptake within the colony (Grottoli, Rodrigues et al. 2006), but the microbial contribution to digestion is mostly unknown (Agostini, Suzuki et al. 2012, Leal, Nejstgaard et al. 2014). Thus, understanding the role of different microhabitats within coral colonies and the structure of microbial communities is a critical component of deciphering the microbial role in coral health and stability.

The bacterial community associated with the coral polyp has also been investigated in the cold-water corals *Lophelia pertusa* and *Madrepora oculata* (Meistertzheim, Lartaud et al. 2016), as well as in tropical coral *Galaxea fascicularis* (Agostini, Suzuki et al. 2012). Polyp-associated bacterial communities were found to be species-specific in cold-water corals, in the coral *M. oculata* the polyp communities were both temporal and spatially stable and found to be dominated by genus *Endozoicomonas*. In the coral *L. pertusa*, the bacterial community was found to be highly variable between polyps, colonies and seasons, suggesting the importance of environmental drivers in structuring the microbial communities on both micro and macro scales (Meistertzheim, Lartaud et al. 2016). The polyp-associated microbial community in *G. fascicularis* was also found to be as highly abundant and structurally similar as that observed in gut microbiome of other vertebrates and invertebrates (Agostini, Suzuki et al. 2012). While the biological, physical and chemical characteristics of the polyp and the coenosarc tissues have been investigated; to date, no study has evaluated the microbial community structure of the polyp and coenosarc tissue compartments under different environmental conditions. To test

if microhabitats within corals harbour distinct microbial communities, I compared bacterial composition and community structure in polyps and coenosarc tissues (Figure 6-2) from ten *P. damicornis* colonies collected from 2 reef habitats, the reef crest and reef slope.

Materials and Methods

Coral collection and preservation

Fragments (<5 cm long) of branching coral *Pocillopora damicornis* were collected in January 2015 from the reef flat (*n*=5, 1-2 m depth) and reef slope (*n*=5, 10-12m depth) at Coral Gardens reef, adjacent to Heron Island Research Station, Australia (23°26.5248'S, 151°54.754'E). Fragments were collected using a hammer and chisel from five different colonies separated by >3 m. After collection, fragments were held in seawater and immediately transported to the laboratory for preservation. Samples were fixed in 4% Paraformaldehyde solution (PFA) and stored at 4°C for 14h, then stored in sterile 3x phosphate buffered saline at 4°C. Both 4% Paraformaldehyde solution and 3x phosphate buffered saline were prepared on the same day of coral collection using DNA/RNA-free water. Fragments were shipped to James Cook University, Townsville, Australia and stored at 4°C until processing. Coral collections were accomplished under permits supplied by the Great Barrier Reef Marine National Park Authority (Townsville, Australia, G15/37488.1).

Sample decalcification and tissue separation

Coral samples were decalcified with repetitive washes of DNA/RNA-free 20% EDTA at 4°C over a 2-week period. After decalcification for each of the coral fragments, 1-2 cm tissue of the tip of the coral branch was cut axially. Tissue fragments were cut longitudinally and spread out in a petri dish (Figure 6-1A). One polyp and one 1.0mm diameter circle of coenosarc tissue were extracted from each colony using 1.0 mm biopsy punch with a plunger (ProSciTech Pty

Ltd, QLD, Australia) (Figure 6-1B, C). Individual polyps and circles of coenosarc tissues from each colony were then used in DNA extraction. Petri dishes and biopsy punches were sterile and single use. Scission of polyps and coenosarcs and DNA extractions were carried out in sterile conditions under laminar flow.

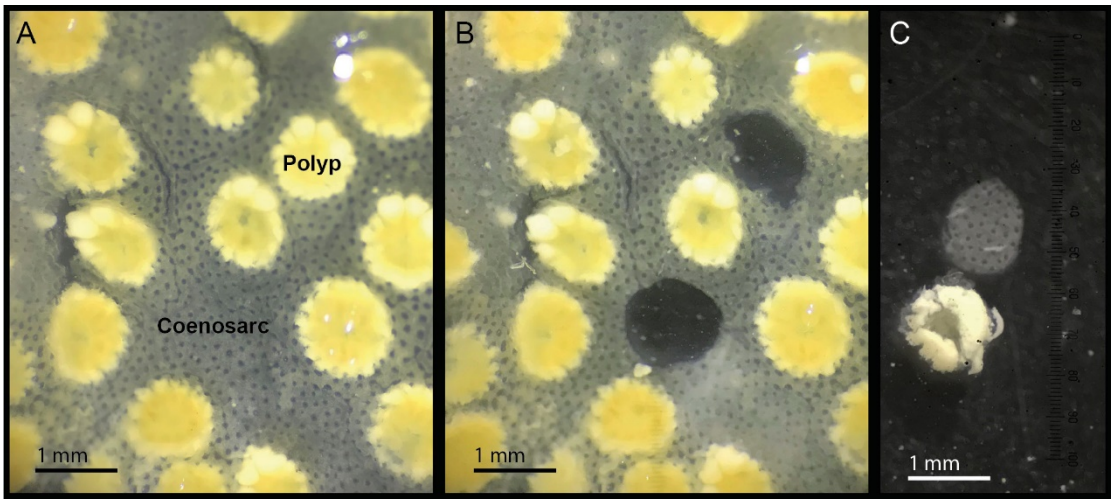


Figure 6-2: Subsampling of polyp and coenosarc. After decalcification coral tissue was extended in a Petri plate (A). Using a biopsy punch, polyp and coenosarc were extracted from the tissue (B) for individual DNA extraction (C).

DNA extraction, amplification and sequencing protocol

Polyps and coenosarcs tissues were individually placed in 1.5 ml tubes, and DNA was extracted per manufacturer's protocol for isolation of genomic DNA from Laser-Microdissected tissues (QIAmp® DNA Mini Extraction kit, QIAGEN). The concentration of extracted DNA was quantified using Qubit Fluorometer and Qubit® dsDNA High-sensitivity Assay Kit (Life Technologies, NSW, Australia). DNA was stored at -20°C before PCR amplification and sequencing. DNA was also extracted, amplified and sequenced in negative controls (no sample template, as suggested in (Salter, Cox et al. 2014)) as part of each samples batch and under the same conditions that other.

Bacterial DNA was amplified using 16S rRNA gene amplicons, primers 27F/519R (v1-v3 region) and barcode on the forward primer in a 30-cycle PCR using HotStarTaq plus master mix kit (QIAGEN, USA). Conditions for PCR were as follows: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds, and 72°C for 1 min, and final elongation at 72°C for 5 minutes. Amplification products were verified in 2% agarose gel and based on molecular weight and DNA concentration, multiple samples were pooled and purified using calibrated Ampured XP beads. Products from pooled and purified PCR products were used to prepare DNA libraries following the Illumina TruSeq DNA library preparation protocol. Sequencing was performed on an Illumina MiSeq platform per manufacturer's protocol at MR DNA (Shallowater, Texas, USA). Raw sequences are available in the National Center for Biotechnology Information (NCBI) Short Read Archive (SRA) under the project number PRJNA435850.

Sequence analysis

Sequence analysis was carried out using the open-source software Quantitative Insights Into Microbial Ecology (QIIME, v. 1.9) (Caporaso, Kuczynski et al. 2010). Barcodes, ambiguous base calls, sequences below 200 bp and homopolymer runs were eliminated from raw sequence data. Chimeras were also removed from raw data using Usearch61 (Edgar, Haas et al. 2011). Representative Operational Taxonomic Units (OTUs) were defined and taxonomically classified using uclust method (Edgar 2010) and the curated open reference Greengenes database (v. 13_8) (DeSantis, Hugenholtz et al. 2006) with 97% of cluster similarity. Chloroplasts, mitochondria (order Rickettsiales) and unassigned OTUs were removed from resulting OTU tables.

Statistical analyses

Differences between microhabitats were analyzed using PRIMER v7 and PERMANOVA+ (Anderson, Gorley et al. 2008). Bacterial communities associated with polyps and coenosarcs were evaluated based on number of sequences, number of OTUs, richness (Margalef's index, *d*), diversity (Shannon-Weaner, *H'*), evenness (Pielou's evenness, *J'* and Simpson evenness, *D*), taxonomic breadth (Average (Δ^+) and Variation (Λ^+) of taxonomic distinctness), composition and structure. Analyses were run on the whole community, including singletons and low read OTUs since their importance has been previously demonstrated in corals (Ainsworth, Krause et al. 2015) and other systems (Sogin, Morrison et al. 2006, Taylor, Tsai et al. 2013, Shade, Jones et al. 2014). To evaluate the composition of the bacterial assemblages, the OTU table (https://figshare.com/s/9b5df5b4b21c8cfc0fb9) was converted to presence/absence, whereas for relative abundance analyses the OTU table was transformed and standardized using a fourth root transformation and standardization by sample by the total. Differences between microhabitats were evaluated using a two-factor design: *Microhabitats* (fixed, 2 levels: coenosarc and polyp) and *Depth* (random, 2 levels: reef flat and reef slope). Permutational multivariate analysis of variance (PERMANOVA) was used to test differences based on dissimilarity matrices. Dissimilarity matrices were generated using Sorensen distance on presence/absence composition data, Gamma+ (Γ+) dissimilarity (Izsak and Price 2001) on composition data to evaluate taxonomic composition, and Bray-Curtis (BC) dissimilarity on relative abundance data to evaluate the structure. Univariate analysis on Euclidean distances was used to evaluate differences in the number of sequences and OTUs and other diversity metrics between microhabitats and depth. Type III (partial) sums of squares, fixed effects sum to zero for mixed terms, permutation of residuals under a reduced model and 9,999 permutations were used as parameters of PERMANOVA analyses. To evaluate the dispersion of bacterial communities, Permutational analysis of multivariate dispersions (PERMDISP,

(Anderson 2001)) was used with 9,999 permutations. Two-dimensional nonmetric dimensional scaling (nMDS) plots (Clarke 1993) are used to illustrate PERMANOVA and PERMDISP results. Composition and structure graphs are presented at Class and Family level to illustrate similarities. Supporting analyses and nMDS plots of composition and structure are based on OTUs.

To determine phylotypes ubiquitous with each microhabitat, I identified a core 100% microbiome. Using the list of OTUs for each of the microhabitats, Venn diagrams were produced using Venn diagram software (Bioinformatics and Evolutionary Genomics, http://bioinformatics.psb.ugent.be/webtools/Venn/). Based on Venn diagram results, OTUs were classified as phylotypes from core microbiome that are not shared (unique to the coenosarc or the polyp tissue) and shared phylotypes (intersection in Venn diagram), and matrices of abundance were generated. To produce a prediction of the metagenomic functional content, I used the matrices in the Galaxy web version of Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt (Langille, Zaneveld et al. 2013)). Using Greengenes database (v. 13_8 (DeSantis, Hugenholtz et al. 2006)) as the reference, each matrix was normalized by copy number, and metagenome prediction was produced using level 2 of KEGG Orthology groups (KOs) of KEGG-Pathway. All graphs, excepting nMDS, were produced using 'ggplot2' package (Wickham 2016) in R (Team 2013).

Results

Number of sequences, Operational Taxonomic Units (OTU) and diversity metrics

A total of 533,701 high quality reads clustered in 4,284 OTUs were retrieved from coenosarc and polyp tissues (Supplementary Table F-1). Negative controls (no sample template) manipulated under the same conditions and with the same reagents failed DNA amplification,

and no sequences were generated from their sequencing. Coral microhabitats differed only in diversity ($p < 0.05$, Figure 6-3, SupplementaryTables F-2 and F-3), being coenosarc more diverse than polyps. Coenosarcs also showed a higher number of sequences, OTUs (27,760.9 vs. 25,609.2 sequences per sample; 599.8 vs. 570.1 OTUs per sample, respectively), and higher richness and evenness (Figure 6-3). However, no significant differences were detected between either microhabitats or reef depth in these community attributes ($p > 0.05$, Supplementary Figure F-1, Supplementary Tables F-2 and F-3). Bacterial communities in both microhabitats also harbour similar in taxonomic breadth ($p > 0.05$, Supplementary Table F-2 and F-3). Diversity metrics, number of sequences and OTUs were highly variable between individuals (Figure 6-3); however, variability was higher in polyps than in coenosarcs.

Community composition and structure

No significant differences were detected for bacterial community structure or composition between polyps and coenosarcs between reef flats and slope ($p > 0.05$ PERMANOVA, Figure 6-3B and Figure 6-4, Supplementary Figures F-2 and F-3, Supplementary Table F-4, F-6, and F-8). Microhabitats shared over half of their OTUs pool (1,764 OTUs, 59.6% and 57.1% of OTUs in coenosarcs and polyps, respectively). Analysis of the community composition and structure evidenced similar microbial communities in polyps and coenosarcs (Figure 6-4A, Supplementary Figure F-3, Supplementary Table F-7 and F-10). However, both composition and structure were significantly more variable in polyps than in coenosarcs (dispersion of points in Figure 6-4B and Supplementary Figure F-3B, $p < 0.05$ PERMDISP, Supplementary Table F-5 and F-9). Phylotypes present in polyps and coenosarcs belong principally to classes Bacilli (1,071 and 889 OTUs, respectively), Actinobacteria (478 and 539 OTUs), Gamma- (536 and 550 OTUs) and Betaproteobacteria (196 and 199). Other classes as Alphaproteobacteria, Clostridia, Saprospirae, Synechococcophycideae, Thermoleophilia and TM7.3, had a smaller

representation in the number of phylotypes (Figure 6-4A). The same bacterial classes dominated the community structure across reef habitats (Supplementary Figure F-3): Bacilli (36.6% and 32.1% of relative abundance in polyps and coenosarcs, respectively), Actinobacteria (17.8% and 19.4%), Gamma- (15% and 15.1%) and Betaproteobacteria (7.8% and 7.4%). Seven dominant families accounted for over 50% of the relative abundance in both microhabitats (Figure 6-5, Supplementary Table F-11): Aerococcaceae (9.3% and 9.3% of relative abundance in polyps and coenosarcs, respectively), Pseudomonadaceae (6.5% and 8.7%), Propionibacteriaceae (9.2% and 8.6%), Staphylococcaceae (9.9% and 7.6%), Corynebacteriaceae (5.5% and 7.5%), Comamonadaceae (5.7% and 5.9%), and Streptococcaceae (10.7% and 5.6%). *Propionibacterium acnes, Staphylococcus epidermis, Streptococcus* sp. and a phylotype from the family Aerococcaceae dominate bacterial assemblages of both microhabitats (Supplementary Table F-12). A phylotype from the family Endozoicomonadaceae (NROTU28) was also found as part of the top 30 most dominant OTUs in both microhabitats, with higher relative abundance in polyps that in coenosarcs (1.63 ± 2.3 and 0.25 ± 0.5, respectively).

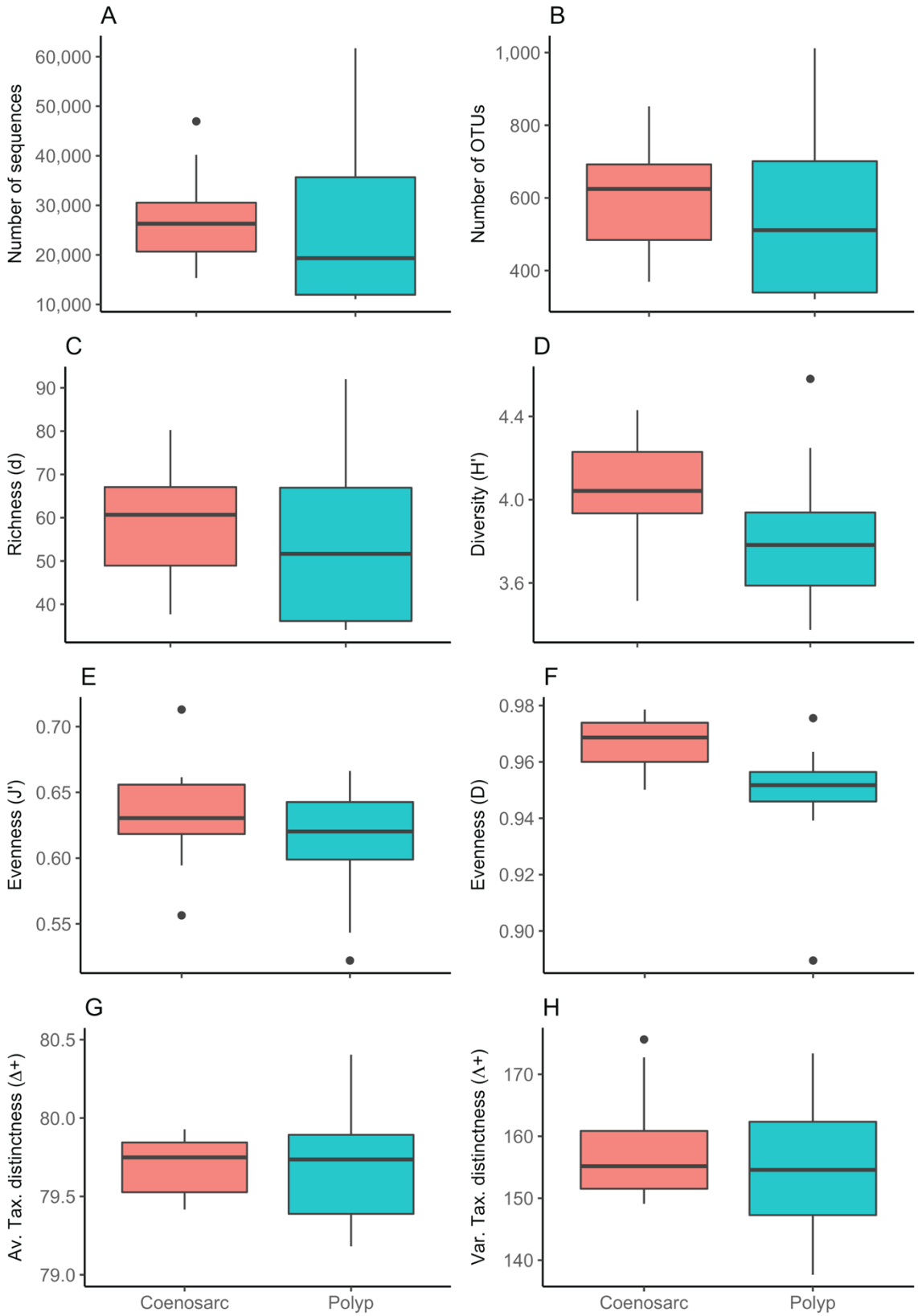


Figure 6-3: Diversity metrics per microhabitat. Data used for boxplots excludes chloroplast, non-identified and unassigned OTUs. Microhabitats only differ in diversity (PERMANOVA, $p < 0.01$, Supplementary Table F-3). Richness (d): Margalef's index, Diversity (H'): Shannon diversity, Evenness

(J'): Pielou's evenness, Evenness (D): Simpson evenness, Av. Tax. Distinctness (Δ^+): Average of taxonomic distinctness, Var. Tax. Distinctness (Λ^+): Variation of taxonomic distinctness. Data used for boxplots exclude chloroplast, mitochondria and unassigned OTUs (see Supplementary Table F-1 and F-2).

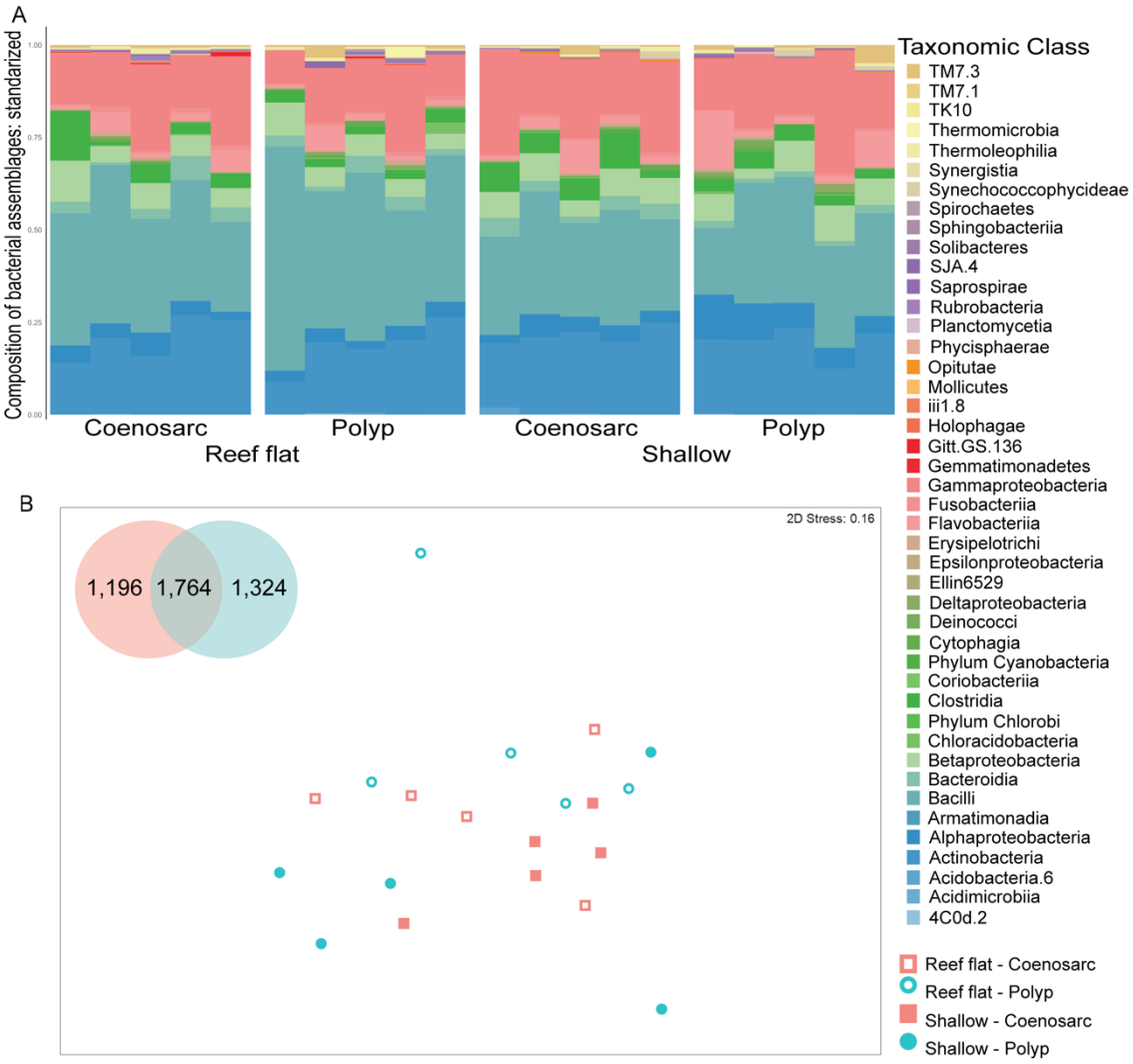


Figure 6-4: Bacterial communities associated with polyps and coenosarcs have a similar composition. Coral microhabitats share ~58% of their associated bacterial community (1,764 OTUs, Venn diagram in B) and show similarities in the overall composition (B). Taxonomic classes have similar representation (in number of OTUs) between microhabitats across samples (A). Non-metric MDS based on Sorensen dissimilarity on composition data. For statistical analyses on composition and taxonomic composition, see Supplementary Tables F4-F7 and Supplementary Figure F-2.

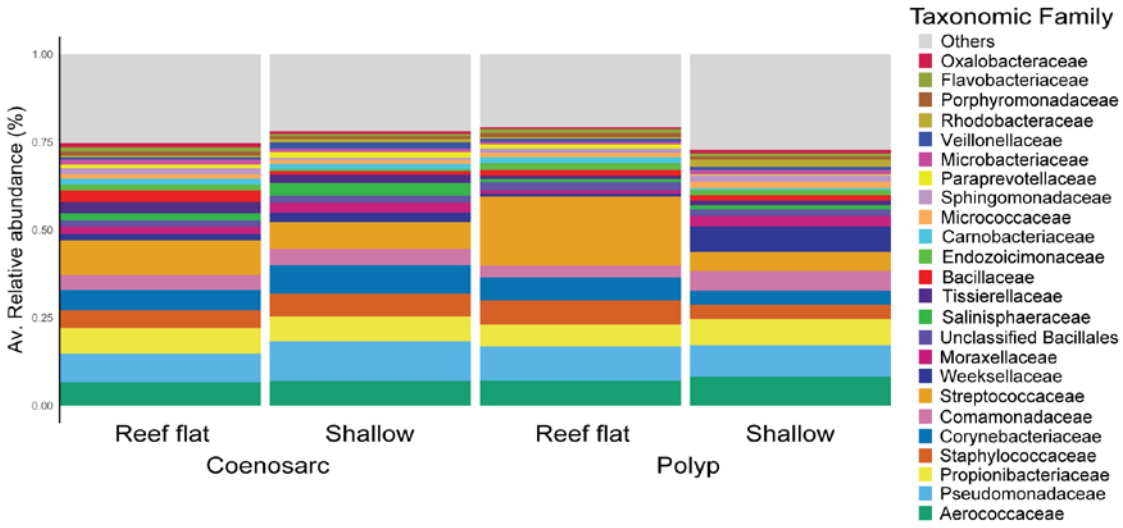


Figure 6-5: Bacterial community structure is similar in polyps and coenosarcs. Both microhabitats harbour same dominant families (Supplementary Table F-11), although the taxonomic structure at family level was highly variable across individuals (Supplementary Figure F-4). For statistical analyses on community structure, see Supplementary Tables F-8 and F-9.

Core microbiome

52 and 38 bacterial phylotypes were identified as core microbiome (100% occurrence across individuals; 100% core microbiome) in coenosarcs and polyps, respectively. Relative abundance of these bacterial phylotypes across samples was variable, ranging from low (0.003%) to highly abundant (25.2%), and varying independently of the total number of sequences per sample (Supplementary Figure F-5, Supplementary Table F-13). 64% and 87% of the core (highly persistent) bacterial phylotypes associated with coenosarcs and polyps were found to be shared between the two tissue types (intersection in Venn diagram, top of Figure 6-6). 4 of the 33 persistent and shared phylotypes were highly abundant with relative abundances above 2% in both microhabitats (Supplementary Table F-13), and the composition of the shared core community reflected the composition observed for the whole bacterial community (compare center of Figure 6-6 with Figure 6-4A). Comparatively, the polyp highly persistent phylotypes were smaller in richness and composition than that of the coenosarc (Venn diagram, top Figure 6-6).

Only five phylotypes were persistently found in all the polyps investigated (no persistently associated to coenosarcs). These phylotypes belong to classes Actinobacteria and Bacilli and two of them, from the genera *Corynebacterium* and *Propionibacterium*, are rare-low abundance phylotypes with relative abundance below 1% (Supplementary Table F-13). Highly persistent phylotypes in coenosarcs were more diverse than in polyps, and the composition reflected that observed in the whole bacterial community of the coenosarc, including one phylotype from the class Thermoleophilia. All the phylotypes highly persistent in the coenosarc are rare-low abundance bacteria in the whole community framework.

A prediction of the functional content in phylotypes of the core microbiome showed in the three groups (shared phylotypes; core microbiome of coenosarc; core microbiome of polyps) a high abundance of genes related to membrane transport, followed by replication and repair, carbohydrate and amino acid metabolism (bottom Figure 6-6). No significant differences were detected between either microhabitats or reef depth in the predicted functions ($p > 0.05$ PERMANOVA, Supplementary Table F-14).

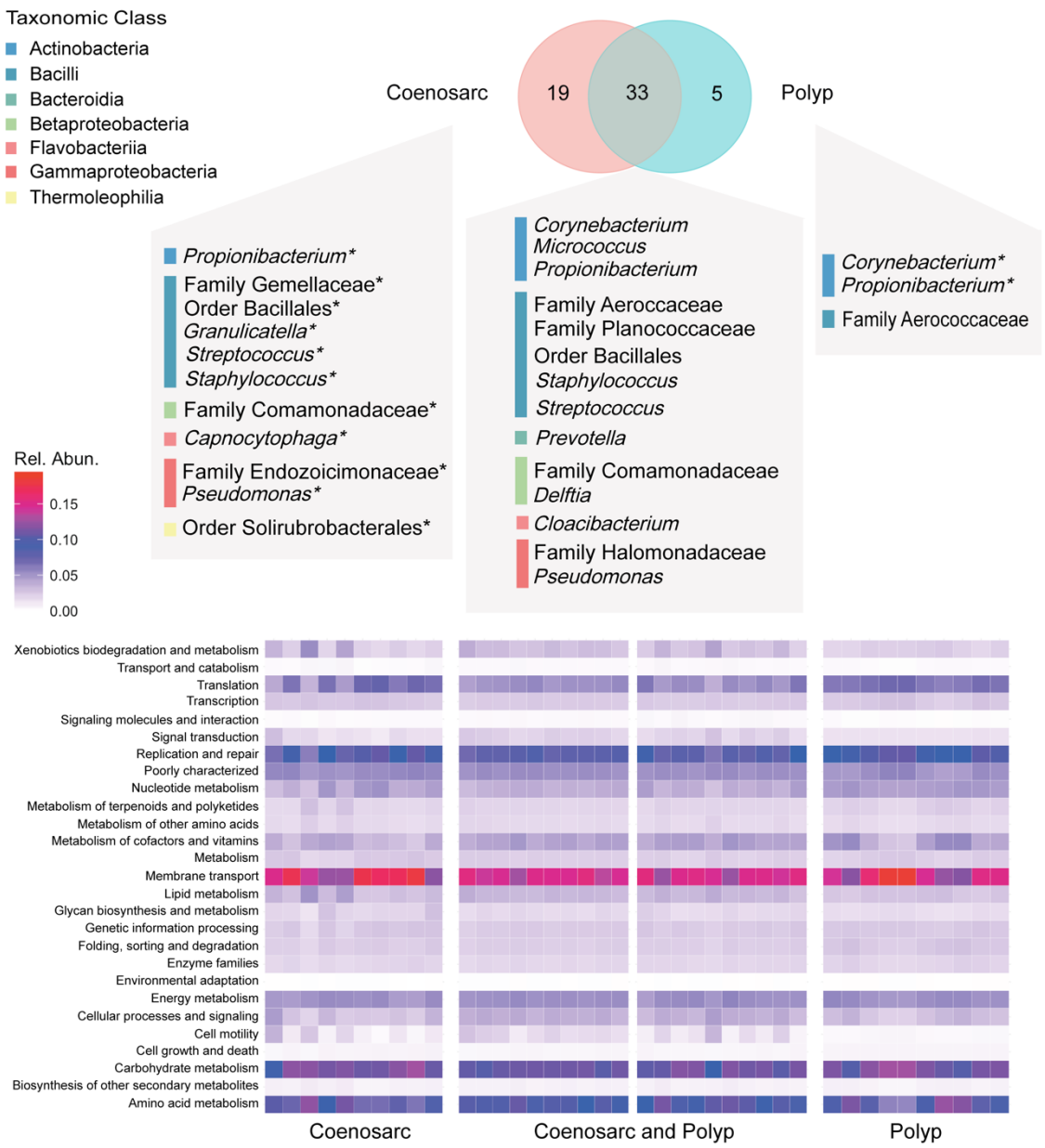


Figure 6-6: Composition and functional prediction of core microbiome. Top of the figure: Venn diagram of highly persistent phylotypes (present in 100% of the samples). Centre: lower taxonomic identification of highly persistent phylotypes, in colours its taxonomic class. Phylotypes with relative abundance below 1% are identified with asterisks. Bottom of the figure: prediction of functional content based on relative abundance of KEGG KO genes (standardized by total). For statistical analyses on functional prediction, see Supplementary Table F-14.

Discussion

While the coral microbiome literature has broadly explored structure and composition of microbial assemblages in coral individuals (see review in Chapter 2), this is the first study to compare microbial communities between the polyp and coenosarc microhabitats within coral individuals. Interestingly, comparison of bacterial assemblages of the coenosarc and polyp tissues in healthy branching corals of *P. damicornis* revealed no differences in composition and structure between these microhabitats when the whole community was evaluated. However, these results found differences in the diversity and variability of the bacterial communities of these compartments and the rare, low-abundance, core bacteria.

Bacterial communities in coral coenosarcs and polyps are comparable in the number of sequences and OTUs, richness, evenness and taxonomic breadth and with ~58% of OTUs in common, they harbour similar overall composition and structure. The same taxonomic classes and families, dominate the number of OTUs and the relative abundance in both microhabitats. The similar structure of the polyp and coenosarc microbiome may be reflective of common tissue layers, and niche habitats shared among the two microhabitats; the compartments surface mucus layer and skeleton, as well as the epidermis, mesoglea, gastrodermis and calicoblastic epithelium, are common habitats between the two microhabitats. The dominance of classes Bacilli and Actinobacteria in polyp and coenosarc contrasts with previous coral microbiome reports. In many species, including *P. damicornis*, class Gammaproteobacteria dominates bacterial assemblages' structure on healthy corals (Lee, Yang et al. 2012, Bourne, Dennis et al. 2013, Blackall, Wilson et al. 2015, Zhang, Ling et al. 2015). Bacilli and Actinobacteria are less referenced in the literature as dominant groups of the coral microbiome; this could be reflective of the differences in the preparation methods used to study bacterial assemblage in tissues (tissue slurry in (Ceh, Raina et al. 2012, Bourne, Dennis et al. 2013, Tout, Siboni et al. 2015,

Zhang, Ling et al. 2015) vs. decalcification, in this study). Alternatively, the dominance of these two classes may be related to the assemblage variability across reef locations. From the seven dominant families, Pseudomonadaceae and Comamonadaceae have been found with different importance in dominance in bacterial assemblages of healthy, diseased and disturbed corals (Barott, Rodriguez-Brito et al. 2011, Morrow, Moss et al. 2012, Closek, Sunagawa et al. 2014, Morrow, Bourne et al. 2014, Rothig, Ochsenkuhn et al. 2016, Hadaidi, Rothig et al. 2017). Further, family Pseudomonadaceae has recently been suggested as a potentially crucial group in coral microbiome assembly, due to the high number of positive co-occurrence correlations with other bacterial members (Leite, Salles et al. 2018).

Differences in diversity and dispersion of bacterial communities between the two compartments indicate that polyps are less diverse and more variable in composition and structure than the coenosarc bacterial community. Similarities in dominant bacteria may be reflective of the similarities in tissues layers in the two microhabitats. However, these results suggest that less represented bacteria might be driving the differences in diversity and variability between polyps and coenosarcs. This hypothesis is compatible with the fact that coenosarc and polyps are different environments with different functional roles. The coenosarc is an open environment (Galloway, Work et al. 2006, Tambutté, Allemand et al. 2007), in that it is a niche with an open interchange with the water column, exposed to high light irradiance and in contact with other organisms. A polyp is a semi-open microhabitat (Galloway, Work et al. 2006, Tambutté, Allemand et al. 2007), with constant water circulation but with the capacity to retract the tentacles, diminishing the water flux, the light irradiance and the contact with other organisms. I hypothesize that together these results may indicate a certain degree of individuality in polyps; as such, each polyp drives bacterial communities that may be distinct.

Coral microbiome literature usually focuses on understanding patterns of dominant (high abundance) bacteria. However, in other systems, like soil, rare-low abundance bacteria have proven to have a crucial role in the ecosystem functioning due to their metabolic versatility and fast growth rates (Baldrian, Kolarik et al. 2012, Kurm, Putten et al. 2017). Interesting insights into the coral microbiome can be gained by investigating the less represented classes, rare or low-abundance bacteria and the core microbiome. Highly persistent bacteria (coral core microbiome) common for the two microhabitats were highly abundant, and their taxonomic composition reflected that observed in the whole bacterial assemblage. Thus, these bacteria are likely to be part of the community associated with the coral tissues in common between the two microhabitats, where these habitats form a continuous and stable niche. Interestingly, the core microbiome of coenosarc and polyps, in isolation, compromise a small group of rare bacteria, 19 and 5 OTUs, respectively, whose relative abundance was below 1%. This result highlights the rare members of the bacterial community and the relevance of exploring coral-associated bacterial communities from alternative approaches other than abundance and encourages further testing results from taxonomic profiling studies with alternative methodologies (e.g. fluorescence *in situ* hybridization to localize low abundance/highly persistent bacteria in specific compartments). These rare, low-abundance bacteria were from less represented classes (in the coenosarc) and would be easily overlooked with traditional approaches of focusing on bacteria found in high relative abundance. Composition in the core microbiome of the coenosarc also reflects the composition of the whole assemblage, however, one phylotype of the class Thermoleophilia, was consistently found in individuals (100% core microbiome) but had low representation in the whole assemblage. Furthermore, the family Endozoicomonadaceae was part of the core of the coenosarc, supporting the importance of this group (particularly genus *Endozoicomonas*) within the *Pocillopora* microbiome (Tout, Siboni et al. 2015, Neave, Michell et al. 2017, Neave, Rachmawati et al. 2017, Ziegler, Seneca et al.

2017, Pogoreutz, Rädecker et al. 2018). The polyp core microbiome was smaller in number and composition, with two of the OTUs from the class Actinobacteria (genera *Corynebacterium* and *Propionibacterium*) and one from class Bacilli. Thus, along with diversity and dispersion results, the difference in the richness and composition of the core microbiome between microhabitats further strengthen the hypothesis of polyps as a semi-open and selective microhabitat.

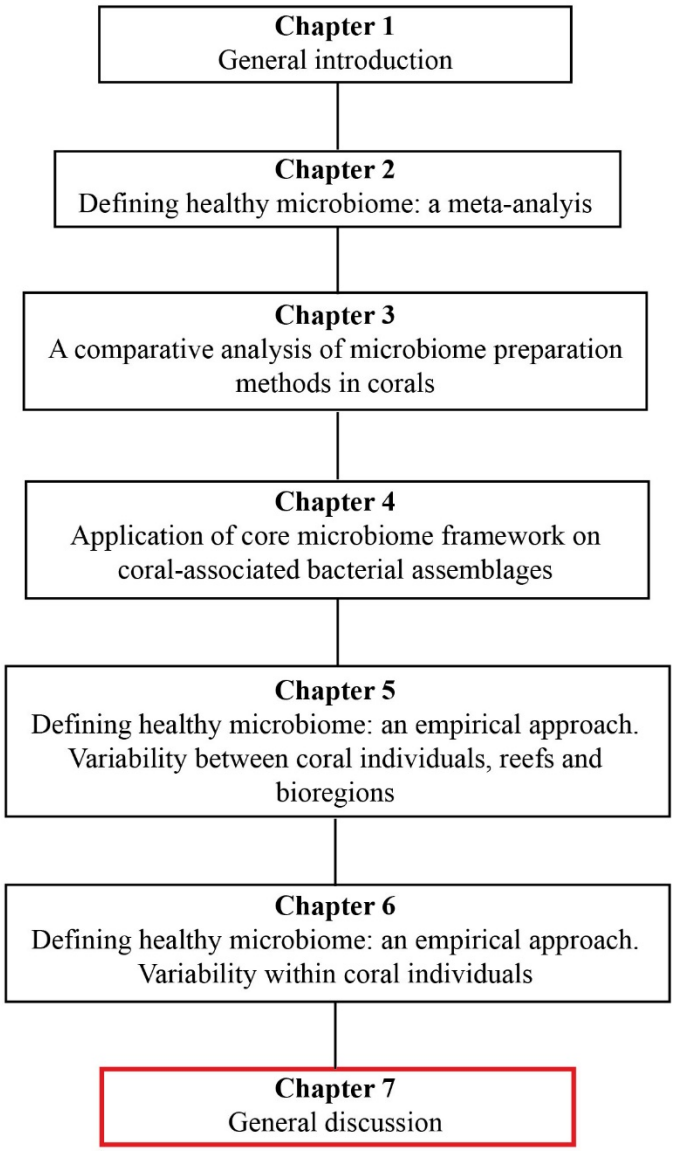
A functional prediction on the highly persistent bacteria (the core of coenosarc and polyp, and shared phylotypes) also provides some preliminary evidence that genes related to membrane transport are enriched within the tissue-associated microbiomes, along with replication and repair, carbohydrate metabolism and amino acid metabolism. Notably, no enrichment of these genes is higher in one microhabitat compared to the other. It is important to note that both taxonomic structure and functional profile in coral polyps are likely to be variable between coral species, polyp size, and in response to bleaching scenarios when heterotrophic feeding is vital to coral's resilience. The use of functional prediction based on 16s data has been a subject of extensive discussion in microbiome research field. Certainly, the functional prediction is unreliable to detect species-specific functional roles within the functional profile of the whole microbial community. However, in uncharacterized systems it offers a preliminary profile of the community functions, serving as an extra layer to classify samples based on its similarity and to identify potential differences in function of the overall bacterial community. In previous studies, the use of functional prediction in coral microbiome data has offered an insight on the potential functional roles of key members of the community (Morrow, Bourne et al. 2014, Ainsworth, Krause et al. 2015) and changes in community functional profile in response to environmental changes (e.g. salinity (Rothig, Ochsenkuhn et al. 2016) and temperature (Ziegler, Seneca et al. 2017)).

Some of bacterial OTUs and families described in this study as important due to their high occurrence (*Corynebacterium* and *Propionibacterium*) or high relative abundance (Family Aerococcaceae) have been previously identified as contaminants in distinct stages of sample preparation (Salter, Cox et al. 2014). However, this contradicts with the lack of sequences in negative controls used in this study (i.e. no sample template). Currently, there is a lack of consensus regarding the treatment of sequences obtained from the sequencing of negative controls (Pollock, Glendinning et al. 2018). Particularly for corals, distinguishing contaminants from members of the communities is particularly challenging for two reasons. First, the coral microbiome is an open system with evidence of horizontal bacterial acquisition (Apprill, Marlow et al. 2009, Lema, Bourne et al. 2014). This characteristic of coral microbiome could contribute to confounding distinct members of the bacterial community with contaminants. Second, members of the class Actinobacteria listed as a contaminant by Salter, Cox et al. (2014) have been found present in the symbiosome, in close proximity to *Symbiodinium* (Ainsworth, Krause et al. 2015). Localization of these bacteria might be indicating a symbiosis with the zooxanthellae and functional importance in the translocation of zooxanthellae products to the host, and they were rare-low abundant members of the whole bacterial community. This evidences that exclusion of particular groups of bacteria identified as contaminants might not be straightforward in corals and highlights the necessity of further discussion of this topic in coral microbiome research field.

In conclusion, here I show that despite the lack of differences in the whole microbial assemblage, coral polyps and coenosarcs of healthy *P. damicornis* differ in diversity, dispersion and core and rare bacterial associations. These results suggest differential assembly mechanisms in the bacterial communities within these microhabitats, likely to be constrained by the host and functional properties of these distinct niches. I suggest that further bacterial community profiling, combined with methods allowing localize and decipher functionality of

bacteria, will contribute to the understanding of assembly processes driving bacterial micro-niches at the individual level, and along with the functional contribution of individual bacteria to the coral host.

Chapter 7: General Discussion



In review as: **Hernandez-Agreda A**, Ainsworth TD. A place for taxonomic profiling in the study of the coral microbiome. *FEMS Microbiology Letters.*

Why taxonomic profiling still matters: The coral microbiome in a chaotic reef environment

Corals are described as a complex system, with an immense variability, diversity and abundance (Rohwer et al., 2002; Blackall et al., 2015). However, there are knowledge gaps regarding;

1) The processes and mechanisms driving the assembly of the coral microbiome within the coral reef environment,

2) The functional roles of microbes within the coral holobiont, and

3) The extent of its importance on the health and resilience of corals and coral reefs ecosystems.

Particularly, accurately identifying the healthy symbiotic state becomes a crucial component of understanding the biology and ecology of corals when we consider the current frequency and severity of environmental pressures within reef habitats (Hughes et al., 2017; Hughes et al., 2018) (Figure 7-1). Establishing the characteristics of a symbiotic versus dysbiotic state in corals is the foundation that is required to facilitate advances in the enhancement of coral reef resilience and facilitation of their recovery.

The goal of this thesis was to develop an understanding of the healthy coral microbiome, by:

1) Defining the characteristics of a healthy coral microbiome,

2) Evaluating the presence of universal bacterial symbionts in coral-associated bacterial assemblages, and

3) Identifying and quantifying natural and artificial factors generating variability in assessments of coral-associated bacterial communities.

This was achieved through a broad-scale evaluation of bacterial communities across coral species, spatial scales (from within-colony to between geographic regions) and ecological habitats (from shallow to lower mesophotic depths) to identify the relevant drivers of variation.

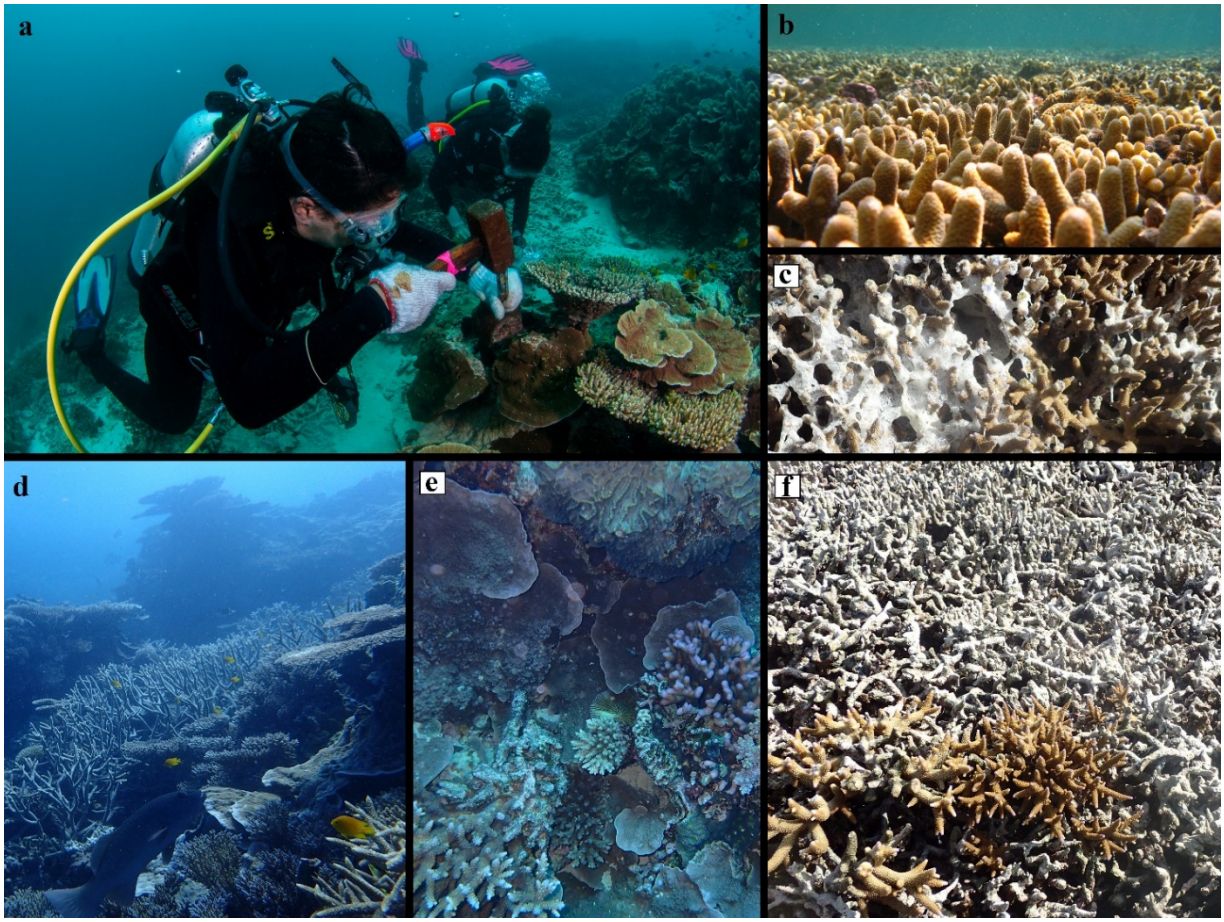


Figure 7-1: Diverse states of coral reefs observed during the course of the thesis. Sampling the healthy corals from Corals Gardens reef slope (12 m), close to Heron Island, Australia (a, summer 2017). Signs of stress (c) observed in remaining corals (f, from previous stress episodes) in the reef flat of the same location during the period of massive bleaching 2016 (March). Healthy corals observed in the reef flat of Harry's bommie (b) on November 2015, and in the reef slope of the same location right after the bleaching (d,e; April 2016). While the north of the GBR suffer a massive bleaching event in 2016, the south of the Great Barrier Reef, where Heron Island is located, received the ex-tropical cyclone Winston, bringing clouds and rain and decreasing the temperature (Hughes, Kerry et al. 2017). Photo credits: a) Ed Roberts, b) Helios Martinez, c and f) Connor Gervais, d, e) Floriaan Devloo-Delva.

A meta-analysis of the coral microbiome literature demonstrated that in over 25 coral species, bacterial communities are dominated by Gamma- and Alphaproteobacteria ((Blackall et al. (2015) and Supplementary Table B-1), are strongly responsive to the reef environment (Barott et al., 2011; Morrow et al., 2014; Lee et al., 2015); yet retain species-specific associations (Ritchie and Smith, 1997; Rohwer et al., 2001; Rohwer et al., 2002; Morrow et al., 2012) (*see* Chapter 2). Until now, dominant bacterial associations have been assumed to be characteristic of healthy, symbiotic states in corals (Littman et al., 2011; Cardenas et al., 2012; Croquer et al., 2013; Lee et al., 2015). However, the high variability observed across coral microbiomes and weaknesses associated with the current gold-standard approach (16s rRNA, *see* Chapter 2 and 3, (Soergel et al., 2012; Ghyselinck et al., 2013; Robertson et al., 2016)), hamper our ability to identify bacteria that are in a stable symbiosis with corals and play a significant functional role. Therefore, alternative approaches are required to explore bacterial communities in corals and establish patterns to identify the mechanisms and drivers influencing communities.

One of those alternative approaches is the core microbiome framework (reviewed in Chapter 2). This conceptual framework was initially developed to study the human microbiome and is now broadly used across many microbiome systems (Turnbaugh and Gordon, 2009; Lundberg et al., 2012; Newton et al., 2013). The core microbiome concept was initially based on the identification of phylotypes consistently found across individuals (Turnbaugh et al., 2007) and then applied to the identification of persistent genes (Turnbaugh et al., 2009; Qin et al., 2010). This framework has proven to be applicable across phyla for investigating the microbiome and identifying possible symbioses. In this thesis, it has been applied to the coral microbiome and determining the structure of bacterial communities in healthy corals (*see* Chapter 2 (Hernandez-Agreda et al., 2017); Chapters 3-6, (Hernandez-Agreda et al., 2016)).

Evaluating the approach

Taxonomic profiling has been the most commonly used technique to explore bacterial communities in corals (Shokralla et al., 2012; D'Amore et al., 2016; Lear et al., 2018). However, as yet, there are no standardized protocols for profiling these communities, despite the widely acknowledged biases that occur through 16s rRNA amplicon sequencing, data analysis and sample processing (Ghyselinck et al., 2013; McMurdie and Holmes, 2014; Rocha et al., 2014). For example, Ghyselinck et al. (2013) evaluated OTU richness, taxonomic assignment and coverage rate of 10 primers targeting regions V1-V9, concluding that primers vary in their performance and suggesting only two of them (both from region V4) as the most reliable. Weber et al. (2017) demonstrated that rare microbiota is more accurately represented when DNA extraction protocols include exhaustive lysis. Comparisons between homogenization methods, and chemical preservation methods (salt saturated dimethyl sulfoxide (DMSO) – EDTA and 4% paraformaldehyde solution) with the more widely used snap-freezing in liquid nitrogen, have shown that easy-to-use and quick preparation methods can be uniformly taken up (Chapter 3). For example, using chemical preservation methods and bead beating as homogenization, it is possible to extend sampling efforts across more of the reef environment and increase replication, while diminishing sample handling and processing time. Doing so will mean it is possible to utilise taxonomic profiling as a tool to identify community patterns far more broadly, with fewer logistical limitations, and resulting in directly comparable datasets that have been undertaken to date. Furthermore, standardization of profiling methodology is necessary to encourage regional collaborations that permit more robust approaches to addressing the enormous variability of microbial communities in corals.

Use of core microbiome framework as a tool to understand coral microbiome structure

Currently, the core microbiome framework has been applied to the coral microbiome as a tool to identify highly persistent bacterial phylotypes across coral individuals ((Chu and Vollmer, 2016; Lawler et al., 2016; van de Water et al., 2016), reviewed in Chapter 2). Recently, the power of this concept has been tested in sponges concluding that while cautiously interpreted, the core microbiome provides a robust approach for evaluating host specificity and environmental quality (Astudillo-Garcia et al., 2017). Throughout this thesis, the core microbiome framework has been applied to identify bacteria, which could be overlooked if we would only consider high relative abundance. For example, no differences were found between microhabitats, polyps and coenosarcs, when the whole bacterial community in *P. damicornis* was evaluated (Chapter 6). However, differences were found between these microhabitats when using the core microbiome framework, along with the evaluation of diversity and dispersion. Bacteria persistently found in both coenosarc and polyp are low abundance bacteria (<1% of relative abundance) and in the coenosarc, included one unrecognised phylotype in the coral microbiome, Thermoleophilia. Interestingly, rare bacteria are often excluded from the characterization of bacterial communities in coral as a result of the rarefaction method used to standardize sequence number per samples (McMurdie and Holmes, 2014) or due to the analysis focused on highly abundant phylotypes (e.g. studies in Supplementary Table B-1). However, the importance of rare bacteria in corals as in other systems has been widely discussed (Sogin et al., 2006; Taylor et al., 2013; Shade et al., 2014; Ainsworth et al., 2015; Ziegler et al., 2017a). The core microbiome framework is a useful tool to understand coral microbiome structure and overcome this bias towards highly abundant phylotypes. Other similar methods have been used in microbial ecology to identify bacterial with the potential functional contribution, including the Abundance-Ubiquity (AU) Test (Hester et al., 2015) and Indicator value analysis (De Cáceres and Legendre, 2009). Both alternative approaches utilise persistence to determine

common phylotypes across species, habitat, location and individuals. These approaches have however provided invaluable insights into the structure, and function of the coral microbiome, as applied within the current thesis.

The healthy coral microbiome

Evidence in the literature suggests that microbial communities are highly fractionated with distinct microhabitats of the host coral (*see* Chapter 2, (Hernandez-Agreda et al., 2017)). For example, bacteria communities in coral mucus, tissue, and skeleton differ from each other and are distinct to bacteria in the water column (Rosenberg et al., 2007; Sweet et al., 2011; Apprill et al., 2016). Recently, Apprill et al. (2016) found that regardless of the location, the water column, mucus and tissue, of five Caribbean coral species harbour different bacterial communities, with a minimal fraction of the phylotypes (<10%) shared between mucus and tissue microhabitats. Coral-associated microbial aggregates (CAMAs, also known as bacterial aggregations), principally from class Gammaproteobacteria, have also been found in both epidermis and gastrodermis in over 20 coral species from different bioregions (Ainsworth et al., 2006; Ainsworth and Hoegh-Guldberg, 2009; Work and Aeby, 2014; van de Water et al., 2015). In both the coral tissue layers, *Endozoicomonas* aggregations have also been detected in *Stylophora pistillata* and *Pocillopora verrucosa* (Neave et al., 2016). A reduced bacterial community composed by phylotypes from the phylum *Actinobacteria* and genus *Ralstonia sp.* have also been shown in the perialgal space of *Symbiodinium* (symbiosome) (Ainsworth et al., 2015). Other bacterial species highlighted in the literature that have been suggested as possible universal symbionts include members of the genus *Endozoicomonas,* which has been described as a globally ubiquitous symbiont in corals, due to its stability in symbiosis even in severely bleaching colonies (Tout et al., 2015b; Apprill et al., 2016; Neave et al., 2016; Neave et al., 2017; Ziegler et al., 2017b; Pogoreutz et al., 2018). However, substantial variation in

occurrence and abundance of this species has also been demonstrated in the literature (Bayer et al., 2013; Meyer et al., 2014; Morrow et al., 2014; Apprill et al., 2016), and in the current study. Class Endozoicimonaceae (or genus *Endozoicomonas*) was present in the *I. palifera* and *A. aculeus* core microbiome (Chapter 3 and 5) and also in the core microbiome of *P. damicornis* coenosarc (Chapter 6). The relative abundance of these phylotypes varied from rare (i.e. in *P. damicornis* coenosarc) to highly abundant (i.e. in *A. aculeus*). This genus was only occasionally found in *P. speciosa*, *M. elephantotus* and *G. edwardsi*. The results in this study suggest that the genus *Endozoicomonas* may be part of the *resident community* that varies in abundance and occurrence between individuals.

Results of this thesis further contribute to the understanding of the structure of the coral microbiome (all microhabitats together; mucus, tissue and skeleton). It demonstrates that the composition and structure of associated bacterial communities in the coral holobiont differ between coral species (in Chapter 3 and 5) and between reef locations (in Chapter 4 and 5). It also provides evidence for similarities and dissimilarities, in the response of the whole bacterial community to those environments. For example, Chapter 5 shows that in the three coral species, *P. speciosa*, *M. elephantotus* and *A. aculeus*, the coral microbiome is reflective of changes in reef environment between bioregions and reef sites; but structural changes along reef depths varied between coral species and the different coral reefs.

In undertaking this work, I have further proposed a new conceptual framework under which to consider the coral microbiome. This framework proposes the coral microbiome to be considered in the following fractions;

1) *Environmental responsive community*: the coral-associated bacterial community as comprising ~20 thousand phylotypes per coral species with some of the phylotypes shared between them, transient and highly variable across coral individuals.

2) *Resident/individual microbiome*: the fraction of the bacterial community which, on average, is associated with an individual coral (i.e. colony) and which varies in structure and composition between individuals. This fraction is composed of ~600 phylotypes (as seen in taxonomic composition at the individual level in Chapters 3-6), as identified across the six coral species evaluated in Chapters 3-6 (*G. edwardsi, I. palifera, P. speciosa, M. elephantotus, A. aculeus* and *P. damicornis*).

3) *Core microbiome:* a small, conserved fraction of the community that is highly persistent between individuals (e.g. less than 50 phylotypes of ~20 thousand phylotypes), and can vary between coral species although some of the phylotypes can be shared between species (Chapter 4 and 5).

The differences in bacterial composition detected between species, reefs and individuals ultimately inflate diversity estimates (both, alpha and beta-diversity), creating the perception of coral as a highly complex biosphere. The healthy coral microbiome is indeed highly diverse with an enormous abundance of phylotypes. However, the results presented in this thesis, provide the first evidence for the coral microbiome as a structured meta-organism, arguing that it is similar to other systems studied to date.

First, the pattern of dominance based on numerical abundance by Gamma- and Alphaproteobacteria (Blackall et al., 2015; Bourne et al., 2016) is also observed in the taxonomic composition of the whole bacterial community in the six coral species evaluated within this thesis (Chapter 3-6). For example, evidence for taxonomic redundancy was identified in the three coral species evaluated at different spatial scales and across a large depth gradient (*P. speciosa*, *M. elephantotus*, and *A. aculeus* in Chapter 5). Applying indices of taxonomic relatedness (average of taxonomic distinctness, Δ^+, (Clarke and Warwick, 1998)) and taxonomic evenness (variation of taxonomic distinctness, Λ^+,(Clarke and Warwick, 2001))

further revealed a consistent pattern between coral species and reef environments, and such that bacterial communities with higher taxonomic relatedness are less taxonomically even, and vice-versa (Chapter 5). Thus, the taxonomic breadth of coral-associated bacteria community varies between species, for example, more in *A. aculeus* than in *M. elephantotus* and *P. speciosa*, despite phylotypes belonging majorly to 2-3 classes. Finally, the stability in beta-diversity further supports a hypothesis of taxonomic redundancy in the coral microbiome (Chapter 5). Beta-diversity, evaluated as turnover across spatial scales demonstrated that, for the three coral species evaluated, 23-50% of the microbial pool is conserved at the same reef and 18-27% between reefs, illustrating that there is some percentage of the bacterial community conserved within the host regardless of environment.

However, understanding the biological relevance of taxonomic redundancy is the ultimate question that needs to be addressed. As such I suggest that there is a high likelihood for functional redundancy within the coral microbiome. I investigated the *core microbiome* of *P. speciosa*, *M. elephantotus*, and *A. aculeus* across the depth, reef and bioregions in which the corals reside (Chapter 5). Bacterial phylotypes representing the multivariate pattern observed in each reef were selected for each species and, along with the *core microbiome*, evaluated taxonomically and functionally. Both the core microbiome and representative phylotypes per reef showed similar functional prediction across reefs and depth. While predictive functional estimation is limited in their power and reliability due to the extent of published literature (Koo et al., 2017), studies to date have shown this approach can provide accurate insights into differing functional roles between communities (e.g. (Odamaki et al., 2016)). In the current study, I find very few differences in predicted function between the core microbiomes generated in the 3 coral species investigated. These results provide early evidence to suggest that the *resident community* and *core microbiome* are likely to be composed by different phylotypes performing the same functional roles.

Results of this work can contribute to the efforts in manipulating coral microbiome to enhance host resistance and resilience in current environmental conditions. The concept of Beneficial Microorganisms for Corals (BMC, (Peixoto, Rosado et al. 2017)) has been recently suggested as an approach to manipulate bacteria contributing to coral health. Through the use of core microbiome concept, persistent phylotypes would be identified in healthy assemblages. Further research in the functional contribution of persistent phylotypes would then permit to identify bacteria able to play those functional roles more efficiently in extreme conditions (e.g. high temperature, high turbidity). In a preventive strategy, these core phylotypes could be included in the BMC consortiums to enhance those functional activities in the microbiome of environmental challenged corals. The taxonomic structure identified in this study (Chapter 5) could be one of the traits taken into account in the design of BMC consortiums. In cases for bioremediation, the application of BMC consortiums having the taxonomic structure of a healthy microbiome could stimulate restoration of healthy states in dysbiotic (bleached or diseased) microbiomes.

Mechanisms driving bacterial communities in corals

The evidence for a structured bacterial microbiome in corals provides several avenues from which to investigate the potential mechanisms driving these communities in corals. Some of the mechanisms discussed here are also processes that have been observed in systems such as the as human gut and the plant rhizosphere. As mentioned in Chapter 2, human and coral microbiomes differ in their interaction with the environment (corals are an open system) and the factors generating different microhabitats are distinct. However, structurally these two systems mirror each other in the presence of a mucus layer as an external barrier (Ritchie, 2006; Bythell and Wild, 2011; Tuddenham and Sears, 2015). Similarly, corals and the rhizosphere system in plants are both openly exposed to a high nutrient environment in which the activities

of other organisms and the host, produces a dynamic and rapidly changing microbial system. Here I compare and contrast current understanding of the establishment of the coral microbiome, from studies undertaken across phyla and provide insight into the factors which drive the microbial community structure within the coral system.

1) Mechanisms of bacteria acquisition

The acquisition of bacteria into the coral meta-organism could occur in, at least, three ways via;

1) Vertical transmission, inherited from parental corals,

2) Horizontal transmission in the form of uptake of individual bacteria from the water column, and

3) Horizontal transmission through the inclusion of bacteria through the uptake of *Symbiodinium*.

The vertical transmission of bacteria has been demonstrated in newly released planulae of the brooding coral *Porites astreoides* (Sharp et al., 2012) and gametes of spawner *Mussismilia hispida* (Leite et al., 2017). The horizontal acquisition has been observed in fully developed planulae (79 h) of the broadcast-spawner *Pocillopora meandrina* (Apprill et al., 2009), and in early development stages of the broadcast-spawner *Acropora millepora* (Lema et al., 2014). As such, there is no consensus as yet, between mode of acquisition and the host corals reproductive strategy. However, bacterial communities in the early stages of development converge as a low diversity and abundance system, differing from the adult-associated bacterial community. Furthermore, during early life stages, the genus *Roseobacter* is often a recurrent and highly abundant member of the microbial community (Apprill et al., 2009; Sharp et al.,

2012; Lema et al., 2014). The third proposed mechanism, the uptake of bacteria to coral tissues during the establishment of symbiosis with *Symbiodinium*, has not yet been proven. In aquatic environments, phytoplankton and bacteria interact in close proximity, influencing performance and health status of both partners via broad range relationships, from mutualism to competition (Seymour et al., 2017). Phylotypes from the genera *Labrenzia* (Alphaproteobacteria), *Marinobacter* and Chromatiaceae (Gammaproteobacteria) have been found associated to 18 types from 5 clades of cultured *Symbiodinium*, suggesting a physiological contribution of bacteria to zooxanthellae functioning (Lawson et al., 2018). While in symbiosis with corals, genera *Actinobacter* and *Ralstonia* have been detected inhabiting the gastrodermal cells with the endosymbiotic dinoflagellates (Ainsworth et al., 2015). Thus, if *Symbiodinium* and bacteria are in symbiosis in the water column and reef sediments where they exist in free-living form, the entrance of specific bacteria to the coral system may be mediated through the horizontal acquisition of zooxanthellae. This acquisition is likely to drive bacterial community found in the symbiosome, closely related to the *Symbiodinium*, rather than impact the overall bacterial community in corals (Littman et al., 2009). Most likely, the bacterial acquisition may be occurring as a combination of all the strategies (Bourne et al., 2016). The initial restricted bacterial community acquired from parental transmission or inclusion via *Symbiodinium* and the subsequent evolution, in structure and composition, to a mature, diverse and abundant bacterial community (Apprill et al., 2009; Littman et al., 2009; Sharp et al., 2012; Williams et al., 2015) with the acquisition and constant replacement of bacterial members via horizontal uptake or due to *Symbiodinium* switching (Baker 2003). Under this hypothesis, coral microbiome would be much like what has been described with the establishment of the human microbiome (Spor et al., 2011).

2) Chemical mechanisms controlling bacterial community structure

The difference in bacterial community structure and diversity between early life stages coral and mature corals (Apprill et al., 2009; Littman et al., 2009; Sharp et al., 2012; Williams et al., 2015) suggest that horizontal acquisition has a relevant role in the assembly of coral-associated microbial communities. Horizontal acquisition of bacteria could be responsible for the differences in the structure of bacterial communities within coral species among reef environments, generating taxonomical and functional redundancy (Chapter 4 and 5). Corals may be controlling bacterial communities through chemotaxis, as well as physical and immunological barriers. Coral mucus is an extremely nutritive environment for bacteria, enriched with carbohydrates, amino acids and inorganic nutrients (Wild et al., 2005; Raina et al., 2009; Raina et al., 2010; Raina et al., 2013; Garren et al., 2014). The surrounding space, continuous to the mucus layer, is also a niche different from that of the overlying water column and comprising a nutritive and chemical gradient from the surface of the mucus layer, creating a niche habitat (Tout et al., 2014) which is detected by bacteria through chemotaxis (Garren et al., 2014; Tout et al., 2015a). Dimethylsulfoniopropionate (DMSP; an organic sulphur compound present in the mucus) and amino acids (tryptophan, aspartic and casamino acid) produce a chemotactic response in both pathogenic and non-pathogenic coral-associated bacteria as well as non-coral-associated bacteria (Garren et al., 2014; Tout et al., 2015a). As occurs in plants (Bulgarelli et al., 2013), corals could be attracting bacteria present in the water column (through the production of DMSP, amino acids and carbons) to establish symbiosis. However, since the bacterial community varies at reef scales (Morrow et al., 2012; Pantos et al., 2015), the phylotypes attracted to the coral from the water column, may be taxonomically different between reefs, but have equivalent metabolic capabilities, facilitating the functional redundancy in *core microbiome*, *resident* and *environmental responsive community*. Differences in the diversity, dispersion and core microbiome in *P. damicornis* coenosarc and

polyps (Chapter 6) reflect how differences between microniches (at the scale of millimetres) within the coral colony can generate variations in a fraction of the bacterial community.

3) Physical and immunological barriers

Physical and immunological barriers likely play important roles in controlling the bacterial community structure of corals. The concentration of bacteria in coral mucus is 100-1000 fold higher than that of the adjacent water column (Rosenberg et al., 2007) and less than 10% of the bacteria present in the mucus are in the coral tissues (Apprill et al., 2016). Thus, as observed in the human gut, coral mucus may act not only as a particle trap (Wild et al., 2004) but also as a trap for bacteria in the water column. Mucus properties are likely to differ in viscosity, thickness and composition between coral species, which consequently, will impact the role of this matrix as a physical barrier and the composition of the bacterial community in corals (e.g. mucus shedding (Glasl, Herndl et al. 2016)). This, in turn, will also likely impact the immune function and the role of immunological barriers, in controlling the coral-bacteria interaction across different species. Immunological processes driving bacterial communities in corals are understudied, and therefore it is unknown how these interact with the coral host (Bourne et al., 2016). However, immunological control is likely to occur in all coral microhabitats given the observed difference in the abundance and diversity of bacteria among microhabitats (from mucus to cellular spaces in tissues). Similarly, strict control on the microbial community has been found in the epithelium of other cnidarians (reviewed in Bosch and Miller (2016)). Substances such as microbe-associated molecular patterns (MAMPs) are recognized by receptors (Toll-like receptors, pattern recognition receptors PRRs) localized in the epithelium of cnidarians, triggering immunological responses for the production of antimicrobial peptides (Bosch et al., 2009). Some microbes are also likely able to evade these process and to establish

stable symbiosis through other recognized receptors, as occurs with *Symbiodinium* (Davy et al., 2012).

In fact, further understanding of the taxonomical and functional redundancy in coral-associated microbial communities may be informed through our knowledge on the coral-*Symbiodinium* symbiosis. Corals can establish symbiosis with distinct clades and types of the endosymbiotic dinoflagellates *Symbiodinium* (>100 ITS2 types, (Pochon, Montoya-Burgos et al. 2006, Blackall, Wilson et al. 2015)). Within each coral species, and each individual coral, there are patterns of dominance and conserved, ubiquitous interactions, and our understanding of both dominance and diversity, contribute to comprehending important traits exhibited by the host coral (Pochon, Pawlowski et al. 2001, Baker 2003, Cooper, Berkelmans et al. 2011). The endosymbiotic association influences host growth, thermal resilience, and recovery from stress (Little, van Oppen et al. 2004, Abrego, Ulstrup et al. 2008, Howells, Beltran et al. 2012, Hume, D'angelo et al. 2013). However, the endosymbiosis with this population of eukaryotic microbes is one in which the community provides the same pre-dominant functional role in the host, that being the supply of carbon (Muscatine, McCloskey et al. 1981, Muscatine, Falkowski et al. 1984). The dominant type, and diversity of symbiosis results in differing performance and ultimately can lead to a change in the dominant symbiont preference following disturbance to the symbiosis (Little, van Oppen et al. 2004, Abrego, Ulstrup et al. 2008, Hsu, Keshavmurthy et al. 2012). Considering the eukaryote-eukaryote symbiosis of corals in this way, and applying a similar approach to the eukaryote-prokaryote symbiosis, it may be that the bacterial community dynamics in many ways, resemble that of the endosymbiotic *Symbiodinium* assembly.

Conclusions and future research

Herein, I have identified and described the structure of the healthy coral microbiome, contributing to our understanding of the mechanisms driving coral resistance and resilience to environmental change. The core microbiome framework has demonstrated to be a useful tool for identifying subtle differences in microbial communities and documenting persistent phylotypes within the coral holobiont. From here further challenges remain, including to determining the stability of potential symbioses over time, the localization of these bacteria within coral microhabitats, and the characterisation of the symbiotic relationship across the symbiotic-dysbiotic continuum. Addressing these questions will, without doubt, require a coupling taxonomic profiling with novel multidisciplinary approaches, which consequently represent the collaboration of different groups and the standardization of protocols. The proposal of a structured coral microbiome, as identified by combining the core microbiome framework coupled with ecological tools, opens a new avenue for research to further address the taxonomic, and potentially functional, redundancy of microbial communities in corals. Ultimately, this knowledge will be crucial in efforts enhancing the resilience of coral communities, whether it is directly (through manipulation of the microbiome) or indirectly (or through targeted protection of ecological communities).

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." Proceedings of the Royal Society B: Biological Sciences **275**(1648): 2273-2282.

Adam, B., I. Klawonn, J. B. Sveden, J. Bergkvist, N. Nahar, J. Walve, S. Littmann, M. J. Whitehouse, G. Lavik, M. M. Kuypers and H. Ploug (2016). "N2-fixation, ammonium release and N-transfer to the microbial and classical food web within a plankton community." The ISME Journal **10**(2): 450-459.

Agostini, S., Y. Suzuki, T. Higuchi, B. E. Casareto, K. Yoshinaga, Y. Nakano and H. Fujimura (2012). "Biological and chemical characteristics of the coral gastric cavity." Coral Reefs **31**(1): 147-156.

Ainsworth, T. and O. Hoegh-Guldberg (2009). "Bacterial communities closely associated with coral tissues vary under experimental and natural reef conditions and thermal stress." Aquatic Biology **4**(3): 289-296.

Ainsworth, T. D., M. Fine, L. L. Blackall and O. Hoegh-Guldberg (2006). "Fluorescence *in situ* hybridization and spectral imaging of coral-associated bacterial communities." Applied and Environmental Microbiology **72**(4): 3016-3020.

Ainsworth, T. D. and R. D. Gates (2016). "Corals' microbial sentinels." Science **352**(6293): 1518-1519.

Ainsworth, T. D., L. Krause, T. Bridge, G. Torda, J. B. Raina, M. Zakrzewski, R. D. Gates, J. L. Padilla-Gamino, H. L. Spalding, C. Smith, E. S. Woolsey, D. G. Bourne, P. Bongaerts, O. Hoegh-Guldberg and W. Leggat (2015). "The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts." The ISME Journal **9**(10): 2261-2274.

Ainsworth, T. D., R. V. Thurber and R. D. Gates (2010). "The future of coral reefs: a microbial perspective." Trends in Ecology & Evolution **25**(4): 233-240.

Alahuhta, J., M. Toivanen, J. Hjort, F. Ecke, L. B. Johnson, L. Sass and J. Heino (2017). "Species richness and taxonomic distinctness of lake macrophytes along environmental gradients in two continents." Freshwater biology **62**(7): 1194-1206.

Anderson, M., R. N. Gorley and R. K. Clarke (2008). Permanova+ for Primer: Guide to software and statistical methods. Plymouth, Primer-E Limited.

Anderson, M. J. (2001). "A new method for non-parametric multivariate analysis of variance." Austral Ecology **26**(1): 32-46.

Angly, F. E., C. Heath, T. C. Morgan, H. Tonin, V. Rich, B. Schaffelke, D. G. Bourne and G. W. Tyson (2016). "Marine microbial communities of the Great Barrier Reef lagoon are influenced by riverine floodwaters and seasonal weather events." PeerJ **4**: e1511.

Apprill, A., K. Hughen and T. Mincer (2013). "Major similarities in the bacterial communities associated with lesioned and healthy Fungiidae corals." Environmental Microbiology **15**(7): 2063-2072.

Apprill, A., H. Q. Marlow, M. Q. Martindale and M. S. Rappe (2009). "The onset of microbial associations in the coral *Pocillopora meandrina*." The ISME Journal **3**(6): 685-699.

Apprill, A., H. Q. Marlow, M. Q. Martindale and M. S. Rappe (2012). "Specificity of associations between bacteria and the coral *Pocillopora meandrina* during early development." Applied and Environmental Microbiology **78**(20): 7467-7475.

Apprill, A., L. G. Weber and A. E. Santoro (2016). "Distinguishing between microbial habitats unravels ecological complexity in coral microbiomes." mSystems **1**(5): e00143-00116.

Arencibia, A. D., F. Vinagre, Y. Estevez, A. Bernal, J. Perez, J. Cavalcanti, I. Santana and A. S. Hemerly (2006). "*Gluconoacetobacter diazotrophicus* elicitate a sugarcane defense response against a pathogenic bacteria *Xanthomonas albilineans*." Plant signaling & behavior **1**(5): 265-273.

Ashton, M., W. Rosado, N. S. Govind and T. R. Tosteson (2003). "Culturable and nonculturable bacterial symbionts in the toxic benthic dinoflagellate *Ostreopsis lenticularis*." Toxicon **42**(4): 419-424.

Augustin, R., S. Fraune, S. Franzenburg and T. C. G. Bosch (2012). Where simplicity meets complexity: *Hydra*, a model for host–microbe interactions. New York, NY, Springer New York.

Backhed, F., C. M. Fraser, Y. Ringel, M. E. Sanders, R. B. Sartor, P. M. Sherman, J. Versalovic, V. Young and B. B. Finlay (2012). "Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications." Cell Host Microbe **12**(5): 611-622.

Baird, A. and P. Marshall (2002). "Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef." Marine Ecology Progress Series **237**: 133-141.

Baker, A. C. (2003). "Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of Symbiodinium." Annual Review of Ecology, Evolution and Systematics **34**(1): 661-689.

Baldrian, P., M. Kolarik, M. Stursova, J. Kopecky, V. Valaskova, T. Vetrovsky, L. Zifcakova, J. Snajdr, J. Ridl, C. Vlcek and J. Voriskova (2012). "Active and total microbial communities in forest soil are largely different and highly stratified during decomposition." The ISME Journal **6**(2): 248-258.

Barott, K. L., B. Rodriguez-Brito, J. Janouskovec, K. L. Marhaver, J. E. Smith, P. Keeling and F. L. Rohwer (2011). "Microbial diversity associated with four functional groups of benthic reef algae and the reef-building coral *Montastraea annularis*." Environmental Microbiology **13**(5): 1192-1204.

Barott, K. L., B. Rodriguez-Mueller, M. Youle, K. L. Marhaver, M. J. A. Vermeij, J. E. Smith and F. L. Rohwer (2012). "Microbial to reef scale interactions between the reef-building coral *Montastraea annularis* and benthic algae." Proceedings of the Royal Society B: Biological Sciences **279**: 1655–1664.

Baselga, A. (2010). "Partitioning the turnover and nestedness components of beta diversity." Global Ecology and Biogeography **19**(1): 134-143.

Baselga, A. and C. D. L. Orme (2012). "Betapart: an R package for the study of beta diversity." Methods in Ecology and Evolution **3**(5): 808-812.

Baselga, A., D. Orme, S. Villeger, J. De Bortoli and F. Leprieur (2012). "Partitioning beta diversity into turnover and nestedness components." Package 'betapart', Version **1**.

Bates, S. T., J. C. Clemente, G. E. Flores, W. A. Walters, L. W. Parfrey, R. Knight and N. Fierer (2013). "Global biogeography of highly diverse protistan communities in soil." The ISME Journal **7**(3): 652-659.

Bayer, T., M. J. Neave, A. Alsheikh-Hussain, M. Aranda, L. K. Yum, T. Mincer, K. Hughen, A. Apprill and C. R. Voolstra (2013). "The microbiome of the Red Sea coral *Stylophora pistillata* is dominated by tissue-associated *Endozoicomonas* bacteria." Applied and Environmental Microbiology **79**(15): 4759-4762.

Beaman, R. (2010). Project 3DGBR: A high-resolution depth model for the Great Barrier Reef and Coral Sea. Cairns, Australia, Marine and Tropical Sciences Research Facility (MTSRF). **Project 2.5i.1a Final Report:** 13 plus Appendix 11.

Belizario, J. E. and M. Napolitano (2015). "Human microbiomes and their roles in dysbiosis, common diseases, and novel therapeutic approaches." Frontiers in Microbiology **6**: 1050.

Bellwood, D. R., T. P. Hughes, C. Folke and M. Nyström (2004). "Confronting the coral reef crisis." Nature **429**(6994): 827-833.

Bennett, J. R. and B. Gilbert (2016). "Contrasting beta diversity among regions: how do classical and multivariate approaches compare?" Global Ecology and Biogeography **25**(3): 368-377.

Blackall, L. L., B. Wilson and M. J. van Oppen (2015). "Coral-the world's most diverse symbiotic ecosystem." Molecular Ecology **24**(21): 5330-5347.

Bolhuis, H., M. S. Cretoiu and L. J. Stal (2014). "Molecular ecology of microbial mats." FEMS Microbiology Ecology **90**(2): 335-350.

Bongaerts, P., T. C. L. Bridge, D. I. Kline, P. R. Muir, C. C. Wallace, R. J. Beaman and O. Hoegh-Guldberg (2011). "Mesophotic coral ecosystems on the walls of Coral Sea atolls." Coral Reefs **30**(2): 335-335.

Bongaerts, P., P. R. Frade, K. B. Hay, N. Englebert, K. R. W. Latijnhouwers, R. P. M. Bak, M. J. A. Vermeij and O. Hoegh-Guldberg (2015). "Deep down on a Caribbean reef: lower mesophotic depths harbor a specialized coral-endosymbiont community." Scientific Reports **5**: 7652.

Bongaerts, P., P. R. Frade, J. J. Ogier, K. B. Hay, J. van Bleijswijk, N. Englebert, M. J. A. Vermeij, R. P. M. Bak, P. M. Visser and O. Hoegh-Guldberg (2013). "Sharing the slope: depth partitioning of agariciid corals and associated *Symbiodinium* across shallow and mesophotic habitats (2-60 m) on a Caribbean reef." BMC Evolutionary Biology **13**: 205.

Bongaerts, P., C. Riginos, T. Ridgway, E. M. Sampayo, M. J. H. van Oppen, N. Englebert, F. Vermeulen and O. Hoegh-Guldberg (2010). "Genetic divergence across habitats in the widespread coral *Seriatopora hystrix* and Its associated *Symbiodinium*." PLoS One **5**(5): e10871.

Bordenstein, S. R. and K. R. Theis (2015). "Host biology in light of the microbiome: ten principles of holobionts and hologenomes." PLoS Biology **13**(8): e1002226.

Bosch, T. C. G. (2012). "Understanding complex host-microbe interactions in *Hydra*." Gut Microbes **3**(4): 345-351.

Bourne, D., Y. Iida, S. Uthicke and C. Smith-Keune (2008). "Changes in coral-associated microbial communities during a bleaching event." The ISME Journal **2**(4): 350-363.

Bourne, D. and C. Munn (2005). "Diversity of bacteria associated with the coral *Pocillopora damicornis* from the Great Barrier Reef." Environmental Microbiology **7**(8): 1162-1174.

Bourne, D. G., P. G. Dennis, S. Uthicke, R. M. Soo, G. W. Tyson and N. Webster (2013). "Coral reef invertebrate microbiomes correlate with the presence of photosymbionts." The ISME Journal **7**(7): 1452-1458.

Bourne, D. G., K. M. Morrow and N. S. Webster (2016). "Insights into the coral microbiome: underpinning the health and resilience of reef ecosystems." Annual Review of Microbiology **70**: 317-340.

Brown, B. E. and J. C. Bythell (2005). "Perspectives on mucus secretion in reef corals." Marine Ecology Progress Series **296**: 291-309.

Bruck, T. B., W. M. Bruck, L. Z. Santiago-Vazquez, P. J. McCarthy and R. G. Kerr (2007). "Diversity of the bacterial communities associated with the azooxanthellate deep water octocorals *Leptogorgia minimata*, *Iciligorgia schrammi*, and *Swiftia exertia*." Marine Biotechnology **9**(5): 561-576.

Bruder, L. M., M. Dorkes, B. M. Fuchs, W. Ludwig and W. Liebl (2016). "Flow cytometric sorting of fecal bacteria after in situ hybridization with polynucleotide probes." Systematic and Applied Microbiology **39**(7): 464-475.

Bulgarelli, D., M. Rott, K. Schlaeppi, E. Ver Loren van Themaat, N. Ahmadinejad, F. Assenza, P. Rauf, B. Huettel, R. Reinhardt, E. Schmelzer, J. Peplies, F. O. Gloeckner, R. Amann, T. Eickhorst and P. Schulze-Lefert (2012). "Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota." Nature **488**(7409): 91-95.

Bulgarelli, D., K. Schlaeppi, S. Spaepen, E. V. L. van Themaat and P. Schulze-Lefert (2013). "Structure and functions of the bacterial microbiota of plants." Annual Review of Plant Biology **64**: 807-838.

Bythell, J. C., M. R. Barer, R. P. Cooney, J. R. Guest, A. G. O'Donnell, O. Pantos and M. D. Le Tissier (2002). "Histopathological methods for the investigation of microbial communities associated with disease lesions in reef corals." Letters in Applied Microbiology **34**(5): 359-364.

Bythell, J. C. and C. Wild (2011). "Biology and ecology of coral mucus release." Journal of Experimental Marine Biology and Ecology **408**(1-2): 88-93.

Cameron, E. A., M. A. Maynard, C. J. Smith, T. J. Smith, N. M. Koropatkin and E. C. Martens (2012). "Multidomain carbohydrate-binding proteins Involved in *Bacteroides thetaiotaomicron* starch metabolism." Journal of Biological Chemistry **287**(41): 34614-34625.

Canani, R. B., M. D. Costanzo, L. Leone, M. Pedata, R. Meli and A. Calignano (2011). "Potential beneficial effects of butyrate in intestinal and extraintestinal diseases." World Journal of Gastroenterology **17**(12): 1519-1528.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Pena, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Tumbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld and R. Knight (2010). "QIIME allows analysis of high-throughput community sequencing data." Nature Methods **7**(5): 335-336.

Cardenas, A., L. M. Rodriguez, V. Pizarro, L. F. Cadavid and C. Arevalo-Ferro (2012). "Shifts in bacterial communities of two caribbean reef-building coral species affected by white plague disease." The ISME Journal **6**(3): 502-512.

Carlos, C., T. T. Torres and L. M. Ottoboni (2013). "Bacterial communities and species-specific associations with the mucus of Brazilian coral species." Scientific Reports **3**: 1624-1630.

Carmona, C. P., F. M. Azcarate, F. de Bello, H. S. Ollero, J. Leps and B. Peco (2012). "Taxonomical and functional diversity turnover in Mediterranean grasslands: interactions between grazing, habitat type and rainfall." Journal of Applied Ecology **49**(5): 1084-1093.

Carvalho, T. L. G., E. Balsemao-Pires, R. M. Saraiva, P. C. G. Ferreira and A. S. Hemerly (2014). "Nitrogen signalling in plant interactions with associative and endophytic diazotrophic bacteria." Journal of Experimental Botany **65**(19): 5631-5642.

Castillo, I., C. Lodeiros, M. Nunez and I. Campos (2001). "*In vitro* evaluation of antibacterial substances produced by bacteria isolated from different marine organisms." Revista de Biologia Tropical **49**(3-4): 1213-1221.

Ceh, J., M. R. Kilburn, J. B. Cliff, J. B. Raina, M. van Keulen and D. G. Bourne (2013). "Nutrient cycling in early coral life stages: *Pocillopora damicornis* larvae provide their algal symbiont (*Symbiodinium*) with nitrogen acquired from bacterial associates." Ecology and Evolution **3**(8): 2393-2400.

Ceh, J., J. B. Raina, R. M. Soo, M. van Keulen and D. G. Bourne (2012). "Coral-bacterial communities before and after a coral mass spawning event on Ningaloo Reef." PLoS One **7**(5): e36920.

Ceh, J., M. van Keulen and D. G. Bourne (2011). "Coral-associated bacterial communities on Ningaloo Reef, Western Australia." FEMS Microbiology Ecology **75**(1): 134-144.

Ceh, J., M. van Keulen and D. G. Bourne (2013). "Intergenerational transfer of specific bacteria in corals and possible implications for offspring fitness." Microbial Ecology **65**(1): 227-231.

Chaparro, J. M., D. V. Badri, M. G. Bakker, A. Sugiyama, D. K. Manter and J. M. Vivanco (2013). "Root exudation of phytochemicals in *Arabidopsis* follows specific patterns that are developmentally programmed and correlate with soil microbial functions." PLoS One **8**(2): e55731.

Chen, C.-P., C.-H. Tseng, C. A. Chen and S.-L. Tang (2011). "The dynamics of microbial partnerships in the coral *Isopora palifera*." The ISME Journal **5**(4): 728-740.

Chu, N. D. and S. V. Vollmer (2016). "Caribbean corals house shared and host-specific microbial symbionts over time and space." Environmental Microbiology Reports **8**(4): 493-500.

Claesson, M. J., Q. Wang, O. O'Sullivan, R. Greene-Diniz, J. R. Cole, R. P. Ross and P. W. O'Toole (2010). "Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16s rRNA gene regions." Nucleic Acids Research **38**(22): e200.

Clarke, K. and R. Warwick (1998). "Quantifying structural redundancy in ecological communities." Oecologia **113**(2): 278-289.

Clarke, K. R. (1993). "Non-parametric multivariate analyses of changes in community structure." Austral Ecology **18**(1): 117-143.

Clarke, K. R., R. N. Gorley and R. M. Warwick (2014). Change in marine communities: An approach to statistical analysis and interpretation. Plymouth, UK, PRIMER-E.

Clarke, K. R., P. J. Somerfield, L. Airoldi and R. M. Warwick (2006). "Exploring interactions by second-stage community analyses." Journal of Experimental Marine Biology and Ecology **338**(2): 179-192.

Clarke, K. R. and R. M. Warwick (1998). "A taxonomic distinctness index and its statistical properties." Journal of Applied Ecology **35**(4): 523-531.

Clarke, K. R. and R. M. Warwick (2001). "A further biodiversity index applicable to species lists: variation in taxonomic distinctness." Marine Ecology Progress Series **216**: 265-278.

Closek, C. J., S. Sunagawa, M. K. DeSalvo, Y. M. Piceno, T. Z. DeSantis, E. L. Brodie, M. X. Weber, C. R. Voolstra, G. L. Andersen and M. Medina (2014). "Coral transcriptome and bacterial community profiles reveal distinct Yellow Band Disease states in Orbicella faveolata." The ISME Journal.

Condit, R., N. Pitman, E. G. Leigh, J. Chave, J. Terborgh, R. B. Foster, P. Núñez, S. Aguilar, R. Valencia, G. Villa, H. C. Muller-Landau, E. Losos and S. P. Hubbell (2002). "Beta-diversity in tropical forest trees." Science **295**(5555): 666-669.

Cooper, T. F., R. Berkelmans, K. E. Ulstrup, S. Weeks, B. Radford, A. M. Jones, J. Doyle, M. Canto, R. A. O'Leary and M. J. van Oppen (2011). "Environmental factors controlling the distribution of Symbiodinium harboured by the coral *Acropora millepora* on the Great Barrier Reef." PLoS One **6**(10): e25536.

Costello, E. K., K. Stagaman, L. Dethlefsen, B. J. Bohannan and D. A. Relman (2012). "The application of ecological theory toward an understanding of the human microbiome." Science **336**(6086): 1255-1262.

Croquer, A., C. Bastidas, A. Elliott and M. Sweet (2013). "Bacterial assemblages shifts from healthy to yellow band disease states in the dominant reef coral *Montastraea faveolata*." Environmental Microbiology Reports **5**(1): 90-96.

Curson, A. R. J., R. Rogers, J. D. Todd, C. A. Brearley and A. W. B. Johnston (2008). "Molecular genetic analysis of a dimethylsulfoniopropionate lyase that liberates the climate-changing gas dimethylsulfide in several marine alpha-proteobacteria and *Rhodobacter sphaeroides*." Environmental Microbiology **10**(3): 757-767.

D'Amore, R., U. Z. Ijaz, M. Schirmer, J. G. Kenny, R. Gregory, A. C. Darby, M. Shakya, M. Podar, C. Quince and N. Hall (2016). "A comprehensive benchmarking study of protocols and sequencing platforms for 16S rRNA community profiling." BMC Genomics **17**(1): 55.

Davy, S. K., D. Allemand and V. M. Weis (2012). "Cell biology of cnidarian-dinoflagellate symbiosis." Microbiology and Molecular Biology Reviews **76**(2): 229-261.

Dawson, M. N., K. A. Raskoff and D. K. Jacobs (1998). "Field preservation of marine invertebrate tissue for DNA analyses." Molecular marine biology and biotechnology **7**(2): 145-152.

De Cáceres, M. and P. Legendre (2009). "Associations between species and groups of sites: indices and statistical inference." Ecology **90**(12): 3566-3574.

Dennehy, J. J. (2014). "What ecologists can tell virologists." Annual Review of Microbiology **68**(1): 117-135.

DeSantis, T. Z., P. Hugenholtz, N. Larsen, M. Rojas, E. L. Brodie, K. Keller, T. Huber, D. Dalevi, P. Hu and G. L. Andersen (2006). "Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB." Applied and Environmental microbiology **72**(7): 5069-5072.

DeVantier, L. and E. Turak (2017). "Species richness and relative abundance of reef-building corals in the Indo-West Pacific." Diversity **9**(3): 25.

Dickschat, J. S., S. Wickel, C. J. Bolten, T. Nawrath, S. Schulz and C. Wittmann (2010). "Pyrazine biosynthesis in *Corynebacterium glutamicum*." European Journal of Organic Chemistry(14): 2687-2695.

Dinsdale, E. A., R. A. Edwards, D. Hall, F. Angly, M. Breitbart, J. M. Brulc, M. Furlan, C. Desnues, M. Haynes, L. Li, L. McDaniel, M. A. Moran, K. E. Nelson, C. Nilsson, R. Olson, J. Paul, B. R. Brito, Y. Ruan, B. K. Swan, R. Stevens, D. L. Valentine, R. V. Thurber, L. Wegley, B. A. White and F. Rohwer (2008). "Functional metagenomic profiling of nine biomes." Nature **452**(7187): 629-632.

Dinsdale, E. A., O. Pantos, S. Smriga, R. A. Edwards, F. Angly, L. Wegley, M. Hatay, D. Hall, E. Brown, M. Haynes, L. Krause, E. Sala, S. A. Sandin, R. V. Thurber, B. L. Willis, F. Azam, N. Knowlton and F. Rohwer (2008). "Microbial ecology of four coral atolls in the Northern Line Islands." PLoS One **3**(2).

Duerkop, B. A., S. Vaishnava and L. V. Hooper (2009). "Immune responses to the microbiota at the intestinal mucosal surface." Immunity **31**(3): 368-376.

Eakin, C. M., J. A. Morgan, S. F. Heron, T. B. Smith, G. Liu, L. Alvarez-Filip, B. Baca, E. Bartels, C. Bastidas, C. Bouchon, M. Brandt, A. W. Bruckner, L. Bunkley-Williams, A. Cameron, B. D. Causey, M. Chiappone, T. R. L. Christensen, M. J. C. Crabbe, O. Day, E. de la Guardia, G. Díaz-Pulido, D. DiResta, D. L. Gil-Agudelo, D. S. Gilliam, R. N. Ginsburg, S. Gore, H. M. Guzmán, J. C. Hendee, E. A. Hernández-Delgado, E. Husain, C. F. G. Jeffrey, R. J. Jones, E. Jordán-Dahlgren, L. S. Kaufman, D. I. Kline, P. A. Kramer, J. C. Lang, D. Lirman, J. Mallela, C. Manfrino, J.-P. Maréchal, K. Marks, J. Mihaly, W. J. Miller, E. M. Mueller, E. M. Muller, C. A. Orozco Toro, H. A. Oxenford, D. Ponce-Taylor, N. Quinn, K. B. Ritchie, S. Rodríguez, A. R. Ramírez, S. Romano, J. F. Samhouri, J. A. Sánchez, G. P. Schmahl, B. V. Shank, W. J. Skirving, S. C. C. Steiner, E. Villamizar, S. M. Walsh, C. Walter, E. Weil, E. H. Williams, K. W. Roberson and Y. Yusuf (2010). "Caribbean corals in crisis: record thermal stress, bleaching, and mortality in 2005." PLoS One **5**(11): e13969.

Edgar, R. C. (2010). "Search and clustering orders of magnitude faster than BLAST." Bioinformatics **26**(19): 2460-2461.

Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince and R. Knight (2011). "UCHIME improves sensitivity and speed of chimera detection." Bioinformatics **27**(16): 2194-2200.

Edwards, J., C. Johnson, C. Santos-Medellín, E. Lurie, N. K. Podishetty, S. Bhatnagar, J. A. Eisen and V. Sundaresan (2015). "Structure, variation, and assembly of the root-associated microbiomes of rice." Proceedings of the National Academy of Sciences of the United States of America **112**(8): E911-E920.

Einbinder, S., T. Mass, E. Brokovich, Z. Dubinsky, J. Erez and D. Tchernov (2009). "Changes in morphology and diet of the coral *Stylophora pistillata* along a depth gradient." Marine Ecology Progress Series **381**: 167-174.

Elbrecht, V. and F. Leese (2015). "Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass—sequence relationships with an innovative metabarcoding protocol." PLoS One **10**(7): e0130324.

Englebert, N., P. Bongaerts, P. R. Muir, K. B. Hay, M. Pichon and O. Hoegh-Guldberg (2017). "Lower mesophotic coral communities (60-125 m depth) of the northern Great Barrier Reef and Coral Sea." PLoS One **12**(2): e0170336.

Faul, F., E. Erdfelder, A.-G. Lang and A. Buchner (2007). "G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences." Behavior Research Methods **39**(2): 175-191.

Fierer, N., M. Hamady, C. L. Lauber and R. Knight (2008). "The influence of sex, handedness, and washing on the diversity of hand surface bacteria." Proceedings of the National Academy of Sciences of the United States of America **105**(46): 17994-17999.

Fine, M. and Y. Loya (2002). "Endolithic algae: an alternative source of photoassimilates during coral bleaching." Proceedings of the Royal Society of London. Series B, Biological Sciences **269**(1497): 1205-1210.

Fouhy, F., J. Deane, M. C. Rea, Ó. O'Sullivan, R. P. Ross, G. O'Callaghan, B. J. Plant and C. Stanton (2015). "The effects of freezing on faecal microbiota as determined using MiSeq sequencing and culture-based investigations." PLoS One **10**(3): e0119355.

Frade, P., F. De Jongh, F. Vermeulen, J. Van Bleijswijk and R. Bak (2008). "Variation in symbiont distribution between closely related coral species over large depth ranges." Molecular Ecology **17**(2): 691-703.

Franzosa, E. A., T. Hsu, A. Sirota-Madi, A. Shafquat, G. Abu-Ali, X. C. Morgan and C. Huttenhower (2015). "Sequencing and beyond: integrating molecular 'omics' for microbial community profiling." Nature Reviews: Microbiology **13**(6): 360-372.

Frias-Lopez, J., A. L. Zerkle, G. T. Bonheyo and B. W. Fouke (2002). "Partitioning of bacterial communities between seawater and healthy, black band diseased, and dead coral surfaces." Applied and Environmental Microbiology **68**(5): 2214-2228.

Galloway, S., T. Work, V. Bochsler, R. Harley, E. Kramarsky-Winters, S. Mc Laughlin, C. Meteyer, J. Morado, J. Nicholson and P. Parnell (2006). "A report of the CDHC coral histopathology workshop II." National Oceanic and Atmospheric Administration, Silver Spring **88**: 2280-2292.

Garcia, A., A. Croquer and N. Malaver (2004). "Some functional characteristics of bacterial communities in mucus associated to healthy tissue and yellow blotch syndrome in *Montastraea annularis*." Interciencia **29**(1): 39-45.

Garren, M. and F. Azam (2012). "New directions in coral reef microbial ecology." Environmental Microbiology **14**(4): 833-844.

Ghyselinck, J., S. Pfeiffer, K. Heylen, A. Sessitsch and P. De Vos (2013). "The effect of primer choice and short read sequences on the outcome of 16S rRNA gene based diversity studies." PLoS One **8**(8): e71360.

Gibson, R., M. Barnes and R. Atkinson (2001). "Practical measures of marine biodiversity based on relatedness of species." Oceanography and Marine Biology **39**: 207-231.

Gil-Turnes, M. S., M. E. Hay and W. Fenical (1989). "Symbiotic marine bacteria chemically defend crustacean embryos from a pathogenic fungus." Science **246**(4926): 116-118.

Gilbert, J. A., R. A. Quinn, J. Debelius, Z. Z. Xu, J. Morton, N. Garg, J. K. Jansson, P. C. Dorrestein and R. Knight (2016). "Microbiome-wide association studies link dynamic microbial consortia to disease." Nature **535**(7610): 94-103.

Glasl, B., P. Bongaerts, N. H. Elisabeth, O. Hoegh-Guldberg, G. J. Herndl and P. R. Frade (2017). "Microbiome variation in corals with distinct depth distribution ranges across a shallow–mesophotic gradient (15–85 m)." Coral Reefs: 1-6.

Glasl, B., G. J. Herndl and P. R. Frade (2016). "The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance." The ISME Journal **10**: 2280-2292.

Glynn, P. W. (1984). "Widespread coral mortality and the 1982–83 El Niño warming event." Environmental Conservation **11**(2): 133-146.

Gonzalez-Zapata, F. L., P. Bongaerts, C. Ramírez-Portilla, B. Adu-Oppong, G. Walljasper, A. Reyes and J. A. Sanchez (2018). "Holobiont diversity in a reef-building coral over its entire depth range in the Mesophotic Zone." Frontiers in Marine Science **5**(29).

Graham, N. A. J., T. D. Ainsworth, A. H. Baird, N. C. Ban and L. K. Bay (2011). "From microbes to people: tractable benefits of no-take areas for coral reefs." Oceanography and Marine Biology: An Annual Review **49**: 105-136.

Gray, M. A., Z. A. Pratte and C. A. Kellogg (2013). "Comparison of DNA preservation methods for environmental bacterial community samples." FEMS Microbiology Ecology **83**(2): 468-477.

Green, J. L., A. J. Holmes, M. Westoby, I. Oliver, D. Briscoe, M. Dangerfield, M. Gillings and A. J. Beattie (2004). "Spatial scaling of microbial eukaryote diversity." Nature **432**(7018): 747-750.

Grottoli, A. G., P. Dalcin Martins, M. J. Wilkins, M. D. Johnston, M. E. Warner, W.-J. Cai, T. F. Melman, K. D. Hoadley, D. T. Pettay, S. Levas and V. Schoepf (2018). "Coral physiology and microbiome dynamics under combined warming and ocean acidification." PLoS One **13**(1): e0191156.

Grottoli, A. G., L. J. Rodrigues and J. E. Palardy (2006). "Heterotrophic plasticity and resilience in bleached corals." Nature **440**: 1186-1189.

Guerrero-Feijóo, E., E. Sintes, G. J. Herndl and M. M. Varela (2017). "High dark inorganic carbon fixation rates by specific microbial groups in the Atlantic off the Galician coast (NW Iberian margin)." Environmental Microbiology.

Hadaidi, G., T. Rothig, L. K. Yum, M. Ziegler, C. Arif, C. Roder, J. Burt and C. R. Voolstra (2017). "Stable mucus-associated bacterial communities in bleached and healthy corals of *Porites lobata* from the Arabian Seas." Scientific Reports **7**.

Hamady, M. and R. Knight (2009). "Microbial community profiling for human microbiome projects: Tools, techniques, and challenges." Genome Research **19**(7): 1141-1152.

Harasti, D., H. Malcolm, C. Gallen, M. A. Coleman, A. Jordan and N. A. Knott (2015). "Appropriate set times to represent patterns of rocky reef fishes using baited video." Journal of Experimental Marine Biology and Ecology **463**: 173-180.

Hernandez-Agreda, A., R. D. Gates and T. D. Ainsworth (2017). "Defining the core microbiome in corals' microbial soup." Trends in Microbiology **25**(2): 125-140.

Hernandez-Agreda, A., W. Leggat, P. Bongaerts and T. D. Ainsworth (2016). "The microbial signature provides insight into the mechanistic basis of coral success across reef habitats." mBio **7**(4): e00560-00516.

Herre, E. A., N. Knowlton, U. G. Mueller and S. A. Rehner (1999). "The evolution of mutualisms: exploring the paths between conflict and cooperation." Trends in Ecology & Evolution **14**(2): 49-53.

Hester, E. R., K. L. Barott, J. Nulton, M. J. A. Vermeij and F. L. Rohwer (2015). "Stable and sporadic symbiotic communities of coral and algal holobionts." The ISME Journal: 1-13.

Houlbreque, F. and C. Ferrier-Pages (2009). "Heterotrophy in tropical scleractinian corals." Biological Reviews **84**(1): 1-17.

Howells, E., V. Beltran, N. Larsen, L. Bay, B. Willis and M. Van Oppen (2012). "Coral thermal tolerance shaped by local adaptation of photosymbionts." Nature Climate Change **2**(2): 116.

Hsu, C.-M., S. Keshavmurthy, V. Denis, C.-Y. Kuo, J.-T. Wang, P.-J. Meng and C. A. Chen (2012). "Temporal and spatial variations in symbiont communities of catch bowl coral *Isopora palifera* (Scleractinia: Acroporidae) on reefs in Kenting National Park, Taiwan." Zoological Studies **51**(8): 1343-1353.

Hughes, T. P., K. D. Anderson, S. R. Connolly, S. F. Heron, J. T. Kerry, J. M. Lough, A. H. Baird, J. K. Baum, M. L. Berumen, T. C. Bridge, D. C. Claar, C. M. Eakin, J. P. Gilmour, N. A. J. Graham, H. Harrison, J.-P. A. Hobbs, A. S. Hoey, M. Hoogenboom, R. J. Lowe, M. T. McCulloch, J. M. Pandolfi, M. Pratchett, V. Schoepf, G. Torda and S. K. Wilson (2018). "Spatial and temporal patterns of mass bleaching of corals in the Anthropocene." Science **359**(6371): 80-83.

Hughes, T. P., J. T. Kerry, M. Alvarez-Noriega, J. G. Alvarez-Romero, K. D. Anderson, A. H. Baird, R. C. Babcock, M. Beger, D. R. Bellwood, R. Berkelmans, T. C. Bridge, I. R. Butler, M. Byrne, N. E. Cantin, S. Comeau, S. R. Connolly, G. S. Cumming, S. J. Dalton, G. Diaz-Pulido, C. M. Eakin, W. F. Figueira, J. P. Gilmour, H. B. Harrison, S. F. Heron, A. S. Hoey, J. A. Hobbs, M. O. Hoogenboom, E. V. Kennedy, C. Y. Kuo, J. M. Lough, R. J. Lowe, G. Liu, M. T. McCulloch, H. A. Malcolm, M. J. McWilliam, J. M. Pandolfi, R. J. Pears, M. S. Pratchett, V. Schoepf, T. Simpson, W. J. Skirving, B. Sommer, G. Torda, D. R. Wachenfeld, B. L. Willis and S. K. Wilson (2017). "Global warming and recurrent mass bleaching of corals." Nature **543**(7645): 373-377.

Hume, B., C. D'angelo, J. Burt, A. Baker, B. Riegl and J. Wiedenmann (2013). "Corals from the Persian/Arabian Gulf as models for thermotolerant reef-builders: prevalence of clade C3 Symbiodinium, host fluorescence and ex situ temperature tolerance." Marine pollution bulletin **72**(2): 313-322.

Hurlbert, S. H. (1984). "Pseudoreplication and the design of ecological field experiments." Ecological Monographs **54**(2): 187-211.

Huttenhower, C., D. Gevers, R. Knight, S. Abubucker, J. H. Badger, A. T. Chinwalla, H. H. Creasy, A. M. Earl, M. G. FitzGerald and R. S. Fulton (2012). "Structure, function and diversity of the healthy human microbiome." Nature **486**(7402): 207.

Izsak, C. and A. R. G. Price (2001). "Measuring beta-diversity using a taxonomic similarity index, and its relation to spatial scale." Marine Ecology Progress Series **215**: 69-77.

Jandhyala, S. M., R. Talukdar, C. Subramanyam, H. Vuyyuru, M. Sasikala and D. N. Reddy (2015). "Role of the normal gut microbiota." World Journal of Gastroenterology **21**(29): 8787-8803.

Johansson, M. E. V., M. Phillipson, J. Petersson, A. Velcich, L. Holm and G. C. Hansson (2008). "The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria." Proceedings of the National Academy of Sciences of the United States of America **105**(39): 15064-15069.

Johnson, P. C. D., S. J. E. Barry, H. M. Ferguson and P. Muller (2015). "Power analysis for generalized linear mixed models in ecology and evolution." Methods in Ecology and Evolution **6**(2): 133-142.

Kahng, S. E., J. R. Garcia-Sais, H. L. Spalding, E. Brokovich, D. Wagner, E. Weil, L. Hinderstein and R. J. Toonen (2010). "Community ecology of mesophotic coral reef ecosystems." Coral Reefs **29**(2): 255-275.

Karch, H., P. I. Tarr and M. Bielaszewska (2005). "Enterohaemorrhagic *Escherichia coli* in human medicine." International Journal of Medical Microbiology **295**(6): 405-418.

Kazandjian, A., V. A. Shepherd, M. Rodriguez-Lanetty, W. Nordemeier, A. W. D. Larkum and R. G. Quinnell (2008). "Isolation of symbiosomes and the symbiosome membrane complex from the zoanthid *Zoanthus robustus*." Phycologia **47**(3): 294-306.

Kembel, S. W., T. K. O'Connor, H. K. Arnold, S. P. Hubbell, S. J. Wright and J. L. Green (2014). "Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest." Proceedings of the National Academy of Sciences of the United States of America **111**(38): 13715-13720.

Kemp, D. W., A. R. Rivers, K. M. Kemp, E. K. Lipp, J. W. Porter and J. P. Wares (2015). "Spatial homogeneity of bacterial communities associated with the surface mucus layer of the reef-building coral *Acropora palmata*." PLoS One **10**(12): e0143790.

Kim, B. H. and G. M. Gadd (2008). Introduction to bacterial physiology and metabolims. Bacterial Physiology and Metabolism, Cambridge University Press.

Kimes, N. E., W. R. Johnson, M. Torralba, K. E. Nelson, E. Weil and P. J. Morris (2013). "The *Montastraea faveolata* microbiome: ecological and temporal influences on a Caribbean reef-building coral in decline." Environmental Microbiology **15**(7): 2082-2094.

Knowlton, N. and F. Rohwer (2003). "Multispecies microbial mutualisms on coral reefs: The host as a habitat." American Naturalist **162**(4): S51-S62.

Koenig, J. E., A. Spor, N. Scalfone, A. D. Fricker, J. Stombaugh, R. Knight, L. T. Angenent and R. E. Ley (2011). "Succession of microbial consortia in the developing infant gut microbiome." Proceedings of the National Academy of Sciences of the United States of America **108**(Supplement 1): 4578-4585.

Koleff, P., K. J. Gaston and J. J. Lennon (2003). "Measuring beta diversity for presence-absence data." Journal of Animal Ecology **72**(3): 367-382.

Konopka, A. (2009). "What is microbial community ecology?" The ISME Journal **3**(11): 1223-1230.

Koren, O. and E. Rosenberg (2006). "Bacteria associated with mucus and tissues of the coral *Oculina patagonica* in summer and winter." Applied and Environmental Microbiology **72**(8): 5254-5259.

Kurm, V., W. H. Putten, W. Boer, S. Naus-Wiezer and W. Hol (2017). "Low abundant soil bacteria can be metabolically versatile and fast growing." Ecology **98**(2): 555-564.

Langille, M. G. I., J. Zaneveld, J. G. Caporaso, D. McDonald, D. Knights, J. A. Reyes, J. C. Clemente, D. E. Burkepile, R. L. V. Thurber, R. Knight, R. G. Beiko and C. Huttenhower (2013). "Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences." Nature Biotechnology **31**(9): 814-821.

Lawler, S. N., C. A. Kellogg, S. C. France, R. W. Clostio, S. D. Brooke and S. W. Ross (2016) "Coral-associated bacterial diversity is conserved across two deep-sea *Anthothela* species." Frontiers in Microbiology **7**, 458 DOI: 10.3389/fmicb.2016.00458.

Le Campion-Alsumard, T., S. Golubic and P. Hutchings (1995). "Microbial endoliths in the skeletons of live and dead corals: *Porites lobata* (Moorea, French Polynesia)." Marine Ecology Progress Series **117**(1-3): 149-157.

Le Campion-Alsumard, T., S. Golubic and K. Priess (1995). "Fungi in corals: symbiosis or disease? Interaction between polyps and fungi causes pearl-like skeleton biomineralization." Marine Ecology Progress Series **117**(1): 137-147.

Leal, M., J. Nejstgaard, R. Calado, M. Thompson and M. Frischer (2014). "Molecular assessment of heterotrophy and prey digestion in zooxanthellate cnidarians." Molecular ecology **23**(15): 3838-3848.

Lear, G., I. Dickie, J. Banks, S. Boyer, H. L. Buckley, T. R. Buckley, R. Cruickshank, A. Dopheide, K. M. Handley and S. Hermans (2018). "Methods for the extraction, storage, amplification and sequencing of DNA from environmental samples." New Zealand Journal of Ecology **42**(1): 10-50A.

Lee, O. O., J. K. Yang, S. Bougouffa, Y. Wang, Z. Batang, R. M. Tian, A. Al-Suwailem and P. Y. Qian (2012). "Spatial and species variations in bacterial communities associated with corals from the Red Sea as revealed by pyrosequencing." Applied and Environmental Microbiology **78**(20): 7173-7184.

Lee, S. T. M., S. K. Davy, S. L. Tang, T. Y. Fan and P. S. Kench (2015). "Successive shifts in the microbial community of the surface mucus layer and tissues of the coral *Acropora muricata* under thermal stress." FEMS Microbiology Ecology **91**(12): fiv142.

Legendre, P., D. Borcard and P. R. Peres-Neto (2005). "Analyzing beta diversity: Partitioning the spatial variation of community composition data." Ecological Monographs **75**(4): 435-450.

Leigh, E. G., Jr. (2010). "The evolution of mutualism." Journal of Evolutionary Biology **23**(12): 2507-2528.

Leite, D. C., J. F. Salles, E. N. Calderon, J. D. Elsas and R. S. Peixoto (2018). "Specific plasmid patterns and high rates of bacterial co-occurrence within the coral holobiont." Ecology and Evolution.

Leite, D. C. A., P. Leão, A. G. Garrido, U. Lins, H. F. Santos, D. O. Pires, C. B. Castro, J. D. van Elsas, C. Zilberberg, A. S. Rosado and R. S. Peixoto (2017). "Broadcast spawning coral *Mussismilia hispida* can vertically transfer its associated bacterial core." Frontiers in Microbiology **8**: 176.

Lema, K. A., D. G. Bourne and B. L. Willis (2014). "Onset and establishment of diazotrophs and other bacterial associates in the early life history stages of the coral *Acropora millepora*." Molecular Ecology **23**(19): 4682-4695.

Lema, K. A., B. L. Willis and D. G. Bourne (2012). "Corals form characteristic associations with symbiotic nitrogen-fixing bacteria." Applied and Environmental Microbiology **78**(9): 3136-3144.

Lema, K. A., B. L. Willis and D. G. Bourne (2014). "Amplicon pyrosequencing reveals spatial and temporal consistency in diazotroph assemblages of the *Acropora millepora* microbiome." Environmental Microbiology **16**(10): 3345-3359.

Lesser, M. P., L. I. Falcon, A. Rodriguez-Roman, S. Enriquez, O. Hoegh-Guldberg and R. Iglesias-Prieto (2007). "Nitrogen fixation by symbiotic cyanobacteria provides a source of nitrogen for the scleractinian coral *Montastraea cavernosa*." Marine Ecology Progress Series **346**: 143-152.

Lesser, M. P. and J. K. Jarett (2014). "Culture-dependent and culture-independent analyses reveal no prokaryotic community shifts or recovery of *Serratia marcescens* in *Acropora palmata* with white pox disease." FEMS Microbiology Ecology **88**(3): 457-467.

Lesser, M. P., C. H. Mazel, M. Y. Gorbunov and P. G. Falkowski (2004). "Discovery of symbiotic nitrogen-fixing cyanobacteria in corals." Science **305**(5686): 997-1000.

Lesser, M. P., M. Slattery, M. Stat, M. Ojimi, R. D. Gates and A. Grottoli (2010). "Photoacclimatization by the coral *Montastraea cavernosa* in the mesophotic zone: light, food, and genetics." Ecology **91**(4): 990-1003.

Letunic, I. and P. Bork (2007). "Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation." Bioinformatics **23**(1): 127-128.

Letunic, I. and P. Bork (2011). "Interactive Tree Of Life v2: online annotation and display of phylogenetic trees made easy." Nucleic Acids Research **39**: W475-W478.

Li, J., Q. Chen, L.-J. Long, J.-D. Dong, J. Yang and S. Zhang (2014). "Bacterial dynamics within the mucus, tissue and skeleton of the coral *Porites lutea* during different seasons." Scientific reports **4**: 7320-7328.

Li, J., Q. Chen, S. Zhang, H. Huang, J. Yang, X.-P. Tian and L.-J. Long (2013) "Highly heterogeneous bacterial communities associated with the South China Sea Reef corals *Porites lutea*, *Galaxea fascicularis* and *Acropora millepora*." PLoS One **8**, e71301 DOI: 10.1371/journal.pone.0071301.

Li, M. M., G. S. Li, L. Y. Zhu, Y. S. Yin, X. L. Zhao, C. Xiang, G. L. Yu and X. Wang (2014) "Isolation and characterization of an Agaro-Oligosaccharide (AO)-hydrolyzing bacterium from the gut microflora of chinese individuals." PLoS One **9** DOI: 10.1371/journal.pone.0091106.

Liang, J., K. Yu, Y. Wang, X. Huang, W. Huang, Z. Qin, Z. Pan, Q. Yao, W. Wang and Z. Wu (2017). "Distinct bacterial communities associated with massive and branching scleractinian corals and potential linkages to coral susceptibility to thermal or cold stress." Frontiers in Microbiology **8**: 979.

Little, A. F., M. J. H. van Oppen and B. L. Willis (2004). "Flexibility in algal endosymbioses shapes growth in reef corals." Science **304**(5676): 1492-1494.

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." Environmental Microbiology Reports **3**(6): 651-660.

Littman, R. A., B. L. Willis and D. G. Bourne (2009). "Bacterial communities of juvenile corals infected with different *Symbiodinium* (dinoflagellate) clades." Marine Ecology Progress Series **389**: 45-59.

Logue, J. B., N. Mouquet, H. Peter and H. Hillebrand (2011). "Empirical approaches to metacommunities: a review and comparison with theory." Trends in Ecology & Evolution **26**(9): 482-491.

Lozupone, C. A., J. I. Stombaugh, J. I. Gordon, J. K. Jansson and R. Knight (2012). "Diversity, stability and resilience of the human gut microbiota." Nature **489**(7415): 220-230.

Lundberg, D. S., S. L. Lebeis, S. H. Paredes, S. Yourstone, J. Gehring, S. Malfatti, J. Tremblay, A. Engelbrektson, V. Kunin, T. G. d. Rio, R. C. Edgar, T. Eickhorst, R. E. Ley, P. Hugenholtz, S. G. Tringe and J. L. Dangl (2012). "Defining the core *Arabidopsis thaliana* root microbiome." Nature **488**(7409): 86-90.

Luo, C., D. Tsementzi, N. Kyrpides, T. Read and K. T. Konstantinidis (2012). "Direct comparisons of Illumina vs. Roche 454 sequencing technologies on the same microbial community DNA sample." PLoS One **7**(2): e30087.

Lynch, M. D. J. and J. D. Neufeld (2015). "Ecology and exploration of the rare biosphere." Nature Reviews: Microbiology **13**(4): 217-229.

Macpherson, A. J., M. B. Geuking, E. Slack, S. Hapfelmeier and K. D. McCoy (2012). "The habitat, double life, citizenship, and forgetfulness of IgA." Immunological Reviews **245**: 132-146.

Madin, J. S., K. D. Anderson, M. H. Andreasen, T. C. L. Bridge, S. D. Cairns, S. R. Connolly, E. S. Darling, M. Diaz, D. S. Falster, E. C. Franklin, R. D. Gates, M. O. Hoogenboom, D. Huang, S. A. Keith, M. A. Kosnik, C.-Y. Kuo, J. M. Lough, C. E. Lovelock, O. Luiz, J. Martinelli, T. Mizerek, J. M. Pandolfi, X. Pochon, M. S. Pratchett, H. M. Putnam, T. E. Roberts, M. Stat, C. C. Wallace, E. Widman and A. H. Baird (2016). "The Coral Trait Database, a curated database of trait information for coral species from the global oceans." Scientific Data **3**: 160017.

Majik, M. S., C. Rodrigues, S. Mascarenhas and L. D'Souza (2014). "Design and synthesis of marine natural product-based 1H-indole-2,3-dione scaffold as a new antifouling/antibacterial agent against fouling bacteria." Bioorganic Chemistry **54**: 89-95.

McClanahan, T. R., M. Ateweberhan, N. A. J. Graham, S. K. Wilson, Sebasti, xe, C. R. n, M. M. M. Guillaume and J. H. Bruggemann (2007). "Western Indian Ocean coral communities bleaching responses and susceptibility to extinction." Marine Ecology Progress Series **337**: 1-13.

McFall-Ngai, M. (2008). "Are biologists in 'future shock'? Symbiosis integrates biology across domains." Nature Reviews: Microbiology **6**(10): 789-792.

McMurdie, P. J. and S. Holmes (2014). "Waste not, want not: why rarefying microbiome data is inadmissible." PLoS Computational Biology **10**(4): e1003531.

Meistertzheim, A.-L., F. Lartaud, S. Arnaud-Haond, D. Kalenitchenko, M. Bessalam, N. Le Bris and P. E. Galand (2016). "Patterns of bacteria-host associations suggest different ecological strategies between two reef building cold-water coral species." Deep Sea Research Part I: Oceanographic Research Papers **114**: 12-22.

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.

Meyer, J. L., J. M. Rodgers, B. A. Dillard, V. J. Paul and M. Teplitski (2016) "Epimicrobiota associated with the decay and recovery of *Orbicella* corals exhibiting Dark Spot Syndrome." Frontiers in Microbiology **7** DOI: 10.3389/fmicb.2016.00893.

Mihaljevic, J. R. (2012). "Linking metacommunity theory and symbiont evolutionary ecology." Trends in Ecology & Evolution **27**(6): 323-329.

Miller, A. W. and L. L. Richardson (2011). "A meta-analysis of 16S rRNA gene clone libraries from the polymicrobial black band disease of corals." FEMS Microbiology Ecology **75**(2): 231-241.

Miquel, S., M. Leclerc, R. Martin, F. Chain, M. Lenoir, S. Raguideau, S. Hudault, C. Bridonneau, T. Northen, B. Bowen, L. G. Bermudez-Humaran, H. Sokol, M. Thomas and P. Langella (2015) "Identification of metabolic signatures linked to anti-inflammatory effects of *Faecalibacterium prausnitzii*." mBio **6** DOI: 10.1128/mBio.00300-15.

Miquel, S., R. Martín, O. Rossi, L. G. Bermúdez-Humarán, J. M. Chatel, H. Sokol, M. Thomas, J. M. Wells and P. Langella (2013). "*Faecalibacterium prausnitzii* and human intestinal health." Current Opinion in Microbiology **16**(3): 255-261.

Montilla, L. M., R. Ramos, E. Garcia and A. Croquer (2016). "Caribbean yellow band disease compromises the activity of catalase and glutathione S-transferase in the reef-building coral *Orbicella faveolata* exposed to anthracene." Diseases of Aquatic Organisms **119**(2): 153-161.

Moretti, M., F. de Bello, S. P. M. Roberts and S. G. Potts (2009). "Taxonomical vs. functional responses of bee communities to fire in two contrasting climatic regions." Journal of Animal Ecology **78**(1): 98-108.

Morrow, K. M., D. G. Bourne, C. Humphrey, E. S. Botte, P. Laffy, J. Zaneveld, S. Uthicke, K. E. Fabricius and N. S. Webster (2014). "Natural volcanic CO2 seeps reveal future trajectories for host-microbial associations in corals and sponges." The ISME Journal **9**: 894-908.

Morrow, K. M., A. G. Moss, N. E. Chadwick and M. R. Liles (2012). "Bacterial associates of two Caribbean coral species reveal species-specific distribution and geographic variability." Applied and Environmental Microbiology **78**(18): 6438-6449.

Moss, J. A., A. Nocker, J. E. Lepo and R. A. Snyder (2006). "Stability and change in estuarine biofilm bacterial community diversity." Applied and Environmental Microbiology **72**(9): 5679-5688.

Mouchka, M. E., I. Hewson and C. D. Harvell (2010). "Coral-associated bacterial assemblages: current knowledge and the potential for climate-driven impacts." Integrative and Comparative Biology **50**(4): 662-674.

Muscatine, L., P. Falkowski, J. Porter and Z. Dubinsky (1984). "Fate of photosynthetic fixed carbon in light-and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*." Proceedings of the Royal Society B: Biological Sciences **222**(1227): 181-202.

Muscatine, L., R. McCloskey and E. Marian (1981). "Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration." Limnology and Oceanography **26**(4): 601-611.

Nagy, Z. T. (2010). "A hands-on overview of tissue preservation methods for molecular genetic analyses." Organisms, Diversity & Evolution **10**(1): 91-105.

Neave, M. J., C. T. Michell, A. Apprill and C. R. Voolstra (2017). "*Endozoicomonas* genomes reveal functional adaptation and plasticity in bacterial strains symbiotically associated with diverse marine hosts." Scientific Reports **7**: 40579.

Neave, M. J., R. Rachmawati, L. Xun, C. T. Michell, D. G. Bourne, A. Apprill and C. R. Voolstra (2016). "Differential specificity between closely related corals and abundant *Endozoicomonas* endosymbionts across global scales." The ISME Journal(19): 8315-8324.

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." The ISME Journal **11**: 186-200.

Newton, R. J., S. M. Huse, H. G. Morrison, C. S. Peake, M. L. Sogin and S. L. McLellan (2013) "Shifts in the microbial community composition of gulf coast beaches following beach oiling." PLoS One **8**, e74265 DOI: 10.1371/journal.pone.0074265.

Ng, J. C. Y., Y. Chan, H. M. Tun, F. C. C. Leung, P. K. S. Shin and J. M. Y. Chiu (2015) "Pyrosequencing of the bacteria associated with *Platygyra camosus* corals with skeletal growth anomalies reveals differences in bacterial community composition in apparently healthy and diseased tissues." Frontiers in Microbiology **6** DOI: 10.3389/fmicb.2015.01142.

Nyholm, S. V. and M. McFall-Ngai (2004). "The winnowing: establishing the squid-vibrio symbiosis." Nature Reviews: Microbiology **2**(8): 632-642.

Olson, J. B. and C. A. Kellogg (2010). "Microbial ecology of corals, sponges, and algae in mesophotic coral environments." FEMS Microbiology Ecology **73**(1): 17-30.

Olson, N. D., T. D. Ainsworth, R. D. Gates and M. Takabayashi (2009). "Diazotrophic bacteria associated with Hawaiian *Montipora* corals: Diversity and abundance in correlation with symbiotic dinoflagellates." Journal of Experimental Marine Biology and Ecology **371**(2): 140-146.

Pantos, O., P. Bongaerts, P. G. Dennis, G. W. Tyson and O. Hoegh-Guldberg (2015). "Habitat-specific environmental conditions primarily control the microbiomes of the coral *Seriatopora hystrix*." The ISME Journal **9**(9): 1916-1927.

Pedros-Alio, C. (2006). "Marine microbial diversity: can it be determined?" Trends in Microbiology **14**(6): 257-263.

Peixoto, R. S., P. M. Rosado, D. C. d. A. Leite, A. S. Rosado and D. G. Bourne (2017). "Beneficial Microorganisms for Corals (BMC): Proposed Mechanisms for Coral Health and Resilience." Frontiers in Microbiology **8**: 341.

Pinto, A. J. and L. Raskin (2012). "PCR biases distort bacterial and archaeal community structure in pyrosequencing datasets." PLoS One **7**(8): e43093.

Pochon, X., J. I. Montoya-Burgos, B. Stadelmann and J. Pawlowski (2006). "Molecular phylogeny, evolutionary rates, and divergence timing of the symbiotic dinoflagellate genus *Symbiodinium*." Molecular phylogenetics and evolution **38**(1): 20-30.

Pochon, X., J. Pawlowski, L. Zaninetti and R. Rowan (2001). "High genetic diversity and relative specificity among *Symbiodinium*-like endosymbiotic dinoflagellates in soritid foraminiferans." Marine Biology **139**(6): 1069-1078.

Pogoreutz, C., N. Rädecker, A. Cárdenas, A. Gärdes, C. Wild and C. R. Voolstra (2018). "Dominance of *Endozoicomonas* bacteria throughout coral bleaching and mortality suggests structural inflexibility of the *Pocillopora verrucosa* microbiome." Ecology and Evolution **8**(4): 2240-2252.

Pollock, J., L. Glendinning, T. Wisedchanwet and M. Watson (2018). "The madness of microbiome: Attempting to find consensus “best practice” for 16S microbiome studies." Applied and environmental microbiology **84**(7): e02627-02617.

Putnam, H. M., K. L. Barott, T. D. Ainsworth and R. D. Gates (2017). "The vulnerability and resilience of reef-building corals." Current Biology **27**(11): R528-R540.

Qin, J., R. Li, J. Raes, M. Arumugam, K. S. Burgdorf, C. Manichanh, T. Nielsen, N. Pons, F. Levenez, T. Yamada, D. R. Mende, J. Li, J. Xu, S. Li, D. Li, J. Cao, B. Wang, H. Liang, H. Zheng, Y. Xie, J. Tap, P. Lepage, M. Bertalan, J.-M. Batto, T. Hansen, D. Le Paslier, A. Linneberg, H. B. Nielsen, E. Pelletier, P. Renault, T. Sicheritz-Ponten, K. Turner, H. Zhu, C. Yu, S. Li, M. Jian, Y. Zhou, Y. Li, X. Zhang, S. Li, N. Qin, H. Yang, J. Wang, S. Brunak, J. Dore, F. Guarner, K. Kristiansen, O. Pedersen, J. Parkhill, J. Weissenbach, P. Bork, S. D. Ehrlich, J. Wang and H. I. T. C. Meta (2010). "A human gut microbial gene catalogue established by metagenomic sequencing." Nature **464**(7285): 59-U70.

Quevrain, E., M. A. Maubert, C. Michon, F. Chain, R. Marquant, J. Tailhades, S. Miquel, L. Carlier, L. G. Bermudez-Humaran, B. Pigneur, O. Lequin, P. Kharrat, G. Thomas, D. Rainteau, C. Aubry, N. Breyner, C. Afonso, S. Lavielle, J. P. Grill, G. Chassaing, J. M. Chatel, G. Trugnan, R. Xavier, P. Langella, H. Sokol and P. Seksik (2016). "Identification of an anti-inflammatory protein from *Faecalibacterium prausnitzii*, a commensal bacterium deficient in Crohn's disease." Gut **65**(3): 415-425.

Rädecker, N., C. Pogoreutz, C. R. Voolstra, J. Wiedenmann and C. Wild (2015). "Nitrogen cycling in corals: the key to understanding holobiont functioning?" Trends in Microbiology **23**(8): 490-497.

Raina, J. B., E. A. Dinsdale, B. L. Willis and D. G. Bourne (2010). "Do the organic sulfur compounds DMSP and DMS drive coral microbial associations?" Trends in Microbiology **18**(3): 101-108.

Raina, J. B., D. Tapiolas, B. L. Willis and D. G. Bourne (2009). "Coral-associated bacteria and their role in the biogeochemical cycling of sulfur." Applied and Environmental Microbiology **75**(11): 3492-3501.

Raina, J. B., D. M. Tapiolas, S. Foret, A. Lutz, D. Abrego, J. Ceh, F. O. Seneca, P. L. Clode, D. G. Bourne, B. L. Willis and C. A. Motti (2013). "DMSP biosynthesis by an animal and its role in coral thermal stress response." Nature **502**(7473): 677-680.

Rajendhran, J. and P. Gunasekaran (2011). "Microbial phylogeny and diversity: Small subunit ribosomal RNA sequence analysis and beyond." Microbiological Research **166**(2): 99-110.

Reis, A. M. M., S. D. Araujo, R. L. Moura, R. B. Francini, G. Pappas, A. M. A. Coelho, R. H. Kruger and F. L. Thompson (2009). "Bacterial diversity associated with the Brazilian endemic reef coral *Mussismilia braziliensis*." Journal of Applied Microbiology **106**(4): 1378-1387.

Relman, D. A. (2008). "'Til death do us part': coming to terms with symbiotic relationships." Nature Reviews: Microbiology **6**(10): 721-724.

Renouf, M. and S. Hendrich (2011). "*Bacteroides uniformis* is a putative bacterial species associated with the degradation of the isoflavone genistein in human feces." Journal of Nutrition **141**(6): 1120-1126.

Ritchie, K. B. (2006). "Regulation of microbial populations by coral surface mucus and mucus-associated bacteria." Marine Ecology Progress Series **322**: 1-14.

Ritchie, K. B. and G. Smith (1997). Physiological comparison of bacterial communities from various species of scleractinian corals. 8th International Coral Reef Symposium, Panama, Proceedings of the Eight International Coral Reef Symposium.

Robertson, V., B. Haltli, E. P. McCauley, D. P. Overy and R. G. Kerr (2016) "Highly variable bacterial communities associated with the octocoral *Antillogorgia elisabethae*." Microorganisms **4**, 23 DOI: 10.3390/microorganisms4030023.

Rocha, J., F. J. R. C. Coelho, L. Peixe, N. C. M. Gomes and R. Calado (2014). "Optimization of preservation and processing of sea anemones for microbial community analysis using molecular tools." Scientific Reports **4**: 6986.

Rodriguez-Lanetty, M., C. Granados-Cifuentes, A. Barberan, A. J. Bellantuono and C. Bastidas (2013). "Ecological inferences from a deep screening of the complex bacterial consortia associated with the coral, *Porites astreoides*." Molecular Ecology **22**(16): 4349-4362.

Rohwer, F., M. Breitbart, J. Jara, F. Azam and N. Knowlton (2001). "Diversity of bacteria associated with the Caribbean coral *Montastraea franksi*." Coral Reefs **20**(1): 85-91.

Rohwer, F., V. Seguritan, F. Azam and N. Knowlton (2002). "Diversity and distribution of coral-associated bacteria." Marine Ecology Progress Series **243**: 1-10.

Rosenberg, E., O. Koren, L. Reshef, R. Efrony and I. Zilber-Rosenberg (2007). "The role of microorganisms in coral health, disease and evolution." Nature Reviews: Microbiology **5**(5): 355-362.

Rothig, T., M. A. Ochsenkuhn, A. Roik, R. van der Merwe and C. R. Voolstra (2016). "Long-term salinity tolerance is accompanied by major restructuring of the coral bacterial microbiome." Molecular Ecology **25**(6): 1308-1323.

Ruppert, E. E., R. S. Fox and R. D. Barnes (2009). Invertebrate Zoology; a Functional Evolutionary Approach. Belmont, CA, Cengage Learning.

Salter, S. J., M. J. Cox, E. M. Turek, S. T. Calus, W. O. Cookson, M. F. Moffatt, P. Turner, J. Parkhill, N. J. Loman and A. W. Walker (2014). "Reagent and laboratory contamination can critically impact sequence-based microbiome analyses." BMC Biology **12**(1): 87.

Samodha, F., J. Wang, K. Sparling, G. D. Garcia, R. B. Francini, R. L. de Moura, R. Paranhos, F. L. Thompson and J. R. Thompson (2015). "Microbiota of the major south Atlantic reef building coral *Mussismilia*." Microbial Ecology **69**(2): 267-280.

Santiago-Vazquez, L. Z., T. B. Bruck, W. M. Bruck, A. P. Duque-Alarcon, P. J. McCarthy and R. G. Kerr (2007). "The diversity of the bacterial communities associated with the azooxanthellate hexacoral *Cirrhipathes lutkeni*." The ISME Journal **1**(7): 654-659.

Sato, Y., B. L. Willis and D. G. Bourne (2013). "Pyrosequencing-based profiling of archaeal and bacterial 16S rRNA genes identifies a novel archaeon associated with black band disease in corals." Environmental Microbiology: 2994–3007.

Schlaeppi, K., N. Dombrowski, R. G. Oter, E. Ver Loren van Themaat and P. Schulze-Lefert (2014). "Quantitative divergence of the bacterial root microbiota in *Arabidopsis thaliana* relatives." Proceedings of the National Academy of Sciences of the United States of America **111**(2): 585-592.

Schmitt, S., P. Tsai, J. Bell, J. Fromont, M. Ilan, N. Lindquist, T. Perez, A. Rodrigo, P. J. Schupp, J. Vacelet, N. Webster, U. Hentschel and M. W. Taylor (2012). "Assessing the complex sponge microbiota: core, variable and species-specific bacterial communities in marine sponges." The ISME Journal **6**(3): 564-576.

Schwartzman, J. A. and E. G. Ruby (2016). "Stress as a normal cue in the symbiotic environment." Trends in Microbiology **24**(5): 414-424.

Selvakumar, G., P. Panneerselvam and A. N. Ganeshamurthy (2012). Bacterial mediated alleviation of abiotic stress in crops. Bacteria in Agrobiology: Stress Management. K. D. Maheshwari. Berlin, Heidelberg, Springer**:** 205-224.

Séré, M. G., P. Tortosa, P. Chabanet, J. Turquet, J.-P. Quod and M. H. Schleyer (2013). "Bacterial communities associated with *Porites* White Patch Syndrome (PWPS) on three Western Indian Ocean (WIO) coral reefs." PLoS One **8**(12): e83746.

Seutin, G., B. N. White and P. T. Boag (1991). "Preservation of avian blood and tissue samples for DNA analyses." Canadian Journal of Zoology **69**(1): 82-90.

Shade, A. and J. Handelsman (2012). "Beyond the Venn diagram: the hunt for a core microbiome." Environmental Microbiology **14**(1): 4-12.

Shade, A., S. E. Jones, J. G. Caporaso, J. Handelsman, R. Knight, N. Fierer and J. A. Gilbert (2014). "Conditionally rare taxa disproportionately contribute to temporal changes in microbial diversity." mBio **5**(4): e01371-01314.

Sharp, K. H., D. Distel and V. J. Paul (2012). "Diversity and dynamics of bacterial communities in early life stages of the Caribbean coral *Porites astreoides*." The ISME Journal **6**(4): 790-801.

Sharp, K. H. and K. B. Ritchie (2012). "Multi-partner interactions in corals in the face of climate change." The Biological Bulletin **223**(1): 66-77.

Shashar, N., A. Banaszak, M. Lesser and D. Amrami (1997). "Coral endolithic algae: life in a protected environment." Pacific Science **51**(2): 167-173.

Shashar, N., Y. Cohen, Y. Loya and N. Sar (1994). "Nitrogen-fixation (acetylene-reduction) in stony corals - evidence for coral-bacteria interactions." Marine Ecology Progress Series **111**(3): 259-264.

Shendure, J. and H. Ji (2008). "Next-generation DNA sequencing." Nature Biotechnology **26**(10): 1135-1145.

Shokralla, S., J. L. Spall, J. F. Gibson and M. Hajibabaei (2012). "Next-generation sequencing technologies for environmental DNA research." Molecular ecology **21**(8): 1794-1805.

Shore-Maggio, A., C. M. Runyon, B. Ushijima, G. S. Aeby and S. M. Callahan (2015). "Differences in bacterial community structure in two color morphs of the hawaiian reef coral *Montipora capitata*." Applied and Environmental Microbiology **81**(20): 7312-7318.

Siewe, R. M., B. Weil, A. Burkovski, L. Eggeling, R. Kramer and T. Jahns (1998). "Urea uptake and urease activity in *Corynebacterium glutamicum*." Archives of Microbiology **169**(5): 411-416.

Soergel, D. A., N. Dey, R. Knight and S. E. Brenner (2012). "Selection of primers for optimal taxonomic classification of environmental 16S rRNA gene sequences." The ISME Journal **6**(7): 1440-1444.

Sogin, M. L., H. G. Morrison, J. A. Huber, D. Mark Welch, S. M. Huse, P. R. Neal, J. M. Arrieta and G. J. Herndl (2006). "Microbial diversity in the deep sea and the underexplored "rare biosphere"." Proceedings of the National Academy of Sciences of the United States of America **103**(32): 12115-12120.

Somerfield, P. J. and K. R. Clarke (1995). "Taxonomic levels, in marine community studies, revisited." Marine Ecology Progress Series **127**: 113-119.

Speck, M. D. and S. P. Donachie (2012). "Widespread Oceanospirillaceae bacteria in *Porites* spp." Journal of Marine Biology **2012**: 7.

Spor, A., O. Koren and R. Ley (2011). "Unravelling the effects of the environment and host genotype on the gut microbiome." Nature Reviews: Microbiology **9**(4): 279-290.

Staley, C., T. J. Gould, P. Wang, J. Phillips, J. B. Cotner and M. J. Sadowsky (2014). "Core functional traits of bacterial communities in the Upper Mississippi River show limited variation in response to land cover." Frontiers in Microbiology **5**: 414.

Sunagawa, S., T. Z. DeSantis, Y. M. Piceno, E. L. Brodie, M. K. DeSalvo, C. R. Voolstra, E. Weil, G. L. Andersen and M. Medina (2009). "Bacterial diversity and White Plague Disease-associated community changes in the Caribbean coral *Montastraea faveolata*." The ISME Journal **3**(5): 512-521.

Sunagawa, S., C. M. Woodley and M. Medina (2010). "Threatened corals provide underexplored microbial habitats." PLoS One **5**(3): e9554.

Sweet, M. J., A. Croquer and J. C. Bythell (2011). "Bacterial assemblages differ between compartments within the coral holobiont." Coral Reefs **30**(1): 39-52.

Tambutté, E., D. Allemand, D. Zoccola, A. Meibom, S. Lotto, N. Caminiti and S. Tambutté (2007). "Observations of the tissue-skeleton interface in the scleractinian coral *Stylophora pistillata*." Coral Reefs **26**(3): 517-529.

Tang, S. L., M. J. Hong, M. H. Liao, W. N. Jane, P. W. Chiang, C. B. Chen and C. A. Chen (2011). "Bacteria associated with an encrusting sponge (*Terpios hoshinota*) and the corals partially covered by the sponge." Environmental Microbiology **13**(5): 1179-1191.

Taylor, M. W., R. Radax, D. Steger and M. Wagner (2007). "Sponge-associated microorganisms: evolution, ecology, and biotechnological potential." Microbiology and Molecular Biology Reviews **71**(2): 295-347.

Taylor, M. W., P. Tsai, R. L. Simister, P. Deines, E. Botte, G. Ericson, S. Schmitt and N. S. Webster (2013). "'Sponge-specific' bacteria are widespread (but rare) in diverse marine environments." The ISME Journal **7**(2): 438-443.

Team, R. C. (2013). R v. 3.0. 2: A language and environment for statistical computing. Vienna, R Foundation for Statistical Computing.

Thompson, L. R., J. G. Sanders, D. McDonald, A. Amir, J. Ladau, K. J. Locey, R. J. Prill, A. Tripathi, S. M. Gibbons and G. Ackermann (2017). "A communal catalogue reveals Earth's multiscale microbial diversity." Nature **551**(7681).

Thrush, S. F., J. E. Hewitt, A. Norkko, P. E. Nicholls, G. A. Funnell and J. I. Ellis (2003). "Habitat change in estuaries: predicting broad-scale responses of intertidal macrofauna to sediment mud content." Marine Ecology Progress Series **263**: 101-112.

Thurber, R. V., D. E. Burkepile, A. M. S. Correa, A. R. Thurber, A. A. Shantz, R. Welsh, C. Pritchard and S. Rosales (2012). "Macroalgae decrease growth and alter microbial community structure of the reef-building coral, *Porites astreoides*." PLoS One **7**(9): e44246.

Thurber, R. V., D. Willner-Hall, B. Rodriguez-Mueller, C. Desnues, R. A. Edwards, F. Angly, E. Dinsdale, L. Kelly and F. Rohwer (2009). "Metagenomic analysis of stressed coral holobionts." Environmental Microbiology **11**(8): 2148–2163.

Tims, S., E. G. Zoetendal, W. M. de Vos and M. Kleerebezem (2011). Host genotype and the effect on microbial communities. Metagenomics of the Human Body. K. E. Nelson. New York, Springer**:** 15-41.

Tóthmérész, B. (1995). "Comparison of different methods for diversity ordering." Journal of Vegetation Science **6**(2): 283-290.

Tout, J., N. Siboni, L. F. Messer, M. Garren, R. Stocker, N. S. Webster, P. J. Ralph and J. R. Seymour (2015) "Increased seawater temperature increases the abundance and alters the structure of natural *Vibrio* populations associated with the coral *Pocillopora damicornis*." Frontiers in Microbiology **6**, 432 DOI: 10.3389/fmicb.2015.00432.

Tuddenham, S. and C. L. Sears (2015). "The intestinal microbiome and health." Current Opinion in Infectious Diseases **28**(5): 464-470.

Turnbaugh, P. J. and J. I. Gordon (2009). "The core gut microbiome, energy balance and obesity." The Journal of Physiology **587**(17): 4153-4158.

Turnbaugh, P. J., M. Hamady, T. Yatsunenko, B. L. Cantarel, A. Duncan, R. E. Ley, M. L. Sogin, W. J. Jones, B. A. Roe, J. P. Affourtit, M. Egholm, B. Henrissat, A. C. Heath, R. Knight and J. I. Gordon (2009). "A core gut microbiome in obese and lean twins." Nature **457**(7228): 480-485.

Turnbaugh, P. J., R. E. Ley, M. Hamady, C. M. Fraser-Liggett, R. Knight and J. I. Gordon (2007). "The human microbiome project." Nature **449**(7164): 804-810.

Turnbaugh, P. J., R. E. Ley, M. A. Mahowald, V. Magrini, E. R. Mardis and J. I. Gordon (2006). "An obesity-associated gut microbiome with increased capacity for energy harvest." Nature **444**(7122): 1027-1031.

Ulstrup, K. E., P. J. Ralph, A. W. D. Larkum and M. Kuhl (2006). "Intra-colonial variability in light acclimation of zooxanthellae in coral tissues of *Pocillopora damicornis*." Marine Biology **149**(6): 1325-1335.

van de Water, J., T. D. Ainsworth, W. Leggat, D. G. Bourne, B. L. Willis and M. J. H. van Oppen (2015). "The coral immune response facilitates protection against microbes during tissue regeneration." Molecular Ecology **24**(13): 3390-3404.

van de Water, J. A., R. Melkonian, H. Junca, C. R. Voolstra, S. Reynaud, D. Allemand and C. Ferrier-Pages (2016) "Spirochaetes dominate the microbial community associated with the red coral *Corallium rubrum* on a broad geographic scale." Scientific Reports **6**, 27277 DOI: 10.1038/srep27277.

Vandeputte, D., R. Y. Tito, R. Vanleeuwen, G. Falony and J. Raes (2017). "Practical considerations for large-scale gut microbiome studies." FEMS Microbiology Reviews **41**(Supp_1): S154-S167.

Varyukhina, S., M. Freitas, S. Bardin, E. Robillard, E. Tavan, C. Sapin, J.-P. Grill and G. Trugnan (2012). "Glycan-modifying bacteria-derived soluble factors from *Bacteroides thetaiotaomicron* and *Lactobacillus casei* inhibit rotavirus infection in human intestinal cells." Microbes and Infection **14**(3): 273-278.

Veron, J. (2000). *Pachyseris speciosa*. Corals of the World. M. Stafford-Smith. Townsville, Australia, Australian Institute of Marine Science. **2:** 228-229.

Vik, U., R. Logares, R. Blaalid, R. Halvorsen, T. Carlsen, I. Bakke, A.-B. Kolstø, O. A. Økstad and H. Kauserud (2013) "Different bacterial communities in ectomycorrhizae and surrounding soil." Scientific Reports **3** DOI: 10.1038/srep03471.

Vlčková, K., J. Mrázek, J. Kopečný and K. J. Petrželková (2012). "Evaluation of different storage methods to characterize the fecal bacterial communities of captive western lowland gorillas (*Gorilla gorilla gorilla*)." Journal of Microbiological Methods **91**(1): 45-51.

Wang, Q., G. M. Garrity, J. M. Tiedje and J. R. Cole (2007). "Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy." Applied and Environmental Microbiology **73**(16): 5261-5267.

Wangpraseurt, D., A. W. Larkum, P. J. Ralph and M. Kuhl (2012) "Light gradients and optical microniches in coral tissues." Frontiers in Microbiology **3** DOI: 10.3389/fmicb.2012.00316.

Wangpraseurt, D., M. Pernice, P. Guagliardo, M. R. Kilburn, P. L. Clode, L. Polerecky and M. Kühl (2015). "Light microenvironment and single-cell gradients of carbon fixation in tissues of symbiont-bearing corals." The ISME Journal **10**: 788-792.

Weber, L., E. DeForce and A. Apprill (2017). "Optimization of DNA extraction for advancing coral microbiota investigations." Microbiome **5**(1): 18.

Wegley, L., R. Edwards, B. Rodriguez-Brito, H. Liu and F. Rohwer (2007). "Metagenomic analysis of the microbial community associated with the coral *Porites astreoides*." Environmental Microbiology **9**(11): 2707-2719.

Wickham, H. (2016). ggplot2: elegant graphics for data analysis. New York City, Springer.

Williams, A. D., B. E. Brown, L. Putchim and M. J. Sweet (2015) "Age-related shifts in bacterial diversity in a reef coral." PLoS One **10** DOI: 10.1371/journal.pone.0144902.

Wilson, B., G. S. Aeby, T. M. Work and D. G. Bourne (2012). "Bacterial communities associated with healthy and *Acropora* white syndrome-affected corals from American Samoa." FEMS Microbiology Ecology **80**(2): 509-520.

Wu, G. D., J. D. Lewis, C. Hoffmann, Y.-Y. Chen, R. Knight, K. Bittinger, J. Hwang, J. Chen, R. Berkowsky, L. Nessel, H. Li and F. D. Bushman (2010). "Sampling and pyrosequencing methods for characterizing bacterial communities in the human gut using 16s sequence tags." BMC Microbiology **10**: 1-14.

Xu, H., Y. Jiang, K. A. S. Al-Rasheid, S. A. Al-Farraj and W. Song (2011). "Application of an indicator based on taxonomic relatedness of ciliated protozoan assemblages for marine environmental assessment." Environmental Science and Pollution Research **18**(7): 1213-1221.

Yang, S. H., S. T. M. Lee, C. R. Huang, C. H. Tseng, P. W. Chiang, C. P. Chen, H. J. Chen and S. L. Tang (2016). "Prevalence of potential nitrogen-fixing, green sulfur bacteria in the skeleton of reef-building coral I*sopora palifera*." Limnology and Oceanography **61**(3): 1078-1086.

Yellowlees, D., T. A. V. Rees and W. Leggat (2008). "Metabolic interactions between algal symbionts and invertebrate hosts." Plant, Cell and Environment **31**(5): 679-694.

Ze, X. L., S. H. Duncan, P. Louis and H. J. Flint (2012). "*Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon." The ISME Journal **6**(8): 1535-1543.

Zhang, F., M. Berg, K. Dierking, M.-A. Félix, M. Shapira, B. S. Samuel and H. Schulenburg (2017). "*Caenorhabditis elegans* as a model for microbiome research." Frontiers in Microbiology **8**: 485.

Zhang, Y. Y., J. Ling, Q. S. Yang, Y. S. Wang, C. C. Sun, H. Y. Sun, J. B. Feng, Y. F. Jiang, Y. Z. Zhang, M. L. Wu and J. D. Dong (2015). "The diversity of coral associated bacteria and the environmental factors affect their community variation." Ecotoxicology **24**(7-8): 1467-1477.

Zhang, Y. Y., J. Ling, Q. S. Yang, C. Q. Wen, Q. Y. Yan, H. Y. Sun, J. D. Van Nostrand, Z. Shi, J. Z. Zhou and J. D. Dong (2015). "The functional gene composition and metabolic potential of coral-associated microbial communities." Scientific Reports **5**: 1-11.

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." Nature Communications **8**: 14213.

Appendix A: Chapter 2 – Glossary of Terms

Abundance: number of individuals of a species in an area, population or community. Based on 16s rRNA amplicon, sequences are considered as a proxy of individuals.

Commensal: a symbiotic relationship in which one organism is benefited and the other is not benefited or harmed.

Community: groups of organisms coexisting in the same habitat.

Community structure: composition and abundance of species in a community.

Composition: species present in a community.

Diazotrophic bacteria: bacteria capable of fixing gaseous nitrogen (N_2).

Ecosystem: interactions and dynamics of physical, chemical and biological components in an area (Shade and Handelsman 2012).

Endolithic: organisms boring and living in pore of corals' skeleton.

Function: metabolic role.

Habitat: the physical space with characteristics that define niches to be occupied by organisms (Shade and Handelsman 2012).

Holobiont: collective term to refer to a host and its symbionts from specific taxonomic groups.

Host: organism where a symbiont lives.

Microbiome: an assemblage of microorganisms, including their genomes, associated with any system (e.g. human, water column, coral).

Microhabitat: physical space in corals with characteristics that provide niches for bacteria.

Mutualistic: a symbiotic relationship in which both partners benefit.

Niche: ecological role and space of an organism in a community (Dennehy 2014).

Operational taxonomic units (OTU): operational proxy of taxon when the definition of taxonomic entities is based on DNA sequences differences. OTU taxonomical definition is based on nucleotide identity (usually 97% for 16s rRNA), reflecting any taxonomic level (from phyla to species) (Lynch and Neufeld 2015).

Phylotypes: DNA sequences grouped as similar based on a specific gene marker (e.g. 16s rRNA). Individuals in the group are approximately 97-99% similar. Synonym for Operational taxonomic units (OTU).

Rare (species): community members with very low (<1%) abundance.

Symbiont: an organism that lives in a host and benefits from its properties.

Symbiosis: biological relationship (commensalism or mutualism) among two or more organisms of different species.

Appendix B: Chapter 2 – Supplementary table

Table B - 1: Summary of Next Generation Sequencing Meta-analysis of the Coral Microbiome. Meta-analysis was conducted through a search in the database Web of Science using the keywords: Bacterial communities, coral, 16s rRNA. From the pool of papers obtained (*n*=187), books, reviews and conferences were excluded. Only were considered studies in hard and hermatypic corals using next generation sequences (NGS), 16s rRNA amplicon pyrosequencing. Results related with coral microbiome and derived from NGS are reflected in this table, despite that some studies can consider other organisms and contain partial results based on previous techniques (DGGE, TRFLP, culturing, microscopy).

Focus	Sequencing platform	Host	Depth (m)	Geographic location	Habitat within coral	Sampling effort	Average number of sequences[a]	Average number of OTUs[a]	Major results	Reference
Characterization of bacterial community structure on healthy corals										
	454	*Porites astreoides*	NR	Bocas del Toro, Panama	Whole coral	10 colonies	4,542	NR	Bacterial community was functionally heterotrophic, with pathways for degradation of aromatic compounds. Prominent bacterial groups: Proteobacteria, Firmicutes, Cyanobacteria and Actinobacteria.	(Wegley, Edwards et al. 2007)
	454	*Porites lutea*, *Galaxea fascicularis*, *Acropora millepora*	3-5	Luhuitou Reef, Sanya, China	Whole coral	3 replicates per species	*P. lutea*: 4,696 *G. fascicularis:* 2,951 *A. millepora*: 1,280	*P. lutea*: 1,623 *G. fascicularis*: 1,181 *A. millepora*: 523	Bacterial communities were highly diverse. Alphaproteobacteria was the dominant class in *P. lutea* and *G. fascicularis*; whereas Betaproteobacteria was more dominant in *A. millepora*. *P. lutea* and *G. fascicularis* had similar bacterial communities and different to the *A. millepora* bacteria community. The three coral species showed bacterial species-specific associations. Bacterial species-specific community was larger than the common bacterial community.	(Li, Chen et al. 2013)*
	454	*Galaxea astreata*, *P. lutea*, *Porites andrewsi*, *Pavona decussata*	5-10	Luhuitou Reef, Sanya, China	Whole coral	3 colonies per species	7,414 per sample	*G. astreata*: 1,891, *P. andrewsi*: 910, *P. decussata* 1,243, *P. lutea* 1,522	Proteobacteria was the most abundant phyla, Gamma- and Alphaproteobacteria as the dominants groups. Functional profiles were different between coral species. There were detected genes related to nitrogen transformation, carbon and sulfur cycling, organic remediation and antibiotics resistance. Bacterial communities involved in dioxide carbon fixation differed between species, whereas microbial composition related to sulfur cycling differed between corals except between *G. astreata* and *P. lutea*. Composition and functional profiles of microbial communities were positive correlated to Chlorophyll a and dissolved oxygen. Further, functional gene composition was also correlated to inorganic nitrogen and phosphate concentrations. Taxonomy composition is driven by variations between coral species, whereas	(Zhang, Ling et al. 2015)

Focus	Sequencing platform	Host	Depth (m)	Geographic location	Habitat within coral	Sampling effort	Average number of sequences[a]	Average number of OTUs[a]	Major results	Reference
									functional gene composition is explained by environmental variables.	
Examination of *Endozoicomonas* association in corals	454	*Stylophora pistillata, Acropora humilis, Pocillopora damicornis*	2-5	Five sites in the Red Sea	Tissue	*S. pistillata*: 5 colonies, *A. humilis* and *P. damicornis*: 3 colonies per species	*S. pistillata* 26,284 (average)	*S. pistillata* 289 (average)	Genera *Endozoicomonas* and *Burkholderia* dominated the bacterial community in *S. pistillata*. Based on full-length sequences and phylogenetic analysis, *Endozoicomonas* was similar to other *Endozoicomonas* species cultivated or reported in other soft, hard corals and marine invertebrates. Genus *Burkolderia* was clustered with fungus sequences. In analysis of the bacterial small-subunit (SSU) rRNA genes, *Endozoicomonas* was identified as the most abundant bacteria in *P. damicornis* and *A. humilis*, and its gene sequences were found associated to other 14 coral species in different geographical regions. Hybridization in *S. pistillata* tissues showed *Endozoicomonas* close to the zooxanthellae, in the endoderm.	(Bayer, Neave et al. 2013)
Evaluation of structure of coral microbiota and their variations based on lineage, environment and disease/health status	454	*Mussismilia harttii, Mussismilia hispida, Mussismilia braziliensis*	5-8	Sebastiao Gomes Reef and Parcel dos Abrolhos, Brazil	Whole coral	3 colonies per coral species and *M. braziliensis*: 6 healthy colonies and 3 diseased colonies (white plage)	*M. braziliensis*: 10,976 *M. hispida*: 6,993 *M. harttii*: 11,841. 12,200 per sample	*M. brasiliensis*: 640; *M. hispida*: 795; *M. harttii*: 680	In healthy fragments, Bacteriodetes, Firmicutes, Proteobacteria and Cyanobacteria dominated bacterial community composition. Bacterial composition in diseased fragments was different from healthy fragments, dominated by Bacteriodetes and Proteobacteria. Proportion of the genus *Vibrio* was higher in diseased than in healthy corals. Healthy corals had three ubiquitous OTUs, two Proteobacteria and one Alphaproteobacteria. Microbiota from *M. braziliensis* was more similar between samples than with other two *Mussusmilia* species.	(Samodha, Wang et al. 2015)
Coral host influence on associated bacterial community										
Determination of the influence of coral	454	*Orbicella faveolata,*	1.5-5.5	Crawl Cay reef, Bocas	Whole coral	5 colonies per coral species	NR	*O. faveolata*: 1,553 *O. franksi*: 2,050	Rare bacteria taxa in seawater are highly abundant in corals. Massive corals (*Orbicella* spp. and *D. strigosa*) are more diverse than branching (*Acropora*). Based on abundance, closely related corals in the same genus or	(Sunagawa, Woodley

Focus	Sequencing platform	Host	Depth (m)	Geographic location	Habitat within coral	Sampling effort	Average number of sequences[a]	Average number of OTUs[a]	Major results	Reference
phylogenetic relationships on bacterial communities		*Orbicella franksi*, *Diploria strigosa*, *Acropora palmata*, *Acropora cervicornis*, *P. astreoides*		del Toro, Panama				*D. strigosa*: 1,759 *A. palmata*: 1,671 *A. cervicornis*: 1,616 *P. astreoides*: 1,340	family have more similar bacteria communities. Bacteria community in corals is highly diverse but species-specific.	et al. 2010)*
Comparison of bacterial communities between coral color morphs	454	*Montipora capitata*	1-3	Moku O Loe, Kaneohe Bay, Hawaii	Whole coral	5 colonies per morph	Orange morph: 19,009 Red morph: 15,596. 17,302 per sample	Orange morph: 587 Red morph: 534	Bacterial diversity and richness were similar between red and orange morph, eight OTUs were present in all the samples. Gamma-, Beta-, Alphaproteobacteria and Firmicutes dominated both morphs. However, bacterial community composition was different between morphs, orange morph had more variable bacterial community than red morphs.	(Shore-Maggio, Runyon et al. 2015)
Influence of other coral's symbionts on bacterial community structure										
Determination of the influence of photosynthetic symbionts	454	*A. millepora* *P. damicornis* *Seriatopora hystrix* Also considered other 13 invertebrates with and without	5-10	Davies Reef, Great Barrier Reef, Australia	Tissue	3 replicates per species	*A. millepora*: 9,391 *P. damicornis*: 11,423 *S. hystrix*: 11,372	NR	Photosynthetic symbionts influenced the composition of species of invertebrate-associated microbiomes, but not theirs richness, evenness and phylogenetic diversity. Gammaproteobacteria and Alphaproteobacteria were the dominant classes in *A. millepora* and *P. damicornis*; whereas Flavobacteria were dominant in *S. hystrix*.	(Bourne, Dennis et al. 2013)

Focus	Sequencing platform	Host	Depth (m)	Geographic location	Habitat within coral	Sampling effort	Average number of sequences[a]	Average number of OTUs[a]	Major results	Reference
		photosynthetic symbionts								
Determination of the influence of host genotype, *Symbiodinium* subclade genotype, depth and geographic location on bacterial communities	454	*S. hystrix*	2, 6, and 27	Yonge Reef, Day Reef and Lizard Island, Great Barrier Reef, Australia	Tissue	33 colonies	1,900	NR	Bacterial communities are different between sites and depth but host and *Symbiodinium* genotype do not affect bacterial community structure in corals. Bacteriodetes, Alpha- and Gammaproteobacteria dominated all locations and depths.	(Pantos, Bongaerts et al. 2015)
Spatial scales: variations in the structure of coral-associated bacteria community at different spatial scales										
Comparisons between corals species from different locations and environmental conditions	454	*Pocillopora verrucosa*, *Astreopora myriophthalma*, *S. pistillata*	8-19	Three locations: two close to the mouth of Sharm Obhur; and the third, at 50km northwest of Sharm Obhur. Red Sea coast, Arabia Saudi	Whole coral	*P. verrucosa:* 1 colony per site. *A. myriophthalma:* 1 colony in site 1 and 3 *S. pistillata:* 1 colony in site 3	*P. verrucosa*: 4,697 *A. myriophthalma*: 4,897 *S. pistillata* 2,947	*P. verrucosa*: 335 *A. myriophthalma*: 207 *S. pistillata*: 230	Coral species sampled in different locations were different. Proteobacteria was the dominant phyla. Replication is not appropriate to consider environmental effects on the bacterial community.	(Lee, Yang et al. 2012)

Focus	Sequencing platform	Host	Depth (m)	Geographic location	Habitat within coral	Sampling effort	Average number of sequences[a]	Average number of OTUs[a]	Major results	Reference
Description of bacterial communities at different spatial scales and anthropogenic disturbances	DGGE and 454	*O. faveolata* *P. astreoides*	5-15	St. Thomas, United States Virgin Island; inshore reef Summerland Key, Florida, United States; Carrie Bow Cay Field Station, Smithsonian Institution, Belize	Mucus	2 colonies per coral specie per site. Except *O. faveolata* in Belize, just 1 colony	777	NR	Bacterial diversity was higher in *O. faveolata* than *P. astreoides*, both coral species with characteristic bacterial communities. The specificity varies between sites and species. Alpha-, Beta- and Gammaproteobacteria were the dominant classes. Genus *Endozoicomonas* was a dominant genus in *P. astreoides.* Both coral species had more diverse and abundant disease-related bacteria near to the coast.	(Morrow, Moss et al. 2012)*
Characterization spatial variability and within-colony differences in bacterial assemblages	454	*A. palmata*	3	Looe Key, Florida Keys National Marine Sanctuary. United States	Mucus	Uppermost and underside zone: 4 colonies, base: 3 colonies	NR	Uppermost: 183 Underside: 183 Base: 182	Bacterial community composition is different between coral, seawater and sediment. There are no differences in community assemblages between the three coral regions (uppermost, underside and base). Dominate groups: Cyanobacteria, Bacteriodetes, Deltaproteobacteria and Alphaproteobacteria.	(Kemp, Rivers et al. 2015)
Temporal scales: variations in the structure of coral-associated bacteria community at different temporal scales										
Characterization of bacterial communities through seasonal changes	454	*M. hispida* *Tubastraea coccinea* *Madracis decactis*	9 and 11	Sao Sebastiao Channel and Buzios Island, Sao Paulo, Brazil	Mucus	2 colonies per species per season; except *M. decactis* in summer,	*M. hispida*: winter: 6,433; summer: 17,254. *T. coccinea*: winter: 15,204;	*M. hispida*: winter: 1150; summer: 2154. *T. coccinea*: winter: 1389; summer:2122.	Based on OTU composition and abundances, there are not differences between the bacteria community in mucus of different species and between seasons. However, species-specific associations were detected with low abundances. Gammaproteobacteria dominated mucus samples of *T. coccinea* and *M. hispida*. The majority of bacteria associated to mucus were aerobic and heterotrophic.	(Carlos, Torres et al. 2013)*

Focus	Sequencing platform	Host	Depth (m)	Geographic location	Habitat within coral	Sampling effort	Average number of sequences[a]	Average number of OTUs[a]	Major results	Reference
						with 1 colony.	summer: 13,085. *M. decactis*: winter: 5,051; summer: 23,931.	*M. decactis*: winter: 788; summer: 3155.		
	454	*P. lutea*	3-5	Luhuitou Reef, Sanya, China	Mucus, tissue and skeleton	3 colonies per sampling, 4 samplings every 3 months. Tissue was not sampled in February	Mucus: 9,347 Tissue: 10,533 Skeleton: 12,092	Mucus: 1,139 Tissue: 847 Skeleton: 1,227	Proteobacteria was the dominant class in *P. lutea*. Seasonal changes influenced bacterial structure rather than compartments. Composition and dominant bacteria were different between compartments and varied between months. Both environmental factors as compartment affect coral-associated composition. Dissolved oxygen and rainfall strongly influence coral-bacteria association. Each compartment showed compartment-specific bacterial associations.	(Li, Chen et al. 2014)
Evaluation of patterns in diazotrophic and general bacterial community through seasons and between inshore-offshore reefs	Pyrosequencing (platform non identified)	*A. millepora*	Fringing reef in Cattle Bay and mid-shelf platform in Trunk Reef	Cattle Bay (Orpheus Island) and Trunk Reef, Great Barrier Reef, Australia	Tissue	3 colonies in each site, sampled repeatedly 4 times during a year. 3 fragments per season per colony. Except: Cattle Bay Autumn, Trunk Reef: summer	Cattle Bay: Spring: 1,597; Summer: 1,514; Autumn: 1,700; Winter: 2,340. Trunk Reef: Spring: 718; Summer: 373; Autumn: 1,078;	Cattle Bay: Spring: 34; Summer: 35; Autumn: 34; Winter: 22. Trunk Reef: Spring: 33; Summer: 39; Autumn: 34; Winter: 60.	Richness of diazotrophic communities were similarly low and did not vary consistently spatial or temporally. Dominant diazotrophic bacteria belong to Alphaproteobacteria class, order Rhizobiales. Overall bacteria community is highly affect by the location. Gammaproteobacteria class dominated bacteria community in corals from Cattle Bay; whereas bacterial community in corals from Trunk Reef was more variable. Bacterial communities were not affected by seasons.	(Lema, Willis et al. 2014)

Focus	Sequencing platform	Host	Depth (m)	Geographic location	Habitat within coral	Sampling effort	Average number of sequences[a]	Average number of OTUs[a]	Major results	Reference
						and autumn	Winter: 1,466			
Determination of spatial and co-occurrence patterns and correlation with *Symbiodinium*	DGGE and Illumina	*P. astreoides*	5-10	3 sites in Water Factory, Snake Bay and Playa Jeremi, Curacao, Nertherlands Antilles	Tissue	5 replicates per site, except Water Factory 2 and Snake Bay with 4 replicates	31,071 average per sample	Water Factory 1: 300 Water Factory 2: 275 Water Factory 3: 125 Snake Bay: 217 Playa Jeremi: 269.	The most abundant phylum was Proteobacteria, Gammaproteobacteria. Two OTUs from the Oceanospirillales order compromised 75% of bacterial abundance. Diversity varied between sites. Rare species are highly diverse and differ in composition and abundance in regard to geographical location. The microbial network is highly connected; rare bacteria are highly connected whereas the most abundant bacterium is not.	(Rodriguez-Lanetty, Granados-Cifuentes et al. 2013)
Temperature increase: shifts in microbial communities due to an increase of temperature (bleached corals)										
	454	*A. millepora*	1.5-3	Nelly Bay, Magnetic Island, Great Barrier Reef, Australia	Whole coral	5 colonies sampled before and after a bleaching event	NR	NR	Photosynthesis and respiration genes decreased in bleached corals bacteria community in regard to healthy corals, which suggest a change in microbial metabolism from autotrophy to heterotrophy. Genes associated with virulence, stress responses and secondary metabolism were higher in bleached than healthy stage. Virulence genes of Firmicutes increased in bleached corals.	(Littman, Willis et al. 2011)
	454	*Acropora muricata*	8-10	Kenting National Park, Nan-wan, Taiwan	Mucus and tissue	10 replicates per treatment (26°C, 27°C, 29°C, 30°C, 31°C, 33°C) from 5 colonies	NR	NR	Gammaproteobacteria dominated bacterial community in mucus and tissue, genera *Endozoicomonas* and *Vibrio* with the highest values of relative abundance. In regard to control treatment (26°C), at high temperature (31°C) Alphaproteoacteria and Verrucomicrobiaceae increased their abundances in both mucus and tissue, whereas Gammaproteobacteria decreased. Relative abundance of *Vibrio* sp. increased in both mucus and tissue at high temperature (31°C). *Endozoicomonas* relative abundance did not change in mucus but decreased in coral tissues at high temperature (31°C).	(Lee, Davy et al. 2015)

Focus	Sequencing platform	Host	Depth (m)	Geographic location	Habitat within coral	Sampling effort	Average number of sequences[a]	Average number of OTUs[a]	Major results	Reference
	Illumina	*P. damicornis*	1-3	Heron Island, Great Barrier Reef, Australia	Tissue	24 fragments in each treatment, from 3 colonies	NR	NR	Fragments under control and heat stress treatment had different bacterial community composition. Before to start the experiment, bacterial community was dominated by Oceanospirillales, principally Endozoicomonacea. At the end of the experiment, both control as coral fragments under heat stress treatment showed an increase of relative occurrence of Rhodobacterales and Flavobacteriales. Further, occurrence of Oceanospirillales decreased in control corals, whereas Vibrionales increased in corals under heat stress. In regard to control corals, *Vibrio* spp. and *Vibrio coralliilyticus* abundance increased two and four orders of magnitude, respectively, in corals under heat stress.	(Tout, Siboni et al. 2015)
Changes in other environmental variables: Effect of increasing temperature, nutrient enrichment, reduced pH and dissolved organic carbon loading on microbial community										
	454	*Porites compressa*	1	Hawaii Institute of Marine Biology, Hawaii	Whole coral	3 colonies	62,664	NR	The stressors affect microbial community, changing the composition from a healthy-associated community (mutualistic and/or commensalistic) to a community similar to diseases corals (pathogenic and opportunist). Stressors increase the abundance of microbial genes involved in virulence, stress resistance, sulphur and nitrogen metabolism, motility and chemotaxis, fatty acid and lipid utilization, and secondary metabolism. High temperature increased *Vibrio* virulence genes.	(Thurber, Willner-Hall et al. 2009)
Evaluation of microbial associations sensitivity to high PCO2/ low pH	454	*A. millepora*, *Porites cylindrica*	3-4	Upa Upasina, D´Entrecast eaux Island, Papa New Guinea	Tissue	*A. millepora* 10 replicates and *P. cylindrica* 5 replicates	*A. millepora*: control 6,488; high CO_2: 3,463. *P. cylindrica*: control	*A. millepora:* 1,528 *P. cylindrica*: 2,794	Proteobacteria was the dominant class in both coral species. *P. cylindrica* was more diverse than *A. millepora*. At the species level the bacterial composition was different between control and low PCO2/pH in both coral species. In both coral species the relative abundance of Proteobacteria, except Gammapreoteobacteria (*Endozoicomonas* sp.), was high at the low PCO2/pH site. In both species, cyanobacteria-affiliated OTUs were proportionally higher in the low PCO2/pH site in regard to control site.	(Morrow, Bourne et al. 2014)

Focus	Sequencing platform	Host	Depth (m)	Geographic location	Habitat within coral	Sampling effort	Average number of sequences[a]	Average number of OTUs[a]	Major results	Reference
							10,274, high CO_2: 5,848			
Competition with algae for space: differences in the bacterial community structure of corals, algae and corals close to algae										
Description of differences between coral-associated and algae-associated bacteria	454	*Orbicella annularis*	8-10	Two sites in Curacao, The Netherlands Antilles	Tissue	25 colonies	58,420	NR, expressed in a figure	Proteobacteria, Bacteriodetes, Firmicutes, and Actinobacteria dominated coral-associated bacteria. Functionally, bacteria in corals were majority facultative anaerobes and heterotrophs. Algae-associated bacteria are more diverse than bacteria in the coral.	(Barott, Rodriguez-Brito et al. 2011)
Examination of interactions between corals and four functional groups of benthic algae	454	*O. annularis*	8-10	Water Factory, Curacao, The Netherlands Antilles	Tissue	5 replicates per zone per coral-algal interaction type	Coral-interactions with: CCA: 64,383 *Dictyota*: 40,831 *Halimeda*: 49,662 Turf: 61,912	Coral-interactions with: CCA: 576 *Dictyota*: 713 *Halimeda*: 694 Turf: 770	In coral tissue, bacterial diversity increased near all types of algae, except in *Halimeda opuntia*. Some bacteria taxa were over-represented at or near the algal interfaces, the number varied depending on the type of algae. Metabolic capabilities of the coral-associated bacteria also were altered in proximity of algal interfaces. In the same zone, pathways related to membrane transport, stress response, aromatic catabolism and flagellar motility were under-represented; whereas metabolism of single-carbon compounds, fatty acids, potassium and purines and virulence were increased.	(Barott, Rodriguez-Mueller et al. 2012)
Reproduction: change in the bacterial community in response to spawning and bacterial community structure in early life stages										
Evaluation of bacterial community structure before and after a mass spawning event	454	*Acropora tenuis*, *P. damicornis*, *Tubastraea faulkneri*	5-6	Near Coral Bay, Ningaloo Reef, Australia	Tissue	2 colonies per coral species, except *P. damicornis* (n=1)	Before coral mass spawning: 1,081 After coral mass spawning: 1,301	Before coral mass spawning: 191 After coral mass spawning: 258	Bacterial diversity increased in all coral species after mass spawning and planulation, but no major changes were detected at class level before and after reproduction event. Only minor changes in abundance were detected in few bacteria classes. Gammaproteobacteria is the dominant class in all corals. Based on taxonomic assignment at level genus, coral species are different to each other without differences before and after reproduction event.	(Ceh, Raina et al. 2012)

Focus	Sequencing platform	Host	Depth (m)	Geographic location	Habitat within coral	Sampling effort	Average number of sequences[a]	Average number of OTUs[a]	Major results	Reference
Description of diazotrophic communities in early coral life stages	Pyrosequencing (platform non identified)	*A. millepora*	Gametes from water column	Pelorus Island, Great Barrier Reef, Australia	Whole coral; note that early stages are just larvae without skeleton	Planulae: 3 sets of 20 replicates together; 1 week: 3 sets of 5 replicates together; 2 weeks: 2 sets of 5 replicates together; 3, 6 and 12 months: 3 replicates	Planulae: 2,874 1 week: 3,208 2 weeks: 2,942 3 months: 3,080 6 months: 2,132 1 year: 2,344	Planulae: 123 1 week: 172 2 weeks: 320 3 months: 451 6 months: 412 1 year: 377	Diazotrophic communities had low diversity in all early life stages. Alphaproteobacteria class dominated both diazotrophic bacteria and overall community in all early stages of development. Cyanobacteria increased in relative abundance when coral were transplanted to the field. Richness increased from laboratory stages to field stages. In both diazotrophic and overall bacteria community, samples were grouped in three groups of different composition: larvae, early stage (1 and 2 weeks) and late-stage (3, 6 and 12 months). Rhizobiales is an important component in early stages of development according both approaches.	(Lema, Bourne et al. 2014)
Injuries and diseases: differences in the bacterial community of healthy and injured or diseased corals										
Evaluation of changes in the bacterial community on injured corals	454 and FISH	*Acropora aspera*	1-2	Heron Island, Great Barrier Reef, Australia	Whole coral	Healthy corals: 3-6 replicates, Injured corals: 2-5 replicates, from 2 genotypes	Day 2, H: 3,794, I: 3,495 Day 3 H: 2,594, I: 3,103 Day 4 H: 2,420; I: 2,617 Day 7 H: 2,486, I: 3,350; Day 10 H: 1,427, I: 3,385	Day 2, Healhty(H): 19, Injured(I): 30 Day 3 H: 10, I: 31 Day 4 H: 26; I: 22 Day 7 H: 26, I: 23 Day 10 H: 66, I: 51	Tissue in healthy and injured corals did not have changes in the number and size of bacterial aggregations. Richness and abundance of coral-associated bacteria did not show differences due to tissue damage.	(van de Water, Ainsworth et al. 2015)

Focus	Sequencing platform	Host	Depth (m)	Geographic location	Habitat within coral	Sampling effort	Average number of sequences[a]	Average number of OTUs[a]	Major results	Reference
Comparison between healthy and white plague disease (WPD) affected tissue	Culture and 454	*Siderastrea siderea*, *D. strigosa*	15	Aguja Island, Tayrona National Park, Colombia	Whole coral	5 replicates per condition per species	Total: 20,410	*S. siderea*: healthy: 378, diseased: 319. *D. strigosa*: healthy: 256, diseased: 372.	Healthy *S. siderea* was more diverse than WPD-affected; whereas in *D. strigosa* occurred the opposite. However, these differences were not significant. Proteobacteria was the dominant phyla in all samples, showing differences in relative abundance between healthy and WPD-affected corals. In both corals species, Alphaproteobacteria decreased in WPD-affected in regard to healthy corals whereas Beta- and Gammaproteobacteria increased. These changes are not consistent between species when higher taxonomic levels are considered.	(Cardenas, Rodriguez et al. 2012)
Characterization of microbial community structure in Black Band Disease (BBD) lesions and in cyanobacterial patches (CP), precursors of BBD	454	*Montipora hispida*	2.5-3	Pelorus Island, Great Barrier Reef, Australia	Whole coral	3 colonies (sampled in both status: cyanobacterial patches and BBD)	CP: 3,556 BBD: 4,051	NR	Both CP and BBD had highly diverse bacterial communities. The progression from CP to BBD developed a shift in taxonomic composition and dominance in bacterial community; cyanobacteria *Oscillatoria* sp. dominated BBD samples; whereas *Trichodesmium* dominated CP samples. Alphaproteobacteria were lower in BBD samples than CP samples.	(Sato, Willis et al. 2013)
Comparison of microbial community structure between Yellow Band Disease (YBD) lesions and healthy corals	454	*Herpolitha limax*, *Ctenactis crassa*	5-10	Eight sites in the Red Sea: *C. crassa*: Jazir Sila, Pele2, Poppocamera, Aptonaythe; *H.limax*: Coast Guard2,	Tissue	1 colony per health state per site	Total: 47,011	Total: 9,707	Bacterial community is highly variable. In *C. crassa* Gammaproteobacteria dominated bacterial community, whereas *H. limax* was dominanted by Gammaproteobacteria and Alphaproteobacteria. Genus *Endozoicomonas* was consistently present in both healthy species and genus *Vibrio* was not abundant in infected corals. Bacterial community structure was not different between health states, but between reefs.	(Apprill, Hughen et al. 2013)

Focus	Sequencing platform	Host	Depth (m)	Geographic location	Habitat within coral	Sampling effort	Average number of sequences[a]	Average number of OTUs[a]	Major results	Reference
				Canyon, Long, AQ3						
Evaluation of Dark Spot Syndrome (DSS) as precursor of BBD	Illumina	*O. annularis*, *O. faveolata*	< 20	Carrie Bow Cay, Belize	Mucus and Tissue	*O. annularis*: 5 colonies, *O. faveolata*: 3 colonies	6,576 to 1,029,276 sequencing reads per sample	Total: 36,878	Bacterial community in BBD fragments were different to healthy and DSS infected fragments. Relative abundance did not show differences between healthy and DSS lesion samples. Abundant genera in healthy and DSS lesion: *Halomonas*, *Moritella*, *Pseudomonas*, *Acinetobacter* and two unclassified genera (Gammaproteobacteria and Rhodobacteraceae). BBD tissue dominated by *Roseofilum reptotaenium*, and some BBD consortium members were detected at lower levels in healthy tissues and DSS lesions. Analyses of disease progression showed that in DSS lesions, genus *Halomonas* decrease in abundance while the unclassified gammaproteobacterial genus increase.	(Meyer, Rodgers et al. 2016)
Determination of the role of *Serratia marcescens* as a casual agent of White Pox Disease (WP)	Culture and 454	*A. palmata*	1-3	Elkhorn Reef, Bahamas	Mucus and Tissue	3 colonies	Total: 247,841	Total: 757	Cultured bacteria were identified as members of the genera *Pseudomonas*, *Xanthomonas* and *Stenotrophomonas* in diseased areas; and in healthy areas as genera *Psedomonas*, *Sediminibacterium*, *Sphingobacterium* and a bacterium from the Family Saprospraceae. Diseased and healthy samples did not show the presence of *Serratia marcescens*. Based on pyrosequencing, coral samples were dominated by Gammaproteobacteria, represented by families Endozoicomonaceae (genus *Endozoicomonas*) and Vibrionaceae (genera *Vibrio* and *Photobacterium*) and order Alteromonadales. Bacteria belonging to Epsilon- and Alphaproteobacteria were also present in minor percentages. In community structure, tissue samples were highly variable, different between each other and different to mucus and water samples. Microbial communities were not different between health states.	(Lesser and Jarett 2014)

Focus	Sequencing platform	Host	Depth (m)	Geographic location	Habitat within coral	Sampling effort	Average number of sequences[a]	Average number of OTUs[a]	Major results	Reference
Characterization of bacterial community in healthy and Skeletal growth anomalies (coral tumors) corals	454	*Platygyra carnosa*	NR	Hoi Ha Wan Marine Park, Hong Kong	Whole coral	4 colonies, healthy and diseases tissues were collected in pairs from same colonies	Total: healthy: 45,077; diseased: 62,398	Total: healthy: 785, diseased: 1,023	Healthy fragments were dominated by Alphaproteobacteria, Bacteriodetes and Gammaproteobacteria, whereas diseased fragments by Proteobacteria. Composition was different between healthy and diseased fragments; however, healthy fragments were highly variable. Proteobacteria was the phylum that contributed more to the differences between healthy and diseased condition. There are 788 OTUs found exclusively in diseased colonies, 42 of them are consistently detected in all the replicates.	(Ng, Chan et al. 2015)

[a] Note that some of these values depend on rarefaction analyses.

* Species-specific bacteria have been detected in this study.

Whole coral: represent all coral microhabitats together (crushed samples, homogenate).

NR**:** non-reported.

CCA: crustose coralline algae.

DGGE: Denaturing gradient gel electrophoresis.

PCO_2: partial pressure of carbon dioxide.

FISH: Fluorescence *in situ* hybridization.

Appendix C: Chapter 3 – Supplementary tables and figures.

Table C - 1: Permutational analysis of variance (univariate PERMANOVA) on the number of sequences for *G. edwardsi*. Test based on Euclidean distances, performed using 9,999 permutations to compare Preservation and Homogenization methods. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Preservation (Pr)	1	3.06E+08	3.06E+08	0.27877	0.6128	9845	0.612	0
Homogenization (Ho)	1	7.86E+08	7.86E+08	0.71521	0.409	9820	0.4055	0
PrxHo	1	7.61E+08	7.61E+08	0.69291	0.4252	9854	0.4168	0
Residual	19	2.09E+10	1.10E+09					100
Total	23	2.30E+10						

Table C - 2: Permutational analysis of variance (univariate PERMANOVA) on number of OTUs for *G. edwardsi*. Test based on Euclidean distances, performed using 9,999 permutations to compare Preservation and Homogenization methods. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique perms**	**P(MC)**	**ECV(%)**
Preservation (Pr)	1	1.20E+06	1.20E+06	0.48789	0.5233	9875	0.4902	0
Homogenization (Ho)	1	46754	46754	0.018994	0.898	9847	0.891	0
PrxHo	1	1.29E+06	1.29E+06	0.52233	0.4855	9834	0.4776	0
Residual	19	4.68E+07	2.46E+06					100
Total	23	5.11E+07						

Table C - 3: Permutational analysis of variance (univariate PERMANOVA) on number of sequences for *I. palifera*. Test based on Euclidean distances, performed using 9,999 permutations to compare Preservation and Homogenization methods. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Preservation (Pr)	1	4.62E+07	4.62E+07	0.075667	0.7913	9850	0.7844	0.0
Homogenization (Ho)	1	6.10E+08	6.10E+08	1.0003	0.3367	9831	0.3293	0.5
PrxHo	1	1.92E+07	1.92E+07	0.031458	0.8572	9816	0.8612	0.0
Residual	20	1.22E+10	6.10E+08					99.5
Total	24	2.77E+10						

Table C - 4: Permutational analysis of variance (univariate PERMANOVA) on number of OTUs for *I. palifera*. Test based on Euclidean distances, performed using 9,999 permutations to compare Preservationand Homogenization methods. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Preservation (Pr)	1	1.78E+05	1.78E+05	0.23918	0.6498	9855	0.625	0.00
Homogenization (Ho)	1	6.85E+05	6.85E+05	0.92203	0.3737	9857	0.3485	0.00
PrxHo	1	1.16E+06	1.16E+06	1.5567	0.2434	9855	0.223	25.02
Residual	20	1.49E+07	7.43E+05					74.98
Total	24	1.89E+07						

Table C - 5: Permutational multivariate analysis of variance (PERMANOVA) for the relative abundance data based on Bray-Curtis dissimilarities. Test performed using 9,999 permutations. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Preservation (Pr)	1	5179.3	5179.3	1.1766	0.3041	16	0.3173	6.19
Homogenization (Ho)	1	4083.8	4083.8	1.0305	0.3068	16	0.4659	2.44
Coral species (Co)	1	12353	12353	3.0905	**0.0001**	9826	0.0001	18.53
PrxHo	1	3992.5	3992.5	1.0395	0.3862	214	0.4611	3.87
PrxCo	1	4401.9	4401.9	1.1013	0.2163	9784	0.3159	5.43
HoxCo	1	3963	3963	0.99148	0.5047	9773	0.4766	0.00
PrxHoxCo	1	3840.6	3840.6	0.96087	0.5968	9786	0.5248	0.00
Residual	39	1.56E+05	3997.1					63.56
Total	48	2.08E+05						

Table C - 6: Permutational multivariate analysis of variance (PERMANOVA) for the compositional (Presence/Absence) data based on Sorensen dissimilarities. Test performed using 9,999 permutations. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Preservation (Pr)	1	5005.1	5005.1	1.1692	0.2934	16	0.3206	6.13
Homogenization (Ho)	1	3965.3	3965.3	1.007	0.2994	16	0.4866	1.20
Coral species (Co)	1	11242	11242	2.7887	**0.0001**	9800	0.0001	18.80
PrxHo	1	3917	3917	1.0434	0.3592	213	0.4552	4.11
PrxCo	1	4280.9	4280.9	1.0619	0.2941	9761	0.3642	5.09
HoxCo	1	3937.6	3937.6	0.97673	0.5685	9781	0.5047	0.00
PrxHoxCo	1	3754.2	3754.2	0.93124	0.7071	9766	0.5765	0.00
Residual	39	1.57E+05	4031.4					64.68
Total	48	2.07E+05						

Table C - 7: Permutational multivariate analysis of variance (PERMANOVA) for the relative abundance of bacterial community associated with *G. edwardsi*. Analysis based on Bray-Curtis dissimilarities, excluding PFA-PBS treated samples. Test performed using 9,999 permutations. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Preservation (Pr)	1	4514.4	4514.4	1.0527	0.3572	9832	0.4055	6.77
Homogenization (Ho)	1	3602.4	3602.4	0.84004	0.8334	9853	0.6247	0.00
PrxHo	1	4025.7	4025.7	0.93876	0.622	9836	0.5231	0.00
Residual	16	68613	4288.3					93.23
Total	19	80756						

Table C - 8: Permutational multivariate analysis of variance (PERMANOVA) for the composition (Presence/Absence) of bacterial community associated to *G. edwardsi*. Analysis based on Sorensen dissimilarities, excluding PFA-PBS treated samples. Test performed using 9,999 permutations. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Preservation (Pr)	1	4484.9	4484.9	1.0529	0.3477	9833	0.3947	6.78
Homogenization (Ho)	1	3544	3544	0.83205	0.8586	9827	0.6439	0.00
PrxHo	1	3836.7	3836.7	0.90075	0.7387	9798	0.5674	0.00
Residual	16	68151	4259.4					93.22
Total	19	80017						

Table C - 9: Permutational multivariate analysis of variance (PERMANOVA) for the relative abundance to compare bacterial community associated to *G. edwardsi* preserved with PFA versus the other preservation and homogenization methods. Analysis based on Bray-Curtis dissimilarities. Test performed using 9,999 permutations. Bonferroni *p*-value for four comparisons 0.0125. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

PFA vs.	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
DMSO-BB	Treatment	1	4648.8	4648.8	1.0934	0.2454	126	0.3854	12.66
	Residual	7	29761	4251.6					87.34
	Total	8	34410						
DMSO-Cr	Treatment	1	4316.1	4316.1	1.0147	0.4036	126	0.4353	5.44
	Residual	7	29775	4253.6					94.56
	Total	8	34091						
LN-BB	Treatment	1	4949.4	4949.4	1.1884	0.1163	126	0.3238	17.07
	Residual	7	29152	4164.6					82.93
	Total	8	34102						
LN-Cr	Treatment	1	4914.1	4914.1	1.1736	0.0908	126	0.3316	16.50
	Residual	7	29309	4187.1					83.50
	Total	8	34224						

Table C - 10: Permutational multivariate analysis of variance (PERMANOVA) for the composition (Presence/Absence) to compare bacterial community associated with *G. edwardsi* preserved with PFA versus the other preservation and homogenization methods. Analysis based on Bray-Curtis dissimilarities. Test performed using 9,999 permutations. Bonferroni *p*-value for four comparisons 0.0125. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

PFA vs.	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
DMSO-BB	Treatment	1	4588.7	4588.7	1.0794	0.2471	126	0.3883	11.79
	Residual	7	29759	4251.3					88.21
	Total	8	34347						
DMSO-Cr	Treatment	1	4256	4256	0.99783	0.4214	126	0.4346	0
	Residual	7	29857	4265.3					100
	Total	8	34113						
LN-BB	Treatment	1	4688.5	4688.5	1.1157	0.2009	126	0.3706	13.89
	Residual	7	29417	4202.5					86.11
	Total	8	34106						
LN-Cr	Treatment	1	4741.6	4741.6	1.1335	0.1133	126	0.3474	14.77
	Residual	7	29281	4183					85.23
	Total	8	34023						

Table C - 11: Permutational multivariate analysis of variance (PERMANOVA) for the relative abundance of bacterial community associated to *I. palifera*. Analysis based on Bray-Curtis dissimilarities, excluding PFA-PBS treated samples. Test performed using 9,999 permutations. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Preservation (Pr)	1	5066.8	5066.8	1.2934	0.0547	9838	0.2088	13.31
Homogenization (Ho)	1	4444.5	4444.5	1.1345	0.1967	9817	0.3241	9.01
PrxHo	1	3807.4	3807.4	0.97188	0.5673	9823	0.4794	0.00
Residual	16	62681	3917.6					77.69
Total	19	76000						

Table C - 12: Permutational multivariate analysis of variance (PERMANOVA) for the composition (Presence/Absence) of bacterial community associated to *I. palifera*. Analysis based on Sorensen dissimilarities, excluding PFA-PBS treated samples. Test performed using 9,999 permutations. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Preservation (Pr)	1	4801	4801	1.2141	0.0974	9816	0.2584	11.73
Homogenization (Ho)	1	4358.8	4358.8	1.1023	0.231	9821	0.3562	8.11
PrxHo	1	3834.5	3834.5	0.96968	0.6081	9808	0.4921	0.00
Residual	16	63271	3954.4					80.16
Total	19	76265						

Table C - 13: Permutational multivariate analysis of variance (PERMANOVA) for the relative abundance to compare bacterial community associated to *I. palifera* preserved with PFA versus the other preservation and homogenization methods. Analysis based on Bray-Curtis dissimilarities. Test performed using 9,999 permutations. Bonferroni *p*-value for four comparisons 0.0125. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

PFA vs.	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
DMSO-BB	Treatment	1	8919.3	8919.3	2.5163	0.0087	126	**0.0262**	35.51
	Residual	8	28357	3544.6					64.49
	Total	9	37276						
DMSO-Cr	Treatment	1	8187.1	8187.1	2.3587	0.0079	126	**0.0338**	34.27
	Residual	8	27768	3471					65.73
	Total	9	35955						
LN-BB	Treatment	1	6419.8	6419.8	1.7634	0.0138	125	0.1034	28.10
	Residual	8	29124	3640.5					71.90
	Total	9	35544						
LN-Cr	Treatment	1	5040.4	5040.4	1.5267	0.0322	126	0.1708	24.50
	Residual	8	26412	3301.5					75.50
	Total	9	31452						

Table C - 14: Permutational multivariate analysis of variance (PERMANOVA) for the composition (Presence/Absence) to compare bacterial community associated to *I. palifera* preserved with PFA versus the other preservation and homogenization methods. Analysis based on Bray-Curtis dissimilarities. Test performed using 9,999 permutations. Bonferroni *p*-value for four comparisons 0.0125. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

PFA vs.	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
DMSO-BB	Treatment	1	8112.6	8112.6	2.1895	0.0092	126	**0.0384**	32.78
	Residual	8	29642	3705.2					67.22
	Total	9	37755						
DMSO-Cr	Treatment	1	7580.1	7580.1	2.1124	0.0083	126	**0.0471**	32.05
	Residual	8	28708	3588.5					67.95
	Total	9	36288						
LN-BB	Treatment	1	6074.1	6074.1	1.6129	0.0175	126	0.1348	25.93
	Residual	8	30128	3766					74.07
	Total	9	36202						
LN-Cr	Treatment	1	5247.1	5247.1	1.5081	0.0212	126	0.1701	24.17
	Residual	8	27834	3479.3					75.83
	Total	9	33081						

Table C - 15: Number of bacterial phylotypes per percentage of occurrence. *For *G. edwardsi* in the treatment PFA-decalcified *n*=4, thus percentages of occurrence are 25%, 50%, 75%, 100%. OTUs: Operational Taxonomic Units.

Coral species	Method		Singleton OTUs	Number of OTUs per percentage of occurrence					Total OTUs
	Preservation	Homogenization		20%	40%	60%	80%	100%	
G. edwardsi	DMSO	Bead beating	5,513	8,668	639	163	37	18	9,525
	DMSO	Crushing	3,869	6,174	517	101	27	7	6,826
	Liquid nitrogen	Bead beating	5,245	8,924	595	94	38	8	9,659
	Liquid nitrogen	Crushing	6,412	9,421	1,010	269	73	20	10,793
	PFA	Decalcified	3,058	4,342*	406*	63*	15*		4,826
I. palifera	DMSO	Bead beating	2,892	4,142	291	113	47	21	4,614
	DMSO	Crushing	2,888	4,381	378	130	67	23	4,979
	Liquid nitrogen	Bead beating	4,253	7,063	589	103	22	13	7,790
	Liquid nitrogen	Crushing	2,233	3,174	273	114	44	42	3,647
	PFA	Decalcified	4,803	6,715	595	204	123	134	7,771

Table C - 16: Taxonomic identification of OTUs part of the Core 100% (A), dominant phylotypes (relative abundance ≥ 0.05, B) and top 10 dominant phylotypes (C) in *G. edwardsi* bacterial assemblage. OTU: Operational Taxonomic Units.

A) Core 100% - *G. edwardsi*

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Anoxybacillus kestanbolensis					OTU_1211, OTU_1300, OTU_2851
Bacteroides uniformis				OTU_2317	
Class Alphaproteobacteria	OTU_44776, OTU_44920				OTU_33247
Family Aerococcaceae	OTU_1854				
Family Endozoicimonaceae	OTU_207	OTU_207	OTU_54, OTU_207	OTU_54, OTU_1924	OTU_207
Family Phyllobacteriaceae		OTU_284			OTU_56231
Family Rhodobacteraceae					OTU_6265
Family Ruminococcaceae					OTU_757
Family Spirochaetaceae					OTU_7124
Genus *Bacteroides*				OTU_9499	
Genus *Diaphorobacter*	OTU_3474	OTU_3474		OTU_3474	OTU_3474
Genus *Erythrobacter*	OTU_769			OTU_769	
Genus *Halomicronema*	OTU_169, OTU_748, OTU_21418				

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Genus *Marinomonas*	OTU_946				
Genus *Muricauda*	OTU_957				
Genus *Ruegeria*					OTU_63604
Genus SGUS912		OTU_73, OTU_6055	OTU_73	OTU_73, OTU_896, OTU_6009, OTU_6055, OTU_6132, OTU_15786, OTU_15792, OTU_15979, OTU_16008	
Order Gemellales	OTU_6137				
Order Kiloniellales	OTU_256, OTU_44796		OTU_256		OTU_256
Propionibacterium acnes	OTU_5472, OTU_29486, OTU_33911, OTU_34038	OTU_5472, OTU_32607	OTU_5472, OTU_34191	OTU_5472, OTU_29486, OTU_33913	OTU_5472
Pseudomonas veronii			OTU_7093, OTU_19203	OTU_7093	OTU_7093
Staphylococcus epidermidis					OTU_2781
Stenotrophomonas geniculata				OTU_5826	

B) Dominant phylotypes - *G. edwardsi*

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Acinetobacter johnsonii	OTU_15208				
Acinetobacter lwoffii		OTU_5687, OTU_12395			OTU_5687
Anoxybacillus kestanbolensis		OTU_1211, OTU_1223, OTU_1300, OTU_2563, OTU_2842, OTU_2843, OTU_2846, OTU_2847, OTU_2851, OTU_2859, OTU_2913, OTU_2915, OTU_4436			OTU_1211, OTU_1223, OTU_1300, OTU_2563, OTU_2842, OTU_2843, OTU_2845, OTU_2846, OTU_2847, OTU_2848, OTU_2850, OTU_2851, OTU_2856, OTU_2859, OTU_2909, OTU_2910, OTU_2913, OTU_2914, OTU_2915, OTU_2916, OTU_3522, OTU_3583, OTU_3584, OTU_3593, OTU_3958, OTU_3966, OTU_3983, OTU_3991, OTU_4003, OTU_4004, OTU_4012, OTU_4030, OTU_4042, OTU_4064, OTU_4077, OTU_4088, OTU_4093, OTU_4095, OTU_4108, OTU_4119, OTU_4135, OTU_4141, OTU_4166, OTU_4170, OTU_4182, OTU_4186, OTU_4203, OTU_4204, OTU_4205, OTU_4207, OTU_4213, OTU_4214, OTU_4216, OTU_4238, OTU_4240, OTU_4243, OTU_4244, OTU_4422, OTU_4427, OTU_4430, OTU_4431, OTU_4436, OTU_4452, OTU_4455,

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
					OTU_4511, OTU_5246, OTU_5643, OTU_7321
Ascidianibacter aurantiacus		OTU_6506		OTU_6506, OTU_7050	
Bacillus cereus		OTU_2673			
Bacillus thermoamylovorans	OTU_2883				
Bacteroides ovatus		OTU_6383			OTU_6383
Bacteroides uniformis			OTU_2317	OTU_2317	OTU_2317
Bifidobacterium pseudolongum			OTU_15681		
Brevibacterium aureum			OTU_34884		
Brevundimonas diminuta		OTU_23567			OTU_23567
Class Alphaproteobacteria	OTU_43157	OTU_43157, OTU_44776, OTU_44920, OTU_46992	OTU_29134, OTU_33653, OTU_44920, OTU_50122	OTU_33247, OTU_33653, OTU_34012, OTU_34292, OTU_52955, OTU_71948	OTU_29134, OTU_33247, OTU_34886, OTU_40855, OTU_43157, OTU_43513, OTU_43789, OTU_43801, OTU_44776, OTU_50121, OTU_56285, OTU_64829, OTU_69119
Class Gammaproteobacteria			OTU_13285		
Class ML635J-21		OTU_46929			
Class Mollicutes			OTU_37		
Class SJA-4			OTU_23110		
Clostridium perfringens			OTU_39062		

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Coccinimonas marina	OTU_9053		OTU_9053	OTU_9053	
Coralibacter albidoflavus			OTU_63		
Desulfovibrio capillatus		OTU_1207		OTU_604, OTU_1207	
Endozoicomonas montiporae					OTU_1494
Enterovibrio coralii					OTU_2229
Eubacterium dolichum		OTU_2899			
Family A4b	OTU_10191				
Family Aerococcaceae	OTU_1854, OTU_2944	OTU_10, OTU_786, OTU_1854, OTU_2720, OTU_2891, OTU_2944, OTU_3060	OTU_10, OTU_786, OTU_1854, OTU_2369, OTU_2718, OTU_2720, OTU_2750, OTU_2944, OTU_3559, OTU_3659	OTU_786, OTU_1854, OTU_2720, OTU_2944, OTU_3060, OTU_4643	OTU_2944
Family Alteromonadaceae			OTU_32037		
Family Anaplasmataceae				OTU_40563	
Family Bacillaceae					OTU_29137
Family Bacteriovoracaceae			OTU_2388		
Family Beijerinckiaceae		OTU_22295			
Family Chromatiaceae				OTU_31368	
Family Cohaesibacteraceae		OTU_43697, OTU_46932			OTU_899
Family Coxiellaceae			OTU_7718		

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Family Desulfobulbaceae		OTU_6087		OTU_6087	
Family Desulfovibrionaceae			OTU_3832		OTU_16242, OTU_21775
Family Endozoicimonaceae	OTU_54, OTU_207	OTU_54, OTU_207	OTU_54, OTU_157, OTU_207, OTU_208, OTU_209, OTU_210, OTU_211, OTU_212, OTU_215, OTU_226, OTU_229, OTU_230, OTU_255, OTU_277, OTU_297, OTU_300, OTU_333, OTU_334, OTU_338, OTU_344, OTU_348, OTU_363, OTU_375, OTU_398, OTU_409, OTU_432, OTU_437, OTU_441, OTU_456, OTU_459, OTU_460, OTU_478, OTU_483, OTU_492, OTU_505, OTU_577, OTU_579, OTU_597, OTU_598, OTU_612, OTU_1587, OTU_1730, OTU_1775, OTU_1793, OTU_1924, OTU_7561, OTU_7735	OTU_54, OTU_157, OTU_207, OTU_209, OTU_210, OTU_211, OTU_212, OTU_229, OTU_230, OTU_255, OTU_297, OTU_300, OTU_333, OTU_334, OTU_338, OTU_344, OTU_348, OTU_363, OTU_375, OTU_398, OTU_422, OTU_432, OTU_437, OTU_441, OTU_456, OTU_459, OTU_474, OTU_483, OTU_490, OTU_492, OTU_577, OTU_597, OTU_598, OTU_612, OTU_1730, OTU_1793, OTU_1924, OTU_1926, OTU_6488, OTU_6989, OTU_7268, OTU_7561, OTU_9073, OTU_9124, OTU_12314, OTU_12701, OTU_12702	OTU_207, OTU_1793, OTU_3485, OTU_7268, OTU_7561
Family Enterobacteriaceae			OTU_6441		
Family Flammeovirgaceae	OTU_17, OTU_5773, OTU_19149, OTU_22521, OTU_25155, OTU_26892, OTU_27759, OTU_28368	OTU_17, OTU_5773, OTU_7264, OTU_16509, OTU_22521	OTU_17, OTU_5773, OTU_22521, OTU_23170	OTU_17, OTU_15950, OTU_27543	OTU_17, OTU_5773, OTU_7264, OTU_14964, OTU_21595, OTU_22521, OTU_26892, OTU_27759, OTU_28148, OTU_28149
Family Flavobacteriaceae		OTU_166, OTU_23510	OTU_166, OTU_778, OTU_6266, OTU_19177	OTU_778, OTU_7593, OTU_11206, OTU_11573, OTU_16725, OTU_27531,	OTU_5821, OTU_16774

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
				OTU_30712, OTU_30720, OTU_30722, OTU_30743	
Family Halomonadaceae				OTU_2294	
Family Helicobacteraceae					OTU_33792
Family Hyphomicrobiaceae	OTU_33305	OTU_33305	OTU_47287	OTU_47287	OTU_63617, OTU_69340
Family Ktedonobacteraceae	OTU_31667				
Family Lachnospiraceae		OTU_2043			
Family Lentisphaeraceae			OTU_45330		OTU_43097
Family Methylobacteriaceae		OTU_15477			
Family Neisseriaceae				OTU_6489	
Family Peptostreptococcaceae					OTU_45883, OTU_47374
Family Phyllobacteriaceae	OTU_284, OTU_33251	OTU_284, OTU_33251, OTU_71604	OTU_284, OTU_33251, OTU_34933, OTU_49682	OTU_284, OTU_26957, OTU_33251	OTU_284, OTU_17868, OTU_43816, OTU_56231, OTU_63620, OTU_66109
Family Pirellulaceae		OTU_19684, OTU_26983			
Family Piscirickettsiaceae			OTU_2331, OTU_13280	OTU_2331	
Family Porphyromonadaceae	OTU_6666				
Family Propionibacteriaceae	OTU_22509				
Family Pseudanabaenaceae	OTU_58847			OTU_24704	

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Family Pseudoalteromonadaceae			OTU_2193		OTU_2193, OTU_3100, OTU_3219
Family Pseudomonadaceae					OTU_2900, OTU_6533
Family Rhodobacteraceae	OTU_6265, OTU_46927, OTU_63155, OTU_66625	OTU_6265, OTU_31981, OTU_33726, OTU_43572, OTU_53182, OTU_59408	OTU_6265, OTU_25157, OTU_31981, OTU_43777, OTU_46927, OTU_67656, OTU_68288, OTU_73060, OTU_43300	OTU_6265, OTU_8247, OTU_25157, OTU_47292, OTU_47497, OTU_65604, OTU_72843	OTU_6265, OTU_46927, OTU_59408, OTU_65604, OTU_68288
Family Rhodospirillaceae			OTU_43300		OTU_48923, OTU_49577
Family Rikenellaceae			OTU_2323	OTU_2323	
Family Ruminococcaceae		OTU_5732			OTU_757, OTU_5732
Family Spirochaetaceae	OTU_1269, OTU_3183, OTU_7124	OTU_1269		OTU_1269, OTU_3183, OTU_8938	OTU_1269, OTU_3183, OTU_7124, OTU_7263, OTU_10594, OTU_11472, OTU_11931, OTU_12162
Family Vibrionaceae					OTU_2872, OTU_3211, OTU_6066, OTU_7269
Family Weeksellaceae		OTU_10762, OTU_15533			
Family Xenococcaceae			OTU_885, OTU_44423, OTU_44455, OTU_46980	OTU_885, OTU_44423, OTU_44455	
Genus 02d06			OTU_39190		OTU_39282
Genus *Acinetobacter*	OTU_12387				OTU_6019
Genus *Actinomyces*				OTU_16723	

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Genus *Alcanivorax*					OTU_7429
Genus *Anabaena*			OTU_63517		
Genus *Anaerococcus*			OTU_23868, OTU_28565		
Genus *Anaerospora*		OTU_32870	OTU_32870		OTU_32870
Genus *Aquimarina*			OTU_9048	OTU_9048	
Genus *Bacillus*					OTU_3193, OTU_4174, OTU_5133, OTU_5145
Genus *Bacteroides*		OTU_6168, OTU_9499		OTU_9499	
Genus Candidatus Portiera				OTU_18933	
Genus *Capnocytophaga*				OTU_11205, OTU_27542	
Genus *Chryseobacterium*	OTU_25925				
Genus *Cloacibacterium*		OTU_970, OTU_22145	OTU_22145		OTU_970, OTU_1513, OTU_10346, OTU_23664
Genus *Clostridium*			OTU_12675, OTU_38759, OTU_39191		
Genus *Comamonas*				OTU_16055, OTU_19845, OTU_21444	
Genus *Congregibacter*			OTU_19301	OTU_6508, OTU_21388	OTU_19830, OTU_21388
Genus *Coprococcus*				OTU_30745	

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Genus *Corynebacterium*	OTU_6790, OTU_33744, OTU_36716	OTU_6790, OTU_33744, OTU_34477	OTU_12410, OTU_30862, OTU_37089	OTU_12410, OTU_16057, OTU_30862	OTU_6790, OTU_12410, OTU_17918, OTU_33744, OTU_6790
Genus *Delftia*			OTU_18355	OTU_18355, OTU_25878	
Genus *Diaphorobacter*	OTU_3474	OTU_969, OTU_3474, OTU_18563, OTU_19565	OTU_3474, OTU_18563	OTU_3474	OTU_3474
Genus *Enhydrobacter*		OTU_23505			
Genus *Erythrobacter*	OTU_769	OTU_769	OTU_769, OTU_63345	OTU_769	
Genus *Exiguobacterium*			OTU_164, OTU_926		OTU_164
Genus *Ferrimonas*					OTU_2238
Genus *Frankia*	OTU_43530				
Genus *Fulvivirga*		OTU_19475			
Genus *Fusobacterium*				OTU_44093	OTU_44093
Genus *Garciella*	OTU_33821				
Genus *Glaciecola*			OTU_17735		OTU_17735, OTU_23420
Genus *Granulicatella*		OTU_2806, OTU_3637			
Genus *Haliangium*			OTU_1565		
Genus *Halomicronema*	OTU_169, OTU_748, OTU_21418, OTU_23662, OTU_29504, OTU_39562, OTU_43191	OTU_748, OTU_21418, OTU_29504, OTU_34682, OTU_44107, OTU_62583		OTU_21418, OTU_23662, OTU_29504, OTU_34682	

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Genus *Herbaspirillum*			OTU_11487		
Genus *Hyphomicrobium*		OTU_46545			
Genus *Inquilinus*		OTU_70486			OTU_44130, OTU_53914, OTU_58822
Genus *Lactobacillus*			OTU_9		OTU_9, OTU_13, OTU_1003
Genus *Lampropedia*				OTU_20315	
Genus *Leptolyngbya*	OTU_39407, OTU_40001			OTU_32585	
Genus *Leptonema*			OTU_8849		
Genus *Lewinella*			OTU_13286		
Genus *Marinomonas*	OTU_946				
Genus *Moraxella*	OTU_15816			OTU_15816, OTU_16724, OTU_20316, OTU_20322, OTU_21925, OTU_21971	
Genus *Muricauda*	OTU_957		OTU_880, OTU_957, OTU_7598	OTU_589, OTU_957, OTU_7598	
Genus *Nisaea*	OTU_26873			OTU_26873	OTU_26873
Genus *Paracoccus*				OTU_71952	
Genus *Pelomonas*		OTU_28818	OTU_28818	OTU_28818	
Genus *Peptoniphilus*			OTU_22978		
Genus *Phaeobacter*			OTU_63336		

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Genus *Photobacterium*					OTU_2228, OTU_3218
Genus *Planctomyces*		OTU_12245			
Genus *Porphyromonas*				OTU_27540	
Genus *Prevotella*	OTU_6343			OTU_6343, OTU_7309	
Genus *Pseudomonas*	OTU_16280	OTU_15232, OTU_19927	OTU_15232, OTU_19071, OTU_19888, OTU_19927, OTU_20648	OTU_15696, OTU_20319, OTU_33629	OTU_15232, OTU_19927
Genus *Pseudoruegeria*	OTU_44674, OTU_63624	OTU_44674		OTU_67172	
Genus *Ralstonia*		OTU_10704			
Genus *Rivularia*			OTU_1568	OTU_1568	
Genus *Roseivirga*		OTU_16590			
Genus *Rubritalea*					OTU_23419
Genus *Ruegeria*					OTU_63604, OTU_66706,
Genus *Ruminococcus*		OTU_33514			
Genus *Salinisphaera*			OTU_6543, OTU_19107, OTU_19115	OTU_6821	
Genus SC3-56		OTU_236	OTU_236, OTU_9114, OTU_15982, OTU_16003, OTU_19141	OTU_236	OTU_236
Genus *Schlegelella*				OTU_20314	

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Genus SGUS912	OTU_73, OTU_896, OTU_6055, OTU_15792, OTU_15979, OTU_15987, OTU_16008	OTU_73, OTU_896, OTU_6010, OTU_6055, OTU_6132, OTU_8534, OTU_15792, OTU_15979, OTU_15987, OTU_15998, OTU_16008, OTU_16320	OTU_73, OTU_896, OTU_2796, OTU_6009, OTU_6055, OTU_8534, OTU_11195, OTU_11526, OTU_11563, OTU_11619, OTU_15562, OTU_15786, OTU_15792, OTU_15979, OTU_15984, OTU_15987, OTU_15994, OTU_15998, OTU_16008, OTU_16016, OTU_16038, OTU_16349, OTU_16364, OTU_17412, OTU_18914, OTU_20251, OTU_20574, OTU_23166	OTU_73, OTU_896, OTU_5755, OTU_5771, OTU_6009, OTU_6055, OTU_6132, OTU_8534, OTU_11195, OTU_11526, OTU_11563, OTU_15562, OTU_15786, OTU_15792, OTU_15979, OTU_15984, OTU_15987, OTU_15994, OTU_15998, OTU_16008, OTU_16038, OTU_16349, OTU_19169, OTU_19332, OTU_20251, OTU_21282, OTU_21643	OTU_73, OTU_896, OTU_6055, OTU_8534, OTU_15792, OTU_15979, OTU_15987, OTU_15998, OTU_16008
Genus *Shewanella*					OTU_7254, OTU_7279
Genus SMB53			OTU_45438		OTU_45438
Genus *Sphingomonas*		OTU_44009, OTU_44034			OTU_62969
Genus *Spirochaeta*					OTU_7110
Genus *Staphylococcus*			OTU_1800		
Genus *Streptococcus*	OTU_194, OTU_3059	OTU_194, OTU_1258, OTU_2949, OTU_3059, OTU_3312, OTU_3313, OTU_3314, OTU_4336, OTU_4588, OTU_4610, OTU_4881	OTU_194, OTU_2949, OTU_3059		OTU_3351
Genus *Thalassomonas*					OTU_6065, OTU_14965
Genus *Tenacibaculum*					OTU_12015, OTU_17919, OTU_17926
Genus *Turicibacter*					OTU_38107

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Genus vadinHB04		OTU_1845			
Genus *Xenococcus*					
Kingdom Bacteria			OTU_13045		OTU_33386, OTU_34520
Lysinibacillus boronitolerans		OTU_293			
Massilia alkalitolerans		OTU_16361			
Massilia haematophila			OTU_15956		
Methylobacterium mesophilicum				OTU_67462	
Methylotenera mobilis		OTU_16274			
Microbacterium chocolatum				OTU_37411	
Micrococcus luteus	OTU_2030			OTU_2030, OTU_37019	OTU_2030, OTU_37019, OTU_37470
Nautella italica			OTU_67439		
Neisseria bacilliformis				OTU_8585, OTU_8588	
Neisseria subflava		OTU_6574, OTU_9257			
Order Burkholderiales			OTU_16039		
Order Chroococcales	OTU_60114				
Order Clostridiales			OTU_18895, OTU_31315	OTU_22705, OTU_29027	
Order Entomoplasmatales			OTU_18908	OTU_18908, OTU_24457	

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Order Flavobacteriales			OTU_24408	OTU_24408, OTU_24467, OTU_28814	
Order Gemellales	OTU_3309, OTU_3325, OTU_4356, OTU_6137	OTU_2171, OTU_3065, OTU_4724, OTU_6137	OTU_2171, OTU_3065, OTU_4541, OTU_5012, OTU_6137		OTU_3325, OTU_5062
Order Kiloniellales	OTU_256, OTU_2053, OTU_22449, OTU_25250, OTU_44796, OTU_56905	OTU_256	OTU_256, OTU_1204, OTU_2053, OTU_2854, OTU_22449, OTU_25250, OTU_29130, OTU_29131, OTU_32433, OTU_32484, OTU_33712, OTU_43582, OTU_43584, OTU_43589, OTU_43919	OTU_256, OTU_1204, OTU_33712	OTU_256, OTU_1204, OTU_1531, OTU_2053, OTU_2854, OTU_18287, OTU_18315, OTU_21723, OTU_22449, OTU_23390, OTU_25250, OTU_27638, OTU_27825, OTU_28514, OTU_28515, OTU_28516, OTU_28995, OTU_29007, OTU_29130, OTU_29131, OTU_29132, OTU_29194, OTU_30944, OTU_32000, OTU_32006, OTU_32174, OTU_32178, OTU_32429, OTU_32433, OTU_32484, OTU_32873, OTU_32876, OTU_32877, OTU_34524, OTU_36049, OTU_36685, OTU_43582, OTU_43584, OTU_43589, OTU_43591, OTU_43609, OTU_43815, OTU_43818, OTU_43823, OTU_43919, OTU_43923, OTU_43925 ,OTU_44181, OTU_44188, OTU_49659
Order Legionellales			OTU_16041		
Order Myxococcales		OTU_2260, OTU_6239	OTU_2770, OTU_6239, OTU_6366, OTU_17310	OTU_19, OTU_2770, OTU_3457, OTU_5868, OTU_6239, OTU_6282, OTU_6302, OTU_6512,	

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
				OTU_7168, OTU_16464 OTU_16899, OTU_22422	
Order Oceanospirillales			OTU_214, OTU_340, OTU_499, OTU_596	OTU_214, OTU_499, OTU_596, OTU_12700	OTU_5804, OTU_6331, OTU_7887, OTU_7890, OTU_7891, OTU_7897, OTU_7900, OTU_7902, OTU_7905, OTU_7908, OTU_8118
Order Phycisphaerales			OTU_25186		
Order RF39				OTU_4	
Order Rhizobiales		OTU_27728, OTU_43799, OTU_44232, OTU_44663	OTU_48912	OTU_44232, OTU_59120, OTU_65671, OTU_66673, OTU_66681	OTU_44232, OTU_66667
Order Rhodospirillales				OTU_2347	OTU_11309, OTU_19828
Order Rickettsiales				OTU_47971, OTU_58188	
Order Roseiflexales	OTU_617	OTU_617		OTU_617	OTU_617
Order Sphingomonadales			OTU_32801	OTU_32801	
Order Vibrionales					OTU_17916, OTU_19379
Paenibacillus barengoltzii	OTU_2256				
Photobacterium damselae					OTU_3083, OTU_3213
Photobacterium rosenbergii	OTU_2052				
Phylum Bacteroidetes		OTU_7566	OTU_7566, OTU_18917, OTU_22021, OTU_22692, OTU_24444, OTU_25190	OTU_7566, OTU_15782, OTU_18917, OTU_22021, OTU_22692, OTU_22693,	OTU_7566, OTU_18917

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
				OTU_24444, OTU_24505, OTU_25190	
Phylum Spirochaetes					OTU_38106
Phylum SR1				OTU_33993, OTU_35595	
Propionibacterium acnes	OTU_5472, OTU_29486, OTU_32607, OTU_33911, OTU_33913, OTU_34038, OTU_34191	OTU_2942, OTU_5472, OTU_12964, OTU_15337, OTU_15466, OTU_29486, OTU_31721, OTU_32607, OTU_33702, OTU_33906, OTU_33911, OTU_33913, OTU_33935, OTU_33942, OTU_34029, OTU_34038, OTU_34191, OTU_34233, OTU_34236, OTU_34654, OTU_43531	OTU_2942, OTU_5472, OTU_12964, OTU_15337, OTU_15345, OTU_15466, OTU_29486, OTU_31721, OTU_32607, OTU_32890, OTU_33513, OTU_33702, OTU_33906, OTU_33911, OTU_33912, OTU_33913, OTU_33935, OTU_33942, OTU_34029, OTU_34038, OTU_34188, OTU_34191, OTU_34225, OTU_34233, OTU_34236, OTU_34654, OTU_34863, OTU_35438, OTU_36466, OTU_43531	OTU_5472, OTU_15466, OTU_29486, OTU_32607, OTU_33702, OTU_33906, OTU_33911, OTU_33913, OTU_33942, OTU_34038, OTU_34191, OTU_34233, OTU_34236	OTU_2942, OTU_5472, OTU_12964, OTU_15337, OTU_26885, OTU_29486, OTU_32607, OTU_33702, OTU_33911, OTU_33913, OTU_33935, OTU_33942, OTU_34029, OTU_34038, OTU_34188, OTU_34191, OTU_34233
Propionibacterium granulosum		OTU_35447	OTU_35447		
Pseudomonas fragi		OTU_15598	OTU_15598		OTU_15598
Pseudomonas stutzeri				OTU_15254	
Pseudomonas veronii	OTU_7093	OTU_910, OTU_7093, OTU_19203, OTU_19833	OTU_7093, OTU_19203, OTU_19833	OTU_7093	OTU_910, OTU_7093, OTU_15922, OTU_19203, OTU_19833
Pseudoxanthomonas mexicana				OTU_8590	
Roseomonas aerilata		OTU_58103			
Ruminococcus gnavus				OTU_3491	

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Spirochaeta halophila	OTU_9094			OTU_9094	OTU_9094, OTU_11465, OTU_11912, OTU_11917, OTU_11939, OTU_12017
Staphylococcus epidermidis	OTU_2781	OTU_2781	OTU_2781, OTU_3780		OTU_2175, OTU_2781, OTU_2945, OTU_3279, OTU_4637, OTU_4760
Stenotrophomonas geniculata	OTU_5826	OTU_5826	OTU_5826	OTU_5826, OTU_8385, OTU_10466	
Veillonella dispar		OTU_753	OTU_753		
Vibrio harveyi					OTU_3210
Xanthobacillum maris				OTU_16726	

C) Top 10 dominant phylotypes - *G. edwardsi*

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Anoxybacillus kestanbolensis		OTU_1211, OTU_1223, OTU_1300			OTU_1211, OTU_1223, OTU_1300, OTU_2847
Family Aerococcaceae			OTU_1854		
Family Endozoicimonaceae			OTU_54	OTU_54, OTU_300, OTU_1793	
Family Flammeovirgaceae	OTU_17, OTU_5773	OTU_17			
Family Flavobacteriaceae				OTU_11206	
Family Neisseriaceae				OTU_6489	
Genus *Bacillus*					OTU_3193
Genus *Diaphorobacter*	OTU_3474	OTU_3474		OTU_3474	
Genus *Halomicronema*	OTU_43191				
Genus SC3-56			OTU_236		
Genus SGUS912	OTU_73	OTU_73	OTU_73, OTU_896, OTU_6055	OTU_73, OTU_6055	OTU_73
Genus *Streptococcus*	OTU_194	OTU_194			
Order Kiloniellales	OTU_256		OTU_256		OTU_256
Order Oceanospirillales					OTU_6331
Phylum Bacteroidetes				OTU_7566	

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Propionibacterium acnes	OTU_5472	OTU_5472, OTU_29486	OTU_5472, OTU_29486	OTU_5472	OTU_5472
Pseudomonas veronii	OTU_7093	OTU_7093	OTU_7093		OTU_7093
Staphylococcus epidermidis	OTU_2781				

Table C - 17: Taxonomic identification of OTUs part of the Core 100% (A), dominant phylotypes (relative abundance ≥ 0.1, B) and top 10 dominant phylotypes (C) in *I. palifera* bacterial assemblage. Dominant phylotypes are presented as a count of number of OTU per taxonomic classification. OTU: Operational Taxonomic Units.

A) Core 100% - *I. palifera*

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Anoxybacillus kestanbolensis		OTU_1300			OTU_1300
Brevundimonas diminuta		OTU_67153			
Endozoicomonas montiporae				OTU_2218, OTU_2328, OTU_2342, OTU_2457, OTU_2502	OTU_2218, OTU_2328, OTU_2342, OTU_2434, OTU_2435, OTU_2457, OTU_2465, OTU_2487, OTU_2502, OTU_2536, OTU_2538, OTU_2566, OTU_2573, OTU_2585, OTU_2606, OTU_7199
Family Aerococcaceae	OTU_1854	OTU_2715		OTU_10, OTU_1854	
Family Endozoicimonaceae		OTU_207	OTU_207, OTU_1775	OTU_207, OTU_265, OTU_1087, OTU_1399, OTU_1439, OTU_1587, OTU_1602, OTU_1775, OTU_1799, OTU_1957, OTU_2286, OTU_2411, OTU_2422, OTU_2432, OTU_2445, OTU_2479, OTU_2501, OTU_2523, OTU_2526, OTU_2529, OTU_2540, OTU_2575, OTU_2607, OTU_2623, OTU_2627	OTU_54, OTU_173, OTU_187, OTU_207, OTU_264, OTU_265, OTU_287, OTU_904, OTU_1087, OTU_1399, OTU_1439, OTU_1447, OTU_1480, OTU_1587, OTU_1602, OTU_1775, OTU_1777, OTU_1778, OTU_1784, OTU_1786, OTU_1798, OTU_1799, OTU_1802, OTU_1922, OTU_1957, OTU_2226, OTU_2286, OTU_2372, OTU_2411, OTU_2416, OTU_2419, OTU_2422, OTU_2427,

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
					OTU_2432, OTU_2440, OTU_2445, OTU_2460, OTU_2466, OTU_2467, OTU_2468, OTU_2470, OTU_2479, OTU_2481, OTU_2486, OTU_2490, OTU_2495, OTU_2501, OTU_2507, OTU_2522, OTU_2523, OTU_2524, OTU_2526, OTU_2529, OTU_2540, OTU_2544, OTU_2545, OTU_2549, OTU_2553, OTU_2567, OTU_2568, OTU_2575, OTU_2578, OTU_2579, OTU_2586, OTU_2605, OTU_2607, OTU_2614, OTU_2623, OTU_2626, OTU_2627, OTU_2636, OTU_2639, OTU_2643, OTU_2646, OTU_2648, OTU_2819, OTU_6534, OTU_6604, OTU_7185, OTU_7197, OTU_7209, OTU_7213, OTU_7239, OTU_7770, OTU_7781, OTU_7811, OTU_9317, OTU_9366, OTU_9384, OTU_11763, OTU_14003
Family Methylobacteriaceae		OTU_15477			
Family Phyllobacteriaceae					OTU_284
Family Ruminococcaceae					OTU_933, OTU_5732
Genus *Bacteroides*		OTU_6168			OTU_9499, OTU_12526
Genus *Delftia*				OTU_18355	

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Genus *Diaphorobacter*	OTU_3474, OTU_18563	OTU_969, OTU_3474, OTU_13531, OTU_18563, OTU_19565	OTU_969, OTU_3474, OTU_18563	OTU_969, OTU_3474	OTU_3474
Genus *Klebsiella*		OTU_7973			
Genus *Lactobacillus*					OTU_13, OTU_66, OTU_152, OTU_273
Genus *Marinomonas*					OTU_268
Genus *Parabacteroides*					OTU_8387
Genus *Pseudomonas*			OTU_15232		
Genus *Reinekea*					OTU_2658
Genus SGUS912					OTU_73
Genus *Sphingobium*	OTU_28287				
Genus *Streptococcus*			OTU_194		
Order Clostridiales					OTU_31, OTU_1876
Order Entomoplasmatales					OTU_1641
Order Kiloniellales					OTU_256
Order Myxococcales				OTU_6302, OTU_6539	OTU_237, OTU_239, OTU_6302, OTU_6539
Propionibacterium acnes	OTU_5472, OTU_11342, OTU_29486, OTU_32607, OTU_33702, OTU_33906, OTU_33912, OTU_33935,	OTU_5472, OTU_15337, OTU_29486, OTU_32607, OTU_33911, OTU_33913,	OTU_5472, OTU_33913, OTU_34191	OTU_5472, OTU_33911, OTU_33935	OTU_5472, OTU_33913

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
	OTU_33942, OTU_34029, OTU_34038, OTU_34191, OTU_34233, OTU_34236, OTU_43531	OTU_34029, OTU_34038, OTU_34191			
Pseudomonas fragi			OTU_15598		
Pseudomonas veronii	OTU_7093		OTU_7093, OTU_19203	OTU_7093, OTU_19203	OTU_7093, OTU_19203
Staphylococcus epidermidis	OTU_2781	OTU_2781, OTU_3064			

B) Dominant - *I. palifera*

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Acinetobacter lwoffii	OTU_5687, OTU_10899, OTU_10903		OTU_5687		
Bacillus agaradhaerens	OTU_198				
Brevundimonas diminuta		OTU_23567, OTU_59723			
Class Bacilli	OTU_78161				
Escherichia coli			OTU_507		

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Family Aerococcaceae	OTU_10, OTU_786, OTU_1854, OTU_2369, OTU_2718, OTU_3060, OTU_4731	OTU_10, OTU_786, OTU_1854, OTU_2369, OTU_2715, OTU_2750, OTU_2944, OTU_3060	OTU_10, OTU_786, OTU_1854, OTU_2369, OTU_2944, OTU_3060	OTU_10, OTU_187, OTU_194, OTU_205, OTU_207, OTU_239, OTU_265, OTU_287, OTU_904, OTU_969, OTU_1087, OTU_1399, OTU_1439, OTU_1587, OTU_1602, OTU_1641, OTU_1775, OTU_1777, OTU_1799, OTU_1854, OTU_1957, OTU_2030, OTU_2175, OTU_2218, OTU_2286, OTU_2294, OTU_2328, OTU_2342, OTU_2411 OTU_2422, OTU_2432, OTU_2435, OTU_2445, OTU_2457, OTU_2479, OTU_2501, OTU_2502, OTU_2523, OTU_2524, OTU_2526, OTU_2529, OTU_2540, OTU_2573, OTU_2575, OTU_2607, OTU_2614, OTU_2623, OTU_2627, OTU_2648, OTU_2750, OTU_2781, OTU_2944, OTU_3474, OTU_3793, OTU_5472, OTU_5998, OTU_6302, OTU_6481, OTU_6539, OTU_6575, OTU_7093, OTU_7095, OTU_7168, OTU_9207, OTU_12964, OTU_15232, OTU_15598, OTU_17706, OTU_18355, OTU_18563, OTU_19203, OTU_19313, OTU_19600, OTU_19833, OTU_21605, OTU_21606, OTU_22509, OTU_23229, OTU_23967, OTU_24995, OTU_26885, OTU_28287, OTU_28818,	OTU_13, OTU_31, OTU_187, OTU_207, OTU_226, OTU_239, OTU_264, OTU_265, OTU_273, OTU_287, OTU_904, OTU_1009, OTU_1081, OTU_1087, OTU_1284, OTU_1286, OTU_1395, OTU_1399, OTU_1423, OTU_1439, OTU_1440, OTU_1448, OTU_1463, OTU_1602, OTU_1641, OTU_1775, OTU_1777, OTU_1778, OTU_1798, OTU_1799, OTU_1800, OTU_1802, OTU_1876, OTU_1922, OTU_1957, OTU_2218O, TU_2226, OTU_2286, OTU_2328, OTU_2342, OTU_2411, OTU_2416, OTU_2422, OTU_2432, OTU_2434, OTU_2435 OTU_2445, OTU_2457, OTU_2479, OTU_2490, OTU_2495, OTU_2501, OTU_2502, OTU_2507, OTU_2523, OTU_2524, OTU_2526, OTU_2529, OTU_2540, OTU_2553, OTU_2567, OTU_2573, OTU_2575, OTU_2578, OTU_2605, OTU_2607, OTU_2614, OTU_2623, OTU_2627, OTU_2636, OTU_2639, OTU_2648, OTU_5472, OTU_5732, OTU_6302, OTU_6534, OTU_6539, OTU_6604, OTU_7093, OTU_9499, OTU_17706

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
				OTU_29486, OTU_32268, OTU_32607, OTU_32890, OTU_33702, OTU_33744, OTU_33906, OTU_33911, OTU_33913, OTU_33935, OTU_34029, OTU_34038, OTU_34191, OTU_34233, OTU_34477, OTU_34665, OTU_44527, OTU_45686, OTU_47320, OTU_47497, OTU_59064, OTU_63506	
Family Bifidobacteriaceae	OTU_37174				
Family Endozoicimonaceae	OTU_207	OTU_207, OTU_287	OTU_207, OTU_1775		
Family Halomonadaceae		OTU_2294			
Family Methylobacteriaceae		OTU_15477, OTU_50253			
Family Oxalobacteraceae	OTU_23821	OTU_15600			
Family Planococcaceae	OTU_4257				
Family Porphyromonadaceae	OTU_6666				
Family Propionibacteriaceae	OTU_22509, OTU_39510				
Family Salinisphaeraceae	OTU_34146				
Genus *Acinetobacter*	OTU_16417, OTU_32647	OTU_10931	OTU_6019		
Genus *Bacillus*	OTU_3405, OTU_5688		OTU_2394		
Genus *Bacteroides*		OTU_6168	OTU_6168, OTU_7465		
Genus *Bifidobacterium*	OTU_32201				

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Genus *Cloacibacterium*	OTU_970, OTU_1513, OTU_22145	OTU_22145, OTU_12410	OTU_970		
Genus *Coprococcus*	OTU_30073				
Genus *Corynebacterium*	OTU_6147, OTU_6790, OTU_12410, OTU_16057, OTU_30862, OTU_33744	0	OTU_12410, OTU_30862, OTU_33744		
Genus *Delftia*	OTU_18355, OTU_23836	OTU_18355			
Genus *Diaphorobacter*	OTU_969, OTU_3474, OTU_13531, OTU_18563, OTU_19565, OTU_19601	OTU_3474, OTU_18563, OTU_19565	OTU_3474, OTU_18563, OTU_19565		
Genus *Exiguobacterium*			OTU_164		
Genus *Finegoldia*	OTU_28291, OTU_28300, OTU_31095				
Genus *Granulicatella*			OTU_2806, OTU_3637		
Genus *Halomicronema*		OTU_43191			
Genus *Hydrogenophaga*	OTU_25858				
Genus *Janthinobacterium*	OTU_24708				
Genus KD1-23	OTU_21877				
Genus *Klebsiella*		OTU_7973			
Genus *Lactobacillus*			OTU_13		
Genus *Lactococcus*			OTU_3012		

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Genus *Massilia*	OTU_22484, OTU_25857				
Genus *Prevotella*	OTU_2717, OTU_31030	OTU_2717			
Genus *Pseudomonas*	OTU_15770, OTU_16293		OTU_15232		
Genus *Pseudoruegeria*		OTU_44674			
Genus *Ralstonia*		OTU_6575			
Genus *Rubrobacter*	OTU_7066				
Genus *Salinisphaera*		OTU_6821			
Genus *Sphingobium*	OTU_28287	OTU_28287			
Genus *Staphylococcus*			OTU_1800		
Genus *Stenotrophomonas*		OTU_6113			
Genus *Streptococcus*	OTU_194	OTU_194, OTU_3059	OTU_194, OTU_1274		
Haemophilus parainfluenzae	OTU_6570				
Micrococcus luteus	OTU_2030, OTU_44076				
Order Actinomycetales	OTU_46520				
Order Chroococcales			OTU_53053		
Order Clostridiales			OTU_11351		
Order Entomoplasmatales	OTU_1641	OTU_1641, OTU_17706			

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Order Myxococcales		OTU_239, OTU_6302, OTU_6539	OTU_6302		
Order Salinisphaerales	OTU_12046				
Propionibacterium acnes	OTU_5472, OTU_11342, OTU_29486, OTU_31721, OTU_32607, OTU_32964, OTU_33513, OTU_33702, OTU_33906, OTU_33911, OTU_33912, OTU_33913, OTU_33935, OTU_33942, OTU_34029, OTU_34038, OTU_34191, OTU_34233, OTU_34236, OTU_34654, OTU_34964, OTU_35482, OTU_36335, OTU_36408, OTU_43531, OTU_44274	OTU_5472, OTU_12964, OTU_15337, OTU_29486, OTU_32607, OTU_33702, OTU_33906, OTU_33911, OTU_33913, OTU_33935, OTU_33942, OTU_34029, OTU_34038, OTU_34191, OTU_34233	OTU_5472, OTU_29486, OTU_32607, OTU_33702, OTU_33906, OTU_33911, OTU_33913, OTU_33935, OTU_33942, OTU_34029, OTU_34191		
Pseudomonas fragi	OTU_15598		OTU_15598		
Pseudomonas mendocina	OTU_21772				
Pseudomonas veronii	OTU_4847, OTU_7093, OTU_16278, OTU_22183, OTU_29222	OTU_7093	OTU_7093, OTU_19203		
Staphylococcus epidermidis	OTU_2781, OTU_3279, OTU_4991	OTU_2781	OTU_2175, OTU_2781		
Stenotrophomonas geniculata	OTU_5826	OTU_5826, OTU_6660			
Veillonella dispar		OTU_753	OTU_753		

C) Top 10 dominant phylotypes - *I. palifera*

Taxa	DMSO-BB	DMSO-Cr	LL-BB	LL-Cr	PFA
Family Aerococcaceae	OTU_970, OTU_1854, OTU_2781, OTU_3474, OTU_5472, OTU_7093, OTU_12410, OTU_18563, OTU_29486, OTU_32607	OTU_194, OTU_1641, OTU_1854, OTU_2781, OTU_2944, OTU_3474, OTU_5472, OTU_5826, OTU_15477, OTU_23567	OTU_207, OTU_970, OTU_2781, OTU_2806, OTU_2944, OTU_3474, OTU_5472, OTU_6168, OTU_7093, OTU_29486	OTU_207, OTU_239, OTU_1641, OTU_1775, OTU_1854, OTU_3474, OTU_5472, OTU_6302, OTU_6539, OTU_7093	OTU_207, OTU_287, OTU_1439, OTU_1602, OTU_1775, OTU_2218, OTU_2411, OTU_2422, OTU_2502, OTU_6302

Table C - 18: Diversity indexes of bacterial assemblages. Average (Av.) and Variation (Var.) of taxonomic (Tax.) distinctness between treatments.

Coral species	Method		Av. Tax. Distinctness (Δ+)	Var. Tax. Distinctness (Λ+)
	Preservation	Homogenization		
G. edwardsi	DMSO	Bead beating	80.67	113.72
	DMSO	Crushing	80.46	117.88
	Liquid nitrogen	Bead beating	81.10	107.13
	Liquid nitrogen	Crushing	80.80	107.44
	PFA	Decalcified	80.05	116.38
I. palifera	DMSO	Bead beating	79.42	136.66
	DMSO	Crushing	79.40	134.01
	Liquid nitrogen	Bead beating	80.56	124.92
	Liquid nitrogen	Crushing	80.19	124.85
	PFA	Decalcified	80.69	113.36

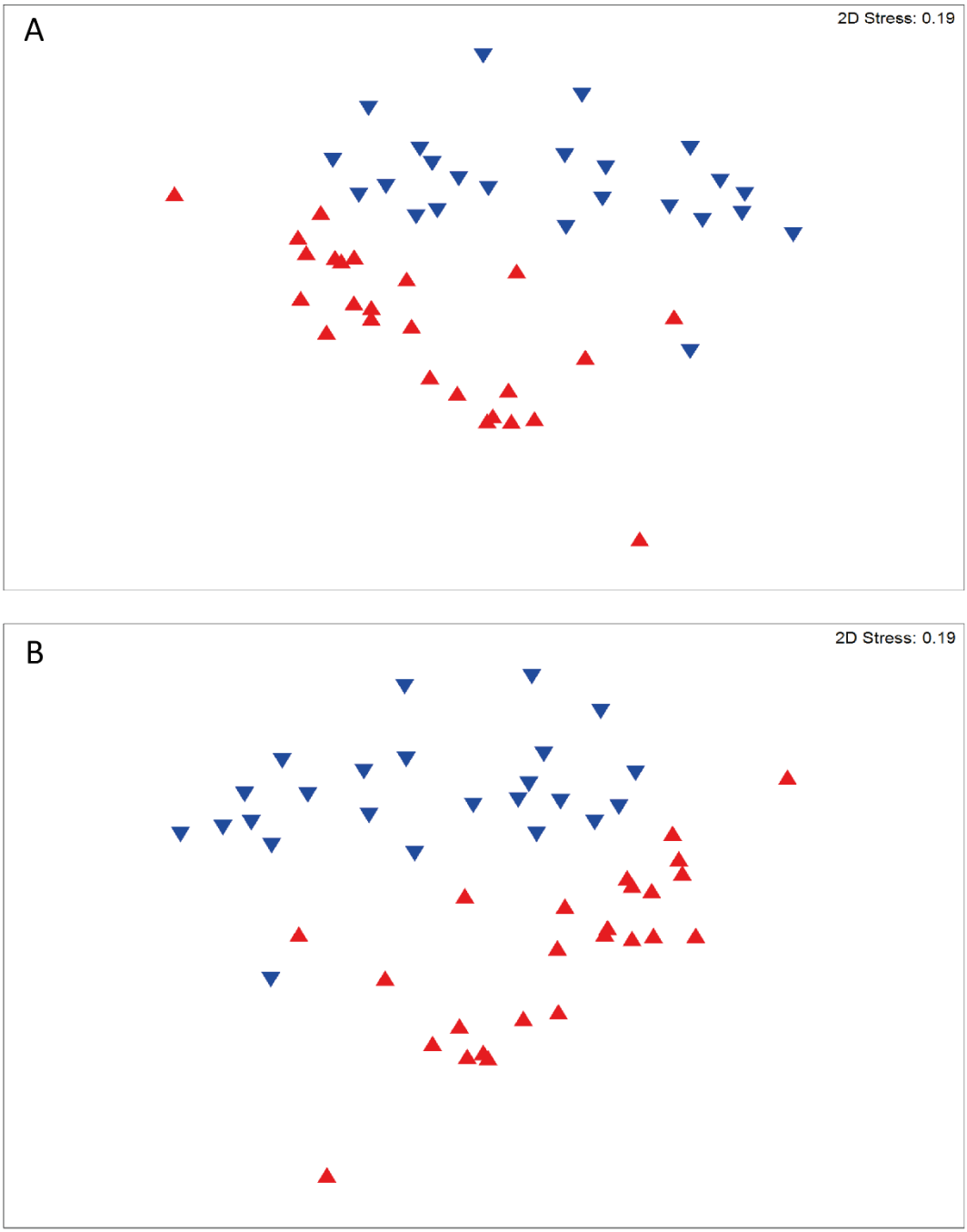


Figure C - 1: Bacterial communities are different between coral species. Non-metric MDS based on relative abundance (A) and presence/absence data (B). NMDS are based on Bray-Curtis dissimilarity of fourth root-transformed data (A) and Sorensen dissimilarity (B). Bacterial assemblage structure is different among coral species. Blue: *G. edwardsi*, red: *I. palifera*. Statistical analysis in Supplementary Table C-5, C-6.

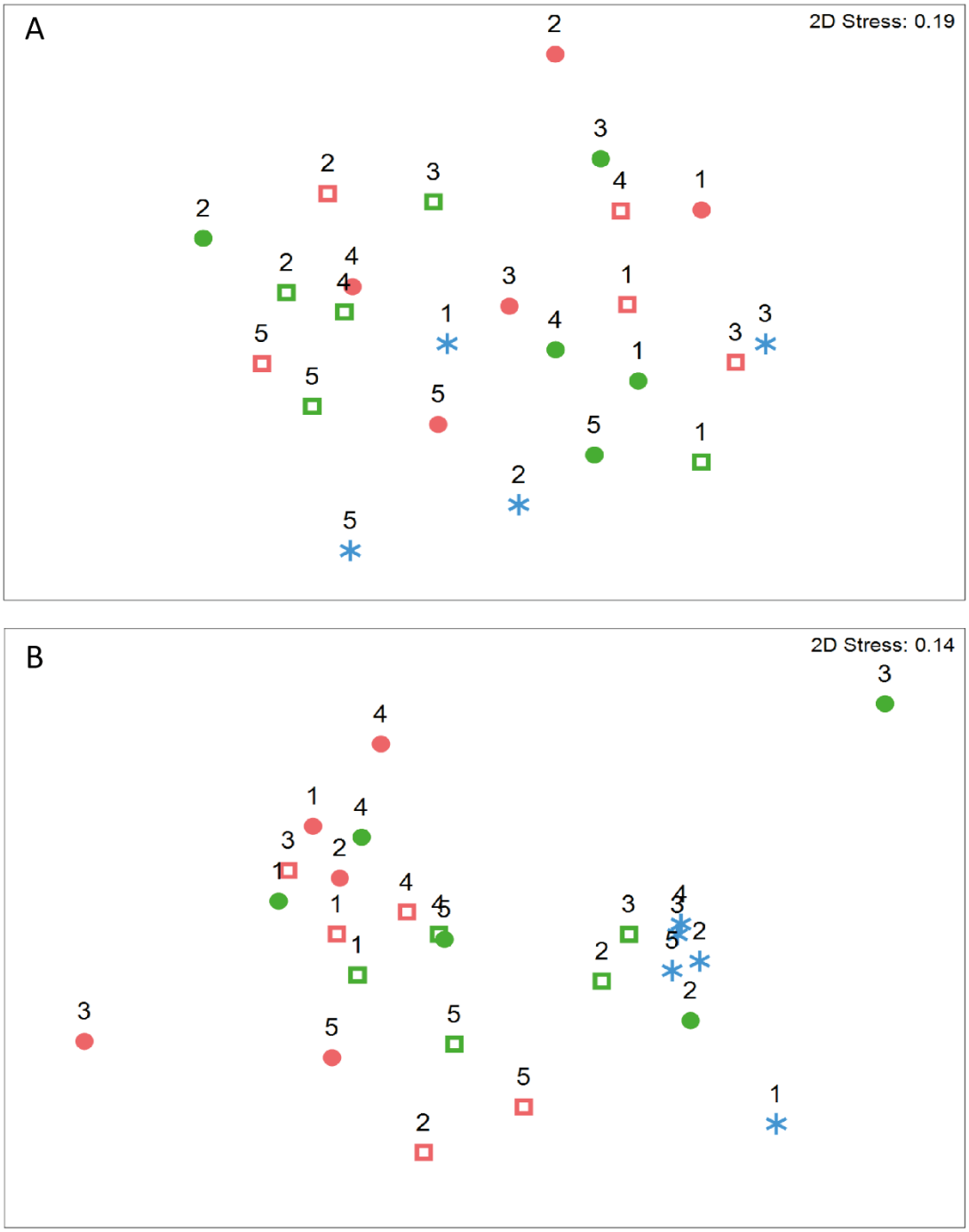


Figure C - 2: Non-metric MDS based on presence/absence data for *G. edwardsi* (A) and *I. palifera* (B). NMDS are based on Sorensen dissimilarity. Green: Liquid nitrogen, red: DMSO, circles: bead beating, squares: crushing, blue stars: PFA-decalcified. Colonies indicated with numbers. Supporting analyses in Supplementary Table C-8, C-10, C-12, C-14. No differences are observed in the composition of *G. edwardsi*. For *I. palifera*, there are differences in composition between fragments treated with PFA-decalcification (blue starts) and fragments preserved with DMSO, regardless the homogenization method.

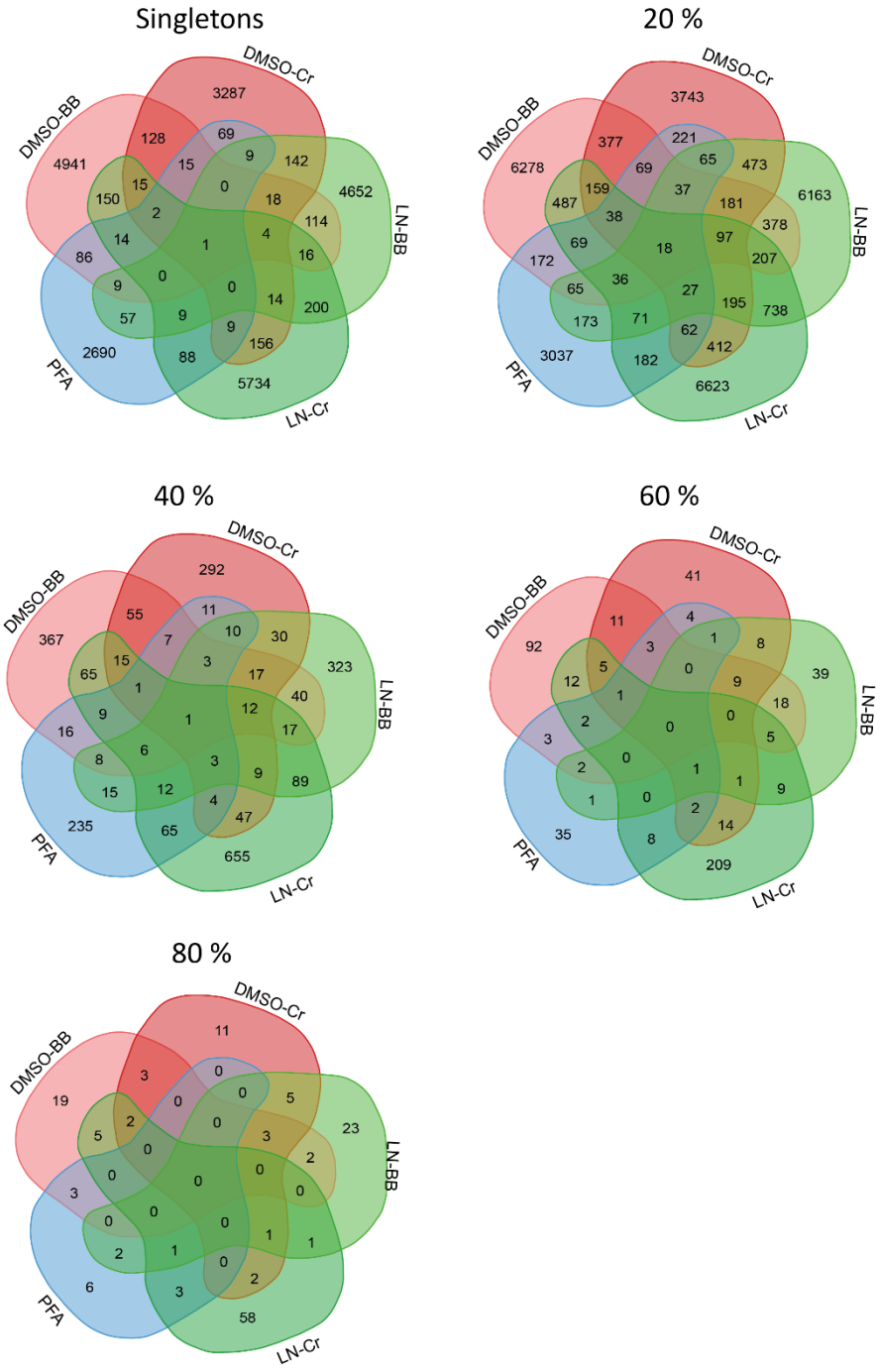


Figure C - 3: Venn diagram for singletons and bacterial assemblages persistent at 20, 40, 60, 80% of the samples of each methodology considered for preservation and homogenization in *G. edwardsi*. Bacterial phylotypes analysed at different percentages of persistence seems to show distinct bacterial assemblages, since number of phylotypes detected by only one combination of preservation and homogenization method is superior to those shared between distinct methods. However, analyses of structure of bacterial assemblages (Figure 3-3A) demonstrate that there are no differences and there are common phylotypes among methods, but their persistence and relative abundance vary among preservation and homogenization treatments (Figure 3-4A, C, E, G, I).

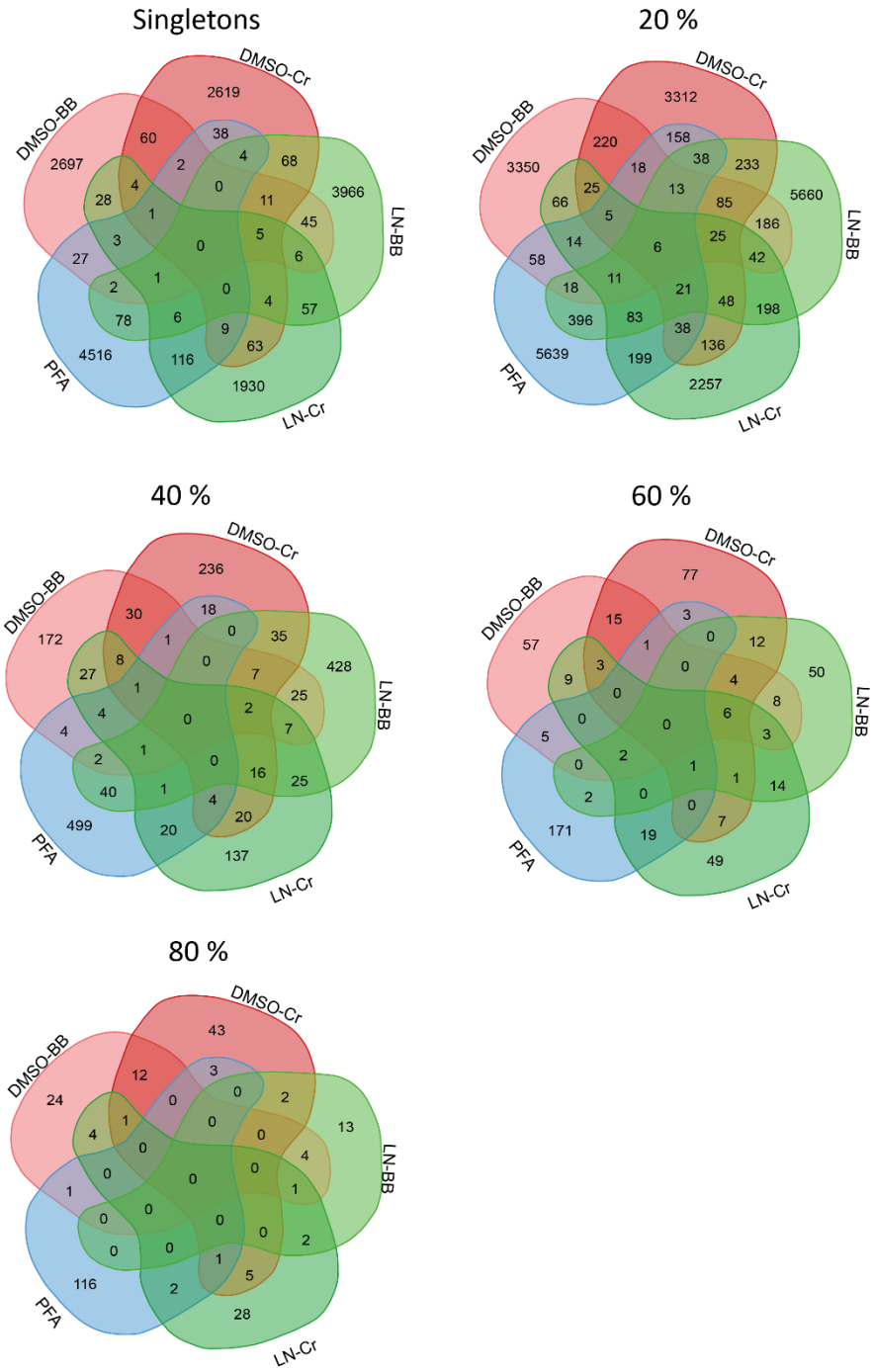


Figure C - 4: Venn diagram for singletons and bacterial assemblages persistent at 20, 40, 60, 80% of the samples of each methodology considered for preservation and homogenization in *I. palifera*. As observed in *G. edwardsi* (previous image) analysis of bacterial phylotypes considering different percentages of persistence indicate distinct bacterial assemblages. However, community structure analysis only detected differences between PFA-decalcified and both homogenization treatments preserved in DMSO (Figure 3-3B). Bacterial assemblages preserved with PFA and liquid nitrogen have shared phylotypes, but their persistence and relative abundance differ between treatments (Figure 3-4B, D, F, H, J).

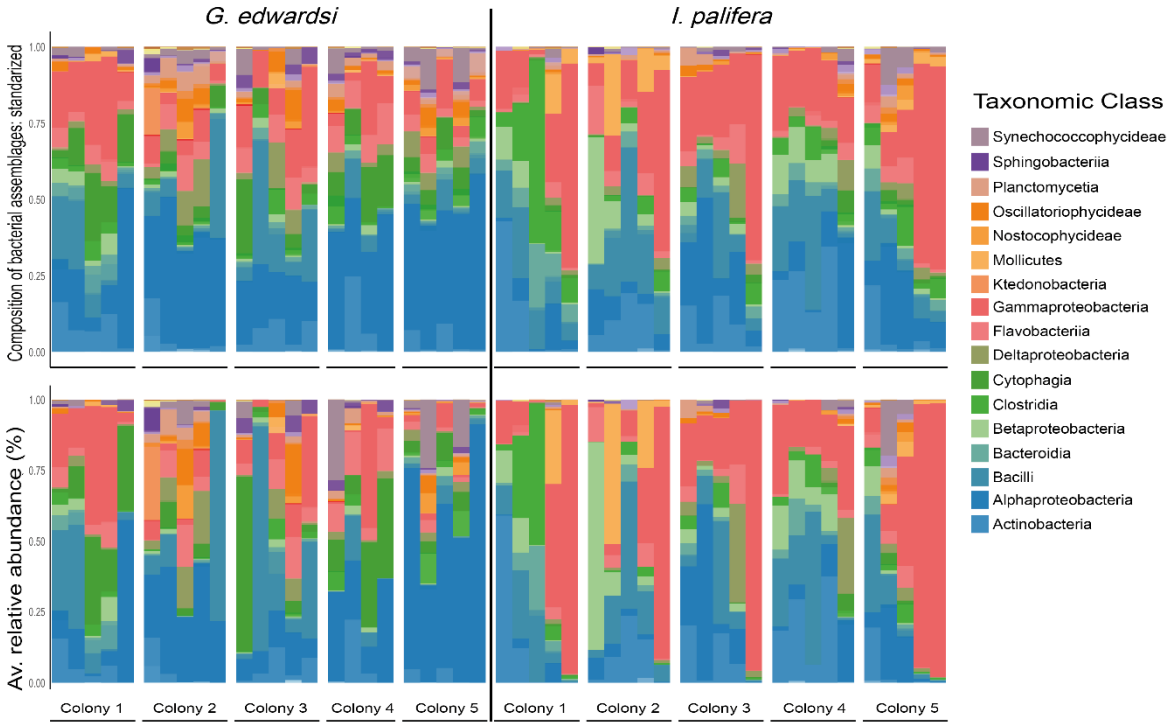


Figure C - 5: Variation of taxonomic composition (top) and structure (bottom) among preservation and homogenization methods by colony. For each colony columns are ordered as follows: DMSO-Bead beating, DMSO-crushing, LN-Bead beating, LN-crushing, PFA-decalcification. Major taxonomic classes are presented in the legend, for complete legend *see* Supplementary Figure C-6. There is a high variability across colonies in taxonomic composition and structure. Analysing each colony individually, the comparison among preservation and homogenization methods shows a consistent composition among them for *G. edwardsi* (top left) despite patterns in the structure (bottom left) are not. For *I. palifera*, patterns in taxonomic composition and structure differ among colonies, but colonies 3-5 show consistency in dominant classes among preservation and homogenization methods (top and bottom right).

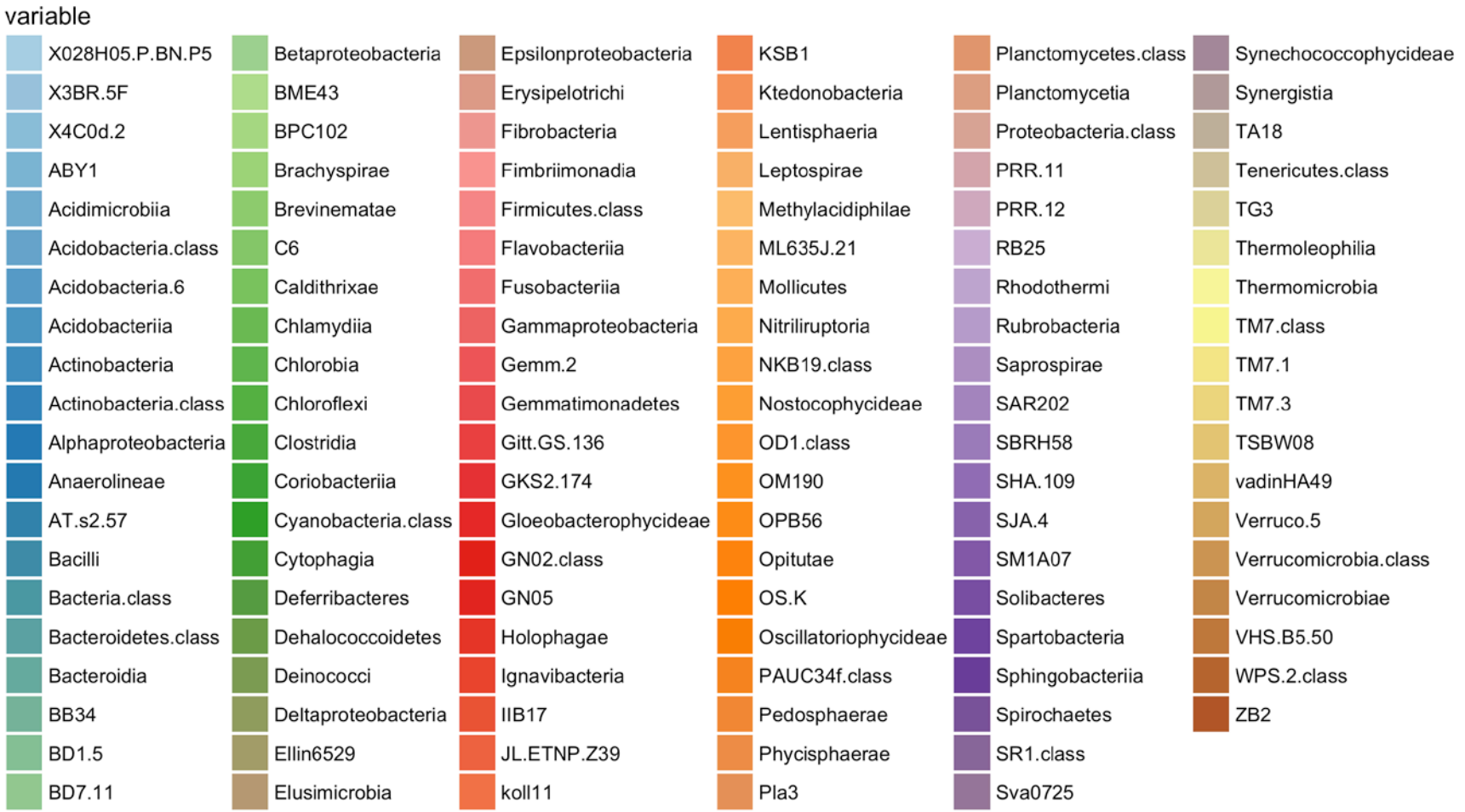


Figure C - 6: Legend of all taxonomic classes considered in Figure 3-6 and Supplementary Figure C-5.

Appendix D: Chapter 4 – Supplementary tables and figures

Table D - 1: Sampling design and collection dates

Region	Reef	Depth (m)	N. samples	Collection date
Great Barrier Reef	Great Detached	10	5	13-Dec-2012
		40	5	11-Dec-2012
	Tijou Reef	10	5	14,15-Dec-2012
		40	5	14-Dec-2012
	Yonge Reef	10	4	21-Oct, 20-Dec-2012
		40	5	19-Dec-2012
	Myrmidon Reef	10	5	28-Sep-2012
		40	5	26-Sep-2012
Coral Sea	Dutch Towers (Osprey 1)	10	5	24,25-Oct-2012
		20	4	24-Oct-2012
		40	4	25-Oct-2012
		60-80	5	26-Nov-2013
	Halfway Wall (Osprey 2)	10	4	26-Oct-2012
		20	5	27,28-Oct-2012
		40	5	26-Oct-2012
		60-80	5	24-Nov-2013
	Bigeye Ledge (Osprey 3)	10	5	30-Oct-2012
		20	5	30-Oct-2012
		40	5	29-Oct-2012
		60-80	4	25-Nov-2013
	Holmes Reef	10	5	19,20-Sep-2012
		40	5	19-Sep-2012
		60-80	5	19,20-Sep-2012
	Flinders Reef	10	5	23-Sep-2012
		40	4	23-Sep-2012
		60-80	4	23,24-Sep-2012

Table D - 2: Pairwise comparisons from permutational multivariate analysis of variance (PERMANOVA) using Bray-Curtis distances for the interaction Depth x Reef (Region), Presence/Absence data. P(perm): *P*-value based in permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value.

Depth (m)		10 vs. 20				10 vs. 40				10 vs. 60-80				20 vs. 40				20 vs. 60-80				40 vs. 60-80			
Region	Reef	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)
Great Barrier Reef	G. Detached	-				1.1842	**0.0426**	126	0.2098	-				-				-				-			
	Tijou Reef	-				1.0691	0.1303	126	0.3461	-				-				-				-			
	Yonge Reef	-				1.2503	**0.0189**	126	0.168	-				-				-				-			
	Myrmidon Reef	-				1.2249	0.0515	126	0.1761	-				-				-				-			
Coral Sea	Osprey 1	1.2504	0.0533	126	0.175	1.085	0.1955	126	0.3351	1.4823	**0.0078**	126	0.0455	1.0913	0.2852	35	0.322	0.96708	0.5403	126	0.4813	1.1361	0.1311	126	0.2709
	Osprey 2	1.1083	0.1547	126	0.2968	1.1551	0.0708	126	0.252	1.5653	0.0224	126	0.0416	1.0479	0.2684	126	0.3763	1.5731	**0.0076**	126	0.0301	1.1598	0.0723	126	0.2279
	Osprey 3	1.1499	0.0642	126	0.2405	1.2651	0.0284	126	0.1487	1.1875	0.0548	126	0.2047	1.0792	0.182	126	0.3242	1.214	0.0217	126	0.1895	1.2964	**0.016**	126	0.1375
	Holmes Reef	-				1.078	0.234	126	0.3348	1.2128	0.0801	126	0.1966	-				-				0.97571	0.51	126	0.473
	Flinders Reef	-				1.4327	**0.0169**	126	0.0703	1.4555	**0.0086**	126	0.0649	-				-				1.0413	0.3993	35	0.3814

Table D - 3: Pairwise comparisons from permutational multivariate analysis of variance (PERMANOVA) using Bray-Curtis distances for the factor Reef (Region) in the Region Great Barrier Reef, Presence/Absence data. P(perm): *P*-value based in permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value.

	Great Detached				Tijou Reef				Yonge Reef			
	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)
Tijou Reef	1.1034	0.1061	9858	0.2568	-				-			
Yonge Reef	1.3909	**0.0003**	9861	0.0294	1.1944	0.023	9847	0.1489	-			
Myrmidon Reef	1.4151	**0.0004**	9841	0.0211	1.1555	0.0502	9840	0.1871	1.4136	**0.0011**	9864	0.0294

Table D - 4: Pairwise comparisons from permutational multivariate analysis of variance (PERMANOVA) using Bray-Curtis distances for the factor Reef (Region) in the Region Coral Sea, Presence/Absence data. P(perm): *P*-value based in permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value.

	Osprey 1				Osprey 2				Osprey 3				Holmes Reef			
	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)
Osprey 2	1.3694	**0.0029**	9864	0.0209	-				-				-			
Osprey 3	1.896	**0.0001**	9856	0.0001	1.5906	**0.0001**	9872	0.0011	-				-			
Holmes Reef	1.4150	**0.0061**	9877	0.0187	1.4906	**0.0035**	9897	0.0098	1.9558	**0.0001**	9878	0.0002	-			
Flinders Reef	1.2923	0.0212	9860	0.0518	1.4789	**0.0004**	9867	0.0065	1.8461	**0.0001**	9863	0.0003	1.2965	0.0348	9877	0.0628

Table D - 5: Pairwise comparisons from permutational multivariate analysis of variance (PERMANOVA) using Bray-Curtis distances for the interaction Depth x Reef (Region), Abundance data. *P*-value based in permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value.

Depth (m)		10 vs. 20				10 vs. 40				10 vs. 60-80				20 vs. 40				20 vs. 60-80				40 vs. 60-80			
Region	Reef	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)
Great Barrier Reef	G. Detached	-				1.1293	0.0683	126	0.2709	-				-				-				-			
	Tijou Reef	-				1.0783	0.0963	126	0.3318	-				-				-				-			
	Yonge Reef	-				1.2365	0.0083	126	0.1761	-				-				-				-			
	Myrmidon Reef	-				1.1968	0.0764	126	0.1925	-				-				-				-			
Coral Sea	Osprey 1	1.2501	0.0646	126	0.1709	1.1185	0.1542	126	0.2846	1.4149	**0.0078**	126	0.071	1.1363	0.2274	35	0.282	0.96912	0.4682	126	0.4739	1.135	0.1362	126	0.2699
	Osprey 2	1.0969	0.175	126	0.3208	1.1325	0.0726	126	0.2743	1.4826	0.0239	126	0.0565	1.0415	0.2495	126	0.3845	1.524	**0.0095**	126	0.0405	1.14	0.0981	126	0.2542
	Osprey 3	1.137	0.0826	126	0.259	1.2781	**0.0178**	126	0.1328	1.1655	0.0492	126	0.2423	1.062	0.2343	126	0.3564	1.1652	0.0232	126	0.2337	1.2752	**0.0175**	126	0.1474
	Holmes Reef	-				1.0607	0.2807	126	0.3435	1.1841	0.1076	126	0.2149	-				-				0.98751	0.4678	126	0.4453
	Flinders Reef	-				1.4106	**0.0146**	126	0.0772	1.4368	**0.0089**	126	0.0662	-				-				1.0159	0.3428	35	0.425

Table D - 6: Pairwise comparisons from permutational multivariate analysis of variance (PERMANOVA) using Bray-Curtis distances for the factor Reef (Region) in the Region Great Barrier Reef, Abundance data. P(perm): *P*-value based in permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value.

	Great Detached				Tijou Reef				Yonge Reef			
	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)
Tijou Reef	1.1142	0.0771	9840	0.2346	-				-			
Yonge Reef	1.3864	**0.0004**	9850	0.0288	1.1888	0.0225	9849	0.1459	-			
Myrmidon Reef	1.3915	**0.0006**	9818	0.0239	1.1913	0.0266	9830	0.14	1.3664	**0.004**	9852	0.0414

Table D - 7: Pairwise comparisons from permutational multivariate analysis of variance (PERMANOVA) using Bray-Curtis distances for the factor Reef (Region) in the Region Coral Sea, Abundance data. P(perm): *P*-value based in permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*-value.

	Osprey 1				Osprey 2				Osprey 3				Holmes Reef			
	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)
Osprey 2	1.3449	**0.004**	9872	0.0245	-				-				-			
Osprey 3	1.72	**0.0001**	9846	0.0001	1.4614	**0.0003**	9857	0.0053	-				-			
Holmes Reef	1.4158	**0.0043**	9868	0.0161	1.4477	**0.0064**	9875	0.0145	1.8041	**0.0001**	9867	0.0004	-			
Flinders Reef	1.2836	0.0217	9892	0.061	1.4191	**0.0006**	9869	0.0126	1.6826	**0.0001**	9856	0.0003	1.2818	0.0344	9900	0.0742

Table D - 8: Taxonomic identification assigned to Operational Taxonomic Units (OTU) present in the 50% coral core microbiome. * OTU present in the 80% coral core microbiome. ** OTU present in the 80% coral core microbiome and define as highly persistent bacteria (reported in all the reefs and depths).

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Specie
142 **	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales			
157 **	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Gluconacetobacter*	
180	Bacteria	Bacteroidetes					
262	Bacteria	Proteobacteria	Gammaproteobacteria				
282	Bacteria						
289	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Weeksellaceae		
293	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Weeksellaceae		
304	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales			
306 **	Bacteria	Bacteroidetes					
409	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Nonlabens*	*sediminis*
414	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*veronii*
548	Bacteria	Proteobacteria	Alphaproteobacteria				
623	Bacteria						
692	Bacteria	Proteobacteria	Gammaproteobacteria				
727	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicimonaceae		
824	Bacteria						
916	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	*Mycobacterium*	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Specie
1087	Bacteria						
1159	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Weeksellaceae	*Cloacibacterium*	
1310	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Weeksellaceae	*Cloacibacterium*	
1323 **	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Alteromonas*	
1450	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	*fortis*
3128	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Alcanivoracaceae	*Alcanivorax*	
3174	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Alteromonas*	
3489	Bacteria	Bacteroidetes					
4919	Bacteria	Bacteroidetes	Cytophagia	Cytophagales			
6312	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	*epidermidis*
7956	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Alteromonas*	
11132	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Alteromonas*	
13855	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Ralstonia*	
14049	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Gluconacetobacter*	
14183	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Pseudoalteromonadaceae	*Pseudoalteromonas*	
14330 **	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	*Corynebacterium*	
14345	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Pseudoalteromonadaceae	*Pseudoalteromonas*	
14379 *	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Gluconacetobacter*	
15394	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales			

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Specie
15551	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	*Corynebacterium*	
15896	Bacteria	Proteobacteria	Alphaproteobacteria				
16005	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae		
16616	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales			
16983	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae		
16991	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Gluconacetobacter*	
17550	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Gluconacetobacter*	
17607	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Gluconacetobacter*	
17643	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Gluconacetobacter*	
17680	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Gluconacetobacter*	
17701	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*	*guillouiae*
18224	Bacteria	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	*Alloiococcus*	
18545	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	*Corynebacterium*	
18722	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Weeksellaceae		
18723	Bacteria	Bacteroidetes					
18991	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae		
19526	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales			
19568	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Gluconacetobacter*	
19690	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Amoebophilaceae		

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Specie
19786	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Alcanivoracaceae	*Alcanivorax*	
19790	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Pseudoalteromonadaceae	*Pseudoalteromonas*	
19806	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Gluconacetobacter*	
20017	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Gluconacetobacter*	
20801	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Gluconacetobacter*	
21048	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Gluconacetobacter*	
21122	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Amoebophilaceae	*SGUS912*	
21136	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	
22721	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	
25296 **	Bacteria	Proteobacteria	Deltaproteobacteria				
26567	Bacteria						
26725	Bacteria	Proteobacteria	Gammaproteobacteria				
30079	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*	*rhizosphaerae*
34075	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	*Propionibacterium*	*acnes*
38184	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae		
48791	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Acidovorax*	*delafieldii*
48792	Bacteria						
50396	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		
58340	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	*Corynebacterium*	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Specie
59777	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	*Mycobacterium*	
65268 **	Bacteria	Proteobacteria	Alphaproteobacteria				
82086	Bacteria	Cyanobacteria	Synechococcophycideae	Synechococcales	Synechococcaceae	*Synechococcus*	
84944 **	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae		
87311	Bacteria	Proteobacteria	Deltaproteobacteria				
87630	Bacteria	Proteobacteria	Deltaproteobacteria				
88352	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae		
89274	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Ruegeria*	
89625	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	*Propionibacterium*	*acnes*
92525	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales			
92538	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales			
95024	Bacteria	Proteobacteria	Alphaproteobacteria				
99751	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	*Mycobacterium*	
100174	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	*Mycobacterium*	
110333	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Ruegeria*	
111353	Bacteria	Proteobacteria	Deltaproteobacteria				
111355	Bacteria	Proteobacteria	Deltaproteobacteria				
111398	Bacteria	Proteobacteria	Deltaproteobacteria				
111436	Bacteria	Proteobacteria					

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Specie
111556	Bacteria						
112169	Bacteria	Proteobacteria	Deltaproteobacteria				
113899	Bacteria	Proteobacteria	Deltaproteobacteria				
114062	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingobium*	

Table D - 9: Association of bacteria part of the 50% coral core microbiome with coral bacteria reported in published literature. OTU: Operational Taxonomic Units (OTU), P. occurrence: Percentage of occurrence (%), Avg. rel. abundance: Average of relative abundance, Max. value abundance: Maximum value of abundance.

Symbiotic: reported in coral tissue, Endosymbiotic: present in coral endodermals cells, Holobiont: reported as part of the whole bacterial assemblage. HT: healthy tissue, DT: Diseased tissue, MHC: mucus from healthy coral colony, MDC: mucus from diseased coral colony, SCC: Sponge-covered tissue (competition), OB: Oocyte bundies, P: Planulae. *A. granulosa*: *Acropora granulosa*, *M. braziliensis*: *Mussismilia braziliensis*, *P. lutea*: *Porites lutea*, *P. compressa*: *Porites compressa*, *O. faveolata*: *Orbicella faveolata*, *D. strigosa*: *Diploria strigosa*, *P. meandrina*: *Pocillopora meandrina*, *A. pruinosa*: *Acropora pruinosa*, *O. franksi*: *Orbicella franksi*.

* Direct submission: JQ347405.1. Xu,C.Y., Huang,H. and Yang,J.H.

OTU	P. occurrence (%)	Avg. rel. abundance	Max. value abundance	Symbiotic	Endosymbiont	Holobiont
306	99.19	3.81	48.93			
25296	97.56	3.32	31.74			
1323	96.75	1.34	11.33			*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
14330	95.93	3.10	32.99		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	
65268	95.12	2.11	48.20			*M. braziliensis* (MDC)(Reis, Araujo et al. 2009)
142	93.50	1.62	21.08			
84944	91.87	0.97	56.08			*P. lutea* (HT)(Séré, Tortosa et al. 2013)
157	91.06	1.03	24.84			
14379	90.24	0.83	18.23			
21136	87.80	0.74	21.99	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
59777	86.99	0.75	16.39	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
50396	85.37	0.82	75.02			*P. lutea* (HT)(Séré, Tortosa et al. 2013)
304	82.11	0.17	2.11			
18723	82.11	0.17	1.99			
22721	82.11	0.41	7.33			
26725	82.11	0.71	18.57			
14049	81.30	0.45	6.00			
34075	81.30	0.45	5.92	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)

OTU	P. occurrence (%)	Avg. rel. abundance	Max. value abundance	Symbiotic	Endosymbiont	Holobiont
16991	80.49	0.40	6.43			
88352	80.49	0.37	22.85			*P. lutea* (HT)(Séré, Tortosa et al. 2013)
7956	78.86	0.13	2.01			*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
82086	78.86	0.33	16.79	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
293	78.05	1.57	18.06		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	
17643	78.05	0.04	0.39			
623	77.24	2.69	81.56			*P. compressa* (MHC)(Speck and Donachie 2012), *P. lutea* (SCC)(Tang, Hong et al. 2011)
13855	77.24	0.44	9.32	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
180	76.42	0.16	1.79			
1450	76.42	0.50	8.67			*A. granulosa* (HT)(Ainsworth, Krause et al. 2015), *P. lutea* (DT)(Séré, Tortosa et al. 2013)
18991	75.61	0.45	4.83	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
1159	74.80	1.60	17.31	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
11132	74.80	0.07	0.63			*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
15896	74.80	0.21	4.89			*M. braziliensis* (MDC)(Reis, Araujo et al. 2009)
6312	73.98	0.43	14.59	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
87630	73.98	0.08	0.77			
16983	73.17	0.49	11.05			*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
30079	73.17	0.44	13.12	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
727	72.36	0.41	7.32			*A. granulosa* (HT)(Ainsworth, Krause et al. 2015), *P. lutea* (HT)(Séré, Tortosa et al. 2013)
18545	71.54	0.14	1.28		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	
916	70.73	0.08	1.47	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)

OTU	P. occurrence (%)	Avg. rel. abundance	Max. value abundance	Symbiotic	Endosymbiont	Holobiont
38184	69.92	0.42	5.51			
262	68.29	0.91	23.61			
113899	68.29	0.05	0.54			
692	67.48	0.78	14.85			
14183	67.48	0.30	7.52		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
58340	67.48	0.05	0.84		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	
87311	67.48	0.08	1.67			
114062	67.48	0.28	4.80	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
26567	66.67	0.62	17.35			
548	65.85	0.51	15.15			*A. granulosa* (HT)(Ainsworth, Krause et al. 2015), *O. faveolata* (DT)(Sunagawa, DeSantis et al. 2009); *D. strigosa* (HT)(Sunagawa, Woodley et al. 2010)
3128	65.04	0.29	29.02			
3489	65.04	0.88	38.84			
20017	64.23	0.02	0.20			
89625	64.23	0.08	2.11	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
48791	63.41	0.25	4.33			
110333	63.41	0.45	36.10			*A. granulosa* (HT)(Ainsworth, Krause et al. 2015), *P. lutea* (DT)(Séré, Tortosa et al. 2013), *P. meandrina* (P)(Apprill, Marlow et al. 2009)
17680	61.79	0.03	0.41			
99751	61.79	0.02	0.32	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
111436	61.79	0.05	0.62			
14345	60.98	0.21	4.69		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015), *P. lutea* (HT)(Séré, Tortosa et al. 2013)
19690	60.98	0.51	22.59			*O. faveolata* (HT)(Sunagawa, DeSantis et al. 2009)
414	60.16	0.07	2.29	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
18224	60.16	0.22	3.48			
1087	59.35	0.27	10.59			

OTU	P. occurrence (%)	Avg. rel. abundance	Max. value abundance	Symbiotic	Endosymbiont	Holobiont
19568	59.35	0.01	0.19			
15394	57.72	0.07	0.96			
17701	57.72	0.23	4.08			
95024	57.72	0.02	0.25			*M. braziliensis* (MDC)(Reis, Araujo et al. 2009)
19790	56.91	0.11	4.66		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
100174	56.91	0.02	0.19	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
111355	56.91	0.04	0.75			
824	56.10	0.13	3.69			*P. compressa* (MHC)(Speck and Donachie 2012), *P. lutea* (SCC)(Tang, Hong et al. 2011)
16005	56.10	0.33	37.62		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	
18722	56.10	0.32	4.84		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	
19786	56.10	0.25	11.43			
92538	56.10	0.02	0.59			
3174	55.28	0.16	2.13			*A. granulosa* (HT)(Ainsworth, Krause et al. 2015), *P. lutea* (DT)(Séré, Tortosa et al. 2013)
15551	55.28	0.18	1.88		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	
19526	55.28	0.02	0.73			
89274	55.28	0.22	6.60			*A. granulosa* (HT)(Ainsworth, Krause et al. 2015), *A. pruinosa**
92525	55.28	0.04	0.55			
111353	55.28	0.03	0.33			
289	54.47	0.12	2.20		*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	
111398	54.47	0.02	0.33			
16616	53.66	0.31	20.09			
282	52.85	0.12	2.40			
1310	52.85	0.10	0.96	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)	*A. granulosa* (HT)(Ainsworth, Krause et al. 2015), *P. meandrina* (OB)(Apprill, Marlow et al. 2009)
17550	52.85	0.02	0.53			

OTU	P. occurrence (%)	Avg. rel. abundance	Max. value abundance	Symbiotic	Endosymbiont	Holobiont
20801	52.85	0.01	0.16			
17607	52.03	0.02	0.46			
21122	52.03	0.20	5.11			*A. granulosa* (HT)(Ainsworth, Krause et al. 2015), *O. franksi* (HT)(Sunagawa, Woodley et al. 2010)
112169	52.03	0.02	0.42			
409	51.22	0.39	24.57			*A. granulosa* (HT)(Ainsworth, Krause et al. 2015)
4919	51.22	0.49	50.51			
21048	51.22	0.01	0.14			
48792	51.22	0.16	4.29			*P. compressa* (MHC)(Speck and Donachie 2012), *P. lutea* (SCC)(Tang, Hong et al. 2011)
111556	51.22	0.02	0.26			
19806	50.41	0.02	0.23			

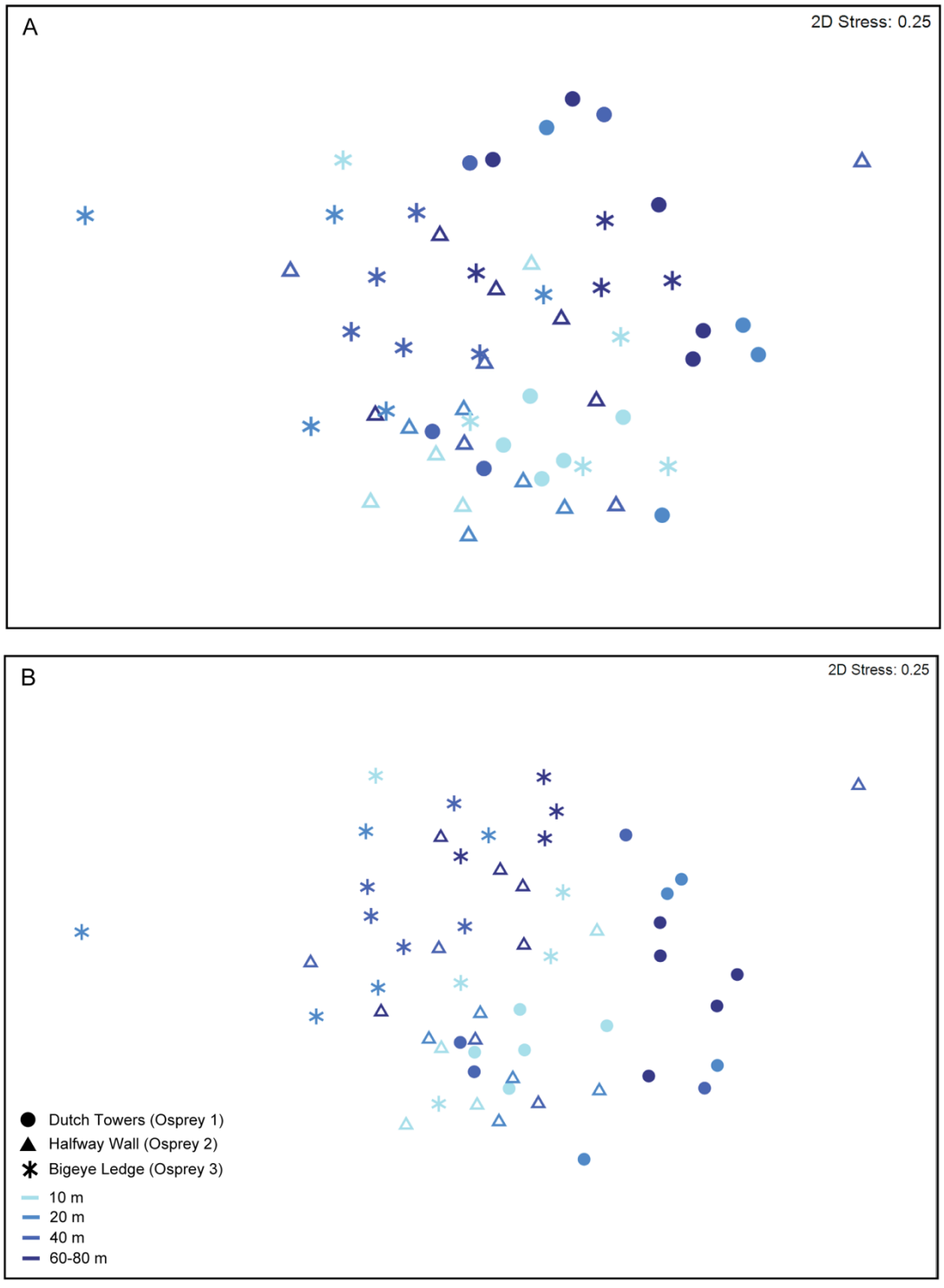


Figure D - 1: Non-metric MDS based on structure (A) and composition (B) data to illustrate no clustering by sampling date. NMDS based on Bray-Curtis (A) and Sorensen (B) dissimilarity data.

Appendix E: Chapter 5 – Supplementary tables and figures.

Table E - 1: Sampling design and collection dates.

M. elephantotus (*n*=95)

Region	Reef	Depth (m)	N. samples	Collection date
Great Barrier Reef	Great Detached	10	5	11,12-Dec-2012
		40	4	12,13-Dec-2012
	Tijou Reef	10	4	15,16-Dec-2012
		40	5	14,15-Dec-2012
	Yonge Reef	10	5	20,21-Dec-2012
		40	5	19,20-Dec-2012
	Myrmidon Reef	40	4	26,27-Sep-2012
Coral Sea	Dutch Towers (Osprey 1)	10	5	23-Oct-2012
		40	4	25-Oct-2012
		60-80	5	25-Oct-2012, 26-Nov-2013
	Halfway Wall (Osprey 2)	10	5	26,27-Oct-2012
		20	5	27,28-Oct-2012
		40	4	26,28-Oct-2012
		60-80	5	24-Nov-2013
	Bigeye Ledge (Osprey 3)	10	5	29,30-Oct-2012
		20	5	31-Oct-2012
		40	5	30-Oct-2012
		60-80	5	25-Nov-2013
	Flinders Reef	10	5	24-Sep-2012
		40	5	24-Sep-2012

A. aculeus (*n*=91)

Region	Reef	Depth (m)	N. samples	Collection date
Great Barrier Reef	Great Detached	10	5	12,13-Dec-2012
		40	4	12,13-Dec-2012
	Day Reef	10	5	21-Oct-2012, 18-Dec-2012
	Yonge Reef	10	5	19,20,21-Dec-2012
		40	4	20,21-Dec-2012
	Myrmidon Reef	10	8	26,27-Sep-2012
		40	8	26,27-Sep-2012
Coral Sea	Dutch Towers (Osprey 1)	20	4	25-Oct-2012
		40	7	23,24,25-Oct-2012
	Halfway Wall (Osprey 2)	10	6	26,28-Oct-2012
		40	6	26,28-Oct-2012
	Bigeye Ledge (Osprey 3)	10	5	30,31-Oct-2012
		20	6	31-Oct-2012
		40	5	30,31-Oct-2012
	Holmes Reef	10	3	19,20-Dec-2012
	Flinders Reef	10	5	23,24-Sep-2012
		40	5	22,23,24-Sep-2012

Table E - 2: Raw data depuration and division between the three coral species. *Excluding* column reflect groups eliminated in the data of the previous row. First row represents raw data after the elimination of ambiguous base calls, homopolymer, barcodes and primers.

Coral species	Excluding	N. samples	N. sequences (total)	N. sequences (av. by samples)	N. OTUs
3 coral species	-	311	17,210,426	55,338.99	79,724
	Chloroplast and None	311	10,449,737	33,600.441	70,932
	Low count samples	309	10,376,444	33,580.725	70,932
	Unassigned	309	6,425,024	20,792.958	49,044
P. speciosa	-	123	2,606,830	21,193.74	25,124
M. elephantotus	-	95	1,274,202	13,412.653	19,964
A. aculeus	-	91	2,543,992	27,955.956	19,875

Table E - 3: Bacterial phylotypes comprising the core microbiome. Rel. abundance: Relative abundance, Per. Occurrence: percentage of occurrence, Taxonomical ID: lower taxonomical level of identification. NROTU: New Reference OTU, NCROTU: New Clean up Reference OTU

P. speciosa

OTU	**Rel. Abundance**	**Per. Occurrence**	**Taxonomical ID.**
580295	1.59	99.19	Genus *Gluconacetobacter*
439036	5.11	98.37	Genus *Corynebacterium*
806717	1.59	97.56	Family Alteromonadaceae
NROTU1421	2.01	96.75	Order Campylobacterales
NROTU839	1.97	96.75	Genus *Rhodobacter*
4331183	0.93	95.12	Genus *Gluconacetobacter*
NROTU2242	2.32	93.50	Family Hyphomicrobiaceae
555495	0.57	87.80	Genus *Mycobacterium*
396109	3.00	86.18	Genus *Cloacibacterium*
NROTU491	0.90	86.18	Family Rhodobacteraceae
613414	0.59	86.18	*Acinetobacter rhizosphaerae*
1088265	0.44	86.18	*Propionibacterium acnes*
NROTU352	2.49	84.55	Family Weeksellaceae
543864	0.71	84.55	Genus *Pseudomonas*
170405	0.44	84.55	Genus *Pseudomonas*
309489	0.72	83.74	Genus *Pseudoalteromonas*
NROTU1103	1.37	82.93	Order Campylobacterales

M. elephantotus

OTU	Rel. Abundance	Per. Occurrence	Taxonomical ID.
NROTU1987	16.85	100.00	Order EC94
NROTU377	11.83	98.95	Order Kiloniellales
NROTU681	9.03	98.95	Family Ellin6075
251481	1.02	97.89	Family Rhodobacteraceae
806717	0.78	97.89	Family Alteromonadaceae
NROTU839	3.44	95.79	Genus *Rhodobacter*
NROTU1950	2.26	95.79	Order Kiloniellales
NROTU703	1.16	95.79	Class Alphaproteobacteria
549595	1.22	86.32	Family Nitrospiraceae
NROTU1261	3.19	83.16	Order EC94
NROTU2624	1.47	81.05	Order Kiloniellales

A. aculeus

OTU	Rel. Abundance	Per. Occurrence	Taxonomical ID.
806717	3.83	100.00	Family Alteromonadaceae
4435279	3.31	100.00	Family Flavobacteriaceae
NROTU998	4.82	98.90	Family Endozoicimonaceae
221108	1.74	98.90	Family Endozoicimonaceae
4314827	1.37	97.80	Family Flavobacteriaceae
562126	1.21	97.80	Family Rhodobacteraceae
543999	0.38	97.80	Family Alteromonadaceae
309489	0.69	96.70	Genus *Pseudoalteromonas*
318171	0.19	96.70	Family Alteromonadaceae
NROTU235	1.65	95.60	Family Flavobacteriaceae
2999126	0.71	95.60	Family Rhodobacteraceae

OTU	Rel. Abundance	Per. Occurrence	Taxonomical ID.
557211	0.46	95.60	Genus Synechococcus
3991527	2.89	93.41	Genus Alicyclobacillus
355538	0.68	92.31	Genus Prochlorococcus
NROTU1152	0.86	91.21	Family Endozoicimonaceae
NCROTU817811	0.69	91.21	Family Endozoicimonaceae
1106960	0.60	91.21	Family Alteromonadaceae
1088265	0.31	90.11	*Propionibacterium acnes*
4302976	0.47	87.91	Family Flavobacteriaceae
250136	1.30	86.81	Family Rhodobacteraceae
276493	0.36	86.81	Family Pseudoalteromonadaceae
NROTU2323	0.45	85.71	Family Endozoicimonaceae
550168	0.15	85.71	Genus Synechococcus
NROTU1528	0.84	84.62	Family Endozoicimonaceae
91492	0.37	84.62	Genus Alteromonas
NROTU1465	0.95	83.52	Family Endozoicimonaceae
169836	0.43	83.52	Vibrio fortis
634455	0.24	83.52	Genus Synechococcus
145419	1.20	82.42	Family Flavobacteriaceae
NROTU1322	0.57	82.42	Family Rhodobacteraceae
543864	0.22	82.42	Genus *Pseudomonas*
NROTU2362	0.34	81.32	Family Endozoicimonaceae
1784974	0.30	81.32	Genus Oleibacter
251481	0.40	80.22	Family Rhodobacteraceae

Table E - 4: Pairwise comparisons from permutational multivariate analysis of variance (PERMANOVA) for the factor coral species. P/A data represents composition, analysed based on Sorensen and Unweighted Unifrac; whereas Rel. Abundance stands for relative abundance, evaluated with Bray-Curtis and Weighted Unifrac. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value.

Data	Distance	Groups	t	P(perm)	Unique perms	P(MC)
P/A	Sorensen	*P.spe, M.ele*	2.5165	**0.0028**	9940	0.0001
		P.spe, A.acu	2.5515	**0.0009**	9944	0.0001
		M.ele, A.acu	2.2811	**0.0035**	9936	0.0001
	Unweighted Unifrac	*P.spe, M.ele*	2.9161	**0.0018**	9937	0.0001
		P.spe, A.acu	2.6282	**0.0001**	9945	0.0001
		M.ele, A.acu	2.505	**0.0026**	9955	0.0001
Rel. Abundance	Bray-Curtis	*P.spe, M.ele*	2.4119	**0.0026**	9961	0.0001
		P.spe, A.acu	2.4559	**0.0007**	9937	0.0001
		M.ele, A.acu	2.2091	**0.0049**	9948	0.0001
Abundance	Weighted Unifrac	*P.spe, M.ele*	2.1465	**0.0114**	9947	0.0001
		P.spe, A.acu	2.2804	**0.0054**	9966	0.0001
		M.ele, A.acu	1.6938	**0.0413**	9950	0.003

Table E - 5: Permutational multivariate analysis of variance (PERMANOVA) for the compositional data (Presence/Absence) based on Sorensen distance for the three coral species. Note the similarity between the three coral species in the percentage of estimated components of variation. Test performed using 9,999 permutations. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation

P. speciosa

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Region	1	15066	15066	3.0665	**0.0238**	8378	0.0001	15.24
Depth	3	12077	4025.7	1.0495	0.3783	9825	0.3059	2.64
Reef(Region)	7	38552	5507.4	1.5915	**0.0001**	9381	0.0001	12.15
RegionxDepth**	1	4081.5	4081.5	1.0609	0.4107	9903	0.3317	3.29
Reef(Region)xDepth**	13	49906	3838.9	1.1093	**0.0006**	9125	0.0118	8.83
Residual	97	3.36E+05	3460.5					57.85
Total	122	4.56E+05						

M. elephantotus

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Region	1	11814	11814	2.605	**0.0061**	8720	0.0001	14.74
Depth	3	14548	4849.5	1.1565	0.2307	9855	0.1359	5.70
Reef(Region)	6	29905	4984.2	1.4357	**0.0001**	9569	0.0002	11.27
RegionxDepth**	1	3891.3	3891.3	0.93495	0.5425	9925	0.5809	0.00
Reef(Region)xDepth**	8	33430	4178.7	1.2037	**0.0012**	9522	0.0084	11.70
Residual	75	2.60E+05	3471.6					56.59
Total	94	3.56E+05						

A. aculeus

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Region	1	12103	12103	2.5908	**0.0084**	9637	0.0001	14.48
Depth	2	7930.6	3965.3	1.0642	0.4174	9917	0.3278	3.17
Reef(Region)	7	35513	5073.3	1.5974	**0.0001**	9485	0.0001	13.69
RegionxDepth**	1	4414	4414	1.1869	0.3482	9938	0.1881	6.17
Reef(Region)xDepth**	5	18638	3727.6	1.1737	**0.0054**	9525	0.0351	9.55
Residual	74	2.35E+05	3176					52.94
Total	90	3.18E+05						

Table E - 6: Permutational multivariate analysis of variance (PERMANOVA) for the compositional data (Presence/Absence) based on Unweighted Unifrac distance on data for the three coral species. Note the similarity between the three coral species in the percentage of estimated components of variation. Test performed using 9,999 permutations. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation

P. speciosa

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Region	1	0.95417	0.95417	3.2842	**0.0134**	8411	0.0001	16.12
Depth	3	0.77272	0.25757	1.084	0.3085	9842	0.2431	3.54
Reef(Region)	7	2.2547	0.3221	1.5097	**0.0001**	9549	0.0001	11.59
RegionxDepth**	1	0.21717	0.21717	0.91295	0.5858	9914	0.6464	0.00
Reef(Region)xDepth**	13	3.0916	0.23782	1.1147	**0.0099**	9418	0.0336	9.29
Residual	97	20.696	0.21336					59.45
Total	122	28.025						

M. elephantotus

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Region	1	0.93476	0.93476	3.1297	**0.005**	8777	0.0002	16.86
Depth	3	0.92826	0.30942	1.149	0.2584	9891	0.1915	5.45
Reef(Region)	6	1.9586	0.32644	1.4015	**0.0003**	9704	0.0016	10.84
RegionxDepth**	1	0.2575	0.2575	0.96213	0.5028	9926	0.4961	0.00
Reef(Region)xDepth**	8	2.1478	0.26848	1.1527	**0.0318**	9594	0.0623	10.15
Residual	75	17.469	0.23292					56.70
Total	94	23.803						

A. aculeus

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Region	1	0.85227	0.85227	2.847	**0.0079**	9631	0.0001	15.28
Depth	2	0.5465	0.27325	1.3071	0.2032	9909	0.0539	6.35
Reef(Region)	7	2.3041	0.32916	1.7462	**0.0001**	9536	0.0001	14.42
RegionxDepth**	1	0.26683	0.26683	1.278	0.3012	9939	0.1129	6.89
Reef(Region)xDepth**	5	1.0456	0.20911	1.1093	**0.0434**	9570	0.1243	7.14
Residual	74	13.949	0.1885					49.90
Total	90	19.15						

Table E - 7: Permutational multivariate analysis of variance (PERMANOVA) for relative abundance based on Bray-Curtis distance for the three coral species. Similarity in the response of the three species is evident in the percentage of estimated components of variation. Test performed using 9,999 permutations. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation

P. speciosa

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Region	1	13404	13404	2.7065	**0.015**	8411	0.0001	13.79
Depth	3	12657	4218.9	1.0466	0.3771	9818	0.3072	2.60
Reef(Region)	7	38437	5491	1.51	**0.0001**	9371	0.0001	11.47
RegionxDepth**	1	4453.4	4453.4	1.101	0.3595	9904	0.2537	4.31
Reef(Region)xDepth**	13	52447	4034.4	1.1094	**0.0005**	9145	0.0123	8.99
Residual	97	3.53E+05	3636.5					58.83
Total	122	4.74E+05						

M. elephantotus

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Region	1	11477	11477	2.4973	**0.0097**	8712	0.0004	14.24
Depth	3	14882	4960.6	1.1271	0.2755	9861	0.1823	5.23
Reef(Region)	6	30186	5031	1.4114	**0.0001**	9605	0.0002	11.03
RegionxDepth**	1	3851.9	3851.9	0.88209	0.6041	9919	0.6933	0.00
Reef(Region)xDepth**	8	35090	4386.2	1.2305	**0.0004**	9487	0.0041	12.53
Residual	75	2.67E+05	3564.5					56.97
Total	94	3.64E+05						

A. aculeus

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Region	1	10627	10627	2.2309	**0.0095**	9604	0.0001	12.35
Depth	2	8190.6	4095.3	1.0589	0.4308	9915	0.3439	2.97
Reef(Region)	7	36097	5156.7	1.5621	**0.0001**	9452	0.0001	13.00
RegionxDepth**	1	4463.8	4463.8	1.1563	0.3701	9946	0.2195	5.52
Reef(Region)xDepth**	5	19346	3869.2	1.172	**0.0024**	9449	0.0322	9.30
Residual	74	2.44E+05	3301.3					51.83
Total	90	3.27E+05						

Table E - 8: Permutational multivariate analysis of variance (PERMANOVA) for the relative abundance based on Weighted Unifrac for the three coral species. Note the difference in the response in relation to the relative abundance with Bray-Curtis (previous table). Test performed using 9,999 permutations. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation

P. speciosa

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Region	1	0.63752	0.63752	3.9452	**0.0001**	8502	0.001	17.93
Depth	3	0.28221	0.094069	0.75011	0.8125	9906	0.8275	0.00
Reef(Region)	7	1.2561	0.17944	1.5209	**0.0063**	9843	0.0099	11.45
RegionxDepth**	1	0.15036	0.15036	1.1947	0.2927	9927	0.2709	5.79
Reef(Region)xDepth**	13	1.6311	0.12547	1.0635	**0.297**	9770	0.3038	6.76
Residual	97	11.444	0.11798					58.07
Total	122	15.542						

M. elephantotus

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Region	1	0.18042	0.18042	1.0497	0.2743	8739	0.3829	2.97
Depth	3	0.46222	0.15407	1.247	0.2151	9913	0.2072	7.24
Reef(Region)	6	1.1669	0.19448	1.6431	**0.0025**	9837	0.0032	14.90
RegionxDepth**	1	0.15205	0.15205	1.2352	0.2877	9945	0.2633	7.62
Reef(Region)xDepth**	8	0.98569	0.12321	1.041	0.3732	9812	0.3831	5.71
Residual	75	8.877	0.11836					61.56
Total	94	11.98						

A. aculeus

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Region	1	0.1533	0.1533	0.8003	0.5574	9632	0.6225	0.00
Depth	2	0.25473	0.12736	0.66143	0.6848	9947	0.8443	0.00
Reef(Region)	7	1.5262	0.21803	2.3429	**0.0001**	9847	0.0001	19.84
RegionxDepth**	1	0.21249	0.21249	1.1109	0.3638	9950	0.3435	6.08
Reef(Region)xDepth**	5	0.96424	0.19285	2.0723	**0.0002**	9825	0.0006	22.93
Residual	74	6.8864	0.09306					51.16
Total	90	10.205						

Table E - 9: Pairwise comparisons from permutational multivariate analysis of variance (PERMANOVA) for the interaction ReefxDepth: composition data per coral species based on Sorensen distances. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value.

P. speciosa

Depth (m)		10 vs. 20				10 vs. 40				10 vs. 60-80				20 vs. 40				20 vs. 60-80				40 vs. 60-80			
Region	Reef	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)
Great Barrier Reef	G. Detached	-				0.98302	0.6353	126	0.4775	-				-				-				-			
	Tijou Reef	-				1.054	0.0809	126	0.3608	-				-				-				-			
	Yonge Reef	-				1.017	0.2808	126	0.4196	-				-				-				-			
	Myrmidon Reef	-				1.0524	0.1316	126	0.3618	-				-				-				-			
Coral Sea	Dutch Towers	1.1078	0.1032	126	0.2978	1.1012	0.0501	126	0.3171	1.0931	0.0803	126	0.3104	1.1145	0.1406	35	0.2961	1.0004	0.3981	126	0.4434	1.0706	0.1728	126	0.3493
	Halfway Wall	1.0027	0.4597	126	0.4359	1.0585	0.0665	126	0.364	1.3243	**0.0079**	125	0.1076	1.0216	0.3022	126	0.4093	1.3433	**0.0089**	126	0.091	1.144	0.0251	126	0.2465
	Bigeye Ledge	0.98532	0.5432	126	0.4612	0.9756	0.637	126	0.4842	1.0319	0.19	126	0.4026	0.92741	0.9265	126	0.5612	1.0564	0.0988	126	0.3697	1.0739	0.032	126	0.3422
	Holmes Reef	-				0.96928	0.6103	126	0.4964	1.0285	0.2669	126	0.4027	-				-				1.004	0.3707	126	0.4404
	Flinders Reef	-				1.1706	0.0336	126	0.2359	1.157	**0.0163**	126	0.2387	-				-				0.96868	0.5152	35	0.4805

M. elephantotus

Depth (m)		10 vs. 20				10 vs. 40				10 vs. 60-80				20 vs. 40				20 vs. 60-80				40 vs. 60-80			
Region	Reef	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)
GBR	G. Detached	-				1.0951	0.0651	125	0.3079	-				-				-				-			
	Tijou Reef	-				1.0352	0.2041	126	0.4013	-				-				-				-			
	Yonge Reef	-				1.023	0.2954	126	0.4076	-				-				-				-			
Coral Sea	Dutch Towers	-				0.94	0.8011	126	0.5307	1.0856	0.0985	126	0.3219	-				-				1.112	0.1198	126	0.2942
	Halfway Wall	1.1194	**0.0076**	126	0.2785	0.96621	0.7915	126	0.4911	1.1783	**0.0075**	126	0.2108	1.0291	0.2736	126	0.3907	1.0758	0.1523	126	0.3282	1.0129	0.3489	126	0.4201
	Bigeye Ledge	1.2109	**0.0152**	126	0.1795	1.4719	0.0227	126	0.0547	1.4099	**0.0078**	126	0.066	1.1574	0.1463	126	0.2328	1.1415	0.1671	125	0.2549	1.258	0.0856	126	0.1511
	Flinders Reef	-				1.1053	0.1517	126	0.2928	-				-				-				-			

A. aculeus

Depth (m)		10 vs. 20				10 vs. 40				20 vs. 40			
Region	Reef	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)
Great Barrier Reef	G. Detached	-				0.98875	0.5373	126	0.4586	-			
	Yonge Reef	-				1.0552	0.1607	126	0.3649	-			
	Myrmidon Reef	-				1.5791	**0.0002**	5015	0.0069	-			
Coral Sea	Dutch Towers	-				-				0.932	0.8092	330	0.556
	Halfway Wall	-				0.95913	0.5475	461	0.5075	-			
	Bigeye Ledge	1.2265	**0.0103**	461	0.1591	1.1813	**0.0151**	126	0.2099	1.0214	0.2989	462	0.4003
	Flinders Reef	-				1.0518	0.184	126	0.369	-			

Table E - 10: Pairwise comparisons from permutational multivariate analysis of variance (PERMANOVA) for the interaction ReefxDepth: composition data per coral species based on Unweighted Unifrac distances. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value.

P. speciosa

Depth (m)		10 vs. 20				10 vs. 40				10 vs. 60-80				20 vs. 40				20 vs. 60-80				40 vs. 60-80			
Region	Reef	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)
Great Barrier Reef	G. Detached	-				0.9836	0.5401	126	0.4695	-				-				-				-			
	Tijou Reef	-				0.93241	0.9012	126	0.5576	-				-				-				-			
	Yonge Reef	-				1.024	0.3418	126	0.4057	-				-				-				-			
	Myrmidon Reef	-				0.96722	0.5981	126	0.4859	-				-				-				-			
Coral Sea	Dutch Towers	1.1064	0.1521	126	0.2985	1.1688	0.0488	126	0.2386	1.0632	0.1604	126	0.3564	1.2278	0.0285	35	0.193	0.99619	0.3706	126	0.4411	1.0439	0.3109	126	0.3777
	Halfway Wall	0.97016	0.6523	126	0.482	0.97206	0.7282	126	0.4816	1.2072	**0.0077**	126	0.1897	1.1688	0.0262	126	0.224	1.4065	**0.0081**	125	0.0602	1.0885	0.0743	126	0.328
	Bigeye Ledge	1.0248	0.3409	126	0.4015	0.96295	0.5661	126	0.496	0.99605	0.4835	126	0.4434	0.91289	0.8435	126	0.5809	1.2175	0.0632	125	0.1944	1.2109	0.0438	126	0.1948
	Holmes Reef	-				0.87529	0.9677	126	0.6334	1.0247	0.2958	126	0.4056	-				-				0.95571	0.6728	126	0.5063
	Flinders Reef	-				1.2043	0.0552	126	0.1958	1.208	0.0544	126	0.1958	-				-				0.98864	0.5122	35	0.4615

M. elephantotus

Depth (m)		10 vs. 20				10 vs. 40				10 vs. 60-80				20 vs. 40				20 vs. 60-80				40 vs. 60-80			
Region	Reef	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)
GBR	G. Detached	-				1.0237	0.36	126	0.4082	-				-				-				-			
	Tijou Reef	-				0.98852	0.5119	126	0.4629	-				-				-				-			
	Yonge Reef	-				1.0067	0.373	126	0.4301	-				-				-				-			
Coral Sea	Dutch Towers	-				0.94998	0.6096	126	0.5081	1.0576	0.2455	126	0.3608	-				-				1.1212	0.1621	126	0.2903
	Halfway Wall	1.093	0.1231	126	0.3164	0.92607	0.8446	126	0.5508	1.1101	0.1012	126	0.2944	1.0662	0.1988	126	0.356	1.1794	0.0709	126	0.2146	1.0037	0.39	126	0.4343
	Bigeye Ledge	1.2375	0.0373	126	0.161	1.614	0.0227	126	0.0293	1.2795	**0.0082**	126	0.1229	1.1178	0.1719	126	0.2782	0.95664	0.5927	126	0.4883	1.1937	0.1556	126	0.1988
	Flinders Reef	-				1.0497	0.2395	126	0.3653	-				-				-				-			

A. aculeus

Depth (m)		10 vs. 20				10 vs. 40				20 vs. 40			
Region	Reef	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)
Great Barrier Reef	G. Detached	-				0.98847	0.5019	126	0.4587	-			
	Yonge Reef	-				1.0543	0.2066	126	0.3543	-			
	Myrmidon Reef	-				1.4702	**0.0003**	5001	0.017	-			
Coral Sea	Dutch Towers	-				-				0.99057	0.5259	330	0.4561
	Halfway Wall	-				1.0286	0.3083	462	0.4025	-			
	Bigeye Ledge	1.2544	**0.016**	462	0.1306	1.2595	**0.0072**	126	0.1447	1.1727	**0.0186**	460	0.2141
	Flinders Reef	-				0.91964	0.8508	125	0.568	-			

Table E - 11: Pairwise comparisons from permutational multivariate analysis of variance (PERMANOVA) for the interaction ReefxDepth: relative abundance data per coral species based on Bray-Curtis distances. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value.

P. speciosa

Depth (m)		10 vs. 20				10 vs. 40				10 vs. 60-80				20 vs. 40				20 vs. 60-80				40 vs. 60-80			
Region	Reef	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)
Great Barrier Reef	G. Detached	-				0.98407	0.5485	126	0.4751	-				-				-				-			
	Tijou Reef	-				1.053	0.0712	126	0.3655	-				-				-				-			
	Yonge Reef	-				1.0222	0.2968	126	0.4208	-				-				-				-			
	Myrmidon Reef	-				1.0865	0.0531	126	0.3147	-				-				-				-			
Coral Sea	Dutch Towers	1.1328	0.0967	126	0.271	1.1327	**0.017**	126	1.1327	1.1258	0.0503	126	0.2663	1.162	0.0867	35	0.254	0.99945	0.3668	126	0.4361	1.1039	0.1058	126	0.3061
	Halfway Wall	1.027	0.2885	125	0.405	1.0514	0.1136	126	1.0514	1.2529	**0.0072**	126	0.1548	1.0266	0.2955	126	0.4035	1.2675	**0.0086**	126	0.1327	1.076	0.0964	126	0.3393
	Bigeye Ledge	1.0065	0.3469	126	0.4261	0.99589	0.4593	126	0.99589	1.0257	0.2093	126	0.4105	0.96043	0.8077	126	0.5108	1.0588	0.1117	126	0.3507	1.1003	0.039	126	0.3132
	Holmes Reef	-				0.94354	0.767	126	0.5275	1.0017	0.3955	126	0.4447	-				-							
	Flinders Reef	-				1.2013	0.0398	125	0.1988	1.1691	**0.0082**	126	0.237	-				-							

M. elephantotus

Depth (m)		10 vs. 20				10 vs. 40				10 vs. 60-80				20 vs. 40				20 vs. 60-80				40 vs. 60-80			
Region	Reef	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)
GBR	G. Detached	-				1.1035	0.0558	126	0.3138	-				-				-				-			
	Tijou Reef	-				1.0076	0.3457	126	0.4344	-				-				-				-			
	Yonge Reef	-				1.0113	0.3432	126	0.4308	-				-				-				-			
Coral Sea	Dutch Towers	-				0.94692	0.7816	126	0.5261	1.1029	0.1017	126	0.2931	-				-				1.1273	0.1096	126	0.2792
	Halfway Wall	1.2029	**0.0151**	126	0.1917	0.99758	0.5317	125	0.4495	1.2264	**0.0094**	125	0.1709	1.0431	0.2391	125	0.384	1.0821	0.1574	126	0.3139	1.0223	0.2545	126	0.4052
	Bigeye Ledge	1.2032	**0.0084**	126	0.1929	1.5006	0.0232	126	0.0469	1.4112	**0.0096**	126	0.0594	1.1491	0.1945	126	0.2458	1.114	0.2153	126	0.2916	1.2424	0.0727	126	0.1629
	Flinders Reef	-				1.1292	0.1068	126	0.2744	-				-				-				-			

A. aculeus

Depth (m)		10 vs. 20				10 vs. 40				20 vs. 40			
Region	Reef	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)
Great Barrier Reef	G. Detached	-				0.99842	0.4106	126	0.4498	-			
	Yonge Reef	-				1.0666	0.1479	126	0.355	-			
	Myrmidon Reef	-				1.5666	**0.0002**	5005	0.0064	-			
Coral Sea	Dutch Towers	-				-				0.94225	0.7329	330	0.5267
	Halfway Wall	-				0.96824	0.5372	462	0.4974	-			
	Bigeye Ledge	1.1915	**0.0216**	462	0.1928	1.1571	**0.017**	126	0.2392	1.0103	0.3677	462	0.4313
	Flinders Reef	-				1.0665	0.1598	126	0.3532	-			

Table E - 12: Pairwise comparisons from permutational multivariate analysis of variance (PERMANOVA) for the interaction ReefxDepth: relative abundance data per coral species based on Weighted Unifrac distances. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value

A. aculeus

Depth (m)		10 vs. 20				10 vs. 40				20 vs. 40			
Region	Reef	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)	t	P(perm)	U. perms	P(MC)
Great Barrier Reef	G. Detached	-				1.1967	0.0979	126	0.2204	-			
	Yonge Reef	-				0.89674	0.6532	126	0.5127	-			
	Myrmidon Reef	-				3.4194	**0.0001**	5089	0.0001	-			
Coral Sea	Dutch Towers	-				-				1.2715	0.1373	329	0.1626
	Halfway Wall	-				1.1843	0.1362	462	0.2289	-			
	Bigeye Ledge	0.79435	0.7383	462	0.6413	1.0401	0.3537	126	0.3639	0.77208	0.8822	462	0.72
	Flinders Reef	-				1.0015	0.4558	126	0.4178	-			

Table E - 13: Indexes Average (Δ+) and variation (Λ+) of taxonomic distinctness for core microbiome of the three species.

Coral species	N. of OTUs	Av. Tax. Distinctiness (Δ+)	Var. Tax. Distinctiness (Λ+)
P. speciosa	17	76.47	225.32
M. elephantotus	11	71.59	383.26
A. aculeus	34	71.86	528.51

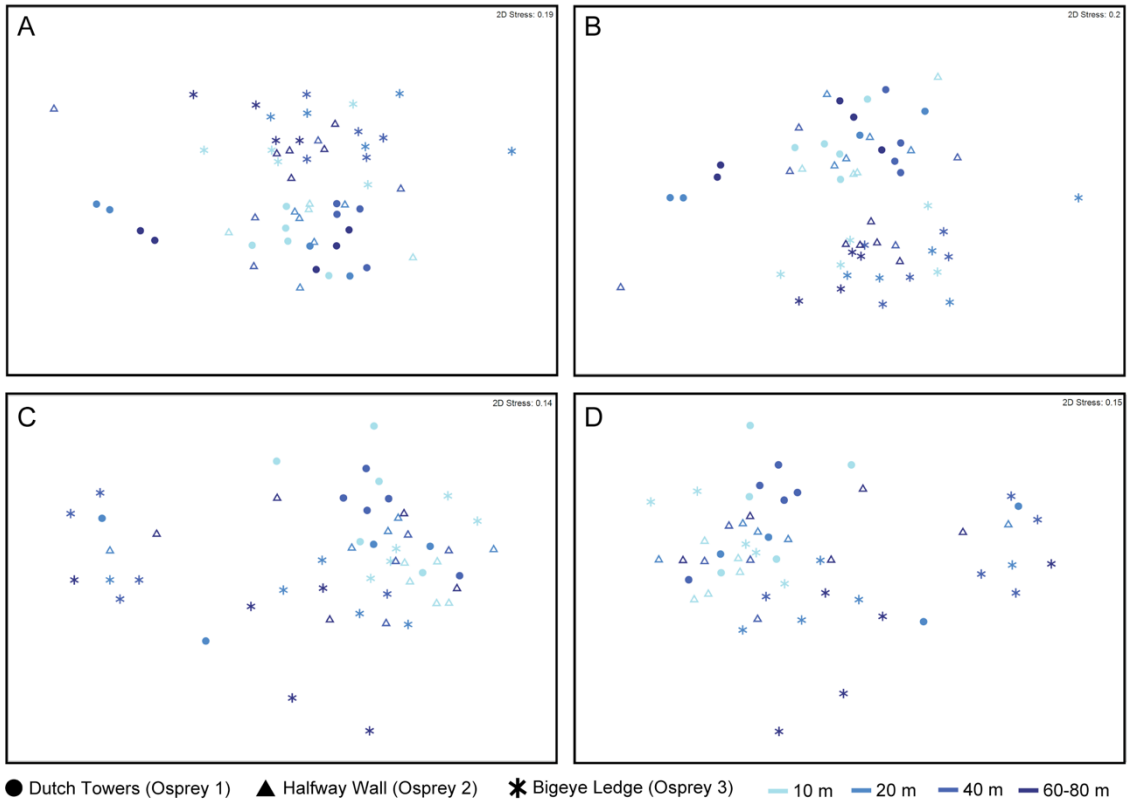


Figure E - 1: Non-metric MDS based on structure (A,C) and composition (B,D) data to illustrate no clustering by sampling date. A,B) *P. speciosa*, C,D) *M. elephatotus*. NMDS based on Bray-Curtis (A,C) and Sorensen (B,D) dissimilarity data.

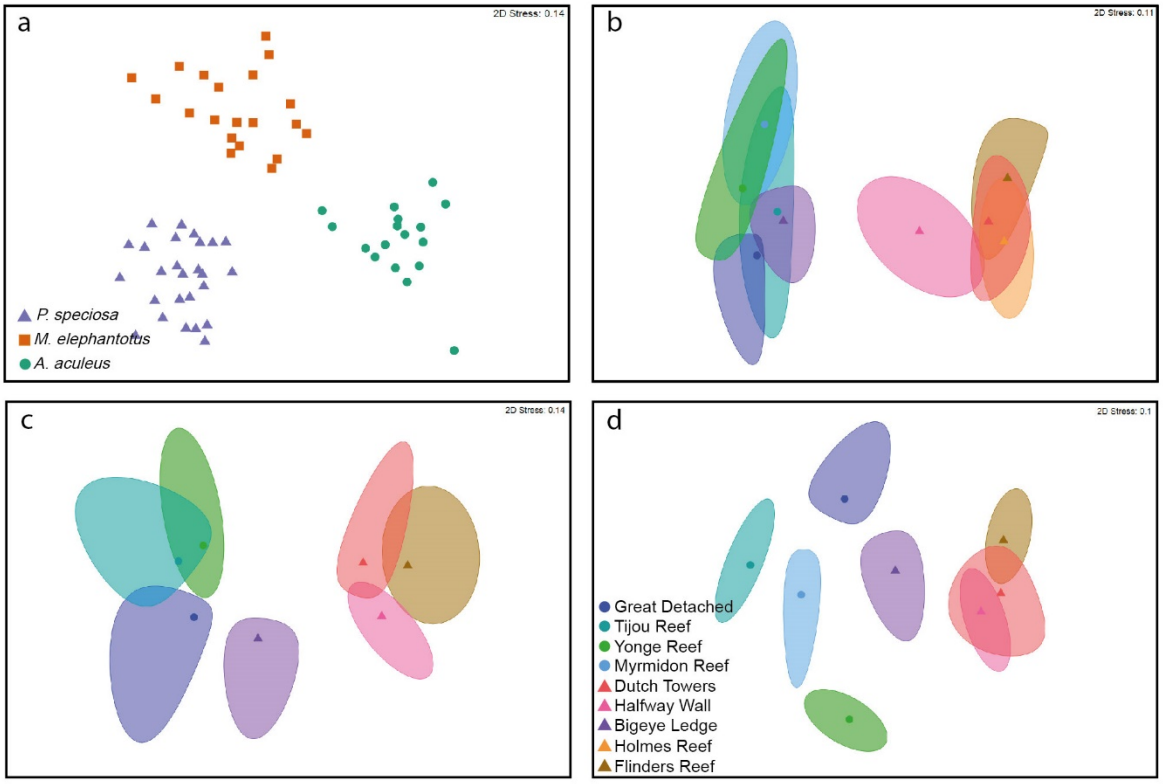


Figure E - 2: Non-metric MDS based on composition data to illustrate differences between coral species (a; PERMANOVA, $p<0.01$, Supplementary Table E-4) and between reefs for *P. speciosa* (b), *M. elephantotus* (c; excluding Myrmidon reef), and *A. aculeus* (d; excluding Holmes reef). NMDS based on Sorensen dissimilarity data. (a) Centroids, (b-d) bootstrap area and average for reefs. Circles denote GBR reefs, triangles CS reefs.

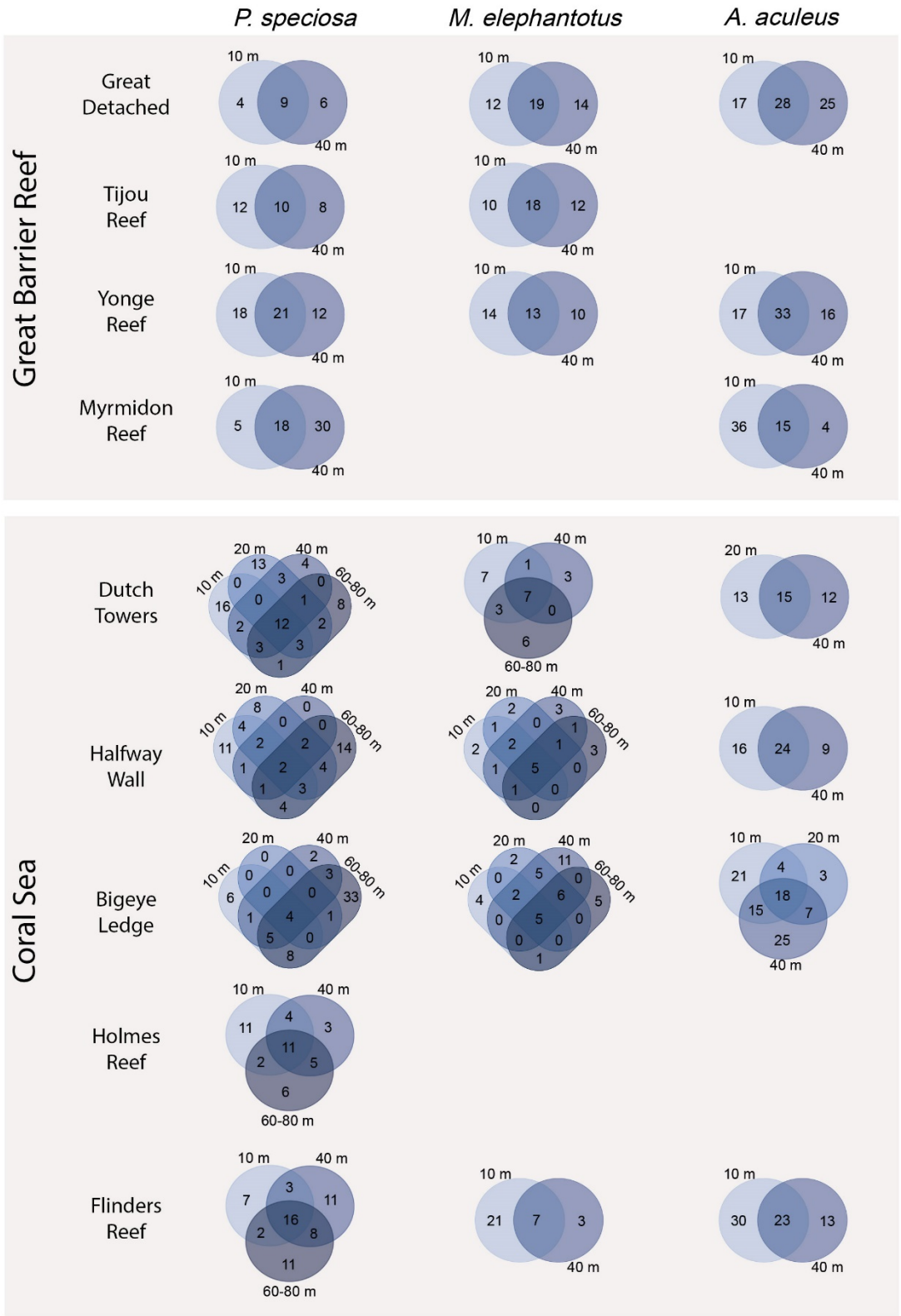


Figure E - 3: Core microbiome of the lowest level of the design (reef x depth) showed bacteria phylotypes in common between the depths (intersection of the Venn diagrams).

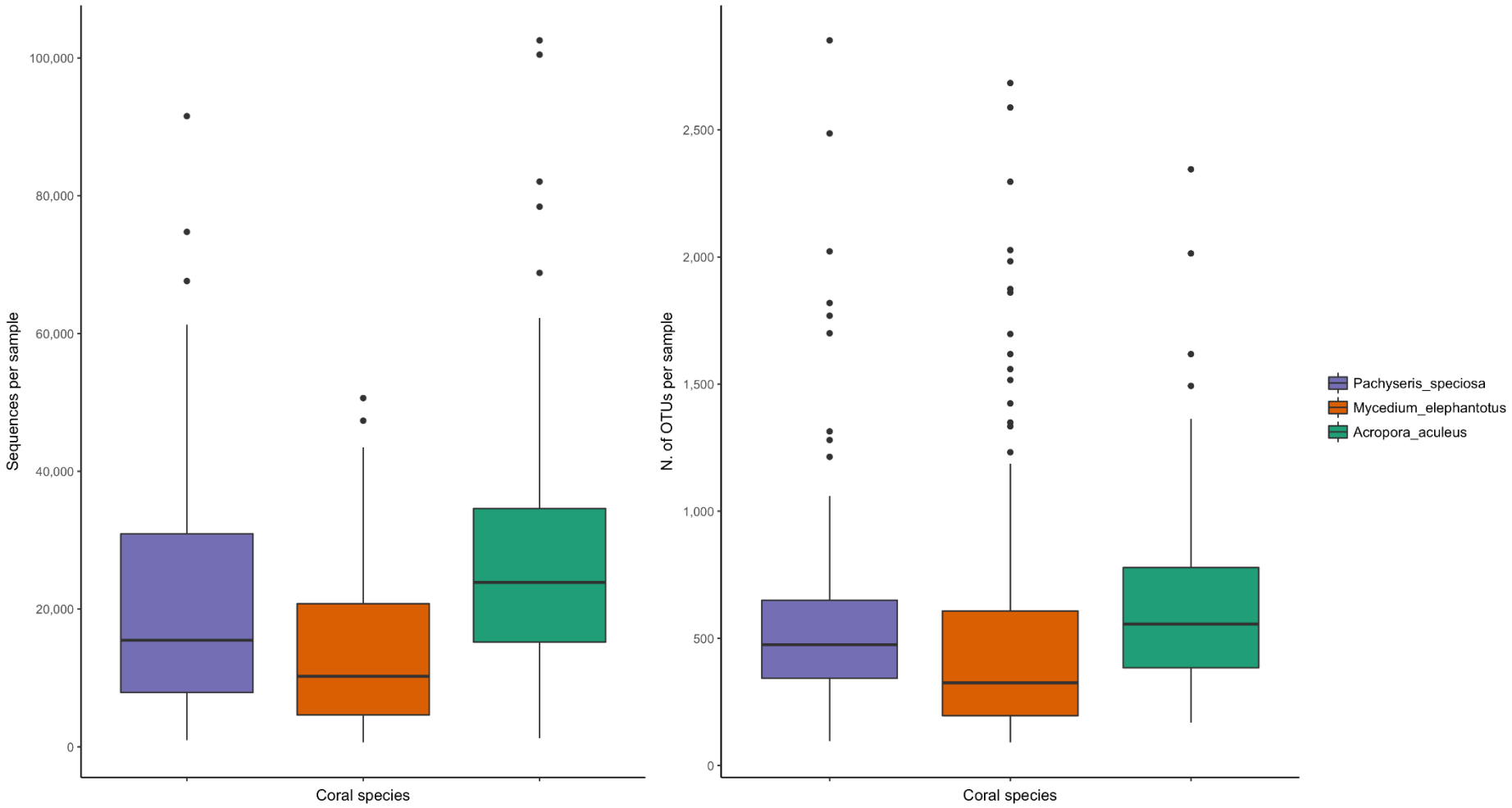


Figure E - 4: Distribution of sequences and number of OTUs per sample per species. Boxplots are constructed with raw data after the elimination of chloroplast, non-identified and unassigned sequences (6.4 millions of sequences, 49 thousands of OTUs, Supplementary Table E-2).

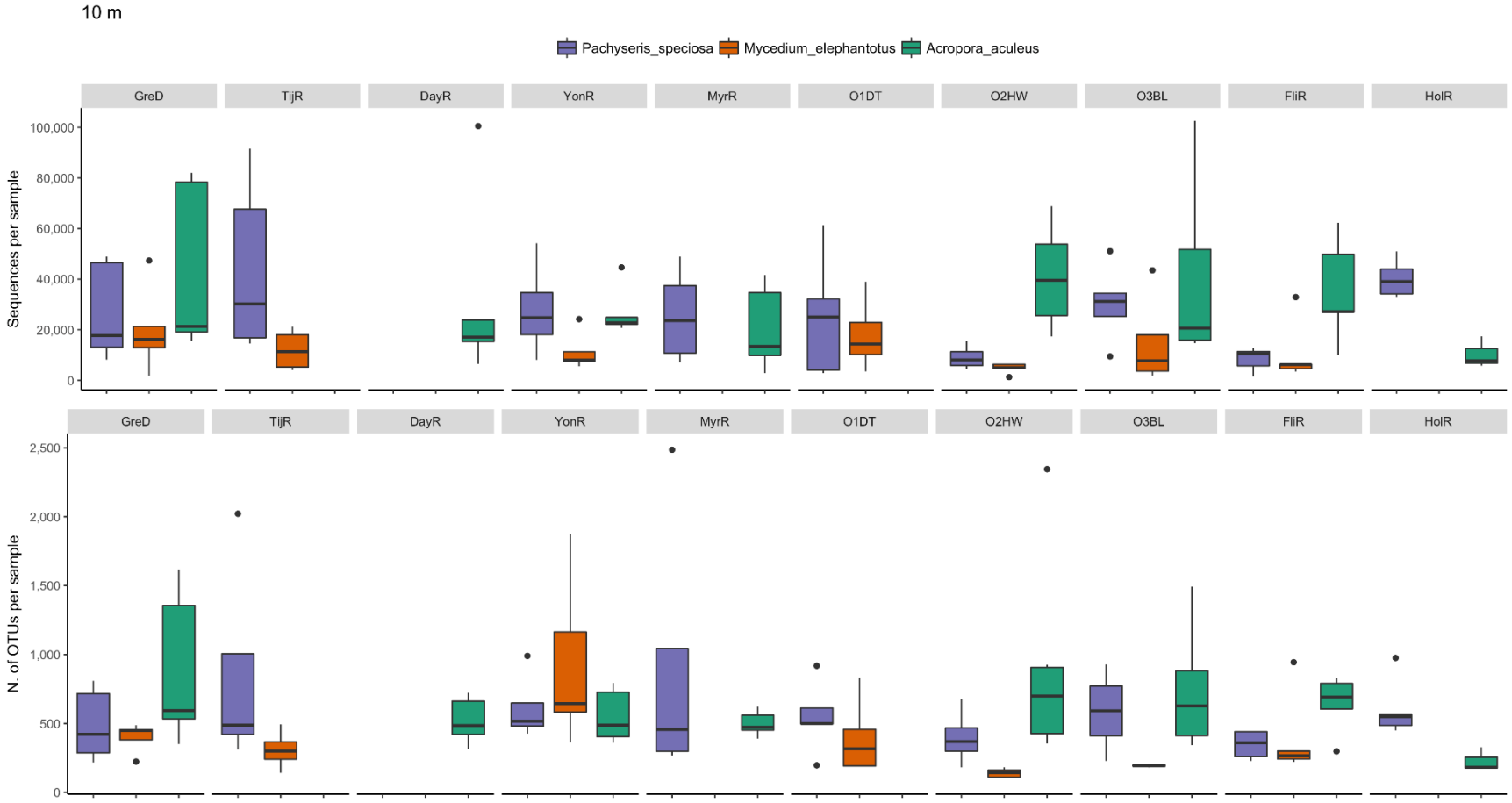


Figure E - 5: Distribution of sequences and number of OTUs per sample per reef at 10 m. Patterns in number of sequences and number of OTUs per sample are similar. Few exceptions are in *M. elephantotus* and *A. aculeus* at Yonge Reef, *A. aculeus* at Myrmidon Reef and *P. speciosa* at Dutch Towers. Note the difference in scale and median between this graph and the other depts. GreD: Great Detached, TijR: Tijou Reef, DayR: Day Reef, YonR: Yonge Reef, MyrR: Myrmidon Reef, O1DT: Dutch Towers (Osprey), O2HW: Halfway Wall (Osprey), O3BL: Bigeye Ledge (Osprey), FliR: Flinders Reef, HolR: Holmes Reef.

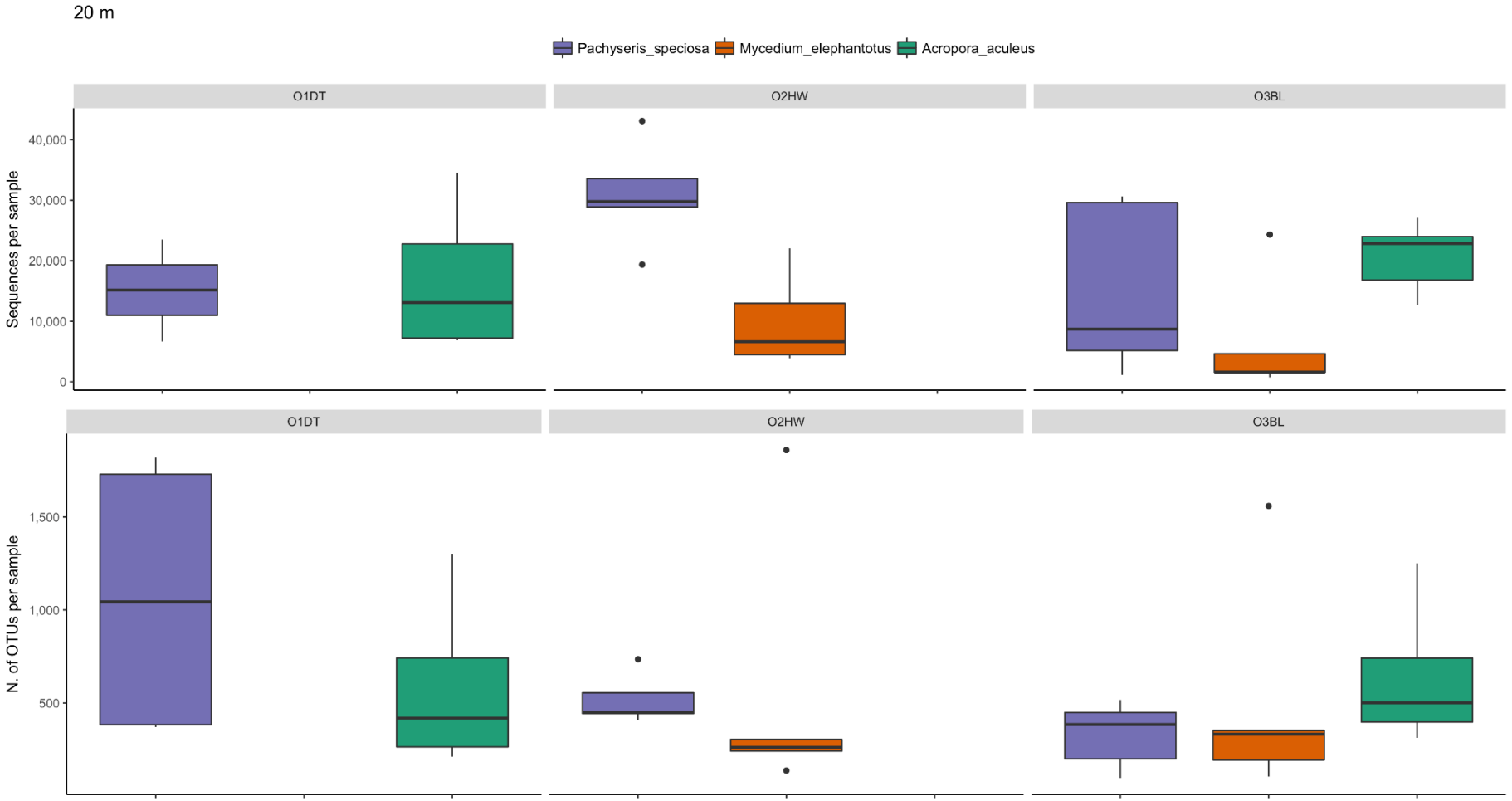


Figure E - 6: Distribution of sequences and number of OTUs per sample per reef at 20 m. As seen in previous figures, patterns observed in sequences number are reflective of the trend in number of OTUs. Exceptions are observed in variability of *P. speciosa* Dutch Towers and Bigeye Ledge. Note the difference in scale in regards to other depths. O1DT: Dutch Towers (Osprey), O2HW: Halfway Wall (Osprey), O3BL: Bigeye Ledge (Osprey).

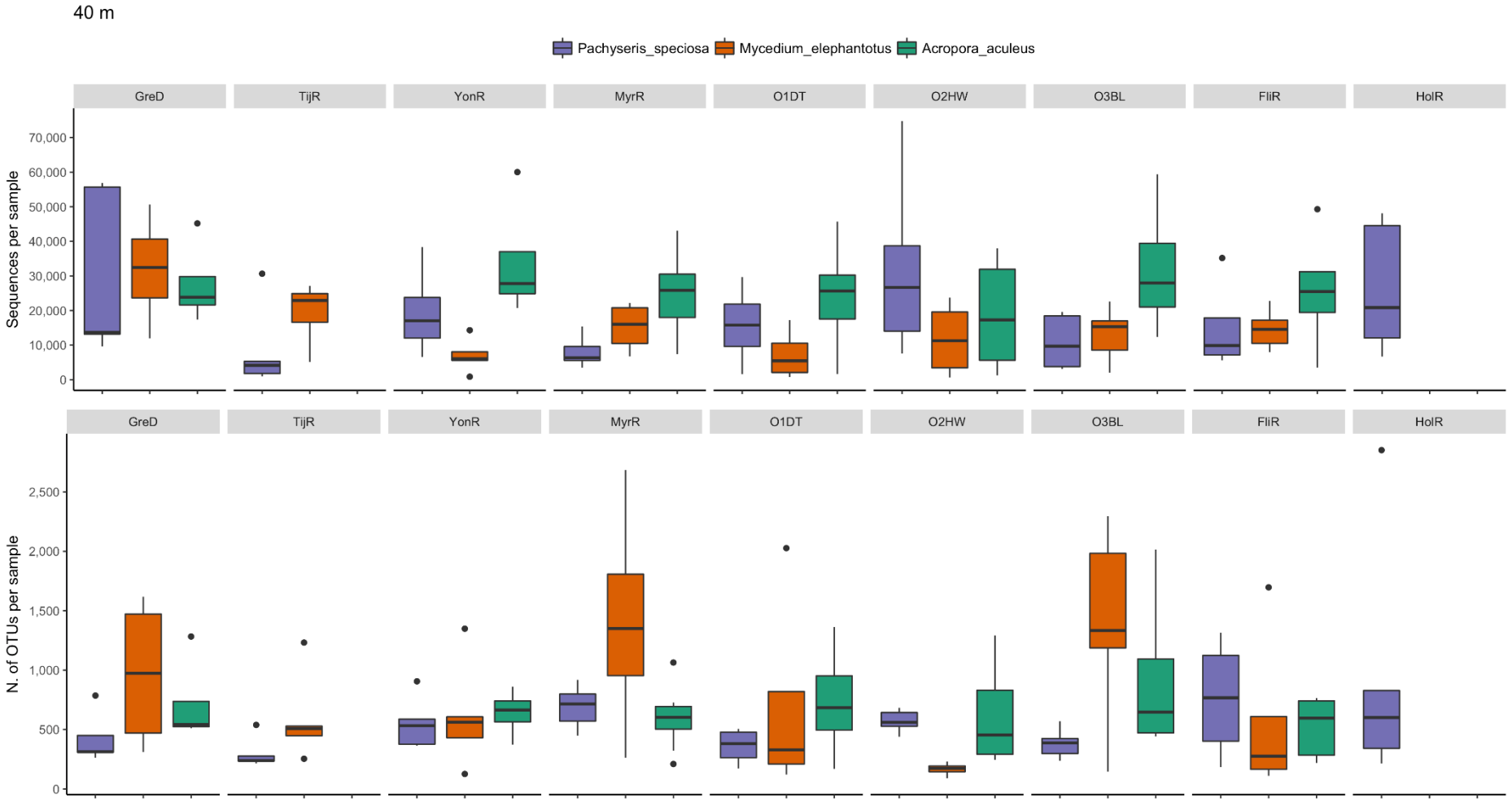


Figure E - 7: Distribution of sequences and number of OTUs per sample per reef at 40 m. Pattern in number of sequences per samples are also observed in number of OTUs per sample. *M. elephantotus* at Myrmidon Reef and Bigeye Ledge and *P. speciosa* Halfway Wall are exceptions to this observation. Note the difference in the scales among depths. GreD: Great Detached, TijR: Tijou Reef, YonR: Yonge Reef, MyrR: Myrmidon Reef, O1DT: Dutch Towers (Osprey), O2HW: Halfway Wall (Osprey), O3BL: Bigeye Ledge (Osprey), FliR: Flinders Reef, HolR: Holmes Reef.

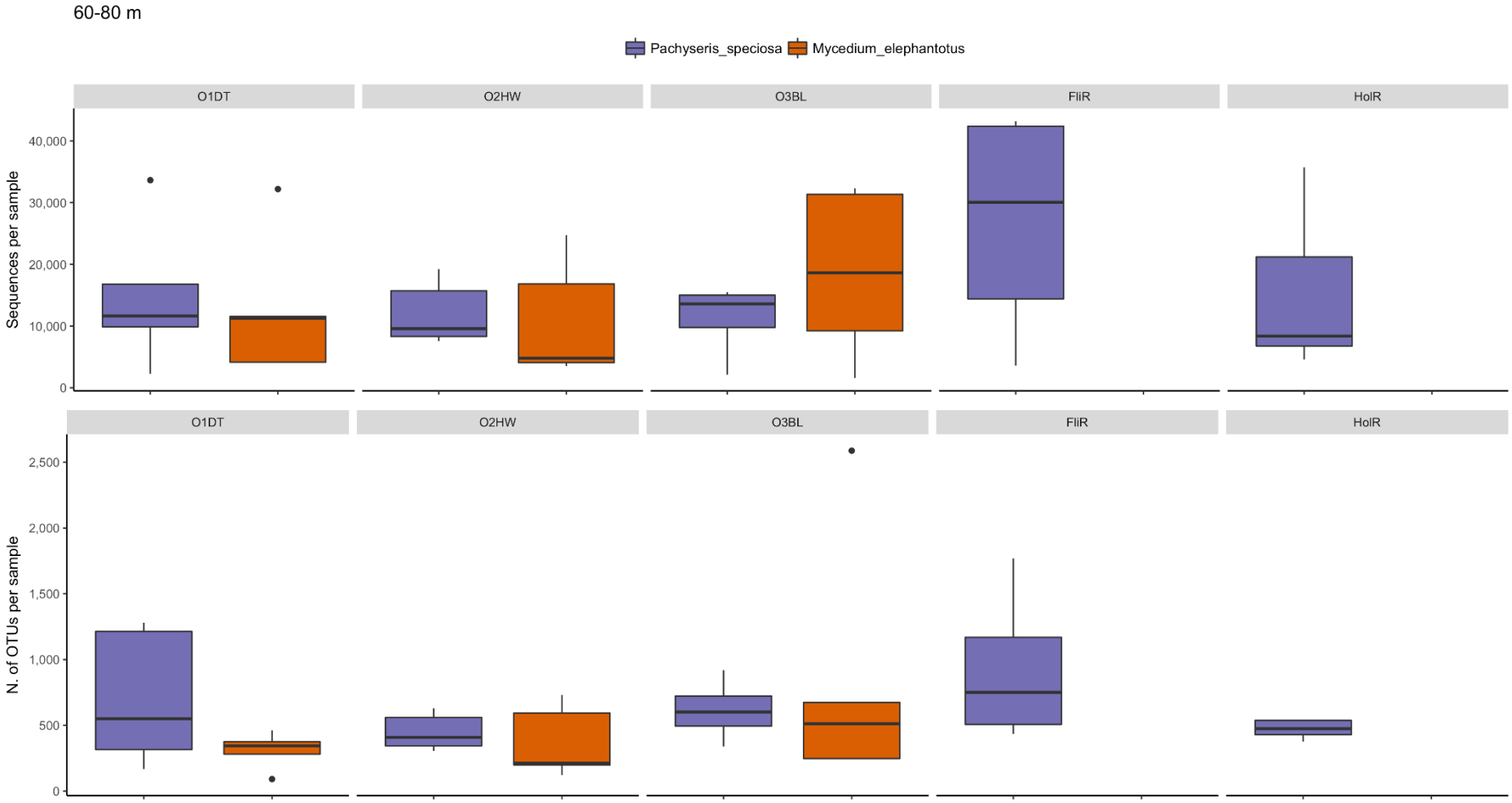


Figure E - 8: Distribution of sequences and number of OTUs per sample per reef at 60-80 m. Relatives differences observed in number of sequences were also presented in number of OTUs per sample. Note the difference in scale in regards to other depths (Supplementary Figure E-5-7). O1DT: Dutch Towers (Osprey), O2HW: Halfway Wall (Osprey), O3BL: Bigeye Ledge (Osprey), FliR: Flinders Reef, HolR: Holmes Reef.

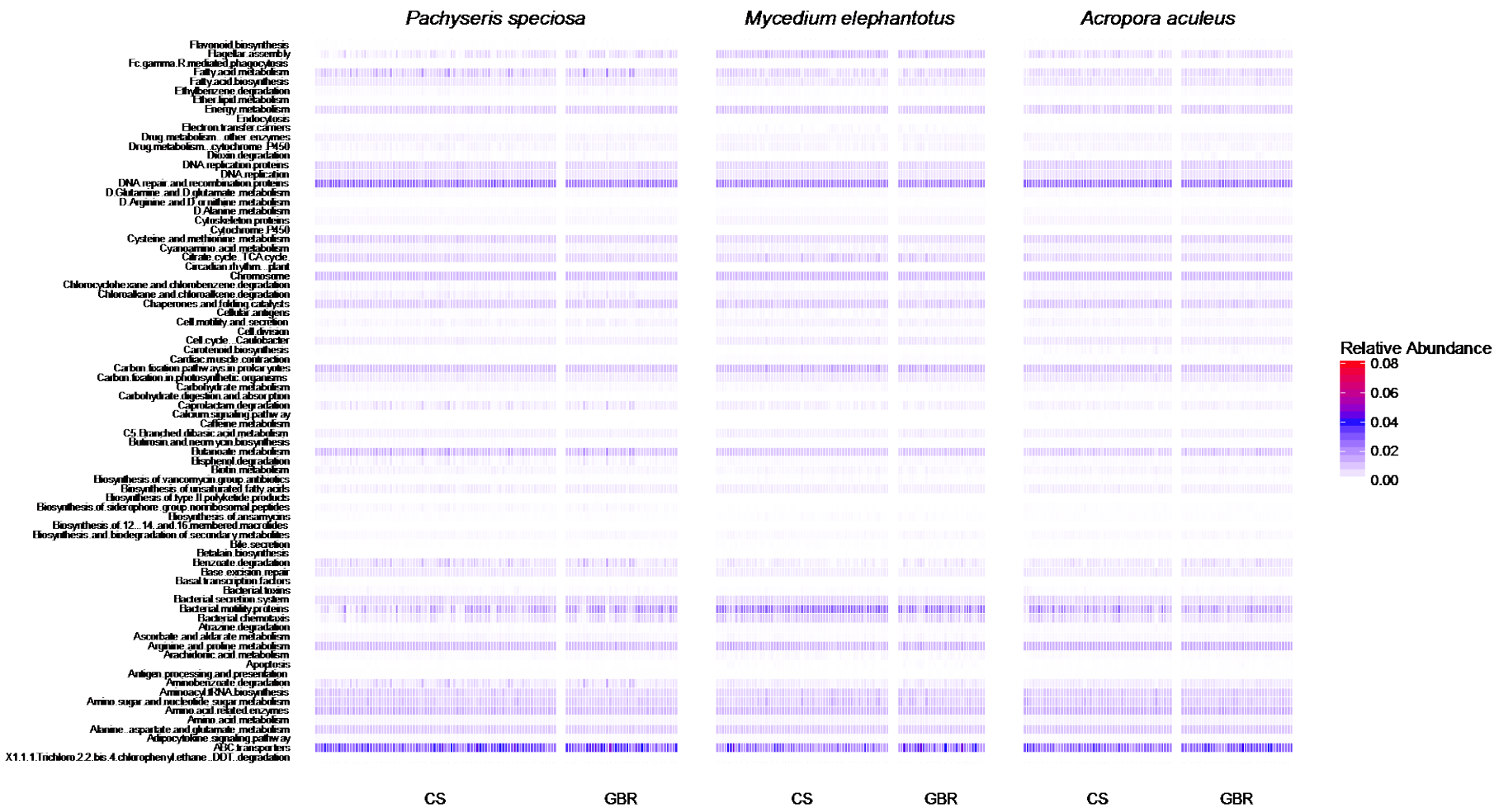


Figure E - 9: Functional prediction for content for core microbiome per species – set 1. Functional prediction content generated from relative abundance of KEGG KO genes, normalized, and standardized by sample by total.

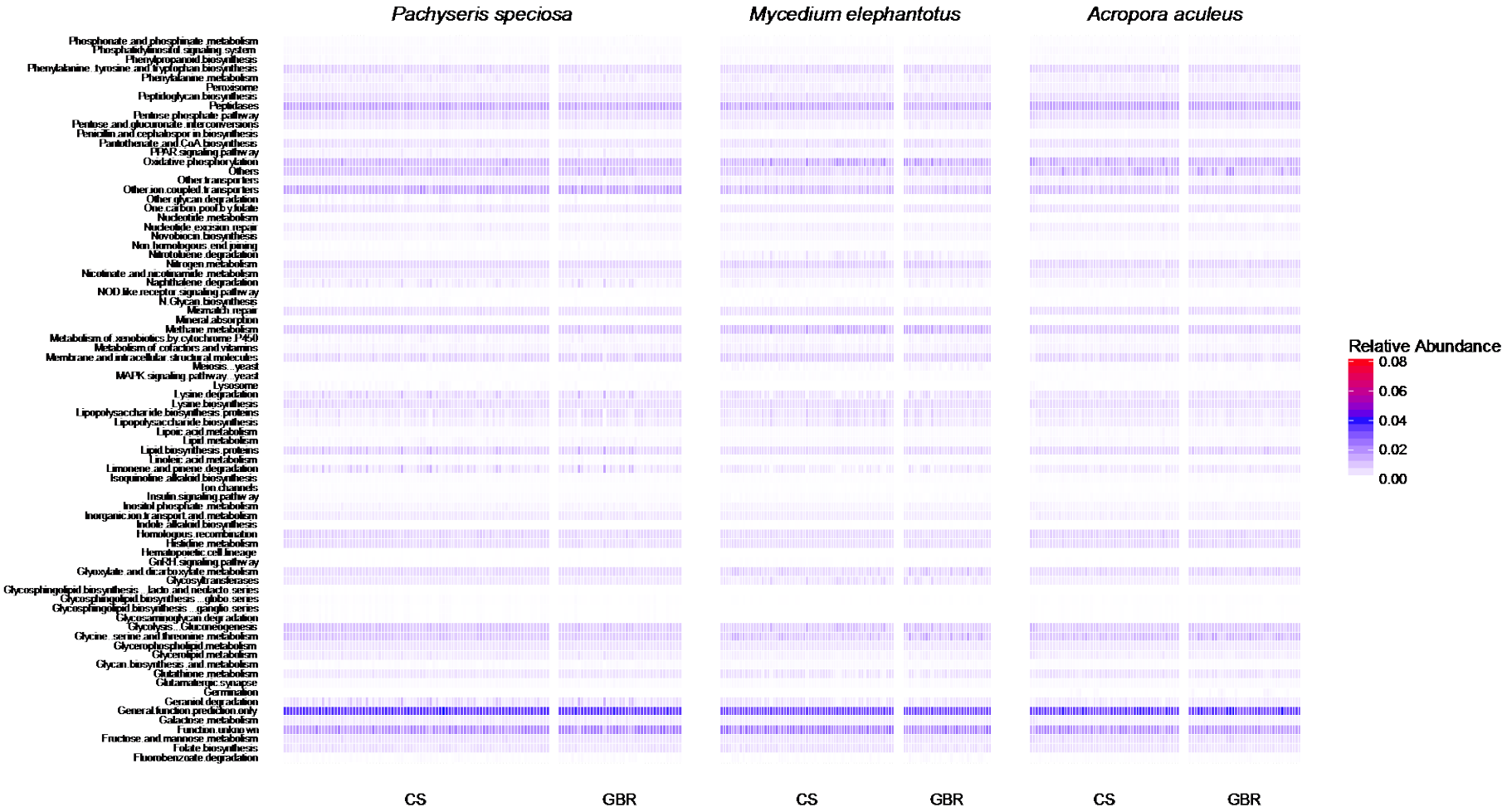


Figure E - 10: Functional prediction for content for core microbiome per species – set 2. Functional prediction content generated from relative abundance of KEGG KO genes, normalized, and standardized by sample by total.

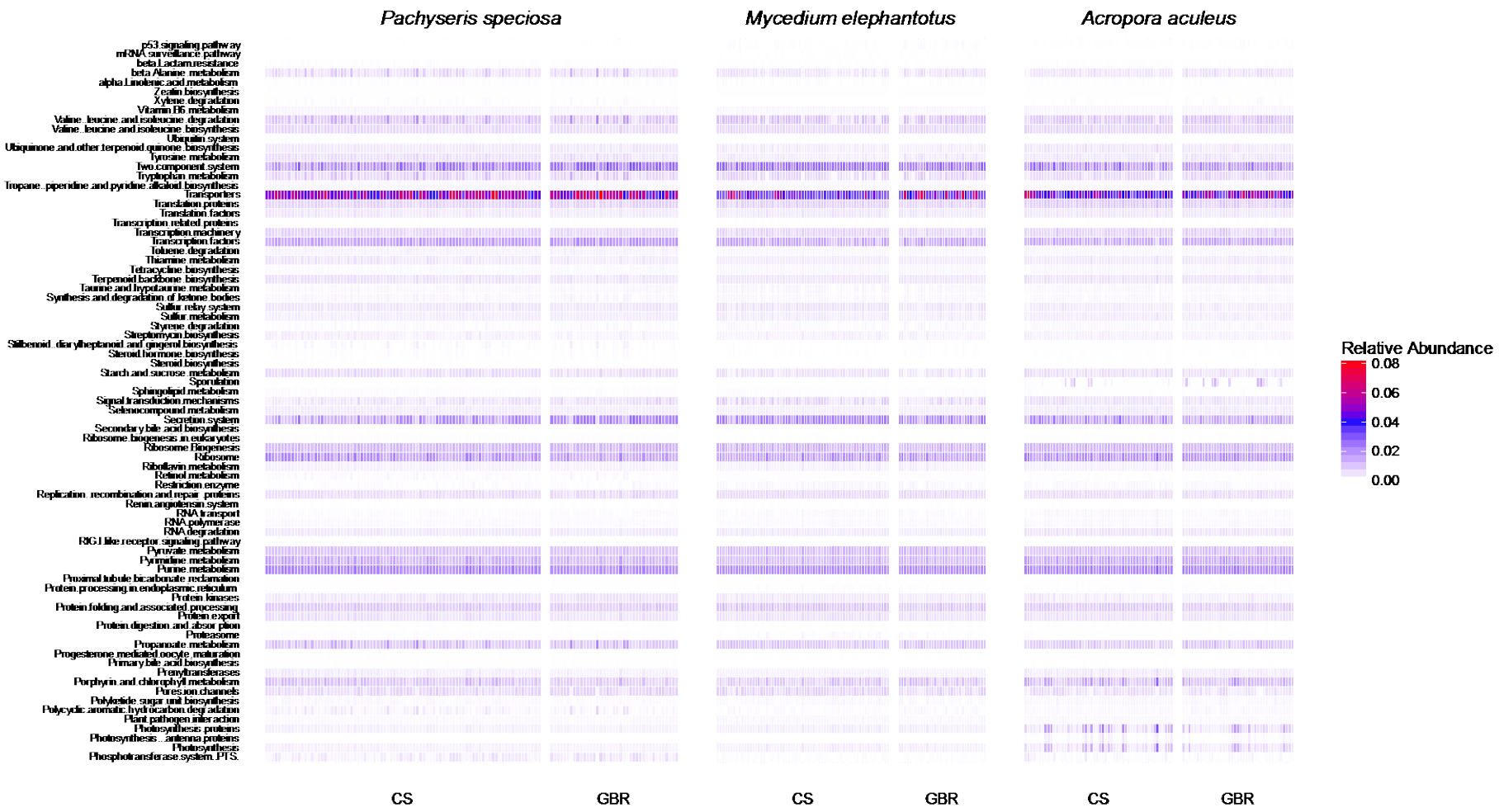


Figure E - 11: Functional prediction for content for core microbiome per species – set 3. Functional prediction content generated from relative abundance of KEGG KO genes, normalized, and standardized by sample by total.

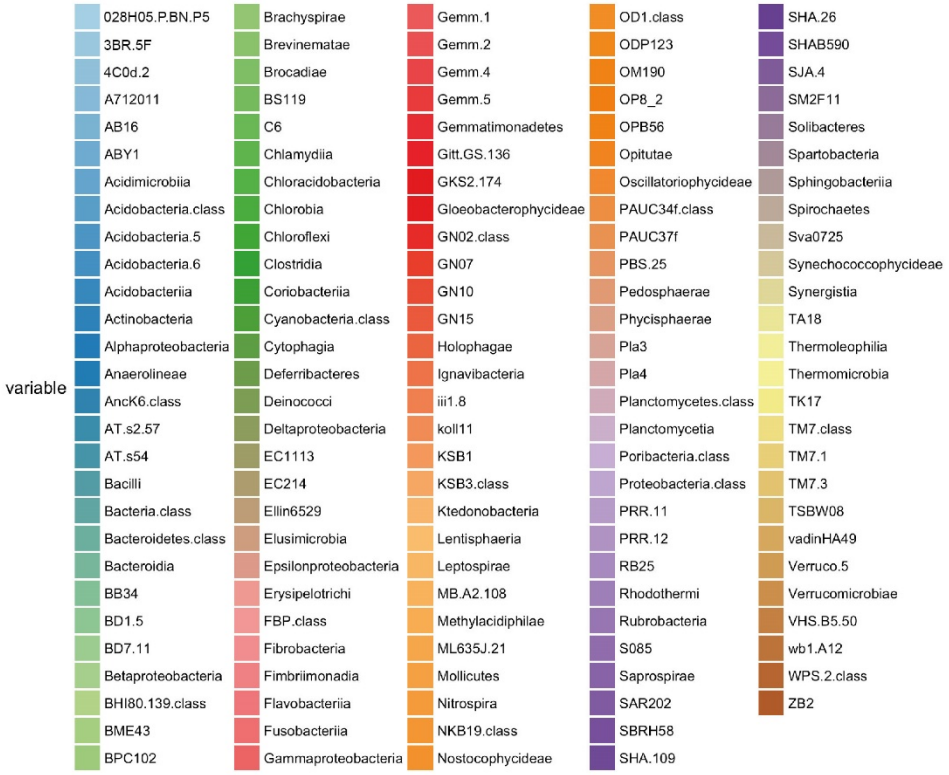


Figure E - 12: Legend of all taxonomic classes considered in Figure 5-3 and 5-5.

Appendix F: Chapter 6 - Supplementary tables and figures.

Table F- 1: Raw data depuration and division between the two microhabitats. *Excluding* column reflect groups eliminated in the data of the previous row. The first row represents raw data after the elimination of ambiguous base calls, homopolymer, barcodes and primers. Av: average per sample, s.d: standard deviation, Unassigned: not assigned to Bacteria kingdom.

Microhabitat	Excluding	N. samples	N. sequences (total)	N. sequences (Av. ± s. d.)	N. OTUs (total)	N. OTUs (Av. ± s. d.)
Both	-	20	631,915	31,595.8 ± 13,698.9	5,346	715.9 ± 204.6
	Chloroplast and None	20	616,197	30,809.9 ± 13,219.8	5,288	706.1 ± 200.9
	Unassigned	20	601,249	30,062.5 ± 13,330.2	4,607	629.5 ± 184.3
	Mitochondria (Order Rickettsiales)	20	533,701	26,685.1 ±13,466.6	4,284	585 ± 209.7
Coenosarc	-	10	277,609	27,760.9 ± 9,917.2	2,960	599.8 ± 154.9
Polyp	-	10	256,092	25,609.2 ± 16,790.6	3,088	570.1 ± 261.5

Table F- 2: Diversity indices per sample. Richness (d): Margalef's index, Diversity (H'): Shannon index, Evenness (J'): Pielou's evenness, Evenness (D): Simpson's index, Av. Tax. Distinctness (Δ^+): Average of taxonomic distinctness, Var. Tax. Distinctness (Λ^+): Variation of taxonomic distinctness.

Microhabitat	Individual	N. OTUs	N. sequences	Richness (d)	Diversity (H')	Evenness (J')	Evenness (D)	Av. Tax. Distinctness (Δ^+)	Var. Tax. Distinctness (Λ^+)
Coenosarc	25	369	17,442	37.68	3.51	0.59	0.95	79.75	151.25
	27	442	15,353	45.75	4.34	0.71	0.98	79.80	152.35
	31	852	40,202	80.27	4.43	0.66	0.98	79.86	155.12
	32	610	24,727	60.20	4.24	0.66	0.97	79.92	155.21
	131	630	46,951	58.47	3.59	0.56	0.95	79.46	161.94
	212	713	30,457	68.97	4.06	0.62	0.97	79.42	157.61
	213	735	30,563	71.07	4.19	0.63	0.97	79.93	149.96
	214	621	25,347	61.14	4.03	0.63	0.97	79.42	172.73
	221	628	27,248	61.39	4.00	0.62	0.96	79.75	149.10
	226	398	19,319	40.23	3.91	0.65	0.97	79.73	175.63
Polyp	25	321	11,923	34.09	3.57	0.62	0.95	80.24	144.06
	27	721	33,691	69.06	3.91	0.59	0.96	80.40	137.64
	31	1012	61,699	91.66	4.25	0.61	0.98	79.93	146.61
	32	458	16,862	46.95	3.95	0.64	0.96	79.71	171.13

Microhabitat	Individual	N. OTUs	N. sequences	Richness (d)	Diversity (H')	Evenness (J')	Evenness (D)	Av. Tax. Distinctness (Δ^+)	Var. Tax. Distinctness (Λ^+)
	131	564	21,816	56.35	3.44	0.54	0.89	79.18	160.89
	212	967	36,323	92.00	4.58	0.67	0.96	79.77	150.54
	213	332	11,057	35.55	3.86	0.66	0.95	79.36	162.82
	214	336	11,513	35.82	3.71	0.64	0.95	79.49	158.61
	221	641	39,133	60.52	3.37	0.52	0.94	79.77	149.26
	226	349	12,075	37.03	3.64	0.62	0.95	79.33	173.38

Table F- 3: Permutational analysis of variance (univariate PERMANOVA) on diversity indices. Test based on Euclidean distances, performed using 9,999 permutations to compare microhabitats and depth. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

Number of sequences

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Microhabitats (Mi)	1	2.31E+07	2.31E+07	0.7939	0.4916	3	0.536	0
Depth (De)	1	1.13E+08	1.13E+08	0.55336	0.4848	9843	0.4724	0
MixDe	1	2.92E+07	2.92E+07	0.14224	0.7136	9803	0.7131	0
Residual	16	3.28E+09	2.05E+08					100
Total	19	3.45E+09						

Number of OTUs

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Microhabitats (Mi)	1	4410.5	4410.5	0.21335	0.5104	3	0.7175	0
Depth (De)	1	3354	3354	0.066461	0.7975	9825	0.7994	0
MixDe	1	20672	20672	0.40963	0.5296	9846	0.529	0
Residual	16	8.07E+05	50466					100
Total	19	8.36E+05						

Richness (d) - Margalef's index

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Microhabitats (Mi)	1	34.151	34.151	0.20568	0.5026	3	0.731	0
Depth (De)	1	14.085	14.085	0.037466	0.8535	9854	0.8517	0
MixDe	1	166.04	166.04	0.44165	0.5174	9824	0.5083	0
Residual	16	6015.2	375.95					100
Total	19	6229.5						

Diversity (H') - Shannon index

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Microhabitats (Mi)	1	0.20439	0.20439	9771.3	0.2456	3	**0.0078**	28.6
Depth (De)	1	0.00063506	0.00063506	0.0049743	0.9442	9851	0.944	0.0
MixDe	1	2.09E-05	2.09E-05	0.00016384	0.9901	9847	0.9907	0.0
Residual	16	2.0427	0.12767					71.4
Total	19	2.2477						

Evenness (J') - Pielou's evenness

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Microhabitats (Mi)	1	0.0021674	0.0021674	2.6507	0.2447	3	0.3479	19.8
Depth (De)	1	0.00023684	0.00023684	0.10641	0.7535	9846	0.7494	0.0
MixDe	1	0.00081765	0.00081765	0.36736	0.5448	9822	0.544	0.0
Residual	16	0.035612	0.0022258					80.2
Total	19	0.038834						

Evenness (D) - Simpson's index

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Microhabitats (Mi)	1	0.0018391	0.0018391	80.613	0.2517	3	0.0714	42.0
Depth (De)	1	2.72E-05	2.72E-05	0.07867	0.8128	9883	0.7807	0.0
MixDe	1	2.28E-05	2.28E-05	0.066057	0.8372	9874	0.8064	0.0
Residual	16	0.0055258	0.00034536					58.0
Total	19	0.0074149						

Av. Tax. Distinctness (Δ^+) - Average of taxonomic distinctness

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Microhabitats (Mi)	1	0.00093565	0.00093565	0.01297	0.5011	3	0.9255	0.0
Depth (De)	1	0.26255	0.26255	2.9235	0.1016	9829	0.1068	30.5
MixDe	1	0.072142	0.072142	0.8033	0.3868	9839	0.3773	0.0
Residual	16	1.4369	0.089807					69.5
Total	19	1.7725						

Var. Tax. Distinctness (Λ^+) - Variation of taxonomic distinctness

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Microhabitats (Mi)	1	33.714	33.714	25.694	0.2546	3	0.1237	11.7
Depth (De)	1	201.22	201.22	1.7627	0.2036	9808	0.2033	19.1
MixDe	1	1.3121	1.3121	0.011494	0.9158	9825	0.9155	0.0
Residual	16	1826.4	114.15					69.2
Total	19	2062.7						

Table F- 4: Permutational multivariate analysis of variance (PERMANOVA) for the composition (Presence/Absence) of bacterial community associated to distinct microhabitats. Analysis based on Sorensen dissimilarities and performed using 9,999 permutations. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Microhabitats (Mi)	1	2353.8	2353.8	0.90676	0.4974	3	0.5702	0
Depth (De)	1	2694.9	2694.9	1.0783	0.2973	9842	0.3752	7.5
MixDe	1	2595.8	2595.8	1.0387	0.3938	9841	0.4125	7.5
Residual	16	39986	2499.1					85.0
Total	19	47630						

Table F- 5: Test of homogeneity of dispersion (PERMDISP) for the bacterial community composition (Presence/Absence). Analysis based on Sorensen dissimilarities and performed using 9,999 permutations. P(perm): *P*-value based on permutations, s.e: standard error.

Microhabitats

Source	**df**	**F**	**P(perm)**
Microhabitats (Mi)	1	7.0122	**0.017**
Residual	18		

Depth

Source	**df**	**F**	**P(perm)**
Depth (De)	1	0.13417	0.7258
Residual	18		

Distances from centroid

Source	**Group**	**Size**	**Distance (Av. ± s. e.)**
Microhabitat	Coenosarc	10	46.1 ± 0.7
	Polyp	10	48.9 ± 0.8
Depth	Reef flat	10	47.1 ± 0.8
	Shallow	10	47.5 ± 0.8

Table F- 6: Permutational multivariate analysis of variance (PERMANOVA) for the taxonomic composition of bacterial community associated to distinct microhabitats. Analysis based on Gamma^{+} dissimilarities and performed using 9,999 permutations. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Microhabitats (Mi)	1	133.6	133.6	0.7741	0.501	3	0.6651	0
Depth (De)	1	106.79	106.79	0.7806	0.7784	9872	0.6532	0
MixDe	1	172.59	172.59	1.2615	0.1667	9896	0.2424	18.6
Residual	16	2188.9	136.81					81.4
Total	19	2601.9						

Table F- 7: Test of homogeneity of dispersion (PERMDISP) for the bacterial community composition (Presence/Absence). Analysis based on Gamma^{+} dissimilarities and performed using 9,999 permutations. P(perm): *P*-value based on permutations, s.e: standard error.

Microhabitats

Source	df	F	P(perm)
Microhabitats (Mi)	1	3.6884	0.0979
Residual	18		

Depth

Source	df	F	P(perm)
Depth (De)	1	0.02292	0.8972
Residual	18		

Distances from centroid

Source	Group	Size	Distance (Av. ± s. e.)
Microhabitat	Coenosarc	10	10.2 ± 0.5
	Polyp	10	11.7 ± 0.6
Depth	Reef flat	10	11 ± 0.6
	Shallow	10	11.1 ± 0.6

Table F- 8: Permutational multivariate analysis of variance (PERMANOVA) for the relative abundance of bacterial community associated to distinct microhabitats. Analysis based on Bray-Curtis dissimilarities and performed using 9,999 permutations. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Microhabitats (Mi)	1	2376.7	2376.7	0.85931	0.4973	3	0.6139	0
Depth (De)	1	2788.2	2788.2	1.0755	0.3031	9809	0.3795	7.2
MixDe	1	2765.9	2765.9	1.0669	0.3033	9846	0.3811	9.6
Residual	16	41479	2592.4					83.2
Total	19	49409						

Table F- 9: Test of homogeneity of dispersion (PERMDISP) for the bacterial community structure (Relative abundance). Analysis based on Bray-Curtis dissimilarities and performed using 9,999 permutations. P(perm): *P*-value based on permutations, s.e: standard error.

Microhabitats

Source	**df**	**F**	**P(perm)**
Microhabitats (Mi)	1	5.842	**0.0272**
Residual	18		

Depth

Source	**df**	**F**	**P(perm)**
Depth (De)	1	0.0349	0.8575
Residual	18		

Distances from centroid

Source	**Group**	**Size**	**Distance (Av. ± s. e.)**
Microhabitat	Coenosarc	10	46.8 ± 0.8
	Polyp	10	50 ± 1.0
Depth	Reef flat	10	48.1 ± 0.8
	Shallow	10	48.3 ± 1

Table F- 10: Number of OTUs and average of relative abundance per taxonomic Class. Av: average of relative abundance, s.d: standard deviation.

Class	Coenosarc		Polyp	
	N. OTUs	Av. ± s.d.	N. OTUs	Av. ± s.d.
Bacilli	889	32.1 ± 5.2	1,071	36.6 ± 14.9
Actinobacteria	539	19.4 ± 4.6	478	17.8 ± 6.4
Gammaproteobacteria	550	15.1 ± 4.5	536	15.0 ± 7.4
Betaproteobacteria	199	7.4 ± 4.4	196	7.8 ± 4.9
Flavobacteriia	100	4.1 ± 5.5	184	6.3 ± 8.8
Alphaproteobacteria	136	3.4 ± 2.1	205	6.6 ± 6.1
Clostridia	249	5.9 ± 4.1	76	2.1 ± 2.6
Bacteroidia	127	5.0 ± 3.8	85	2.1 ± 2.5
Fusobacteriia	18	2.2 ± 2.2	17	0.5 ± 1.2
Cytophagia	10	1.1 ± 1.9	19	0.8 ± 0.7
TM7-3	22	0.7 ± 1.1	33	0.8 ± 1.0
Thermoleophilia	16	0.8 ± 1.1	15	0.1 ± 0.1
Erysipelotrichi	7	0.5 ± 0.8	11	0.3 ± 1.0
Synechococcophycideae	9	0.2 ± 0.7	9	0.3 ± 0.7
Saprospirae	3	0.4 ± 1.2	5	0.2 ± 0.4
Acidobacteria-6	16	0.2 ± 0.5	2	0.1 ± 0.3
Rubrobacteria	7	0.1 ± 0.2	5	0.3 ± 0.8
Opitutae	3	0.2 ± 0.5	1	0.2 ± 0.5
Thermomicrobia	3	0.002 ± 0.004	29	0.3 ± 0.6
Chloracidobacteria	4	0.002 ± 0.003	16	0.3 ± 0.8
Deltaproteobacteria	4	0.002 ± 0.004	29	0.3 ± 0.4
Armatimonadia	1	0.0004 ± 0.001	1	0.3 ± 0.8
Deinococci	4	0.1 ± 0.2	8	0.1 ± 0.3
SJA-4	1	0.001 ± 0.001	7	0.2 ± 0.6
Coriobacteriia	2	0.1 ± 0.5	4	0.1 ± 0.3

Class	Coenosarc		Polyp	
	N. OTUs	Av. ± s.d.	N. OTUs	Av. ± s.d.
Holophagae	2	0.2 ± 0.6	1	0.001 ± 0.002
Epsilonproteobacteria	3	0.1 ± 0.2	5	0.1 ± 0.2
Mollicutes	1	0.0002 ± 0.0008	1	0.2 ± 0.5
Sphingobacteriia	2	0.003 ± 0.01	1	0.1 ± 0.5
Planctomycetia	2	0.1 ± 0.4	2	0.002 ± 0.01
iii1-8	1	0.1 ± 0.4	Absent	
Phylum Cyanobacteria - unclassified	7	0.1 ± 0.3	1	0.0003 ± 0.001
Ellin6529	5	0.1 ± 0.1	1	0.01 ± 0.04
Solibacteres	7	0.01 ± 0.03	13	0.1 ± 0.1
Phycisphaerae	1	0.1 ± 0.2	Absent	
Spirochaetes	2	0.001 ± 0.002	5	0.1 ± 0.2
Gemmatimonadetes	8	0.1 ± 0.1	2	0.003 ± 0.01
Acidimicrobiia	Absent		5	0.03 ± 0.1
TK10	Absent		1	0.02 ± 0.1
TM7-1	Absent		1	0.02 ± 0.1
Gitt-GS-136	Absent		4	0.02 ± 0.1
Synergistia	Absent		1	0.01 ± 0.03
4C0d-2	Absent		1	0.003 ± 0.01
Phylum Chlorobi - unclassified	Absent		1	0.001 ± 0.002

Table F- 11: Number of OTUs and average of relative abundance per taxonomic Family. Av: average of relative abundance, s.d: standard deviation.

Family	Coenosarc	Polyp
Aerococcaceae	9.3 ± 5.5	9.3 ± 6.2
Pseudomonadaceae	8.7 ± 4.6	6.5 ± 7.3
Propionibacteriaceae	8.6 ± 2.8	9.2 ± 5.5
Staphylococcaceae	7.6 ± 3.5	9.9 ± 7.7
Corynebacteriaceae	7.5 ± 4.6	5.5 ± 4.4
Comamonadaceae	5.9 ± 3.8	5.7 ± 4.4
Streptococcaceae	5.6 ± 3.5	10.7 ± 14.8
Prevotellaceae	2.9 ± 3.1	1.4 ± 2.3
Tissierellaceae	2.8 ± 2.4	1.1 ± 2.2
Weeksellaceae	2.5 ± 4.8	5 ± 8.7
Fusobacteriaceae	2.1 ± 2.2	0.4 ± 1.1
Planococcaceae	1.9 ± 1.6	1.3 ± 1.9
Bacillaceae	1.8 ± 4.9	0.9 ± 1.3
Moraxellaceae	1.6 ± 1.9	2.2 ± 2.3
Carnobacteriaceae	1.6 ± 2.2	1.0 ± 1.8
Porphyromonadaceae	1.6 ± 2.3	0.6 ± 1
Gemellaceae	1.6 ± 3.3	0.5 ± 0.8
Flavobacteriaceae	1.6 ± 2.8	1.3 ± 2.4
Order Bacillales - unclassified	1.5 ± 0.8	1.9 ± 1.6
Veillonellaceae	1.5 ± 1.6	0.4 ± 0.4
Halomonadaceae	1.3 ± 1.1	1.1 ± 0.9
Acetobacteraceae	1.1 ± 1.6	1.8 ± 3.4
Endozoicimonaceae	1.0 ± 2.5	2.2 ± 2.4
Microbacteriaceae	0.9 ± 1.4	0.4 ± 0.7
Sphingomonadaceae	0.9 ± 1.2	1.9 ± 3.1
Salinisphaeraceae	0.9 ± 1	0.3 ± 0.3

Family	Coenosarc	Polyp
Amoebophilaceae	0.8 ± 1.6	0.002 ± 0.004
Lachnospiraceae	0.7 ± 1.6	0.3 ± 0.4
Peptostreptococcaceae	0.7 ± 1.1	0.09 ± 0.2
Order EW055 - unclassified	0.6 ± 1.1	0.5 ± 0.9
Neisseriaceae	0.6 ± 1.3	0.7 ± 1.7
Nocardioidaceae	0.6 ± 0.9	0.3 ± 0.2
Micrococcaceae	0.6 ± 0.6	1.4 ± 1.1
Xanthomonadaceae	0.5 ± 0.8	0.3 ± 0.7
Order Solirubrobacterales - unclassified	0.5 ± 0.9	0.05 ± 0.1
Lactobacillaceae	0.5 ± 0.9	0.2 ± 0.5
Oxalobacteraceae	0.5 ± 0.6	0.2 ± 0.3
Rickettsiaceae	0.5 ± 1.0	0.6 ± 1.2
Erysipelotrichaceae	0.5 ± 0.8	0.3 ± 1.0
S24-7	0.4 ± 1.3	0.005 ± 0.005
Pasteurellaceae	0.4 ± 0.9	0.7 ± 1.4
Rhodobacteraceae	0.4 ± 0.7	0.7 ± 1.0
Chitinophagaceae	0.4 ± 1.2	0.2 ± 0.4
Thermoactinomycetaceae	0.3 ± 1.0	0.2 ± 0.6
Intrasporangiaceae	0.3± 0.4	0.4 ± 0.5
Leuconostocaceae	0.3 ± 0.8	0.5 ± 1.0
Gaiellaceae	0.3 ± 0.8	0.01 ± 0.03
Clostridiaceae	0.3 ± 0.6	0.004 ± 0.005
iii1-15 family	0.2 ± 0.5	0.1 ± 0.3
Alcanivoracaceae	0.2 ± 0.5	0.09 ± 0.3
Cytophagaceae	0.2 ± 0.6	0.6 ± 0.8
Synechococcaceae	0.2 ± 0.7	0.3 ± 0.7
Enterobacteriaceae	0.2 ± 0.6	0.8 ± 1.5

Family	Coenosarc	Polyp
Actinomycetaceae	0.2 ± 0.2	0.04 ± 0.06
Rhizobiaceae	0.2 ± 0.4	0.2 ± 0.6
Holophagaceae	0.2 ± 0.6	0.0009 ± 0.002
Pelagicoccaceae	0.2 ± 0.5	Absent
Coriobacteriaceae	0.1 ± 0.5	0.08 ± 0.2
Rhodocyclaceae	0.1 ± 0.3	0.1 ± 0.3
Dietziaceae	0.1 ± 0.5	0.01 ± 0.05
Paenibacillaceae	0.1 ± 0.4	0.02 ± 0.02
Planctomycetaceae	0.1 ± 0.4	0.002 ± 0.005
Order Pseudomonadales - unclassified	0.1 ± 0.4	0.04 ± 0.1
Leptotrichiaceae	0.1 ± 0.2	0.1 ± 0.3
Order DS-18 - unclassified	0.1 ± 0.4	Absent
Geodermatophilaceae	0.1 ± 0.2	0.2 ± 0.3
Bradyrhizobiaceae	0.1 ± 0.4	0.05 ± 0.1
Methylophilaceae	0.1 ± 0.2	0.1 ± 0.3
Mycobacteriaceae	0.1 ± 0.3	0.07 ± 0.2
Beijerinckiaceae	0.1 ± 0.3	0.01 ± 0.04
Pseudonocardiaceae	0.09 ± 0.2	0.01 ± 0.05
Phylum Cyanobacteria - unclassified	0.09 ± 0.3	0.0002 ± 0.0008
Deinococcaceae	0.09 ± 0.2	0.08 ± 0.2
Class TM7-3 - unclassified	0.08 ± 0.2	0.1 ± 0.3
Campylobacteraceae	0.07 ± 0.2	0.1 ± 0.2
Caulobacteraceae	0.07 ± 0.2	0.02 ± 0.07
Order WD2101 - unclassified	0.07 ± 0.2	Absent
Class Ellin6529 - unclassified	0.07 ± 0.1	0.01 ± 0.04
Bifidobacteriaceae	0.07 ± 0.2	0.09 ± 0.2
Rubrobacteraceae	0.07 ± 0.2	0.3 ± 0.8

Family	Coenosarc	Polyp
Burkholderiaceae	0.06 ± 0.2	0.8 ± 2.2
Order Gemmatimonadales - unclassified	0.054 ± 0.1	0.003 ± 0.009
Sporichthyaceae	0.05 ± 0.1	0.02 ± 0.05
Enterococcaceae	0.05 ± 0.1	0.2 ± 0.4
Order Micrococcales - unclassified	0.04 ± 0.1	0.0006 ± 0.002
Order Actinomycetales - unclassified	0.04 ± 0.05	0.03 ± 0.04
Brucellaceae	0.04 ± 0.1	0.3 ± 0.7
Thermaceae	0.02 ± 0.03	0.07 ± 0.2
Rikenellaceae	0.02 ± 0.05	Absent
Aeromonadaceae	0.02 ± 0.05	Absent
Ruminococcaceae	0.02 ± 0.05	0.006 ± 0.02
Order Cardiobacteriales - unclassified	0.02 ± 0.05	Absent
Chromatiaceae	0.02 ± 0.02	0.2 ± 0.7
Order Solibacterales - unclassified	0.01 ± 0.03	0.07 ± 0.1
Class Gammaproteobacteria - unclassified	0.009 ± 0.01	0.006 ± 0.006
Erythrobacteraceae	0.009 ± 0.03	0.009 ± 0.03
Methylobacteriaceae	0.007 ± 0.006	0.5 ± 1.5
Gordoniaceae	0.006 ± 0.02	0.009 ± 0.03
Alcaligenaceae	0.005 ± 0.01	0.0007 ± 0.001
Pseudoalteromonadaceae	0.005 ± 0.02	Absent
Hyphomicrobiaceae	0.005 ± 0.01	0.2 ± 0.5
Micromonosporaceae	0.003 ± 0.009	Absent
Order Burkholderiales - unclassified	0.003 ± 0.008	0.001 ± 0.002
Sphingobacteriaceae	0.003 ± 0.007	0.15 ± 0.5
Class Gemmatimonadetes - unclassified	0.002 ± 0.008	Absent
Sanguibacteraceae	0.002 ± 0.005	Absent
Order JG30-KF-CM45 - unclassified	0.002 ± 0.004	0.3 ± 0.6

Family	Coenosarc	Polyp
Order RB41 - unclassified	0.002 ± 0.003	0.3 ± 0.8
Order Lactobacillales - unclassified	0.006 ± 0.003	0.0002 ± 0.0005
Order Rhizobiales - unclassified	0.001 ± 0.002	0.1 ± 0.4
Bdellovibrionaceae	0.001 ± 0.004	0.2 ± 0.4
Shewanellaceae	0.001 ± 0.002	0.3 ± 0.9
Class Bacilli - unclassified	0.0013 ± 0.002	0.0006 ± 0.001
Puniceicoccaceae	0.001 ± 0.002	0.2 ± 0.5
Sporolactobacillaceae	0.001 ± 0.002	0.1 ± 0.4
Order Clostridiales - unclassified	0.001 ± 0.002	0.1 ± 0.4
Spirochaetaceae	0.001 ± 0.002	0.07 ± 0.2
Class SJA-4 - unclassified	0.0007 ± 0.001	0.2 ± 0.6
Order Gemellales - unclassified	0.0006 ± 0.002	0.0002 ± 0.0005
Promicromonosporaceae	0.0004 ± 0.001	0.03 ± 0.07
Streptomycetaceae	0.0004 ± 0.001	0.02 ± 0.06
Order Blgi18 - unclassified	0.0004 ± 0.001	0.1 ± 0.4
Vibrionaceae	0.0004 ± 0.001	0.03 ± 0.1
Armatimonadaceae	0.0004 ± 0.001	0.3 ± 0.8
Brevibacteriaceae	0.0004 ± 0.001	0.05 ± 0.1
Class Alphaproteobacteria - unclassified	0.00034 ± 0.001	0.03 ± 0.09
Listeriaceae	0.0003 ± 0.001	0.02 ± 0.07
Syntrophobacteraceae	0.0003 ± 0.001	0.02 ± 0.06
Mycoplasmataceae	0.0003 ± 0.0008	0.2 ± 0.5
Order Legionellales - unclassified	0.0002± 0.0008	0.03 ± 0.09
Class Betaproteobacteria - unclassified	0.0002 ± 0.0008	0.08 ± 0.2
Thermomonosporaceae	0.0002 ± 0.0007	0.005 ±,0.02
Flammeovirgaceae	0.0002 ± 0.0007	0.1 ± 0.4
Order SC-I-84 - unclassified	Absent	0.03 ± 0.09

Family	Coenosarc	Polyp
Aurantimonadaceae	Absent	0.1 ± 0.3
Order Saprospirales - unclassified	Absent	0.005 ± 0.01
Cellulomonadaceae	Absent	0.009 ± 0.03
Order MLE1-12 - unclassified	Absent	0.0023 ± 0.008
Dethiosulfovibrionaceae	Absent	0.009 ± 0.03
AKIW874	Absent	0.03 ± 0.09
Class Gitt-GS-136 - unclassified	Absent	0.02 ± 0.06
Methylocystaceae	Absent	0.02 ± 0.06
Order Myxococcales - unclassified	Absent	0.03 ± 0.1
Order Sphingomonadales - unclassified	Absent	0.002 ± 0.005
Order B07_WMSP1 - unclassified	Absent	0.02 ± 0.06
Bacteriovoracaceae	Absent	0.003 ± 0.009
Hyphomonadaceae	Absent	0.0008± 0.003
Phylum Chlorobi - unclassified	Absent	0.0006 ± 0.002
HTCC2089	Absent	0.02 ± 0.05
Order CW040 - unclassified	Absent	0.01 ± 0.04
Sinobacteraceae	Absent	0.2 ± 0.7
Actinosynnemataceae	Absent	0.02 ± 0.06
Class TM7-1 - unclassified	Absent	0.02 ± 0.06

Table F- 12: Average of the relative abundance of the 30 most abundant OTUs in each microhabitat. ID: Lower taxonomic identification. NROTU: New Reference OTU.

OTU	ID	Coenosarc	Polyp
1088265	*Propionibacterium acnes*	6.26 ± 1.9	7.01 ± 4.2
1098410	*Staphylococcus epidermidis*	4.88 ± 2.8	6.07 ± 5.2
1110381	Family Aerococcaceae - unclassified	5.03 ± 4.3	4.55 ± 3.8
1078207	*Streptococcus* sp.	3.00 ± 2.2	6.15 ± 9.6
69980	Family Aerococcaceae - unclassified	2.87 ± 2.0	1.52 ± 1.7
396109	*Cloacibacterium* sp.	1.55 ± 2.6	2.72 ± 5.7
543864	*Pseudomonas* sp.	2.21 ± 2.5	1.99 ± 3.4
750018	*Pseudomonas veronii*	2.55 ± 2.8	1.19 ± 1.4
525199	*Delftia* sp.	1.36 ± 0.8	2.25 ± 4.0
377613	*Corynebacterium* sp.	2.48 ± 2.2	1.11 ± 1.7
1056769	*Staphylococcus* sp.	1.45 ± 0.9	1.87 ± 1.6
4465204	*Prevotella tannerae*	2.09 ± 2.5	0.98 ± 1.6
439036	*Corynebacterium* sp.	1.19 ± 2.0	1.86 ± 3.7
1056626	Order Bacillales - unclassified	1.30 ± 0.7	1.69 ± 1.5
1020410	Family Planococcaceae – unclassified	1.55 ± 1.4	1.32 ± 1.9
846710	Family Comamonadaceae – unclassified	1.83 ± 3.4	0.89 ± 1.4
979107	Family Halomonadaceae - unclassified	1.26 ± 1.2	1.04 ± 0.9
530966	Family Aerococcaceae - unclassified	0.38 ± 0.6	1.85 ± 2.5
1084738	*Propionibacterium acnes*	1.02 ± 0.4	1.10 ± 0.7
546165	Family Comamonadaceae - unclassified	1.10 ± 1.2	0.85 ± 0.8
NROTU28	Family Endozoicomonadaceae - unclassified	0.25 ± 0.5	1.63 ± 2.3
4392229	*Pseudomonas* sp.	1.09 ± 2.1	0.77 ± 1.6
925707	Family Gemellaceae - unclassified	1.29 ± 2.7	0.38 ± 0.6
561294	*Pseudomonas* sp.	0.87 ± 1.3	0.79 ± 1.0
1011954	*Corynebacterium* sp.	1.05 ± 1.0	0.59 ± 0.6
444857	*Fusobacterium* sp.	1.21 ± 1.8	0.37 ± 1.1

OTU	ID	Coenosarc	Polyp
NROTU148	*Acidisoma* sp.	0.12 ± 0.4	1.25 ± 3.4
1047041	*Corynebacterium* sp.	0.68 ± 1.0	0.59 ± 1.6
945455	*Micrococcus luteus*	0.32 ± 0.5	0.94 ± 1.0
384716	*Staphylococcus* sp.	0.47 ± 1.4	0.71 ± 1.4
851704	*Parvimonas* sp.	0.90 ± 1.5	0.25 ± 0.5
102348	Family Acetobacteraceae - unclassified	0.88 ± 1.3	0.26 ± 0.5
574102	*Enhydrobacter* sp.	0.10 ± 0.3	0.95 ± 1.3
1084865	*Staphylococcus* sp.	0.29 ± 0.6	0.70 ± 1.1
1040713	*Corynebacterium* sp.	0.70 ± 0.8	0.28 ± 0.4
463607	*Sphingomonas wittichii*	0.002 ± 0.003	0.94 ± 3.0
4320518	*Selenomonas* sp.	0.67 ± 1.0	0.19 ± 0.2
583197	Genus SGUS912	0.83 ± 1.6	0.002 ± 0.003
1033473	*Streptococcus* sp.	0.01 ± 0.01	0.77 ± 2.4

Table F- 13: Average of the relative abundance of the core microbiome OTUs in each microhabitat. ID: Lower taxonomic identification. NROTU: New Reference OTU.

OTU	ID	Core	Coenosarc	Polyp
1084865	*Staphylococcus* sp.	Coenosarc-Polyp	0.29 ± 0.6	0.58 ± 1
377613	*Corynebacterium* sp.	Coenosarc-Polyp	2.46 ± 2.2	1.10 ± 1.7
992354	*Propionibacterium acnes*	Coenosarc-Polyp	0.10 ± 0.04	0.11 ± 0.06
1034344	*Propionibacterium acnes*	Coenosarc-Polyp	0.07 ± 0.03	0.08 ± 0.07
945455	*Micrococcus luteus*	Coenosarc-Polyp	0.26 ± 0.4	0.84 ± 0.9
1056626	Order Bacillales - unclassified	Coenosarc-Polyp	1.06 ± 0.6	1.38 ± 1.1
1098410	*Staphylococcus epidermidis*	Coenosarc-Polyp	3.92 ± 2.1	4.96 ± 3.9
1011954	*Corynebacterium* sp.	Coenosarc-Polyp	1.03 ± 1.0	0.55 ± 0.6
4392229	*Pseudomonas* sp.	Coenosarc-Polyp	1.07 ± 2.1	0.77 ± 1.6
1084738	*Propionibacterium acnes*	Coenosarc-Polyp	0.85 ± 0.2	0.91 ± 0.5
1056769	*Staphylococcus* sp.	Coenosarc-Polyp	1.18 ± 0.7	1.53 ± 1.2
1110381	Family Aerococcaceae - unclassified	Coenosarc-Polyp	4.34 ± 4.3	4.05 ± 3.5
525199	*Delftia* sp.	Coenosarc-Polyp	1.17 ± 0.8	2.17 ± 4.0
543864	*Pseudomonas* sp.	Coenosarc-Polyp	1.87 ± 1.9	1.82 ± 3.3
750018	*Pseudomonas veronii*	Coenosarc-Polyp	1.90 ± 1.5	1.06 ± 1.4
852665	*Propionibacterium acnes*	Coenosarc-Polyp	0.01 ± 0.01	0.02 ± 0.01
927202	*Propionibacterium acnes*	Coenosarc-Polyp	0.03 ± 0.01	0.04 ± 0.02
1067574	*Propionibacterium acnes*	Coenosarc-Polyp	0.07 ± 0.04	0.08 ± 0.04
870106	*Propionibacterium acnes*	Coenosarc-Polyp	0.02 ± 0.01	0.03 ± 0.02
902903	*Propionibacterium acnes*	Coenosarc-Polyp	0.02 ± 0.02	0.02 ± 0.01
396109	*Cloacibacterium* sp.	Coenosarc-Polyp	1.41 ± 2.6	2.42 ± 5.0
4465204	*Prevotella tannerae*	Coenosarc-Polyp	2.07 ± 2.5	0.97 ± 1.6
69980	Family Aerococcaceae - unclassified	Coenosarc-Polyp	2.52 ± 1.7	1.36 ± 1.6
1020410	Family Planococcaceae - unclassified	Coenosarc-Polyp	1.42 ± 1.2	1.04 ± 1.5
384716	*Staphylococcus* sp.	Coenosarc-Polyp	0.47 ± 1.4	0.69 ± 1.4
846710	Family Comamonadaceae - unclassified	Coenosarc-Polyp	1.55 ± 2.7	0.78 ± 1.4
1088265	*Propionibacterium acnes*	Coenosarc-Polyp	5.32 ± 1.6	5.87 ± 3.0

OTU	ID	Core	Coenosarc	Polyp
1078207	*Streptococcus* sp.	Coenosarc-Polyp	2.62 ± 1.9	5.53 ± 8.3
791973	*Pseudomonas* sp.	Coenosarc-Polyp	0.54 ± 1.1	0.42 ± 0.8
979107	Family Halomonadaceae - unclassified	Coenosarc-Polyp	1.13 ± 1.2	0.93 ± 0.8
439036	*Corynebacterium* sp.	Coenosarc-Polyp	1.06 ± 2	1.71 ± 3.5
972955	*Propionibacterium acnes*	Coenosarc-Polyp	0.10 ± 0.04	0.11 ± 0.06
546165	Family Comamonadaceae - unclassified	Coenosarc-Polyp	0.98 ± 1.2	0.74 ± 0.6
NROTU28	Family Endozoicomonadaceae - unclassified	Coenosarc	0.23 ± 0.5	1.44 ± 2
992022	*Propionibacterium acnes*	Coenosarc	0.02 ± 0.01	0.02 ± 0.02
1055132	Order Bacillales - unclassified	Coenosarc	0.01 ± 0.01	0.02 ± 0.01
373689	Order Bacillales - unclassified	Coenosarc	0.01 ± 0.01	0.02 ± 0.02
870751	Order Bacillales - unclassified	Coenosarc	0.02 ± 0.01	0.02 ± 0.02
4438988	*Streptococcus* sp.	Coenosarc	0.04 ± 0.04	0.17 ± 0.4
NROTU93	Family Comamonadaceae - unclassified	Coenosarc	0.36 ± 0.4	0.27 ± 0.3
4399761	*Streptococcus* sp.	Coenosarc	0.03 ± 0.02	0.06 ± 0.1
200629	Order Solirubrobacterales - unclassified	Coenosarc	0.22 ± 0.5	0.003 ± 0.003
859700	*Staphylococcus* sp.	Coenosarc	0.01 ± 0.01	0.01 ± 0.01
4366487	*Capnocytophaga* sp.	Coenosarc	0.33 ± 0.5	0.21 ± 0.3
993647	*Granulicatella* sp.	Coenosarc	0.58 ± 1.0	0.64 ± 1.2
152823	*Streptococcus* sp.	Coenosarc	0.08 ± 0.2	0.21 ± 0.3
552449	*Pseudomonas* sp.	Coenosarc	0.07 ± 0.1	0.06 ± 0.1
925707	Family Gemellaceae - unclassified	Coenosarc	0.83 ± 1.8	0.29 ± 0.4
918733	*Propionibacterium acnes*	Coenosarc	0.01 ± 0.01	0.01 ± 0.01
975755	*Propionibacterium acnes*	Coenosarc	0.01 ± 0.01	0.01 ± 0.01
861595	*Propionibacterium acnes*	Coenosarc	0.01 ± 0.003	0.01 ± 0.01
563273	*Pseudomonas* sp.	Coenosarc	0.08 ± 0.08	0.11 ± 0.2
530966	Family Aerococcaceae - unclassified	Polyp	0.35 ± 0.6	1.67 ± 2.5
858026	*Corynebacterium* sp.	Polyp	0.32 ± 0.3	0.15 ± 0.2
1047041	*Corynebacterium* sp.	Polyp	0.54 ± 0.7	0.49 ± 0.5

OTU	ID	Core	Coenosarc	Polyp
370772	*Propionibacterium acnes*	Polyp	0.01 ± 0.01	0.02 ± 0.02
1040713	*Corynebacterium* sp.	Polyp	0.70 ± 0.8	0.28 ± 0.4

Table F- 14: Permutational multivariate analysis of variance (PERMANOVA) on the prediction of functional profiling. Analysis based on Bray-Curtis dissimilarities and performed using 9,999 permutations. P(perm): *P*-value based on permutations, U. perms: Unique permutations, P(MC): Monte Carlo *P*- value, ECV(%): Estimated components of variation.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)	ECV(%)
Microhabitats (Mi)	1	135.33	135.33	4.7344	0.2536	3	0.2464	17.1
Depth (De)	1	14.765	14.765	0.059234	0.8828	9917	0.8771	0.0
MixDe	1	28.584	28.584	0.11468	0.7962	9931	0.7892	0.0
Residual	16	3988.1	249.26					82.9
Total	19	4166.8						

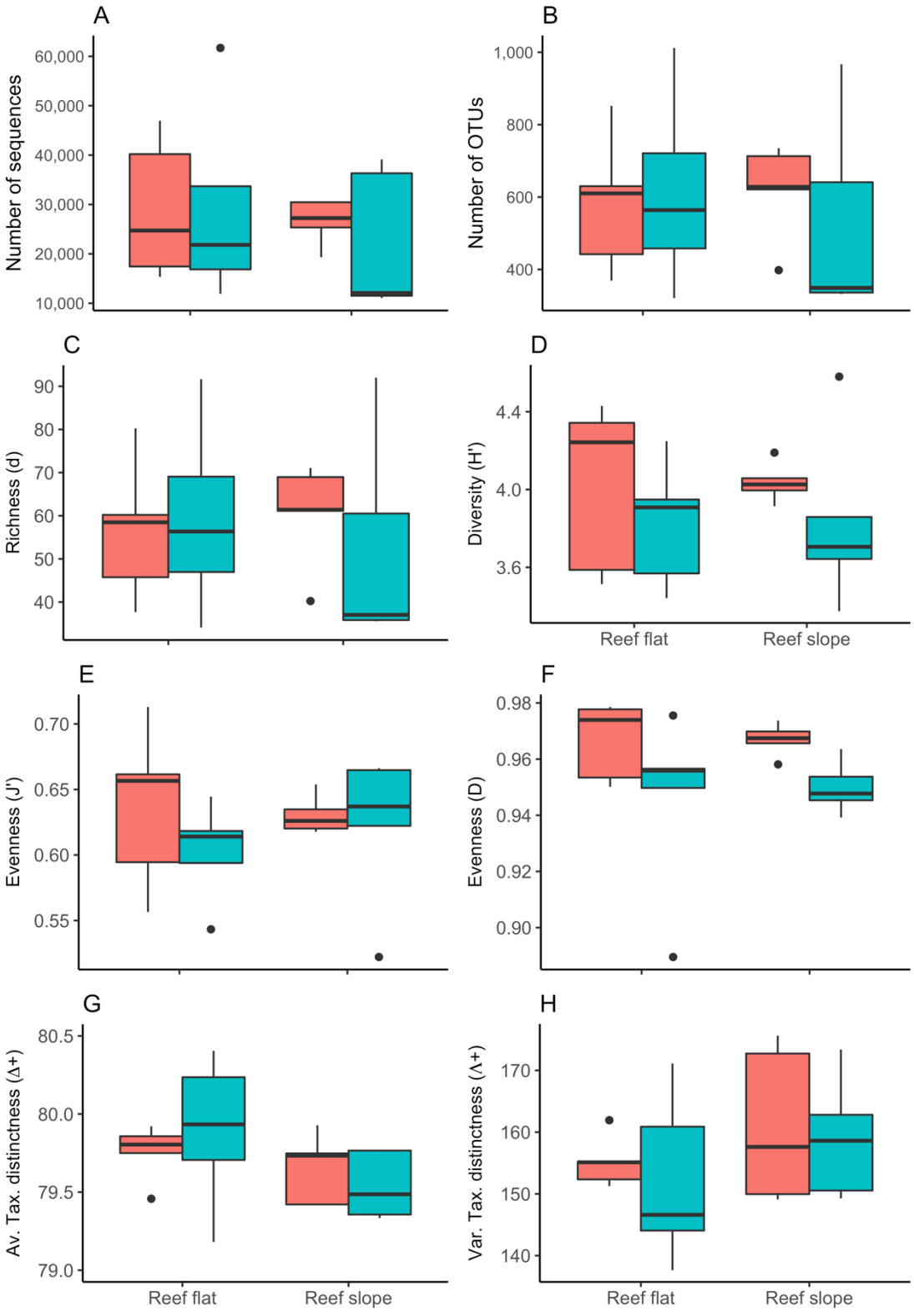


Figure F - 1: Diversity indices per microhabitat and depth. Microhabitats only differ in diversity; no significant differences were detected between considered depths (see Table S3). Pink: coenosarc, blue: polyp. Richness (d): Margalef's index, Diversity (H'): Shannon diversity, Evenness (J'): Pielou's evenness, Evenness (D): Simpson's evenness, Av. Tax. Distinctness (Δ^+): Average of taxonomic distinctness, Var. Tax. Distinctness (Λ^+): Variation of taxonomic distinctness. Data used for boxplots exclude chloroplast, mitochondria, non-identified and unassigned OTUs (see Supplementaty Table F-1).

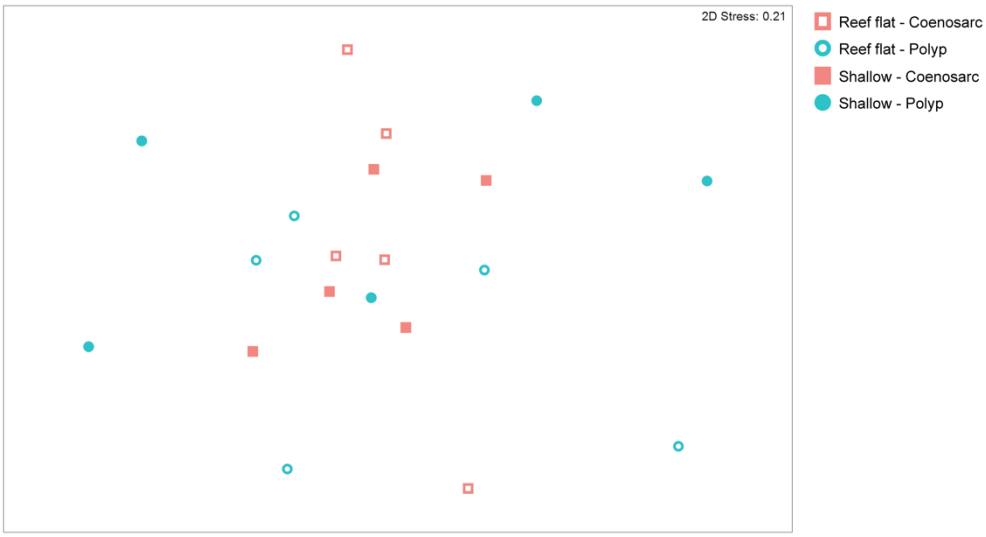


Figure F - 2: Non-metric MDS demonstrate similarities in taxonomic composition between the microhabitats. Non-metric MDS based on Gamma^{+} dissimilarity on taxonomic composition data. For statistical analyses on taxonomic composition, *see* Supplementary Tables F-6 and F-7.

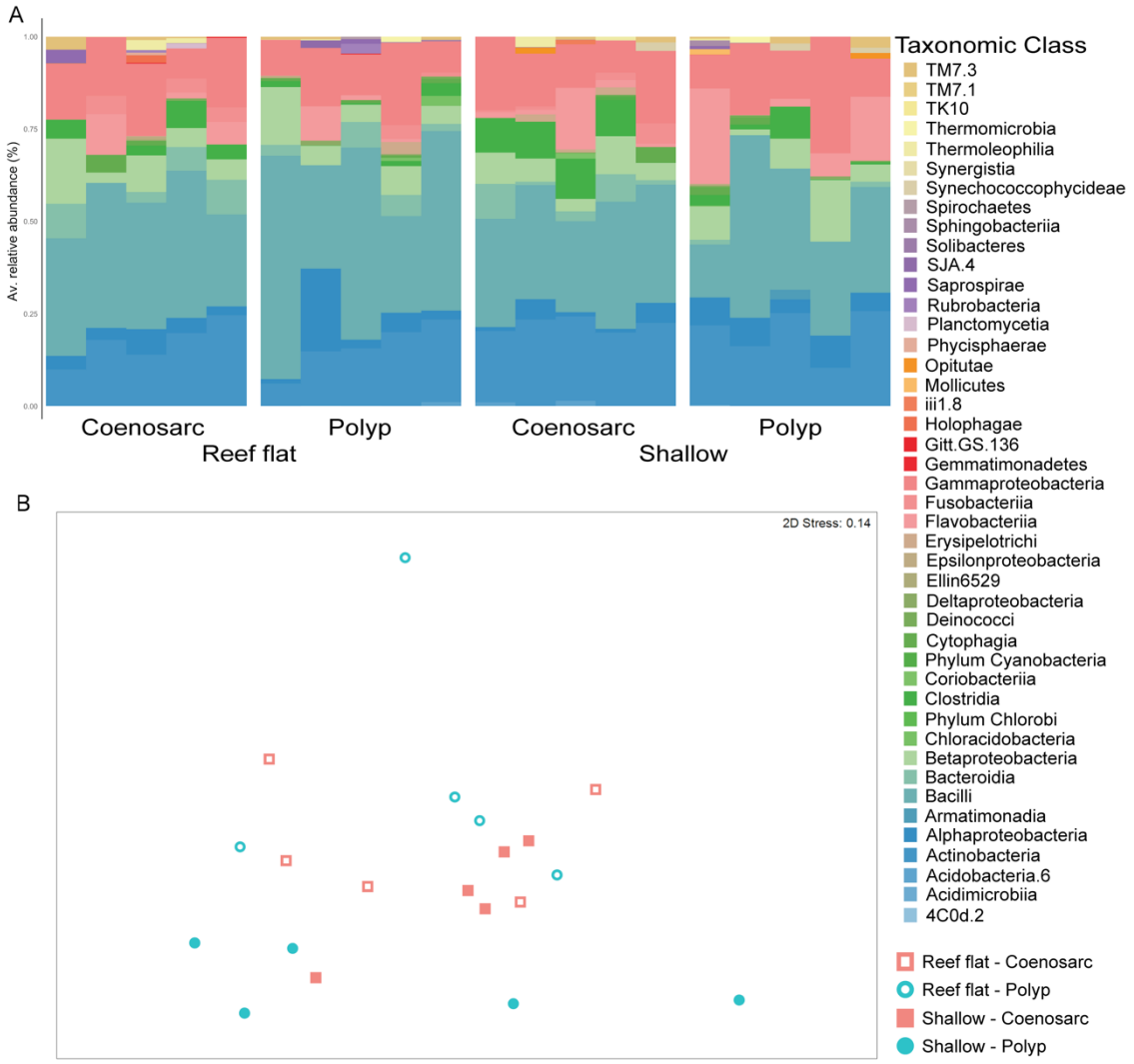


Figure F - 3: Polyps and coenosarcs harbour bacterial communities structurally similar. The taxonomic structure was highly variable across individuals, but dominant classes were consistent across microhabitats (A). Structural similarity between distinct microhabitats and variability between samples are evident in the Non-metric MDS (B). Non-metric MDS based on Bray-Curtis dissimilarity on relative abundance data (fourth root-transformed). For statistical analyses on community structure, *see* Supplementary Tables F-8 and F-9.

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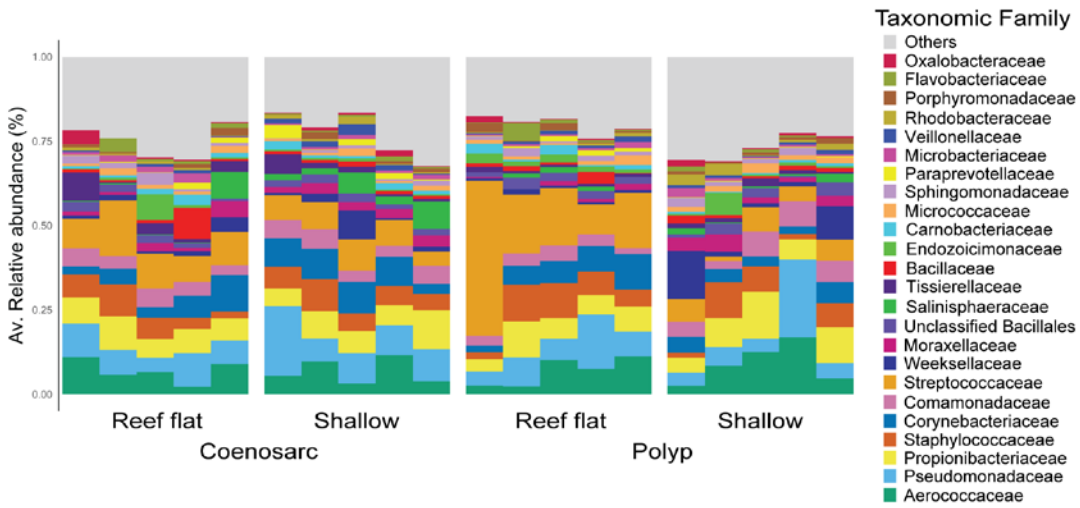

Figure F - 4: Structure of bacterial community per sample. For statistical analyses on community structure, see Tables S8 and S9.

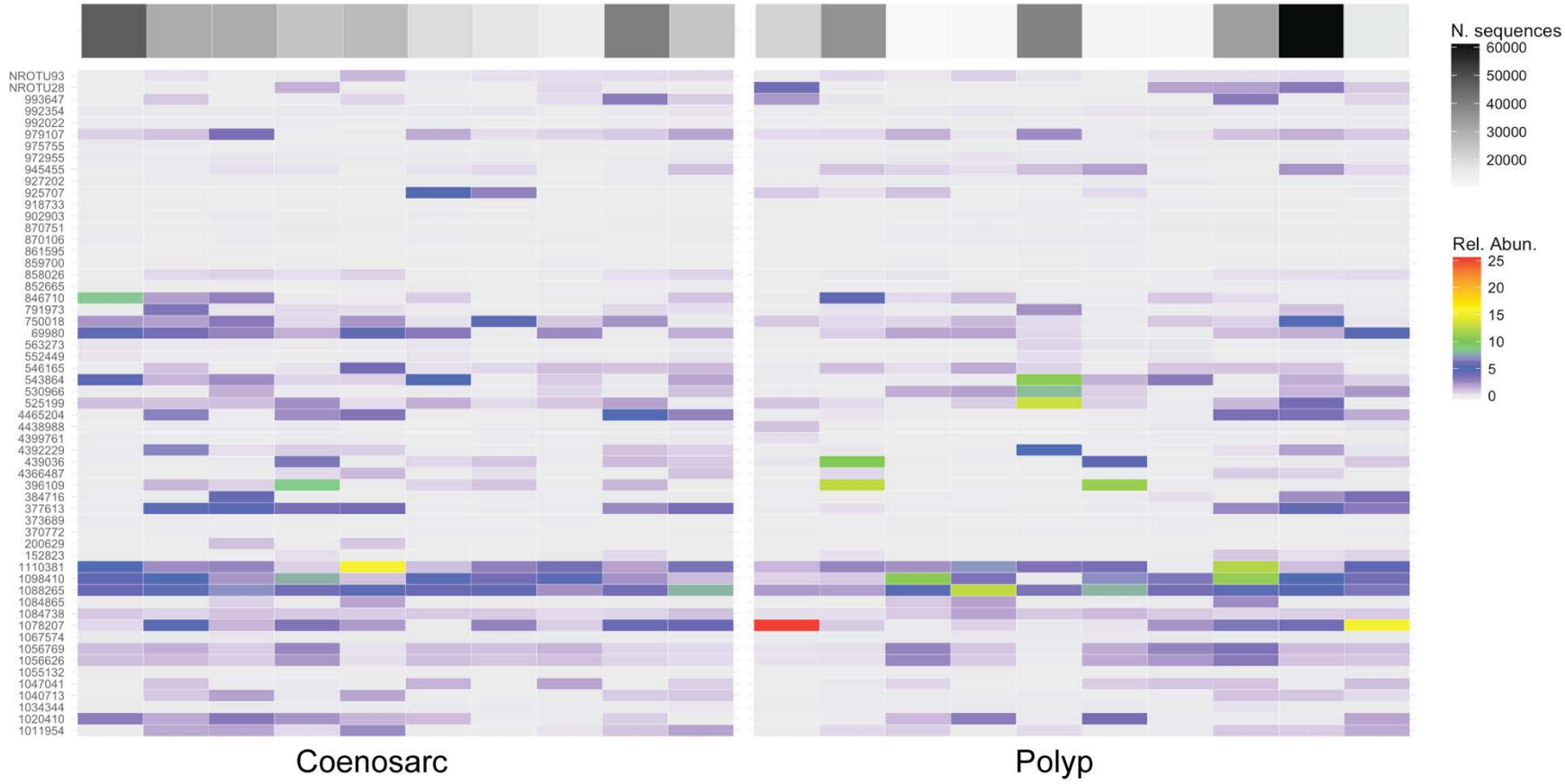

Figure F - 5: Total number of sequences per sample and relative abundance of core microbiome OTUs. The abundance of core microbiome OTUs is highly variable across samples, indicating these phylotypes are only consistent in occurrence.