A Pilot Study of the Barbadian Reef Microbiome: New Approaches for Comparative Analyses

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Abstract

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Coral reef systems are fundamentally important ecosystems for the island of Barbados, supporting a highly diverse marine fauna along while providing critical resources and services which residents of the island are dependent on through means such as food and employment. Coral reefs also play a key role in the contribution to the economies of the Caribbean small island developing states such as Barbados, where tourism is the main economic driver. Over the past five decades the Barbadian coral reef systems have been impacted by global (eg climate change) and local stressors (eg anthropogenic runoffs) causing dynamic changes in the ecosystems such as coral bleaching and benthic algal domination, leading to loss of coral cover and an increase in coral mortality. Here we conducted a pilot study into the marine microbial communities that inhabit the seawater of two coral reef systems located on the west coast of Barbados that have different structural features, ecological features and local stressor exposure. By incorporating modern and novel analysis approaches we revealed that the two reef systems have distinct microbial ecology compositions that reflect the ecosystems ecological differences and effects by stressors. Our data gives insights into the microbial microbiome that interacts with other microbiomes (eg coral holobiont) within the Barbadian reef ecosystems, creating a baseline for future studies and surveying efforts.

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Contribution of Authors

The table below describes the contributions of each member of this project. M Hallett obtained the appropriate CITES permit for this work. S Mahon (ex-director of the Bellairs Research Station and director of the Coral Reef Restoration Alliance, CORALL) assisted with the logistics of sample collection and transportation to Canada. M Medina (Pennsylvania State University) collected the samples taken at the Maycocks reef, and A Ramachandran (Concordia) collected the samples at Bellairs. Ramachandran filtered and preserved the samples for transport to Canada. S Kramer (Concordia) extracted DNA and prepared samples for Illumina sequencing. The McGill-Genome Quebec Innovation Centre sequenced the samples. H Valles (University of West Indies - Cave Hill) assisted with evaluation of the sampling sites and provided metadata and water chemistry regarding these sites. V Dumeaux (Concordia) assisted with our analytic pipelines especially with respect to de novo assemblies and subsequent analyses. V Bettauer (Concordia) made many contributions throughout all the analysis steps of this project, assisted with the preparation of the manuscript and designed the differential programming (aka deep neural network) approaches for new sequence identification. D Walsh provided guidance for the project and supported the sequencing. M Hallett designed and supervised the project, contributing to the analyses, manuscript preparation and funding. I assisted with sample preparation, conducted the microscopy and measurement of physio-chemical variables in the study, conducted statistical analyses of the data and synthesized knowledge from the literature into the manuscript. This work was supported by grants awarded to M Hallett from the NSERC Discovery and the Canadian Research Chairs programs.

Name	Collection/ Site assessment	Sample Prep.	Micro-scopy chemistry	Data Science	Deep learning	Analysis	Manuscript preparation	Funding
Simpson								
Bettauer								
Mahon								
Medina								
Ramachand.								
Kramer								
Valles								
Walsh								
Dumeaux								
Hallett								

Table of Contents

Lists of Figures

List of Tables

Introduction

Importance of coral reef systems both for the global environment and Barbados.

Coral reefs are some of the most important ecosystems within the global oceans. These systems contain a wealth of marine biodiversity with hundreds of thousands of species, while only occupying approximately 0.1% of the global oceans seafloor (Hoegh-Guldberg et al. 2017; Kaimba, de Villiers, and Wambua 2019). Coral reefs are also economically important to millions of people, with a worldwide economic value of USD $375 billion annually (Hoegh-Guldberg et al. 2017). The value of coral systems to islands such as Barbados and other Caribbean nations is extremely high. These islands rely on coral reef systems for coastal protection from hurricanes and storm surges, for economic growth through tourism, and as a source of food and employment through fisheries and other water activities (H.A.Oxenford et al. 2007; Hoegh-Guldberg 2011; Kirkbride-Smith, Wheeler, and Johnson 2016; Cermes 2018). Barbados reef systems consist of near-shore patch reefs and fringing reefs, along with offshore bank reefs which sit in deeper water (Cermes 2018).

Functioning Coral Reef Systems

Tropical coral reef systems require precisely tuned conditions for efficient ecological functionality and survival (Henkel 2010). Corals inhabit shallow water environments where they are able to access adequate sunlight for their algal endosymbionts called Zooxanthellae (Kuanui et al. 2020). Zooxanthellae are the photosynthetic dinoflagellates living within the coral's tissue, and conduct photosynthesis to produce nutrients which corals depend on (Wooldridge 2020). Another key factor in reef systems is clean water. It allows sunlight to easily reach the corals, while opaque water creates conditions that inhibit photosynthesis (Jones et al. 2020). Coral reef systems ideally require oligotrophic water conditions, as corals require nutrient poor environments for sustainable growth and ecological competition (Hughes et al. 2020). Corals continuously grow by secreting layers of calcium carbonate that builds upon their skeleton of the same chemical build. Corals rely on this process of calcification for growth which efficiently occurs in water conditions where salinity ranges from 32-40psu and water temperatures of 23-29°C (Ibarbalz et al. 2019). Coral larval recruitment and settlement is a crucial ecological process for coral reef longevity (Nietzer et al. 2018). Pelagic coral larvae from other reef systems can settle within new reef systems, forming new coral colonies, adding to the local diversity (Cameron and Harrison 2020). Within a thriving coral reef system there is continuous nutrient uptake, retention and recycling by free living and host associated microorganisms, which attributes to the survival of benthic organisms within the ecosystem (Vanwonterghem and Webster 2020). Herbivory plays a key ecological process within a functioning coral reef system. It plays a key role in nutrient cycling, regulating species diversity and productivity, and controls habitat shift regimes such as sustaining a coral dominant reef (Robinson et al. 2020).

Factors threatening coral reef health

Coral reefs are one of the most threatened ecosystems with wide-spread reports of unhealthy coral and coral cover loss (Camp et al. 2018). It was estimated that 70-90% of reefs worldwide could be gone by 2050 due to several factors and influences impinging on the ecological state of coral reefs (Dance 2019). Climate change, other global stressors and local stressors have impacted the coral reef systems of the Caribbean region significantly over the last 50 years; it has seen an approximately 80% loss in coral cover (H.A.Oxenford and Vallès 2016; Ladd, Burkepile, and Shantz 2019). As a result of the combination of these stressors, reef systems located on the south and west of Barbados have experienced a decline in the abundance of hard corals over the last decade (Cermes 2018).

Climate change

Climate change is an established threat to coral reef ecosystems (Boström-Einarsson et al. 2020). Increased sea temperatures and ocean acidification have been linked to coral reef stress, which in turn induces coral bleaching. Coral bleaching is the loss of a critical algal symbiosis within corals (Camp et al. 2018). Small island development states such as Barbados are extremely vulnerable to their marine environments being impacted by increased sea temperature, ocean acidification or increased intensity of storms (Pulwarty, Nurse, and Trotz 2010; Monnereau et al. 2017). Barbados experienced a mass coral bleaching event during 2005 where 70% of coral colonies at six study sites experienced bleaching (H.A.Oxenford et al. 2007). This mass bleaching event occurred due to elevated sea water temperatures, which plagued the island again in 2010. However, coral bleaching and mortality were lower (H.A.Oxenford and Vallès 2016). Hurricanes and tropical storms have impacted Caribbean coral reef systems for decades, primarily causing mechanical damage to the reefs (Scoffin 1993; Fabricius et al. 2008). Recently, more powerful cyclone systems have caused more disruption on already degraded coral systems (Edmunds 2019).

Local stressors

Local stressors of coral reef systems are abiotic and biotic drivers that occur at a local scale, causing a further decline of coral heath and increasing mortality (McLean et al. 2016; Weijerman et al. 2018). This includes overfishing (Aronson and Precht 2006), eutrophication due to agriculture runoffs, sewage, wastewater and other forms of anthropogenic pollution (Bonkosky et al. 2009; Bell, Elmetri, and Lapointe 2014; Hafezi et al. 2020), mechanical disturbances from water-based activities and poor water quality. Runoffs and freshwater outflows are linked to eutrophication within coral reef systems. The inundation of nutrients (eg nitrogen) into coral reef systems creates the risk of an algal bloom of harmful planktonic organisms, which will reduce light and enhance the growth of coral benthic competitors such as macroalgae and turf algae (Lapointe et al. 2019). Along with nutrient filled water, freshwater outflows also discharge sediment into the marine environments. This affects water quality by reducing light and affects coral health directly

by introducing terrestrial microbes that become opportunistic pathogens. Overfishing impacts the coral reef systems, especially by removal of important grazers (eg herbivorous fish) which are primary drivers of the benthic community structure within the ecosystem (Zaneveld et al. 2016; Shantz, Ladd, and Burkepile 2020). Decline in the biomass of grazers erodes coral reef resilience and contributes to the phase shift of coral dominant reefs to algae dominant which has taken place within the Caribbean (Shantz, Ladd, and Burkepile 2020).

Other global stressors

Global oceans are influx with 4.8 to 12.7 million metric tons of plastic annually. Plastic waste and debris cause coral stress by causing light deprivation, toxin release, inducing anoxia and opening the door to microbial colonization by pathogens resulting in disease (Lamb et al. 2018). Within the Caribbean, the effects of global plastic pollution have been identified in various marine environments (Diez et al. 2019). Due to global ocean current dynamics, potential threats or stressors can originate from one geographic location and drift into another location inhabited by coral reef systems. Since 2011, Caribbean islands have experienced repeated golden tidal events caused by Sargassum seaweed (S. natans & S. fluitans) originating from the tropical Atlantic ocean, east of Brazil (Langin 2018). In Barbados these golden tides are a threat to fragile coral reef systems, where they stress the ecosystems by light reduction, oxygen depletion, increasing nutrient levels and mortality of associated reef fish (Hinds et al. 2016; van Tussenbroek et al. 2017). Influences from South America also occur via water outflow by the Amazon river which is transported into the southeastern Caribbean and can have significant impact in marine environments. For example, a transient dark green water mass originating from the Amazon river served as a protective measure for corals during a warm ocean temperature event, reducing the level of coral bleaching and mortality in Barbados (H.A.Oxenford and Vallès 2016). However this transient water mass could also be bringing in an influx of nutrients and opportunistic pathogens (Mendoza et al. 2009; Pawlik, Burkepile, and Thurber 2016; Correa-Ramirez et al. 2020).

Coral reef health

Caribbean coral reef systems have seen an increase in coral diseases, coral bleaching and shifts from once coral dominance to now algal dominant systems (T. P. Hughes 1994; Mumby, Hastings, and Edwards 2007; Mumby and Steneck 2008; H.A.Oxenford and Vallès 2016; Steneck et al. 2018; Camacho et al. 2020). Mortality of coral colonies and the decline in coral larvae settlement and survival has become common traits worldwide in coral reef ecosystems, which hinders the stability and recovery of these impacted environments (Magel et al. 2019; Vargas-Ángel et al. 2019; Cameron and Harrison 2020).

Coral mortality and effects

Coral death can be caused by many factors and influences such as predation, disease, suffocation, growth anomalies and physical disturbances (Ladd and Shantz 2020).Though the act of temperature induced coral bleaching has been seen as one of biggest concerns faced by key corals species in the last couple of decades it does not directly lead to coral death, however like a human with a weak immune system bleached corals are more susceptible to diseases caused

by pathogens (Mao-Jones et al. 2010; Tout et al. 2015). Corals are unique in that they are able to experience partial tissue death caused by diseases and still be alive, however if the tissue loss is severe it can lead to coral death (Hamman 2019). Once the coral tissue is dead the exposed skeleton can be invaded by macroalgae and other bioeroding organisms (Glynn and Manzello 2015). Coral mortality and health could be described by different stages such as recent tissue mortality, old mortality, standing dead, coral bleached and coral diseased. Based on visual appearances healthy corals have none to very minimal tissue damage (exposure to disturbances). Recent tissue mortality is where the coral's tissue has recently died (within the past minutes to days) and the underlying corallite skeletal structure is exposed and still species recognisable, the exposed skeleton can become covered with a thin layer of algae, sediment or bacteria within days of the coral's transition. Old mortality refers to non-living parts of a coral where the corallite structure is no longer recognisable (past tissue death occurred month to years) or is covered by coraline organisms such as algae and sponges. Standing dead refers to corals that are completely dead with zero living tissue present, just the morphological still part of the reef's benthic body ("The Atlantic and Gulf Rapid Reef Assessment Program." n.d.). Some well-known Caribbean corals diseases and their pathogens are black band disease (Phormidium corallyticum, Desulfovibrio,Beggiaotoa), white band (Gram negative bacterium, vibrio carchariae), white pox (serratia marcescens), red band (Oscillatoria sp.), yellow botch (Vibrio sp), dark spots (Vibrio sp.), white plague (Aurantimonas coralicida), bacterial bleaching (vibrio coralliilyticus, vibrio shiloi) and aspergillosis (Aspergillus sydowii) (Harvell et al. 2007). Corallivory, the predation of coral, is a common chronic source of coral tissue loss, corallivores such as fish and invertebrates can induce extensive wounds that can result in reduced coral growth and coral mortality (Rice, Ezzat, and Burkepile 2019). In the Caribbean parrotfish species Sparisoma aurofrenatum, Sparisoma viride, Scarus guacamaia, Scarus taeniopterus and Scarus vetula are known coral predators of reef building corals, where their predation is capable of having a negative effect on coral survival (Roff et al. 2011; Burkepile et al. 2019; Shantz, Ladd, and Burkepile 2020). With the combination of anthropogenic stressors, corallivory can inhibit corals from recovery of these stressors (eg temperature induced bleaching and high nutrient environments) driving corals to mortality directly through predation or indirectly gatewaying for opportunistic microorganisms to infiltrate the coral's microbiome (Rice et al. 2019). The ability for coral colonies to recover from tissue damage caused by natural sources has become challenging due to the presence of anthropogenic sources and factors in the environment, tissue regeneration is inhibited due to decrease in colony fitness and resulting in the wounds then becoming sources of infection and algal overgrowth (Counsell, Johnston, and Sale 2019). This inhibition of tissue regeneration affects coral recovery, coral reproduction and restoration efforts of coral ecosystems, means of combating this inhibition and inducing tissue healing is of great importance (Contardi et al. 2020).

Traditional methods of monitoring and assessing reef health

Coral reef health determination and monitoring systems are traditionally based on methods of physical ecological assessments. Reef substrate evaluation of coral and benthic coverage and using measures that may also consider visual signs of tissue damage, coral disease and bleaching are well established (Harvell et al. 2007; Vallès, Oxenford, and Henderson 2019). Reef

diversity measures are also used to record counts and abundance of fish and other known coral reefs inhabiting species. Counts and abundance statistics of species such as herbivorous fish, other grazers and sponges are used as indicators of a thriving reef system (Aronson and Precht 2006; Bellwood, Hughes, and Hoey 2006; Lang et al. 2017). The combination of above methods produce data that can be used in reef health indexes, scales and models (Teichberg et al. 2018; Camacho et al. 2020). The Barbados government committed to a long-term monitoring program of coral community health in collaboration with the University of the West Indies, Centre for Resource Management and Environmental Studies. This monitoring program was first implemented in 1982 since then 43 permanent reef sites have been established where surveys are performed every five years (Cermes 2018). This program documents the nature and changes of coral reef systems on the west and south coasts of Barbados by relying on key indicators such as the abundance of hard corals, algal species, sponges, soft corals, reef fish, coral recruits, sea urchins (Diadema antillarum), the condition of hard corals considering size and presence of bleaching or disease and the height of turf and macroalgae (Cermes 2018).

The Coral Reef Microbiome

The understanding of microbiomes of marine animals and their inhabiting ecosystems is a growing research area within marine science (Apprill 2017). The microbiome refers to and encompasses all microbial taxa and genes within a given environment along with biotic and abiotic factors (Tipton, Darcy, and Hynson 2019). Microorganisms account for approximately 90% of the global oceans biomass playing integral roles in fundamental biogeochemical processes of maintaining marine environments (Alvarez-Yela et al. 2019). Within each microbiome is a complexity of interactions and relationships among microbial organisms and their environment (Wilkins et al. 2019). Marine microbial communities consist of diverse species of bacteria, eukaryotes, fungi, archaea and viruses (T. D. Ainsworth, Fordyce, and Camp 2017). These microorganisms have been found to also associate with marine animals either within the animal or on the animals exterior; existing a part of the organism's microbiome. Some of these microorganisms are thought to originate from the surrounding supply of seawater which the marine animals inhabit while other cells exist through generational inheritance of the host (Sharp et al. 2007; Apprill 2017). Microbiomes influence and reflect the environment they inhabit, establishing the links of microbial communities, microbial processes and ecosystems processes allows for better understanding of the given ecosystems (Hall et al. 2018). Understanding how these microorganisms play their roles in the global oceans ecosystems is of key importance to understanding how climate change and anthropogenic influences impact marine environments (Shinichi Sunagawa et al. 2020).

The Global Ocean Microbiome

The global ocean ecosystem which comprises diverse habitats and marine environments have been studied holistically to reveal and better understand the morphological, genetic and functional biodiversity of marine microorganisms and how they relate to the physico-chemical changes occurring within the ocean (Karsenti et al. 2011). Joint global scientific studies such as the Tara Ocean Projects and the Sorcerer II Global Ocean Sampling expedition, implemented large scale

oceanic microbial investigations, sampling and metadata collection at various depths within arounds the world's ocean, revealing how a global context of the ubiquitous planktonic communities in different regions of the world's oceans and linkage of taxa and genes to biogeochemical cycles suchs as carbon, nitrogen and sulphur cycling (Rusch et al. 2007; Karsenti et al. 2011; S. Sunagawa et al. 2015). The water column of the photic-zone which tropical coral reef systems inhabit comprise of a high biodiversity of planktonic organisms of different size fractions: pico-nanoplankton (0.8 to 5µm), nanoplankton (5 to 20µm), microplankton (20 to 180µm) and mesoplankton (180 to 2000µm) (Vargas et al. 2015). Open oceanic water like the water column of the photic-zone host key microorganisms associated with marine organisms and their survival eg. corals and dinoflagellate species of Symbiodinium (Decelle et al. 2018).

Marine Microbial Relationships, Associations and Interactions

Symbiosis plays a major role in how some marine organisms survive in marine environments and provides the means and mechanisms for these organisms to compete and adapt to changes within their environments (Apprill 2020). Symbiosis can be seen as a persistent relationship between two or more organisms in which at least one benefits from the other, which usually occurs within marine environments as either parasitism(one benefits and other is harm) or commensalism (both organisms benefit) (Wilkins et al. 2019). Endosymbiosis and ectosymbiosis of microorganisms as seen in corals, sponges, protists, and other marine organisms play direct roles in primary productivity, nutritional acquisition and other biogeochemical processes that are vital for survival within their given ecosystems (Wilkins et al. 2019). However non-symbiotic microbial interactions and associates also play an active role within marine environments, heterotrophic bacteria within the water column are known to interact with eukaryotic phytoplankton within their surrounding microenvironment engaging in beneficial and exploitative productivity such as the exchange of dissolved organic matter for essential nutrients (Weber et al. 2019). These types of interactions between microbial organisms play crucial roles in defining microbial community functions within a given ecosystem (Datta et al. 2016; Pacheco and Segrè 2019). Microbial interactions within marine ecosystems in major processes such as the microbial loop and viral shunt along with microbe competition and allelopathy dictates the community structure, availability of resources and survival conditions of all other organisms within the given ecosystem (Pourtois, Tarnita, and Bonachela 2020; Zoccarato et al. 2020).

The Coral Holobiont

It has been established that corals have formed a close association with not just zooxanthellae but other organisms such as fungi, endolithic algae, bacteria, archaea and viruses forming a communal system known as the coral holobiont which caters to the coral’s functional needs for survival (Littman, Willis, and Bourne 2011). These microbes inhabit the coral’s surface mucus layer, coral tissue and the coral’s skeleton subdivided into distinct microbial communities (M. J. Sweet, Croquer, and Bythell 2011). Extensive research into the coral holobiont has highlighted how this complex symbiosis of the coral-microbial interactions are affected by climate drivers and other disruptive mechanisms (Bourne et al. 2009). Microbial associates within the holobiont are known to play important roles in the coral physiology and health such as nutrient cycling, nitrogen fixation and antibacterial activity (protection from pathogens), environmental stress can cause dissociation of these microbes leading to coral disease and opportunistic infections (M. J. Sweet,

Croquer, and Bythell 2011; Ricci et al. 2019). This network of microorganisms is an integral component of the coral microbiome, the interactions and effects of external biotic and abiotic factors are vital to the understanding of coral resilience. (Hernandez-Agreda et al. 2018) proposed that the bacterial community within the coral microbiome can be divided into three distinct community layers 1) the environmentally responsive community which consist mainly of transient bacteria that where very few are associated with single host individual 2) the resident community consisting of bacteria mainly from Alphaproteobacteria, Gammaproteobacteria and Flavobacteria 3) the core microbiome consisting of bacteria that common and potentially symbiotic. Factors within the water column and surrounding biological community can lead coral microbiome dysbiosis which is the shift in the microbial community structure or the loss of the microbial symbionts, a key player in the global decline of coral health (Lima et al. 2020). The surface mucus layer microbiome is the most influenced microhabitat of the coral microbiome as it acts as an interface between the coral and the environment along with the microbial community within the water column (Apprill, Weber, and Santoro 2016).

Marine Microbial Indicators of Environmental Biotic and Abiotic factors

Within the water column of marine environments such as coral reef systems, the presence of a microbial taxa in a high percentage or low percentage as well as the absence of a microbial taxa or multiple taxa can indicate the conditions or community structure of a given environment (Glasl et al. 2019). As highlighted above coral reefs are impacted by various global and local stressors which can severely alter the natural conditions of the environment and microorganisms are able to respond to these influences instantly so the understanding their natural variability along with shifting community gradients allows for the identification of environmental disturbances with precision (Glasl, Webster, and Bourne 2017). The presence of microbes such as Escherichia coli, Streptococcus spp, Staphylococcus aureus, Pseudomonas aeruginosa, Candida spp, hepatitis, herpes viruses, influenza and enterococci within marine waters bodies have traditionally been used as an indicator for fecal and sewage pollution (Paulino et al. 2020; Verga et al. 2020). The presence and microbial prevalence of heterotrophic taxa such as Pseudomonas sp, Burkholderia sp, Vibrio sp, Legionella sp in coral systems tend to indicate the influence of water sources, for these microorganisms are terrestrial niche specific or non-marine (Stewart et al. 2008; Roitman et al. 2020). With the increase in nutrients within the water column via water sources, the microbial community structure becomes more complex and the prevalence or dominance of heterotrophic microorganisms Is seen (Dinsdale et al. 2008). The conditions of the water quality allows for rapid growth and dominance of heterotrophs due their ability to synthesize and utilise the influx of nutrients (nitrogen, phosphate) compared to most ubiquitous microbes of coral reef systems (Silveira et al. 2019). Increase in water temperatures is known to affect corals by causing bleaching, an indication of increase temperature can be seen within the water column microbial community where mainly heat tolerant microorganisms from bacterial families such as Rhodobacteraceae, Cryomorphaceace, Synechococcaeae, Flavobacterium and opportunistic Vibrio pathogens are seen in high prevalence. Microorganisms from the bacterial family Pelagibacteriaceae and the genus Prochloroccus are indicative of the opposite, where these species are more prevalent at low sea water temperature (Glasl et al. 2019). High prevalence of Pelagibacteriaceace and Prochlorococcus taxa are also indicative of an oligotrophic ocean environment (Robidart et al. 2019). The microbial community can also be indicative of benthic

conditions, such as in algal dominated marine environments where epiphytic microorganisms, mixotrophic and heterotrophic are in high abundance within the water column (Irola-Sansores et al. 2018).

Coral Reef Microbiome Research

Coral Reef Microbiome studies have been conducted globally in the efforts of understanding coral reef systems, their environments, ecological structures, sub-microbiomes and how these main features change due to biotic and abiotic influences, unravelling key links to environment variables (Michael J. Sweet and Bulling 2017). Some past studies have been performed in Australia on the great barrier reef (Glasl et al. 2020), Hawaii (Quinlan et al. 2019), Indo-Pacific (Cleary et al. 2019), Indonesia (Kegler et al. 2017), Northern Line Islands (Dinsdale et al. 2008), Western Indian Ocean(Wambua et al. 2020). Within the Caribbean region some studies have looked at the effect of saharan dust deposition on the microbial community structure in reef surface water (Borchardt et al. 2020), bacterial communities between seawater, black band diseased and dead coral surfaces (Frias-Lopez et al. 2002), diversity of bacteria associated with the Caribbean coral Montastraea franksi (F. Rohwer et al. 2001), microbial signatures of protected and impacted northern Caribbean reefs based on microbial composition and biogeochemistry (Weber et al. 2020), The characterization of bacterial communities present in shallow water Caribbean gorgonian octocorals over time exposed environmental influences (McCauley, Jackson, and Goulet 2020), shift in bacterial of communities of healthy and white plague diseased reef building corals (Cárdenas et al. 2012) and the role of eukaryotic microbes within the coral reef microbiome their part in ecosystem change (T. D. Ainsworth, Fordyce, and Camp 2017). Recent coral reef microbiome studies have relied on the power of metagenomics, utilizing high throughput sequencing to give insights into microbiome compositions, metabolic pathways, microbial functionality and how they link to environmental metadata (Tracy D. Ainsworth, Thurber, and Gates 2010; Glasl et al. 2020).

Why conduct this study?

The health status of coral reef systems surrounding Barbados have relied on traditional methods of visual indicators and measurements of the benthic features to give indication of how local and global factors are affecting the systems. The microbial community within the water of these coral systems, surrounding and interacting corals and other important benthic organisms is relatively unknown. This study will be able to give insight into the inherent microbial composition and diversity inhabiting these marine systems. A baseline knowledge will be generated allowing for the understanding of existing marine microbial communities, how they may relate to present environment factors and creating an initial starting point to track environmental changes in the future (*Microbiology Society* 2019). This study can be the gateway to establishing a core-microbial community of Barbadian coral reef systems allowing for future studies to identify biotic and abiotic drivers of the coral reef microbiome improving the ability of diagnosing coral reef health. This study will also be able to act as an educational tool for the residents of the island, to inform them more about the dynamics of coral reef systems. General population knowledge of coral reef systems is mainly limited to benthic organisms and fish, this study will reveal another layer of these systems and possibly gage the public on how their actions (eg agricultural practices) may influence factors within these ecosystems. By utilising whole genome shotgun sequencing instead

of 16S/18S amplicon sequencing, this study will be able to detect high levels of microorganisms species diversity and genes with high accuracy (Ranjan et al. 2016). This further allows for established DNA profiles found within these marine ecosystems to possibly be used as biomarkers of reef health, progression, adaptation and evolution for future environment monitoring and survey assessment (Parkinson et al. 2020). Predictive measures of changing reef dynamics can be a major source towards specific interventions and key forms of coral reef restoration (Vanwonterghem and Webster 2020).

Study Objective

The purpose of this pilot study was to create a baseline of the coral reef microbiome of the Barbadian reef systems. Water samples were taken from two coral reef systems of different structural features, ecological features and different levels of exposure to local stressors. The assessment of the microbial communities within these coral reef systems will provide insights into the possible state of the coral reefs, where the microbial composition reflects reef health, structure and impact of local and global influences.

Results

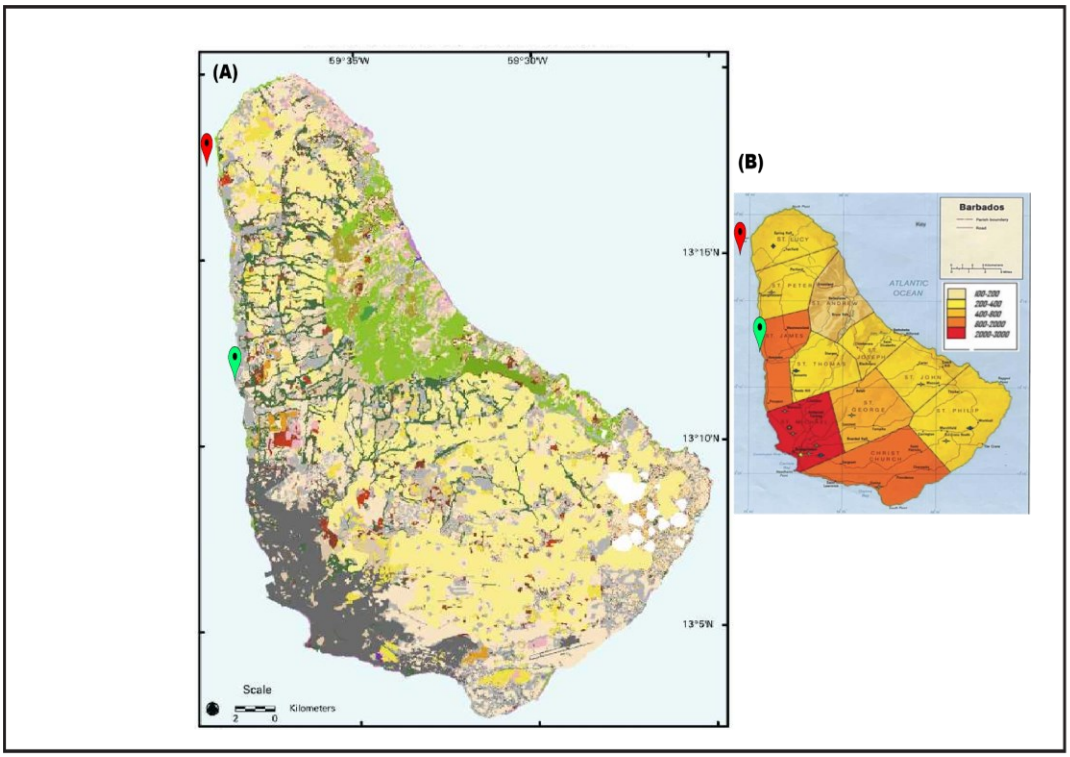
The two reef sites differ in a range of ecological, environmental and health metrics.

Most of the west coast of Barbados have seen extensive development over the years under a tourist driven industry, especially between the northern town of Speightstown to the capital Bridgetown in the south. The Bellairs reef sits centrally in this tourism area within the Folkestone Marine Park in the town of Holetown **(Figure 1)**. This reef site is located in one the most populated parishes (Saint James) on the island where the surrounding coastal area is densely built-up with hotels, restaurants, residential homes and other urban buildings (Helmer et al. 2008). Several runoffs flow through Holetown into the sea, especially the Holetown Watershed which brings freshwater from the inland area of the island (Leitch and Harbor 1999). Though Bellairs reef is located within a protected area of the Folkestone Marine Park the waterfront and surrounding waters are exposed to high human activity.

North of Speightstown, the Maycocks reef is located in a lowly populated parish (Saint Lucy). The surrounding coastal area has little to none urban development other than the nearby Arawak cement plant, south of the reef site (Helmer et al. 2008). A small residential community also exists but inland away from the beachfront. Human activity is significantly low and there is one runoff adjacent to the cement plant. The Bellairs site is the back-reef zone of a fringing coral reef directly adjacent to the shore in shallow water (above 10 m). Maycocks is a bank reef located in deeper water (below 15m) approximately 1.5 km from shore.

Both sites were included in a study two years prior to our sampling that recorded the diversity and relative abundance of corals after bleaching events (H. A. Oxenford and Vallès 2016). This study focused on the five most abundant coral species among their study sites. Porities astreoides was the most abundant species at the Bellairs site with the four other species Orbicella annularis, Diploria strigosa, Montastraea cavernos and Siderastrea siderea having a low abundance. The Maycocks reef is noted to have a more equally distributed across the five

species, with Porities asteroides the most abundant coral. At the end of the two year study, the final transect assessment of the five dominant species among the two sites showed a total of 518 colonies at the Bellairs site across all transacts while at Maycocks they were 673 colonies across all transacts (H.A.Oxenford and Vallès 2016). The average percentage of recently dead coral across all transacts was 0.70% at Bellairs and 0.62% at Maycocks. Based on the Barbados Coral Monitoring Programme 2017 benthic assessment the benthic composition between the two sites shows a significant difference in substrate dominance **(Table 1)**. Bellairs reef substrate was shown to be algal dominated, where filamentous algae covered 54.84% of substratum, 15.85% was covered by hard corals, 13.32% by Coralline algae and 11.5% by sponges. Maycocks reef substrate showed a more level benthic composition in comparison, 26.94% of reef was covered with hard corals, filamentous algae also covered 26.84% followed by coralline algae with 22.48% and sponges with 21%. The average reef fish biomass across the 11 recorded species at Bellairs was 755.10 ($g/100m^2$) while Maycocks it was 806.87 ($g/100m^2$). The sea surface temperature at the time of sampling was estimated to 27 degrees Celsius between the sampling both days.



Urban or Built-up Land
- High-Medium Density Urban or Built-up Land
- Low Density Urban or Built-up Land (Rural or Residential)

Agricultural Land
- Sugar cane
- Mixed and Woody agriculture
- Minor crops

Rangeland, Grassland, Shrubland
- Pasture, Hay or Inactive Agriculture (e.g abandoned sugar cane)
- Pasture, Hay or other Grassy Areas
- Golf course
- Drought Deciduous Woodland

Forested and Non-Forested Wetland
- Emergent Wetland
- Mangrove
- Clouds in imagery

Other
- Quarries
- Coastal Sand and Rock
- Bare Soil (including bulldozed land)
- Water - Permanent

Drought Deciduous or Semi-Deciduous Forest, Lowland or Submontane (Dry, Dry-Moist)
- Deciduous Evergreen Coastal and Mixed Forest or Shrubland, with or without Succulents (includes some Drought Deciduous forest/shrub)
- Drought Deciduous Forest/Shrub
- Semi-Deciduous and Drought Decidouous Forest on Limestone (includes Semi-Evergreen Forest
- Semi-Deciduous and Drought Decidouous Forest (includes Semi-Evergreen Forest

Seasonal Evergreen and Evergreen Forest, Lowland or Submontane (Moist, Wet, Rain)
- Seasonal Evergreen Forest

Figure 1 A. Map of land cover and forest formation of Barbados with colour denotation below. (Helmer et al. 2008). **B.** Map of Barbados showing population density where light yellow is low density and red is high (George 2015).

		Bellairs	Maycocks
Gorgonians		0.14%	0.83%
Hard Coral		15.85%	26.94%
Sponges		11.50%	20.99%
Fleshy/Filamentous algae		54.84%	26.86%
Coralline algae		13.32%	22.48%
Sand/Rubble		4.0%	0.99%
Filamentous Cyanobacteria		0.21%	0.83%
Zoanthid		0.14%	0.08%

Table 1. The benthic composition of the Bellairs and Maycocks Reef based on the percentage cover of major benthic categories on the substratum. This data was collected during the 2017 assessment by the Barbados Coral Reef Monitoring Programme (Cermes 2018).

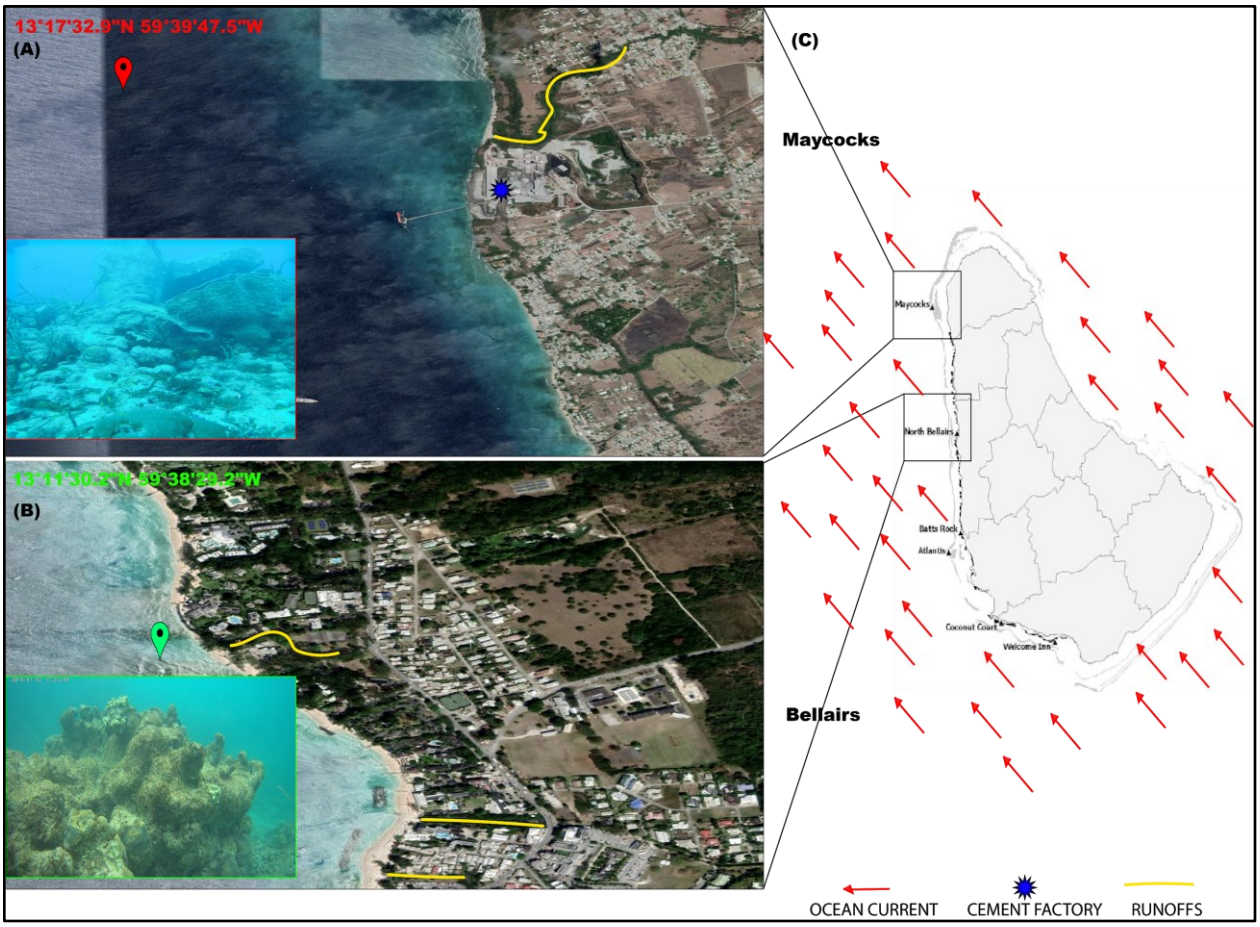


Figure 2 **A.** Aerial view of the Maycocks site and surrounding area with a view of the benthic surface of the bank reef. **B.** Aerial view of the Bellairs site and surrounding area with a view of the benthic surface of the fringing reef. **C.** Map of Barbados, highlighting the extent of coral reefs around the island and some known study sites (H.A.Oxenford et al. 2007) with the sea current direction which occurred during sampling.

Whole genome sequencing of the microbiome of reef water from the two sites.

Water samples were collected from both coral reef sites at different depths but approximately 1m above the reef. Both samples were filtered twice to remove organisms larger than 3µm and smaller than 0.22µm respectively. DNA was extracted and prepared for whole genome shotgun sequencing (NovaSeq PE150, Illumina Inc., **Methods 2-4**). Sequencing generated approximately 19 million and 30.5 million 2x150bp pair-end reads at Bellairs and Maycocks respectively (**Table 2**). Unpaired, short or poor-quality reads were removed - that is 4.1% and 4.5% of all reads for Bellairs and Maycocks, respectively (**Table 2, Methods 5**). Although several methods were used to map reads to reference genome databases (**Figure 3**), the *k*-mer-based classification method implemented in Kraken2 (Wood and Salzberg 2014) refined with Bracken (Lu et al. 2017) was most central to our analysis. Briefly, Kraken2 assigns a query sequence with the lowest common ancestor of all species that share the sequence and Bracken uses a Bayesian method to estimate how much of each species is present among a set of ambiguous species classifications. For our study, we used species in the NCBI database (Pruitt et al. 2012) and Mar marine reference database (Klemetsen et al. 2018) (**Table 1**, **Methods 6**). Read counts from both sites were mapped to nodes of the tree of life created using the NCBI Taxonomy database (Sayers et al. 2009; Benson et al. 2009) **(Methods 7-8)**. Our data is inherently compositional in nature, and our analysis adheres to the so-called Compositional Dataset best practices (**Methods 9**).

			Bellairs	Maycocks	FC
1	Number of raw pair-end reads		19,401,043	30,522,653	1.57
2	Number of raw bases (Methods 2-4)		5,859,144,986	9,217,841,206	1.57
3	Number reads removed (Methods 5)		789,516	1,380,903	1.75
4	**Number of pair-end reads remaining**		**18,611,527**	**29,141,750**	**1.57**
5	Number of reads classified (Kraken)		9,894,597	18,076,397	1.16[a]
6	Number of reads unclassified (Kraken)		8,716,930	11,065,353	0.79[a]
7	Number of species above threshold (Bracken)		10,301	10,919	1.06
9	Number of species below threshold (Bracken)		27,171	29,682	1.09
10	Total number of reads in sample (Bracken)		16,157,053	24,265,733	1.50
11	Reads kept at species level (Bracken)		5,752,498	9,871,052	1.72
12	Reads not distributed		12,340	13,217	1.07
13	Reads discarded based on threshold (Bracken)		53,332	59,891	1.12
14	Number of taxa before removing contaminants		18,807	20,078	1.07
15	**Number of reads before removing contaminants**		**7,374,451**	**13,127,272**	**1.78**
16	Number of Metazoan reads		1,703,655 (23%)	2,320,936 (18%)	1.36

17	Number of Embryophyta reads		1,130,893 (15%)	1,563,503 (12%)	1.38
18	Number of reads mapping to other multicellular taxa		53,900 (<1%)	48,297 (<1%)	0.90
19	**Final number of reads**		**4,486,003**	**9,194,536**	**2.05**
20	**Final number of taxa**		**14,639**	**15,151**	**1.03**
21	**Final number of genera**		**2,429**	**2,509**	**1.03[b]**
22	**Final number of species**		**9,089**	**9,461**	**1.04**

Table 2. Read counts obtained from next generation sequencing at Bellairs and Maycocks.
a. Here the relative fold change incorporating the total number of reads at both sites is reported.
b. The number of reads discarded from species with read totals less than 10 c. 2,317 genera in common. The site-specific genera are typically in low abundance and discussed below.

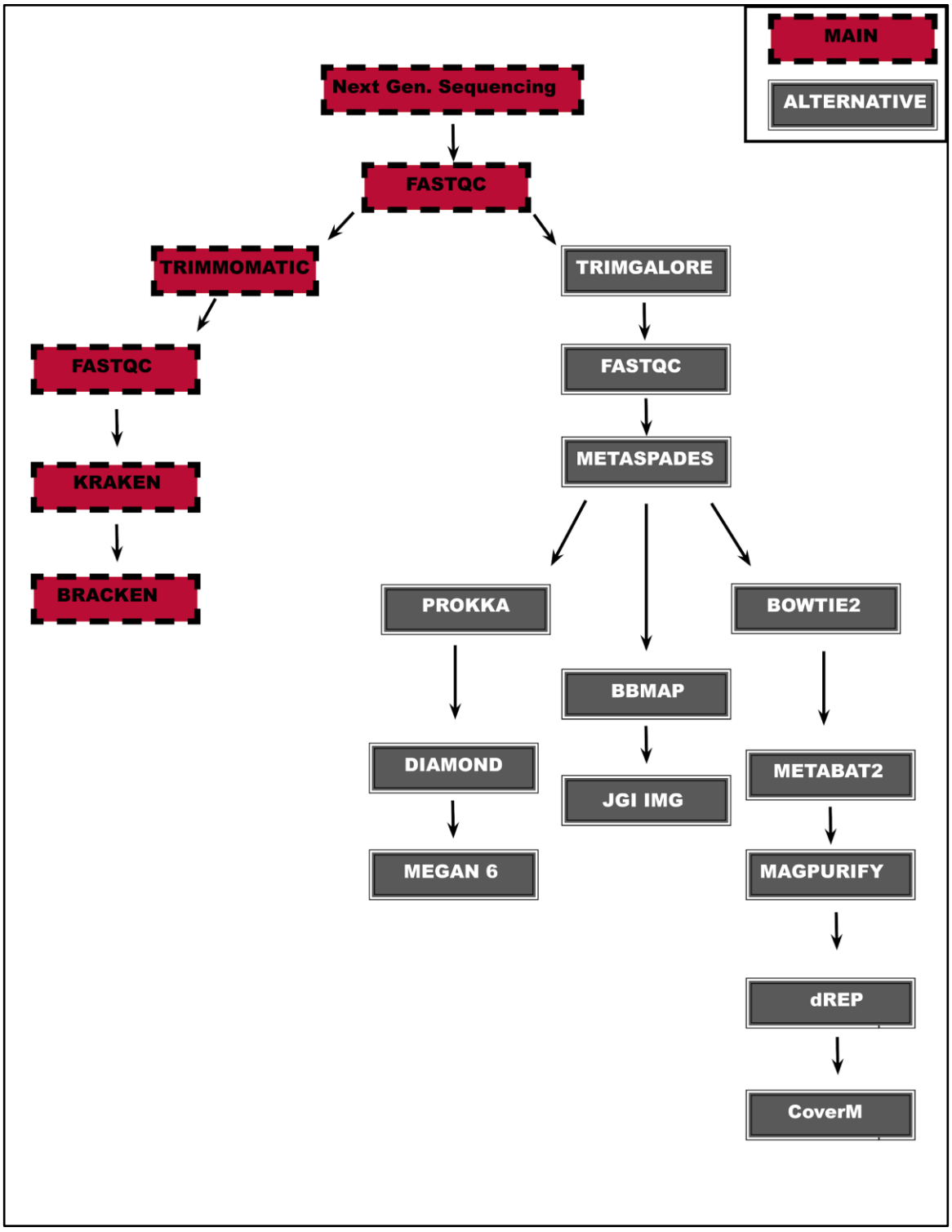


Figure 3. A flowchart describing the main and alternative pipeline used to analyse the whole genome sequencing of the reef microbiome.

Sequence profiles captured a significant number of multicellular organisms

Although our samples were filtered to remove large cells, we nevertheless observed reads mapping to non-microbial taxa such as Metazoa, Embryophyta, and multicellular fungi (**Table 2, rows 15-17**). The genomes of these organisms tend to be orders of magnitude larger in size than Bacteria and Archaebacteria. Therefore even a few such Eukaryotic cells escaping our filtering would provide a disproportionate amount of gDNA in our samples and garner a significant number of sequencing reads. Before removing these organisms from our analysis, we first asked what taxa were identified at the sites. Trace cells will likely not give accurate estimates of relative frequencies, but the existence of marine and related species likely indigenous to the reef system would provide supporting evidence towards the technical validity of our approach to detect marine species.

Multicellular content: Metazoa are primarily marine-related and often indigenous to Barbados

An examination of Metazoa (**Figure 4**) identified several species of fish at both sites including Taki fugu, Sparisoma viride, Latimeria menadoensis, and Megalobrama amblycephala. Although these taxa are indiginous to the Barbadian reef system, most of the remaining species identified did not have a clear link to Barbados, although they remained marine related. We hypothesize that very few reads mapped to very large genomes were not sufficient to reliably differentiate between lower levels in the tree of life. For example, several other species of fish were identified including carp, salmon and atlantic mackerel. Several corals (eg Orbicella faveolata and Porites lobata), Cnidaria (Nematostella vectensis, Hydractinia symbiolongicarpus), sponges (Amphimedon queenslandica), tunicates (Halocynthia roretzi) shrimp (Macrobrachium nipponsense), a tunicate (Oikopleura doica), a sea snail (Haliotis discus), a water flea (Daphnia magna), the tortoise bug (Eurygaster master), several species of (marine) worms, and many basal free-living multicellular Eukaryota (eg Trichoplax adhaerens). Both sites had DNA from nematodes, snakes, insects (including several species of flies) and birds. Both sites had a significant number of reads mapped to human, (Procavia capensis) and green monkey (an old-world primate common across the island), sheep, possum, and rock hyrax. The latter two species have close evolutionary relatives distributed across the island. Reads mapped to several non-indigenous marine mammals, especially at the Maycocks site including seal, walrus. Reads from all Metazoa were removed from our dataset, the relative frequencies of all taxa were recomputed.

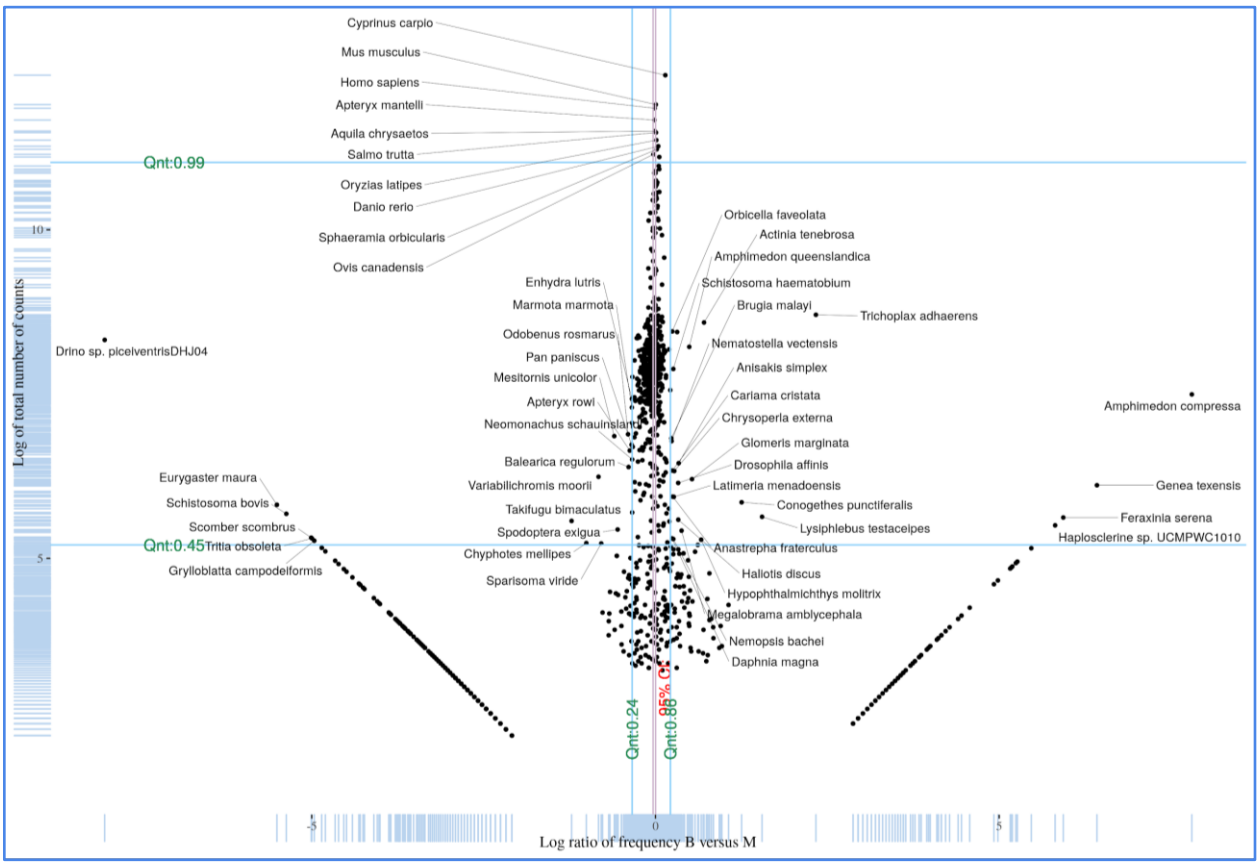


Figure 4. The distribution of Metazoan species. Here the x-axis corresponds to the log ratio of the fraction of reads mapped to a species at Bellairs (relative to the total number of reads mapped to Metazoa at Bellairs) versus the fraction of reads mapped to a species at Maycocks (relative to the total number of Metazoan reads at Maycocks). The y-axis is the log-sum of reads across both sites. Blue lines denote quantiles. The red lines denote a 95% confidence interval for the mean of the distribution.

Multicellular content: Embryophyta correspond to marine, crop or ornamental plants common to the island

An examination of the multicellular Embryophta (**Figure 5**) identified several marine-related species including flowers (Papaver somniferum, Helianthus annuus) and trees. We stress again that the genomes of these taxa are very large but contribute just trace amounts of DNA to our samples. The number of reads is likely insufficient to identify the precise species or genera of tree. However, we did identify coastal salt-tolerant plants including Cynomorium coccineum and Paspalum vaginatum in the Maycocks sample, consistent with the observation that Maycocks has retained a wild grassy beach whereas the Bellairs beaches is adjacent to a research institute and hotels. The presence of Paspalum vaginatum on the island was reported recently and is likely associated with the development of golf courses (McGroary et al. 2014). DNA that may have originated from agricultural crops directly inland from Maycocks were detected including wheat/rich (Triticum timopheevil, Oryza glaberrima), ginseng (Panax ginseng), bamboo (Phyllostachys edulis), and jute (Corchorus capsularis). Several species were identified only at Bellairs including climbing fig (Ficus pumila), loquot (Eriobotrya Japonica), Platycarya tree (Platycarya strobilacea), tropical fern (Nephrolepis biserrata) and the muku tree (Aphanthe aspera). Other than the tropical fern (Nephrolepis biserrata) these species could not be directly identified as being present on shore at Bellairs. Some plant species that can be found on the shore of Bellairs include West Indian mahogany (Swietenia mahagoni), flamboyant tree (Delonix regia), yellow-flamboyant (Peltophorum pterocarpum), Macarthur palm (Ptychosperma macarthurii) golden cane palm (Dypsis lutescens), manchineel tree (Hippomane mancinella), coconut tree (Cocos nucifera) and the whistling pine tree (Casuarina equisetifolia). Although not directly identified in our analysis some of these plant species that are present on the shore come from similar clades and orders of the species identified only at Bellairs. The species C. equisetifolia belongs to the order Fagales of which P. strobilacea is also a member. While species S. mahagoni, D. regia, P. pterocarpum, H. mancinella belong to the Rosids clade of which E. japonica and A. aspera are members of. We note that sequencing at the Bellairs site produced far fewer overall reads that Maycocks (**Table 1**, **row 1**). Therefore, even for a rare species, it is unlikely to be detected at Bellairs but not at Maycocks.

After removing these taxa, we also removed reads mapped to several multicellular fungi and algae, in addition to plasmids and artificial sequences (**row 17**). The parameters of our final dataset are given in **Table 2, rows 18-21**.

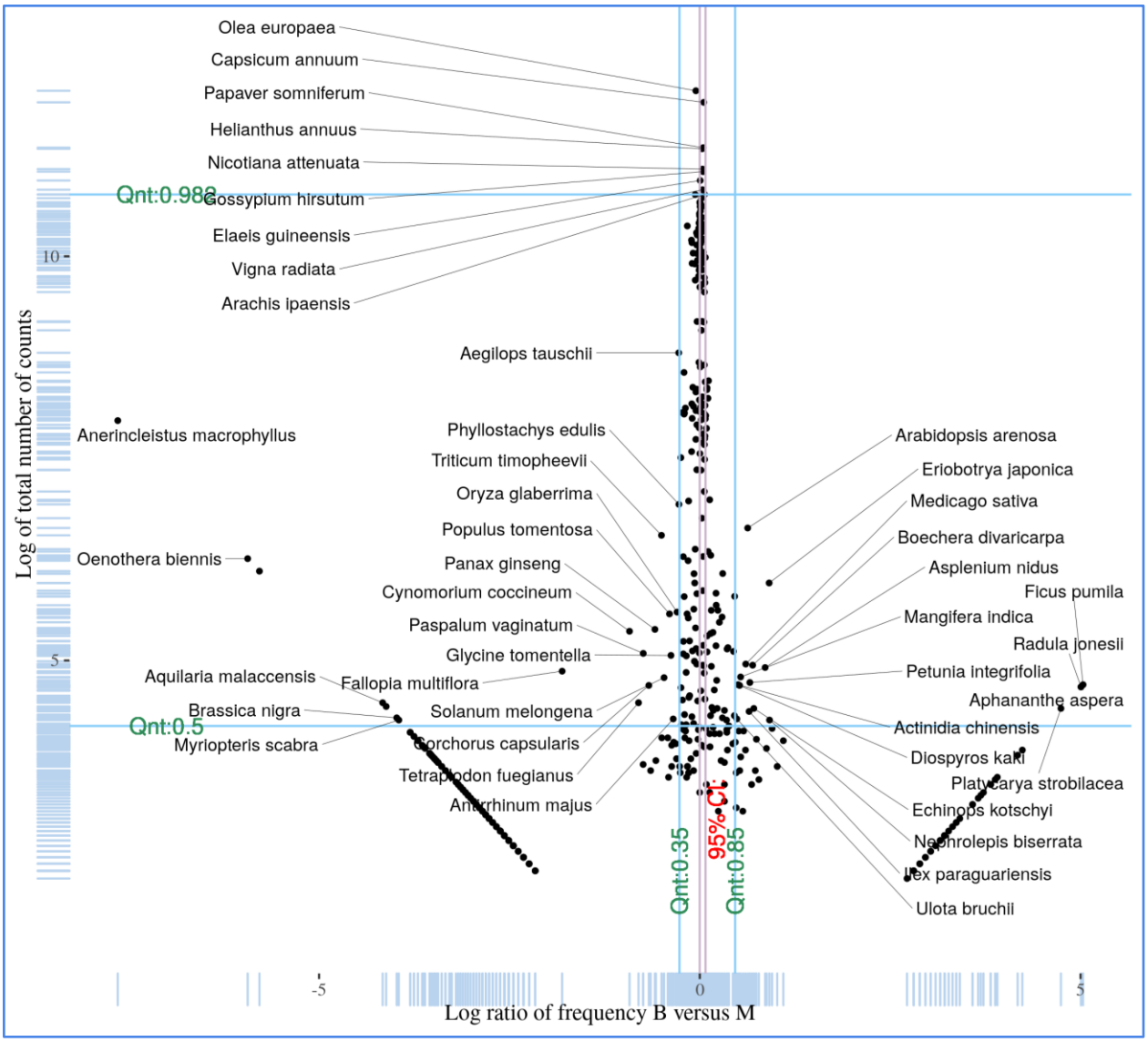


Figure 5. The distribution of Embryophyta species. Here the x-axis corresponds to the log ratio of the fraction of reads mapped to a species at Bellairs (relative to the total number of reads mapped to Metazoa at Bellairs) versus the fraction of reads mapped to a species at Maycocks (relative to the total number of Embryophyta reads at Maycocks). The y-axis is the log-sum of reads across both sites. Blue lines denote quantiles. The red lines denote a 95% confidence interval for the mean of the distribution.

Global differences between the Bellairs and Maycocks sites.

Figure 6 highlights the final distribution of identified taxa between the two sites. At the root level, 93.6% of Bellairs and 95.6% of Maycocks reads mapped to cellular organisms. In the interests of readability, we abbreviate this statement as (93.6% B vs 95.6% M) throughout this manuscript. There were also differences in the number of reads mapped to viruses (5.8% B vs 3.6% M) and for unclassified reads (0.6% B vs 0.8% M). The null hypothesis of a standard Pearson's χ^2 test states that the marginal probabilities for each of these three sub-taxa (cellular, virus, unclassified) are identical between the two sites (**Methods 9a**). Our observed differences are sufficient to reject the null hypothesis in favour of an alternative that there exist differences in the distributions. The estimated p-value is far below 0.01 (written as $p << 0.01$ throughout the manuscript).

Bacteria receive the largest fraction of reads mapping to cellular organisms (78% B vs 83.9% M), representing a 5.9% enrichment at Maycocks. Eukaryota received the second largest fraction (11.9% B vs 9.8% M) followed by Archaea (3.7% B vs 1.9% M). The distribution across the three domains is significantly different (Pearson χ^2 test, $p << 0.01$).

The log-log plot of **Figure 7** provides an overview of the species across all cellular organisms. Here the log-ratio of the fraction of counts at Bellairs versus the fraction of counts at Maycocks is plotted on the x-axis. The (log) total number of counts mapped to the species is plotted along the y-axis. Some such as Candidatus Pelagibacter and Micromonas pusilla are frequently identified at both sites. Bacteria such as Procholorococcus and Synechococcus are enriched at Maycocks, whereas several species of Vibrio are enriched at Bellairs. Archaea such as Marine Group II are biased towards Maycocks whereas Nitrosopumilus and related genera are biased towards Bellairs. Some Eukaryota such as Chrysochromulina tobini and Minutocellus polymorphus only appear at Maycocks and Bellairs, respectively.

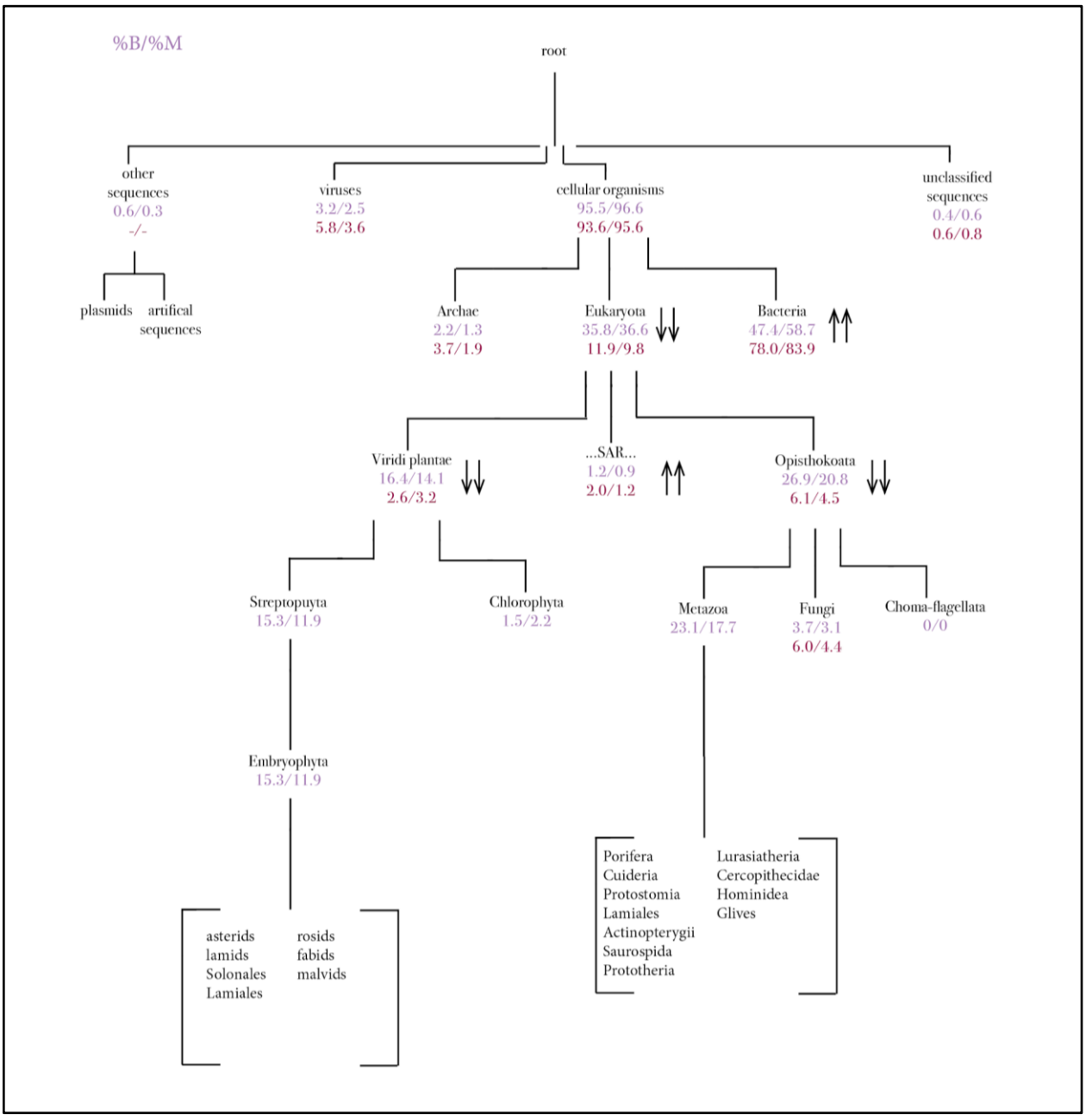


Figure 6. Global differences in percentage read counts at the root of the tree of life in our curated dataset. Percentages were computed after removing contaminants.

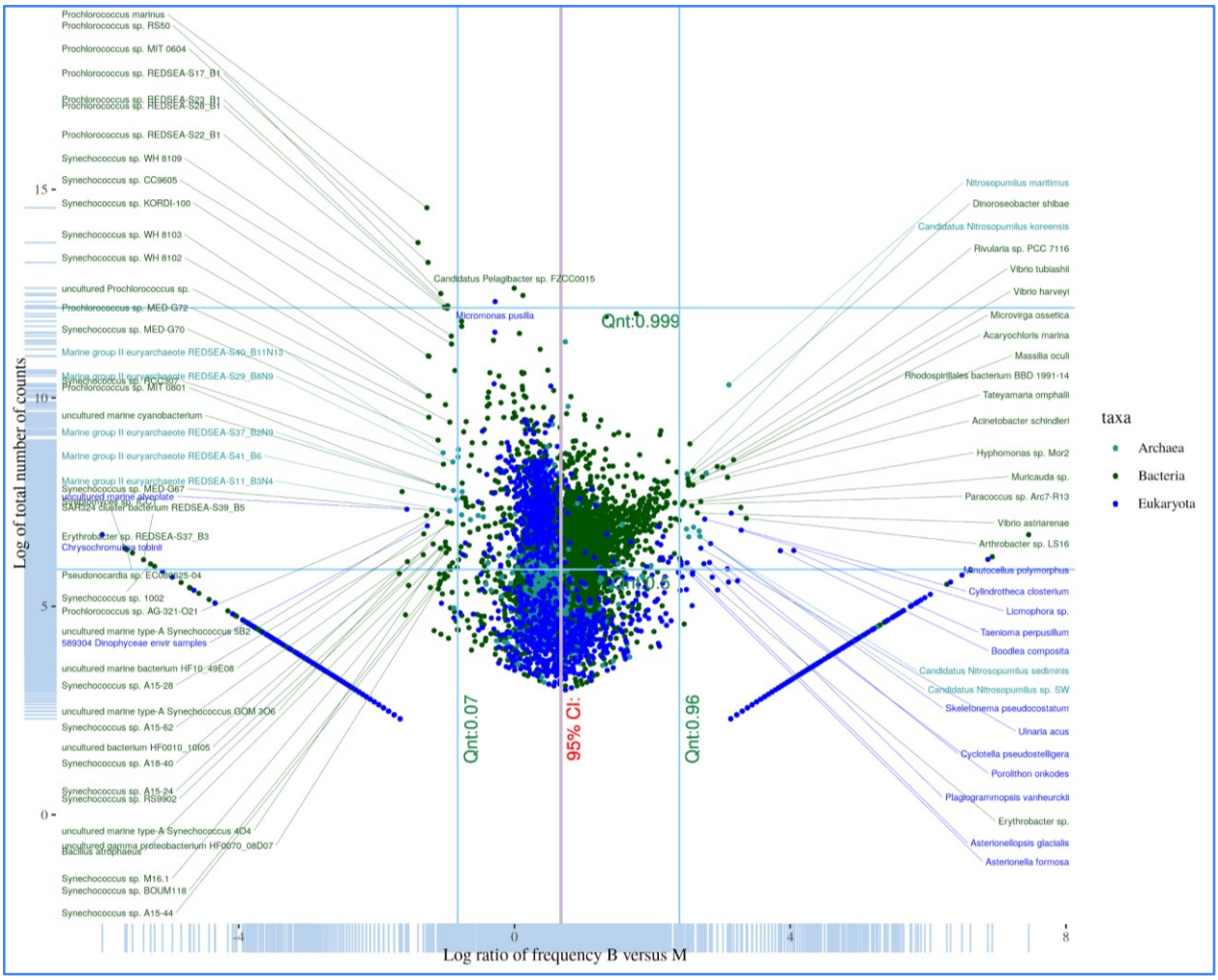


Figure 7. All species of cellular organisms. Log ratio of frequency of species in Bellairs (B) and Maycocks (M) versus the log of the total number of reads for the species at both sites. Red bars denote a 95% confidence interval for the mean of the log-ratios. Blue lines denote quantiles.

Biodiversity can be measured in many different ways including taxonomic diversity (the presence of different species), phylogenetic diversity (the presence of different evolutionary lineages), or functional diversity (the variety of growth forms and resource use strategies) (Le Bagousse-Pinguet et al. 2019). *Abundance* refers to the fraction of each species at a site, and since we are in CoDa setting, this is inherently a relative abundance (**Methods 9**). Diversity is a measure of the distribution of these abundances. Throughout this manuscript we use the Shannon index as our measure of diversity (Tucker et al. 2017) with a permutation based approach to estimate p-values (see **Methods 10**). Briefly, there is an increase in the Shannon index (entropy) as the distribution of abundances approaches a "flat" uniform distribution, and decreased entropy as the frequency of one (or a few species) approaches one.

Richness is defined as the number of species within a specified clade at a site. We asked if there was a difference in richness between Bellairs and Maycocks. However, we must first adjust for the fact that Maycocks and Bellairs had different levels of sequencing coverage. More specifically, budget and technical limitations imply that the number of draws made by the sequencer from the urn is finite although large. The sequencing coverage (total number of reads) may not be sufficiently large to identify with high probability rare species in the sample. For instance, a species whose DNA contributes only 1 read to an urn with 10M reads is unlikely to be identified, if the sequencing coverage is only 1M. In our data, we obtained ~4.5M and ~9.2M mappable paired-end reads from Bellairs and Maycocks respectively (**Table 2, line 19**). Therefore, we expect to identify more taxa in Maycocks than in Bellairs due to this reason alone. In other words, species richness increases with sample size, and differences in richness may be due to differences in sample size.

To address this, we first downsampled from the Illumina paired-end reads from 1-50%, and counted the number of identified species and genera (**Figure 8. A, B**). After only ~3M reads at Maycocks and ~1.5M reads at Bellairs (<⅓ of total in both cases), all taxa have been identified. Maycocks however converges 272 more species (64 more genera) than Bellairs.

Rarefaction provides a second approach to exploring this issue. A statistical correction is computed that estimates the number of taxa we would have observed at the sites if we had sequenced Maycocks to the same coverage as Bellairs (**Methods 10**). Consistent with your downsampling approach, this statistic suggested that no change in the number of identified species. This suggests our sequencing coverage is sufficient for all but the most extremely rare organisms.

In ecological communities including marine, most species are rare (Darwin 1859). Preston argued that this implies that richness would follow a truncated log normal distribution (Preston 1948). This is true for the relative frequencies obtained for our data as depicted in **Figure 8. C, D.** This allows us to estimate the theoretical richness at both sites, the so-called Preston veil (Magurran 2004; Oksanen et al. 2019). Specifically, by integrating the fitted log-normal, the Preston veil speculates how long the right tail is if we had infinite sequencing data. However, consistent with the analysis above, the Preston veil did not predict any new species would have been identified (it predicted 0.28 more species above the 9089 species observed). The results for Maycocks were equally insignificant.

If there is a large difference in the sequencing coverage between sites (as is our case), the presence zeros in the shallower site can have non-intuitive effects on analyses especially with

respect to distance measures and clustering. Moreover, CoDa analysis (such as ours here) often relies on log-ratios of the form $log\ (\frac{p_{B,t}}{p_{M,t}})$ where $p_{B,t}$and $p_{M,t}$are the estimates of the frequency of taxa t at Bellairs and Maycocks respectively. This is referred to as the zero-handling problem (Quinn et al. 2019). We applied a Bayesian-multiplicative replacement strategy that adjusts the count matrix (for all taxa at both sites) in a manner that preserves the ratios between the non-zero components (Martín-Fernández, Palarea-Albaladejo, and Olea 2011). Importantly, the transformation did not have an extreme effect on our count matrix (**Methods 10**).
This analysis is available in `experiments/exp-2-vegan`.

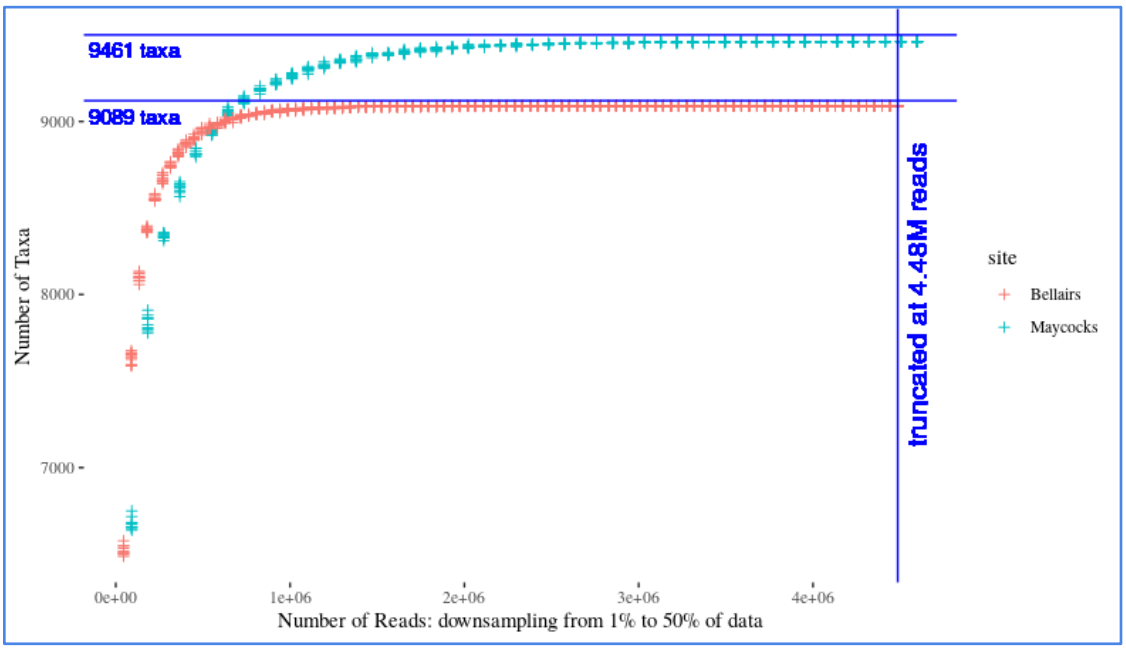


Figure 8 A. Downsampling of the Illumina paired end reads highlighting the number of species identified.

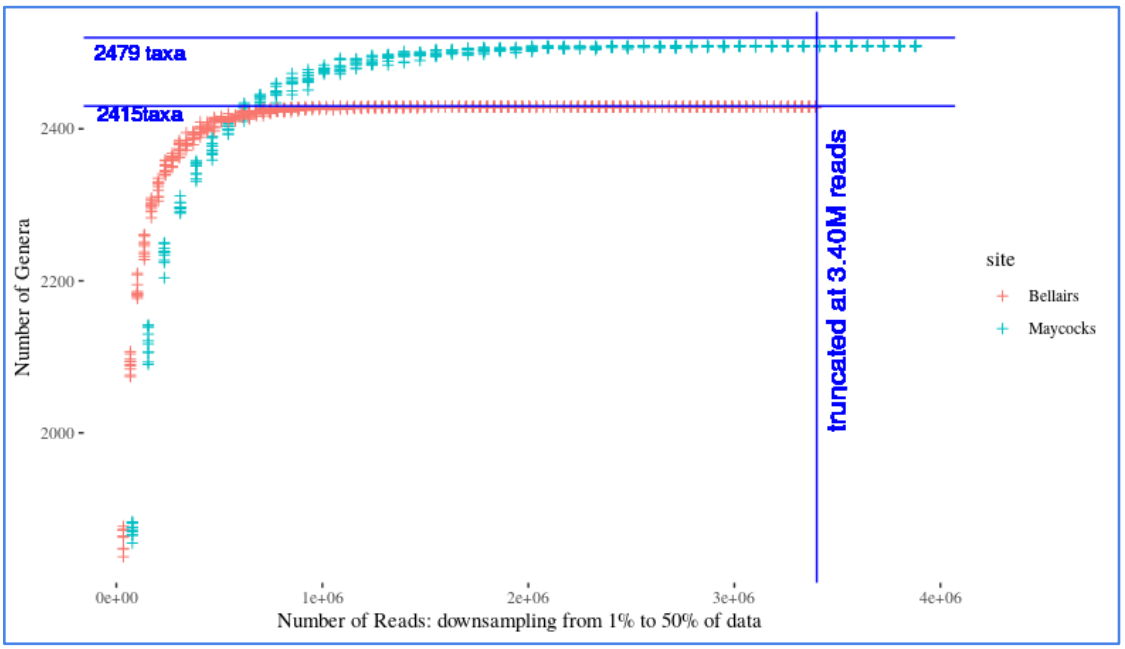


Figure 8 B. Downsampling of the Illumina paired end reads highlighting the number of genera identified.

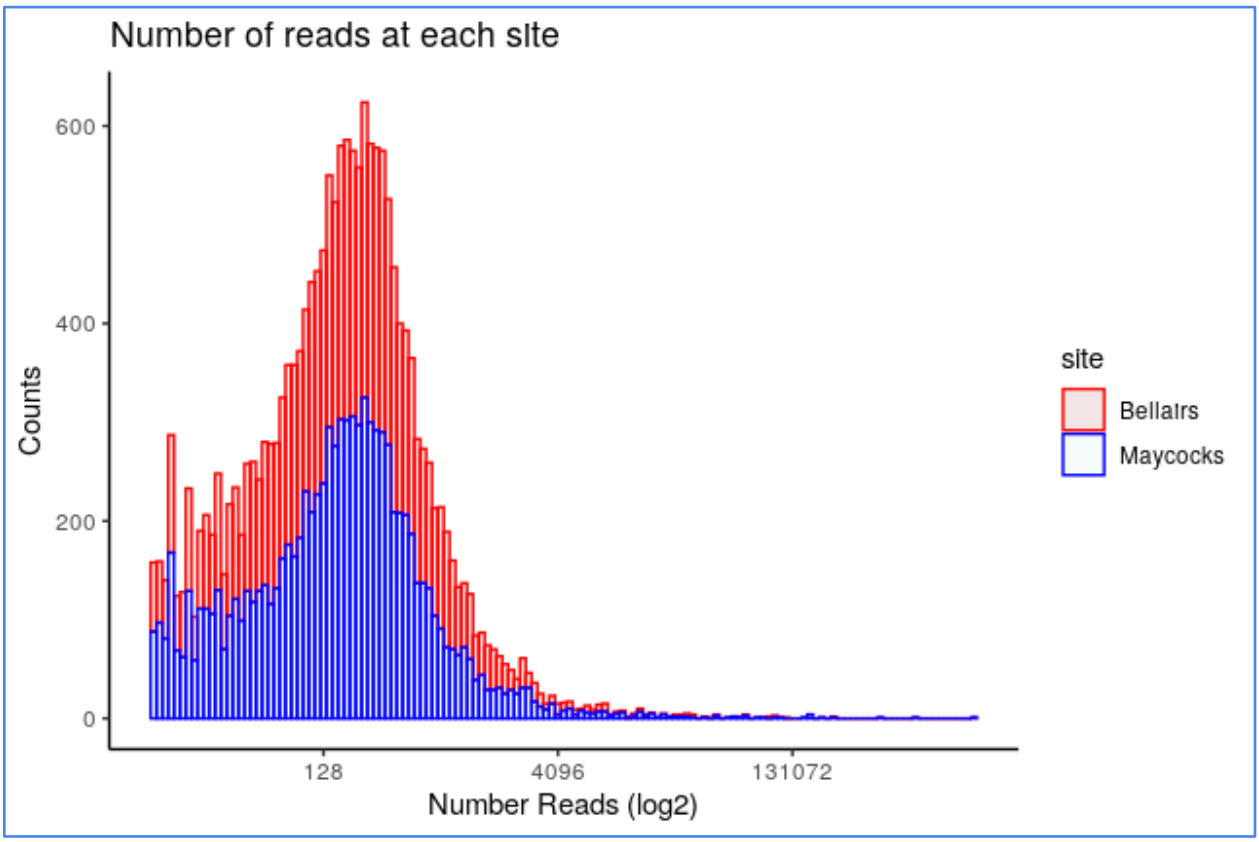


Figure 8 C. Rarefaction highlighting species counts across reads

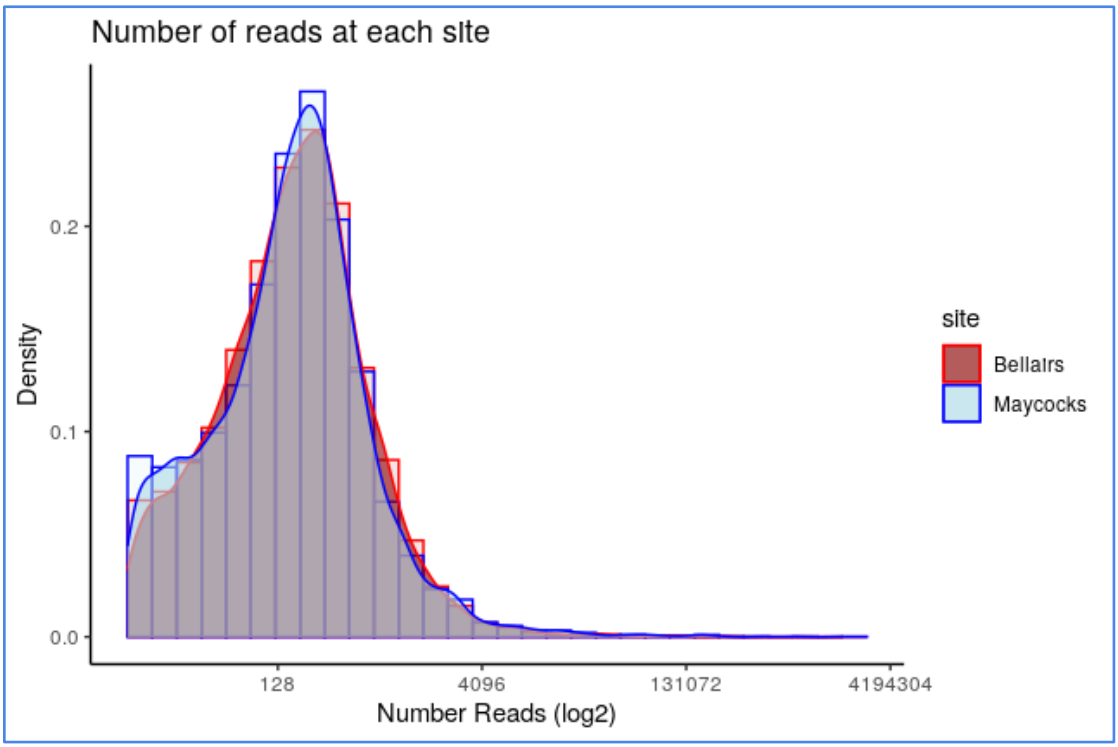


Figure 8 D. Rarefaction highlighting species density across reads

The relationship between genome size and read counts

We asked if there was a correlation between genome size and number of reads aligned to each species across Archaea, Bacteria, Eukaryota and Viruses. The log-log scatterplots of **Figure 10. A-D** and **Figure 12.** depicts these relationships across all species stratified by domain or unstratified respectively. Visual inspection suggests a weak correlation between log genome size and log read count for Eukaryota, but otherwise there is a clear trend for larger genomes to have larger read counts, as expected. To adjust for this effect, we fit a linear model of the form

$$log\ (f_{s,t})\ \backsim\ log\ (g_s)\ +\ \varepsilon$$

where $f_{s,t}$ is the fraction of all reads mapped to taxon t at site s, and g_s is the genome size (Mbp) for species s, and ε is a normally distributed random variable. An implicit assumption in this simple model is that the vast majority of taxa have approximately the same fraction of read counts $f_{s,t}$. The parameters of the fit were then used to correct the observed read counts. Given the compositional nature of our data, any investigations in this manuscript that seek to compare two taxa *within* the same site must first adjust read counts using the linear model.

The corrected relative abundance estimates of all species are depicted in **Figure 11**. The adjustments highlight a high abundance of the bacteria Candidatus Pelagibacter and Prochlorococcus. Several Archaea including the Marine Group II/III and Cand. Poseidoniales appear on magnitude below, followed by viruses that inflect Prochlorococcus and lastly one the Eukaryota Micromonas commoda appear.

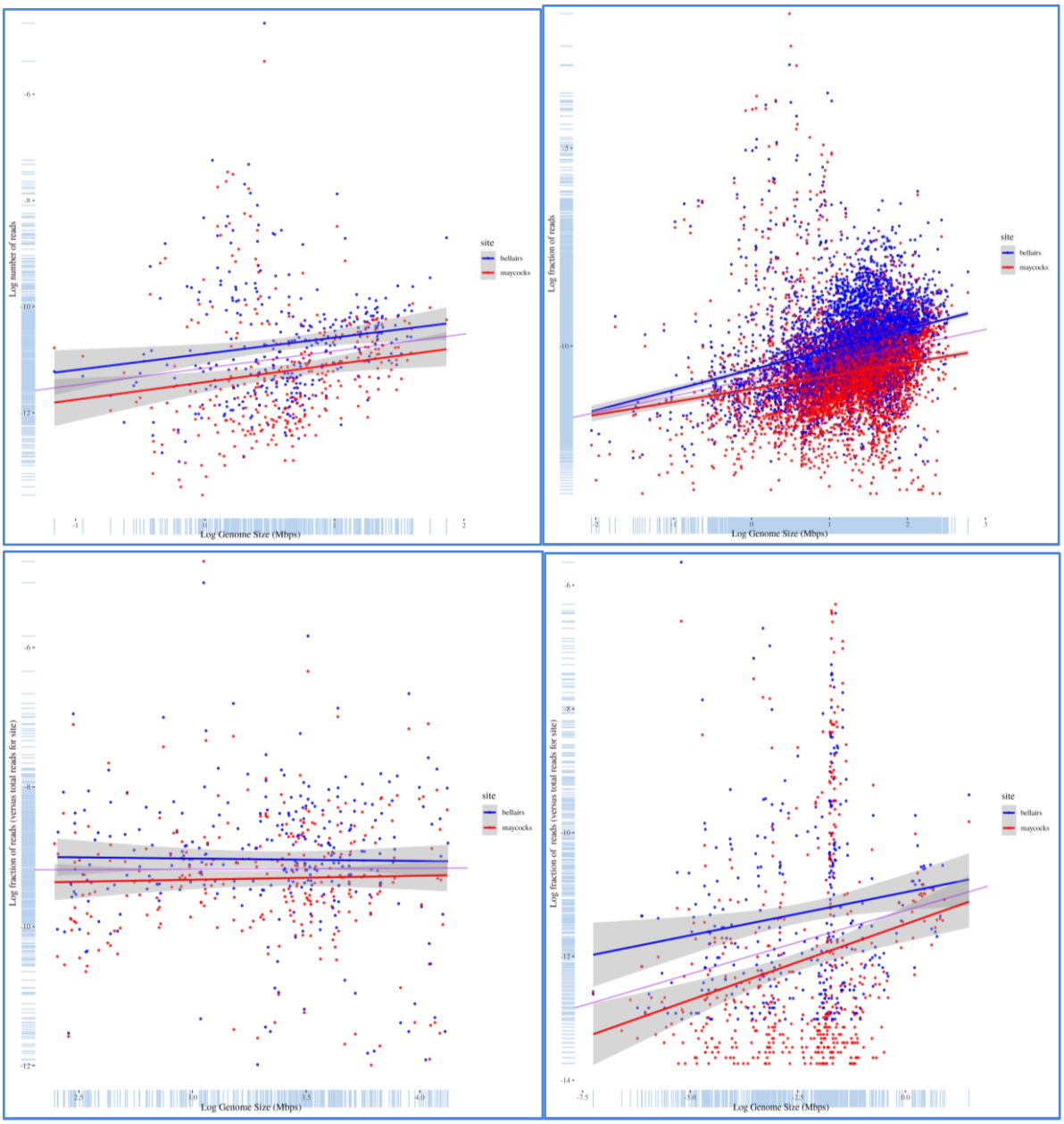


Figure 10. The relationship between genome size and number of reads mapped to the genome. The x-axis corresponds to the log ratio of the fraction of reads mapped to a species at Bellairs and the fraction of reads mapped to a species at Maycocks. The y-axis depicts the log number of reads assigned to a species. **A** Archaea, **B** Bacteria, **C** Eukaryota, **D** Viruses.

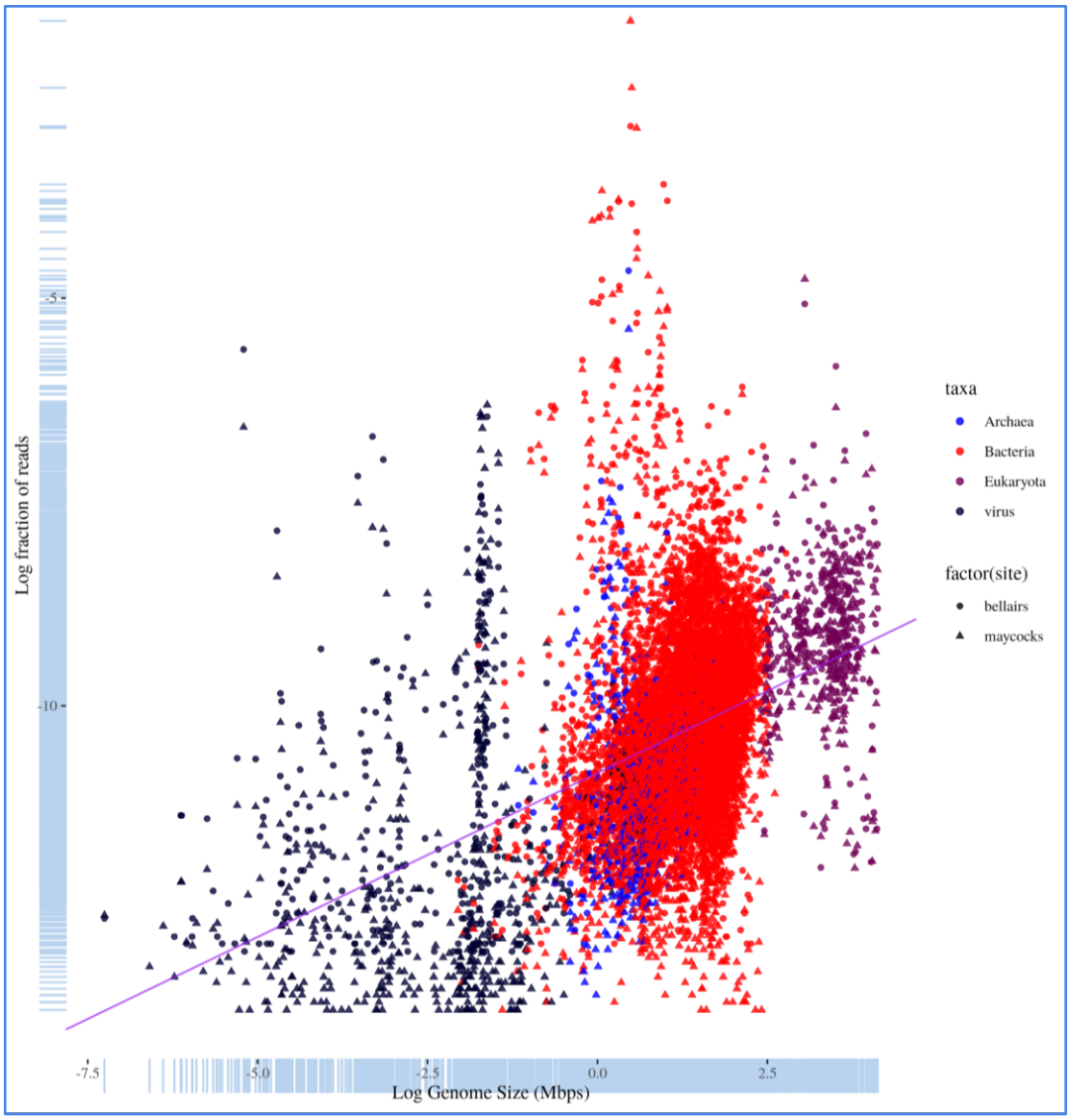


Figure 11. The relationship between genome size and number of reads mapped to the genome as in **Figure 10** but for all species across all domains.

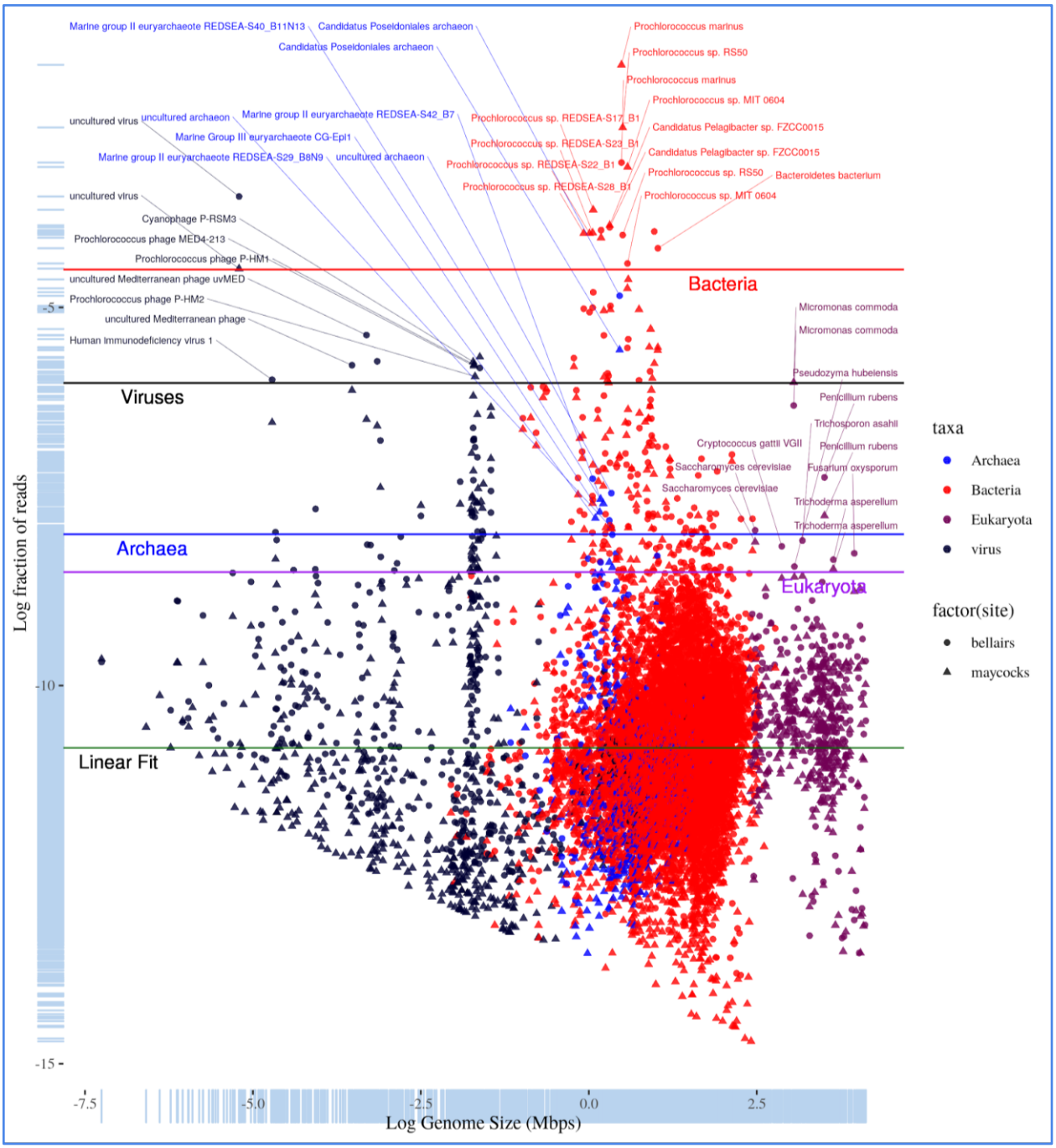


Figure 12. The relationship between genome size and number of reads mapped to the genome as in **Figure 11** but for all species across all domains after adjusting by the slope obtained from a linear model across all species.

Comparison with the Tara Oceans Project (2009-2013)

The Tara Oceans project combines ecology, systems biology and oceanography to study marine ecosystems (Karsenti et al. 2011; Pesant et al. 2015). The study is unique in both its scale and complexity. We focus here on the Tara Oceans 2009-2013 project which performed a comprehensive worldwide sampling of plankton in the upper layers of the ocean (down to 200m and twilight zone) from 210 sites, using standardized protocols (Kultima et al. 2012; Alberti et al. 2017; Pesant et al. 2015) to capture the morphological and genetic diversity of the planktonic community from viruses to small zooplankton. In addition to robust whole genome shotgun profiles from whole DNA, they also measured key physical, chemical and in situ hydrographic parameters of the environmental context of each sample. Tara Oceans did not sample in the vicinity of Barbados, although there is one sample near the coast of Panama (#141), several samples along the eastern North American Atlantic coast (#142, 145, 146), Bermuda (#148), several from the southern Atlantic including (#72, open ocean) and (#76 ,Brazil).

Approximately 81% of all reads were mapped to Bacteria in both our dataset and Tara Oceans. Similarly, both datasets identified approximately 3% of all reads as Archaea. The two datasets diverge more significantly for Viruses (7.5% Tara vs 4.7% Barbados) and Eukaryota (4.6% Tara vs 10.9% Barbados) (S. Sunagawa et al. 2015).

Bacteria

In total, 78% B and 84% M of all reads were mapped to Bacteria (**Figure 6**). Within this domain, the Bellairs site were enriched in gram negative Proteobacteria (48% B vs 25% M) and the Fibriobacteres-Chlorobi-Bacteroidetes group (10 % B, 3% M) (**Figure 13**). The Maycocks site had a strong preference for the Terrabacteria group (37% B vs 69% M). The remaining subtaxa of bacteria had small numbers of reads assigned to them (<1%). The PVC group accounted for 1.6 % B and 0.6% at M of all reads mapped to Bacteria. The differences between all four groups are highly significant (all tests, $p << 0.01$, **Methods 9a-c**).

Figures 14 and **15** establish that the vast majority of differentially abundant genera and species correspond to Proteobacteria and Terrabacteria. We observe a strong global shift of the Terrabacteria towards Maycocks and a counter-shift of Proteobacteria towards Bellairs (KW, $p << 0.01$).

There was evidence for 6,279 species at Bellairs and 6,459 species at Maycocks across 1,434 distinct bacterial genera. The increased richness at Maycocks is consistent with the fact that we have a 1.57 fold increase in the number of reads at this site compared to Bellairs. The deeper sequencing increases the change of detecting rare species.

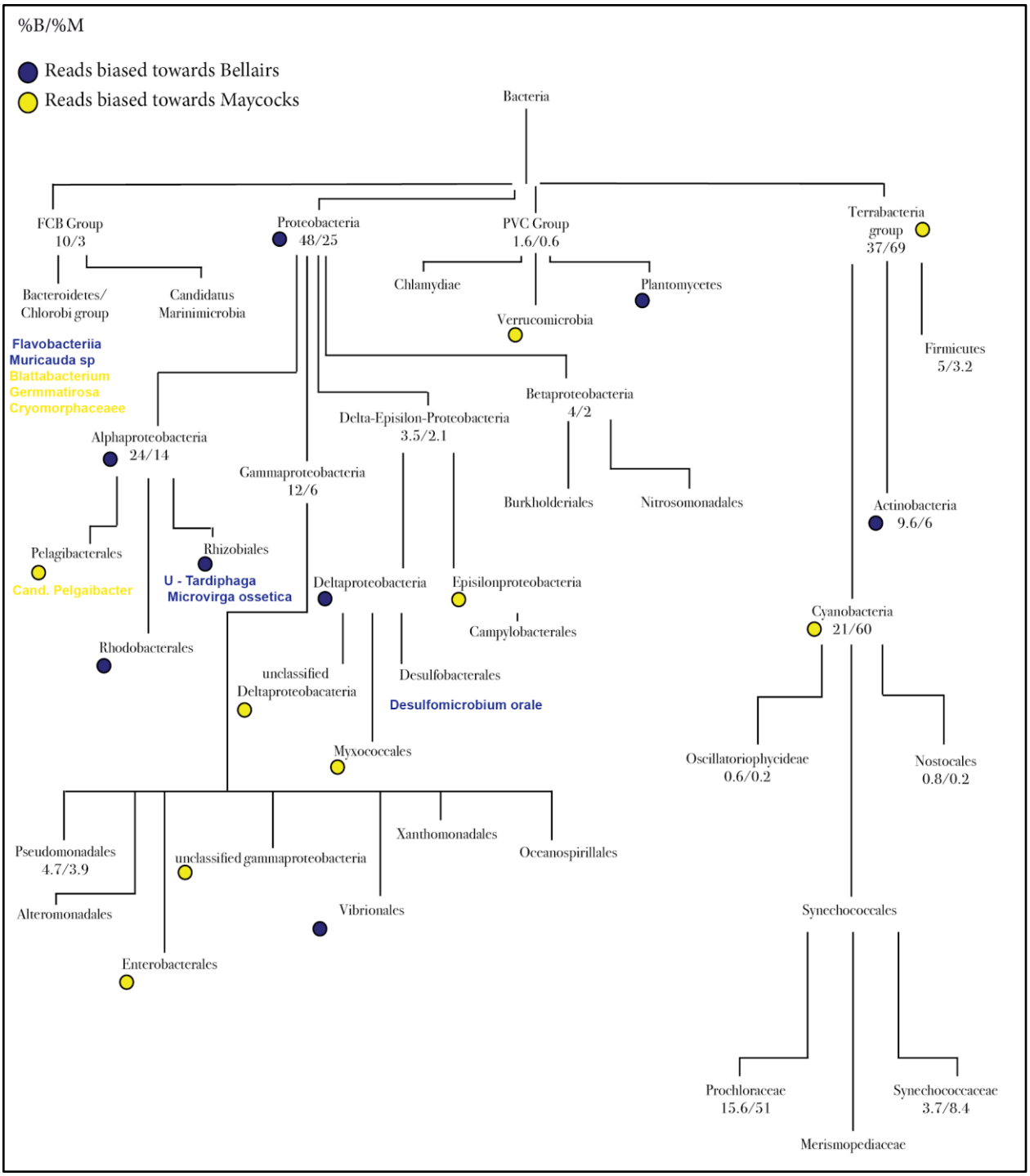


Figure 13. A schematic of the major Bacteria taxa identified at Bellairs and Maycocks. Here the numbers are the percentage of all bacterial reads mapped to the taxa (B/M). Blue and yellow circles indicate taxa where reads are significantly biased towards Bellairs and Maycocks respectively as determined via the K-W test (**Methods 9c**).

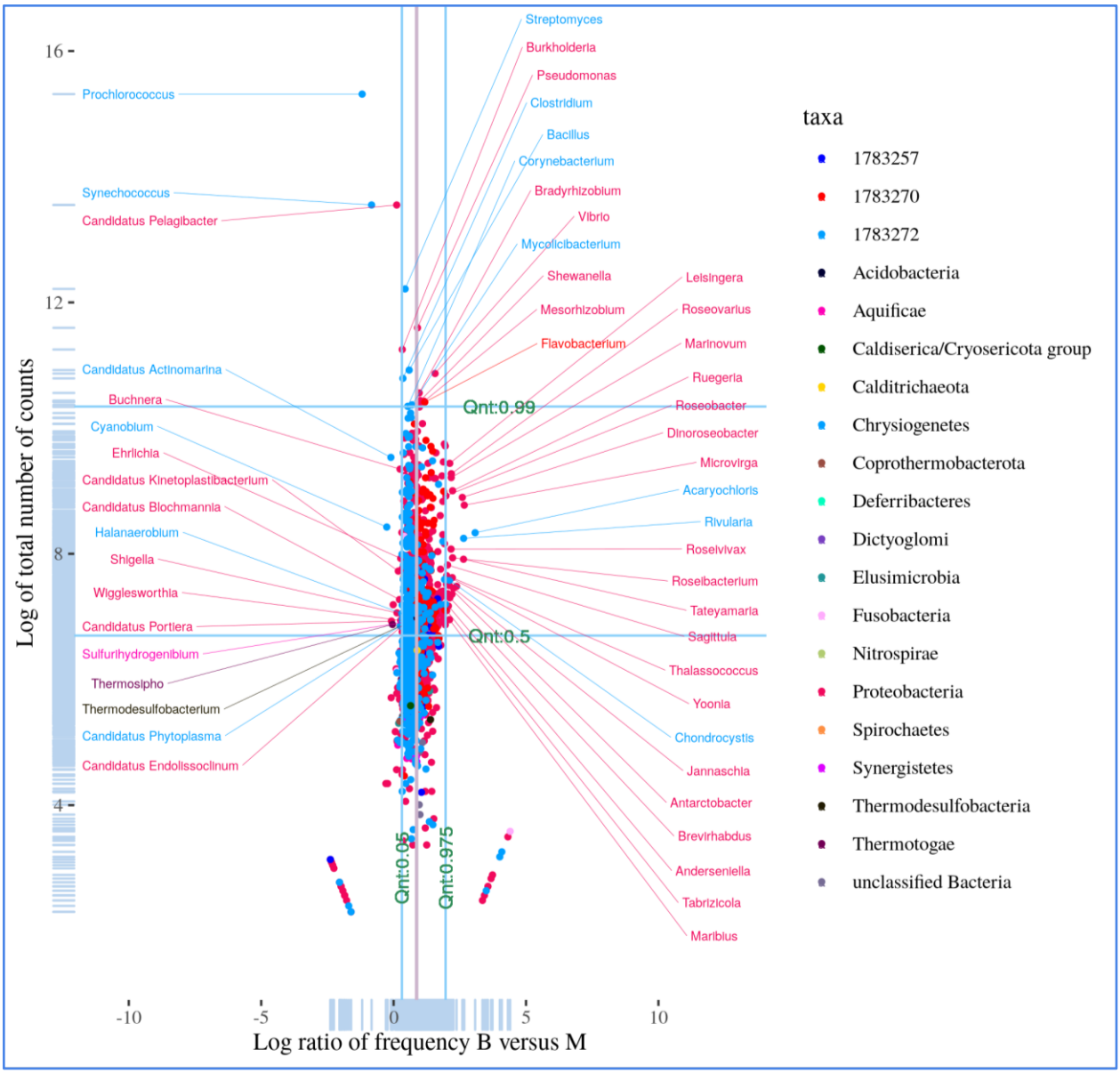


Figure 14. Analogous to the log-log scatter plot of **Figure 15** across all of the bacterial domain but at the genus level.

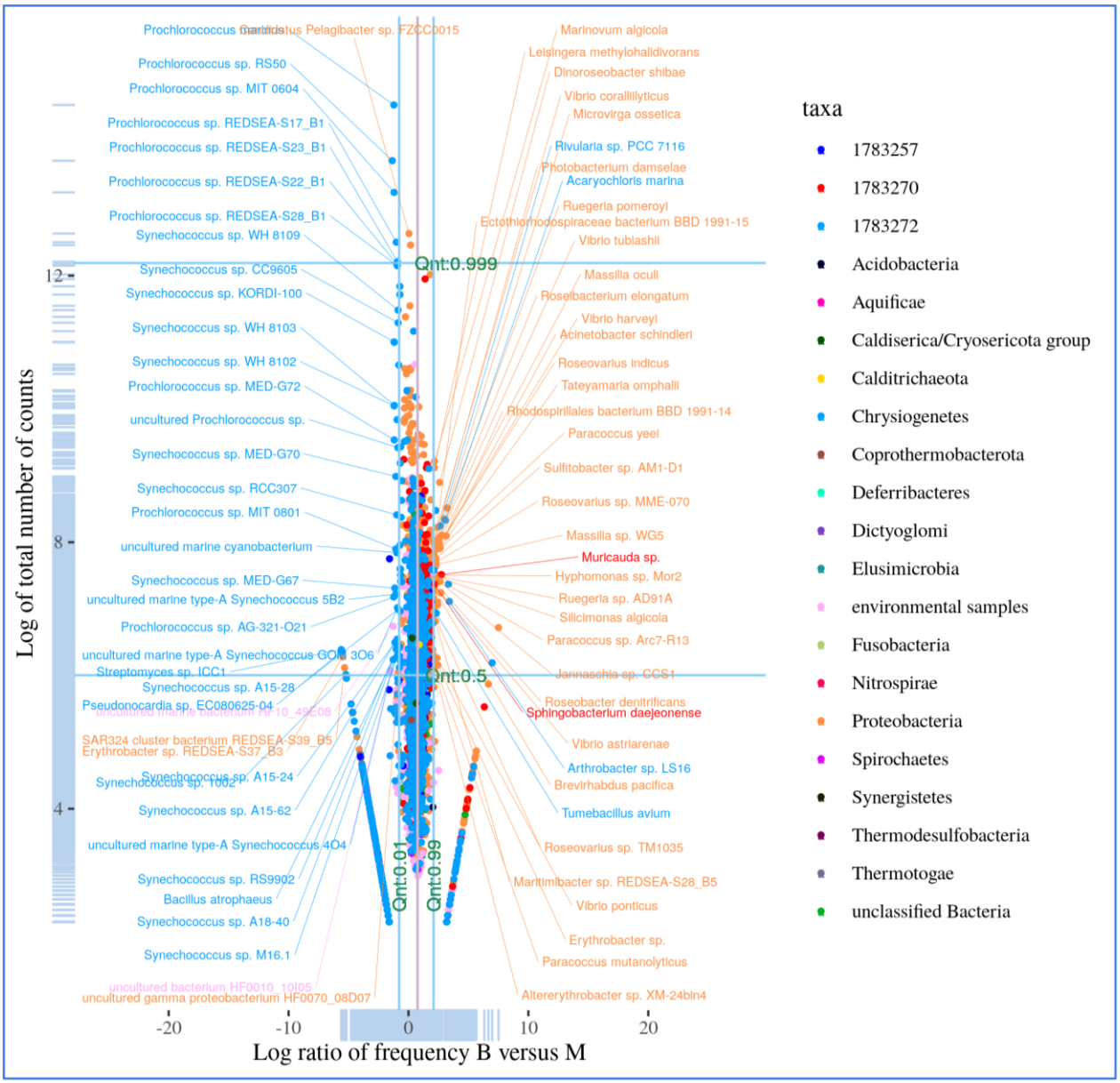


Figure 15. A log-log scatter plot of the log ratio of reads for all bacterial species versus the total number the total number of reads for the species.

Bacteria: Comparison with the Tara Oceans data

We analyzed here a subset of the Tara Oceans data consisting of 243 samples from 68 locations in epipelagic and mesopelagic waters (S. Sunagawa et al. 2015) but further restricted our attention to profiles that had been filtered to capture only organisms ranging in size from 0.02 μm to 3μm to match our approach (**Methods 2**), and to only bacterial taxa (n=139). The original analysis of the Tara Oceans data identified significant fractions of Alpha- and Gamma-proteobacteria with high levels of SAR11 and SAR86 as expected. They also identified an abundance of Cyanobacteria and Deferribacteres albeit with less taxonomic richness. **Figure 16.** is adapted from (S. Sunagawa et al. 2015) and compares the relative abundance and richness across the two datasets at the phylum level (class level for Proteobacteria). **Methods 10** describes the normalization and transformations in more detail, however briefly here we computed the estimations of the relative abundance and richness (total number of species) for each taxon from the estimations of abundance for genera provided by the Tara Oceans effort. Since the figures from Sunagawa et al. covered all prokaryotes, we also performed analyses of our data relative to all bacterial and archaeal reads here. We observe general agreement between our data.

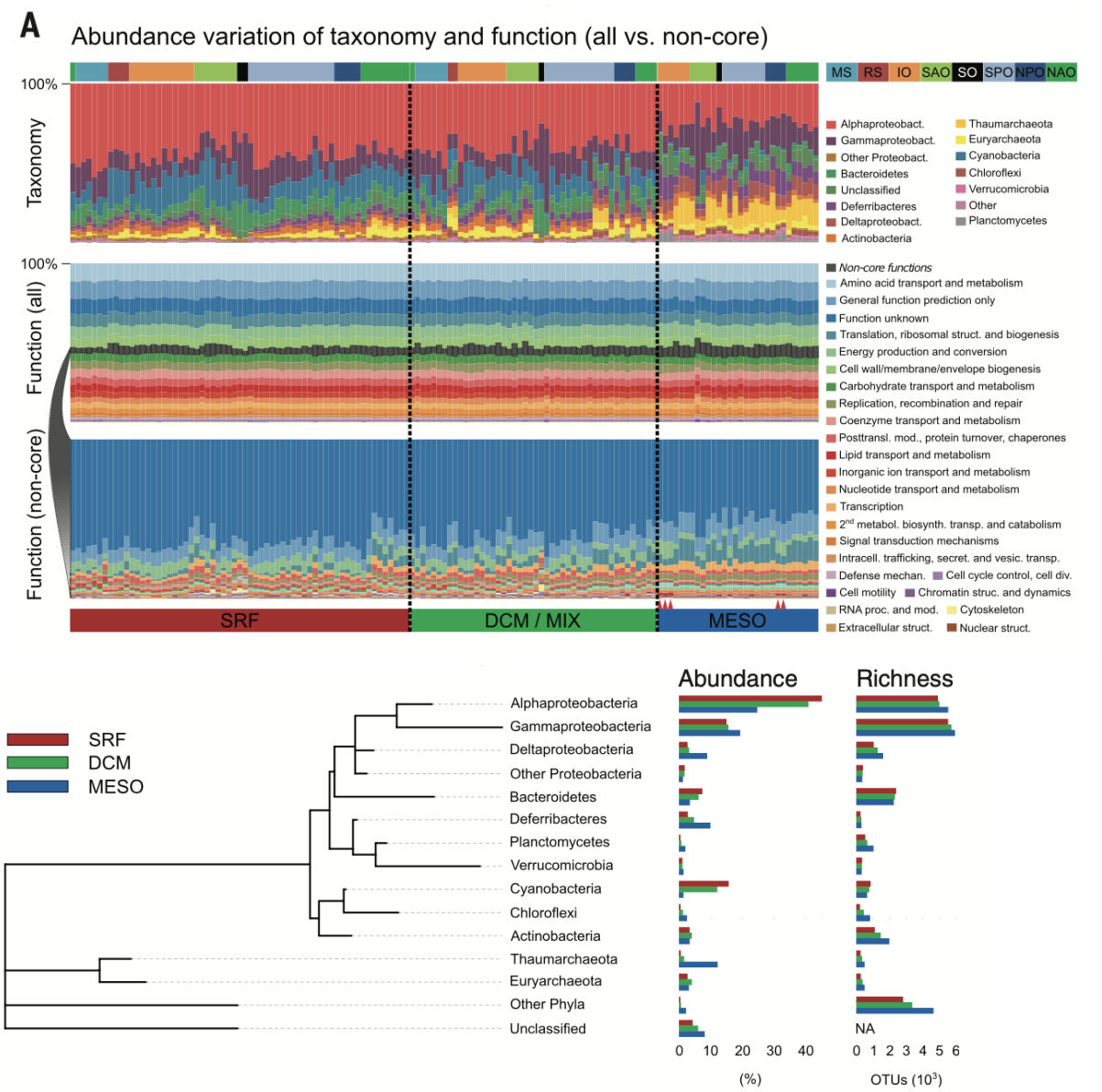


Fig. 2. Taxonomic breakdown of *Tara* Oceans samples. A phylum-level (class-level for Proteobacteria) breakdown of relative abundances is shown for all prokaryotic samples from three depth layers along with the number of detected taxa at the OTU level. SRF, surface water layer; DCM, deep chlorophyll maximum layer; MESO, mesopelagic zone.

Figure 16. Modified from Sunagawa et al.

Bacteria: Unsupervised analysis with Tara Oceans classifies our samples primarily by temperature

We also performed unsupervised analysis to investigate how our sites compare across the global sites sampled by Tara Oceans. Briefly, we selected the most abundant genera across all sites, ranked the total collection of genera at each site, and performed two-dimensional hierarchical clustering with the Kendall τ distance metric. **Figure 17.** depicts the relationships between the sites both with respect to the relative abundance measurements and the associated physio-chemical-hydrographic attributes. We observe that subtree rooted by the least common ancestor of Bellairs and Maycocks (denoted by a start) consists exclusively of samples harvested from the surface (denoted SRF) or from the deep chlorophyll maximum layer (DCM), and depleted for samples from the mesopelagic and epipelagic zones, as expected. This subtree also has lower richness, Chao1, and Shannon entropy, than remaining samples (left subtree from root), and far from polar sites. The same subtree is also enriched for autotrophs. This is consistent with the fact that Barbados is a hot climate with oligotrophic waters.

The Bellairs site co-clusters with samples obtained from the trades and coastal biome, as expected. These nearest neighbours also originate from warm climates (Indian Ocean and Red Sea) and from surface samples. This is consistent with the finding from Sunagawa et al. that depth is the single most important factor that determines species abundance as it explains 75% of variation via Principal Coordinate Analysis (S. Sunagawa et al. 2015). Both sites co-cluster with samples with low concentrations of nitrates, N02, PO4, NO2NO3, and SI.

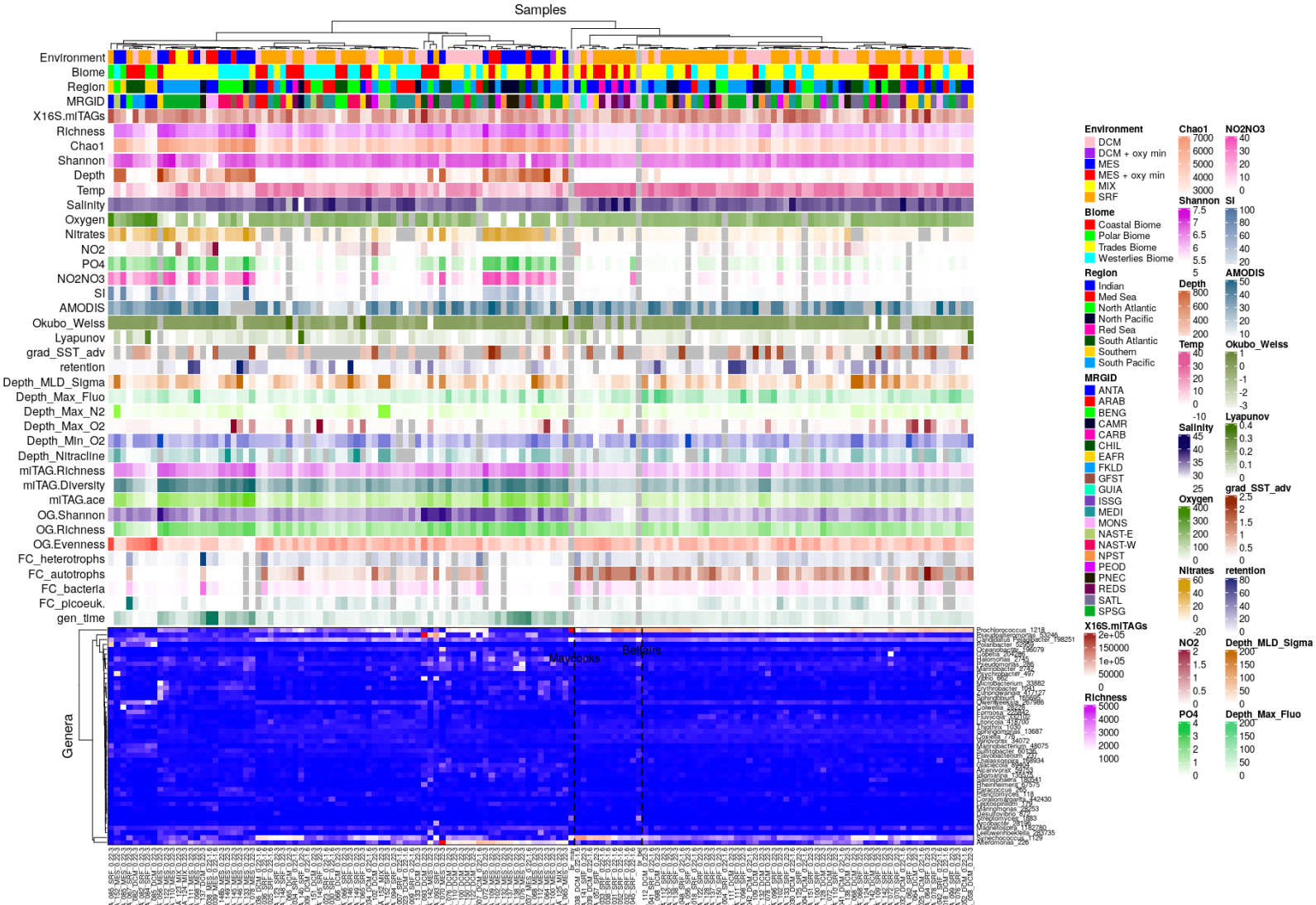

Samples
Environment
Biome
Region
MRGID
X16S.mITAGs
Richness
Chao1
Shannon
Depth
Temp
Salinity
Oxygen
Nitrates
NO2
PO4
NO2NO3
SI
AMODIS
Okubo_Weiss
Lyapunov
grad_SST_adv
retention
Depth_MLD_Sigma
Depth_Max_Fluo
Depth_Max_N2
Depth_Max_O2
Depth_Min_O2
Depth_NitraCline
mITAG.Richness
mITAG.Diversity
mITAG.ace
OG.Shannon
OG.Richness
OG.Evenness
FC_heterotrophs
FC_autotrophs
FC_bacteria
FC_picoeuk.
gen_time
Genera
Maycocks
Bellairs
Environment
DCM
DCM + oxy min
MES
MES + oxy min
MIX
SRF
Biome
Coastal Biome
Polar Biome
Trades Biome
Westerlies Biome
Region
Indian
Med Sea
North Atlantic
North Pacific
Red Sea
South Atlantic
Southern
South Pacific
MRGID
ANTA
ARAB
BENG
CAMR
CARB
CHIL
EAFR
FKLD
GFST
GUIA
ISSG
MEDI
MONS
NAST-E
NAST-W
NPST
PEOD
PNEC
REDS
SATL
SPSG
X16S.mITAGs
Richness
Chao1
Shannon
Depth
Temp
Salinity
Oxygen
Nitrates
NO2
PO4
NO2NO3
SI
AMODIS
Okubo_Weiss
Lyapunov
grad_SST_adv
retention
Depth_MLD_Sigma
Depth_Max_Fluo

Figure 17. Heat Map depicting the Unsupervised classification of Maycocks and Bellairs against Global sampling sites of Tara Oceans and the sites' respective metadata**.**

Bacteria, PVC superphylum: Bellairs is enriched for taxa associated with coral decay and algae cover

Within the PVC, the Planctomycetes were more prevalent at Bellairs than Maycocks (KW, $p << 0.01$) with the genus Rhodopirellula exhibiting differentially abundance (**Figure 18**). Rhodopirellula is a widely distributed marine genus that plays an important role in global carbon and nitrogen cycling (Žure et al. 2017). Rhodopirellula species are known to be abundant in degraded coral reefs where turf algae is dominant (Walsh et al. 2017). The Bellairs site also has a large increase in the abundance of the macroalgae associated species Mariniblastus fucicola (Lage et al. 2017; Faria et al. 2018) and the marine halophilic species Gimesia maris (Ferreira et al. 2016) (**Figure 19**). The elevated abundances of these taxa are consistent with the reduced health of the Bellairs reef.

The Verrucomicrobia phylum was present at both sites albeit in low abundance (<1% both sites) with a relative shift in abundance towards Maycocks (KW, $p << 0.01$)**.** The low abundance in our data is surprising given that Verrucomicrobia are considered the fourth most abundant bacterial phylum in the world's oceans (Freitas et al. 2012). Several of these species were only identified at Maycocks (represented by the left wing of the "V" in **Figure 19**. All of the Verrucomicrobia outliers are currently poorly characterized. The phylum Chlamydia was at low abundance and equally distributed between the two sites.

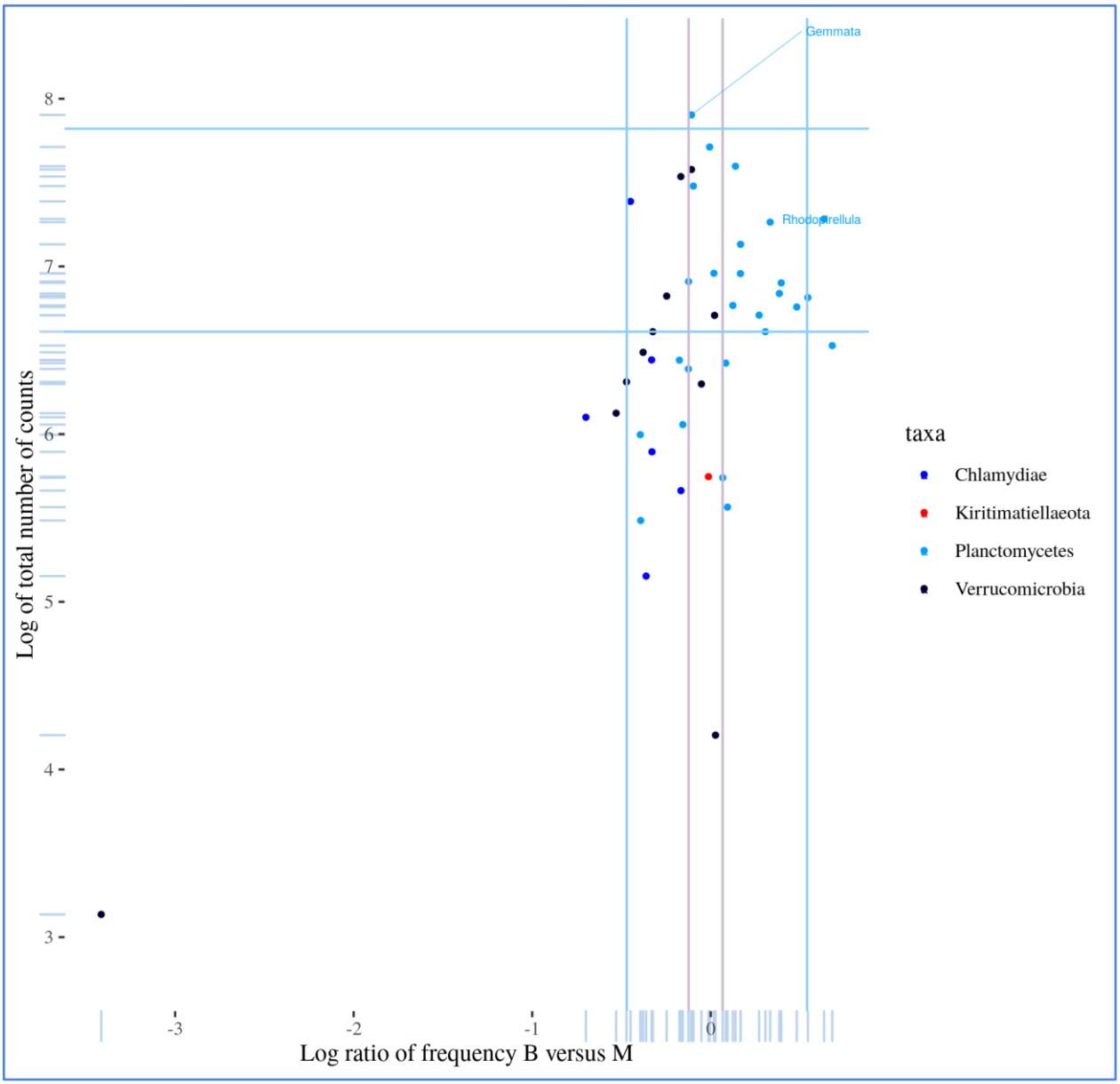


Figure 18. Analogous to the log-log scatter plot of (**Figure 19**) across all of the bacterial domain but at the genus level.

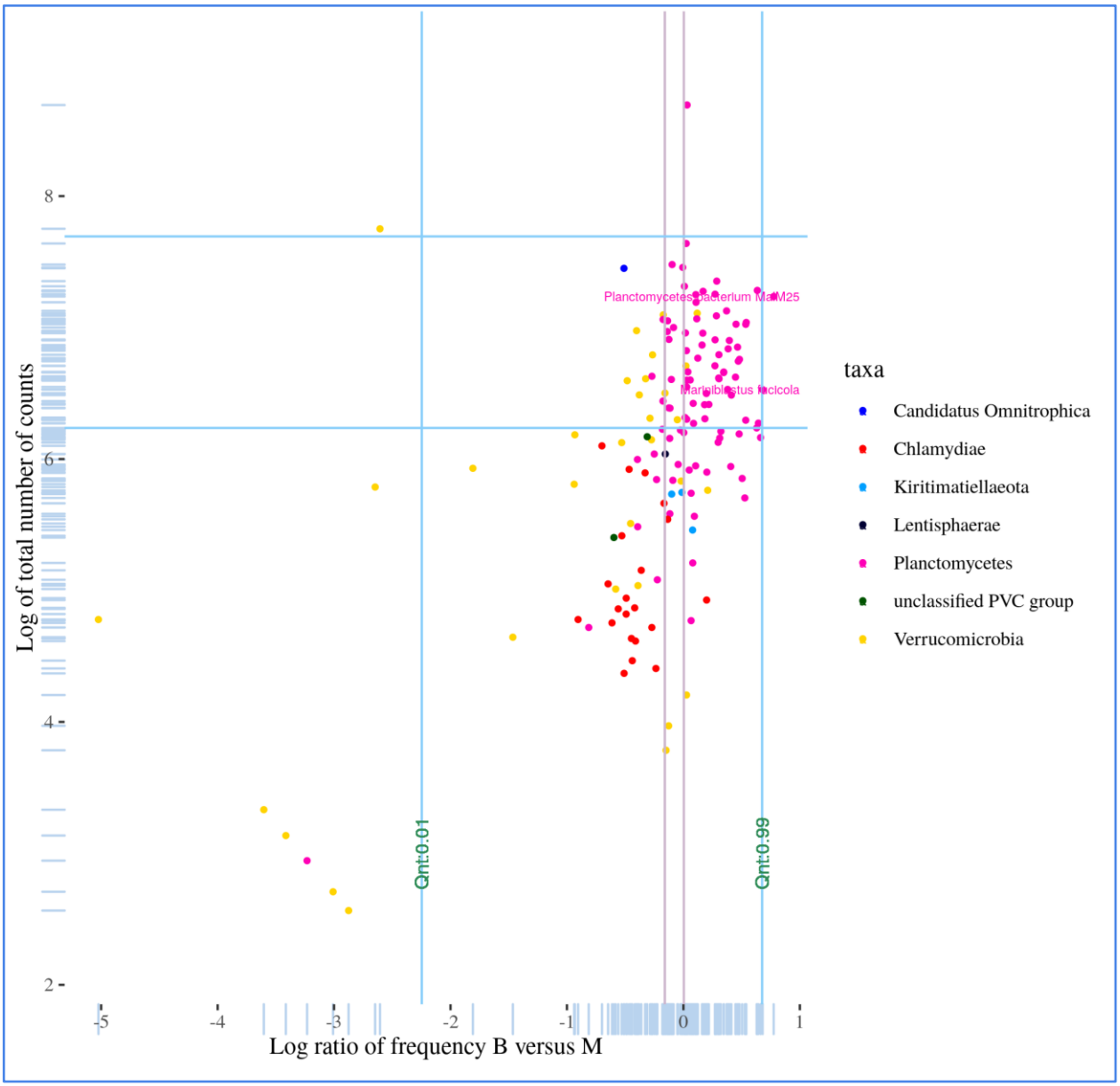


Figure 19. A log-log scatter plot of the log ratio of reads for all PVC species versus the total number the total number of reads for the species.

Bacteria, The Fibrobacteres-Chlorobi-Bacteroidetes superphylum: Bellairs is enriched for organisms involved in the degradation of organic matter

Bacteroidetes (8.2% B vs 2.8% M) are gram-negative non-spore forming bacteria that are often detected in the gut of animals but also seawater where they degrade polymeric organic matter. The difference in read percentage is highly significant under all statistics (**Methods 9a-c**). The remaining percentage of reads map almost exclusively to the Candidatus Marinimicrobia phylum (<1% both sites). Marinimicrobia have also been implicated as key drivers of biogeochemical change via networks of metabolite exchange along energetic gradients. They are often located in oxygen minimum zones with roles in carbon and dissimilatory inorganic nitrogen and sulfur cycling (Hawley et al. 2017).

Bacteria, The Terrabacteria superphylum

As noted above, a large fraction of all bacterial reads are mapped to the Terrabacteria in Maycocks. In fact, Terrabacteria is the most discordant of all taxa in our dataset between the two sites (37% B vs 69% M of all bacterial reads). Within Terrabacteria, the vast majority of the Maycocks reads (60 of 69%) are mapped to Cyanobacteria with smaller fractions aligning to Firmicutes (3 of 69%) and Actinobacteria (6 of 69%). At Bellairs, the reads are more diverse, distributing more uniformly across these three phyla with 21% (of 37%) to Cyanobacteria, 9.6% to Actinobacteria and 5% to Firmicutes amongst others (**Figure 13**).

Bacteria: Terrabacteria: Maycocks is strongly enriched for cyanobacteria

Cyanobacteria are photosynthetic bacteria that synthesize organic compounds from carbon dioxide and produce oxygen as a by-product. They are extremely abundant in warm nutrient-poor waters in the tropical and sub-tropical ocean. Some families are also able to fix atmospheric nitrogen (Pierella Karlusich, Ibarbalz, and Bowler 2020). Prochlorococcus and Synechococcus, two autotrophic genera of the order Synechococcales, account for over 98% of the total Cyanobacteria identified in our study. The genera are recognized as one of the most important primary producers in reef ecosystems (Weber et al. 2020). The relationship between these two picoplankton are usually dictated by the nutrient levels in their environment; Prochlorococcus is typically more prevalent in nutrient poor water likely due to its small size (Partensky, Hess, and Vaulot 1999). Synechococcus is more predominant in eutrophic water (Dinsdale et al. 2008) and coastal plumes of rivers, likely due to increased nitrate and phosphate levels (Wawrik et al. 2003). This is consistent with our data (21% B vs 16% M), as Bellairs is down current from several river outlets (<1km) and directly offshore from tourist resorts. Overall Prochlorococcus was more predominant at both sites. At Bellairs, the ratio of Prochlorococcus to Synechococcus was 78:21 ~ 3.7, while at Maycocks we observe a ratio of 84:16 ~5.3. This is consistent with the oligotrophic nature of the Barbadian marine environment (Biller et al. 2015).

A study of the coral reef systems in the Caribbean waters of Curaçao also reported a high percentage of Cyanobacteria (30-43%) well beyond studies from the north Atlantic Ocean, Mediterranean Sea and Pacific Ocean (Frias-Lopez et al. 2002). They reasoned that this high percentage may be due to ecological differences between offshore reefs and near-shore reefs, methodological differences and environmental differences based on geographic location. Levels of cyanobacteria are also extreme in our data when compared against these sites (**Figure 17)**. Moreover, a more recent study of northern Caribbean reef systems also highlights high abundance of Cyanobacteria among their study sites with a relative abundance ranging from 13.2% to 29.7% of all bacterial and archaeal phyla. This high cyanobacteria abundance was mainly due to the presence of Prochlorococcus and Synechococcus (Weber et al. 2020), although the finding was reproducible. Our observed frequencies for Cyanobacteria are more extreme.

In a study of several sites in the Northern Line islands in the central Pacific (Dinsdale et al. 2008) Prochlorococococcus was the most common bacterial autotroph at two sites (75%, 91% of Cyanobacteria), while Synechococcus was most common at two other sites (64% and 66% of Cyanobacteria). Across the large metagenomic fraction of the four sites in the study, the ratio of proportion of sequences between Prochlorococcus and Synechococcus were 9%:1%, 2%:0.6%, 0.3%:0.7% and 1%:2%.

Along with Prochlorococcus and Synechococcus, the genus Cyanobium was also differentially identified at the Maycocks site but with low counts (**Figure 20**).

Cyanobium species are picocyanobacteria known to occur in open ocean and coastal waters globally, they also contribute to primary production (Costa et al. 2015).

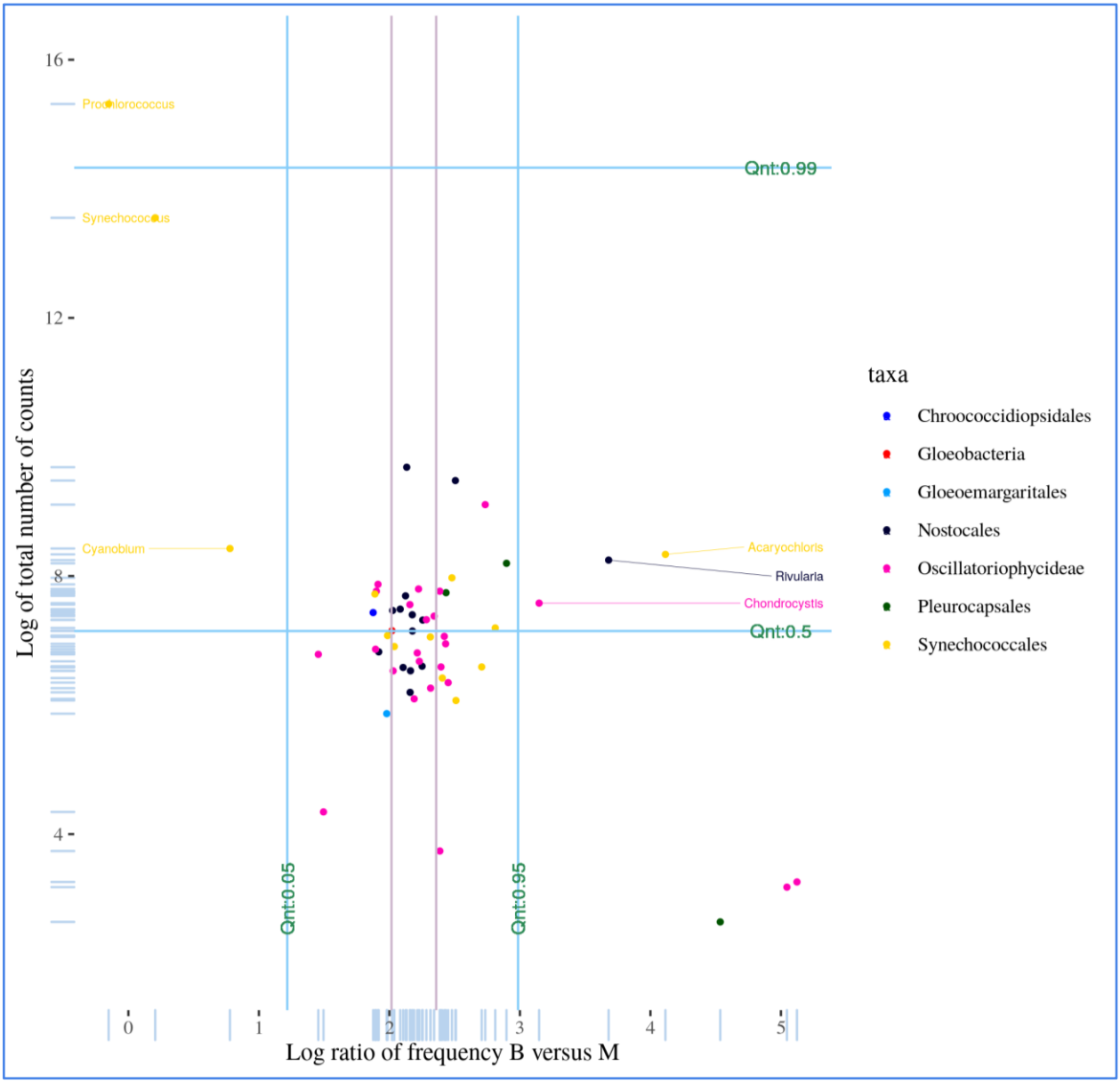


Figure 20. Analogous to the log-log scatter plot of (**Figure 21**) across all of the bacterial domain but at the genus level.

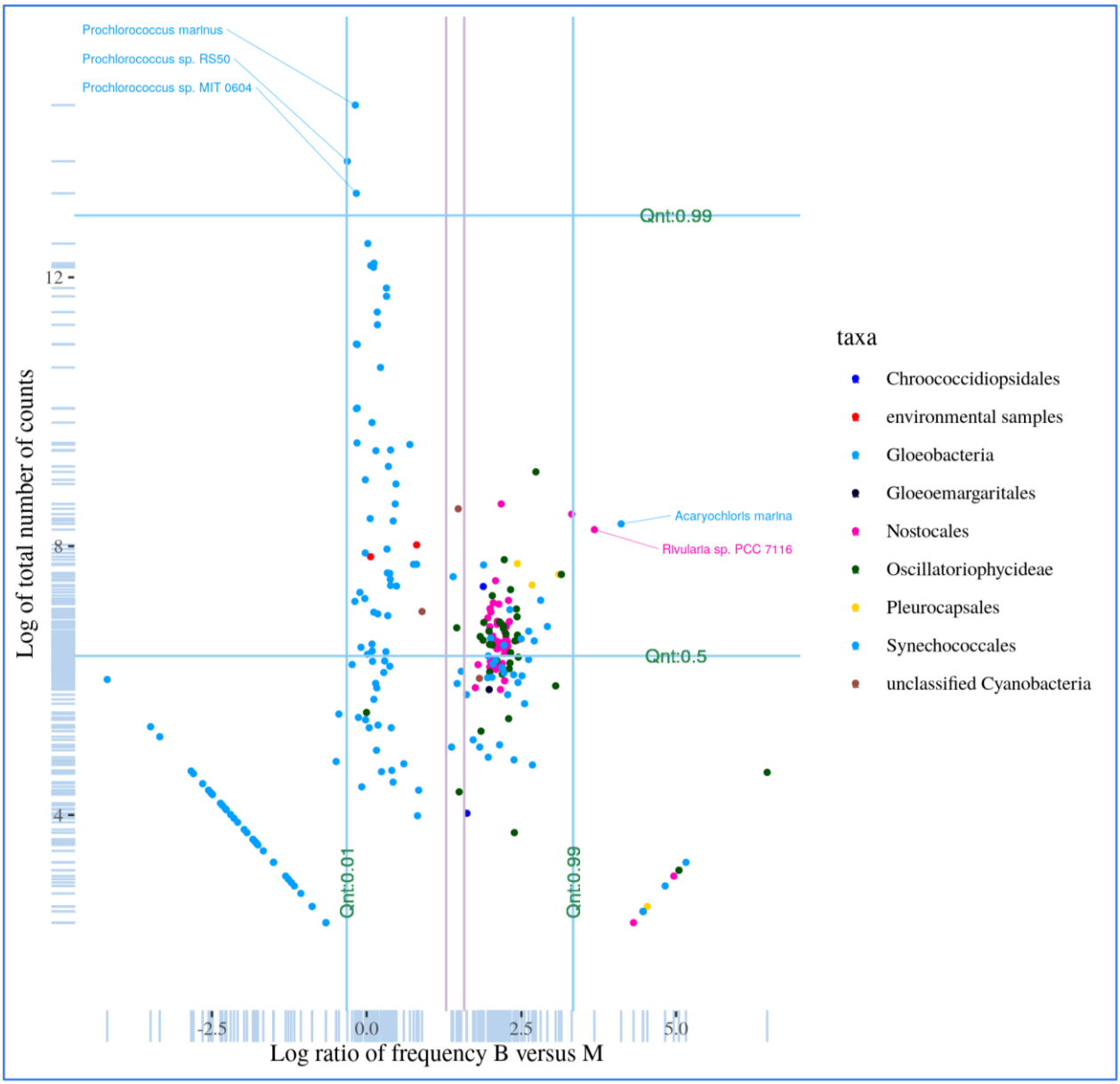


Figure 21. A log-log scatter plot of the log ratio of reads for all Cyanobacteria species versus the total number the total number of reads for the species.

Bacteria, Terrabacteria: Bellairs is enriched for Firmicutes able to survive in extreme environments

The Terrabacteria include the Firmicutes, whose members are extremely diverse in terms of their biochemical, physiological and ecological properties and are able to survive in a variety of extreme environments due to their unique ability to form endospores. They are often found in nutrient rich environments (Galperin 2013). Firmicutes species were differentially identified at both Bellairs and Maycocks. The genera Tumebacillus, Hungatella and Fictibacillus were more abundant at Bellairs but with low counts (**Figure 22**).

Tumebacillus are gram-positive spore forming sulphur-oxidising bacteria that have been isolated from diverse environments such as algal scum, freshwater, soil and mangroves (Bulat et al. 2018; Torres et al. 2019; Carper et al. 2020). Tumebacillus species have been found within the skeletal mucus of Caribbean coral porites and are halotolerant (Manrique et al. 2012; Apprill 2020). The species T. avium was differentially identified at Bellairs (**Figure 23**).

The human pathogen Hungatella hathewayi was differentially identified at Bellairs. Hungatella are anaerobic bacteria closely related to Clostridium (Kaur et al. 2014; Elsayed and Zhang 2004).

Fictibacillus are aerobic bacteria which have been identified in different environments such as soil, freshwater and marine sediment (Wang, Zhang, and Sun 2018) and in coral microbiomes. Some of these species possess protease-producing abilities that play a major role in the biodegradation of corals (Rosales et al. 2019; Su et al. 2020). The species F. arsenicus was differentially identified at Bellairs (**Figure 23**).

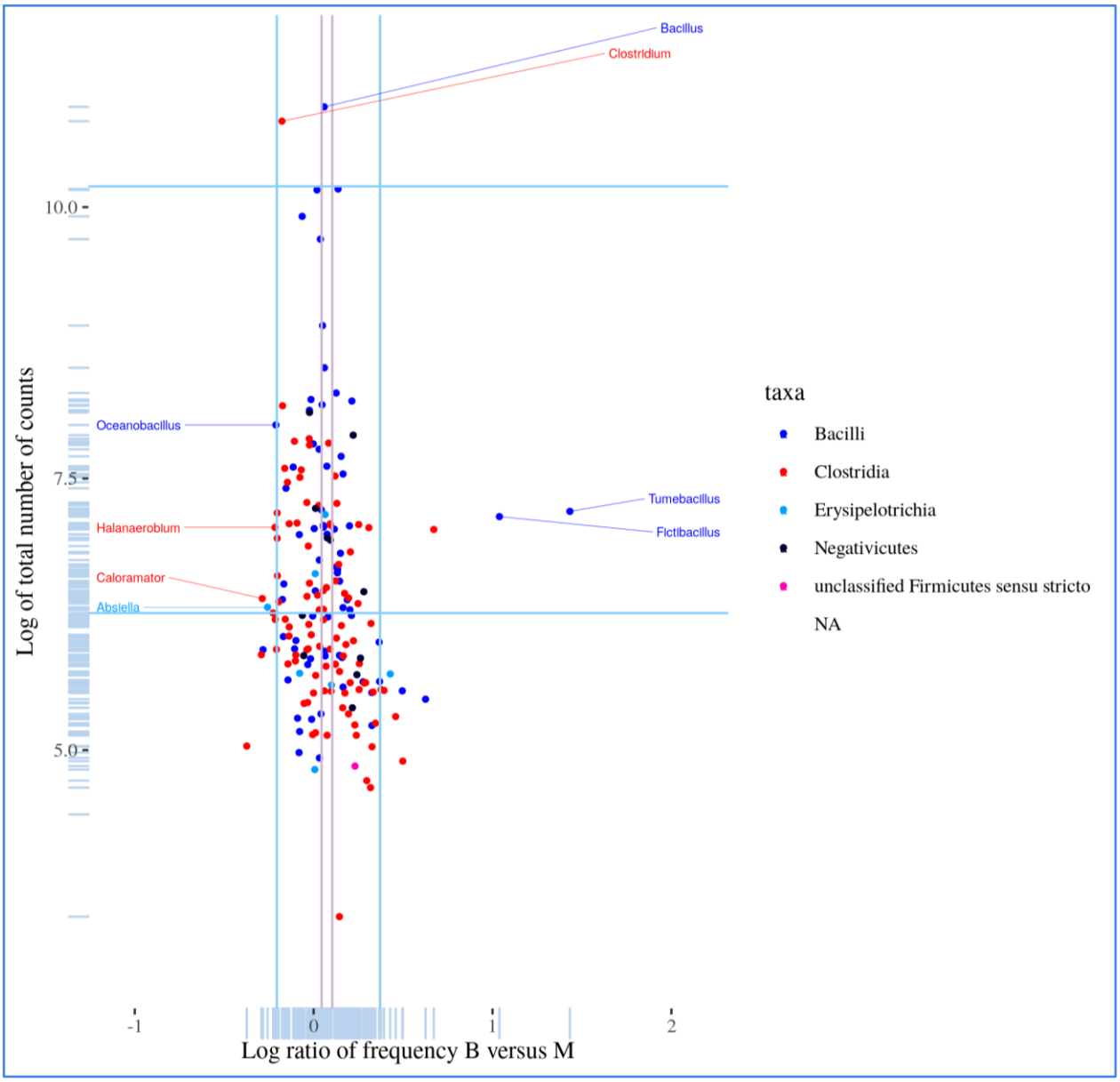


Figure 22. Analogous to the log-log scatter plot of (**Figure 23**) across all of the bacterial domain but at the genus level.

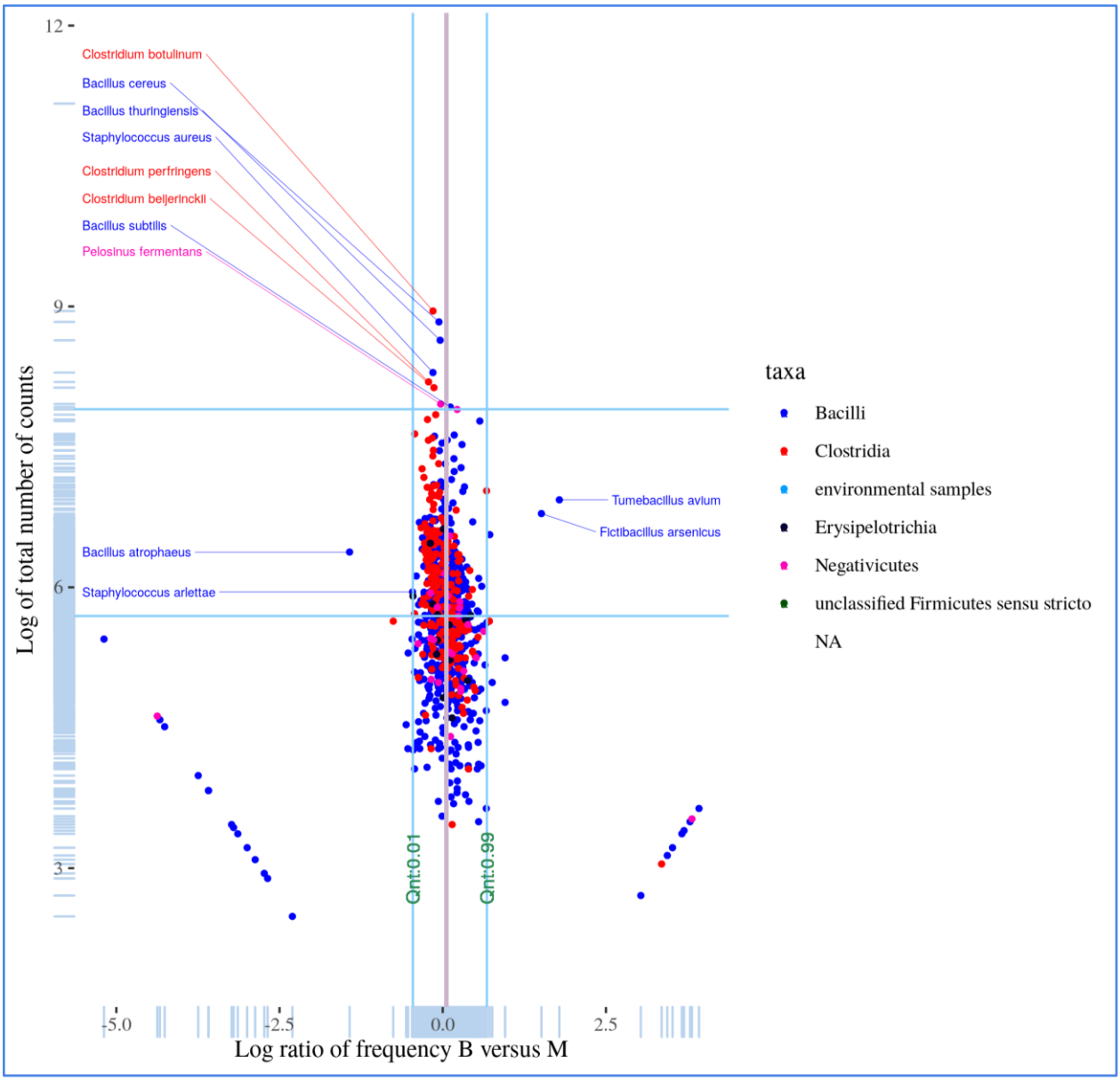


Figure 23. A log-log scatter plot of the log ratio of reads for all Firmicutes species versus the total number the total number of reads for the species.

Bacteria, Terrabacteria: Bellairs is enriched for Actinobacteria involved in recycling refractory biomaterials

Actinobacteria are commonly found in the soil of marine ecosystems where they play a role in recycling refractory biomaterials from dead plants, fungi and animals. They behave much like fungi in soil, decomposing organic matter in a manner suitable for plant root systems and providing nitrogen fixation in exchange for the saccharides of the plant (Ranjani, Dhanasekaran, and Gopinath 2016).

Several species within Actinobacteria have considerable enrichment at Bellairs including Illumatobacter coccineus and Arthrobacter sp. LS16. This enrichment is consistent with the fact that the Bellairs site is more affected by runoff from inland rivers and coastal settlements.

However, many species of Actinobacteria are abundant at both sites, including several Streptomyces lividans, venezuela and cyaneogriseus. Streptomyces species are known to inhabit and thrive in diverse ecosystems such as soil, freshwater and marine environments (Lewin et al. 2016). Some terrestrial Streptomyces species have adapted to marine conditions and play a prominent role in the microbiota of sponges (Chater 2016).

The genus Candidatus Actinomarina was differentially identified at the Maycocks site with low counts. The genus consists of ultra-small free living species that are found globally and known to mirror similar geographic distributions to picocyanobacteria species (Ghai et al. 2013). These species are also known to perform photoheterotrophic metabolism (Reza et al. 2018). Marine pseudonocardia species are known to be coral-associated with antibacterial activities that contribute to coral health (Kuang et al. 2015).

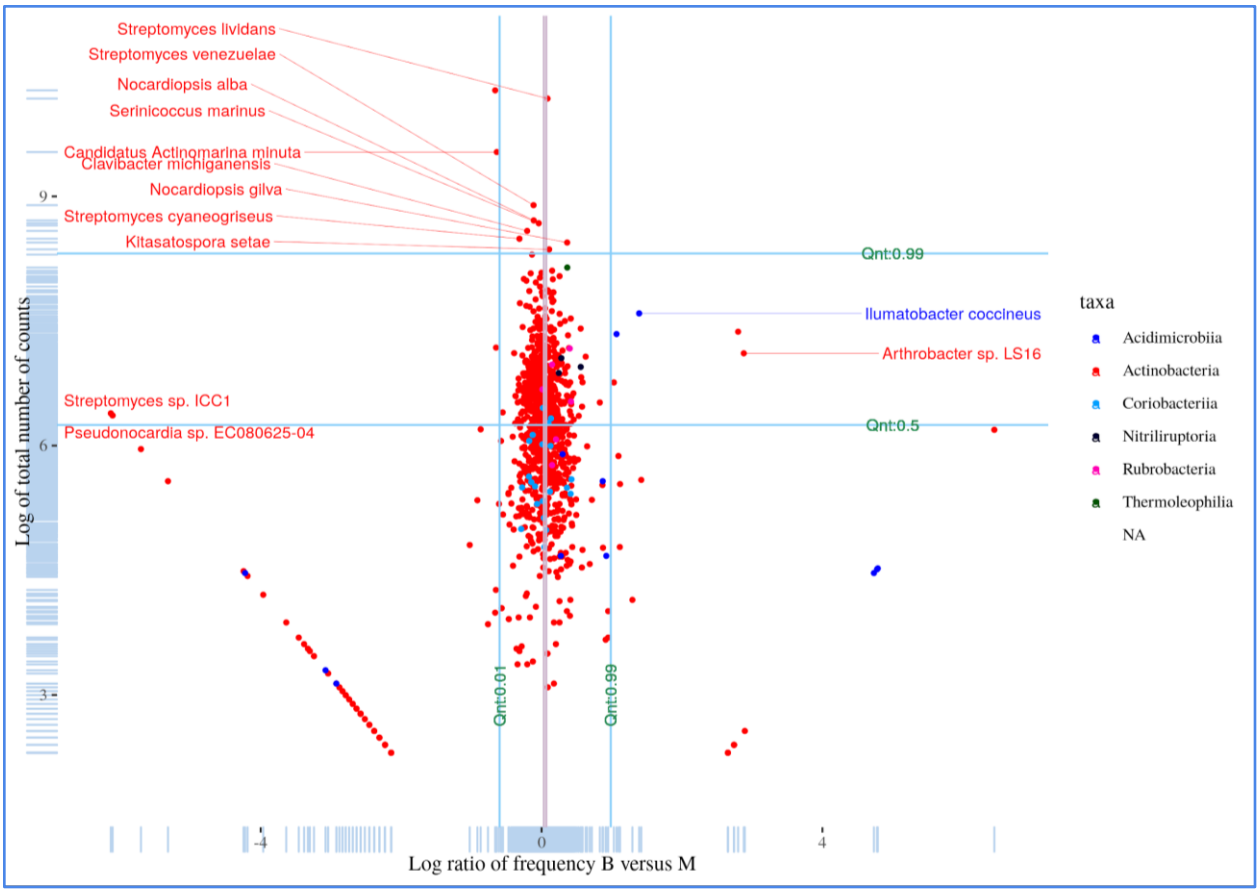


Figure 49. A log-log scatter plot of the log ratio of reads for all Actinobacteria species versus the total number the total number of reads for the species.

Whereas Maycocks is biased towards Terrabacteria (specifically Cyanobacteria), Bellairs appears to be biased towards Proteobacteria, the second most frequent bacterial superphylum (48% B vs 25% M) **(Figure 13**). Within Proteobacteria, the Alpha and Gamma subtaxa contribute many bacterioplankton to ocean waters compared to the Beta-, Delta-, and Epsilon-proteobacteria. This is consistent with their relative abundances in our data (Alpha- 24% B vs 15% M; Gamma- 12% B vs 6% M of all bacterial reads.

Figure 24 depicts the distribution of genera. It suggests that Alphaproteobacteria are largely more abundant at Bellairs, whereas Gammaproteobacteria and Betaproteobacteria show a slight preference for Maycocks (KW and Dunn's test, $p << 0.01$, **Methods 9c**).

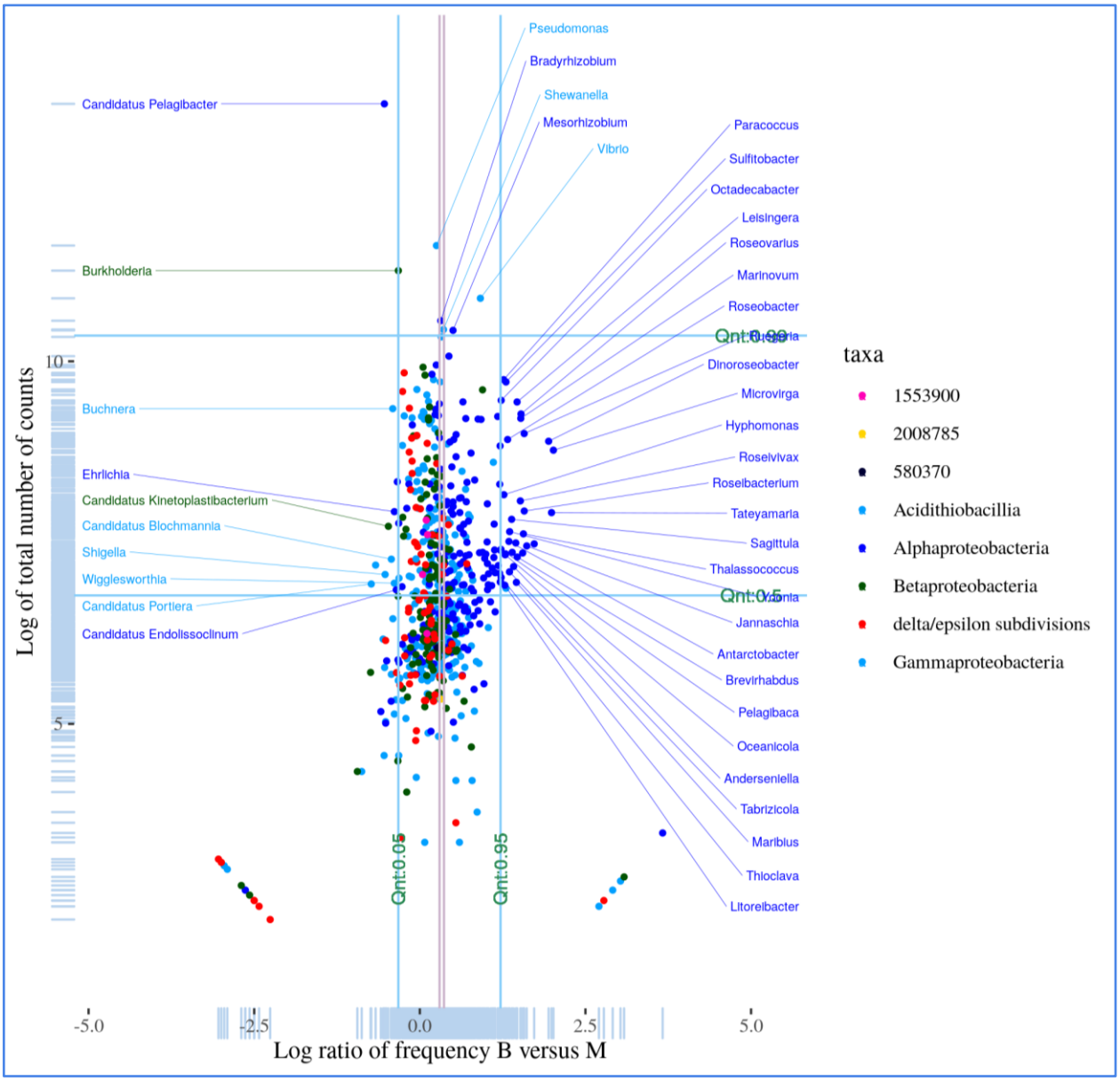


Figure 24. A log-log scatter plot of the log ratio of reads for all Proteobacteria species versus the total number the total number of reads for the species across all of the bacterial domain but at the genus level.

Alphaproteobacteria: Maycocks is enriched for oligotrophs, Bellairs is enriched for organisms associated with coral, algae and ocean sediment

The oligotrophic genus Candidatus Pelagibacter represents one of the most abundant taxa genera in our study and is significantly shifted towards Macocks (KW test, $p << 0.01$; **Figure 24**. They are small free-living heterotrophic species usually found thriving in low-nutrient environments, which play a significant role in carbon cycling, feeding on dissolved organic carbon and nitrogen (Dinasquet, Landa, and Obernosterer 2019; Tout et al. 2014; Zhao et al. 2017). This species Cand. P. ubique belongs to the SAR11 clade of the most abundant group of bacteria in the world's oceans (Steindler et al. 2011). We observed significantly less SAR116, typically more prevalent than SAR11 in coastal waters (1.3% B vs 1.1% M).

Rhodobacterales can utilize various organic and inorganic compounds and carry out sulfur oxidation, aerobic anoxygenic photosynthesis, carbon monoxide oxidation and the production of secondary metabolites (Pohlner et al. 2019). Although several Rhodobacteraceae bacterium strains were highly abundant and biased towards Maycocks, the vast majority of genera showed a general trend towards the Bellairs site (KW test, $p << 0.01$) highlighting genera Dinoroseobacter, Tateyamaria and Jannaschia (**Figure 25**). Dinorosebacter are aerobic anoxygenic phototrophic bacteria, known to be highly abundant in marine turf algae (Meirelles et al. 2018). Some Dinoroseobacter species form epibiotic relationships with red tide dinoflagellates (Wagner-Döbler et al. 2010). Tateyamaria is a genus of marine gram-negative aerobic bacteria isolated from coastal marine environments (Kurahashi and Yokota 2007). Tateyamaria have been identified as components of soft corals and coralline alga microbiomes. Within the alga microbiome, Tateyamaria species are able to survive and increase abundances under acidification conditions (Chen et al. 2012; Huggett, McMahon, and Bernasconi 2018). Jannaschia are aerobic anoxygenic phototrophic bacteria. Some species play a role in transport and nitrate reduction (Moran et al. 2007). Other species appear to be part of microbial communities associated with corals (Apprill et al. 2009). Pohlner and colleagues provide evidence that members of Rhodobacterales correlate with the sedimentary setting(Pohlner et al. 2019).

Within the Rhizobiales, Microvirga is highly abundant and enriched at Bellairs (**Figure 25**). Members of this genus are often found in marine and terrestrial environments, however the specific species detected M. ossetica is a soil bacterium (Z. Liu et al. 2016; Safronova et al. 2017). Liberibacter was differentially identified at Maycocks in addition to the Rickettsiales genus Ehrlichia. Although the Erythrobacter or Roseobacter clades are both highly abundant and widely distributed in ocean systems, we observe very few reads mapped to their taxa at either site.

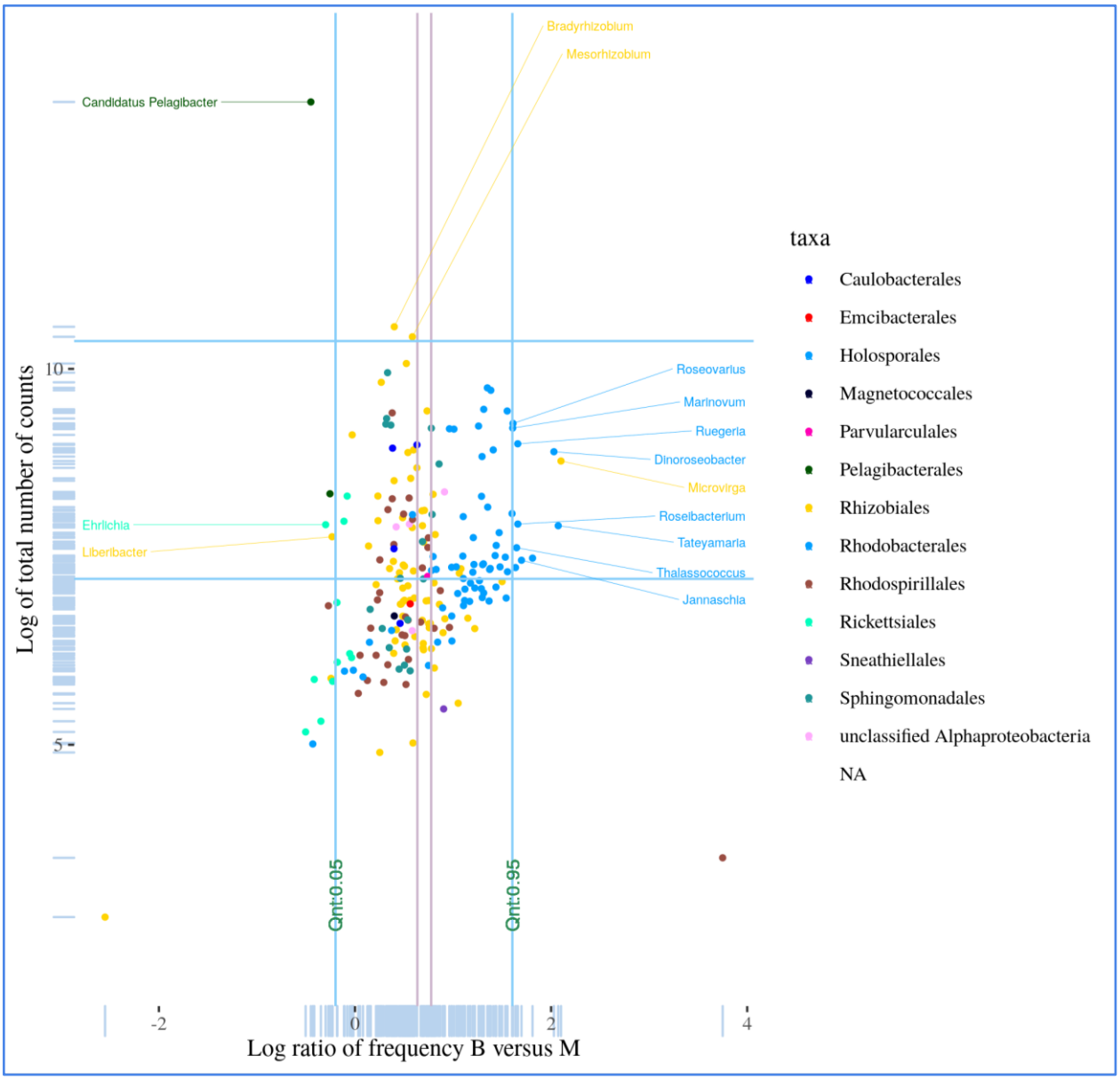


Figure 25. A log-log scatter plot of the log ratio of reads for all Alphaproteobacteria species versus the total number the total number of reads for the species across all of the bacterial domain but at the genus level.

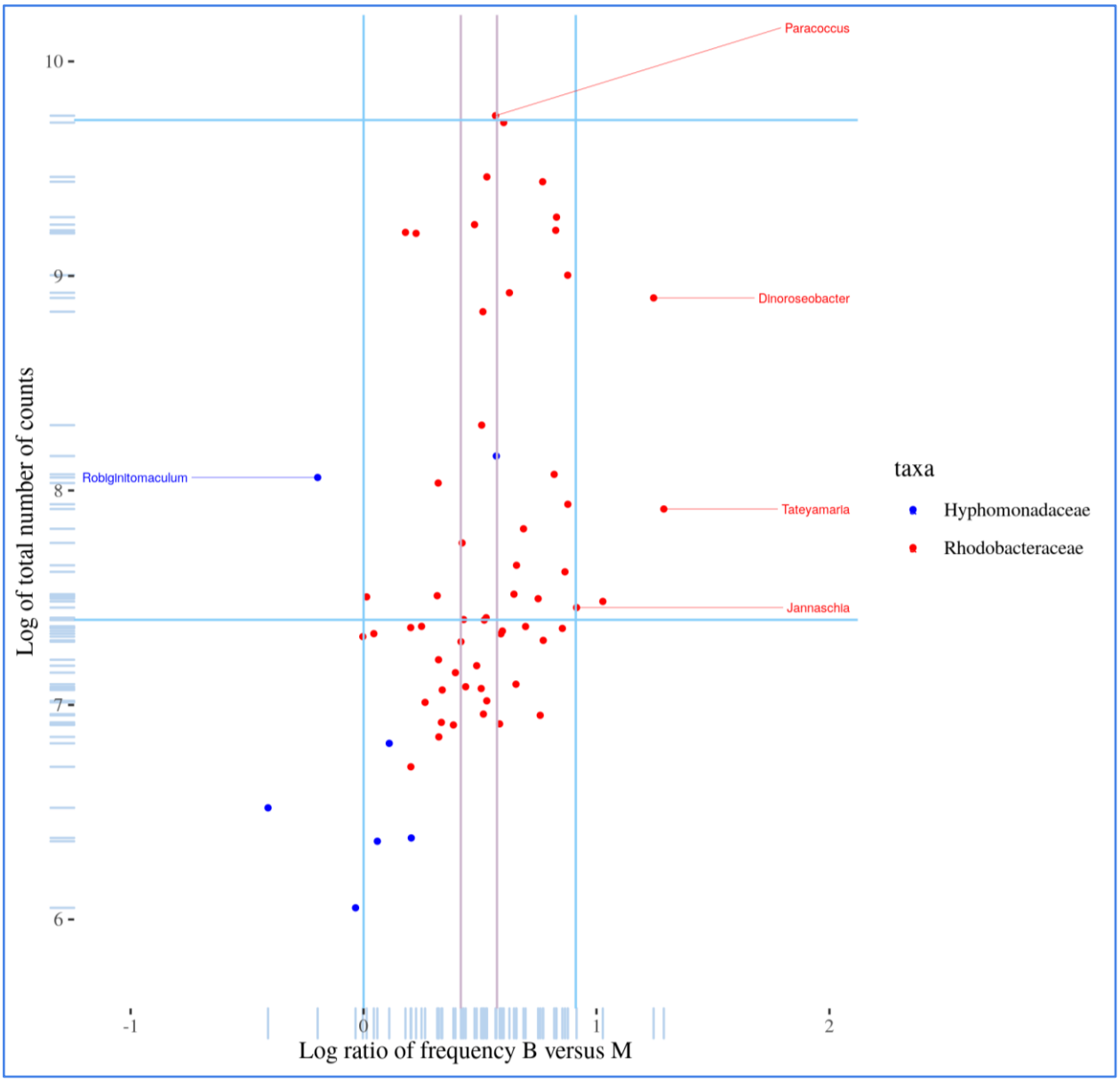


Figure 26. A log-log scatter plot of the log ratio of reads for all Rhodobacterales species versus the total number the total number of reads for the species across all of the bacterial domain but at the genus level.

Gammaproteobacteria: Bellairs is highly enriched for bacterial pathogens for almost all dimensions of the reef biosystem

Vibrionales. The genus Vibrio was one of the most highly abundant genera in our dataset and exhibits a strong skew towards Bellairs (KW, $p << 0.01$, **Figure 27**) involving many species including corallilyticus, tubiashii, harveyi, astriarenae, nigripulchritudo, and ponticus. Vibrio are gram-negative motile bacteria with a curved-rod shape commonly found in marine environments. They are facultative anaerobes, capable of producing ATP by aerobic respiration if oxygen is present, but also able to switch to fermentation. In addition to its role as a human pathogen (eg V cholerae). Vibrio species play a significant causative role in coral diseases and disrupt corals symbiotic relationship with zooxanthellae. There are indications that high nutrient levels promote pathogenic bacteria including Vibrio spp. to dominate in healthy coral reef systems (Morrow et al. 2012). This includes V. corallilyticus which is implicated in white band syndrome (Munn 2015). and V. harveyi which is linked to yellow spot syndrome (Cervino et al. 2008). V. harveyi is also present in healthy corals albeit less frequently (Cróquer et al. 2013). V. tubiashii has been implicated in shellfish vibriosis worldwide (Elston et al. 2008). It may also be a virulence factor in diseases of scleractinian corals, where it plays a role in photoinactivation of the coral (Sussman et al. 2009). V. astriarenae appears as a lowly abundant generalist in many reef systems (Amin et al. 2016), however it is poorly studied to date. V. nigripulchritudo is a shrimp pathogen with major impact on farms in Japan and New Caledonia (Goarant et al. 2006) and V. ponticus is a fish pathogen (Xie et al. 2007).

Photobacterium, also a genus of Vibrionales, are common in marine environments and can survive in both aerobic and anaerobic environments. P. damselae, which was more abundant at Bellairs, is a well-studied pathogen of marine organisms including fish and has made significant negative financial impact on fisheries world-wide (Rivas, Lemos, and Osorio 2013)

Enterobacterales. There is a moderate shift of the Enterobacterales towards Maycocks (KW, $p < 0.01$, **Figure 27**). This bacteria family has been used as a proxy for anthropogenic pollution within coral reef systems mainly by sewage and fecal matter where they established a positive correlation between Enterobacteriaceae and the levels of nitrogen present within the reef water (Leite et al. 2018). The Shigella genus in particular is more abundant at Maycocks. Shigella contains the causative agent of shigellosis (Kotloff et al. 2018). Shigella species have been identified within coral reef microbial communities in the water column and within the coral mucus (Kegler et al. 2017).

Several unclassified Gammaproteobacteria were found to be highly abundant at the Maycocks site, including members of the SAR86 clade, which are globally abundant planktonic bacteria (Dupont et al. 2012).

Pseudomonadales. Two species of the genus Acinetobacter within Pseudomonadales are enriched at the Bellairs site in high abundance. In general, Acinetobacter is a gram-negative genus which plays an important role in the mineralization of aromatic compounds within soil including marine systems. Many Acinetobacter species are known to be able to reduce nitrates to nitrites (Doughari et al. 2011). A. schindeleri is an emerging opportunistic human pathogen that can survive in many environments (Choi et al. 2012), although to the best of our knowledge there are no previous reports related to marine environments. A. indicus was originally isolated from a

hydrocarbon dumpsite, but has more recently been identified as a human pathogen which can survive in many environments including marine (Malhotra et al. 2012). Although neither Acinetobacter has a clearly understood role in marine systems, both are witnessed by a significant number of reads in our data (~1K, 2K reads respectively at Bellairs).

Escherichia coli and several species of Pseudomonas (aeruginosa, fluorescens, and putida) are highly abundant at both sites.

Alteromonadales. Many species form the Alteromonadales including some from the genus Shewanella that have high abundance but are equally present at both sites.

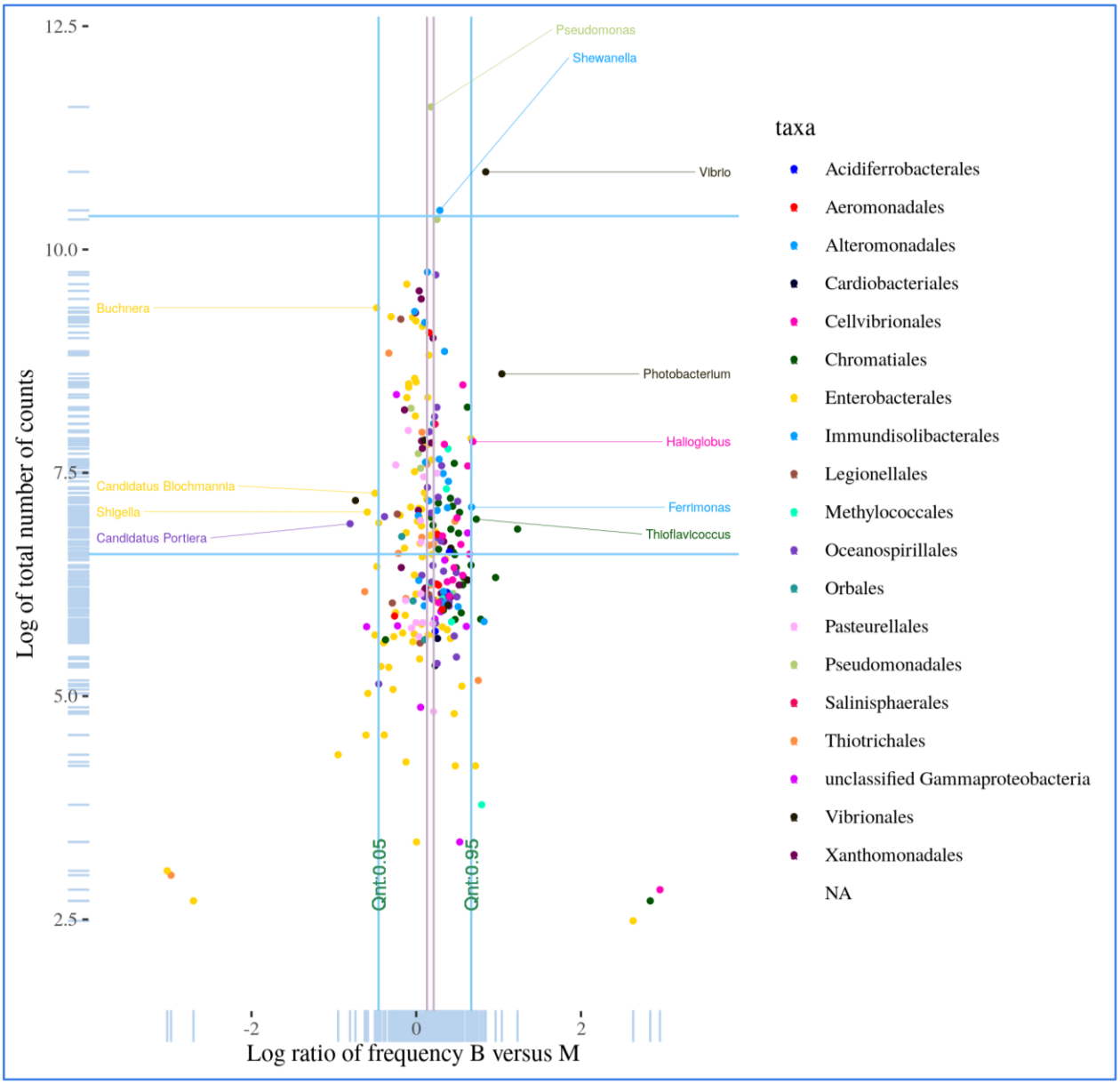


Figure 27. Analogous to the log-log scatter plot of (**Figure 28**) across all of the bacterial domain but at the genus level.

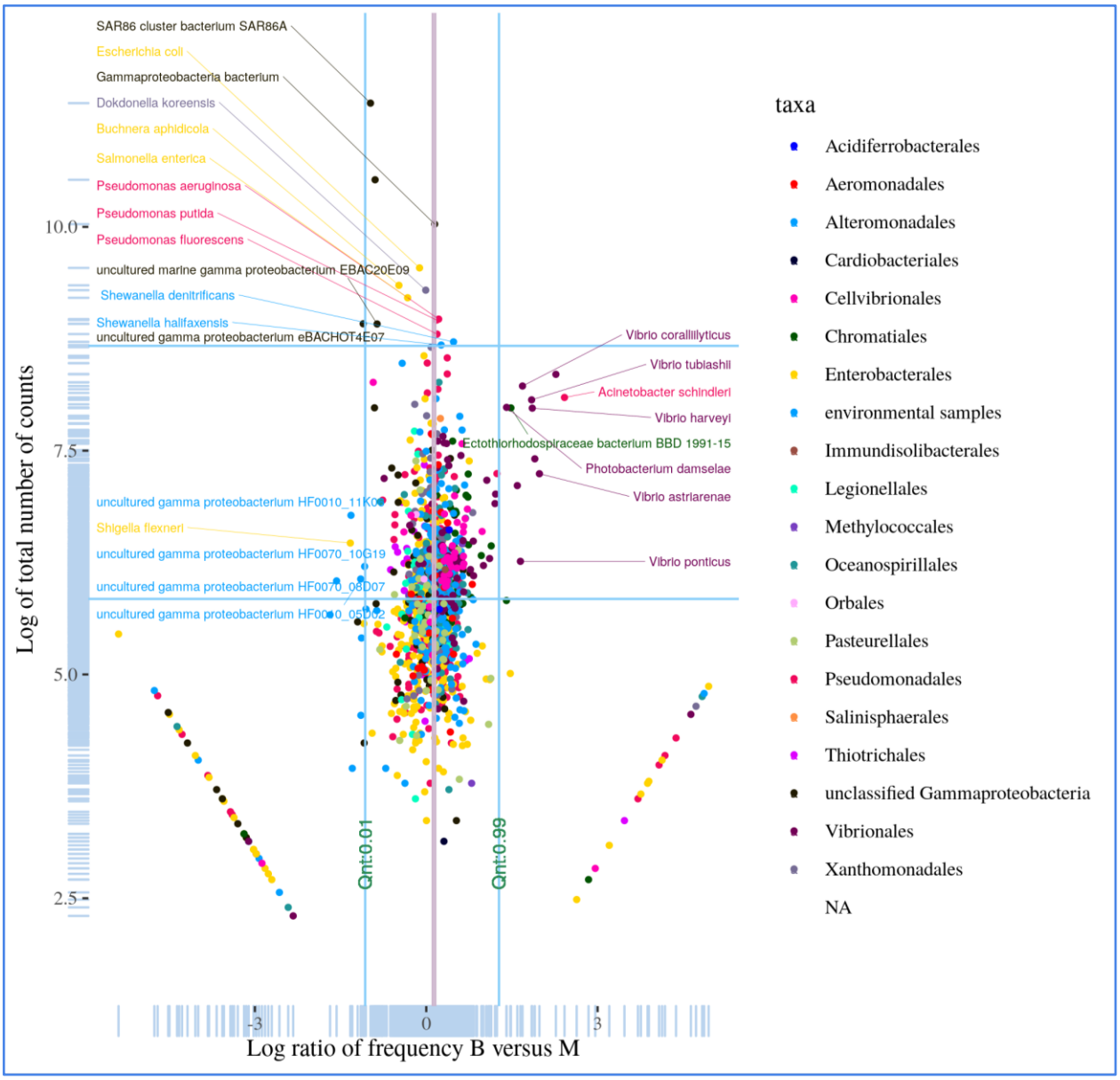


Figure 28. A log-log scatter plot of the log ratio of reads for all Gammaproteobacteria species versus the total number of reads for the species.

Delta/Epsilon-proteobacteria

The Delta-Epsilon subdivisions display a dramatic split in their preference between the two sites (**Figure 30**). Specifically, the Epsilon members are skewed towards Maycocks while Delta members prefer Bellairs almost without exception (KW test, p<<0.01). However, the Epsilonproteobacteria tend without exception to be equally and lowly abundant at both locations. With respect to Deltaproteobacteria, several chemolithotrophic SAR324 species are highly abundant at both locations. However, the genus Desulfomicrobium within the Deltaproteobacteria was weakly differentially identified at Bellairs with low counts. Desulfomicrobium is a genus of sulfate reducing bacteria that thrive in marine anoxic environments and interfaces such as microbial mats (Sass et al. 2002; Miralles et al. 2007) . The species D. orale was differentially identified at Bellairs, this species has been previously identified in Atlantic coastal marine waters (Dias et al. 2008).

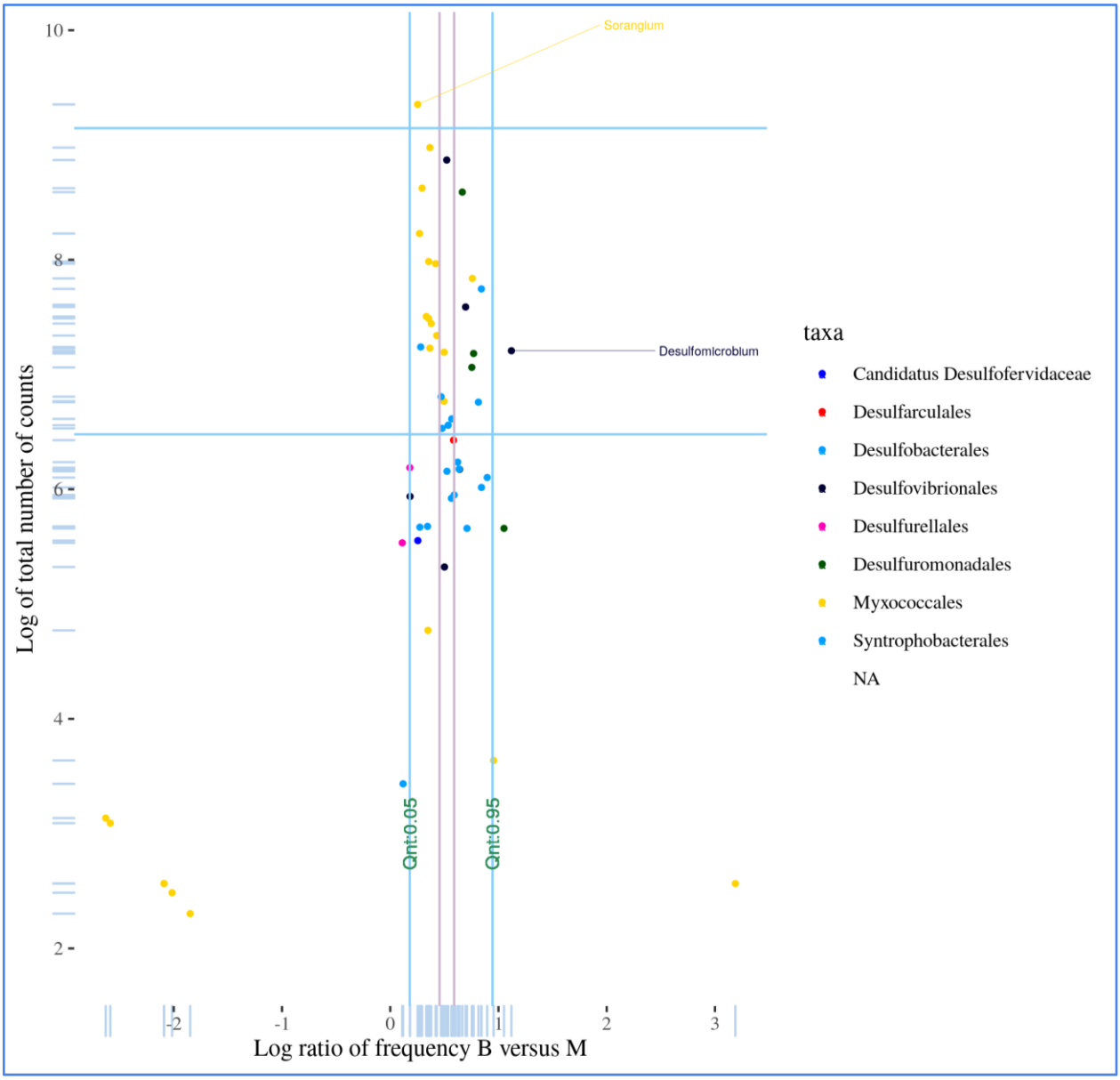


Figure 29. A log-log scatter plot of the log ratio of reads for all Deltaproteobacteria species versus the total number the total number of reads for the species across all of the bacterial domain but at the genus level.

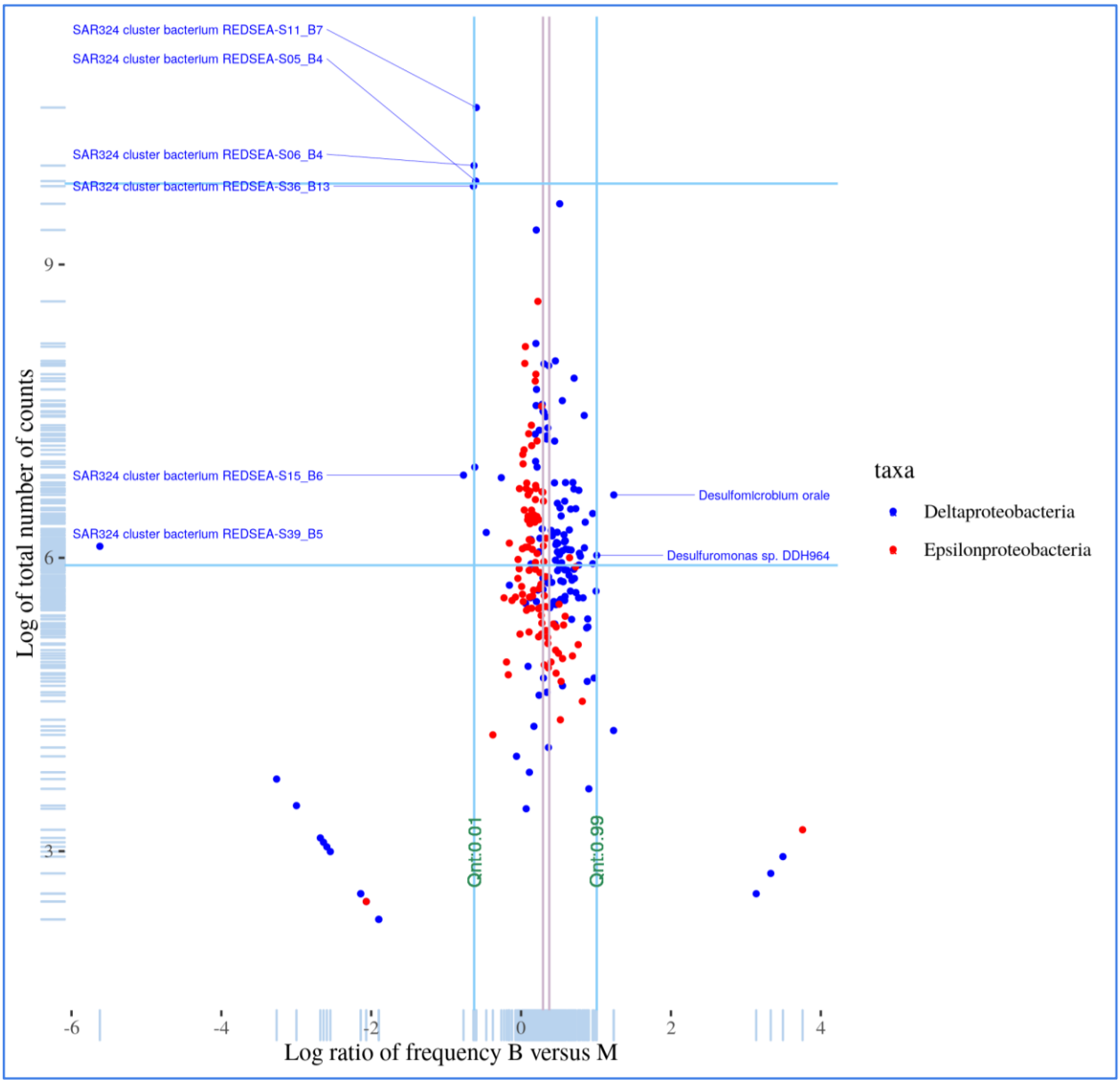


Figure 30. A log-log scatter plot of the log ratio of reads for all Delta-Epsilon Subdivision species versus the total number the total number of reads for the species.

Betaproteobacteria: Maycocks is enriched for bacteria associated with corals and sponges.

Within Betaproteobacteria, members of the order Burkholderiales order were the most abundant but individual genera and species were differentially abundant at each of the sites (**Figure 31**). The genus Massilia, which was more abundant at Bellairs, are aerobic bacteria that have been isolated from freshwater, dust, soil, human skin and marine habitats (Ofek, Hadar, and Minz 2012). This includes species M. oculi, M. sp. WG5, dimethyl disulfide-producing M. putida and M. plicata.

Three Burkholderia species were differentially identified at Maycocks: B. cepacia, B. multivorans, and B. vietnamiensis. Burkholderia can be pathogenic, and are ubiquitous to soil, freshwater, marine environments, humans and other animals (Coenye and Vandamme 2003). They are considered tightly associated with the coral microbiome during early life stages (Leite et al. 2017). B. cepacia, which is highly abundant and biased towards Maycocks, belongs to the Burkholderia cepacia complex (Bcc) that are widely distributed in natural environments, although marine systems are not as common (Eshwar Mahenthiralingam et al. 2006; Vial et al. 2011; Maravić et al. 2013). The species B. multivorans and vietnamiensis are common to natural rhizosphere, soil and water habitats (Vial et al. 2011).

The endosymbiotic genus Candidatus Kinetoplastibacterium was differentially identified at Maycocks. Species of this genus are known mostly as endosymbionts of insect-infecting trypanosomatid flagellates; they have however been identified in coastal surface seawater, likely due to river runoff (Reza et al. 2018). The obligate aerobe Delftia acidovorans and iron-oxidizing species Acidovorax ebreus were also differentially identified at Maycocks (**Figure 31**). D. acidovorans has been identified in various habitats such as soil and freshwater but is also considered a sponge and coral associated bacterium (Forest Rohwer et al. 2002; Karlińska-Batres and Wörheide 2013). Acidovorax species have been previously identified in coastal waters (Peng et al. 2018; Zhang et al. 2020) and are associated with the Caribbean reef building coral Montastraea annularis (Barott et al. 2011).

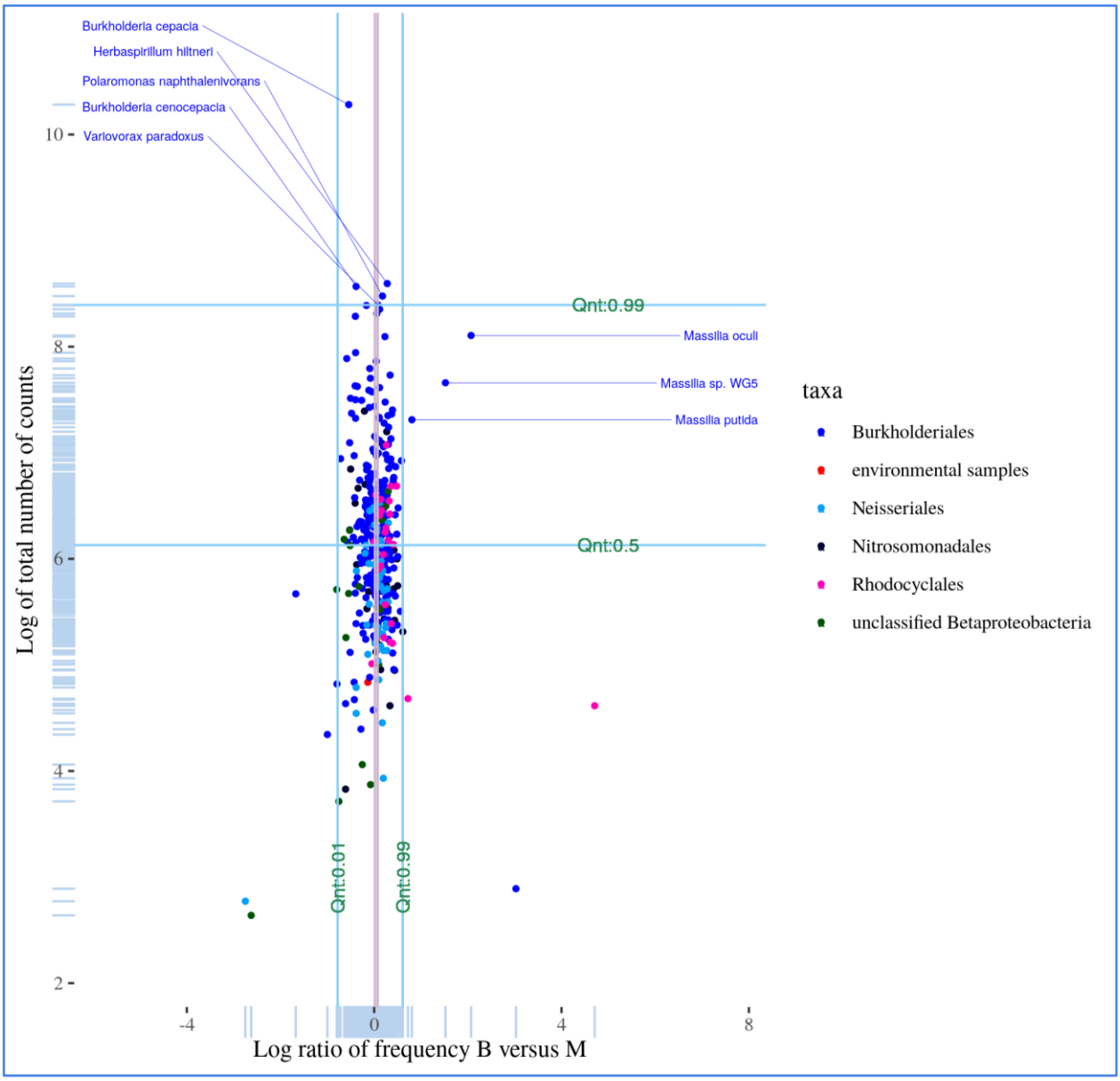


Figure 31. A log-log scatter plot of the log ratio of reads for all Betaproteobacteria species versus the total number the total number of reads for the species.

Archaea

In total, there was evidence of 440 species at Bellairs and 442 species at Maycocks across 122 distinct archaeal genera. A high percentage of all archaeal reads were mapped to Euryarchaeota at both sites, although there is a significantly higher fraction at Maycocks (63% B vs 82% M; **Figure 32**). For both sites, the second highest fraction of archaeal reads were mapped to the Thaumarchaeota (taxon ID 1783275), the ancestor of the Cren-, Cand. Bathy- and Cand. Kor-archaeota phylums, although here there is a significantly higher fraction at Bellairs (32% B versus 13% M). The difference in reads between the two sub-taxa is highly significant (all three tests, $p << 0.01$, **Methods 9a-c**). Thaumarchaeota are estimated to comprise 1% of the sea surface metagenome (Munn 2011); this is consistent with our data at Bellairs (1.2% of all reads) but not at Maycocks (0.2% of all reads). The Thaumarchaeota are chemolithoautotrophic ammonia-oxidizers and play a role in biogeochemical nitrogen and carbon cycles (Doxey et al. 2015; Bayer et al. 2016). Trace amounts of DNA mapped to the remaining five archaeal phyla were identified.

Archaea: photoheterotrophic euryarchaeotes are highly enriched at Maycocks.

The majority of Euryarchaeota reads at Maycocks are mapped to Diaforarchae (32% B vs 44% M) with smaller amounts mapping to the Stenosarchaea group (24% B vs 29% M), and Methanomoda (3% B vs 4% M). These differences are all highly statistically significant (all tests, $p << 0.01$). **Figures 32**, **33** and **34** highlight these biases.

The Diaforarchaea group contains the order Candidatus Poseidoniales, which is recognized as one of the most abundant planktonic archaeons in ocean surface waters (Rinke et al. 2019). This is consistent with the elevated relative abundances in our data (30% B vs 39% M). The ancestor of Cand. Poseidoniales is a motile photoheterotroph, capable of degrading proteins and lipids (Rinke et al. 2019), although there is genus- and family-specific lifestyle and niche partitioning.

Archaea: both sites exhibit high levels of methane producing, anaerobic archaeons

Stenosarchaea and Methanomada groups are in general highly abundant in equal proportions at both sites. The Methanosarcina genus contains anaerobic methanogens that conduct methanogenesis in diverse environments throughout the world including seawater (Maeder et al. 2006). Methanosarcina was also found to be one of the most abundant archaeal groups in the composition of coral rubble (Sánchez-Quinto and Falcón 2019). Methanobrevibacter is a genus of methane producing anaerobic archaea, some known species of this genus inhabit animal intestinal tracts, decaying plants and sewage and are used as indicators of fecal pollution in coastal waters (Ufnar et al. 2006). Methanocella species are known soil and sediment archaeons (Angel, Claus, and Conrad 2012; T. Rodrigues et al. 2016).

Several genera however including Methanoplanus and Methanoshpaerula have significantly higher relative abundance at Maycocks. This genus contains methanogenic species and are known to play endosymbiotic roles with marine ciliates (Hirakata et al. 2015) and other species associated with sponges in anoxic environments (Turque et al. 2010).

Archaea: enrichment for sulphur and iron cycling at Maycocks

Species of Aciduliprofundum are known thermophilic Euryarchaeotes commonly found in proximity deep sea hydrothermal vents environments, involved with sulphur and iron cycling (Schouten et al. 2008). Aciduliprofundum boonei is enriched at Maycocks in addition to the Thermoproteus genus. The sulphur dependent genus Thermoproteus and several additional genera from the Thermoplasmata class exhibit enrichment at the Maycocks sites.

Archaea: Bellairs is enriched for taxa involved in denitrification.

The majority of Thaumarchaeota (taxon ID 1783275) reads at Bellairs are mapped to the Nitrosopumilales order (23% B versus 3% M, Dunn's test, $p << 0.01$; **Figures 33** and **34**). Nitrosopumilus are ammonia-oxidizing archaeons commonly found in marine environments (S.-J. Park et al. 2012; Zhang et al. 2014). The chemolithoautotrophic Nitrosopumilus martitimus species, which is enriched at the Bellairs site, has been established as a dominant contributor to denitrification (Sánchez-Quinto and Falcón 2019). Several additional Nitrosopumilales genera are also differentially identified between Bellairs and Maycocks including Candidatus Nitrosopelagicus, a planktonic pelagic ammonia-oxidizing thaumarchaeon involved in nitrogen and carbon fixation in marine environments (Santoro et al. 2015; Dhal, Kopprio, and Gärdes 2020). The genus was also noted to be one of the most abundant archaeal genera within an aquatic microbiome of mangroves (Dhal, Kopprio, and Gärdes 2020). The Bellairs site is situated offshore from semi-mangrove environments.

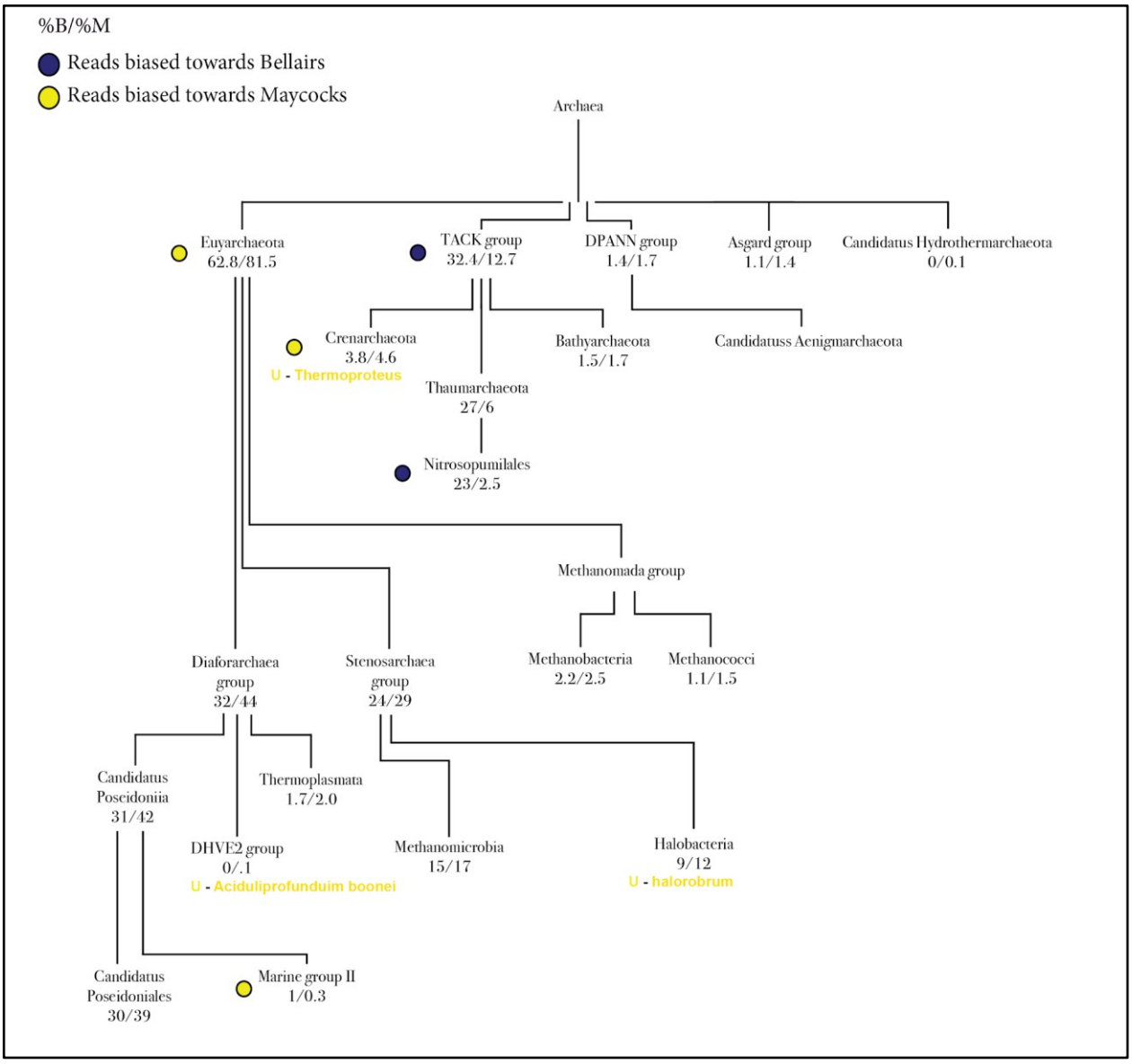


Figure 32. A schematic of the major taxa identified at Bellairs and Maycocks. Here the numbers are the percentage of all archaeal reads mapped to the taxa (B/M). Blue and yellow circles indicate taxa where reads are significantly biased towards Bellairs and Maycocks respectively as determined via the K-W test. (**Methods 9c**). Species uniquely identified at a given site are denoted in blue for Bellairs and yellow for Maycocks.

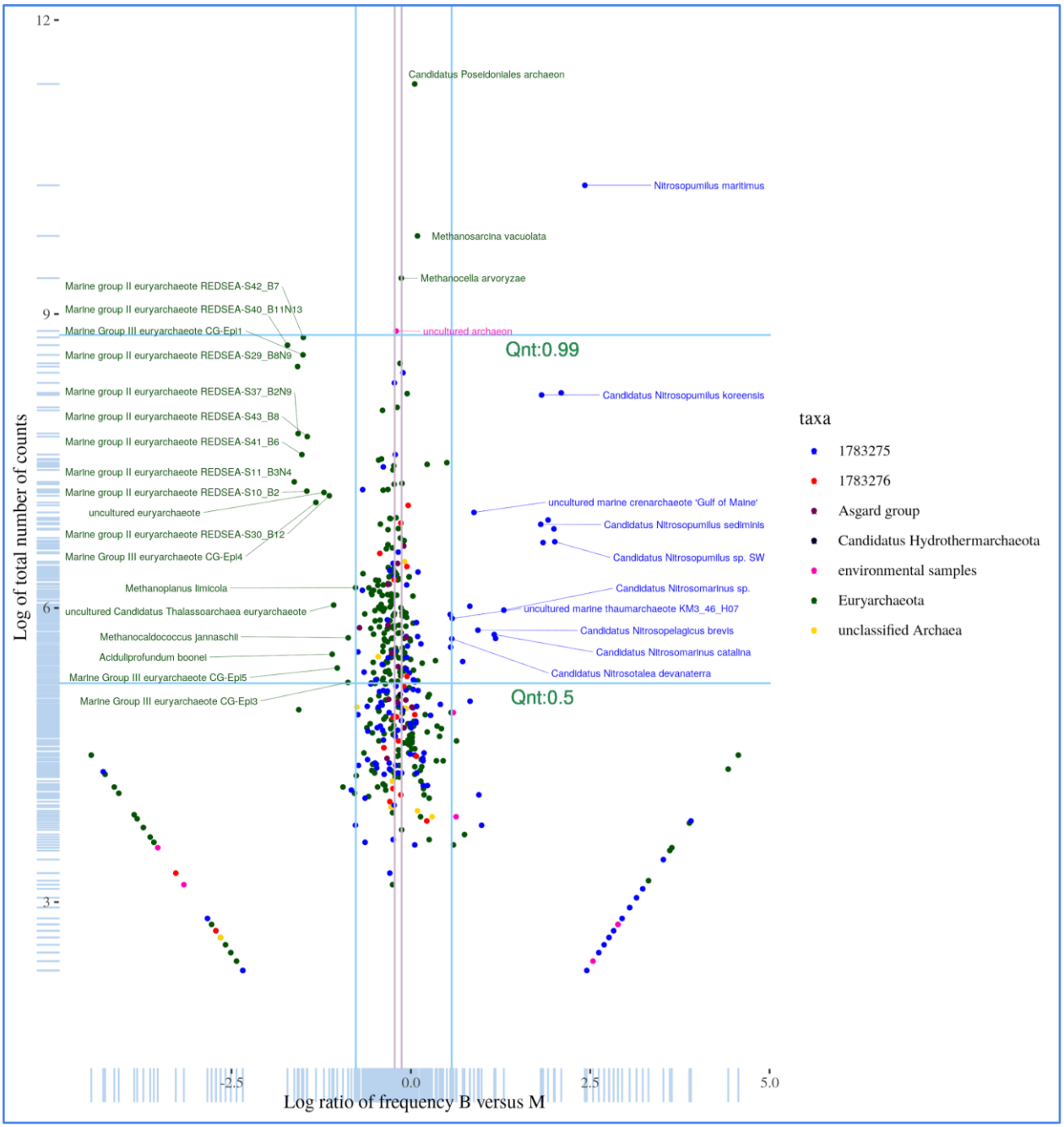


Figure 33. A log-log scatter plot of the log ratio of reads for all archaeal species versus the total number the total number of reads for the species.

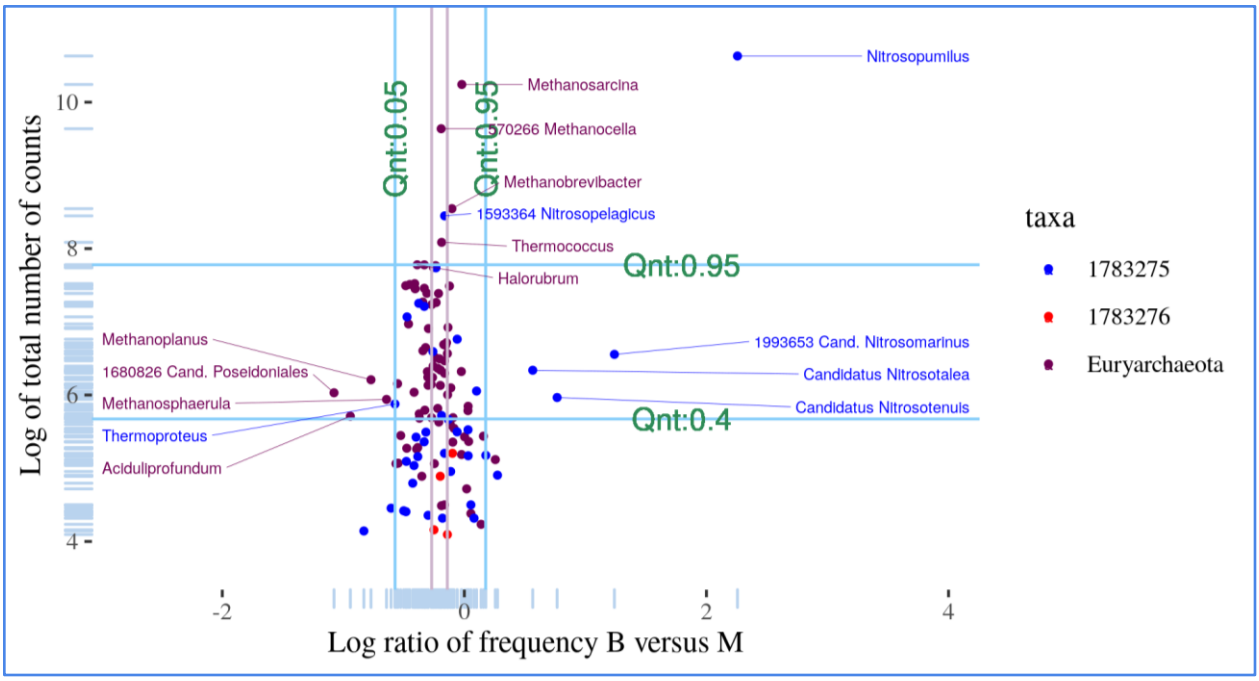


Figure 34. Analogous to the log-log scatter plot of **Figure 33** but at the genus level.

Eukaryota

The two sites identified a similar percentage of Eukaryota (11.9% B vs 9.8% M) and approximately half of these reads were mapped to Opisthokonta, specifically Fungi. The remainder of reads identified Viridiplantae with a slight enrichment at Maycocks (21% B vs 32% M), and several protist and unicellular algae groups including SAR, Rhodophyta and Haptophyta (**Figure 35**). We removed all multicellular organisms from our dataset as best possible (**Methods 8**).

In total, there was evidence for the presence of 579 species at Bellairs and 584 species at Maycocks from a total of 104 genera. **Figure 36** shows a rich representation of Fungi, several of which have very high abundances at both sites. Viridiplantae received a high fraction of reads at both sites Species of Haptophyta are biased towards Maycocks and contribute several outlying genera (Dunn's, $p < 0.01$, **Methods 9c**). Although SAR has low relative abundance at both sites, many different genera are outliers either at Maycocks or Bellairs.

In comparison to Bacteria, Archaea and viruses, there were many species uniquely identified at Bellairs but not at Maycocks. Recall the Maycocks sample generated 1.57 fold more reads. Moreover, the distribution of these unique Bellairs species were clearly not randomly distributed across the tree but localized to less than a dozen nodes (**Figure 35,** dark blue marked with "U"). This difference is or is not explained by the rarefy procedure ($p < 0.01$ or not; **Methods 10**).

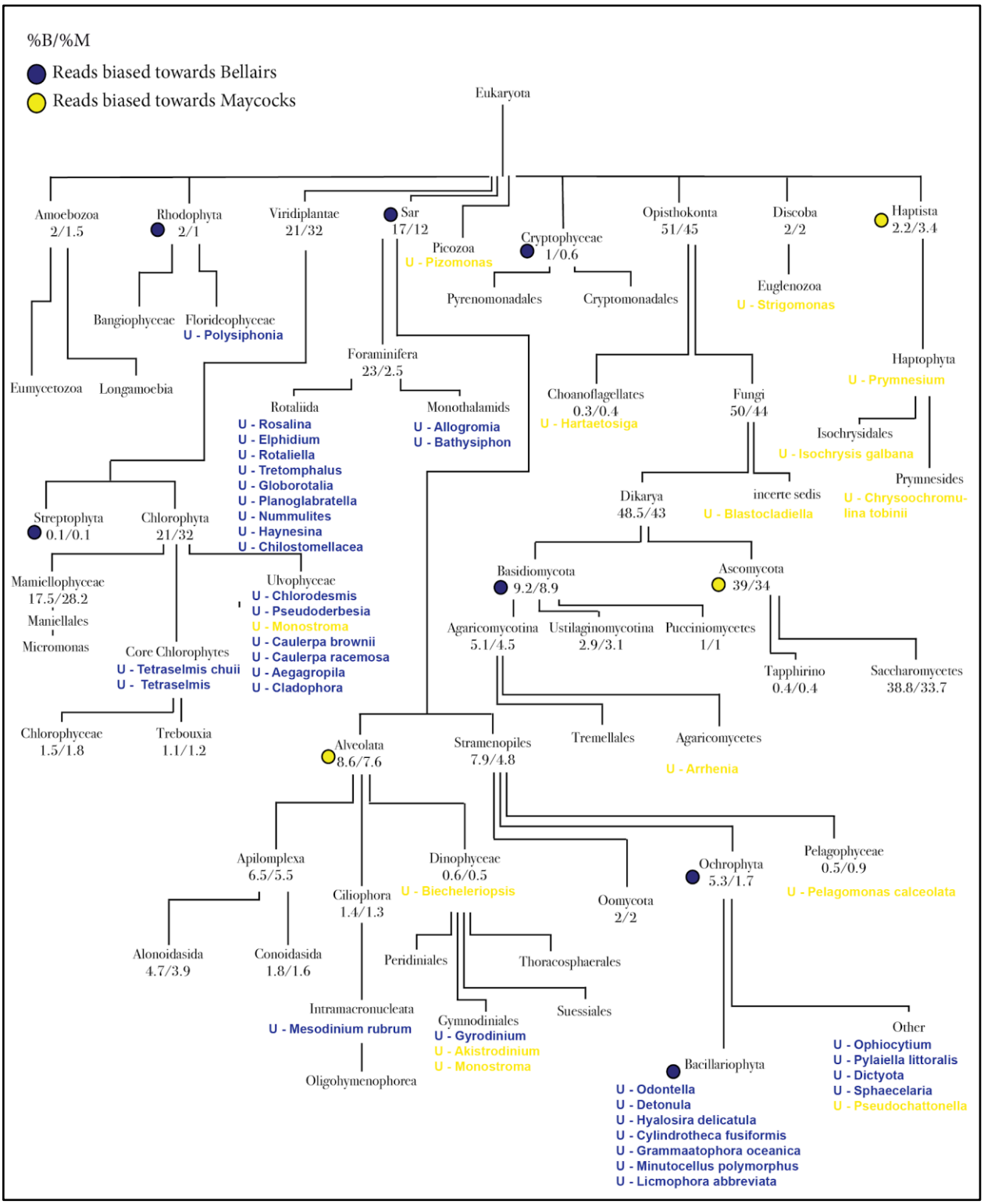


Figure 35. A schematic of the major taxa identified at Bellairs and Maycocks. Here the numbers are the percentage of all eukaryota reads mapped to the taxa (B/M). Blue and yellow circles indicate taxa where reads are significantly biased towards Bellairs and Maycocks

respectively as determined via the K-W test (**Methods 9c**). Species uniquely identified at a given site are denoted in blue for Bellairs and yellow for Maycocks.

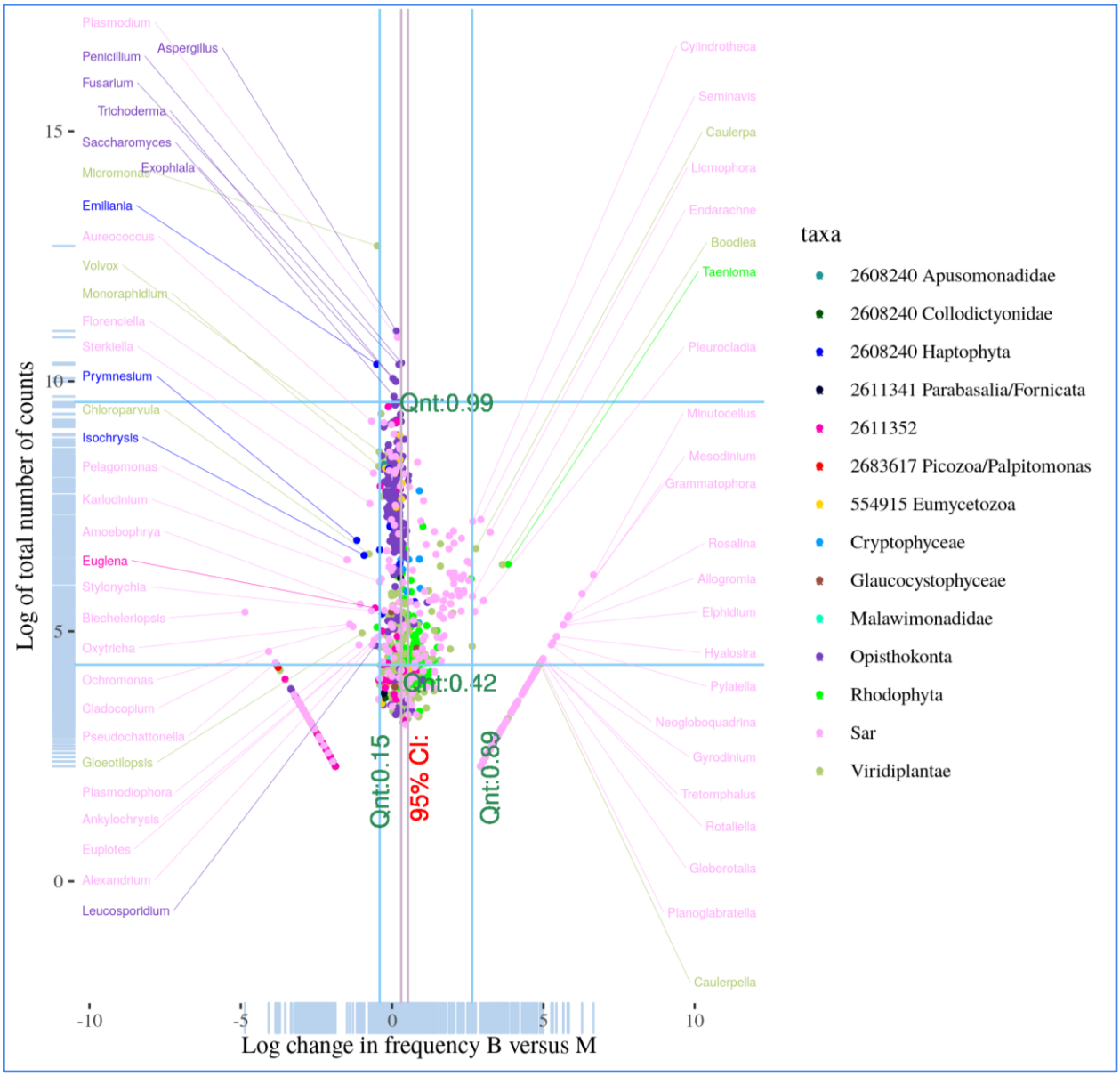


Figure 36. Analogous to the log-log scatter plot of **Figure 37** but at the genus level.

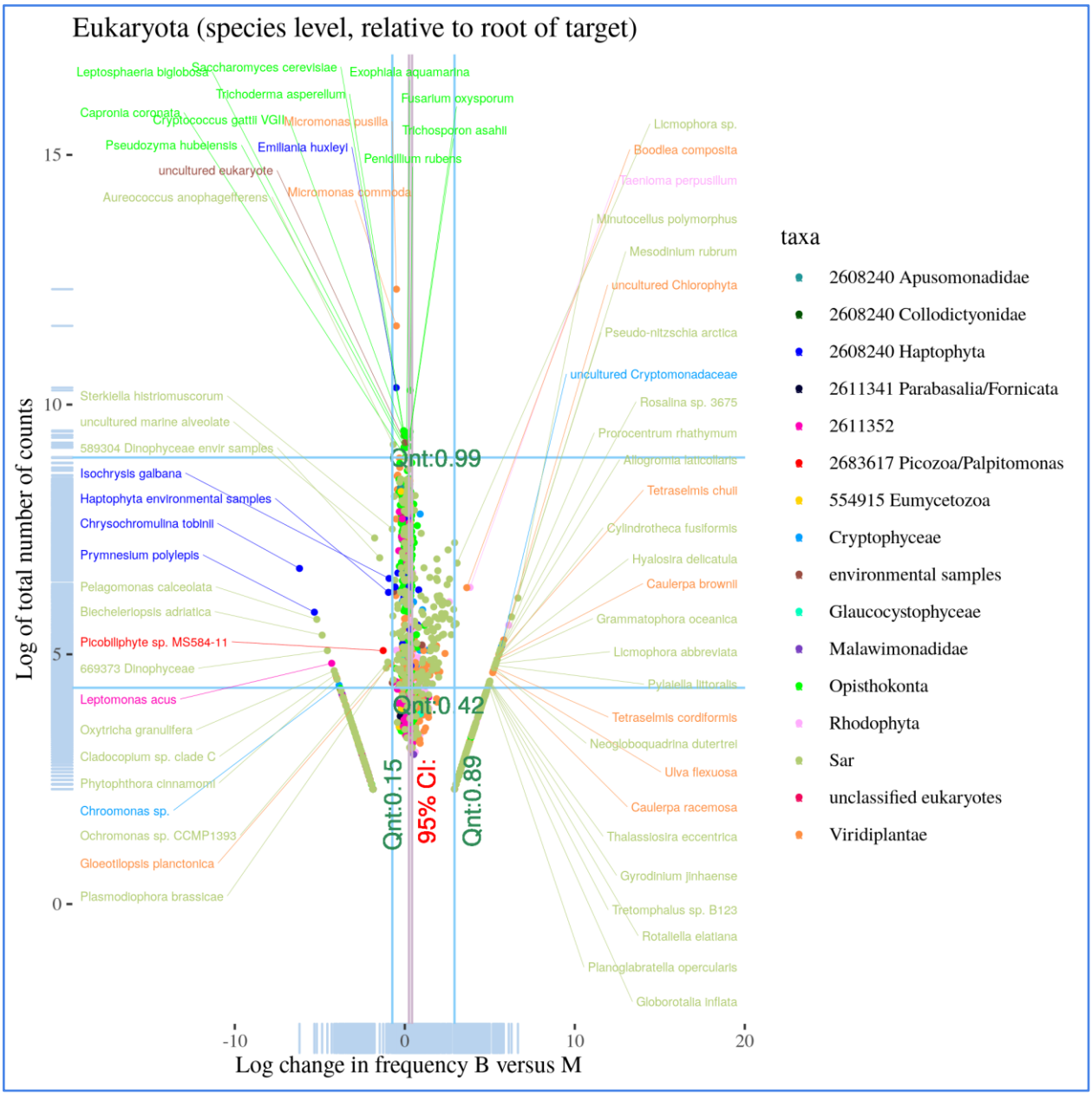


Figure 37. A log-log scatter plot of the log ratio of reads for all eukaryotic species versus the total number the total number of reads for the species.

Eukaryota, Opisthokonta: found to be affluent at both sites

Both sites received a large percentage of all reads mapped to Opisthokonta (51% B vs 45% M), however there were few extreme shifts in species or genera towards either site (**Figure 39**). In turn, the vast majority of these reads are localized to the “true yeasts” saccaryomyceta within Dikarya (fungi).

Rhizosporus microsporus is enriched at Bellairs and with high abundance. However, it is best understood as the pathogen causing rice seedling blight and there is no literature confirming its presence in marine systems. Candida dubliniensis and Candida albicans are also enriched at Bellairs. Both are well studied human pathogens and are capable of surviving in many environments. There is no literature confirming their presence in marine systems to the best of our knowledge. Aspergillus are asexual spore-forming fungi found worldwide in terrestrial and aquatic environments where they can be parasites of sea fans or cause causing blooms impacting coral - dinoflagellate symbiosis (Lee, Park, and Lim 2016; Amend et al. 2019). Specifically, the species A. sydowii is associated with sea fan mortality in the Caribbean, where the species may be an opportunistic pathogen of stressed hosts (Geiser et al. 1998). Aspergillus is thought to be a terrestrial fungus with the ability to survive and grow in marine environments (Soler-Hurtado et al. 2016). Metabolites of Aspergillus and Penicillium species affect the photophysiolocal performance of coral endosymbiont Symbiodinium (Hayashi et al. 2016). We conjecture that the identification of Candida species at Bellairs is due to the high level of evolutionary conservation across the genomes of all these “true yeasts”.

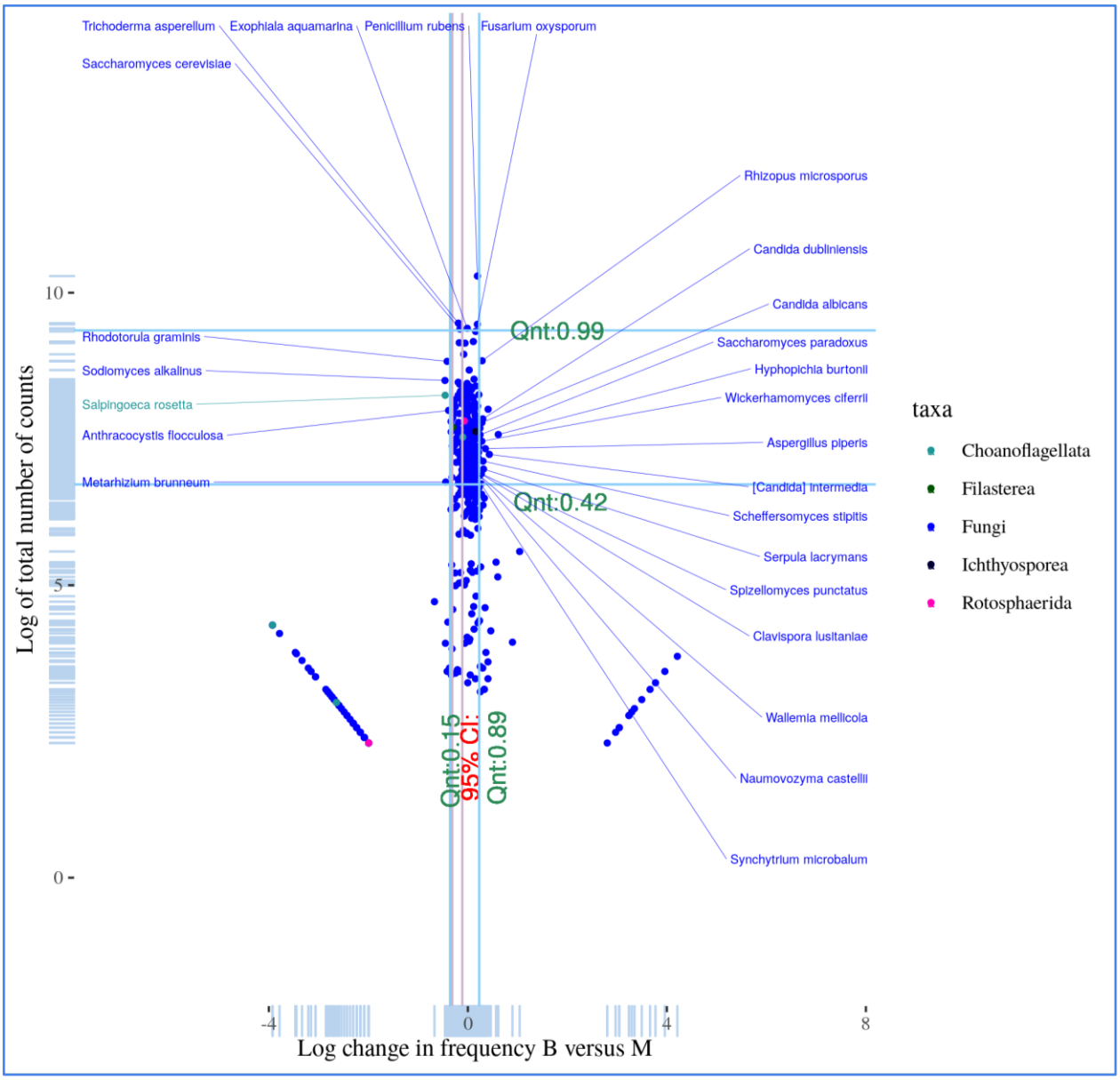


Figure 38. A log-log scatter plot of the log ratio of reads for all Opisthokonta species versus the total number the total number of reads for the species.

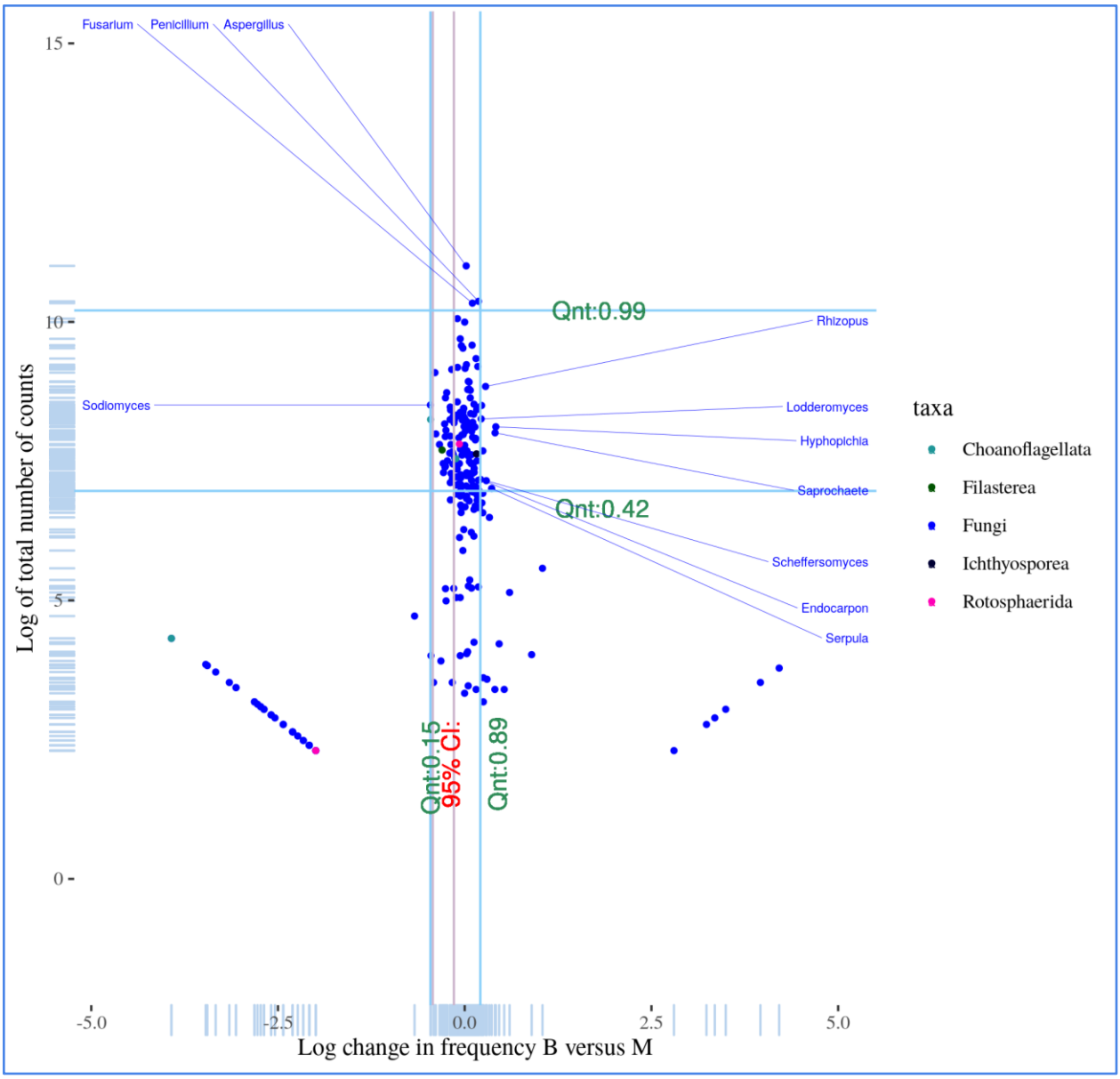


Figure 39. Analogous to the log-log scatter plot of **Figure 38** but at the genus level.

Eukaryota, Viridiplantae: Maycocks is highly enriched in photosynthetic autotrophic Chlorophyta

Over 99% of all reads mapped to Viridiplantae at both sites belong to the autotrophic green algae Chlorophyta. The genus Micromonas was the most highly abundant and preferred Maycocks; this is consistent with a recent pan-Caribbean study highlighting both M. pusilla and M. commoda at several sites (Bakker et al. 2019). Barbados was not profiled in that study. Micromonas contains dominant photosynthetic picoeukaryotes known to thrive globally in tropical marine environments

(Not et al. 2004; Cuvelier et al. 2017) and play a key role in the primary production within the euphotic zone (Šlapeta, López-García, and Moreira 2006).

Boodlea composita, which is abundant at the Bellaris site, is a common green algae in coastal marine environments (Leliaert et al. 2009). These macroalgae are capable of blooms forming dense turf or mats in nutrient rich waters. B. composita growths have been observed to smother coral colonies (Vroom et al. 2009).

Species of the genus Caulerpa were present only at Bellairs. Overall all three species had low abundance, although C. brownii was witnessed by just over 200 reads. Caulerpa is a genus of nitrophilic macroalgae that commonly inhabit tropical marine environments including Caribbean coral reefs (Clifton and Clifton 1999). Like B. composita, some species can bloom creating carpets which smother coral colonies (Smith et al. 2010). Caulerpa species assimilate nutrients from sediment and grow successfully in habitats with anthropogenic disturbance such as waste and stormwater (Crockett and Keough 2014).

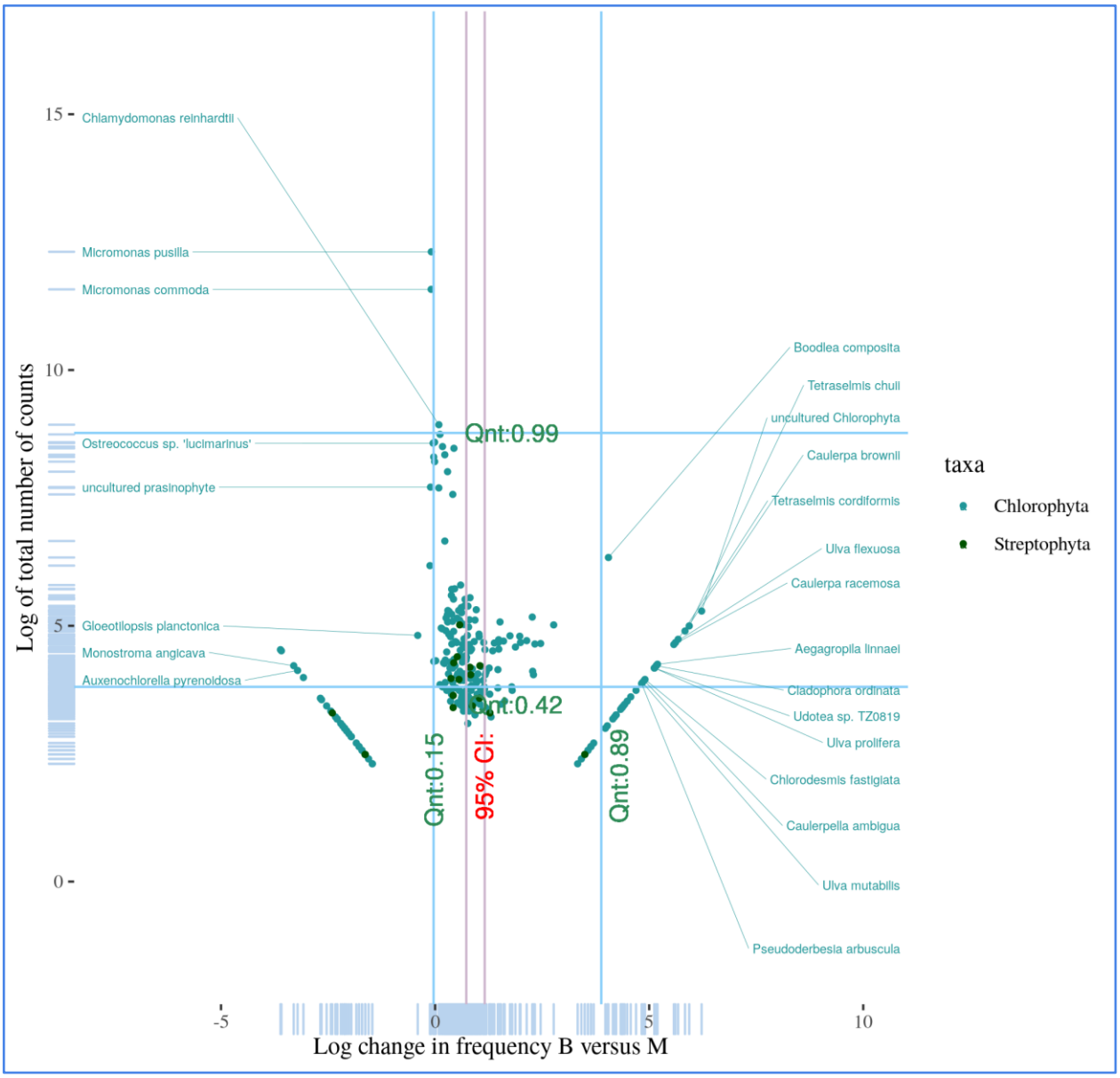


Figure 40. A log-log scatter plot of the log ratio of reads for all Viridiplantae species versus the total number the total number of reads for the species.

Eukaryota, SAR: Bellairs is enriched for benthic epiphytes growing on coral and algae

The Stramenopiles-Alveolates-Rhizaria (SAR) supergroup received 17% B and 12% M of all eukaryotic reads and exhibit large heterogeneity, contributing outlying species and genera at both sites (**Figure 37**). We removed all multicellular Stramenopiles (aka Heterokonts) from our dataset as best possible, leaving only single cell algae and diatoms (Adl et al. 2012). The Alveolates are a broad group of protists that contain the marine plankton Dinoflagellates, the Apicomplexa, and the Ciliates (Apicomplexa, Ciliophora and Dinophyceae in **Figure 35**). The Rhizaria are mostly unicellular non-photosynthetic heterotrophic amoeba and flagellates. Some such as the Radiolaria are marine plankton and, like the Foraminifera, they tend to form symbiotic relationships with marine algae (Moreira et al. 2007).

At Bellairs, Licmophora are marine diatoms that are common epiphytes within marine coastal environments, abundant on natural coral reef substates like filamentous algae (Lobban, Schefter, and Ruck 2011; Macatugal, Tharngan, and Lobban 2019). Some species of Licmophora grow on corals, forming mats which lead to bleaching or smother the coral (Yamashiro, Mikame, and Suzuki 2012). The genus Cylindrotheca consists of marine diatoms which are ubiquitous in coastal areas worldwide (Vanormelingen et al. 2013). Species C. fusiformis has been proposed as a supplementary feed for farmed sea cucumbers because in addition to nutrients for the crop, they may improve water quality by using latent nitrogen and phosphorus nutrients (Li et al. 2015). Seminavis are marine epipelic benthic diatoms found in coastal waters within the benthic community (Danielidis and Mann 2003). Species of this genus are known to associate with seaweed living within coral reef ecosystems (Park, Lobban, and Lee 2018). The genus Gyrodinium consists of marine heterotrophic dinoflagellates which prey on diatoms (Hansen 1992), and can cause red tides (Yim et al. 2007). Pleurocladia is a genus of benthic brown alga epiphyte of macroalgae and Hyalosira is a genus of benthic marine diatoms that are known to attach to seaweeds in intertidal environments (Totti et al. 2009). Endarachne is a monotypic genus of the brown seaweed E. binghamiae commonly found in warm coastal waters (Parente, Neto, and Fletcher 2003). Minutocellus is a genus of marine diatoms and species M. polymorphus is a free living diatom, but is possibly a symbiont of benthic foraminifera (Schmidt et al. 2015). Minutocellus is found to be codominant with brown tide causing Chrysophyte Aureococcus anophagefferens that thrives in eutrophic marine environments (Qiao et al. 2017). Grammatophora is an epiphytic marine genus of diatoms found in coastal marine environments (Sato et al. 2008).

Several of these species are identified uniquely at Bellairs (**Figure 35** denoted with U). Our ecological diversity analysis suggests this is not likely due to the depth of sequencing alone (Section Global Differences above) especially given that Maycocks received 1.57 fold more reads than Bellairs. In fact, the vast majority of uniquely identified organisms at Bellairs are classified as either Foraminifera (discussed in the next subsection) or within the SAR phylum Ochrophyta (11 of all 34 uniquely identified organisms identified at Bellairs, hypergeometric binned by phyla in Eukaryota, $p << 0.01$).

Eukaryota, SAR: Maycocks is enriched for organisms involved in primary production and regulation of the micro-planktonic community

There are many SAR genera and species with abundances shifted towards Maycocks with common ecological roles including primary production. Genus Pelagomonas is monotypic containing only the marine picoplankton P. calceolata (Dimier, Giovanni, et al. 2009), a tiny photosynthetic flagellated alga that contributes to primary production in marine environments (Dimier, Brunet, et al. 2009). Biecheleriopsis contains small marine phototrophic planktonic dinoflagellates (Jang et al. 2015) that play a role as primary producers and symbiotic partners (H. C. Kang et al. 2019). Karlodinium are phytoplanktonic coastal dinoflagellate mixotrophics that rely on both photosynthesis and phagotrophy (Place et al. 2012). They can cause toxic algal blooms when nutrients are scarce within their environments leading to fish mortality (Müller et al. 2019; Lin et al. 2018). Euglena contain unicellular phototrophic euglenoids that are also heterotrophic and able to absorb nutrients. Euglena species are key primary producers within marine ecosystems (Bi et al. 2019). Alexandrium is a genus of planktonic dinoflagellates that contribute to primary production (Toulza et al. 2010; Anderson et al. 2012).

Other species and genera biased towards Maycocks are regulators of the micro-planktonic community or have symbiotic roles with coral and sponges. This includes for example the Stylonychia (Pfister and Arndt 1998), Sterkiella histriomuscorum (X. Chen et al. 2015) formerly known as Oxytricha trifallax, and Oxytricha granulifera (Méndez-Sánchez et al. 2018). Amoebophrya species are marine parasitic dinoflagellates that infect other free-living dinoflagellates that inhabit coastal waters (Kim et al. 2008). Amoebophrya species can be found either in a free-swimming infective stage or as a multinuclear growth phase within an infected dinoflagellate (Velo-Suárez et al. 2013). The golden algae Ochromonas are small unicellular mixotrophic flagellates that play a key role in regulating bacterial abundance (Hu et al. 2015). The dinoflagellates of Cladocopium establish endosymbiosis with cnidarian species such as coral (Vega de Luna et al. 2019). Cladocopium species are the dominant symbionts of some stony corals within the Caribbean including Barbados (Brian, Davy, and Wilkinson 2019; Eckert et al. 2020; Finney et al. 2010). The Euplotes are filter-feeding ciliates that are found worldwide in marine, freshwater and terrestrial ecosystems (Lian et al. 2020). Some free-living marine Euplotes have been linked to the ingestion of coral tissue. This form of pathogenesis from ciliates may lead to disease in corals (M. Sweet and Bythell 2012; M. J. Sweet and Séré 2016).

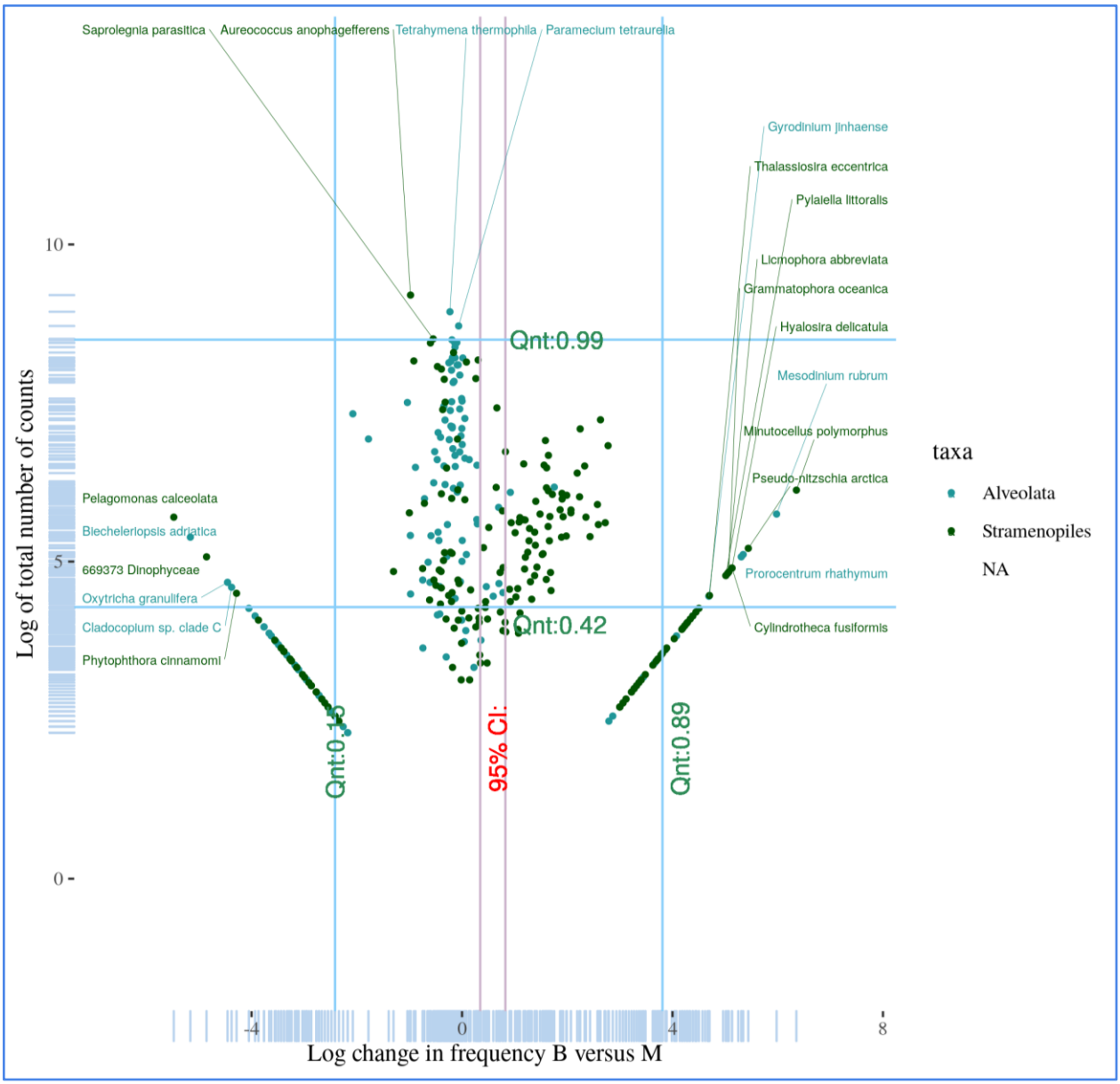


Figure 41. A log-log scatter plot of the log ratio of reads for all SAR species versus the total number the total number of reads for the species.

Eukaryota, Foraminifera: Bellairs exclusively has low levels of species indicative of pollution and natural stress

Across Eukaryota we observed many instances where a species/genus were identified uniquely at Bellairs. This likely cannot be explained by stochastic sampling error, since the Maycocks site received 1.57 fo ld more reads than Bellairs. In fact, 12 of 34 of the genera belonged to Foraminifera ($p << 0.01$, hypergeometric binned by clades directly from the root of Eukaryota). IN fact, 10 of these 12 genera are within the Rotaliida clade. There was no evidence of a Foraminifera species or genus at Maycocks.

Foraminifera are single-celled shelled protists and recognized as one of the most abundant groups of microorganisms in the shallow marine waters. The fact that their size range (100µm-20cm) is well beyond our filtered range may explain why few reads were identified for these taxa. Some amoeboid protists are often benthic or live in the sea sediment; at least 40 morphospecies are planktonic and form symbiotic relationships with marine algae. They are sensitive to the subtle changes in the ambient environment and species are known to survive and increase in numbers in polluted areas (Nigam, Saraswat, and Panchang 2006; Nigam et al. 2009). Planktonic foraminifera play an important role in the carbonate pump, contributing up to 50% of the total carbonate in the ocean sediment (Manno, Morata, and Bellerby 2012).

Genera Neogloboquadrina, Globorotalia, Planoglbratella, Elphidium, Rosalina, Allogromia and Rotaliella are all recognized as planktic. Neogloboquadrina is found globally (Jentzen et al. 2019) with species N. dutertrei recognized as an abundant Caribbean plankton (Hilbrecht 1997; Darling et al. 2006). Tretomphalus is typically a benthic foraminifera, prevalent on tropical reefs (Lipps 1988; Alldredge and King 1977). Planoglabratella is often found in shallow marine waters (Panieri et al. 2005) and species P. opercularis is known to be epiphytic on seagrasses (Takata et al. 2016; Buosi et al. 2020). Some species are widely distributed throughout the Atlantic (Poag and Tresslar 1981). Rotaliella is epiphytic on macroalgae (Wilson and Ramsook 2007). Species of Elphidium form structures on coralline algae (Sarkar, Ghosh, and Narasimha Rao 2016). Rosalina is known to attach to seaweed and other marine benthic surfaces within shallow environments and can also be found unattached within sediment (Todd 1965) and species such as R. leei are able to thrive in ecologically stressed environments (Kurtarkar Raikar et al. 2011). Allogromia is a genus of benthic foraminifera that are part of the benthic foraminifera community in marine habitats (Bernhard and Bowser 1992).

Eukaryota: Other protists and single cell algae

Across the remaining subclades of Eukaryota, the Rhodophyta species Taenoma perpusillum is highly abundant and shifted strongly towards Bellairs. Taenioma is a turf marine algae with finely branched filamentous rhodophytes (Hata, Nishihira, and Kamura 2002). The species T. perpusillum has been identified in Caribbean waters and reefs (Albis-Salas and Gavio 2011; Cetz-Navarro, Quan-Young, and Espinoza-Avalos 2015).

The coccolithophorid genus Emilliania was also abundant but differentially identified at Maycocks. The main species within this genus is E. huxleyi is an unicellular photosynthetic eukaryote that is a key contributor to the oceanic carbon cycle via calcification, photosynthesis and export of inorganic matter to the oceans' interior (Blanco-Ameijeiras et al. 2016) . The planktonic unicellular flagellates Prymnesium are also more abundant at Maycocks. Prymnesium contributes to primary production within marine environments but some species are also known for causing harmful marine algal blooms (Hallegraeff 1992; Cuvelier et al. 2010). Isochrysis is a genus of marine unicellular microalgae which serves as a food for bivalve larvae in commercial settings (J. Liu, Sommerfeld, and Hu 2013).

Viruses

Marine viruses affect microbial populations by releasing carbon and nutrients into the ecosystem through lysis, by complexing nutrients such as iron, through reprogramming of host metabolism and horizontal gene transfer, and via the formation of relationships including for example cyanobacteria-cyanophage relationships which affect CO2 fixation (Breitbart 2012; Breitbart et al. 2018; Brum et al. 2015; Suttle 2007; Bonnain, Breitbart, and Buck 2016). Metagenomic analysis of marine viruses has been investigated including in the context of the Tara Oceans Project (Brum et al. 2015). Brum and colleagues isolated organisms below 0.22 µm and built optimized sequencing and bioinformatics platforms specific for the analysis of viromes. Our investigation here is limited, since we selected for organisms between 0.22 µm and 3 µm. Nevertheless, 5.8% (B) and 3.6% (M) of all reads mapped to viruses (**Figure 42**). Brum and colleagues also built specialized analytic pipelines; we however use the same pipeline for our preliminary investigations here.

Viruses: Bellars is highly enriched for uncharacterized phages

A large fraction of all viral reads were mapped to virus genomes reported first in a study that developed an assembly-free single molecule nanopore sequencing approach for viruses from environmental samples obtained close to Hawaii (Beaulaurier et al. 2020). These so-called assembly-free virus genomes (AVGVs) show a clear preference for Bellairs (KW, $p << 0.01$). Moreover, the original virus-enriched samples sequenced by Beaulaurier were collected at 25, 117, or 250 meters with n=565, 93, 1023 respectively. The samples from Beaulaurier et al. were filtered to remove all organisms > 0.22 µm. Of the 14 Marine virus AFVG in the 97.5% percentile across all viruses in our data (**Figure 43**), 11 were harvested at 25 meters. This depth is closest to our samples harvested just below the surface. In Beaulaurier et al, they estimate that 26% of the AFVGs from 25m correspond to cyanophages, 13.3% to SAR11 phages, 12% to SAR116 phages, and 3% to Vibrio phages.

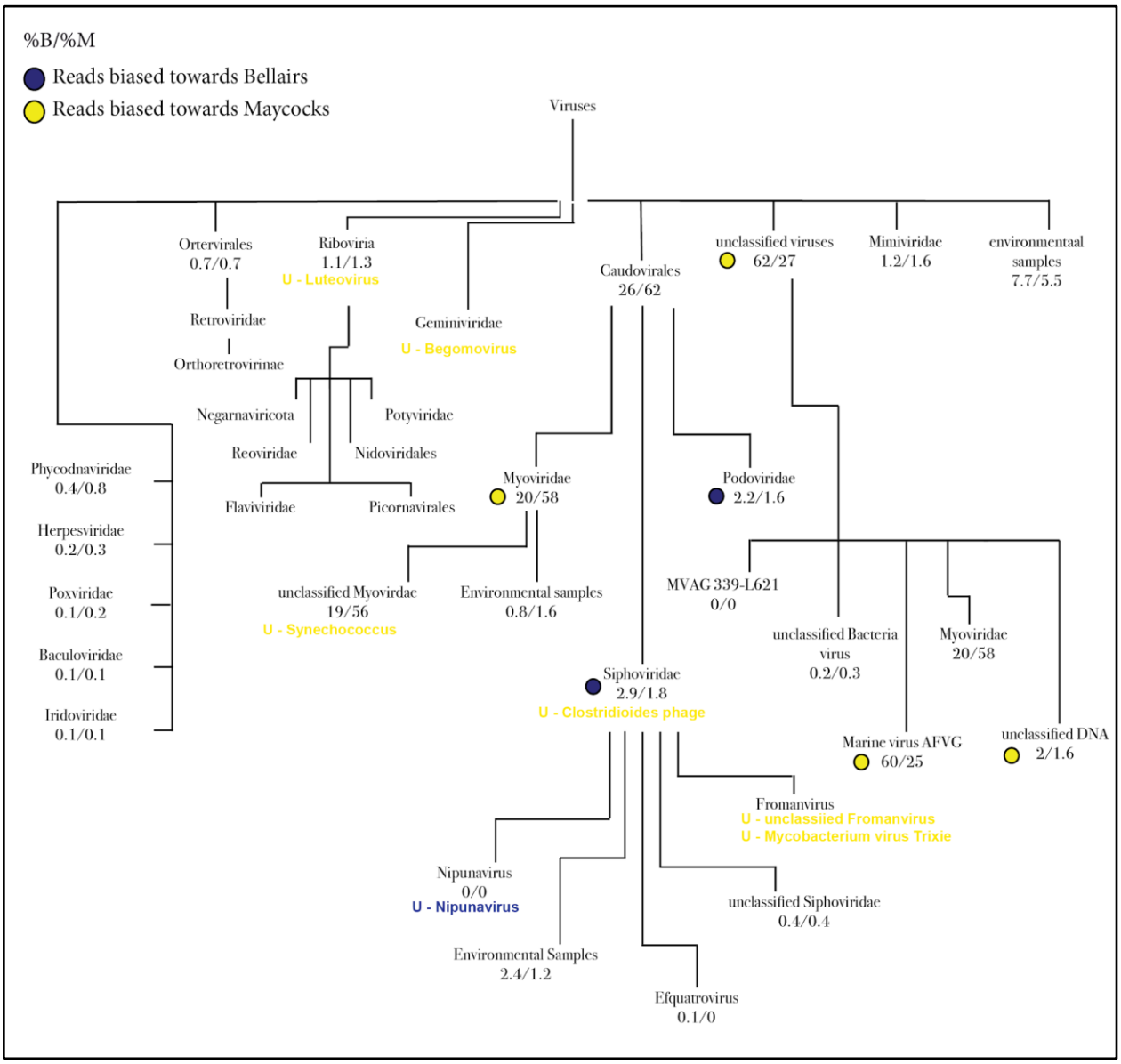


Figure 42. The major taxa identified at the Bellairs and Maycocks sites are annotated with their relative frequencies. Blue and yellow circles indicate taxa where reads are significantly biased towards Bellairs and Maycocks respectively as determined via the K-W test (**Methods 9c**). Species uniquely identified at a given site are denoted in blue for Bellairs and yellow for Maycocks.

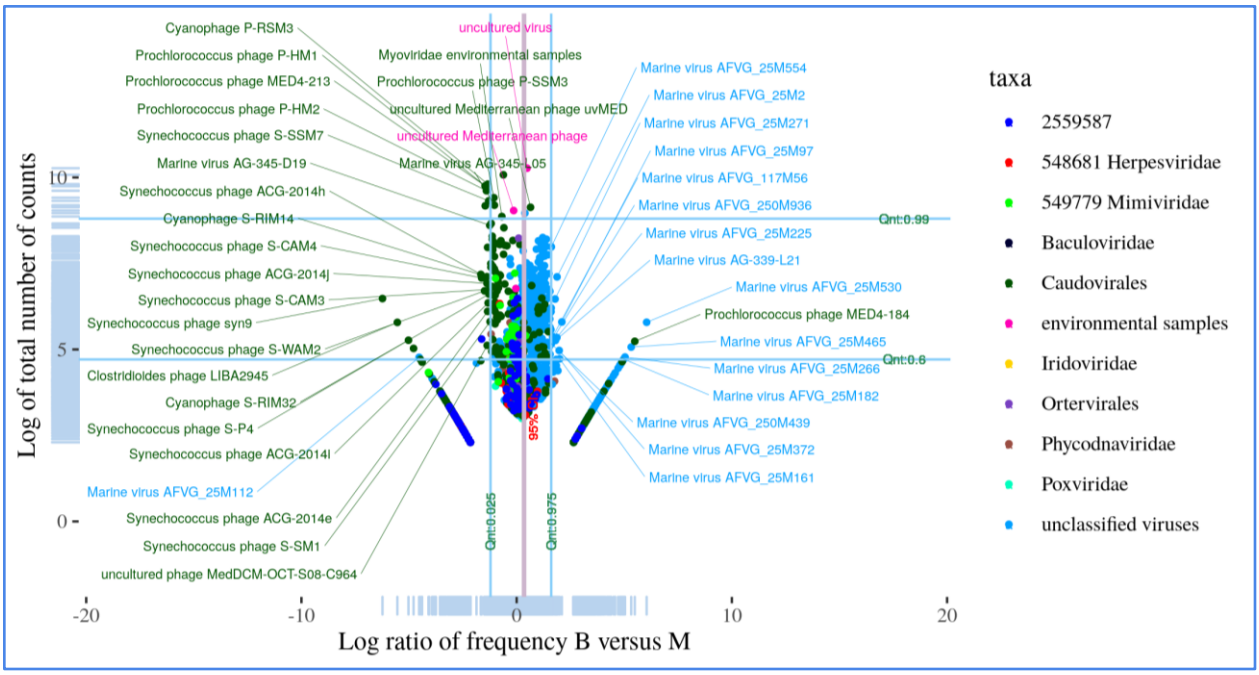


Figure 43. A log-log scatter plot of the log ratio of reads for all virus species versus the total number the total number of reads for the species.

Viruses: Cyanophages are highly enriched at Maycocks, a site highly enriched for cyanobacteria

Consistent with the strong preference for Cyanobacteria (incl. Prochlorococcus and Synechococcus) at Maycocks (26% B vs 62% M), there is a comparably strong preference for cyanophages at Maycocks (KW and Dunn's test for Caudovirales, both p << 0.01, **Figure 43**). Consistent with previous findings (Xiao et al. 2018), Myo-, Sipho- and Podo- viruses are found in our data; these are well established phages for Prochlorococcus and/or Synechococcus.

Viruses: Bellairs is enriched for phages of Pelagibacter

Although the small heterotrophic Pelagibacter, a member of the ubiquitous SAR11 clade, is highly enriched at Maycocks, Podovirus phages of Pelagibacter are systematically shifted towards Bellairs (KW, Dunn's test, p<<0.01).

Viruses: Bellairs is enriched for plant, animal and algae viruses

Several additional plant, animal and algae viruses were enriched at Bellairs including Negarnaviruses (Canine morbillivirus, Salmon isavirus), Potyviruses (Turnip mosaic virus, Sugarcane mosaic virus), Phycodnaviruses and Iridoviruses.

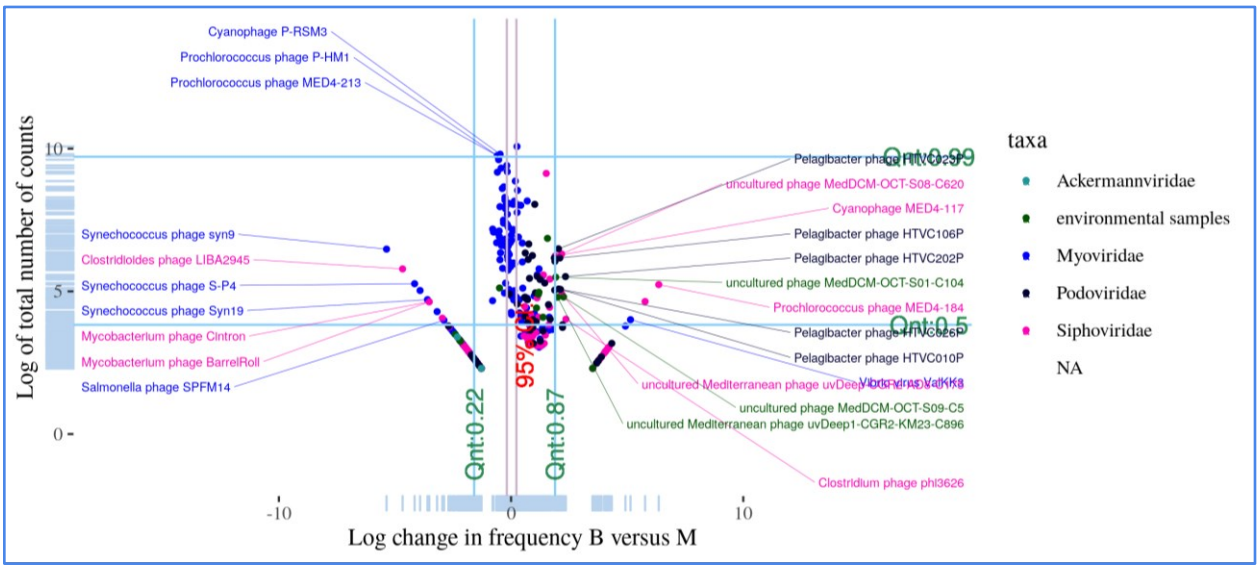


Figure 44. A log-log scatter plot of the log ratio of reads for all Caudovirales species versus the total number the total number of reads for the species.

Viruses: Comparison with the Tara Oceans data

We compared the Shannon index of the Myoviridae, Podoviridae and Giruses against the analogous values reported for the Tara Oceans data (Ibarbalz et al. 2019) (Figure 2A). At 11 degrees latitude, Tara Oceans data established the entropy at 3+/- 0.2 for Myoviridae, whereas

we report a slightly higher entropy at 3.68 (B) and 3.63 (M). For Podoviridae, Tara Oceans reports 5 +/- 0.1, whereas we have a much smaller entropy at 2.94 (B and M). The giant viruses (giruses) have an entropy of 5.5 +/- 0.1 in the Tara Oceans data. As a working definition for viruses, we included any virus specus to the nucleocytoviricota clade. In our data the Shannon index is much lower at 3.19 (B) and 3.2 (M).

Disease and Infections

We next compared the relative abundance of genera and species responsible for human disease obtained in marine environments. This includes bacteria, protozoa or viruses that are directly human pathogens, or bacteria which produce toxins (for example, when they grow in shellfish). We consider autochthonous organisms as well as those introduced by eutrophication eg in sewage.

Disease: Bacterial infections

For bacterial infections, we considered the genera Vibrio (alginolyticus, cholerae, parahaemolyticus, vulnificus, mimicus, hollisae, fluvialis, non-O1 amongst others), Mycobacterium bovi, Salmonella typhi, Ersipilothrix, Clostridium botulinum, Mycobacterium marinum, Staphylococcus iniae, Pseudomonas, and Shigella (Munn 2011; 2015) (Table 11.1 therein) (**Figure 45**). Vibrio species and genera were significantly shifted towards Bellairs (Dunn's test, $p << 0.01$). Conversely, Shigella flexneri and Staphylococcus preferred Maycocks, albeit with overall less abundance.

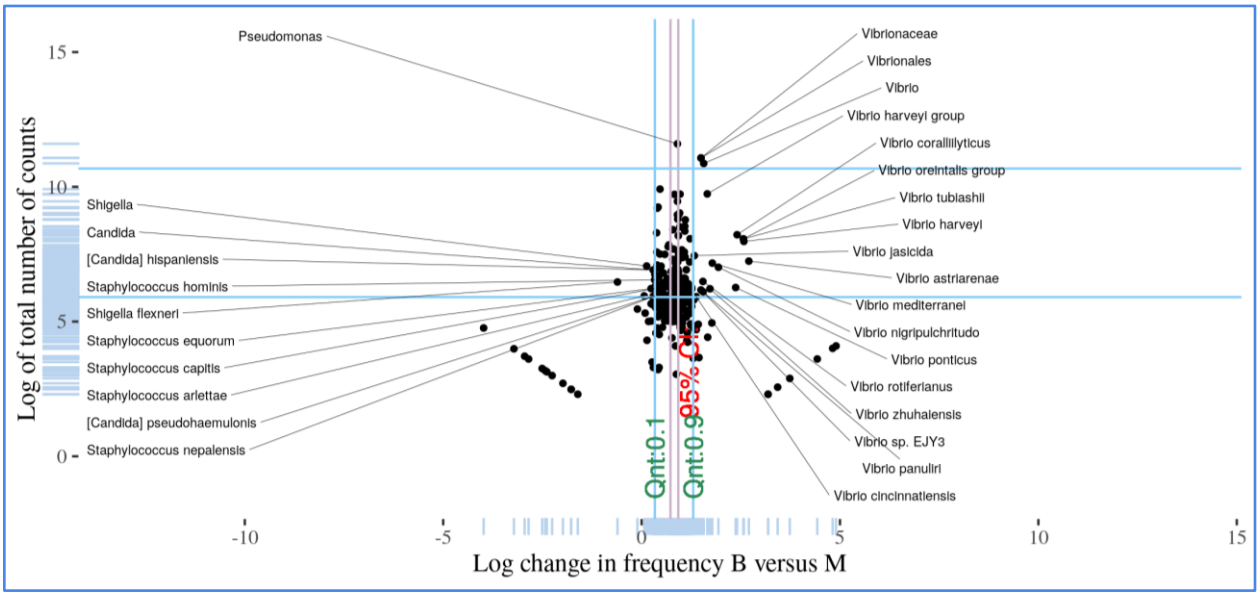


Figure 45. A log-log scatter plot of the log ratio of reads for all bacterial infection-related species versus the total number the total number of reads for the species.

Human Disease: Bacterial intoxications

For bacterial intoxications, we considered species from genera Vibrio, Clostridium botulinum, Shewanella, Morganella, and Photobacterium as a coarse guide for the relative abundance between the two sites (**Figure 46**). Many Vibrio species have increased abundance in addition to Photobacterium damselae at Bellairs. Two Shewanella (baltica and donghaensis) have increased expression at Maycocks as well as Shewanella baltica (KW test, $p < 0.01$).

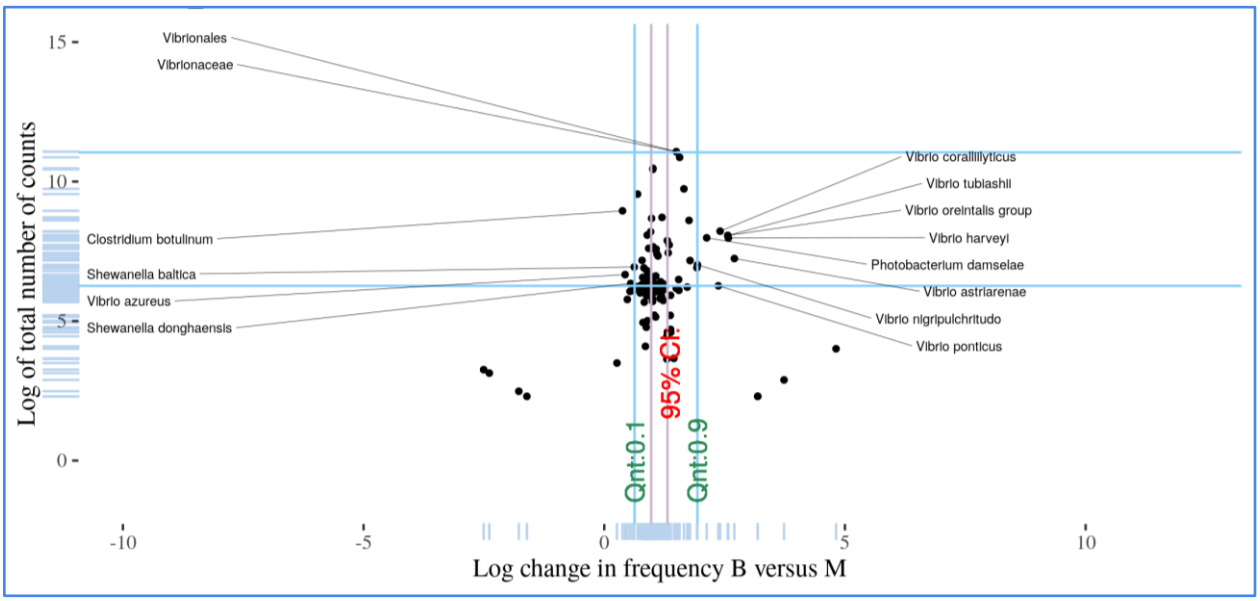


Figure 46.A log-log scatter plot of the log ratio of reads for all bacterial intoxication species versus the total number the total number of reads for the species.

Human disease: Coarse estimation of water quality

Tests for the safety of marine waters are well-established and are based on the concept of "indicator organisms" whose presence implies that there is an increased chance that the waters contain pathogens. As a coarse investigation of our two sites, we examined all species from the following genera Escherichia, Citrobacter, Enterobacter, Erwinia, Hafnia, Klebsiella, Serratia, Yersinia, Bacteroides, Bifidobacterium, Lactobacillus, and Clostridium (**Figure 47**). Although all species were tightly distributed around the mean of the distribution, there were obvious systematic biases with species of Clostridium (n=5) and Lactobacillus (n=3) more abundant at Maycocks. At Bellairs, there was a clear preference for Bacteroides (n=2) and Yersinia (n=2).

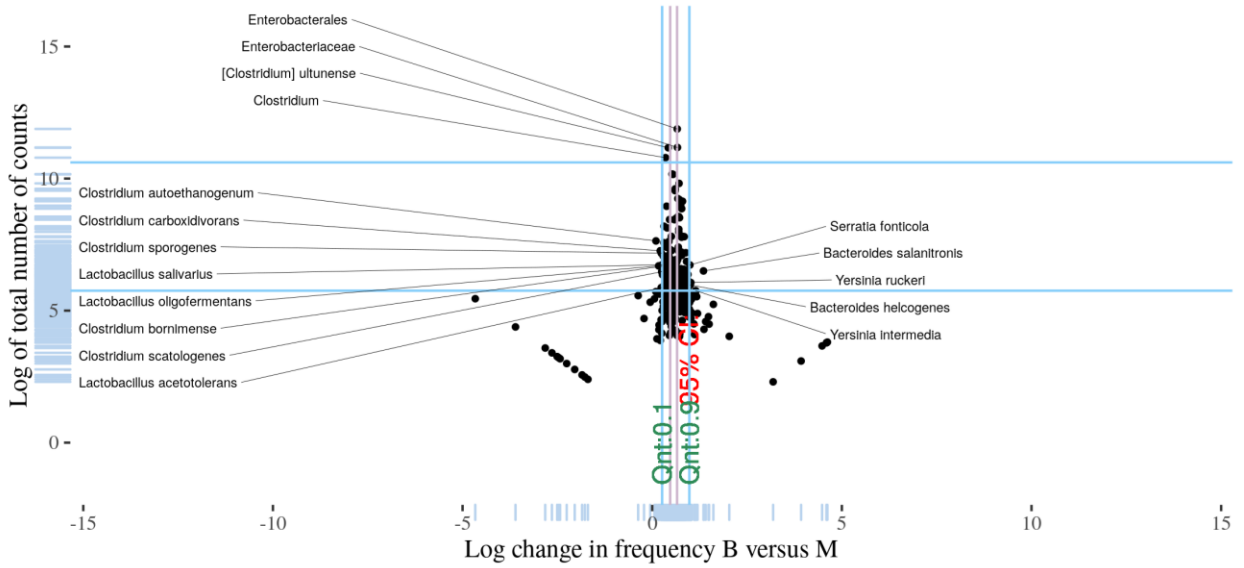


Figure 47. A log-log scatter plot of the log ratio of reads for all pathogenic species used for water quality indication versus the total number the total number of reads for the species.

Human Disease: Viral infections

We considered viruses found in marine environments with established roles in human disease including the Norwalk virus, SSRVs and rotavirus (fecal contamination), A, non-A, non-B hepatitis (swimming), poliovirus (filter feeding molluscs), and influenza virus (captive marine mammals). However, all taxa had very low abundances (< 10 reads) or did not exceed the 95% bootstrap confidence interval for the mean log-ratio of frequencies at Bellairs and Maycocks. We remind the reader that our samples were filtered at 0.22 µm (figure not shown).

Human Disease: Dinoflagellate and diatom intoxications

We included here species from the genus Protoperidinium (grow in filter-feeding shellfish), species Gambierdiscus toxicus (accumulation of ciguatoxins in fish), genus Dinophysis (accumulation of toxic dinoflagellates), and Alexandrium, Gymnodinium, Pyrodinium, Pseudo-nitzschia and Pfiesteria piscicida. However, all genera/species had very little presence in our dataset as most are larger than our filter threshold of 3 µm. Pseudo-nitzschia arctica had a strong preference for Bellairs (figure not shown).

Human disease: Shellfish poisoning

We investigated bacteria associated with paralytic, neurotoxic, and diarrhetic shellfish poisoning but did not find any taxa that had different abundances in our data (figure not shown).

Symbiotic Associations

The relationship between bleaching events and the coral symbiotic dinoflagellate Symbiodinium trenchi has been well-investigated at Barbados including the 2005 event (LaJeunesse et al. 2009). S. trenchi is a potentially opportunistic red algae rarely observed in the Caribbean and which may confirm survival advantages to coral. The average size of Symbiodinium is 6.7-11 μm (Biquand et al. 2017), and therefore it is not surprising that only a few reads are mapped to this genus (187 B vs 351 M total reads) identifying only S. kawagutii, which is thought to primarily form symbiotic relationships with Foraminifera, and not the coral directly (Yuyama, Higuchi, and Mezaki 2016) (**Figure 48**).

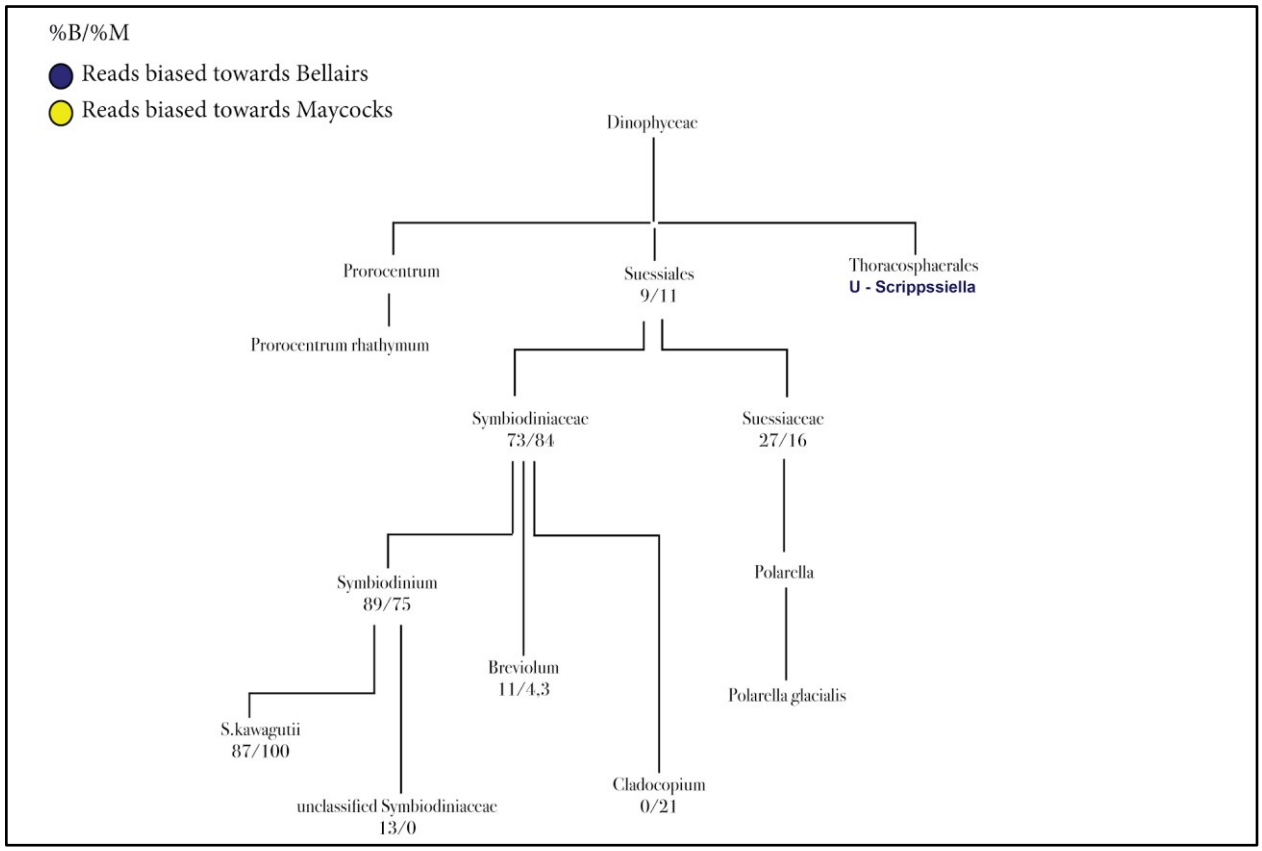


Figure 48. A schematic of the Dinoflagellate taxa identified at Bellairs and Maycocks. Here the numbers are the percentage of all dinoflagellate reads mapped to the taxa (B/M). Blue and yellow circles indicate taxa where reads are significantly biased towards Bellairs and Maycocks respectively as determined via the K-W test (**Methods 9c**). Species uniquely identified at a given site are denoted in blue for Bellairs and yellow for Maycocks.

Abiotic and Biotic Biomass

Cell counts: Prokaryotic and Virus-like Particles

Prokaryotic and virus-like particles (VLP) were imaged and identified across 80 random fields of views for each site. Cells were binned into specific taxa bins based on size to give a hypothetical view on taxa ratios between the two study sites. Cells where bin by taxa based on the following size ranges; VLP (< 0.19µm), C. Pelagibacter (0.2 - 0.49µm), Prochlorococcus (0.5 - 0.89µm), Synechococcus (0.9-1.79µm) and other prokaryotic cells (1.8 - 2.99µm). Counts from each bin were found to have extremely high variance among all fields of view, however.

Nutrient Measures

No significant difference in any of the nutrient measures were identified between Bellairs and Maycocks (Mann-Whitney-Wilcox test, $p > 0.05$). Bellairs showed vastly higher readings for nitrates and nitrites than measurements taken at Maycocks, however phosphate readings were found to be higher at the Maycocks site. These measurements will not reflect the costal microbiome from which our metagenomic analysis represents.

		Bellairs	Maycocks
Dissolved Oxygen (%)		89.73	95.30
Dissolved Oxygen (mg/L)		5.68 (±0.08)	6.04 (±0.03)
Salinity (ppt)		35.03 (±0.48)	34.78 (±0.60)
Temperature (°C)		29.03 (±0.02)	29.13 (±0.06)
Nitrate (NO_3) (mg/L)		26.34 (±3.58)	15.05 (±2.54)
Nitrite (NO_2) (mg/L)		0.44 (±0.12)	0.30 (±0.07)
Phosphate (PO_4) (mg/L)		0.39 (±0.41)	0.81 (±0.08)
Turbidity (NTU)		8 (±2.65)	7 (±1.00)

Table 4. The nutrient measurements of the Bellairs and Maycocks Reef based waters sample replicates and standard deviation.

			Bellairs	Maycocks	FC
	No. of contigs (metaSPAdes)		4,640,479	5,525,054	1.19
	No. of coding Sequences (Prokka)		576329	668933	1.16
	No. of Transfer RNA genes (Prokka)		6078	6328	1.04
	No. of Transfer-messenger RNA (Prokka)		64	96	1.50
	No. of CRISPRs		311	136	0.44
	No. of coding regions successfully mapped to NCBI NR (Diamond)		227538	256509	1.13
	No. of taxa identified (Megan via Diamond)		221	239	1.08
	Average scaffold fold coverage (BBMAP)		2.74	4.91	1.79

Table 3. Basic statistics and counts associated with each step of the alternative bioinformatics processing of the Bellairs and Maycocks samples. Here * indicates the relative fold change incorporating the total number of reads at both sites.

Discussion

The two sites have distinct ecologies

This study focused on two sites on the west coast (Caribbean Sea) of Barbados. The Bellairs site is located approximately one kilometer up current from a heavily populated area (Holetown). Several watersheds originating in the inland hills reach the ocean in Holetown via runoffs. The shoreline from Holetown to just south of the Bellairs site contains a near unbroken chain of houses and resorts, although the Bellairs site is within a federal marine reserve (Folkestone) and is therefore protected from recreational boat traffic and fishing. The beaches are used for swimming and other minor water activities. In general, the water at Bellairs is more turbid than at Maycocks due to the shallowness and its proximity to the shore where there is continuous wave action.The water samples were taken just below the surface, approximately 1 meter from the coral, 50 meters from shore and at a depth of only 2 meters.

Maycocks is further north along the coast from Bellairs. For much of the year the current travels due north, implying that the Holetown river effluence travels through Bellairs to Maycocks. However, at the time of sampling, the current travelled in a north-west direction, a common occurrence in January. Therefore, it is likely the case that the microbiome at Maycocks is significantly affected by events from the south, but our water samples are likely to be diluted of these direct influences. Furthermore, the beach has retained its wild grasses. There is agricultural land of crops common to the island but no significant rivers or streams that empty into the ocean. There is no tourism and only a few homes on the coast of Maycocks. However, there is a cement factory on the coast which is expected to influence the site. The water sample was taken 1 meter from the coral, approximately 1 kilometer from shore, at a depth of 18 meters.

Previous studies and ongoing monitoring of the two sites establish that Maycocks has a greater number of Gorgonians, hard coral, coralline and sponges, while Bellairs is enriched for fleshy and filamentous macroalgae, zoanthid and sand. Maycocks also shows a higher fish biomass.

Ecological differences between the sites are reflected in their microbiomes

Shotgun whole genome DNA-sequencing (Illumina NovaSeq paired-end, 2 x 150bp) was applied to the samples filtered for pico-nano-plankton (0.22-3μm). Nevertheless, our samples did capture some cells from multicellular Eukaryota. It is very unlikely to identify the exact species present in our samples (via Kraken/Bracken analysis), since these organisms tend to have very large genomes (10^7-10^{10} bp), our samples likely contained very few cells, and the number of genomic loci sequenced for each organism was very small. Nevertheless, many of the Viridiplantae (plants) and Opisthokonta (especially from Metazoa) identified at Bellairs were present on the shoreline or have plausibly travelled to the site via inland river systems. The same is true for Maycocks with trace evidence of several crops grown inland from the coast. We also identified old world monkey; the green monkey is the only monkey in Barbados, introduced by the British from Africa.

Overall the Maycocks site has a higher Shannon index compared to Bellairs; this is primarily due to the fact that the distribution is highly concentrated at Maycocks on Cyanobacteria (specifically Prochlorococcus and Synechococuccus) and Candidatus Pelagibacter. Maycocks is moderately richer than Bellairs with ~270 more unique species even after adjustment for the fact

that it received considerably more sequence coverage, although both sites had well over 9,000 species in common. For both sites, we would have identified the same species with high probability even if we had sequenced at only ⅓ of the current coverage, a fact important for the statistical design of future studies. Overall, this suggests that species abundance tends to be slightly more uniformly distributed at Bellairs, whereas the phototrophs at Maycocks are very highly abundant. In fact, whereas the entropy of Bellairs, and relative abundance of cyanobacteria, are well in line with the warm ocean Red Sea samples, Maycocks is a clear outlier across all Tara Ocean samples. The levels of Prochlorococcus and Synechococcus are only matched by studies from a study of nearby Curacao, suggesting that the southern Caribbean constitutes a Cyanobacteria hotspot.

Maycocks as a site for autotrophic photosynthetic organisms

Regardless of superkingdom, the relative abundance of photosynthetic organisms are (dramatically) higher at Maycocks than Bellairs. This includes both the Terrabacteria Prochlorochoccus and Synechococcus, and the oligotrophic Alphaproteobacteria Candidatus Pelagibacter. Within the Euryarchaeota, Maycocks had a high relative abundance of the photoheterotrophic Cand. Poseidoniales. Within the Eukaryota, we observed high abundances of the Micromonas, a photosynthetic picoeukaryotes which is well-established as a dominant primary producer in the euphotic zone. High relative abundance of several other protists supports the autotrophic photosynthetic enrichment at Maycocks. This includes the species Emilliania huxleyi, well-established as a key contributor to the oceanic carbon cycle via calcification, photosynthesis and export of inorganic matter to the oceans' interior, and the unicellular flagellates Prymnesium.

Bellairs as a site for copiotrophs, microalgal symbionts and marine-related disease

Whereas the vast majority of reads at Maycocks are mapped to Cyanobacteria within Terrabacteria, Bellairs has a more uniform distribution across Cyanobacteria, Firmicutes and Actinobacteria. The Firmicutes are often found in nutrient-rich extreme environments including marine sediment or within coral porites. Some including Fictibacillus arsenicus, which was enriched at Bellairs, play a role in coral degradation. Actinobacteria are common in marine sediment where they play a role similar to fungi, decomposing organic matter. Within the PVC Group, Planctomycetes were enriched at Bellairs. In particular, there were several species and genera with established roles in coral degradation that are common in regions where turf algae is dominant. Many Rhodobacterales are enriched at Bellairs; species from this genus can utilize many compounds in a nutrient rich environment for the production of secondary metabolites. This includes the well-studied Dinoroseobacter and at least six other genera commonly found in marine turf algae are also found within soft corals and coralline alga microbiomes and are able to survive in extreme environments and participate in nitrate reduction. The Delta proteobacteria are skewed en masse towards Bellairs. This includes the Desulfomicrobium, a genus of sulfate reducing bacteria that thrive in marine anoxic environments and interfaces such as microbial mats. Within the Gammaproteobacteria, two species of Acinetobacter were enriched at Bellairs. Acinetobacter play a role in the mineralization of aromatic compounds within soil, including within marine systems. Species of the genus Vibrio are some of the most highly abundant taxa in our dataset and strongly and consistently skewed towards Bellairs. The six most differentially abundant Vibrio

species are implicated in fish, sponge, coral and shellfish diseases including white band and yellow spot syndrome. Foraminifera are single-celled shelled protists known to be abundant groups in the shallow marine waters. Their size is typically far beyond our filtering criteria; however, we did identify several species uniquely at Bellairs. Statistical analysis suggests that their unique sighting at Bellairs is not an artifact of the lack of sequencing coverage at Maycocks (also recall that Maycocks received 1.57 fold more reads than Bellairs). Foraminifera amoeboid protists form symbiotic relationships with marine algae and are sensitive to the subtle changes in the ambient environment and foraminifera species are known to survive and increase in numbers in polluted areas.

Within Archaea, several planktonic pelagic ammonia-oxidizing thaumarchaeon involved in denitrification and carbon fixation from the Nitrosopumilales genera are more abundant at Bellairs.

Within Eukaryota, Boodlea composita was also highly abundant and strongly enriched at the Bellairs site. B. composita and several highly nitrophilic macroalgae species within the genus Caulerpa identified uniquely at Bellairs are macroalgae are capable of rapid growth in nutrient rich waters forming blooms that leads to dense turfs which can smother coral colonies. The Stramenopiles-Alveolates-Rhizaria (SAR) supergroup contributed many diatoms and dinoflagellates including the Licmophora, Cylindrotheca, Seminavis, Gyrodinium, Pleurocladia, Minutocellus amongst others enriched at Bellairs. These all have established roles in marine coral reef settings including the ability to form algal mats which smother coral. Protists of the genus Taenioma are also enriched at Bellairs; this is also a turf marine alga with finely branched filamentous rhodophytes.

Correlations between organismal abundances, viruses, bacterial infections and disease

We conjecture that the elevated levels of cyanophages at Maycocks is due to the elevated levels of cyanobacteria. This rationale extends to the Caudovirales (Podo, Myo, Sipho) which are also known to infect cyanobacteria. There is a strong preference for a large set of viruses which were recently identified using (Nanopore whole genome) sequencing near Hawaii but remain largely uncharacterized except that these viruses are 26% correspond to cyanophages, 13.3% to SAR11 phages, 12% to SAR116 phages, and 3% Vibrio phages. This is in line with our observed relative abundances for these hosts at Bellairs. However, Candidatus Peligabacter is a potentially interesting outlier here. C. peligabacter is a member of the SAR11 clade and is highly enriched at Maycocks. However, C. peligabacter/SAR11 phages are enriched at Bellairs, suggesting perhaps a differential rate of infection for these tiny heterotrophs. We remind the reader however that our study was not optimized for capturing nor analyzing the virome.

We applied various signatures for bacterial infections, intoxications, and water quality. These signatures consisted of several genera and species purported to serve as good markers especially with respect to risk to human health. In general, the species of the Vibrio genera dominated in this analysis and in our data, Vibrio is strongly skewed towards Bellairs. The fact that Foraminifera are uniquely identified at Bellairs and are suggested to be very sensitive to subtle changes in marine conditions motivates further study, selecting for the appropriate cellular size range (~100-200μm).

A word of caution when interpreting composition data (CoDa)

The vast majority of taxa are identified at both sites and those taxa identified at only one site have low relative abundance. An inherent property of next generation-based microbiome studies is that reads counts must be interpreted relativistically. That is, the observed read counts across all taxa are generated according to a multidimensional hypergeometric distribution where each taxon t has a true absolute copy number t_n in the sample. Sequencing corresponds to sampling from this multivariate distribution. This is inherently limited. For example, two hypothetical distinct situations for the populations at two sites that appear identical when observed through the lens of next generation sequencing.

Scenario 1. The number of all species at both sites is essentially equal except that at Maycocks a large number of cyanobacteria are “spiked in”. Therefore, the overall concentration of organisms in the sample is higher at Maycocks than Bellairs.

Scenario 2. The increase in the number of cyanobacteria at Maycocks is witnessed by a concomitant and equal decrease in the number of all other taxa. Therefore, for many species, the abundance levels for many species at Maycocks is smaller than at Bellairs.

Scenario 1 would favour a hypothesis that both sites are globally the same with the equivalent metabolic/chemical potential when the site is viewed as a holobiont, with the exception of the molecular consequences as a result of the increase in cyanobacteria. Scenario 2 would favour a hypothesis where the two sites have significantly different metabolic/chemical potentials above and beyond differences due to the increase in cyanobacteria. The two communities would have more fundamental differences in their underlying structure.

Our microscopy-based quantification procedure (limited to Archaea and Bacteria) attempts to resolve this dual polarity as best possible. We find evidence to support Scenario 1, suggesting that the underlying community structure is similar between the two sites. This does not diminish the importance of the identified outliers. Recall that our analysis often relied on KW tests (and Dunn’s test) that ask if a group taxa systematically has differential abundance between the two sites. Such analysis, when based on the log-frequency ratios as we have done in this CoDa setting, should largely be immune from excessive false discoveries here and therefore it is highly likely that the many microalgae, infectious bacteria and parasite outliers discussed above are more prevalent at Bellairs.

Does the microbiome composition reflect a stressed coral reef environment?

Nutrient enrichment of coastal waters is a problem due to anthropogenic pollutants including sewage, animal wastes, and terrestrial run-off from heavily fertilized lands. A widely held hypothesis is that nutrient inputs from sewage or agricultural run-off alter the ratio of particular nutrients and total loading, and likely results in significant changes to the microbiome of coastal waters. Sewage is rich in nitrogen and phosphorus but has low silicon content. One theory is that the rate of growth of phytoplankton is too fast for zooplankton to control it (Roitman et al. 2020). Our data does not allow us to develop a full portrait of protist grazing and symbiotic relationships, as most flagellated protists including dinoflagellates, cryptomonads, euglenoids and ciliates are

in the microplankton range (20-200 um). For example, the genus Symbiodinium had only a few hundred reads total (187 B vs 351 M). Nevertheless, the fact that many (n=12) genus of Foraminifera are uniquely identified at Bellairs (and this was confirmed not likely to be due to under-sequencing), the presence of many algae observed to smother corals, and a host of Vibrio species known to target many marine components including coral, fish, and sponges together confirm the observable degradation in health of the Bellairs reef. Perhaps the observed Bellairs microbiome indicates a shift from coral to macroalgae (Bruno et al. 2009). We hypothesize that the findings at Bellairs capture a reef on the brink of dysbiosis largely caused by anthropogenic interference.

Future Work and Conclusions

This work constitutes a pilot study of the Barbadian reef microbiome. The goal here was to sample from a small number of sites (n=2 due to cost and logistical reasons) to establish that we could sequence the microbiome in a cost effective manner, to obtain a rough estimate of the number and diversity of organisms in the marine systems, and to determine if there were promising differences in richness and abundance between sites. This information is useful for the planning and statistical design of a more robust, integrated and longitudinal study of the island. We sketch here the motivation for our next steps towards these ends.

A more complete sampling of the microbiome

This pilot study was restricted to organisms in the prokaryota-enriched size range of 0.22-3μm, and therefore these samples, which are a subset of the pico-nano-plankton, are not expected to profile the full range of viruses and giruses (<0.22μm), nor the nano- (5-20μm), micro-plankton (20-180μm), and meso-plankton (180-2000μm). We did identify a large number of viruses in our profiles with sizes below our size lower bound. Presumably the viruses escaped filtration by chance, because they had successfully infected their host, or because they had attached to the host cell surface. A modified collection, genomics and bioinformatics protocol following Brum et al. (Brum et al. 2015).

The larger fractions (>3μm) are enriched for protists that fulfill ecological niches through grazing and symbiosis. In our study, less than 500 reads at both sites from Symbiodiniaceae, whose family members form symbiotic relationships with corals and are central in bleaching events. If a filtering step is not used to enforce an upper bound on size, the harvest cells will include many Eukaryota with large genomes and shotgun sequencing approaches, which sample uniformly randomly from the “urn” of DNA fragments, will expend their read budgets primarily on these organisms, missing low abundance organisms with smaller genomes. We could follow the Tara Oceans project which developed a suite of protocols to address such issues, and generally involves 16S/18S ribosomal sequencing and microscopy (Karsenti et al. 2011; Pesant et al. 2015). Data on species abundance is absolutely critical to understanding symbiotic relationships and grazing patterns.

In addition to differences in size, it would also be interesting to sample from a broad range of depths at our sites. This study uses water samples harvested from approximately 1 meter away

from corals. Surface water or conversely water from soil could provide new data points to compare and contrast the sites.

Longitudinal studies of the same two sites are already underway and new samples will be processed from the same sites to compare the microbiomes three years later (January 2018 versus January 2021). A systematic approach that monitors the reef microbiome at more refined intervals (including at different times of the year) would provide important information regarding the dynamics of these ecosystems.

Understanding the interactions and symbioses in coral reef communities is an active area of research. We should consider alternatives to next generation sequencing to ensure sufficient and high-quality data to measure the status of these interactions and symbioses with the ecological structure of the coral reef community. For example, we could consider optimized PCR primers to measure abundance of coral and sponge symbionts, or to better measure lowly abundant species that may be markers of reef stress (eg Foraminifera or Vibrio species).

Better catalog of biogeochemical and ecological metadata for the sites

In order to facilitate comparisons between our data and third-party datasets, we tried to measure the same biogeochemical variables as Tara Oceans. However, some metadata including water chemistry had to be done on freshwater samples taken from the same location but two years later. Moreover, our equipment for measuring the biogeochemical variables differed from the equipment used by Tara Oceans, likely introducing some technical bias into our comparisons. Ideally future studies would seek to more precisely follow the Tara Oceans protocols. Moreover, future efforts would better harmonize with monitoring and conservation efforts such as CARICOMP as our characterizations of the microbiome complement their data and vice versa.

Sample a greater number of diverse Barbadian reef sites

Analysis with only two sites and without technical or biological replicates is clearly limited. Although we can broadly confirm that our sites share many characteristics with other warm surface water sites, a clearer understanding of how environmental and anthropomorphic change affect the Barbadian coral reef microbiome requires that many sites along the reef that present a broad spectrum of phenotypes be studied. Our choice of locations should follow as best possible efforts such as CARICOMP that track many variables including quantification of fish, coral disease and quantity, and specific biogeochemical attributes (Chollett et al. 2017; Vallès, Oxenford, and Henderson 2019). Only through a broad panel of profiles will we be able to point to the most likely causal elements of dysbiosis.

Expanding to adjacent ecosystems that influence our sites

Given the apparent influence of terrestrial run-off especially at the Bellairs site, it would be interesting to study the microbiome of inland rivers including from the subterranean coral cave systems below the island of Barbados. In fact, non-marine microbiome studies of neighbouring crop land or urban spaces from both sites would perhaps provide better context for some of the Bacteria, Archaea and viruses we observed. Moreover, it would help us to link aspects of our microbiome profiles with specific anthropomorphic effects observed on the coasts and inland. This could be an important steppingstone towards the development of classifiers that predict *why* a

particular reef is stressed. For instance, the classifiers would be able to point to specific components of eutrophication that upon intervention would have the highest likelihood of ablating stress at a particular reef site.

Early leads towards microbial-based classifiers of reef stress

Genomics and computational biology have had great successes in human health arenas, where multivariate classifiers have been established based on genomic data (eg polymorphisms and copy number variation in human genomes related to cancer studies) and transcriptomics (eg the development of classifiers of patient benefit to therapy including Oncotype (McVeigh et al. 2014)). The development, testing and acceptance is an arduous and long process. Our goal within this project was primarily to determine if differences in the microbiome between two sites could be identified from shotgun whole-genome DNA sequencing of pico-nano-plankton, and if these differences could be correlated with ecological parameters that differ between the two sites.

Transcriptomics to measure reef stress

Bleaching events are one of the most threatening disruptions to reef ecosystems (Thomas and Palumbi 2017). Coral is able to recover from moderate bleaching events (Mendes and Woodley 2002), however during the recovery phase they find alternative forms of energy, since they cannot rely on the photosynthetically fixed carbon from their algal symbionts, which can decline by up to 90% (L. J. Rodrigues and Grottoli 2007). There are two distinct ways known to achieve this. First, corals rely on energy reserves, slowing their growth and reproduction. This strategy is associated with long recovery times over several months and therefore carries inherent risk if the time between bleaching events disallows sufficient restoration of stores (L. J. Rodrigues and Grottoli 2007). Second, some corals which are able to increase heterotrophy and are therefore able to create the necessary energy themselves. Recovery times associated with this approach are typically much shorter (Grottoli, Rodrigues, and Palardy 2006; A. D. Hughes and Grottoli 2013). There is some indication that at least some Barbados reef sites rely on the first strategy with long periods of decline post-bleaching (H. Oxenford, Roach, and Brathwaite 2010). Longitudinal transcriptomic-based studies which have profiled the reef system post-bleaching have been able to identify stress-response signatures consisting of genes that exhibit differential expression in response to the bleaching event (Thomas and Palumbi 2017). The signatures provide insight into which survival mechanisms the coral is exploiting and how efficate this response is. Some genes remain differential for months post-event. Our plan is to next sequence the transcriptome of the Barbadian coral reef microbiome. Analysis of the holobiont would allow us to create a similar “coping” signature that we could use in a variety of ways including as an early warning system of reef stress.

Development of integrated early warning systems of reef stress

The multi-modal, multi-dimensional longitudinal data additions to our current pilot profiles centered on the microbiome could be integrated to build a holistic portrait of the relative fitness of reefs. Moreover, there is hope that organism abundance in addition to the global functional footprint of the holobiont will provide good predictors for why a specific site is under stress. For

instance, it may be possible to “reverse engineer” from our model the causes of the stress. For instance, we envision classifiers built from our model that identify the specific chemistry that is in disequilibrium due to eutrophication versus shifts in organismal composition due to heat stress versus excessive viral infections. If such classifiers can be discovered in the data and developed into an appropriate ecological test with sufficient efficacy, then the classifier could be used routinely across the island to identify hotspots proactively and suggest human interventions. Our microbiome efforts could be integrated with existing approaches for observation and conservation including CARICOMP (Chollett et al. 2017; Vallès, Oxenford, and Henderson 2019).

Methods

1. Assessment of the two sites of the Barbadian reef

The health status of both coral reef systems was performed in 2017 by the Centre for Resource Management and Environmental Studies (CERMES) under The Barbados Coral Reef Monitoring Programme a few months preceding our sampling in (**Method 2**) (Cermes 2018). We utilized the data collected by them for our analysis.

2. Sample collection and preparation

Samples were collected during the afternoon from two locations along the west coast of Barbados; Folkstone marine park (13°11'30.2"N 59°38'29.2"W) and Hangsman Bay (13°17'32.9"N 59°39'47.5"W) on January 30th and 31st 2018 respectively. At the time of sampling the ocean current was flowing in a northwestern direction between the two sample days ("OSCAR Third Degree Resolution Ocean Surface Currents" 2009). Sea water was collected from the Bellairs reef site located in Folkstone marine park just below the surface. Seawater was collected at 1 metre above the maycock reef located in Hangsman Bay using SCUBA and a boat for reef access. All samples were collected using 7L acid-washed bottles between 1-3pm in the afternoon. The samples were transported back to shore and immediately passed through a 3µm pore polycarbonate membrane filter, followed by 0.22 µm pore Sterivex filter, in both cases using a peristaltic pump (7" of mercury). Organisms captured by the 3µm filter were discarded. RNAlater was added to the 0.22µm filter and stored at -80°C. We therefore expected that organisms smaller than 0.22µm are removed from the analysis. The range of organism size is consistent with the sample collection specifications of the Tara Oceans project (S. Sunagawa et al. 2015; Pierella Karlusich, Ibarbalz, and Bowler 2020). Surface sea water temperature at time of collection was estimated using archived data and images from the NOAA Coral Reef Watch (*NOAA Coral Reef Watch. 2017, Updated Daily.NOAA Coral Reef Watch Version 3.0 Daily Global 5-Km Satellite Virtual Station Time Series Data for Southeast Florida* 2013).

3. Nucleic acid extraction

DNA was extracted from the Sterivex filter using the DNEASY PowerWater Kit (14900, Qiagen Inc.) with an additional 37°C Incubation step. The Sterivex filter was thawed, unfolded and carefully placed into a PowerWater bead tube. To initiate cell lysis, 1 ml of a buffer composed of guanidine thiocyanate (PW1) was preheated to 55°C for 10 minutes and added to the bead tube. The bead tube was placed horizontally to incubate at 65°C for 10 minutes. The bead tube was then vortexed at 3000 RPM for 5 minutes. After vortexing, the bead tube was centrifuged for 1 minute at 3,000g. Once centrifuged, 600-650 µl of supernatant was transferred to a new 2 ml collection tube. Then, 1µl of RNAse A was added and the tube was incubated at 37 °C for 30 minutes. After incubation the tube was centrifuged at 13,000g for 1 minute; the supernatant was then transferred to a new 2 ml collection tube and 200 µl of an IRS solution (PW2) was added. The tube was then vortexed briefly followed by incubation at 4°C for 5 minutes, then centrifuged for 1 minute at 13000g. The supernatant was then transferred to a new 2ml collection tube; 650

µl of a high concentrated salt solution (PW3) was preheated to 55°C and added; the tube was then vortexed briefly. 650 µl of the supernatant was loaded onto a MB Spin Column Filter and centrifuged at 13000g for 1 minute. The flow-through was discarded and centrifugation was repeated until all of the supernatant was processed. The MB Spin Column was placed into a new 2 ml collection tube; 650 µl of an alcohol-based solution (PW4) was added. The tube was then centrifuged for 1 minute at 13000g. The flow-through was discarded and 650 µl of ethanol (PW5) was added. The tube was then centrifuged at 13000g for 1 minute; the flow-through was discarded and the tube was again centrifuged for an additional 2 minutes. The MB Spin Column was then placed into a new 1.5 ml tube lid removed; 50 µl of an elution buffer (PW6) was added and the tube was left to sit for 2 minutes. The tube was centrifuged at 13000g for 1 minute. The supernatant was then transferred to a new 1.5 ml tube (with lid) and stored at -80°C.

4. DNA sequencing

In preparation for sequencing the two DNA extracted samples were thawed and resuspended in 10 mM Tris-HCl pH 8.0 with 0.1mM of EDTA. Next generation DNA-level sequencing was performed on the two samples at the McGill University and Genome Quebec Centre on the NovaSeq PE 150 platform generating 2 x 150bp paired-end reads. Library preparation was performed by Genome Quebec based on the following protocol. gDNA was quantified using Quant-iT™ PicoGreen dsDNA Assay Kit (P11496, Life Technologies). Libraries were generated using NEBNext Ultra II DNA Library Prep Kit for Illumina (E7103, New England Biolabs). The IDT Unique Dual Index adapters and universal primers used were [AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC][AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT]. Size selection of libraries at 360bp was performed using SparQ beads (Qiagen). Libraries were quantified using Kapa Illumina GA with Revised Primers-SYBR Fast Universal kit (Kapa Biosystems Inc.). Average size fragment was determined using a LabChip GX(PerkinElmer) instrument.

5. Quality control of sequencing results

The total number of paired end reads (2 x 150bp), total number of bases and returned by the NovaSeq before any bioinformatics processing is given in **Table 1**. The quality of reads was first assessed using FastQC version 0.11.5 (Simons et al. 2012), which provides several metrics for assessing the overall quality of reads including GC bias, sequence quality, base sequence content, base N content, sequence length distribution, sequence duplication levels, overrepresented sequences, the adapter, the distribution of observed k-mers, and the reliability and quality of all bases at each position along a read. Illumina adapters from the paired-end reads were trimmed using Trimmomatic version 0.38 with parameters 'ILLUMINACLIP:NovaSeq.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 and MINLEN:36' (Bolger, Lohse, and Usadel 2014). Trimmomatic also filtered out 2.1M reads which were either found to be unpaired or had a length less than 36bp (**Table 1)**. Quality of the reads was assessed post-trimming using FastQC.

We also experimented with replacing Trimmomatic with Trimgalore, as the latter is reported to have a more exhaustive approach to removing Illumina associated adapters. We examined the differences between the two approaches but did not observe a meaningful difference.

6. Read alignment & taxa classification via Kraken/Bracken

We used Kraken (Wood and Salzberg 2014) to align and classify the observed sequencing reads against a range of genomes. Kraken compares the distribution of k-mers in a query sequence, which is a read (or pair-end read after pre-processing) (**Methods 6**), against the k-mer distribution of genomes in a database of target taxa. For each query sequence, Kraken identifies the least common ancestor (LCA) of these taxa in the tree defined by the NCBI Taxonomy database (from March 31, 2020) (Federhen 2002). Our target genomes include the `plasmid`, `viral`, `protozoa`, `plant`, `UniVec`, `env_nr`, `nr`, `bacteria`, `archaea` and `fungi` NCBI downloads and the MAR reference database for marine metagenomics (Klemetsen et al. 2018). Bracken was then applied to the taxonomic assignments made by Kraken (Lu et al. 2017). Bracken uses the Kraken assignments in addition to information about the genomes themselves to better estimate abundance at the species level, the genus level, or above. Bracken was used here with a k-mer length of 35 and a read length of 100. Bracken discards reads based on a given threshold. Taxa with total reads less than the set threshold of 10 reads were discarded.

7. Code, analyses and reproducibility

The raw and normalized sequencing data is available at the Gene Expression Omnibus (GEO) under accession code GEOXXXXX. Code and associated data files are available via BitBucket at `git@bitbucket.org:hallettlab/reefmicrobiome.git`. Analyses here were carried out in R version 3.6.3 (R Core Team 2013).

8. Taxonomic analysis

Our in-house analysis made use of the NCBI Taxonomy database (version March 31, 2020) (Federhen 2002). After importing the Taxonomy database into R, we mapped read counts for both sites on the nodes of the tree.

Several organisms that are not microbes were identified in our samples including Metazoa and Embryophyta. Reads from these taxa were removed from our analysis since they tended to have large genomes that disrupt the relativistic proportional analysis across microbes with genomes that are far smaller in size.

9. Compositional Data and Comparative analyses

The use of most modern -omic technologies generates so-called compositional data. In this project, we use next generation sequencing. A sequencer requires a specific concentration of starting DNA, obtained from our starting material, which is a minute sample of the Barbadian ocean water. Therefore, the material sent to the sequencer is a sample of the genomic DNA

extracted from the organisms, which is in turn a sample of all genomic DNA in the Barbadian ocean water. After the DNA is fragmented into small pieces, we can think of the sequencer reaching into this “urn” of DNA and drawing an individual fragment whose nucleotide sequence is to be determined. It is useful but perhaps ultimately incorrect to assume that the choice of DNA fragment is made uniformly randomly. That is, there are no inherent properties of a nucleotide sequence (eg GC composition) that make it more or less likely to be chosen. The use of sequencing in metagenomics is so that we can use these fragments to infer the presence, absence and frequency of every organism. There are many caveats and corrections. For instance, the size of the genome of an organism confounds estimations of frequency (we address this for our data in a later subsection). These facts together imply that our data is compositional in nature (Aitchison 1986; Quinn et al. 2019; Gloor et al. 2017). Our data is an estimate of the relative abundance of each taxon versus all other taxons. In terms of a multinomial distribution with k taxa (components of the vector), the estimate of frequency for any individual taxon influences the estimate of frequency for all remaining taxa and the sum of frequencies must be equal to one. Throughout this manuscript we have attempted to follow so-called CoDa (Compositional Data) best-practice guidelines (Quinn et al. 2019; Gloor et al. 2017).

We often model the relative frequency of taxa at each of the two sites as a multinomial distribution. In particular, for a set of taxa, which are incomparable in the tree of life, we convert the observed counts (number of reads/contigs mapped to each taxa) into a frequency vector.

9a. A standard Pearson’s χ^2 test was used to test for statistically significant differences between two multinomial distributions with p-value thresholds depending on the context (`chisq.test` R function). Here the null distribution is that both sites were generated by a single multinomial distribution

9b. A two-sided binomial test was used to test for statistically significant differences between the observed marginal frequency (for a component of the multinomial) versus its frequency according to the multinomial distribution (function `binom.test` R function).

9c. A Kruskal-Wallis test was used to test for statistically significant differences between two more more groups (taxa, described as multinomial distributions derived from our observed data; `kruskal.test` R function). The null hypothesis is that both sites were generated by the same distribution. When the alternative hypothesis is accepted, it implies that at least one group stochastically dominates another group. Significance was estimated using a p-value threshold. Dunn’s test was used to identify which taxa is stochastically dominant.

9d. We also developed an approach that we term the *decentralization* statistic. The input to our test is two equal length multinomial distributions derived from observed data as described above. The null hypothesis is that both sites were generated by a single multinomial distribution as per a standard Pearson’s χ^2 test. We calculate the number of components along this vector where the marginal probability from the first distribution is greater than the marginal probability from the second distribution (a success) or otherwise (failure). We then estimate a p-value for statistical significance derived from the binomial distribution for the total number of observed successes and failures.

Lastly, we develop a computational approach that "deletes" components of the multinomial distributions (taxa) in order to increase the similarity (minimize distance measures or decrease χ^2 errors) between the two distributions. Informally, this *decentralization* procedure highlights those taxa which, when deleted from the analysis, render the remaining taxa to have almost equal frequencies.

10. Measures of biodiversity: richness, abundance, entropy, rarefaction

Throughout this manuscript, we use the Shannon index defined as

$$H' = -\Sigma_{t \in T}\, p_t \cdot ln\, p_t$$

where $T$is the vector of relative abundance for all taxa and $p_t$is the probability of taxa t (Tucker et al. 2017). The Shannon index is simply the entropy of the system(Ibarbalz et al. 2019).

To rarefy samples from N to n total reads, we used the `rarefy` function in the `vegan` package (Oksanen et al. 2019). We used other `vegan` tools to estimate the theoretical richness at both sites (Magurran 2004; Oksanen et al. 2019). In particular, we extrapolated the number of species at each site individually and also with pooled data using the `prestondistr` function to estimate the log-normal fit, and the `veilespec` function to estimate the integral fitted with `prestondistr`.

To handle zeros, we first experimented with a Bayesian-multiplicative replacement strategy that adjusts the count matrix (for all taxa at both sites) in a manner that preserves the ratios between the non-zero components (Martín-Fernández, Palarea-Albaladejo, and Olea 2011). However, when external datasets (eg Tara Oceans) were included in this analysis with lower sequencing coverage, they had > 50% zeros. We were not able to reach convergence unless we removed taxa. After removing taxa that had zero counts in more than 50% of the samples, we reached convergence, but the adjustment had little effect on the data (data not shown but available in `exp/exp-21-tara-oceans`).

11. Comparison with the Tara Oceans marine metagenomic samples

The metagenomic sequences and the associated metadata were downloaded from the companion website of Sunagawa and colleagues (S. Sunagawa et al. 2015). Samples that were not filtered for organisms in the range of 0.22µm and 3µm were excluded from our analyses. With respect to the metadata, we primarily made use of Tables W1 (description of the sampling sites), W5 (miTAG related data) and W8 (a broad range of molecular concentrations, temperatures, and computational measures of diversity). The miTAG 16S abundance estimations were loaded into our R data frame as described in Methods 8. We first attempted to adjust for zeros in this dataset alongside our two samples using a multiplicate Bayesian approach (Martín-Fernández, Palarea-Albaladejo, and Olea 2011), but could not achieve convergence, since some Tara Oceans samples had zero counts for >50% of the taxa. To achieve convergence, we had to remove taxa, a procedure we deemed undesirable and unnecessary as the observed adjustments were very small. We opted instead to simply add 1 to all entries in the count matrix.

When clustering was performed, we first selected the most abundant taxa for each sample and transformed these abundances to ranks. Then the Kendall τ distance metric was used with Ward's algorithm to construct two dimensional hierarchical clusters. We experimented with alternative non-tree approaches including partial least squares regression, a technique commonly used in metagenomic analysis (Helland 1990), and non-linear machine learning approaches such as UMAP (Becht et al. 2019).

12. Read alignment and taxa classification via alternative tools

We compared our results from **Methods 6** above to other approaches (**Figure 3**). In particular, two alternative approaches were considered: (i) MEGAN6 (Huson et al. 2016) and (ii) the analysis pipeline available from the Joint Genome Institute (JGI) Integrated Microbial Genomes and Microbiomes (IMG/M) Expert Review system (ER) (Grigoriev et al. 2011; 2012). Both approaches made use of metaSPAdes version 3.11.1 to assemble the trimmed pair-end reads for each sample separately with default parameters of kmer sizes of 21, 33 and 55 (Nurk et al. 2017). MetaSPAdes genome assembly outputs sets of overlapping DNA sequences forming contigs and scaffolds. **Table 3** contains summary statistics related to results obtained from metaSPAdes. MetaSpades assemblies were binned using MetaBAT2 (D. D. Kang et al. 2015) with default parameters. Bowtie2 (Langmead and Salzberg 2012) was used to map the trimmed pair-end reads back to the MetaSpades assemblies to determine read coverage which was required for binning. MAGPurify (Nayfach et al. 2019) was used to Bin perform bin refinement on the binned metagenome-assembled genomes (MAGs) produced by MetaBat2. MAGpurify was used based on the standard procedures outlined in the respective article cited. Incomplete bins were then filtered from the clean bins produced by MAGpurify using dRep (Olm et al. 2017). dRep filtered the incomplete bins by incorporating CheckM (Parks et al. 2015) completeness >= 75% and contaminate <=25% followed by identifying groups of genomes with similar DNA content. CoverM (Woodcroft n.d.) was used to count reads in each clean MAG produced by MAGpurify and each de-replicated MAG highlighted by dRep. It was also used to determine the number reads from the MetaSpades contigs produced for each site.

MEGAN6. The resultant assembled DNA sequences within the contig files were annotated using Prokka version 1.13.3 (Seemann 2014). Prokka attempts to identify genomic features including coding regions, ribosomal and transfer RNA genes, non-coding RNAs, and signal leader peptides. We used the predicting coding regions, and aligned the translated sequences against the NCBI Non-redundant protein reference database (January 10, 2019) via Diamond version 0.9.24 (Buchfink, Xie, and Huson 2004). The output from the Diamond was imported into MEGAN6 (community edition, version 6.13.5) (Huson et al. 2016) for further analysis and visualization. **Table 3** contains summary statistics related to the results obtained from Prokka and Diamond.

JGI IMG/M. Annotation, gene prediction and functional analysis with the JGI IMG/M made use of the same assembled contigs from metaSPAdes, however, since a great range of IMG/M functional analyses are enabled if we have estimates of the depth of coverage for each contig, BBMAP version 35.34 was applied to the output from metaSPAdes(Bushnell 2014). **(Table 3)** contains summary statistics related to the application of BBMAP to the output from metaSPAdes.

13. Estimate of absolute microbial content in seawater samples

Direct counts of Bacteria, Archaea and Virus-like particles were taken using the method by (Dinsdale et al. 2008). From the same locations at the same time as the collection of samples for sequencing **(Methods 2)**, seawater samples were collected using acid-washed two 60ml polycarbonate bottles. TE-glycerol to each bottle, they were then immediately stored at -80°C. To estimate the absolute microbial count, the seawater samples were thawed and an aliquot of 6 ml per sample was used. The aliquots were fixed with Electron Microscopy Sciences 4% Paraformaldehyde (5025999, Fisher Scientific) then stained with SYBR™ Gold Nucleic Acid Gel Stain (S11494, ThermoFisher) and centrifuged for 3 hours. The samples were then filtered (placed) onto 0.02 µm Anodisc inorganic filters (WHA68096002, Millipore Sigma) and mounted on glass slides. Counting was done using epifluorescence microscopy, images were captured using a Leica DMi6000 epifluorescent microscope with 63x (NA 1.4) lens and Hamamatsu Orca ER camera. The SYBR™ Gold Nucleic Acid Gel Stain was excited by light from an ExCite bulb filtered through a GFP filter cube (480/20nm ex, 540/40nm em). For each sample 10 images were captured from randomly-spaced points across the filter with 0.3 Z-Spacing, these images were then preprocessed using Fiji ImageJ and further analyzed using R version 3.6.3 (R Core Team 2013) where Bacteria, Archaea and virus-like particles were determined and counted.

14. Nutrient analysis of seawater water

Water measurements were taken using an EXO2 Multiparameter Sonde, 20m apart at a depth of 5m at both sampling sites. This analysis was conducted on September 4th, 2020. Seawater temperature, salinity and dissolved oxygen information was collected (3 sample readings per location). Sea water samples were also collected to conduct analysis of nitrates, nitrites and phosphates. These analyzes were conducted by the Centre for Resource Management and Environmental Studies (CERMES, Barbados). Nitrate and nitrite concentration were determined using the cadmium reduction method, phosphate concentration by the ascorbic acid method, while the turbidity was determined using a turbidity meter.

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