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Master in Marine and Coastal Systems
Work performed under the supervision of:

Dr. Pedro Frade (CCMAR, UAlg, Faro)
Dr. Tânia Aires (CCMAR, UAlg, Faro)

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Jasmine Brooke Haskell

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Resumo

Sargassum é uma macroalga frondosa com uma distribuição global ampla em recifes temperados e tropicais. Esta alga providencia habitat e refúgio a diversas espécies marinhas, mas também afecta outras negativamente. Apesar da sua importância ecológica, muito pouco se sabe sobre a vida microbiana nas superfícies de *Sargassum*. Através do estudo das comunidades microbianas presentes no biofilme da alga *Sargassum* sp., em paralelo com a água e o sedimento, ao longo de uma série temporal, pretende-se com este estudo entender as dinâmicas de sucessão microbianas do biofilme de *Sargassum* sp. e de que forma a comunidade microbiana se altera com as alterações ambientais. A amostragem de *Sargassum* sp., sedimento e água foi feita em Magnetic Island, na Grande Barreira de Coral na Australia, ao longo de um periodo de 13 meses. A sequenciação do gene 16S do rRNA de 30 amostras de *Sargassum* sp., usando a tecnologia Illumina, permitiu uma análise aprofundada da diversidade taxonomica do biofilme, ao mesmo tempo que o possível perfil funcional de taxa bacterianos chave, foi aferido usando o programa FAPROTAX. A implementação de análise estatística multivariada de ordenação, análises de correlação e estatísticas de abundância diferencial, permitiram investigar a resposta das comunidades bacterianas a parâmetros ambientais abióticos tais como: temperatura e nutrientes orgânicos e inorgânicos. Os biofilmes de *Sargassum* sp. revelaram estar dominados por bactérias dos Filos Firmicutes (23-45%), Proteobacteria (35-38%) e Bacteroidetes (13-30%) com diferenças observadas entre o Inverno e o Verão. As comunidades bacterianas da água e do sedimento mantiveram-se estáveis ao longo de toda a série temporal equanto que as comunidades associadas com *Sargassum* sp. revelaram flutuações ao nível da composição bacteriana. Os parâmetros ambientais explicam: 56% da variação da comunidade bacteriana da água, 46% da variação das comunidades associadas com o sedimento e 29% da variação do biofilme de *Sargassum* sp. Estes resultados sugerem que os mecanismos que medeiam as alterações na composição da comunidade microbiana de *Sargassum* sp. podem estar relacionados com factores bióticos tais como interação bactéria-bactéria ou interacção entre o hospedeiro e as bactérias associadas (endo e epifíticas), ou ainda interações com outros macroorganismos e seus microbiomas. Este estudo destaca a importância de examinar simultaneamente factores bióticos e ambientais, como forças condutoras de alteração dos microbiomas associados a espécies estruturantes dos recifes de coral.

Palavras-Chave: Epibiontes de macroalgas; 16S rRNA; recife de coral; holobionte; factores abióticos; FAPROTAX

Abstract

The canopy-forming macroalgae *Sargassum* is widely distributed throughout temperate and tropical reefs globally, playing an important role in habitat/refugia provision for diverse marine species, but also affecting others negatively. Despite its ecological importance, little is known about the microbes living on the alga’s surface. This study aimed to understand microbial successional dynamics within the *Sargassum* sp. biofilm and determine how environmental variations affect the microbial community, by investigating the microbial communities of *Sargassum* sp. biofilms, seawater and sediment in tandem over a time series. *Sargassum* sp., sediment and seawater samples were collected over a 13-month period from Magnetic Island on the Great Barrier Reef. Illumina 16S rRNA gene sequencing of 30 *Sargassum* sp. samples provided an in-depth analysis of the taxonomic diversity existing on the biofilm while putative functional roles of key bacterial taxa were assigned with FAPROTAX. Unconstrained ordination, correlation analyses and differential abundance statistics were used to investigate the response of bacterial communities to measured environmental parameters such as temperature, organic and inorganic nutrients. *Sargassum* sp. biofilms were dominated by bacteria belonging to Firmicutes (23-45%), Proteobacteria (35-38%) and Bacteroidetes (13-30%) phyla, with differences in community structure observed between winter and summer. Microbial communities of seawater and sediment were stable throughout the time series, while the communities associated with *Sargassum* sp. fluctuated in composition. Environmental parameters explained 56% of the variation in microbial community composition for seawater, 46% for sediment-associated communities and 29% for epiphytic *Sargassum* sp. biofilm communities. These results suggest that the *Sargassum* microbiome could be primarily mediated by biotic factors such as bacteria-bacteria interactions or interactions between the host and its associated bacteria (endophytes and epiphytes) and interactions with other species and their microbiomes. This study highlights the importance of simultaneously examining biotic and environmental drivers to determine factors structuring the microbiome of key reef species.

Key-words: Macroalgal epibionts; 16S rRNA; coral reef; holobiont; abiotic factors; FAPROTAX

Resumo Alargado

Os microorganismos são ubíquos nos recifes de coral (Ainsworth et al., 2010) e, com a mudança climática drástica que enfrentamos, perceber os seus nichos ecológicos torna-se cada vez mais relevante. Uma vez que as comunidades microbianas respondem rapidamente a stresses ambientais (Webster & Reusch, 2017), esses microorganismos podem fornecer um sistema de alarme precoce para a saúde dos recifes de coral (Glasl et al., 2018). Enquanto que a maioria dos estudos se focaram nos microbiomas de vários componentes do recife de coral tais como os corais (Barott et al., 2011), as esponjas (Webster & Thomas, 2016) e as comunidades pelágicas (Haas et al., 2016), muito poucos estudos examinaram a comunidade microbiana de macroalgas bentónicas tropicais ao longo de uma série temporal. Algas e corais competem directamente por espaço e, apenas recentemente, os microorganismos foram descritos como mediadores potencialmente cruciais nesse mecanismo (Barott & Rowher, 2012). Os estudos em comunidades microbianas das componentes de recifes de coral tiveram como objectivo caracterizar os principais intervenientes nesse mecanismo. No entanto, apenas quando a totalidade do ecossistema for estudado e entendermos a relação entre todos as componentes, poderemos começar a entender como essa dinâmica pode variar sob stress ambiental. Neste estudo, usou-se como modelo a alga *Sargassum* sp., uma macroalga pseudo-perene comum ao longo da zona costeira da Grande Barreira de Coral (Wernberg et al., 2001; Vuki & Price, 1994). O *Sargassum* é um produtor primário essencial na zona bentónica do recife de coral, proporcionando estrutura, habitat e alimento a uma variedade de organismos (Vuki & Price, 1994), incluindo organismos endo e epifiticos a ele associados (Singh & Reddy, 2014). Este projecto pretende, primeiramente, caracterizar a diversidade taxonómica dos biofilmes de *Sargassum* sp. através da sequenciação do gene 16S do rRNA. Em Geoffrey Bay, Magnetic Island, Australia, colectaram-se amostras de *Sargassum* sp. (n=30), água (n=30) e sedimento (n=30), ao longo de um período temporal de 13 meses. Este regime de amostragem permitiu analisar a sucessão microbiana ao longo de uma série temporal, incorporando a variação sazonal. A resposta da comunidade microbiana a parâmetros ambientais tais como temperatura e concentração de nutrientes orgânicos e inorgânicos, foi interpretada através da implementação de diversas ferramentas estatísticas que permitiram analisar conjuntamente dados ambientais e de sequenciação. O possível perfil funcional de grupos taxonómicos microbianos chave foi determinado usando o software FAPROTAX. Os Filos Proteobacteria e Bacteroidetes foram dominantes em todos os habitats com uma abundancia relativa entre os 35%-63% e 12%-18% da comunidade, respectivamente. As amostras de água encontravam-se dominadas por Cyanobacteria, O filo Firmicutes foi encontrado unicamente no biofilme de *Sargassum* sp. e o sedimento estava dominado por bactérias do filo Planctomycetes. A composição da comunidade bacteriana é claramente diferente entre todos os habitats o que indica que cada um dos três habitats possui uma comunidade única ($F_{(2/87)} = 33.855$, $p = 0.001$, $R^2 = 0.437$). Embora não se tenham encontrado diferenças significativas entre verão e inverno, as comunidades bacterianas do biofilme de *Sargassum* sp. mostraram flutuações ao longo da série temporal de amostragem. Uma vez que tanto as amostras de água como as de sedimento se mantiveram bastante estáveis ao longo da série temporal, o biofilme de *Sargassum* sp foi sujeito a uma análise mais pormenorizada. As análises de abundância diferencial, mostraram que as famílias Bacillaceae e Flavobacteraceae apresentavam tendências opostas na sua abundância relativa e os seus respectivos zOTUs foram frequentemente associados com alterações na comunidade bacteriana entre tratamentos de grupos abióticos. Curiosamente, no seguimento de um evento de branqueamento de coral em 2016, registou-se um aumento na abundância relativa de Pseudoaltermonadaceae, um conhecido patógeno marinho. Os factores ambientais incluídos neste estudo foram responsáveis por 29% da variação observada na comunidade bacteriana do biofilme de *Sargassum* sp. No entanto, esse valor caiu para metade quando apenas se incluíram apenas as zOTUs dominantes na análise. Apesar da comunidade bacteriana no geral ser menos susceptível aos parametros ambientais, diversos taxa bacterianos demonstraram uma correlação forte com a temperatura, concentração de Carbono orgânico e amónio. Usando o FAPROTAX, a estes taxa foram, na sua maioria, atribuídas as funções de quimioheterotróficos e fototróficos e 95% dos taxa encontados neste estudo foram putativamente relacionados com o metabolismo do Carbono. Os resultados deste estudo mostram que, enquanto que a totalidade das comunidades bacterianas epífitas associadas ao *Sargassum* sp. exibem uma resposta muito baixa aos parâmetros ambientais, vários taxa mostram-se sensíveis a esses parâmetros. Em conjunto com outros estudos que investigaram a influencia de potenciais mecanismos bióticos na superfície das algas (tais como

hospedeiro-bactérias, bacteria-bactéria, bactéria-outros epífitos), este estudo, e similares, poderão dar-nos uma visão holística de como estas comunidade bacterianas em mudança poderão afectar toda a comunidade bentónica do recife de coral. Durante um período de grande vulnerabilidade, é imperativo continuar a investigar a influência dos microorganismos nos recifes de coral (e todo esse ecossistema) de modo a preservar a saúde e integridade da Grande Barreira de Coral.

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1 INTRODUCTION

1.1 OBJECTIVES

In this study, I aim to identify the epiphytic bacterial community present on *Sargassum* sp. biofilm samples collected over a 13-month period from an inshore reef site of the Great Barrier Reef (GBR). I will relate the fluctuations observed in bacterial community composition to measured environmental parameters and putatively assign the function of key bacterial members to better understand the role of *Sargassum* surface biofilm communities within the coral reef ecosystem.

1.2 THE CORAL REEF ENVIRONMENT

In an era where coral reef cover around the world is decreasing at an alarming rate (Hoegh-Guldberg et al., 2017) the role of microbes on reefs is one that is often overshadowed by rising sea surface temperatures, pollution, overfishing and storm damage. However, it may be these microbes that could provide us with an early warning system to the health of the coral reef ecosystem (Glasl et al., 2018) as microbial assemblages exhibit a rapid response to environmental stressors (Webster & Reusch, 2017). With a comprehensive understanding of microbial dynamics in the coral reef environment, this invaluable attribute can be used advantageously for coral reef conservation. The coral reef ecosystem is often characterized by space limitation (Benayahu & Loya, 1981) with the two major competitors being reef-building corals and non-calcareous algae (i.e. macroalgae) (Barott & Rohwer, 2012). A consistent feature across degrading reefs worldwide is the observed phase shift from a coral-dominated benthos to an algal-dominated benthos (Smith et al., 2006). These persistent phase shifts can cause a dramatic reduction in biodiversity and ecosystem degradation (Ceccarelli et al., 2020). While local and regional causes of these shifts have been studied, one crucial mechanism by which this competition is mediated has only recently been identified as microbial (Barott & Rohwer, 2012).

All algae (and other primary producers) release dissolved organic carbon (DOC) into the water column (Haas et al., 2011), which goes on to fuel microbial life (Kline et al., 2006). However, the amount of DOC released by turf and fleshy macroalgae is significantly higher than that released by other organisms occupying the reef benthos, such as corals, thus generating a surplus of algal-derived DOC (Haas et al., 2016). This form of DOC enhances the growth of copiotrophic bacteria which exhibit an increased tendency to express virulent genes (Haas et al., 2016). These pathogenic bacteria can cause hypoxic zones on the coral which may cause mortality (Barott & Rohwer, 2012). This bacteria-mediated coral death liberates space on the benthos, allowing for algal species to colonize even more reef substrates (Haas et al., 2016). This positive feed-back loop is known as the DDAM model (DOC, disease, algae, microbes), first described by Barott & Rohwer (2012). This shift from a healthy coral reef ecosystem to a system with higher microbial biomass and energy use (via the copiotrophic bacteria remineralizing DOC) is described as microbialization (Haas et al., 2016).

While DDAM and microbialization exemplify how macroalgal communities contribute to the microbes in the water column, less is known regarding the microbial dynamics on the macroalgal biofilm (Coelho-Souza et al., 2017). A key into understanding the role macroalgae play in the microbialization of the coral reef environment could lie in the microbial community living on its surface (biofilm). It has been shown that the health, functioning, and resilience of macroalgae is largely due in part to their associated microbial communities (Egan et al., 2013). This tightly coupled relationship between a host and its associated microbiome can be described as a single entity known as the holobiont (Margulis et al., 1991; Rohwer et al., 2002).

1.3 MACROALGAL HOLOBIONT AND BIOFILMS

The macroalgal holobiont is considered a single entity composed of a host (basibiont) and associated microorganisms (epibionts and endobionts) (Egan et al., 2013). A variety of prokaryotic and eukaryotic microorganisms, such as bacteria, archaea, microalgae, viruses, fungi and protozoa comprise the holobiont (van der Loos et al., 2019). Bacteria are likely to be the early colonizers on macroalgal surfaces (Wahl et al., 2012) releasing extracellular polymeric substances containing essential building blocks for catalysing the biofilm formation process (Li et al., 2019). Microbial biofilms act as an interface between the host macroorganisms and the surrounding seawater and are thus responsible for energy and material transmission (Wahl et al., 2012). The presence of bacteria in seaweed-associated biofilms is imperative for the successful growth and development of the macroalgae (Singh & Reddy, 2014). Bacteria associated to macroalgal biofilms have been found to modulate key functions such as: protection against pathogens (Egan et al., 2013), nutrient provisioning (Hollants et al., 2013), nitrogen fixation (Goecke et al., 2010), morphogenesis (Matsuo et al., 2003) and zoospore settlement (Singh & Reddy, 2014). In addition, bacteria inhabiting macroalgal biofilms have found to function as biofilters (Riquelme et al., 1997). In return, the algae provide bacteria with an oxic environment, a habitable substrate, and a source of organic substances (Singh & Reddy, 2014; Mei et al., 2019). Due to the unique intersection of macroalgal biofilms, they can provide valuable information regarding the health of benthic macroorganisms and external environmental conditions (Glasl et al., 2019).

1.4 BACTERIAL COMMUNITY RESPONSE TO BIOTIC AND ABIOTIC FACTORS

Akin to bacteria living elsewhere in the biosphere, bacteria living on algal surfaces are quick to respond to changes caused by their host, other microbes and environmental factors (Singh & Reddy, 2014; Glasl et al., 2019). Bacterial communities on algal surfaces are tissue-specific, as shown for different morphological structures of the brown algae *Laminaria saccharina* (Staufenberger et al., 2008). In the green algae *Ulva austalis*, bacterial density increased exponentially from the algal tip towards the base of the organism (Tujula, 2006). The age of the algal tissue influences the species of bacteria that colonize the macroalgae, with an initial colonization by copiotrophic species followed by oligotrophic species which exhibit a higher degree of functional specialization on the surface of *Laminaria hyperborea* (Bengtsson et al., 2012). Competition between bacterial taxa on the macroalgal surface can also cause changes in the bacterial communities (Serebryakova et al., 2018). Additionally, the overall health of the macroalgal host has also proved to be a determinant factor of the bacterial constituents inhabiting surface biofilms of *Ecklonia radiata* (Marzinelli et al., 2015).

With a changing climate it is imperative to understand how the macroalgal holobiont will respond to environmental stressors and the implications this may have on other members of the benthic community. Abiotic factors such as light availability, pollution, wave energy and nutrient concentrations have also been shown to cause changes in macroalgal bacterial biofilm community composition (Bengtsson et al., 2012; Aires et al., 2016; van der Loos et al., 2019). Seasonality was examined by Lachnit et al. (2011) for three algae species sampled over multiple winter and summer seasons, with the resulting microbial assemblages of all three species changing between the seasons. Fluctuations in temperature have also been shown to cause changes in bacterial community composition (Bengtsson et al., 2010; Mancuso et al., 2016;

Serebryakova et al., 2018). External nitrogen deficiency stress caused the epiphytic community of *Sargassum horneri* to be heavily dominated by nitrogen transporters when compared to control conditions (Mei et al., 2019).

1.5 PRIOR STUDIES ON SEAWEED BIOFILMS AND GAPS

The first descriptive study of seaweed biofilms was reported in 1875 (Johansen et al., 1999) with an initial recognition of a symbiosis between macroalgae and bacteria being described in 1903 (Hollants et al., 2013). Rapid improvements in molecular approaches over the last two decades have shed light on the phylogeny and biochemistry of bacterial species commonly found occupying the algae's biofilm (Hollants et al., 2013). Examples of these consistently dominant bacterial phyla, or common bacterial core, are Firmicutes and Proteobacteria (Singh & Reddy, 2014). Epibacterial communities have been shown to vary over time, space, and among macroalgal host species (Lachnit et al., 2011; Egan et al., 2013; Aires et al., 2015) . Most of the bacterial epiphytes found to date are obligate aerobes and photoautotrophs (Li et al., 2019; van der Loos et al., 2019). Divergence from this trend exists in the Bacteroidetes in which several taxa belonging to this phylum are strict anaerobes (Mei et al., 2019). Successional patterns have also been determined, for instance, *Laminaria hyperborea*'s premature biofilm formation in late spring includes bacteria from the phyla Planctomycetes, Verrucomicrobia and Proteobacteria, which are then later supplemented by members of the Cyanobacteria and Bacteroidetes in the fall (Bengtsson et al., 2010). While many seaweed-associated biofilms have been studied across temperate and tropical regions (Goecke et al., 2010; Wahl et al., 2012; Hollants et al., 2013; Singh & Reddy, 2014), there is a research gap regarding the surface biofilms of brown macroalgae and their relationship to the coral reef environment. With the increasing intensity of environmental stressors related to the present climate emergency, the impact this may have on the macroalgae's relationship with its associated microbiome remains uncertain (van der Loos et al., 2019). As part of the DDAM feedback loop, the release of macroalgal polysaccharides into the water column may be accelerated in warming seas, causing further damage to corals (Barott & Rohwer, 2012). Declines in coral cover cooccurring with increases in macroalgal abundance have been reported for a few regions in the Great Barrier Reef (GBR) (Ceccarrelli et al., 2020).

Examining how environmental stressors influences this trend through macroalgal biofilm studies may prove as a valuable model for other vulnerable coral reef ecosystems around the world.

1.6 THE MACROALGAL MODEL - *SARGASSUM*

Sargassum, a brown pseudo-perennial macroalgae (Wernberg et al., 2001), is an essential primary producer and ecosystem engineer (Egan et al., 2013) inhabiting our world's oceans . Like other seaweeds, the canopy-forming *Sargassum* provides structure to the benthos and food for organisms across multiple trophic levels (Vuki & Price, 1994), including microorganisms present in the water column and those living on the *Sargassum*'s surface (Singh & Reddy, 2014). To date 200 species of *Sargassum* are described (Torralba et al., 2017), with distributions ranging from subpolar to tropical regions (Martin-Smith, 1993). Select species of *Sargassum* (namely, *Sargassum muticum*) have been considered invasive in many regions of the world (Gestoso et al., 2012; Engelen et al., 2015; Milledge et al., 2016), with high-density blooms occurring in degraded reef environments impacting coral larvae recruitment, thus further threatening coral reef resilience (Hughes, 1994). However, on Australia's Great Barrier Reef (GBR), *Sargassum* displays a limited geographic range and seasonal sensitivity with increased abundances in the summer (Vuki & Price, 1994). From the austral spring through summer (October-February) is when large fronds and multi-branched individuals thrive. In August (winter), these frond tissues begin to degenerate (Umar et al., 1998; Glasl et al., 2020) with species such as *S. microphyllum* only consisting of the holdfast and main axis with short branches (Umar et al 1998).

1.7 HYPOTHESES

By conducting this study, I expect to find bacterial taxa belonging to the aforementioned common bacterial taxonomic groups (Firmicutes and Proteobacteria) within the epiphytic community of these *Sargassum* sp. biofilm samples. I also anticipate that the bacterial community inhabiting *Sargassum* sp. surface biofilms will vary throughout the entire time series being influenced by environmental parameters. As *Sargassum* has greater biomass during the summer months in the GBR, I expect a fluctuation of copiotrophic bacterial taxa. As the sampling scheme coincides with one of the most intense coral bleaching events recorded during the austral summer of 2016 (Morgan et al., 2017), I expect to see changes in the bacterial community such as an introduction of taxa potentially virulent to corals, during or immediately following these months.

2 MATERIALS AND METHODS

2.1 SAMPLING AND STUDY AREA

Sargassum sp. samples were collected by colleagues at the Australian Institute of Marine Science (AIMS) between February 2016 and March 2017 from three inshore reef sites of the Great Barrier Reef, Queensland, Australia (Figure 1). Samples were collected on a monthly basis from Geoffrey Bay, Magnetic Island, on a periodic basis from Pioneer Bay, Orpheus Island, and a single occurrence from Channel, Orpheus Island, however, only samples collected from Geoffrey Bay were included in this study as the sample distribution was not even between the three sites. For each sampling event, three replicate samples (*Sargassum* thalli located at least 5m apart) were collected for a total sample number of 30 individuals. The samples collected and processed were all composed of *Sargassum* sp. stem, blades, and vesicles (Figure 2). All *Sargassum* sp. samples were obtained at a depth of 3m with sterile scalpels, rinsed with filtered-sterile seawater, and flash frozen in liquid nitrogen.

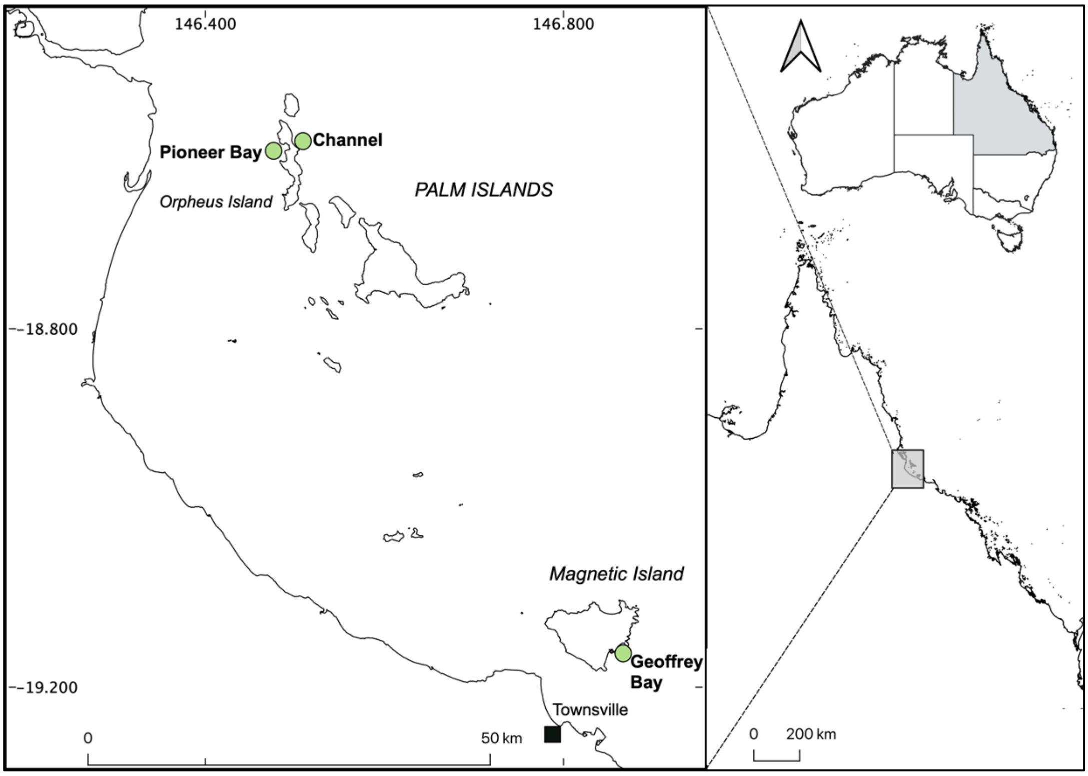


Figure 1: Map displaying the locations of Magnetic and Orpheus islands off the coast of Queensland, Australia. The sampling sites, Geoffrey Bay, Pioneer Bay and Channel, are represented by green circles.

Along with the *Sargassum* sp. sampling, water samples (n=30) were obtained in triplicate with a Niskin bottle at 2m depth to measure the following seawater parameters: dissolved inorganic nutrients (ammonium, nitrate, nitrite, phosphate), total suspended solids (TSS), chlorophyll *a* concentration (Chl *a*), salinity, particulate nitrogen (PN), total nitrogen (TN), non-purgeable organic carbon (NPOC) and non-purgeable inorganic carbon (NPIC). Sediment samples (n=30) were collected in 100mL jars at 2m depth with parameters measured including grain size distribution, total organic carbon (TOC) and total organic nitrogen (TON). Further analysis of the samples was conducted according to standardized procedures set forth by the Australian Institute of Marine Science (AIMS) (Devlin and Lourey, 2000). Seawater temperatures were extracted from AIMS long-term monitoring temperature records (http://eatlas.org.au/).

Figure 2: ***Sargassum*** **sp. sampling from Geoffrey Bay, GBR. Photo provided by Bettina Glasl.**

2.2 DNA EXTRACTION AND 16S RRNA GENE SEQUENCING

For separation of the biofilm from the seaweed tissue, thawed samples were incubated overnight at 200 rpm on a shaking plate in 10 mL of phosphate-saline buffer at 37°C. The biofilm in suspension was transferred to a sterile tube and centrifuged for 10 minutes at 16,000 rcf at 4°C. The resulting pellet was used for DNA extraction using the DNeasy PowerSoil kit (Qiagen) and the DNA extract was stored at -80°C until further processing (Glasl et al., 2019). All samples underwent amplicon sequencing of the 16S rRNA gene amplicon using the 27F (Lane, 1991) and 519R (Turner et al., 1999) primer pairs at the Ramaciotti Centre for Genomics in Sydney, Australia. Sequencing data was analysed as single nucleotide variants following the standardized platform of the Australian Microbiome Initiative (Brown et al., 2018; Glasl et al., 2019), where chimeras were removed and taxonomic affiliation of zero-radius operational taxonomic units (zOTUs) were assigned using the SILVA v132 database (Yilmaz et al., 2014). As we designed this study within a prokaryotic context, the use of the term microbe or microbial is regarding bacteria only and not other microbial organisms (such as fungi, protists, archaea or microalgae).

2.3 STATISTICAL ANALYSIS

Statistical and graphical analyses were performed in R (R Core Team, 2020) using the high-throughput microbiome census data package phyloseq (McMurdie & Holmes, 2013) and graphical package ggplot2 (Wickham, 2011). Chloroplast and mitochondrial sequences were removed prior to analysis and all samples were rarefied to the minimum number of sequences (6800 reads). Non-rarified count data was used for the DESeq2 analysis, while the rarified dataset was used for all steps thereafter. Relative abundances were used for bubble plot generation and correlograms. Three indices for alpha diversity (within host diversity) were calculated for this study: Shannon index, Chao1 and Observed zOTUs. Observed number of zOTUs gives us the actual number of zOTUs detected in the dataset while Chao1 is a richness estimator predicting the number of zOTUs present if sequencing depth was not restricted. Shannon index accounts for zOTU richness and evenness. Singletons, zOTUs with one count, were kept for alpha diversity analysis but were removed for subsequent analysis. To assess beta diversity (among host diversity), a non-metric multidimensional scaling plot (nMDS) based on Bray-Curtis dissimilarity matrices, an unconstrained method of ordination, was created with the vegan package (Oksanen et al., 2017). Significance of community differences between season and habitat were tested with a permutational multivariance analysis of variance (PERMANOVA). Differences in dispersions among habitat groups was tested with homogeneity of multivariate dispersions (PERMDISP). P-values for both PERMANOVA and PERMDSIP were calculated with 999 permutations and adjusted with the Bonferroni method. The VennDiagram package (Chenn, 2018) was used to construct a Venn diagram representing the unique, shared and ubiquitous zOTUs between the three habitats.

Quantile and density distribution plots were created in conjunction with the Shapiro-Wilks test and homogeneity of variances tests to assess the normality for alpha diversity measures and the environmental parameters. Inverse and square root transformations of several alpha diversity measures were required, with only one inverse transformation for environmental data necessary. Parametric or non-parametric methods were then implemented. P-values below an alpha of 0.05 were deemed significant for all the statistical methods herein.

To gain an overview of the relationship between sampling dates and environmental parameters, a Principal Component Analysis (PCA) based on Euclidean distances was conducted with the ggbiplot package (Vu, 2011). This aided in determining which abiotic factors would be relevant to describe differences occurring within each season. To reduce redundancy in abiotic variables, a correlogram with the GGally package (Barret et al., 2020) was generated to eliminate collinear variables from the PCA and further analysis. Variables which shared a Pearson correlation lower than -0.7 or higher than 0.7 were removed from subsequent analysis. All variables were scaled to account for the varying units used.

A distance-based redundancy analysis (db-RDA) was performed with the vegan package to investigate the relationship between the *Sargassum* sp. associated bacterial community and the environmental factors. db-RDA is a form of constrained ordination between the explanatory variables and the response variables which assumes linear relationships. This assumption is appropriate in the context of this study as it is over a limited geographic area. As with the PCA, all environmental variables were scaled. Two db-RDAs were generated: one including the total *Sargassum* sp. associated zOTUs and the other only including the dominant *Sargassum* sp. associated zOTUs. To generate models based on the db-RDA, the function ordiR2step was used within the vegan package followed by a variance partitioning analysis. One-way analysis of variance (ANOVA) was implemented to test the suitability of the models and the environmental parameters included therein.

To analyse seasonal trends in the data, categories were created for summer and winter based on the seasonal definitions set forth by the Australian Bureau of Meteorology (https://bom.gov.au), where dates between November and April were considered summer samples and sampling dates between May and October were considered as winter samples. For the abiotic factors deemed relevant by the db-RDA, high and low categories were created based on median values for each factor. Cut-off values were: 27°C for temperature, 1.33mg/L for NPOC, 0.22μmol/L for NH4 and 0.65mg/L for TSS.

The DESeq2 package (Love et al., 2014) was used to ascertain which zOTUs were responsible for causing shifts in the bacterial community of the *Sargassum* sp. biofilm between seasons and the aforementioned abiotic factor groupings. The output (log2foldchange) quantifies the change in zOTU abundance between experimental groups and is paired with a p-value. However, due to false discovery rate, the p-value must be adjusted. For this study a false discovery rate threshold (padj) was set to 0.01. Correlation heat maps based on the Spearman correlation coefficient were generated using the microbiomeSeq package (Ssekagiri et al., 2017) to relate the most abundant taxa in *Sargassum* sp. biofilms to the aforementioned abiotic factors considered significant by the db-RDA models. P-values were adjusted for multiple comparisons with the Benjamin and Hochberg method (Ssekagiri et al., 2017).

2.4 FUNCTIONAL ASSIGNMENT

Implementation of Functional Annotation of Prokaryotic Taxa v1.2.3 (FAPROTAX) (Louca et al., 2016) was used to map bacterial species from the *Sargassum* sp. biofilm in this dataset to generalized metabolic functions based on pre-existing literature on cultured strains. The functions primarily include metabolism of nitrogen, sulfur and carbon, however, information regarding viral and parasitic strains also exists. The zOTU table of the *Sargassum* sp. samples from the Geoffrey Bay dataset was inputted into the FAPROTAX program and the included python script created putative functional tables split by zOTU and by sample. The results were further split by metabolism type (carbon, nitrogen and sulfur) with the mean and standard deviation calculated for each of the sampling dates. Metabolic functions describing 90% of the output were plotted against the sampling dates with ggplot2 in R. Plots were also generated for the *Sargassum* sp. biofilm samples split into the aforementioned abiotic groupings, with the mean and standard deviation calculated for each metabolic function within each abiotic category.

3 RESULTS

A total of 2,755,858 reads were obtained for this dataset corresponding to 38,677 zOTUs including singletons. Following the removal of singletons, 22,499 zOTUs remained with 6,309 for *Sargassum* sp., 3,035 for seawater and 15,416 for sediment.

3.1 MICROBIAL DIVERSITY

Alpha diversity estimates showed that the bacterial community associated to *Sargassum* sp. had the largest variance in diversity while those from the sediment samples had the highest values of diversity (Figure 3, Table 1). The Kruskal-Wallis test performed for each index (Observed zOTUs, H(2,88) = 61.23, p = $5.1\text{x}10^{-14}$; Chao1, H(2,88) = 60.61, p = $6.90\text{x}10^{-14}$; Shannon, H(2,88) = 59.51, p = $1.2\text{x}10^{-13}$) indicated a significant difference between each habitat group. The Dunn test (with Bonferroni adjustment) confirmed the differences between *Sargassum* sp. and sediment (Observed zOTUs, p = $7.161\text{x}10^{-12}$; Chao1, p = $6.877\text{x}10^{-11}$; Shannon, p = $3.272\text{x}10^{-10}$;) and seawater and sediment (Observed zOTUs, p = $7.224\text{x}10^{-10}$; Chao1, p = $8.417\text{x}10^{-11}$; Shannon, p = $1.689\text{x}10^{-11}$) while the differences between *Sargassum* sp. and seawater diversity resulted as non-significant.

TABLE 1: MEAN AVERAGE ALPHA DIVERSIY INDEX VALUES FOR EACH HABITAT WITH STANDARD DEVIATION

Habitat	Observed	Chao1	Shannon
Sargassum sp.	945 ± 407	1517 ± 556	4.7 ± 1.29
Seawater	780 ± 92	1322 ± 238	4.46 ± 0.22
Sediment	3197 ± 231	5555 ± 705	7.69 ± 0.11

One-way Analysis of Variance (ANOVA) and Kruskal-Wallis results showed no significant differences in indices between summer and winter for *Sargassum* sp. (Observed zOTUs, $F_{(1,28)}$ = 2.368, p = 0.135; Chao1, $F_{(1/28)}$ = 2.869, p = 0.099; Shannon, $F_{(1,28)}$ = 1.888, p = 0.180), sediment (Observed zOTUs, H(1,29) = 0.492, p = 0.483; Chao1, $F_{(1,30)}$ = 0.039, p = 0.845; Shannon, $F_{(1,30)}$ = 0.009, p = 0.926) and seawater samples (Observed zOTUs, H(1,29) = 0.008 p = 0.928; Chao1, H(1,29) = 1.549 p =0.213; Shannon H(1,29) = 0.013, p = 0.910) (Figure 4).

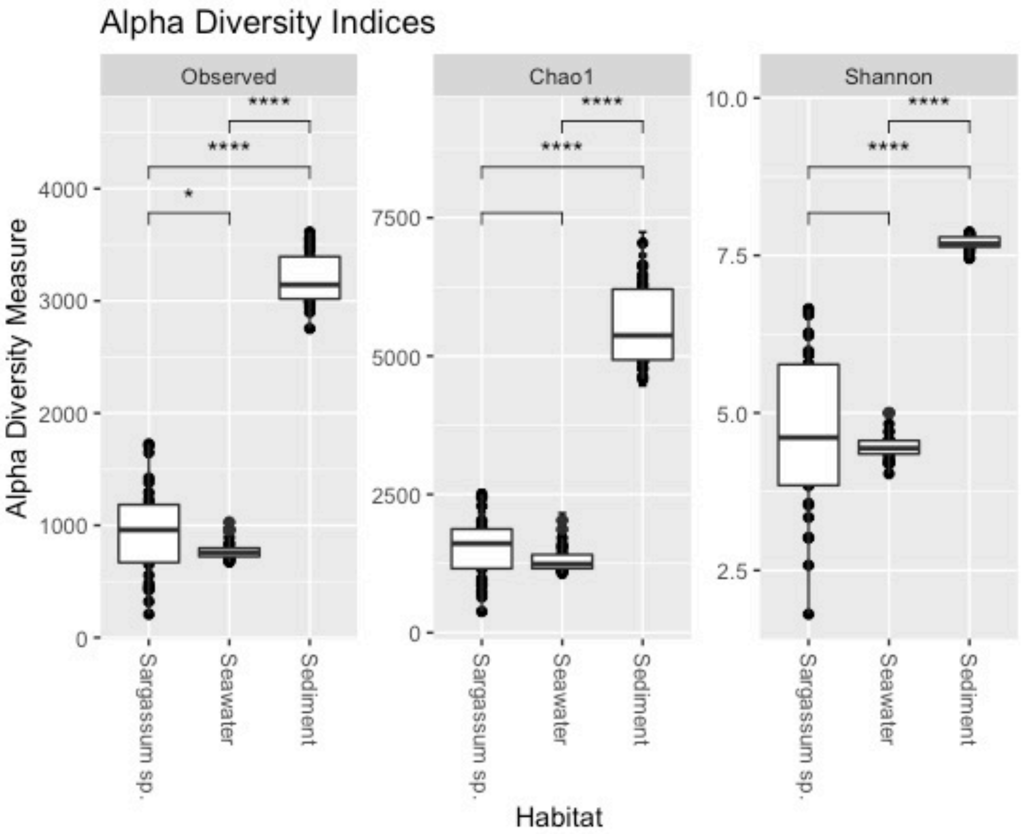
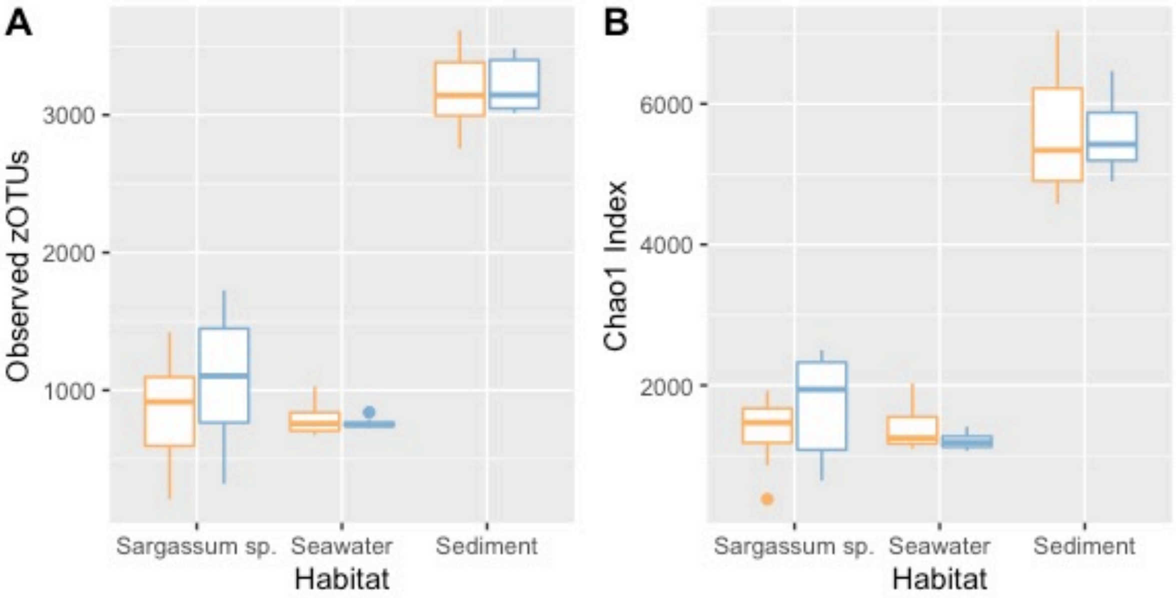


Figure 3: Box plots for alpha diversity measures Observed, Chao1, and Shannon for all habitats at Geoffrey Bay. The boxes represent the interquartile range, where the heavy line denotes the median of the data set. The whiskers extending from the box represent the entire range of the data set. Strength of differences were visualized with a pairwise Wilcoxon test between groups. Numbers of asterisks denote strength of significance (*:p <=0.05, **:p <=0.01, ***: p <=0.001, ****: p <=0.0001).



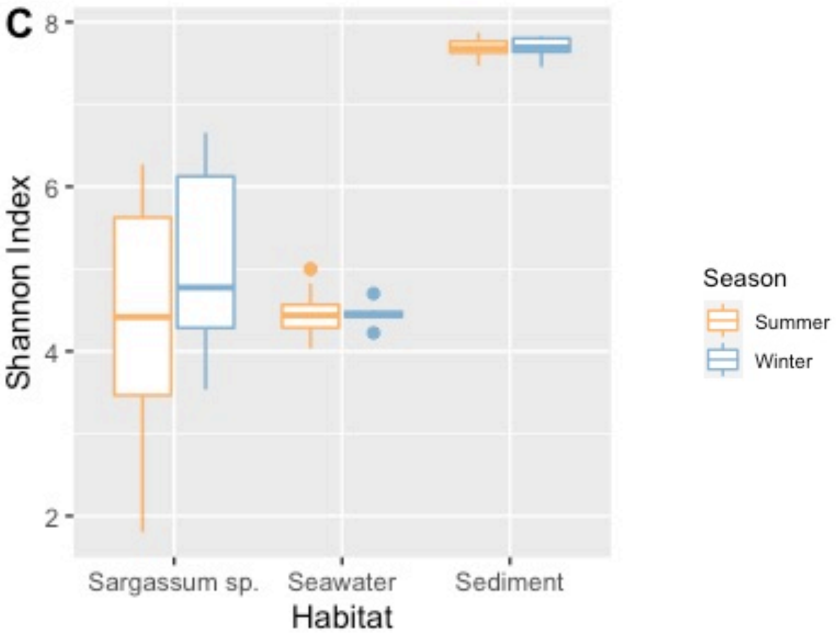


Figure 4: Alpha diversity plots for Observed zOTUs (A), Chao1 Index (B), and Shannon Index (C) across all three habitats for summer (orange) and winter (blue). the boxes represent the interquartile range, where the heavy line denotes the median of the data set. The whiskers extending from the box represent the entire range of the data set.

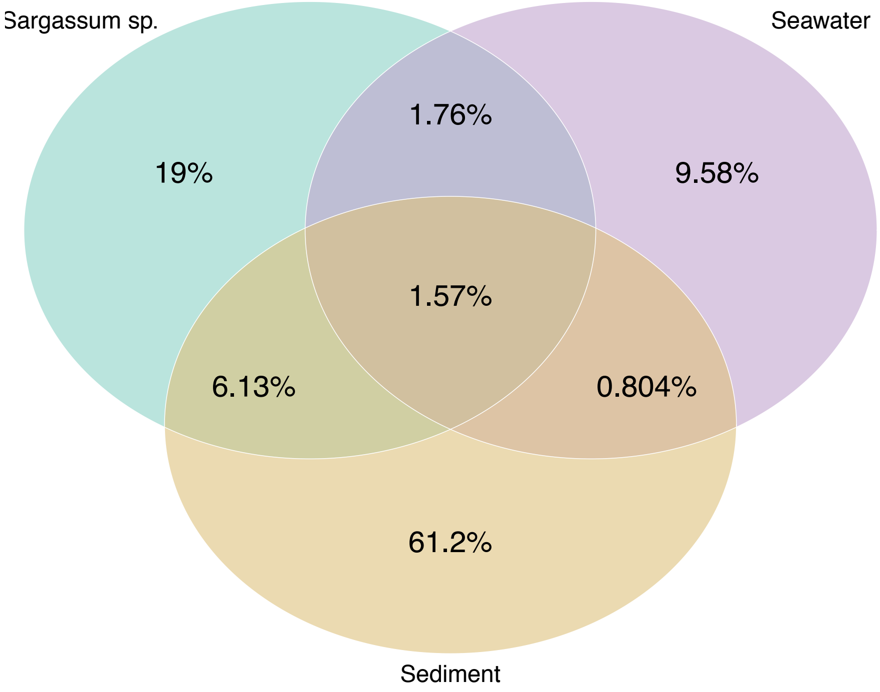


Figure 5: Venn diagram exploring specific and ubiquitous zOTUs between the three habitats at Geoffrey Bay.

Comparing the zOTU diversity between habitats has shown that sediment and *Sargassum* sp. samples have the highest number of shared zOTUs between any two habitats while all three habitats share 1.57% of the total number of zOTUs (Figure 5).

3.2 MICROBIAL SUCCESSION

The nMDS plot exhibits distinct microbial communities among each of the three habitats (PERMANOVA: ($F_{(2/87)} = 33.855$, p = 0.006, $R^2 = 0.437$) (Figure 6) while seasons did not significantly affect any of the habitats (PERMANOVA: $F_{(1/88)} = 1.164$, p = 1.5, $R^2 = 0.0131$). The effect of habitat in bacterial community structure did not differ significantly between seasons (interaction habitat vs season, PERMANOVA: $F_{(1/87)} = 1.697$, p=0.246, R^2= 0.021). There is a seasonal effect in the bacterial community structure of seawater (PERMANOVA: $F_{(1/28)} = 4.204$, p = 0.006, R2 = 0.131) which could be an effect of sample dispersion (PERMDISP: $F_{(1/28)} = 16.471$, p = 0.006). On the other hand, no seasonal differences were found in sediment (PERMANOVA: $F_{(1/28)} = 1.3$, p=0.252, R^2= 0.044) but this comparison is biased by heterogeneous dispersion (PERMDISP: $F_{(1/28)} = 6.70$, p=0.06). There was no seasonal effect in bacterial community structure for *Sargassum* sp. (PERMANOVA: $F_{(1/28)} = 1.6769$, p = 0.096, R2 = 0.057), and this result was not an effect of sample dispersion (PERMDISP: $F_{(1/28)} = 2.468$, p = 0.828).

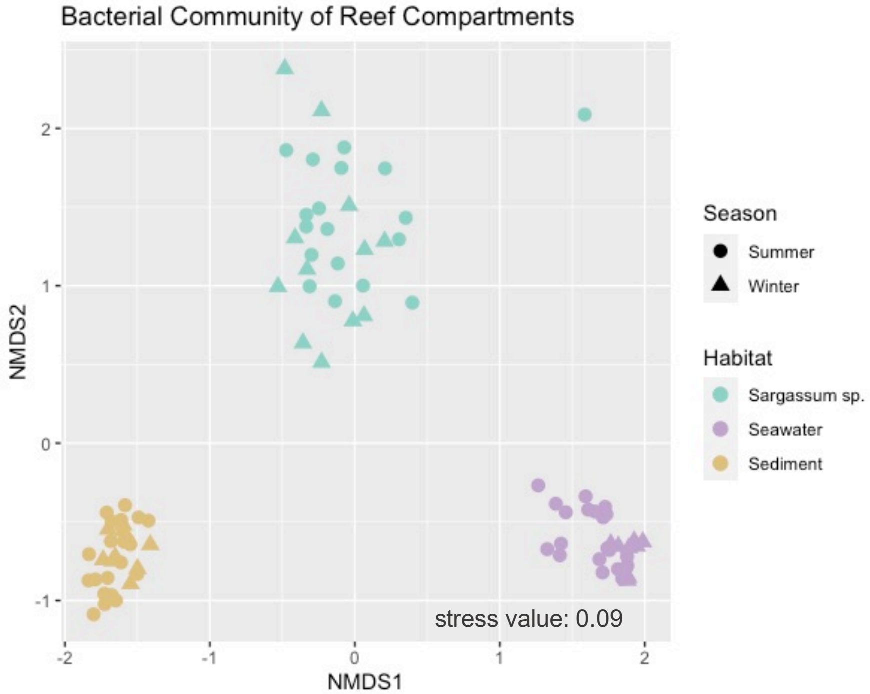


Figure 6: Non metric multi-dimensional scaling ordination based on a Bray-Curtis dissimilarity matrix for seawater (brown), sediment (purple) and Sargassum sp. (green) samples demonstrating the unique bacterial microbiome of each habitat. Stress value of 0.09 indicates that two axis were sufficient to describe the data dispersion.

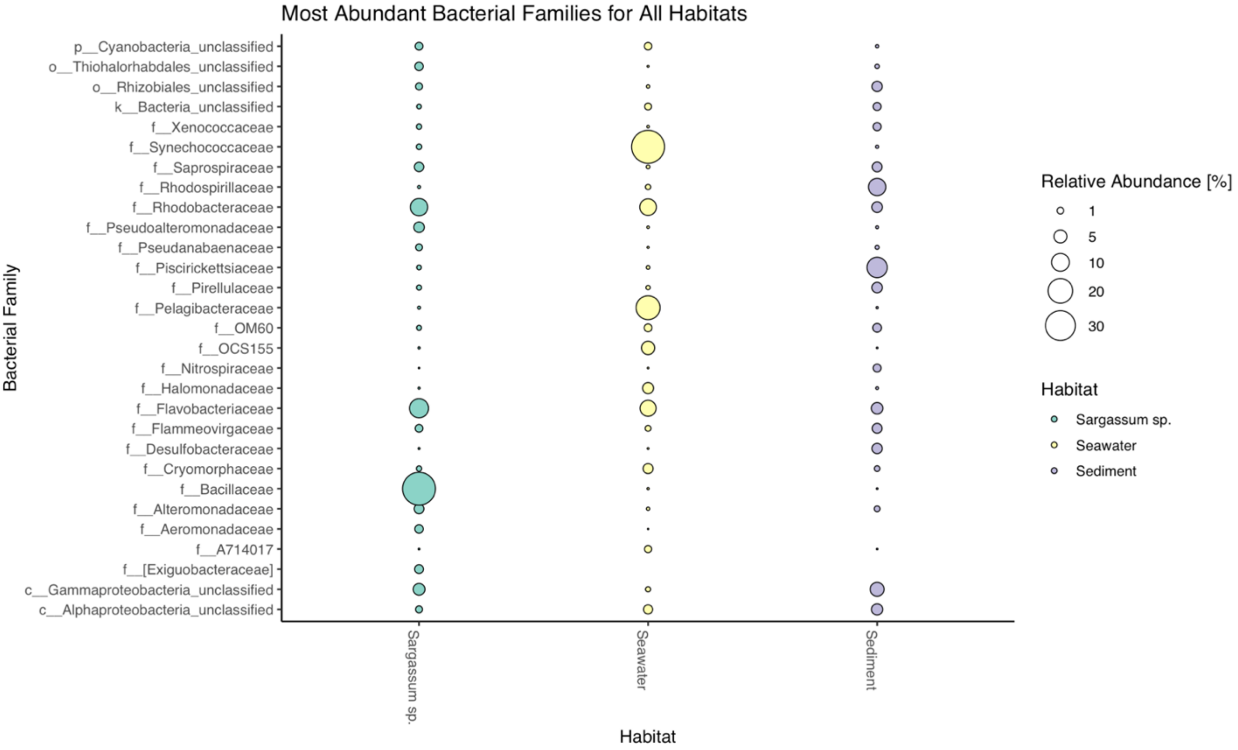


Figure 7: Bubble plot illustrating the most abundant bacterial families for each habitat across the entire time series at Geoffrey Bay.

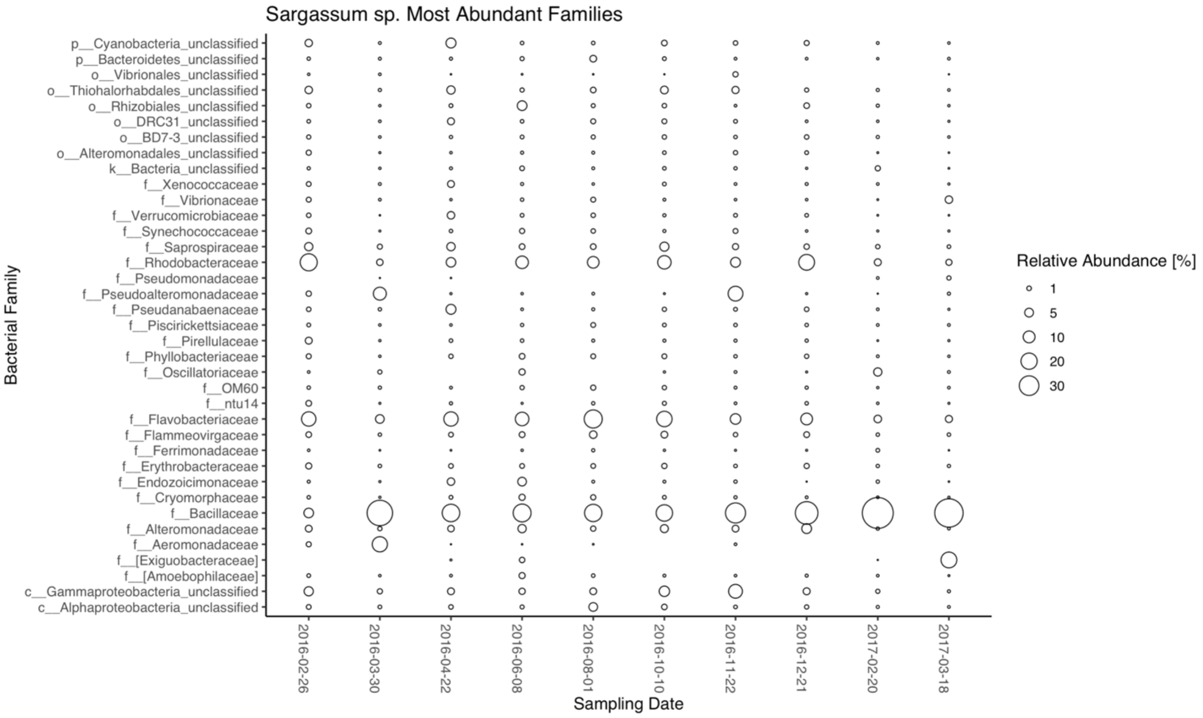


Figure 8: Relative abundances of bacterial families for *Sargassum* sp. samples over the entire time series at Geoffrey Bay.

Sargassum sp. samples were dominated by a few bacterial families while the sediment samples depicted a much more even distribution of several bacterial families. The most abundant bacterial families found within *Sargassum* sp. associated bacterial community were Bacillaceae (35%), Flavobacteriaceae (12%) and Rhodobacteraceae (10%). As for the sediment samples, the dominant bacterial families were Piscirickettsiaceae (13%), Rhodospirillaceae (10%) and unclassified Gammaproteobacteria (6%). For the seawater samples, the most abundant families were Synechococcaceae (37%), Pelagibacteraceae (19%), Rhodobacteraceae (9%) and Flavobacteriaceae (8%) (Figure 7).

Throughout the sampling regime, Bacillaceae stands out as the most dominant bacterial family for *Sargassum* sp. biofilm samples with a reduction in the abundance during the winter months (23% in winter versus 46% in summer). Flavobacteriaceae shows an opposite trend with increasing relative abundance in the winter months (19%) and a reduction in the summer months (9%). While the relative abundance of Rhodobacteraceae changes throughout the sampling scheme, it does not show a clear pattern with seasonality. Spikes in Pseudoalteromonadaceae are observed for the sampling dates 2016-03-30 and 2016-11-22 (Figure 8). Seawater and sediment samples depict a higher degree of stability throughout the year than that of the *Sargassum* sp. samples with only slight changes in the relative abundances of dominant bacterial families (Supplementary Information, Figures S1 and S2). The three habitats shared Flavobacteriaceae, Rhodobacteraceae, Pirellulaceae, and Flammeovirgaceae as the most abundant bacterial families. Flavobacteriaceae showed opposite trends in abundance between sediment and *Sargassum* sp. samples. Rhodobacteraceae showed a contrasting trend between *Sargassum* sp. biofilm samples and the free-living communities. Pirellulaceae showed opposing trends between the seawater and *Sargassum* sp. samples while Flammeovirgaceae had similar trends for both the seawater and *Sargassum* sp. samples.

Following the temporal analysis of the three habitats, a more in-depth community composition analysis was performed for *Sargassum* sp. samples as the bacterial component of the microbiome for the sediment and seawater samples remained relatively stable throughout the sampling regime (Supplementary Information, Figures S1 and S2).

Implementation of DESeq2 between the seasonal groups showed that 48% of the 94 zOTUs responsible for causing shifts in the *Sargassum* sp. biofilm for winter versus summer belonged to the Bacillaceae, Flavobacteriaceae and Rhodobacteraceae families (Figure 9, FDR-adjusted p values ≤ 0.01). Bacillaceae associated zOTUs are preferentially more abundant in summer than in winter, with Rhodobacteraceae and Flavobacteriaceae displaying zOTUs abundant in both winter and summer (Figure 9). The phyla Proteobacteria and Bacteroidetes had several families displaying a seasonal shift while only one family of Firmicutes (i.e., Bacillaceae) exhibited significant differences between the winter and summer seasons (Figure 9).

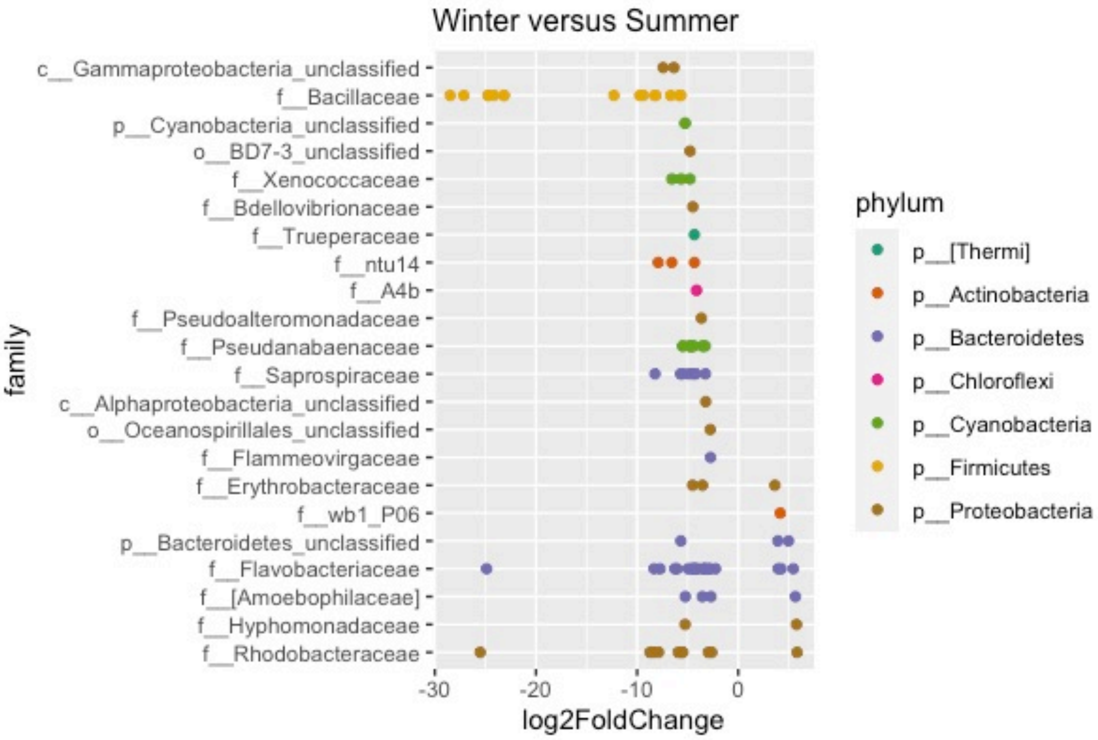


Figure 9: DESeq2 analysis for bacterial families responsible for causing shifts between the winter and summer seasons. Positive values of logfold2 change indicate an increase in zOTU abundance by a multiplicative factor. Fdr-adjusted p value was set to 0.01. Colored dots correspond to an zOTU with the associated phyla described in the legend.

3.3 INFLUENCE OF ABIOTIC FACTORS

Analysis of collinearity between the abiotic factors led to the exclusion of the following variables: POC, Chl *a*, PO4, NO2-NO3 and NO2 (Supplementary Information, Figure S3). The PCA, which included NPOC, NH4, temperature, SiO2, TSS, NPIC, and PN, revealed that the most relevant abiotic factors responsible for sample distribution amongst sampling dates were NPIC, temperature, TSS, PN, SiO2 and NPOC (Figure 10). The first two principal components explained 67.9% of the variance between samples. The variation among summer samples leans on both PC1 and PC2 indicating that the dispersion of these samples can be attributed to fluctuations in temperature, NPOC, SiO2, NH4 and TSS. Variation in winter samples leans more on PC2 than PC1, indicating that the distribution of these samples may be best explained by changes in TSS and SiO2 and NH4.

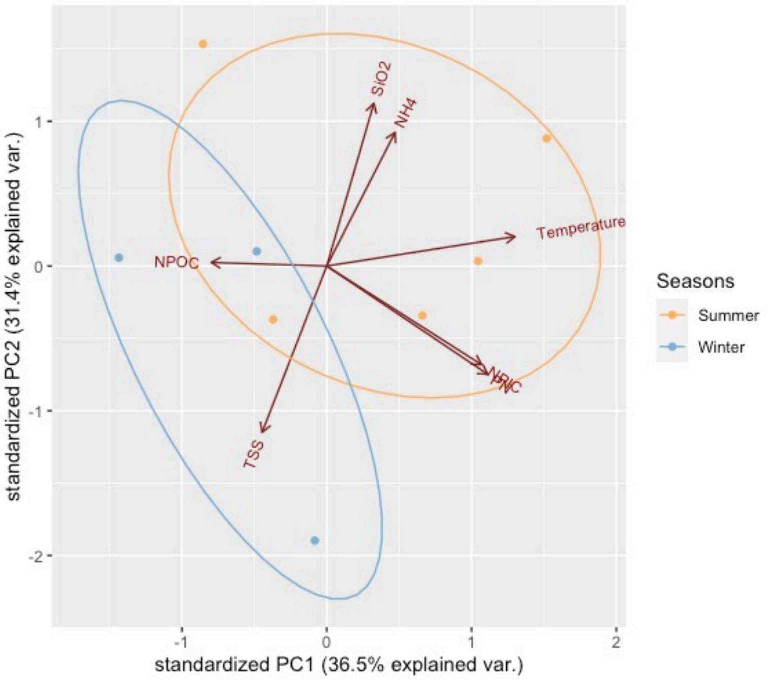


Figure 10: Principal Component Analysis (PCA) for all sampling dates for all habitats divided into winter (blue) and summer (orange) groups with each dot representing a sampling date.

Two db-RDAs were conducted for each habitat, one testing abiotic influence on the dominant zOTUs only, and a second including all *Sargassum* sp. associated zOTUs. The *Sargassum* sp. samples show a clear clustering between the winter and summer seasons for both the dominant zOTUs (n=83) and total zOTUs with the distribution of the dominant zOTUs being dependent on temperature and NPOC (Figure 11B, Supplementary information, Table1). With total zOTUs present in the model, TSS, NH4, SiO2 and NPOC became significant abiotic factors (Figure 11A, Supplementary information, Table1). Distribution of summer samples is dependent on temperature for both dominant and total zOTUs with the distribution of winter bacterial community dependent on NPOC for dominant zOTUs and TSS for total zOTUs (Figures 11A-B). NH4 becomes a relevant factor to summer bacterial community assemblage for total zOTUs (Figure 11A). A variance partitioning analysis suggested that the abiotic factors explained 15% and 29% of community variation for dominant and total zOTUs respectively and explained 62% and 56% of microbial community variation for dominant and total seawater associated zOTUs respectively. As for the sediment, dominant zOTUs were affected by the highest number of abiotic factors when compared to any other habitat and the variance partitioning analysis suggested that the abiotic factors explained 23% and 41% of variation in microbial community for dominant and total sediment associated zOTUs respectively (Supplementary Information, Table 1). All three habitats share temperature as a statistically significant factor for both dominant zOTUs and total zOTUs (Supplementary Information, Table 1). Permutational ANOVA tests for each of the db-RDA-based models indicated their statistical relevance in describing zOTU distribution for each habitat (Supplementary Information, Table 2).

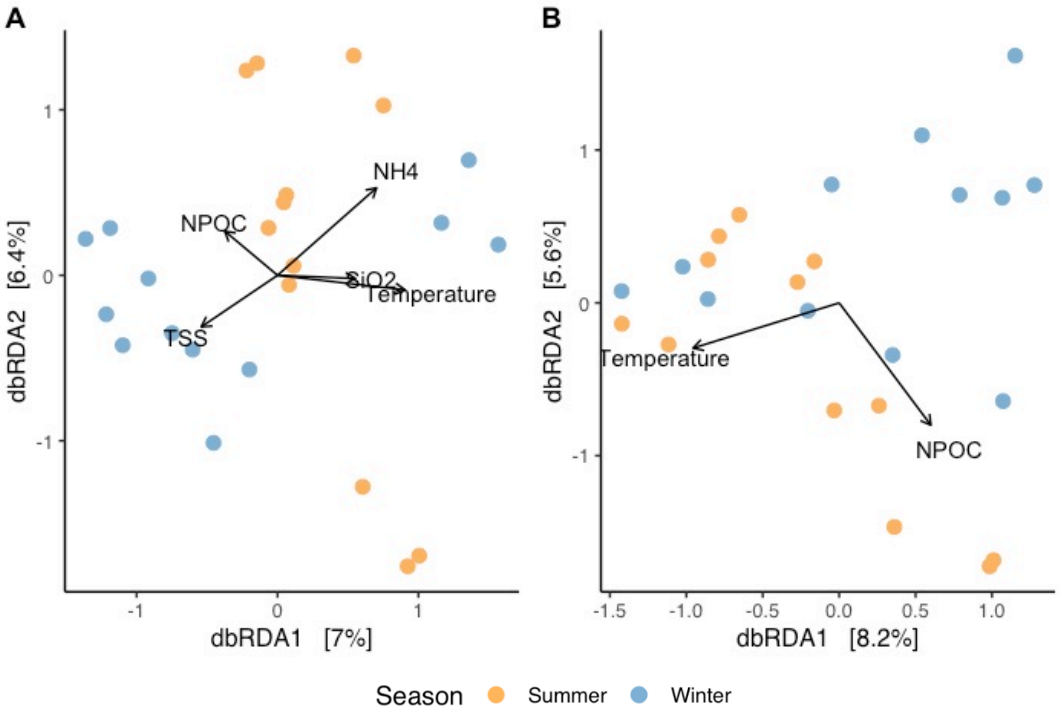


Figure 11: db-RDAs based on Bray-Curtis distancing A) total *Sargassum* sp. zOTUs B) *Sargassum* sp. dominant zOTUs (n = 83). Orange dots denote summer samples and blue dots denote winter samples

Based on the db-RDA models for the total and dominant zOTUs, community correlation heat maps were generated highlighting the significance of unclassified families in the order Thiohalorhabdales, class Gammaproteobacteria, phylum Cyanobacteria, and families Flavobacteriaceae, and Phyllobacteriaceae for both total and dominant *Sargassum* sp. associated zOTUs (Figures 12 and 13). The bacterial families ntu14 and Alteromonadaceae were only significant for dominant *Sargassum* sp. associated zOTUs (Figure 12, $p < 0.05$). Flammeovirgaceae, Saprospiraceae, Endozoicimonaceae, Verrucomicrobiaceae, Rhodobacteraceae and unclassified families in the class Alphaproteobacteria and order Rhizobiales were only significant for total zOTUs (Figure 13, $p < 0.05$) TSS did not show strong correlations to any of the bacterial families (Figure 13).

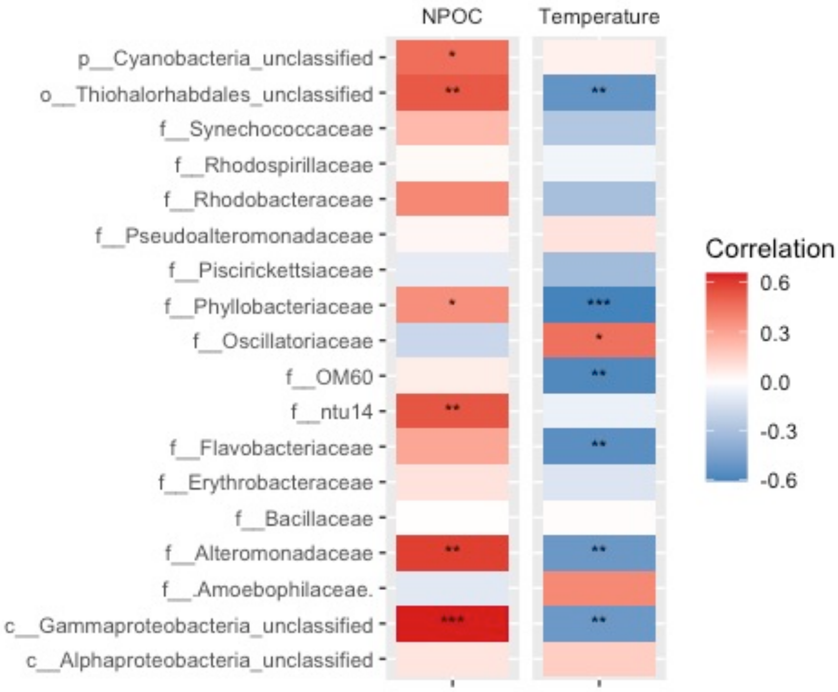


Figure 12: Correlation heat map relating abiotic factors with bacterial families of *Sargassum* sp. for dominant zOTUs at Geoffrey Bay. Spearman ranking , p = 0.05. Red shades indicate a positive correlation while blue shades indicate a negative correlation. Asterisks denote significance.

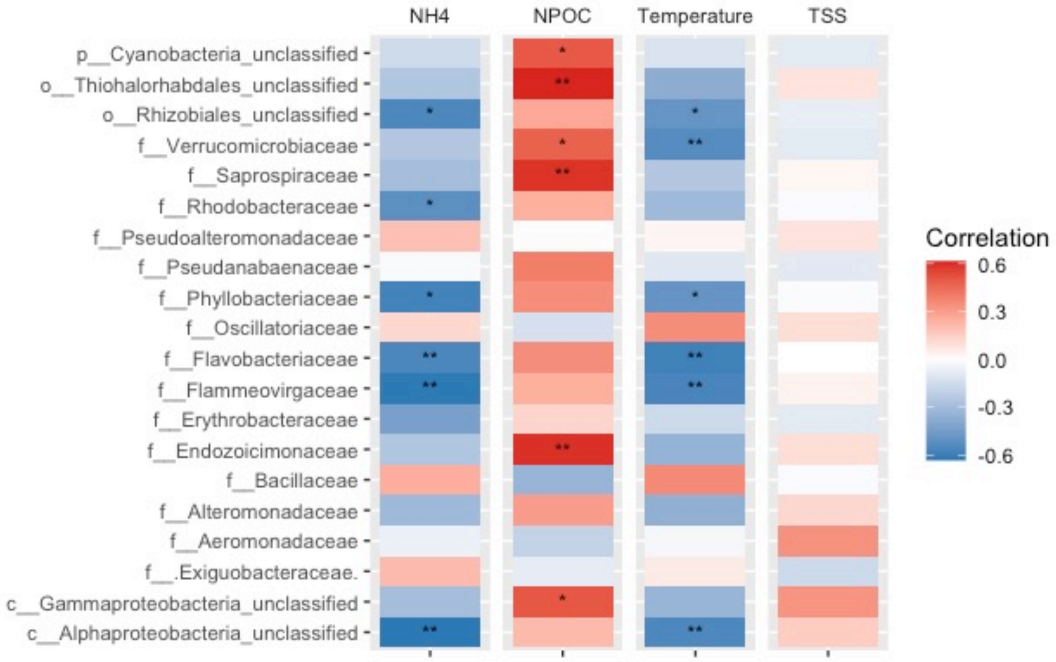
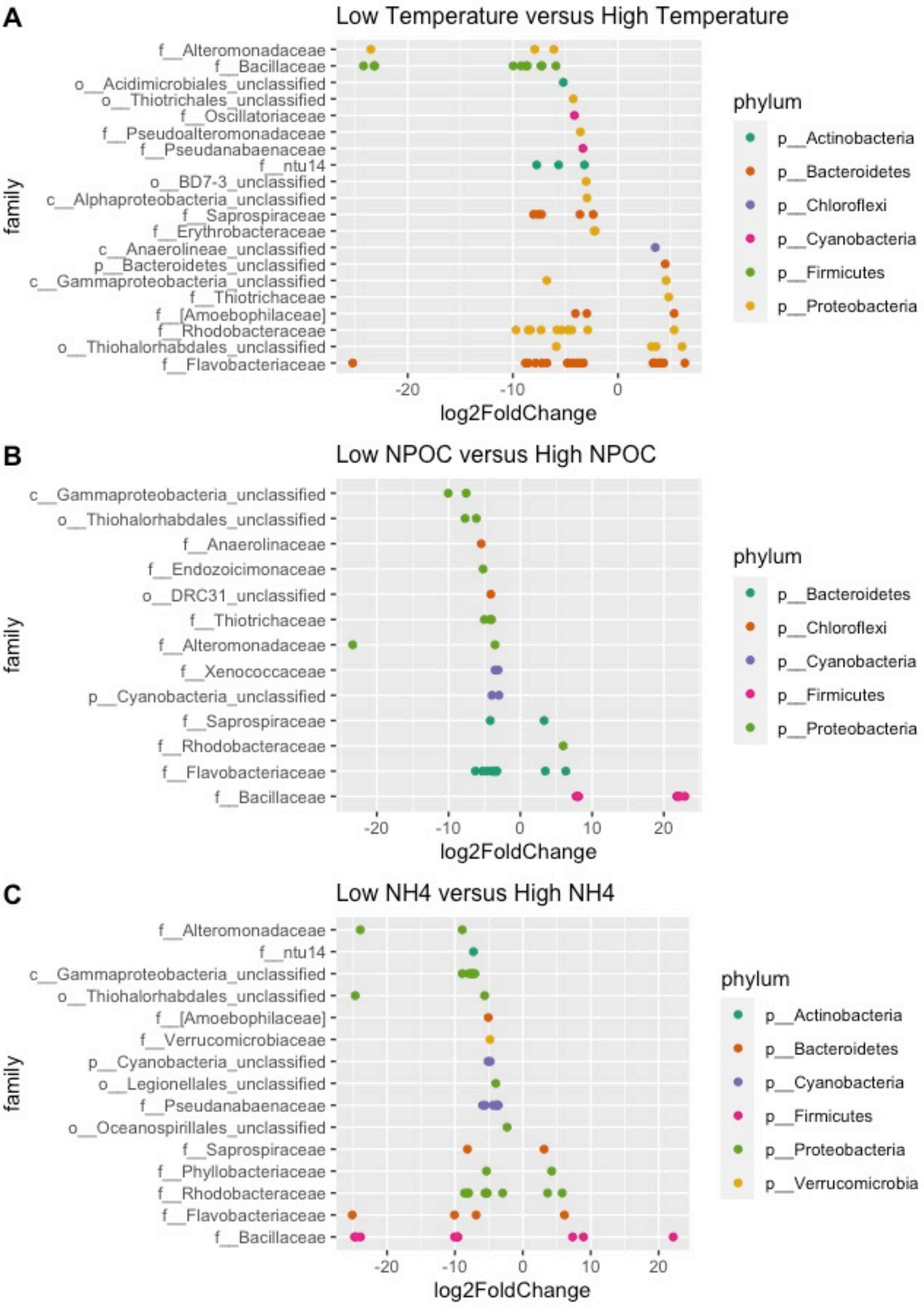


Figure 13: Correlation heat map relating abiotic factors with bacterial families of *Sargassum* sp. for total zOTUs at Geoffrey Bay. Spearman ranking , p = 0.05. Red shades indicate a positive correlation while blue shades indicate a negative correlation. Asterisks denote significance.

The DESeq2 and heat map shared several bacterial families in common and in some instances, the DESeq2 results corroborated patterns observed with the correlation heat map (Figures 14A-D, FDR-adjusted p values ≤ 0.01). 42% of the zOTUs causing statistical differences between low and high temperature groups belonged to the Rhodobacteraceae and Flavobacteriaceae families. 27% of the zOTUs causing statistical differences between low NH4 and high NH4 groups also belonged to Rhodobacteraceae and Flavobacteriaceae. Between 14% and 17% of the zOTUs causing statistical differences between treatment groups of the abiotic factors belonged to Bacillaceae (Figures14A-D).


A
Low Temperature versus High Temperature
family
f__Alteromonadaceae
f__Bacillaceae
o__Acidimicrobiales_unclassified
o__Thiotrichales_unclassified
f__Oscillatoriaceae
f__Pseudoalteromonadaceae
f__Pseudanabaenaceae
f__ntu14
o__BD7-3_unclassified
c__Alphaproteobacteria_unclassified
f__Saprospiraceae
f__Erythrobacteraceae
c__Anaerolineae_unclassified
p__Bacteroidetes_unclassified
c__Gammaproteobacteria_unclassified
f__Thiotrichaceae
f__[Amoebophilaceae]
f__Rhodobacteraceae
o__Thiohalorhabdales_unclassified
f__Flavobacteriaceae
-20
-10
0
log2FoldChange
phylum
p__Actinobacteria
p__Bacteroidetes
p__Chloroflexi
p__Cyanobacteria
p__Firmicutes
p__Proteobacteria
B
Low NPOC versus High NPOC
family
c__Gammaproteobacteria_unclassified
o__Thiohalorhabdales_unclassified
f__Anaerolinaceae
f__Endozoicimonaceae
o__DRC31_unclassified
f__Thiotrichaceae
f__Alteromonadaceae
f__Xenococcaceae
p__Cyanobacteria_unclassified
f__Saprospiraceae
f__Rhodobacteraceae
f__Flavobacteriaceae
f__Bacillaceae
-20
-10
0
10
20
log2FoldChange
phylum
p__Bacteroidetes
p__Chloroflexi
p__Cyanobacteria
p__Firmicutes
p__Proteobacteria
C
Low NH4 versus High NH4
family
f__Alteromonadaceae
f__ntu14
c__Gammaproteobacteria_unclassified
o__Thiohalorhabdales_unclassified
f__[Amoebophilaceae]
f__Verrucomicrobiaceae
p__Cyanobacteria_unclassified
o__Legionellales_unclassified
f__Pseudanabaenaceae
o__Oceanospirillales_unclassified
f__Saprospiraceae
f__Phyllobacteriaceae
f__Rhodobacteraceae
f__Flavobacteriaceae
f__Bacillaceae
-20
-10
0
10
20
log2FoldChange
phylum
p__Actinobacteria
p__Bacteroidetes
p__Cyanobacteria
p__Firmicutes
p__Proteobacteria
p__Verrucomicrobia

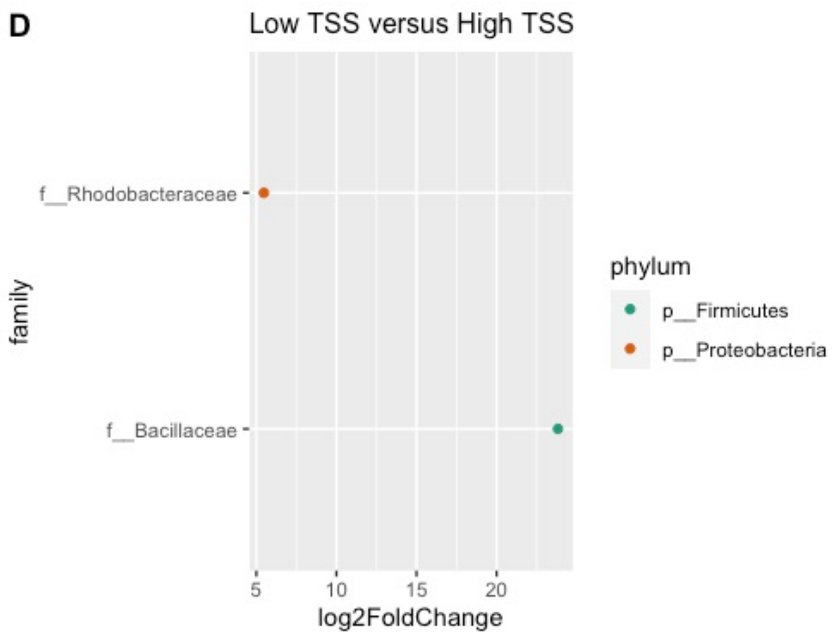


Figure 14: DESeq2 analysis for bacterial families responsible for causing shifts between high and low temperature (14A), low NPOC and high NPOC (14B) low NH4 and high NH4 (14C) and low TSS and high TSS (14D). Positive values of logfold2 change indicate an increase in the zOTU by a multiplicative factor. FDR-adjusted p-value was set to 0.01. Colored dots correspond to a zOTU with the assigned phyla described in the legend.

3.4 MICROBIAL FUNCTION

From the 6309 *Sargassum* sp. zOTUs provided to the FAPROTAX database, 1872 (29.67%) were assigned to at least one of the 35 functional groups represented. With these 1872 zOTUs, 4358 functional assignments were established, which includes overlapping zOTUs between the functional groups. The most frequently observed taxonomic families related to chemoheterotophy belonged to the Bacteroidetes and Proteobacteria phyla, while those related to phototrophy belonged to Cyanobacteria (Table 2). Fermentation and nitrate reduction functions were dominated by bacterial taxa belonging to Proteobacteria, Bacteroidetes and Fusobacteria and intracellular parasites were mostly assigning taxa belonging to Proteobacteria (Table 2). Bacillaceae, the previously described highly abundant bacterial family, was not assigned by FAPROTAX.

Table 2: Metabolic functions representing the majority of the FAPROTAX functional assignments and their associated dominant bacterial families

Metabolic function	% of FAPROTAX functional assignment	Dominant bacterial families
Chemoheterotrophy	62%	Flammeovirgaceae^{+}, Alteromonadaceae*, Flavobacteriaceae^{+}
Phototrophy	21%	Cyanobacteria(unclassified)‡, Xenococcaceae‡, Pseudanabaenaceae‡
Fermentation	2%	Vibrionaceae*, Fusobacteriaceae°
Nitrate reduction	2%	Vibrionaceae*, Flavobacteriaceae^{+}
Intracellular parasites	1%	Pelagibacteraceae*

Bacterial phyla are represented by the following symbols: Bacteroidetes($^{+}$) Proteobacteria(*) Fusobacteria(°) Cyanobacteria(‡)

98.20% of the functions assigned to the *Sargassum* sp. biofilm samples were attributed to carbon metabolism, with only 0.014% and 0.004% attributed to nitrogen and sulfur metabolism respectively. Chemoheterotrophy, oxygenic photoautotrophy, fermentation and phototrophy represented 90% of the carbon metabolism functions present for the *Sargassum* sp. biofilm samples. Trends in chemoheterotropy and photoautrophy show an inverse relationship with the mean percentage of chemoheterotrophic functions increasing from summer 2016 to winter 2016 and reaching a minimum in summer 2017 (Figure 15). Fermentation is relatively stable throughout the sampling regime with slightly increased percentages during summer sampling dates (Figure 15). Nitrate reduction was responsible for 95% of all the nitrogen-related metabolic functions in the *Sargassum* sp. biofilm samples. Similar to trends observed with fermentation, peaks of nitrate reduction occur in the summer months, with an exception for a winter peak on 2016-08-01 (Figure 16). Dark sulfide oxidation, respiration of sulfur compounds and anoxygenic photoautotrophy (S oxidizing) comprised 98% of the sulfur-related metabolic functions. Dark sulfide oxidation and anoxygenic photoautotrophy (S oxidizing) display an inverse relationship with no obvious seasonal pattern. Mean percentages in respiration of sulfuric compounds fluctuate throughout the time series and also lack a distinct seasonal pattern (Figure 17).

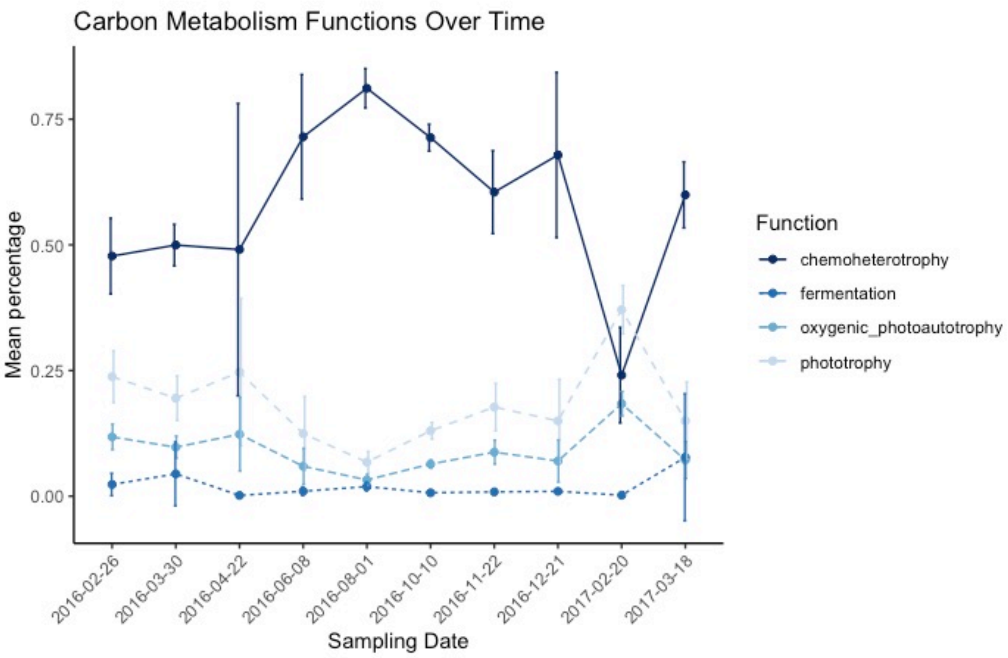


Figure 15: Mean percentage of Carbon-related metabolic functions for all *Sargassum* sp. samples across the sampling regime. Error bars indicate standard deviation.

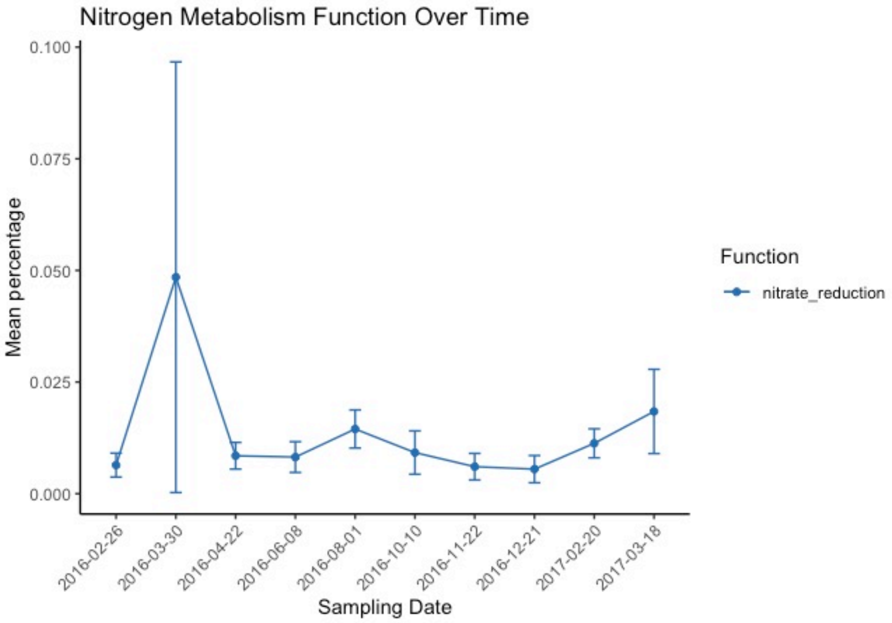


Figure 16: Mean percentage of Nitrogen-related metabolic functions for all *Sargassum* sp. samples across the sampling regime. Error bars indicate standard deviation.

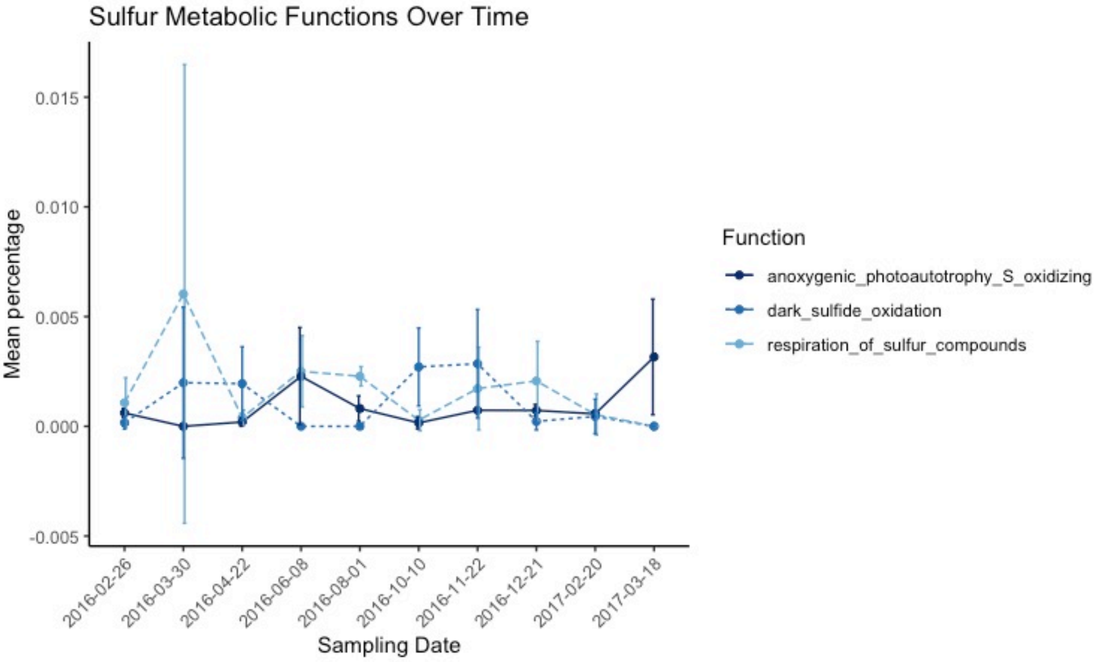


Figure 17: Mean percentage of Sulfur-related metabolic functions for all *Sargassum* sp. samples across the sampling regime. Error bars indicate standard deviation.

4 DISCUSSION

Coral reefs are globally threatened by phase shifts characterized by rapid rates of coral cover decline and its replacement by algal dominated grounds (Kline et al., 2006). This is known to contribute to increasing abundances of copiotriphic bacteria in the water column, which can pose additional threats to corals, a process coined microbialization of reefs (Haas et al., 2016). There remains a paucity of information regarding the role of benthic macroalgae in these shifts and particularly how their association with microorganisms may respond to changing climatic conditions. This study aimed to address this in the context of the Great Barrier Reef (GBR) by characterizing the bacterial biofilm of *Sargassum* sp., a commonly found inhabitant native of inshore reef sites in the region (Vuki & Price, 1994). By combining 16S rRNA gene sequencing data with abiotic metadata, this study was able to successfully relate relative abundances of *Sargassum*-associated bacterial taxa to fluctuations of environmental parameters occurring within the coral reef ecosystem. It is imperative to further our understanding of these relationships as changes in the macroalgal holobiont can affect the health of the coral reef (Barott et al., 2011).

4.1 BACTERIAL COMMUNITY COMPOSITION OF REEF COMPARTMENTS

Bacterial communities from the three habitats in Geoffrey Bay were shown to be unique from each other as previously demonstrated for other locations (Bolinches et al., 1988; Longford et al., 2007; Bengtsson & Øvreås, 2010; van der Loos et al., 2019; James et al., 2020). In this study, the dominant phyla for the seawater samples were Proteobacteria (40%), Cyanobacteria (39%) and Bacteroidetes (12%), which agrees with findings from varying global planktonic communities (Longford et al., 2007). The sediment samples were largely dominated by Proteobacteria (63%) followed by Bacteroidetes (12%) and Planctomycetes (5%), which matches a study conducted by Uthicke & McGuire (2007) on bacterial community assemblages of GBR sediments. The most dominant phyla for the *Sargassum* sp. biofilm samples were Firmicutes (37%), Proteobacteria (35%) and Bacteroidetes (18%). The dominance of these three bacterial phyla are consistent with other research on biofilms from brown algae (de Mesquita et al., 2019). The prominence of epiphytic Proteobacteria and Bacteroidetes found on these *Sargassum* sp. samples is congruous with similar 16S rRNA studies previously published on *Sargassum* biofilms (Serebryakova et al., 2018; Li et al., 2019; Mei et al., 2019). A possible overrepresentation of Firmicutes has been reported in previous studies conducted in the GBR due to the ability of Firmicutes to produce freezing resistant endospores during sampling (cryogenic preservation) conditions as was found with the sponges *Pseudoceratina clavata* and *Rhabdastrella globostellata* (Lafi et al., 2005). While the dominating presence of Firmicutes has been less widely reported for *Sargassum* biofilms, studies on *Sargassum polycystum* in Indonesia (Susilowati et al., 2015) and *Sargassum myriocystum* in India (Chakraborty et al., 2017) confirms the presence of Firmicutes on *Sargassum*, therefore validating the results of this study. Firmicutes has been described in other algal species inhabiting temperate Australia such as the red algae *Delsia pulchra* (Penesyan et al., 2009) and has been found in Australian sponges of the GBR (Lafi et al., 2005). Planctomycetes, which are commonly observed as a dominant phylum in *Sargassum* biofilms (Bondoso et al., 2014; Serebryakova et al., 2018; Li et al., 2019) represented less than 1% of the bacterial component of the biofilm in this study suggesting an effect of geographical variation in the epiphytic bacterial community of *Sargassum* (Lachnit et al., 2011). On a family level, Bacillaceae, Flavobacteriaceae and Rhodobacteraceae were consistently predominant in *Sargassum* sp. biofilm samples throughout the time series. Of particular interest is the increase in the relative abundance of Pseudoalteromonadaceae during the summer months (5%) compared to its relative abundance in the winter (<0.001%).

4.2 HOST COLONIZATION

Bacterial taxa responsible for initially colonizing the biofilm are a function of site-specific environmental conditions (Campbell et al., 2015) as local abiotic factors are a contributing force to the functional requirements of epiphytic communities inhabiting macroalgal biofilms (Morrissey et al., 2019). Initial colonization of *Sargassum* sp. biofilms from this study may occur with microbial species present in the free-living communities which explains why there exists some overlap in the bacterial community composition between sediment and *Sargassum* sp. and between seawater and *Sargassum* sp., namely with the Flavobacteriaceae, Rhodobacteraceae and Flammeovirgaceae families (Figure 7). The copiotrophic bacterial taxa Alteromonadaceae, Rhodobacteraceae and Flavobacteriaceae, all found in this study, have been previously described as pioneer taxa of young brown algae tissues (Bengtsson et al., 2012; Paix et al., 2019, 2020).

Once the *Sargassum* thallus has been initially colonized, the macroalgae may be selecting which bacterial taxa fulfil its functional requirements as was concluded by Mei et al. (2019) on their study of *Sargassum horneri* in China. This selectivity imposed by the macroalgae is governed by multiple factors including host species, geography, environmental factors and morphology (Egan et al., 2013). The stability of the seawater and sediment bacterial communities over the time series in this study suggests that the fluctuation in the relative abundances of bacterial epiphytes already occupying the *Sargassum* sp. biofilm is not exclusively dictated by these free-living communities. Bacterial species occupying functional niches within the macroalgal biofilm vary primarily with species and secondarily with geography (Lachnit et al., 2009). Both of these factors may explain why the bacterial community composition of *Sargassum* sp. biofilms from this study differs from biofilms of other *Sargassum* species, namely in the high relative abundance of Firmicutes observed here. As another example, Planctomycetes have been reported to break down sulfated polymeric carbon in *Sargassum thunbergii* (Li et al., 2019) and *Laminaria hyperborea* (Bengtsson & Øvreås, 2010) and their low abundance (<1%) in this study may be because their role is being occupied by a different bacterial taxa, i.e., the community may display functional redundancy.

4.3 MICROBIAL SUCCESSION

If the *Sargassum* sp. associated bacterial community composition is not stable throughout the sampling regime, what drivers could be causing these fluctuations? As *Sargassum* displays seasonal sensitivity (large canopy-forming thalli in the summer, holdfast and short branches in the winter), the effect of seasonality on bacterial diversity was tested. This study showed that seasonality had little effect on the epiphytic bacterial diversity observed between the winter and summer seasons for the *Sargassum* sp. biofilm, however variations in community composition were observed along the time series. Sediment and seawater samples also did not demonstrate seasonality in bacterial diversity. This finding suggests that community assemblage instead of alpha diversity is responsible for the variation in the *Sargassum* sp. associated bacterial community along the time series, as was found for corals inhabiting different depth ranges (Glasl et al., 2017). A successional pattern exists for these *Sargassum* sp. biofilm samples, but akin to *Cystoseira compressa* in the Mediterranean (Mancuso et al. 2016), the changes in bacterial community composition may be gradual and do not necessarily demonstrate a drastic reorganization, which may be why diversity indices were not significantly different between the seasons.

Temporal fluctuations of bacterial community assemblages inhabiting macroalgal surfaces have previously been described across several habitats such as the coral reef (Glasl et al., 2020), estuaries (Bolinches et al., 1988) and rocky coasts (Mancuso et al., 2016). Research regarding seawater microbial dynamics has demonstrated that microbial community assemblages are more similar during the same season of the year as compared to opposite seasons (6 months apart) (Fuhrman et al., 2015). These successional patterns were also observed over a two-year study for three species of macroalgae in Australia, in which similar epibacterial colonization trends were observed at the same season (Lachnit et al., 2011). These seasonal patterns could also exist for *Sargassum* in Geoffrey Bay. Mechanisms driving these bacterial successional patterns have yet to be explored in the context of this study, however I suggest that it may be related to *Sargassum*'s seasonal growth and degradation cycle. Seaweed-associated biofilms have been proven to induce morphogenesis in their macroalgal hosts (Egan et al., 2013; Singh & Reddy, 2014). Glasl et al. (2020) previously suggested this morphogenic mechanism could be mediated by the shifting relative abundances of Firmicutes and Bacteroidetes between the winter and summer seasons and fluctuations in the relative abundances of these bacterial phyla were notable between seasons in this study. To test this hypothesis, future field studies should be performed over a longer time series to test the relationship between *Sargassum* biomass and bacterial community composition. Axenic culture studies could also be performed on *Sargassum* to test this hypothesis. Related experiments have been conducted for the green alga *Ulva linza* (Marshall et al., 2006) and *Ulva fasciata* (Singh et al., 2011) where it was found that Bacteroidetes and Firmicutes (respectively for each algae species) were required for typical morphogenesis of the host.

4.4 INFLUENCE OF ENVIRONMENTAL FACTORS

Out of the three habitats, community variation in seawater was best explained by the environmental parameters pertinent to this study while bacterial communities in sediment and *Sargassum sp.* demonstrated a comparatively more stable relationship with changing environmental conditions, which agrees with previous studies (Glasl et al. 2019). Although percentages of explained variance were lower for the *Sargassum* sp. biofilm community than for those of the other two habitats, bacterial community structure was altered with respect to fluctuations in temperature, NPOC and NH4 (Figures 12-14). This is congruent with previous studies examining the effect of environmental factors on macroalgal biofilms (Lachnit et al., 2011; Campbell et al., 2015; Aires et al., 2016; Mei et al., 2019). When only the dominant *Sargassum* sp. associated zOTUs were analysed, the variation in community composition explained by environmental factors was halved as compared to that in the total zOTUs. This suggests that the dominant zOTUs present are not heavily influenced by the environment, while the collective bacterial community with the total zOTUs displays an increased sensitivity to environmental factors. Abiotic factors proved to have a degree of influence on the microbial community present on *Sargassum* sp. surface biofilms (Figures 11-14), however due to the low variance partitioning percentage (29%), other drivers must be at play. Due to this low variance, biotic factors are suggested to be the primary mechanism driving changes in the bacterial community for *Sargassum* sp. in this study. Potential biotic mechanisms can include interactions between different bacteria, between bacteria and other epibionts, and/or between bacteria and the host. Interactions between bacterial species can come in the form of quorum sensing (Singh & Reddy, 2014) and production of antibacterial compounds by competing bacterial taxa (de Mesquita et al., 2019). Metabolites released by the macroalgae have also been shown to affect the bacterial community composition (Egan et al., 2013). In the context of the current study, the determinant drivers of biotic factors remain unclear. To test interactions between bacteria and other epibionts, sequencing data on eukaryotes could be gathered and compared to bacterial sequencing data with a MANTEL test as was done in a study conducted by Lopez et al. (2020), where a strong correlation between the community structure of prokaryotes and eukaryotes was shown.

4.5 BACTERIAL FAMILIES RESPONDING TO ENVIRONMENTAL CHANGES

While the total microbial community inhabiting *Sargassum* sp. biofilms was not heavily influenced by the abiotic factors included in this study, several bacterial families displayed significant relationships to temperature, NPOC and NH4. 33% of the zOTUs causing statistical difference between the NPOC treatment groups belonged to Flavobacteriaceae (Figure 14). In estuarine systems, Flavobacteriaceae was favoured in conditions of high nutrient concentrations derived from mineralization of organic matter produced by algae (Bolinches et al., 1988). Results of the current study partially deviate from this observation as Flavobacteriaceae demonstrated a significant inverse relationship with NH4 (Figure 13), however, this could be due to the difference in environment as well as interactions with temperature and mineralization rates. Bacillaceae are not significantly correlated to any of the abiotic factors (Figures 12 & 13), which was unexpected due to its dominance and relevance between treatment groups for the abiotic factors (14-17% of the divergence) (Figures 9 & 14A-D). While Bacillaceae as a family did not respond to abiotic factors, individual zOTUs were responsive to abiotic treatment groups suggesting that these individual zOTUs of Bacillaceae could occupy different niches. Additionally, Bacillaceae's abundance may be related to a different abiotic factor altogether, or is instead mediated by biotic mechanisms such as the seasonal shifting populations of Bacteroidetes (Flavobacteriaceae) and Firmicutes (Bacillaceae) previously mentioned. This assumption may be pre-emptively ascertained by the inverse relationship observed in the relative abundances of these two families (Figure 7). A potential mechanism for the oscillating patterns in relative abundances for Flavobacteriaceae and Bacillaceae may be related to available carbon sources. As Flavobacteriaceae is preferentially abundant in conditions of high NPOC (Figure 14B) it is suggested that the relative abundance of Flavobacteriaceae is related to the availability of NPOC in the water column, possibly thriving on *Sargassum* senescence, instead of carbon originating directly from *Sargassum*. Relative abundance of Bacillaceae may be driven by the increased abundance of *Sargassum* biomass in the summer, and therefore a greater availability of *Sargassum* derived sugars. While Bacillaceae did not show a statistically significant correlation to NPOC (Figure 13), the bacterial family was preferentially abundant in conditions of low NPOC (Figure 14B).

In this study, Alteromonadaceae and Flammeovirgaceae were significantly inversely correlated to temperature for *Sargassum* sp. associated zOTUs (Figures 12 and 13) and showed increased abundances in the winter (Figure 8). This finding is in contrast with Paix et al. (2019) study on the Mediterranean brown algae *Taonia atomaria* which concluded that both Alteromonadaceae and

Flammeovirgaceae displayed higher abundances in the summer. Saprospiraceae in the present study was significantly highly correlated to NPOC for total *Sargassum* sp. associated zOTUs (Figure 13) and displayed a slightly higher relative abundance in summer which was also opposite to the Paix et al. (2019) study. In this study Alteromonadaceae was preferentially present in conditions of high NH4 and high NPOC (Figures 14C and 14D), which is congruent with previous descriptions of Alteromonadaceae (López-Pérez & Rodriguez-Valera, 2014)-. Contrasting trends to previous studies could be due to a multitude of effects including biogeography, different host species, variations in environmental conditions or a functional redundancy occupied by other bacterial taxa.

4.6 PUTATIVE FUNCTIONS OF KEY BACTERIAL TAXA

The most commonly assigned functions by FAPROTAX were those relating to chemoheterotrophy and were dominated by Flavobacteriaceae, Alteromonadaceae and Flammeovirgaceae (Table 2). The dominance of Flavobacteriaceae, a member of the class Flavobacteriia has demonstrated an importance in the degradation of algal tissues (Li et al 2019). This family was significantly negative correlated to temperature (Figures 12 & 13), suggesting that it may have a role in breaking down algal tissues in the winter when *Sargassum* reduces its biomass via frond loss. Relative abundances of Flavobacteriaceae were highest during the winter sampling dates (Figure 8), which corresponds to the peaks observed in chemoheterotrophy (Figure 15). As Flavobacteria was identified by FAPROTAX to be one of the dominant bacterial families responsible for chemoheterotrophy, it may be suggested that the fluctuating abundances of Flavobacteriaceae contribute to the seasonal patterns of chemoheterotrophy observed for the *Sargassum* sp. biofilm samples. Flavobacteria are also known to generate toxin-resistant substances, protecting the algal host from pathogens (Li et al., 2019). However, several taxa from Flavobacteriaceae have been described as opportunistic marine pathogens associated with temperature-induced bleaching on the kelp *Ecklonia radiata* (James et al., 2020). Akin to Flavobacteriaceae previously described, Alteromonadaceae was one of the most dominant bacterial taxa related to chemoheterotrophy. While there does not exist a parallel trend between the relative abundance of Alteromonadaceae (Figure 8) and its chemoheterotrophic function (Figure 15), the peaks observed for the winter dates of chemoheterotrophy validate the inverse relationship between temperature and abundance of Alteromonadaceae (Figure 12). Flammeovirgaceae, a dominant chemoheterotroph in this study (Table 2) has previously demonstrated positive correlations with metabolites acting as macroalgal antifouling defences (Paix et al., 2019).

The increase of taxa performing chemoheterotrophic functions in the winter is contrasting to what was expected, as bacterial heterotrophic functions in pelagic marine systems have demonstrated an increase during summer months (Williams et al., 2016). However, taking into consideration that the FAPROTAX database was unable to assign Bacillaceae, the most dominant bacterial family in this data set and also a known heterotroph (Du et al., 2010), I suggest that this representation of chemoheterotrophy is not complete. This assumption can be verified by referring to the DESeq2 analysis (Figure 9), where Bacillaceae stands out as the bacterial family with the largest log2foldchange value between the summer and winter seasons and its zOTUs explain 16% of the divergence between the seasonal treatment groups. The reduction in their abundance during winter could be related to changes in *Sargassum* biomass, with the amount of organic matter originating from the *Sargassum* decreasing when compared to the summer months. Metagenomic data regarding Firmicutes (Bacillaceae) have suggested that this bacterial phylum is capable of producing antibiotics that act as antagonizing agents against foreign microbes, protecting its host (Glasl et al., 2020). Firmicutes have also proved to metabolize macroalgal-derived sugars and perform other regulatory functions such as biofilm formation and nitrogen utilization (Glasl et al., 2020).

Higher percentages of phototrophic functions were observed during summer sampling dates with the dominant bacterial taxa corresponding to Cyanobacteria (Figure 15 and Table 2). The presence of Cyanobacteria on benthic macroalgal biofilms suggests that as fellow autotrophs, they may aid with the primary production of the algae (Egan et al., 2013) or if in high abundances have suggested a competitive mechanism with the algae as they are competing for the same resources (Li et al., 2019). Cyanobacteria have been reported to carry out nitrogen fixation in other *Sargassum* species (Phlips et al., 1986) and although very few records for nitrogen fixation existed for the current data set, 3 of the 5 records assigned to nitrogen fixation belonged to Cyanobacteria. FAPROTAX results from this study indicated that the sulfurous metabolic functions were performed by Proteobacteria instead of Planctomycetes as previously described in the brown algae *Laminaria hyperborea* (Bengtsson & Øvreås, 2010). Desulfarculales and Desulfobacterales, two taxa assigned to sulfur respiration functions in this study were also found in *Sargassum* samples from the Caribbean (Lopez et al., 2020).

An increase in Pseudoalteromonadaceae on *Sargassum* sp. surface biofilms was noted at the sampling date 30-03-2016 (Figure 8) which corresponds to the 2016 GBR bleaching event (Hughes et al., 2017) and for the sampling date 22-11-2017 which precedes the 2017 GBR bleaching event (Brodnicke et al., 2019) . This bacterial family has been implicated in pathogenicity (Li et al 2019) and demonstrated greater relative abundances in higher temperatures in this study (Figure 14A), suggesting that increased temperatures could possibly introduce more virulent bacterial taxa on macroalgal surfaces. This 30-03-2016 sampling date also corresponds to the outlying data point of the nMDS for the *Sargassum* sp. samples (Supplementary Information, Figure S3).

4.7 PATHOGENICITY

Macroalgae release carbon-derived exudates into the water column that have a higher tendency to fuel the growth of virulent taxa versus exudates released from their coral counterparts (Nelson et al., 2013). Through this DDAM (DOC, disease, algae, microbes) mechanism, macroalgae may fuel copiotrophic bacteria that can adversely affect coral health without having to come into contact with the coral (Barrott & Rowher, 2012). Pathogenic bacterial taxa may also be introduced to corals through direct contact of the macroalgae with the coral surface (Barrott & Rowher, 2012), thereby disturbing the coral holobiont (Glasl et al., 2020). This highlights the importance of investigating bacterial taxa present on algal species within the coral reef environment. In this study, an increase in Pseudoalteromonadaceae on *Sargassum* sp. surface biofilms was noted at the sampling date 30-03-2016 (Figure 8) which corresponds to the 2016 GBR bleaching event. Coral bleaching, and subsequently mortality, frees up space on the benthos for more algal dominance, thereby further exacerbating the DDAM cycle (Haas et al., 2016). The Pseudoalteromonadaceae bacterial family has been implicated in pathogenicity (Li et al., 2019) and demonstrated greater relative abundances in higher temperatures in this study (Figure 14A). Virulent taxa fuelled by macroalgal exudates, such as Vibrionaceae and Pseudoalteromonadaceae (Nelson et al., 2013), were both observed in the current study on *Sargassum* sp. biofilms.

4.8 LIMITATIONS AND FUTURE DIRECTIONS

My research indicated that environmental factors had little effect on the bacterial community composition of *Sargassum* sp. biofilms, especially for dominant zOTUs. Therefore, it is suggested that changes in the bacterial community composition are a response to biotic interactions. Several relationships could be further examined in future studies: interactions between bacterial taxa, interactions between bacteria and other microorganisms (ie: eukaryotes, archaea), and interactions between bacteria and the macroalgal host, *Sargassum*. Of particular interest is the interaction between *Sargassum* biomass and changes in bacterial community composition, (especially of dominant taxa) which would require a long-term sampling scheme including several seasonal cycles. *Sargassum* sampling at multiple time points throughout the day could better aid in the understanding of biotic mechanisms driving changes in microbial community composition as macroalgal surface conditions (such as available carbon and oxygen) fluctuate throughout the day (Egan et al., 2013). Analysing microbial communities of different macroalgal species present in the same geographical location could demonstrate how communities on varying hosts present in the coral reef environment respond to environmental stressors. As only a few environmental factors were tested in this study, future studies could incorporate environmental parameters such as pH and inorganic nutrients in a laboratory setting. Exploring the bacterial community present on differentiated morphological tissues of *Sargassum* could aid in disentangling seasonal changes in bacterial community composition from variations in structural development, as *Sargassum* is pseudo-perennial. A few limitations existing in this study relate to sequencing and sampling. As for all studies using primers, a selection of different primers could yield varying results in the taxonomic identification of biofilm communities. Primers used to amplify the 16S rRNA gene have previously demonstrated a bias towards the underrepresentation of Planctomycetes for instance (Bengtsson et al., 2010). Implementation of a different functional assignment software, such as PiCRUST2, could have been used to better determine the functional role of bacterial taxa. It would have also aided in verifying the results observed from FAPROTAX. Future studies in this topic aim to couple metagenomic data of key bacterial taxa from this study (Bacillaceae and Flavobacteriaceae) with the 16S sequencing data over the time series to further elucidate the function of these bacterial families on the *Sargassum* sp. biofilm.

5 CONCLUSION

This study successfully identified the epiphytic bacterial community present on *Sargassum* sp. biofilm samples from Geoffrey Bay, in the Great Barrier Reef, and examined how the community composition changed over time. Seasonality was deemed not to be a significant driver of changes in the bacterial diversity, however fluctuations in relative abundances of several bacterial taxa were observed over the 13 months of sampling. Bacterial community composition on *Sargassum* sp. surface biofilms were not as responsive to environmental parameters when compared to seawater or sediment associated communities, however, several bacterial taxa showed significant sensitivities to temperature, NH4 and NPOC. With the implementation of FAPROTAX, putative functional assignments were designated to several key bacterial taxa, with trends in these functions changing over the time series. Drivers behind what exactly causes the change in community composition remain ambiguous, however future studies incorporating the use of metagenomics and investigating biotic drivers can aid in understanding what role key bacterial taxa are playing on *Sargassum* surface biofilms. The continued incorporation of microbial dynamics in the context of the coral reef environment remains imperative for preserving the health and resilience of the coral reefs worldwide.

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SUPPLEMENTARY INFORMATION

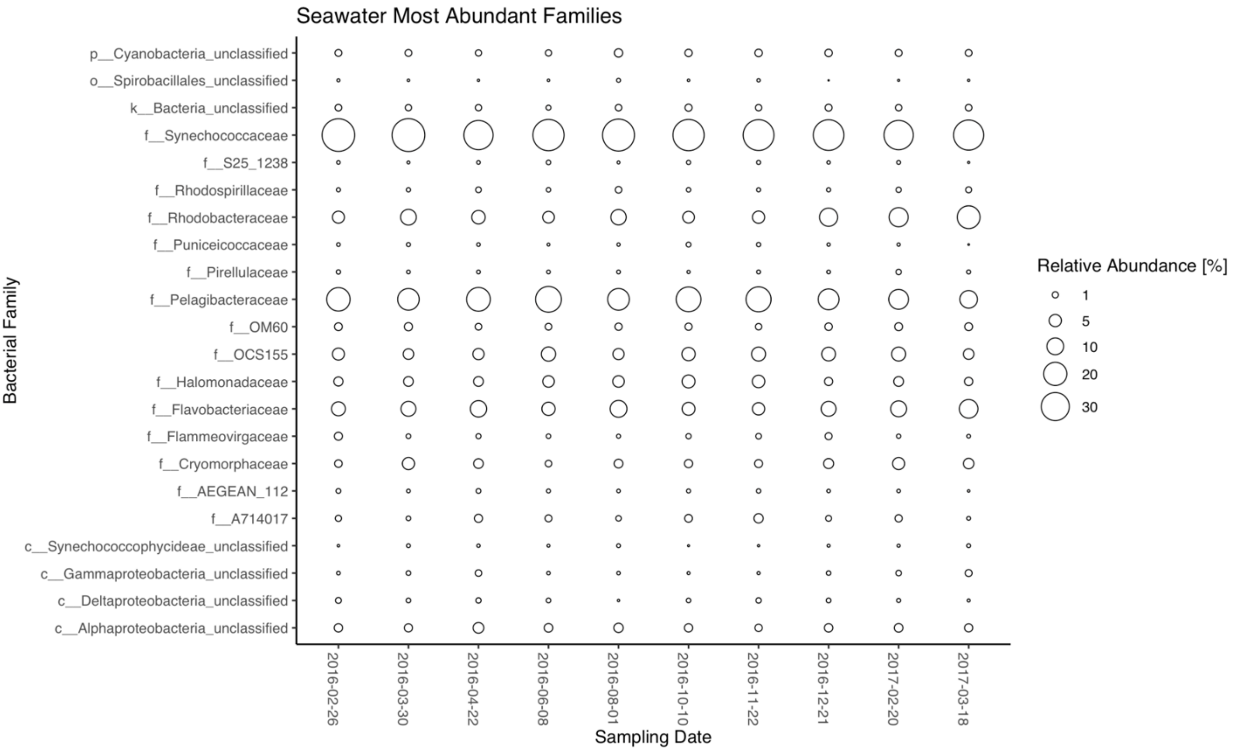

Figure S1: Relative abundances of bacterial families for seawater samples over the time series in Geoffrey Bay.

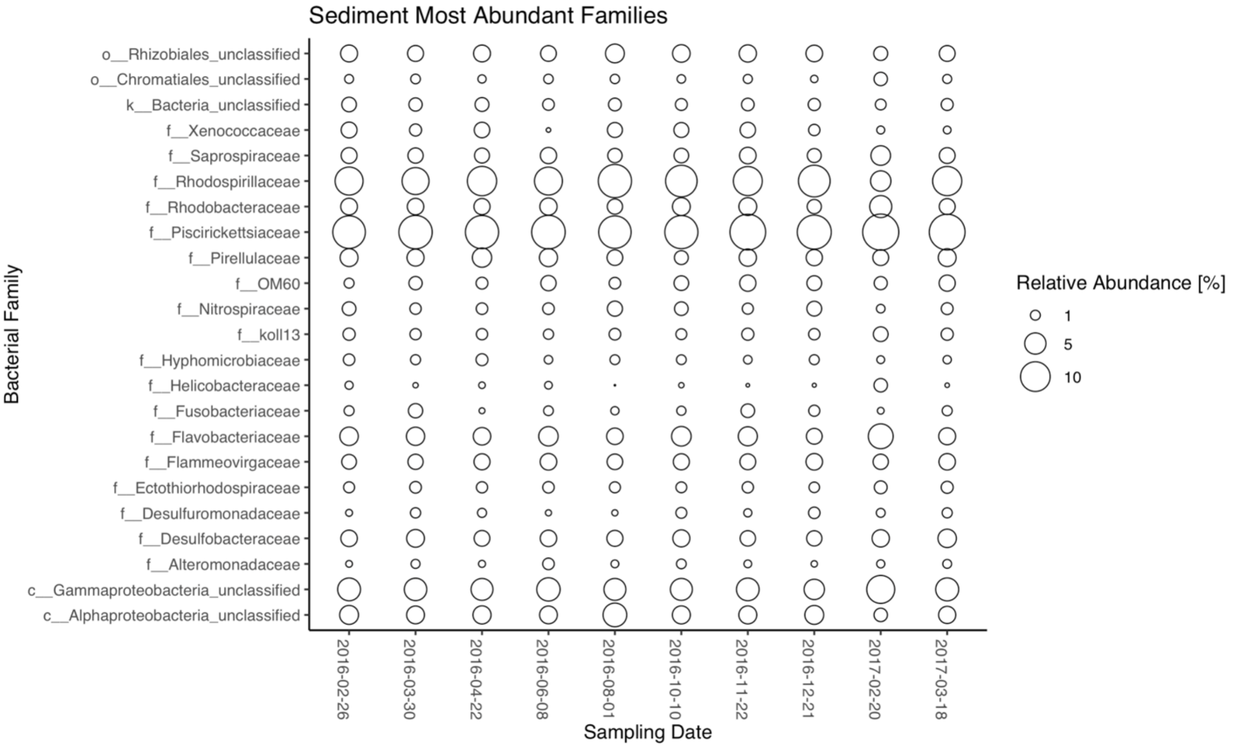

Figure S2: Relative abundances of bacterial families in sediment samples over the time series in Geoffrey Bay.

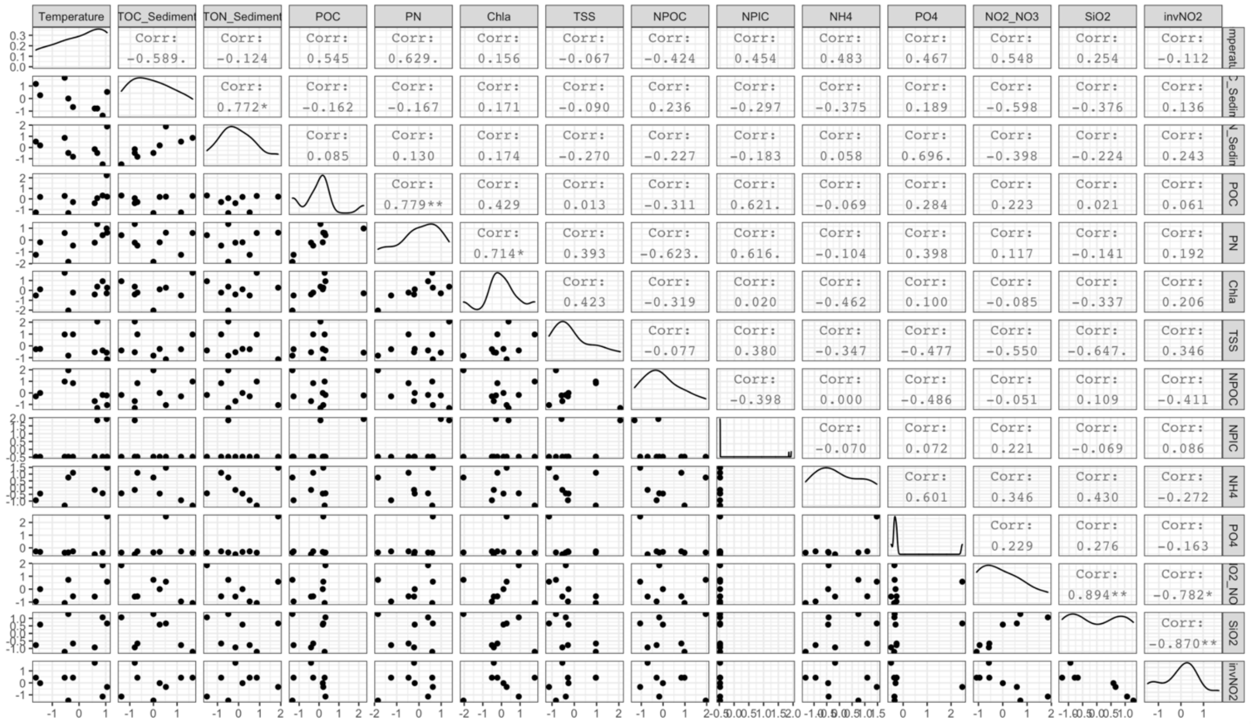

Figure S3: Correlation matrix for environmental parameters collected at Geoffrey Bay computed with Pearson correlation. Correlation threshold set to values equal to or above 0.7 or values equal to or below -0.7. Values meeting these criteria are marked with asterisks.

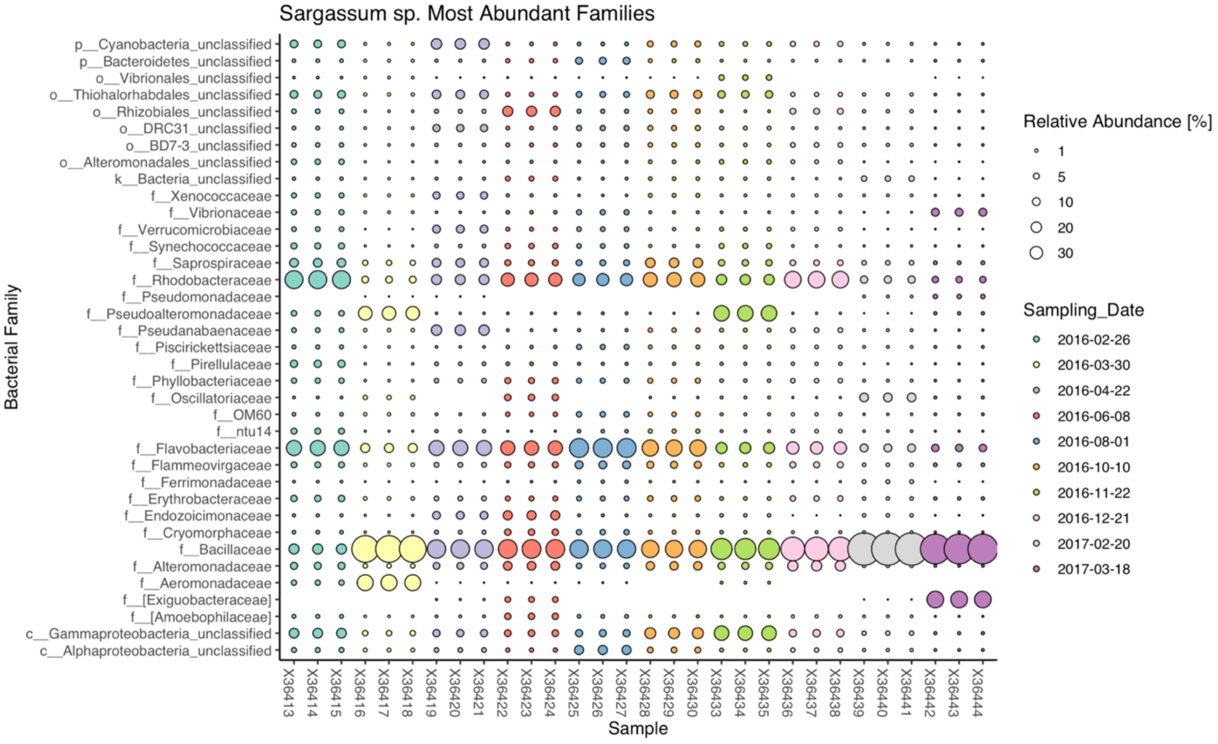

Figure S4: Relative abundances of *Sargassum* sp. bacterial families with all samples. Colors indicate sampling date. This shows that replicate samples were consistent across the sampling scheme.

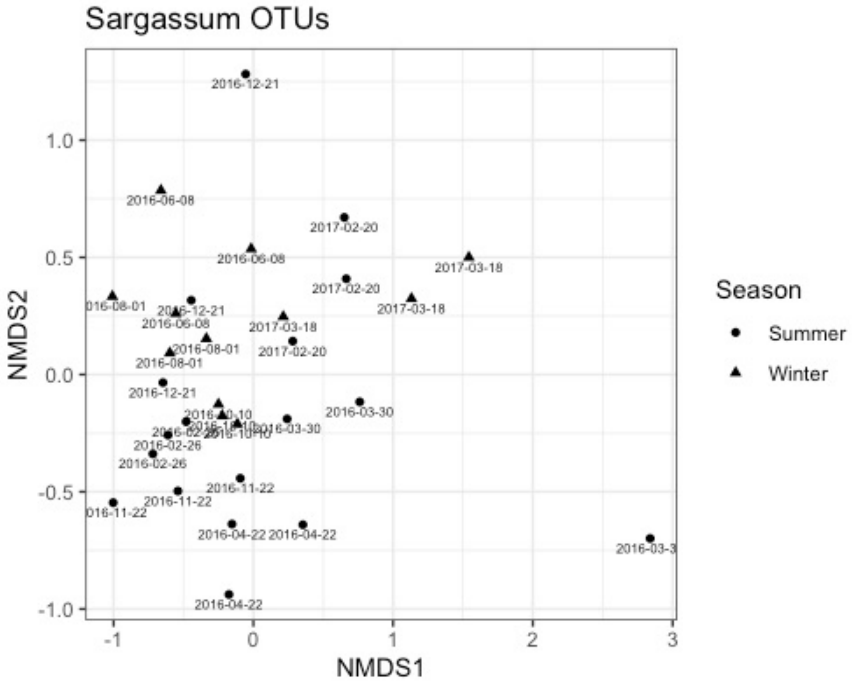

Figure S5: NMDS based on Bray-Curtis distancing matrix for *Sargassum* zOTUs for the sampling dates. Triangles correspond to winter and circles correspond to summer.

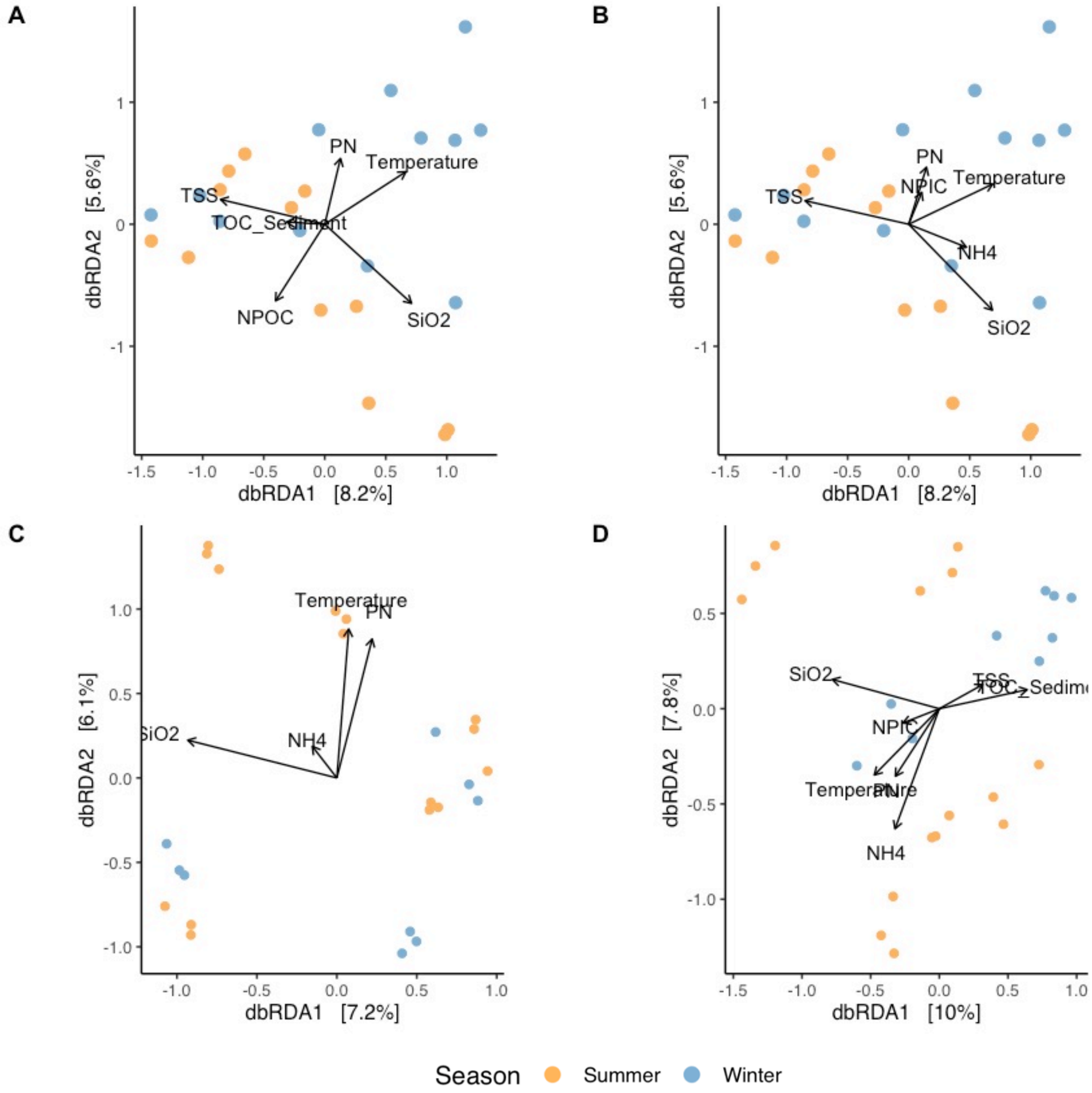

Figure S6: db-RDAs based on Bray-Curtis distancing. A) total seawater zOTUs B) dominant seawater zOTUs C) total sediment zOTUs D) sediment dominant zOTUs. Orange dots denote summer samples and blue dots denote winter samples

Table S1: ANOVA test results for the significance of each abiotic factor in the db-RDA models for *Sargassum* sp., seawater and sediment dominant and all zOTUs. P-values lower than 0.05 deemed significant.

Sargassum sp. - all zOTUs

Variable	R2.adj	Df	AIC	F	Pr(>F)	
Temperature	0.025082	1	57.197	1.5917	0.002	**
NH4	0.041662	1	57.669	1.3806	0.006	**
NPOC	0.056169	1	58.132	1.3228	0.02	*
TSS	0.070895	1	58.524	1.317	0.024	*
SiO2	0.092072	1	58.673	1.4432	0.018	*

Sargassum sp. - dominant zOTUs

Variable	R2.adj	Df	AIC	F	Pr(>F)	
Temperature	0.044781	1	52.041	2.0782	0.004	**
NPOC	0.068861	1	52.311	1.5689	0.02	*

Seawater - all zOTUs

Variable	R2.adj	Df	AIC	F	Pr(>F)	
TSS	0.12667	1	31.823	4.3361	0.002	**
NPOC	0.20298	1	30.512	3.1064	0.002	**
Temperature	0.27069	1	29.211	2.9496	0.002	**
SiO2	0.32479	1	28.13	2.6025	0.002	**
TOC_Sediment	0.35982	1	27.554	2.0397	0.004	**
PN	0.40482	1	26.432	2.3609	0.002	**

Seawater - dominant zOTUs

Variable	R2.adj	Df	AIC	F	Pr(>F)	
TSS	0.16428	1	24.392	5.5211	0.002	**
SiO2	0.24576	1	22.813	3.3768	0.002	**
Temperature	0.33718	1	20.541	3.8965	0.002	**
PN	0.38001	1	19.707	2.3815	0.002	**
NPIC	0.42202	1	18.725	2.381	0.006	**
NH4	0.47927	1	16.85	2.9791	0.002	**

Sediment - all zOTUs

Variable	R2.adj	Df	AIC	F	Pr(>F)	
SiO2	0.025332	1	50.109	1.5978	0.002	**
Temperature	0.043548	1	50.54	1.419	0.002	**
NH4	0.05692	1	51.031	1.2978	0.004	**
PN	0.066178	1	51.564	1.1983	0.018	*

Sediment - dominant zOTUs

Variable	R2.adj	Df	AIC	F	Pr(>F)	
SiO2	0.035238	1	38.187	1.8401	0.002	**
PN	0.061737	1	38.402	1.6213	0.002	**
NH4	0.087545	1	38.562	1.594	0.002	**
Temperature	0.102242	1	38.941	1.3274	0.008	**
TSS	0.117666	1	39.227	1.3321	0.028	*
NPIC	0.135979	1	39.352	1.3815	0.018	*
TOC_Sediment	0.153188	1	39.414	1.3455	0.008	**

Table S2: Permutational ANOVA results testing the suitability of the db-RDA model for each habitat between dominant and all zOTUs.

Sargassum sp. - dominant zOTUs

	Df	Inertia	F	Pr(>F)	
Model	2	1.2665	1.8505	0.01	**
Residual	21	7.1865			

Sargassum sp. - total zOTUs

	Df	Inertia	F	Pr(>F)	
Model	5	2.9718	1.4665	0.01	**
Residual	18	7.2955			

Seawater - dominant zOTUs

	Df	Inertia	F	Pr(>F)	
Model	6	2.2302	3.6073	0.01	**
Residual	17	1.7517			

Seawater - total zOTUs

	Df	Inertia	F	Pr(>F)	
Model	6	1.8779	4.5282	0.01	**
Residual	17	1.175			

Sediment - dominant zOTUs

	Df	Inertia	F	Pr(>F)	
Model	7	1.9309	1.5944	0.01	**
Residual	16	2.7681			

Sediment - total zOTUs

	Df	Inertia	F	Pr(>F)	
Model	4	1.7472	1.4075	0.01	**
Residual	19	5.8966			

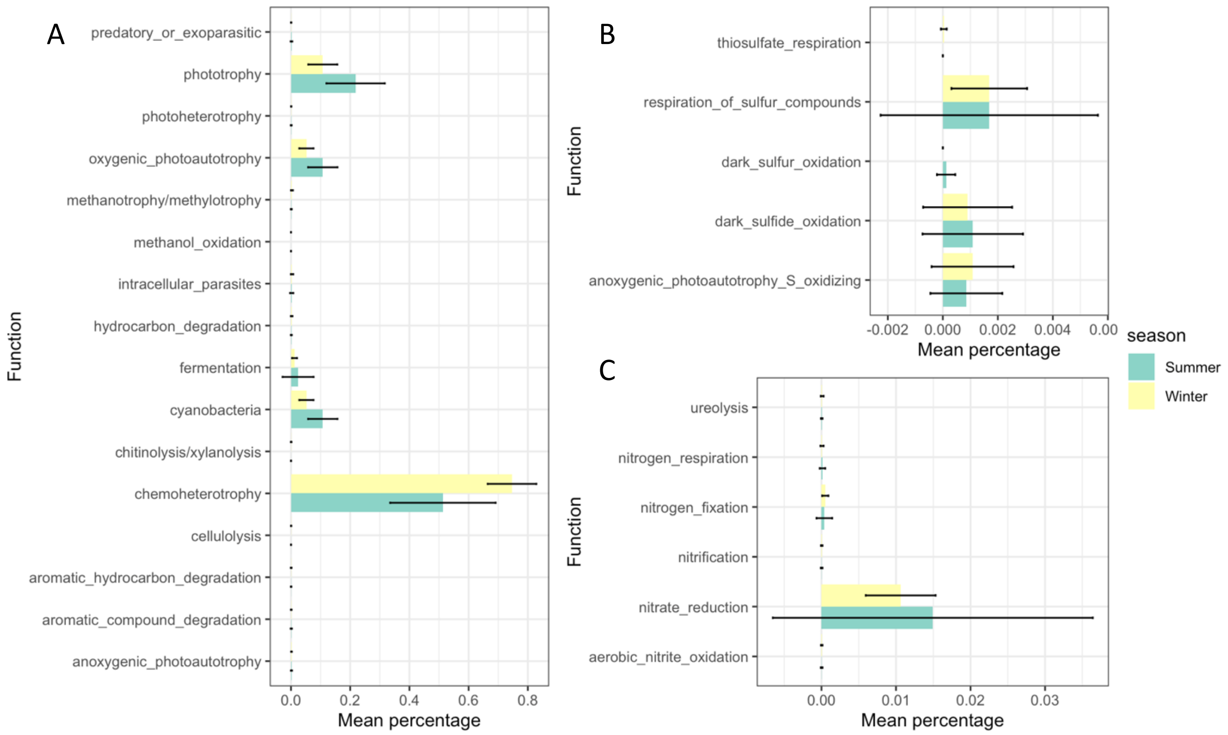

Figure S7: Functions related to Carbon (A), Sulfur (B) and Nitrogen (C) metabolism assigned by FAPROTAX. Mean percentages and standard deviations (error bars) were calculated in R for each the winter (yellow) and summer (green) sample groups.

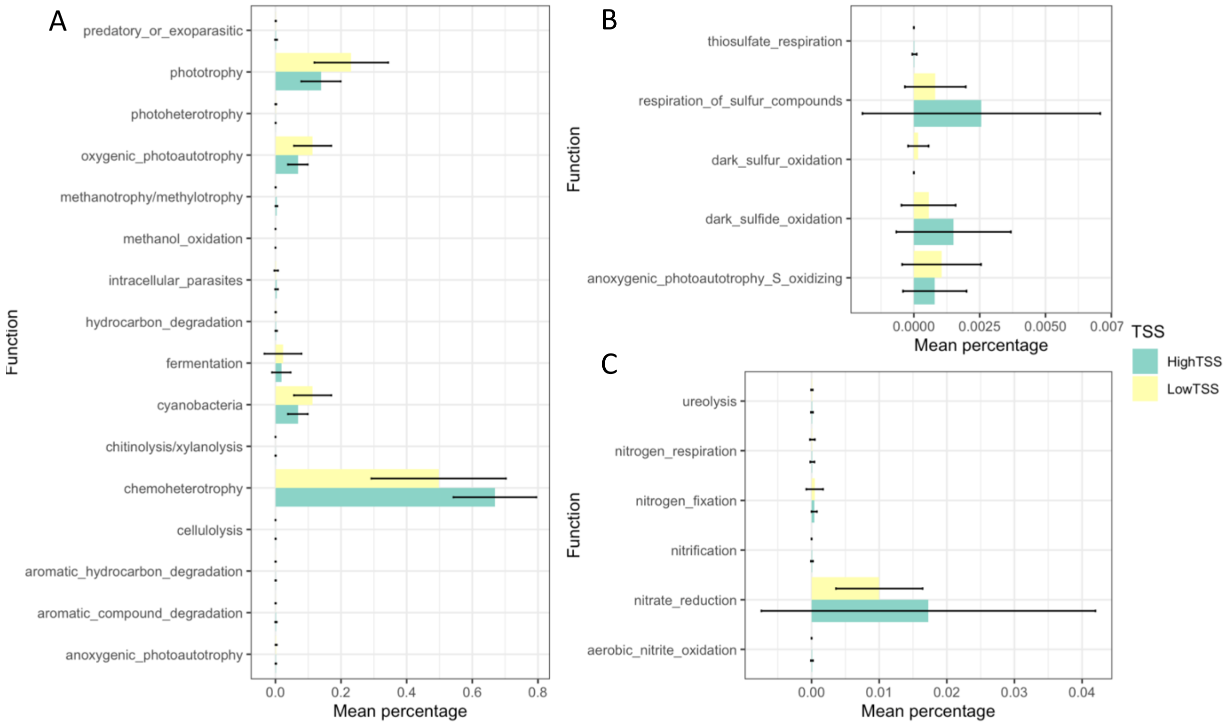

Figure S8: Functions related to Carbon (A), Sulfur (B) and Nitrogen (C) metabolism assigned by FAPROTAX. Mean percentages and standard deviations (error bars) were calculated in R for each low TSS (yellow) and high TSS (green) sample groups.

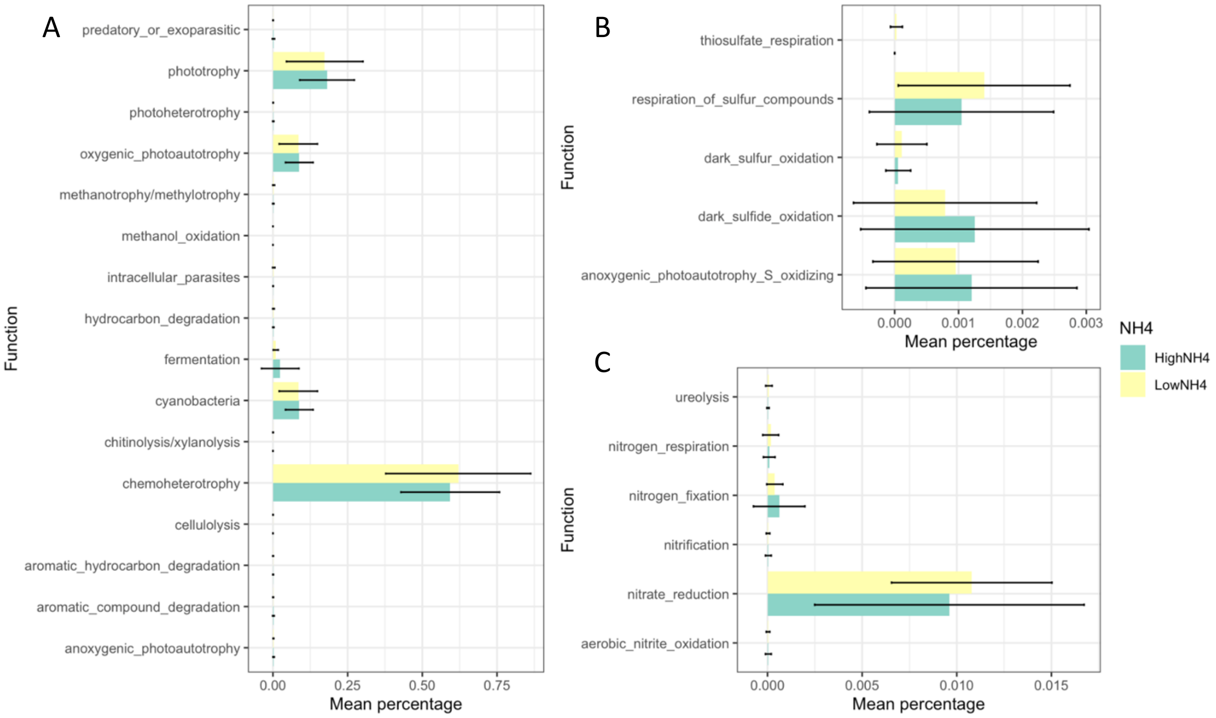

Figure S9: functions related to Carbon (A), Sulfur (B) and Nitrogen (C) metabolism assigned by FAPROTAX. Mean percentages and standard deviations (error bars) were calculated in R for each low NH4 (yellow) and high NH4 (green) sample groups.

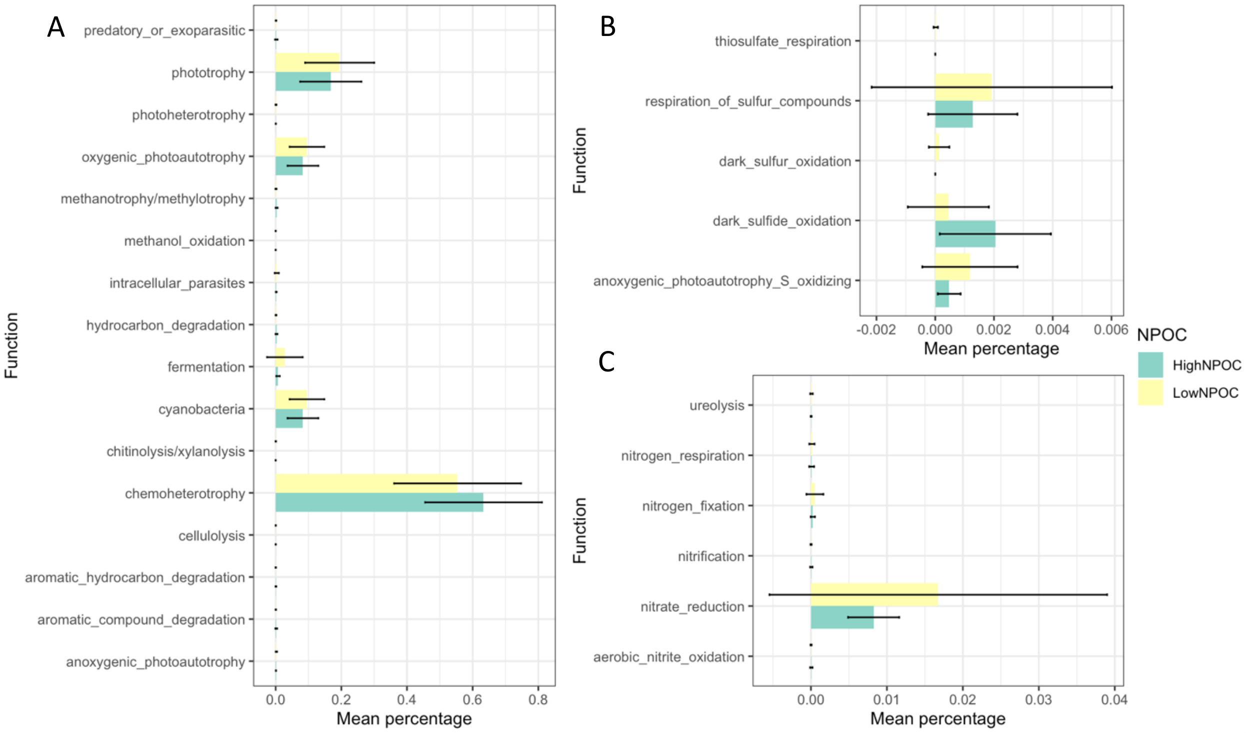

Figure S10: Functions related to Carbon (A), Sulfur (B) and Nitrogen (C) metabolism assigned by FAPROTAX. Mean percentages and standard deviations (error bars) were calculated in R for each low NPOC (yellow) and high NPOC (green) sample groups.

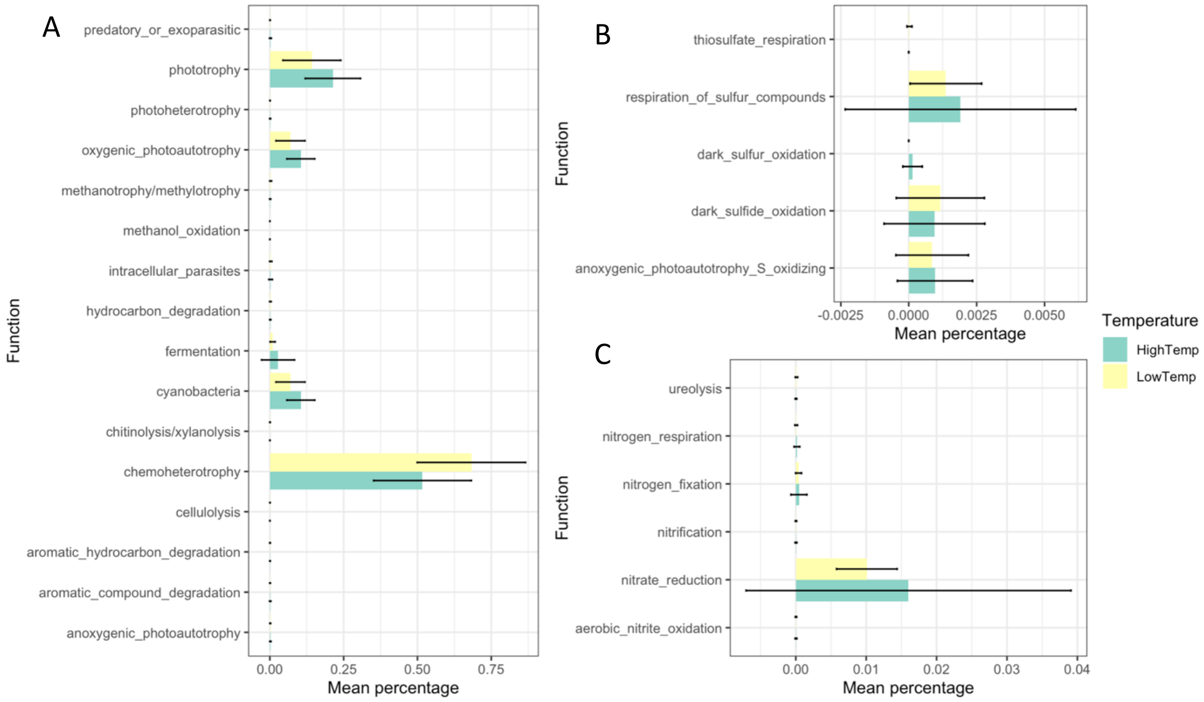

Figure S11: Functions related to Carbon (A), Sulfur (B) and Nitrogen (C) metabolism assigned by FAPROTAX. Mean percentages and standard deviations (error bars) were calculated in R for each low temperature (yellow) and high temperature (green) sample groups.