DISENTANGLING THE ROLE OF PROKARYOTES IN REGULATING EXPORT FLUX VIA SUSPENDED AND SINKING ORGANIC MATTER IN THE SOUTHERN OCEAN

A thesis submitted in fulfilment of the

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DOCTOR OF PHILOSOPHY

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at

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by

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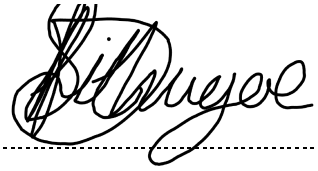
Declaration

I, **Choaro David Dithugoe**, declare that the thesis entitled "*Disentangling the role of prokaryotes in regulating export flux via suspended and sinking organic matter in the Southern Ocean*" is my own research. The thesis has not been submitted for examination to any other university other than Rhodes University, Makhanda (Grahastown), South Africa. Chapter 2 of the thesis has been submitted for publication. My contribution to the publication was the conceptual development of the study, field data collection, bioinformatic analysis and writing with input from the co-authors.



Signature

05 September 2022

Date

Preface

Data for the thesis were collected during two cruises to the Southern Ocean. The first cruise formed part of the Southern Ocean Time Series (SOTS) and was conducted southwest of Tasmania (Australia) over the period March to April 2019. The second cruise formed part of the Seasonal Cycle experiment (SCALE) along the Goodhope line between Cape Town (South Africa) and Antarctica and was conducted in winter (July-August) and spring (October-November) 2019. The main findings of the first cruise have been submitted for publication.

List of publications

1. Dithugoe, C., Bezuidt, O., Cavan, E., Froneman, W., Thomalla, S., Makhalanyane, T. Submitted. **Prokaryotes regulate particulate organic carbon export in suspended and sinking marine particle fractions.**
   Environmental Microbiome.

2. Castillo, D., Dithugoe, C., Bezuidt, O., Makhalanyane T. Submitted. Minireview: **Microbial ecology of the Southern Ocean**.
   FEMS Microbiology Ecology.

3. Castillo, D., Dithugoe, C., Magabotha, S., Mutseka, L., Mabaso, M., Fourie, C., Classen, Z., Makinde, O., Akpudo, Y., Pierneef, R., Bezuidt, O., Van Goethem, M., Makhalanyane, T. Submitted. **Single-cell genomics reveals the metabolic capacity of SAR324 members and their associated viral signatures in abyssopelagic zones.**
   Environmental Microbiology.

Abstract

The role of phytoplankton in regulating atmospheric carbon dioxide in the marine environment has been the subject of extensive research. We lack, however, comparative insights regarding the functional contributions of bacteria, archaea, fungi, and viruses (the microbiota) to organic matter export especially in understudied polar marine environments such as the Southern Ocean. This knowledge deficit is in part due to the high levels of microbial diversity which obscures efforts to study the relationship between diversity and ecosystem functions including their roles in the sequestration of carbon and nitrogen. Elucidating their precise contributions to organic matter export may be central to potential ecosystems feedbacks to global climate change. We examined several factors which may influence organic matter export to depth including net primary production, phytoplankton biomass, temperature, and prokaryotic functional capacity in the Southern Ocean. A Marine Snow Catcher was used to collect suspended and sinking material 10 metres below mixed layer depth at Southern Ocean Time Series (SOTS) in autumn (March-April) and in the Atlantic sector of the Southern Ocean in winter (July-August) and spring (October-November) 2019. The suspended and sinking material was used to determine the particulate organic carbon and nitrogen concentrations which were then used to calculate fluxes and export ratio ((e-ratio) - particulate organic carbon flux divided by net primary production). Additionally, genomic DNA was extracted from the suspended and sinking material and sequenced to obtain Shotgun metagenomic data which was employed to reconstruct metagenome assembled genome (MAGs) and their functional capacity using bioinformatic tools such as DRAM. Data from the Atlantic sector of the Southern Ocean, demonstrate that net primary production and temperature were inversely related to the e-ratio which is consistent with previous findings from the northern region of the Southern Ocean. Genomic functional capacity from SOTS suggested that *r*-strategist (organisms adapted to live in unstable environments) bacteria (e.g., *Gammaproteobacteria*) were prominent in the

suspended pool. By contrast, the sinking particle-pool appeared to be dominated by *K*-strategists (organisms adapted to stable environment). The opposite was true for the archaea. This finding (i.e., bacteria) differs from a previous study in the northern region of the Southern Ocean, showing that microbes with *K*-strategists were more abundant in the suspended fraction. *K*-strategists typically degrade sinking organic matter into suspended organic matter or dissolved organic matter reducing the organic carbon export efficiency. Furthermore, Data from the Atlantic sector of the Southern Ocean revealed that seasonal temperature changes might dictate the rate of regional prokaryotic degradation across the zones. Resulting in rapid degradation at the northerly warmer regions and slow degradation further south. The data further provide evidence of chemolithoautotrophic mechanisms, with prokaryotes harbouring key pathways, required to transform dissolved inorganic carbon into complex organic forms, including recalcitrant dissolved organic carbon. Collectively, the SOTS and Atlantic sector of the Southern Ocean data suggest that shifts in prokaryotic community structure and functional capacity may regulate (either degradation or synthesis of organic matter) carbon export to depth.

"*It always seems impossible until it's done*"

-Nelson Mandela

"*Nothing is impossible. The word itself says 'I'M POSSIBLE!'*"

-Audrey Hepburn

"*All our dreams can come true if we have the courage to pursue them*"

-Walt Disney

This thesis is dedicated to

my loving mother

Pulane Margret Dithugoe

and my late father

Pasane Joseph Selai (02 August 2000)

Acknowledgements

I would like to thank Dr. Sandy Thomalla, Prof. Thulani Makhalanyane, Prof. William Froneman and Dr. Emma Cavan for their constructive criticism throughout my PhD. Thanks to Dr. Sandy Thomalla and Dr. Pedro Monteiro for giving me the opportunity to pursue my PhD in Biological Oceanographer. I appreciate Prof. Thulani Makhalanyane's and Microbiome's group with their weekly journal clubs which helped me analysis research papers critically and being more creative with my own science work. I'm also grateful to Dr. Oliver K.I. Bezuidt mentorship regarding bioinformatics pipeline to improve my results and gain insightful information from shotgun metagenome data. I would also like to thank my SOCCO (mostly Dr. Asmita Singh and soon to be Dr. Laique Djeutchouang). I would like to extend my gratitude to acknowledge Laique Djeutchouang (University of Cape Town/CSIR) and Thomas Ryan-Keogh (CSIR) for assisting with python script to plot the sampling coordinates and Natasha Van Horsten (Stellenbosch University/CSIR) for the water mass python script. I would also like to thank Australia's Integrated Marine Observing System (IMOS) which is enabled by the National Collaborative Research Infrastructure Strategy (NCRIS). It is operated by a consortium of institutions as an unincorporated joint venture, with the University of Tasmania as Lead Agent. The MSC sampling at SOTS was made possible by to Philip Boyd (Institute for Marine and Antarctic Studies, University of Tasmania), Thomas Trull (CSIRO, Tasmania, Australia) and Mathew Bressac (Institute for Marine and Antarctic Studies, University of Tasmania) using the *RV Investigator* IN2019_V02 (GIpr08) research ship. I would also like to thank the Sothern Ocean Seasonal Experiment (SCALE) committee for organising the winter and spring 2019 cruises using *SA Agulhas II*. This work was supported through the CSIR's Southern Ocean and Carbon Climate Observatory (SOCCO) Programme (http://socco.org.za/) funded by the Department of Science and Innovation (DST/CON 0182/2017) and the CSIR's Parliamentary Grant. In addition, I would like to acknowledge funding from the National Research Foundation (NRF) Grant No: 110729.

List of Figures

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List of Tables

NB: The numbering of the tables includes the chapter, section, and figure number (e.g., 2.3-1)

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List of Abbreviations

AAZ – Antarctic Zone

ANI – Average nucleotide identity

AOA – Ammonia oxidizing archaea

BCP – Biological carbon pump

CO_2 – Carbon dioxide

DIC – Dissolved inorganic carbon

DOC – Dissolved organic carbon

e-ratio – export ratio

MAGs – Metagenome-assembled genomes

MCP – Microbial carbon pump

MLD – Mixed layer depth

MSC – Marine Snow Catcher

N_2 – Nitrogen

NCD – Non-cyanobacterial diazotrophs

NOA – Nitrite oxidizing bacteria

NOB – Nitrite oxidizing bacteria

NPP – Net primary production

OTUs – Operational taxonomic units

PF – Polar Front

PFZ – Polar Frontal Zone

POC – Particulate organic carbon

POC:PON ratio – Particulate organic carbon and particulate organic nitrogen ratio

POM – Particulate organic matter

PON – Particulate organic nitrogen

RDOC – Recalcitrant dissolved organic carbon

rDOC – Refractory dissolved organic carbon

RDOM – Recalcitrant dissolved organic matter

SACCZ – Southern Antarctic Circumpolar Current Zone

SAF – Subantarctic Front

SAZ – Sub-Antarctic Zone

SBdy – Southern Boundary

SCFA – Short chain fatty acid

SK – Sinking particle-pool

SOTS – Southern Ocean Time Series

SP – Suspended particle-pool

STF – Subtropical Front

TS – Temperature and salinity

1. Introduction

The Sub-Tropical Front (STF) located at ~40ºS forms the northern boundary of the Southern Ocean (Pollard et al 2002). Despite the Southern Ocean only accounting for ~20% of the world's ocean surface (Deppeler and Davidson 2017), it takes up ~50% of the ocean uptake of anthropogenic CO_2 and it also accounts for ~75% of the total ocean heat uptake generated by the accumulation of anthropogenic CO_2 in the atmosphere (Frölicher et al 2015). As such, the Southern Ocean is considered the most effective oceanic carbon sink as compared to other oceans (DeVries 2014, DeVries et al 2017), making it disproportionately more important when it comes to buffering the impacts of climate change. The effective Southern Ocean carbon sink is governed by two distinct pumps which are the solubility pump and the biological carbon pump. The solubility pump is driven by physicochemical processes that increase the solubility of CO_2 in cold waters. Due to the density of cold water (and the dominance of cold waters at high latitude), the Southern Ocean solubility pump facilitates effective transport of high concentrations of dissolved inorganic carbon (DIC) to the deep ocean (Hauck et al 2018). The effects of climate change on the efficiency of the solubility pump are still unclear as a deepening of the pycnocline linked to an intensification of the westerly winds from a more persistent positive Southern Annular Mode (SAM) (Hauck et al 2015), may reduce temperatures and thus enhance DIC uptake. By contrast, increased temperatures from global warming and wind driven upwelling might reduce DIC uptake and drive more outgassing (Dufour et al 2013, Lovenduski et al 2007).

1.1. Southern Ocean biological carbon pump

The biological carbon pump is the term for a suite of processes by which carbon dioxide that is fixed by phytoplankton in the euphotic zone is exported to the deep ocean (Galbraith and

Skinner 2020, Hauck et al 2015). The seasonal blooms of autotrophic phytoplankton play a key role in the biogeochemical cycling of carbon driven by photosynthesis that transforms DIC into complex organic compounds (carbohydrates, lipids, and proteins) that comprise the bulk of particulate organic matter (POM) (Biersmith and Benner 1998, Thornton 2014). A net result is the transport of carbon from surface waters that may be in equilibrium with atmospheric CO_2, to the deep ocean, thereby 'pumping' (i.e., concentrating) carbon in deeper waters (Ito and Follows 2013) and subsequently aiding the sequestration of carbon from the atmosphere (Buesseler and Boyd 2009). This biological carbon pump is the major contributor to the natural CO_2 sink accounting for ~33% of global organic carbon export from surface waters (Schlitzer 2002) in the form of sinking particles or marine snow. In addition, the biological carbon pump serves as the main food source to other trophic organisms in the marine ecosystem food web (Eppley and Peterson 1979, Hader et al 2014). Finally, the Southern Ocean's biological carbon pump influences productivity at global scales by regulating the supply of nutrients that are transported to all the major oceans around the globe (Sarmiento et al 2004, Sigman and Boyle 2000). Changes to the biological carbon pump are considered to have the potential to be one of the most important positive feedbacks on climate change (Hauck et al 2015), the other being changes to the meridional overturning circulation (DeVries et al 2017). Compounded to this is the fact that the magnitude of the biological carbon pump is predicted to change in response to global climate change, thus altering the ocean's ability to store carbon and hence atmospheric levels of CO_2 (Bopp et al 2013, Boyd et al 2016, Henson et al 2011). However, there is little agreement on the climate sensitivity of the Southern Ocean's biological carbon pump with a lack of consensus in even the direction of predicted change (Hauck et al 2015, Henson et al 2011, Steinacher et al 2010). Quantifying the strength and efficiency of the Southern Ocean biological carbon pump and its sensitivity to predicted changes in the Earth's climate is fundamental to our ability to predict long term changes in the global carbon cycle and by

extension, the impact of continued anthropogenic perturbation of atmospheric CO_2 levels (Henson et al 2011, Laufkötter et al 2017).

The Southern Ocean is considered a high-nutrient low-chlorophyll (HNLC) region driven by high macronutrient availability and summertime light levels, but ultimately constrained by seasonal changes in light and a scarce supply of the essential micronutrient, iron (Boyd 2002, Martin et al 1990). Additional controls include potential silicate limitation of diatom production towards the end of the growing season (Boyd et al 2010, Hutchins et al 2001) and top-down controls by zooplankton grazing (Le Quéré et al 2016, Mayzaud and Pakhomov 2014). The net effect of the interplay of these factors on the biological carbon pump in response to climate change is still unclear (Sarmiento et al 1998). One prediction is an increase in the prevalence of large diatoms (Boyd et al 2019) and net primary production (NPP) favouring a strengthening of biological carbon pump efficiency (Petrou et al 2016) in response to increased iron supply (Henley et al 2020) from enhanced wind driven upwelling, anthropogenic warming (Constable et al 2014) and ocean acidification (Hancock et al 2020). However, an opposing prediction is a dominance of smaller cells and a reduction in NPP resulting in a weakening of the biological carbon pump (Petrou et al 2016) in response to a shoaling of the mixed layer that may reduce the supply of macronutrients and the micronutrient iron. This in addition to a reduction in iron supply either from the loss of sea ice (as a possible iron source) or through the reduction in the bioavailability of iron (from anthropogenic warming and/or ocean acidification) (Henley et al 2020, Petrou et al 2016, Shi et al 2010). Any trajectory in the efficiency of the Southern Ocean's biological carbon pump would necessarily reflect the integrated impact of a complex suite of concurrent physical, chemical, and biological processes making it difficult to predict the sensitivity of its response to climate change (Caron and Hutchins 2013).

In general, only a small fraction of the phytoplankton derived POM reaches the deep ocean or is buried in marine sediments (Giering et al 2014, Martin et al 1987) persisting for hundreds to millions of years (Figure 1.1-1). It remains uncertain what combination of factors regulate variability in the percentage of POM that reaches the deep ocean (i.e. POM export efficiency) on regional and seasonal scales (Cavan et al 2015). Known factors include phytoplankton productivity, particle formation, particle sinking rates (aggregation, fragmentation, senescence, ballasting, grazing), chemical dissolution, prokaryotic degradation, and zooplankton grazing (Fenchel 2008, Richardson and Jackson 2007) (Figure 1.1-1). Transfer efficiency is typically calculated using the power law function described by Martin et al (1987). However, large regional and seasonal variability in the above-mentioned factors means that a single exponential function to describe the decrease in POM with depth is unsuitable for global (or even seasonal) applications and indeed, may not be suitable for the Southern Ocean (Guidi et al 2015).

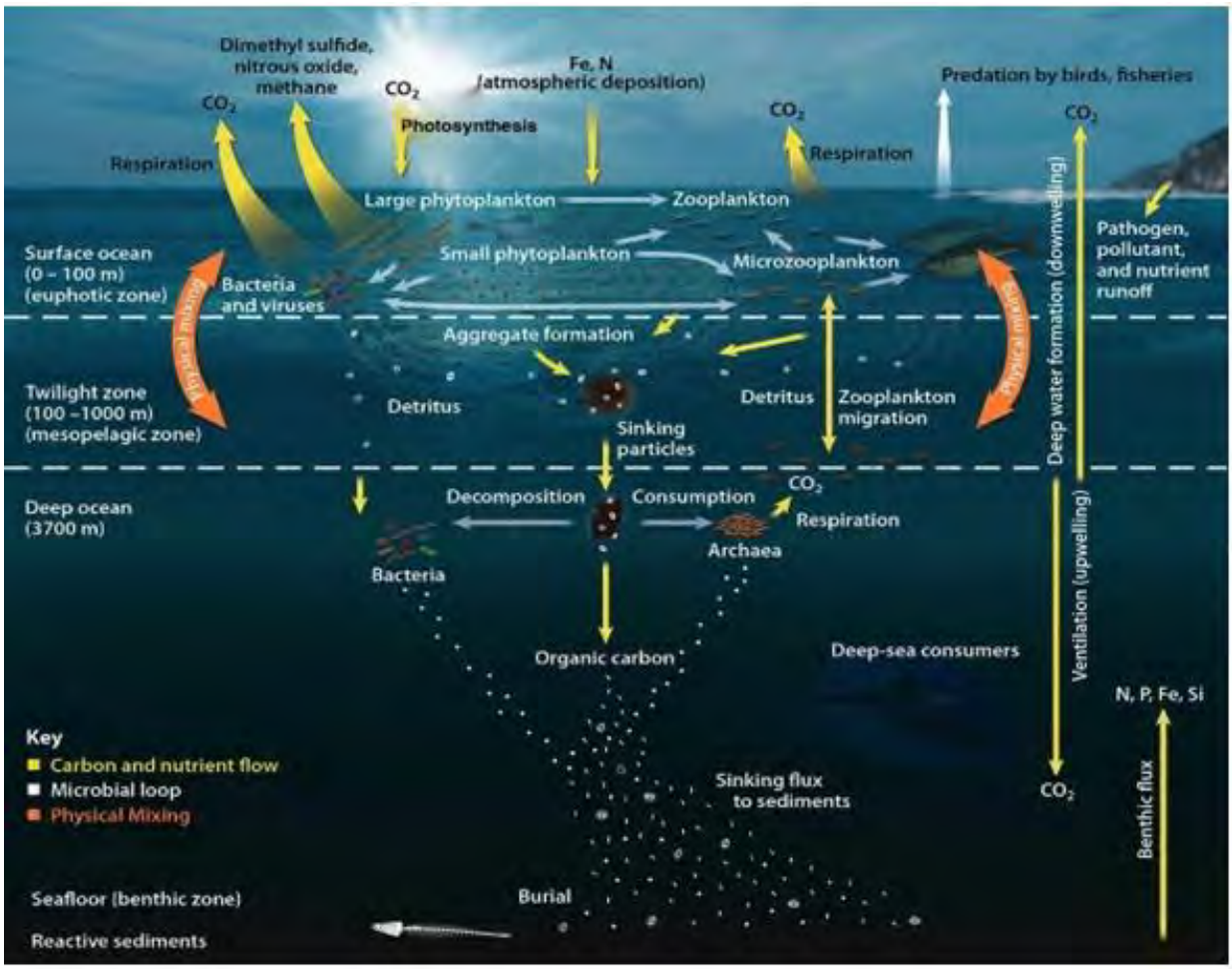

Figure 1.1-1 Detailed biological carbon pump with both physical and biological components involved. Figure and caption extracted from the Office of Biological and Environmental Research of the U.S. Department of Energy Office of Science.

Sinking POM to the deep ocean can be enhanced by zooplankton faecal pellet formation (Turner 2015) and the presence of inorganic minerals (e.g. calcite and opal) (Riley et al 2012), which increases the sinking speed of POM thereby reducing the residence time for bacterial activity and remineralisation. Zooplankton grazing repackages material into a potentially denser, larger and faster sinking form through faecal pellet formation (Liszka et al 2019). However, the export efficiency of zooplankton faecal pellets is affected by abiotic factors including temperature (Bendtsen et al 2015), seasonality (Frangoulis 2001) as well as biotic factors such as microbial colonization and degradation (Belcher et al 2016). Zooplankton faecal

pellets also get ingested (coprophagy), and are prone to fragmentation (coprorhexy), and loosening (coprochaly) (Iversen and Poulsen 2007) enhancing the carbon export (Robinson et al 2010). As such, the contribution of faecal pellets to enhance POC flux can range from very little (e.g. if small and fragile) to significant (i.e. hundreds of meters per day) if large, sturdy, and dense (Ploug et al 2010).

The presence of inorganic minerals provides ballast by increasing POM density and nucleation, while also stabilizing POM and protecting it against degradation (Iversen and Robert 2015, Le Moigne et al 2014). Ballasting in the Southern Ocean is associated primarily with silica due to the prevalent phytoplankton community known as diatoms (Buesseler and Boyd 2009, Le Moigne et al 2014) that are characterised in part by their silica frustule. Other species that contribute to mineral ballasting include coccolithophores (Balch et al 2011), which produce calcite platelets and are typically associated with the Great Calcite Belt in the region of the STF, Subantarctic Front (SAF), and Polar Front (PF) (Balch et al 2016). Coccolithophores overlap with diatoms and together these species are considered key drivers of the high uptake of CO_2 between 30° and 65° S (Balch et al 2016, Smith et al 2017).

1.2. The complex role of microbes in the Southern Ocean biological carbon pump

Marine organic material derived from photosynthesis in the surface ocean serves as substrate supporting a vast heterotrophic prokaryote population. Microbes degrade POC to DOC and simple DOC to DIC (Fenchel 2008, Wu et al 2018) and in so doing reduce the amount of POM exported to depth (Herndl and Reinthaler 2013). Although labile DOC is produced in great abundance it has a very short lifetime (hours to days), being rapidly consumed by heterotrophic prokaryotes and quickly respired back to CO_2 in support of the microbial loop (Azam et al

2016), thus limiting its contribution to the global inventory of DOC. The chemical composition and rate of production of DOM is influenced by the ambient nutrient concentrations and the community composition of the microbial food web (Carlson and Hansell 2015). The particle-associated prokaryotic community (and hence the dominant function of the microbial community) changes with depth due to taxa succession on particles (Datta et al 2016) or continuous association and dissociation of taxa from sinking particles (Duret et al 2019), which complicates efforts to disentangle their biogeochemical contribution towards organic carbon export. Other notable mechanisms that generate DOM is via the viral lysis (Suttle 2007) termed the viral shunt (Figure 1.2-1), which reflects virus mediated lysis of microorganisms that returns POM to the DOM pool and 'Sloppy feeding' by zooplankton grazers, which entails organic matter spill (from released phytoplankton cytosol) in the form of DOM (Jiao et al 2010). Although the greater majority of NPP passes through the labile DOC fraction (Mentges et al 2020), a small fraction of the labile DOM being produced escapes rapid mineralization. A fraction of the labile DOM is transformed (biotically or abiotically) to resistant material and accumulates as residual (Ogawa et al 2001), biologically recalcitrant (RDOM) or refractory DOM (rDOM) mostly from degradation of complex organic material, which contributes to the large ocean inventory of DOC via the microbial pump (Zhang et al 2018a).

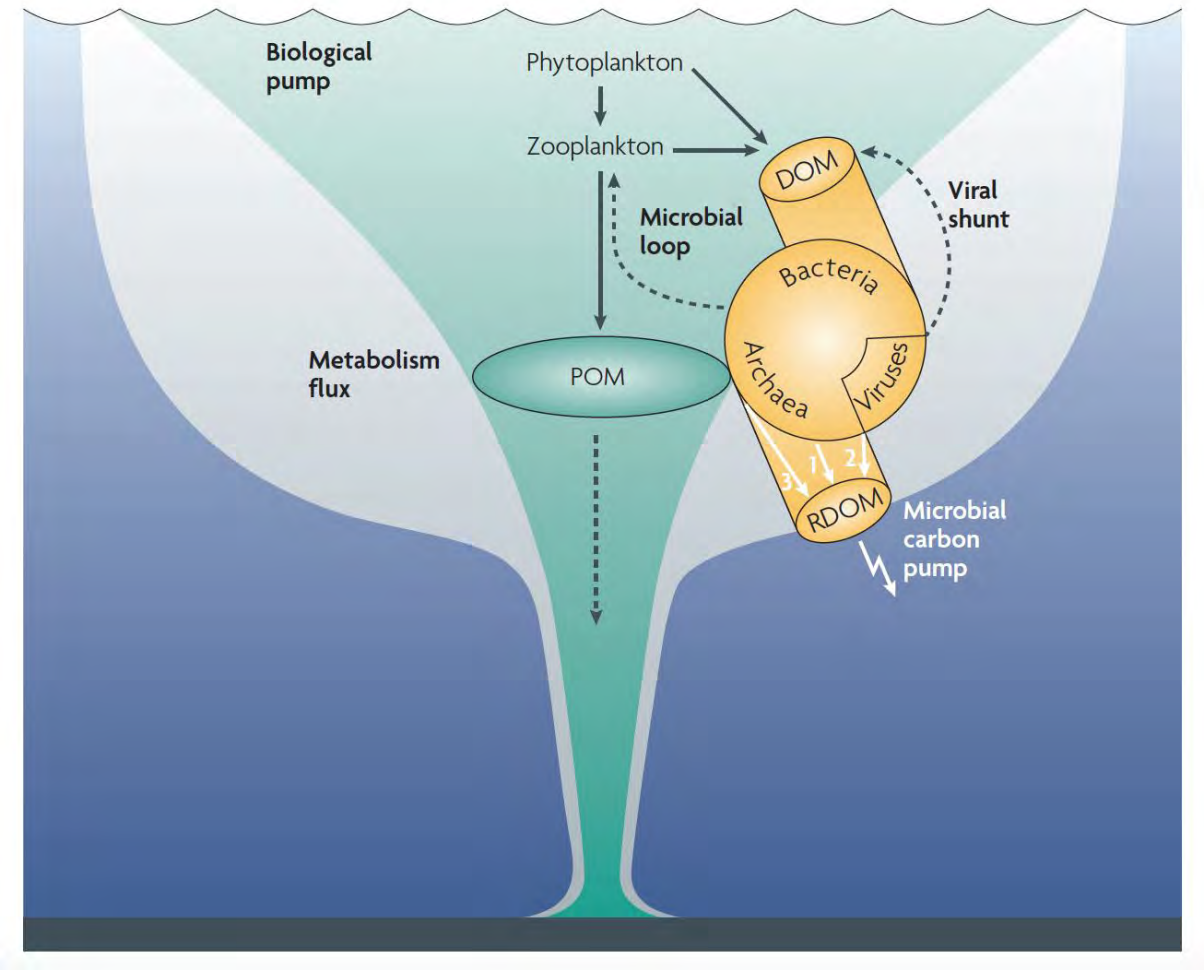

Figure 1.2-1 A simplified schematic of the four major biological processes involved in ocean carbon cycling. 1. The biological carbon pump, whereby CO_2 is fixed by phytoplankton and transported to the deep ocean via grazing and sinking of POM or DOM. 2. The microbial loop, whereby DOM is taken up by the heterotrophic microorganisms (bacteria and archaea) and transported to the grazing food web. 3. The viral shunts, whereby virus-mediated lysis returns POM to the DOM pool and 4. The microbial carbon pump, whereby microbial processes produce RDOM that persists in the ocean for millennia as a reservoir of carbon storage (Jiao et al 2014).

Advancements in molecular techniques for the detection of uncultivable microorganisms has led to an improved understanding of prokaryotic community contribution towards enhancing

POM export efficiency via the microbial carbon pump (Figure 1.2-2) (Jiao et al 2011, Jiao et al 2014, Zhang et al 2018a). Contrary to the historic understanding of the role of microbes in carbon sequestration, which emphasised the role of microbial loop, the microbial pump has more recently been shown to play a substantial role in enhancing POM export through the production of RDOM (Hach et al 2020) and rDOM (Lechtenfeld et al 2015). Prokaryotes produce RDOM by either fixing CO_2 (chemoautotrophs or chemolithotrophs) or utilising labile DOM forming complex high molecular weight (HMW) compounds such as polysaccharides and humic acids (Martin et al 2014), which can only be degraded by specific prokaryotes with extracellular peptidases (Hoarfrost and Arnosti 2017). Additionally, viral lysis of microbes (viral shunt) is another potential source of RDOM, although some of the products released by lysis are labile (Evans et al 2021, Heinrichs et al 2020, Sullivan et al 2005, Zhang et al 2020). The rDOM on the other hand, is a by-product of degraded labile DOM, semi-labile DOM and RDOM (Jiao et al 2011, Osterholz et al 2015), which is resistant to further degradation by prokaryotes (Jiao et al 2014). At the oceans' surface, rDOM can, however, be degraded by abiotic factors such as UV radiation, "Priming", temperature and other biotic factors like heterotrophic community composition and sorption/aggregation with both inorganic and organic particles (Baltar et al 2021). The Southern Ocean microbial carbon pump is very understudied, however, rDOM has been identified (Chen 2011) and the Sargasso Sea rDOM pool at the surface waters was estimated to be ~2500 years old (Bauer et al 1992). In the Southern Ocean rDOM pool accounts for ~75% of the DOM pool in the euphotic zone (Jiao et al 2010). Ocean sediments are also large reservoirs of rDOM containing prokaryote lipids membrane with kerogen (Eglinton 1994, Tegelaar et al 1989).

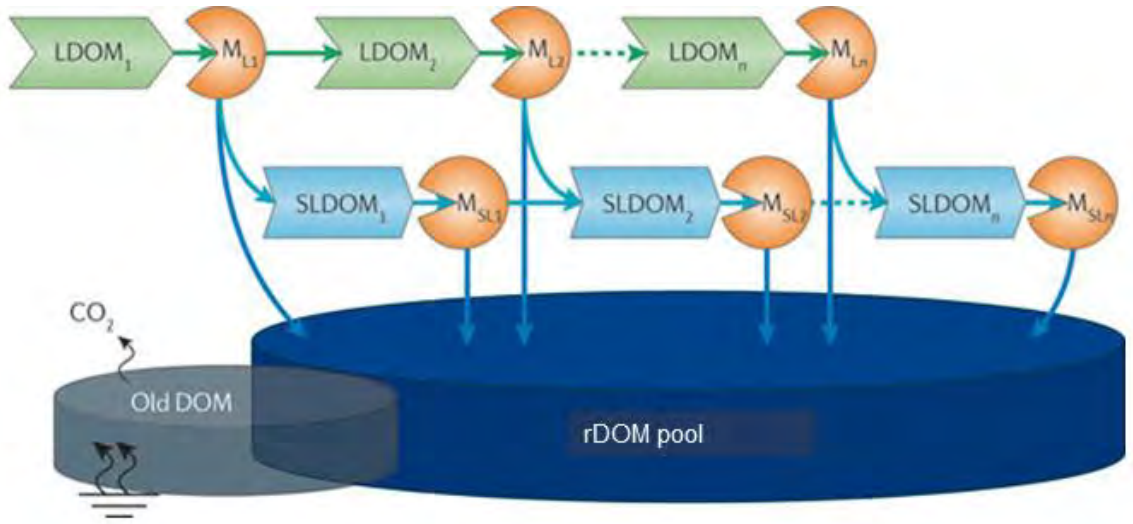

Figure 1.2-2 The overview of microbial carbon pump contribution to organic matter export. Flow of organic matter during degradation increases the rDOM pool. Microbes that utilise labile DOM (LDOM) and semi-labile DOM (SLDOM) are represented as M_L and M_{SL}, respectively. The subscripts (1, 2 or n) represent number of compounds (LDOM and SLDOM) and microbes (M) involved. The extent of recalcitrance is indicated by the colour sequence dark blue > light blue > green. The DOM pools are represented by refractory DOM in dark blue (rDOM) compared to old DOM in grey. Old DOM is from seabed seeps or hydrothermal vents, which are not 100% recalcitrant with some old compounds being readily available to microorganisms for respiration (Jiao et al 2011).

The ability of prokaryotes to utilise labile DOM and produce RDOM is improved by signal transduction systems (TCS) (Dang and Jiao 2014) such as general signal transduction (Laub and Goulian 2007), chemotaxis (Stocker et al 2008) and quorum sensing (West et al 2012). The signal transduction (Figure 1.2-3) occurs when an extracellular signalling molecule stimulates or activates cell surface receptors as receivers and these receptors result in the alteration of intracellular molecules in response to an environmental stimulus (Wuichet et al 2010). About ~95% of the bacterial genome and ~50% of the archaeal genome encodes for the two-component TCS, which enhances their adaptation in response to nutrient changes

(Galperin 2010, Laub and Goulian 2007, Wuichet et al 2010). Some chemotaxis prokaryotes also secrete repellent chemicals to alleviate competition, which is most predominant in high nutrient environments such as the Southern Ocean (Azam et al 1983, Dang and Jiao 2014). Whereas, quorum sensing prokaryotes secrete toxins, extracellular polysaccharides substrates (EPS), biosurfactants, pigments, siderophores and exoenzymes as a repellent (West et al 2012).

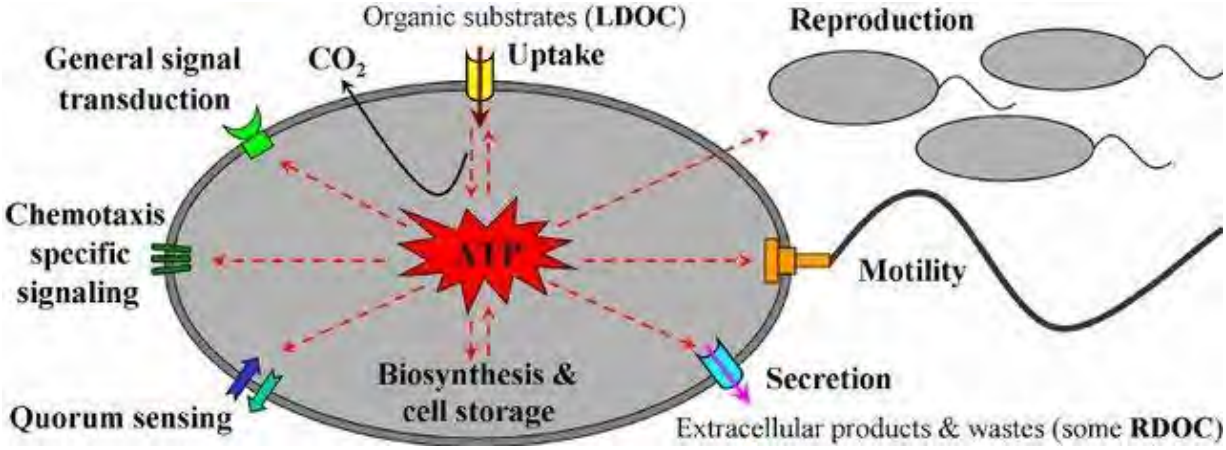

Figure 1.2-3 Prokaryotic adaptation to environmental changes due to signal transduction system (TCS) in response to environmental stimulus (Dang and Jiao 2014).

In addition to RDOM and rDOM, prokaryotes also secrete a diverse array of large complex HMW molecules including EPS and exopolymers (Kumar et al 2007). The secretion of EPS facilitates aggregation of organic debris and leads to the formation of biofilms (Dang and Lovell 2016), organic colloids, transparent exopolymer particles (TEPs) (Pannard et al 2015) and the formation of large aggregates such as marine snow (Quigg et al 2021, Turner 2015). Quorum sensing is mostly active during the formation of biofilms which is produced during high prokaryotic density to facilitate the survival of prokaryotes on sinking POM (Azam and Long 2001, Simon et al 2002). The biofilm is formed by polymerisation of repeating monosaccharide units of sugar moieties and carrier lipid-isoprenoid alcohol phosphates (Quigg et al 2021) such as glycan, peptidoglycan, teichoic acids and lipopolysaccharides, which

polymerise to form complex polysaccharides (Pereira et al 2015). Under extreme environmental (low temperatures) conditions such prokaryotes are known to stabilize their external environment by secreting EPS either as a capsule around their cells or as a larger matrix to form biofilm (Quigg et al 2021). Excessive production of EPS occurs during the later-stage of a phytoplankton bloom as an excess prokaryotic metabolic by-product that drives the release of an organic matter pool that transitions between dissolved-, colloidal- and gel-states (Decho and Gutierrez 2017). Some of these EPS are highly labile DOM, whereas others are refractory (Engel et al 2004b). These gel-like EPS accelerate the aggregation of non-sticky solid particles, thus enhancing marine snow production and the sinking of POM (Engel and Passow 2001, Passow 2002). Some of these EPS such as TEP also allow aggregation between the DOM and the POM continuum (Engel et al 2004a) including abiotic self-assembled dissolved organic colloids or precursors (Orellana and Verdugo 2003). Extracellular polymer substance and TEP production is thought to enhance the export efficiency of POM. However, the potential microbial contribution towards DOM/POM export via EPS/ TEP production is limited by the degradation and consumption of EPS by specific microbes (Arrigo et al 2008, Gardes et al 2011, Ling 2003). The main factors that contribute to the release of EPS in surface waters are; 1) an increase in microbial derived EPS due to high UV radiation (Iuculano et al 2017, Ortega-Retuerta et al 2009); 2) self-aggregation of RDOM and other organic precursors into EPS due to high turbulence (Beauvais et al 2006, Passow 2002); and 3) sinking of TEP produced by autotrophic phytoplankton and prokaryotes and (Zhou et al 1998).

The balance between the biological and microbial carbon pumps is further complicated by viruses (Evans et al 2009, Fuhrman 1999). Virus-host interactions contribute towards nutrient regeneration via the viral shunt (Figure 1.2-4) (Evans et al 2021), and they also enhance POM aggregation contributing to POM export by releasing EPS during the lytic replication cycle via

the viral shuttle (Biggs et al 2021) or by harbouring auxiliary metabolic genes (AMGs) involved in the utilisation of DOM to produce both CO_2 and rDOM (Heinrichs et al 2020, Zhao et al 2019). Indeed, about 150 Gt of DOM is considered to be released annually in the global oceans by viral lysis of their prokaryotic hosts, thus acting as a major contributor to marine DOM/POM flux (Suttle 2005). Viruses manipulate the metabolic machinery of their diverse prokaryotic hosts via AMGs that impact population dynamics, diversity and potential function in the ocean (Hurwitz and U'Ren 2016). The biogeochemical effects of viral lysis are similarly complex, on the one hand, viral lysis generates cell debris providing additional DOC substrate for other bacteria in a semi-closed trophic loop that is associated with additional respiratory losses, inorganic nutrient regeneration and reduced transport of carbon to depth (Fuhrman 1999). On the other hand, released cell contents include gel-like polymers that may facilitate the aggregation and sinking of particles from the euphotic to mesopelagic zones enhancing the transfer of carbon to depth (Breitbart 2012, Fuhrman 1999). Alternatively, viral lysis of prokaryotes within sinking aggregates may convert some sinking material into dissolved material (Proctor and Fuhrman 1991) thus reducing carbon export. Sinking particles may also coincide with increased viral production as metabolic processes of the prokaryotic host become subject to viral control (Gazitua et al 2021, Li et al 2021b, Suttle 2007).

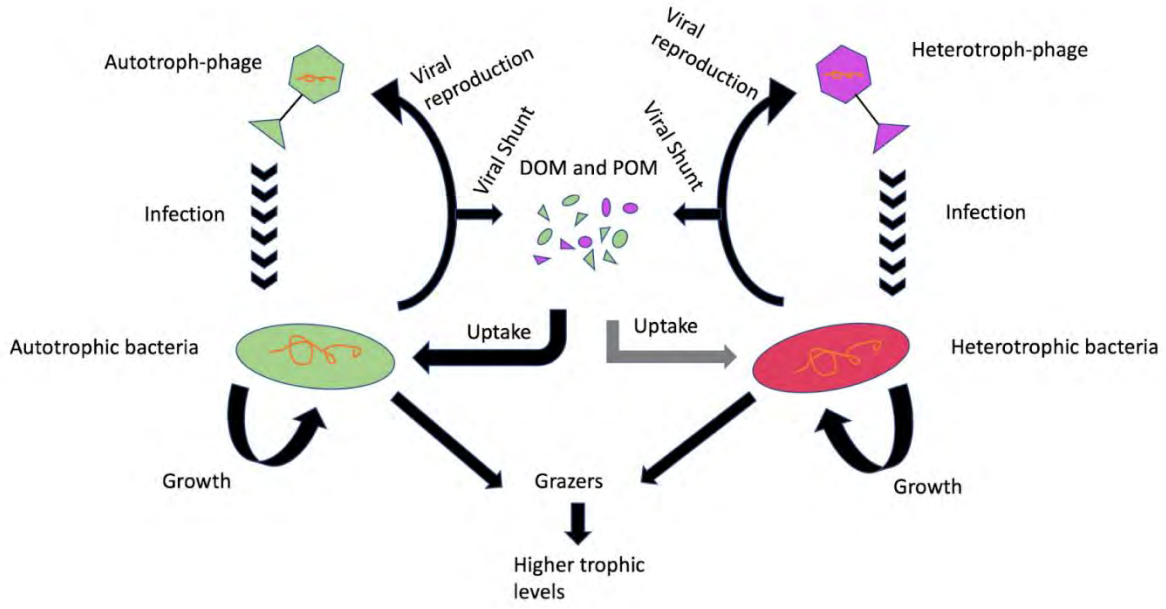

Figure 1.2-4 A simplified schematic of the viral-mediated lysis of autotrophic and heterotrophic microbes' cells releasing DOM and POM back into the ocean via the viral shunt pathway (Jover et al 2014). Lysis of the bacterial cells releases organic matter into the ocean, this organic material is both recalcitrant and labile (Weitz and Wilhelm 2012).

1.3. Connecting the biological and microbial carbon pumps

The microbial carbon pump complements and connects the concepts of the biological carbon pump, the microbial loop and the viral shunt into a more integrated understanding of the cycling of carbon (Zhang et al 2018a). The viral shunt is tightly connected by default because cell lysis transforms living POM into non-living POM and DOM with as much as 25% of carbon fixed by phytoplankton photosynthesis flowing through the viral shunt promoting respiration (Wilhelm and Suttle 1999). When the lysis products are processed by the microbial loop, the more labile DOM is metabolised, releasing inorganic nutrients and enriching the pool of less labile DOC (Zhang et al 2018a, Zhang et al 2020). The balance between the biological and microbial carbon pump is thought to vary both temporally and spatially depending on trophic

environmental conditions (Jiao et al 2010). For example, during nutrient replete conditions (with regards to both macro and micronutrients) the biological carbon pump is said to dominate carbon export, whereby large phytoplankton outcompete their smaller counterparts due to faster growth rates thus increasing the formation of fast sinking POM (Nissen et al 2018). Small phytoplankton (< 20um) species (with a larger surface area to volume ratio) tend to dominate in the oligotrophic regions of the world's oceans (Jardillier et al 2010) where nutrients are limiting and unfavourable light conditions with small, non-sinking particles favour the transfer of energy and organic matter via the microbial loop (Legendre et al 2015). In such instances (i.e., with net community respiration and decreased POC flux), the microbial carbon pump is said to be the dominant mechanism of carbon sequestration (Zhang 2016, Zhang et al 2018a). Furthermore, microbes produce DOM with high C:P and C:N ratios when nutrients are limiting, thus enhancing the microbial carbon pump with RPOC (Polimene et al 2016).

1.4. Future perspectives

The effects of climate change on the microbial community are still unclear, however increased temperature (Cavan and Boyd 2018) and ocean acidification are likely to influence microbial interactions with phytoplankton and increase degradation and remineralisation. There is a positive correlation between temperature and microbial remineralization of POC with an expected 17% decrease in POC export into the mesopelagic zone expected at the end of the century in the world's oceans (Cavan and Boyd 2018, Coelho et al 2013, Constable et al 2014). The microbial response to variability in pH is dependent on seasonal and environmental conditions with an expected increase in pathogenic microbes in warmer oceans (Karvonen et al 2010). Changes in pH also affect the biofilm formation and anticipated ocean acidification may decrease the relative abundance of some taxa (e.g., *Alphaproteobacteria*), relative to other taxa (e.g., *Flavobacteriales*) that thrive at low pH (Deppeler et al 2020, Krause et al 2012, Witt

et al 2011). On the other hand, aerobic anoxygenic phototrophic bacteria (e.g., *Cyanobacteria*) which constitute over ~10% of the microbial community in the open ocean (Jiao et al 2003) are able to adapt to ocean acidification (Brown et al 2012, Hartmann et al 2016), thereby producing organic matter.

The compounding effects of ocean warming (in response to increasing atmospheric CO_2 levels) on microbial community structure and function (i.e., community or taxa specific) is complex but intriguing and requires more experimental studies and data collection. On the one hand, warming may drive an increase in microbial activity and a shift towards a larger conversion of the fraction of fixed carbon (from phytoplankton production) into DOM and its derivative gel phases via (Lønborg et al 2020). What the consequences are of enhanced microbial activity on the microbial carbon pump are unclear, but it is possible that its relevance will become more prominent in future oceanic carbon flow.

1.5. Study aims and hypothesis

The biological carbon pump is the major transporter for POM into the ocean interior via photosynthetic phytoplankton in the global oceans. The effectiveness of the biological carbon pump is reduced by predators such as zooplankton grazing, nekton and heterotrophic microbes. On the other hand, the zooplankton and nekton community utilise POM and produce fecal pellets that might enhance the sinking POM speed while reducing its carbon content. By contrast microbes remineralise labile DOM or POM to DIC reducing the amount of organic carbon exported to depth. However, microbes also generate RDOC, which constitutes the majority of the DOC pool persisting in the ocean for 4000– 6000 years (Bauer et al 1992, Hansell 2013) and thus provide an additional path for carbon sequestration within the marine ocean carbon cycle (Stone 2010). As such, the relationship between the biological and

microbial carbon pump is complicated as both contribute towards altered DOM/POM export efficiency which is essential to carbon sequestration in the Southern Ocean. More specifically, the microbial carbon pump plays a dominant role in three distinct mechanisms; 1) the release of rDOM from extracellular enzymatic activities to degrade complex RDOM into rDOM, 2) the release of RDOM or EPS via secretory pathways and 3) the production of TCS in response to low concentrations of DOM that lower their metabolic efficiency during pre-/post-bloom conditions.

The Southern Ocean prokaryotic community associated with POM and DOM has been poorly studied (Griffiths 2010, Milici et al 2017) and as such, we lack sufficient data to understand their role (through their various trophic interactions) in the biological carbon pump, the microbial pump, the microbial loop and the viral shunt. We also lack information on the taxonomic and functional diversity of the prokaryotic and viral community and their interaction with POM and DOM. It is essential to understand the prokaryotic contribution to Southern Ocean carbon export in order to disentangle their precise ecological contribution to regional and global climate. Despite their important biogeochemical and ecological role, few studies have addressed the taxonomic and functional diversity of the prokaryotic community and their interaction with POM in the suspended and sinking particles in general and in the Southern Ocean in particular (Duret et al 2019, Huston and Deming 2002, Johnson et al 2019). Consequently, we lack fundamental insights regarding the effects of the prokaryotic community on biogeochemical processes and their influence on the fate of sinking particles (Collins et al 2015). Understanding these interactions hinges on identifying and quantifying the diversity and structure of prokaryotic communities, which underpin complex marine food webs.

This study aims to address this knowledge gap utilising a series of marine snow catcher (MSC) deployments that collect suspended and sinking material from the Southern Ocean Times Series (SOTS) in the sub-Antarctic zone (SAZ) south of Tasmania in austral autumn and along the Goodhope Line (from the SAZ to the Marginal Ice Zone (MIZ) south of Cape Town) in winter and spring 2019. The aim is to understand the microbial contribution in the suspended and sinking particle-pool and whether they contribute to POM degradation or synthesis. To achieve this aim we will analyse 1) POC and PON concentration from the suspended and sinking particle-pool from the marine snow catcher, 2) Use POC/PON to calculate the POC/PON ratio and flux and 3) extract DNA from suspended and sinking particle-pool for shotgun sequencing to get the microbial community structure and 4) Reconstruct metagenomic-assembled genomes (MAGs) from metagenomic reads as a representative of the taxon in the water column and 5) Predict the MAGs metabolic capacity in the suspended and sinking particle-pool to understand their potential contribution in the Southern Ocean. We hypothesize that the prokaryotic community (bacteria and archaea) from raw reads and MAGs are phylogenetically diverse, and MAGs potential metabolic capacity will vary according to the POC/PON content in the suspended and sinking particle-pool. Results from this research will provide fundamental insights on the role of prokaryotic activity and their contribution to organic carbon export, specifically through an assessment of prokaryotic functional capacity in sinking and suspended marine particle fractions.

2. Prokaryotes regulate particulate organic carbon export in suspended and sinking marine particle fractions

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Running title: Prokaryotes regulate particulate organic carbon export

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Abstract

In the Southern Ocean, the biological carbon pump (BCP) is driven by phytoplankton productivity and is an effective organic matter sink. There is some evidence showing that sinking particulate organic matter (POM) sustains microorganisms with different ecological strategies (i.e., *r*-/*K*-strategists). However, we lack mechanistic insights regarding the importance of these microorganisms, their diversity and influence on the efficiency of the BCP. Here, we reveal microbial contributions towards POM export in the Southern Ocean by analysing prokaryotic metabolic capacity linked to suspended and sinking marine particles. A Marine Snow Catcher (MSC) was deployed at several Sub-Antarctic Zone (SAZ) stations to obtain suspended and sinking particulate material for determining carbon and nitrogen flux. A combination of unbinned contigs and metagenome assembled genomes (MAGs) showed that both the suspended and sinking particle-pools were dominated by bacteria (*Proteobacteria* and *Bacteroidota*) with metabolic capacity for degrading POM. Archaeal genomes (*Poseidoniia* and *Nitrososphaeria*) appeared to drive nitrogen metabolism via nitrite and ammonia oxidation. *r*-Strategist bacteria were more ubiquitous in the suspended pool while the sinking particle-pool appears to be dominated by *K*-strategists and the opposite was true for archaea. In addition, metabolic reconstructions suggest that prokaryotes harbour substantial genetic capacity for degrading complex POM and chemoautotrophic synthesis of recalcitrant dissolved organic carbon (RDOC) from CO_2. Together, our data suggest that prokaryotes in suspended and sinking particles may enhance POM export via the production of RDOC in the Southern Ocean.

2.1. Introduction

The Southern Ocean plays a significant role in carbon cycling, buffering the impacts of climate change by accounting for 50% of the total oceanic uptake of CO_2 (DeVries et al 2017, Friedlingstein et al 2019). Phytoplankton primary production and carbon export to the deep ocean, i.e. the biological carbon pump (BCP) (Boyd et al 2019, Henson et al 2019) is considered a major contributor to the sink of natural CO_2 removing ~33% of the global organic carbon flux (Schlitzer 2002). However, only a small fraction of the organic carbon fixed by phytoplankton in surface waters ultimately reaches the ocean interior (Giering et al 2014, Martin et al 1987) and it is uncertain what factors control the fraction of production that is exported or how effectively this material is transferred to depth. Factors that regulate phytoplankton growth, particle formation, rates of sinking and remineralisation all modify the extent to which fixed particulate organic carbon (POC) is transformed to dissolved organic carbon (DOC) and effectively exported and hence the efficiency of the BCP (De La Rocha and Passow 2007, Talmy et al 2016).

Marine microbes in particular that associate with both sinking and suspended particles have been shown to mediate key processes linked to the BCP (Azam et al 2016, Cavan et al 2015, Manganelli et al 2009). Microbial diversity influences the composition of DOC, which includes a diverse range of molecules that can be biologically labile (e.g., amino acids and glucose) that are rapidly remineralised by microbes in the surface ocean to produce dissolved inorganic carbon (DIC) thus reducing export efficiencies (the microbial loop) (Azam et al 2016, Fenchel 2008). Alternatively, recalcitrant DOC (RDOC) (e.g., lignin and lipids) is exported to the deep ocean, and therefore longer-term storage becomes possible (Ki et al 2014, Romera-Castillo et al 2019). A small percentage of microbial DOC production is refractory (rDOC) (e.g., ~5-7% derived from glucose), which resists rapid remineralization and further degradation (Gruber et

al 2006, Koch et al 2014, Ogawa et al 2001). The rDOM produced by microbial degradation of complex organic carbon accumulates in the ocean interior accounting for >95% of the large DOC pool (Chen 2011, Jiao et al 2010) and this long-lived reservoir plays an important role in shaping global climate by sequestering CO_2 from the atmosphere. The RDOC and rDOC microbial production form part of the microbial carbon pump (MCP) which enhances export efficiencies by aiding the transfer of DOC to the deep ocean (Jiao et al 2011, Jiao et al 2014, Legendre et al 2015). Indeed, in instances where the microbial loop dominates (i.e., a system with small-celled non-sinking particles and low POC flux) the MCP is considered the prevailing mechanism for carbon sequestration (Zhang et al 2018a). Despite the intricate role of prokaryote diversity and activity on regulating both the BCP and MCP, we lack a mechanistic understanding regarding the phylogeny and function of prokaryotes linked with suspended and sinking particle pools in the ocean.

The composition of the suspended and sinking particle pool (either labile, semi-labile, semi-recalcitrant and recalcitrant) (Kharbush et al 2020) may also determine the ecological growth strategies of prokaryotes (*r*- or *K*-strategist) (Baumas et al 2021, Liu et al 2020a). Prokaryotes which grow rapidly or change in response to labile and semi-labile POM are regarded as *r*-strategists (Duret et al 2019) whereas prokaryotes which slowly degrade complex high molecular weight organic compounds such as RDOC are regarded as *K*-strategists (Wang et al 2021a). As such, a niche differentiation is expected in prokaryotic distribution based on their ability to degrade particulate organic matter (POM) (Teeling et al 2012) with r-strategists being prevalent in the sinking particle-pool where they degrade transient POC, while K-strategists are more likely to exploit the suspended particle-pool (Duret et al 2019).

Here, we present the first assessment of prokaryotic functional capacity in sinking and suspended marine particle fractions collected with a marine snow catcher (MSC) at five stations from the Southern Ocean Time Series (SOTS) site in the sub-Antarctic zone (SAZ) during austral autumn. In addition to determining carbon and nitrogen flux, we specifically elucidate carbon and nitrogen cycling metabolic pathways linked to prokaryotes with different lifestyles (*r*- and *K*-strategists) in the suspended and sinking particle fractions. By using metagenome-assembled genomes (MAGs) to link bacterial and archaeal genomes to POM sequestration we provide insights into the role of *r*-/*K*-strategists towards organic carbon export in the Southern Ocean.

2.2. Material and methods

2.2.1. Site description and cruise details

The SOTS is Australia's contribution to the international Ocean SITES global network of time series observatories (http://www.oceansites.org/). The site is located at 47ºS and 142ºE, approximately 530 km southwest of Tasmania in the Indian/Australian sector of the SAZ (Trull et al 2001). Samples were collected from five MSC stations (Figure 2.3-1A) over the course of 2 weeks between March and April 2019 aboard the RV Investigator. At each station, CTD deployments provided temperature, salinity, and fluorescence derived chlorophyll profiles. The mixed layer depth (MLD) was calculated from temperature profiles with a threshold criterion of 0.2ºC as detailed previously (de Boyer Montégut 2004).

2.2.2. Marine Snow Catcher Sample collection

The Marine Snow Catcher (MSC; locally manufactured) was deployed at five stations (Table 2.3-1) at 10 m below the MLD following the method described by Riley et al (2012). A MSC acts as a settling chamber allowing the separation of suspended (top of the MSC) and sinking (bottom of the MSC) samples after 2 hours of settling. MSC which allows suspended and sinking particles to separate by their sinking velocity (>20 m d^{-1}) which might miss those particles with low sinking rates (<20 m d^{-1}) (García-Martín et al 2021, Puigcorbe et al 2020, Riley et al 2012). After sampling, both the suspended and sinking particle-pools were homogenised and divided using a Folsom splitter for POC, particulate organic nitrogen (PON) and molecular analysis. Sub-samples for POC/PON analysis were filtered onto pre-combusted (450ºC, 12 h) glass fibre filters (47 mm diameter GF/F, Whatman) using a vacuum filtration pump at a pressure of -0.2 bar. Blanks were run for estimation of potential contribution of POC

from a plain nonfiltered filter (i.e., very small signal that may be present on a pre-combusted GFF was subtracted). The resultant filters were placed in sterile petri-dishes and oven dried overnight at 25ºC. This was followed by acid fumigation with concentrated hydrochloric acid overnight to remove inorganic carbon. Filters with samples and blank filters were then punched and folded into aluminium tin cup foils. The samples were analysed in the Department of Archaeology at the University of Cape Town on a Flash 2000 organic elemental analyser (Thermo Fisher Scientific, Waltham, MA, USA). The POC/PON concentrations for the sinking fraction were adjusted based on the assumption that suspended POC/PON was homogenous throughout the MSC before settling (i.e. the concentration of the suspended fraction was subtracted from the sinking fraction) (Riley et al 2012). The POC and PON flux was calculated by dividing the sinking mass (mg) with the MSC area (0.06 m-2). The resulting value was then divided by the settling time (0.0833 days) and multiplied by the ratio of the sampled sinking volume (0.87) modified based on the MSC design (Fontanez et al 2015). The average sinking speed of the particles was calculated by dividing MSC height (1.5 m) with settling time (0.0833 days) resulting in the sinking velocity of 18 m d^{-1} with an average settling velocity of 9 m d^{-1}.

2.2.3. Molecular analysis and sequencing

To explore the composition and function of microorganisms associated with the suspended (including free-living) and sinking particle-pools, 2 litres of water from both fractions was filtered using 0,2 µm pore-size polycarbonate membrane filters (47 mm diameter, Millipore (Burlington, Massachusetts, United States) as detailed previously (Silva et al 2016). The filters were stored at -80ºC until further processing. DNA was extracted using the Power Soil kit (QIAGEN, Hilden, Germany) as described by Hirai et al (2017). The resultant DNA was assessed using Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). High quality DNA was sequenced by Admera Health Biopharma Services (South Plainfield, USA).

Library preparation was performed using the Nextera XT DNA Library Preparation Kit (Illumina, California, USA) as recommended by the manufacturer. The library was quantified using the KAPA SYBR® FAST qPCR with Quant Studio® 5 System (Applied Biosystems). The libraries were pooled (equimolar concentrations) and sequenced using an Illumina® HiSeq (Illumina, California, USA) using the 2x150 paired end chemistry.

2.2.4. Taxonomic classification and MAG reconstruction

The quality of raw metagenomic data was assessed using FastQC (https://github.com/s-andrews/FastQC). The reads were processed to remove sequencing adapters and low-quality reads using Trimmomatic v.0.36 (Bolger et al 2014). These reads were used for taxonomic classification, using the default parameters in SingleM v0.13.2 (https://github.com/wwood/singlem). The ATLAS workflow (Kieser et al 2020) was used to assemble raw reads and for generating MAGs using the default parameter settings. Unbinned metagenomic contigs with length ≥2.5kb were assigned taxonomy using Contig Annotation Tool (CAT) with the default settings (von Meijenfeldt et al 2019). CheckM v1.1.3 was used to assess the quality of MAGs as detailed previously (Parks et al 2015). Following genome reporting standards, MAGs with genome completeness scores >50% and <10% contamination were selected for downstream analysis (Bowers et al 2017). About 24 of our MAGs were medium quality drafts with ≥50% completeness and <10% contamination. The Genome Taxonomy Database toolkit (GTDB-Tk) v1.5.0 was used to assign taxonomy to all MAGs. Phylogenetic diversity of the reconstructed MAGs was inferred against complete archaeal and bacterial genomes acquired from the NCBI RefSeq database using the FastANI v1.32 tool (Jain et al 2018). To estimate the relative abundance of each taxon, MAGs from the suspended and sinking particle-pools were mapped using default parameters in CoverM v0.6.1

(https://github.com/wwood/CoverM). Functional profiles of the unbinned metagenomic contigs and all the MAGs were obtained by using DRAM v1.2.0 (Shaffer et al 2020).

2.3. Results

2.3.1. Ancillary station data

Temperature and salinity (TS) plots for all 5 stations (Appendix I-1A) showed very similar water mass characteristics in the surface 120 m, above the MSC sampling depth (Appendix I-1). Stations 2 and 3 demonstrated slight deviations with increased salinity levels relative to temperature, but only at depths greater than 120 m. As such, any observed variability between the 5 MSC samples was unlikely the result of lateral advection but more a reflection of temporal adjustments in the system over the two-week sampling period. MLD's for all stations were similar and ranged from 100 - 110 m (Table 2.3-1). Chlorophyll profiles, at stations 1 to 3, exhibited a subsurface maximum at ~30 m indicative of surface nutrient limitation. Stations 4 and 5 were homogenous throughout the mixed layer (Appendix I-1B). MLD integrated chlorophyll was highest at stations 1 and 2 (106 and 104 mg m^{-3} , respectively) and lowest at station 4 (86 mg m^{-3}) (Appendix I-1C).

Table 2.3-1 SOTS Marine Snow Catcher sampling site from five stations with date, time, and depth. Sampling station are number based on sampling date and time.

Station	*Latitude*	*Longitude*	*Date*	*Time*	*Sampling Depth (m)*
1	-46.85	142.35	2019/03/19	19:39	110
2	-46.86	141.64	2019/03/21	21:17	100
3	-46.85	141.82	2019/03/24	02:46	100
4	-46.85	142.11	2019/03/28	21:09	110
5	-46.86	142.37	2019/03/30	20:33	110

2.3.2. Variations in POC and PON in the suspended and sinking particle-pools

Despite relatively similar water mass and chlorophyll biomass characteristics between stations, the differences in POC and PON flux and distribution between stations were substantial. The large majority of POC was observed in the suspended particle pool (84% ±11%) with only a small percentage found in the sinking pool (16% ±11%) (Figure 2.3-1B). PON levels demonstrated the opposite trend with the highest values observed in the sinking pool (68% ±14%) compared to the suspended pool (32% ±14%) (Figure 2.3-1C). The distribution of PON between stations was also different, with the highest levels (in both the suspended and sinking fractions) occurring at station 3 (87 and 29 µg l^{-1}, respectively) compared to all other stations (< 20 µg l-1) (Figure 2.3-1D), which drove the highest PON flux at station 3 (31 mg m-2 d-1) (Figure 2.3-1E). For POC, the highest concentrations for sinking pools were found at station 2 (96 µg l^{-1}), which consequently had the highest POC flux (34 mg m^{-2} d^{-1}); an order of magnitude higher than the POC flux observed at station 4 (3 mg m^{-2} d^{-1}). In contrast, suspended POC was highest at stations 2 and 3 (222 and 252 µg l^{-1}, respectively). This variability drove large differences in suspended and sinking POC:PON ratios, which were particularly high in the

suspended fraction, at stations 1 and 2 (31 and 40 POC:PON, respectively) compared to all other stations (<9) (Figure 2.3-1D). In the sinking fraction, the highest ratios were similarly found at stations 1 and 2 (2 and 6 POC:PON, respectively) compared to all other stations (<1).

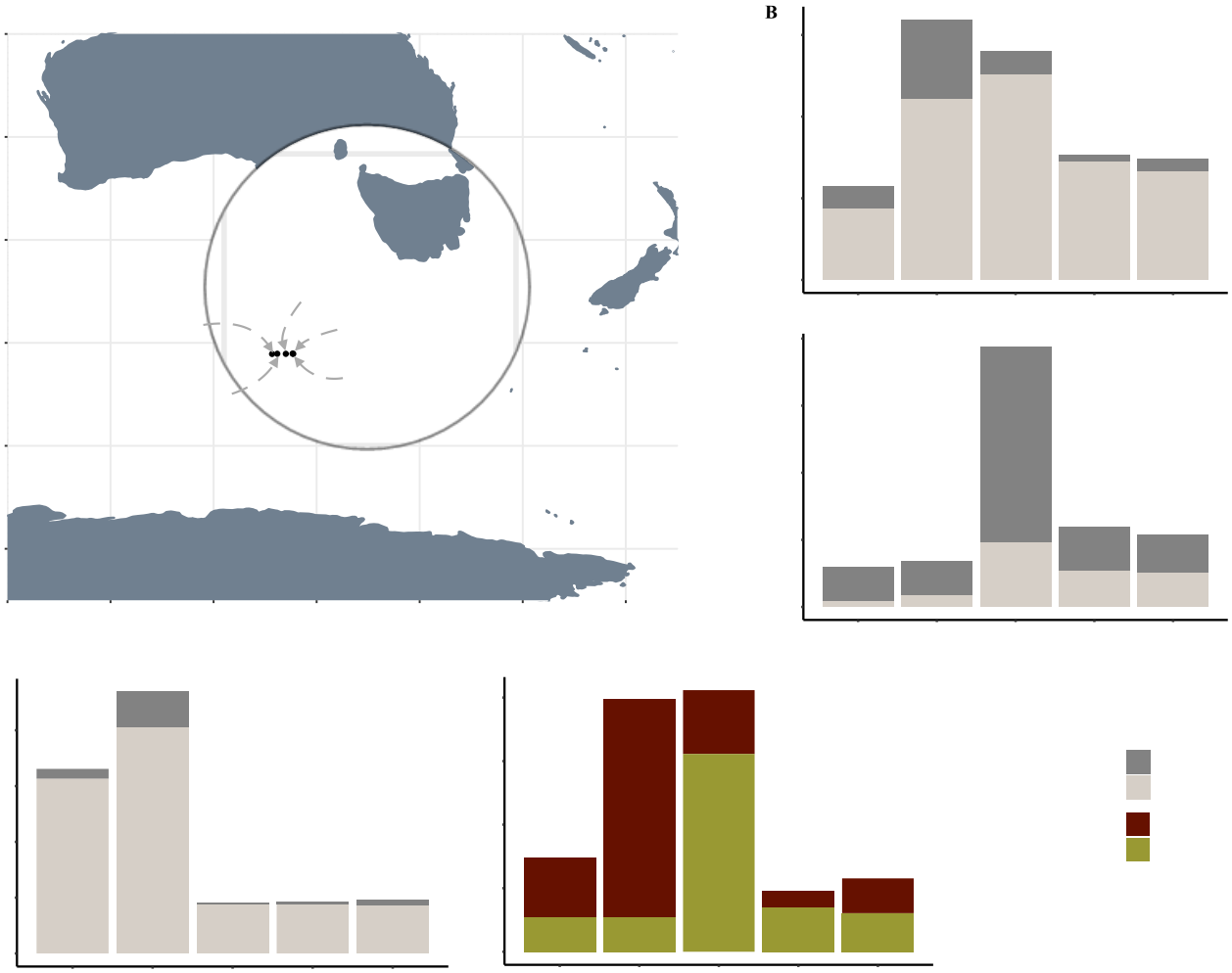


Figure 2.3-1 Marine Snow Catcher (MSC) was deployment at the Southern Ocean Time Series (SOTS) site during the IN2019_V02 using *RV investigator*. Samples for POC and PON from suspended (SP) and sinking (SK) particle-pool. A) The location of the SOTS site relative to Antarctica, Australia and New Zealand, B) POC concentration for SP (green bar) and SK (blue bar), C) PON concentration for SP and SK, D) POC:PON ratio for SP and SK and E) POC and PON export flux at five stations.

2.3.3. Taxonomic profiles using raw sequencing reads

The core shared operational taxonomic units (OTUs) by suspended and sinking particle fractions with 80% frequency cut off in both bacterial and archaeal communities. Bacterial communities shared 81.8% OTUs while ~1.1% (suspended) and ~1.9% (sinking) were unique (Figure 2.3-2A). Similarly, the archaeal community shared 89.1% OTUs while ~4.2% (suspended) and ~1.2% (sinking) were unique (Figure 2.3-2B). Classification at class level revealed that bacterial communities were dominated by *Alphaproteobacteria*, *Gammaproteobacteria* and *Bacteroidia* in all stations for both the sinking and suspended fractions (Figure 2.3-2C). Additionally, *Pelagibacteraceae* (*Alphaproteobacteria*) and *Flavobacteriaceae* (*Bacteroidia*) at family level were the most dominant taxa at all station in both suspended and sinking particle-pools (Appendix I-3A). Very little difference was observed in bacterial community distribution when comparing the suspended and sinking particle-pools across all stations at class level. However, at family level, bacterial communities were diverse with *Nitrospinaceae* (*Nitrospina*) only represented at station 1 and *Cyanobiaceae* (*Oxyphotobacteria*) only present at stations 3 to 5. Inter-station differences, at both class and family levels were more apparent in the distribution of archaeal lineages (**Figure *2.3-2***D; Appendix I-3B). The suspended particle-pool was dominated by *Methanosarcinia*, *Thermoplasmata* and *Archaeoglobi* while the sinking particle-pool was dominated by *Thermoplasmata*, *Thermoprotei* and *Methanomicrobia* (Figure 2.3-2D). Stations 1 and 2 were dominated by *Nitrosophaeria*, *Thermoplasmata* and *Methanomicrobia*. Stations 4 and 5 were also dominated by members of *Nitrosophaeria*, *Thermoplasmata* and *Methanosarcinia*. Similar to bacterial communities, very little difference was observed when comparing archaea in the suspended and sinking particle-pools at class and family level. Archaeal community composition at station 3 was the most diverse and displayed the largest difference between suspended and sinking fractions.

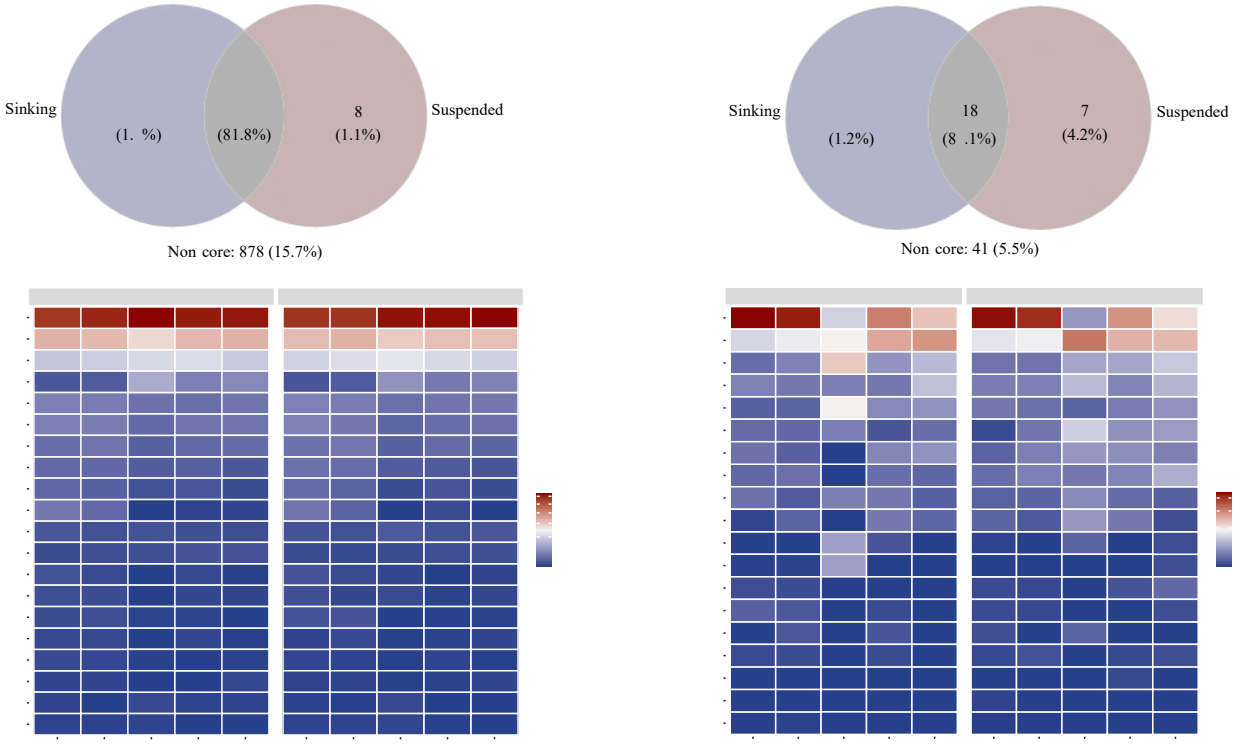


Figure 2.3-2 Taxonomic composition and distribution of the SOTS prokaryotic communities at SOTS site. The phylogenetic affiliation was determined based on single copy marker genes (ribosomal protein genes) using SingleM pipeline. A) The core taxa shared by suspended and sinking bacterial taxonomic composition represented using a Venn diagram. B) Venn diagram the percentage of core shared archaeal OTUs (based on read abundance) in suspended and sinking fractions. C) The proportion of bacteria (at class level) in suspended and sinking fractions at each station. Heatmap showing the percentage read abundances (%) of the bacterial class composition. Heatmap showing the percentage read abundances (%) of the archaeal class in the suspended and sinking at each station. For both C and D, taxa with low abundances are coloured blue while red indicates taxa present at higher relative abundances.

2.3.4. Taxonomic profiles and functional annotation of unbinned metagenomic contigs

Taxonomic assignments using unbinned metagenomic contigs suggests that bacteria were abundant in all samples. Several bacterial phyla including *Proteobacteria*, *Bacteroidota* and *Verrucmicrobia* were overrepresented at station 1, 2 and 4. By contrast, *Cyanobacteria* were dominant in both the suspended and sinking particle pools in station 3 and 5 (Appendix I-3A). These phyla were predicted harbour genes (CAZymes) with functional capacity for degrading high-molecular weight organic compounds and more CAZymes were observed in sinking particle pools (Figure 2.3-3A). Our analysis suggests that these genes use organic compounds generated through the conversion of short-chain fatty acids (SCFA), and alteration of alcohol into D isomers organic compounds or rDOC. All *Proteobacteria* were predicted to be methanotrophs, possessing metabolic capabilities that allow them to convert trimethylamine to dimethylamine in both sinking and suspended particle pools. Both *Actinobacteria* and *Nitrospinae* phyla which were among the least overrepresented taxa were predicted to not possess functional capacity to degrade high-molecular weight compounds.

On the other hand, the archaeal community harboured an abundance of *Thaumarchaeota*. The only exception was for prokaryotes from station 5, which was dominated by *Candidatus Thermoplasmatota* in both the suspended and sinking particle-pools (Appendix I-3B). Interestingly, only *Thaumarchaeota* in station 1 (including suspended and station 2 sinking particle pools) were predicted to possess functional capacity for degrading high-molecular weight compounds such as polyphenolics (Figure 2.3-3B). Additionally, in the same stations both *Thaumarchaeota* and *Candidatus Thermoplasmatota* were predicted to possess the capacity to use organic compounds by converting pyruvate into acetyl-CoA and alcohol production. Overall, both bacterial and archaeal communities were determined to be

chemoautotrophs based on the presence of metabolic genes linked to the Arnon-Buchanan, dicarboxylate-hydroxybutyrate, Wood-Ljungdahl pathway and hydroxypropionate bi-cycle (Appendix I-4).

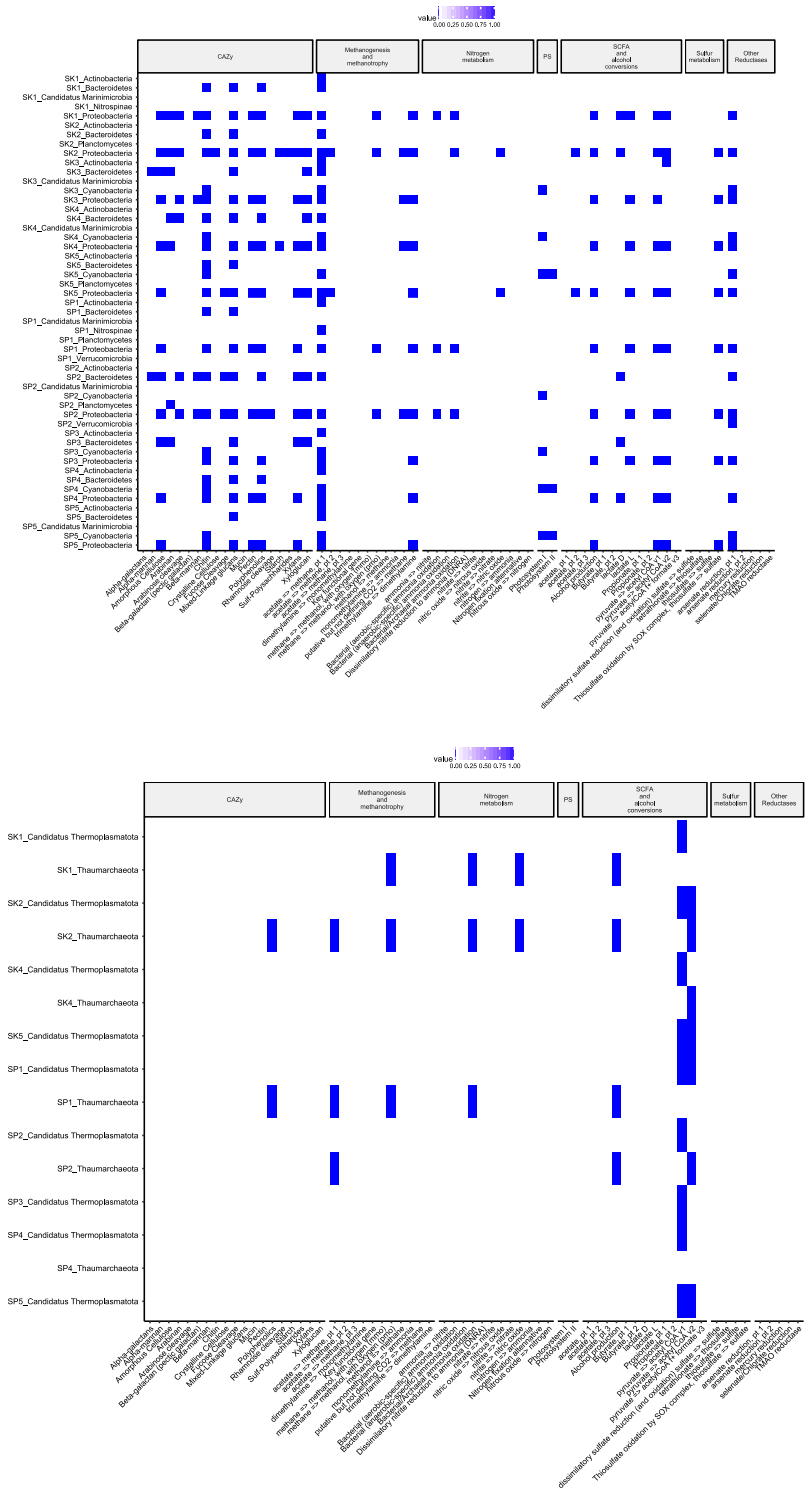


Figure 2.3-3 The functional capacity of bacterial and archaeal communities. The figure is generated using data from unbinned contigs, based on DRAM tool in both the suspended (SP) and sinking (SK) particle-pool. A) Functional annotation of bacterial (A) and (B) archaeal communities.

2.3.5. Genome reconstruction and taxonomic profiles

A total of 28 genomes were recovered, of which 24 were classified as medium quality and 4 were low quality and therefore, excluded from subsequent analysis. The medium quality MAGs included 11 from the suspended and 13 from the sinking particle-pool fractions, respectively. Taxonomic classification revealed that 5 MAGs were affiliated with *Gammaproteobacteria*. Of these MAGS, 4 were retrieved from the sinking particle pool (stations 3 - 5) and only one from metagenomes constructed from the suspended fraction (station 2). In total, 6 MAGs were affiliated with *Cyanobacteriia*, and these were distributed evenly between the sinking and suspended particle pools from stations 3, 4 and 5. The remaining MAGs were classified as *Poseidoniia* (9 in total occurring in both the suspended and sinking fractions at all stations except station 3) and *Nitrososphaeria* (4 in total occurring in both the suspended and sinking fractions at stations 1 and 2). The average nucleotide identity (ANI) scores of all bacterial and archaeal MAGs were below 90%, against 1254 bacterial (*Cyanobacteriia* and *Gammaproteobacteria*) (Figure 2.3-4A) and 4 957 archaeal (*Thermoplasmatota* and *Thaumarchaeota*) (Figure 2.3-4B) RefSeq complete genomes, respectively.

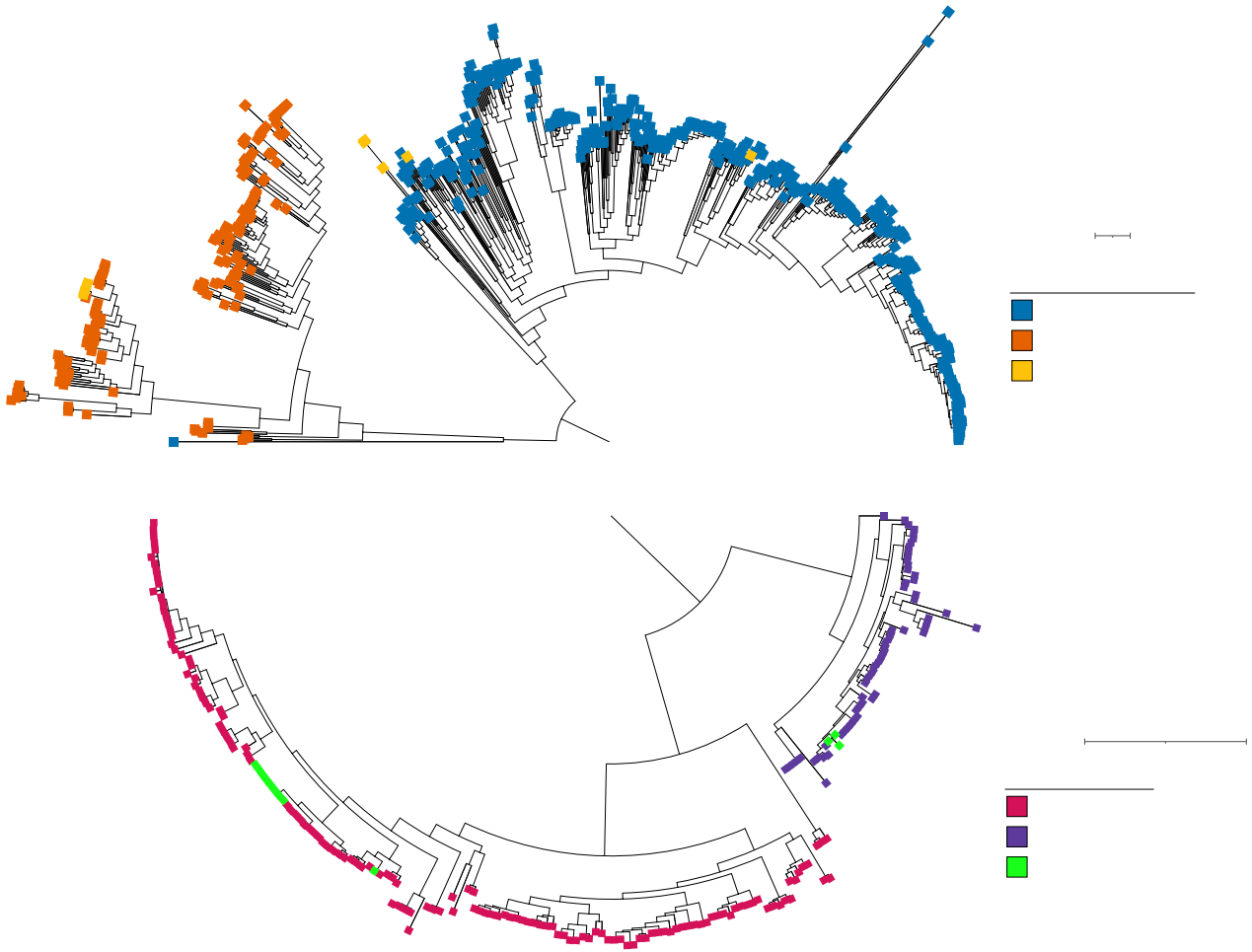


Figure 2.3-4 Phylogenomic inference showing the placement of the 24 metagenome assembled genomes (MAGs). The phylogenomic tree was constructed using an alignment based on 40% of the marker gene present in our MAGs. A) Bacterial MAGs (yellow) against *Gammaproteobacteria* (blue) and *Cyanobacteriia* (orange). B) Archaeal MAGs (green) against *Euryarchaeota* (red) and *Thaumarchaeota* (purple).

2.3.6. Read recruitment and functional profiling of metagenome assembled genomes from the suspended and sinking particle-pools

Raw reads were mapped against reconstructed MAGs using CoverM. For all the 24 MAGs, functional profiles linked to central, nitrogen and carbon metabolism were predicted based on the DRAM pipeline. Read recruitment analysis revealed that *Gammaproteobacteria* MAGs were more abundant at station 1 (suspended and sinking), 4 (suspended) and station 5 (sinking) (Appendix I-5A). Specifically, SK1_Gammaprotebacteria (*Thioglobaceae*) and SK5_Gammaprotebacteria_b (SAR86) MAGs were more abundant at station 1 (suspended), 2 (sinking and suspended) and 5 (sinking). Whereas SP2_Gammaprotebacteria (D2472) and SK5_Gammaprotebacteria_a (D2472) was relatively more abundant in the sinking particle-pool fractions. *Cyanobacterial* (*Cyanobiaceae*) MAGs were relatively abundant at station 3 and present at station 1, 2, 4 and 5. MAGs affiliated with bacterial harboured functions associated with methanotrophs. With the exception of SK5_Gammaproteobacteria_b, these bacterial MAGs also harboured CAZymes, which are involved in the degradation of chitin (Figure 2.3-5A). All MAGs, characterized as bacteria, harboured capacity to convert acetate to methane with the exception of SK1_Gammaproteobacteria (*Thioglobaceae*), which harboured a pathway for converting trimethylamine to dimethylamine. Additionally, *Gammaproteobacterial* MAGs harboured a suite of CAZymes involved in complex POM (e.g., cellulose, chitin, polyphenolics, Fucose and xylans) degradation, most notably linked to the sinking fraction of samples recovered at station 2 and 5. *Gammaproteobacterial* MAGs also harboured genes linked to short chain fatty acid (SCFA) and alcohol conversions for degradation of labile and complex POM. The SK2_Gammaprotebacteria (*Alteromonadaceae*) MAG in particular possessed most CAZymes and was the only MAG involved in nitrogen metabolism, through the use of nitrite oxidoreductases which converts nitrite to nitrite oxide. Bacterial MAGs harboured multiple central and energy metabolic pathways including

glycolysis, Krebs cycle, pentose phosphate pathway, glyoxylate cycle, Calvin cycle, Arnon-Buchanan cycle, dicarboxylate-hydroxybutyrate cycle, Wood-Ljungdahl pathway and hydroxypropionate bi-cycle (Appendix I-6A). Only *Cyanobacteriia* and SK2_*Gammaproteobacteria* possessed genes related to the Entner-Doudoroff pathways, which converts phosphorylated glucose molecules to use as carbon and energy sources.

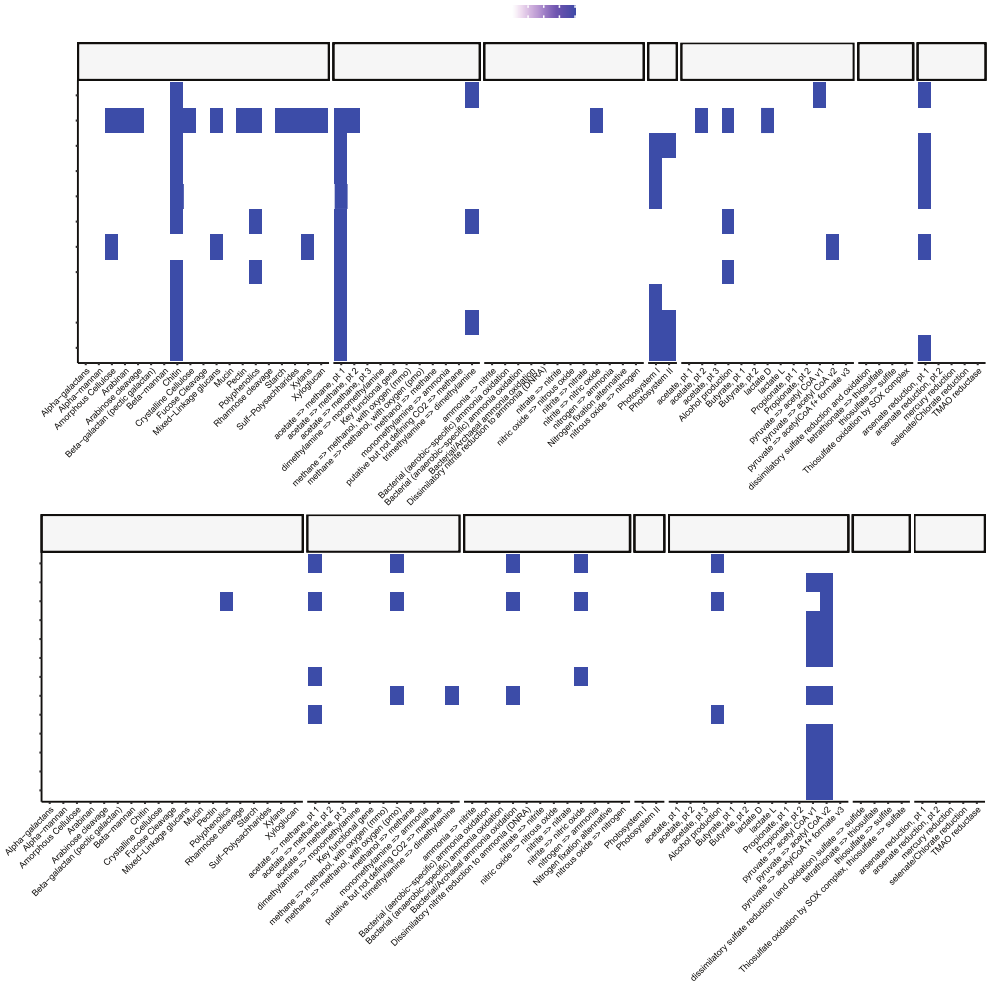


Figure 2.3-5 The figure shows the presence of potential complex metabolisms in the 24 metagenome assembled genomes (MAGs). The heatmap shows metabolisms linked to CAZymes, methanogenesis and methanotrophy, nitrogen and other pathways. The heatmap was generated using the DRAM pipeline. Functional metabolisms linked to A) bacteria and B) archaea are shown.

Poseidoniia (*Thalassoarchaeaceae*) and *Nitrososphaeria* (*Nitrosopumilaceae*) archaeal MAGs were relatively abundant across all samples with the exception of station 3 (absent in both sinking and suspended) (Appendix I-5B). These archaeal MAGs were relatively more abundant in the suspended than the sinking particle-pools at stations 1 and 2 and evenly abundant at station 3. *Nitrososphaeria* MAGs were methanogens/methanotrophs, which were reconstructed at station 1 (sinking and suspended) and station 2 (sinking). These *Nitrososphaeria* MAGs are involved in nitrogen metabolism via nitrite oxidoreductase and ammonia oxidation, and they are involved in the conversion of short chain fatty acids (SCFA) and alcohol (Figure 2.3-5B). Interestingly, a *Nitrososphaeria* MAG from the sinking fraction of station 2 possessed CAZy genes involved in the degradation of polyphenolics and involved in the SCFA and alcohol conversion pathway. Archaeal MAGs from *Poseidoniia* on the other hand are all implicated in the conversion of SCFA and alcohol. *Poseidoniia* MAG on the suspended fraction at station 1 were classified as a methanogen/methanotrophs and contained the nitrite oxidoreductase (Figure 2.3-5B). *Nitrososphaeria* and *Poseidoniia* MAGs harboured a similar central and energy metabolism as bacteria lacking the Entner-Doudoroff pathway. Functional reconstruction suggests that *Nitrososphaeria* MAGs lack the Wood-Ljungdahl pathway (Appendix I-6B). Archaeal MAGs possess methanogenesis pathways, with evidence that they may be able to convert CO_2 to methane, as part of their central metabolism.

2.4. Discussion

Particulate organic carbon (POC) and PON are considered the main resource supporting a diverse microbial community in both the suspended and sinking particle-pools (Baumas et al 2021). The suspended community comprises of free-living prokaryotes and prokaryotes attached to suspended particles and the sinking community refers to prokaryotes attached to sinking particles (i.e., gravitational sinking particles) (Baumas et al 2021). The range of activities associated with this community of microbes subsequently alters the nature of both the particulate (sinking and suspended) and dissolved pools and thus contributes significantly to the quantity of carbon or nitrogen that is effectively exported below the seasonal mixed layer. As such, an understanding of the prokaryotic community composition and their functional capacity on both sinking and suspended particles may facilitate a more mechanistic understanding of microbial contributions to POC/PON degradation and/or synthesis and ultimately to POC/PON export. This study addresses this knowledge gap by providing the first metagenome assembled genomes from suspended and sinking particle pool fractions in the Southern Ocean. The SOTS site southwest of Tasmania is a long-term time series using a set of automated moorings occupying same stations (i.e., slightly similar geographical location) to understand global climate system (Schallenberg et al 2019, Trull et al 2019, Wynn-Edwards et al 2020). We, However, lack microbial community diversity and functional capacity in response to availability of organic matter derived from phytoplankton in the SOTS. However, we acknowledge that the data presented here is limited to 24 medium quality MAGs, which constrains our ability to interpret their functional capacity within the context of POC/PON flux variability at the SOTS.

2.4.1. Differences in prokaryotes may explain the divergence in POC and PON concentration in both the sinking and suspended particle pools

Recent studies have shown a positive relationship between phytoplankton biomass and the magnitude of POM export (Dybwad et al 2021, Hung et al 2021). These findings were corroborated by our data which showed a positive relationship between MLD integrated chlorophyll and POM flux. However, this relationship was poor ($r^2 = 0.33$ for POC and $r^2 = 0.06$ for PON) suggesting that phytoplankton biomass accounts for only ~30% of carbon flux variability and as little as ~6% of nitrogen flux variability. This is perhaps unsurprising when one considers the complex interplay of the many factors that influence the concentration of POM, which is effectively exported out of the surface layer (e.g., sinking rates and prokaryotic activity etc.) (Garcia et al 2018, Moutin and Raimbault 2002). Previous studies have demonstrated that POM content (labile, semi-labile, recalcitrant, or refractory), rather than POM concentration, is the main driver of prokaryotic community structure (Hernandez-Magana et al 2021, Liu et al 2020b). There is also evidence showing that as POM sinks through the mesopelagic zone it is subjected to degradation by several free-living and particle-associated prokaryotes (Duret et al 2019), which alters the chemical and biological properties of the POM (Alldredge and Silver 1988). Prokaryotes may also in turn contribute as secondary drivers of change, by altering their community structure and/or associated activity in response to the altered POM (Jiao et al 2014, Kamalanathan et al 2020). Both suspended and sinking POM serve as the main source of carbon and energy for prokaryotes in the mesopelagic zone (Kong et al 2021, Painter et al 2017) where prokaryotes degrade polysaccharides from POM into labile or semi-labile DOM , while also producing RDOM or rDOM (Antunes et al 2020). These RDOM compounds act as ‘sticky polysaccharides’, which can contribute to the aggregation of RDOM into POM and suspended POM into sinking POM (Hwang et al 2010). Since the sinking POC flux is insufficient to support the carbon demand of prokaryotes,

suspended particles are considered a major sustaining source of organic carbon for microbes in the mesopelagic (Baltar et al 2009, Duret et al 2019). While the concentration of POC in sinking particles decreases exponentially with depth, the concomitant POC concentration in suspended particles remains largely constant and is typically ~1-2 orders of magnitude higher than that of sinking particles (Baltar et al 2010, Duret et al 2019). Our POC data support these findings with concentrations that were substantially higher in the suspended than the sinking fraction. However, the same was not true for PON with more PON concentration at stations 1-3 and similar PON concentration at stations 4 and 5 in the sinking material when compared to the suspended.

Several factors may account for the widespread variability in the POC:PON ratio observed on suspended and sinking samples in this study. These include i) the preferential degradation of nitrogen rich POM (most notably in the suspended samples at stations 1 and 2 where POC:PON ratios were >30), ii) the synthesis of refractory POC resistant to further degradation, which would also drive high POC:PON ratios, iii) chemoautotrophic microbial activity on the POM, increasing the POC:PON ratio and iv) the oxidation of sinking POC by marine microbiota, which may drive a preferential reduction in POC relative to PON, thereby decreasing POC:PON ratios as evidenced in all sinking samples where POC:PON ratios were less than the Redfield ratio of 6.6. Despite the large differences in the distribution of POC:PON ratios between suspended and sinking samples, and between stations, the bacterial community composition was very similar. This suggests that variability in POC:PON ratios may be a product of variability of microbial activity despite similarities in bacterial community structure. On the other hand, differences were observed in archaeal communities such that the PON content might have been different between stations. Alternatively, the composition of the source material may have been similar but may be acted upon differently by the bacteria and

archaea driving secondary changes in their community structure (Bacosa et al 2018, Jiao et al 2014, Manganelli et al 2009). Evidence for this argument can be seen in the relative abundance of bacterial community from phylum *Proteobacteria* (unbinned contigs and MAGs) and *Bacteroidota* (unbinned contigs), which demonstrates variability in bacterial activity between suspended and sinking pools, and between stations, despite similarities in community composition at class level. Our results suggest that differences in prokaryotic activity rather than diversity at class level, particularly in the case of bacteria, impact the signature of POC and PON in both sinking and suspended material. Nevertheless, examples of the impact of archaeal diversity on POC:PON variability (although most likely secondary) are evident when observing *Nitrososphaeria* (*Nitrosopumilaceae*), which were highest at stations 1 and 2 where the highest POC:PON ratios were encountered, together with the highest POC flux (most notably at station 2). This implies that *Nitrososphaeria* may be actively involved in PON degradation, a notion that is corroborated by the presence of nitrogen metabolism in *Nitrososphaeria* at stations 1 and 2. Conversely, station 3 had the least amount of *Nitrososphaeria* and observed a particularly high PON flux relative to all the other stations.

2.4.2. Prokaryotic ecological strategists based on POC and PON content

Prokaryotes may exhibit different ecological strategies in response to POC content (Giovannoni et al 2014, Lauro et al 2009), with degradation capacity of RDOC varying between species, functional groups, and environmental conditions (Carlson and Hansell 2015, Jiao et al 2011). Previous studies suggest that *r*-strategists may be more prevalent in sinking particle-pools where they degrade transient POM (Carreira et al 2021, Lauro et al 2009), whereas *K*-strategists appear to exploit more complex compounds (e.g. RDOM) from the suspended particle-pool (Duret et al 2019, Lauro et al 2009). Despite the limited number of studies focusing on prokaryotes from suspended and sinking particle-pools collected with a

MSC (Baumas et al 2021, Duret et al 2019), these studies nonetheless, suggest that a niche differentiation would be expected in the prokaryotic community between the sinking and suspended pools. Prokaryotes are however also known to detach from sinking particles, thereby potentially enhancing the suspended particle-pool with microbiota that are similar to the sinking particle-pool (Baumas et al 2021). Our data provides some evidence of ecological niche distinction of organic matter between bacterial and archaeal communities. The bacteria phylum from *Proteobacteria* (*Gammaproteobacteria*) MAGs and *Bacteroidota* unbinned contigs had the capacity to degrade polysaccharides derived from phytoplankton with specific CAZymes which is similar to previous findings (Unfried et al 2018). On contrary, our data suggests that the archaeal community had potential to degrade proteins, lipids and carbohydrates from POM based on our data and previous reports (Jain and Krishnan 2021). Therefore, despite the phylogenetically similarities between our MAGs they exhibit ecotypes-ecological differentiation due to its diverse functional capacity which is similar to previous studies.

Our prokaryotic MAGs and unbinned contigs supported an association with *K*-strategists due to their metabolic capacity to fix CO_2 via the Wood-Ljungdahl pathway, the Calvin cycle, Arnon-Buchanan cycle and the Hydroxypropionate-hydroxybutyrate cycle. These pathways condense two molecules of CO_2 as electron acceptor and hydrogen as electron donor into Acetyl-CoA as building blocks for biosynthesis (Hugler and Sievert 2011). In addition to fixing CO_2, chemoautotrophic prokaryotic community resemble of free-living prokaryotes which are typically in the suspended fraction (Bachmann et al 2018). Prokaryotes exhibiting these metabolic capacities are typically chemoautotrophs, which synthesise complex organic carbon such as RDOM or polysaccharides polymers from CO_2 (Kusch et al 2021). In our results, these chemoautotrophic bacteria MAGs, typical of *K*-strategists, were more prevalent in the sinking particle pool than the suspended pool, which is in contrast to previous studies (Duret et al

2019). However, it is likely that any metabolic activity which uses POM to form polymers may consequently initiate aggregation (Jiao et al 2014) and subsequently enhance POM export flux, thus accounting for their presence on sinking material. In such instances, bacterial ecological niche specificity may indeed favour *K*-strategists on sinking POM where RDOC forms part of the sinking fraction [14,72]. Our *Bacteroidota* contigs and Gammaproteobacterial MAGs were more prevalent in the sinking particle-pool which has similarly been previously reported (Boeuf et al 2019, Martinez-Garcia et al 2012). In addition to fixing CO_2, chemoautotrophic bacterial community also had the functional capacity to reduce sulfur, carbon monoxide and methane (Middelburg 2011). Also, these chemoautotrophs can produce labile organic carbon when the organic carbon content in the water column is mostly rDOM carbon thus sustaining the prokaryotic community in the ocean (Middelburg 2011, Taylor et al 2001). The chemoautotrophic bacteria response to carbon utilisation or environmental stimulus is still unclear, therefore, the fate of the POM produced by chemoautotrophic and its contribution to carbon flux is not yet explored (Middelburg 2011).

On the other hand, there was no discernible difference in the functional capacity between suspended and sinking material associated with *r*-strategists and *K*-strategist, respectively. For example, bacterial MAGs including *Gammaproteobacteria* were present in both sinking and suspended samples at station 2 and possessed CAZymes involved in the degradation of labile POM such as diatom-derived POM (Landa et al 2014), grass POM, and virus-induced POC from picocyanobacterial and polysaccharides (Zhao et al 2019), whose expected role would be to reduce carbon flux via particle degradation while sinking into the mesopelagic. Similarly, all bacterial communities from both the unbinned contigs and MAGs were associated with the degradation of chitin, regardless of their association with suspended or sinking material. Chitin is rich in both carbon and nitrogen and can be reintegrated into biomass forming polysaccharide

polymers or remineralized to enrich the water column with dissolved inorganic carbon and nitrogen (Beier and Bertilsson 2013), thus reducing both the POC and PON export flux.

Overall, our results suggest that a combination of microbial driven transition between suspended particles and the formation of aggregates (e.g. via the synthesis of sticky polysaccharides) and the dissociation of microbes from the sinking particle pool to the suspended particle pool (Baumas et al 2021) make it difficult to discern any specific bacterial preference of *r*-/*K*-strategists for one particle type over another. This is contrary to some studies which suggest specific biogeochemical roles for prokaryotes in suspended and sinking particle-pools based on the amplicon data in the marine carbon cycle (Duret et al 2019). However, taxonomic classification of prokaryotes and metabolic capacity is insufficient to alone infer ecological strategies due to the bacterial functional evolution involving gene gain and loss (Iranzo et al 2019). Gene gain and loss can occur through the deletion or insertion of genes from a genome including genomic islands via non-homologous recombination mechanisms and mobile genetic elements (Koonin and Wolf 2008). Therefore, ecological strategy of suspended and sinking associated bacterial can be elucidated via metatranscriptomics data to check the expression level of genes and determine pathway completeness.

Our chemoautotrophic archaeal MAGs from Marine Group I (e.g., *Nitrososphaeria*) specifically those associated with *Nitrosopumilaceae* were more prevalent at stations 1 and 2 (suspended only) while being relatively less abundant at stations 3 to 5 (but present in both suspended and sinking fractions). As with bacteria, chemoautotrophic archaeal MAGs are expected to be *K*-strategists dominating the suspended particle pool, which was indeed the case for our samples at stations 1 and 2. This is more in-line with predicted archaeal MAG distribution that is said to be more dominant in the sinking particle-pool as *r*-strategists that

scavenge labile POM (Li et al 2021a). Previous metagenomic studies reports have shown that the archaeal community has the metabolic capacity to colonise and degrade complex POM (Orsi et al 2015, Tully 2019)., which is typically more common in the suspended particle pool. Moreover, Marine Group II (e.g., *Poseidoniia*) associated with *Thalassoarchaeaceae* were also predicted to play a major role in PON transformations based on the protein degradation pathways recovered (Iverson et al 2012, Orsi et al 2015). These Marine Group II heterotrophs typically utilise low molecular weight PON (Alderkamp et al 2006) and were more dominant on suspended particle-pool. However, there is no direct experimental evidence (e.g., experimental or metatranscriptomics) regarding the role of archaeal in utilising PON (e.g., Protein) and their interactions with RDOC in the ocean.

Furthermore, the ammonia oxidizing archaea (AOA) MAGs were present on sinking samples at stations 1 and 2 (*Nitrososphaeria*) and the suspended sample at station 1 (*Poseidoniia*). These AOA MAGs were able to utilise labile and complex organic nitrogen as their main source of ammonia and nitrite Based on the POC:PON ratio it appears that the suspended particle-pool might be more influenced by AOA resulting in low concentrations of PON relative to POC in the suspended fraction, driving ratios in the suspended material that far exceed Redfield at all stations reaching >30 at stations 1 and 2. Recent studies have shown that Marine Group I dominate the particle-associated AOA community (Cai et al 2019) and are involved in the uptake and assimilation of ammonia (Li et al 2021a) and the release of DON via degradation of particles (Alonso-Saez et al 2012, Qin et al 2014). Surprisingly, *Cyanobacteriia* had no metabolic capacity linked to the nitrogen metabolism pathway. However, *Gammaproteobacteria* from the sinking sample at station 2 possessed the metabolic capacity for denitrification and also has the highest POC:PON ratio. Nitrogen metabolism was also more prevalent in archaeal compared to bacterial MAGs. The presence of AOA

(*Nitrososphaeria* and *Poseidoniia*), NOB (*Gammaproteobacteria*) and NOA (*Nitrososphaeria*) MAGs at station 1 (suspended and sinking) and station 2 (suspended) had the highest POC:PON ratio, the highest POC flux and the lowest PON flux. These NOB/NOA and AOA are obligatory partners where the AOA catalyse the oxidised ammonia released from PON to nitrite and NOB/NOA which is further oxidised to nitrate (Lam and Kuypers 2011). This may explain the decrease in PON export flux observed at station 1 and 2. NOB/NOA, and AOA are also key players in the removal of nitrogen from PON, increasing the POC:PON ratio at station 1 and 2, thereby increasing the POC export flux relative to PON export flux. In addition to preferential degradation of PON, archaeal MAGs may also be involved in the synthesis of RDOC, enriching the water column with organic carbon particles, increasing POC export flux relative to the PON flux (Kim et al 2021).

Although *Cyanobacteriia* (*Cyanobiaceae*) are well known photosynthetic microbes involved in nitrogen fixation, the fact that our unbinned contigs and MAGs showed no evidence for nitrogen fixation is surprising (Momper et al 2015, Wang et al 2021b). A possible reason for this may be the presence of non-cyanobacterial diazotrophs (NCD), such as dinitrogen (N_2) fixing bacteria and archaea (Moisander et al 2017). Indeed, *Gammaproteobacteria* (on the sinking sample at station 2) were the only bacterial MAG containing nitrogen metabolism, while *Nitrososphaeria* were also present at station 1 and 2. The *Poseidoniia* MAG at station 1 (suspended) also had the metabolic capacity for nitrogen metabolism. Coincidentally, these were the two stations with the highest POC:PON ratio (and highest carbon flux) indicative of preferential nitrogen uptake by the prokaryotic community. Since phytoplankton biomass can account for only ~6% of the nitrogen flux, the high PON flux observed in station 3 to 5 might be due to prokaryotic activity on PON by assimilating the available inorganic nitrogen into its biomass (Polerecky et al 2021). The dissimilation of inorganic nitrogen from PON (Mettam et

al 2019) which favours PON export is thus more likely to be a result of prokaryotic activity than phytoplankton biomass.

2.5. Conclusions

Our results suggest that differences in prokaryotic functional capacity, rather than diversity (particularly in the case of bacteria), may impact the signature of POC and PON in both the sinking and suspended material. In addition, our data indicate that PON content (or an altered chemical constituency of POM) may act as a secondary driver of change in archaeal community structure. The variability in archaeal communities and functional traits may impact the POM flux. This is particularly true for nitrogen metabolism from PON by *Nitrososphaeria,* which appears to regulate high POC flux when present as opposed to high PON flux in the absence of nitrogen metabolism. Contrary to previous studies, our data suggest that prokaryotic activity on suspended particles, whose metabolic capacity may act to form aggregates (e.g., via the synthesis of sticky polysaccharides) and their dissociation from sinking to suspended particle-pools may confound the ecological strategies of these microorganisms. On the other hand, our archaeal MAGs were consistent with the predicted dominance of *r*-strategists scavenging PON in the suspended particle-pool. The connection between AOA and NOB/NOA may contribute to the dissimilatory nitrogen metabolism from PON, thus, reducing PON flux relative to POC flux in the Southern Ocean. The relationship between phytoplankton, as primary regulators of POM content, and prokaryotes acting on POM as a secondary driver of change in POM levels complicate efforts to disentangle their precise biogeochemical function in the suspended and sinking particle-pools. However, mechanistic laboratory studies are required to generate further insights regarding phytoplankton and prokaryotes trophic interactions and their ultimate contribution to POM export flux in the ocean.

3. Metagenomic analysis reveals the key role of prokaryotes in organic matter export during pre and early blooms in the Atlantic Southern Ocean

Abstract

Seasonal and regional phytoplankton blooms drive changes in the prokaryotic community and functional capacity. However, we lack mechanistic insights regarding the interaction between phytoplankton productivity and prokaryotic activity that contributes to the observed seasonal and regionally variability in organic carbon export in the Southern Ocean. Here we evaluate the effect of these interactions on particulate organic carbon (POC) flux and its relationship to primary production (the export ratio) by obtaining suspended and sinking particulate material from Marine Snow Catcher (MSC) deployments in winter and spring across all zones of the Atlantic Southern Ocean. Our result revealed that net primary production was inversely related to the export ratio across all stations in both winter and spring. On the other hand, the metagenomic assembled genomes (MAGs) revealed that prokaryotes degrade complex POC in winter than spring, lowering the sinking POC:PON in winter. In addition, in winter the increase in the suspended POC:PON might have been favoured by chemolithoautotrophic capacity in all stations. While in spring, the sinking POC:PON ratios in the more southerly regions were favoured by allochthonous POC, zooplankton community and slow prokaryotic degradation due to cold surface waters. Our data reveal that seasonal temperature changes might dictate the rate of regional prokaryotic degradation across the zones with rapid degradation more at the northerly warmer regions (SAZ and PFZ) compared to slow degradation further south (AAZ, SBdy and SACCZ). Our data suggests that prokaryotic communities and functional capacity respond to temperature changes, and together they alter the POC:PON ratios and regulates (either reduce or enhance) POC flux or export ratio in the Southern Ocean.

3.1. Introduction

Southern Ocean phytoplankton productivity regulates the uptake of CO_2 in the marine food web accounting for up to 75% of global ocean primary and export production (Palter et al 2010, Primeau et al 2013). The Southern Ocean is mostly dominated by large diatoms (> 20 μm) with high sedimentation rates, especially in early spring/summer when light is available and nutrients (including iron) are in abundant supply (Swan et al 2016, Trull et al 2018, Wright et al 2010). These diatom communities are thought to be the main driver of both the extensive accumulation of silica and burial of particulate organic matter (POM) in the sediments and are thus considered the main contributors to organic carbon export in the Southern Ocean (Buitenhuis et al 2013, Pollard et al 2007, Poulton et al 2007, Sarthou et al 2005). Nonetheless, the composition of the Southern Ocean phytoplankton assemblage varies both seasonally and regionally. For example, coccolithophores tend to dominate the Southern Ocean in late summer north of 50ºS when light levels are high, and diatoms are typically limited by silicic acid availability following biological drawdown (Nissen et al 2018). As with the silica frustules of diatoms, coccolithophores have calcite platelets that similarly enhance their density and sinking rates making them important contributors to carbon export (Balch et al 2019, Rigual Hernández et al 2020, Saavedra-Pellitero et al 2014). In the marginal ice zone on the other hand, diatoms dominate in a high light environment in spring/summer when freshwater from melting sea ice results in mixed layers that are shallower and strongly stratified, while *Phaeocystis antarctica* dominate in late winter/early spring in lower light conditions associated with more deeply mixed waters (Eriksen et al 2018). The seasonal variability in phytoplankton community structure dictates changes in POM content (either labile, semi-labile, recalcitrant), which ultimately influences the export efficiency and the effectiveness of the biological carbon pump (BCP) in transporting carbon to depth (Arteaga et al 2020, Deppeler and Davidson 2017). As such, any climate-induced changes in the phytoplankton community structure is likely to alter

the efficiency of the BCP, with feedback to the rate of climate change (Le Quere et al 2007, Matear and Hirst 2016).

In addition to phytoplankton community structure, the rate at which organic matter is produced in the surface waters typically impacts carbon export to depth (Stukel and Ducklow 2017). This can be determined by the export ratio (e-ratio) which represents the fraction of CO_2 transformed into organic matter that is fixed through net primary production (NPP) and exported to the deep ocean (Arteaga et al 2018). In the global ocean, e-ratios typically increase with increasing NPP, however this is not representative of the Southern Ocean where e-ratios have been demonstrated to have a negative relationship with NPP (Maiti et al 2013). This is particularly true in the sub-Antarctic Zone (SAZ) and the Polar Frontal Zone (PFZ) but less so further south (Fan et al 2020). The POM produced in the surface waters by phytoplankton typically has a carbon to nitrogen ratio (POC:PON) of 6.6, referred to as the Redfield ratio (Geider and La Roche 2002, Redfield 1936, Weber and Deutsch 2010). However, when phytoplankton die and either settle through the water column as marine snow, or remain suspended (depending on their size, density and ability sink), prokaryotes degrade the POM, with a preferential degradation for carbon relative to nitrogen thereby reducing the POC:PON ratios of the POM below Redfield (Jover et al 2014). Alternatively, prokaryotes with the ability to produce recalcitrant dissolved organic carbon (RDOC) can aggregate into suspended particles (Hopkinson and Vallino 2005) with a high carbon content. Viral lysis releases vast amounts of DOM and POM containing large polymeric molecules with a high carbon content that forms microgels such as transparent polymeric particles (Suttle 2005, Suttle 2007) thereby increasing POC:PON ratios above the Redfield ratio. Despite these contribution of prokaryotes and viruses on export, there are large gaps in our understanding of the complex interplay between the role of phytoplankton (via the BCP) (Claustre et al 2021), prokaryotes (via the microbial

loop and pump) (Anderson and Ducklow 2001, Jiao et al 2010, Zhang et al 2018a) and viruses (via the viral shunt and shuttle) (Guidi et al 2016, Wilhelm and Suttle 1999) in contributing to the export efficiency of the Southern Ocean.

The fate of the organic carbon fixed by phytoplankton in the ocean is influenced by 1) prokaryotic degradation (Sala et al 2020), 2) accumulation of the suspended and dissolved pools (Arrieta et al 2015), and 3) sinking rates (Fan et al 2020). In the Southern Ocean, the phenomenon of decreasing e-ratios with increasing NPP has been largely attributed to the accumulation of suspended organic matter in surface waters which is influenced by phytoplankton community structure, zooplankton grazing and the degradation of organic matter by prokaryotes (Liu et al 2019, Takahashi et al 2011, Vedenin et al 2019). It is now recognised that seasonal phytoplankton blooms trigger a prokaryotic response in community succession and activity (Teeling et al 2012), with shifts in the prokaryotic community occurring within days to months of the seasonal blooms (Alonso-Saez and Gasol 2007, Lindh et al 2015). The prokaryotic succession is mainly in response to changes in phytoplankton derived-POM concentration and content (Liu et al 2019). Variability in the prokaryotic community succession has mostly been investigated in the Southern Ocean coastal systems (Luria et al 2016), where *Gammaproteobacteria* and *Bacteroidetes* were identified as rapid responders to the accumulation of phytoplankton biomass in spring/summer (Bunse and Pinhassi 2017, Liu et al 2020c). These taxa actively degrade sinking POM to suspended POM thus driving an accumulation of organic matter in surface waters and reducing the e-ratio despite relatively high productivity (Le Moigne et al 2016), while simultaneously adjusting the POC:PON ratios to be lower than Redfield (Cavan et al 2017b, Hach et al 2020, Huang et al 2018, Zakem and Levine 2019). Although seasonal and regional variability in the e-ratio is clearly influenced by

the prokaryote community, little is known about the complex biotic and abiotic interactions that affect their diversity and functional capacity (Sapp et al 2007).

The Southern Ocean plays an important role in global carbon cycling and the importance of prokaryotic functional contribution on organic carbon export has been acknowledged but is poorly understood (Balch et al 2011, Berube et al 2018, Christaki et al 2020, Liu et al 2020c). Primarily, we lack seasonal prokaryotic taxonomy , diversity, and functional capacity in the distinct zones of the Southern Ocean. This is necessary to understand prokaryotic trophic interactions with organic matter derived from phytoplankton in surface waters and their impact on export. The objective in this study is to improve our understanding of the regional and seasonal variability in the Southern Ocean prokaryotic diversity and functional capacity by examining the prokaryotic contribution to suspended and sinking POM relative to the variability observed in e-ratios across distinct water masses of the Southern Ocean during austral winter and spring. We hypothesis that the Southern Ocean prokaryotic community composition and functional capacity play an important role in regulating seasonal and regional export fluxes.

3.2. Material and Methods

3.2.1. Marine Snow Catcher stations and ancillary data

Sampling was carried out during two cruises aboard the *RV SA Agulhas II*. These cruises were part of the Seasonal Cycle Experiment (SCALE), conducted in winter (18 July - 12 August) and spring (12 October - 20 November) of 2019. The cruise transects the GoodHope line between Cape Town and the sea ice at 58º S (in winter) and 5º S (in spring) (Figure 3.3-1A). A total of 12 (6 in winter and 6 in spring) samples, representing suspended and sinking material, were collected by deploying a Snow Catcher (MSC) 10 metres below the mixed layer depth (MLD). CTD deployments coincided with each station and provided water column profiles of temperature and salinity throughout the water column. Niskin bottles on the CTD were sampled for Chlorophyll-a, by filtering ~500 mL of seawater through a 0.3 µm Whatman Glass Fibre filter (Maidstone, United Kingdom) at a pressure of -0.2 bar. Chlorophyll-a was extracted from the filter over 24 hours using 90% acetone at -20ºC in the dark following which it was measured using the non-acidification method (Welschmeyer 1994) on a Turner Designs Triology fluorometer (California, USA), calibrated with a Chlorophyll-a standard (Sigma Aldrich, Missouri, USA). The carbon-based productivity model (CbPM) (Behrenfeld et al 2005) was employed to calculate NPP for each station using surface Chlorophyll-a and backscatter from the CTD, and Photosynthetically Active Radiation (PAR) taken as the maximum PAR from the ship board sensor for that 24 hour period. The NPP was calculated by multiplying phytoplankton carbon biomass (mg C m^{-3}) with phytoplankton growth rate (d^{-1}) by sea surface light intensity (Einstein m^{-2}) and thick layer of depth (m). The CbPM is based on satellite estimates of the Carbon:Chlorophyll-a ratio as indicator for phytoplankton biomass (Pinkerton et al 2021). The MLD was calculated from temperature profiles with a threshold criterion of 0.2ºC (de Boyer Montégut 2004). The position of the oceanic fronts was determined using sea

surface height data from maps of absolute dynamic topography (MADT) from the CLS/AVISO product (Rio et al 2011), using monthly composites for winter (July – August) and spring (October – November) and the MADT values from Swart et al (2010).

3.2.2. Marine Snow Catcher deployment, POC sampling and analysis

The MSC was deployed at 12 stations, 10 m below the MLD following the method described by Riley et al (2012). The MSC acts as a settling chamber allowing sinking particles to settle to the bottom of the chamber over time (Riley et al 2012). MSC which allows suspended and sinking particles to separate by their sinking velocity which might miss those particles with low sinking rates (Puigcorbe et al 2020). After 2 hours on deck, a sample was collected from the suspended fraction. With the exception of water at the bottom, which was retained as the sinking fraction, the entire sample was drained from the MSC and discarded. After sample collection, both the suspended and sinking particle-pool fractions were homogenised. These samples were then separated using a Folsom splitter for POC, particulate organic nitrogen (PON) and metagenomic analyses. Sub-samples for POC/PON analysis were filtered onto pre-combusted (450ºC, 12 h) glass fibre filters (25 mm diameter GF/F, Whatman (Maidstone, United Kingdom)) using a vacuum filtration pump at a pressure of -0.2 bar. Filters were placed in sterile petri-dishes and oven dried overnight at 25ºC. This was followed by acid fumigation with 30-36% concentrated hydrochloric acid overnight to remove inorganic carbon. Filters were then punched and folded into aluminium tin cup foils that were analysed in the Archaeology department at the University of Cape Town. The filters were combusted in a Flash 2000 Organic Elemental Analyser (Thermo Scientific, Bremen, Germany) and the gases passed to a Delta V Plus isotope ratio mass spectrometer (IRMS) (Thermo Scientific, Bremen, Germany) via a Conflo IV (Thermo Scientific, Bremen, Germany) gas control unit. The concentrations of the POC/PON in the sinking fractions were adjusted based on the assumption

that the POC/PON in the suspended fraction was homogenous throughout the MSC before settling detailed in Riley et al (2012). As such, the concentration of the suspended particle-pool was subtracted from the sinking particle-pool to derive the sinking fraction values and the results were expressed as micrograms per litre (μg l^{-1}) (Riley et al 2012). The POC and PON flux was calculated by dividing the sinking mass (mg) with the MSC area (0.06 m^{-2}), dividing the output by the settling time (0.083 days) and multiplying by the ratio of the sampled sinking volume (0.87). The average sinking speed of the particles was calculated by dividing MSC height (1.5 m) with settling time (0.083 days) resulting in the sinking velocity of 18 m d^{-1} with an average settling velocity of 9 m d^{-1}. The export efficiency (e-ratio) was calculated as POC flux divide by NPP to give a value between 0 to 1 (Ducklow et al 2008).

3.2.3. DNA extraction and shotgun sequencing

To determine the composition of bacteria and archaea associated with the suspended and sinking particle-pools, respectively, 2L of MSC seawater was filtered onto a 0,2 μm pore-size polycarbonate membrane filter (47 mm diameter, Millipore (Burlington, Massachusetts, United States)) at vacuum pressure of -0.2 bar. The filters were then placed in sterile petri dishes and stored at -80ºC until further processing in the laboratory. DNA was extracted using a protocol described by Hirai et al (2017). The quantity and quality of the resultant DNA was assessed using Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Fifteen microliters of extracted DNA were aliquoted into sterile centrifuged tubes and submitted to Admera Health Biopharma services (South Plainfield, USA) for library preparation and shotgun metagenomic sequencing. At Admera, the DNA was quantified using a Qubit 2.0 DNA HS Assay (ThermoFisher, Massachusetts, USA). Library preparation was performed using the NexteraXT library kit (Illumina, California, USA) per manufacturer's recommendations. The final library quantity was measured using a KAPA SYBR® FAST qPCR with QuantStudio ®

5 System (Applied Biosystems, California, USA) and the quality of the library was evaluated by TapeStation HSD1000 ScreenTape (Agilent Technologies, CA, USA). Equimolar pooling of libraries was performed based on QC values and sequenced on an Illumina HiSeq (Illumina, California, USA) with a read length configuration of 150 PE for 20 M PE reads (10M in each direction) per sample.

3.2.4. Taxonomy classification and MAG reconstruction

Raw reads received from the sequencing provider were assessed for quality using FastQC (https://github.com/s-andrews/FastQC). These were then processed for quality trimming using Trimmomatic v.0.36 (Bolger et al 2014) to remove sequence adapters and low-quality reads. The quality filtered reads were subsequently assessed for taxonomic classification using SingleM v0.13.2 (https://github.com/wwood/singlem) default parameters. Raw reads were further used for metagenome assembly and binning of MAGs using the ATLAS v2.8 workflow (Kieser et al 2020) default parameters. The quality of the MAGs was determined using CheckM v1.1.3 (Parks et al 2015). Following genome reporting standards (Bowers et al 2017), MAGs with at least >50% completeness and <10% contamination were selected for downstream analysis. The Genome Taxonomy Database toolkit (GTDB-Tk) v1.5.0) (Chaumeil et al 2019) was used to assign taxonomy to all MAGs. Phylogenetic diversity of the winter reconstructed MAGs were inferred against spring reconstructed MAGs using GToTree v1.6.12 (Lee 2019). Functional profiles for all MAGs in winter and spring were obtained using DRAM v1.2.0 (Shaffer et al 2020).

3.3. Results

3.3.1. MSC stations ancillary data

The mean (winter/spring) position of the oceanic fronts were calculated as follows: the Subtropical Front (STF) = 40ºS; the sub-Antarctic Front (SAF) = 44ºS; the Polar Front = 49ºS and the Southern Boundary (SBdy) = 56ºS. These fronts delineated the following zones: the Sub-Antarctic Zone (SAZ), Polar Frontal Zone (PFZ), Antarctic Zone (AAZ) and Southern Antarctic Circumpolar Current Zone (SACCZ) (Figure 3.3-1A). The MSC was deployed within each of the different zones and at the SBdy in both winter and spring. The winter and spring stations were at similar locations except within the SACCZ zone where stations SACCZ_3 and SACCZ_4 was further south in spring due to the retreating ice. POC and PON results for the SACCZ are reported as an average of the two stations within this zone.

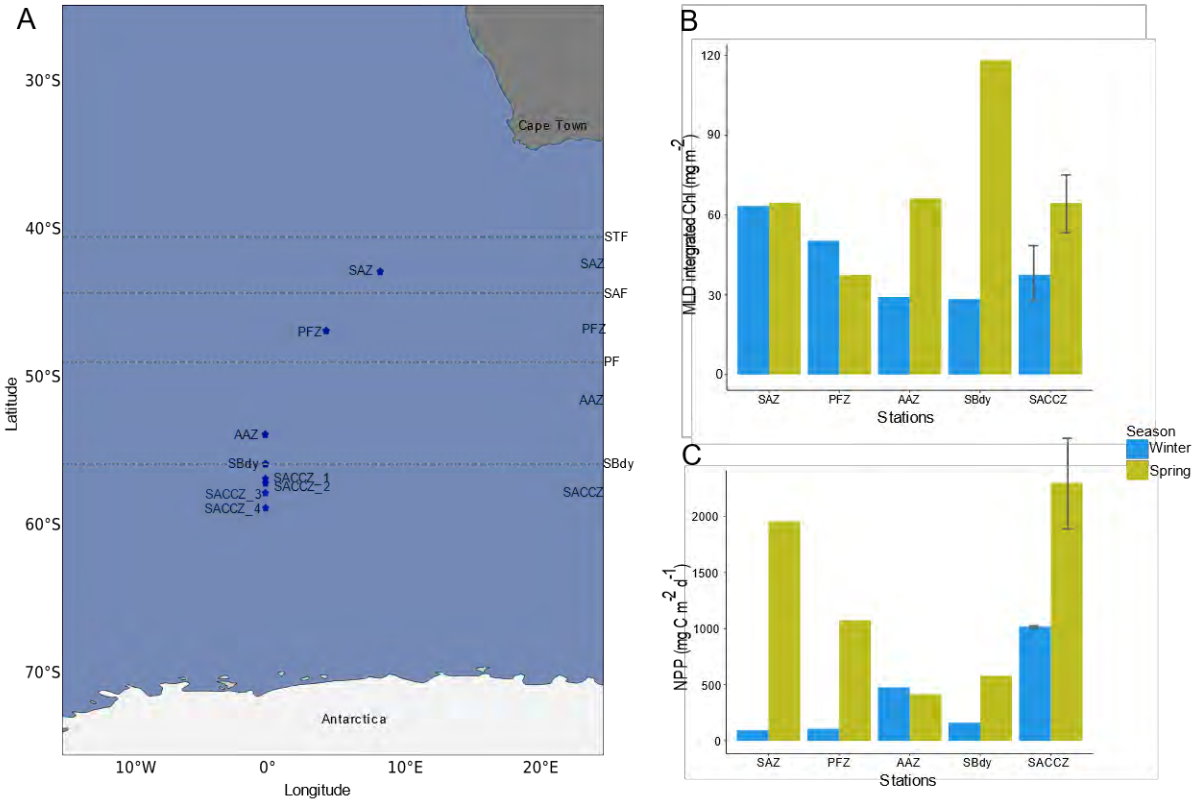


Figure 3.3-1 A) The marine snow catcher (MSC) deployment locations in winter (w) and spring (s) in the different frontal zones as determined by the position of the sub-Tropical Font (STF), sub-Antarctic Font (SAF), Polar Font (PF) and Southern boundary (SBdy). The zones from north to south are the sub-Antarctic zone (SAZ), Polar frontal zone (PFZ), Antarctic Zone (AAZ), SBdy and the sub-Antarctic circumpolar current zone (SACCZ). Ancillary station data derived from coincident CTD profiles in winter (blue) and spring (green) of B) Mixed-layer depth (MLD) integrated chlorophyll-a and C) net primary production (NPP). The SACCZ were combined in winter (SACCZ_1 and SACCZ_2) and spring (SACCZ_3 and SACCZ_4) as one stations, and the standard deviation presented in Table 3.3-1.

Mean temperature within the MLD (determined from CTD profiles) were higher in spring than winter at the northernmost regions of the SAZ (11.05ºC vs 8.65ºC) and PFZ (5.45ºC vs 5.08ºC) (**Table 3.3-1**). There was very little difference in temperatures (<0.5ºC) for the more southerly regions of the AAZ (-0.22ºC vs -0.62ºC), SBdy (-1.32ºC vs -1.70ºC) and SACCZ (-1.55ºC vs -1.74ºC), which were actually slightly colder in spring than winter. MLD integrated Chlorophyll-a concentrations, for all stations combined, showed lower average concentrations in winter (34.80 ±18,69 mg m^{-2}) compared with spring (58.54 ±33.45 mg m^{-2}). However, MLD integrated Chlorophyll-a concentrations were similar for both seasons in the SAZ (64.61 mg m^{-2}) and were higher in the PFZ in winter (50.34 mg m^{-2}) compared to spring (37.50 mg m^{-2}) (Figure 3.3-1B). MLD integrated Chlorophyll-a concentrations tended to decrease, with increasing latitude in winter, but were much more variable in spring. The highest MLD integrated Chlorophyll-a concentrations were found at the SBdy (118.29 mg m^{-2}), lowest at the PFZ (37.50 mg m^{-2}) and were similar for the remaining three regions, ranging from 65.53 to 66.30 mg m^{-2}. For all stations combined, the average NPP was >3 times higher in spring (1053.70 ±561.76 mg C m^{-2} d^{-1}) when compared to winter (308.97 ±208.26 mg C m^{-2} d^{-1}) (Figure 3.3-1C), but this was not reflected in the AAZ, which showed slightly higher NPP in winter (475.86 mg C m^{-2} d^{-1}) when compared to spring (415.63 mg C m^{-2} d^{-1}). NPP did not reflect seasonal or regional patterns in MLD integrated Chlorophyll-a concentrations, particularly during the spring season, which instead showed the highest values of NPP in the SACCZ (2297.45 mg C m^{-2} d^{-1}) and the SAZ (1954.49 mg C m^{-2} d^{-1}) and lowest NPP in the AAZ (415.63 mg C m^{-2} d^{-1}) and at the SBdy (580. 98 mg C m^{-2} d^{-1}).

Table 3.3-1. The MSC station names, ancillary data together with the organic matter concentrations/ratios and fluxes/export ratio (e-ratio) in winter and spring. The chlorophyll-a concentration and surface temperature were determined by mixed layer depth (MLD) integration and mean, respectively. The asterisk (*) indicate that the SACCZ stations were combined with standard deviation provided.

Station	*Season*	*Depth (m)*	*Latitude*	*Longitude*	*MLD integrated chlorophyll-a (mg m^{-2})*	*NPP (mg C m^{-2} d^{-1})*	*Suspended POC:PON*	*Sinking POC:PON*	*POC Flux (mg m^{-2} d^{-1})*	*PON flux (mg m^{-2} d^{-1})*	*Mean MLD Temperature (^{o}C)*	*e-ratio*
SAZ	Spring	110	-43.00	8.51	64.61	1954.49	15.30	4.19	4.53	1.32	11.05	0.002
PFZ	Spring	50	-47.00	4.50	37.50	1073.68	6.46	7.56	6.16	0.10	5.16	0.006
AAZ	Spring	110	-54.01	-0.01	66.30	415.63	6.12	73.02	10.11	2.41	-0.61	0.024
SBdy	Spring	80	-56.00	0.03	118.29	580.98	4.65	21.54	18.83	0.87	-1.70	0.032
SACCZ*	Spring	110	-58.97	0.03	64.53 ±10.27	2297.45 ±342.60	9.92 ±3.61	33.58 ±18.90	36.88 ±14.90	1.47 ±0.49	-1.74 ±0.09	0.016 ±0.031
SAZ	Winter	210	-43.00	8.50	63.39	93.38	15.51	2.70	1.19	0.44	8.65	0.013
PFZ	Winter	150	-47.00	4.50	50.34	106.30	18.94	5.84	4.78	0.82	5.08	0.045
AAZ	Winter	160	-54.00	0.00	29.18	475.86	16.11	2.95	11.29	3.83	-0.08	0.024
SBdy	Winter	110	-56.00	0.00	28.38	161.35	9.08	2.96	7.28	2.46	-1.32	0.045
SACCZ*	Winter	140	-57.30	0.02	37.52 ±9.95	1016.94 ±8.57	22.35 ±2.03	10.69 ±3.00	15.91 ±4.75	2.94 ±0.06	-1.55 ±0.02	0.016 ±0.001

3.3.2. Suspended and sinking POC:PON ratios and flux in winter and spring

The suspended POC:PON ratios were higher in winter (13.66 ±3.86) than in spring (7.07 ±4.40) (Figure 3.3-2A), although the opposite was true for sinking POC:PON ratios, which were higher in spring (23.31 ±26.57) than winter (4.19 ±1.98), particularly south of the PFZ (Figure 3.3-2B) where they reached a maximum at the AAZ (73). In winter POC:PON ratios were typically higher in the suspended fractions (81.99 ±16.40) when compared to the sinking fractions (25.14 ±5.03), while in spring the POC:PON ratios were typically higher in the sinking fraction (139.89 ±27.98 relative to winter 25.14 ±5.03). The POC flux for all stations combined was typically higher in spring (6.74 ±4.03 mg C m^{-2} d^{-1}) than winter 12.75 ±9.28 mg C m^{-2} d^{-1}) (Figure 3.3-2C), whereas PON flux was similar and typically higher in winter (1.75 ±1.23 mg N m^{-2} d^{-1}) when compared to spring (1.01 ±0.84 mg N m^{-2} d^{-1}) (Figure 3.3-2D). POC flux increased with increasing latitude with the highest POC flux in both seasons observed in the SACCZ (15.91 and 36.88 mg C m^{-2} d^{-1} in winter and spring, respectively). No latitudinal gradient was observed in PON flux, which was the highest in both seasons in the AAZ (3.83 and 2.41 mg N m^{-2} d^{-1} in winter and spring, respectively) (Figure 3.3-2D). The e-ratio was substantially higher in winter than in spring at PFZ (0.045 versus 0.006, respectively), SBdy (0.045 versus 0.032, respectively) and SAZ (0.013 versus 0.002, respectively) but similar for both seasons including AAZ (0.024) and SACCZ (0.016) (Figure 3.3-2E).

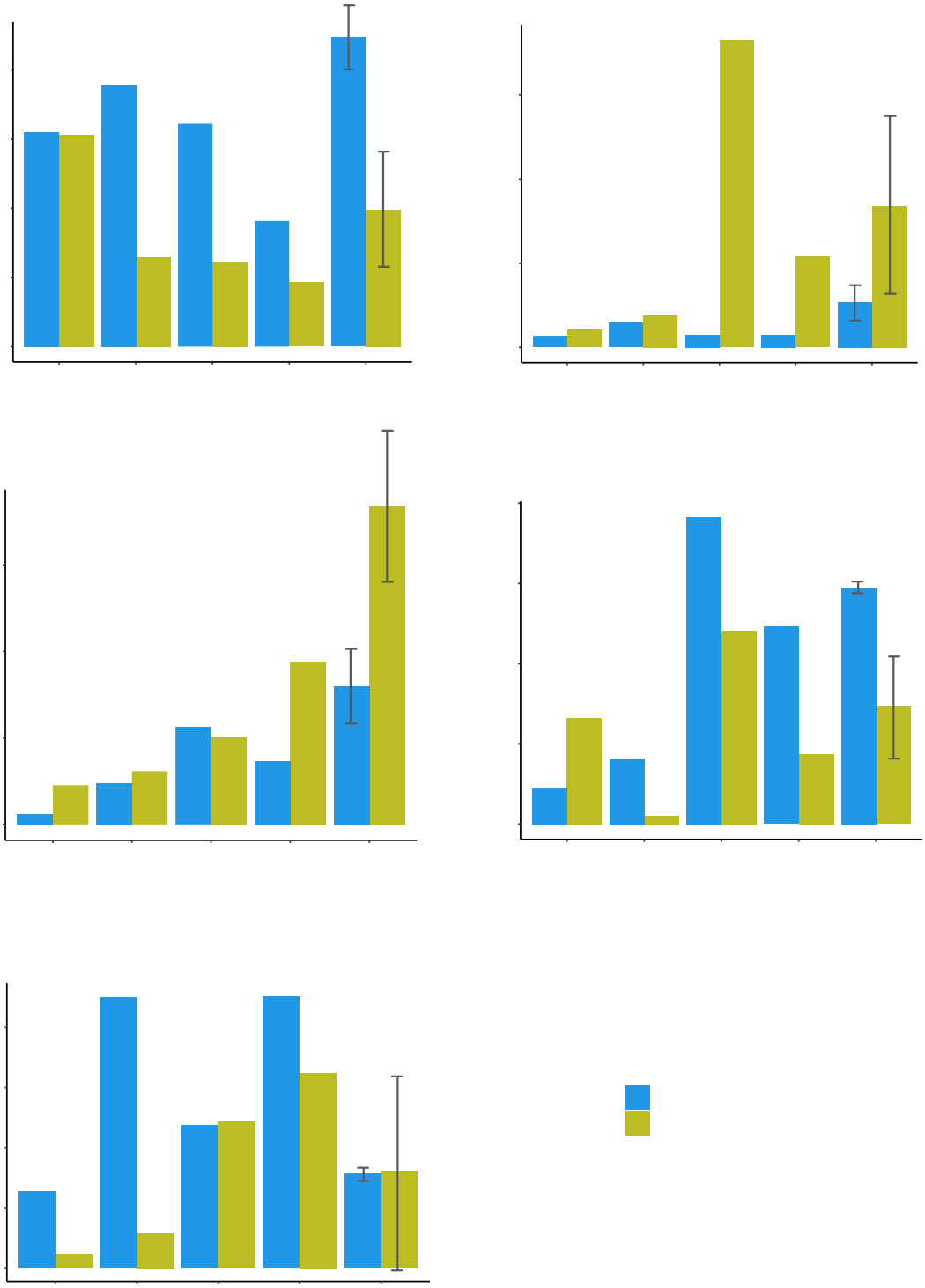


Figure 3.3-2 The MSC derived data in winter (blue) and spring (green) at SAZ, PFZ, AAZ, SBdy and SACCZ. A) Suspended POC: PON ratio and B) sinking POC:PON ratio, C) particulate organic carbon (POC) flux, D) particulate organic nitrogen (PON) flux and E) the e-ratio. Note SACCZ standard deviations are presented in Table 3.3-1.

3.3.3. Taxonomic classification

Bacterial communities in winter shared ~47 (78.7%) core OTUs between the suspended and sinking particle-pool, ~17 (3.1%) and ~5 (1.4%) OTUs were exclusive to the suspended and sinking particle-pool, respectively (Figure 3.3-3A). Whereas in spring ~50 (78.8%) core OTUs were shared between the particle-pools, with ~9 (1.5%) and ~14 (2.8%) OTUs found to be unique for the suspended and sinking particle-pools, respectively (Figure 3.3-3B). Bacterial communities from the winter samples, in both particle-pools across all the stations, were dominated by *Gammaproteobacteria* and *Alphaproteobacteria* followed by *Bacteroidia* (Figure 3.3-3C). *Alphaproteobacteria* typically dominated at all stations in winter, except station AAZ where *Gammaproteobacteria* dominated both the suspended and sinking fractions. In spring, the same three classes were dominant with more *Alphaproteobacteria* than *Gammaproteobacteria* at all stations (Figure 3.3-3D) except at station SACCZ with similar abundances.

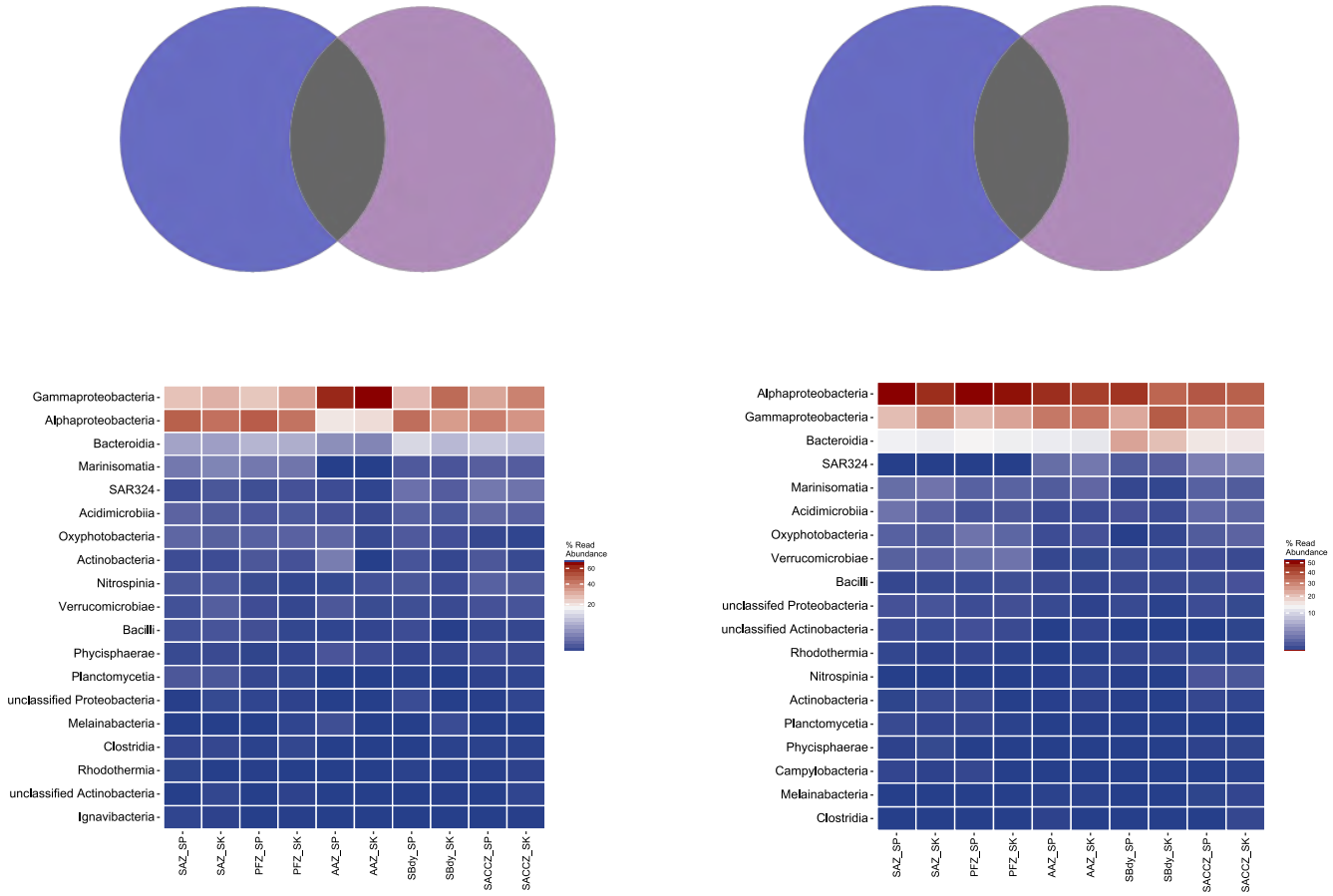


Figure 3.3-3 The bacterial taxonomy from raw metagenomic reads at class level. A) The core shared OTU between the suspended and sinking fraction in winter, B) the core shared OTU between suspended and sinking fraction in spring, C) the top 10 most abundant bacterial class in winter and D) the top 10 most abundant bacterial class in spring.

Archaea shared more OTUs compared with bacteria with ~12 (86.6%) core OTUs being shared between particle-pools in winter, ~4 (3.3%) and ~2 (2.5%) OTUs unique for the suspended and sinking particle-pools, respectively (Figure 3.3-4A). Whereas in spring, ~17 (89.1%) core OTUs were shared with ~5 (5.2%) and ~2 (1.6%) OTUs unique to the suspended and sinking particle-pool, respectively (Figure 3.3-4B). The winter archaeal community across all stations was dominated by *Nitrososphaeria* followed by *Thermoplasmata* (Figure 3.3-4C). This was typically followed by *Methanomicrobia* except at station AAZ (sinking) and SACCZ_2 (sinking) where *Thermoprotei* was instead the third most abundant. Archaeal communities in spring were similarly dominated by *Nitrososphaeria* and *Thermoplasmata* with *Methanomicrobia* shown to be the third most abundant (Figure 3.3-4D). The archaeal communities were, however, more diverse in spring compared with winter. *Thermoplasmata* were dominant at station AAZ (sinking), PFZ (suspended and sinking) and SAZ (suspended and sinking). *Nitrososphaera* were dominant at station AAZ (suspended), in the SACCZ (suspended and sinking) and at the SBdy (suspended and sinking). A large proportion of archaeal communities were attributed to other taxa in spring including *Methanomicrobia*, *Mathanosarcinia* and *Thermoprotei*.

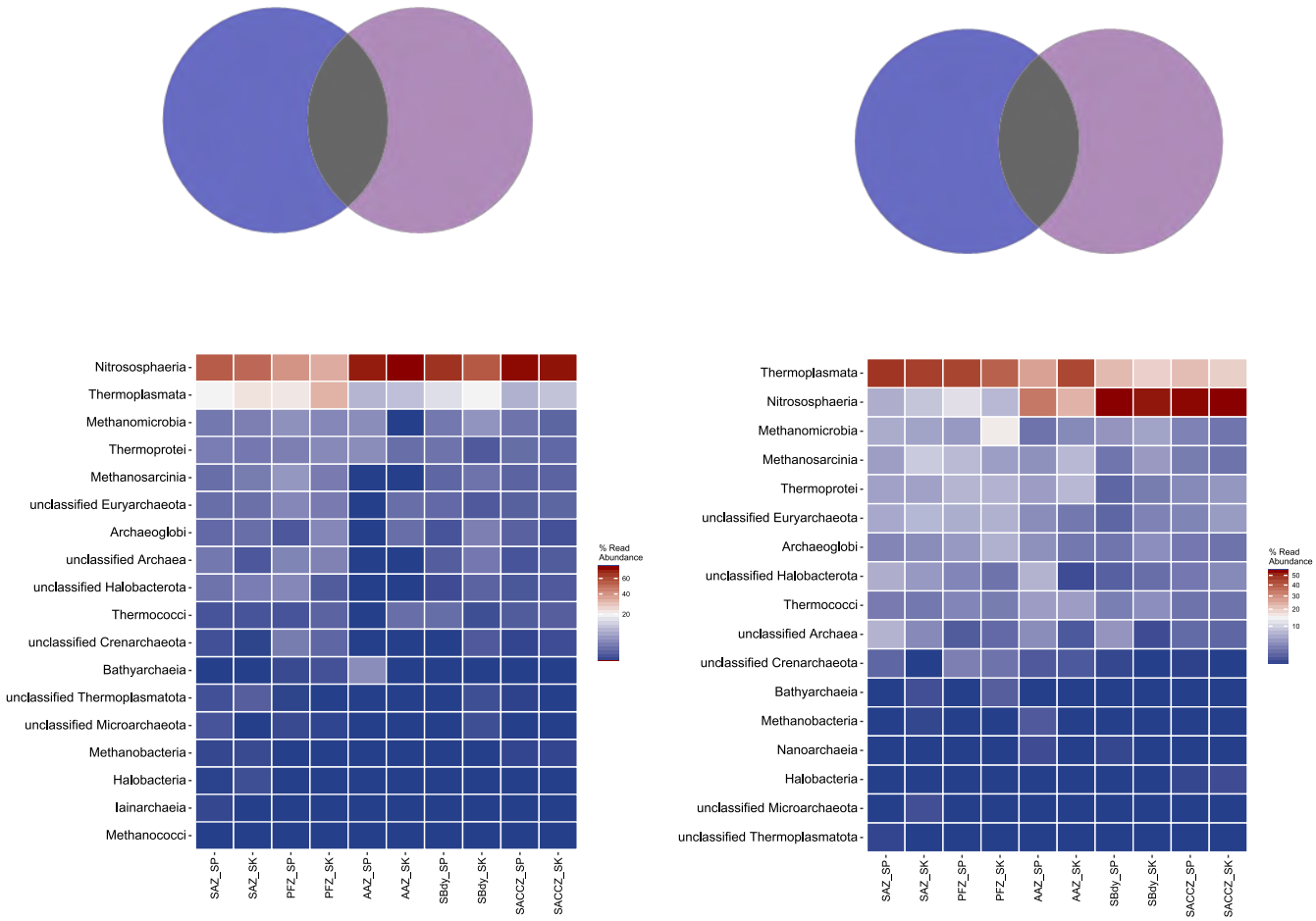


Figure 3.3-4 The archaeal taxonomy from raw metagenomic reads. A) the core shared OTU between the suspended and sinking fraction in winter, B) the core shared OTU between suspended and sinking fraction in spring, C) the top 10 most abundant archaeal class in winter and D) the top 10 most abundant archaeal class in spring.

The phylogenomic tree revealed that some of the bacterial MAGs in both winter and spring shared 30% similarities including *Gammaproteobacteria* (SACCZ and SBdy), *Alphaproteobacteria* (AAZ, SBdy and SACCZ), SAR324 (SACCZ) and *Acidiomicrobiia* (Figure 3.3-5A). While other bacterial taxa in both winter and spring MAGs did not share 30% similarities at SAZ (*Gammaproteobacteria*) and at SBdy only from spring. In spring, *Bacteroidia*, *Cyanobacteriia* and *Verrucomicrobiae* were similar in all stations as separate clusters. Whereas all archaeal MAGs from class *Poseidoniia* shared 30% similarities at PFZ and SACCZ, SBdy and SACCZ, and SAZ and PFZ in both winter and spring (Figure 3.3-5B). *Nitrososphaeria* shared 30% similarities at SBdy and SACCZ in both winter and spring whereas one MAG at SAZ in winter did not share 30% similarities with other MAGs.

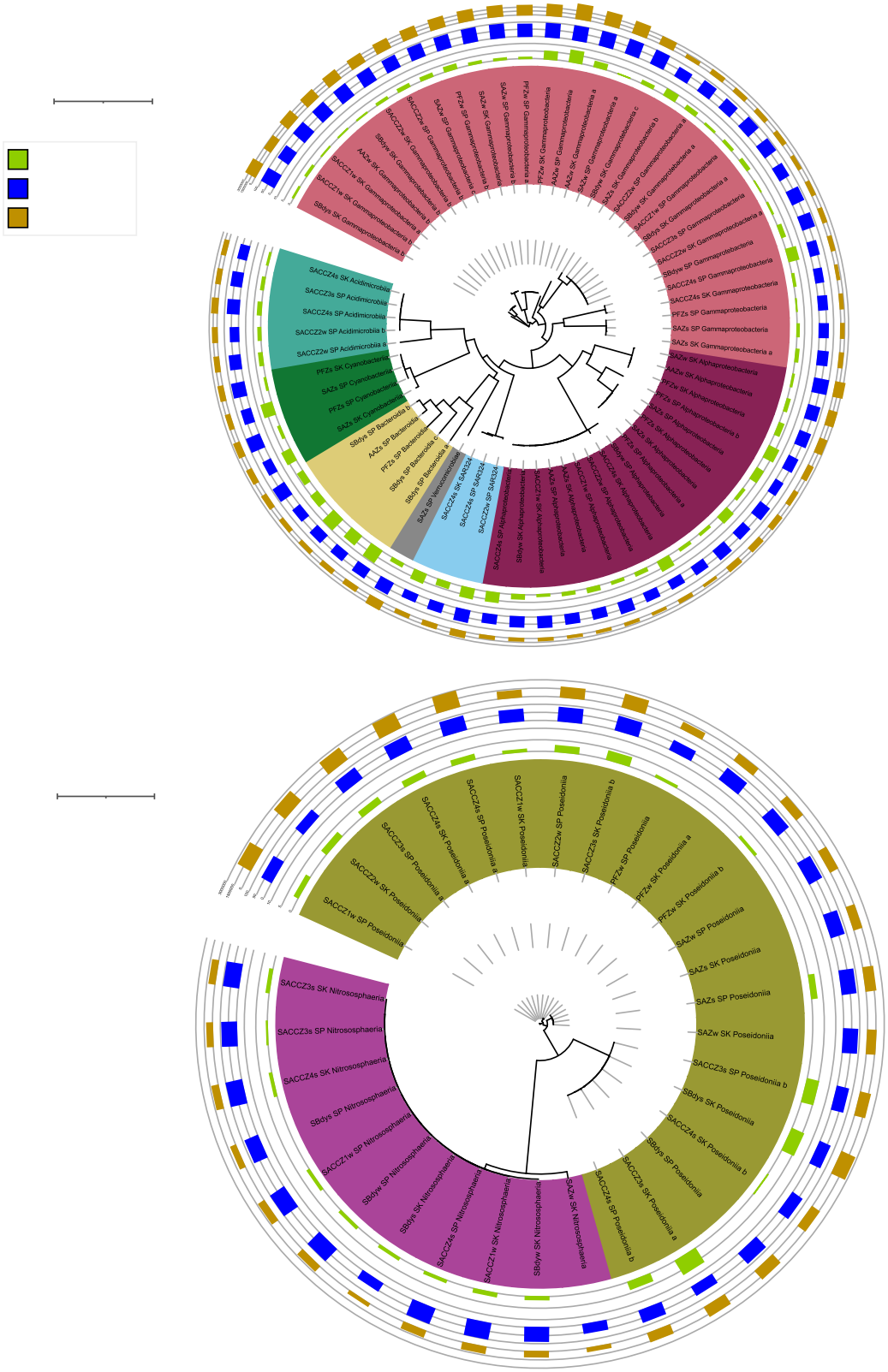


Figure 3.3-5 The phylogenomic tree for the prokaryotic MAGs. A) The bacterial phylogenomic tree from both the winter and spring bacterial MAGs and B) the archaeal phylogenomic tree from both the winter and spring archaeal MAGs.

3.3.4. Prokaryotic MAGs distribution in suspended and sinking particle-pool

Using the standards established by Bowers et al (2017), we recovered 46 MAGs with 15 designated as high quality and 31 medium quality MAGs in winter. In spring, we recovered 51 MAGs with 3 high quality MAGs and 48 medium quality MAGs. In winter, 22 MAGs were retrieved from the suspended particle-pool while 24 were from the sinking particle-pool. In spring, 29 out of the 51 MAGs were from the suspended particle-pool, while the remaining 22 MAGs were from the sinking particle-pool. In winter, 33 MAGs were affiliated to the bacterial classes *Gammaproteobacteria* (*n*=22), *Alphaproteobacteria* (*n*=8), *Acidimicrobiia* (*n*=2) and SAR324 (*n*=1) (Figure 3.3-6A). Additionally, 14 MAGs belonged to the archaeal classes *Poseidoniia* (*n*=9) and *Nitrososphaeria* (*n*=5). In spring, 32 MAGs belonged to the bacterial classes *Alphaproteobacteria* (*n*=9), *Gammaproteobacteria* (*n*=9), *Bacteroidia* (*n*=5), *Cyanobacteriia* (*n*=4), *Acidimicrobiia* (*n*=3) SAR324 (*n*=1) and *Verrucomicrobiae* (*n*=1), while 19 MAGs belonged to the archaeal classes *Poseidoniia* (*n*=13) and *Nitrososphaeria* (*n*=6) (Figure 3.3-6B). Overall, bacterial MAGs from spring metagenomes were more diverse than those recorded in winter in both the suspended and sinking fractions.

Gammaproteobacteria MAGs in winter were found in both the suspended and sinking fractions (Figure 3.4-6A). By contrast the *Alphaproteobacteria* MAGs were largely recovered from the sinking particle fraction in the SAZ, PFZ, AAZ and in both suspended and sinking fractions at the SBdy and SACCZ_1. Only a single *Alphaproteobacteria* was recovered from the suspended fraction samples retrieved from site SACCZ_2. *Acidimicrobiia* and SAR324 MAGs were reconstructed from only the suspended fraction at SACCZ_2. In spring, *Gammaproteobacteria* MAGs were reconstructed in both the suspended and sinking fractions at the SAZ and SACCZ_4 and the suspended fraction at the PFZ and SACCZ_3 and the sinking fraction at the

SBdy (Figure 3.3-6B). The *Alphaproteobacteria* MAGs were reconstructed in both the suspended and sinking fractions at the SAZ, PFZ, AAZ and SACCZ_3. *Acidimicrobiia* MAGs were only reconstructed from both the suspended and sinking fractions at SACCZ_4 and the suspended fraction only at SACCZ_3. The *Bacteroidia* MAGs were reconstructed in the suspended fraction at the PFZ, AAZ and SBdy and the *Cyanobacteriia* MAGs in both suspended and sinking fraction at SAZ and PFZ, whereas the *Verrucomicrobia* MAGs were only in the suspended fraction at SAZ. The *Bacteroidia* (suspended only), *Cyanobacteriia* and *Verrucomicrobia* were uniquely reconstructed in spring and not in winter. The winter archaeal class *Poseidoniia* MAGs were found in both suspended and sinking fractions except AAZ and SBdy stations, while the *Nitrososphaeria* MAGs were only at SBdy and SACCZ suspended and sinking fraction and SAZ sinking fraction (Figure 3.3-6A). In spring, *Poseidoniia* MAGs were found in both suspended and sinking fraction of all stations except PFZ and AAZ, while *Nitrososphaeria* MAGs were only found at SBdy and SACCZ stations (Figure 3.3-6B).

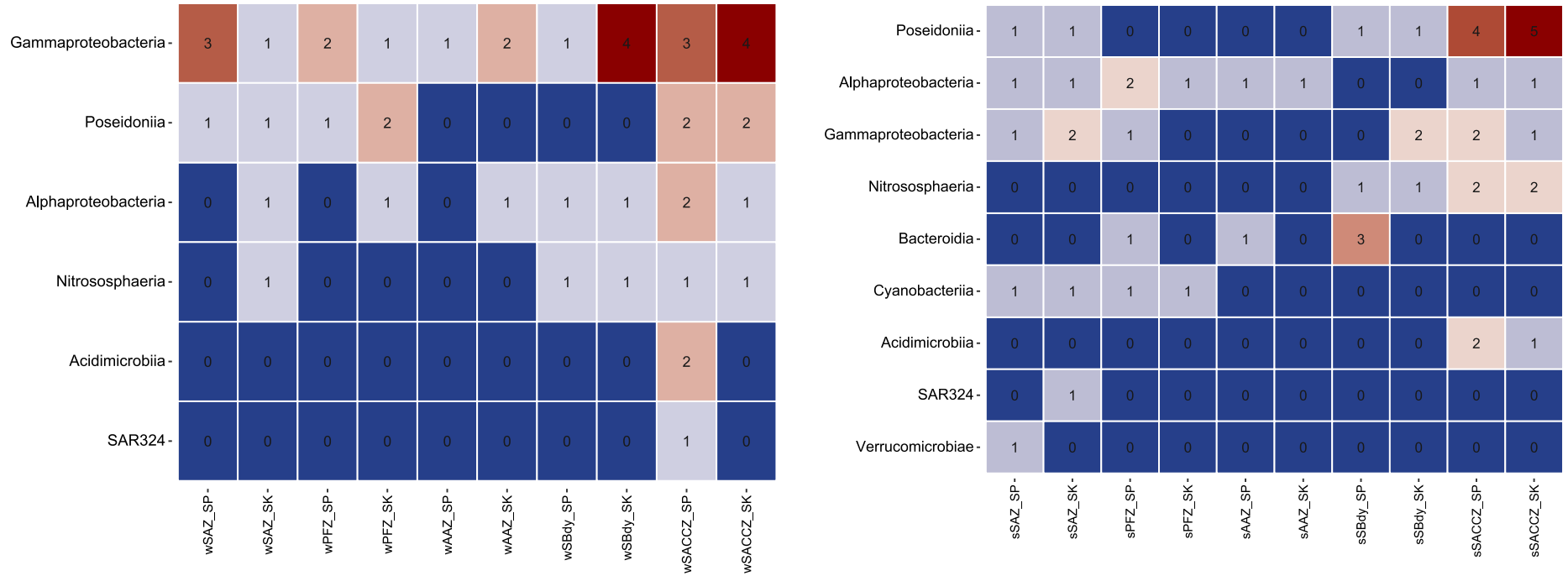


Figure 3.3-6 The prokaryotic MAGs count per station. A) the prokaryotic MAGs reconstructed in winter with the counts inside the block and B) the prokaryotic MAGs reconstructed in spring.

3.3.5. Functional capacity of bacterial and archaeal MAGs in the suspended and sinking particle-pool

In general, the bacterial MAGs harboured more CAZymes, short-chain fatty acid (SCFA) and alcohol conversion pathways in winter than those from spring. Overall, the winter bacterial MAGs (e.g., *Alphaproteobacteria* and *Gammaproteobacteria*) demonstrated a higher functional capacity for degrading high molecular weight organic compounds (Figure 3.3-7A), compared to those from spring (Figure 3.3-7B). These high molecular weight organic compounds include chitin, polyphenols, amorphous cellulose, xyloglucan, xylan, mixed-linked glucan, arabinose, pectin, starch, sulf-polysaccharides and alpha-galactans (Figure 3.3-7). Bacterial MAGs from the SAZ (higher in suspended fraction) and PFZ (evenly distributed in suspended and sinking fraction) had a higher capacity to degrade high molecular weight compounds than those from AAZ, SBdy and SACCZ (Figure 3.3-7A). On the other hand, the southward stations showed similar trend at AAZ (higher in sinking) and SACCZ (one MAG in suspended). The bacterial MAGs in spring, at the southward stations (AAZ, SBdy and SACCZ) also had a higher capacity to degrade high molecular weight organic compounds in the suspended as compared to sinking (Figure 3.3-7B). Bacterial MAGs at the northward stations had a higher capacity to degrade high molecular weight organic compounds in the suspended (PFZ) and sinking (SAZ) particle fraction (Figure 3.3-7B). Bacterial MAGs from spring showed some evidence of methanotrophy with genes linked to the conversion of acetate to methane at all stations, except southward stations including SACCZ_1 in both the suspended and sinking fractions and suspended fraction at SBdy and SACCZ_2. On the contrary, only one MAG in spring (*Gammaproteobacteria*) in the sinking fraction at the SBdy possessed the complete denitrification pathway to convert nitrate through nitrite (reversible reaction), nitric oxide, and nitrous oxide to nitrogen (N_2) (Figure 3.3-7B). There was more capacity for

photosynthesis in spring relative to winter with both *Cyanobacteriia* at PFZ and SAZ both suspended and sinking and only one MAG with the capacity for photosynthesis being found in winter from *Gammaproteobacteria* at PFZ sinking fraction.

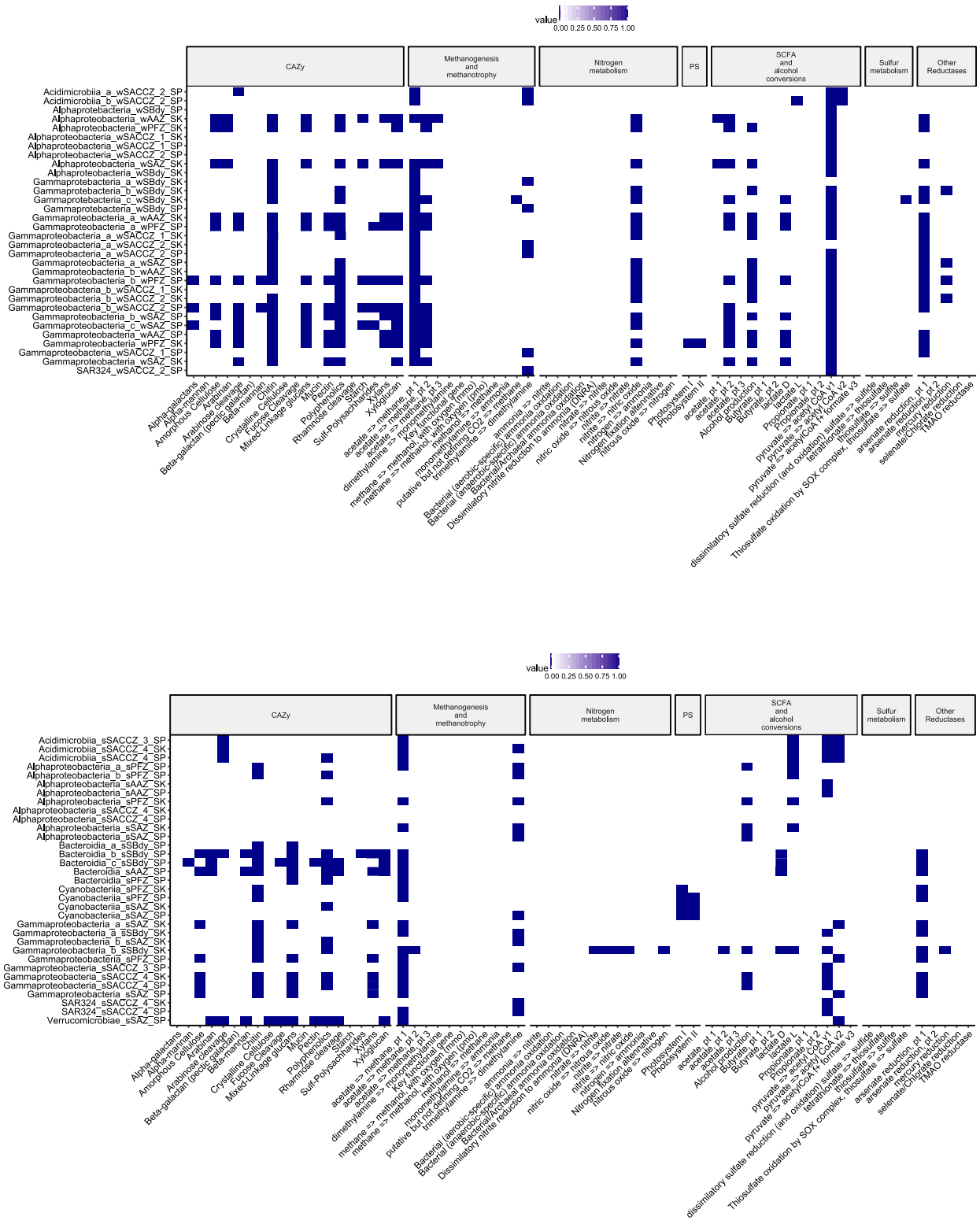


Figure 3.3-7 The bacterial MAGs functional capacity profiling in A) winter and B) spring.

Archaeal MAGs possessed less CAZymes, SCFA and alcohol conversion functional capacity in winter (Figure 3.4-8A) than in spring (Figure 3.3-8B). *Poseidoniia* were the most prominent class of archaeal MAGs that possess the functional capacity to degrade high molecular weight organic compounds in both winter and in spring. *Poseidoniia* MAGs from winter had the functional capacity for degrading high molecular weight organic compounds such as polyphenolics in both the suspended and sinking fraction at PFZ and at SACCZ_1 suspended and sinking fraction SACCZ_2 sinking (Figure 3.3-8A). Whereas *Poseidoniia* MAGs from spring had capacity for degrading arabinan in the suspended and sinking fractions at SACCZ_4 and SBdy suspended fraction (Figure 3.3-8B). These taxa had the capacity to degrade polyphenolics at SACCZ_4 and SACCZ_3 in the suspended and sinking fraction. Additionally, taxa in the suspended fraction at SACCZ_3 had the functional capacity to degrade mixed-linkage glucans. The archaeal MAGs from class *Nitrososphaeria* in both winter and spring, demonstrated functional traits for methanotrophy through conversion of acetate to methane, and methane to methanol. Additionally, *Nitrososphaeria* MAGs had some functional capacity for alcohol production and all the archaeal MAGs had the complete pyruvate to acetyl-CoA pathway in both winter and in spring except the MAG in the sinking fraction at SAZ in winter and also SBdy and SACCZ_3 in spring. *Nitrososphaeria* MAGs were the only genomes with functional capacity for nitrification by ammonia oxidation to nitrite and denitrification by converting nitrite to nitric oxide. *Poseidoniia* MAGs from spring, at sites SAZ (suspended fraction) and SACCZ (both suspended and sinking fraction) had the functional capacity for photosynthesis.

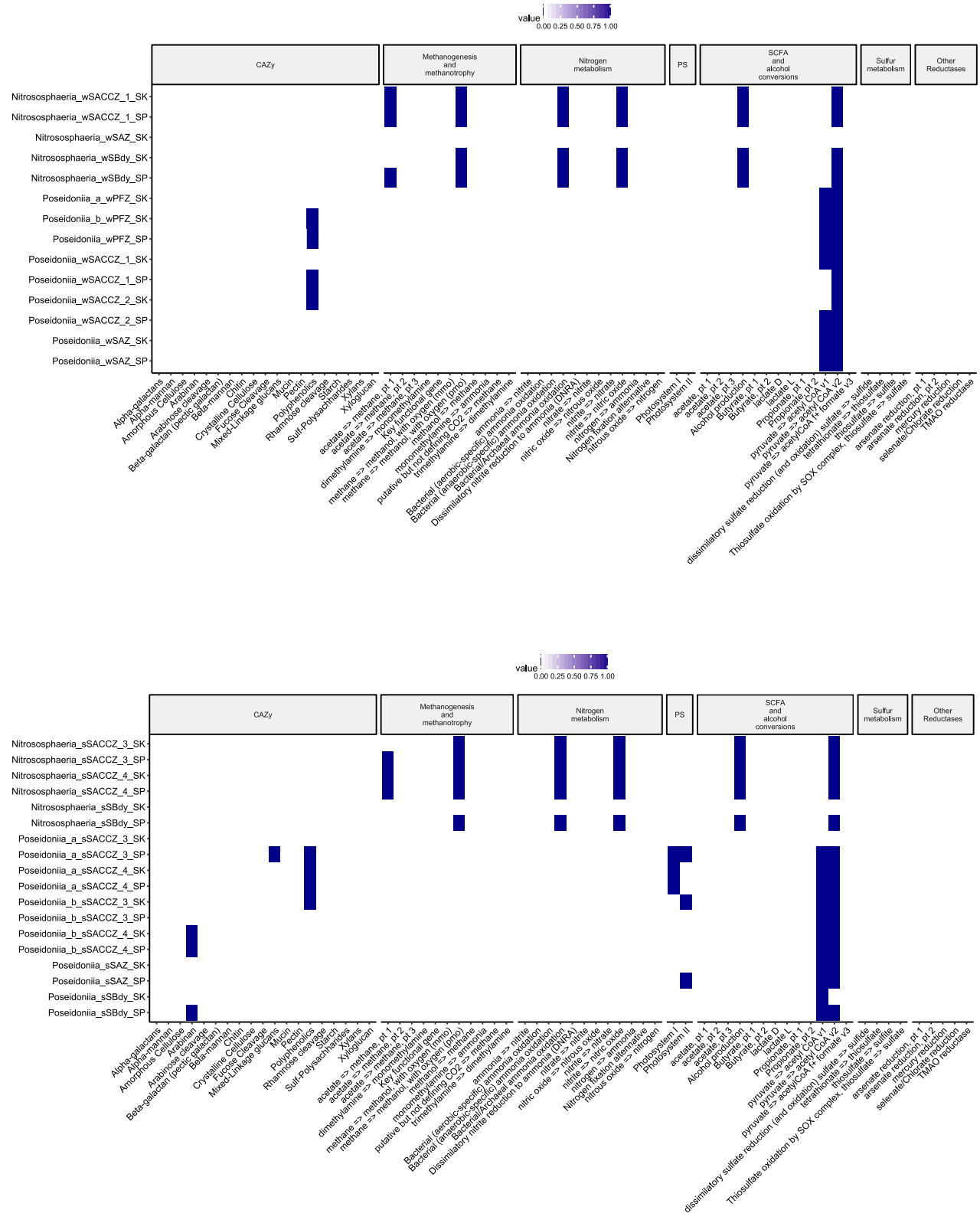


Figure 3.3-8 The archaeal MAGs complex functional capacity profiling in A) winter and B) spring.

3.3.6. Functional capacity of chemolithoautotrophic prokaryotes in the suspended and sinking particle-pool

Genome resolved metagenomics revealed capacity for chemolithoautotrophy in our prokaryotic MAGs. Our data showed that all genomes from winter (Appendix II-1A and II-2A) and spring (Appendix II-1B and II-2B) chemolithoautotrophic prokaryotic MAGs harboured capacity for the Wood-Ljungdahl pathway. The only exception was bacterial MAGs from spring samples with *Acidimicrobiia*, at SACCZ_3 and SACCZ_4 in the suspended fraction and *Bacteroidia* at SBdy and AAZ in the suspended fraction lacking traits associated with the Wood-Ljungdahl pathway. Whereas, the hydroxypropionate-hydroxybutylate cycle, Dicarboxylate-hydroxybutyrate and 3-hydroxypropionate bi-cycle were found at all stations in both the suspended and sinking fraction. MAGs from winter samples, affiliated with the class *Acidimicrobiia* (at SACCZ_2 in the suspended), *Gammaproteobacteria* (at SBdy in the sinking) and SAR324 (at SACCZ_2 in the suspended) showed capacity for methanotrophy via CO_2 to methane pathway (Figure 3.3-7A). MAGs from spring linked to the class *Acidimicrobiia* at SACCZ_3 suspended, SACCZ_4 both the suspended and sinking SBdy suspended and AAZ suspended fraction were capable of converting CO_2 to methane (Figure 3.3-7B). In addition, the archaeal MAGs were methanogens via conversion of CO_2 to methane except *Nitrososphaeria* at the SBdy in the sinking fraction in winter and spring, *Poseidoniia* at SACCZ_2 in winter and SACCZ_3 and SBdy in the sinking fraction. These chemolithoautotrophic pathways were more complete in winter than in spring suggesting that our prokaryotic MAGs were able to produce more high molecular weight organic compounds including RDOM and transparent exopolymer particles (TEP) in winter relative to spring.

3.4. Discussion

Although rates of NPP primarily determine the magnitude of organic material produced in the surface layer, it is not the only factor that dictates the quantity and quality of organic matter which is exported to depth (Henson et al 2019). There are a number of drivers that influence the e-ratio (i.e. the proportion of carbon exported, relative to what is produced), and include top-down impacts of zooplankton grazing and repackaging of particles by zooplankton into faecal pellets (Griffin and Rippingale 2001, Wilson et al 2008) as well as bottom-up (Frederiksen et al 2006, Lynam et al 2017) controls. These controls are associated with particle characteristics, which dictate sinking rates such as size, shape, composition and density of individual cells, faecal pellets or aggregates as well as water column density and stratification (Omand et al 2020). In addition, the prokaryotic communities are considered a key determinant of the e-ratio, as they typically reduce the amount of carbon being exported by degrading POC to DOC and then finally to DIC (Braeckman et al 2019, Hach et al 2020). The seasonal and regional characteristics of the prokaryotic community and functional capacity is driven by both abiotic (e.g., pH and temperature) and biotic factors such as the availability and content of POM (i.e., being labile, semi-labile or recalcitrant) (Quigley et al 2019, Wu et al 2018). The Southern Ocean prokaryotic community is thought to respond primarily to changes in the concentration and composition of POM that are in turn driven by seasonal and regional variability in phytoplankton blooms (Liu et al 2020c). The prokaryotic community subsequently acts upon the POM (either degradation or synthesis of DOM including RDOC) thus altering the characteristics of the sinking and suspended material (e.g., driving POC:PON ratios away from Redfield) (Boeuf et al 2019, Kellogg and Deming 2014, Milici et al 2017). The prokaryotes in the microbial loop are largely involved in the degradation of sinking POM to suspended POM and DOM (which would typically reduce the e-ratio) (Cavan et al 2019, Robinson 2019, Roshan and DeVries 2017). More recent research indicate that prokaryotes

may additionally contribute positively to export through the production of recalcitrant and refractory DOC (RDOC and rDOC, respectively) which avoids further degradation, thus facilitating export to the deep ocean via a process termed the microbial pump (Hansell 2013, Jiao et al 2011, Legendre et al 2015, Zhang et al 2018a). Furthermore, components of the prokaryotic communities are chemolithoautotrophs that possessing the capacity to fix CO_2 to produce RDOC in addition to labile DOC. As such, the role of prokaryotes in determining flux efficiency is complex and hard to disentangle.

The role of the prokaryotic communities in regulating carbon export to depth remains poorly studied in the Southern Ocean. This despite the important role that the Southern Ocean plays in regulating atmospheric CO_2 drawdown via the BCP (Henley et al 2020, Sigman et al 2010). Here we present the main findings of an investigation that assessed the regional and spatial variability in the characteristics of carbon and nitrogen export together with the prokaryotic communities and their functional capacity to better understand how they may influence or be influenced by POM derived from phytoplankton.

3.4.1. Seasonal variability in organic matter export in response to prokaryotic community and functional capacity

Phytoplankton biomass (inferred by MLD integrated Chlorophyll-a concentration), NPP and POC flux are typically higher in spring than in winter, but this is not the case for PON flux or the e-ratio, which is typically higher in winter based on the data. Net primary production (and subsequent Chlorophyll-a concentrations) in winter is primarily limited by light availability through a combination of deep convective mixing that drives deep mixed layers and low solar zenith angle (Arrigo et al 2008, Li and Cassar 2016). In spring, increased solar radiation and a subsequent shoaling of the mixed layer increases light availability which promotes NPP

(Constable et al 2014, Deppeler and Davidson 2017, Pollard et al 2002). Our results revealed that the low POC flux in winter may be influenced by the prokaryotic community (e.g., *Gammaproteobacteria* and *Alphaproteobacteria*), which had more functional capacity to degrade high molecular weight compounds, including carbohydrates, in winter than in spring, thus exacerbating the observed decrease in POC flux in winter. This is potentially due to a lack of labile POM from low rates of NPP in winter (relative to spring). As such, prokaryotic communities' resort to degrading more complex high molecular weight organic material as a food source (Kharbush et al 2020, Thornton 2014, Tisserand et al 2020). Similarly, this functional capacity to degrade complex, carbon rich compounds likely drive the observed low POC:PON ratios of sinking material in winter and consequently the higher observed PON flux in winter relative to spring.

From a global perspective, e-ratios typically increase with increasing NPP (Dunne et al 2005, Henson et al 2011, Laws et al 2011). This pattern is not evident south of 40ºS, where e-ratios can be negatively correlated with NPP (Fan et al 2020, Maiti et al 2013). This is consistent with our results which show typically higher rates of NPP in spring (than in winter) being associated with lower e-ratios and a negative correlation between NPP and e-ratios. Fan et al (2020) proposed that an accumulation of suspended and dissolved organic carbon in the surface waters could be responsible for the low e-ratios despite high NPP in the Southern Ocean. Our results support this hypothesis in part, with higher concentrations of carbon observed in the suspended than in the sinking fraction. However, this was true for both winter and spring (except in the SAZ in spring), and as such cannot alone account for the decline in e-ratios in spring. Furthermore, if higher rates of prokaryotic degradation in spring was responsible for the accumulation of suspended POC and DOC, then one would expect suspended POC:PON ratios to reflect significant prokaryotic degradation, which surprisingly they do not, averaging at near

Redfield ratio of 8.5 for spring when compared to 3.9 for winter. Alternatively, phytoplankton community (i.e., small cells that are not rapidly sinking) or zooplankton grazing (i.e., sloppy feeding on large sinking material to generate smaller suspended material) are the more likely contender for driving the inverse relation between NPP and e-ratios.

Despite e-ratios typically showing little dependence on sea surface temperature when temperatures are low (<6ºC) (Le Moigne et al 2016, Maiti et al 2013), the e-ratio in the Southern Ocean (when observed across all regions and seasons) decreases with an increase in temperature (Fan et al 2020). Our elevated e-ratios in winter are consistent with previous studies that suggest that at warmer temperatures, the rate of heterotrophic degradation increases at a rate that exceeds photosynthesis (Boscolo-Galazzo et al 2018, Cavan et al 2019), leading to a decrease in e-ratios at the SAZ and PFZ in spring. In addition, our mean MLD temperatures were negatively correlated with e-ratios. Although neither the correlations were significant (potentially driven by a limited number of data points), the correlation coefficient was nonetheless higher in spring (r^2 = -0.72, p-value = 0.107) than winter (r^2 = -0.22, p-value = 0.675). On the other hand, the cold spring temperatures at the AAZ and SACCZ could be responsible for slow heterotrophic degradation and increased e-ratios. The synergistic impact of prokaryotic degradation and temperature on e-ratios is especially apparent when comparing the warmer SAZ and the colder SACCZ in spring, which both had the high NPP, but only the SACCZ had high POC flux. This might be due to colder temperatures resulting in slow prokaryotic degradation of POC in the SACCZ allowing organic matter to sink, whereas in the warmer SAZ in spring prokaryotic degradation was likely responsible for the decline in export and e-ratios and the lowest sinking POC:PON ratios (much lower than Redfield).

Interestingly, our heterotrophic prokaryotic community with chemolithoautotrophic capacity was more evident in winter than in spring, which may generate energy by oxidation of inorganic compounds (such as DIC) for biosynthesis of high molecular weight compounds (DeLorenzo et al 2012, Karl et al 1984). In other words, like phytoplankton they are able to generate organic carbon thus contributing to the production of both DOC (mostly in the form of transparent exopolymer substrates), which can be transformed into POC (suspended or sinking) via aggregation or adsorption (Druffel and Williams 1 0, Romera-Castillo et al 201) . More chemolithoautotrophs in winter could thus contribute to the higher suspended POC:PON ratios in winter than spring. Presence of more chemolithoautotrophic prokaryotes in winter relative to summer has been reported to be associated with solar irradiation and primary productivity (Grzymski et al 2012).. Indeed, this is likely to have been triggered by a decrease in NPP and solar irradiation in winter and a subsequent reduction in labile food source stimulating prokaryotic functional capacity to fix DIC thus increasing the suspended POC:PON ratio relative to the sinking POC:PON in winter.

Interestingly, *Bacteroidia*, *Cyanobacteriia* and *Verrucomicrobia* MAGs were unique in spring. *Bacteroidia* was only in the suspended fraction (less at the PFZ and AAZ and more at the SBdy), and has been reported to metabolise fresh DOM substrates (LaBrie et al 2020), which may explain their dominance in spring relative to winter. *Cyanobacteria* (present in the SAZ and PFZ in both suspended and sinking fractions) are photoautotrophs with light harvesting complexes that convert light into energy (Stanier and Cohen-Bazire 1977) to fix DIC thus increasing DOC and POC concentrations. As such, they may play a role in increasing the POC:PON ratio of suspended material, particularly in the SAZ, which revealed the highest suspended POC:PON ratio of 15. *Verrucomicrobia* (found only in the SAZ in the suspended fraction) constitutes only a minor fraction of the bacterial MAGs but are able to degrade very

high-molecular weight POM compounds (Cao et al 2019, Galand et al 2009, Galand et al 2010). *Verrucomicrobia* is the only taxa that can degrade crystalline cellulose containing several hundred and over ten thousand D-glucose molecules (Park et al 2010, Sichert et al 2020), making it a primary contender for driving the high suspended POC:PON ratio in the SAZ in spring.

3.4.2. Regional variability in organic matter export in response to prokaryotic community and functional capacity

In winter, both NPP and the POC flux tended to increase southwards. However, there was no latitudinal gradient in the winter e-ratios. The winter increase in POC flux southwards is likely due to a combination of increased NPP (generating organic material in the surface waters that has the potential to be exported) and chemolithoautotrophic *Gammaproteobacteria* and *Alphaproteobacteria* having more functional capacity to degrade high molecular weight POC at the more northerly SAZ, PFZ and AAZ but less to none at the SBdy and SACCZ. Additionally, the winter chemolithoautotrophic prokaryotic community at the SBdy and SACCZ had the functional capacity to degrade chitin, thus supporting the presence of zooplankton such as Antarctic krill (e.g., *Euphausia superba*) (Wang et al 2013). The presence of Antarctic krill might lead to an increase in POC flux (and high sinking POC:PON ratios) from intensive grazing and fecal pellet production (Atkinson et al 2004, Smetacek and Nicol 2005) promoting efficient sinking of densely packed fecal pellets (Shatova et al 2012) in winter.

Although POC flux also increased southwards in spring, this pattern was not evident in the NPP or e-ratio values. The bacterial communities (e.g., *Gammaproteobacteria*, *Bacteroidia* and *Acidimicrobiia*) and the archaeal community (e.g., *Poseidoniia*) at the SBdy and the SACCZ had a greater capacity to degrade high-molecular weight POC compounds than other

regions. Despite the presence of these MAG's and their preferential degradation of POC (relative to PON), these zones had the highest POC flux, and relatively high e-ratios and sinking POC:PON ratios. This might be due to the impact of sea ice in these regions, which recedes during spring leading to phytoplankton blooms (Assmy et al 2017, Lizotte 2001) and a simultaneous supply of POC as a resource for prokaryotes from both autochthonous (native) and allochthonous (incorporated from snow during ice formation) (Lizotte 2001) origins. This provides an example on how prokaryotic community and functional capacity might respond to the changes in POM content (as their food source) rather than the characteristics of POM reflecting the outcome of functional capacity from the resident prokaryotic community. The lower water temperatures may in addition have negated the high functional capacity of the prokaryotes to degrade high molecular weights (Ntzimani et al 2021) in the more southerly regions, thus enhancing POC flux.

In all three of the southern most regions, *Gammaproteobacteria* and *Alphaproteobacteria* (less at the AAZ and more at the SBdy and SACCZ) were present and had the functional capacity to deaminate amino acids to α-keto acids (Kubota et al 2016), which act as precursors for polymer synthesis (Penteado et al 2019), thus enhancing aggregation of DOC to POC (Wagner et al 2020) and potentially increasing the sinking POC:PON ratio in these zones. Chemolithoautotrophic SAR324 was also present in both the suspended and sinking material in the SACCZ, which is involved in pyruvate conversion to acetyl-CoA. The acetyl-CoA compound is a central precursor metabolite for prokaryotes involved in the biosynthesis of high molecular weight compounds characterised as RDOC (e.g., fatty acids, sterols and polyphenolics) (Nielsen 2014) thus also promoting the aggregation of particles (Ma et al 2021, Strijbis et al 2008, Trudnowska et al 2021) and likely contributing to high POC:PON ratios in the sinking material at SACCZ. The AAZ in particular, had the highest sinking POC:PON

ratios (an order of magnitude higher than Redfield). This station also had the lowest number of MAGs (e.g., *Alphaproteobacteria* and *Bacteroidia*) with little to no functional capacity to degrade POM. However, these *Alphaproteobacteria* and *Bacteroidia* MAGs did have the functional capacity to utilize pyruvate to acetyl-CoA and to produce D-lactate. This RDOC, if aggregated with detritus to form marine snow or incorporated into fecal pellets (Alldredge and Silver 1988, Carlson et al 2010, Kinsey et al 2018, Trudnowska et al 2021) would drive an increase in the sinking POC:PON ratio. D-lactate on the other hand is a refractory organic compound that cannot be further utilised by prokaryotes (Niu et al 2014, Zhang et al 2018b) thus forming part of the rDOM pool. D-lactate can however undergo self-esterification to form bioplastic precursors (e.g., poly-lactic acid) (Datta et al 1995, Zhang et al 2018b) that have a high carbon content and are thus likely to increase POC:PON ratios if incorporated into the sinking material. Although these pathways were also present in spring in all other regions, the prokaryotic community in these regions had the functional capacity to preferentially degrade POC over PON, which was not the case in the AAZ.

In winter, the SACCZ region had the highest suspended POC: PON ratio in conjunction with the highest number of chemolithoautotrophic prokaryote MAGs (e.g., *Gammaproteobacteria*, *Alphaproteobacteria*, *Acidimicrobiia*, SAR324 and *Nitrosopheria*). Increased chemolithoautotrophic functional capacity would typically result in the production of RDOC and TEP, which promotes aggregation of prokaryotic derived DOC into suspended POC (Druffel and Williams 1 0, Romera-Castillo et al 201) , thus potentially increasing the SACCZ suspended POC: PON ratio. Similarly, chemolithoautotrophic SAR324 (Boeuf et al 2021) present in the suspended fraction only at the SACCZ was involved in pyruvate conversion to acetyl-CoA (methanogen), which participate in various biochemical processes including protein, carbohydrates and lipid metabolism (Shi and Tu 2015). In addition, acetyl-

CoA can be utilised to produce complex organic carbon (Nielsen 2014) and promote biofilm formation (i.e., visibly sticky aggregates of prokaryotic cells) (Chen et al 2020, Pisithkul et al 2019) thus also enhancing the suspended POC:PON ratio.

In spring and winter, the SAZ and the SACCZ had the highest suspended POC:PON ratios, which may have been influenced by the presence of chemolithoautotrophic *Poseidoniia,* having Photosystem II in the SAZ (suspended only) and Photosystems I and II in the SACCZ (suspended and sinking). In essence, *Poseidoniia* harness light as their energy source to drive ion-pumps during DIC fixation without the production of oxygen (Frigaard et al 2006), which likely increases both DOC and RDOC production in the SAZ and SACCZ. Aggregation of this material to form suspended POC, would drive an increase in suspended POC:PON ratios. On the other hand, the low suspended POC:PON ratios observed in spring in the AAZ and SBdy may result from prokaryotic degradation of arabinose (e.g., *Acidimicrobiia* and *Bacteroidia* MAGs) and polyphenolics (e.g., *Gammaproteobacteria*, *Alphaproteobacteria* and *Poseidoniia* MAGs). The degradation of arabinose and polyphenols is similar to the degradation of peptides via hydrolysis of ester bonds (Liu et al 2017), which releases D-amino acids that might accumulate as refractory DOC (rDOC) (Jiao et al 2014, Tang et al 2012) in the suspended POM decreasing the suspended POC:PON in spring in the AAZ and at the SBdy.

3.5. Conclusions

Southern Ocean phytoplankton seasonal blooms sustain and influence the suspended and sinking prokaryotic community and their functional capacity. These prokaryotic communities alter the stoichiometry of exported organic matter to either below or above the Redfield ratio of 6.6 thus influencing the proportion of carbon exported to depth. In agreement with previous studies (Arteaga et al 2018), our findings demonstrate a negative correlation between NPP and the e-ratio at the Atlantic Southern Ocean. The e-ratio was also negatively correlated (higher in spring than winter) with temperature. These relationships appear to be influenced by low temperatures that impact degradation rates as well as phytoplankton community structure and zooplankton grazing, which together drive an accumulation of suspended material in surface waters. High suspended POC:PON ratios in winter were thought to be influenced by low NPP and a subsequent reduction in labile food source stimulating chemolithoautotrophic capacity. *Bacteroidia*, *Cyanobacteriia* and *Verrucomicrobia* were unique to spring with DIC fixation, additionally, *Cyanobacteriia* and *Verrucomicrobia* had the ability to degrade high-molecular weight POM compounds, likely driving the observed increase in suspended POC:PON ratios in the SAZ in spring. In spring, the more southerly stations (SBdy and SACCZ) appeared to be to be influenced by an allochthonous supply of high molecular weight POC (evidenced in the disconnect between high POC flux but a coincident capacity to preferentially degrade high-molecular weight POC) and zooplankton communities (evidenced in the functional capacity to degrade chitin), which together account for the strong impact on POC flux and high e-ratios in these regions. The high POC:PON ratios observed in both the suspended fractions in winter at SACCZ was likely due to enhanced aggregation of DOC to POC by 1) deaminating amino acids as precursors for polymer synthesis, 2) chemolithoautotorphic conversion of pyruvate to acetyl-CoA involved in biosynthesis of RDOC and TEP and 3) DOC and RDOC production from *Poseidoniia* having Photosystem II. On the other hand, low suspended POC:PON ratios

in spring at AAZ and SBdy may result from degradation of arabinose producing D-amino acids that accumulate as rDOC. Despite the complexity and multifaceted drivers of seasonal and regional variability in POC flux and the e-ratio, the prokaryotic community and functional capacity were shown to have a definitive impact in regulating the export of organic matter in both winter and spring. Results suggest that in the warmer waters of the SAZ and PFZ in spring microbial degradation likely contributes to low sinking POC:PON and e-ratios, whereas in the colder AAZ, SBDy and SACCZ their chemolithoautotrophic capacity might be responsible for enhancing sinking POC:PON ratios driving high e-ratios and advocating for the important role of the microbial pump.

4. Synthesis

Recent studies have provided evidence regarding the distribution of prokaryotic communities in the suspended and sinking particle-pools in the North Atlantic and Southern Ocean (Scotia Sea) (Baumas et al 2021, Duret et al 2019). These studies have applied amplicon sequencing to provide additional understanding of how the structure and phylogenetic composition of microbiota affect the vertical flux of marine snow particles, which increases the efficiency of the BCP. These studies suggest a negative correlation between prokaryotic carbon losses and microbial species richness. The functional repertoire of microbiota associated with sinking and suspended particles remains, unknown. Here, the data provide insights into the functional capacity of bacteria and archaea associated with suspended and sinking material. By using shotgun metagenomic data, this study presented evidence of seasonal and regional variability in the microbial contributions to organic matter export in the Southern Ocean. The study focused on four key regional, seasonal and intra-seasonal aspects; 1) Variability in NPP (where available) and MLD integrated chlorophyll-a concentration as proxy for phytoplankton biomass, 2) Export of organic matter out of the mixed layer and its relationship to NPP (e-ratios), 3) POC:PON ratios of sinking and suspended material and 4) prokaryotic community and functional capacity from reconstructed prokaryotic MAGs.

4.1. Significance of the research

The main findings of this investigation provide important contributions to our understanding of the role of microbes in influencing the export of material in the Southern Ocean. These include *inter alia* **1**) NPP and temperature are inversely related to e-ratios in the Atlantic sector of the Southern Ocean (Chapter 3), **2**) Prokaryote community and functional capacity in both the suspended and sinking fractions likely impacts organic matter export (Chapter 2 and 3), **3**)

Prokaryotic functional capacity rather than diversity impacts the POC and PON signature of suspended material (in spring) and sinking material (in autumn and winter) (Chapter 2 and 3), **4**) Bacteria as *r*-strategists dominate the suspended pool whereas *K*-strategists dominate the sinking particle-pool, while the opposite is true for archaea (Chapter 2) and **5**) Chemoautotrophic and Chemolithoautotrophic prokaryotic communities have the capacity to enhance carbon export to depth , particularly at reduced temperatures (Chapter 2 and 3).

4.1.1. The NPP, temperature and e-ratio

A simplistic interpretation of the BCP suggests that only a small fraction of the organic matter produced in surface waters by NPP is exported to deeper regions of the world's oceans (Brewin et al 2021, Honjo et al 2014). The low rates of carbon export to depth is primarily attributed to temperature dependent heterotrophic activity associated with sinking organic material, which is composed of dead or senescent phytoplankton cells and zooplankton faecal pellets (Cavan et al 2015, Cavan et al 2017a, Cavan et al 2017b, Melo Viríssimo et al 2022). Global observations show a positive correlation between NPP and e-ratios, and a negative correlation between temperature and e-ratios (Dunne et al 2005, Henson et al 2011, Laws et al 2011). However, in the Southern Ocean, NPP has been shown to be inversely correlated with e-ratios (Le Moigne et al 2016, Maiti et al 2013), while e-ratios demonstrate little temperature dependence at temperatures below 6ºC (Fan et al 2020). Our results, which expand the latitudinal extent of the Atlantic Southern Ocean across two seasons, supports the previously observed inverse relation between NPP and e-ratios, while also supporting a negative relationship between temperature and e-ratios (mostly in spring) despite temperatures being substantially colder than 6ºC. Additionally, our data point to at an allochthonous top-down supply of high molecular weight organic material likely derived from sea-ice melt and the heterotrophic activity of zooplankton (faecal pellets) that may play an important role in

controlling e-ratios in the seasonally productive low temperature regions of the Southern Ocean during spring at southerly most stations.

4.1.2. Prokaryotic communities and functional capacity in the suspended and sinking fractions

It is generally accepted that prokaryotic communities associated with the microbial loop reduce the e-ratio by remineralising POM to DIC (Anderson and Ducklow 2001, Azam et al 2016, Fenchel 2008, Martin et al 2012). However, this *status quo* has been challenged by the more recent concept of the microbial carbon pump, which emphasises the role of the microbial transformation of suspended or sinking organic matter from labile or semi-labile forms to refractory, which ultimately determines its residence time in the ocean (Jiao et al 2011, Jiao and Zheng 2011). Results from previous studies suggest that prokaryotic communities have different ecological niches with *K*-strategists occurring predominantly in the suspended fraction (with high molecular weight compounds), while *r*-strategists dominate the sinking fraction (with more labile organic material) (Duret et al 2019). The archaeal functional capacity from our SOTS results (Atlantic SAZ in autumn), confirm this finding for POC, however, the bacterial functional capacity suggests the opposite, with *K*-strategists found predominantly in the sinking fraction. The *K*-strategist prokaryotic communities actively degrade sinking POC to suspended POC, which might suggest that the sinking fraction had more complex high molecular weight compounds than the suspended fraction. Additionally, MAGs from the Atlantic Southern Ocean prokaryotic communities suggest degradation of high molecular weight compounds mostly in sinking POC in winter and suspended POC in spring, which results in the production of rDOC including D-lactate and D-amino acids (Benner and Amon 2015, Zhang et al 2018a) with important implications for carbon export and deep ocean carbon storage. Prokaryotic communities and functional capacity from both sites (SOTS and Atlantic

Southern Ocean) reveal that functional redundancy rather than diversity impacts the characteristics of POM as either labile, semi-labile, recalcitrant or refractory thus impacting the POC:PON signature of sinking and suspended material. In addition, a holistic view of the characteristics of the functional capacity of the microbial community together with the characteristics of the sinking and suspended material, export flux and e-ratios reveal that lower temperatures may negate prokaryotic functional capacity at the southerly station at Atlantic Southern Ocean. However, even though prokaryotes reduce e-ratios at high temperatures, they nonetheless sequester organic material as rDOM, thereby, regulating the characteristics of exported material that is stored in the deep ocean as DOC for millennia.

4.1.3. Prokaryotic chemoautotrophic and chemolithoautotrophic capacity

Photosynthetic phytoplankton play an important role in the transfer of carbon from the ocean surface to depth, thus sustaining the marine food web and impacting global climate (Strzepek et al 2019). Historically phytoplankton communities, through the process of photosynthesis, were thought to be the only organisms capable of transforming inorganic carbon into organic carbon in shallower waters (Arteaga et al 2020, Parekh et al 2006), with microbial DOC production occurring only in the deep ocean (e.g. >1000m) and in the vicinity of hydrothermal vents (Nakagawa and Takai 2008, Sievert and Vetriani 2012). More recently it has been revealed that prokaryotes possess the capacity to generate more complex DOC via their chemolithoautotrophic capacity in the aphotic and photic zones in the Eastern South Pacific Ocean (Farías et al 2009). Results from the two cruises revealed that the prokaryotic community had chemolithoautotrophic functional capacity to fix DIC to more complex DOC in the upper water column . This result provides supporting evidence of chemolithoautotrophic capacity at shallower waters <500m in the Southern Ocean. Chemolithotrophic capacity was

mostly seen in winter than spring at the Atlantic sector of the Southern Ocean whereas chemolithoautotrophic capacity was observed in autumn at SOTS sites. Production of DOC from prokaryotic communities needs to be considered in the ocean carbon budget (Middelburg 2011). Indeed, our chemoauto- and chemolithoautotrophic prokaryotes are the first to be identified in the suspended and sinking fraction of material from the SOTS and Atlantic sector of the Southern Ocean. These communities produce more complex DOC including RDOC and TEP, which aggregate to each other or with detritus to form dense biofilm structures that ultimately sink (Jennings et al 2017, Jiao et al 2014). This implies that models which estimate carbon export using NPP and phytoplankton biomass as proxies for carbon export may overlook the important contributions of the prokaryotic community to vertical carbon flux and thus potentially underestimate organic matter export within the region.

4.2. Future Perspectives

4.2.1. Extending measurements of phytoplankton community

Phytoplankton NPP and biomass, both based on measurements of chlorophyll, do not provide insights into phytoplankton community structure, which can include diatom and coccolithophores to small flagellates (Håkanson 2005). Given the important role of phytoplankton community structure in dictating the characteristics of the suspended and sinking material, it is advisable that they be investigated in tandem with NPP, biomass, export and microbes for a more holistic view of the BCP (Kramer et al 2020, Riegman 2001). Although on the winter and spring cruises of the Atlantic Southern Ocean these data were collected (i.e. community composition and size distribution from HPLC, coulter counter and imaging flow cytometry), the complete data set was not yet ready for inclusion as part of this thesis These data, together with information of the biogenic silica and inorganic carbon content

of the suspended and setting material, will provide a better overview of the phytoplankton community composition and its impact on particle content and ballasting potential (Lombard et al 2013, Riley et al 2012). Furthermore, genomic measurements on eukaryotes will provide information on the phytoplankton community taxonomic and functional capabilities in the Southern Ocean, which will complement the reports by Duret et al (2020) who investigated the eukaryotic community associated with suspended and sinking particle-pool in the mixed layer and upper mesopelagic of the Scotia Sea, Southern Ocean. This would change the way we study the phytoplankton community and will allow us to focus on their contribution at a molecular level providing a more holistic understanding of the BCP when combined with the suite of biogeochemical and physical oceanography measurements.

4.2.2. Expanding microbial community sequencing for more comprehensive functionality

The main findings of this thesis showed the importance of prokaryotic community and functional capacity as a 2-Dimensional (2D) model (shotgun data), whereas Duret et al (2019) and Baumas et al (2021) presented a (1D) model (amplicon data). Nonetheless, our data reveal only the skeletal structure (as 2D) and functional capacity of the prokaryotic on the suspended and sinking POM across the Southern Ocean. Therefore, we require more advanced state of the art approaches to better understand the active microbial community structure and their functional diversity including gene expression and regulation. This advanced approach could include metatranscriptomics (3D model) and metaproteomics (4D model) (Buttigieg et al 2018), which compliment amplicon and shotgun metagenomic sequencing (Aguiar-Pulido et al 2016, Crovadore et al 2017, Georges et al 2014, Saminathan et al 2018). The 3D model will provide the level of genes, which are then transcribed, whereas the 4D model, will provide the level of expressed genes as well as functional enzymes. Providing a spatiotemporal coherent

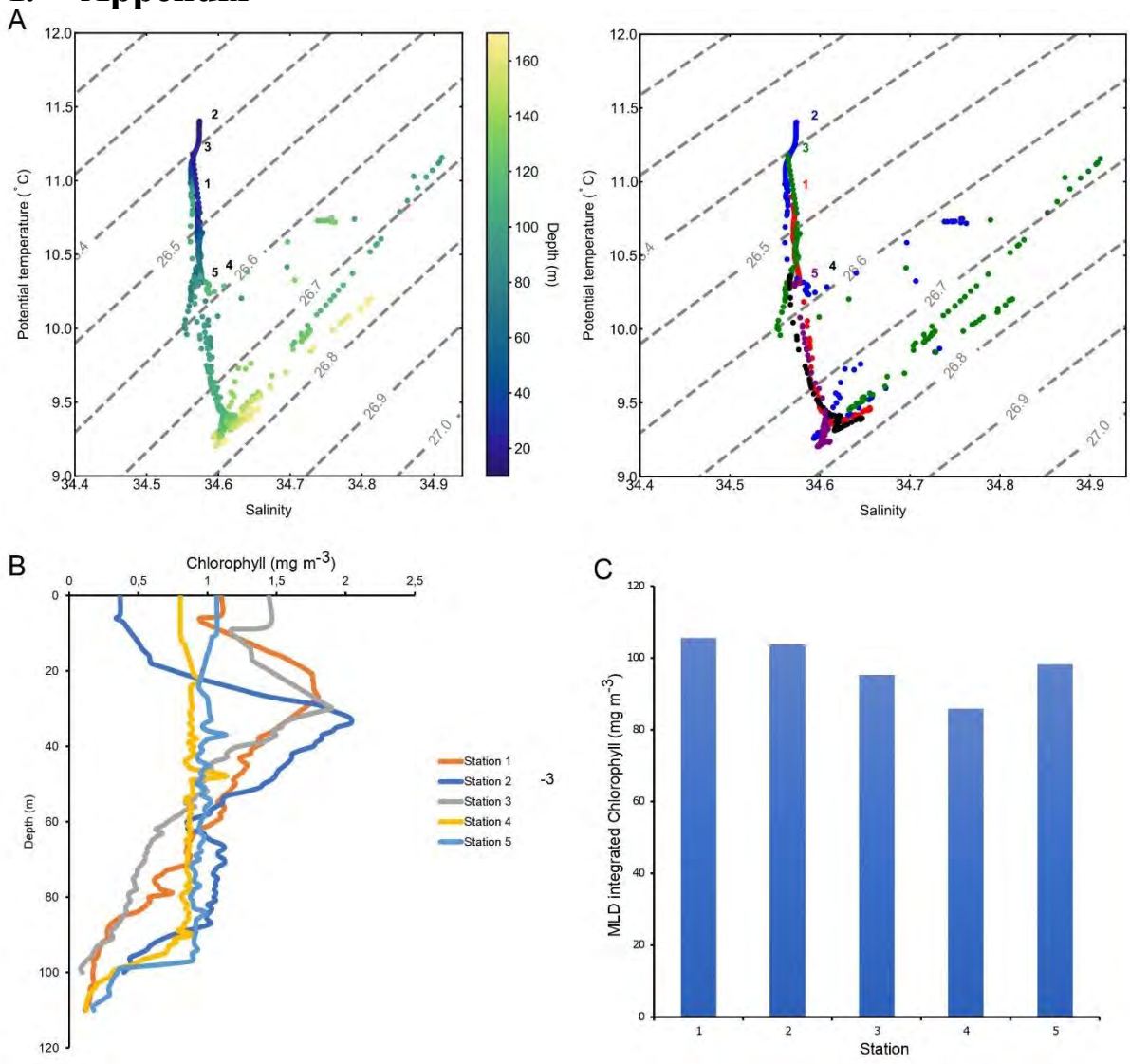
and comprehensive functionality of the microbial community would assist in disentangling their full functional capabilities for determining their role in Southern Ocean biogeochemical cycling.

4.2.3. Expanding taxonomic assessments of microbial community to include viruses

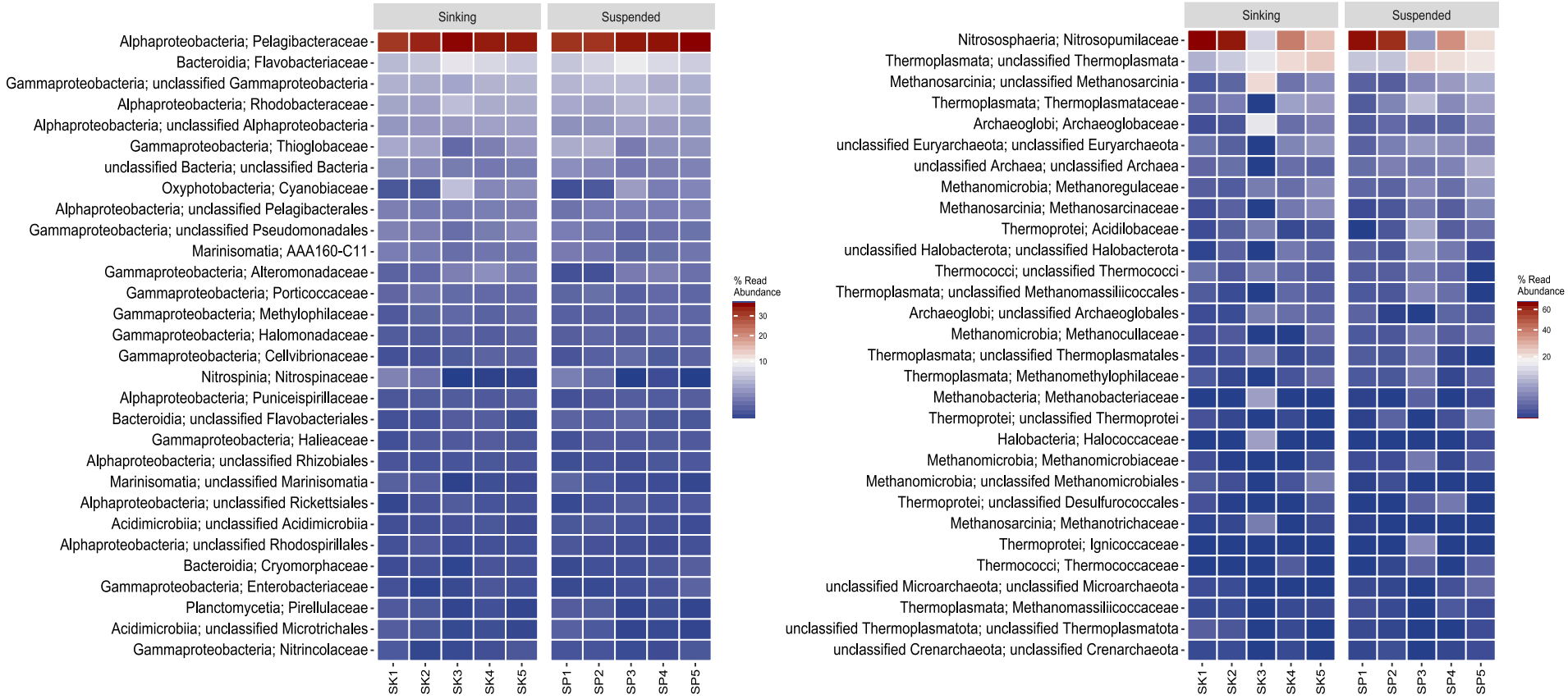
The balance between phytoplankton and microbial communities is complicated by viruses (Evans et al 2009, Fuhrman 1999). Virus-host interactions contribute towards regeneration of organic material via the viral shunt (Evans et al 2021), while also enhancing POM aggregation which ultimately contributes to POM export to depth. This is achieved by releasing EPS during the lytic replication cycle via the viral shuttle (Biggs et al 2021) or by harbouring AMGs involved in the utilisation of DOM to produce rDOM (Heinrichs et al 2020, Zhao et al 2019). Intracellular materials and cell detritus released during lysis (Proctor and Fuhrman 1991, Suttle 2007) changes the stoichiometry of DOM/POM (Jover et al 2014) by redistributing the stored organic material into the ocean DOM/POM pool, thus altering the DOM/POM content and enhancing DOM/POM sequestration (Weinbauer et al 2011). About 150 Gt of DOM/POM is thought to released annually by viral lysis of their prokaryotic hosts and is considered a major contributor to marine organic matter flux in the ocean (Suttle 2005). The molecular nature of DOM/POM and its original contributors (i.e., phytoplankton, prokaryotic or viral derived-DOM/POM) can be assessed by advanced analytical mass spectrometry such as ultrahigh-resolution electrospray ionization (ESI) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) (Xu et al 2020). Mass spectrometry has revealed that DOM/POM consist of complex marine organic molecules (Hertkorn et al 2013) and prokaryotic organic material (Koch et al 2014, Lechtenfeld et al 2015), which suggests that viral lysis or viral

induced DOM contributes to the DOM/POM pool in the deep ocean (Zhao et al 2019). Viral induced DOM/POM also results in alterations in the prokaryotic community structure and chemodiversity (small molecules with signalling function e.g., TCS) (Zhao et al 2019). Changes in POM chemical composition via phytoplankton community structure and virus-prokaryotic interaction dictates aggregation into sinking POM that can enhance POM export efficiency. The specific role of viral trophic interactions is difficult to disentangle (Brussaard et al 2008, Evans and Brussaard 2012) making their precise contribution to the Southern Ocean biogeochemical cycle difficult to determine. As part of the Atlantic Southern Ocean winter and spring cruises, we obtained putative viral sequences identified using Virsorter2 from unbinned metagenomic contigs (Guo et al 2021). This data, although outside of the scope of the PhD thesis, is currently being investigated. However, these viral contigs will only serve as a 2D model for viruses as they are incomplete viral sequences, and some have less information regarding AMGs. Future studies should incorporate the viral flocculation experiments (Poulos et al 2018) on both the suspended and sinking fractions. In order to recover active viruses to complement the organic matter and MAGs data collected. This will better our understanding on the role of viral-host interaction contributions to the suspended and sinking POM in the Southern Ocean.

I. Appendix

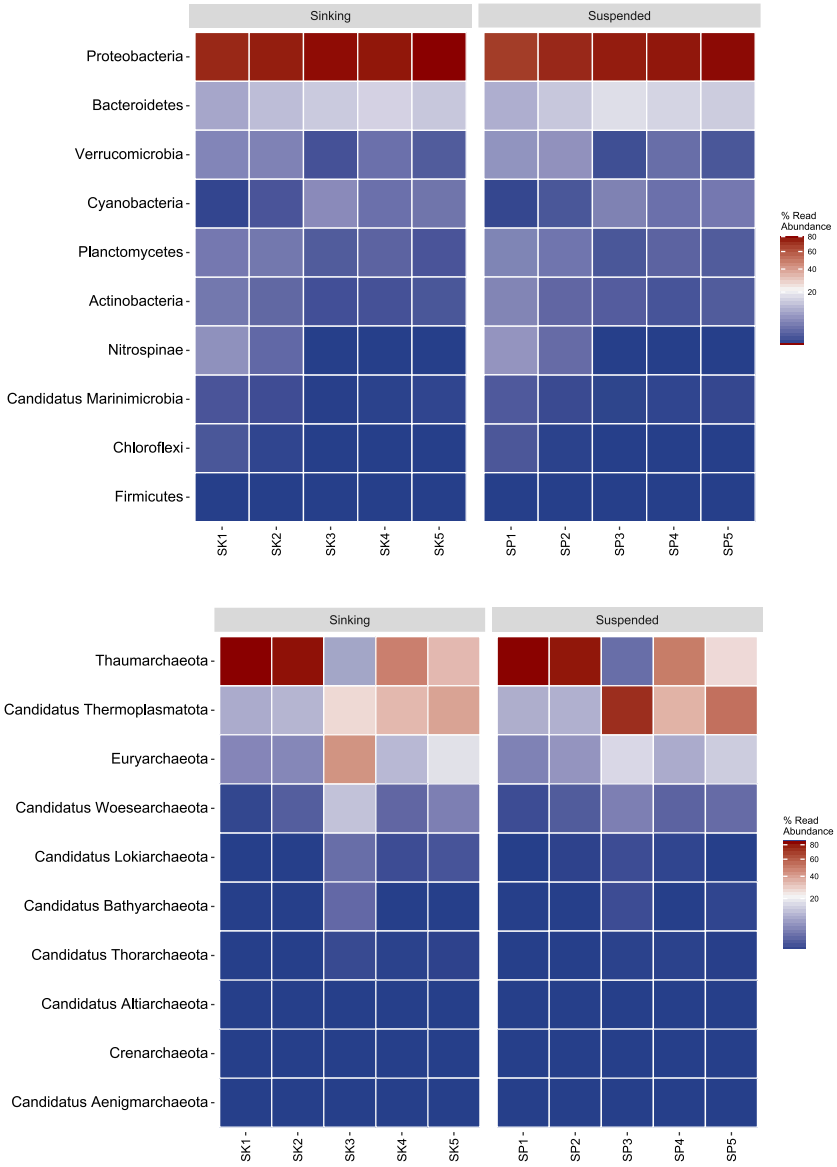


Appendix I-1 Ancillary data from the SOTS sites obtained from CTD data. A) Temperature (in ºC) and salinity plot showing the five sampling stations. B) Chlorophyll depth profile for five stations by determined by CTD fluorescence sensor and C) the MLD integrated Chlorophyll.

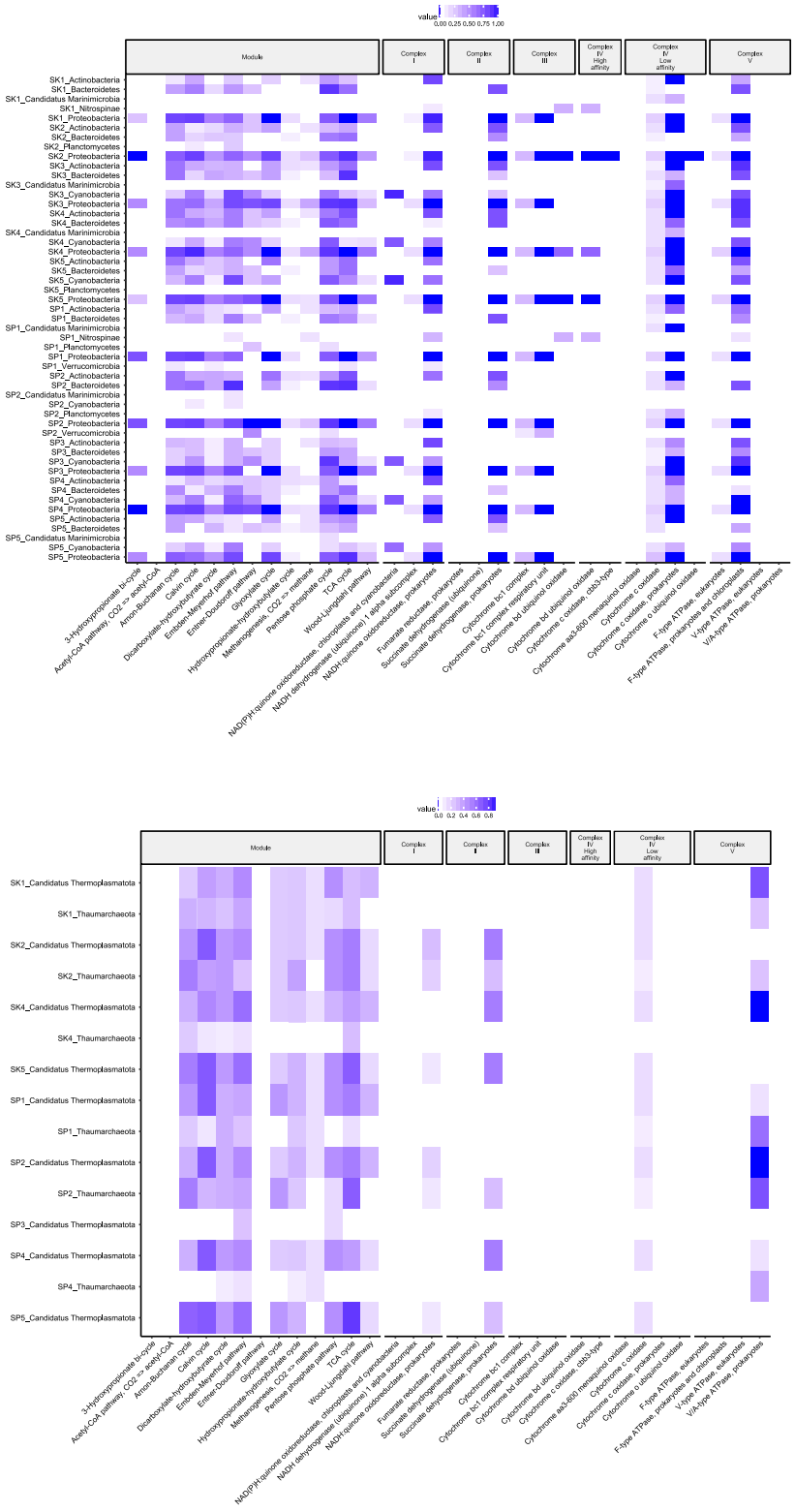


Appendix I-2 The overview of the Prokaryotic community composition for five stations at Class and Family level for SP and SK particle-pool.

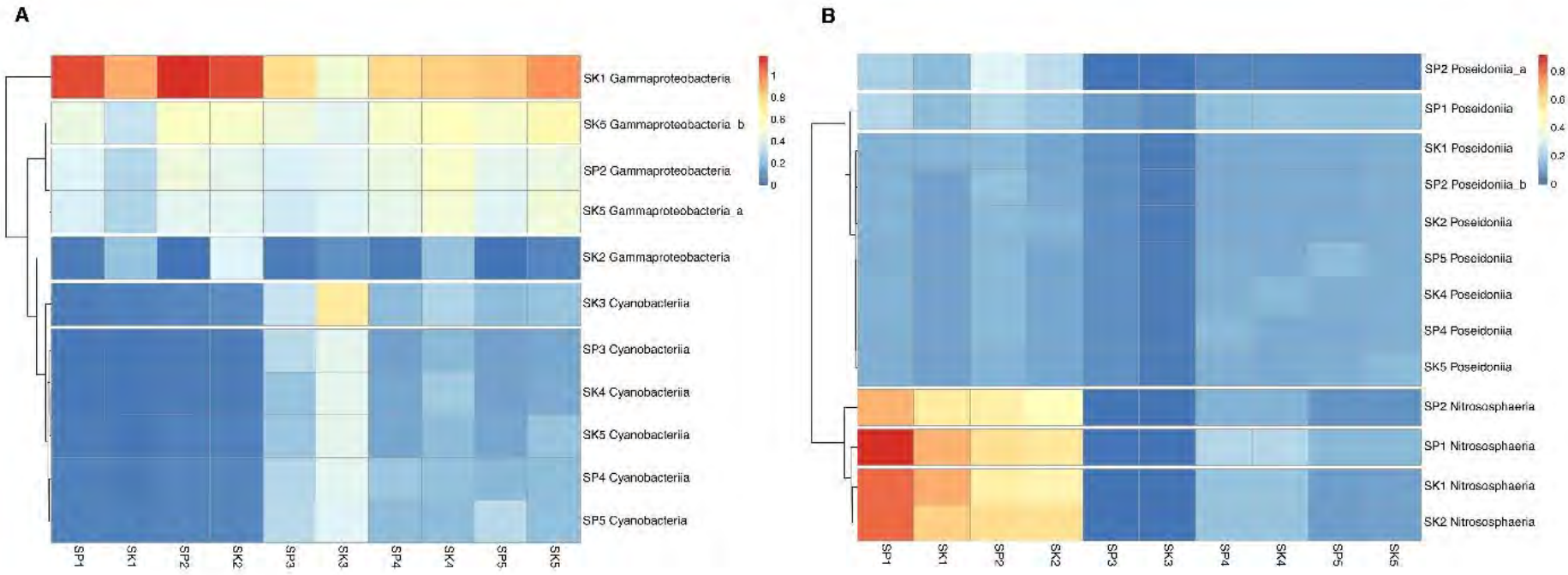
A) Bacterial community composition and B) Archaeal community composition at both class and family level.



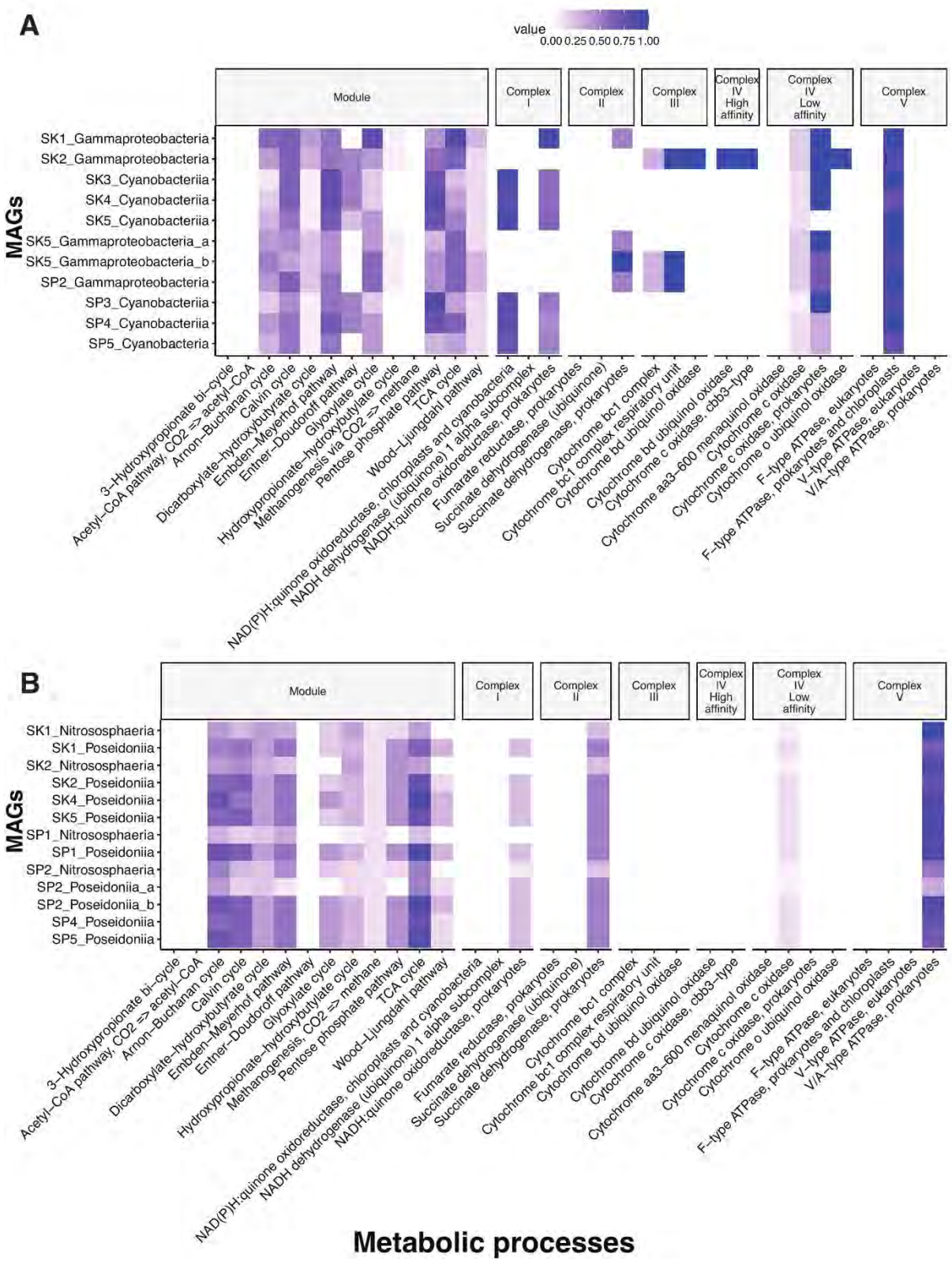
Appendix I-3 The taxonomic classification of the prokaryotic community from unbinned contigs at Phylum level. A) Bacterial community and B) Archaeal community presented as relative abundance.



Appendix I-4 The central functional capacity of the prokaryotic community from unbinned contigs. A) Bacterial central metabolism and electron transport chain (ETC) complexes and B) Archaeal central metabolism and ETC complexes.

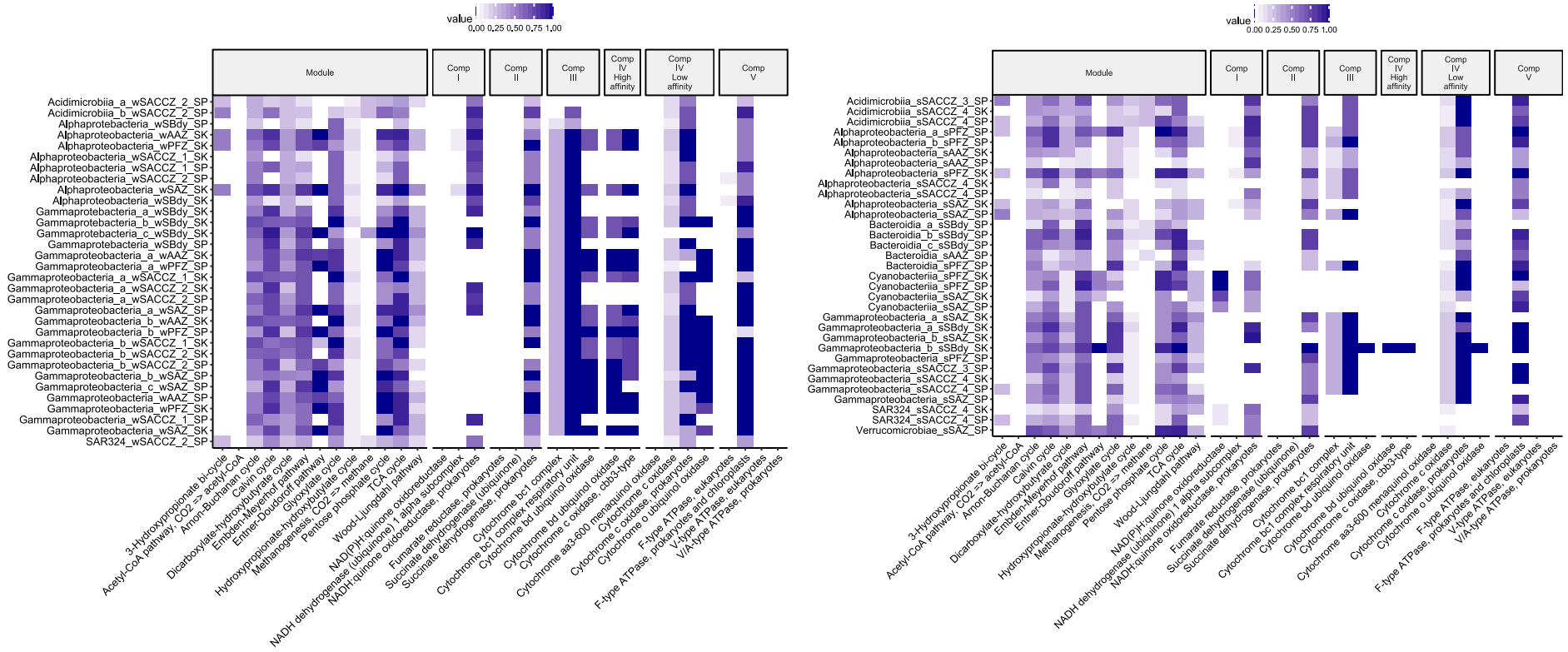


Appendix I-5 Read recruitment for our 24 MAGs against the 10 raw shotgun metagenomic reads. A) Bacterial read recruitment represented as relative abundance and B) Archaeal read recruitment.

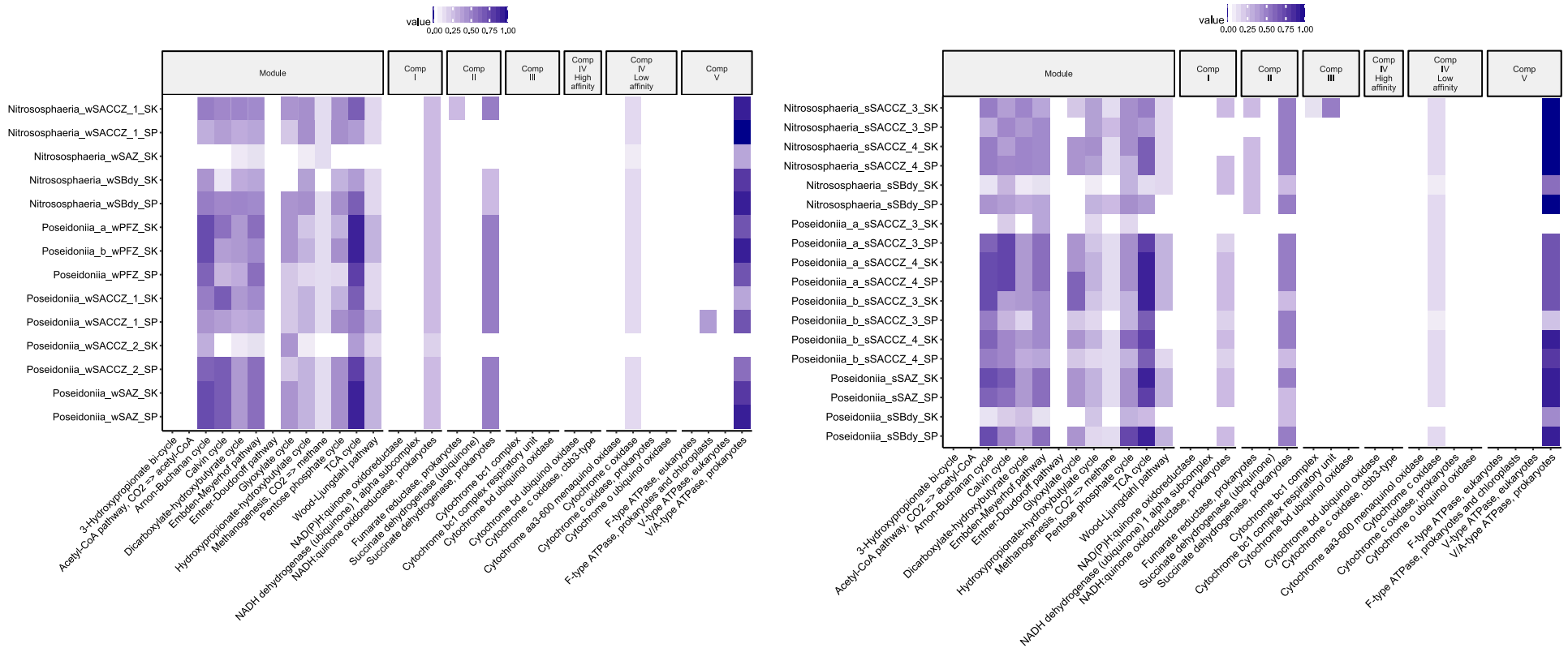


Appendix I-6 The central metabolic function and electron transport chain (ETC) complexes present on our MAGs. A) Bacterial central metabolism and ETC complexes and B) Archaeal central metabolism and ETC complexes.

II. Appendix



Appendix II-1 The bacterial MAGs central functional capacity profiling and the electron transport chain (ETC) in A) winter and B) spring.



Appendix II-2 The archaeal MAGs central functional capacity profiling and the electron transport chain (ETC) in A) winter and B) spring.

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