

Microbial oceanography of southern hemisphere seamounts and hydrothermal vents

Anni Djurhuus



Thesis submitted in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy

University of Oxford
Department of Zoology

12 June 2015

Declaration

I hereby declare that I conducted the work presented in this thesis under the supervision of professor Alex D. Rogers. Contributions by others have been specifically acknowledged and clearly indicated where appropriate.

Chapter 2 was co-authored by myself, professor Alex D. Rogers, and Jane F. Read. This chapter has been submitted for publication in Deep Sea Research Part II as a part of a special issue on seamounts in the Southwest Indian Ridge. Jane F. Read calculated the sea surface height of fig. 2.1. Chapter 3 was co-authored with Jeff Hawkes, Alistair Lough, Douglas Connelly, Pamela Rossel, Eric Achterberg and Thorsten Dittmar. All samples were collected by me on the East Scotia Ridge expedition and by Jeff Hawkes on the Caribbean expedition. The particulate organic carbon analysis was done by myself. Jeff Hawkes was the lead author on the chapter, although I analysed all data relating to particulate organic carbon and contributed to writing of the whole chapter. Chapter 5 was co-authored with professor Alex D. Rogers, Svein-Ole Mikalsen, and Helge Ansgar-Giebel. Helge Ansgar-Giebel did the flow cytometry analysis of this chapter. Leig Marsh was kind enough to lend me one of her high resolution bathymetric maps of the East Scotia Ridge stations, featured in fig. 5.1.

I hereby confirm that no part of my thesis has been accepted or is currently being submitted for any degree, diploma, or certificate or other qualification in this University or elsewhere.

Anni Djurhuus
June 2015
OSS Student No. 575693

Abstract

Microbial biogeography is being increasingly more studied, both in terms of genetic divisions and 'ecotype' variation. This thesis investigates the regional (100-1000s km) and local (10s m) distribution and diversity of microorganisms around hydrothermal vents and seamounts at the East Scotia Ridge and the Southwest Indian Ridge.

Microbial communities were characterized using Illumina dye sequencing to determine taxon richness and diversity and flow cytometry to obtain cell counts. In addition I investigated the physicochemical environment (nutrients, organic carbon, salinity and temperature) in which the microorganisms persist.

Typical deep-sea microorganisms were abundant at vents and below the euphotic zone on the seamounts. The surface layer of the seamounts contained typical open-ocean photoautotrophic organisms. Microbial communities were correlated to organic carbon on both hydrothermal vents and seamounts. With microorganisms possibly having a large influence on carbon sequestration into the deep-sea from hydrothermal vents.

On a local scale the hydrothermal vents had a relatively higher abundance of chemosynthetic Epsilonproteobacteria and the Gammaproteobacteria family SUP05, which were closely correlated to the redox potential in the vent effluent. This was prominent in both plumes from the East Scotia Ridge and the Southwest Indian Ridge, which also had distinct microbial community structures.

Across the Southwest Indian Ocean the microbial communities were firstly segregated by depth. However, on a regional scale their physical environment primarily divided the microbial communities into three biological regimes the sub-tropical, convergence zone and the sub-Antarctic.

The microbial community structure and biogeography is influenced by steep environmental gradients, displaying a distance-decay relationship between sampling locations. With environmental conditions persisting at different scales, from local (10s m) around the hydrothermal vents to regional (100-1000 km) between the seamounts,

driving the microbial community patterns. The same horizontal and vertical patterns for microorganisms and metazoans points to fundamental differences throughout all parts of the ecosystem/food web.

Acknowledgements

This thesis would not have been successfully completed without the support and friendship of the people surrounding me (ongin nevndur, ongin gloymdur).

In particular I would like to thank Alex Rogers for his role as my principal supervisor and for ensuring my place as a participant on research expeditions to the Southwest Indian Ridge and the East Scotia Ridge creating the fundament upon which my work is based. And to my co-supervisor Svein-Ole Mikalsen and former lecturer Hóraldur Joensen who fought for me being able to bridge the funding gap enabling me to finish my degree and for being great mentors for the duration of my BSc and my DPhil. The Anglo-Danish society, University of the Faroe Islands and the Faroese Research Council provided maintenance funding. The Faroese Research Council and St. Hilda's college funded conference trips and additional research expenses. The Earth Microbiome Project generously sequenced all genetics samples and Helge-Ansgar Giebel and Ian Salter helped with analysis of flow cytometry samples. From the Earth Microbiome project Jack Gilbert, Jarrad Hampton-Marcell and Iratxe Zarraonaindia were a great help with bioinformatics and sequencing. Mya Breitbart provided me with office space away from home and a professional environment where I could develop ideas for my thesis.

To all my friends and colleagues who believed in me and lent me their ear countless times. To my friends who have been so far away yet so close Julia, Anna, and Johanna. To Caitlin for applying her ninja editing skills on my manuscripts and chapters. Vanessa for unicorns and rainbows. Catherine for sharing my rants and Michella for never doubting my abilities as a researcher. To Philipp for reading my thesis and helping me reach my full potential as a scientist!

Lastly and most importantly, I would like to express my deepest gratitude to those closest to me for their love, patience and reassurance. The Boersch-Supaners for their understanding and support. To my grand parents omma abbi líð og omma abbi norð for taking interest in my project. For great inspiration from my almost-big-brothers

Eiríkur and Hannís and our great adventures through black holes and climbing cliffs. To my sisters Kára og Ángela for making my life filled with joy and great purpose. To my parents Emly og Níels Pauli, you have filled my life with possibilities and opportunities and always supported my dreams through the best and worst of times. Last but certainly not least to my dearest husband Philipp for his great friendship, making even this challenging time a happy time. Eg elski teg.

Contents

Declaration	iii
Abstract	v
Acknowledgements	vii
List of Figures	xiii
List of Tables	xix
1 Introduction to the ecology and biogeography of marine microorganisms	1
1.1 Introduction	3
1.2 Marine microorganisms	4
1.2.1 Advancements in the field of marine microbiology	4
1.2.2 Bacteria	6
1.2.3 Archaea	7
1.3 Ecological roles of marine microorganisms	8
1.4 Biogeography of microorganisms	9
1.4.1 Bacterial and archaeal biogeography	11
1.4.2 Seamount biogeography of microorganisms	14
1.4.3 Hydrothermal vent biogeography of microorganisms	14
1.5 Study sites	16
1.5.1 The South West Indian Ridge	16
1.5.2 The East Scotia Ridge	18
1.6 Project aims and objectives	19
1.6.1 Seamounts	20
1.6.2 Hydrothermal vents	20
2 The spatial distribution of particulate organic carbon and microorganisms on seamounts of the South West Indian Ridge	23
2.1 Introduction	25
2.2 Methods	28
2.2.1 Sampling	28
2.2.2 Transmission measurements	29
2.2.3 Elemental analysis	30

Contents

2.2.4	Fluorescence activated cell sorting	31
2.2.5	Microplankton biomass	32
2.2.6	Data and statistical analysis	32
2.3	Results	33
2.3.1	Hydrographic setting	33
2.3.2	POC:cp regression	34
2.3.3	POC and pPOC concentrations	35
2.3.4	Microbial abundance and biomass	40
2.4	Discussion	43
2.4.1	Local and regional distribution of POC	43
2.4.2	Provenance of POC	45
2.4.3	Seamount influence on particle and POC dispersal	48
2.5	Conclusion	49
3	The biogeography of prokaryotes around seamounts in a dynamic frontal system	51
3.1	Introduction	53
3.2	Materials and methods	56
3.2.1	Sampling	56
3.2.2	Elemental analysis and Fluorescence activated cell sorting . . .	57
3.2.3	Illumina MiSeq sequencing and preparation	57
3.2.4	Bioinformatics	58
3.2.5	Data analysis	59
3.3	Results	60
3.3.1	Depth distribution of microbial communities	60
3.3.2	Regional biogeography between seamount microorganisms . .	64
3.4	Discussion	71
3.4.1	Depth distribution of microbial communities	71
3.4.2	Regional biogeography between seamount microorganisms . .	76
4	Organic matter in deep-ocean hydrothermal systems	79
4.1	Introduction	81
4.2	Methods	82
4.2.1	Sampling strategy and methods	82
4.2.2	Analysis	86
4.3	Results	90
4.3.1	Vent geochemistry	90
4.3.2	Organic carbon and nitrogen concentrations	90
4.3.3	Molecular composition of SPE-DOM	91
4.4	Discussion	96
4.4.1	Organic carbon and nitrogen concentrations	96
4.4.2	Molecular characteristics of SPE-DOM	99

4.5	Conclusions	100
5	Cutting through the smoke: Free-living bacterial diversity in deep-sea hydrothermal plumes	101
5.1	Introduction	103
5.1.1	Hydrothermal vents – importance of microbiota	103
5.1.2	Vent endemism and patterns of biogeography	105
5.2	Methods	106
5.2.1	Field site	106
5.2.2	Sample collection	108
5.2.3	Elemental Analysis	109
5.2.4	Flow cytometry	110
5.2.5	Illumina MiSeq sequencing and preparation	110
5.2.6	Bioinformatics of sequencing data	111
5.2.7	Data analyses	111
5.3	Results	112
5.3.1	Vent setting and bacterial abundance	112
5.3.2	16S rRNA gene-based assessment of bacterioplankton community structures	113
5.4	Discussion	122
5.4.1	Ecological impact on vent microbial communities	122
5.4.2	Diluting microbial communities	124
5.4.3	Vent biogeography	125
6	Final remarks	127
6.1	Key findings	129
6.1.1	Spatial scales of microbial community turnover	129
6.2	Outlook	133
	Bibliography	137
A	Supplementary material for chapter 3	165
B	Supplementary material for chapter 5	169

Contents

List of Figures

1.1	A simplified model of the ocean's food web showing the dominant role of the microbial loop. The major fluxes of carbon and energy are delineated by solid lines and the mixing/transport of matter is in dashed lines. Nutrients are in blue boxes. Consumers of primary producers are in red boxes. The boxes represent parts of the microbial loop (green=photosynthetic and yellow=heterotrophic) (Pomeroy and Darwin, 2007).	10
1.2	The effect of the processes on the relationship between compositional similarity and spatial distance. A) Selection and drift increase the strength of the distance-decay relationship. B) Dispersal weakens the distance-decay relationship (flattens the slope). C) Mutation decreases the similarity between locations, regardless of the distance between them. Figure is from Hanson et al. (2012)	11
1.3	Relationship between genetic and geographic distance ($R^2 = 0.98$). π , number of base substitutions per site from sequences. Figure is from Mino et al. (2013).	16
1.4	Map of the study area with the Polar front (PF), sub-Antarctic front (SAF), and sub-tropical front (STF). Hydrothermal vent stations are white triangles. Seamounts are black triangles and background is white circle. BVD: Beebe and Von Damm. E2 is the segment with hydrothermal vent investigated on the East Scotia Ridge. SWIR: South West Indian Ridge.	18
2.1	Eight-day composite (24 November 2011) of sea-surface height from AVISO. On RRS James Cook cruise 66, sub-surface data were not collected across the fronts but data collected in 2009 (Read and Pollard, In press) found the boundaries of the Agulhas Return Current (ARC), Subtropical Front (STF) and SubAntarctic Front (SAF) to be roughly synonymous with the 90-110 cm, 30 cm, 10 cm, and -10 cm isolines, respectively, indicated here with bold dashed lines. The topography is outlined by the 3000 m (solid) contours. The axis of the Southwest Indian Ridge runs from Coral Seamount to Atlantis Bank.	30

List of Figures

2.2	Hydrological conditions of Coral, Melville, Middle of What and Atlantis seamounts. The lower measurements of salinity and temperature are seen on Coral (A). The fluorescence is portrayed to 200 m (B), with the deep chlorophyll maximum of Atlantis seen at 90 m and the highest maximum of Coral at ~40m. Figure C and D show the average nutrient and c_p (particle) concentrations of all casts on all seamounts down to 2000 m. NB: Dashed and solid lines represent different variables.	36
2.3	Linear models of microbial abundance and the beam attenuation coefficient, c_p (A), Shallow POC and c_p (B) and deep POC and c_p (C). Formulas for the linear models are portrayed on the individual figures, with the microbial abundance and shallow carbon models being significant. The solid lines signify the model formula with the dashed lines representing the 95% confidence intervals. The colours represent the four different seamounts. MoW: Middle of What. POC: Particulate Organic Carbon.	37
2.4	Boxplot showing the three vertical layers sampled for POC measurements on the four seamounts. Dark grey box is in the SW, light grey symbolizes the convergence zone and white is the NE. POC: Observed POC, F_{max} : Fluorescence maximum, EZ: Euphotic Zone, Cor: Coral, Mel: Melville, MoW: Middle of What, Atl: Atlantis. NB: y-axis range is different between top figure and the other two.	39
2.5	Abundance of <i>Prochlorococcus</i> and <i>Synechococcus</i> . Bar plot with the absolute abundances of <i>Prochlorococcus</i> and <i>Synechococcus</i> bacteria on the four seamounts, presented from North to South. The bars represent the standard deviations of the mean abundance.	40
2.6	NMDS ordination plot of the phytoplankton community composition grouped according to geographical zone (Stress=0.06). The shaded area represents the 95% confidence interval.	41
2.7	From south to north (Coral, Melville, Middle of What and Atlantis) predicted POC at the top 200 m above all four seamounts, with six transmission profiles from surface to bottom on each seamount. The grey shaded area roughly represents the shapes of the seamounts. The horizontal dashed lines on each seamount represent the carboclines. N.B. The transmission profiles have different scales in the euphotic zone and below the euphotic zone. There is a gap in the depth profile between the upper 200 m and the sea-bed.	42
3.1	Map showing the sampling locations for the four sampling stations on the South West Indian Ridge. The solid line represents the 1000 m contour. The dashed lines are the Agulhas return current, sub-Antarctic and sub-tropical front (from top to bottom). MoW is Middle of What.	57

3.2	Abundance of microorganisms between the four seamounts, Coral (Cor), Melville (Mel), Middle of What (MoW) and Atlantis (Atl). Boxplot is separated into the shallow (A, 40-80 m), middle (B, 200 m) and deep (C, >200 m) water column. Sapmer is not visualized.	62
3.3	Richness plot of all samples from the four seamounts, Coral (Cor), Melville (Mel), Middle of What (MoW) and Atlantis (Atl). The richness plot is separated into the shallow (A, 40-80 m), middle (B, 200 m) and deep (C, >200 m) water column. Sapmer is not visualized.	65
3.4	Species accumulation curve on a per sample basis for all five seamounts.	66
3.5	NMDS plot between the three depth layers (shallow=40-80 m, middle= 200 m, deep=>200 m). The ellipses represent the 99% standard error of the individual depth layers. MANOVA = Multivariate Analysis of Variance. Stress = 0.1225.	67
3.6	Barplot of the relatively most abundant microbial classes between depths (shallow=40-80 m, middle= 200 m, deep=>200 m) on all seamounts. MoW is Middle of What.	68
3.7	A) Multivariate Regression Tree (MRT) on all biological and physical variables measured in this study. Latitude and depth were the only significant variables creating five clusters, presented on the seamount. B) samples are coloured according to the clustering by the MRT. . . .	69
3.8	Canonical correlation analysis (CCA) divided into the three depth layers (shallow=40-80 m, middle= 200 m, deep=>200 m), coloured by station. Each CCA in a depth layer is followed by the environmental variables correlated to the microbial communities on those seamounts. Seamounts and environmental variables are plotted on the same scale on each depth. Only variables with a higher significance than 0.01 were visualised. The ellipses represent the 99% standard error. MANOVA = Multivariate Analysis of Variance.	70
3.9	Rank abundance between the 50 most abundant OTUs on Coral, Melville, Middle of What and Atlantis. The rank abundance plots are divided between the three depth layers (shallow=40-80 m, middle= 200 m, deep=>200 m). Taxonomy of certain OTUs is specified on the left side of the figure. The letter for each OTU changes according to rank (in brackets) on that specific seamount. The line thickness values are based on a rescaling of all OTU values for all stations to each other represents the change in relative abundance of an OTU from one station to the next (see legend).	73
4.1	A: Map showing the location of the three vent sites and a background site studied in our research. B: Photograph taken by Remotely Operated Vehicle (ROV) showing the sampling of a high temperature vent at the Beebe vent site.	84

List of Figures

4.2	Concentrations of various analytes in the dispersing plumes of the three sites. All plots are shown vs. $\log[\text{ppm vent fluid}]$, and the fluid samples taken by ROV are separated by a dashed line. A) TOC and DOC (note log scale). The red line shows predicted conservative mixing at the Beebe site. Errors are smaller than the data symbols. B) SPE-DOC and extraction efficiency. C) POC. D) NO_3^- and TON.	92
4.3	Box plot comparing the distribution of TOC concentrations taken by CTD only at the four sites. The background site samples were taken at depths similar to the Beebe site. The E2 site is in the Atlantic sector of the Southern Ocean and has lower ambient TOC concentrations. . . .	93
4.4	Trends in DOM molecular composition compared with hydrothermal influence (PC1) in the sample set. Green = plume samples from all three sites, black = E2, red = Beebe, blue = Von Damm. A: average molecular weight and average number of sulphur atoms per molecule B: average number of carbon atoms per molecule and average number of oxygen atoms per molecule C: Average double bond equivalence (closed symbols) and average modified aromaticity index.	94
4.5	A) Principle Component separation of the sample set. B) Correlation of individual molecular formula with PC1 (top panel) and PC2 (lower panel) in Van Krevelen space - red signifies positive correlation along the PC, blue negative.	95
4.6	Close up view of TOC concentrations at Beebe (black diamonds) and DOC concentrations where available (TOC-POC). The predicted concentration is shown as a dashed line, based on mixing between the hydrothermal and background end-members, with the first and third quartile of the background samples also modelled as dotted lines. The first to third quartile of the Beebe TOC data are shown as a separate bar in blue.	96
4.7	Van Krevlen diagrams showing changes to molecular composition between a typical plume sample and high temperature fluid sample at E2. A: Molecules lost from seawater (not present in fluid) B: Molecules present in fluid but not plume water. C: Molecules present in both – red colouring signifies a trend (significant to 0.05 level) to higher concentrations (SPEDOC corrected) in the fluid, blue signifies a trend to higher concentrations in the plume.	97
5.1	A) Map showing the locations of samples in the southern ocean and Indian Ocean. PF: Polar Front. B) Shows the topography of the ESR vents at the E2 segment. The two vent stations are 43 and 44, the two diffuse vent stations are 2 and 130.	107

5.2	Eh, LSS and temperature from CTD casts from the SWIR and the ESR. Figure a) shows the concentrations of LSS and temperature at the Longqi vent and background. Figure b), c) and d) show the temperature and Eh potential at the background, diffuse vents (cast 2 and 130) and Dog's Head (cast 43 and 44), respectively.	114
5.3	The boxplots show the abundance of bacteria between the Longqi and Dog's Head vent fields and their respective background samples (a), and between the four CTD casts collected from the ESR representing diffuse venting and black smoker venting (b).	115
5.4	Hierarchical clustering dendrogram with 0.2 μm and 3.0 μm filters. We designate the dendrogram into three groups, which represent background samples (a), vent samples (b) and diffuse vent samples (c). Barplots for each sample show the community composition of the most abundant phyla. For clarity only OTUs that were observed more than two times and in more that 50% of the samples are shown.	116
5.5	Barplot of Gamma- (a) and Epsilonproteobacteria (b) abundances from background to diffuse vent to black smoker vents according to increase in Eh potential. For clarity only OTUs that were observed more than two times and in more that 50% of the samples are shown.	117
5.6	Linear model of Epsilonproteobacteria sequences and Eh between the two Dog's Head casts from the ESR.	118
5.7	a) shows the ordination of all seven CTD casts with their 99% standard error (ellipses). Figure b) shows the four CTD casts at the ESR and their environmental variables.	120
6.1	Non-metric multidimensional scaling plot of all microbial community samples collected from this study, showing four clusters, mostly by depth layer and vent samples.	133
A.1	Multivariate Regression tree error output. The top plot is a histogram of how often the optimal tree is selected. The bottom graph displays the cross-validated error between the tree sizes. Optimal tree sizes were between 5-11. The most parsimonious were tree sizes 5 and 9. . . .	167
B.1	Species accumulation curves from the vents and backgrounds (bg) of the East Scotia Ridge (ESR) and Southwest Indian Ridge (SWIR)	171
B.2	Boxplots of richness between the individual CTD casts from the East Scotia Ridge (2,130,43 and 44) and the South West Indian Ridge (1, 11, and 18)	171
B.3	Barplot of proteobacteria (a) and the Gammaproteobacteria family SUP05 (b) between all samples.	172
B.4	Unifrac PCoA plot between all samples colored by filter type.	173

List of Figures

List of Tables

2.1	The table lists the location and how many samples where collected on each seamount. POC: Particulate organic carbon. FC: Samples for flow cytometry counts.	29
2.2	The table shows the concentrations of the integrated predicted POC (pPOC) per cubic meter in the euphotic zone and the total observed POC for the fluorescence maximum (f_{max}) and bottom of euphotic zone (EZ) layers. Presented below the total POC is the relative carbon, calculated from the microbial cells, to the total POC.	38
2.3	Relative amount of POC near seabed on seamounts. The table shows the water depth at the position the CTD cast was done and the per cent POC at seabed relative to surface POC of that cast. WD: Water Depth. MoW: Middle of What.	38
3.1	Overview of samples collected at the five seamount locations. FC is flow cytometry samples. DNA, FC and Nutr. (nutrients) are number of samples. WM: Water Mass. Subant.: Subantarctic, subtrop.: Subtropical/	60
3.2	Overview of the most abundant orders-families of OTUs in the three depth layers and stations. Sequences identified to family are in italics. Letters in brackets represent unique OTUs of the same order/family but different species (undetected). The two most abundant OTUs found in this study are in bold.	63
3.3	Bray-Curtis dissimilarity index between shallow depth strata of the different stations. Values scale from 0-1, with 0 being the most similar and 1 the least.	72
3.4	Bray-Curtis dissimilarity index between middle depth strata of the different stations. Values scale from 0-1, with 0 being the most similar and 1 the least.	72
3.5	Bray-Curtis dissimilarity index between deep depth strata of the different stations. Values scale from 0-1, with 0 being the most similar and 1 the least.	72
4.1	End-member concentrations and samples taken from the three vent fields. VD: Von Damm. SW: Seawater.	90

List of Tables

5.1	Properties of vent chemistry at Dog's Head, Longqi and the ESR diffuse vent. Data is from (Bach et al., 2002; Peng et al., 2011; James et al., 2014; Tao et al., 2012)	108
5.2	Sample overview. T is temperature, FC is flow cytometry, POC is Particulate Organic Carbon and bg are background samples. Depth is the vent depth or seafloor depth for background sites.	109
5.3	Jaccard and Bray Curtis (brackets) similarity indices between averaged samples of each cast indices scale from 0-1, 0 being most similar and 1 having no shared OTU's. Bg: background; DV: diffuse vent. The highest and lowest similarity between casts is highlighted in bold type.	121

Chapter 1

Introduction to the ecology and biogeography of marine microorganisms

Chapter 1 Introduction

1.1 Introduction

The ocean covers approximately 71% of the Earth's surface and the deep oceans represent approximately 90% of the habitable volume for life on Earth. The biodiversity and ecology of these ecosystems are poorly understood. The world's oceans are teeming with microscopic life forms, representing an enormous pool of diverse microbes with a great variety of functions. Communities of bacteria, archaea, and phytoplankton account for most oceanic biomass (Kirchman, 2008; Alderkamp et al., 2006). Ecologically, microbes play a vital role in marine food chains and global nutrient cycling and are involved in virtually all geochemical reactions occurring in the oceans (Arrigo, 2005; Rinta-Kanto et al., 2011). Although these marine microbes account for very large fluxes of energy and matter, which influence the whole biosphere (e.g through oxygen production), their genetic and metabolic diversity are just starting to be explored, making it possible to start connecting different microbes to ecological questions (DeLong et al., 2006; Pommier et al., 2007; Rusch et al., 2007; Sunagawa et al., 2015). Bacterial cell counts exceed 10^6 cells/ml in surface seawater. It has been estimated that in total the oceans harbour 3.6×10^{29} microbial cells with a total cellular carbon content of $\sim 3 \times 10^{17}$ g (Kirchman et al., 2009; Whitman et al., 1998). Some of the organic matter fixed by marine autotrophic microbes enters the food webs, where it remains for variable time periods adding to other processes which drive the oceans to act as carbon sinks. Transfer of organic carbon from the dissolved fraction to higher trophic levels in a linear food chain via bacteria, heterotrophic protists, and ciliates was formalized as the microbial loop concept (Azam et al., 1983). Bacterioplankton constitute a potentially important food resource that, in terms of total biomass, is on par with phytoplankton (Kirchman et al., 2009; Kirchman, 2008). In environments where chlorophyll is low, such as the deep sea, bacterial biomass becomes an increasingly important component of the total plankton biomass (Kallmeyer et al., 2012; Mendonca et al., 2009).

Despite recent progress, our understanding of the biological properties and vertical and geographical distributions of planktonic microbial groups is far from complete. Acknowledging the importance of marine microbes to food chains, nutrient processing and their effect on the whole ecosystem, this project seeks to investigate marine microbial community structure across multiple spatial scales in the ocean. The data gathered from this aims to fill gaps in our knowledge of the marine ecosystem in gen-

eral but focuses on the South West Indian Ocean as a region particularly suited to examination of variation in biological communities across different water masses and different local conditions. In addition, the East Scotia Ridge hydrothermal vents provide an ideal study site, where distribution of microorganisms can be directly observed on a local scale in hydrothermal plumes.

1.2 Marine microorganisms

1.2.1 Advancements in the field of marine microbiology

Microbial life in the oceans comprises diverse communities of living organisms including viruses, bacteria, Archaea, fungi, nanoflagellates and phytoplankton. Until recently, our understanding of microbial taxonomical, physiological and biochemical diversity has largely come from the less than 1% of microorganisms, which can be maintained by enrichment or cultivation (Rusch et al., 2007). To date several attempts have been made to divide prokaryotes into species or functional groups (Kirchman, 2008). Most of the best-known prokaryotes have been given names using the binomial system, according to this division there are more than 80 phyla with ~7,000 species of bacteria and archaea described. The current estimate of the total number of prokaryotic “species” lies somewhere between 100,000-1,000,000, which indicates, that an accurate estimate of prokaryotic species is really beyond comprehension given our current understanding and technology (Clark et al., 2009). Gene surveys are increasingly used to study highly diverse microbial communities with next generation sequencing based on the highly conserved 16S rRNA gene (Caporaso et al., 2011). In spite of technical advances that have provided increases in orders of magnitude in sequencing coverage, microbial ecologists still struggle with how to interpret the genetic diversity represented by the 16S rRNA gene. Two widely used approaches put sequences into groups based on either their similarity to reference sequences (i.e., phylotyping) or their similarity to other sequences in the community (i.e., Operational Taxonomic Units (OTUs)).

The 16S rRNA gene can be used as a phylogenetic marker to classify organisms and place them in an evolutionary context and commonly, organisms whose 16S rRNA gene sequences are $\geq 97\%$ identical, are considered to be the same Operational Tax-

onomic Unit (OTU) (Stackebrandt and Goebel, 1994), often referred to as “species”, ribotypes, or phylotypes (Santoferrara et al., 2014; Rusch et al., 2007). The relationship between sequence abundance and cell abundance is not always straightforward and the problem of multiple copies of the 16S rRNA gene from the same cell on the evaluation of abundance has been raised. However, most sequencing errors are removed by subsequent analysis, although multiple copy numbers has not yet been addressed (Caporaso et al., 2011; Schloss et al., 2011; Quince et al., 2011). So called next generation sequencing techniques produce thousands of short (~150 basepair) sequence reads requiring bioinformatical tools to align and identify these reads (Caporaso et al., 2010b). After pre-processing and alignment the high quality reads can be taxonomically assigned by comparing to reference databases, such as the ribosomal database project (RDP)(Huse et al., 2008) to obtain community diversity and an approximate relative abundance of the different OTUs. Another issue with next generation sequencing is the specificity of the primers, which does not pick up 100% of the different microbial species. Also, during sequencing, singletons (unique individual sequences) will be abundant in a microbial dataset. Because of the less than 100% sample coverage from sequencing, and abundant singletons, patterns of the rare microbiome is problematic to address. However, the abundant microbiome should be well represented in a sequence dataset as the likelihood of an abundant microorganisms DNA to start amplifying during sequencing is high (Caporaso et al., 2010b). Using next generation sequencing the quantitative abundance of the microbial communities is compromised. Unable to know what the actual abundances of the individual OTUs are, we suggest coupling sequencing with quantitative counts. For abundance measures flow cytometry is the most common, efficient, and accurate method. However, there are limitations on the taxonomic resolution from a flow cytometer and this only gives us abundance estimates of large groups such as nanoeukaryotes, picoeukaryotes, heterotrophs, and autotrophs. From counts of individual microbial groups information such as carbon can be extracted using previously found conversion factors. These conversion factors allow us to get general comparable data between the groups, although these are not based on the individual cells, which vary a lot depending on shape and species. Any flow cytometric analysis can be verified using traditional methods such as epifluorescence microscopy. However, because of the high counts and diversity of individual cells in environmental samples it is incredibly labourious to count and measure the individuals for carbon content estimates. Thus, the preferred methods

used are general conversion factors for carbon estimates coupled with flow cytometric counts, complemented by large scale sequencing for a more detailed perspective on the microbial communities in oceanic environments.

1.2.2 Bacteria

Autotrophic bacteria

Autotrophic bacterial carbon metabolism is based on either energy from light (photoautotrophy) or energy from chemical substances (chemoautotrophy). These organisms fix carbon from inorganic compounds, such as CO₂.

In the euphotic zone where light levels are sufficient for photosynthesis to occur, the cyanobacteria (e.g. *Prochlorococcus* and *Synechococcus*) are abundant. They comprise up to 25% of all microorganisms on Earth (Ladau et al., 2013). In terms of genetics and ecology the marine cyanobacteria are probably the best understood group of marine bacteria. Many members of these groups have not only been cultivated, but have also had their entire genome sequenced (Honda et al., 2014). All known members of this group possess chlorophyll *a* and perform oxygenic photosynthesis (Ting et al., 2002). From the genus *Prochlorococcus* cell abundances have been reported in the order of 10⁵ cells/mL in some warm oligotrophic environments mostly ranging from the subtropics to tropical seas. *Prochlorococcus* are small (0.4-0.8 µm) and have a cell carbon content that ranges from 45-94 fg C/cell (Sieracki et al., 1994). Genotypic and phenotypic properties of vertically stratified *Prochlorococcus* “ecotypes” are suggestive of depth variable adaptation to light intensity and nutrient availability (Rocap et al., 2003; Scanlan and West, 2002). *Synechococcus*, a close relative of *Prochlorococcus*, is also common in seawater but has slightly larger cells averaging at 0.9 µm (Sieracki et al., 1994). Most *Prochlorococcus* and *Synechococcus* are thought to have different ecological strategies (Scanlan and West, 2002). *Prochlorococcus* is able to compete successfully against other bacterioplankton for the uptake of amino acids and might therefore be considered somewhat photoheterotrophic (Kirchman et al., 2009). Typically chemoautotrophic bacteria, such as nitrifying or sulphur-oxidising bacteria endure in extreme environments, e.g. hydrothermal vents and sulphur springs. Noteworthy examples of these are bacteria from the phyla Gammaproteobacteria and Epsilonproteobacteria. These are persistently found around hydrothermal vents and are

important resource providers for hydrothermal vent animals. In similar ways both chemotrophs and phototrophs play vital roles in the marine ecosystem carbon fixation processes within marine ecosystems, as they are predominant primary producers in their respective habitats.

Heterotrophic bacteria

Heterotrophic bacteria are the most abundant bacteria in the ocean. As much as 86% of the total planktonic community on seamounts in the Northeast Atlantic was comprised of heterotrophic bacteria during summer and winter months (Mendonça et al., 2012). Of all the heterotrophic bacteria the dominating bacterioplankton in surface marine environments are the Alphaproteobacteria, Gammaproteobacteria, and Bacterioidetes (Fuhrman et al., 2006; Nelson et al., 2014; Lefort and Gasol, 2013; Alonso Gutiérrez et al., 2009; Alonso-Sáez et al., 2007b; West et al., 2008). Heterotrophs can be divided into photoheterotrophs, chemoheterotrophs and organotrophs, all consume energy from organic carbon compounds pre-formed by other organisms. Photoheterotrophic organisms include the facultative heterotrophic autotrophs (e.g. some *Synechococcus* and *Prochlorococcus*), the Aerobic Anoxygenic Phototrophs and the group that uses bacterial rhodopsin as their light-harvesting pigments. Chemoheterotrophs are unable to fix carbon to form their own organic compounds and must utilise inorganic compounds for growth and proliferation. An important member of the Chemoheterotrophs is the ubiquitous SAR11 of the Alphaproteobacteria (Carini et al., 2014). The organotrophic microorganisms get their nutrition from organic material, e.g. from faeces or by breaking down decaying matter of other organisms. The families Pseudoalteromonadaceae and Alteromonadaceae from the Gammaproteobacteria and the Crenarchaeota are important microorganisms in this group (Ivanova et al., 2004).

1.2.3 Archaea

Microorganisms from the archaea domain are phenotypically diverse organisms. Planktonic marine archaea are divided into four major groups within the two phyla, *Crenarchaeota* and *Euryarchaeota*. The most abundant of these groups are the Marine Group I (*Crenarchaeota*) and Marine Group II (*Euryarchaeota*).

Surveys in temperate waters off the coast of California have shown that planktonic *Crenarchaeota* are most abundant below 100 m. Similar studies have demonstrated that *Crenarchaeota* represent more than 20% of the total microbial cells in water from 80-3,000 m and more than 30% from 200-5,000 m (DeLong, 2003) (Chapter 3). These studies strongly indicate that *Crenarchaeota* account for a large fraction of the total microbial biomass and archaeal contribution to carbon cycling and the microbial loop of deep oceans might be significantly higher than previously estimated (Azam and Malfatti, 2007).

1.3 Ecological roles of marine microorganisms

As obligate osmotrophs bacteria and archaea are confined to utilise relatively small solutes (<500 Dalton). Upwelling from the deep sea brings inorganic nutrients to areas with sufficient light for phytoplanktonic and photosynthetic bacterial growth. These two groups of organisms release DOC (low molecular weight), which in turn are highly labile substrates for heterotrophic bacteria. Organisms of higher trophic levels then consume the different microorganisms. As cells decompose they often aggregate into particulate organic matter (POM), from which eventually compounds are recycled back into the pools of dissolved organic matter (DOM) and nutrients, usually in the mesopelagic or deep-sea (Anderson and Tang, 2010). The DOM can be further decomposed by bacterioplankton or spontaneously form POM by aggregating together with metazoan faeces (Anderson and Tang, 2010). Thus energy released as DOM by phytoplankton is rather inefficiently returned to the main food chain via a microbial loop of bacteria-flagellates-microzooplankton (Azam, 1983) (Fig. 1.1).

Polymers such as polysaccharides and protein comprise a large (20-30%) fraction of the bulk pool of dissolved organic matter in the oceans. The complexity and size of these biopolymers precludes direct microbial transport, requiring mobilization of hydrolytic enzymes to cleave the polymeric substrates into monomers that are readily transported into the cell. The labile nutrients in seawater thus affect the growth of microorganisms and their necessity for producing extracellular enzymes. Of the bacterial biomass, up to 10-20% may be attached to particles; thus the majority of the bacterial fraction is free-living bacterioplankton (Ghiglione et al., 2007). However, the contribution of attached bacteria to total bacterial production can be high (>50%)

(Ghiglione et al., 2007). The rate at which sinking POM is solubilized and mineralized affects the length scale of carbon delivery in the water column, and thus has a major impact on ocean carbon fluxes (Anderson and Tang, 2010). Attached bacteria present a range of extracellular enzymes, therefore the bacterial hydrolysis and degradation of POM plays an important role in those fluxes. POM accumulates bacteria that mobilize enzymes and begin to digest the particle while multiplying (De La Rocha and Passow, 2007). The largest, heaviest POM particles fall into the depths of the ocean before being utilized by sediment bacteria, but the majority disintegrate in the upper 50–100 meters.

1.4 Biogeography of microorganisms

Biogeography is the descriptive and explanatory study of spatial patterns and processes in the distribution of biodiversity. A prominent idea among microbiologists since the 1930s was that free-living marine microorganisms should, because of their small size, great abundance, and easy dispersal be cosmopolitan, and consequently their global diversity should be low (Fenchel and Finlay, 2004). The proclamation ‘everything is everywhere: but the environment selects’ was propagated by the Dutch microbiologist Martinus Wilhelm Beijerinck in the early twentieth century (De Wit and Bouvier, 2006). In 1934 his compatriot, Lourens G. M. Baas Becking, further developed this concept and throughout the twentieth century this paradigm has been widely used and accepted by microbiologists. In recent years a number of studies have refuted this paradigm by not only demonstrating patterns in microbial distribution but also genetic diversity in microbial populations that are structured by geography as well as environmental parameters (Mino et al., 2013; Read et al., 2015; Kallmeyer et al., 2012; Zinger et al., 2011). Nesbø et al. (2006) suggested that the study of microbial biogeography should find a basis in global gene distribution instead of species distribution. Spatial changes in biodiversity may be examined from gene to community levels, and from local to global scales. Two well-defined spatial patterns of diversity can be observed for all domains of life, including microorganisms: First, increasing taxon richness has been repeatedly observed when considering increasingly larger areas (Ladau et al., 2013; Hatosy et al., 2013a), a pattern referred to as the taxa-area relationship (Zinger et al., 2014). Second, the taxonomic composition of biological assemblages is often

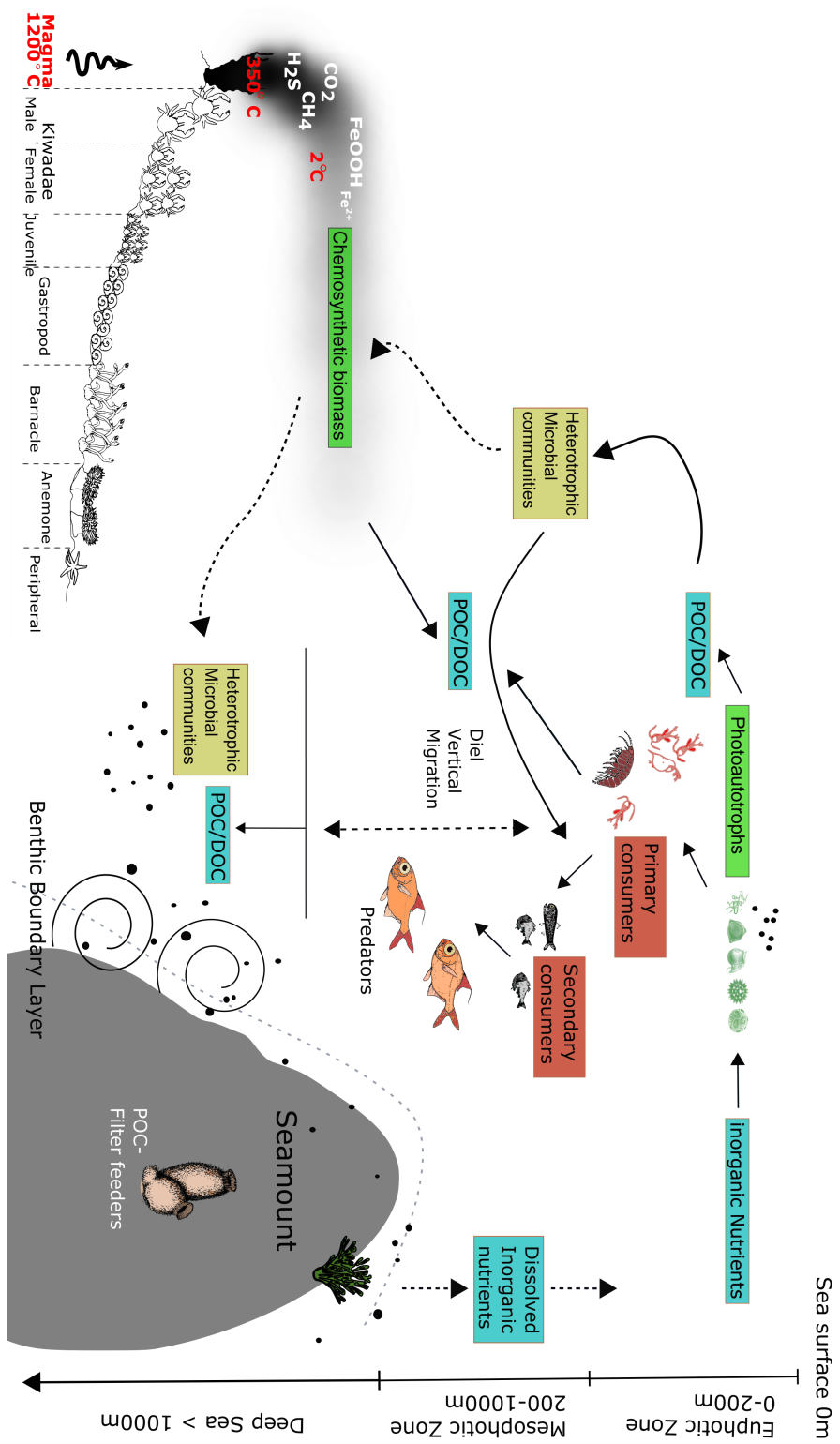


Figure 1.1: A simplified model of the ocean's food web showing the dominant role of the microbial loop. The major fluxes of carbon and energy are delineated by solid lines and the mixing/transport of matter is in dashed lines. Nutrients are in blue boxes. Consumers of primary producers are in red boxes. The boxes represent parts of the microbial loop (green=photosynthetic and yellow=heterotrophic) (Pomeroy and Darwin, 2007).

1.4 Biogeography of microorganisms

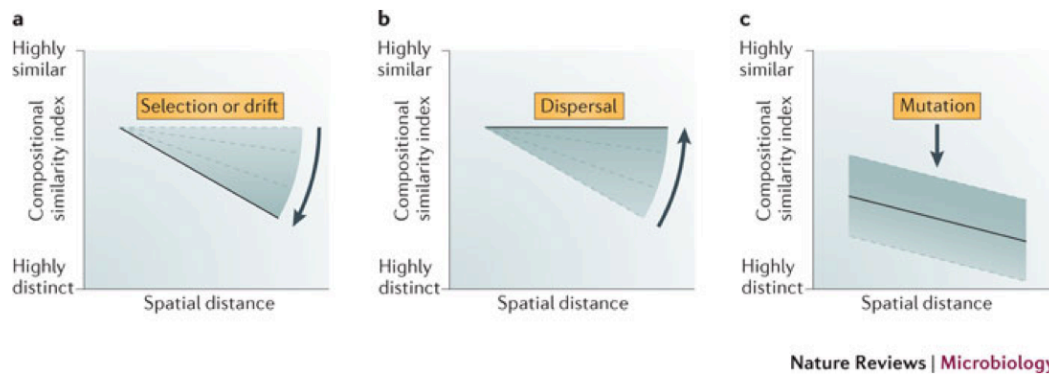


Figure 1.2: The effect of the processes on the relationship between compositional similarity and spatial distance. A) Selection and drift increase the strength of the distance-decay relationship. B) Dispersal weakens the distance-decay relationship (flattens the slope). C) Mutation decreases the similarity between locations, regardless of the distance between them. Figure is from Hanson et al. (2012)

observed as becoming increasingly more dissimilar with increasing geographic distance, a pattern designated as the distance-decay of compositional similarity (Nekola and White, 1999). The biogeography of microorganisms is undoubtedly directed by the evolutionary and ecological interactions of selection, genetic drift, dispersal and genetic mutation (Hanson et al., 2012) (Fig. 1.2). According to Hanson et al. (2012) the distance-decay relationship, should be relatively weak in habitats where dispersal is high, such as in pelagic marine environment, where ocean currents facilitate microbial dispersal. It has also been proposed that genetic drift has a larger impact on microbial biogeography when dispersal is low and populations are small. Distance-decay and taxa-area relationships are frequently observed on local and global scales, this will be investigated in Chapter 3. However, regional biogeography of pelagic environments has not been clearly demonstrated until recently and will be expanded upon in Chapter 5.

1.4.1 Bacterial and archaeal biogeography

Near-surface photoheterotrophic bacteria are dominated by the phyla Alphaproteobacteria, Gammaproteobacteria and Bacteroidetes (Villar et al., 2015; Lima-Mendez et al., 2015; Rusch et al., 2007; Hatosy et al., 2013b). Within the Alphaproteobacteria, the SAR11 and Roseobacter clades are very abundant. Within the Gammaproteobacteria,

the SAR86 and OM60 clades are prominent components of the community, and from the phylum Bacterioidetes, the Flavobacteria group is ubiquitous. Amongst the relatively well-known photoautotrophic bacteria in marine pelagic environments the cyanobacteria, *Prochlorococcus* and *Synechococcus*, are most abundant (Partensky et al., 1999). Bacteria have shown variation in diversity across ocean fronts (Pinhassi and Berman, 2003) and along salinity gradients (Bouvier and del Giorgio, 2002; Crump et al., 2004). Species of *Roseobacter* and *Vibrio*, as well as *Alteromonas*, all have salt requirements for growth and it has been seen in the Baltic sea, where salinity is low, that instead of these species members of the genera *Pseudomonas*, *Shewanella*, and *Sphingomonas* are common (Hagström et al., 2000).

For a few microbial groups, vertical and lateral distributions have been studied. While picoplankton abundance decreases with depth from the euphotic to the bathypelagic layer by about 1 or 2 orders of magnitude to 10^4 cells ml^{-1} , the SAR11 clade and Archaea have been correlated to depth stratification, with SAR11 decreasing and Archaea increasing (Tripp et al., 2008; Giebel et al., 2011; Giovannoni and Stingl, 2005; Giovannoni and Vergin, 2012). The exact nature of the latitudinal gradient in bacteria is still somewhat uncertain because of the substantial unexplained variation seen in OTU richness. However, OTU richness has been correlated with temperature, distance to thermocline and phosphate concentrations (Lin et al., 2012; Ladau et al., 2013; Hatosy et al., 2013a; Sunagawa et al., 2015). Furthermore, bacteria also show seasonal patterns of abundance (Azam, 1983; Fuhrman et al., 2006). Seasonal dynamics of bacterial phyla in the Baltic Sea suggest that alphaproteobacteria are better adapted to low nutrient conditions whilst members of the Flavobacteria are adapted to areas of higher productivity (Pinhassi et al., 2004). In some locations, Archaeal abundance appears to vary with the seasons. For instance, off the Antarctic Peninsula, the pelagic Crenarchaeota (Group I) appear more abundant in surface winter waters and subsurface summer waters. In temperate seas, Marine Group II Euryarchaeota, unlike Marine Group I Crenarchaeota, seem to be more abundant at the surface. The SUP05 and another group of uncultivated sulphur-oxidizing bacteria, SAR324 Deltaproteobacteria, are abundant in the dark pelagic oceans, where they play important roles in linking the sulfur and nitrogen cycles (Ghiglione et al., 2012). Finally, *Halomonas* and *Marinobacter* species are present throughout the oceans and hydrothermal plumes (Kaye et al., 2011). As reported above, studies frequently find both ubiquitous and restricted taxa. Although we must take into account that micro-

1.4 Biogeography of microorganisms

bial endemism is difficult to confirm because many rare taxa will not be detected in a sample because of the limitations in technology for taxon (OTU) identification (Nemergut et al., 2011; Quince et al., 2011). It is important to keep in mind that even if the presence of a taxon is reported, its activity, either as dormant transient or highly active member of the community is not known using OTU-based approaches alone (Hanson et al., 2012). The global patterns described in the literature give an idea of distribution on the scale of thousands of kilometres. In trying to understand the mechanisms that generate and maintain diversity, including OTU richness and community composition, many microbial studies now focus on the processes that drive biogeographical patterns. Microbial biogeography is being increasingly more studied, both in terms of genetic divisions and 'ecotype' variation. Although the existence of microbial biogeography patterns is now well established, little is understood about the processes underlying it (Hanson et al., 2012). However, Finley and Fenchel (2004) showed that most microorganisms that are <1mm in size occur wherever their required habitats are found complying with Bass-Beckings argument '... but the environment selects'. Finley (2002) states that abundance is inversely proportional to body size: and so, for example, the abundance of organisms measuring 10 μm will be about 12 orders of magnitude higher than that of organisms measuring 10 cm. Lawton Jobbágy and Jackson (2000) presumed that the transition phase between small, ubiquitous, and large, restricted organisms to be in the region of 1 mm. Based on a study by Finley and Finlay and Fenchel (2004) the transition phase indeed appears to be within the range of 1-10mm. However, the biodiversity patterns described on morphospecies identification may include different genotypically determined physiological forms, thus it would be rather challenging to apply morphospecies identification of the smallest microorganisms, such as bacteria and Archaea. Bacteria are approximately 1000 times more abundant and 1000 times smaller than the smallest protozoa (Finlay and Esteban, 2001) and, mainly because of the difficulty in deciding what a species is, similar studies as in Finley and Fenchel (2004) with bacteria and Archaea would require alternative methods. Patterns of bacterial plankton abundances and the degree of cosmopolitanism can be collected from studying the abundance of bacterial OTUs. Sogin et al. (2006) performed a study based on 454 pyrosequencing and showed sequencing showed that 33% of the individual sequencing reads were unique, revealing that most bacteria are rare. Similar results were seen in metagenomic shotgun sequencing by Rusch et al. (2007), where 7.7 million sequencing reads were obtained, whereof 57%

were unique OTUs.

As documented by Sunagawa et al. (2015) geographical patterns in microbial distribution do appear and the distance-decay relationship may replace Beijerinck's and Baas Becking's principle as microbiogeography's background assumption (Hanson et al., 2012). The accumulation of genetic mutations in bacteria and Archaea during the past 3.5 billion years should have led to very high levels of genetic variation among marine microorganisms and hitherto, there is evidence of unrecognized physiological groups among the planktonic microbes (Rinta-Kanto et al., 2011; DeLong et al., 2006; Venter, 2004).

1.4.2 Seamount biogeography of microorganisms

Seamounts have not received much attention in the quest to disentangle the biogeography of microorganisms (Mendonça et al., 2012). Several studies have discussed the effect of seamounts as isolating features for macrofaunal distribution, and endemism is rare and restricted to a few sessile taxa with non-dispersive life histories (McClain et al., 2009). Mendonça et al. (2012) report differences in microbial composition between two seamounts and non-seamount samples (Mendonça et al., 2012). However, they found same species at all sampling locations commonly found in the open-ocean and deep sea. Seamount microorganisms are not isolated from the rest of the open-ocean, especially considering microorganisms being passively transported by currents over the seamounts. However, given that seamounts most often occur in offshore, highly oligotrophic waters, away from continental influence, enhancement of vertical fluxes, often associated with steep ocean bottom features, may lead to the injection of nutrients into the near-surface layer, vital to microorganisms (Rogers, 1994) (Chapter 2).

1.4.3 Hydrothermal vent biogeography of microorganisms

Deep-sea hydrothermal plumes occur where seafloor vents inject hydrothermal fluids with potential microbial energy sources such as H_2S , Fe, Mn, CH_4 , and H_2 into the deep oceans. These hot, chemically reduced fluids rapidly mix with cold, oxidizing seawater, forming hydrothermal plumes that rise hundreds of meters off the seafloor and disperse hundreds of kilometres away from their source (Hawkes et al., 2013). The

1.4 Biogeography of microorganisms

impact of deep-sea hydrothermal vents on ocean chemistry involves several processes that are influenced by microbial activities in hydrothermal plumes. First, vents are thought to be a significant source of Fe and Mn to the oceans because their concentrations in hydrothermal fluids are up to 10^6 times that of background seawater (Sander and Koschinsky, 2011; Wankel et al., 2011). A process by which plume microorganisms mediate broader ocean biogeochemistry is chemosynthetic fixation of carbon. Chemosynthetic activity at vents was recognized upon the initial discovery of deep-sea hydrothermal vent ecosystems (Jannasch and Wirsén, 1979) and in early plume studies (Winn et al., 1986) yet the magnitude of chemosynthesis in plumes remains poorly constrained. There is growing recognition that chemical species fuel microbial growth in hydrothermal plumes (H_2 , various sulfur species, ammonium, and iron) (Sylvan et al., 2009). Because only a small fraction of surface organic carbon reaches the deep oceans, the hydrothermal contribution could represent up to 25% of the global deep ocean organic carbon inventory. Indeed, plumes are enriched with organic carbon and disperse them kilometres away from vent sites (Bennett et al., 2011b,a). In Chapter 4 I demonstrate how dissolved organic and inorganic carbon disperses with hydrothermal plume.

Despite the well-recognized importance of microorganisms in the biogeochemistry of hydrothermal plumes, few studies have characterized microbial communities that inhabit them. Thus the physical source, taxonomic composition, and ecological nature of these organisms remain poorly understood. In Chapter 5 I show that the plume-adapted microorganisms dilute linearly with plume concentration. Most commonly the distribution of sulfur-oxidizing Gammaproteobacteria and Epsilonproteobacteria is seen in hydrothermal plumes and background environments. (Fuhrman et al., 2006; Nelson et al., 2014; Lefort and Gasol, 2013; Alonso Gutiérrez et al., 2009; Alonso-Sáez et al., 2007b; West et al., 2008) noted partitioning of distinct clades of sulfur-oxidizing Gammaproteobacteria in vent environments (SUP05 in plumes and animal symbioses) versus others (Arctic96BD-19 in background) and noted that sulfide concentration likely controls the balance of Gammaproteobacteria versus Epsilonproteobacteria. These two groups have also been identified in the dark pelagic ocean (Ghiglione et al., 2012).

Unlike seamounts, the biogeography of deep-sea hydrothermal vents has been relatively well studied and their role as isolating features of microbial communities is increasingly well understood Mino et al. (2013) showed that for some hydrothermal vent

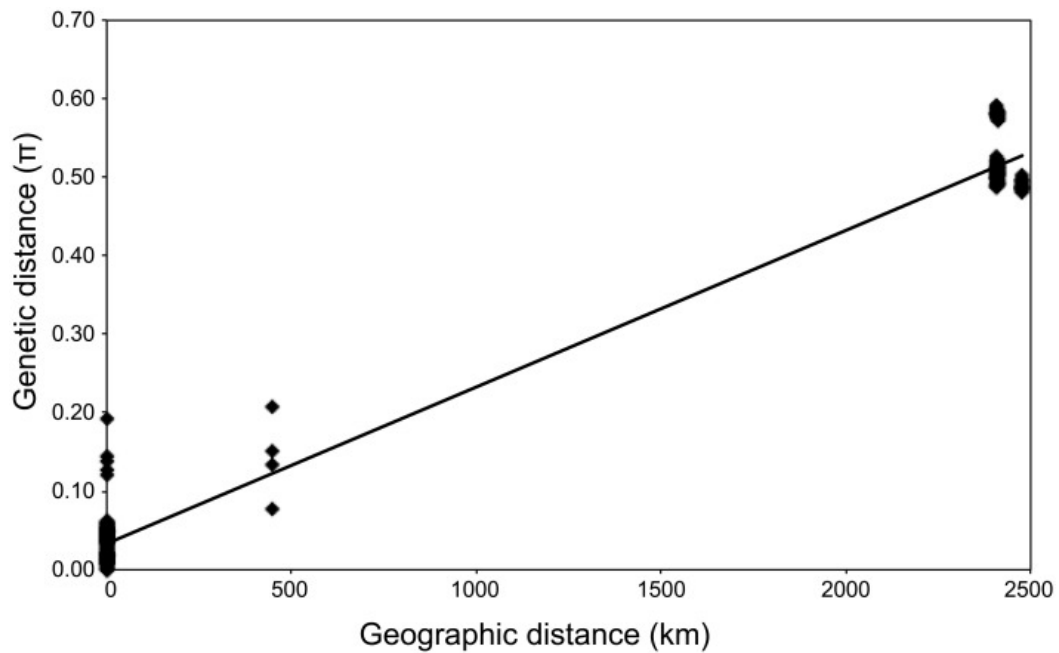


Figure 1.3: Relationship between genetic and geographic distance ($R^2 = 0.98$). π , number of base substitutions per site from sequences. Figure is from Mino et al. (2013).

microorganisms genetic distance was correlated with geographic distance (Fig. 1.3), as has also been described before for thermophilic archaea (Flores et al., 2012; Whitaker et al., 2003).

1.5 Study sites

1.5.1 The South West Indian Ridge

The SWIR is a major oceanic ridge that extends south–westwards from its junction with the Central Indian and Southeast Indian ridges, at approximately 25° S 75° E (Fig. 1.4), to the region between South Africa and Antarctica where it adjoins with the Mid-Atlantic Ridge at the complex Bouvet Triple Junction (Ligi et al., 1999). There are numerous seamounts, banks, and ridge features along its length and hydrothermal activity has been detected at several locations (German et al., 1998). The NE part of the ridge lies beneath the centre of the South Indian Ocean Gyre in a region of weak currents and low primary production. The central and SW sections of the ridge, how-

ever, are influenced by higher current velocities and enhanced primary production. The enhanced primary production results from upwelling in a region of turbulent mixing between the east-going Agulhas Return Current (ARC) and the Southern Ocean circulation (Longhurst, 1995).

This region has thus far been poorly studied (Rogers, 1994) but is notable as an area where the ARC, the Sub-Tropical Front (STF) and the Sub-Antarctic Front (SAF), further to the south, create one of the most energetic and important hydrographic regions of the global ocean (Read et al., 2000) with marked seasonal variation in primary production occurring in the region (Read et al., 2000). In the frontal zone, peak chlorophyll concentrations of $>1 \mu\text{g l}^{-1}$ has been recorded. Outside this region, chlorophyll concentrations have been measured at $<0.9 \mu\text{g l}^{-1}$ (Bornmann et al., in press). It is thought that the stability of the water column, the availability of nutrients (especially iron), and the accumulation of phytoplankton cells all contribute to elevated chlorophyll measurements at the STF and SAF (Lutjeharms, 1985; Weeks and Shillington, 1996).

Hydrological data from the SWIR collected during previous expeditions and as part of the work for the present thesis suggest complex local hydrodynamics (Read et al., 2000; Read and Pollard, In press, Chapter 2). These result in high biological variability with marked changes in the structure of the deep scattering layers (Boersch-Supan et al., 2012), phytoplankton (Mendonça et al., 2012), zooplankton (Martin and Christiansen, 2009), and micronekton communities, communities of aquatic predators (seabirds) and aggregating behaviour of fish (Fock et al., 2002). The seamounts of the ridge lie within an area of complex biogeochemistry, phytoplankton composition and productivity associated with the transition from sub-tropical conditions to sub-Antarctic (Hense et al., 2000; Read and Pollard, In press)). The sub-tropical/sub-Antarctic front is also thought to represent a major biogeographic boundary in the Southern Indian Ocean dividing two distinct faunal provinces (Vierros et al., 2009). In deeper water, the SWIR acts as a major physical barrier to the flow of deep water masses and separates areas of deep-sea floor on the Enderby Abyssal Plain, the Agulhas Basin and the Crozet Basin. Thus, seamounts along the SWIR are likely to be in contrasting productivity regimes and water masses depending on their proximity to the sub-tropical convergence zone and the sub-Antarctic front.

The hydrothermal vent field explored on the SWIR is at the approximate center of the SWIR at $37^{\circ}47'S$ and $49^{\circ}39'E$. The vent field named, called Longqi, was first discovered by the Chinese vessel D/V *Dayang Yihao* in 2007 (Tao et al., 2012) but not

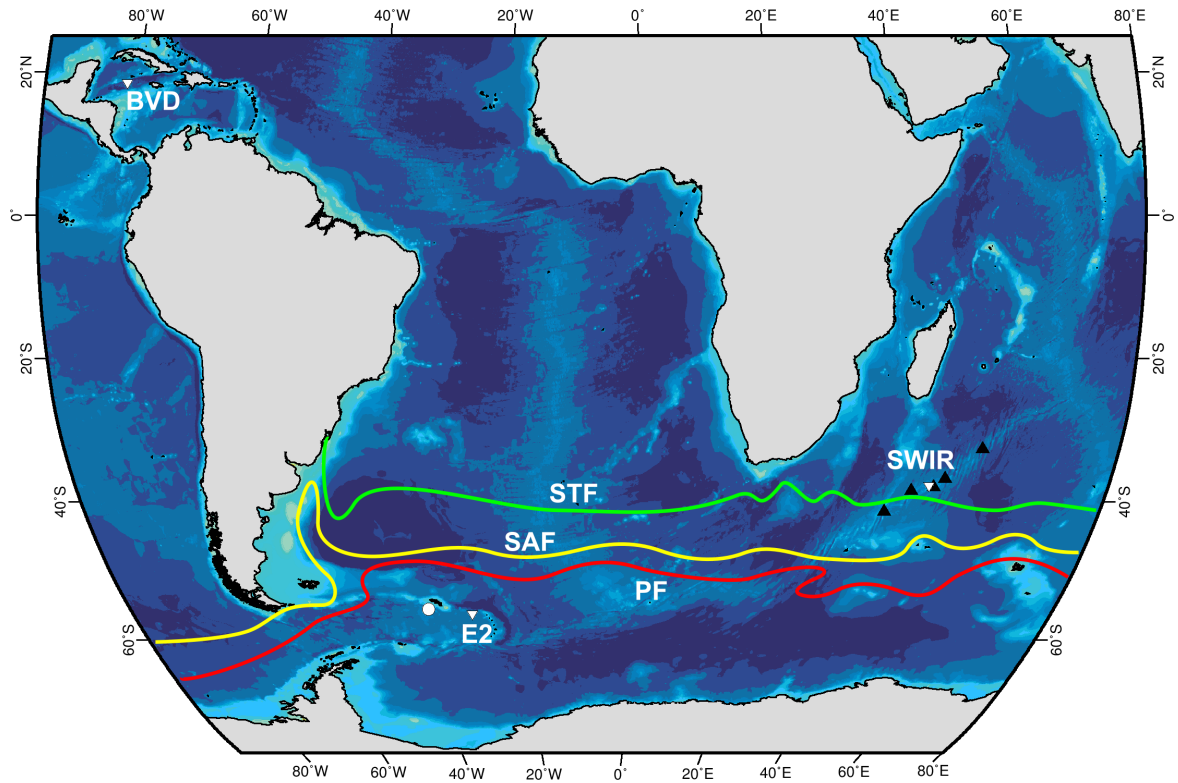


Figure 1.4: Map of the study area with the Polar front (PF), sub-Antarctic front (SAF), and sub-tropical front (STF). Hydrothermal vent stations are white triangles. Seamounts are black triangles and background is white circle. BVD: Beebe and Von Damm. E2 is the segment with hydrothermal vent investigated on the East Scotia Ridge. SWIR: South West Indian Ridge.

surveyed until the JC66/67 cruise where the samples for this thesis were collected. The Longqi vent field is of particular interest because the SWIR is an ultraslow spreading ridge, where hydrothermal activity is expected to remain for a very long time, possibly centuries. Ultraslow spreading ridges were long believed not to sustain hydrothermal activity because of the lack of magmatic supply to the underlying crust. Currently the only other two ultraslow spreading ridges known are the Cayman Trough and the Arctic Mid-Ocean Ridge (Connelly et al., 2012; Pedersen et al., 2010).

1.5.2 The East Scotia Ridge

The Scotia Sea is defined by a loop of shallow banks and islands, known as the Scotia Arc, that extends eastwards from Cape Horn, south of the Falkland Islands (Burdwood

1.6 Project aims and objectives

Bank, Shag Rocks, and South Georgia), then southwards along the South Sandwich Arc, and westwards along the South Scotia Ridge, including the South Orkney Islands, to the tip of the Antarctic Peninsula near Elephant Island. With exception of these peripheral ridges, much of the Scotia Sea extends to depths in excess of 3,000 m. West of the ESR, the floor of the Scotia Sea forms part of the Scotia Plate. The ESR is ~500 km long, and spreading was initiated more than 15 million years ago and is presently proceeding at an average full spreading rate of ~70 mm y⁻¹ (Larter et al., 2003). The ESR consists of nine second-order ridge segments (E1-E9), separated by non-transform discontinuities (Livermore, 2003). E2 and E9 are characterised by smooth volcanic highs, typical of faster-spreading mid-ocean ridges. An axial magma chamber is known to underlie segment E2 (Livermore et al., 1997). The first evidence of hydrothermal activity along the ESR was from data obtained by a light-scattering sensor attached to the Towed Ocean Bottom Instrument, a deep-towed sonar system, during a geophysical mapping survey along the ESR in 1999 (German et al., 2000). Additional evidence was obtained from conductivity, temperature and depth (CTD) profiles and manganese anomalies in water samples collected at depth during that survey (Rogers et al., 2012). For this project I only collected data from the E2 segment of the ESR at vent a vent site between 56° 5.2' and 56° 5.4' S and between 30° 19' and 30° 19.35W at ~2600 m depth. For a comparison between vent fields Beebe and Von Damm of the Caribbean Sea were chosen as outliers. These vent fields are most likely to show contrasting patterns to the ESR vent field based on faster spreading ridge and sedimentation on the crustal geochemistry (Reeves et al., 2014a).

1.6 Project aims and objectives

During austral summer of 2011 and 2012 expeditions (JC66/67 and JC80) were made to the South West Indian Ridge (SWIR) and the East Scotia Ridge (ESR) respectively. The former was a part of the project 'Benthic Biodiversity of Seamounts on the SWIR' and the latter of the 'Chemosynthetic Ecosystems of the Southern Ocean' project.

During recent development in the field of marine microbiology it has been demonstrated that the ocean's microorganisms account for the majority of genetic and metabolic variation in the ocean and the genetic diversity, community composition, relative abundance, and distribution in the sea remain under-sampled. The presented

thesis reports on a quantitative analysis of organic carbon and microbial community structure, diversity, and abundance across four seamounts at the SWIR and two hydrothermal vent systems, one at the SWIR and one at the ESR with a comparative site in the Caribbean. (Fig. 1.4). The thesis is mainly separated into two parts:

1) Microbial biogeography of seamounts and their role in carbon sequestration and distribution.

2) Microbial biogeography of hydrothermal vents and the influence through hydrothermal plumes on ambient water.

We do a comparative study between hydrothermal vents and seamounts where we draw in the environmental parameters, specifically focused on carbon, and microbial communities of these two different systems. From the seamounts and vents we can do a comparison between environmental factors with large scale (seamounts) and small scale (vent plumes) microbial biogeography.

1.6.1 Seamounts

Chapters 2 and 3 explore the general ecology and distribution of particulate organic carbon on four seamounts in the SWIR. This work principally demonstrates how both particles and microorganisms are distributed over the summit and flanks of seamounts, both laterally and vertically. Seamounts along the SWIR are in contrasting productivity regimes depending on their proximity to the sub-tropical convergence and the sub-Antarctic front. Based on the work in this thesis I elucidate how the dominant microbial communities and particulate carbon distribute along this contrasting productivity regime. I investigate regional community differences of microbial communities and how the abundances of the microorganisms contribute to the particulate carbon across the seamounts consisting with the primary productivity regimes suggested previously.

1.6.2 Hydrothermal vents

Chapters 4 and 5 are concerned with the dispersal of carbon and microorganisms from hydrothermal vent plumes. Hydrothermal plumes are contrasting environments to the ambient deep-sea, presenting an ideal study site for habitat adapted microorganisms and the distance-decay relationship with that habitat. I had a similar approach with the organic carbon in these systems. In addition I compare the community structure of

1.6 Project aims and objectives

microorganisms between two different hydrothermal vent fields at the SWIR and ESR to examine the similarities between different vent sites separated at large spatial scales in the Southern Hemisphere. For the carbon measurements the ESR and Beebe and Von Damm at the Caribbean were examined as contrasting sites with the Caribbean vents as outliers to the other sampling stations.

It is hoped therefore that this body of work will contribute to the field of seamount and hydrothermal vent ecology and biogeography at a local and regional scale by examining the general distribution of fundamental participants of these fascinating ecosystems.

Chapter 1 Introduction

Chapter 2

The spatial distribution of particulate organic carbon and microorganisms on seamounts of the South West Indian Ridge

Abstract The ecology and biogeochemistry of oceanic seamounts is not very well understood and explored. Pelagic ecosystems above summits and flanks of seamounts have been observed to show different biological or biogeochemical properties to off-seamount areas, such as enhanced chlorophyll concentrations, a phenomenon referred to as the “seamount effect”. In addition, seamount biogeography has been hypothesised to be similar to islands where community structure differences in multiple organisms have been shown to change between seamounts and across frontal systems.

We used elemental analysis, to measure particulate organic carbon (POC), and flow cytometry, to estimate abundance of microorganisms from above four seamounts (Coral, Melville, Middle of What and Atlantis) along the South West Indian Ridge (SWIR) from latitude 32.6°S to 41.3°S, longitude 57.1°E to 42.7°E. Samples were collected from the surface to the bottom using a CTD fitted with optical sensors. POC was predicted from models created from in-situ transmission (optical) data (c_p). The high resolution predicted POC in the euphotic zone showed a heterogeneous distribution on individual and between seamounts. The shallow penetration of two of the seamounts displayed an effect on the POC concentration in the euphotic zone depleting the layer around the summit. The transmission data showed higher concentrations of particles towards the surface, caused by primary production, and near to the seabed, probably resulting from re-suspension of sediments. The POC concentrations and microbial abundance were positively correlated to c_p , with microorganisms accounting for ~50% of the observed POC. Based on the non-metric multidimensional scaling three microbial clusters strongly indicate three separate biological regimes associated with northeastern, central and southwestern zones of the SWIR. This biological zonation is associated with physical oceanographic boundaries represented by the subtropical and sub-Antarctic fronts, forming three distinct “biogeographical” regions.

Chapter 2 Particulate organic carbon on seamounts

2.1 Introduction

There are more than one hundred thousand seamounts and knolls in the world's oceans (Rogers, 1994; Yesson et al., 2011). However, little is known about their ecology and influence on ocean biogeochemistry. Although data from seamounts are limited, they are considered unique ecosystems, in terms of the structure and sometimes high biomass of the benthic and pelagic biological communities they host (McClain et al., 2009; McClain and Hardy, 2010; Rowden et al., 2010; Priede and Froese, 2013). Pelagic ecosystems above the summit or flanks of seamounts have been observed to show different biological or biogeochemical properties compared to off-seamount areas (Rogers, 1994). For example, observations of chlorophyll concentrations in the waters above individual seamounts or seamount chains have occasionally revealed reduced or enhanced primary production compared to off-seamount sites (Genin and Boehlert, 1985; Dower et al., 1992; Venrick, 1991; Lopukhin, 1986; Meredith et al., 2003), although these have frequently been perceived temporally unstable. Differences in phytoplankton communities has been explained through the effect of seamounts on current flow, leading to localised hydrodynamic phenomena such as Taylor Columns or Cones, doming of density surfaces or isotherms, closed circulation cells, or enhanced vertical mixing, which in turn affects nutrient availability causing enhanced primary productivity (e.g. White, M et al., 2008). Increases of biomass have been observed in other parts of the pelagic community extending from heterotrophic microorganisms (e.g. Mendonça et al., 2012) to zooplankton (Fedosova, 1974; Zaika and Kovalev, 1985; Martin and Christiansen, 2009), micronekton (Nellen, 1973; Boehlert and Yoklavich, 1984; Genin et al., 1988) and large predators (Parin and Prut'ko, 1985; Holland and Grubbs, 2007; Litvinov, 2008; Kaschner and Pitcher, 2007; Thompson and Campanis, 2007; Morato et al., 2010; Louzao et al., 2011).

Whilst the ecology of seamounts has been subject to relatively little research, studies focusing on lower trophic levels and microbial processes on seamounts, which have a strong influence on biogeochemistry, are notably lacking (Vilas et al., 2009) with a few exceptions (Moiseyev, 1986; Smith and Batiza, 1989; Wishner et al., 1995; von Bröckel and Meyerhöfer, 1999; Mendonça et al., 2012; Abell et al., 2013). Most organic matter in open-ocean marine ecosystems originates from primary production by phytoplankton (Raven and Giordano, 2009; Hügler and Sievert, 2011). Organic matter is released into seawater as particulate organic matter (POM) or dissolved or-

ganic matter (DOM), the latter being material that passes through a 0.7 μm pore size filter. POM is formed mainly by sinking phytoplankton cells and other microorganisms, as well as messy feeding, faecal pellets, mucous feeding nets, structural materials (i.e. chitin from the exoskeletons of crustaceans) and dead organisms (Herring, 2002; Zsolnay, 2003). The fixation of CO_2 through photosynthesis and passage of this carbon towards the deep ocean is known as the biological carbon pump (Volk and Hoffert, 1985).

DOM is formed mainly as a by-product of carbon fixation by phytoplankton and subsequent food-web interactions, as well as the dissolution of POM from microbial activity (Azam et al., 1983; Hansell, 2013; Hansell and Carlson, 2012). A fraction of the POM sinks through the water column where it predominantly is consumed and remineralised by heterotrophs and possibly laterally advected by ocean currents or re-suspended after it has settled on the seabed. Consequently microbial activity and abundance is greater with higher POM and phytoplankton abundances. In general, the closer the POM is generated to the seabed the greater will be its input into seabed-associated communities.

Explanations for decreased or enhanced densities of planktonic and nektonic organisms around seamounts range from effects arising from the physical obstruction of a seamount on vertically migrating species, hydrodynamic mechanisms that increase nutrients, or enhance densities of pelagic species by entrapment, to active migration to seamounts for foraging purposes (e.g. Rogers, 1994; Genin, 2004; Louzao et al., 2011). Given the relatively few examples of enhancement of primary production around oceanic seamounts, it would seem likely that high densities of benthic and pelagic organisms result from processes other than physical obstruction of ocean currents. An obvious contributory factor is that seamount communities live closer to the surface waters than other deep-sea communities and thus benefit from higher supplies of sinking primary production. The negative correlation between supply of POM with depth and the resultant decrease in faunal biomass moving from shallow to deep waters is well understood (Smith and Demopoulos, 2003; Rex et al., 2006).

Another possible explanation, or at least a contributory factor, could be the enhanced supply or trapping of POM through localised hydrodynamic mechanisms associated with the seamount summit or flanks (Kiriakoulakis et al., 2009). Support for such a mechanism was obtained during the OASIS project, which focused on Seine and Sedlo seamounts in the NE Atlantic. Here, although data showed a high degree

of spatial and temporal variability, there was evidence of enhanced concentrations of organic matter in the waters above the seamounts, particularly close to the seabed (Kiriakoulakis et al., 2009; Vilas et al., 2009). In addition to hydrodynamic mechanisms, diel vertical migration of zooplankton and micronekton may play a role through two mechanisms. Firstly, by feeding in surface waters at night and digesting surface-generated POM at mesopelagic depths during the day, migrators may actively transport POM to the mesopelagic zone, repackaging POM as faecal pellets. Secondly, the interception of organisms undertaking diel vertical migration by predators associated with seamounts, through messy feeding and, again digestion and defecation, may also contribute to localised concentration of POM. Evidence of the latter mechanism comes from acoustic and dietary studies of predatory fish that aggregate around seamounts (Isaacs and Schwartzlose, 1965; Gorelova and Prut'ko, 1985; Genin et al., 1988, 1994; Fock et al., 2002) and also from observations of high numbers of carcasses of metazoan plankton over seamounts compared to off-seamount localities (e.g. Haury et al., 1995).

The South West Indian Ridge is an area where the Agulhas Return Current, the sub-Tropical front and the sub-Antarctic front, further to the south, create one of the most energetic and productive hydrographic regions of the global ocean (Read et al., 2000). The NE part of the ridge lies beneath the centre of the South Indian Ocean Gyre in a region of weak currents and low primary production. The central and SW sections of the ridge, however, are influenced by higher current velocities and enhanced primary production. The enhanced primary production results from upwelling in the region of turbulent mixing between the east-going Agulhas Return Current (ARC) and the Southern Ocean circulation (Longhurst, 1995). This region has so far been poorly studied (Rogers, 1994) but In the frontal zone, peak chlorophyll concentrations of $>1 \mu\text{g l}^{-1}$ have been recorded. Outside this region, chlorophyll concentrations have been measured at $<0.9 \mu\text{g l}^{-1}$, It is thought that the stability of the water column, the availability of nutrients (especially iron), and the accumulation of phytoplankton cells all contribute to elevated chlorophyll measurements at the sub-Antarctic front and sub-tropical front (Lutjeharms, 1985; Weeks and Shillington, 1996). Thus, seamounts along the SWIR are likely to be in contrasting productivity regimes and water masses depending on their proximity to the sub-tropical convergence zone and the sub-Antarctic front.

The present study reports on a quantitative analysis of particulate organic carbon (POC) and microbial community abundance across four seamounts of the South West

Indian Ridge (SWIR) (Fig. 2.1). Specifically we investigated the quantity of suspended POC and microorganisms in the waters around four seamounts during austral summer (November - December) of 2011. The aim was to elucidate how POC supplies and microbial abundance were related and how surface primary produced biomass influenced them; in addition to whether there was evidence of a seamount influenced distribution of POC quantities above the summit and flanks of each seamount. We hypothesise that the seamounts influence the distribution of particles and secondarily carbon above the features. Secondly we hypothesise that the carbon concentrations and microbial abundances will reflect the trophic level of the ecosystem.

2.2 Methods

2.2.1 Sampling

All sampling was carried out during the *RRS James Cook* voyage JC66 from November-December 2011. During the fieldwork, a total of 154 samples were collected for POC elemental analysis and 213 samples were collected for quantification of microorganisms through flow cytometry (Table 2.1). Conductivity, temperature, and depth (CTD) profiles, as well as all water samples, were collected with a SeaBird Electronics SBE 9plus CTD and rosette fitted with 24, 10 l Niskin bottles across transects of six deployments on the four seamounts Coral, Melville, Middle of What and Atlantis, along the SWIR (Fig. 2.1). An in-situ fluorometer measured Chlorophyll *a* fluorescence to a maximum depth of about 300 m (Hundahl and Holck, 1980) on all CTD deployments and density was calculated from temperature and salinity. Dissolved oxygen and salinity samples were collected to calibrate the CTD sensors.

The depths chosen for sampling from each deployment were most frequently at the fluorescence maximum (f_{max}) (~40 m) or immediately below (~80-90 m), at the boundary of the euphotic zone (EZ; ~200 m depth), and as close to the seafloor as possible (10-20 m from the bottom). Two samples were collected for each depth from separate Niskin bottles. For every CTD deployment, nutrients were collected at the

Table 2.1: The table lists the location and how many samples where collected on each seamount. POC: Particulate organic carbon. FC: Samples for flow cytometry counts.

Station	Longitude	Latitude	Summit (m)	Samples (POC)	Samples (FC)
Coral	42°50'31"E	41°21'23"S	198	39	77
Melville	46°45'74"E	38°31'56"S	120	32	65
MoW	50°22'16"E	37°56'76"S	1078	37	74
Atlantis	57°17'26" E	32°42'01"S	713	33	66

same depths as POC and flow cytometry samples as well as at intermediate depths. We collected 173 samples for macronutrients, nitrite, phosphate and silicate. They were analysed using a 5 channel Technicon AAll segmented flow analyser (Grasshoff et al., 1999). Results were all corrected, quality controlled, and checked against KANSO certified nutrient reference materials.

Upper ocean currents direction and speed were measured using a hull mounted 75kHz acoustic Doppler current profiler (ADCP) and were averaged over the upper 250 m.

2.2.2 Transmission measurements

The CTD was fitted with a SeaTech transmissometer, which measured beam attenuation in the red spectral band ($\lambda = 660$ nm) throughout the deployment and recovery of the instrument. In brief, the per cent transmission (Tr) of light was measured and converted to beam attenuation by particles (c_p) using the equation

$$c_p = -r^{-1} \ln(Tr)$$

, attenuation of the light beam (r) across the transmissometer was 25cm path length (Pak et al., 1988). Beam attenuation (c) is the sum of attenuation resulting from particles (c_p), water (c_w), and coloured dissolved organic matter (c_{cdom}). According to several studies, c_{cdom} is small enough to be ignored in measurements at 660nm wavelength in open waters (Pak et al., 1988; Bartz et al., 1978). Attenuation by water c_w is essentially constant for this instrument at a factory-calibrated value ($0.364m^{-1}$); our study will be focusing on the attenuation caused by particles (c_p). In some occasions Tr was more than 100%, thus all individual profiles were normalised by shifting the maximum

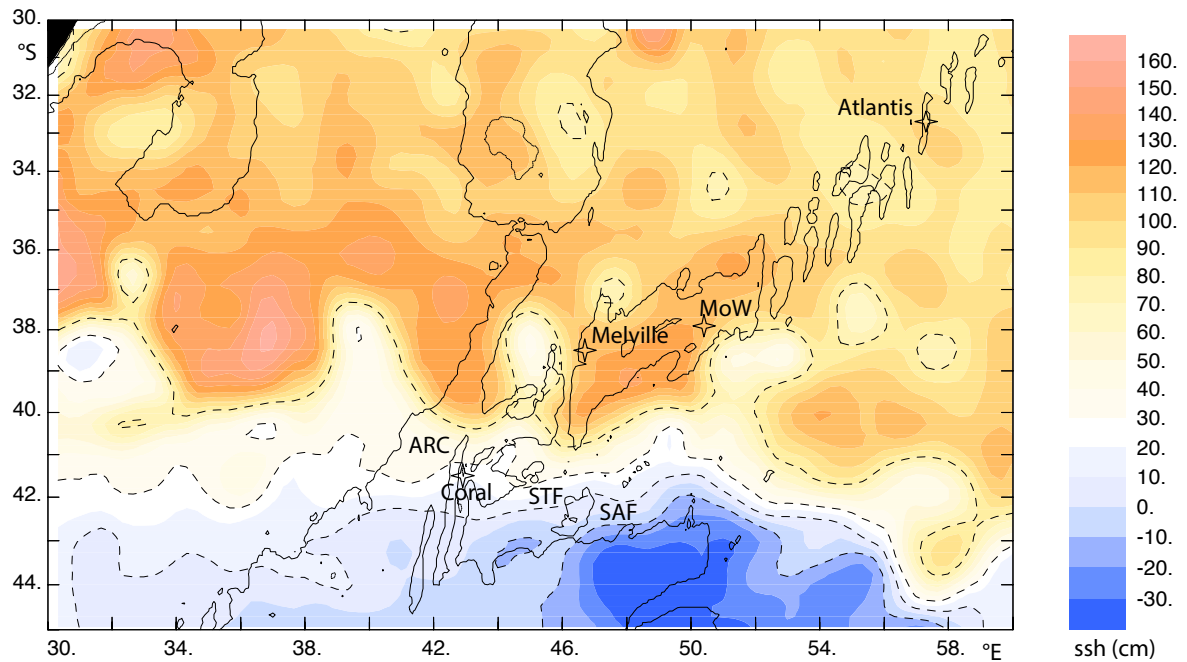


Figure 2.1: Eight-day composite (24 November 2011) of sea-surface height from AVISO. On RRS James Cook cruise 66, sub-surface data were not collected across the fronts but data collected in 2009 (Read and Pollard, In press) found the boundaries of the Agulhas Return Current (ARC), Subtropical Front (STF) and SubAntarctic Front (SAF) to be roughly synonymous with the 90-110 cm, 30 cm, 10 cm, and -10 cm isolines, respectively, indicated here with bold dashed lines. The topography is outlined by the 3000 m (solid) contours. The axis of the Southwest Indian Ridge runs from Coral Seamount to Atlantis Bank.

measured Tr to 100% and all other profiles used in this study relatively to the maximum. Normalization was done to allow comparison of carbon concentrations between the seamounts. Because of interference by bubbles and condensation in the sensors of the instrument until equilibration in the water, the data from the first 15 m were cut from the CTD transmission profiles. The raw data from downcast of the transmissometer was quality checked with the R package 'oce' (Kelley, 2014). Quality check included trimming, removal of large spikes and smoothing by 5-point averages. The smooth trimmed data was binned into depth averages of 2-meter intervals.

2.2.3 Elemental analysis

At least one litre of seawater was filtered through a pre-combusted 0.7 μm glass microfiber filter for each sample. The filters were analyzed by combustion with a CE440

elemental analyzer (Exeter analytical), according to Knap et al. (1996). Filters were measured for particles of a nominal size $>0.7 \mu\text{m}$ for POC, referred to here as observed POC. Before analysis, the frozen filters were thawed, dried overnight (50-55°C), and placed in a desiccator with concentrated hydrochloric acid to remove carbonates for approximately 2 hours. Following the acid bath, the filters were dried again (50-55°C) for at least 6 hours. After drying and cooling, the filters were folded gently and placed into nickel sleeves. For every eight nickel sleeves with samples, one nickel sleeve with a pre-combusted (blank) filter was added as a reference. A filter kit was used to pack the filters. Adsorption of DOC and colloidal organic carbon onto filters could have introduced a positive bias in the POC values (Moran et al., 1999; Gardner et al., 2003). However, this effect is most notable at low concentrations ($\sim 2 \mu\text{mol l}^{-1}$) (Gardner et al., 2003). Gardner et al. (2003) suggests that the protocols for POC filtration should include a blank that account for DOC adsorption onto filters. Such a method was used by Kiriakoulakis et al. (2009) to compensate for DOC adsorption but the concentrations remained generally the same. Some of the differences between this study and similar studies of seamount POC are possibly related to different sampling strategies (*in situ* filtration vs Niskin bottles). However, a planktonic carbon concentration estimate made by Landry and Kirchman (2002) indicated that POC concentration measured from bottles are closer to the true POC concentrations, especially in cold water (Gardner et al., 2003). The DOC could unfortunately not be sampled in this project, but based on the precise measurements of the elemental analyser used and based on the experiment performed by Landry and Kirchman (2002) we believe that our measurements are satisfactory and that the DOC adsorption would not have made a significant difference, especially not when doing intra-seamount comparisons, where relative measures are of greater value.

2.2.4 Fluorescence activated cell sorting

Sub-samples (4.5 ml of seawater) were collected from each Niskin bottle. Each sample was fixed with glutaraldehyde (final solution of 1.0%), mixed by shaking gently, incubated for 15 minutes at 4°C, and stored at -80°C or in liquid nitrogen pending flow cytometry analysis of phytoplankton and bacteria (Vaulot et al., 1989). At least two samples were collected at each sampling point from each Niskin bottle.

Samples were defrosted at room temperature and analyzed using a FACSCanto flow

cytometer (BD-Biosciences) equipped with fiberoptic-emitted light (407, 488 and 633 nm). Fluorescent 1.002 μm beads (Polysciences Inc., Europe) were added to each sample as an internal standard to normalize cell properties and to compare cell populations. Accurate analyzed volumes and subsequent estimations of cell concentrations were calculated using Becton-Dickinson Trucount™ beads. Phytoplankton cells were discriminated and enumerated according to their right-angle light scattering properties and orange (560-620 nm) and red (>670 nm) fluorescence from phycoerythrin and chlorophyll pigments, respectively. After thawing samples for bacterial counts, 1 ml sub-samples were incubated with the nucleic acid stain SYBRGreen I (Molecular Probes) for 15 minutes at room temperature in the dark

(Marie et al., 1997). Total bacterial cells were enumerated and two clusters (i.e. High Nucleic Acid content and Low Nucleic Acid content bacteria) were differentiated according to the variations of light scatter properties and green fluorescence related to nucleic acid content.

2.2.5 Microplankton biomass

With the flow cytometer, we obtained abundances of nanoeukaryotes, picoeukaryotes, *Prochlorococcus*, *Synechococcus*, and heterotrophic bacteria. The plankton biomass was calculated according to Mendonça et al. (2012). Heterotrophic bacteria abundances were converted to biomass using a factor of 11.5 fg C cell⁻¹ (Zubkov et al., 2000). *Prochlorococcus* cell numbers were converted to a biovolume of 0.1 μm^3 cell⁻¹ and then carbon using a conversion factor of 290 fg C μm^{-3} (Sieracki et al., 1994). *Synechococcus* and picoeukaryote cell numbers were converted to biomass by using a conversion factor of 100 fg C cell⁻¹ and 1500 fg C cell⁻¹, respectively (Kana and Glibert, 1987; Li et al., 1992; Campbell et al., 1997) (Table 2.2). All nanoplankton were assumed a volume of 14 μm^3 cell⁻¹ and converted to biomass with a factor of 3080 fg C cell⁻¹ (Børsheim and Bratbak, 1987).

2.2.6 Data and statistical analysis

Simple linear models were used to estimate POC for the water column in-between collected samples, based on measured POC concentrations and in-situ transmission data (c_p) for the EZ. POC concentrations were then predicted with the model based

on the high-resolution c_p profiles. The predicted POC will be referred to as pPOC. The profiles of pPOC were interpolated and visualized using the 'akima' package in R (Akima, 1978; Akima et al., 2011). The pPOC was subsequently differentiated to find the depth of biggest change in carbon concentrations down to 200 m, which from here on is referred to as the carbocline. The same was done to the c_p data below 200 m, to find the depth of biggest change (increase) in particle concentration.

A linear mixed effects model was executed to test for significant differences in carbon at different depths between the stations with temperature and fluorescence, keeping the cast as a random effect of the model to prohibit pseudoreplication within the same seamount. The water column was divided into three zones, the shallow (<100 m), mid (100-500 m), and deep (>500 m). All data were log transformed before analysis. A non-metric multidimensional scaling (NMDS) plot was used to visualize the microbial communities and an analysis of similarity (ANOSIM) was executed to test for similarities in the geographical clustering of the microbial communities. The NMDS was performed on abundance measures above 200 m to include photosynthetic microorganisms.

Maps of sea-surface height (mean absolute dynamic topography, MADT) were obtained from the SSALTO/DUACS (Segment Sol multimissions d'ALTimétrie, d'Orbitographie et de localisation précise/Developing Use of Altimetry for Climate Studies) multimission altimeter data processing system of CLS/AVISO (collecte Localis Satellites-/Archivage, Validation, Interprétation des données des Satellite Océanographiques). The mapped product was created from eight days of data merged from all available satellites (Envisat, Jason-1 and Jason-2) gridded at 1/3 degree resolution on a Mercator grid.

The statistical programme R v2.12 (R Core Team, 2013) was used for all the statistical analysis and most of the data visualization.

2.3 Results

2.3.1 Hydrographic setting

For all variables (temperature, salinity fluorescence, nitrite, phosphate, and silicate), except c_p the measurements between Melville and Middle of What were very similar.

Coral had lower temperature and salinity but higher phosphate, nitrite, silicate, and fluorescence than the other seamounts. The opposite is seen on Atlantis, with higher temperature but lower nutrients (Fig. 2.2). The surface (0-250 m) currents over Middle of What were particularly strong, $\sim 66.0 \text{ cm s}^{-1}$ in a northern direction, while currents over Melville Bank were $\sim 30.9 \text{ cm s}^{-1}$ and over Coral Seamount were $\sim 17.6 \text{ cm s}^{-1}$ in an average east/southeast direction.

The four seamounts had sea-surface temperatures ranging from 20°C on Atlantis to 12°C on Coral, with the greater part of the difference occurring across the STF and Agulhas return current similar to measurements taken on the *RV Dr Fridtjof Nansen* Cruise 410 in 2009 (Read and Pollard, in press). However, the temperature was considerably lower in the NE and higher in the SW than in 2009. The DCM on Atlantis Bank is most likely caused by deeper pycnocline, where the surface mixed layer is heated more extensively than in the SW and convergence zone. On Atlantis Bank, a large reduction in temperature was visible from surface to $\sim 100 \text{ m}$ (Fig. 2.2a) due to seasonal heating, where after it decreases very slowly, the plateau in temperature seems to follow a similar plateau in density (Fig. 2.2b). The temperature on Atlantis reaches a lower temperature than Melville and Middle of What below 50 m . Close to the surface, Coral Seamount had a temperature change at $\sim 50 \text{ m}$, which is also visible from the density, Melville Bank and Middle of What Seamount had the biggest temperature changes above $\sim 1000 \text{ m}$ depth. From the density, the water on Coral is stratified at approximately 50 m (Fig. 2.2). This stratification is clearly reflected in the c_p data, whereas the c_p results on the other seamounts show relatively uniform mixing, down to their respective stratification zone (Fig. 2.7).

Based on our data, Coral Seamount had a distinctly different physical setting from the other three seamounts with higher nutrient concentrations, higher fluorescence, and lower salinity. Atlantis Bank also had some distinct features with highest surface temperature but the lowest and deepest fluorescence maximum. However, apart from temperature and fluorescence, Atlantis Bank had similar concentrations of nutrients and salinity to Melville Bank and Middle of What Seamount (Fig. 2.2).

2.3.2 POC: c_p regression

Models were produced based on c_p profiles correlated with the POC from all seamounts and presented here as scatterplots. The regression between carbon and c_p was highly

significant in the euphotic zone ($p < 0.001$) (Fig. 2.3b), but in the deeper water, the model fit was not significant ($p = 0.24$) (Fig. 2.3c) and therefore not useable for carbon predictions below the euphotic zone. However, the c_p profiles showed that there were increases in particles of between 3-5 fold in c_p value, from the minimum (mid water-column), towards the bottom in each profile. The c_p profiles had higher values at the surface where the chlorophyll *a* fluorescence was highest, corresponding to our POC. Below the euphotic zone, the c_p values were stable, approaching zero until at ~1000 m depth or within ~100 m of the seabed on profiles shallower than 1000 m. Atlantis was the only seamount with higher c_p concentrations at the base of the seamount than at the surface.

Creating a model for four seamounts set in the diverse physical conditions of the SWIR was a compromise. Apparent from the shallow carbon model scatterplot, the pPOC on Coral Seamount was largely underestimated while the pPOC from Atlantis was overestimated and for the two middle seamounts the model appeared to be a relatively good fit.

2.3.3 POC and pPOC concentrations

The POC concentrations varied between the seamounts at the fluorescence maximum ($f_{\max}$) (Fig. 2.4), but generally showed the same trend with higher concentrations at the surface and decreasing concentrations with depth. The average carboclines on the individual seamounts were at approximately the same depth as the fluorescence maximum, from North to South: Atlantis = 101 ± 19 m, Middle of What = 91 ± 20 m, Melville = 85 ± 27 m and Coral = 53 ± 20 m, however, unlike the other three Atlantis did not show a decline but rather an incline caused by the deep chlorophyll maximum. According to the mixed effects model, fluorescence and temperature significantly explain the carbon concentration at the surface ($p < 0.001$). Below the surface, temperature still explained some of the variation ($p < 0.001$) on Middle of What, but in the deep zone our variables didn't explain the variation. The mixed effects model presented that Atlantis surface carbon concentrations were significantly different from all other seamounts. Middle of What was significantly different from Melville and the middle column (bottom of euphotic zone) on Middle of What was significantly different from the others.

Coral Seamount had the highest average POC at the $f_{\max}$ of $10.5 \mu\text{M C}$ ($138.4 \mu\text{g}$

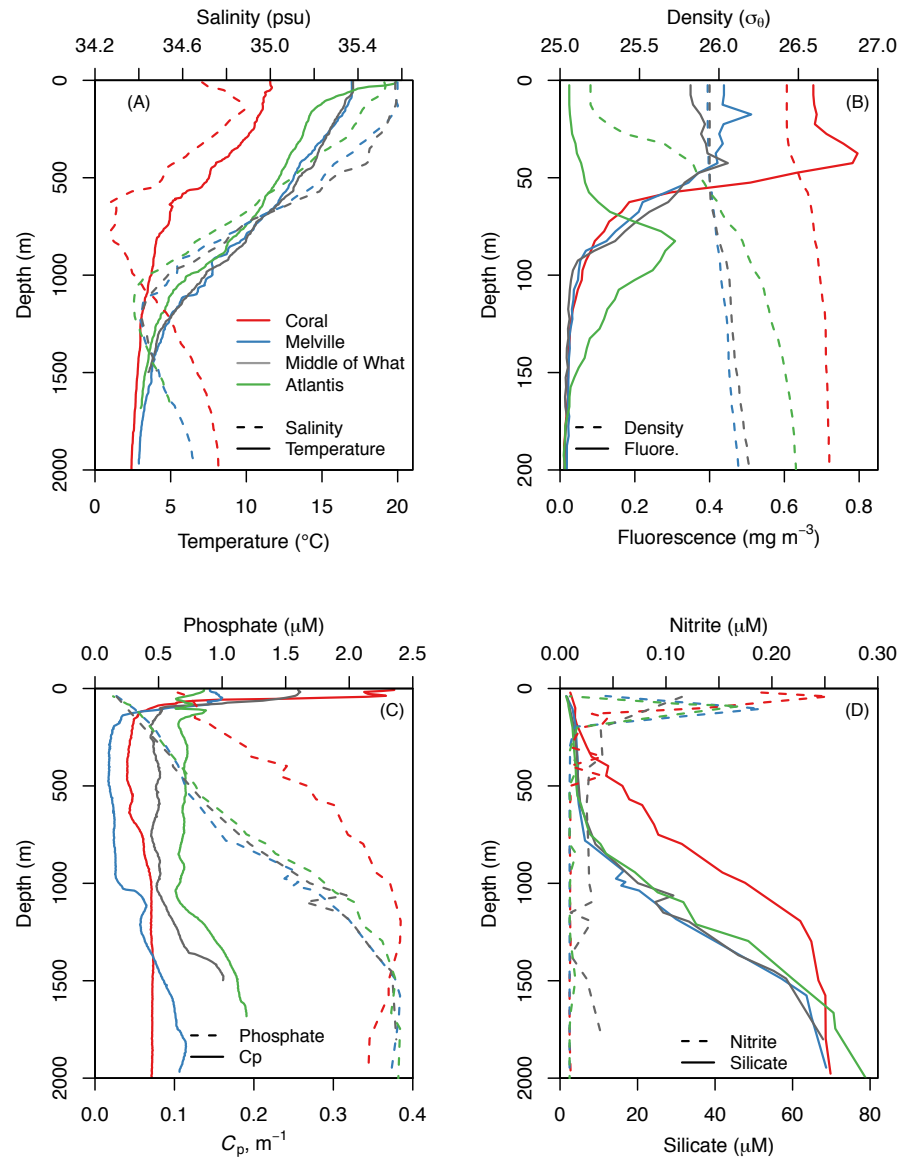


Figure 2.2: Hydrological conditions of Coral, Melville, Middle of What and Atlantis seamounts. The lower measurements of salinity and temperature are seen on Coral (A). The fluorescence is portrayed to 200 m (B), with the deep chlorophyll maximum of Atlantis seen at 90 m and the highest maximum of Coral at ~40m. Figure C and D show the average nutrient and c_p (particle) concentrations of all casts on all seamounts down to 2000 m. NB: Dashed and solid lines represent different variables.

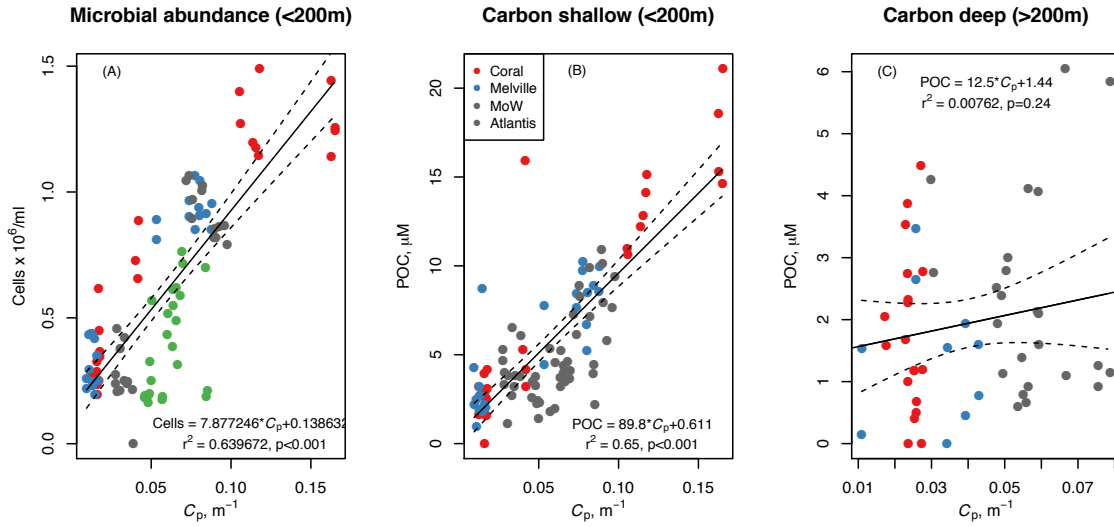


Figure 2.3: Linear models of microbial abundance and the beam attenuation coefficient, c_p (A), Shallow POC and c_p (B) and deep POC and c_p (C). Formulas for the linear models are portrayed on the individual figures, with the microbial abundance and shallow carbon models being significant. The solid lines signify the model formula with the dashed lines representing the 95% confidence intervals. The colours represent the four different seamounts. MoW: Middle of What. POC: Particulate Organic Carbon.

C l^{-1}), followed by Melville and Middle of What with $7.5 \mu\text{M C}$ ($96.1 \mu\text{g C l}^{-1}$) and $8.4 \mu\text{M C}$ ($99.5 \mu\text{g C l}^{-1}$), respectively, and Atlantis Bank the lowest of $3.8 \mu\text{M C}$ ($45.9 \mu\text{g C l}^{-1}$) (Table 2.2; Fig. 2.4). At the f_{max} there was a significant difference between Coral Seamount and Melville Bank ($p=0.036$), Coral Seamount and Atlantis Bank ($p=0.00$), and Atlantis Bank and Middle of What Seamount ($p=0.038$). The POC at the bottom of the euphotic zone and below were relatively uniform across all four seamounts with no significant differences. However, Middle of What Seamount had a higher absolute median at the end of euphotic zone and in the deep below the euphotic zone. Middle of What Seamount also had the highest integrated pPOC concentration ($\sim 25.4 \text{ mg C m}^{-2}$) in the euphotic zone, probably because values were integrated over the entire euphotic zone (0-200 m), and although concentrations are not the highest at the f_{max} POC is accumulated below the f_{max} on Middle of What. Although Coral Seamount had the absolute highest POC and fluorescence of the four seamounts, it had the third-highest integrated pPOC ($\sim 19.8 \text{ mg C m}^{-2}$), accumulating at the top 50 m of the water column.

Chapter 2 Particulate organic carbon on seamounts

Table 2.2: The table shows the concentrations of the integrated predicted POC (pPOC) per cubic meter in the euphotic zone and the total observed POC for the fluorescence maximum ($f_{\max}$) and bottom of euphotic zone (EZ) layers. Presented below the total POC is the relative carbon, calculated from the microbial cells, to the total POC.

	Coral	Melville	MoW	Atlantis
pPOC (g C m^{-3})	19.801 (1.46)	16.629 (0.77)	25.407 (0.50)	21.657 (0.30)
POC ($f_{\max}$ + EZ) ($\mu\text{g C m}^{-3}$)	84.37	65.70	73.93	41.15
C_{microbial}POC (%)				
Heterotrophs	9.06	10.46	8.92	10.31
<i>Prochlorococcus</i>	0.01	0.03	0.19	1.82
<i>Synechococcus</i>	1.84	2.18	1.42	0.45
Picoeukaryotes	4.17	2.94	1.84	0.88
Nano-eukaryotes	27.5	39.7	32.1	44.5
Total (%)	42.4	55.6	44.4	57.9

Table 2.3: Relative amount of POC near seabed on seamounts. The table shows the water depth at the position the CTD cast was done and the per cent POC at seabed relative to surface POC of that cast. WD: Water Depth. MoW: Middle of What.

Cast	WD (m)	Cor (%)	WD (m)	Mel (%)	WD (m)	MoW (%)	WD (m)	Atl (%)
1	1988	8.2	1960	14.7	1490	52.1	1674	76.0
2	955	4.0	1195	7.8	1378	36.1	952	24.2
3	198	16.6	550	13.8	1078	33.4	713	57.1
4	580	16.8	120	27.5	1186	20.7	728	75.2
5	1198	5.6	1035	51.2	1450	29.3	1220	19.6
6	1599	21.3	1978	13.8	1810	27.8	2115	33.6
Mean		12.1		21.5		33.2		47.6

Atlantis Bank had the second highest pPOC of $\sim 21.7 \text{ mg C m}^{-2}$. On Atlantis Bank the pPOC is quite high and shows little patchiness in the upper 200 m of the water column (Table 2.2). The two most dissimilar seamounts in terms of pPOC concentration were Melville Bank and Middle of What Seamount, although both are situated in similar physical regions but Middle of What Seamount. These two seamounts had relatively high carbon values in the upper 100 m of the water column, but the concentration of pPOC decreased markedly below 100 m depth on Melville Bank, but not on Middle of What Seamount, causing the large difference in carbon concentration (Fig. 2.7).

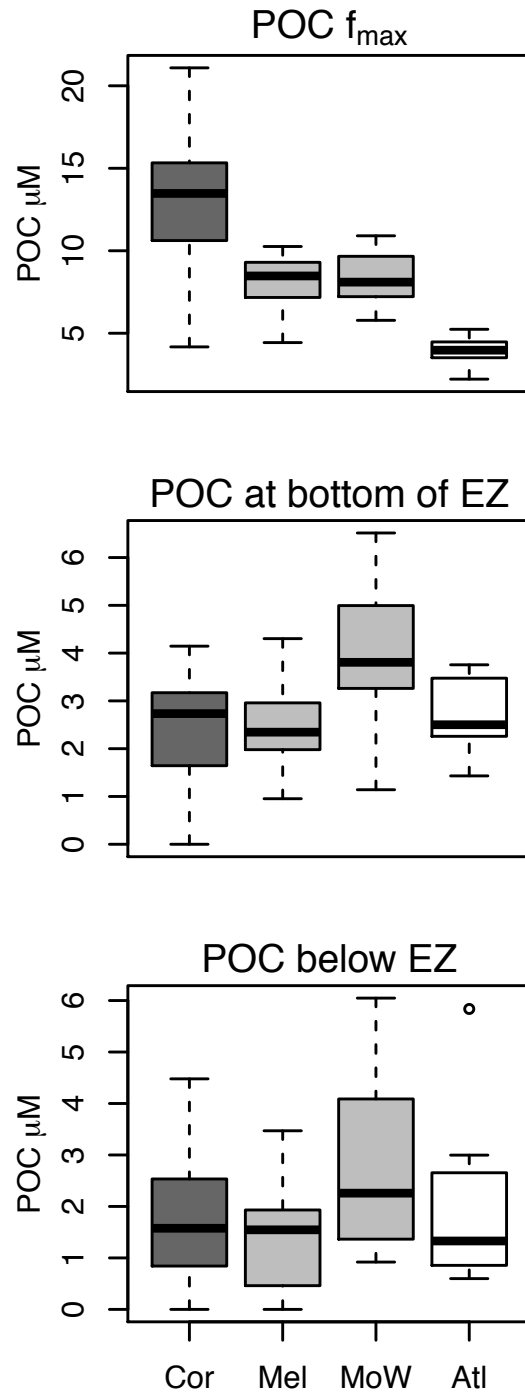


Figure 2.4: Boxplot showing the three vertical layers sampled for POC measurements on the four seamounts. Dark grey box is in the SW, light grey symbolizes the convergence zone and white is the NE. POC: Observed POC, f_{max} : Fluorescence maximum, EZ: Euphotic Zone, Cor: Coral, Mel: Melville, MoW: Middle of What, Atl: Atlantis. NB: y-axis range is different between top figure and the other two.

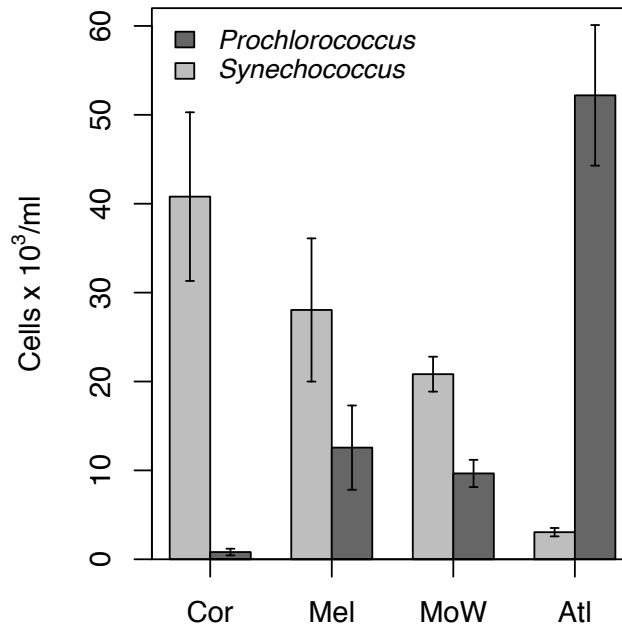


Figure 2.5: Abundance of *Prochlorococcus* and *Synechococcus*. Bar plot with the absolute abundances of *Prochlorococcus* and *Synechococcus* bacteria on the four seamounts, presented from North to South. The bars represent the standard deviations of the mean abundance.

2.3.4 Microbial abundance and biomass

The microbial abundance correlates with the quantity of POC (Fig. 2.3c), and the flow cytometry analysis measured highest total abundance of microbial cells on Coral Seamount where the POC at the surface also was highest. However, the abundances were not uniformly distributed within and across all four seamounts. *Prochlorococcus* and *Synechococcus* cells presented opposite trends with *Prochlorococcus* being most abundant in the NW and *Synechococcus* cells most abundant in the SE (Fig. 2.5). Most of the POC was related to microorganisms (Table 2.2), specifically nanoeukaryotes, forming 27-44% of the total POC in the euphotic zone. Nanoeukaryotes represented more of the POC on Atlantis Bank than Coral Seamount, while picoeukaryotes were

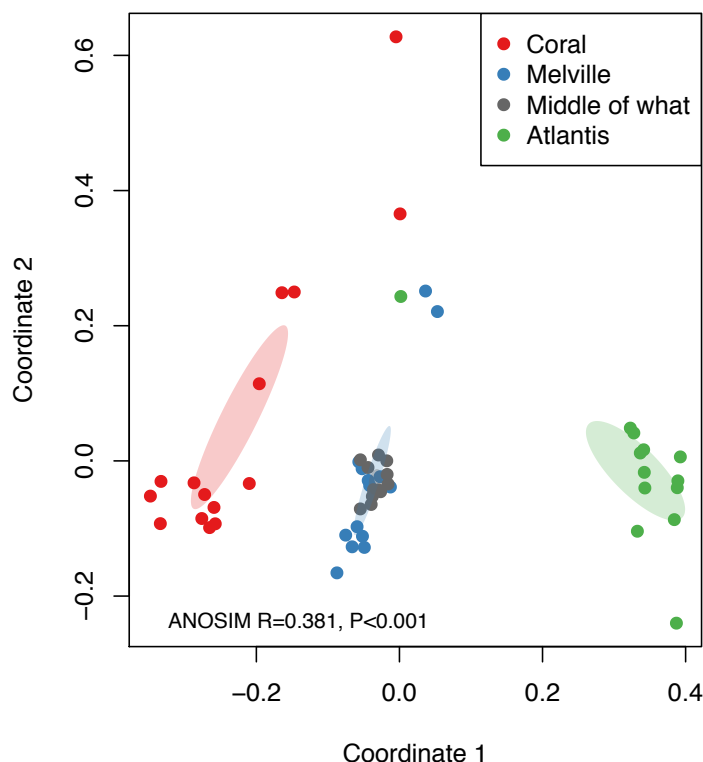


Figure 2.6: NMDS ordination plot of the phytoplankton community composition grouped according to geographical zone (Stress=0.06). The shaded area represents the 95% confidence interval.

more abundant on Coral Seamount and decreased with decreasing latitude, with the lowest concentration apparent on Atlantis Bank. *Synechococcus* bacteria show the same trend as picoeukaryotes, whereas *Prochlorococcus* have the opposite trend, similar to that of the nanoeukaryotes. Heterotrophic bacteria constituted approximately the same relative amount of total POC on all seamounts.

Although only based on five groups of microorganisms our non-metric multidimensional scaling (NMDS) clearly shows that the microbial communities differ in abundance composition down to 200 m from north to south, with three distinct regions, the northeast, the central convergence zone, and the Southwest. The abundance of the microbial components in these three regions is significantly different from each other based on an ANOSIM ($R=0.381$, $p<0.001$; Fig. 2.6).

Chapter 2 Particulate organic carbon on seamounts

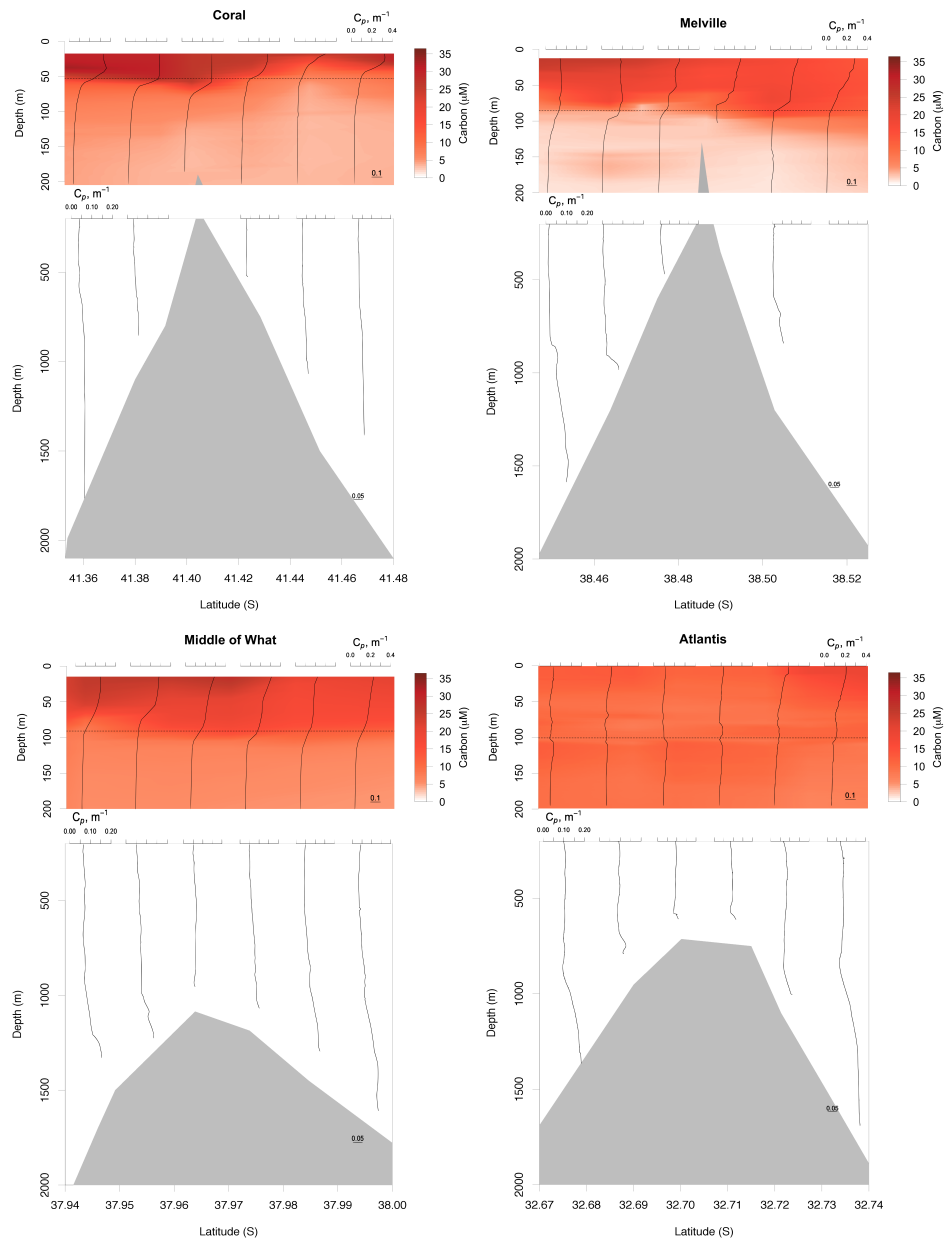


Figure 2.7: From south to north (Coral, Melville, Middle of What and Atlantis) predicted POC at the top 200 m above all four seamounts, with six transmission profiles from surface to bottom on each seamount. The grey shaded area roughly represents the shapes of the seamounts. The horizontal dashed lines on each seamount represent the carboclines. N.B. The transmission profiles have different scales in the euphotic zone and below the euphotic zone. There is a gap in the depth profile between the upper 200 m and the sea-bed.

2.4 Discussion

2.4.1 Local and regional distribution of POC

The seamounts in the SW and within the convergence zone have higher concentrations of POC than in the NE. This result reflects the higher nutrient availability and possibly the local effects of ocean currents, with more turbulent waters causing more upwelling supporting higher levels of primary production and therefore microbial biomass as indexed by POC (Fig. 2.2 – Fig. 2.4) (Read et al., 2000). Compared to two other seamounts, Seine and Sedlo, in oligotrophic regimes in eastern North Atlantic Ocean (Kiriakoulakis et al., 2009; Vilas et al., 2009), the concentrations of POC on Coral are relatively high. Atlantis however, has similar concentrations to those found on the comparative seamounts, reflecting the fact that it is situated in the oligotrophic sub-tropical gyre in contrast to the other seamounts.

We found a heterogeneous distribution of POC on and between all four seamounts. Read and Pollard Read and Pollard (in press) showed that the four seamounts at the SWIR have near identical water masses in the pycnocline, except for Coral Seamount, reflecting the westward drift between Atlantis Bank and the seamounts near the Agulhas return current and the partial isolation of Coral Seamount south of the subtropical front and on the edge of the sub-antarctic front (Fig. 2.1). However, although Atlantis Bank has a similar water mass to the convergence zone seamounts, its POC distribution showed the most dissimilar pattern from the other seamounts. It had a slightly uniform mixing of POC with no visible stratified layer and not a very clear carbocline. The NMDS, with Bray-Curtis distance measures, clearly shows four distinct microbial clusters whereas the seamounts in the convergence zone are completely overlapping. The three groups were statistically distinct from each other, strongly indicating three separate regimes of the sampled part of the SWIR exist; the NE, the convergence zone, and the SW. The analysis, simply based on the abundance patterns of five microbial groups, shows clear boundaries despite the taxonomically coarse analysis of the microbial community. Similar to these results, other studies in the same region have shown comparable biological zonation with physical oceanographic boundaries (Boersch-Supan et al., 2012) being constrained by the STF and SAF, forming three distinct “biogeographical” regions (Letessier et al., In press).

Although Coral Seamount appears to have the most distinct physical environment

from the other three seamounts, Atlantis Bank also presents unique biological conditions from the other seamounts in this and other studies, separated by the dynamic frontal systems in the convergence zone, which further supports the hypothesis of three distinct biological regimes (Fig. 2.2). These differences in the physical environment exert influence across several trophic levels, including microorganisms, micronekton (Letessier et al., In press), cephalopods (Laptikovsky et al., In press), fish (Boersch-Supan et al., In press), and seabirds (LeCorre, pers. com.). Overall, the physical environment emphasises the impact of water mass and other aspects of the oceanographic regime on the structure of pelagic communities in the vicinity of seamounts. Such a finding is encouraging in light of recent efforts to undertake bioregional classification of pelagic and benthic deep-sea communities, including seamounts (Rowden et al., 2005; Vierros et al., 2009; Clark et al., 2011).

The convergence zone has on several occasions been demonstrated as an important foraging area for large animals (McIntyre et al., 2012; Boersch-Supan et al., 2012; Barange et al., 1998a; Pakhomov et al., 1994). Boersch-Supan et al. (In press) found an increase in scattering layers at the SAF and STF in 2009, which may indicate a response of higher trophic levels (fish, micronekton) (Longhurst, 1995). Primary production enhances POC and thus feeding resources for macro fauna that actively transport carbon to deeper layers of the water column by vertical migration and defecation. For POC, this active transport can include up to 15-20% of the gravitational flux and, in areas with high zooplankton abundance, active transport is comparable to POC transport by sinking (Al-Mutairi and Landry, 2001; Hannides et al., 2009; Robinson et al., 2010). Presumably, areas with higher abundance of mesopelagic fish and zooplankton, such as the convergence zone, will have higher relative concentration of POC at greater depths as a result of active transportation. Although, this is not clear in our data, there is certainly some indication of relatively higher POC near the seabed in the convergence zone than in the SW (Table 2.3). However, the NE has the highest relative POC of surface concentrations at the seabed. The pattern seen in the NE is likely due to less primary production and, because of i.e. higher temperatures (higher microbial activity) and smaller cells (slower sinking rates), remineralisation is more efficient and leads to a lower export of POC to the abyss (Fig. 2.3a). This results in more of the minute stock of sinking POC to be left when it reaches the bottom (i.e. a higher transfer efficiency). There is possibly a larger difference between the surface and the bottom POC, but the POC concentrations on Atlantis are so low that in some cases

they are below the detection levels of the elemental analyser. Concentrations of POC are mostly uniform between the four seamounts at the seabed; it is the surface layers that show a heterogeneous pattern; consequently, where there is more POC at the surface there will be relatively less at the bottom, as is clearly seen on Coral seamount. However, relatively nutrient rich waters tend to have high rates of new production, relatively to regenerated production in nutrient poor regions. Lower latitude regions with low nutrient concentrations, will contain a relatively active microbial loop that ensures remineralisation and recycling before being exported from the euphotic zone (Azam, 1983). The nutrient rich regions will often have relatively large primary producing organisms that sink faster through the water column, yielding a relatively export efficient water column (Henson et al., 2012). However, the nature of the organisms reproducing in these areas makes them prone to degradation and rapid remineralisation after leaving the euphotic zone. This results in relatively low transfer efficiency to the benthos from the surface (Henson et al., 2012). Thus Coral and Atlantis have similar carbon concentrations in the deep layer even though they are set in different biological regimes.

2.4.2 Provenance of POC

Where POC is higher, the particle concentration (c_p) is elevated (Fig. 2.3b). The correlation between the amount of particles and microbial abundance is also well demonstrated (Fig. 2.3a), denoting that POC concentrations and microbial abundance are positively correlated to c_p and fluctuate with particle abundance. Evidently, more POC from primary production creates an elevated food resource for bacterial organisms attaching to the particles, resulting in a greater abundance of microorganisms (Azam and Malfatti, 2007; Ghiglione et al., 2007). Generally, the biggest portion of POC on the four seamounts was from nanoeukaryotes. However, the clear latitudinal pattern of the microbial groups strongly indicates a niche adaptation, similar to what was found in phytoplankton by Bornmann et al. (in press). This result is most likely because of nutrient conditions, particularly considering *Prochlorococcus* and *Synechococcus* bacteria. The *Synechococcus* cells are larger and have a competitive advantage over *Prochlorococcus* in more nutrient rich waters. *Prochlorococcus* however, is adapted to low nutrients. Higher abundance and more carbon resulted from *Prochlorococcus* presence at lower latitudes (Atlantis Bank), agreeing with previous

studies (Partensky and Garczarek, 2010; Malmstrom et al., 2010). The heterotrophic bacteria show approximately the same relative concentration on all seamounts (Table 2.3, Fig. 2.5).

Because c_p is a function of particle size, shape, and index of refraction, we expect the beam c_p to vary with different plankton community structures and size spectra (Gardner et al., 2006). The scatter of light from the beam attenuation increases with higher abundance of small phytoplankton (i.e. coccolithophores) (Baker and Lavelle, 1984; De La Rocha and Passow, 2007) and is relatively highest on Atlantis, likely because of the niche adaptations of the smaller organisms to the oligotrophic region. Atlantis shows very low POC but relatively high pPOC, since the refracted light causes an increased c_p concentration, resulting in higher predicted POC. We can accordingly determine that the pPOC on Atlantis Bank is overestimated because of the differing microbial communities and overestimation from the statistical model. Considering that the flow Cytometry method does not give us estimates of the microphytoplankton and large nanoplankton, which also contribute to the c_p data, some of the carbon caused by larger planktonic organisms will be present, which we have not taken into consideration. According to our calculations with the carbon conversion factors of microorganisms' abundance, the carbon concentrations are up to 50% of the total carbon, where the bigger organisms and detrital material would likely comprise the other half of the total carbon.

When predicting POC, making a single model based on all samples from the four seamounts is a compromise. On the scatterplot (Fig. 2.3b), it is clear that Atlantis will be overestimated and Coral mostly underestimated by the model. (Bishop, 1999) promoted an approach with all POC: c_p regressed to make a global model; however, this is not well supported when looking at our data, where Atlantis Bank would not show any significant relationship between c_p and POC on its own because of the uniform distribution of very low POC concentrations and clear overestimation. To create a global model, such as that found in Bishop (1999) and Gardner et al. (2006), this approach may be suitable; however, we advise caution when exploiting total POC: c_p regression involving regional data where the compared regions are in differing physical and biological regimes. Considering the occurrences described in (Gardner et al., 2006), there is a tongue of low- c_p , high-POC and high-chlorophyll starting from South Africa, extending to the Kerguelen Islands, especially prominent during austral summer, which may skew models that predict POC from c_p .

It has been shown that ~98% of POC remineralises during transit through the meso- and bathypelagic water column independent of production in the surface (Sarmiento and Gruber, 2006). Because of disruption of currents by seamounts, downwelling of water could increase the downward flow of POC from the surface to benthic communities on the summit seabed (Brink, 1995; Mullineau and Mills, 1997), further enhancing benthic recruitment and causing a retention of particles, visible from our data. Both the pelagic and benthic communities rely on primary production, in addition to the formation and sinking of POC. The minor portion of POC that reaches the seafloor is mostly remineralised by benthic biological activity. When taking into account the similar concentrations of POC below the euphotic zone on the four seamounts, the data indicated that, generally, the organic material is recycled in the upper water column. Although microbes accumulate on POM, they simultaneously remineralise and release nutrients, breaking down the particles, which may lead to a fairly constant carbon concentration after a particle leaves the euphotic zone. The seabed c_p increase is not visible in the carbon measurements, as is obvious from the deep carbon model (Fig. 2.3c). On the SWIR sub-Antarctic Mode Water mass is found down to about 500m depth. Below it occurs Antarctic Intermediate Water, which is identified by a salinity minimum (McDonagh et al., 2008). Underlying this water mass at a depth of about 1000 m is the Upper Deep Water, it flows south and forms part of the Indian Ocean overturning circulation. This water mass comprises mainly Indian deep water, exhibiting an oxygen minimum and high levels of inorganic nutrients. This layer is clearly seen from ~1000 m depth by the increasing inorganic nutrient concentrations on Melville, Middle of What and Atlantis and could partly explain the increased concentrations in c_p , from the persisting water masses, which penetrates to about a 2000 m depth (McDonagh et al., 2008). The deep accumulation of particles (visible from the c_p data), above the seabed, especially in the convergence zone and NE seamounts, is undoubtedly detrital mixed with resuspended material, since it does not coincide with the POC measurements (Fig. 2.3c). The benthic habitat is hence very different from the surface, as the elemental composition of particles in the latter is probably comprised of mostly fresh organic carbon derived from the surface generated primary producers. Instead, the benthic habitat probably includes resuspended organic matter and inorganic material (Fig. 2.3c) from the sediments and the Upper Deep Water, potentially constituting an important food resource to the benthic fauna. It is very unlikely that the POC will sediment at the seabed directly below where it was gener-

ated, reinforcing the importance of resuspended particulate matter within the benthic boundary layer of seamounts.

2.4.3 Seamount influence on particle and POC dispersal

Results from various studies on primary production do not show a clear image of phytoplankton biomass accumulation on or close to seamounts. The most common view invokes enhanced primary productivity over seamounts initiated by local upwelling and entrapment of nutrients by Taylor columns (Taylor, 1923; Uda and Ishino, 1958; Hogg, 1973; Huppert and Bryan, 1976; Boehlert and Genin, 1987; Dower et al., 1992; Nylander and Lacasce, 2004), though direct evidence for entrapment of nutrients seems difficult to determine for this study (for a review see Genin, 2004).

As proposed by Goldner and Chapman (1997) retention of particles on the seabed over seamount summits can be caused by Taylor columns or trapped benthic waves. In our case, however, there was no evidence of Taylor columns/caps or eddies on any of the seamounts (Read and Pollard, *In press*), although there was prominent patchiness of pPOC above the seamounts, mainly visible on the shallow seamounts in the south, Melville and Coral. In contrast, the deep seamounts had an elevated particle concentration towards the summit, although this appears to be a product of depth rather than a distinct summit accumulation, as discussed above.

Comparing Melville Bank and Middle of What Seamount, the pPOC (Fig. 2.7) concentrations were elevated below 100 m on the latter compared to the former. The integrated value of carbon was highest on Middle of What and lowest on Melville, even though these seamounts were set in most similar physical conditions. The summits of Middle of What and Melville are ~1000 m and ~120 m deep, respectively (Table 2.2). The shallow peak of Melville appears to interfere with the water column, causing turbulence in the pPOC spatial distribution. Stratification appears at ~100 m on both Middle of What and Melville, but below this depth the POC is almost depleted from Melville Bank. A similar pattern is noticeable on Coral Seamount, revealing the importance in the protrusion of the seamount into the euphotic zone. It appears that Middle of What Seamount has the highest integrated pPOC in the euphotic zone as a result of low concentrations of POC below 100 m, although, Coral Seamount has the highest discrete POC concentrations (Fig. 2.4). The depleted layer on Melville Bank and Coral Seamount below 100 m depth may have been caused by POC feeding

organisms.

The mechanisms for the enrichment of POC concentration on top and around the seamounts seem spatially variable and are not easily discerned from short-term surveys. However, seamounts and their surrounding ocean seem to be a location of patchily enriched POC concentrations, which may play an important role in the functioning of marine ecosystems (Mullineau and Mills, 1997). Considering the time constraint of the expedition, we were unable to collect off-ridge samples and thus it is not possible to get proper estimates of reduced or enhanced POC on the seamounts compared to the neighbouring open ocean. However, Vilas et al. (2009) discuss the possibility of an intra-seamount decrease on certain sites, where the Seine and Sedlo seamounts favoured differential enhancements of organic matter around certain sites of the seamounts, which corresponds with the patchiness visible on the convergence zone and SW seamounts. In particular this correspondence implies an intra-seamount effect on the SWIR seamounts.

Our study confirms the conclusions of Karageorgis and Anagnostou (2001); the particulate matter distribution appears to be a function of three principal factors: (i) the proximity to the sources, (ii) the general water circulation patterns, and (iii) the bottom topography. Nonetheless, we would recommend an additional two sub factors, when considering oceanic seamounts, concerning the (iv) proximity to dynamic systems, such as frontal zones, especially in respect to nutrient concentrations and (v) the height of seamount summit (penetration into the euphotic zone).

2.5 Conclusion

The highest absolute POC concentration was measured on the nutrient rich Coral seamount, with more than half of the POC explained by microorganisms. The two convergence zone seamounts had similar values of all physical variables measured. This reflects the water mass characteristics that are also supported by distinct compositions of microorganisms. As a result the seamounts can be separated into three biological regimes, north of the sub-tropical front, convergence zone, and south of the sub-tropical front.

Integrated predicted carbon concentrations of the euphotic zone show an unexpected highest concentration on Middle of What compared to the other seamounts.

Chapter 2 Particulate organic carbon on seamounts

The large difference in integrated POC between Melville and Middle of What is most likely caused by the difference of summit depth between the seamounts. The two shallower seamounts, Coral and Melville, are observed to have a dome feature around their summits depleted of POC.

We conclude that most of the POC production occurs in the shallow layer in the euphotic zone, however, where a summit protrudes into the euphotic zone the productive layer will be disturbed and become depleted of POC. However, this does not appear to have an effect on the POC concentration deeper in the water column. All seamounts had similar POC concentrations in the deep layer, although Coral had a much higher POC concentration at the surface than Atlantis, indicating higher transfer efficiency to deep waters and a more rapid remineralisation in nutrient rich environments compared to oligotrophic seamounts.

Chapter 3

The biogeography of prokaryotes around seamounts in a dynamic frontal system

Abstract Biogeography of microorganisms has been widely discussed in recent years and many local and global studies have demonstrated geographical patterns. Local-scale (cm-m) biogeography is frequently attributed to aggregation of microorganisms, while global scale (1000s km) is described by the different ecosystem in which the microorganisms persist. However, the regional scale (100s km) in microbial distribution and geographical trends commonly shows little to no pattern. However, differences in microbial communities with depth have been well demonstrated. We present a study on seamounts located in a dynamic frontal zone of the South West Indian Ridge, investigating the regional effect of ocean water masses and frontal dynamics on microbial communities.

We collected samples across four seamounts using a CTD fitted with niskin bottles for water filtration at three depths, 1) the chlorophyll maximum, 2) the bottom of the euphotic zone and 3) as close to the seabed as possible. The filters were sequenced for the 16S rRNA gene using an Illumina MiSeq sequencer. In addition, we measured abundance of microorganisms in our samples using flow cytometry. We show a similar composition of phyla across depth strata between seamounts, mostly dominated by Gammaproteobacteria, Thaumarchaeota, and Deltaproteobacteria. Depth was observed to be the most significant divider of microbial communities, supported by a non-metric multidimensional scaling plot and a multivariate regression tree. Richness was highest at the bottom of the euphotic zone and in the convergence zone of the South West Indian Ridge. Our study strongly indicates that ocean fronts create a dynamic, well mixed habitat different from the adjacent water masses. The South West Indian Ridge appears to be clearly divided through the frontal zone, clearly demonstrating a biogeographical pattern and substantiating three biological regimes of sub-Antarctic, convergence zone and sub-tropical zones.

Chapter 3 Microbial oceanography of seamounts

3.1 Introduction

The world's oceans are teeming with an enormous pool of diverse microscopic life forms. Communities of bacteria, archaea, protists, and unicellular fungi account for most oceanic biomass of a total cellular carbon content of $\sim 3 \times 10^{17}$ g (Schattenhofer, 2009; Kirchman, 2008). Ecologically, microbes play a vital role in marine food chains and global nutrient cycling and are involved in virtually all geochemical reactions occurring in the oceans (Arrigo, 2005; Teeling et al., 2012). (Giovanonni and Rappé, 2000) summarised nine dominant groups of prokaryotes in the ocean; *Roseobacter* and SAR11/pelagibacteracea (class Alphaproteobacteria) the SAR86 and OM60 clades (class Gammaproteobacteria), SAR324 (class Deltaproteobacteria), marine Actinobacteria (phylum Actinobacteria), SAR202 (phylum Chloroflexi), SAR406 (phylum Fibrobacteres), and Picophytoplankton such as *Prochlorococcus* and *Synechococcus* (phylum Cyanobacteria). Also very common in marine environments are the Antarctic pelagic Crenarchaeota (Marine Group I), which appear more abundant in surface winter waters and subsurface summer waters. In temperate seas, Marine Group II Euryarchaeota, unlike Marine Group I Crenarchaeota, seem to be more abundant at the surface (Partensky and Garczarek, 2010; Malmstrom et al., 2010; Morris et al., 2005; Suzuki et al., 2001; Fuhrman et al., 2006; Alonso-Sáez et al., 2007a; West et al., 2008; Alonso Gutiérrez et al., 2009). The interplay between these microorganisms and their community structure with depth and environment is not very well known (Sunagawa et al., 2015).

For a few microbial groups, depth and geographical distributions have been studied, however, their effects on the surrounding environment is relatively unknown. While total picoplankton abundance decreases from 10^6 cells ml^{-1} in the euphotic zone (<200 m) to 10^4 cells ml^{-1} in the bathypelagic zone (>1000 m). The SAR11 clade and archaea exhibit opposite vertical abundance trends, with SAR11 decreasing and archaea increasing with depth (Giebel et al., 2011; Alderkamp et al., 2006; Yokokawa et al., 2013). It has been demonstrated that species richness varies with season and peaks in high-latitude waters during winter (Ladau et al., 2013; Hatosy et al., 2013a; Gilbert et al., 2012). However, it has also been demonstrated that Operational Taxonomic Unit (OTU) richness peaks at lower latitudes, independently of season (Sul et al., 2013). Through all seasons *Prochlorococcus* and *Synechococcus* typically exhibit a core distribution from the equator, with *Prochlorococcus* dominating at lower lati-

tudes with the contrary pattern for *Synechococcus*. Sul et al. (2013) showed that most microbial OTUs did not appear to have a bipolar distribution and argued that their findings suggest that bacteria follow biogeographic patterns more typical of macroscopic organisms, and that dispersal limitation, not just environmental selection, likely plays an important role. The exact nature of the latitudinal gradients in richness, abundance and diversity in bacteria is still uncertain because of the substantial unexplained spatial and temporal variation of OTUs. OTU richness has been shown to correlate with temperature, salinity, primary productivity, and depth (Lin et al., 2012; Kirchman et al., 2007; Fuhrman et al., 2006; Alves Junior et al., 2015; Sunagawa et al., 2015). Changes in ocean currents and productivity may therefore be responsible for changes in observed bacterial and archaeal diversity.

In the first global study of prokaryotic microbes, Pommier et al. (2007) collected samples of marine surface water and performed species richness and abundance analyses. In their research, only two taxa, the Alphaproteobacterium, *Pelagibacter ubique*, and the photosynthetic cyanobacterial genus, *Synechococcus*, were found to be cosmopolitan. Furthermore, 69% of the identified OTUs were unique to each collection location.

The biogeography of microorganisms is undoubtedly directed by the evolutionary and ecological interaction of selection, genetic drift, dispersal and genetic mutation (Hanson et al., 2012). According to Hanson et al. (2012) the distance-decay relationship, which states that the connection between two locations declines as geographical distance increases, should be relatively weak in habitats where dispersal is high, such as in the pelagic environment, where ocean currents facilitate microbial dispersal. Becking's and Beijerinck's conjecture of microbial cosmopolitanism stating that 'everything is everywhere, but the environment selects' may be replaced by the distance-decay relationship proposed by Hanson et al. (2012) which has been demonstrated on multiple occasions (Mino et al., 2013; Read et al., 2015; Aguilar and Lado, 2012; Nemerget et al., 2011), Chapter 6). A distance effect on microbial communities is most often observed at small (0–1 km) or very large, basin-scale (>5,000 km) scales. This is highlighted by several studies that directly compared multiple spatial scales. For example, dispersal-related factors in diatoms were found to increase in relative importance with increasing maximum distance between lakes (Verleyen et al., 2009). Similarly, a distance-decay pattern was apparent at only the largest spatial scales (1000s km) for freshwater bacteria (Soininen et al., 2011; Van der Gucht et al., 2007). By con-

trast, community composition was related to distance at only the smallest scale (<5 km) for salt marsh bacteria, not at the regional or global scale (Martiny et al., 2011). Such a small-scale distance effect might be the result of microbial aggregation (Morlon et al., 2008), which can be caused by dispersal limitation. Investigating microbial communities at regional scales (100s km) is imperative as different ocean masses present contrasting physical conditions and thus contrasting microbial communities (Bardgett et al., 2008).

Seamounts, globally ubiquitous sub-sea features (Yesson et al., 2011), are considered to be “hotspots” of marine life but their role in ocean primary production is still under discussion (Rowden et al., 2010). Seamounts interact with the water column by obstructing currents, resulting in the mixing of sediments with surrounding water and the re-suspension of material from the benthos (Chapter 2), they are considered unique ecosystems in terms of their structure and sometimes high biomass of the benthic and pelagic biological communities (McClain et al., 2009; McClain and Hardy, 2010; Rowden et al., 2010; Priede and Froese, 2013). While the ecology of metazoans on seamounts has received considerable attention, studies focusing on lower trophic levels, and microbial processes on seamounts in particular are lacking, despite their potential strong influence on biogeochemistry (Vilas et al., 2009; Mendonça et al., 2012; Abell et al., 2013). Seamounts are very dynamic hydrological habitats and may in some instances have local enhancement of large autotrophic cells and picoplankton (i.e. the summit or flanks of seamounts) (Mendonça et al., 2012). Open-ocean seamounts have not been thoroughly surveyed for microbial composition. Using seamounts on the Southwest Indian Ridge, one of the world’s most hydrologically dynamic regions (Pollard and Read, 2001; Read et al., 2002) we present a comparative study on microbial biogeography around and between seamounts in differing environments.

This study focuses on differences in community composition on local/depth (1 km) and regional (100s km) patterns with the aim of addressing the following hypotheses.

- a. Does microbial OTU richness increase in convergence zones compared to subtropical or sub-Antarctic regions?
- b. Do OTUs rank with respect to relative abundance in a similar way across seamounts located geographically close but in clearly different temperature regions?

- c. Is the dominant microbial community in demersal samples distinct from pelagic samples?
- d. Does the dominant pelagic community near the surface vary with distance to the summit?
- e. Is the biogeography of dominant microorganisms different on regional (100s of km, between seamounts) and local (10s of km, within seamount stations) scales?

3.2 Materials and methods

3.2.1 Sampling

Sampling was carried out during the *RRS James Cook* voyage JC66 from 4th November – 20th December 2011. Conductivity, temperature, and depth (CTD) profiles, as well as all water samples, were collected with a SeaBird Electronics SBE +911 CTD and rosette fitted with Niskin bottles of 10 l volume. Samples were collected along transects of six CTD deployments on Coral, Melville, Middle of What, and Atlantis seamounts and a single CTD deployment on the summit of Sapmer seamount (Fig. 3.1). Sapmer seamount is not included in all data analyses because of the small number of samples. An in-situ fluorometer measured Chlorophyll *a* fluorescence to a maximum depth of about 300 m (Hundahl and Holck, 1980) on all CTD deployments.

1 l of water was filtered on a 0.2 µm filter from each CTD deployment at the chlorophyll maximum (40 - 80 m, referred to as shallow column), at the boundary of the euphotic zone (~200 m depth, referred to as mid column), and 10-20 m from the seafloor (referred to as deep column). Two samples were collected from each depth layer from separate Niskin bottles. For every CTD deployment, water samples were collected for nutrient analysis were collected at the same depths as POC and FC samples (see Chapter 2). We collected 173 samples for macronutrients, nitrite, nitrite+nitrate, phosphate and silicate. They were analysed using a 5 channel Technicon AAII segmented flow analyser (Grasshoff et al., 1999). Results were all calibrated, quality controlled, and checked against KANSO-certified nutrient reference materials.

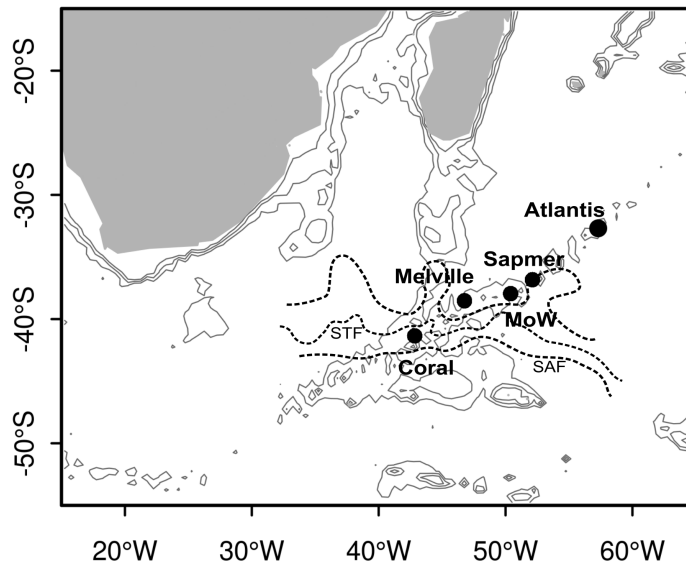


Figure 3.1: Map showing the sampling locations for the four sampling stations on the South West Indian Ridge. The solid line represents the 1000 m contour. The dashed lines are the Agulhas return current, sub-Antarctic and sub-tropical front (from top to bottom). MoW is Middle of What.

3.2.2 Elemental analysis and Fluorescence activated cell sorting

Elemental analysis and fluorescence activated cell sorting were conducted using the procedures described in Chapter 2, to obtain abundances for heterotrophic, autotrophic (*Prochlorococcus* and *Synechococcus*, Picoeukaryotes and Nanoeukaryotes).

3.2.3 Illumina MiSeq sequencing and preparation

DNA extraction, PCR and sequencing were performed using a modified version of the protocol presented in (Caporaso et al., 2010a), adapted for the Illumina MiSeq ac-

cording to the Earth Microbiome Project (EMP) standards¹ (Caporaso et al., 2012a). In brief, the genomic DNA was extracted from sub-samples from the water filters using a Powersoil-htp 96 Well DNA isolation kit (MoBio) with a 10 min. (65°C) incubation step modification². The V4-V5 region of the 16S rRNA gene was amplified with the 515F/806R primers with 12 base pair (bp) barcodes. The amplification primers were adapted from the (Caporaso et al., 2012a) protocol to include nine extra bases in the adapter region of the forward amplification primer that support paired-end sequencing on the MiSeq. Amplifications were done in triplicate and followed the EMP PCR protocol³. PCR products were pooled at equimolar concentrations and cleaned using the UltraClean® PCR Clean-Up Kit (MoBio). 16S rRNA amplicon sequencing was conducted at the IGSB Next Generation Sequencing Core at Argonne National Laboratory using 151 bp paired-end sequencing on an Illumina MiSeq instrument.

Quality filtering of reads was applied, as described previously (Caporaso et al., 2012a). Reads shorter than 75 bases and reads whose barcode did not match with an expected barcode were discarded.

3.2.4 Bioinformatics

Forward and reverse raw sequences were combined. Joined reads were demultiplexed and quality trimmed using QIIME 1.8 (Caporaso et al., 2010b). An open-reference OTU picking strategy was used, where OTUs were clustered against the GreenGenes 13_8 reference sequences using uclust ref (Edgar, 2010) and reads with no hit to the reference sequence collection were subsequently clustered de novo at the 97% similarity level using uclust (Edgar, 2010). Reads were assigned to OTUs based on their best hit to this database at greater than or equal to 97% sequence identity. PYNAST (Caporaso et al., 2010a) was used to align OTU sequences and OTU taxonomy was assessed using the RDP classifier retrained towards the GreenGenes database (97% similarity) (Wang et al., 2007). Median sequence counts per sample after OTU picking were 22,522 with a standard deviation of 8,321. To generate a final OTU table, sequences not aligning in the PYNAST step were removed, and a subsampled OTU table was created by random sampling to an even depth of 11,270 sequences per

¹<http://www.earthmicrobiome.org/emp-standard-protocols>

²<http://www.earthmicrobiome.org/emp-standard-protocols/dna-extraction-protocol/>

³<http://www.earthmicrobiome.org/emp-standard-protocols/16s/>

sample.

Taxonomy was assigned to each read by accepting the Greengenes taxonomy string of the best matching Greengenes sequence. All bioinformatics were conducted using QIIME (Caporaso et al., 2010b). To eliminate sequence artifacts on the biogeographical patterns the rare biosphere was not considered and we only performed the forthcoming statistical test with the dominant (top 50 taxa, >90% of sequences) microbiome except the CCA analysis.

3.2.5 Data analysis

The statistical programme R v2.12 (R Core Team, 2013) was used for all the statistical analysis. For all ordination and richness analyses, the R package *vegan* was used (Oksanen et al., 2013).

For an estimate of the fraction of OTUs covered by the sampling, species accumulation curves were constructed on a sample basis. For species richness estimates we used observed richness. Differences in bacterial abundances and richness between stations were compared using an ANOVA and *post hoc* Tukey HSD test.

Variation of microbial community structure with depth was assessed using non-metric multidimensional scaling (NMDS) analysis using Bray-Curtis similarities, with 1,000 random permutations. Canonical correspondence analyses (CCA) were conducted for each depth stratum to understand the geographical clustering and environmental drivers of the bacterial communities. Comparisons between the seamounts were performed with permutational multivariate analysis of variance (permutation MANOVA) using distance matrices with the function *adonis* from the R *vegan* package (Ramette, 2007). Jaccard and Bray-Curtis distances were calculated between samples from each depth stratum between all seamounts (Table 3.3-3.5).

Multivariate Regression Tree (MRT) analysis (De'ath, 2002) was used to identify driving environmental factors and their individual contribution to microbial community structure. This method performs hierarchical dichotomous clustering of community data by selecting environmental parameters (including depth, latitude, temperature, salinity, NO_3^- , NO_2^- , phosphate, silicate and oxygen) that maximize the homogeneity within groups of samples. Accordingly, these clusters are characterized by both a homogenous assemblage structure and similar ecological parameters. MRTs are not based on traditional significance testing but on 10-fold cross-validation (CV) in order

Table 3.1: Overview of samples collected at the five seamount locations. FC is flow cytometry samples. DNA, FC and Nutr. (nutrients) are number of samples. WM: Water Mass. Subant.: Subantarctic, subtrop.: Subtropical/

Station	Longitude	Latitude	Summit (m)	WM	DNA	FC	Nutr
Coral	42°50'31"E	41°21'23"S	198	Sub-Ant.	77	79	86
Melville	46°45'74"E	38°31'56"S	120	CZ	65	64	73
MoW	50°22'16"E	37°56'76"S	1078	CZ	74	74	81
Sapmer	52°07'24"E	36°49'63"S	446	CZ	11	12	53
Atlantis	57°17'26" E	32°42'01"S	713	Sub-trop.	66	66	91

to determine the optimal number of dichotomous splits and the importance of predictor variables (De'ath, 2002). We used the R package mvpart 1.6–0 (Therneau et al., 2012) to perform the analyses with 1,000 cross-validation runs on Bray-Curtis transformed data and the first tree below 1 standard error with cross-validation was picked as the optimal tree. Additionally, Indicator Species Analysis (ISA) (Dufrêne and Legendre, 2008) was performed using the R package MvPARTwrap 0.1–9.2 (Ouellette et al., 2012) in order to identify microbial taxa associated with splits derived from the MRT analyses. This method aims at detecting OTUs that represent distinct ecological settings and indicate location-specific community types.

Bar plot and rank abundance plots were visualized using the phyloseq package (McMurdie and Holmes, 2013) and code from Marcus W. Beck's R blog⁴ was used for rank abundance plotting.

3.3 Results

During the fieldwork, a total of 149 samples were collected for sequencing of microorganisms and 223 samples were collected for quantification of microorganisms through flow cytometry (Table 3.1).

3.3.1 Depth distribution of microbial communities

Marked differences were observed in chemical and biological parameters between the different locations, reflecting the wide latitudinal gradient (32°S–41°S) covered in this

⁴Code is from github (source_gist('5281518'))

study. The microbial abundances differed with depth, where the shallow layer (~40-80 m) had the most abundant microorganisms (Fig. 3.2). In addition, there are large differences between the shallow water abundance between seamounts with Coral (the southernmost seamount with lowest temperature) having the highest average abundance (1.16×10^6 cells/ml) and Atlantis (northern most seamount with highest temperature) the lowest (0.50×10^6 cells/ml) (Tukey HSD, $df=2$, adjusted $p < 0.001$), Melville and Middle of What had similar average abundances in the shallow layer (0.926 and 0.925×10^6 cells/ml, respectively) that were significantly different to Coral (Melville-Coral, Tukey HSD, $df=2$, adjusted $p = 0.008$, MoW-Coral ANOVA, Tukey HSD, $df=2$, adjusted $p = 0.006$) and Atlantis seamounts (Melville-Atlantis, Tukey HSD, $df=2$, adjusted $p < 0.001$, MoW-Atlantis, Tukey HSD, $df=2$, adjusted $p < 0.001$).

Per seamount we found roughly the same total OTU abundance as in surface and deep pelagic layers, of 19,841 OTUs, found by Zinger et al. (2011). In total we observed 69,687 OTUs. On Coral, we observed a total of 27,544 OTUs, while Melville had 28,821 OTUs, Middle of What had 31,996 OTUs, and Atlantis had 21,988 OTUs. The observed OTU richness varied between seamounts with no significant pattern from north to south (Tukey HSD, $df=2$, $p > 0.05$) (Fig. 3.3). Although Melville and Middle of What seamounts had persistently higher median richness per sample (shallow = 1486-1530/sample, middle = 1780-1805/sample, deep = 1433-1451/sample) than Coral (shallow = 1410/sample, middle = 1134/sample, deep = 1224/sample) and Atlantis (shallow = 1421/sample, middle = 1502/sample, deep = 994/sample), which can also be observed on the species accumulation curves between the stations (Fig. 3.4). However, the middle depth column appears richer than the shallow and deep column with middle and deep column significantly different (Tukey HSD $p = 0.00045$). The depth layers correspondingly contained differences in the microbial community composition supported by the NMDS (Fig. 3.5) (MANOVA $p < 0.01$) with clear separation between the depth strata. Gammaproteobacteria dominated all depth layers, but the shallow layer clearly had a higher abundance of the photoautotrophic class Synechococcophyceae compared to the middle and deep layers. In addition the classes Flavobacteriia and Acidimicrobiia were more abundant in the shallow layer. Thaumarchaeota and Deltaproteobacteria both increased from shallow to deep layers (Fig. 3.6).

Bacteria accounted for 86.5 % and archaea 13.5 % of all sequences. The most abundant phylum was Proteobacteria (61.3 %) with classes Gammaproteobacteria (26.6 %), Alphaproteobacteria (16.8 %), and Deltaproteobacteria (8.7 %) being the

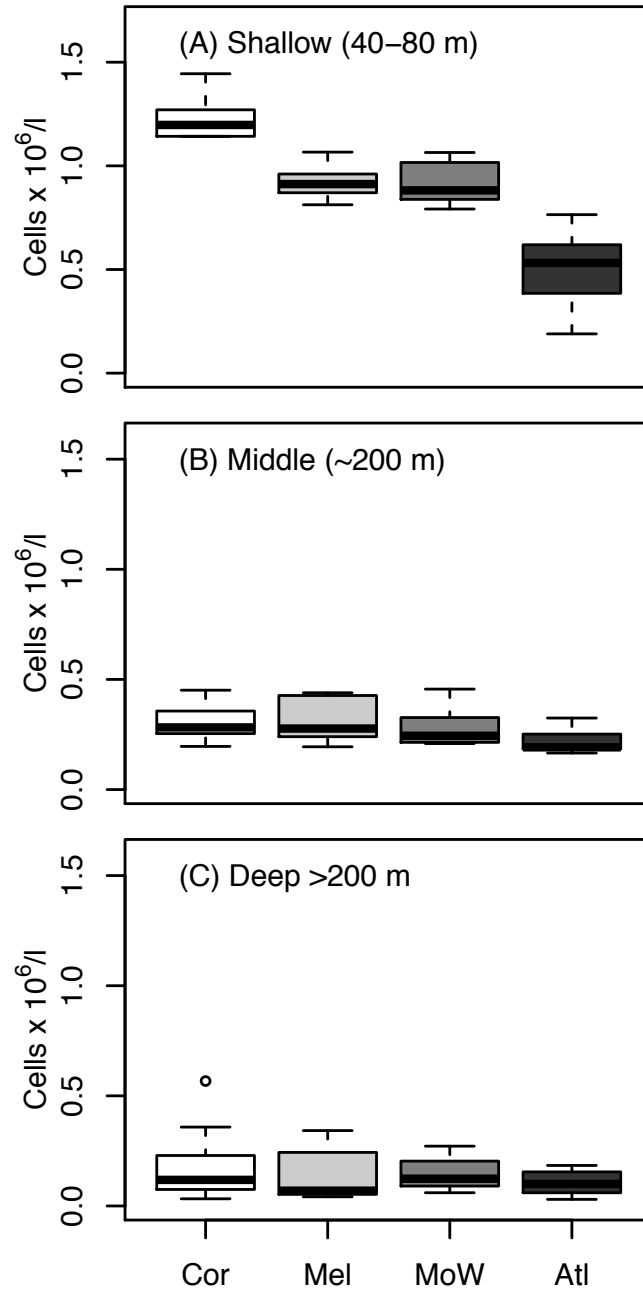


Figure 3.2: Abundance of microorganisms between the four seamounts, Coral (Cor), Melville (Mel), Middle of What (MoW) and Atlantis (Atl). Boxplot is separated into the shallow (A, 40–80 m), middle (B, 200 m) and deep (C, >200 m) water column. Sapmer is not visualized.

Table 3.2: Overview of the most abundant orders-families of OTUs in the three depth layers and stations. Sequences identified to family are in italics. Letters in brackets represent unique OTUs of the same order/family but different species (undetected). The two most abundant OTUs found in this study are in bold.

	Coral	Melville	MoW	Atlantis
Shallow (40-80 m)	<i>Synechococcaceae</i> (a)	<i>Synechococcaceae</i> (a)	OCS155(b)	OCS155(b)
	Oceanospirillales	OCS155(b)	SAR324	<i>Synechococcaceae</i> (c)
	OCS155(a)	<i>Synechococcaceae</i> (b)	<i>Synechococcaceae</i> (a)	<i>Pelagibacteraceae</i> (2)
	<i>Halomonadaceae</i>	OCS155(c)	Oceanospirillales	<i>Synechococcaceae</i> (d)
Middle (~200 m)	SAR324	<i>Flavobacteriaceae</i>	OCS155(c)	<i>Rhodobacteraceae</i>
	Oceanospirillales	<i>Cenarchaeaceae</i> (c)	<i>Cenarchaeaceae</i> (c)	<i>Cenarchaeaceae</i> 1)
	SAR324	OCS155(b)	Oceanospirillales	SAR324
	<i>Cenarchaeaceae</i> (a)	Oceanospirillales	SAR324	<i>Pseudoalteromonadaceae</i>
	<i>Cenarchaeaceae</i> (b)	<i>Pelagibacteraceae</i>	OCS155 (b)	<i>Cenarchaeaceae</i> (d)
	<i>Pelagibacteraceae</i>	<i>Cenarchaeaceae</i> (a)	<i>Pelagibacteraceae</i>	<i>Pelagibacteraceae</i>
Deep (>200 m)	Oceanospirillales	SAR324	SAR324	SAR324
	SAR324	Oceanospirillales	Oceanospirillales	<i>Pseudoalteromonadaceae</i>
	<i>Cenarchaeaceae</i> (b)	<i>Cenarchaeaceae</i> (b)	<i>Cenarchaeaceae</i> (b)	Oceanospirillales
	<i>Halomonadaceae</i>	<i>Halomonadaceae</i>	<i>Halomonadaceae</i>	<i>Cenarchaeaceae</i> (b)
	<i>Cenarchaeaceae</i> (e)	<i>Cenarchaeaceae</i> (f)	<i>Cenarchaeaceae</i> (f)	<i>Moraxellaceae</i>

most abundant. Other abundant classes were the Thaumarchaeota (9.7%), Acidimicrobiia (5.7 %), and Synechococcophycideae (3.5 %, Fig. 3.6). Thaumarchaeota accounted for 72% of all archaea sequences. Two OTUs dominated in terms of abundance and accounted for approximately 10% of all sequences: One is classified in the family SAR324 from the order Sva0853 (Deltaproteobacteria, Table 3.2). The other is from the order Oceanospirillales (Gammaproteobacteria). Both have a high rank abundance at all seamounts and all depths (Fig. 3.6).

Based on the multivariate regression analysis, the optimal tree size, based on cross-validation error, varied between 5 and 11 (Appendix. A.1), but the smallest most parsimonious within ± 1 SE of the optimal tree had 5 clusters. It revealed that depth, and latitude together explained 66% of variation in community composition (Fig. 3.7). As indicated by the hierarchical order of splits and branch lengths of the MRT, depth was the main explanatory factor (split 1; Fig. 3.7), separating all seamount samples below 560 m from the rest. The cluster shallower than 560 m was again separated at 125 m, distinguishing the middle layer and shallow layer. In the middle column, Coral seamount was distinct from all other stations and in the shallow layer (chlorophyll maximum) Atlantis clustered separately from all other stations.

The ISA revealed that the first split was mainly associated with the relative abundance of SAR324 (OTU 833419) and Oceanospirillales (OTU 884345) explaining 46.19% and 14.40% of the variation, respectively (permutation test, $p = 0.001$). Both SAR324 and Oceanospirillales exhibited the highest rank abundance in the deep layer (Table 3.2 and Fig. 3.9). Synechococcaceae (a) (explaining 15.68% of variation) and Crenarchaeacea (17.41% of explained variation) represent the split between the shallow and middle layer with *Synechococcus* being more abundant in the shallow layer and Crenarchaeacea in the middle layer. The third split of Atlantis shallow layer from the other seamounts is associated with Synechococcaceae (a) (22.83%), SAR324 (11.64%) and Synechococcaceae (c) (12.95%). The last split between Coral middle layer from the other seamounts is associated with SAR324 (20.52%), Crenarchaeacea (14.83%) and Oceanospirillales (37.41%).

3.3.2 Regional biogeography between seamount microorganisms

As demonstrated by the multivariate regression tree, depth is the main predictor of the microbial community structure across survey area. However, separating the data into

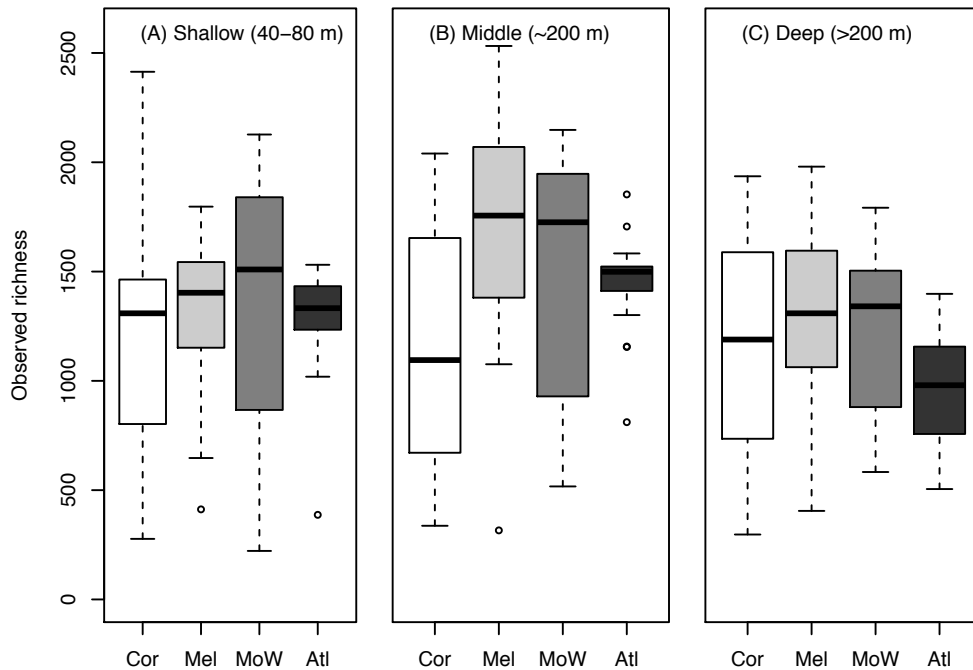


Figure 3.3: Richness plot of all samples from the four seamounts, Coral (Cor), Melville (Mel), Middle of What (MoW) and Atlantis (Atl). The richness plot is separated into the shallow (A, 40–80 m), middle (B, 200 m) and deep (C, >200 m) water column. Sapmer is not visualized.

the three depth strata of the MRT analysis, the CCA revealed additional segregation of the community composition between the seamounts at the OTU level. In the shallow layer, Atlantis and Coral show very different structures to Melville, Middle of What and Sapmer (MANOVA $p < 0.01$). In the middle layer Atlantis is constrained within its own ordination space, while samples from Coral and Middle of What are less well separated than in the shallow layer, but Melville, Middle of What, and Sapmer still greatly overlap (MANOVA $p < 0.01$). In the deep layer Middle of What and Coral co-cluster, while Atlantis, Melville and Sapmer create three distinct clusters (MANOVA $p < 0.01$). Sapmer clearly shows the biggest variation between individual samples of all the seamounts, even though it has the fewest samples per depth stratum.

In the shallow layer, *Synechococcus*, nanoeukaryotes, total microbial abundance, picoeukaryotes, Particulate Organic Carbon (POC) and Fluorescence were positively correlated with the communities at seamounts in the convergence zone and Coral (Fig. 3.8). In contrast, the cluster at Atlantis was correlated with the abundance of

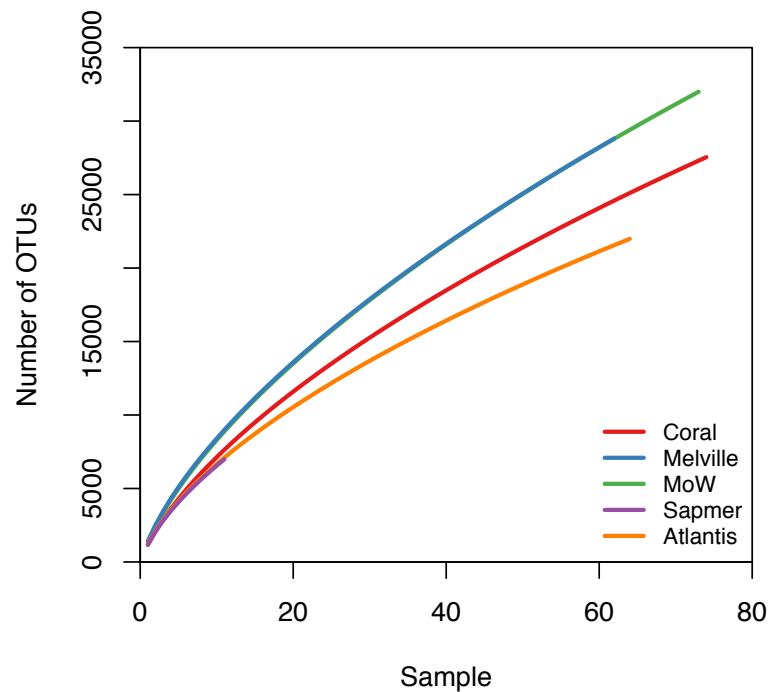


Figure 3.4: Species accumulation curve on a per sample basis for all five seamounts.

Prochlorococcus (from flow cytometry) communities at Atlantis were correlated with particulate hydrogen through all strata. Silicate, nitrite, phosphate and oxygen explain most variation between microbial communities in the shallow and middle layer of Coral, while salinity, temperature and particulate hydrogen explain variance at Melville, Middle of What and Atlantis. All variables visualized on the CCAs were statistically significant at $p < 0.01$.

The abundance of microbes between the seamounts differs at the phylum (Fig. 3.6) and OTU levels (Fig. 3.9). Overall, the rank abundance of OTUs changes substantially between Coral and Melville and again between Middle of What and Atlantis. The largest changes in ranks between Melville and Middle of What can be seen in the deep layer (Fig. 3.9). However, in the shallow layer the ranks of Synecococcaceae (a) and Halomonadaceae decrease substantially from Coral (ranks 1 and 3, respectively) to Atlantis (ranks 32 and 42, respectively). The opposite is observed for OCS155 and

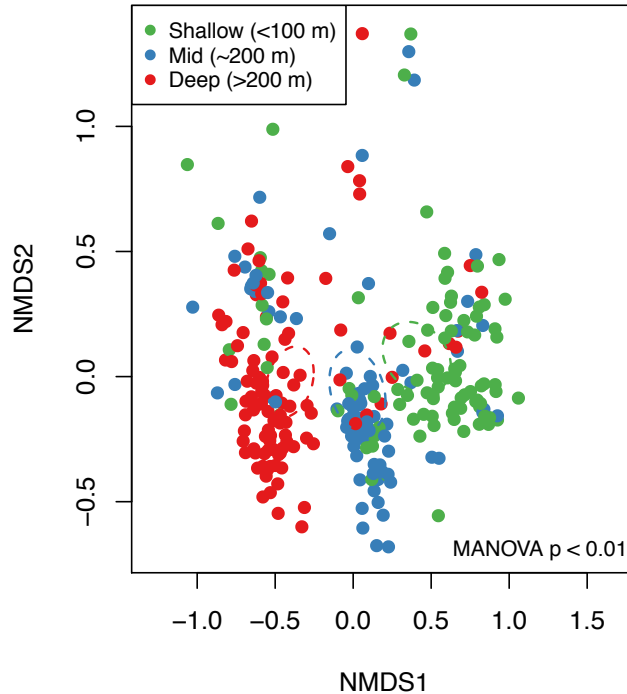


Figure 3.5: NMDS plot between the three depth layers (shallow=40-80 m, middle= 200 m, deep=>200 m). The ellipses represent the 99% standard error of the individual depth layers. MANOVA = Multivariate Analysis of Variance. Stress = 0.1225.

Synechococcus (Prochl.) from ranks 21 and 50, respectively to ranks 2 and 1, respectively. In the middle layer the changes in rank abundance are similar to the shallow layer, with the most abundant OTUs on Coral becoming the least abundant on Atlantis and vice versa for rank changes occurring on Melville and Middle of What. A large section of OTUs in the middle layer decreases in relative abundance from Coral to Melville (Fig. 3.9c). These OTUs include Alteromonadales, HTCC 2188, Flavobacteriaceae, 2A3409c, A714017, Pelagibacteraceae, Marine Group II, Crenarchaeacea, and Pseudoalteromonadaceae. The cluster occupies high ranks on Coral, but most of its OTUs are of lower rank on all other seamounts. The deep layer exhibits fewer rank changes than the shallower strata. The five most abundant OTUs in the deep layer are highly ranked throughout the seamounts, and are unchanging between Melville and

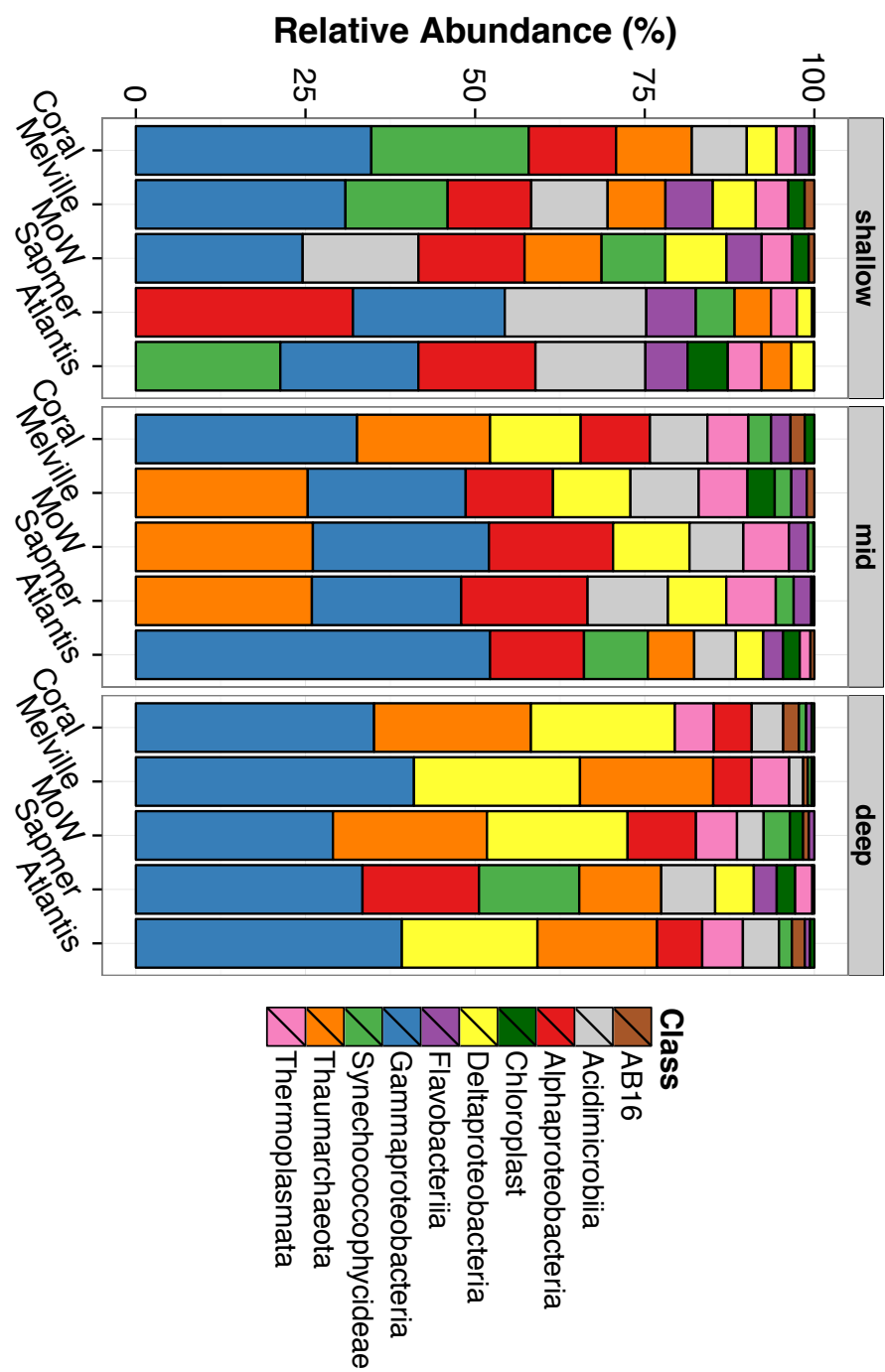


Figure 3.6: Barplot of the relatively most abundant microbial classes between depths (shallow=40-80 m, middle= 200 m, deep=>200 m) on all seamounts. MoW is Middle of What.

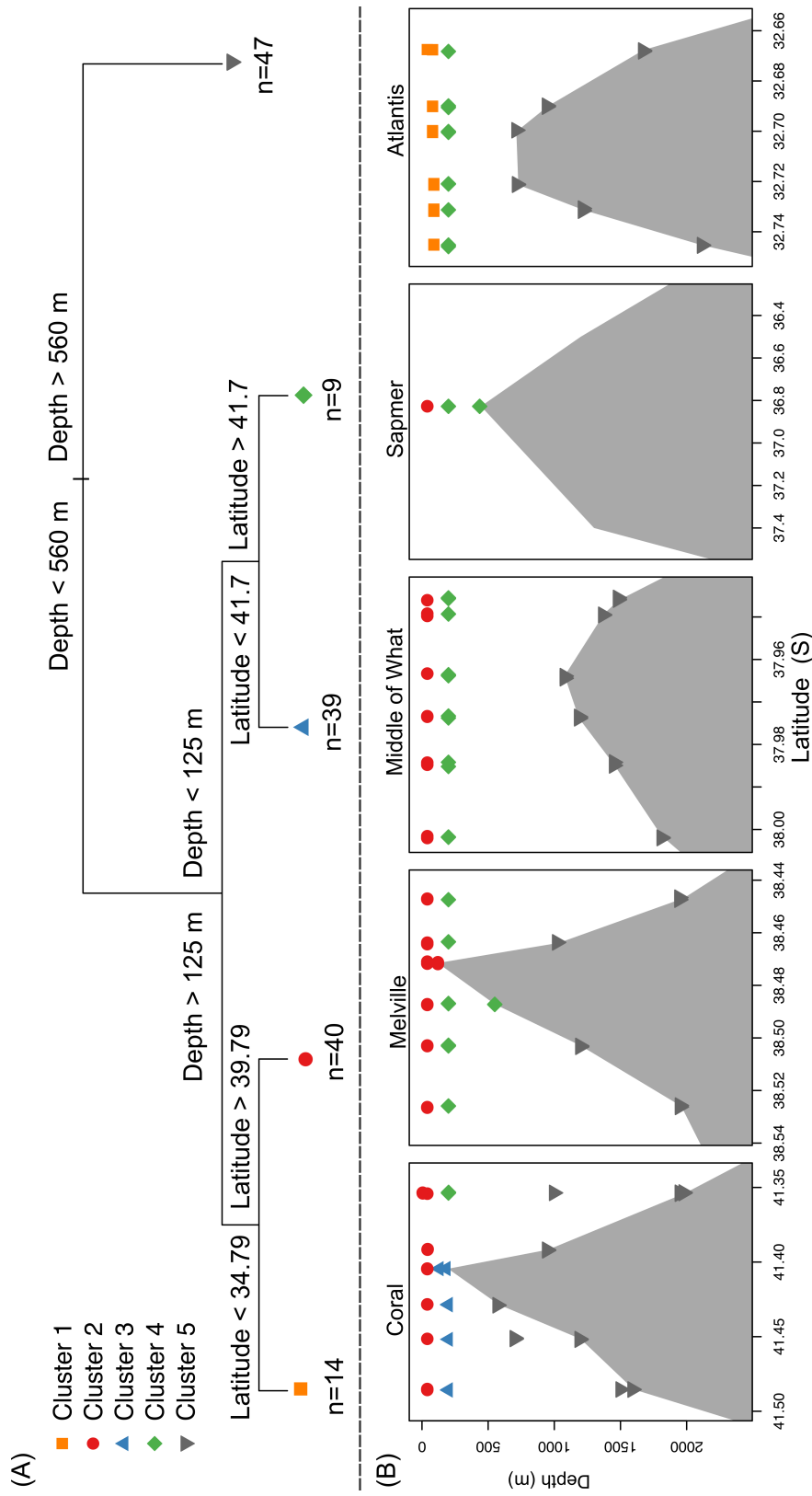


Figure 3.7: A) Multivariate Regression Tree (MRT) on all biological and physical variables measured in this study. Latitude and depth were the only significant variables creating five clusters. B) samples are coloured according to the clustering by the MRT.

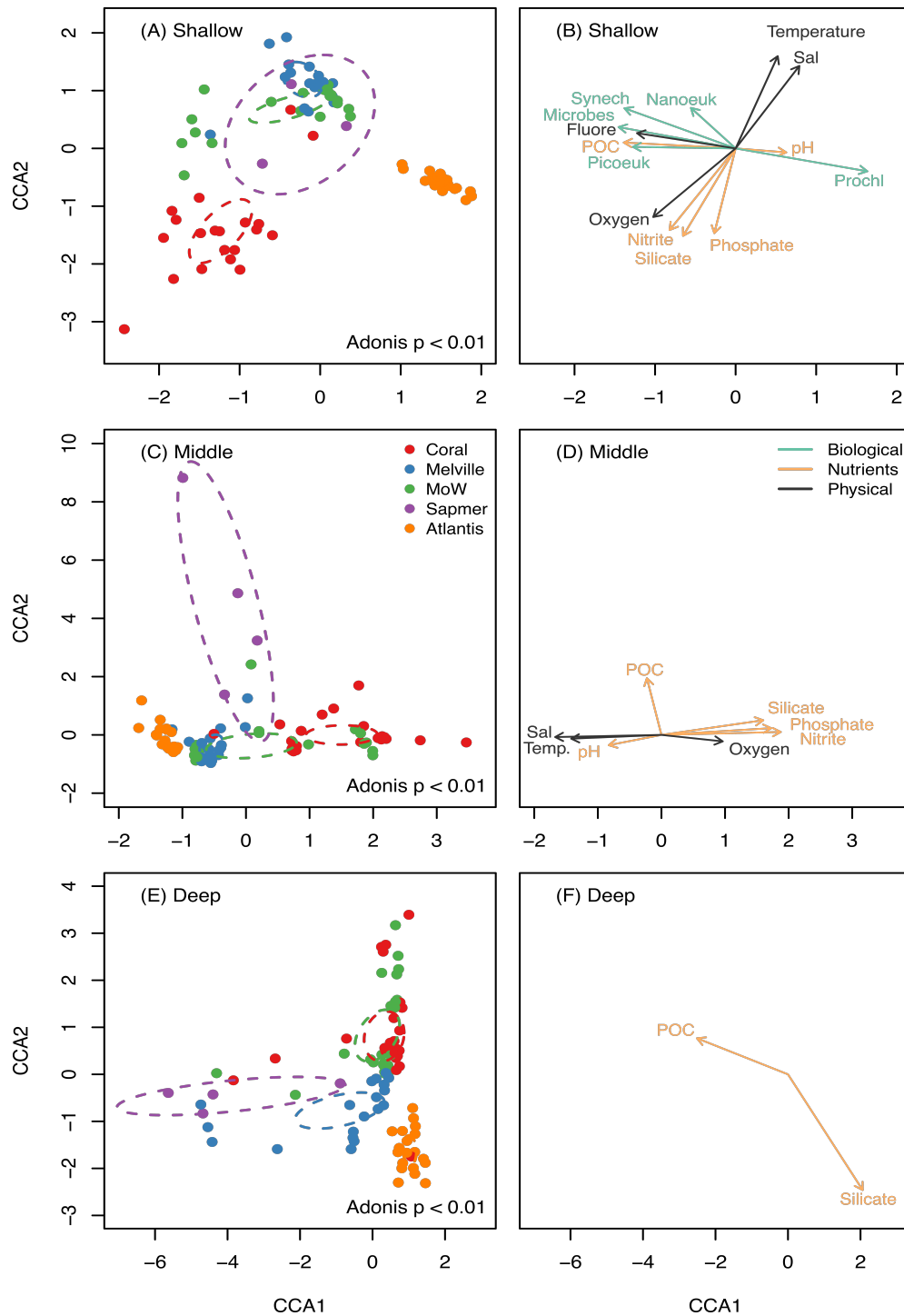


Figure 3.8: Canonical correlation analysis (CCA) divided into the three depth layers (shallow=40-80 m, middle= 200 m, deep= ≥ 200 m), coloured by station. Each CCA in a depth layer is followed by the environmental variables correlated to the microbial communities on those seamounts. Seamounts and environmental variables are plotted on the same scale on each depth. Only variables with a higher significance than 0.01 were visualised. The ellipses represent the 99% standard error. MANOVA = Multivariate Analysis of Variance.

Middle of What.

3.4 Discussion

3.4.1 Depth distribution of microbial communities

Our study reveals that the community composition of microorganisms is similar to open-ocean and deep-sea environments elsewhere (Sunagawa et al., 2015), with similar phyla being abundant, particularly Alphaproteobacteria and Gammaproteobacteria, throughout the water column. We found very similar relative abundances of all phyla to Sunagawa et al. (2015), with the exception of much lower abundance of Alphaproteobacteria in our samples. In our study, the dominating primary producers are the Synechococcaceae, which have been found in similar abundances globally (Zubkov et al., 2000; Zwirgmaier et al., 2007) (Chapter 2). This is in contrast to Alves Junior et al. (2015) who found members from the order Prochlorales dominating the surface and chlorophyll maximum layer in the southwest Atlantic Ocean. In the shallow layer we also found the Actinobacteria, OCS155, which are heterotrophic and prolific producers of secondary compounds (Ward and Bora, 2006), likely relying on excretion of organic compounds from primary producing organisms.

We find similar relative abundances of total Gammaproteobacteria in the deep layers to Alves Junior et al. (2015), although in our study it is dominated by the order Oceanospirillales, and not Alteromonadales. The MRT analysis suggested distinct differences in community composition at depths >560 m with characteristic deep-sea microorganisms SAR324 and Oceanospirillales. SAR324 has been implicated in sulphur oxidation, carbon fixation and heterotrophy. The versatile metabolisms of SAR324 (lithotrophy, heterotrophy and alkane oxidation) all operate simultaneously, and may explain SAR324's ubiquity in deep oceans (Sheik et al., 2014). The order Oceanospirillales, being the other of the two dominating OTUs, is indicative of the first split of the MRT. Oceanospirillales is a psychropiezophilic microorganism, which explains its preference for the deep sea, and colder environments, such as Coral seamount rather than Atlantis (Cao et al., 2014). The surface split in the MRT is caused by another abundant family in our samples, Crenarchaeaceae, which is a very common member of deep-sea communities (Auguet et al., 2010) and Synechococcaceae a common mem-

Table 3.3: Bray-Curtis dissimilarity index between shallow depth strata of the different stations. Values scale from 0-1, with 0 being the most similar and 1 the least.

Shallow	Coral	Melville	MoW	Sap
Melville	0.4728957			
MoW	0.4244729	0.313426		
Sapmer	0.7913352	0.8063409	0.7990701	
Atlantis	0.6357124	0.4921935	0.4955671	0.8486491

Table 3.4: Bray-Curtis dissimilarity index between middle depth strata of the different stations. Values scale from 0-1, with 0 being the most similar and 1 the least.

Middle	Coral	Melville	MoW	Sap
Melville	0.4952477			
MoW	0.3935434	0.2904049		
Sapmer	0.7911203	0.7658903	0.7913297	
Atlantis	0.6000634	0.3645212	0.4170942	0.7611666

Table 3.5: Bray-Curtis dissimilarity index between deep depth strata of the different stations. Values scale from 0-1, with 0 being the most similar and 1 the least.

Deep	Coral	Melville	MoW	Sap
Melville	0.4179698			
MoW	0.2582076	0.3806682		
Sapmer	0.8113728	0.6868022	0.7920829	
Atlantis	0.4364046	0.346313	0.4414915	0.8269666

3.4 Discussion

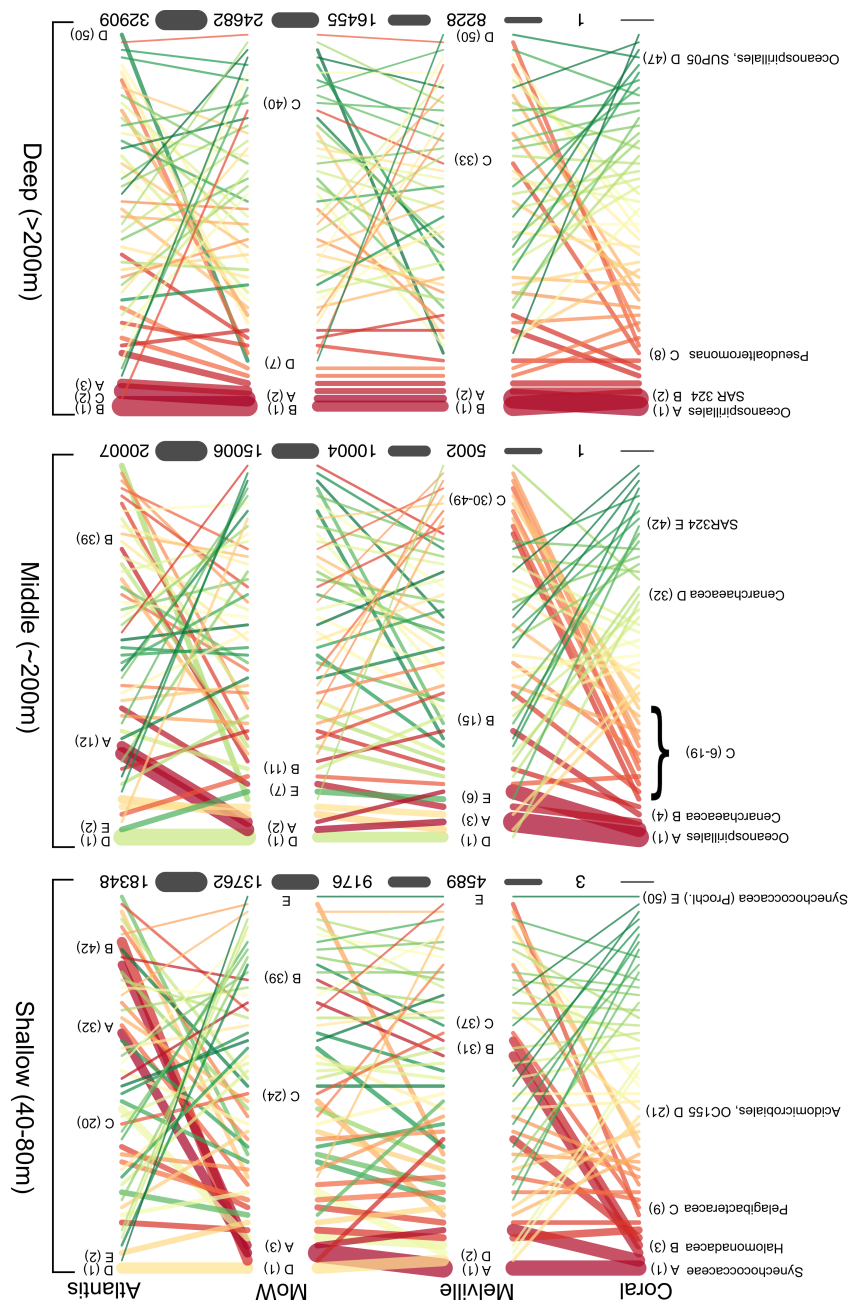


Figure 3.9: Rank abundance between the 50 most abundant OTUs on Coral, Melville, Middle of What and Atlantis. The rank abundance plots are divided between the three depth layers (shallow=40-80 m, middle= 200 m, deep=>200 m). Taxonomy of certain OTUs is specified on the left side of the figure. The letter for each OTU changes according to rank (in brackets) on that specific seamount. The line thickness values are based on a rescaling of all OTU values for all stations to each other represents the change in relative abundance of an OTU from one station to the next (see legend).

ber of the surface realm (Scanlan et al., 2009). In contrast to Alves Junior et al. (2015) we have samples at 200 m and, which show higher abundance of Thaumarchaeota at depths just below the chlorophyll maximum, which was also demonstrated by Sunagawa et al. (2015).

Overall, we found that most of the abundant microorganisms in the deep layer were microorganisms with a wide range of life strategies, enabling widespread geographic distribution. Similarly, at the surface, we found a high abundance of microorganisms adapted to high light conditions, such as Synechococcacea and the Actinobacterium, OCS155. The middle layer appears to be a mixture between the deep and shallow communities. It has a high abundance of several groups common in the deep (e.g. Thaumarchaeota and Deltaproteobacteria) and shallow (Flavobacteriia, Alphaproteobacteria and Synechococcacea). The layer below the chlorophyll maximum has been established as the area where most re-mineralisation takes place in the water column, which may explain the presence of high abundances of heterotrophic microorganisms such as Pelagibacteraceae capable of degrading dimethylsulfoniopropionate (DMSP) released by decaying phytoplankton (Cui et al., 2015). This might explain the pattern of higher species richness seen in the middle (Fig. 3.3) with a habitat structure similar to that of the deep-sea but a stronger influence from the surface, creating an intermediate habitat where psychrophilic, piezophilic, and primary producing microorganisms can co-exist: some of them thriving and some existing as transient members, sinking away from the surface.

For marine macrofauna depth is a stronger predictor of metazoan community structure than geographical location (Sutton, 2013). Some recent studies showed depth variation in microorganisms, but most focus has been on coastal areas or open-ocean within the upper 100-300 m (Sunagawa et al., 2015) with exception of a few open-ocean research (Alves Junior et al., 2015). In our study we demonstrate that, like macrofauna, the microbial community is segregated by depth (Auguet et al., 2010; Sunagawa et al., 2015; Alves Junior et al., 2015). Because temperature decreases with depth, this might obscure the temperature effect on the microbial communities as observed by Sunagawa et al. (2015), because lower temperatures are in deep layer and higher temperatures at the surface. However, temperature is often merely an indicator of water mass, and can be confounded within location when investigating biogeography on a global level, or between two contrasting locations. In addition, depth appeared to be a stronger predictor of microbial community structure than geographi-

cal location, although within the layer above 125 m, location predicts a clear pattern of geographical separation in microbial composition. Microbial taxa are sensitive to temperature; therefore, two locations with the same temperature are likely to have a more similar microbial composition to one another than between two locations with different temperatures.

Studies suggest that different seamount morphologies, as well as the variability of impinging currents, result in a broad range of hydrodynamic patterns, the relative strength and persistence of which may vary greatly in space and time (Clark et al., 2010). Consequently, the integral effect of seamounts on biological communities becomes highly intermittent and difficult to observe on the spatial and temporal scales accessible by vessel-based research. Mendonça et al. (2012) observed a clear effect on the summit of Seine and Sedlo seamounts in the North Atlantic Ocean, with higher biomass and abundance on the summit compared to a reference background sample. Unfortunately, we did not have reference locations away from the seamounts and therefore cannot provide insight into potential differences between on-seamount and off-seamount samples. However, we collected multiple samples along transects across all but one seamount (Sapmer) and were able to compare within seamount differences. All seamounts were relatively homogeneous within each depth layer and the MRT did not separate the summit or benthos of individual seamounts from the remainder of the samples. Although significant heterogeneity of microbial community composition has been described on local scales (1-10s km) (Martiny et al., 2011), the depth zonation of our samples is greater than the between sample differences at the same depth at geographical distances in the order of (10-100s km), in other words, we demonstrate geographical distance patterns between depth layers of the seamounts. On these seamounts it has been shown that the demersal stratum is dominated by benthos-associated zooplankton (Letessier et al., In press). The differences between demersal and pelagic microbial species are not well known and there were no clear effects of distance to seabed within a depth layer, although comparative samples are only available for Coral and Melville, given the deep summits of the other seamounts. It has been shown before that the particulate organic carbon can be depleted on the summit of a seamount (Chapter 2), however, no effect is seen by obstruction of summit with differences between the summit/flank microorganisms. Given the sampling design of our study, distance to seabed is furthermore confounded with depth and so the study cannot address the differences of benthic boundary layer compared to open

ocean microbial communities at similar depth.

3.4.2 Regional biogeography between seamount microorganisms

The SWIR is in an area where the Agulhas Return Current (ARC), the Sub-Tropical Front (STF) and the Sub-Antarctic Front (SAF), further to the south, create one of the most energetic and important hydrographic regions of the global ocean (Read et al., 2000). In the frontal zone (Melville and Middle of What), peak chlorophyll concentration of $>1 \mu\text{g l}^{-1}$ has been recorded (Read et al., 2000). Outside this region, chlorophyll concentrations have been measured at $<0.9 \mu\text{g l}^{-1}$ (Bornmann et al., in press). Thus, seamounts along the SWIR are in contrasting productivity regimes and water masses depending on their proximity to the sub-tropical convergence zone and the sub-Antarctic front (Chapter 2). This trend is also reflected by the abundance of microorganisms, with higher abundances south of the sub-Antarctic front (Coral) and lowest abundances in the sub-tropical north (Atlantis) of the sub-tropical front (Melville and Middle of What).

Microbial communities of corresponding depth layers between north (Atlantis), convergence zone (Melville and Middle of What) and south (Coral) have similar abundant microorganisms at the order and phyla level, indicating adaptation to habitat rather than location for similar types of organisms. However, on an OTU level, the microbial communities show quite large differences between each location based on rank abundance, the MRT and the CCAs. The MRT divides all deep seamount samples into one cluster, while subdividing the middle layer of Coral into one cluster, and the surface layer of Atlantis into another. As seen in the rank abundance, the surface layer on Coral is clearly dominated by a *Synechococcaceae* OTU, which also dominates Melville and Middle of What, while Atlantis is dominated by a different OTU from the *Synechococcaceae*. Both *Synechococcus* and *Prochlorococcus* are in this family, and, as shown in Chapter 2, the dominant cyanobacteria at the surface of these seamounts are *Synechococcus* and *Prochlorococcus*, respectively. The family OCS155 is also dominant on Atlantis, and, together with *Prochlorococcus*, explains the distinct clusters from in MRT. In the middle layer there is a clear cluster that decreases from high rank abundance on Coral to low rank abundance on the other seamounts. The middle layer on Coral seamount was weakly stratified with maxima and minima temperature and salinity at 250 m depth, respectively (Read and Pollard, In press). The deep layer formed a single cluster signifying the stability of this environment with the deep

Antarctic current dominating below 1000 m on all seamounts (McDonagh et al., 2008). Atlantis was the seamount in the most strongly stratified waters (Read and Pollard, In press) creating a very stable environment. As depth is the strongest predictor of microbial community structures, the stable stratified environment (i.e. no mixing between depth strata) on Atlantis would explain the largest difference seen in microbial community structure within the deep and shallow layers on Atlantis (Table 3.2) between all samples, again indicating a depth effect that is stronger than a geographical location effect.

Following the segregation of the complete data set into the five clusters by the MRT, the depth strata were separated, and emerged as a segregation factor for the community structure on an OTU level according to the CCA. The rank abundance plots clearly demonstrate the shifts between the microbial communities from south to north, through the convergence zone. The most abundant taxa on Coral are low on Melville, with a similar shift between Middle of What and Atlantis, demonstrating the community shift with oceanic water mass and habitat. This is evidence that water masses influence prokaryotic community composition and are considered barriers to microbial dispersal (Acha et al., 2015). In the deep sea, habitats have relatively little environmental variation (e.g., temperature, salinity) has led to the evolution of species which have broad horizontal ranges. However, because abiotic and biotic factors vary greatly with depth, many species possess restricted vertical ranges. Structuring of the microbial diversity is related to the physical, chemical, and biological features of the water masses (Acha et al., 2015); however, the definition of water masses by physical properties can be enhanced by the microbial ecology component as highlighted in this study. The water mass separation based on temperature and salinity are in agreement with our grouping based on microbial diversity (Read and Pollard, in press). Based on the microbial communities the north, convergence zone, and south could be considered three biogeographic zones, as previously suggested by macrofauna and megafauna, chemical, and physical studies (Laptikovskiy et al., In press; Letessier et al., In press; Read and Pollard, in press; Boersch-Supan et al., In press) (Chapter 2). Such clear water mass separation has not been found previously in microbial communities (Alves Junior et al., 2015). However, the ability to detect a biogeographic pattern may depend on taxonomic resolution. Biogeographic patterns can exist in the absence of endemism. As demonstrated in this study, the distance–decay relationship is clear between the most abundant taxa. Accordingly, the latter half of Baas-Becking princi-

ple ‘the environment selects’ combined with distance-decay, might be appropriate in this example where currents might facilitate adequate dispersal between seamounts to continuously distribute microorganisms, but restriction of differing environments will compromise the success of the specific microbial taxa. However, the dispersal is not high enough to counteract the compositional differentiation imposed by the distance-decay relationship (Hanson et al., 2012) and, contrary to ideas previously suggested by Hanson et al. (2012), the distance-decay relationship is strong in the pelagic environment between seamounts in differing environmental setting (water-masses). This demonstrates a regional biogeography, on major taxa, with semi-restricted dispersal by currents and lack of water mass mixing.

Chapter 4

Organic matter in deep-ocean hydrothermal systems

Abstract In this study we investigated the concentration and composition of dissolved organic matter and related compounds in three hydrothermal environments. Our samples were taken from deep ocean high-temperature hydrothermal vents and the plumes emanating from the fluid discharge. We analysed organic carbon and nitrogen concentrations as well as hydrothermal constituents (Mn, CH₄), and characterised solid phase extracted dissolved organic matter (SPE-DOM) by high-resolution mass spectrometry (FT-ICR-MS). We found no systematic variation in carbon concentrations in the hydrothermal plumes, but some evidence to suggest a regional increase (~1.7 µM) in the concentration of total organic carbon around the vent sites. We also found large changes in concentration and molecular composition in the high-temperature fluids at all three sites, with the fluids containing lighter, more H, N, S rich and O depleted molecular formulas with less aromaticity and unsaturation. Our data suggest that while crustal heating destroys most DOM in circulating fluids, interaction of the plumes with deep ocean water stimulates the production of organic carbon in the water column in the region of venting. The effect on the global DOC budget of the latter process may be a thousand times greater than the former, leading to some net deep ocean production of DOM, roughly equating to 1% of the export from surface primary production to deep waters.

Chapter 4 Organic matter in deep-ocean hydrothermal systems

4.1 Introduction

Dissolved organic matter (DOM) is present throughout the deep oceans at relatively uniform concentrations of 34 to 50 μM carbon (Hansell, 2013), and is composed of billions of individual molecules at vanishingly small concentrations. Most of deep ocean DOM is refractory, and has accumulated in the oceans over thousands of years. This refractory DOM plays an important role in the long-term storage of carbon in the deep oceans (Hansell, 2013; Dittmar and Koch, 2006; Jiao et al., 2010), and the biotic and abiotic mediation of carbon between inorganic and organic pools in seawater is a critical control on atmospheric and marine concentrations of CO_2 , O_2 and marine nutrient supply (Jiao et al., 2010; Sexton et al., 2011; Giering et al., 2014; Meador et al., 2010).

The concentration and composition of DOM in natural waters is most variable over steep geochemical gradients such as in estuaries (Ward et al., 2013; Roth et al., 2013) and other redox interfaces (Riedel et al., 2013, 2012). In this study we consider the changes to DOM during high temperature hydrothermal circulation, an extremely dynamic environment in the typically stable and unreactive deep ocean environment.

The concentration of DOC can be higher or lower than ambient seawater in high temperature hydrothermal fluids (Lang et al., 2010, 2006), and this depends on the geochemistry of venting and the extent of sedimentation in the subsurface crust and overlying the vent orifices (Lang et al., 2006). The molecular changes to DOM (such as changes to the reactivity of refractive DOM) during hydrothermal circulation are under-investigated to date. The interpretation of hydrothermal fluid analysis is challenging because hydrothermal vents are not simply flow-through systems for existing DOM; there is also production of new DOM through interaction of microbes with magmatically sourced methane, hydrogen and sulphide (Crépeau et al., 2011; De Angelis et al., 1993; Teske et al., 2002), abiotic synthesis of small DOM compounds (Lang et al., 2010; Shock and Schulte, 1998; Martin and Russell, 2007; McCollom et al., 2010; McCollom, 2000), reduction of dissolved inorganic carbon and interaction (desorption/adsorption) of DOM with host rocks and sediments (Simoneit, 2002, 1983; Delacour et al., 2008).

The effect of high temperature systems on global DOM distributions may be smaller along oceanic ridge axes (e.g. < 1 Ma crust (Stein and Stein, 1994)) than low temperature systems at ridge flanks (McCarthy et al., 2011; Lang et al., 2006), but the paucity

of dissolved organic carbon and nitrogen (DOC/DON) concentration data available from axial high temperature fluids and hydrothermal plumes currently precludes assessment of the importance of these systems for marine DOM. The higher temperature of these systems may also allow greater alteration to the molecular composition of DOM in the circulating seawater.

In this study we analysed DOC and DON concentrations and determined mass distributions of solid phase extracted dissolved organic matter (SPE-DOM (Dittmar et al., 2008)) in high temperature hydrothermal fluids from three sites with very diverse geological setting and fluid chemistry. We also measured total and particulate organic carbon and total nitrogen (TOC, POC, TN), and characterised SPE-DOM in a suite of samples from the dispersing plumes from the three sites. We compared trends in the data with concentrations of manganese and methane – typical tracers of hydrothermal influence on seawater. Our aim was to investigate whether high-temperature hydrothermal circulation (and plume formation) was a source or sink of total DOC and DON, and whether any organic compound classes could be identified that were removed from, altered in or provided to ocean water at these sites. We focus on large (>150 Da), long-lived forms of DOM and how they are altered in high temperature settings. We hypothesise that through venting hydrothermal systems break down carbon in the ambient water, which then becomes freely available to microbial production. We expect the two compared systems to most likely be dissimilar given the geographical distance and physical/chemical differences.

4.2 Methods

4.2.1 Sampling strategy and methods

Sample collection equipment

Samples were collected at sea during two cruises on the Royal Research Ship (RRS) *James Cook* to the East Scotia Ridge, Southern Ocean (JC080) Mount Dent, Caribbean Sea (JC082) and the deep Cayman Trough, Caribbean Sea (JC082). Three vent sites were sampled (E2 Vent Field, Von Damm Vent Field and Beebe Vent Field (AKA Piccard Vent Field), respectively; Fig. 4.1) and samples were taken from plumes and vent orifices according to water column anomalies using a Conductivity, Temperature and

Depth (CTD) profiler and by real-time camera observation and sampler manipulation using a remotely operated vehicle (ROV) *Isis*.

The Conductivity, Temperature and Depth (CTD) profiler (Seabird +911) was mounted on a titanium frame that holds 24 externally sprung 10 litre OTE (Ocean Test Equipment) sampling bottles. These bottles are specifically designed for the sampling of waters with low levels of trace metals, organic carbon and nutrients, and are Polytetrafluoroethylene (PTFE) lined with PTFE taps. The frame was also equipped with a light scattering sensor (LSS) and a bespoke Eh detector (Ko-ichi Nakamura) for detecting hydrothermal anomalies.

The ROV *Isis* was equipped with 6 OTE internally sprung bottles (1.2 litre capacity) mounted on a carousel at the back of the vehicle next to a CTD system (Seabird FastCAT SBE 49) and a low resolution/high range LSS sensor (0-1000 NTU, ECO-NTU, Planet Ocean Ltd.). A camera was also positioned to observe the bottles, allowing triggering of the closing mechanisms when the bottles (and not just the sensors) were in the black smoker plumes or shimmering diffuse waters. On the RRS *James Cook* (JC) 080 (E2 Vent Field), the ROV was equipped with two 2x750 ml capacity titanium samplers, which could be accurately positioned into vent orifices for sampling with real-time temperature logging. On JC082 (Von Damm and Beebe vent fields) a similar system was used but with lower capacity gas-tight samplers (200 ml).

CTD profiler and ROV bottle sampling

Total Organic Carbon and Nitrogen (TOC/TN) samples were taken directly from the Ocean Test Equipment (OTE) bottle PTFE stopcock. Dissolved organic carbon and total dissolved nitrogen (DOC/TDN) were sampled through a 0.7 μm pre-combusted GF/F filter directly from the OTE bottle. Sample vials (ICHEM certified low level total organic carbon) were rinsed with the sample seawater and were filled with 20 ml sample. Particulate Organic Carbon and Nitrogen (POC/PON) were taken by vacuum filtration of a larger quantity of seawater (3-5 litres) through a pre-combusted GF/F filter (47 mm, Whatman). When filtering was completed, the filter was placed in a tin foil envelope and put in the oven at 55°C until dry (~10-15 minutes). Samples were then frozen at -20°C in the tin foil envelope. Samples (400 ml) for characterisation of dissolved organic matter were taken directly from the OTE bottles and frozen immediately (without filtration). Samples for methane were taken into 500 ml Evarex

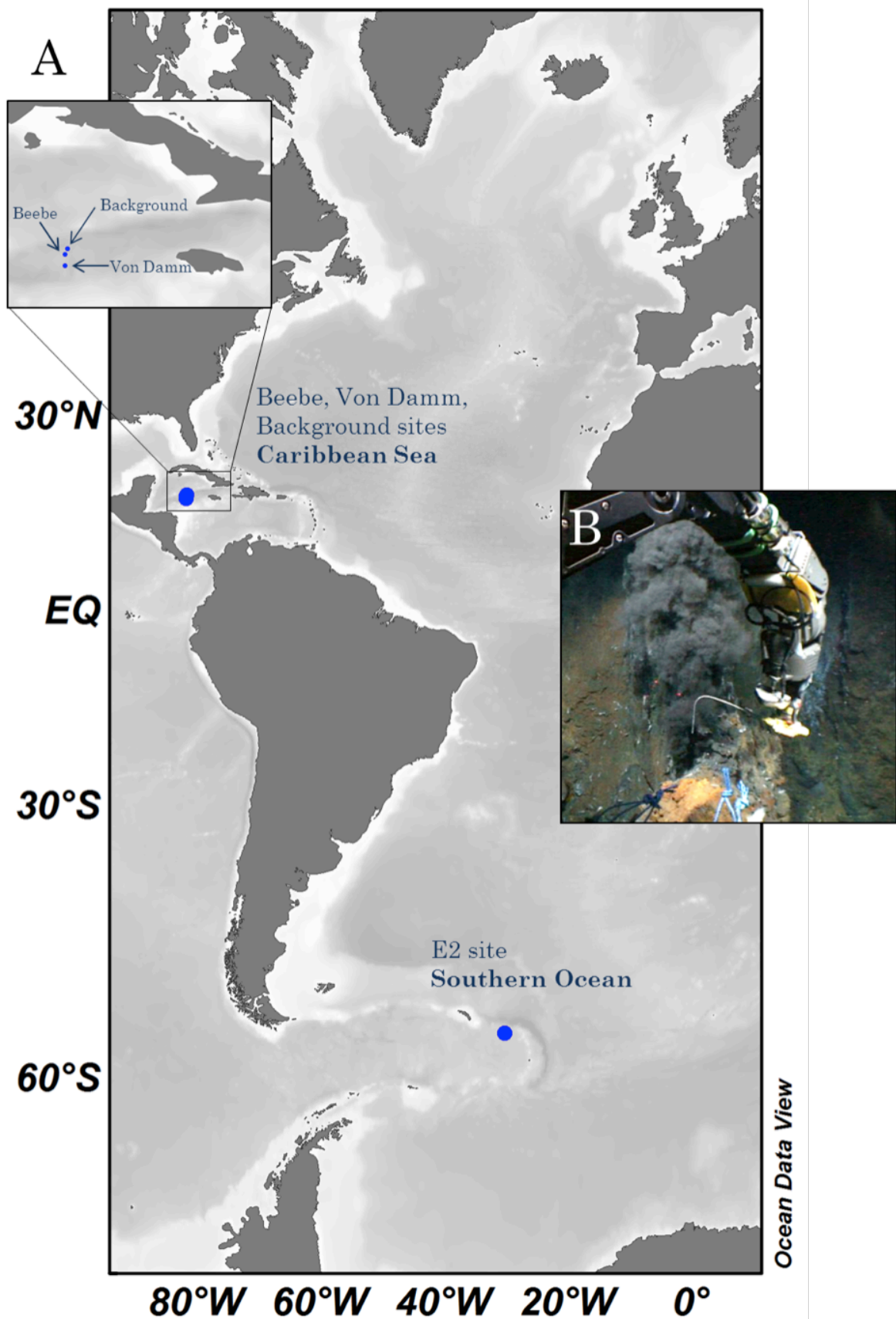


Figure 4.1: A: Map showing the location of the three vent sites and a background site studied in our research. B: Photograph taken by Remotely Operated Vehicle (ROV) showing the sampling of a high temperature vent at the Beebe vent site.

triple layer Barrier Bags (Oxford Nutrition, Devon, U.K.) for CTD samples and 21 ml glass vials for ROV OTE bottle samples. Samples for manganese were taken into acid cleaned LDPE bottles in a dedicated clean van, without filtration. The samples were acidified to pH 1.9 with sub-boiled HNO_3 and stored for at least six months before analysis.

ROV fluid sampling

Upon recovery, the samplers were rinsed with deionised water and the fluid was taken as quickly as possible. Time sensitive parameters such as alkalinity, pH, gases and H_2S were taken first, then samples were taken for anions and cations. These were analysed shipboard or at the National Oceanography Centre (NOC) Southampton, UK, as described previously (James et al., 2014), except gases from JC082 (Von Damm and Beebe), which were analysed at Géosciences Environment Toulouse, France. Except for one sample from the E2 site, only DOC/TDN were taken from the high temperature fluids because of the high concentration of poly-metallic sulfide particles, which may have affected the analysis of TOC/TN. The sample volumes were not high enough for filtration/analysis of POC/PON. At the E2 vent field, where 750 ml titanium ‘majors’ samplers were used, the sampling tube was connected to an HDPE Luer-lock fitting which was connected to the polypropylene filter housing as described above. At the Von Damm and Beebe vent fields, where 200 ml gas-tight samplers were used, DOC/TDN samples were drawn into a sterile polypropylene syringe, and the sample was then filtered through the GF/F filter by direct attachment of the syringe to the polypropylene filter housing. Blank analysis suggested that contamination from collection and filtration was $<3 \mu\text{M C}$; far lower than the scale of concentration differences we found in the fluid samples. Samples for characterisation of dissolved organic matter (400 ml at E2, 35-40 ml at Von Damm and Beebe) were taken directly from the samplers and frozen immediately (without filtration).

All DOC/TDN and TOC/TN samples, along with shipboard deionised water blanks, were acidified to pH 2 with 50 μl 8M HCl (Fisher Scientific; sub boiled in deionised water, MilliQ, Millipore). They were stored in the dark at 4°C onboard the RRS *James Cook*, and were analysed at the NOC Southampton, UK within 8 months of sampling.

4.2.2 Analysis

TOC, DOC, TN and TDN

The acidified samples were analysed by oxidative combustion using a Shimadzu TOC-V and TNM-1 simultaneous analyser. The organic carbon in each sample (TOC or DOC) was measured by infrared analysis of CO₂ after purging of inorganic carbon from the acidified sample and oxidative combustion using a platinum catalyst in a combustion tube set to 720°C. The total nitrogen in each sample (TN or TDN) was analysed by chemiluminescence after reaction of the oxidised sample nitrogen (as NO) with ozone (generated from air) to form NO₂ and light. Organic nitrogen was then calculated by subtraction of nitrate + nitrite concentration. External standards were prepared from furnace dried (110°C, 1 hour) glycine (C and N) and furnace dried potassium hydrogen phthalate (C only) in deionised water (MilliQ, Millipore). The standards were prepared to range 25-125 µM (carbon) and 8-36 µM (nitrogen). A larger standard range (13.5 - 675 µM C, 4.0 - 200 µM N) was also prepared for analysis of the high temperature vent fluids, which had much more variable concentrations than the plume samples. During the analysis, 3 injections of seawater (100 µl) were measured after two automated washes of the Shimadzu syringe, and if the coefficient of variance was greater than 2% for carbon or 4% for nitrogen a fourth injection was measured. The average of the three injections with the least variance was then taken. Certified reference materials (Low Carbon Water (LCW; lot 12-01-B2) and Deep Seawater Reference (DSR; lot 12-08), Hansell Laboratory, University of Miami) were analysed after every 8 samples, and the Shimadzu TOC-V combustion tube was changed and a new calibration was conducted if the DSR carbon was not between 40-44 µM (certified value 41-43 µM). This was typically after ~100 seawater samples due to the accumulation of sea salt on the platinum catalyst. To avoid contamination, samples were individually fed to the instrument and purging of inorganic carbon was conducted in the internal syringe of the instrument (i.e. the auto-sampler carousel was not used).

DOC/TN quality control

The method blank was checked by acidifying shipboard Milli-Q water followed by storage and analysis as described above. Two blanks contained 2.64 ± 0.73 and 3.33 ± 0.44 µM carbon; nitrogen was below the detection limit of 0.4 µM. The shipboard

MilliQ water was not used in any part of the sampling process, so these samples should be considered maximum estimates of the method blank, and no blank is subtracted from the reported concentrations. Reproducibility was checked by measuring 5 TOC samples from one CTD OTE bottle. The coefficient of variation was 1.3% for carbon, 4.0% for nitrogen. The variability of the DSR blank was similar: 1.48% for carbon, 1.42% for nitrogen ($n=54$). Results are reported with conservative errors (E) as the root sum of squares of the reproducibility error (E_R) and the coefficient of variation between the three sample injections (E_S) (i.e. $E = \sqrt{E_R^2 + E_S^2}$). Introduction of contamination from the internally sprung OTE bottles on *Isis* was estimated by filling the bottles with seawater from the CTD profiler and comparing the TOC content in the two bottle types after 4 hours of storage at 4°C and 2 hours of storage at 22°C (typical conditions for samples taken by *Isis*). The *Isis* bottle samples averaged $3.05 \pm 1.16 \mu\text{M}$ (mean $\pm$ SD, $n=3$) more than the OTE bottle samples. This blank is subtracted from the presented data for these samples, and the error bars incorporate this bottle error (E_B) (i.e. $E = \sqrt{E_R^2 + E_S^2 + E_B^2}$).

Our obtained values for DOC were usually slightly higher in carbon (1-3 μM) than TOC for the same sample, indicating a small but important amount of contamination in the filtration process. We therefore only report TOC/TN values for the CTD and ROV OTE bottle samples. Only DOC was taken for most of the high temperature fluids, but the variability we found in these fluids was far greater than this contamination value. The reported concentrations were corrected for the daily average of DSR analyses (i.e. all DSR samples were assumed to have equal DOC concentrations) in order to account for small daily differences in the instrumental blank.

Particulate organic matter (POM)

Before analysis, the frozen filters were thawed, dried overnight (50-55°C) and placed in an desiccator with concentrated hydrochloric acid for approximately 2 hours to remove carbonates. Following the acid bath, the filters were dried again (50-55°C) for at least 6 hours. After drying and cooling the filters were folded and placed into nickel sleeves. For every sample, one nickel sleeve with a pre-combusted blank filter was also analysed. The samples were analysed using a CE440 Elemental Analyser (Exeter Analytical) by combustion and elemental analysis of the gas phase.

SPE-DOM characterisation

Samples for dissolved organic matter characterisation were filtered ($<0.2\ \mu\text{m}$, GHP Acrodisc 25mm, PALL Life Sciences) and de-salted / pre-concentrated using PPL solid phase extraction cartridges (Varian) as described in detail elsewhere (Dittmar et al., 2008). Briefly, the samples were acidified to pH 2 and passed over individual columns (0.1 g sorbent) at $\sim 10\ \text{ml min}^{-1}$. The columns were thoroughly rinsed with acidified de-ionised water, dried with argon and the SPE-DOM was eluted with 0.6 ml HPLC-grade methanol (Sigma-Aldrich). The samples were stored at -20°C in amber vials until analysis. An aliquot was quantified for carbon (SPE-DOC) by Shimadzu TOC-V after drying of methanol and re-dissolution with 10 ml acidified (pH 2) deionised water. For the high-temperature fluid samples, which had lower SPE-DOC concentrations, SPE-DOC was analysed in a more concentrated 1ml sample (Stubbins and Dittmar, 2012). SPE-DOC concentrations are reported here as equivalents in seawater (considering pre-concentration). Characterisation of SPE-DOM was conducted in negative ionisation mode by Electrospray Ionisation - Fourier Transform - Ion Cyclotron Resonance - Mass Spectrometry (ESI-FT-ICR-MS) using a 15 Tesla (0.1 mDa resolution) Solarix system (Bruker Daltonics, USA). The extracts were filtered (0.2 μm PTFE syringe filter) and diluted to approximately 15 ppm C in a 1:1 mixture of ultrapure water and methanol before analysis. A total of 200 scans were accumulated per run, and each sample was analysed three times. The vent fluid extracts contained considerably less carbon than the plume samples, and the settings were changed slightly for their analysis so that the ions were accumulated before separation and mass spectrometry for 1.2 seconds instead of 0.2 seconds. Spectra were measured over the mass window 150–2000 Da, and were individually calibrated with an internal calibration list using the Bruker Daltonics Data Analysis software package. Molecular formulae were assigned (Koch et al., 2005) to peaks that were significantly greater than the instrument noise and which occurred in at least two of the three repeat analyses. Formulas up to $\text{C}_{40}\text{H}_{100}\text{O}_{50}\text{N}_4\text{S}_4\text{P}$ were considered, and formulas were disregarded for unlikely combinations of heteroatoms (N,S,P). In cases of multiple formula assignments to one mass, P containing formulas were removed because of ambiguity with Cl^- and formulas only containing C, H and O were preferred over those containing S or N. For each molecular formula we calculated the double bond equivalents ($\text{DBE} = 1 + \frac{1}{2}(2\text{C}-\text{H} + \text{N} + \text{P})$) to examine the degree of unsaturation and the modified Aromaticity Index (AI_{mod}

$= (1 + C - \frac{1}{2}O - S - \frac{1}{2}H) / (C - \frac{1}{2}O - S - N - P)$ to assess the presence and extent of aromatic structures (Koch and Dittmar, 2006). Molecules were further assigned as black carbon ($AI_{mod} > 0.666$), polyphenols ($0.5 < AI_{mod} < 0.666$), highly unsaturated lignin-like ($AI_{mod} < 0.5$, $H/C < 1.5$, $O/C < 0.9$), unsaturated aliphatics ($1.5 < H/C < 2.0$, $O/C < 0.9$, no N), peptides ($1.5 < H/C < 2.0$, $O/C < 0.9$, with N), saturated fatty acids ($H/C > 2$, $O/C < 0.9$) and sugars ($O/C > 0.9$). Quality control was monitored with an in-house standard of deep-sea Pacific “NELHA Pool” water and deionised water blanks, which were put through the extraction/elution procedure.

CH₄, Mn and nutrients

Seawater CH₄ concentrations were determined on board after one-hour equilibration of the sample (500 ml for CTD, 21 ml for *Isis* OTE bottles) into a high purity nitrogen headspace (60 ml, 10 ml), after vigorous shaking. A 2 ml subsample of the equilibrated headspace was analysed by GC-FID (Agilent 7890 equipped with a 6 ft. 2mm i.d. stainless steel column with 80/100 mesh HayeSep Q support, isocratic at 60 °C). The instrument was calibrated with 10, 20 and 100 ppm CH₄ standards (S.D. 1%, $n=3$). Seawater CH₄ concentrations were calculated with consideration of sample temperature and pressure.

Manganese was measured in unfiltered samples after dilution in 1M sub-boiled HNO₃ using an Element 2 (Thermo Scientific) ICP-MS at the NOC Southampton, UK following the method of Milne et al. (2010). Mn concentrations were calculated from the slope of standard additions. Procedural blanks were calculated by repeat analysis of blank 1M sub-boiled HNO₃ solution ($n=10$). Limit of detection, calculated as 3σ of the blank, was 0.005 nM. Near shore seawater CASS-4 (NRCC) was analysed to assess accuracy, measured as 50.0 ± 2.8 nM compared to certified value of 50.6 ± 0.34 nM.

Nitrate and nitrite were measured by Seal QuAAtro at the NOC Southampton, UK for the CTD and ROV OTE bottle samples, and by small volume UV-vis spectrophotometry (Schnetger and Lehnert, 2014) at the ICBM, Oldenburg, Germany for the fluid samples.

Table 4.1: End-member concentrations and samples taken from the three vent fields. VD: Von Damm. SW: Seawater.

Site	Analyte	Fluid (μM)	SW (nM)	ppm (OTE)	ppm (Fluid)	Max ppm fluid
E2	Mn, Si	2050	0.1	0-6,952	47,011-999,000	999,000
VD	SO_4 , Si	14	0.1	0-14,079	250,274-748,214	740,317
Beebe	Mn,Si	674	0.1	0-1,150	97,106-990,418	842,153

4.3 Results

4.3.1 Vent geochemistry

This study used samples from three different vent sites. The E2 site is hosted on a back-arc basin spreading centre (Rogers et al., 2012), and is fairly typical globally in depth (2400 m), temperature (351°C) and major element concentrations (James et al., 2014). The Beebe vent site is the deepest discovered to date at ~5000 m, has a correspondingly high temperature (~401°C), and is hosted on a slow-spreading mid-ocean ridge axis (Kinsey and German, 2013). The Von Damm vent field is hosted on an ocean core complex at 2280 m, and vents methane rich, metal poor, 212°C fluids that precipitate talc, not sulfides or carbonates as found previously at deep-ocean hydrothermal vents. Between these three sites, a wide representation of global hydrothermal geochemistry was sampled (Table 4.1).

In the rest of this study, we consider samples according to their parts per million (ppm) vent fluid in background seawater. This is calculated using manganese, SO_4^{2-} or silica concentrations as follows (where X is the analyte):

4.3.2 Organic carbon and nitrogen concentrations

Concentrations of TOC (40-50 μM) POC (0.7-4.4 nM) or TON (2-7 μM , higher variation at E2 as a result of large nitrate subtraction) did not vary systematically throughout the dispersing plume, even over a very large range of the fluid-seawater gradient (Fig. 4.2). The concentration of DOC in the high-temperature fluids was much more variable, reaching as high as 761 μM at Von Damm, 246 μM at Beebe, and as low as 19.7 μM at E2. No simple mixing gradient was found at any of the three sites, and at Beebe it appeared that much (>50%) of the increased DOC precipitated with sul-

phides during the first few seconds of mixing (Fig. 4.2) – i.e. the relationship between ppm fluid and DOC was not conservative. DON (total dissolved nitrogen – NO_3^- and NO_2^-) concentrations increased up to 65 μM in the Beebe fluids, up to 25.2 μM in the Von Damm fluids, and remained below 5 μM (as in background seawater) in the E2 fluids.

Interestingly, while there were no systematic changes in TOC concentration in the Beebe plume, the samples contained more TOC than a set of 16 background samples taken in the region (Fig. 4.3), all of which were at least ten nautical miles away from the hydrothermally active site (see supplementary information). Our background sample set is only about half what it should be (16 instead of 30) for this result to be statistically robust, but ‘boot-strapping’ the Beebe samples into random sets of 16 for direct comparison by Student’s t-test always gives a significant difference between the two sets. Our data suggest that the plume-influenced region around the Beebe vent site contained about 1.7 μM more TOC than the background regions. The Von Damm and E2 vent fields are bathed in different water masses and cannot be as easily compared with this set of background samples.

4.3.3 Molecular composition of SPE-DOM

Extraction efficiency was $55 \pm 10\%$ (mean $\pm$ SD) in the CTD/ROV OTE samples and $14 \pm 12\%$ (mean $\pm$ SD) in the high temperature fluids (Fig. 4.2b). On average, 2521 ± 351 (mean $\pm$ SD) individual molecular formulas were assigned in the CTD and ROV-OTE bottle samples, and 1698 ± 456 (mean $\pm$ SD) formulas (i.e. significantly less) were assigned for the high temperature fluid samples. The average molecular weight of samples extracted from plume water was about 406 Da, and this trended downwards to 345 Da in the high temperature fluids (Fig. 4.4). The average carbon and oxygen content per molecule also decreased, from about 19.4 to 16.5 and 9 to 6.5, respectively. The average hydrogen and nitrogen content did not significantly change (so the H:C and N:C ratios increased) and the average sulphur content increased ten times from about 0.04 to 0.4 per molecule. The average double bond equivalence decreased from ~ 8.5 to 5 and the modified aromaticity index (Koch and Dittmar, 2006) decreased from 0.25 to 0.15, both indicating an increase in saturation of DOM. Note that all of these averages were weighted by signal intensity in order to devalue the peaks that occurred around the detection limit. This approach will over-represent easily ionisable

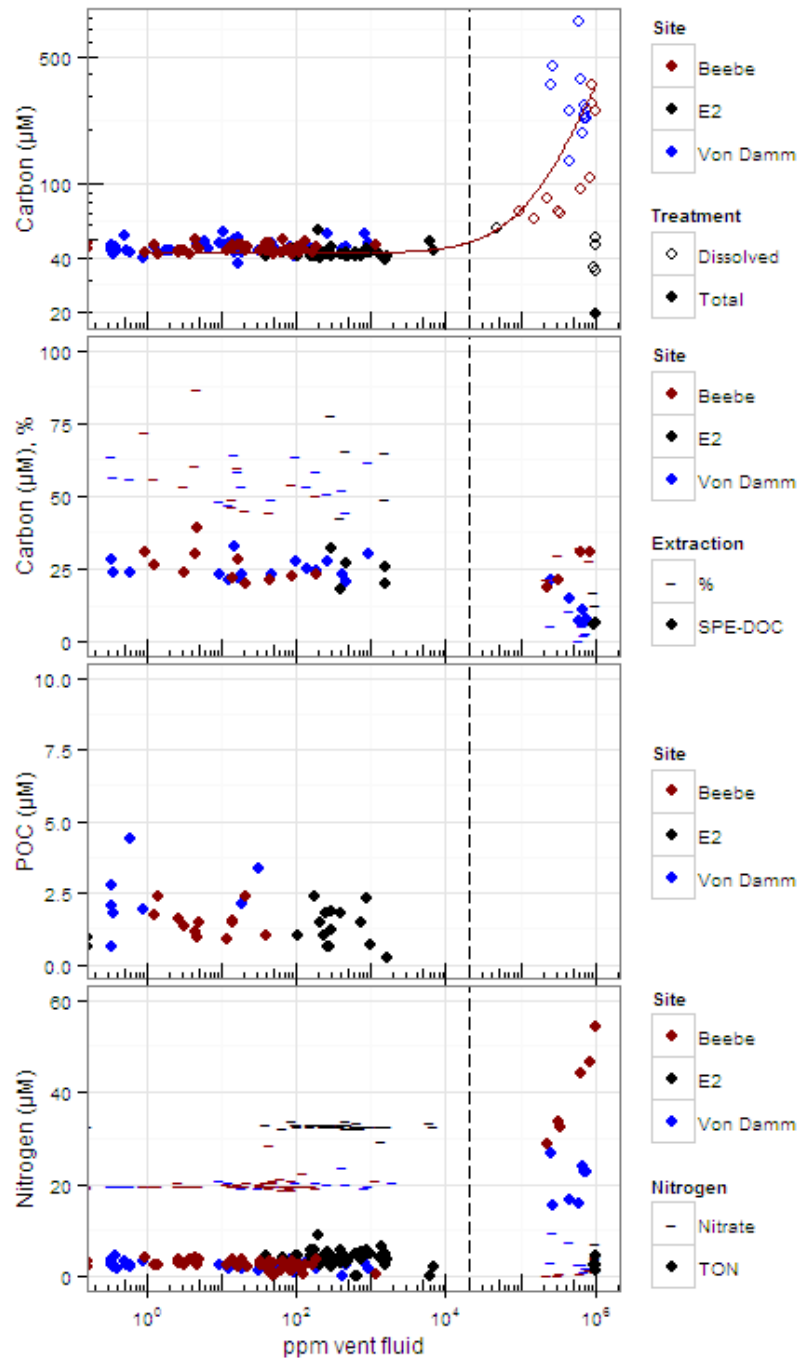


Figure 4.2: Concentrations of various analytes in the dispersing plumes of the three sites. All plots are shown vs. $\log[\text{ppm vent fluid}]$, and the fluid samples taken by ROV are separated by a dashed line. A) TOC and DOC (note log scale). The red line shows predicted conservative mixing at the Beebe site. Errors are smaller than the data symbols. B) SPE-DOC and extraction efficiency. C) POC. D) NO_3^- and TON.

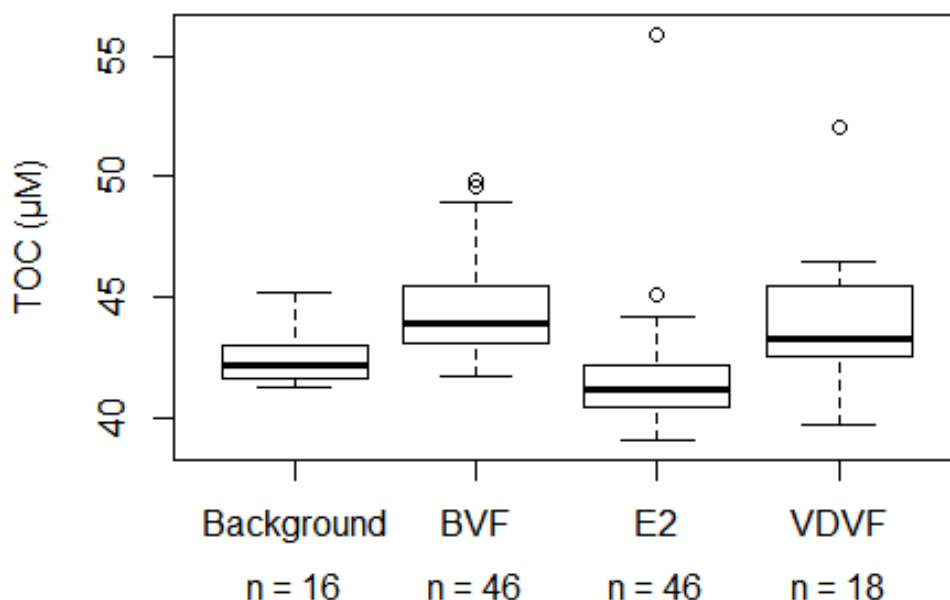


Figure 4.3: Box plot comparing the distribution of TOC concentrations taken by CTD only at the four sites. The background site samples were taken at depths similar to the Beebe site. The E2 site is in the Atlantic sector of the Southern Ocean and has lower ambient TOC concentrations.

molecules, but serves as a reasonable guide to overall trends. The weighted average formula in the plume samples was $C_{19.4}H_{24.6}O_{9.0}N_{0.34}S_{0.036}P_{0.005}$ and in the purest high-temperature fluid sample (*Isis190-Y2*, E2 site) was $C_{16.6}H_{25.8}O_{6.3}N_{0.45}S_{0.40}P_{0.014}$. The normalised molecular weight distributions were analysed by multivariate statistics and 90% of the differences between the samples could be described by five principle components. PC1 correlated strongly with ppm vent fluid, and described 58% of the variability in the data, and PC2 correlated well with the vent site, and described a further 16% of the variability (Fig. 4.5a). The other principle components did not seem to correlate to any environmental factors and the variability in the samples at this level was only as large as typical instrumental variability (<10% Bray-Curtis dissimilarity). For comparison, the fluid samples were up to 70% dissimilar to the plume samples

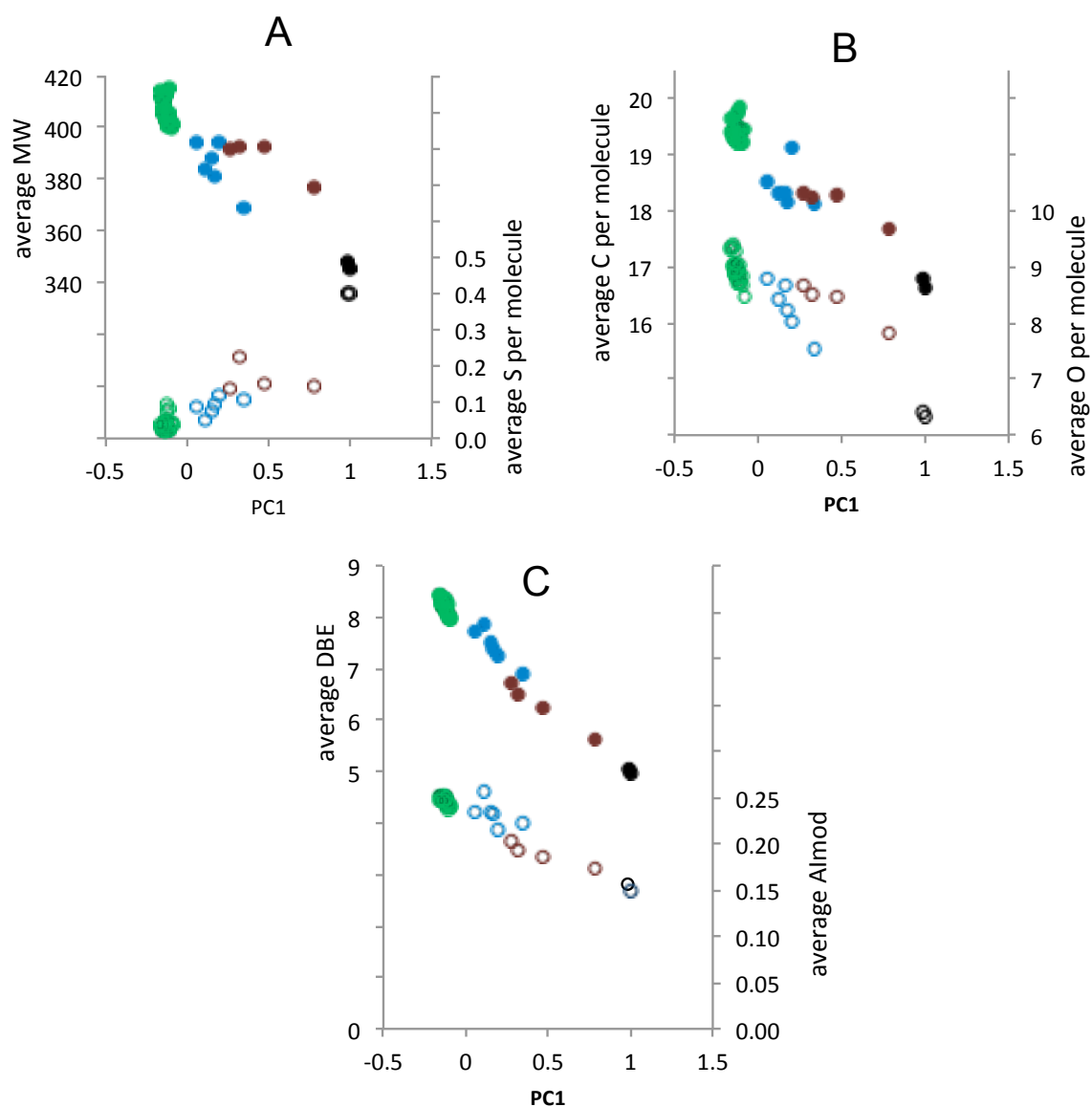


Figure 4.4: Trends in DOM molecular composition compared with hydrothermal influence (PC1) in the sample set. Green = plume samples from all three sites, black = E2, red = Beebe, blue = Von Damm. A: average molecular weight and average number of sulphur atoms per molecule B: average number of carbon atoms per molecule and average number of oxygen atoms per molecule C: Average double bond equivalence (closed symbols) and average modified aromaticity index.

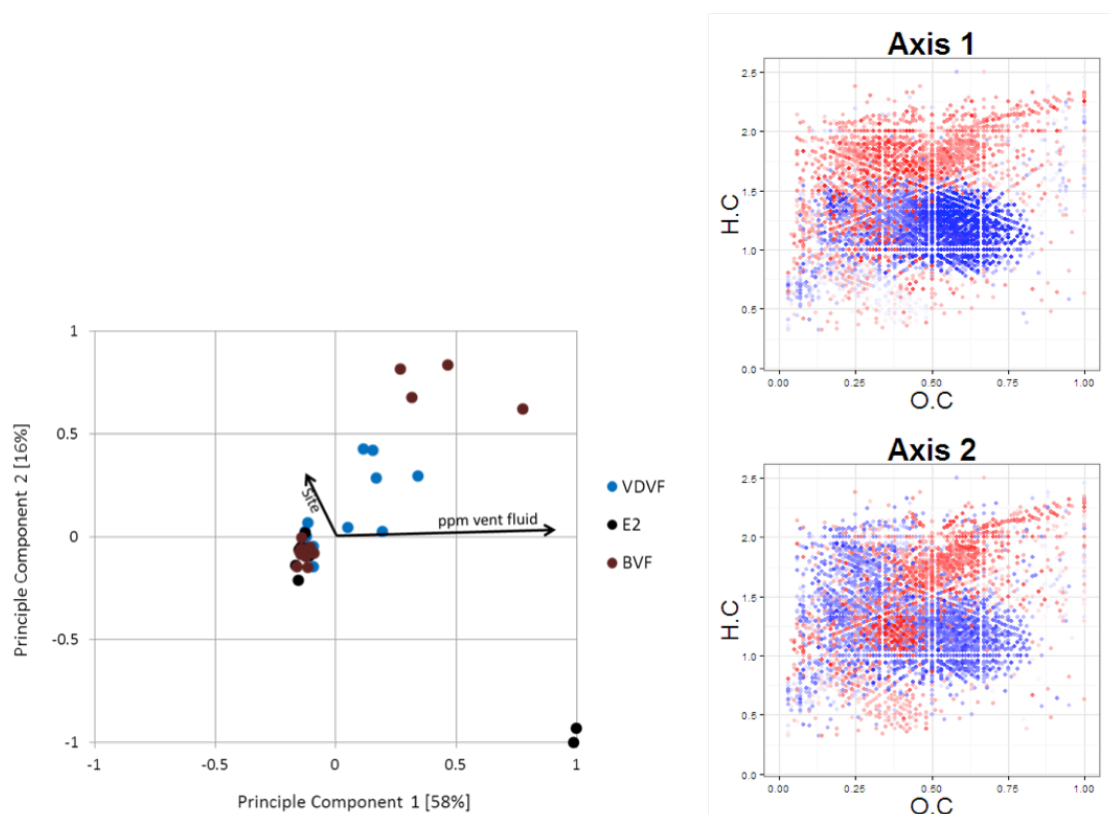


Figure 4.5: A) Principle Component separation of the sample set. B) Correlation of individual molecular formula with PC1 (top panel) and PC2 (lower panel) in Van Krevelen space - red signifies positive correlation along the PC, blue negative.

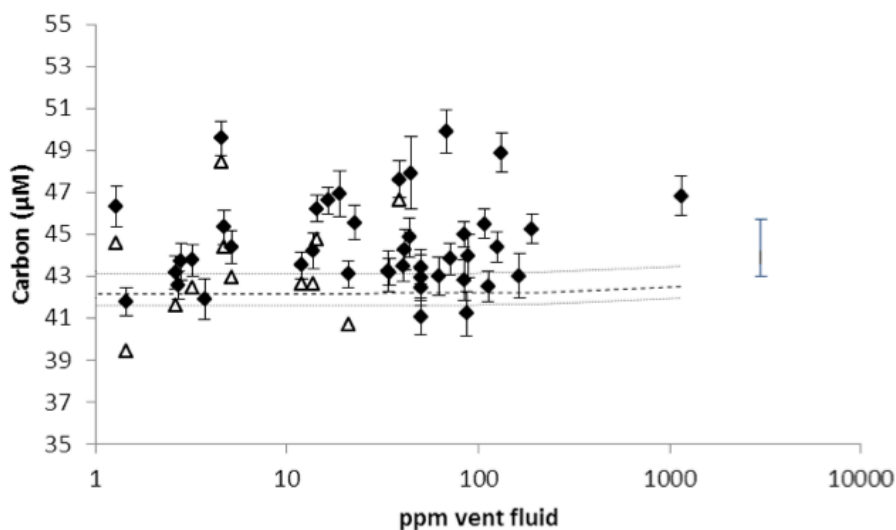


Figure 4.6: Close up view of TOC concentrations at Beebe (black diamonds) and DOC concentrations where available (TOC-POC). The predicted concentration is shown as a dashed line, based on mixing between the hydrothermal and background end-members, with the first and third quartile of the background samples also modelled as dotted lines. The first to third quartile of the Beebe TOC data are shown as a separate bar in blue.

(over PC1).

4.4 Discussion

4.4.1 Organic carbon and nitrogen concentrations

We found that SPE-DOC concentrations (a proxy for deep ocean DOM) were generally depleted in the vent fluids. The concentrations were as low as 6.2 μM at the E2 end-member fluids, corresponding to a loss of about 29 μM DOC when considering the typical extraction efficiency (55%) of the SPE technique. This is likely the result of thermal decomposition and sorption to mineral surfaces (Lang et al., 2006; Delacour et al., 2008). End member concentrations were similar at the Von Damm site, and higher (possibly indicating no net loss of SPE-DOC) at Beebe (Fig. 4.2). At the two Caribbean sites (Beebe and Von Damm), total DOC concentrations were increased in the vent fluid (above the SPE-DOC background) suggesting inputs of thermogenically or abiotically produced compounds (Reeves et al., 2014b) that were not extractable by

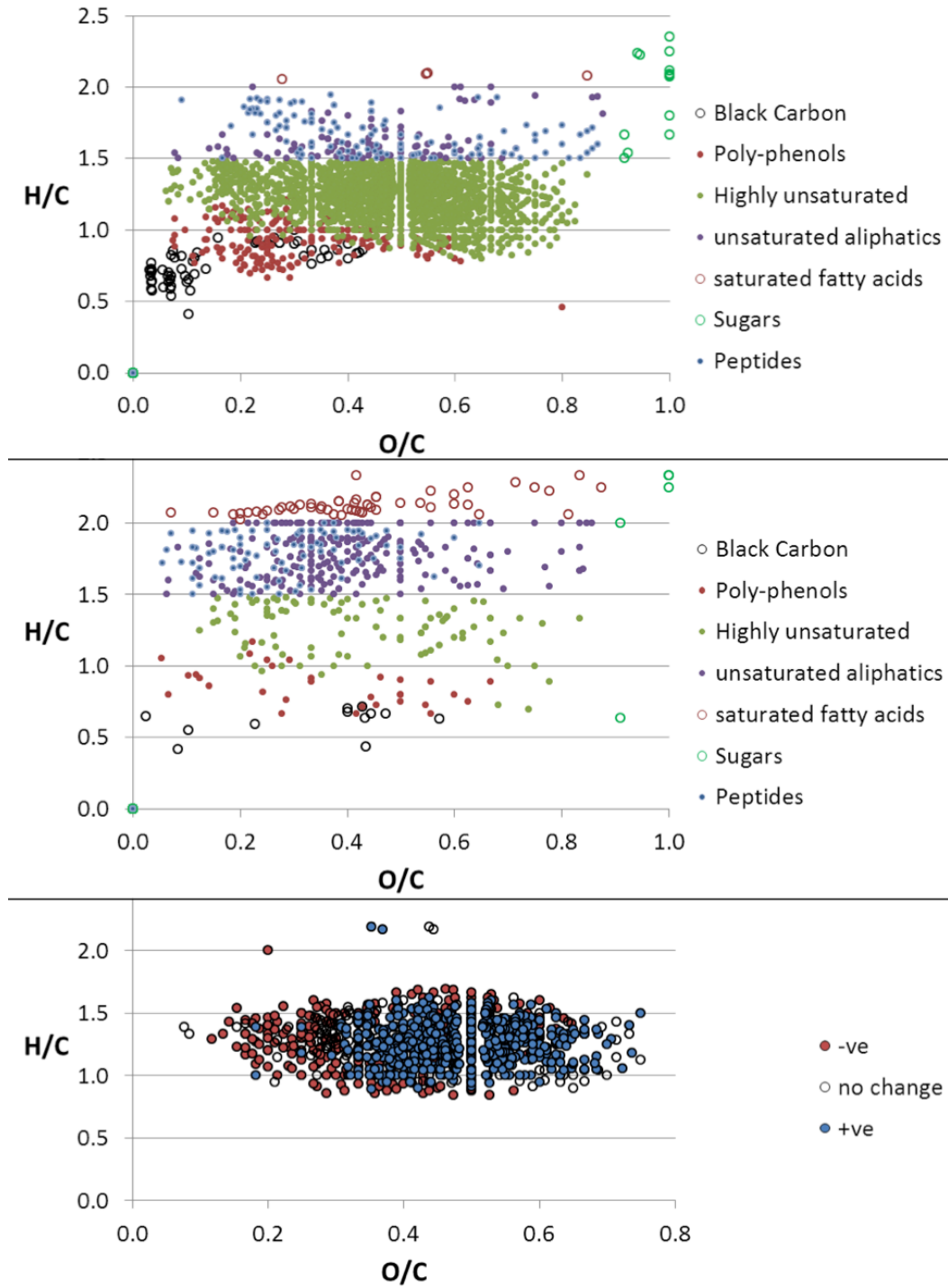


Figure 4.7: Van Krevelen diagrams showing changes to molecular composition between a typical plume sample and high temperature fluid sample at E2. A: Molecules lost from seawater (not present in fluid) B: Molecules present in fluid but not plume water. C: Molecules present in both – red colouring signifies a trend (significant to 0.05 level) to higher concentrations (SPEDOC corrected) in the fluid, blue signifies a trend to higher concentrations in the plume.

our technique. This may include very water-soluble organic acids (Lang et al., 2010), methanethiol (Reeves et al., 2014a) or insoluble petroleum hydrocarbons (Simoneit, 2002; Didyk and Simoneit, 1990), which would tend to precipitate from solution during un-acidified storage, and may explain the sharp decrease in DOC with seawater mixing at the Beebe site (Fig. 4.2a). No such loss of DOC was found at Von Damm, possibly suggesting greater bacterial re-working of DOC in the early stages of venting. The lower temperature of venting and greater concentration of methane and subsurface processes may facilitate continued DOC production at this site (Wankel et al., 2011).

The main difference at the Caribbean sites compared with E2 is the very slow spreading rate (Kinsey and German, 2013) and relative importance of sedimentation on the crustal geochemistry (Reeves et al., 2014a), and we suggest that increased interaction of high temperature fluids with relatively carbon-rich sediments leads to the observed inputs of new DOC at the Caribbean sites. TOC and POC concentrations were highly variable at the three sites, indicating some heterogeneity in productivity in and around the plumes. We took a set of 16 background samples near the Beebe site where no hydrothermal anomalies were found by CTD, over ten nautical miles away from the vent site. The on-site samples contained significantly more TOC than the background samples, and we tentatively suggest that the provision of reduced gases and metals, and the general perturbation of chemistry in the area was enough to slightly increase regional bacterial productivity. This result has been predicted previously (McCollom, 2000; Cowen et al., 2001; Dick et al., 2009) and merits further constraint as a back of the envelope calculation¹ suggests that this type of increase could globally represent $\sim 1.5 \times 10^{12}$ g DOC yr⁻¹, roughly 1% of the scale of annual export production that reaches >500 m depth¹. This may be a very important source of fresh organic carbon along the ridge axis, though it is important to note that we did not find significant molecular level differences in SPE-DOC amongst the plume samples correlated with TOC concentration. The scale of regional stimulation may be much larger than the loss of DOM to high temperature venting, which considered globally may amount to just 0.025×10^{12} g C yr⁻¹, if the E2 site (the site where we found the greatest loss of SPE-DOC) is considered typical. This calculation is based on a global hydrothermal flux of 7.2×10^{12} kg yr⁻¹, and the result is similar to that found by Lang et al.

¹If the 10,000 kg of seawater (McDuff, 1995) influenced by 1 kg of vent fluid contains on average 1.7 $\mu\text{mol kg}^{-1}$ new DOC and global vent fluid flux is 7.2×10^{12} kg yr⁻¹ (Nielsen et al., 2006)

(2006). POC concentrations were also highly variable within the plumes at the three sites (Fig. 4.2c), and may account for some of the increased TOC at Beebe (Fig. 4.6). Plume POC may include bacteria (Dick and Tebo, 2010; Anantharaman et al., 2013) or DOC adsorbed onto plume particles (German et al., 2011; Bennett et al., 2011b). Either way, some new production of DOC is needed to account for the raised TOC concentrations.

4.4.2 Molecular characteristics of SPE-DOM

Over 70% of the total formulas (~2150/3000) detected in the plume samples were not present in the high temperature fluids at E2, having been destroyed or altered during hydrothermal circulation. There was no obvious selectivity in atomic ratios (H/C or O/C) in the lost formulas (Fig. 4.7a). The remaining 30% were present in all samples at E2, and consisted of highly unsaturated lignin like formulas (Fig. 4.7c). A selection of 536 (generally) hydrogen-rich formulas dominated by saturated fatty acids, peptides and unsaturated aliphatic compounds was found in the two fluid samples at E2 and not in the seawater samples, suggesting very low concentrations (quickly diluted) or high lability (Fig. 4.7). It is unlikely that these compounds are important in oceanic cycling of DOM as they were not present in our seawater samples. Interestingly a direct elevation of hydrothermal vent specific microorganisms with distance from these vents has been clearly shown previously (Chapter 5). What role these chemicals play in microbial cycles is still somewhat unknown, but because of the strong correlation to the redox potential of vents, the carbon from plumes could spike microbial reproduction in the vicinity (Chapter 5). There was a broad shift to lower O:C and higher H:C ratios (Fig. 4.5b, principle component 1), and an appearance of several hundred new sulphur and nitrogen containing molecules. Surprisingly, we saw a decrease in the aromaticity index; a measure of the number of aromatic and condensed aromatic rings in the formulae. We also found no evidence for an increase in the occurrence of black carbon or poly-aromatic phenols, suggesting that hydrothermal vents are not a source of dissolved poly-aromatic hydrocarbon (PAH) molecules to ocean water, as hypothesised previously (Dittmar and Koch, 2006). It is clearly possible, even likely, that PAH compounds (which are typically surface active and insoluble) co-precipitated from solution or were insoluble upon entry into seawater (Brault et al., 1989), in which case they may be incorporated into the sulfide-dominated solid phase recovered from

the samplers. We plan to investigate this in the near future.

4.5 Conclusions

We have demonstrated that high-temperature hydrothermal circulation destroys most deep ocean DOM that passes through the axial crust (a loss of ~ 2.5 million kg DOC yr^{-1}), confirming previous research at other vent sites. Some labile or insoluble compounds are also produced during venting, especially at sedimented sites. These compounds, along with reduced gases and metals, may help to provide stimulation for further reworking of DOM by plume bacteria, allowing the production of fresh DOM (possibly 1.5 billion kg DOC yr^{-1}) in the deep ocean. We show that through these simultaneous processes, hydrothermal venting serves to re-work deep ocean DOM, certainly on a local scale, leading to a more reduced, more saturated and more sulfonated mixture of DOM. The relatively high concentration and uniformity of deep-ocean DOM has made our investigation very challenging, and the use of state-of-the-art mass spectrometry instrumentation has been crucial in our research.

Chapter 5

Cutting through the smoke: Free-living bacterial diversity in deep-sea hydrothermal plumes

Abstract Microbial communities of hydrothermal vents have become increasingly studied as the development of technology (e.g. through use of remotely operated vehicles) has both simplified access to these extreme ecosystems, thereby enabling researchers to get more detailed compositional data on the complex communities of bacteria and archaea (e.g. through next generation sequencing).

Here we explore two hydrothermal vent fields in the Southern hemisphere one in the Indian Ocean (Longqi), the other in the Southern Ocean (E2). We collected samples across vent fields at varying horizontal and vertical distances from plumes. The two vent fields showed differences in the composition of microbial communities on an Operational Taxonomic Unit (OTU) level. The E2 vent field, showed significant differences between diffuse vent sites and black smoker plumes. Samples collected above black smokers were found to have a substantial amount of vent-specific microorganisms, including several members of Epsilonproteobacteria class, with the genera *Arcobacter*, *Caminibacter* and *Sulfurimonas* completely dominating around the vents. They were barely found in the background samples. Also, the most abundant Gammaproteobacteria genera differed substantially between the black smokers, diffuse vents and background samples. The abundance of vent-specific Epsilonproteobacteria decreases linearly with distance from the plume, measured by the Eh redox potential. To our knowledge this is the first study to investigate how the community composition of vent microorganisms changes spatially around hydrothermal vent plumes.

Chapter 5 Microbial succession in hydrothermal plumes

5.1 Introduction

5.1.1 Hydrothermal vents – importance of microbiota

Hydrothermal vents are ubiquitous along mid-ocean ridges, back-arc basins and volcanically-active seamounts. These relatively “food-rich” deep-sea ecosystems host animals, which are often endemic and in high abundances, although not vastly species rich. The fairly recent discovery of hydrothermal vents (Corliss et al., 1979) has transformed our view of the functional ecology of microorganisms. Chemosynthetic organisms likely supply the base of the food web for the numerous animals associated with deep-sea hydrothermal vents. They use electrons from reduced inorganic compounds to supply their electron transport chains, thereby giving energy to fixate inorganic carbon into organic molecules (i.e. H_2S , Fe^{2+} , NH_4^+ , and $\text{S}_2\text{O}_3^{2-}$). Hydrothermal vent systems offer a range of habitats for microorganisms, with a rich variety of environmental conditions and steep physical and chemical gradients (Reysenbach and Shock, 2002), which allow several niches to be realised. Chemosynthetic symbiosis is frequently the preferred method of food supply for vent megafauna (Dubilier et al., 2008). The symbiotic microbiome can comprise different microbial taxa. Generally, hydrothermal vent-endemic invertebrates hold complex arrays of sulphur reducing bacteria attached to various parts of their body, most frequently from the Epsilon- and Gammaproteobacteria classes (Zbinden et al., 2008; Goffredi et al., 2008; Urakawa et al., 2005).

Although abundant, the diversity and function of non-symbiotic (free-living and mat-producing) hydrothermal vent microorganisms is not well known (Forget and Kim Juniper, 2013). Most commonly, these microorganisms include chemolithotrophs or heterotrophs. Chemolithotrophic organisms face the challenge of extracting energy from narrow redox zones in marine environments. Thus non-symbiotic chemolithotrophic microorganisms typically occur in biofilms on sulfidic rocks or in filamentous mats, e.g. *Beggiatoa* spp., absorbing reduced compounds from the substrata below and oxygen from the water above. Contrary to mat-producing bacteria, the free-living organisms (non-mat-producing and non-symbiotic) around the vents dwell in the unpredictable fluid environment around the vents, with unstable physicochemical conditions and immense variation in temperature and availability of compounds. These conditions offer a variety of microhabitats that potentially could be occupied by diverse microorganisms.

Although a large amount of primary production in hydrothermal-vent environments is chemosynthetic (Jannasch, 1983), much of the microbial biomass in these extreme environments is accounted for by heterotrophic prokaryotes, which use organic matter released from animals and other microbes as a food source (Campbell and Cary, 2004). In recent years, several studies have documented the diversity of free-living bacteria and archaea in the ambient water of vent discharge (Huber et al., 2003, 2010b; Rogers et al., 2012; Millard et al., 2014), these studies have found remarkable diversity of microorganisms inhabiting this environment with evidence that vents with different chemical characteristics harbour distinct microbial populations. However, data are scarce on the roles of dispersion and nutrition, by which the free-living microorganisms provide food to the surrounding ecosystem (Vrijenhoek, 2010; Nakagawa et al., 2005).

The most abundant (>70%) bacterial phylum around vent plumes is the Proteobacteria. The Epsilon- and Gammaproteobacteria within this phylum are often abundant around vent fields, particularly the taxa with the metabolic flexibility of rapid adaptation to changing geochemical conditions (Campbell et al., 2006). Epsilonproteobacteria are found in almost all habitats surrounding hydrothermal vents, including symbiotic and free-living environments (Nakagawa et al., 2005; Watsuji et al., 2012; Cr  peau et al., 2011; Goffredi, 2010). The Epsilonproteobacteria are known to have diverse metabolic properties but are most commonly thermophilic hydrogen and sulphur-oxidizing chemolithoautotrophs (Campbell et al., 2006). Because of their physical preferences and high abundance in hydrothermal vent systems, Epsilonproteobacteria serve as an ideal taxa for examining vent-specific microbial community structures (Huber et al., 2010b). Other common free-living bacterial taxa found around vents are Zetaproteobacteria and Firmicutes, in addition to typical oceanic microorganisms, such as Bacteroidetes and SAR11 (Alphaproteobacteria), which are most likely introduced from the surrounding environment (Rogers et al., 2012).

By cloning and metagenomic sequencing techniques microbial diversity was investigated in hydrothermal plumes in the Indian Ocean at the South West Indian Ridge (SWIR) and in the Southern Ocean at the East Scotia Ridge (ESR) (Cao et al., 2014; Rogers et al., 2012), whereas in the Southern Ocean (vent fields E2 and E9), Gammaproteobacteria were the most abundant phylum constituting more than half of all buoyant plume sequences with high resemblances to the endo- and episymbionts of the respective hydrothermal vent fauna (Rogers et al., 2012). Similarly, the Longqi vent field in the Indian Ocean exhibited a dominance of Gammaproteobacteria in

addition to Deltaproteobacteria. But however, neither study examined the microbial community structure throughout the plume in detail.

5.1.2 Vent endemism and patterns of biogeography

Hydrothermal vents are globally distributed but because of geological/environmental barriers, individual fields are regarded as unique ecosystems. Recent exploration has led to a biological clarification of 11 hydrothermal vent provinces based on the biogeography of vent metazoan macrofauna and megafauna (Rogers et al., 2012; Bachraty et al., 2009). These provinces were based on multicellular organisms only, although microorganisms have been suggested to show unique patterns around vents. It has also been suggested that arc and backarc hydrothermal systems may represent bacterial hotspots of diversity in the ocean (Davis and Moyer, 2008), emphasising the importance of including the complex communities of microorganisms around vents into biogeographic classification schemes.

Recent studies have explored the effects of dispersal limitation on microbial biogeography. Huber et al. (2010b) compared five different vent locations with similar chemistry on the Mariana Arc, specifically looking at Epsilonproteobacteria from diffuse vents. They found distinct assemblages with location but not with chemical parameters. Biogeographical signals have also been found for hyperthermophilic archaea, such as the deep-sea hydrothermal vent euryarchaeota 2, and sulfate-reducing bacteria in similar environments (Whitaker et al., 2003; Flores et al., 2012). One detailed biogeographic study has been conducted on the hyperthermophilic chemoautotrophic genus *Persephonella* (phylum Aquificae) (Mino et al., 2013). Interestingly the authors of this study found distinct biogeographic patterns in a relatively small area of the Western Pacific, even at the genus level. Mino et al. (2013) suggested that since the *Persephonella* members are hyperthermophilic chemoautotrophs, endemic to deep-sea vents, considerable dispersal barriers for the migration to spatially distinct habitats should exist. These studies have shown that as with metazoans, the genetic similarity of microorganisms negatively correlates with geographical distance i.e. distance-decay relationship (Mino et al., 2013; Whitaker et al., 2003). Therefore, we can presume that hydrothermal plumes with distinct chemical characteristics and geographically separated would host different microbial communities.

The vast majority of research on vents, seeps, and organic falls has taken place

in equatorial to temperate regions. Much less work has been conducted at latitudes greater than 40°, partly because of the challenges of ship-borne scientific work at higher latitudes where extreme weather and/or ice conditions persist. In the case of hydrothermal vents, almost the entire SW and SE Indian Ridges are un-sampled as well as large parts of the East Scotia Ridge (Rogers et al., 2012; Cao et al., 2014). Hydrothermal plumes can influence growth, diversity, and distribution of allochthonous heterotrophic and autochthonous autotrophic prokaryotes, even in areas relatively distant (100s of metres) from active seepage fields (Manini et al., 2008).

However, to our knowledge, no study has yet investigated the change of microbial community composition, as vent effluents are diluted with distance from vent plumes. In addition for the first time we report a study between deep-sea vent sites of the Southern hemisphere. We compare free-living microorganisms from two deep-sea hydrothermal vents at the South West Indian Ridge and the East Scotia Ridge. We investigate the spatial pattern of microorganisms between these two vent fields and, by strategic sampling, show how the microbial community structure changes across the vent plume, i.e. the dilution of hydrothermal-specific microbial communities with those of the background seawater. We hypothesise the microbial communities to not be the same between the ESR and SWIR. In addition we expect the hydrothermal plumes to effect the local environment stimulating vent-specific microbial community growth.

5.2 Methods

5.2.1 Field site

We conducted a comparative study of two vent sites in the southern hemisphere: the E2 site (30°19.1'W, 56°05.3'S) from East Scotia Ridge (ESR) and the Lonqi vent field (49°38.942'E, 37°47.022'S) from the South West Indian Ridge (SWIR) (Fig. 5.1). The E2 segment is slow spreading at ~65-70 mm y⁻¹ but, because of the large magma chambers underneath the vent field, it has similar characteristics to a faster spreading ridge (Bruguier and Livermore, 2001; Livermore, 2003). Extinct and actively venting chimneys are found along the fissures in an elevated section of the E2 segment. The investigated vent site Dog's Head, consists of a complex of four chimneys up to ~12

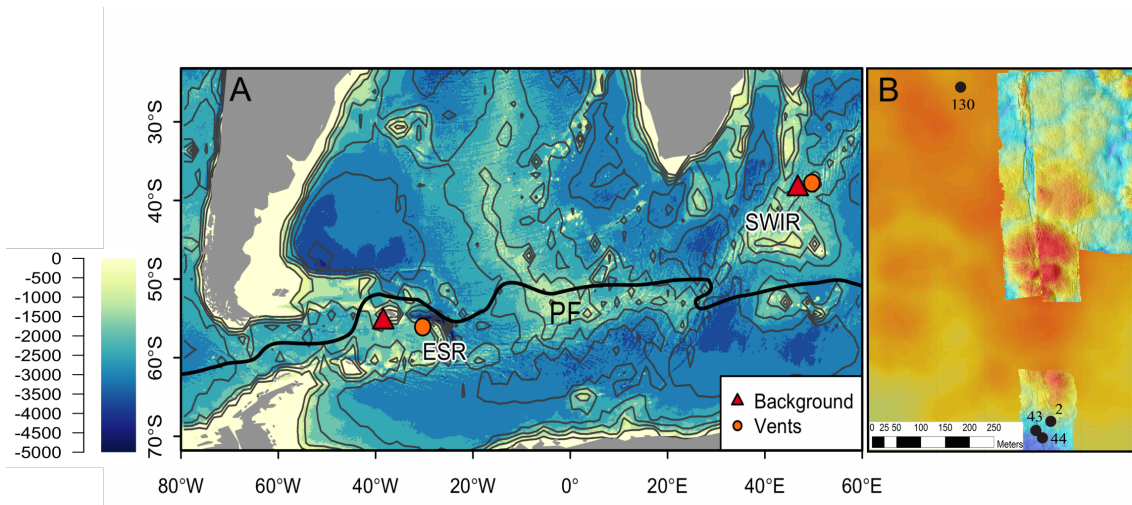


Figure 5.1: A) Map showing the locations of samples in the southern ocean and Indian Ocean. PF: Polar Front. B) Shows the topography of the ESR vents at the E2 segment. The two vent stations are 43 and 44, the two diffuse vent stations are 2 and 130.

m high that actively vent black smoker fluids at temperatures of up to 351°C (James et al., 2014).

The Lonqi vent field, discovered in 2009, is the first active seepage found at a ultra-slow spreading ridge ($<16 \text{ mm y}^{-1}$) (Tao et al., 2012; Cao et al., 2014; Peng et al., 2011). This vent field is constructed of chalcopyrite (CuFeS_2), kusachiite (CuBi_2O_4), pyrite (FeS_2) and manganese phosphate hydrate (Table 5.1). Remarkably high concentrations of sulfate has been documented in some areas (Cao et al., 2014; Peng et al., 2011).

The E2 vent fields were discovered in 2009 and, since then, these hydrothermal chimneys have been visited by several research cruises with diverse purposes (Aquilina et al., 2013, 2014; James et al., 2014; Hawkes et al., 2013; Cole et al., 2014; Connelly et al., 2012; Rogers et al., 2012). The chemical and physical environment as well as the megafaunal compositions of these vents is relatively well known. The chemical composition of fluids from E2 consists of chloride (Cl^-) concentrations similar to that of seawater and, consequently, have higher concentrations of the major cations, including sodium, and lower concentrations of volatiles such as hydrogen sulphide (H_2S) (Table 5.1), (Rogers et al., 2012; Aquilina et al., 2013; Connelly et al., 2012; Livermore, 2003; Millard et al., 2014; Marsh et al., 2012; Reid et al., 2013).

Table 5.1: Properties of vent chemistry at Dog's Head, Longqi and the ESR diffuse vent. Data is from (Bach et al., 2002; Peng et al., 2011; James et al., 2014; Tao et al., 2012)

Environment	Chemistry	Longqi	Diffuse vent ESR	Dog's Head
Chimney	Sulfate	Very high	-	-
	Pyrite (FeS ₂)	high	-	low
	Kusachiite (CuBi ₂ O ₄)	high	-	-
	Chalcopyrite (CuFeS ₂)	high	-	high
	Anhydrite	absent	-	present
End-member	Max temp. (°C)	low	3.5	351
	H ₂ S (mM)	low	0.1	7
	SO ₄ (mM)	-	-	3.6
	Si (mM)	high	1.26	17.7-22.6
	Cl (mM)	-	536	536
	Fe (mmol/kg)	-	2.17	1,280
	Mn (mmol/kg)	-	9.76	2,050

5.2.2 Sample collection

Sampling was carried out during Royal Research Ship (RRS) James Cook voyages JC67 and JC80 during austral summer from November 4th – December 21st 2011 and December 1st – 30th 2012, respectively (Fig. 5.1a). For sample collection, we deployed a Seabird +911 Conductivity, Temperature and Depth (CTD) profiler mounted on a titanium frame holding 24 externally sprung 10 litre niskin bottles. The profiler was equipped with a light scattering sensor (LSS) and a bespoke Eh electrode (Ko-ichi Nakamura) for detecting hydrothermal anomalies and reduced chemical species. The former was only available for JC80.

The samples were collected around vent sites from high temperature fluids, diffuse areas of (low temperature) venting, buoyant hydrothermal plumes, neutrally buoyant plumes, and background seawater. At the ESR we collected samples from four different CTD casts: two were the same vent location designated as Dog's Head (cast 43 and 44, Fig. 5.2d) and the other two were from different diffuse vents located close by (casts 2 and 130, Fig. 5.2c). At the SWIR we collected samples from a single CTD cast through the plume of a high temperature vent (Table 5.2).

The plume from Dog's Head rises to 350-400 m from the seafloor (Hawkes et al., 2013), thus all samples collected at E2 and Longqi were deeper than 1900 m, a depth which is approximately 600 m from the seabed to ensure collection of non-vent af-

Table 5.2: Sample overview. T is temperature, FC is flow cytometry, POC is Particulate Organic Carbon and bg are background samples. Depth is the vent depth or seafloor depth for background sites.

Station	Cast	Latitude	Longitude	Cells/mL	Depth (m)	3.0 μ m	samples		
							0.2 μ m	FC	POC
Bg ESR	1	55°26'66"S	38°25'63"W	51,000	2500	6	6	12	6
Bg SWIR	11	38°31'56"S	46°45'74"E	96,440	1950	4	4	8	4
Dog's Head	43, 44	56°05'35"S	30°19'13"W	16,581	2607	18	16	32	17
Longqi	18	49°38'94"S	37°47'02"E	106,124	2750	6	6	12	3
DV ESR	2, 130	56°04'62"S	30°19.41'W	25,122	2610	15	15	32	15

fectured samples. Fluid samples collected over the hydrothermal vents were taken according to water column anomalies from the Eh detector on the CTD (Fig. 5.2a). At each vent field, samples were collected along observed Eh (JC80), or LSS (JC67) profiles to detect microbial community structures relative to distance from plume. High Eh is correlated with distance from origins of the plume and is thus used as the estimate of plume distance. Because the CTD used at the SWIR was not equipped with an Eh sensor, the distance from the Longqi vent will be defined with lower values of the LSS sensor.

At each sampling point 1 l of seawater was collected for particulate organic carbon (POC) and microbial community structure (DNA) analysis in duplicates from two different bottles, and for flow cytometry analysis in triplicates from each bottle, respectively. All DNA samples were pre-filtered through 3.0 μ m filter (representing particle-associated bacterial community) and subsequently free-living microbial cells were collected on 0.2 μ m pore size filters (polycarbonate membranes, 4.5 cm diameter, Sigma-Aldrich). Both filters were sequenced and included in analyses.

5.2.3 Elemental Analysis

Samples for Particulate Organic Carbon (POC), Particulate Nitrogen (PN) and Particulate Hydrogen (PH) were taken by vacuum filtration (3 litres of seawater) through a pre-combusted 0.7 μ m glass microfibre GF/F filter (47 mm, Whatman). The filters were analyzed by combustion in excess of O₂ with a CE440 elemental analyzer (Exeter analytical), according to Knap et al. (1996).

Before analysis, the frozen filters were thawed, dried overnight at 50-55°C and placed in a desiccator for 2 hours with concentrated hydrochloric acid to remove

carbonates. Following the acid bath, the filters were dried again at 50-55°C for at least 6 hours. After drying and cooling, the filters were folded and placed into nickel sleeves. For every eight nickel sleeves with samples, one nickel sleeve with a pre-combusted blank filter was added as a reference.

5.2.4 Flow cytometry

Subsamples for flow cytometry were taken directly from the Niskin bottles, fixed with glutaraldehyde (1.0% final concentration), incubated for 15 min. at 4°C and finally stored at -80°C until further analysis. Bacterial cell numbers were determined using an Accuri C6 flow cytometer (BD Biosciences) by SybrGreen I (SGI, Invitrogen) staining. 1 µm Multicolour latex beads (Polysciences Europe, Eppelheim, Germany) were used as an internal standard. After 30 minutes of incubation in the dark, each sample was analyzed using a flow rate of 14 µl min⁻¹. Bacteria were detected using manual gating after visual inspection of the dot plots of the green vs. red fluorescence and forward vs. sideward scatters (FSC, SSC) and their histogram plots, respectively. The internal fluidics calibration of the device was used for volume calibration and verified by Tru-Count beads (BD) as described previously (Giebel et al., 2009). Data were processed by BD Accuri C6 C-Flow software (version 1.026421).

5.2.5 Illumina MiSeq sequencing and preparation

DNA extraction, PCR and sequencing were performed using a modified version of the protocol presented in Caporaso et al. (2010a), adapted for the Illumina MiSeq, according to the Earth Microbiome Project (EMP) standards ¹www.earthmicrobiome.org/emp-standard-protocols (Caporaso et al., 2010a). In brief, the genomic DNA was extracted from subsamples of the water filters using a Powersoil-htp 96 Well DNA isolation kit (MoBio) with a 10 min. (65°C) incubation step modification ². The V4-V5 region of the 16S rRNA gene was amplified with the 515F/806R primers with 12 base pair (bp) barcodes. The amplification primers were adapted from Caporaso et al. (2010b) to include nine extra bases in the adapter region of the forward amplification primer that support paired-end sequencing on the MiSeq. Amplifications were done in triplicates

¹<http://www.earthmicrobiome.org/emp-standard-protocols>

²<http://www.earthmicrobiome.org/emp-standard-protocols/dna-extraction-protocol/>

and followed the EMP PCR protocol³. PCR products were pooled at equimolar concentrations and cleaned using the UltraClean® PCR Clean-Up Kit (MoBio). 16S rRNA amplicon sequencing was conducted at the IGSB Next Generation Sequencing Core at Argonne National Laboratory, using 151 bp paired-end sequencing on an illumina MiSeq instrument.

Quality filtering of reads was applied, as described previously (Caporaso et al., 2010b). Reads shorter than 75 bases and reads whose barcode did not match with an expected barcode were discarded.

5.2.6 Bioinformatics of sequencing data

Forward and reverse raw sequences were combined. Joined reads were demultiplexed and quality trimmed using QIIME 1.8 (Caporaso et al., 2010b). An open-reference OTU (Operational Taxonomic Units) picking strategy was used, where OTUs were clustered against the GreenGenes 13_8 reference sequences using uclust ref (Edgar, 2010) and reads with no hit to the reference sequence collection were subsequently clustered *de novo* at the 97% similarity level using uclust (Edgar, 2010). Reads were assigned to OTUs based on their best hit to this database at greater than or equal to 97% sequence identity. PyNASt (Caporaso et al., 2010a) was used to align OTU sequences and OTU taxonomy was assessed using the RDP classifier retrained towards the GreenGenes database (97% similarity) (Wang et al., 2007). Median sequence counts per sample after OTU picking were 22,522 (SD 8,321). To generate a final OTU table, sequences not aligning in the PYNAST step were removed, and a subsampled OTU table was created by random sampling to an even depth of 11,270 sequences per sample.

Taxonomy was assigned to each read by accepting the Greengenes taxonomy string of the best matching Greengenes sequence. All bioinformatics were conducted using QIIME (Caporaso et al., 2010b).

5.2.7 Data analyses

For an estimate of the fraction of OTUs covered by the sampling, species accumulation curves were constructed on a sample basis. Species richness was estimated from

³<http://www.earthmicrobiome.org/emp-standard-protocols/16s/>

observed richness. Differences in bacterial abundances and richness between casts were calculated using an ANOVA and *post hoc* Tukey HSD test.

Canonical correspondence analysis (CCA) was conducted to understand the clustering and environmental drivers of the bacterial communities; a permutation test (10,000 permutations) was performed to check the significance of the CCA models. For all ordination and richness analyses, the R package *vegan* was used (Oksanen et al., 2013). *Post hoc* analyses between the seven CTD casts (background ESR, background Longqi, ESR vent 43 and 44, ESR diffuse vent 2 and 130, and Longqi vent) were performed with a permutational multivariate analysis of variance (permutation MANOVA) using distance matrices with the function *adonis* from the R *vegan* package (Ramette, 2007). Similarly to the CCA weighted and unweighted Unifrac distances were computed between all samples, and principal coordinates analysis was applied to visualize the results using the R package *phyloseq* (McMurdie and Holmes, 2013).

We calculated a divisive hierarchical clustering of all 96 samples using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method using the *cluster*, and *ape* packages (Paradis et al., 2004; Maechler et al., 2007; McMurdie and Holmes, 2013). The relationship between sequence abundance of Epsilonproteobacteria and Eh redox potential was investigated using ordinary least square linear regression. All CTD data were analysed with the R package '*oce*' (Kelley, 2012).

All data was analysed using R version 3.0.1 (R Core team 2015).

5.3 Results

5.3.1 Vent setting and bacterial abundance

The environmental setting at the SWIR and ESR vents reflects their respective positions north and south of the polar front based on temperature (Fig. 5.1 - Fig. 5.2) (Pollard and Read, 2001). The background samples from both ridges show no hydrothermal vent signal and a stable declining temperature throughout the deep-water column (Fig. 5.2a-b). Where samples collected from the Longqi vent the LSS evidently shows anomalies when approaching the hydrothermal vent (Fig. 5.2a). The two diffuse vent casts show clear Eh signals, more prominently from cast 2 than 130 (Fig. 5.2c). Dog's Head casts are very similar to each other (Fig. 5.2d). There is a notably higher concentration of

reducing chemical species at the vent sites compared to the diffuse vents and background at the ESR based on Eh potential.

Microbial abundances at the Longqi vent field averaged 106,124 cells ml⁻¹ and 96,440 in the background. At a lower abundance, the ESR vent samples averaged 20,900 cells ml⁻¹ and 51,000 cells ml⁻¹ in the background (Fig. 5.3; Table 5.1). The bacterial abundances were significantly different between the background and vent samples at both locations (Tukey HSD, $p < 0.01$).

5.3.2 16S rRNA gene-based assessment of bacterioplankton community structures

After filtering and quality checking, we had a total of 1.5 million sequences from the 96 samples averaging at 21,800±5,100 sequences per sample. In total 25,700 taxa were determined based on a 97% similarity threshold. The total taxon abundance for the ESR and SWIR backgrounds was lower with 4,970 and 5,000, respectively, compared to the vent taxa abundance with 7,190 for the SWIR and 18,650 for the ESR (Appendix B.1), although these values are skewed towards locations with a greater sampling effort (i.e. vents at the ESR; Appendix B.1). The mean observed richness by location showed similar richness across the locations with a slightly lower variance of richness in the Dog's Head samples (Appendix B.2), however based on an ANOVA, these patterns were not significant.

The RDP classifier assigned most of the 16S rRNA sequences to Bacteria with only 14% classified as archaea (Fig. 5.3). Proteobacteria was the most dominant phylum in all samples (58%); other abundant phyla include the Bacteroidetes (7.1%), Marine Group A (6.9%) (Wright et al., 2014), and Actinobacteria (5.7%, Fig. 5.4). Gamma- and Alphaproteobacteria represent the most abundant classes of the Proteobacteria of 53% and 24%, respectively, followed by Delta- (20%), Epsilon- (1.3%) and Betaproteobacteria (1.1%, Appendix B.3). While most of the taxa detected in all samples are typical of deep-sea water, the Epsilon- and Gammaproteobacteria are indicative of microaerobic, reducing environments in the water column, suggesting that samples containing those taxa were specifically collected from vent-influenced water. Half (49%) of the Gammaproteobacteria and Epsilonproteobacteria sequences were classified to genus level; of the Gammaproteobacteria sequences the HTCC 2207, *Candidatus Portiera*, *Halomonas* and *Pseudoalteromonas* represented 75% of the sequences

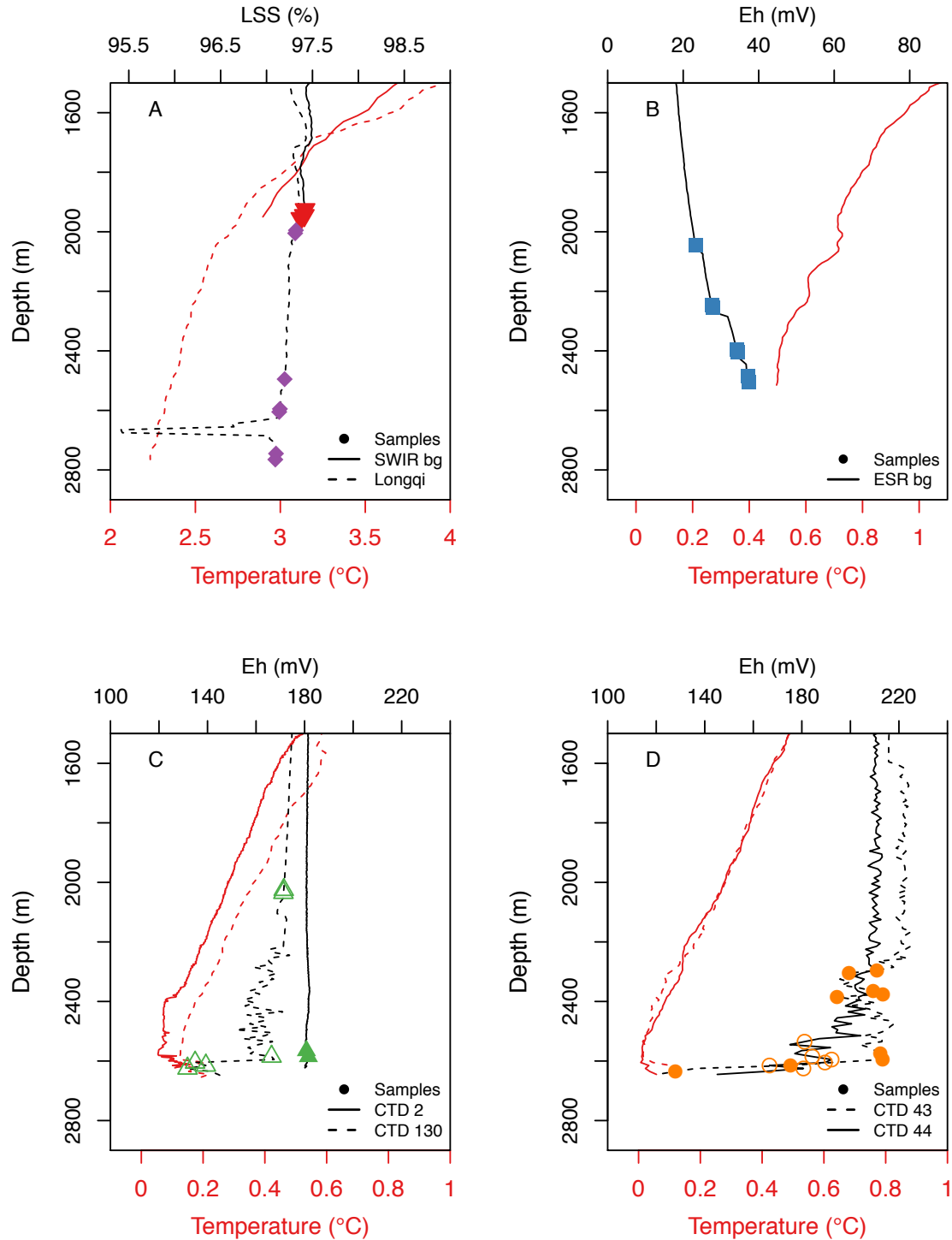


Figure 5.2: Eh, LSS and temperature from CTD casts from the SWIR and the ESR. Figure a) shows the concentrations of LSS and temperature at the Longqi vent and background. Figure b), c) and d) show the temperature and Eh potential at the background, diffuse vents (cast 2 and 130) and Dog's Head (cast 43 and 44), respectively.

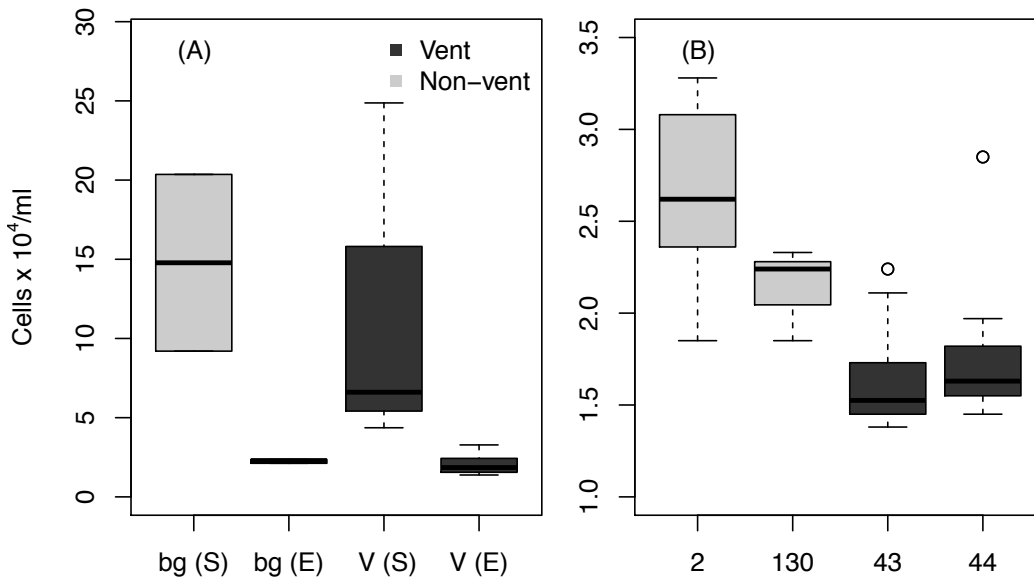


Figure 5.3: The boxplots show the abundance of bacteria between the Longqi and Dog's Head vent fields and their respective background samples (a), and between the four CTD casts collected from the ESR representing diffuse venting and black smoker venting (b).

(Fig. 5.5a). Among the samples containing Epsilonproteobacteria the genera *Arcobacter*, *Sulfurimonas* and *Caminibacter* were clearly dominant representing 99% of sequences assigned to Genus.

ESR diversity – Dilution of microbial communities

From the four ESR casts the community structure can be divided into two, the diffuse vents (cast 2 and 130) and the black smoker vents (casts 43 and 44, both at Dog's Head), referred to as diffuse vents and Dog's Head. They are both dominated by similar phyla (Fig. 5.4) but the two distinct CTD casts from the diffuse vents had a much higher abundance of bacteria than the Dog's Head samples with cast 43 significantly different from cast 2 (Tukey HSD p -value < 0.01) and cast 130 (Tukey HSD p -value = 0.0245) and cast 44 significantly different from cast 130 (Tukey HSD p -value < 0.01) (Fig. 5.3a).

Casts from Dog's Head casts were very similar to each other while the diffuse vent casts were more dispersed across the phyla. At the diffuse vent Epsilonproteobacteria was barely present and *Psychrobacter* and *Pseudomonas* of the Gammaproteobacteria

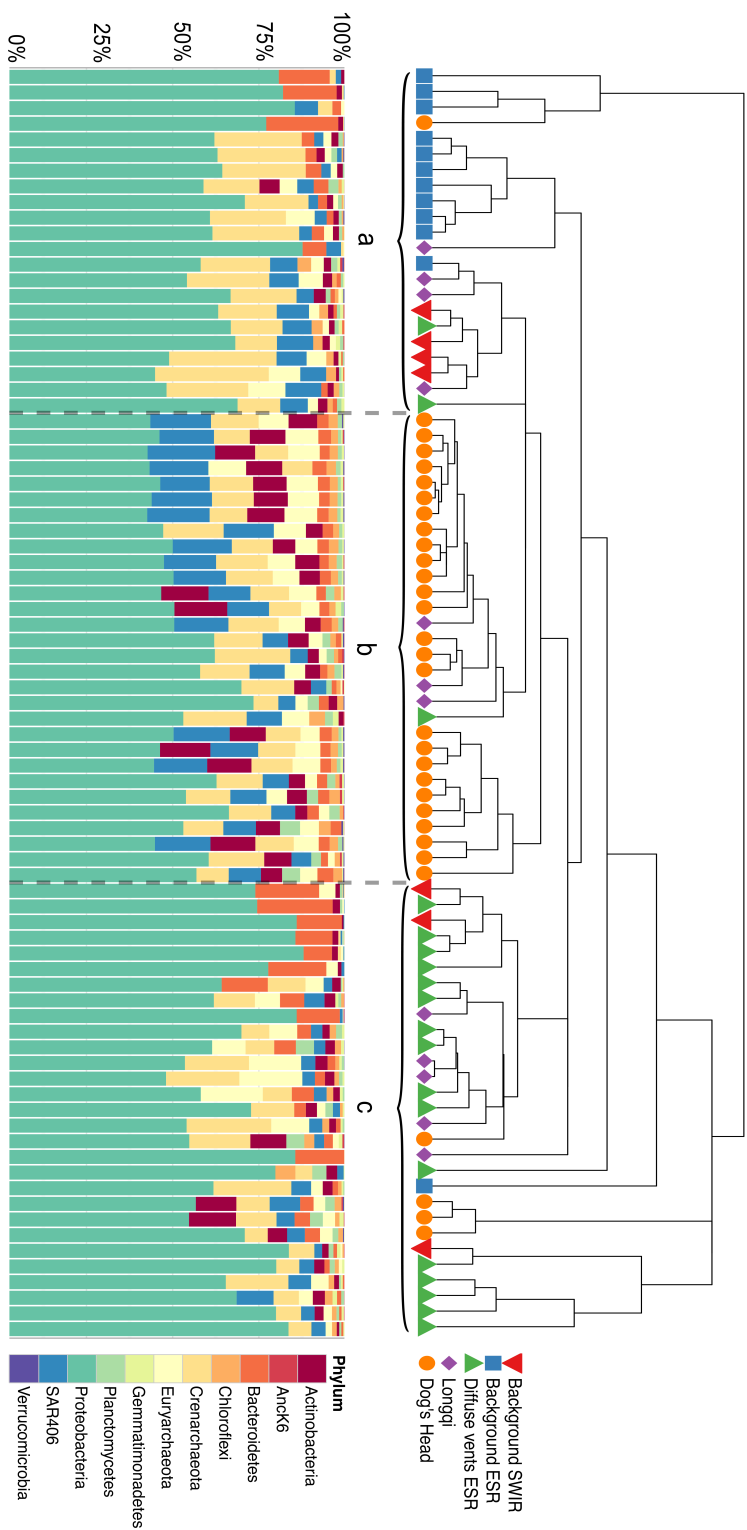


Figure 5.4: Hierarchical clustering dendrogram with 0.2 μm and 3.0 μm filters. We designate the dendrogram into three groups, which represent background samples (a), vent samples (b) and diffuse vent samples (c). Barplots for each sample show the community composition of the most abundant phyla. For clarity only OTUs that were observed more than two times and in more than 50% of the samples are shown.

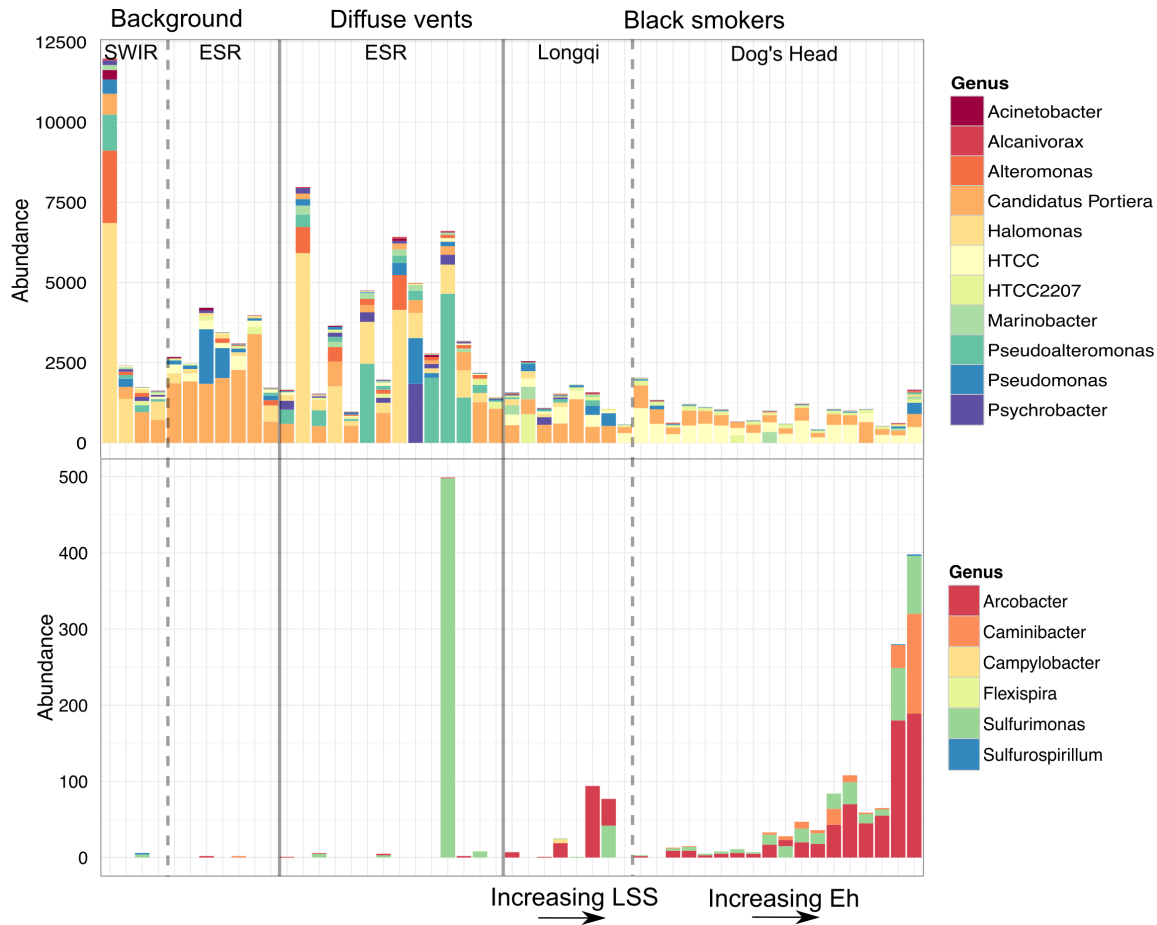


Figure 5.5: Barplot of Gamma- (a) and Epsilonproteobacteria (b) abundances from background to diffuse vent to black smoker vents according to increase in Eh potential. For clarity only OTUs that were observed more than two times and in more that 50% of the samples are shown.

completely dominated the community, in contrast to the Gammaproteobacteria HTCC 2207 and Epsilonproteobacteria dominating at Dog's Head. From the Gammaproteobacteria the sulphur oxidizing family SUP05 was high in the vent samples, but not found in the background samples. The hydrothermal community at Dog's Head seems to be forced by the reduction potential Eh (MANOVA $p < 0.01$), higher hydrogen concentrations (MANOVA $p < 0.01$), and carbon (MANOVA $p = 0.0129$), while the diffuse vent is positively correlated to a higher salinity (MANOVA $p < 0.01$) and concentration Nitrogen (MANOVA $p < 0.01$) as well as a higher abundance of bacteria (MANOVA $p < 0.01$) (Fig. 5.3 and (Fig. 5.6).

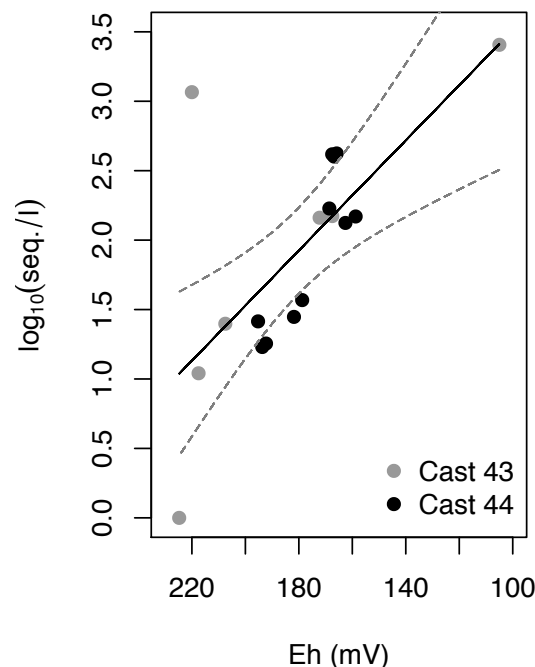


Figure 5.6: Linear model of Epsilonproteobacteria sequences and Eh between the two Dog's Head casts from the ESR.

The Epsilonproteobacteria from Dog's Head show a declining abundance with distance from vent, indicating dilution of the community by the background community. Epsilonproteobacteria abundance shows a log-linear relationship to increasing Eh (Fig. 5.7) with a p -value of 0.0002 ($r^2=0.766$) from a Pearson correlation test. A similar relationship with Epsilonproteobacteria seems apparent for Longqi with LSS increase, however, we do not have the sample size to explore this with a linear model. Contrary to the Epsilonproteobacteria the Gammaproteobacteria seem to have a stable abundance with distance from vent and rather show an increase in abundance outside the black smoker flourishing at the diffuse vents with distinct taxa, such as *Pseudoalteromonas*, *Halomonas* and *Psychrobacter*.

Community differences between vent fields

Background samples from the ESR and SWIR contained a similar composition of phyla to the vent samples but with fewer Marine Group A and Actinobacteria and relatively more Proteobacteria (Fig. 5.4). At the class level, the ESR showed a higher abundance of Betaproteobacteria and both backgrounds had no Epsilonproteobacteria (Fig. B.3). However, these two backgrounds do show differing patterns in the Gammaproteobacteria genera, with ESR background being completely dominated by *Candidatus Portiera* at substantially higher relative abundances than the background of the SWIR. The background at the SWIR was mostly dominated by *Candidatus Portiera*, but also had high abundances of *Halomonas*. One sample, in particular, had a high abundance of genera *Halomonas* and *Alteromonas*, which resembles some of the diffuse vent samples from the ESR. According to the CCA, the background samples from the SWIR resemble the diffuse flow samples of the ESR better than the background sample of the ESR, while the ESR sample is completely isolated from all other locations (Fig. 5.7a). The hierarchical clustering divided the data between background, ESR diffuse vents and the Longqi samples. The amount of samples at Longqi were not numerous enough to show any distinct patterns, however, both the communities on the 3.0 μm and 0.2 μm show a similar way of clustering between the two filter types, with filter type not strictly separating the microbial communities by size (Fig. 5.4 and Fig. B.4)

The most prominent difference between Dog's Head and Longqi is the high abundances of HTCC and HTCC 2207 at the former. These genera are most commonly found in surface areas with light as they contain proteorhodopsin; however, they have recently been found in areas without photosynthetic active radiation (Stingl et al., 2007). According to the CCA, the communities at Longqi overlap slightly with the ordination dimension of some ESR background samples, but are otherwise separate from the other locations (MANOVA $p < 0.01$). Unsurprisingly the Jaccard diversity index showed that the two casts from Dog's Head were most similar, however the least similar locations were Dog's Head 44 to diffuse vent 130 (Table 5.2).

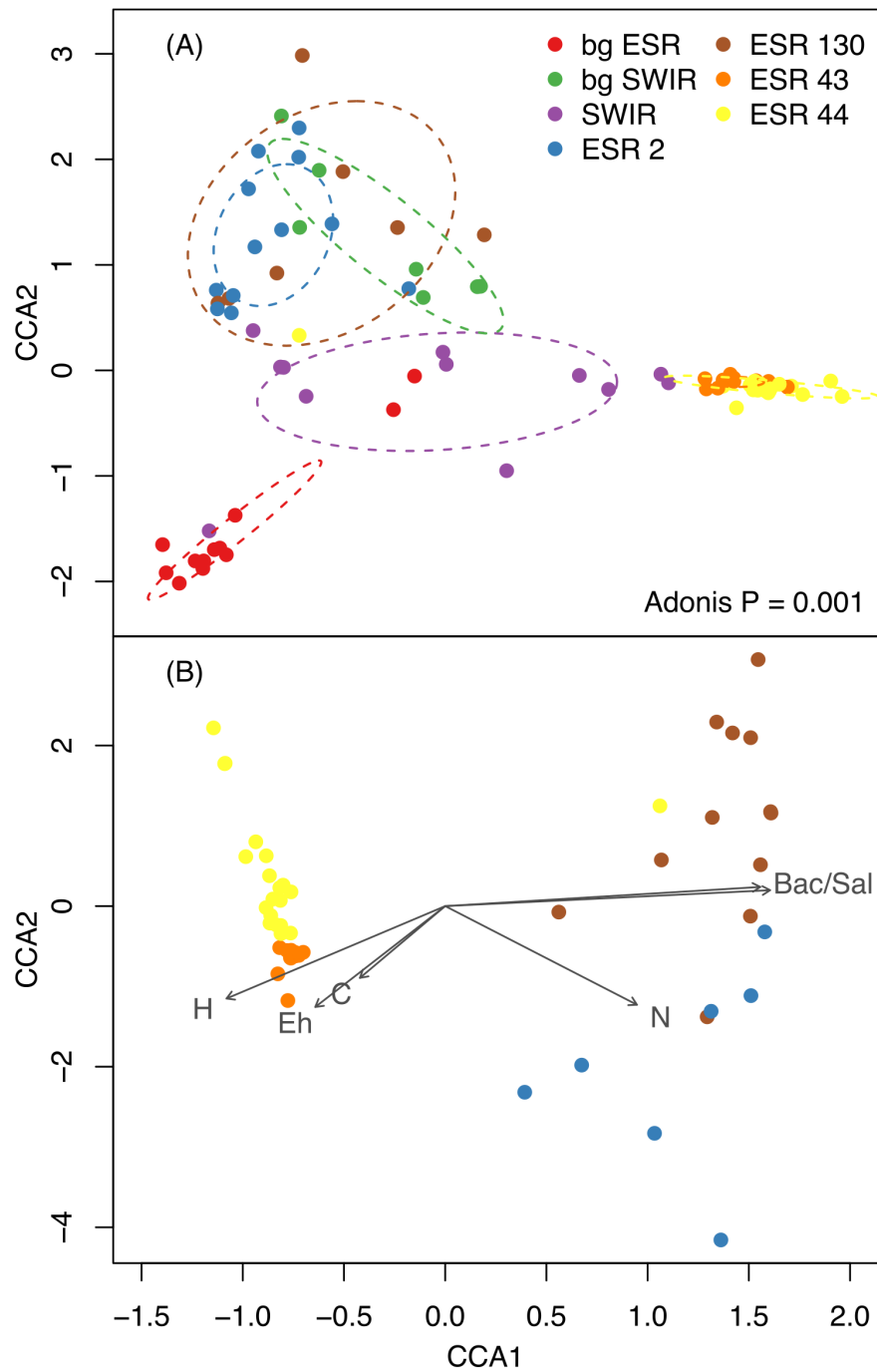


Figure 5.7: a) shows the ordination of all seven CTD casts with their 99% standard error (ellipses). Figure b) shows the four CTD casts at the ESR and their environmental variables.

Table 5.3: Jaccard and Bray Curtis (brackets) similarity indices between averaged samples of each cast indices scale from 0-1, 0 being most similar and 1 having no shared OTU's. Bg: background; DV: diffuse vent. The highest and lowest similarity between casts is highlighted in bold type.

	Bg ESR								
Bg SWIR	0.806 (0.676)	Bg SWIR							
Longqi	0.665 (0.498)	0.695 (0.533)	Longqi						
Dv 2	0.719 (0.562)	0.606 (0.434)	0.676 (0.511)	DV 2					
DV 130	0.805 (0.673)	0.631 (0.461)	0.669 (0.503)	0.611 (0.440)	DV 130				
Vent 43	0.852 (0.743)	0.787 (0.648)	0.670 (0.504)	0.851 (0.739)	0.829 (0.709)	Vent (43)			
Vent 44	0.843 (0.728)	0.817 (0.690)	0.723 (0.566)	0.845 (0.731)	0.859 (0.752)	0.535 (0.365)			

5.4 Discussion

5.4.1 Ecological impact on vent microbial communities

Hydrothermal fluids are the product of chemical exchange between seawater and the ocean lithosphere at high temperature in the deepest part of the hydrothermal convection cell. Sulfate-reducing microorganisms commonly use hydrogen and/or dissolved organic matter as electron donors, both of which have been detected in hydrothermal fluids (Lang et al., 2010; Cao et al., 2014). Our results strongly indicate that the bacterial community compositions at our sampling locations were related to reducing chemical species, which is in agreement with previous research on the drivers of bacterial assemblages and dispersion close to vent locations (Huber et al., 2010a; Forget and Kim Juniper, 2013; Peng et al., 2011; Cao et al., 2014). The bacterial assemblages described in this study are well documented at hydrothermal vent and other deep-sea environments (Huber et al., 2003, 2007; Holden et al., 1998; Dick and Tebo, 2010; Crépeau et al., 2011; Karl et al., 1988; Zwirgmaier et al., 2014). While most of them are common deep-sea bacteria, such as *Halomonas* and *Marinobacter*, there are specific samples with very abundant Epsilonproteobacteria such as *Arcobacter*, *Sulfurimonas* and *Caminibacter*. Epsilonproteobacteria are wide-spread at deep-sea hydrothermal vents, both as endo- and epibionts, and free-living (Huber et al., 2010b; Watsuji et al., 2012; Longnecker and Reysenbach, 2001). Recently members of this group were cultured and found to exhibit both heterotrophic and autotrophic sulphur reduction, moderate thermophily, and the ability to use hydrogen as an electron donor (Campbell et al., 2006). The ability of certain Gammaproteobacteria, such as the family SUP05, to utilize hydrogen has also been widely demonstrated (Anantharaman et al., 2013) (Marshall and Morris, 2013). The family SUP05 had a much higher abundance around vent influenced water, which is consistent with the correspondence between elevated concentration of hydrogen and the Dog's Head samples correlated by the CCA (Fig. 5.7a). In addition, previously measured carbon at the ESR vents indicated breaking down of carbon, making it freely available to microbial production (Chapter 4). This may indicate that vent deposits directly sustain the Epsilon- and Gammaproteobacteria. Clone libraries of the genus *Arcobacter* have also been shown to perform sulfur oxidation with denitrification in pure cultures (e.g. Gevertz et al., 2000) and in the environment (Lavik et al., 2009). When hydrothermal fluids and

seawater mix, either in the subsurface or by advection across deposit walls, neutrality of the fluids is quickly reached, resulting in most marine hydrothermal vent habitats being circumneutral. This is evident from the diffuse vents and the less-hydrothermally affected samples, which have a community structure that is more similar to the background samples than the vents. The low direct temperatures measured during the CTD casts can also indicate the degree of vent-fluid to seawater neutrality where samples were collected. Considering the rapid mixing between the surrounding water and the hydrothermal fluids, the large peaks in SUP05 and *Sulfurimonas* (Fig. 5.5 and Appendix: B.3) in one sample from the diffuse vent at the ESR can mark a signal from a vent field, which has not yet been discovered. We did not deploy the ROV on this diffuse vent, and could not say with certainty that there is not vent present in this location. Given the linear trend with distance to vent from the Epsilonproteobacteria and the absence of SUP05 in other non-vent samples, we believe this to be an area influenced by higher concentrations of nutrients vital to chemosynthesis and possibly hydrothermal activity.

Interestingly the bacterial composition is different between the vents and the diffuse flow (Fig. 5.4 and Fig. 5.7) at the ESR. In addition the abundances are significantly higher at the diffuse vent from Dog's Head (Tukey HSD $p < 0.05$) (Fig. 5.3). A lower concentration of particulate hydrogen has the potential to impact the energy available for microbial populations at the vent site, with low hydrogen sulfide and therefore low microbial abundance (Butterfield et al., 2003). (Huber et al., 2003) suggested the Epsilonproteobacteria are favored by conditions caused by greater seawater mixing with hydrothermal fluids resulting in a lower temperature and increased levels of seawater electron acceptors. The mixed conditions above the vents with relatively low temperatures have been suggested to lead to an increase in habitat volume for organisms with an oxidative metabolism (Huber et al., 2003). The temperatures at Longqi were lower than at Dog's Head (Tao et al., 2012), as were the Epsilonproteobacteria abundances. It is widely known that the initial high temperatures from vents create the underlying chemical environment for microbial growth and thus influence the abundance of these microorganisms. (Huber et al., 2003) suggest that the abundance of Epsilonproteobacteria in the core of the plume close to the vent orifice would show a lower abundance as a result of a lack of electron acceptors. In this study Epsilonproteobacterial abundance increased across the full range of observed Eh values. However, although efforts were made to collect samples with maximum influence of hydrothermal fluids, sam-

ples collected with a CTD cannot substitute the precision of ROV or HOV collected samples, and may be some distance away from the core of the plume.

5.4.2 Diluting microbial communities

Similar to Read et al. (2015) for riverine systems, we also propose that the observed pattern in bacterial community composition is related to water residence time or current speed and to changing resource availability (Tao et al., 2012, 2011). Firstly, hydrothermal plumes vary in their dispersion depending on the temperature of the plume origins, current speed and direction. As water transitions away from the plume the bacterial community becomes dispersed and resource limited. This is supported by the decrease in abundance of known vent taxa with distance from plume (Fig. 5.5). The pattern is especially clear for the succession of the Epsilonproteobacteria composition with distance from plume, transitioning from high abundance closer to the vent to a lower abundance farther away (Fig. 5.6), which has also been observed as patchiness in vent sediment samples (Nakagawa et al., 2005). The hydrothermal vent plume environment is a novel environment with potentially lower levels of competition, which would favour rapidly growing species that can utilise available resources quickly. Indeed, Epsilonproteobacteria, here used as a vent indicative class, have previously been found to be fast growing. Moreover, the background seawater microorganisms can serve as indicators of mixing between the plumes and surrounding water to track this trend. However, when the plume community is mixed with the background community the latter can dominate in terms of abundance over the vent-specific bacteria. In our study the class level patterns (Appendix: B.3) do not show a clear trend between the vents, diffuse vents and background samples. When looking closely it is possible to observe the Epsilonproteobacteria in the vent samples without extracting the vent-specific microorganisms and analysing them separately. Thus when using relative abundances to locate the vent-community it becomes easy to miss vent specific microbes because they are relatively rare compared to the ambient deep-sea community, and are thus much harder to detect with the employed sampling approach.

The relationship between sequence abundance and cell abundance is not always straightforward and the problem of multiple copies of the 16S rRNA gene from the same cell on the evaluation of abundance has been raised (Caporaso et al., 2012b). However, with the sequencing depth achieved here, we can distinguish between the

main bacterial players in and between the samples and locations and would argue that the abundance of Epsilonproteobacteria to vent-distance pattern is representative, given the 100-300 fold increase of sequences between the plume and the background (Fig. 5.6).

5.4.3 Vent biogeography

Weak biogeographical signals in microbial communities are usually explained by the hypothesis of microbial cosmopolitanism formulated by Bass Becking (De Wit and Bouvier, 2006). However, recent studies have explored the effects of dispersal limitation on microbial biogeography. Like macroorganisms, the genetic similarity was negatively correlated with geographical distance, exhibiting a distance-decay relationship for cyanobacteria (Papke et al., 2003), sulfate-reducing bacteria (Mino et al., 2013), marine planktonic bacteria, and hyperthermophilic archaea (Flores et al., 2012; Whitaker et al., 2003). The biogeography of hydrothermal vent-associated microbial community has been studied before (Takai and Horikoshi, 1999; Kato et al., 2010; Flores et al., 2012; Mino et al., 2013; Nakagawa et al., 2005) and members of the genera presented in this study have been found globally at hydrothermal vent fields (Huber et al., 2010a). However, their relative abundance and heterogeneities between vent fields are poorly understood. Hydrothermal vent microbial ecology has been mostly focused on the epi- and endosymbionts of the metazoan vent fauna and sediment microorganisms (Sanders et al., 2013; Polz and Cavanaugh, 1995; Buckeridge et al., 2013; Herrera et al., 2015). The free-living microorganisms in the water column above hydrothermal plumes have only been studied on few occasions (Huber et al., 2003, 2010b, 2002; Sylvan et al., 2009, 2012) and never at the vent fields in the ESR and SWIR. The curious discoveries of our study are the dissimilarities between Dog's Head and Longqi with other well-studied plumes. The two vents clearly show a great difference in their community structure (Fig. 5.7). While the most common genera are the same between them, their relative abundances rank differently. At Dog's Head Rogers et al. (2012) previously found members of the phylum Deferribacteres, which is a chemoorganotrophic heterotroph that respire anaerobically. The Deferribacteres phyla was not found again in our study, possibly because of sample volume or because we did not use a direct plume sampling probe.

Compared to other vents the genus *Arcobacter* of the Epsilonproteobacteria was

very abundant and common genera such as *Thioreductor*, *Sulfurovum* and *Lebetimonas* were not found in our samples (Huber et al., 2010b). The Epsilonproteobacteria genera not found in our samples were targeted with different primers using the 16S rRNA gene region V6. This may result in differences in the detectability, in addition to the fact that most samples from vents elsewhere have been sequenced subsequently to cloning, which may introduce a bias for specific strains of microorganisms (e.g. *Defferibacter* in Rogers et al., 2012). For future research it would be interesting to investigate the global biogeography of free-living hydrothermal vent microorganisms using directly comparable genetic sequencing techniques and compare the microbial biogeography to that of metazoan vent communities (Rogers et al., 2012; Bachraty et al., 2009).

The idea of Baas Becking was that while all microbial life is distributed worldwide, in a given environmental setting most of the microbial species are only latently present. Hence, on a small scale, most microbial biodiversity is hidden for our observation, because most species will occur at densities below our limit of detection. The Epsilonproteobacteria dilution from the plume may be a good demonstration of the dispersion of bacteria in the water column and a perfect example of the limitations of bacterial niche-specificity to their habitat, supporting the idea of environmental selection suggested by Baas-Becking. We also demonstrate a clear distance-decay relationship based on abundance of niche-specific bacteria (De Wit and Bouvier, 2006).

Chapter 6

Final remarks

Chapter 6 Final remarks

6.1 Key findings

The sequencing results of Sogin et al. (Sogin et al., 2006) and Rusch et al. (Rusch et al., 2007) generated many questions with respect to the global distribution of marine microorganisms both on smaller (10s cm) and larger scales (1000s cm). From recent advancements in the field of marine microorganisms we have learned that microbes account for the majority of genetic and metabolic variation in the oceans and the genetic diversity, community composition, relative abundance, and distribution of microbes in the sea remain under-sampled. In this project I set out to investigate microorganisms, their distribution and biogeographic patterns on local (10-100 m) and regional spatial scales (10-1000 km). For the first time I examined organic carbon and microbial communities in two hydrothermal vent plumes and seamounts in the Southern Hemisphere. The hydrothermal vent organic carbon was compared to two Caribbean vent fields and the five seamounts were compared across different water masses. I investigated the physical and chemical properties of the studied plumes and seamounts in an attempt to link these parameters to the microbial community structure to better understand the ecology of the respective organisms. Research presented here had an emphasis on the use of Illumina MiSeq sequencing, but also employed flow cytometry, nutrient analysis, and elemental analysis of environmental samples. In addition we analysed physical parameters collected *in-situ* using CTD profiles.

6.1.1 Spatial scales of microbial community turnover

In Chapter 2 and 4 I examined the spatial patterns of particulate organic carbon concentrations and microorganisms on seamounts and hydrothermal vents, respectively. The measurements showed a heterogeneous distribution of microbial communities, POC and DOC with depth and spatial distance. I show that hydrothermal vents and seamounts greatly influence the distribution of carbon, by physical forcing, in the surrounding environment and correlation of microbial communities are strongly affected by their environment.

Regional patterns

The analysis of POC between the seamounts showed that most of the surface POC is associated with fluorescence, a proxy for primary productivity, which was highest on

Chapter 6 Final remarks

the southernmost (Coral) of the investigated seamounts. This is not surprising considering that Coral is in a eutrophic/mesotrophic environment with turbulent mixing that replenishes the surface layer with nutrients. Autotrophic organisms benefit from this influx of nutrients, which enhances the microbial biomass and the POC stock on Coral (Chapter 2 and 3).

Different microorganisms dwell in the surface of the seamounts and it is clear that most autotrophic bacteria in this layer are *Synechococcus* (southern seamount, Coral) and *Prochlorococcus* (northern seamount, Atlantis). The regional pattern of physical parameters in the surface layers of the seamounts divides the South West Indian Ridge (SWIR) into three biological regimes, the oligotrophic north (Atlantis), the mesotrophic convergence zone (Melville, Middle of What, and Sapmer) and the mostly eutrophic south (Coral). POC and the abundance of microorganisms in the shallow layer also reflect this separation of the SWIR.

In Chapter 3 I further resolve the regime division between the seamounts across depth layers. The differences between the surface environments of the seamounts are large and can be explained by primary productivity and differences between environmental settings around the seamounts, depending on light, temperature and salinity. At mesopelagic depths (>200 m) the environmental gradient is less steep between seamounts and in the deepest layer the nutrient concentrations and temperature are similar across all seamounts. The microbial communities in the deep layer are separated by geographic location. It would be expected that the diversity of taxa at the sub-tropical convergence-zone peaks as a result of mixing of taxa from the different water masses and biochemical conditions which lead to a peak in plankton and metazoan diversity. The same horizontal and vertical patterns for microorganisms and metazoans points to fundamental differences throughout all parts of the ecosystem/food web across this frontal zone (Barange et al., 1998b; Longhurst et al., 1995; Bornmann et al., in press; Letessier et al., In press). Thus, seamounts along the SWIR are in contrasting productivity regimes depending on their proximity to the sub-tropical convergence and the sub-Antarctic front with steep environmental changes within the surface layer corresponding to the largest differences in microbial community.

I cannot say much about the processes of dispersal, but the microbial communities in the convergence zone are most likely affected by mixing from the north and south causing higher richness in the convergence zone. The microbial community composition is stable between the convergence zone seamounts (Melville and Mid-

dle of What) and taxa turnover occurs between the convergence zone - sub-Antarctic and convergence zone - sub-tropical (Chapter 3) at all depths. The separation of the communities between the regions depends on their environment, causing stability in the three biological regimes. In marine microbial communities it is common to have a large proportion of rare taxa (Huse et al., 2008; Sogin et al., 2006), and this is confirmed by my samples where ~70% of OTUs are rare, respectively. Given the sampling strategy I was only able to get a snapshot of microbial diversity over the seamounts, and from this it is impossible to say if population sizes of rare taxa are a result of limited dispersal, caused by interspecies competition, or environmental constraints. I do not know at which rates taxa disperse, but if dispersion is high and/or selection weak the similarity should consequently be high and if selection is strong and dispersal is weak, similarity should be low.

Local patterns

Below the photic zone parameters did not predict any variation in POC. POC was very similar between all stations at mesopelagic and bathypelagic depths, most likely as a result of efficient remineralisation near the surface. However, the concentration of particles on the seamounts increased towards the seabed. POC is both a substrate and product of microbial productivity and aggregation; as POC decreases with depth there is a steep change in environmental conditions, such as nutrients (Chapter 2). This change is more pronounced with depth than with latitude resulting in environmental selection of microbial communities, primarily with depth, most likely because of lack of vertical water mixing between the depth layers. This is consistent with a vertical stratification of microbial taxa according to changes in physicochemical parameters (Sunagawa et al., 2015). Together depth and water mass drive the community structure at the South West Indian Ocean seamounts, which is in accordance with microbial communities largely being controlled by deep-sea circulation and hydrography elsewhere (Ghiglione et al., 2012). The separation of microbial communities at 500 m might tie in with wider changes in biogeochemistry or pelagic macro and megafaunal communities, however sampling intervals would need to be increased to get a higher resolution of microbial communities with depth. This would increase our knowledge about which depth the community structure changes and which environmental feature it was related to.

Chapter 6 *Final remarks*

I have demonstrated that the demersal environment in the deep-sea is affected by the resuspension of material, although this material does not contain a large organic fraction (Chapter 2). In contrast to the seamounts, I confirm previous observations that hydrothermal vents degrade the DOM passing through the axial crust. The resulting compounds are readily available to the microbial communities persisting around the plume, which may take opportunistic advantage of these compounds. The reworking of degenerated DOM with other compounds by plume bacteria, allows the release of newly produced DOM into the deep-ocean. The amount equivalent to approximately 1% of total organic carbon fixed at the surface (Chapter 4). I was unable to obtain samples from the end-member fluids of the plumes for POC or microbial measurements, because of the relatively small (750 ml) amount of water collected by the ROV Niskins, and because a CTD is not able to accurately sample the end-member vent fluids. Samples for the microbial diversity estimates were collected at the same time as the POC samples but not as the DOM, and are thus not directly comparable.

The increase of DOM closer to the plume origin is very clear at the Beebe and Von Damm vents, in the Caribbean, but not so clear in the E2 vent field, in the southern hemisphere. However, it is evident that the microbial communities adapted to hydrothermal vent environments are more abundant closer to the plume origin at E2 and are displaying a distance-decay relationship at a scale of 10s-100s of m from the plume origin (Chapter 5). Huber et al. (2003) suggested an optimum habitat for microorganisms 10s of centimeters from the vent orifice, I thus expect the interaction between the degenerated DOM and the deep ocean water to stimulate the production of organic carbon by microbial activity in the water column in the region of venting. The process by which the microorganisms in vent plumes disperse is well known, and it is also known that the plumes affect the deep-ocean several kilometers away from the plume origin (German et al., 2011; Hawkes et al., 2013). The basin scale difference between the hydrothermal vents of the East Scotia Ridge and the South West Indian Ridge are most likely a result of mutation processes in the absence of gene flow. This has been demonstrated previously within the *Persephonella* genus (Mino et al., 2013) where hydrothermal vent strains are specific to an area of low dispersion and high selection. However, on a global scale, the microorganisms at hydrothermal vents and at seamounts cluster within the respective environments, indicating environmental selection of microbial communities (Fig. 6.1). Overall, I show that through the simultaneous geochemical process of degenerating DOM and flow of hydrogen

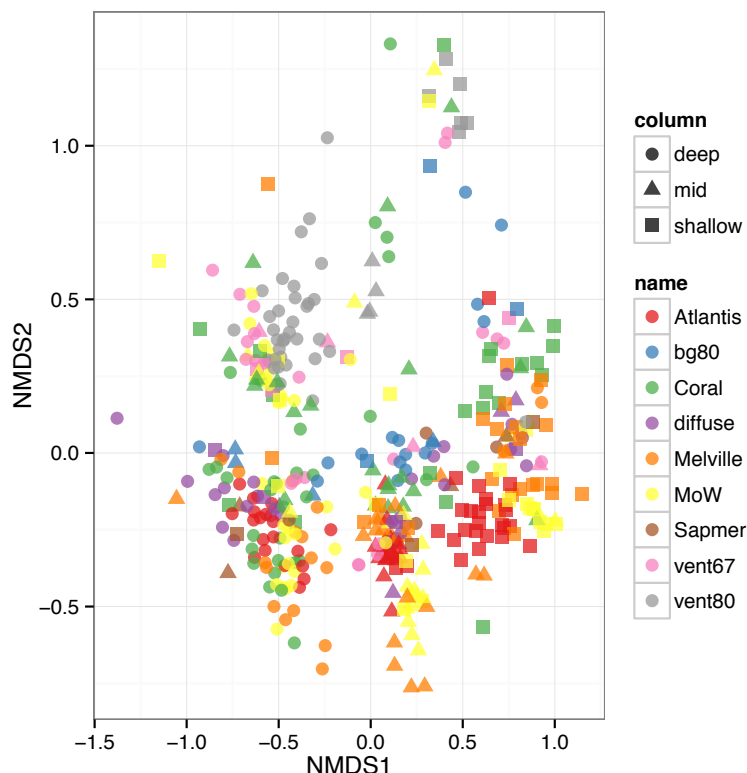


Figure 6.1: Non-metric multidimensional scaling plot of all microbial community samples collected from this study, showing four clusters, mostly by depth layer and vent samples.

and nitrogen, hydrothermal venting serves to re-work deep ocean DOM, certainly on a local scale, leading to a more reduced, more saturated and more sulfonated mixture of DOM, which in turn enables highly adapted microorganisms to flourish.

6.2 Outlook

In this thesis I did not consider the role of damaged sequences introduced by PCR or sequencing errors, which are known to affect both alpha- and beta-diversity estimates (Schloss et al., 2011), for which no computationally efficient solutions exist yet (Schloss et al., 2011; Coissac et al., 2012). Nevertheless, these errors are likely to generate OTUs of low abundance (Kunin et al., 2010; Quince et al., 2011; Coissac et al., 2012) and to be of the same magnitude across all samples, because all processing

Chapter 6 Final remarks

was identical from amplification to sequencing. As a result I assume a low impact on the diversity patterns and ecological interpretation (Zinger et al., 2014; Schloss et al., 2011; Santoferrara et al., 2014). My results show a very clear pattern in microbial biogeography on differing levels and spatial scales and this is unlikely to be caused by technical artifacts alone.

The likelihood of detecting a distance effect of microbial community structure increases with increasing resolution (more in-depth sequencing). Whole-genome or multilocus genotyping techniques and full-sequence comparisons were more likely to detect a distance effect than studies using single-gene fingerprinting techniques or morphological techniques (Hanson et al., 2012). From metagenomic studies I would get a measure for gene function in the microbial taxa present in the samples. This would give me a better overview of the functioning of different taxa and their relation to their respective environment and selection towards that environment. I thus suggest using metagenomics or transcriptomics in future regional studies of microbial communities on seamounts to better understand of expression of microbial communities across physical barriers.

This thesis only had a spatial element and did not investigate the temporal factors of microbial community variation. It would be preferable to study within station temporal variation, however, shipborne research in rough seas, e.g. Southern Ocean and South West Indian Ridge, far away from land is logistically challenging.

An uncertainty presented in the sampling of hydrothermal vents is the distance to vent estimate, which was based on electrochemical Eh or light scattering sensor measurements, which can suffer from sensor drift. The degree of vent influence could be better estimated using the iron to manganese ratio from the same water samples. However, this is a very laborious and costly procedure, which was not within the scope of this thesis.

Going forward with open-ocean and deep-sea microbial community research I would suggest continuing this work along three questions (Dick et al., 2013).

- (i) Define how the interplay between microbiology and geochemistry in hydrothermal plumes extends to global-scale interactions between mid-ocean ridges and deep-sea microbiology, and to which degree this influences chemosynthesis outside hydrothermal plumes
- (ii) The deep oceans hold the largest reservoir of rapidly exchangeable inorganic

carbon on the Earth's surface and our data suggest that microbial autotrophy potentially converts dissolved inorganic carbon into the organic phase through the vent plume. Microbial communities consist of both seafloor and pelagic environments, but to what extent does chemoautotrophic and heterotrophic growth influence the broader deep oceans?

- (iii) Further investigation on the inter relationship between plume microbial activity and hydrocarbon degradation will provide better understanding of the impact of hydrothermal plumes on the deep-ocean carbon cycling.

These suggestions would further increase our knowledge on dispersal of microorganisms from deep-sea environments and the interplay between chemosynthetic, photoautotrophic and heterotrophic microorganisms in the world's ocean.

Chapter 6 Final remarks

Bibliography

- Abell, R. E., T. Brand, A. C. Dale, and G. H. Tilstone, 2013. Variability of particulate flux over the Mid-Atlantic Ridge. *Deep Sea Research Part II: Topical Studies in Oceanography* **98**:257–268.
- Acha, E. M., A. Piola, O. Iribarne, and H. Mianzan, 2015. Biology of Fronts, vol. Chapter 3. Springer International Publishing.
- Aguilar, M. and C. Lado, 2012. Ecological niche models reveal the importance of climate variability for the biogeography of protosteloid amoebae. *The ISME Journal* **6**:1506–1514.
- Akima, H., 1978. A method of bivariate interpolation and smooth surface fitting for irregularly distributed data points. *Soviet Journal of Marine Biology* **4**:148–159.
- Akima, H., A. Gebhardt, T. Petzoldt, and M. Maechler, 2011. akima: Interpolation of irregularly spaced data. R package version 0.5-4.
- Al-Mutairi, H. and M. R. Landry, 2001. Active export of carbon and nitrogen at Station ALOHA by diel migrant zooplankton. *Deep Sea Research Part II: Topical Studies in Oceanography* **48**:2083–2103.
- Alderkamp, A. C., E. Sintes, and G. J. Herndl, 2006. Abundance and activity of major groups of prokaryotic plankton in the coastal North Sea during spring and summer. *Aquatic Microbial Ecology* **45**:237–246.
- Alonso Gutiérrez, J., I. Lekunberri, E. Teira, J. M. Gasol, A. Figueras, and B. Novoa, 2009. Bacterioplankton composition of the coastal upwelling system of 'Ría de Vigo', NW Spain. *FEMS Microbiology Ecology* **70**:493–505.
- Alonso-Sáez, L., J. Arístegui, and J. Pinhassi, 2007a. Bacterial assemblage structure and carbon metabolism along a productivity gradient in the NE Atlantic Ocean. *Aquatic Microbial Ecology* **46**:43–53.
- Alonso-Sáez, L., J. M. Gasol, and J. Arístegui, 2007b. Large-scale variability in surface bacterial carbon demand and growth efficiency in the subtropical northeast Atlantic Ocean. *Limnology and Oceanography* **52**:533–546.
- Alves Junior, N., P. M. Meirelles, E. de Oliveira Santos, B. Dutilh, G. G. Z. Silva, R. Paranhos, A. S. Cabral, C. Rezende, T. Iida, R. L. de Moura, R. H. Kruger, R. C. Pereira, R. Valle, T. Sawabe, C. Thompson, and F. Thompson, 2015. Microbial community diversity and physical-chemical features of the Southwestern Atlantic Ocean. *Archives of Microbiology* **197**:165–179.

Bibliography

- Anantharaman, K., J. A. Breier, C. S. Sheik, and G. J. Dick, 2013. Evidence for hydrogen oxidation and metabolic plasticity in widespread deep-sea sulfur-oxidizing bacteria. *Proceedings of the National Academy of Sciences of the United States of America* **110**:330–335.
- Anderson, T. R. and K. W. Tang, 2010. Carbon cycling and poc turnover in the mesopelagic zone of the ocean: Insights from a simple model. *Deep-Sea Research Part II: Topical Studies in Oceanography* **57**:1581–1592.
- Aquilina, A., D. P. Connelly, J. T. Copley, D. R. H. Green, J. A. Hawkes, L. E. Hepburn, V. A. I. Huvenne, L. Marsh, R. A. Mills, and P. A. Tyler, 2013. Geochemical and Visual Indicators of Hydrothermal Fluid Flow through a Sediment-Hosted Volcanic Ridge in the Central Bransfield Basin (Antarctica). *PLoS ONE* **8**:e54686.
- Aquilina, A., W. B. Homoky, J. A. Hawkes, T. W. Lyons, and R. A. Mills, 2014. Hydrothermal sediments are a source of water column Fe and Mn in the Bransfield Strait, Antarctica. *Geochimica et Cosmochimica Acta* **137**:64–80.
- Arrigo, K. R., 2005. Marine microorganisms and global nutrient cycles. *Nature* **437**:349–355.
- Auguet, J.-C., A. Barberan, and E. O. Casamayor, 2010. Global ecological patterns in uncultured Archaea. *The ISME Journal* **4**:182–190.
- Azam, F., 1983. Growth of bacteria, and its relationship to the production of organic matter in the sea. JE Robbie and PJ leB. Williams [eds.].
- Azam, F., T. Fenchel, J. G. Field, and J. S. Gray, 1983. The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series* **10**:257–263.
- Azam, F. and F. Malfatti, 2007. Microbial structuring of marine ecosystems. *Nature reviews. Microbiology* **5**:782–791.
- Bach, W., N. R. Banerjee, H. J. B. Dick, and E. T. Baker, 2002. Discovery of ancient and active hydrothermal systems along the ultra-slow spreading Southwest Indian Ridge 10°–16°E. *Geochemistry, Geophysics, Geosystems* **3**:1–14.
- Bachraty, C., P. Legendre, and D. Desbruyères, 2009. Biogeographic relationships among deep-sea hydrothermal vent faunas at global scale. *Deep Sea Research Part I: Oceanographic Research Papers* **56**:1371–1378.
- Baker, E. T. and J. W. Lavelle, 1984. The effect of particle size on the light attenuation coefficient of natural suspensions. *Journal of Geophysical Research* **89**:8197–8203.
- Barange, M., E. A. Pakhomov, and R. Perissinotto, 1998a. Pelagic community structure of the subtropical convergence region south of Africa and in the mid-Atlantic Ocean. *Deep-Sea Research Part II: Topical Studies in Oceanography* **45**:1663–1687.
- Barange, M., S. C. Pillar, and I. Hampton, 1998b. Distribution patterns, stock size and life-history strategies of Cape horse mackerel *Trachurus trachurus capensis*, based on bottom trawl and acoustic surveys. *South African Journal of Marine Science* **19**:433–447.

- Bardgett, R. D., C. Freeman, and N. J. Ostle, 2008. Microbial contributions to climate change through carbon cycle feedbacks. *The ISME Journal* **2**:805–814.
- Bartz, R., J. Ronald, V. Zaneveld, and H. Pak, 1978. A Transmissometer For Profiling And Moored Observations In Water. *22nd Annual Technical Symposium* **0160**:102–109.
- Bennett, S. A., R. L. Hansman, A. L. Sessions, K.-i. Nakamura, and K. J. Edwards, 2011a. Tracing iron-fueled microbial carbon production within the hydrothermal plume at the Loihi seamount. *Geochimica et Cosmochimica Acta* **75**:5526–5539.
- Bennett, S. A., P. J. Statham, D. R. H. Green, N. Le Bris, J. M. McDermott, F. Prado, O. J. Rouxel, K. Von Damm, and C. R. German, 2011b. Dissolved and particulate organic carbon in hydrothermal plumes from the East Pacific Rise, 9°50'N. *Deep Sea Research Part I: Oceanographic Research Papers* **58**:922–931.
- Bishop, J., 1999. Instruments and methods-Transmissometer measurement of POC. *Deep Sea Research Part I: Oceanographic Research Papers* **46**:355.
- Boehlert, G. W. and A. Genin, 1987. A review of the effects of seamounts on biological processes. Wiley Online Library.
- Boehlert, G. W. and M. M. Yoklavich, 1984. Reproduction, embryonic energetics, and the maternal-fetal relationship in the viviparous genus *Sebastes* (Pisces: Scorpaenidae). *The Biological Bulletin* **167**:354–370.
- Boersch-Supan, P. H., L. Boehme, and J. F. Read, 2012. Elephant seal foraging dives track prey distribution, not temperature: Comment on McIntyre et al.(2011). *Marine Ecology Progress Series* **461**:293–298.
- Boersch-Supan, P. H., A. D. Rogers, and A. S. Brierley, In press. Scattering layers of six south-west indian ocean seamounts. *Deep-Sea Research Part II: Topical Studies in Oceanography* .
- Bornmann, T. G., M. J. Sonnekus, and E. E. Campbell, in press. Phytoplankton and nutrient dynamics of six south idian ocean seamounts. *Deep-Sea Research Part II: Topical Studies in Oceanography* .
- Børsheim, K. Y. and G. Bratbak, 1987. Cell volume to cell carbon conversion factors for a bacterivorous (*Monas* sp.) enriched from seawater. *Marine Ecology Progress Series* **36**:171–175.
- Bouvier, T. C. and P. A. del Giorgio, 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. *Limnology and oceanography* **47**:453–470.
- Brault, M., B. Simoneit, and A. Saliot, 1989. Trace petroliferous organic-matter associated with massive hydrothermal sulfides from the east pacific rise at 13-degrees-n and 21-degrees-n. *Oceanologica Acta* **12**.

Bibliography

- Brink, K. H., 1995. Tidal and lower frequency currents above Fieberling Guyot. *Journal of Geophysical Research: Oceans* **100**:10817–10832.
- von Bröckel, K. and M. Meyerhöfer, 1999. Impact of the Rocks of Sao Pedro and Sao Paul upon the quantity and quality of suspended organic matter. *Archive of Fishery and Marine Research* **47**.
- Bruguier, N. J. and R. A. Livermore, 2001. Enhanced magma supply at the southern East Scotia Ridge: evidence for mantle flow around the subducting slab? *Earth and Planetary Science Letters* **191**:129–144.
- Buckeridge, J. S., K. Linse, and J. A. Jackson, 2013. *Vulcanolepas scotiaensis* sp. nov., a new deep-sea scalpelliform barnacle (Eolepadidae: Neolepadinae) from hydrothermal vents in the Scotia Sea, Antarctica. *Zootaxa* **5**:551–568.
- Butterfield, D. A., W. E. Seyfried Jr, and M. D. Lilley, 2003. Energy and mass transfer in marine hydrothermal systems. composition and evolution of hydrothermal fluids. Tech. rep., Dahlem Workshop report.
- Campbell, B. J. and S. C. Cary, 2004. Abundance of Reverse Tricarboxylic Acid Cycle Genes in Free-Living Microorganisms at Deep-Sea Hydrothermal Vents. *Applied and Environmental Microbiology* **70**:6282–6289.
- Campbell, B. J., A. S. Engel, M. L. Porter, and K. Takai, 2006. The versatile Epsilonproteobacteria: key players in sulphidic habitats. *Nature reviews. Microbiology* **4**:458–468.
- Campbell, L., H. Liu, H. A. Nolla, and D. Vault, 1997. Annual variability of phytoplankton and bacteria in the subtropical North Pacific Ocean at Station ALOHA during the 1991–1994 ENSO event. *Deep Sea Research Part I: Oceanographic Research Papers* **44**:167–192.
- Cao, H., Y. Wang, O. O. Lee, X. Zeng, Z. Shao, and P.-Y. Qian, 2014. Microbial Sulfur Cycle in Two Hydrothermal Chimneys on the Southwest Indian Ridge. *mBio* **5**:e00980–13.
- Caporaso, J. G., K. Bittinger, F. D. Bushman, T. Z. DeSantis, G. L. Andersen, and R. Knight, 2010a. PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* **26**:266–267.
- Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight, 2010b. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**:335–336.
- Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S. M. Owens, J. Betley, L. Fraser, M. Bauer, N. Gormley, J. A. Gilbert, G. Smith, and R. Knight, 2012a. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal* **6**:1621–1624.

- , 2012b. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal* **6**:1621–1624.
- Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer, and R. Knight, 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America* **108**:4516–4522.
- Carini, P., A. E. White, E. O. Campbell, and S. J. Giovannoni, 2014. Methane production by phosphate-starved SAR11 chemoheterotrophic marine bacteria. *Nature Communications* **5**:4346.
- Clark, D. P., P. Dunlap, M. Madigan, and J. Martinko, 2009. Brock Biology of Microorganisms. Prentice Hall Upper Saddle River, NJ.
- Clark, M. R., A. A. Rowden, T. Schlacher, A. Williams, M. Consalvey, K. I. Stocks, D. Amon, T. D. O'Hara, M. White, T. M. Shank, and J. M. Hall-Spencer, 2010. The ecology of seamounts: structure, function, and human impacts. *Annual Review of Marine Science* **2**:253–278.
- Clark, M. R., L. Watling, A. A. Rowden, and J. M. Guinotte, 2011. A global seamount classification to aid the scientific design of marine protected area networks. *Ocean & Coastal Management* **54**:19–36.
- Coissac, E., T. Riaz, and N. Puillandre, 2012. Bioinformatic challenges for DNA metabarcoding of plants and animals. *Molecular ecology* **21**:1834–1847.
- Cole, C. S., R. H. James, D. P. Connelly, and E. C. Hathorne, 2014. Rare earth elements as indicators of hydrothermal processes within the East Scotia subduction zone system. *Geochimica et Cosmochimica Acta* **140**:20–38.
- Connelly, D. P., J. T. Copley, B. J. Murton, K. Stansfield, P. A. Tyler, C. R. German, C. L. Van Dover, D. Amon, M. Furlong, N. Grindlay, N. Hayman, V. Hühnerbach, M. Judge, T. Le Bas, S. McPhail, A. Meier, K.-i. Nakamura, V. Nye, M. Pebody, R. B. Pedersen, S. Plouviez, C. Sands, R. C. Searle, P. Stevenson, S. Taws, and S. Wilcox, 2012. Hydrothermal vent fields and chemosynthetic biota on the world's deepest seafloor spreading centre. *Nature Communications* **3**:620.
- Corliss, J. B., J. Dymond, L. I. Gordon, J. M. Edmond, R. P. von Herzen, R. D. Ballard, K. Green, D. Williams, A. Bainbridge, K. Crane, and T. H. van Andel, 1979. Submarine thermal springs on the galapagos rift. *Science* **203**:1073–1083.
- Cowen, J. P., M. A. Bertram, S. G. Wakeham, R. E. Thomson, J. William Lavelle, E. T. Baker, and R. A. Feely, 2001. Ascending and descending particle flux from hydrothermal plumes at Endeavour Segment, Juan de Fuca Ridge. *Deep Sea Research Part I: Oceanographic Research Papers* **48**:1093–1120.

Bibliography

- Crépeau, V., M.-A. Cambon Bonavita, F. Lesongeur, H. Randrianalivelo, P.-M. Sarradin, J. Sarrazin, and A. Godfroy, 2011. Diversity and function in microbial mats from the Lucky Strike hydrothermal vent field. *FEMS Microbiology Ecology* **76**:524–540.
- Crump, B. C., C. S. Hopkinson, M. L. Sogin, and J. E. Hobbie, 2004. Microbial biogeography along an estuarine salinity gradient: combined influences of bacterial growth and residence time. *Applied and environmental microbiology* **70**:1494–1505.
- Cui, Y., S. Suzuki, Y. Omori, S.-K. Wong, M. Ijichi, R. Kaneko, S. Kameyama, H. Tanimoto, and K. Hamasaki, 2015. Abundance and Distribution of Dimethylsulfoniopropionate Degradation Genes and the Corresponding Bacterial Community Structure at Dimethyl Sulfide Hot Spots in the Tropical and Subtropical Pacific Ocean. *Applied and Environmental Microbiology* **81**:4184–4194.
- Davis, R. E. and C. L. Moyer, 2008. Extreme spatial and temporal variability of hydrothermal microbial mat communities along the Mariana Island Arc and southern Mariana back-arc system. *Journal of Geophysical Research: Oceans* **113**:B08S15.
- De Angelis, M. A., M. D. Lilley, and J. A. Baross, 1993. Methane oxidation in deep-sea hydrothermal plumes of the endeavour segment of the Juan de Fuca Ridge. *Deep Sea Research Part I: Oceanographic Research Papers* **40**:1169–1186.
- De La Rocha, C. L. and U. Passow, 2007. Factors influencing the sinking of POC and the efficiency of the biological carbon pump. *Deep Sea Research Part II: Topical Studies in Oceanography* **54**:639–658.
- De Wit, R. and T. Bouvier, 2006. ‘Everything is everywhere, but, the environment selects’; what did Baas Becking and Beijerinck really say? *Environmental Microbiology* **8**:755–758.
- De’ath, G., 2002. Multivariate regression trees: A new technique for modeling species-environment relationships **83**:1105–1117.
- Delacour, A., G. L. Früh-Green, S. M. Bernasconi, P. Schaeffer, and D. S. Kelley, 2008. Carbon geochemistry of serpentinites in the Lost City Hydrothermal System (30°N, MAR). *Geochimica et Cosmochimica Acta* **72**:3681–3702.
- DeLong, E. F., 2003. Oceans of archaea. *American Society for Microbiology* **69**:503–511.
- DeLong, E. F., C. M. Preston, T. Mincer, V. Rich, and S. J. Hallam, 2006. Community genomics among stratified microbial assemblages in the ocean’s interior. *Science* **311**:496–503.
- Dick, G. J., K. Anantharaman, B. J. Baker, M. Li, D. C. Reed, and C. S. Sheik, 2013. The microbiology of deep-sea hydrothermal vent plumes: ecological and biogeographic linkages to seafloor and water column habitats. *Frontiers in microbiology* **4**:124.
- Dick, G. J., B. G. Clement, S. M. Webb, and F. J. Fodrie, 2009. Enzymatic microbial Mn (II) oxidation and Mn biooxide production in the Guaymas Basin deep-sea hydrothermal plume. *Geochimica et Cosmochimica Acta* **73**:6517–6530.

- Dick, G. J. and B. M. Tebo, 2010. Microbial diversity and biogeochemistry of the Guaymas Basin deep-sea hydrothermal plume. *Environmental Microbiology* **12**:1334–1347.
- Didyk, B. M. and B. R. T. Simoneit, 1990. Petroleum characteristics of the oil in a Guaymas Basin hydrothermal chimney. *Applied Geochemistry* **5**:29–40.
- Dittmar, T., B. Koch, N. Hertkorn, and G. Kattner, 2008. A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. *Limnology and Oceanography: Methods* **6**:230–235.
- Dittmar, T. and B. P. Koch, 2006. Thermogenic organic matter dissolved in the abyssal ocean. *Marine Chemistry* **102**:208–217.
- Dower, J., H. Freeland, and K. Juniper, 1992. A strong biological response to oceanic flow past Cobb Seamount. *Deep Sea Research Part I: Oceanographic Research Papers* **39**:1139–1145.
- Dubilier, N., C. Bergin, and C. Lott, 2008. Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. *Nature reviews. Microbiology* **6**:725–740.
- Dufrêne, M. and P. Legendre, 2008. Species Assemblages and Indicator Species: The need for a flexible asymmetrical approach. *Ecological Monographs* **67**:345–366.
- Edgar, R. C., 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**:2460–2461.
- Fedosova, R. A., 1974. Distribution of some copepod species in vicinity of underwater Hawaiian ridge. *Oceanology of the Academy of Sciences of the USSR* **14**:724–728.
- Fenchel, T. and B. J. Finlay, 2004. The ubiquity of small species: patterns of local and global diversity. *Bioscience* **54**:777–784.
- Finlay, B. J. and G. F. Esteban, 2001. Ubiquitous microbes and ecosystem function. *Limnetica* **21**:31–43.
- Finlay, B. J. and T. Fenchel, 2004. Cosmopolitan Metapopulations of Free-Living Microbial Eukaryotes. Supplementary Information. *Protist* **155**:e1–e38.
- Flores, G. E., I. D. Wagner, Y. Liu, and A.-L. Reysenbach, 2012. Distribution, abundance, and diversity patterns of the thermoacidophilic "deep-sea hydrothermal vent euryarchaeota 2". *Frontiers in Microbiology* **3**:47.
- Fock, H. O., B. Matthiessen, and H. Zidowitz, 2002. Diel and habitat-dependent resource utilisation by deep-sea fishes at the Great Meteor seamount: niche overlap and support for the sound scattering layer interception hypothesis. *Marine Ecology Progress Series* **244**:217–233.
- Forget, N. L. and S. Kim Juniper, 2013. Free-living bacterial communities associated with tube-worm (*Ridgeia piscesae*) aggregations in contrasting diffuse flow hydrothermal vent habitats at the Main Endeavour Field, Juan de Fuca Ridge. *Microbiology Open* **2**:259–275.

Bibliography

- Fuhrman, J. A., I. Hewson, M. S. Schwalbach, J. A. Steele, M. V. Brown, and S. Naeem, 2006. Annually reoccurring bacterial communities are predictable from ocean conditions. *Proceedings of the National Academy of Sciences of the United States of America* **103**:13104–13109.
- Gardner, W. D., A. V. Mishonov, and M. J. Richardson, 2003. Global POC distribution using Ocean Color and WOCE/JGOFS Beam Attenuation and POC Data. *Progress in Oceanography* **53**:718–740.
- , 2006. Global POC concentrations from in-situ and satellite data. *Deep Sea Research Part II: Topical Studies in Oceanography* **53**:718–740.
- Genin, A., 2004. Bio-physical coupling in the formation of zooplankton and fish aggregations over abrupt topographies. *Journal of Marine Systems* **50**:3–20.
- Genin, A. and G. W. Boehlert, 1985. Dynamics of temperature and chlorophyll structures above a seamount: An oceanic experiment. *Journal of Marine Research* **43**:907–924.
- Genin, A., C. Greene, L. Haury, P. Wiebe, G. Gal, S. Kaartvedt, E. Meir, C. Fey, and J. Dawson, 1994. Zooplankton patch dynamics: daily gap formation over abrupt topography. *Deep Sea Research Part I: Oceanographic Research Papers* **41**:941–951.
- Genin, A., L. Haury, and P. Greenblatt, 1988. Interactions of migrating zooplankton with shallow topography: predation by rockfishes and intensification of patchiness. *Deep Sea Research Part I: Oceanographic Research Papers* **35**:151–175.
- German, C. R., E. T. Baker, C. Mevel, and K. Tamaki, 1998. Hydrothermal activity along the southwest Indian ridge. *Nature* **395**:490–493.
- German, C. R., R. A. Livermore, E. T. Baker, N. I. Bruguier, D. P. Connelly, A. P. Cunningham, P. Morris, I. P. Rouse, P. J. Statham, and P. A. Tyler, 2000. Hydrothermal plumes above the East Scotia Ridge: an isolated high-latitude back-arc spreading centre. *Earth and Planetary Science Letters* **184**:241–250.
- German, C. R., E. Ramirez-Llodra, M. C. Baker, P. A. Tyler, and the ChEss Scientific Steering Committee, 2011. Deep-water chemosynthetic ecosystem research during the census of marine life decade and beyond: a proposed deep-ocean road map. *PLoS ONE* **6**:e23259.
- Gevertz, D., A. J. Telang, G. Voordouw, and G. E. Jenneman, 2000. Isolation and characterization of strains cvo and fwko b, two novel nitrate-reducing, sulfide-oxidizing bacteria isolated from oil field brine. *Applied and Environmental Microbiology* **66**:2491–2501.
- Ghiglione, J.-F., P. E. Galand, T. Pommier, C. Pedrós-Alió, E. W. Maas, K. Bakker, S. Bertilson, D. L. Kirchman, C. Lovejoy, P. L. Yager, and A. E. Murray, 2012. Pole-to-pole biogeography of surface and deep marine bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America* **109**:17633–17638.
- Ghiglione, J. F., G. Mevel, M. Pujo-Pay, L. Mousseau, P. Lebaron, and M. Goutx, 2007. Diel and seasonal variations in abundance, activity, and community structure of particle-attached and free-living bacteria in nw mediterranean sea. *Microbial Ecology* **54**:217–231.

- Giebel, H.-A., T. Brinkhoff, W. Zwisler, N. Selje, and M. Simon, 2009. Distribution of Roseobacter RCA and SAR11 lineages and distinct bacterial communities from the subtropics to the Southern Ocean. *Environmental Microbiology* **11**:2164–2178.
- Giebel, H.-A., D. Kalhoefer, A. Lemke, S. Thole, R. Gahl-Janssen, M. Simon, and T. Brinkhoff, 2011. Distribution of Roseobacter RCA and SAR11 lineages in the North Sea and characteristics of an abundant RCA isolate. *The ISME Journal* **5**:8–19.
- Giering, S. L. C., R. Sanders, R. S. Lampitt, T. R. Anderson, C. Tamburini, M. Boutrif, M. V. Zubkov, C. M. Marsay, S. A. Henson, K. Saw, K. Cook, and D. J. Mayor, 2014. Reconciliation of the carbon budget in the ocean's twilight zone. *Nature* **507**:480–483.
- Gilbert, J. A., J. A. Steele, J. G. Caporaso, and L. Steinbrück, 2012. Defining seasonal marine microbial community dynamics. *The ISME Journal* **6**:298–308.
- Giovannoni, S. J. and U. Stingl, 2005. Molecular diversity and ecology of microbial plankton. *Nature* **437**:343–348.
- Giovannoni, S. J. and K. L. Vergin, 2012. Seasonality in ocean microbial communities. *Science* **335**:671–676.
- Giovanonni, S. and M. Rappé, 2000. Microbial Ecology of the Oceans. Wiley Online Library.
- Goffredi, S. K., 2010. Indigenous ectosymbiotic bacteria associated with diverse hydrothermal vent invertebrates. *Environmental microbiology reports* **2**:479–488.
- Goffredi, S. K., W. J. Jones, and H. Erhlich, 2008. Epibiotic bacteria associated with the recently discovered Yeti crab, Kiwa hirsuta. *Environmental Microbiology* **10**:2623–2634.
- Goldner, D. R. and D. C. Chapman, 1997. Flow and particle motion induced above a tall seamount by steady and tidal background currents. *Deep Sea Research Part I: Oceanographic Research Papers* **44**:719–744.
- Gorelova, T. A. and V. G. Prut'ko, 1985. Feeding of Diaphus suborbitalis (Myctophidae, Pisces) in the Equatorial Indian Ocean. *Oceanology of the Academy of Sciences of the USSR* **25**:523–529.
- Grasshoff, K., K. Kremling, and M. Ehrhardt, 1999. Methods of seawater analysis. WILEY-VCH, Weinheim.
- Van der Gucht, K., K. Cottenie, K. Muylaert, N. Vloemans, S. Cousin, S. Declerck, E. Jeppesen, J.-M. Conde-Porcuna, K. Schwenk, G. Zwart, H. Degans, W. Vyverman, and L. De Meester, 2007. The power of species sorting: local factors drive bacterial community composition over a wide range of spatial scales. *Proceedings of the National Academy of Sciences of the United States of America* **104**:20404–20409.
- Hagström, Ä., J. Pinhassi, and U. L. Zweifel, 2000. Biogeographical diversity among marine bacterioplankton. *Aquatic Microbial Ecology* **21**:231–244.

Bibliography

- Hannides, C., M. R. Landry, and C. R. Benitez-Nelson, 2009. Export stoichiometry and migrant-mediated flux of phosphorus in the North Pacific Subtropical Gyre. *Deep Sea Research Part II: Topical Studies in Oceanography* **56**:73–88.
- Hansell, D. A., 2013. Recalcitrant dissolved organic carbon fractions. *Annual Review of Marine Science* **5**:421–445.
- Hansell, D. A. and C. A. Carlson, 2012. Net removal of major marine dissolved organic carbon fractions in the subsurface ocean. *Global Biogeochemical Cycles* **26**:1–9.
- Hanson, C. A., J. A. Fuhrman, M. C. Horner-Devine, and J. B. H. Martiny, 2012. Beyond biogeographic patterns: processes shaping the microbial landscape. *Nature reviews. Microbiology* **10**:497–506.
- Hatosy, S. M., J. B. H. Martiny, R. Sachdeva, J. Steele, J. A. Fuhrman, and A. C. Martiny, 2013a. Beta diversity of marine bacteria depends on temporal scale. *Ecology* **94**:1898–1904.
- , 2013b. Beta diversity of marine bacteria depends on temporal scale. *Ecology* **94**:1898–1904.
- Haury, L., C. Fey, G. Gal, A. Hobday, and A. Genin, 1995. Copepod carcasses in the ocean. I. Over seamounts. *Marine Ecology Progress Series* **123**:57–63.
- Hawkes, J. A., D. P. Connelly, M. Gledhill, and E. P. Achterberg, 2013. The stabilisation and transportation of dissolved iron from high temperature hydrothermal vent systems. *Earth and Planetary Science Letters* **375**:280–290.
- Hense, I., U. V. Bathmann, and R. Timmermann, 2000. Plankton dynamics in frontal systems of the Southern Ocean. *Journal of Marine Systems* **27**:235–252.
- Henson, S. A., R. Sanders, and E. Madsen, 2012. Global patterns in efficiency of particulate organic carbon export and transfer to the deep ocean. *Global Biogeochemical Cycles* **26**:n/a–n/a.
- Herrera, S., H. Watanabe, and T. M. Shank, 2015. Evolutionary and biogeographical patterns of barnacles from deep-sea hydrothermal vents. *Molecular ecology* **24**:673–689.
- Herring, P., 2002. *The Biology of the Deep Ocean*. Oxford University Press.
- Hogg, N. G., 1973. On the stratified Taylor column. *Journal of Fluid Mechanics* **58**:517–537.
- Holden, J. F., M. Summit, and J. A. Baross, 1998. Thermophilic and hyperthermophilic microorganisms in 3–30° C hydrothermal fluids following a deep-sea volcanic eruption. *FEMS Microbiology Ecology* **25**:33–41.
- Holland, K. N. and R. D. Grubbs, 2007. *Seamounts: Ecology, Fisheries and Conservation*. Fish Visitors to Seamounts: Tunas and Bill Fish at Seamounts. Blackwell Publishing Ltd, Oxford, UK.

- Honda, T., Y. Liang, D. Arai, Y. Ito, T. Yoshino, and T. Tanaka, 2014. Draft Genome Sequence of Marine Cyanobacterium *Synechococcus* sp. Strain NKBG042902, Which Harbors a Homogeneous Plasmid Available for Metabolic Engineering. *Genome announcements* **2**:e00704–14.
- Huber, J. A., D. A. Butterfield, and J. A. Baross, 2002. Temporal Changes in Archaeal Diversity and Chemistry in a Mid-Ocean Ridge Subseafloor Habitat. *Applied and Environmental Microbiology* **68**:1585–1594.
- , 2003. Bacterial diversity in a subseafloor habitat following a deep-sea volcanic eruption. *FEMS Microbiology Ecology* **43**:393–409.
- Huber, J. A., H. V. Cantin, and S. M. Huse, 2010a. Isolated communities of Epsilonproteobacteria in hydrothermal vent fluids of the Mariana Arc seamounts. *FEMS Microbiology Ecology* **73**:538–549.
- Huber, J. A., H. V. Cantin, S. M. Huse, D. B. M. Welch, M. L. Sogin, and D. A. Butterfield, 2010b. Isolated communities of Epsilonproteobacteria in hydrothermal vent fluids of the Mariana Arc seamounts. *FEMS Microbiology Ecology* **73**:538–549.
- Huber, J. A., D. B. Mark Welch, H. G. Morrison, S. M. Huse, P. R. Neal, D. A. Butterfield, and M. L. Sogin, 2007. Microbial population structures in the deep marine biosphere. *Science* **318**:97–100.
- Hügler, M. and S. M. Sievert, 2011. Beyond the Calvin cycle: autotrophic carbon fixation in the ocean. *Marine Science* **3**:261–289.
- Hundahl, H. and J. Holck, 1980. The optical instruments developed at the Institute of Physical Oceanography University of Copenhagen. Tech. rep., University of Copenhagen.
- Huppert, H. E. and K. Bryan, 1976. Topographically generated eddies. *Deep Sea Research and Oceanographic: Abstracts* **23**:655–679.
- Huse, S. M., L. Dethlefsen, J. A. Huber, D. Mark Welch, D. M. Welch, D. A. Relman, and M. L. Sogin, 2008. Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing. *PLoS Genetics* **4**:e1000255.
- Isaacs, J. D. and R. A. Schwartzlose, 1965. Migrant sound scatterers: interaction with the sea floor. *Science* **150**:1810–1813.
- Ivanova, E. P., S. Flavier, and R. Christen, 2004. Phylogenetic relationships among marine *Alteromonas*-like proteobacteria: emended description of the family Alteromonadaceae and proposal of Pseudoalteromonadaceae fam. nov., Colwelliaceae fam. nov., Shewanellaceae fam. nov., Moritellaceae fam. nov., Ferrimonadaceae fam. nov., Idiomarinaceae fam. nov. and Psychromonadaceae fam. nov. *International Journal of Systematic and Evolutionary Microbiology* **54**:1773–1788.

Bibliography

- James, R. H., D. R. H. Green, M. J. Stock, B. J. Alker, N. R. Banerjee, C. Cole, C. R. German, V. A. I. Huvenne, A. M. Powell, and D. P. Connelly, 2014. Composition of hydrothermal fluids and mineralogy of associated chimney material on the East Scotia Ridge back-arc spreading centre. *Geochimica et Cosmochimica Acta* **139**:47–71.
- Jannasch, H. W., 1983. *Microbial Processes at Deep Sea Hydrothermal Vents*. Springer US, Boston, MA.
- Jannasch, H. W. and C. O. Wirsen, 1979. Chemosynthetic Primary Production at East Pacific Sea Floor Spreading Centers. *Bioscience* **29**:592–598.
- Jiao, N., F. Azam, M. W. Group, and S. Wg134, 2010. Microbial Carbon Pump —A New Mechanism for Long-Term Carbon Storage in the Global Ocean (Invited). *AGU Fall Meeting Abstracts* **1**:0396.
- Jobbágy, E. G. and R. B. Jackson, 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological applications* **10**:423–436.
- Kallmeyer, J., R. Pockalny, R. R. Adhikari, D. C. Smith, and S. D'Hondt, 2012. Global distribution of microbial abundance and biomass in subseafloor sediment. *Proceedings of the National Academy of Sciences of the United States of America* **109**:16213–16216.
- Kana, T. M. and P. M. Glibert, 1987. Effect of irradiances up to 2000 $\mu\text{E m}^{-2} \text{ s}^{-1}$ on marine *Synechococcus* WH7803—II. Photosynthetic responses and mechanisms. *Deep Sea Research Part I: Oceanographic Research Papers* **34**:497–516.
- Karageorgis, A. P. and C. L. Anagnostou, 2001. Particulate matter spatial–temporal distribution and associated surface sediment properties: Thermaikos Gulf and Sporades Basin, NW Aegean Sea. *Continental Shelf Research* **21**:2141–2153.
- Karl, D. M., G. T. Taylor, J. A. Novitsky, H. W. Jannasch, C. O. Wirsen, N. R. Pace, D. J. Lane, G. J. Olsen, and S. J. Giovannoni, 1988. A microbiological study of Guaymas Basin high temperature hydrothermal vents. *Deep Sea Research Part I. Oceanographic Research Papers* **35**:777–791.
- Kaschner, K. and T. J. Pitcher, 2007. Air-breathing visitors to seamounts: Marine mammals. *In* *Seamounts: Ecology, Fisheries and Conservation*. Blackwell Publishing.
- Kato, S., Y. Takano, T. Kakegawa, H. Oba, K. Inoue, C. Kobayashi, M. Utsumi, K. Marumo, K. Kobayashi, Y. Ito, J.-i. Ishibashi, and A. Yamagishi, 2010. Biogeography and biodiversity in sulfide structures of active and inactive vents at deep-sea hydrothermal fields of the Southern Mariana Trough. *Applied and Environmental Microbiology* **76**:2968–2979.
- Kaye, J. Z., J. B. Sylvan, K. J. Edwards, and J. A. Baross, 2011. *Halomonas* and *Marinobacter* ecotypes from hydrothermal vent, subseafloor and deep-sea environments. *FEMS Microbiology Ecology* **75**:123–133.
- Kelley, D., 2012. *Oce: Analysis of Oceanographic Data*. R Package Version 0.8-9.

- Kinsey, J. C. and C. R. German, 2013. Sustained volcanically-hosted venting at ultraslow ridges: Piccard Hydrothermal Field, Mid-Cayman Rise. *Earth and Planetary Science Letters* **380**:162–168.
- Kirchman, D. L., 2008. *Microbial Ecology of the Oceans*. John Wiley & Sons, Inc., Hoboken, NJ, USA.
- Kirchman, D. L., H. Elifantz, and A. I. Dittel, 2007. Standing stocks and activity of Archaea and Bacteria in the western Arctic Ocean. *Limnology and Oceanography* **52**:495–507.
- Kirchman, D. L., V. Hill, M. T. Cottrell, and R. Gradinger, 2009. Standing stocks, production, and respiration of phytoplankton and heterotrophic bacteria in the western Arctic Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography* **56**:1237–1248.
- Kiriakoulakis, K., J. C. Vilas, and S. J. Blackbird, 2009. Seamounts and organic matter—Is there an effect? The case of Sedlo and Seine seamounts, Part 2. Composition of suspended particulate organic matter. *Deep Sea Research Part II: Topical Studies in Oceanography* **56**.
- Knap, A. H., A. Michaels, A. R. Close, and H. Ducklow, 1996. Protocols for the joint global ocean flux study (JGOFS) core measurements. Tech. rep., JGOFS.
- Koch, B. P. and T. Dittmar, 2006. From mass to structure: an aromaticity index for high-resolution mass data of natural organic matter. *Rapid Communications in Mass Spectrometry* **20**:926–932.
- Koch, B. P., M. Witt, R. Engbrodt, T. Dittmar, and G. Kattner, 2005. Molecular formulae of marine and terrigenous dissolved organic matter detected by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Geochimica et Cosmochimica Acta* **69**:3299–3308.
- Kunin, V., A. Engelbrektson, H. Ochman, and P. Hugenholtz, 2010. Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates. *Environmental Microbiology* **12**:118–123.
- Ladau, J., T. J. Sharpton, M. M. Finucane, G. Jospin, S. W. Kembel, J. O'Dwyer, A. F. Koeppel, J. L. Green, and K. S. Pollard, 2013. Global marine bacterial diversity peaks at high latitudes in winter. *The ISME Journal* **7**:1669–1677.
- Lang, S. Q., D. A. Butterfield, M. D. Lilley, H. Paul Johnson, and J. I. Hedges, 2006. Dissolved organic carbon in ridge-axis and ridge-flank hydrothermal systems. *Geochimica et Cosmochimica Acta* **70**:3830–3842.
- Lang, S. Q., D. A. Butterfield, M. Schulte, D. S. Kelley, and M. D. Lilley, 2010. Elevated concentrations of formate, acetate and dissolved organic carbon found at the Lost City hydrothermal field. *Geochimica et Cosmochimica Acta* **74**:941–952.
- Laptikovsky, V., P. Boersch-Supan, K. Kemp, T. Letessier, and A. D. Rogers, In press. Cephalopods of the Southwest Indian Ocean Ridge: a hotspot of extreme biological diversity and absence of endemism. *Deep Sea Research Part II: Topical Studies in Oceanography* .

Bibliography

- Larter, R. D., L. E. Vanneste, P. Morris, and D. K. Smythe, 2003. Structure and tectonic evolution of the South Sandwich arc. *Geological Society, London, Special Publications* **219**:255–284.
- Lavik, G., T. Stührmann, V. Brüchert, A. Van der Plas, V. Mohrholz, P. Lam, M. Mußmann, B. M. Fuchs, R. Amann, U. Lass, et al., 2009. Detoxification of sulphidic African shelf waters by blooming chemolithotrophs. *Nature* **457**:581–584.
- Lefort, T. and J. M. Gasol, 2013. Global-scale distributions of marine surface bacterioplankton groups along gradients of salinity, temperature, and chlorophyll: a meta-analysis of fluorescence in situ hybridization studies. *Aquatic Microbial Ecology* **70**:111–130.
- Letessier, T. B., S. De Grave, P. H. Boersch-Supan, K. M. Kemp, A. S. Brierley, and A. D. Rogers, In press. Seamount influence on mid-water shrimp (decapoda) and gnathophausiids (lophogastridea) of the south-west indian ridge. *Deep Sea Research Part II: Topical Studies in Oceanography*.
- Li, W. K. W., P. M. Dickie, B. D. Irwin, and A. M. Wood, 1992. Biomass of bacteria, cyanobacteria, prochlorophytes and photosynthetic eukaryotes in the Sargasso Sea. *Deep Sea Research Part I. Oceanographic Research Papers* **39**:501–519.
- Ligi, M., E. Bonatti, G. Bortoluzzi, G. Carrara, and P. Fabretti, 1999. Bouvet triple junction in the south atlantic: Geology and evolution. *Journal of Geophysical Research* **104**:26,365–29,386.
- Lima-Mendez, G., K. Faust, N. Henry, J. Decelle, S. Colin, F. Carcillo, S. Chaffron, J. C. Ignacio-Espinosa, S. Roux, F. Vincent, L. Bittner, Y. Darzi, J. Wang, S. Audic, L. Berline, G. Bontempi, A. M. Cabello, L. Coppola, F. M. Cornejo-Castillo, F. d'Ovidio, L. De Meester, I. Ferrera, M.-J. Garet-Delmas, L. Guidi, E. Lara, S. Pesant, M. Royo-Lluch, G. Salazar, P. Sánchez, M. Sebastian, C. Souffreau, C. Dimier, M. Picheral, S. Searson, S. Kandels-Lewis, Tara Oceans Coordinators, G. Gorsky, F. Not, H. Ogata, S. Speich, L. Stemmann, J. Weissenbach, P. Wincker, S. G. Acinas, S. Sunagawa, P. Bork, M. B. Sullivan, E. Karsenti, C. Bowler, C. de Vargas, and J. Raes, 2015. Ocean plankton. Determinants of community structure in the global plankton interactome. *Science* **348**:1262073–1262073.
- Lin, L., J. He, Y. Zhao, F. Zhang, and M. Cai, 2012. Flow cytometry investigation of picoplankton across latitudes and along the circum Antarctic Ocean. *Princeton University Press* **31**:134–142.
- Litvinov, F., 2008. Fish Visitors to Seamounts: Aggregations of Large Pelagic Sharks Above Seamounts. Blackwell Publishing Ltd, Oxford, UK.
- Livermore, R., 2003. Back-arc spreading and mantle flow in the East Scotia Sea. *Geological Society, London, Special Publications* **219**:315–331.
- Livermore, R., A. Cunningham, L. Vanneste, and R. Larter, 1997. Subduction influence on magma supply at the East Scotia Ridge. *Earth and Planetary Science Letters* **150**:261–275.

- Longhurst, A., 1995. Seasonal cycles of pelagic production and consumption. *Progress in Oceanography* **36**:77–167.
- Longhurst, A., S. Sathyendranath, T. Platt, and C. Caverhill, 1995. An estimate of global primary production in the ocean from satellite radiometer data. *Journal of Plankton Research* **17**:1245–1271.
- Longnecker, K. and A. L. Reysenbach, 2001. Expansion of the geographic distribution of a novel lineage of ϵ -Proteobacteria to a hydrothermal vent site on the Southern East Pacific Rise. *FEMS Microbiology Ecology* **35**:287–293.
- Lopukhin, A. S., 1986. Distribution of ATP concentration above seamounts in the Atlantic Ocean. *Oceanology of the Academy of Sciences of the USSR* **26**.
- Louzao, M., J. Navarro, M. G. Forero, and J. M. Igual, 2011. Exploiting the closest productive area: geographical segregation of foraging grounds in a critically endangered seabird. *Marine Ecology Progress Series* **429**:291–301.
- Lutjeharms, J., 1985. Location of frontal systems between Africa and Antarctica: some preliminary results. *Deep Sea Research Part I: Oceanographic Research Papers* **32**:1499–1509.
- Maechler, M., A. Struyf, and M. Maechler, 2007. The cluster package.
- Malmstrom, R. R., A. Coe, G. C. Kettler, A. C. Martiny, J. Frias-Lopez, E. R. Zinser, and S. W. Chisholm, 2010. Temporal dynamics of Prochlorococcus ecotypes in the Atlantic and Pacific oceans. *The ISME Journal* **4**:1252–1264.
- Manini, E., G. M. Luna, C. Corinaldesi, D. Zeppilli, G. Bortoluzzi, G. Caramanna, F. Raffa, and R. Danovaro, 2008. Prokaryote Diversity and Virus Abundance in Shallow Hydrothermal Vents of the Mediterranean Sea (Panarea Island) and the Pacific Ocean (North Sulawesi-Indonesia). *Microbial Ecology* **55**:626–639.
- Marie, D., F. Partensky, S. Jacquet, and D. Vaulot, 1997. Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. *Applied Environmental Microbiol* **63**:186–193.
- Marsh, L., J. T. Copley, V. A. I. Huvenne, K. Linse, W. D. K. Reid, D. Amon, C. J. Sweeting, and P. A. Tyler, 2012. Microdistribution of Faunal Assemblages at Deep-Sea Hydrothermal Vents in the Southern Ocean. *PLoS ONE* **7**:e48348–19.
- Marshall, K. T. and R. M. Morris, 2013. Isolation of an aerobic sulfur oxidizer from the sup05/arctic96bd-19 clade. *The ISME journal* **7**:452–455.
- Martin, B. and B. Christiansen, 2009. Distribution of zooplankton biomass at three seamounts in the NE Atlantic. *Deep Sea Research Part II: Topical Studies in Oceanography* **56**:2671–2682.

Bibliography

- Martin, W. and M. J. Russell, 2007. On the origin of biochemistry at an alkaline hydrothermal vent. *Philosophical Transactions of the Royal Society B: Biological Sciences* **362**:1887–1925.
- Martiny, J. B. H., J. A. Eisen, K. Penn, S. D. Allison, and M. C. Horner-Devine, 2011. Drivers of bacterial beta-diversity depend on spatial scale. *Proceedings of the National Academy of Sciences of the United States of America* **108**:7850–7854.
- McCarthy, M. D., S. R. Beaupré, B. D. Walker, I. Voparil, T. P. Guilderson, and E. R. M. Druffel, 2011. Chemosynthetic origin of ¹⁴C-depleted dissolved organic matter in a ridge-flank hydrothermal system. *Nature Geoscience* **4**:32–36.
- McClain, C. R. and S. M. Hardy, 2010. The dynamics of biogeographic ranges in the deep sea. *Proceedings of the Royal Society of London Series B* **277**:3533–3546.
- McClain, C. R., L. Lundsten, M. Ream, J. Barry, and A. DeVogelaere, 2009. Endemicity, Biogeography, Composition, and Community Structure On a Northeast Pacific Seamount. *PLoS ONE* **4**:e4141.
- McCollom, T. M., 2000. Geochemical constraints on primary productivity in submarine hydrothermal vent plumes. *Deep Sea Research Part I: Oceanographic Research Papers* **47**:85–101.
- McCollom, T. M., B. S. Lollar, G. Lacrampe-Couloume, and J. S. Seewald, 2010. The influence of carbon source on abiotic organic synthesis and carbon isotope fractionation under hydrothermal conditions. *Geochimica et Cosmochimica Acta* **74**:2717–2740.
- McDonagh, E. L., H. L. Bryden, B. A. King, and R. J. Sanders, 2008. The circulation of the Indian Ocean at 32°S. *Progress in Oceanography* **79**:20–36.
- McIntyre, T., I. J. Ansorge, H. Bornemann, and J. Plötz, 2012. Elephant seal foraging dives do indeed track prey distribution, but temperature influences the distribution of prey: Reply to Boersch-Supan et al.(2012). *Marine Ecology Progress Series* **461**:299–303.
- McMurdie, P. J. and S. Holmes, 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* **8**:e61217.
- Meador, T. B., A. Gogou, G. Spyres, G. J. Herndl, E. Krasakopoulou, S. Psarra, T. Yokokawa, D. De Corte, V. Zervakis, and D. J. Repeta, 2010. Biogeochemical relationships between ultrafiltered dissolved organic matter and picoplankton activity in the Eastern Mediterranean Sea. *Deep Sea Research Part II: Topical Studies in Oceanography* **57**:1460–1477.
- Mendonça, A., J. M. Gasol, C. M. Duarte, and G. J. Herndl, 2009. Microbial oceanography of the dark ocean's pelagic realm. *Limnology and Oceanography* **54**:1501–1529.
- Mendonça, A., A. Mendonça, J. C. Vilas, M. F. Montero, A. Ojeda, M. Espino, and A. Martins, 2012. Is there a seamount effect on microbial community structure and biomass? The case study of Seine and Sedlo seamounts (northeast Atlantic). *PLoS ONE* **7**:e29526.

- Meredith, M. P., J. L. Watkins, and E. J. Murphy, 2003. An anticyclonic circulation above the northwest Georgia rise, Southern Ocean. *Geophysical research letters* **30**.
- Millard, A. D., I. Hands-Portman, and K. Zwirgmaier, 2014. Morphotypes of virus-like particles in two hydrothermal vent fields on the East Scotia Ridge, Antarctica. *Bacteriophage* **4**:e28732–7.
- Milne, S. B., K. A. Tallman, R. Serwa, C. A. Rouzer, M. D. Armstrong, L. J. Marnett, C. M. Lukehart, N. A. Porter, and H. A. Brown, 2010. Capture and release of alkyne-derivatized glycerophospholipids using cobalt chemistry. *Nature Chemical Biology* **6**:205–207.
- Mino, S., H. Makita, T. Toki, J. Miyazaki, S. Kato, H. Watanabe, H. Imachi, T.-o. Watsuji, T. Nunoura, S. Kojima, T. Sawabe, K. Takai, and S. Nakagawa, 2013. Biogeography of *Persephonella* in deep-sea hydrothermal vents of the Western Pacific. *Frontiers in Microbiology* **4**:107.
- Moiseyev, Y. V., 1986. Moiseyev: Distribution of protozoans near seamounts in the western Indian Ocean. *Oceanology of the Academy of Sciences of the USSR* **26**:86–90.
- Moran, S. B., M. A. Charette, S. M. Pike, and C. A. Wicklund, 1999. Differences in seawater particulate organic carbon concentration in samples collected using small-and large-volume methods: the importance of DOC adsorption to the filter blank. *Marine Chemistry* **67**:33–42.
- Morato, T., S. D. Hoyle, and V. Allain, 2010. Seamounts are hotspots of pelagic biodiversity in the open ocean. *Proceedings of the Royal Society of London Series B* **107**:9707–9711.
- Morlon, H., G. Chuyong, R. Condit, S. Hubbell, D. Kenfack, D. Thomas, R. Valencia, and J. L. Green, 2008. A general framework for the distance–decay of similarity in ecological communities. *Ecology Letters* **11**:904–917.
- Morris, R. M., K. L. Vergin, J. C. Cho, and M. S. Rappé, 2005. Temporal and spatial response of bacterioplankton lineages to annual convective overturn at the Bermuda Atlantic Time-series Study site. *Limnology Oceanography* **50**:1687–1695.
- Mullineau, L. S. and S. W. Mills, 1997. A test of the larval retention hypothesis in seamount-generated flows. *Deep Sea Research Part I: Oceanographic Research Papers* **44**:745–770.
- Nakagawa, S., K. Takai, F. Inagaki, H. Hirayama, T. Nunoura, K. Horikoshi, and Y. Sako, 2005. Distribution, phylogenetic diversity and physiological characteristics of epsilon-Proteobacteria in a deep-sea hydrothermal field. *Environmental Microbiology* **7**:1619–1632.
- Nekola, J. C. and P. S. White, 1999. The distance decay of similarity in biogeography and ecology. *Journal of Biogeography* **26**:867–878.
- Nellen, W., 1973. Kinds and abundance of fish larvae in the Arabian Sea and the Persian Gulf. *In The Biology of the Indian Ocean*, vol. 3, pp. 415–430. Springer-Verlag.

Bibliography

- Nelson, C. E., C. A. Carlson, C. S. Ewart, and E. R. Halewood, 2014. Community differentiation and population enrichment of Sargasso Sea bacterioplankton in the euphotic zone of a mesoscale mode-water eddy. *Environmental Microbiology* **16**:871–887.
- Nemergut, D. R., E. K. Costello, M. Hamady, C. Lozupone, L. Jiang, S. K. Schmidt, N. Fierer, A. R. Townsend, C. C. Cleveland, L. Stanish, and R. Knight, 2011. Global patterns in the biogeography of bacterial taxa. *Environmental Microbiology* **13**:135–144.
- Nesbø, C. L., M. Dlutek, and W. F. Doolittle, 2006. Recombination in *Thermotoga*: implications for species concepts and biogeography. *Genetics* **172**:759–769.
- Nielsen, S. G., M. Rehkämper, D. A. Teagle, D. A. Butterfield, J. C. Alt, and A. N. Halliday, 2006. Hydrothermal fluid fluxes calculated from the isotopic mass balance of thallium in the ocean crust. *Earth and Planetary Science Letters* **251**:120–133.
- Nycander, J. and J. H. Lacasce, 2004. Stable and unstable vortices attached to seamounts. *Journal of Fluid Mechanics* **507**:71–94.
- Oksanen, J., F. G. Blanchet, R. Kindt, and P. Legendre, 2013. Vegan: community ecology. R package version 1.18-28/r1569.
- Ouellette, M. H., P. Legendre, and D. Borcard, 2012. Cascade multivariate regression tree: a novel approach for modelling nested explanatory sets. *Methods in Ecology and Evolution* **3**:234–244.
- Pak, H., D. A. Kiefer, and J. C. Kitchen, 1988. Meridional variations in the concentration of chlorophyll and microparticles in the North Pacific Ocean. *Deep Sea Research Part I: Oceanographic Research Papers* **35**:1151–1171.
- Pakhomov, E. A., R. Perissinotto, and C. D. McQuaid, 1994. Comparative structure of the macrozooplankton/micronekton communities of the Subtropical and Antarctic Polar Fronts. *Marine Ecology Progress Series* **111**:155–169.
- Papke, R. T., N. B. Ramsing, M. M. Bateson, and D. M. Ward, 2003. Geographical isolation in hot spring cyanobacteria. *Environmental Microbiology* **5**:650–659.
- Paradis, E., J. Claude, and K. Strimmer, 2004. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* **20**:289–290.
- Parin, N. V. and V. G. Prut'ko, 1985. The thalassial mesobenthopelagic ichthyocoene above the Equator Seamount in the western tropical Indian Ocean. *Oceanology* **25**:781–783.
- Partensky, F., J. Blanchot, and D. Vaultot, 1999. Differential distribution and ecology of prochlorococcus and synechococcus in oceanic waters: a review. *Bulletin-institut oceanographique monaco-numero special* pp. 457–476.
- Partensky, F. and L. Garczarek, 2010. Prochlorococcus: advantages and limits of minimalism. *Annual Review of Marine Science* **2**:305–331.

- Pedersen, R. B., H. T. Rapp, I. H. Thorseth, M. D. Lilley, F. J. A. S. Barriga, T. Baumberger, K. Flesland, R. Fonseca, G. L. Früh-Green, and S. L. Jorgensen, 2010. Discovery of a black smoker vent field and vent fauna at the Arctic Mid-Ocean Ridge. *Nature Communications* **1**:126.
- Peng, X., S. Chen, H. Zhou, L. Zhang, and Z. Wu, 2011. Diversity of biogenic minerals in low-temperature Si-rich deposits from a newly discovered hydrothermal field on the ultraslow spreading Southwest Indian Ridge. *Journal of Geophysical Research* .
- Pinhassi, J. and T. Berman, 2003. Differential growth response of colony-forming α - and γ -proteobacteria in dilution culture and nutrient addition experiments from lake kinneret (israel), the eastern mediterranean sea, and the gulf of eilat. *Applied and Environmental Microbiology* **69**:199–211.
- Pinhassi, J., M. M. Sala, H. Havskum, F. Peters, O. Guadayol, A. Malits, and C. Marrasé, 2004. Changes in bacterioplankton composition under different phytoplankton regimens. *Applied and Environmental Microbiology* **70**:6753–6766.
- Pollard, R. T. and J. F. Read, 2001. Circulation pathways and transports of the Southern Ocean in the vicinity of the Southwest Indian Ridge. *Journal of Geophysical Research: Oceans (1978–2012)* **106**:2881–2898.
- Polz, M. F. and C. M. Cavanaugh, 1995. Dominance of one bacterial phylotype at a Mid-Atlantic Ridge hydrothermal vent site. *Proceedings of the National Academy of Sciences* **92**:7232–7236.
- Pomeroy, L. and C. Darwin, 2007. The microbial loop. *Oceanography* .
- Pommier, T., B. Canbäck, L. Riemann, K. H. Boström, K. Simu, P. Lundberg, A. Tunlid, and Å. Hagström, 2007. Global patterns of diversity and community structure in marine bacterioplankton. *Molecular Ecology* **16**:867–880.
- Priede, I. G. and R. Froese, 2013. Colonization of the deep sea by fishes. *Journal of fish biology* **83**:1528–1550.
- Quince, C., A. Lanzen, R. J. Davenport, and P. J. Turnbaugh, 2011. Removing noise from pyrosequenced amplicons. *BMC bioinformatics* **12**:38.
- Ramette, A., 2007. Multivariate analyses in microbial ecology. *FEMS Microbiology Ecology* **62**:142–160.
- Raven, J. A. and M. Giordano, 2009. Biomineralization by photosynthetic organisms: Evidence of coevolution of the organisms and their environment? *Geobiology* **7**:140–154.
- Read, D. S., H. S. Gweon, M. J. Bowes, L. K. Newbold, D. Field, M. J. Bailey, and R. I. Griffiths, 2015. Catchment-scale biogeography of riverine bacterioplankton. *The ISME Journal* **9**:516–526.

Bibliography

- Read, J. and R. Pollard, in press. Circulation, stratification and seamounts in the South West Indian Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography* pp. 1–16.
- , In press. An introduction to the physical oceanography of six seamounts in the south-west indian ocean. *Deep Sea Research Part II: Topical Studies in Oceanography* .
- Read, J. F., M. I. Lucas, S. E. Holley, and R. T. Pollard, 2000. Phytoplankton, nutrients and hydrography in the frontal zone between the Southwest Indian Subtropical gyre and the Southern Ocean. *Deep Sea Research Part I: Oceanographic Research Papers* **47**:2341–2367.
- Read, J. F., R. T. Pollard, and U. Bathmann, 2002. Physical and biological patchiness of an upper ocean transect from South Africa to the ice edge near the Greenwich Meridian. *Deep Sea Research Part II: Topical Studies in Oceanography* **49**:3713–3733.
- Reeves, E. P., J. M. McDermott, and J. S. Seewald, 2014a. The origin of methanethiol in midocean ridge hydrothermal fluids. *Proceedings of the National Academy of Sciences of the United States of America* **111**:5474–5479.
- , 2014b. The origin of methanethiol in midocean ridge hydrothermal fluids. *Proceedings of the National Academy of Sciences of the United States of America* **111**:5474–5479.
- Reid, W. D. K., C. J. Sweeting, B. D. Wigham, K. Zwirgmaier, J. A. Hawkes, R. A. R. McGill, K. Linse, and N. V. C. Polunin, 2013. Spatial differences in east scotia ridge hydrothermal vent food webs: Influences of chemistry, microbiology and predation on trophodynamics. *PLoS ONE* **8**:e65553–11.
- Rex, M. A., R. J. Etter, J. S. Morris, and J. Crouse, 2006. Global bathymetric patterns of standing stock and body size in the deep-sea benthos. *Marine Ecology Progress Series* **317**:1–8.
- Reysenbach, A.-L. and E. Shock, 2002. Merging genomes with geochemistry in hydrothermal ecosystems. *Science* **296**:1077–1082.
- Riedel, T., H. Biester, and T. Dittmar, 2012. Molecular fractionation of dissolved organic matter with metal salts. *Environmental science & technology* **46**:4419–4426.
- Riedel, T., D. Zak, H. Biester, and T. Dittmar, 2013. Iron traps terrestrially derived dissolved organic matter at redox interfaces. *Proceedings of the National Academy of Sciences of the United States of America* **110**:10101–10105.
- Rinta-Kanto, J. M., S. Sun, S. Sharma, R. P. Kiene, and M. A. Moran, 2011. Bacterial community transcription patterns during a marine phytoplankton bloom. *Environmental Microbiology* **14**:228–239.
- Robinson, C., D. K. Steinberg, and T. R. Anderson, 2010. Mesopelagic zone ecology and biogeochemistry—a synthesis. *Deep Sea Research Part II: Topical Studies in Oceanography* **57**:1504–1518.

- Rocap, G., F. W. Larimer, J. Lamerdin, S. Malfatti, P. Chain, N. A. Ahlgren, A. Arellano, M. Coleman, L. Hauser, W. R. Hess, Z. I. Johnson, M. Land, D. Lindell, A. F. Post, W. Regala, M. Shah, S. L. Shaw, C. Steglich, M. B. Sullivan, C. S. Ting, A. Tolonen, E. A. Webb, E. R. Zinser, and S. W. Chisholm, 2003. Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. *Nature* **424**:1042–1047.
- Rogers, A. D., 1994. The Biology of Seamounts. *Advances in Marine Biology* **30**:305–350.
- Rogers, A. D., P. A. Tyler, D. P. Connelly, and J. T. Copley, 2012. The Discovery of New Deep-Sea Hydrothermal Vent Communities in the Southern. *PLoS Biology* **10**:e1001234.
- Roth, V.-N., T. Dittmar, R. Gaupp, and G. Gleixner, 2013. Latitude and pH driven trends in the molecular composition of DOM across a north south transect along the Yenisei River. *Geochimica et Cosmochimica Acta* **123**:93–105.
- Rowden, A. A., M. R. Clark, and I. C. Wright, 2005. Physical characterisation and a biologically focused classification of “seamounts” in the New Zealand region. *New Zealand Journal of Marine and Freshwater Research* **39**:1039–1059.
- Rowden, A. A., T. A. Schlacher, and A. Williams, 2010. A test of the seamount oasis hypothesis: seamounts support higher epibenthic megafaunal biomass than adjacent slopes. *Marine Chemistry* **31**:95–106.
- Rusch, D. B., A. L. Halpern, G. Sutton, K. B. Heidelberg, S. Williamson, S. Yooseph, D. Wu, J. A. Eisen, J. M. Hoffman, K. Remington, K. Beeson, B. Tran, H. Smith, H. Baden-Tillson, C. Stewart, J. Thorpe, J. Freeman, C. Andrews-Pfannkoch, J. E. Venter, K. Li, S. Kravitz, J. F. Heidelberg, T. Utterback, Y.-H. Rogers, L. I. Falcón, V. Souza, G. Bonilla-Rosso, L. E. Eguarte, D. M. Karl, S. Sathyendranath, T. Platt, E. Bermingham, V. Gallardo, G. Tamayo-Castillo, M. R. Ferrari, R. L. Strausberg, K. Nealson, R. Friedman, M. Frazier, and J. C. Venter, 2007. The Sorcerer II Global Ocean Sampling expedition: northwest Atlantic through eastern tropical Pacific. *PLoS biology* **5**:e77.
- Sander, S. G. and A. Koschinsky, 2011. Metal flux from hydrothermal vents increased by organic complexation. *Nature Geoscience* **4**:145–150.
- Sanders, J. G., R. A. Beinart, F. J. Stewart, E. F. DeLong, and P. R. Girguis, 2013. Metatranscriptomics reveal differences in in situ energy and nitrogen metabolism among hydrothermal vent snail symbionts. *The ISME Journal* **7**:1556–1567.
- Santoferrara, L. F., J.-D. Grattepanche, L. A. Katz, and G. B. McManus, 2014. Pyrosequencing for assessing diversity of eukaryotic microbes: analysis of data on marine planktonic ciliates and comparison with traditional methods. *Environmental Microbiology* **16**:2752–2763.
- Sarmiento, J. and N. Gruber, 2006. Ocean Biogeochemical Dynamics. Princeton University Press.

Bibliography

- Scanlan, D. J., M. Ostrowski, S. Mazard, A. Dufresne, L. Garczarek, W. R. Hess, A. F. Post, M. Hagemann, I. Paulsen, and F. Partensky, 2009. Ecological Genomics of Marine Pico-cyanobacteria. *Microbiology and Molecular Biology Reviews* **73**:249–299.
- Scanlan, D. J. and N. J. West, 2002. Molecular ecology of the marine cyanobacterial genera *Prochlorococcus* and *Synechococcus*. *FEMS Microbiology Ecology* **40**:1–12.
- Schattenhofer, M., 2009. Distribution of Major Bacterioplankton Groups in the Atlantic Ocean. Tech. rep.
- Schloss, P. D., D. Gevers, and S. L. Westcott, 2011. Reducing the Effects of PCR Amplification and Sequencing Artifacts on 16S rRNA-Based Studies. *PLoS ONE* **6**:e27310.
- Schnetger, B. and C. Lehnert, 2014. Determination of nitrate plus nitrite in small volume marine water samples using vanadium(III)chloride as a reduction agent. *Marine Chemistry* **160**:91–98.
- Sexton, P. F., R. D. Norris, P. A. Wilson, H. Pälike, T. Westerhold, U. Röhl, C. T. Bolton, and S. Gibbs, 2011. Eocene global warming events driven by ventilation of oceanic dissolved organic carbon. *Nature* **471**:349–352.
- Sheik, C. S., S. Jain, and G. J. Dick, 2014. Metabolic flexibility of enigmatic SAR324 revealed through metagenomics and metatranscriptomics. *Environmental Microbiology* **16**:304–317.
- Shock, E. L. and M. D. Schulte, 1998. Organic synthesis during fluid mixing in hydrothermal systems. *Journal of Geophysical Research: Oceans (1978–2012)* **103**:28513–28527.
- Sieracki, M. E., E. M. Haugen, and T. L. Cucci, 1994. Overestimation of heterotrophic bacteria in the Sargasso Sea: direct evidence by flow and imaging cytometry. *Deep-Sea Research I: Oceanographic Research Papers* **42**:1399–1409.
- Simoneit, B. R. T., 1983. Effects of Hydrothermal Activity on Sedimentary Organic Matter: Guaymas Basin, Gulf of California — Petroleum Genesis and Proto-Kerogen Degradation. In B. R. T. Simoneit (ed.) *Hydrothermal Processes at Seafloor Spreading Centers*, pp. 451–471. Springer US, Boston, MA.
- , 2002. Biomass burning — a review of organic tracers for smoke from incomplete combustion. *Applied Geochemistry* **17**:129–162.
- Smith, C. R. and A. Demopoulos, 2003. The deep Pacific ocean floor. *Ecosystems of the World* pp. 179–218.
- Smith, T. L. and R. Batiza, 1989. New field and laboratory evidence for the origin of hyaloclastite flows on seamount summits. *Bulletin of Volcanology* **51**:96–114.
- Sogin, M. L., H. G. Morrison, J. A. Huber, D. Mark Welch, S. M. Huse, P. R. Neal, J. M. Arrieta, and G. J. Herndl, 2006. Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proceedings of the National Academy of Sciences* **103**:12115–12120.

- Soininen, J., J. J. Korhonen, J. Karhu, and A. Vetterli, 2011. Disentangling the spatial patterns in community composition of prokaryotic and eukaryotic lake plankton. *Limnology and Oceanography* **56**:508–520.
- Stackebrandt, E. and B. M. Goebel, 1994. Taxonomic Note: A Place for DNA-DNA Reassociation and 16S rRNA Sequence Analysis in the Present Species Definition in Bacteriology. *International Journal of Systematic and Evolutionary Microbiology* **44**:846–849.
- Stein, C. A. and S. Stein, 1994. Constraints on hydrothermal heat flux through the oceanic lithosphere from global heat flow. *Journal of Geophysical Research: Oceans (1978–2012)* **99**:3081–3095.
- Stingl, U., R. A. Desiderio, J.-C. Cho, K. L. Vergin, and S. J. Giovannoni, 2007. The SAR92 clade: an abundant coastal clade of culturable marine bacteria possessing proteorhodopsin. *Applied and Environmental Microbiology* **73**:2290–2296.
- Stubbins, A. and T. Dittmar, 2012. Low volume quantification of dissolved organic carbon and dissolved nitrogen. *Limnology and Oceanography: Methods* **10**:347–352.
- Sul, W. J., T. A. Oliver, H. W. Ducklow, L. A. Amaral-Zettler, and M. L. Sogin, 2013. Marine bacteria exhibit a bipolar distribution. *Proceedings of the National Academy of Sciences of the United States of America* **110**:2342–2347.
- Sunagawa, S., L. P. Coelho, S. Chaffron, J. R. Kultima, K. Labadie, G. Salazar, B. Djahanschiri, G. Zeller, D. R. Mende, A. Alberti, F. M. Cornejo-Castillo, P. I. Costea, C. Cruaud, F. d'Ovidio, S. Engelen, I. Ferrera, J. M. Gasol, L. Guidi, F. Hildebrand, F. Kokoszka, C. Lepoivre, G. Lima-Mendez, J. Poulain, B. T. Poulos, M. Royo-Llonch, H. Sarmiento, S. Vieira-Silva, C. Dimier, M. Picheral, S. Searson, S. Kandels-Lewis, Tara Oceans Coordinators, C. Bowler, C. de Vargas, G. Gorsky, N. Grimsley, P. Hingamp, D. Iudicone, O. Jaillon, F. Not, H. Ogata, S. Pesant, S. Speich, L. Stemann, M. B. Sullivan, J. Weissenbach, P. Wincker, E. Karsenti, J. Raes, S. G. Acinas, and P. Bork, 2015. Ocean plankton. Structure and function of the global ocean microbiome. *Science* **348**:1261359–1261359.
- Sutton, T. T., 2013. Vertical ecology of the pelagic ocean: classical patterns and new perspectives. *Journal of Fish Biology* **83**:1508–1527.
- Suzuki, M., Y. Nakagawa, S. Harayama, and S. Yamamoto, 2001. Phylogenetic analysis and taxonomic study of marine Cytophaga-like bacteria: proposal for Tenacibaculum gen. nov. with Tenacibaculum maritimum comb. nov. and Tenacibaculum ovolyticum comb. nov., and description of Tenacibaculum mesophilum sp. nov. and Tenacibaculum amyolyticum sp. nov. *International Journal of Systematic and Evolutionary Microbiology* **51**:1639–1652.
- Sylvan, J. B., B. C. Pyenson, O. Rouxel, C. R. German, and K. J. Edwards, 2012. Time-series analysis of two hydrothermal plumes at 9°50'N East Pacific Rise reveals distinct, heterogeneous bacterial populations. *Geobiology* **10**:178–192.

Bibliography

- Sylvan, J. B., B. M. Toner, P. R. Girguis, and K. J. Edwards, 2009. Bacterial community composition in hydrothermal plume environments is heterogeneous and distinct. *AGU Fall Meeting* **1**:1739.
- Takai, K. and K. Horikoshi, 1999. Genetic diversity of archaea in deep-sea hydrothermal vent environments. *Genetics* **152**:1285–1297.
- Tao, C., H. Li, W. Huang, X. Han, G. Wu, X. Su, N. Zhou, J. Lin, Y. He, and J. Zhou, 2011. Mineralogical and geochemical features of sulfide chimneys from the 49°39'E hydrothermal field on the Southwest Indian Ridge and their geological inferences. *Chinese Science Bulletin* **56**:2828–2838.
- Tao, C., J. Lin, S. Guo, Y. J. Chen, G. Wu, X. Han, C. R. German, D. R. Yoerger, N. Zhou, H. Li, X. Su, J. Zhu, DY115-19 (Legs 1 –2), and DY115-20 (Legs 4-7) Science Parties, 2012. First active hydrothermal vents on an ultraslow-spreading center: Southwest Indian Ridge. *Geology* **40**:47–50.
- Taylor, G. I., 1923. The motion of ellipsoidal particles in a viscous fluid. *Proceedings of the Royal Society of London. Series A* pp. 58–61.
- Teeling, H., B. M. Fuchs, D. Becher, C. Klockow, A. Gardebrecht, C. M. Bennke, M. Kassabgy, S. Huang, A. J. Mann, J. Waldmann, M. Weber, A. Klindworth, A. Otto, J. Lange, J. Bernhardt, C. Reinsch, M. Hecker, J. Peplies, F. D. Bockelmann, U. Callies, G. Gerds, A. Wichels, K. H. Wiltshire, F. O. Glöckner, T. Schweder, and R. Amann, 2012. Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science* **336**:608–611.
- Teske, A., K.-U. Hinrichs, V. Edgcomb, A. de Vera Gomez, D. Kysela, S. P. Sylva, M. L. Sogin, and H. W. Jannasch, 2002. Microbial diversity of hydrothermal sediments in the Guaymas Basin: evidence for anaerobic methanotrophic communities. *Applied and Environmental Microbiology* **68**:1994–2007.
- Therneau, T. M., B. Atkinson, B. Ripley, and J. Oksanen, 2012. Mvpart: multivariate partitioning. R package version 1.6-0.
- Thompson, A. B. and G. M. Campanis, 2007. Information on fishing on and around the four closed seamount areas in the NRA. *NAFO Scientific Council Meeting Documents* **7**.
- Ting, C. S., G. Rocap, J. King, and S. W. Chisholm, 2002. Cyanobacterial photosynthesis in the oceans: the origins and significance of divergent light-harvesting strategies. *Trends in microbiology* **10**:134–142.
- Tripp, H. J., J. B. Kitner, M. S. Schwalbach, J. W. H. Dacey, L. J. Wilhelm, and S. J. Giovannoni, 2008. SAR11 marine bacteria require exogenous reduced sulphur for growth. *Nature* **452**:741–744.
- Uda, M. and M. Ishino, 1958. Enrichment pattern resulting from eddy systems in relation to fishing grounds. *Journal of Tokyo University* **44**.

- Urakawa, H., N. Dubilier, Y. Fujiwara, D. E. Cunningham, S. Kojima, and D. A. Stahl, 2005. Hydrothermal vent gastropods from the same family (Provannidae) harbour ϵ and γ -proteobacterial endosymbionts. *Environmental Microbiology* **7**:750–754.
- Vaulot, D., C. Courties, and F. Partensky, 1989. A simple method to preserve oceanic phytoplankton for flow cytometric analyses. *Cytometry* **10**:629–635.
- Venrick, E. L., 1991. Mid-ocean ridges and their influence on the large-scale patterns of chlorophyll and production in the North Pacific. *Deep Sea Research Part I: Oceanographic Research Papers* **38**:S83–S102.
- Venter, J. C., 2004. Environmental Genome Shotgun Sequencing of the Sargasso Sea. *Science* **304**:66–74.
- Verleyen, E., W. Vyverman, M. Sterken, D. A. Hodgson, A. De Wever, S. Juggins, B. Van de Vijver, V. J. Jones, P. Vanormelingen, D. Roberts, R. Flower, C. Kilroy, C. Souffreau, and K. Sabbe, 2009. The importance of dispersal related and local factors in shaping the taxonomic structure of diatom metacommunities. *Oikos* **118**:1239–1249.
- Vierros, M., I. Cresswell, J. Rice, and J. Ardron, 2009. Global open oceans and deep seabed (Good): Bio-geographic classification.
- Vilas, J. C., J. Arístegui, K. Kiriakoulakis, and G. A. Wolff, 2009. Seamounts and organic matter—Is there an effect? The case of Sedlo and Seine Seamounts: Part 1. Distributions of dissolved and particulate organic matter. *Deep Sea Research Part II: Topical Studies in Oceanography* **56**:2618–2630.
- Villar, E., G. K. Farrant, M. Follows, L. Garczarek, S. Speich, S. Audic, L. Bittner, B. Blanke, J. R. Brum, C. Brunet, R. Casotti, A. Chase, J. R. Dolan, F. d’Ortenzio, J.-P. Gattuso, N. Grima, L. Guidi, C. N. Hill, O. Jahn, J.-L. Jamet, H. Le Goff, C. Lepoivre, S. Malviya, E. Pelletier, J.-B. Romagnan, S. Roux, S. Santini, E. Scalco, S. M. Schwenck, A. Tanaka, P. Testor, T. Vannier, F. Vincent, A. Zingone, C. Dimier, M. Picheral, S. Searson, S. Kandels-Lewis, T. O. Coordinators, S. G. Acinas, P. Bork, E. Boss, C. de Vargas, G. Gorsky, H. Ogata, S. Pesant, M. B. Sullivan, S. Sunagawa, P. Wincker, E. Karsenti, C. Bowler, F. Not, P. Hingamp, and D. Iudicone, 2015. Environmental characteristics of Agulhas rings affect interocean plankton transport. *Science* **348**:1261447–1261447.
- Volk, T. and M. I. Hoffert, 1985. Ocean Carbon Pumps: Analysis of Relative Strengths and Efficiencies in Ocean-Driven Atmospheric CO₂ Changes, *Geophysical Monograph Series*, vol. 32. American Geophysical Union, Washington, D. C.
- Vrijenhoek, R. C., 2010. Genetics and Evolution of Deep-Sea Chemosynthetic Bacteria and Their Invertebrate Hosts. *In* The Vent and Seep Biota, pp. 15–49. Springer Netherlands, Dordrecht.
- Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole, 2007. Naive bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* **73**:5261–5267.

Bibliography

- Wankel, S. D., L. N. Germanovich, M. D. Lilley, G. Genc, C. J. DiPerna, A. S. Bradley, E. J. Olson, and P. R. Girguis, 2011. Influence of subsurface biosphere on geochemical fluxes from diffuse hydrothermal fluids. *Nature Geoscience* **4**:461–468.
- Ward, A. C. and N. Bora, 2006. Diversity and biogeography of marine actinobacteria. *Current Opinion in Microbiology* **9**:279–286.
- Ward, N. D., R. G. Keil, P. M. Medeiros, D. C. Brito, A. C. Cunha, T. Dittmar, P. L. Yager, A. V. Krusche, and J. E. Richey, 2013. Degradation of terrestrially derived macromolecules in the Amazon River. *Nature Geoscience* **6**:530–533.
- Watsuji, T., M. Nishizawa, Y. Morono, and H. Hirayama, 2012. Cell-specific thioautotrophic productivity of epsilon-proteobacterial epibionts associated with *Shinkaia crosnieri*. *PLoS ONE* **70**:3082–3090.
- Weeks, S. J. and F. A. Shillington, 1996. Phytoplankton pigment distribution and frontal structure in the subtropical convergence region south of Africa. *Deep Sea Research Part I: Oceanographic Research Papers* **43**:739–768.
- West, N. J., I. Obernosterer, O. Zemb, and P. Lebaron, 2008. Major differences of bacterial diversity and activity inside and outside of a natural iron-fertilized phytoplankton bloom in the Southern Ocean. *Environmental Microbiology* **10**:738–756.
- Whitaker, R. J., D. W. Grogan, and J. W. Taylor, 2003. Geographic barriers isolate endemic populations of hyperthermophilic archaea. *Science* **301**:976–978.
- White, M, Bashmachnikov, I, and Arístegui, J, 2008. Seamounts. In T. J. Pitcher (ed.) *Seamounts: Ecology, Fisheries and Conservation*. Blackwell Publishing.
- Whitman, W. B., D. C. Coleman, and W. J. Wiebe, 1998. Prokaryotes: The unseen majority. *Proceedings of the National Academy of Sciences* **95**:6578–6583.
- Winn, C. D., D. M. Karl, and G. J. Massoth, 1986. Microorganisms in deep-sea hydrothermal plumes. *Letters to Nature* **320**:744–746.
- Wishner, K. F., C. J. Ashjian, C. Gelfman, M. M. Gowing, L. Kann, L. A. Levin, L. S. Mullineaux, and J. Saltzman, 1995. Pelagic and benthic ecology of the lower interface of the Eastern Tropical Pacific oxygen minimum zone. *Deep Sea Research Part I: Oceanographic Research Papers* **42**:93–115.
- Wright, J. J., K. Mewis, N. W. Hanson, K. M. Konwar, K. R. Maas, and S. J. Hallam, 2014. Genomic properties of Marine Group A bacteria indicate a role in the marine sulfur cycle. *The ISME Journal* **8**:455–468.
- Yesson, C., M. R. Clark, M. L. Taylor, and A. D. Rogers, 2011. The global distribution of seamounts based on 30 arc seconds bathymetry data. *Deep Sea Research Part I: Oceanographic Research Papers* **58**:442–453.

- Yokokawa, T., Y. Yang, C. Motegi, and T. Nagata, 2013. Large-scale geographical variation in prokaryotic abundance and production in meso- and bathypelagic zones of the central Pacific and Southern Ocean. *Limnology and Oceanography* **58**:61–73.
- Zaika, V. E. and A. V. Kovalev, 1985. Investigation of ecosystems of submarine elevations. *Soviet Journal of Marine Biology* **10**:301–306.
- Zbinden, M., B. Shillito, and N. Le Bris, 2008. New insights on the metabolic diversity among the epibiotic microbial community of the hydrothermal shrimp *Rimicaris exoculata*. *Journal of experimental marine biology and ecology* **359**:131–140.
- Zinger, L., L. A. Amaral-Zettler, J. A. Fuhrman, M. C. Horner-Devine, S. M. Huse, D. B. M. Welch, J. B. H. Martiny, M. Sogin, A. Boetius, and A. Ramette, 2011. Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. *PLoS ONE* **6**:e24570.
- Zinger, L., A. Boetius, and A. Ramette, 2014. Bacterial taxa-area and distance-decay relationships in marine environments. *Molecular Ecology* **23**:954–964.
- Zsolnay, A., 2003. Dissolved organic matter: artefacts, definitions, and functions. *Geoderma* **113**:187–209.
- Zubkov, M. V., M. A. Sleight, and P. H. Burkill, 2000. Assaying picoplankton distribution by flow cytometry of underway samples collected along a meridional transect across the Atlantic Ocean. *Aquatic Microbial Ecology* **21**:13–20.
- Zwirgmaier, K., J. L. Heywood, K. Chamberlain, E. M. S. Woodward, M. V. Zubkov, and D. J. Scanlan, 2007. Basin-scale distribution patterns of picocyanobacterial lineages in the Atlantic Ocean. *Environmental Microbiology* **9**:1278–1290.
- Zwirgmaier, K., W. D. K. Reid, J. Heywood, C. J. Sweeting, B. D. Wigham, N. V. C. Polunin, J. A. Hawkes, D. P. Connelly, D. Pearce, and K. Linse, 2014. Linking regional variation of epibiotic bacterial diversity and trophic ecology in a new species of Kiwaidae (Decapoda, Anomura) from East Scotia Ridge (Antarctica) hydrothermal vents. *Microbiology Open* **4**:136–150.

•

Bibliography

Appendix A

Supplementary material for chapter 3

Appendix A Supplementary material for chapter 3

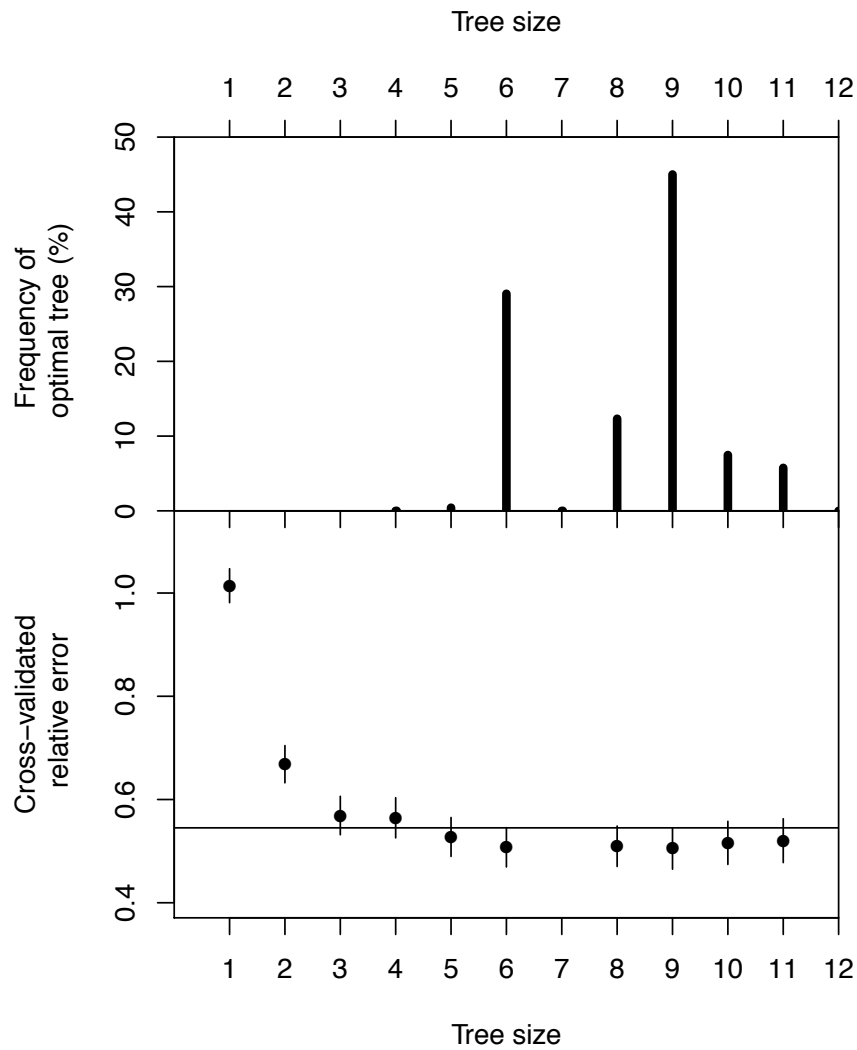


Figure A.1: Multivariate Regression tree error output. The top plot is a histogram of how often the optimal tree is selected. The bottom graph displays the cross-validated error between the tree sizes. Optimal tree sizes were between 5-11. The most parsimonious were tree sizes 5 and 9.

Appendix A Supplementary material for chapter 3

Appendix B

Supplementary material for chapter 5

Appendix B Supplementary material for chapter 5

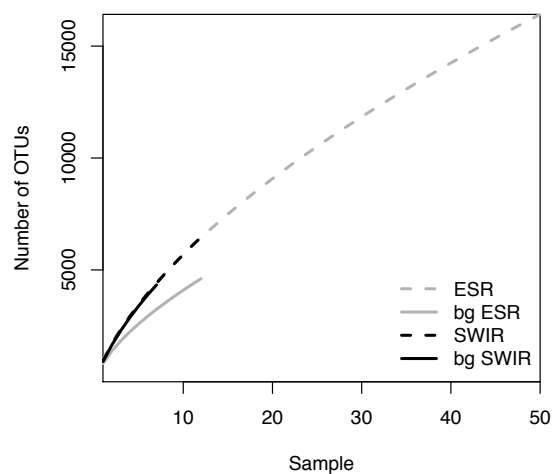


Figure B.1: Species accumulation curves from the vents and backgrounds (bg) of the East Scotia Ridge (ESR) and Southwest Indian Ridge (SWIR)

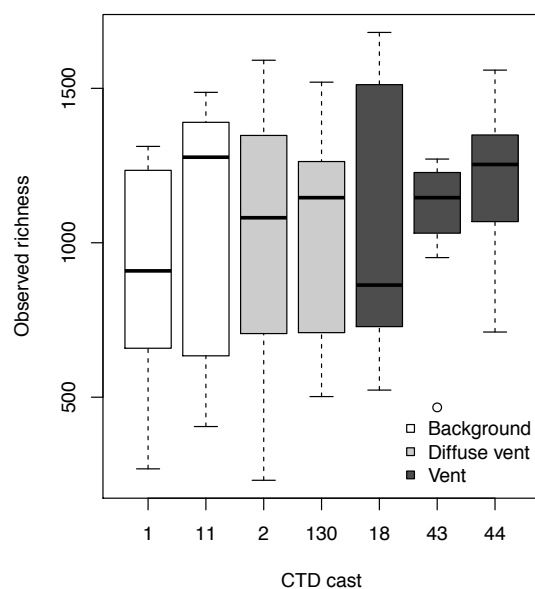


Figure B.2: Boxplots of richness between the individual CTD casts from the East Scotia Ridge (2,130,43 and 44) and the South West Indian Ridge (1, 11, and 18)

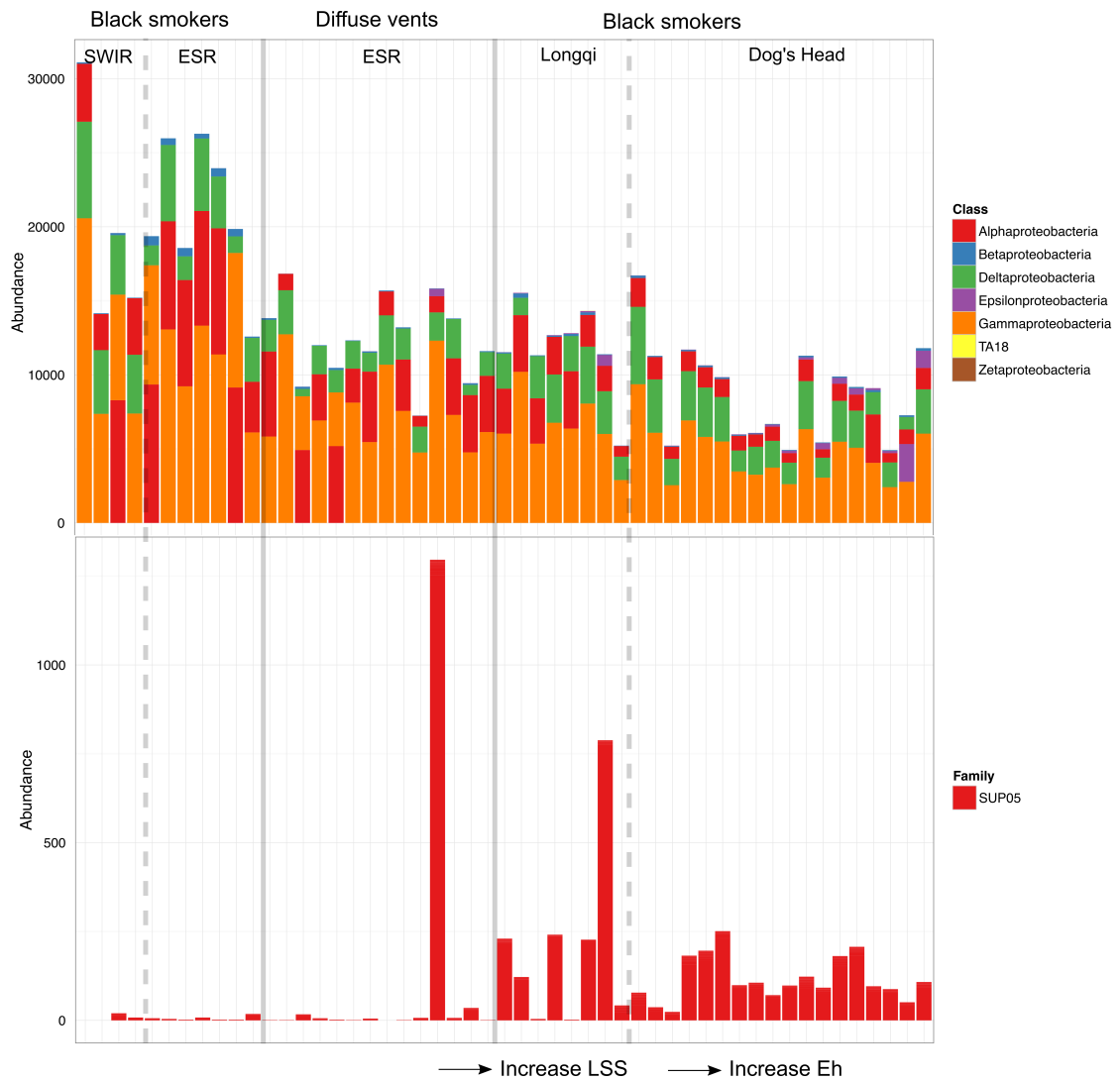


Figure B.3: Barplot of proteobacteria (a) and the Gammaproteobacteria family SUP05 (b) between all samples.

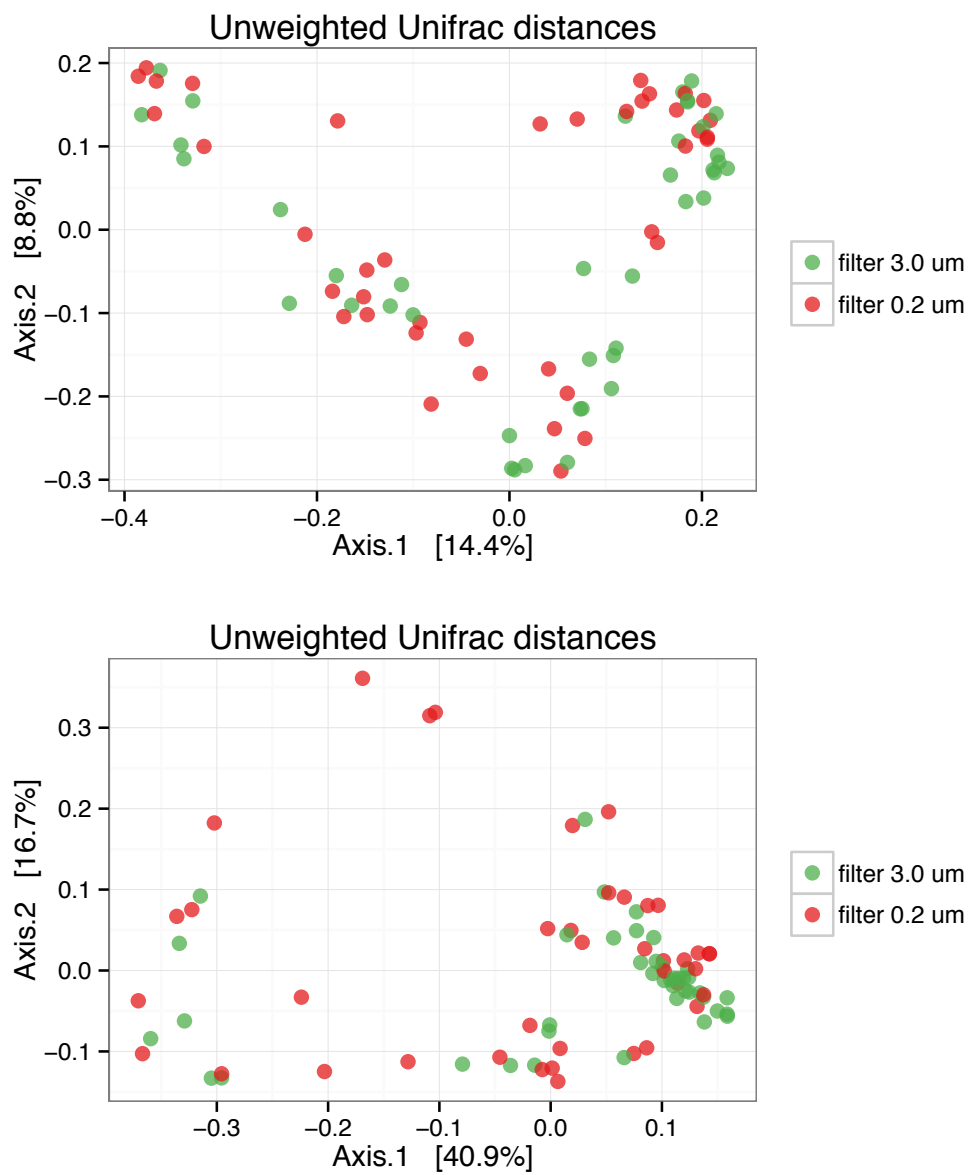


Figure B.4: Unifrac PCoA plot between all samples colored by filter type.

Appendix B Supplementary material for chapter 5

Revisions

Action points

1. The thesis would benefit from tying the chapters together better perhaps changing the chapter titles, hypotheses and introductions slightly to give a logical flow.

According to the following action points hypotheses have been added to each chapter and aims and objectives have been clarified in the general introduction. To give a more logical flow, information from other chapters have been added into some chapter introductions and discussions, especially to connect the habitats to each other (seamounts and vents). We also tried to connect the chapters by adding more information to the aims and objectives.

2. Add clear hypotheses.

We have added hypotheses at the end of the introduction of each chapter.

3. A fundamental flaw throughout is the lack of an 'off site comparison'. Whilst this can be impossible to obtain, it should be made clear that the effects of seamount and hydrothermal vents are in comparison to other such features and non on/off system. AP – add an explanation in the introduction/summary and check throughout that it is clear you are doing a comparative study. Use of appropriate hypotheses will also add clarity here.

We mostly addressed this issue while clarifying action point 1, 2, and 3 (Otherwise see, Page 56).

4. Following up on the above point, you cannot really talk of a 'seamount effect' if you cannot show that there is one. AP – remove reference to a 'seamount effect' except perhaps in speculative discussion.

The seamount effect was previously mentioned in the introduction and discussion of the second chapter, this has been changed (Page 46-47).

5. Regional biogeography comparisons without 100% coverage can be problematical

Appendix B Supplementary material for chapter 5

(as you cannot demonstrate that a sequence is not present just because you haven't detected it). You could check observations with Q-PCR or FISH probes, but these too have their limitations. AP – the thesis needs a paragraph explaining why regional biogeography can be compared with less than 100% coverage (you can use other studies to justify the approach but it needs to be stated explicitly).

A short paragraph about the sequence method had been added (Page 4-5) in addition to clarifying the hypotheses (Page 19)

6. The Carribean vs Antarctic comparison is clearly opportunistic and to describe it as a valid comparison is somewhat unconvincing. AP – I suggest describing it specifically as an outlier – i.e. a comparison with a system most unlikely to show the same patterns. Again, the use of appropriate hypotheses will help to clarify this point.

Hypothesis have been added (see above) and the Carribean station has been clarified as an outlier (Page 18-20 and 80).

7. A paragraph needs to be added to each key method stating its limitations and the potential influence on the onward interpretation of the data.

In the section 'Advancements in the field of marine microbiology' in the introduction a paragraph has been added about the methods used. In addition, the methodological limitations have been clarified in the discussion of most chapters.

8. Throughout the thesis, others were involved in the work (e.g. the calculation of sea surface heights) and it is not always clear what contribution was made by you and what contribution was made by the collaborators. Although I do not see this it is a problem, it needs to be clarified. AP – state the exact contribution by others.

We added more details into the declaration of the thesis and have clarified who did what and exactly what was added by other authors.

9. Throughout words like 'shown' and 'demonstrate' are used. E.g. where communities are strongly influenced by the environment' this implies it is known from your work, where actually a 'strong correlation' is shown. AP – where correlations are shown, please refer to them as such.

Shown and demonstrate have been exchanged with correlated where appropriate.

Other points

1. The reference section is sloppy. There are many issues and this requires attention.

The bibliography has been revised.

2. *Some of the figures need clarification (e.g. p68) as they appear to have been produced in a hurry and some are very poor quality (e.g. P90/91).*

The higher resolution figures have been used in the thesis (Page 90/91) and the labels have been made clearer on the other (Page 68)

3. *Typos need checking (e.g. Flavobacteriia p72).*

The class Flavobacteriia has not been misspelled. We added the word "class" in front of Flavobacteriia to avoid any confusion.

4. *Not sure on the relevance of the Faroe paper, other than to demonstrate that you can publish your techniques. Did you learn anything from the approach you took in that study that you applied to the thesis?*

The Faroe paper was taken out of the thesis.

5. *Page vii) "professional" is misspelled*

Spelling corrected (Page 30)

6. *Page 27) Explain the normalization process in more detail and why it is appropriate.*

Normalization process has been explained in more detail. (Page ??)

7. *The axis labels on many of the figures are nonsensical due to what I presume is a Latex error (i.e. cells3/ml). All of these should be fixed.*

All labels now say cells x 10.

8. *Fig. 3.2 Labels on the x axis are missing.*

x-axis label added.

9. *Page 79, third line) the 'possibly billions' statement is does not make sense. Please reword.*

"Possibly" was deleted.

10. *Fig. 5.1 The colorbar is missing.*

A colorbar has been added.

11. *Page 108: 'threshold of 900' What are the units?*

Sentence has been changed.

12. *Page 111, second paragraph: Please use an appropriate number of significant digits in each of these averages.*

The averages have been given appropriate digits.

13. *Figure 5.2: should the x-□ axis label read "Relative temperature" instead?*

The temperature is the measured temperature by the CTD in-situ.

Appendix B Supplementary material for chapter 5

14. *Figure 5.3: the figure caption does not make sense (it has been copied from another figure)*

Figure caption has been changed for the correct one. (Page 115)

15. *Figure 5.6: Write out the base of the log.*

The log 10 was written to the y-axis label.

16. *Figure 5.7: You are missing the x-□ axis label.*

x-axis is fixed (Page 120).

17. *Page 121: chemosynthesis is misspelled.*

Changed.

18. *Page 127: References on first line are repeated twice. Fix 'in an attempted to link'.*

References and sentence have been corrected.

19. *Page 129, penultimate line: 'more precisely the splits' has a typo and more generally needs to be reworded.*

Typo has been corrected and the sentence reworded (Page 131)

20. *Page 132: reword 'few opportunities allow such expeditions'*

We deleted the latter part of this sentence.

21. *Page 133, last line: typo: reword to 'world's oceans'.*

Reworded