MICROBIAL COMMUNITIES IN CANADIAN COASTAL SEDIMENTS

by

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Abstract

Prokaryotes are the most abundant organisms on Earth. These microorganisms play an integral role in maintaining Earth's habitability through their role as catalysts in global biogeochemical cycling. While microorganisms have been identified in almost every environment on Earth, the most populous environments are the open ocean, soil, and oceanic subsurface (coastal and deep-sea sediments). Notably, coastal sediments play an outsized role in global biogeochemical cycling, as they host some of the largest microbial communities on Earth despite comprising a relatively small fraction of the Earth's surface area. To date, however, there is a lack of knowledge on the microbial ecology of these sediments. This thesis investigates the microbial community diversity, composition, and structure in 10 geographically disparate Canadian sites. Community profiling using 16S rRNA sequencing, reveals a core microbial community shared among all sediments studied and an accessory community that displays biogeographical variation. Quantitative analyses of population sizes based on direct cell counting and qPCR suggests that core members of coastal sediment communities may be among the most abundant organisms on Earth. This information on coastal sediment microbial communities represents the first step towards linking coastal sediment biogeochemical cycling to underlying microbial community metabolism.

Lay summary

Prokaryotes are the most abundant organisms on Earth and have been found in almost every environment on Earth. Coastal sediments are one of the largest prokaryote habitats, despite their relatively small fraction of Earth's surface area. The prokaryotes in coastal sediments play an important role in controlling the distribution and movement of essential nutrients between Earth's oceans, atmosphere, and crust through biogeochemical cycling. In spite of the importance of coastal sediments to these processes, little is known about the abundance, species composition, or diversity of the prokaryotes they host. This thesis addresses this knowledge gap by determining prokaryote abundance, diversity, and composition in sediments recovered from the west coast of British Columbia and the Arctic ocean. This new knowledge reveals that coastal sediments are among the most biologically diverse habitats on Earth and that they host some of the most abundant organisms on the planet.

Preface

This work was made possible through the contributions and dedication of many collaborators. Dr. Sean A. Crowe, as the research advisor, was involved in all aspects of this work including experimental design, data analysis and interpretation, and writing.

- Chapter 1: Jenifer S. Spence wrote the main text with editorial support from Dr. Sean A. Crowe.
- Chapter 2: Jenifer S. Spence wrote the main text with editorial support from Dr. Sean A. Crowe. Dr. Crowe designed the research. Dr. Crowe, Céline Michiels, Dr. Rachel Simister, Kohen Bauer, Ashley Davidson, Kate Thompson, Alfonso Mucci, and Cedric Magen collected samples. Jenifer S. Spence, Céline Michiels, and Kohen Bauer performed laboratory work, Jenifer S. Spence and Dr. Sean A. Crowe analyzed and interpreted the data.
- Chapter 3: Jenifer S. Spence wrote the main text with editorial support from Dr. Sean A. Crowe.

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To Jack,

You stood by me through thick and thin. Thank you for loving me endlessly, supporting me continuously, and always bringing a smile to my face and warmth to my heart.

To my parents; Ron and Arlene Spence,

Through the years you have provided me with unwavering love and support in all my ventures and believed in me when I wasn't able to do so myself. You shaped me into who I am today, and I am lucky to have had you both as role models growing up. I owe all that I achieve to you.

"You can only eat an elephant one bite at a time."

Chapter 1: Introduction

Prokaryotic microorganisms are the most populous life form on Earth[1]. With an estimated 10^{30} cells globally[1,2], microorganisms have been observed in every explored environment, from the deep terrestrial subsurface to the upper levels of Earth's atmosphere[3-5]. Notably, the open ocean, soil, terrestrial subsurface, and oceanic subsurface support the most populous microbial communities on Earth (1×10^{29}, 3×10^{29}, $0.3\text{-}3 \times 10^{30}$, 4×10^{30} cells, respectively)[1]. The enormous abundance of microorganisms in these environments, their widespread distribution, and sustained metabolic activities over geologic time-scales has led to global-scale changes of Earth's physical and chemical properties and its evolution from a barren planet in the Hadean Eon to its modern state that supports the three diverse domains, including complex multi-cellular life [6,7].

Microbially mediated biogeochemical cycles have influenced Earth's geochemical properties throughout Earth's history, maintaining planetary habitability and promoting a protracted and sustained evolution and proliferation of life[8-10]. The biogeochemical cycling of key elements—for example carbon (C), oxygen (O_2), nitrogen (N), and sulphur (S)—are underpinned by reactions catalyzed by microbial communities inhabiting soils, oceans, and the subsurface. Over the course of Earth's 4.6 billion year history, for example, microbial metabolisms combined with geophysical processes promoted the accumulation of molecular oxygen in the atmosphere as the ultimate result of oxygenic photosynthetic primary production[11]. This introduction of O_2 to the atmosphere transformed the nature and activity of life and ultimately gave rise to the emergence of complex, multicellular life and animals[10]. Modern microbial metabolisms continue to cycle C, O_2, S, and N between the atmosphere, hydrosphere, lithosphere and biosphere. In this way, microorganisms have shaped and continue to shape biogeochemical cycles ultimately driving the co-evolution of Earth surface chemistry and life.

Coastal sediments play an outsized role in global biogeochemical cycling and as a habitat for microorganisms. These sediments house approximately 20% (10^{29} cells) of the global population of prokaryotes, despite comprising only 7-10% of the marine subsurface[1,2,12]. Collectively, the metabolic potential of sediment microbial communities supports biogeochemical processes including C, N, and S cycling. Microbial respiration in coastal sediments, for example, controls rates of sedimentary carbon burial. Given that about 80% of global carbon burial takes place in these sediments, the resident microbial communities control the strength of the marine carbon sink and its corresponding effects on atmospheric chemistry[13]. Furthermore, coastal sediments contribute up to about 50 % of global denitrification[14], which is an anaerobic microbial metabolism that removes bioessential N from the ocean and thus influences rates of marine primary productivity. Much (50%) of the respiration in coastal sediments also proceeds through sulphate (SO_4^{2-}) reduction, which is an anaerobic microbial metabolism that reduces SO_4^{2-} to sulphide (H_2S)[15]. Coastal sediments, and their microbial communities, are thus important sources and sinks for biologically active elements from the oceans, and therefore support many key biogeochemical cycles.

Studies to date suggest that globally, coastal sediments harbor diverse microbial communities comprised of the same principle phyla, similar to soils. Among these are the Proteobacteria, Bacteroidetes, and Verrucomicrobia phyla, which have been consistently identified as the most abundant phyla in coastal sediment microbial communities[16-25]. Existing estimates of microbial species richness imply that coastal sediments harbor between 400-27,000 species, rivaling soils in terms of their diversity (Table 1.1). Much of this previous work on coastal sediments, however, is difficult to compare across sites, as it has been conducted using inconsistent approaches and outdated platforms and methodologies, rendering global inferences highly tenuous. Instead, available information using more modern approaches comes mostly from

anomalous sediment environments through studies motivated largely by the desire to constrain responses of these sediments to perturbations—like oil spills, for example, or by the desire to study specific taxa and metabolisms of interest, such as hydrocarbon degradation, despite their rather unusual biogeochemical properties in comparison to coastal sediments more broadly[26,27]. There is thus a need for new knowledge on coastal sediment microbial communities from representative and geographically distant locations to supplement existing data sets, and in principle, such new knowledge can be acquired using modern techniques that more broadly extensible across systems.

To create new knowledge on coastal sediment microbial communities, I analysed sediments from western and northern Canada including those recovered from: The Strait of Georgia; Jervis Inlet; Saanich Inlet; and the southern Beaufort Sea. I determined sediment microbial community diversity, composition, and structure in these coastal sediments, placed these community properties in the context of sediment biogeochemical features, and, where possible, compared microbial communities from Canadian sediments with communities previously described from other locations. In this introduction, I provide a brief overview of the biogeochemistry and microbiology of coastal sediments and highlight gaps in our current knowledge of the resident microbial communities. This work represents a key first step towards linking the role of these sediments in global biogeochemical cycles to the underlying microbial metabolisms.

Table 1.1. Global review of current literature that employed next generation sequencing of the 16S rRNA gene. Diversity estimates are indicated where available, the listed microbial taxa are at the highest taxonomic resolution classified. Water depth is measured in meters below sea level (mbsl).

Author(s)	Location	Water depth (mbsl)	Primers	Sequencing technology	Dominant taxa listed	Species richness estimate (OTU)
Broman *et al.* (2017)[28]	Baltic Sea	31	Bacterial: 341F/805R	Illumina MiSeq	Proteobacteria, Bacteroidetes, Chloroflexi	-
Broman *et al.* (2018)[29]	Baltic Sea	6.5	Bacterial: 341F/805R	Illumina MiSeq	Proteobacteria, Bacteroidetes, Chloroflexi, Planctomycetes	-
Ciobanu *et al.* (2014)[3]	Canterbury Basin, New Zealand	344	Bacterial: V4/V5 Archaeal: V1/V3	454 pyrosequencing	Thaumarchaeota, Euryarchaeota Chloroflexi, Proteobacteria	<150
Dong *et al.* (2017)[30]	Western Arctic Ocean, South region of Chuckhi Shelf	46-338	Bacterial V6 967F/1046R	Illumina HiSeq	Gammaproteobacteria, Deltaproteobacteria, Verrucomicrobia, Unclassified	6,693-10,002
Dyksma *et al.* (2016)[31]	Western Europe Australia	20-29 (sublittoral sands), sandy intertidal sediments	Bacterial V3-V4	454 pyrosequencing Illumina MiSeq	Gammaproteobacteria, Bacteroidetes, Deltaproteobacteria, Alphaproteobacteria, Planctomycetes, Actinobacteria, Chloroflexi	7,026 +/- 3,697
Kumbhare *et al.* (2014)[32]	Agatti Island	1-40	Bacterial V3	Illumina MiSeq	Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Cyanobacteria, Spirochaetes	3,420

Author(s)	Location	Water depth (mbsl)	Primers used	Sequencing technology	Dominant taxa listed	Species richness estimate (OTU)
Learman *et al*. (2016)[33]	Western Antarctic Peninsula	223-800	Bacterial/archaeal V4 515F/806R	Illumina MiSeq	Proteobacteria, Thaumarchaeota, Bacteroidetes, Verrucomicrobia, Planctomycetes, Actinobacteria, Acidobacteria, Gemmatimonadetes	9,000-26,900
O'Reilly *et al.* (2016)[34]	Western Irish Sea	12-111 (surface sediments)	Bacterial 909F/1492R	454 pyrosequencing	Proteobacteria, Bacteroidetes (Flavobacteriia, Firmicutes (Clostridia), Acidobacteria, Verrucomicrobia	<400
Probandt *et al*. (2017)[35]	North Sea	-	Bacterial 341F/785R	Illumina MiSeq	JTB255, Ectothiorhodospiraceae, Alteromonadaceae, Deltaproteobacterial Sh765B-TzT-29, Sandaracinaceae, Flavobacteraceae, Planctomycetaceae, Phycisphaeraceae	5,300 +/-3,400 (0-1 cmbsf) 7,500 +/-4,000 (1-2 cmbsf)
Wang *et al.* (2012)[36]	Yam O Wan, Hong Kong	-	Bacterial V6 967F/1046R	Illumina HiSeq	Proteobacteria *(Cycloclastious, Pelobacter, Desulfobulbus, Desulfobacterium, Acinetobacter* sp.*),* Planctomycetes (*Planctomyces* sp.), Actinobacteria (*Gp10* sp.), Acidobacteria (*Gp22* sp.), Firmicutes, Chloroflexi, Verrucomicrobia (*Rubitralia* sp.), *Caldithrix* sp.	13,300

1.1 Sediment biogeochemistry

1.1.1 Coastal sediment geochemical processes

Biogeochemical cycling in coastal sediments is the result of the complex interplay between geophysical and biological processes. Geophysical processes, such as diffusion and sedimentation, supply microbial substrates and non-reactive particles to sediments, thus regulating the activity of sedimentary microorganisms. For example, sedimentation of sinking particulate organic carbon (C_{org}) – in essence the remains of primary photosynthetic and secondary biomass – from the overlying water column to the sediment-water interface delivers a strong supply of electron donor (typically >0.01 g C cm^{-2} yr^{-1}) to coastal sediments[37] and fuels sedimentary heterotrophic respiration. Generally high rates of marine primary productivity in coastal waters, coupled with their shallow depths (0-600 meters below sea level (mbsl)), sustain this high rate of carbon deposition to coastal sediments, and lead to relatively high rates of respiration, compared to deeper sediments further off shore[13,14]. While sedimentation of C_{org} is the main source of electron donor to coastal sediments, electron acceptors, like O_2 and sulphate (SO_4^{2-}), are primarily supplied through diffusion from the overlying water into the sediment. Consumption of electron acceptors through respiration in the sediment leads to a concentration gradient across the sediment water interface and this concentration gradient drives diffusive fluxes of electron acceptors into the sediment. A combination of sedimentation, microbial respiration, and diffusion thus supports biogeochemical reactions and fuels microbial growth in sediments.

Both electron donors and electron acceptors are supplied from the overlying water column and are consumed within the sediment, and thus concentrations of both donors and acceptors tend to decrease with increasing depth in the sediment. Respiratory microorganisms grow through redox coupling of an electron donor and electron acceptor pair, which are preferentially used based on the energy yielded from this coupling[13]. Electron acceptors and donors are generally depleted

sequentially in order of decreasing free energy yields (most thermodynamically favorable to least thermodynamically favorable). This results in a decrease in both electron donor and acceptor availability as sediment depth increases[38]. O_2 and C_{org}—supplied to the sediment through diffusion and sedimentation, respectively—are the most thermodynamically favorable electron donor-acceptor pair, and fuel aerobic microbial communities in the upper reaches of coastal sediments[13,38]. Heterotrophic microorganisms, that grow through aerobic respiration for example, gain energy from the reduction of O_2 and produce carbon dioxide (CO_2) as the product of C_{org} oxidation. Chemoautotrophs, on the other hand, use inorganic energy sources (electron donors)—such as sulphide (H_2S) or ammonium (NH_4^+) compounds—to fuel carbon fixation through O_2 reduction[13]. Notably, most of the electron donors that are used by chemoautotrophs are generated as the products of heterotrophic respiration and these diffuse through the sediments along concentration gradients. Sulphur-oxidizing bacteria are prominent sediment chemoautotrophs, and they gain energy by coupling reduction of O_2 in the upper reaches of the sediment to the oxidation of H_2S produced via heterotrophic SO_4^{2-} reduction and supplied through diffusion from below. This energy is used to synthesize biomass from CO_2. In coastal sediments, O_2 consuming processes, both heterotrophic and chemoautotrophic, can outpace the diffusive supply rate of O_2 from overlying water, leading to its depletion and the development of anoxia, which in coastal sediments generally results in O_2 penetration to depths of <1 cm[13] below the sediment-water interface. In the absence of O_2, microorganisms can use alternative terminal electron acceptors, such as $NO_3^-$and SO_4^{2-} for respiration of organic matter, and in oxidation of reduced inorganic species[38]. The physical supply of microbial substrates to the sediments thus sets up a geochemically-induced stratification of microorganisms, leading to aerobic processes in the oxic upper reaches of the sediment, and anaerobic processes in the anoxic deeper sediments.

1.1.2 Anaerobic metabolisms and carbon burial

Organic carbon respiration, as well as chemotrophic metabolisms, below the depth of O_2 penetration are supported by alternative electron acceptors that are generally used in order of decreasing free energy yields (O_2>NO_3^->Fe^{3+}>SO_4^{2-}) (Figure 1.1)[38]. Like O_2, NO_3^- and SO_4^{2-} are primarily delivered to the sediment through diffusion from the overlying water[39], though NO_3^- and SO_4^{2-} are also produced through microbial oxidation of ammonium (NH_4^+) and H_2S in the upper oxic sediments, as described above. Denitrification (NO_3^- respiration) is one key anaerobic respiration pathway, and it results in the conversion of bioavailable (i.e. fixed) N to much less biologically available nitrogen gas (N_2), which is ultimately lost from the sediment to the oceans and atmosphere above. NO_3^- can be respired through organotrophic or lithotrophic pathways, coupled to either the oxidation of C_{org} or inorganic compounds such as H_2S, respectively. Since denitrification leads to the loss of bioavailable N, it represents a negative feedback on marine biological productivity[13,40]. Following NO_3^-, SO_4^{2-} is generally the most favorable electron acceptor in marine sediments, though iron (Fe) and manganese (Mn) oxides are important in some settings[38]. SO_4^{2-} reduction occurs in anoxic sediments where it is an important contributor to C_{org} oxidation, second only to aerobic respiration[15,41]. Through the respiration of SO_4^{2-}, both H_2S and CO_2 are formed, and these can diffuse along concentration gradients within sediment porewaters. H_2S, as mentioned above, can react with electron acceptors in overlying sediments, where it supports processes such as denitrification, or conversely, it can react with Fe in the sediment, forming pyrite (FeS_2). Meanwhile, CO_2 either diffuses upwards through the sediment and into overlying seawater where it can exchange with the atmosphere and provide a carbon source to fuel primary production, or it interacts with porewater calcium (Ca) and magnesium (Mg) to form carbonate minerals, thereby contributing to inorganic carbon burial[40,42]. Anaerobic metabolisms thus play important roles in supporting microbial growth in coastal sediments, where they

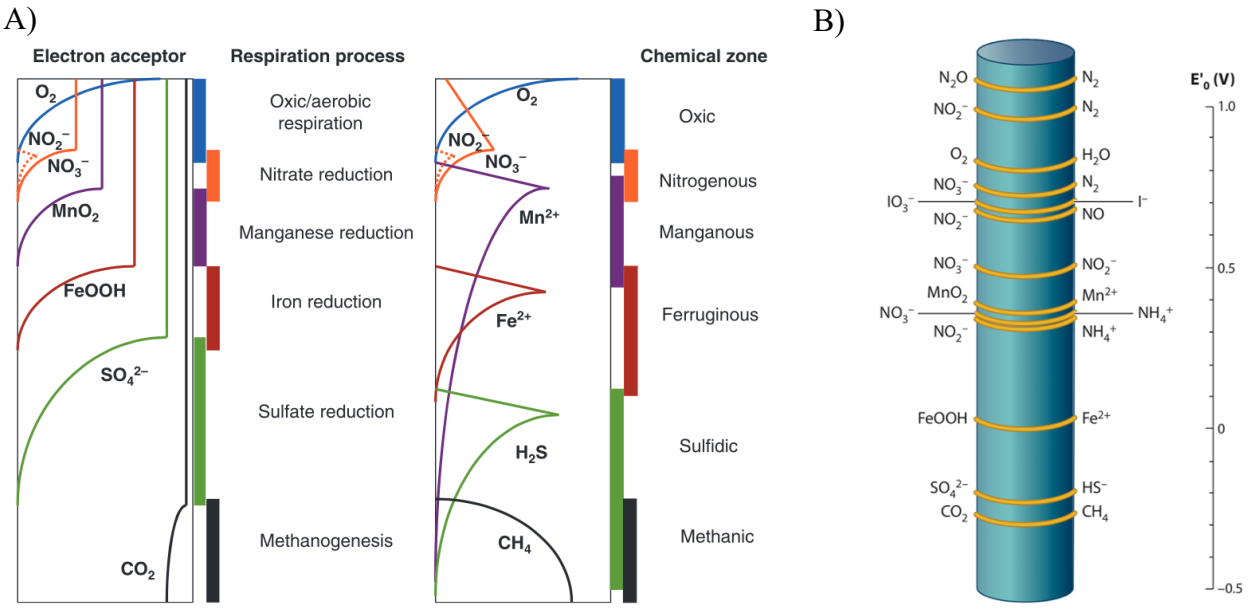

Figure 1.1: A) Depth distribution of common electron acceptors in sediments and the names used to represent the zones where these different electron acceptors are used[43]. B) The electron tower of the electron potentials (E'_o) of various redox couples found in sediments[44].

contribute to coupled biogeochemical cycling of C, N and S, and influence carbon burial in both organic and inorganic forms.

Carbon burial in marine sediments is influenced by sedimentation rates and microbial respiration (aerobic and anaerobic). Sedimentation rates directly influence the amount of C_{org} that is buried in sediments, with areas where sedimentation rate is high correlating to a higher rate of carbon burial[13]. This is seen in the coastal oceans, where high sedimentation rates lead to rapid deposition of C_{org}, limiting the amount of time this carbon is exposed to O_2 and thus its degradation through aerobic respiration[13]. Coastal sediments have a typical sedimentation rate of approximately 1 mm y^{-1}, whereas deep-sea sediments are often characterized by much lower sedimentation rates (<0.01 mm yr^{-1})[37]. Deep-sea sediments have correspondingly lower C_{org} burial rates, and, despite their much greater areal extent than coastal sediments, only account for 4% of the global net C_{org} burial[45]. Coastal sediments, in contrast, are responsible for about 86% of the

C_{org} buried in sediments globally[45]. Organic carbon burial is attenuated by microbial respiration in the sediment, which remineralizes C_{org} into inorganic carbon forms, such as CO_2 and carbonate minerals. Organic carbon remineralization in coastal sediments is driven by both aerobic and anaerobic respiration, though anaerobic remineralization is often considered less efficient. The time it takes for C_{org} to remineralize into inorganic carbon forms is often referred to as carbon turnover. Organic carbon in sediments that is not remineralized is buried in the sediments along with carbonate minerals, which are ultimately subducted into the mantle[13]. By controlling the efficiency and extent of C_{org} remineralization through aerobic and anaerobic respiration, the geophysical processes of sedimentation and diffusion thus combine with microbial physiology and dictate burial rates of organic and inorganic carbon. Coastal sediments play an outsized role in this C_{org} burial, since high sedimentation rates restrict exposure to O_2 and channel somewhat diminished carbon mineralization through anaerobic pathways.

1.1.3 Nitrogen and sulphur cycling in coastal sediments

Coastal sediments are a strong sink for fixed N, which is converted to N_2 gas through microbial NO_3^- respiration. There are two modes of NO_3^- metabolism that contribute to N_2 production in coastal sediments: denitrification and anammox (Eq. 1, 2; Fig. 1.2).

$$5CH3OH + 6NO_3^- \rightarrow 5CO_2 + 3N_2 + 7H_2O + 6OH^- \quad [1]$$

$$NH_4^+ + NO_2^- \rightarrow N_2 + H_2O \quad [2]$$

While these metabolisms are biochemically different, the geochemical outcome is similar, and thus for the remainder of this discussion I'll refer to combined N_2 production from both metabolisms as denitrification[14,46]. Denitrification in coastal sediments is driven primarily by N supplied through organic matter deposition from the overlying water column. NH_4^+ is released

from this organic matter through ammonification, which is then oxidized to NO_3^- through microbial nitrification coupled to O_2 reduction. This NO_3^- diffuses into underlying anoxic sediments where it supports denitrifying microorganisms that respire NO_3^- and produce N_2. This N_2 is not readily consumed through other biological processes, aside from N fixation (the conversion of N_2 to a bioavailable N species), and thus diffuses into overlying seawater where it exchanges with the atmosphere[14]. Since the rate of sedimentation in coastal oceans is high, and sediment oxygen penetration is shallow, a large supply rate of organic N translates to high activities of denitrifying microorganisms. Indeed, coastal sediments are estimated to contribute up to 44% of global denitrification, and thus act as major global sinks of fixed N[14,46].

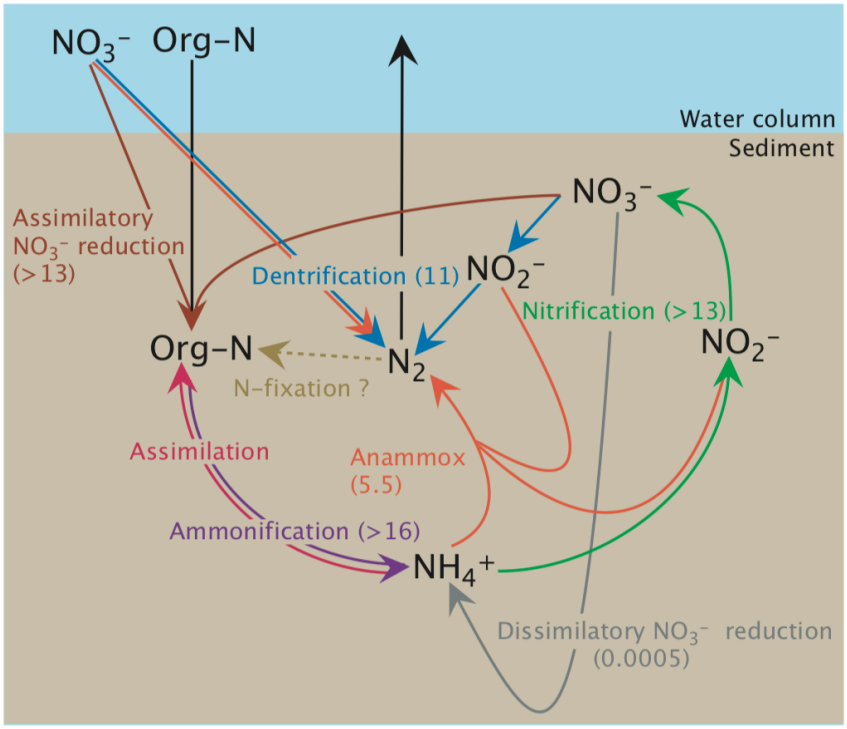

Figure 1.2: Sedimentary nitrogen cycle. The light blue section represents the overlying water, the beige section represents the sediment nitrogen cycles. The numbers in parenthesis are the rates in μmol m^{-2} h^{-1} [47].

In addition to acting as an important sink for fixed N, microbial metabolisms in coastal sediments also play an import role in the S cycle. SO_4^{2-} reduction, for example, accounts for 50%

of C_{org} remineralization in coastal sediments, with aerobic respiration accounting for most of the remainder[15]. SO_4^{2-} reduction (Eq. 3) occurs after all the other electron acceptors have been consumed, and its importance to C_{org} remineralization is due to the high concentrations of SO_4^{2-} (28 mM) in seawater, which makes SO_4^{2-} up to 50-100x more abundant than all other the electron acceptors combined[13,48]. This high concentration of SO_4^{2-} in seawater supports strong diffusive fluxes of SO_4^{2-} into sediments in response to SO_4^{2-} consumption by respiration[48]. High sedimentation rates in coastal sediments contribute to shallow oxygen penetration depths, which promote SO_4^{2-} reduction in underlying sediments and relatively high activities of SO_4^{2-} reducing bacteria compared to deep sea sediments offshore. Indeed, the rate of SO_4^{2-} reduction in coastal sediments is commonly >1 mmol cm^{-2} y^{-1}, whereas in deep sea sediments it can be $<10^{-5}$ μmol cm^{-2} y^{-1} [49]. The high rate of SO_4^{2-} reduction in coastal sediments has important effects on the activity of other metabolisms. This is because the product of SO_4^{2-} reduction, H_2S, is almost completely re-oxidized back to SO_4^{2-} through chemoautotrophic metabolisms in the upper reaches of the sediment using electron acceptors including O_2 and NO_3^- [13]. The H_2S produced also reacts with sedimentary Fe to form the mineral FeS_2 (Eq. 4, 5). Pyrite burial in coastal sediments represents a net sink for reduced equivalents from organic matter originally fixed during primary production. Burial of these reduced equivalents as FeS_2 effectively precludes their back-reaction with O_2 and thus represents an important net source O_2 to Earth's surface environments and atmosphere[50]. SO_4^{2-} reduction in coastal sediments thus represents a key process in coupled C and S cycling and, through FeS_2 burial, plays an important role in regulating the O_2 content of Earth's oceans and atmosphere.

$$2CH_2O + SO_4^{2-} \rightarrow H_2S^- + 2HCO_3^- \quad [3]$$

$$Fe^{2+} + H_2S \rightarrow FeS + 2H^+ \quad [4]$$

$$FeS + S^o \rightarrow FeS_2 \quad [5]$$

Biogeochemical processes in coastal sediments thus play a large role in regulating Earth's surface chemistry and climate, and these processes are coupled to the metabolism and growth of sediment microorganisms. Interactions between geophysical processes, including sedimentation and diffusion, with microbial physiology control material fluxes between the oceans and sediments, and ultimately between Earth's surface and the mantle a result of sediment subduction. These material fluxes, in turn, influence the activity of life in the oceans and have effects on climate. High sedimentation rates and productivity in the coastal oceans give rise to coastal sediments harboring microbial communities that play a disproportionate role in these biogeochemical processes. While abundant information constrains rates and pathways of microbial metabolisms in coastal sediments, far less is known about the composition, diversity, and metabolic potential of the specific microorganisms the inhabit coastal sediments and drive biogeochemical cycling.

1.2 Diversity, composition, structure, and function of coastal sediment microbial communities

1.2.1 Sediment microbial community abundance and diversity

Coastal sediments house one of the largest microbial communities on Earth. It is estimated that up to 20% of global prokaryotes reside in coastal sediments, despite only comprising about 7-10% (~36 x 10^{12} m^2) of ocean area[1,2,12,51]. In comparison, terrestrial soil covers 120 x 10^{12} m^2, yet only houses an estimated 4-6% of the global population of prokaryotes. Microbial community abundances in coastal sediments range from 10^6-10^9 cells cm^{-3} up to 50 cm below seafloor (cmbsf) in sediments ranging from 2-850 meters below sea-level (mbsl)[19,52-55]. In addition to differing abundances between sediment sites and with water depth, there is also a link between microbial abundance and depth below the sediment-water interface[55-59]. Geochemical stratification of

microbial substrates, as discussed in 1.1, leads to changes in electron donor and acceptor concentrations with increasing depth[38]. The decreasing free energy yield of electron donor-acceptor pairs with increasing depth likely exerts influence on the abundances of microorganisms in coastal sediments[57,60]. For example, the availability of organic matter directly influences prokaryotic abundance, with higher organic matter contents generally leading to higher abundances of microorganisms and, correspondingly, decreasing availability of organic matter with increasing depth is thought to result in a decrease in microorganisms abundance[57]. Microbial abundances are thus shaped by the geochemical features of coastal sediments, which ultimately support the largest microbial communities on Earth.

Current estimates of coastal sediment microbial diversity are limited in number compared to similar estimates made for soils, however, recent data now suggests that diversity in coastal sediments is comparable to soils. Descriptions of diversity commonly consider either the number of species and their proportion within a single community or the dissimilarity between two or more communities (alpha or beta diversity, respectively). Alpha diversity describes the number and distribution of taxa within a community and is commonly estimated through diversity indices that signify species richness like Chao1, the Shannon, or the Simpson indices[61]. Beta diversity describes dissimilarity between communities using diversity indices including Bray-Curtis dissimilarity or the Jaccard index, with higher values for these indices indicating greater dissimilarity between communities (i.e. fewer shared taxa)[61]. Coastal sediments have historically been considered relatively diverse ecosystems, with estimates of species richness ranging from 70-1,300 and 400-1,100 in sediments sourced from the Mediterranean Sea and the Antarctic, respectively[22,24]. That being said, the outdated methods used in these studies, such as denaturing gel gradient electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) are known to underestimate diversity in complex communities, and are semi-quantitative

at best[62-64]. High-throughput methods now suggest much higher diversity with estimates of species richness ranging from 400-27,000 (Table 1.1). In comparison, global analysis of soil microbial communities, generally thought of as the most diverse environment on Earth, report species richness of 800-20,000 [65-67]. Given that coastal sediments house a larger prokaryotic community than soils (20% and 5% of the global community, respectively), suggests that they may harbour an even larger fraction of the microbial diversity on Earth[1]. However, further work is required to better constrain these estimates as current high-throughput data sets for soils greatly outnumber those of coastal sediments.

Coastal sediment community diversity is strongly influenced by environmental variables. Sediment lithology and chemistry can influence microbial diversity. For example, sediment permeability and grain size, such as fine-grained (impermeable) versus coarse-grained (permeable) sediments, influence species richness. Course-grained sediments reportedly harbour a less diverse microbial community (~3,800 OTUs), whereas finer-grained sediments house a more diverse microbial community (~6,800-11,300 OTUs)[35]. These course-grained sediments allow for the transport of oxygenated porewater vertically through the sediment, thus allowing for a deeper O_2 penetration depth than what is typically observed in coarser-grained sediments[68]. Although the mechanism is uncertain, the deeper O_2 penetration depth in coarse-grained sediments in comparison to fine-grained sediments appears to influence microbial diversity. Despite the apparent role sediment lithology can play in influencing microbial diversity, further work remains to be performed to fully describe the interactions between environmental variables and the microbial community and determine how this shapes sediment community diversity. More mechanistic knowledge on the controls of microbial community diversity in coastal sediments would require systematic exploration across depositional environments along with detailed biogeochemical characterization.

1.2.2 Sediment microbial community composition, structure, and function

Analyses to date suggest that coastal sediment microbial communities are comprised of the same key taxa regardless of location. Classical techniques, such as clone library sequencing, revealed that Planctomycetes, Acidobacteria, Actinobacteria, Bacteroidetes, and Verrucomicrobia are the most abundant phyla in coastal sediments deposited around the world, including sites from the Antarctic and the Mediterranean Sea[16-25]. Limited application of high-throughput sequencing techniques (Roche 454, Illumina) confirm that coastal sediment microbial communities are comprised of core phyla including Proteobacteria, Bacteroidetes, and Verrucomicrobia which are shared amongst coastal sediments globally (Table 1.1)[3,28-36]. While archaeal lineages in coastal sediments tend to be under-reported compared to bacterial lineages, there is a growing number of studies indicating that archaea represent an important component of coastal sediment communities. For example, Thaumarchaeota are often the most abundant archaeal phylum in these sediments, and Euryarchaeota, miscellaneous crenarchaeotal group (more recently reclassified as Bathyarchaeota), and marine benthic group D (now known as the Thermoprofundales order), have also been observed, however at generally lower abundances than the Thaumarchaeota[33,69,70]. Together, reports on the taxonomic composition of bacteria and archaea in coastal sediments suggest a core group of key taxa that are ubiquitous and abundant.

Although vertical stratification of microbial substrates is widely observed in coastal sediments, only a limited number of reports link microbial community compositions to this geochemical stratification. Geophysical processes supply electron donors and acceptors to sediments from overlying waters and their consumption through microbial metabolism within the sediment sets up vertical gradients in substrate availability and niche space that are expected to shape microbial community composition and structure (see 1.1). Geochemical stratification can be linked to the community composition at varying depths in the sediment profile, thought to be

caused by the terminal oxidases that are associated with these specific zones[59]. For example, since SO_4^{2-} concentrations are highest in the upper layers of sediments, SO_4^{2-} reducing bacteria have been reported to be the most abundant in the surface layers of the sediment and subsequently decrease with increasing depth. Additionally, total N, C_{org}, and redox potential have been identified as important variables in the vertical structuring the sediment community composition[71,72]. While there are numerous such anecdotal observations, there remains a lack of supporting data that firmly links vertical stratification of microbial substrates to corresponding structure in microbial community composition. Furthermore, there have been reports of vertical overlap between microorganisms that were originally thought to occupy different metabolic niches which may contradict this general asuumption[59]. The primary controls on sediment microbial community composition and structure, whether it be geochemical zonation or not, remain uncertain.

While environmental and geochemical parameters may drive community composition variation, this variation may not be accurately captured in existing data sets. Coastal sediment microbiological studies typically report microbial community taxonomy to the phyla or class level (Table 1.1). Such low level taxonomic resolution provides a broad overview of the community composition, however, some reports suggest that most taxonomic variation as function of depth is expressed at higher resolution taxonomy (species level), while relative abundances at phylum level are constant[73]. This may not always be the case, however, as stratification of major phylogenetic groups has also been observed[74]. Given the uncertainty in the taxonomic level where variation in community composition manifests, the role of environmental and geochemical parameters in sediment microbial community may remain obscured.

Bioturbation (sediment reworking and irrigation by macrofaunal) appreciably influences the sediment microbial community composition. Bioturbation and macrofaunal movement introduce burrows into the sediment, expanding the area of the sediment-water interface and

introducing O_2 to sediment that would otherwise be anoxic. This leads to dynamics in the geochemical conditions in bioturbated zones of sediment; with the presence of O_2 at the surface of these burrows, electron acceptors such as NO_3^- and SO_4^{2-} extend deeper into the sediment. Indeed, the microbial community at the surfaces of these burrows is highly similar to the microbial community at the sediment-water interface, and different than the microbial community at deeper depths in the sediment[75]. Bioturbation has also been suggested as a driver in microbial community structure by influencing the abundances of Bacteria and Archaea in the top 20 cmbsf of coastal sediment[55]. Through its control on microbial substrates in sediments, bioturbation thus appreciably influences coastal sediment microbial communities.

The coastal sediment microbial community diversity, composition, and structure is dependent on the complex interplay of both geophysical and biological processes. To date, researchers have only recently begun to capture the diversity of these communities and their compositions through the use of high-throughput sequencing methods. Geophysical, environmental, and biological sources of variability that underpin sediment community diversity and composition have yet to be completely accounted for in existing literature and is an area of active research. Additionally, current reports on coastal sediment microbial communities are largely focused on the environmental parameters, as well as taxa, or metabolisms of interest. As a result, the ecological controls the influence how coastal sediment microbial communities shape global biogeochemical cycling are not yet well constrained. A census of the sediment microbial communities is a key first step in determining how geophysical and geochemical processes effect microorganisms, and how microbial ecology underpins global biogeochemical cycling.

1.3 Summary

The main objective of my thesis is to determine the diversity, composition, and structure of microbial communities in Canadian coastal sediments, including those from the Strait of Georgia, Jervis Inlet, Saanich Inlet, and the southern Beaufort Sea. In so doing, I test the hypotheses that sediments host diverse microbial communities; these communities are structured by vertical gradients in sediment properties; and the communities exhibit differences due to geography. I test these hypotheses by combining 16S rRNA gene amplicon sequencing with analyses of microbial abundance and place these data in biogeochemical context through process rate measurements and geochemical analyses. Given the lack of existing information from coastal sediments around the world, I evaluate the extent to which information gained by studying Canadian coastal sediment sites can be made more broadly extensible to coastal sediments deposited globally. This new knowledge on coastal microbial communities can be leveraged in future studies connecting biogeochemical processes to underlying microbial metabolisms with potential to improve knowledge on global elemental cycles, climate regulation, and planetary habitability.

Chapter 2: Microbial communities in Canadian coastal sediments

2.1 Introduction

There are an estimated 10^{30} microorganisms[1] on Earth and their collective metabolisms underpin the biogeochemical cycles that regulate climate and maintain Earth's habitability[76]. The rise of complex life—including plants and animals—was dependent on biogeochemical cycles set in motion by microorganisms, and now interacts with microorganisms to maintain the conditions life collectively depends on[1,10]. Vascular plants, for example, play a key role in the global production of O_2, which in turn fuels respiration by both macro- and microorganisms across the Earth's surface[77,78]. The environments with the most populous microbial communities are the open ocean, soil, and oceanic subsurface (coastal marine, deep ocean, and coastal plain sediments), accounting for approximately 3, 5, and 79% of the global microbial community, respectively[1]. While microbial communities in soils are well studied[66,67,79,80], coastal marine sediments and the deep ocean subsurface remain relatively underexplored. The deep subsurface is difficult to access and though it houses a very large microbial community (about 3×10^{30} cells), this community is depauperate and has very low metabolic activity[1]. Many coastal sediments, on the other hand, are teeming with microbial life and are readily sampled through standard oceanographic sediment coring techniques. These sediments are estimated to support on the order of 8×10^{29} prokaryotic cells—upwards of 20% of the total global microbial community, and are thus one of the largest single microbial habitats on Earth[1]. Collectively, coastal sediments and their microbial communities support much of the total global organic carbon and sedimentary pyrite (FeS_2) burial, and thus play a critical role in modulating atmospheric O_2 and CO_2 concentrations over geological time[81,82].

Coastal sediments studied from distant regions of the global ocean including those from Antarctic, Arctic, and Pacific Oceans, as well as the Mediterranean Sea[16-25] appear to host

microbial communities with similar taxonomic compositions comprised predominantly of Proteobacteria, Planctomycetes, Acidobacteria, Actinobacteria, Bacteroidetes, and Verrucomicrobia (Table 1.1)[3,16-25,28-36]. By comparison, these phyla also make up abundant components of soil microbial communities[66]. Early estimates of microbial diversity in these sediments implied between 70-1,300 taxa based on clone library sequencing and denaturing gradient gel electrophoresis techniques, known to under-sample diversity[22,24], while more recent studies using high throughput sequencing platforms (Roche 454, Illumina) estimate that sediments contain 400-27,000 taxa (Table 1.1). This level of diversity appears to approach that found in microbial ecosystems like soils – thought to be among the most diverse ecosystems on Earth – which have an estimated species richness ranging from 800-20,000 taxa[65-67]. There are a number of factors that may influence the coastal sediment microbial community. For example, microbial community compositions in vertically stratified environments, like coastal sediments, are expected to vary as a function of depth due to gradients in the availability of substrates. Microbial communities in coastal sediments indeed display vertical structure linked to redox potential and microbial substrate availability[38,57,59,71,72]. Coastal sediments are also subject to mixing through bioturbation, which is thought to homogenize communities in the uppermost sediments with overlying bottom seawater, thereby overprinting or muting vertical community structure[75]. While existing data suggest that coastal sediments around the world host diverse microbial communities with the same key phyla as soils and some degree of vertical structure that results primarily from substrate availability, there remain few studies that have systematically explored community composition and diversity across depth, at multiple geographical areas, and using high-throughput techniques that allow quantitative determination of the abundance and distribution of relevant taxa.

Some recent work on coastal sediment communities using high-throughput sequencing has targeted sediments with unusual chemical and physical properties or microbial taxa of specific

interest or rarity[31,83-85]. For example, petroleum-contaminated sediments have been studied in connection with remediation efforts and these sediments are often characterized by large proportions of hydrocarbon and a higher diversity of hydrocarbon degrading bacteria[26,27,86]. Focus on specific microbial taxa or metabolisms—like anaerobic methanotroph (ANME) archaea or anaerobic hydrocarbon degradation[87-90]—marginalizes the broader sediment microbial community that underpins background sediment metabolism. It is this background metabolism in coastal sediments that is responsible for global biogeochemical cycling, however, it remains largely unexplored through modern high throughput sequencing approaches.

To create new knowledge on microbial community diversity, composition, and structure in coastal sediments, I profiled microbial communities in Canadian coastal sediments using SSU rRNA gene amplicon sequencing, quantified microbial abundances in these sediments with qPCR and direct cell counting, and placed this microbiological data in biogeochemical context through process rate measurements and geochemical analyses. These microbial community analyses provide quantitative assessments of sediment community diversity and taxonomic composition, revealing that coastal sediments are comprised of a core group of abundant cosmopolitan taxa, as well as a broad diversity of accessory taxa that exhibit biogeographical variation. This work provides new knowledge on Canadian coastal sediment microbial communities and represents the first step towards linking coastal sediment biogeochemical cycling to the underlying microbial metabolisms.

2.2 Materials and methods

2.2.1 Study sites and sample collection

Samples were collected from the southern Beaufort Sea (Canadian Arctic Shelf Exchange Study (CASES) 109, 200, 312, 409, 415, 600; October 2003-August 2004)[91], Saanich Inlet (SI-1, SI-2;

August 2015), the Strait of Georgia (BC; September 2016), and Jervis Inlet (JV; September 2016). CASES sediments were collected and sampled as described previously[91]. Sediments were recovered with the ROV Hercules from sites in Saanich Inlet with anoxic (SI-1) and oxic (SI-2) overlying water during a cruise aboard the E/V Nautilus. SI-1 was recovered at a position of 48.58 °N, -123.5 °W, and a water depth of 220 mbsl. SI-2 was recovered at a position of 48.64 °N, -123.5 °W and a depth of 100 mbsl. The sediments from sites BC and JV were collected using a multi-corer during a cruise on the R/V Vector. BC sediments were recovered at 49 °N, 123 °W, at a depth of 360 mbsl. JV sediments were recovered at 50.07 °N, 123.8 °W, at a depth of 510 mbsl. JV, BC, SI-1, and SI-2 sediments were sectioned at pre-defined depth intervals within a glove bag continuously flushed with nitrogen gas (N_2)[92]. Following sectioning, all samples were stored at -20 °C until further analysis. Water from sites BC and JV was collected using Niskin bottles™ housed in a rosette, up to depths of 350 mbsl and 500 mbsl, respectively. All maps were generated using Ocean Data View (Fig. 2.4)[93].

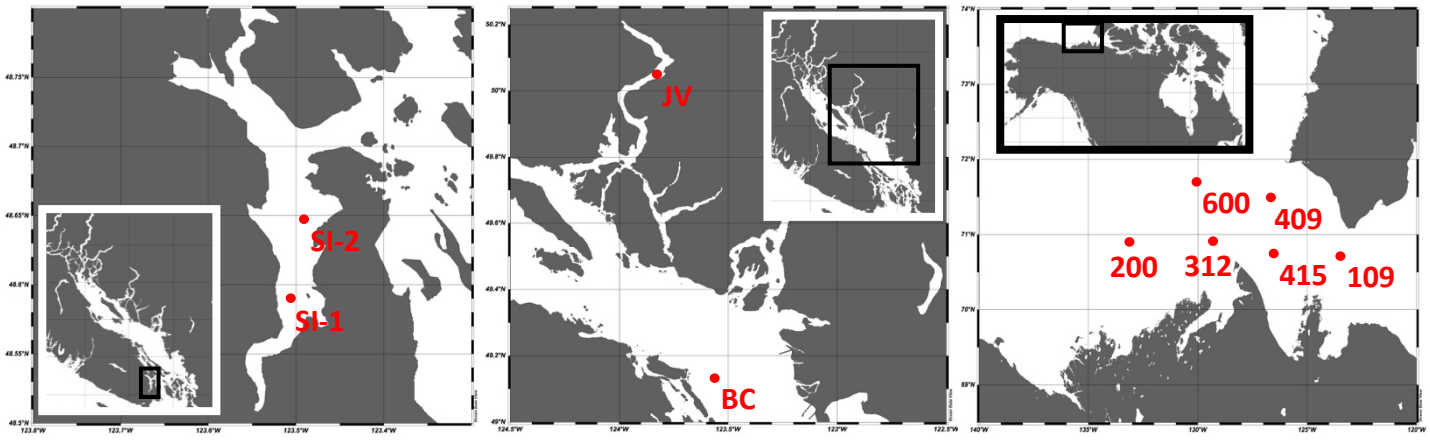

Figure 2.4: A) Map of Saanich inlet. SI-1 (48.58 °N, -123.5 °W) and SI-2 (48.64 °N, -123.5 °W) denote the sediment sites with anoxic and oxic overlying water, respectively. B) Map of the coast of British Columbia, including the Strait of Georgia (BC) and Jervis Inlet (JV) with coordinates (49 °N, 123 °W) and (50.07 °N, 123.8 °W), respectively. C) Map of the Southern Beaufort Sea, including the CASES sediment sites 109 (70.8 °N, -121.28 °W), 200 (70.98 °N, -133.79 °W), 312 (71.51 °N, -127.09 °W), 409 (71.51 °N, -127.09 °W), 415 (70.45 °N, -121.28 °W), 600 (71.66 °N, -130.66 °W).

2.2.2 Porosity & total organic carbon

CASES sediments were prepared and measured for porosity and total organic carbon (TOC) as described in Magen *et al.* (2010)[91]. JV, BC, SI-1, and SI-2 sediments were weighed while frozen and freeze-dried at -40 °C for 7 days. Porosity was calculated as:

$$\varphi = (Mw/\rho H2O)/[(Md/\rho) + (Mw/\rho H2O)]$$

where M_w and M_d are the weights of interstitial water and dry sediment, respectively, ρ_{H2O} is the density of water (1.00 g cm^{-3}) and ρ is the density of dry sediment (2.65 g cm^{-3})[94]. Sediment TOC was determined for the JV, BC, SI-1, and SI-2 sediments using by coulometery (UIC, Inc. Model 5014™) as described previously[91].

2.2.3 Oxygen profiles and uptake rates

Vertical distributions of dissolved O_2 concentrations in porewaters were determined onboard (JV and BC), and in the laboratory (SI-1 and SI-2) using a retractable needle optode (Pyrosciences®). Vertical penetration of dissolved O_2 was averaged across 3 and 4 cores for JV and BC respectively, and 1 core for each SI site. Rates of total oxygen uptake (TOU) were measured for sediment sites JV and BC in 6 undisturbed intact cores for each site. The cores were incubated for 6-24 hours and were maintained in the dark at 4 °C and the overlying water was stirred at 30 revolutions min^{-1} using a magnetic stir bar suspended 5 cm above the sediment-water interface. TOU rates were determined using robust oxygen optodes (Pyrosciences®) that were sealed into the tops of the core tubes with thick rubber stoppers. Optodes for vertical O_2 profiles and TOU rates were calibrated with a two-point calibration including 0% O_2 water made anoxic with the addition of dithionite, and water saturated in O_2 with respect to air made by equilibrating seawater with the atmosphere through vigorous shaking. Temperature corrections were based on continuous monitoring using a Pyrosciences ® temperature probe.

2.2.4 Cell counts

Sediment from the JV and BC cores was sampled for cell counting on-site. These samples were stored in 2.5% glutaraldehyde and frozen at -20 °C until analysis. Cells were separated using a previously described protocol with a 50% Nycodenz gradient[95]. The separated cells were diluted either 1/100 or 1/10, and fluorescently dyed using SYBR Green with a 10x final concentration. Cells were counted on a Guava EasyCyte HT flow cytometer (Millipore™) and quantified using the EasyCyte data analysis program.

2.2.5 DNA extraction and quantification

Microbial DNA was extracted from approximately 0.25g sediment using the Mobio PowerMax Soil DNA Isolation Kit™ as per manufacturer's instructions and stored at -20 °C. The quality of genomic DNA was assessed by spectrophotometry with NanoDrop ND-1000 (Thermo Fisher Scientific™). Bacterial and archaeal DNA was quantified by quantitative polymerase-chain reaction (qPCR) using the SsoFast™ EvaGreen assay (Bio-Rad™) and a CFX96 Real-Time Detection System (Bio-Rad™). The 16S rRNA gene was targeted using either bacterial-specific (27F, 5′-AGAGTTTGATCCTGGCTCAG) or archaeal-specific forward primers (20F, 5′-TTCCGGTTGATCCYGCCRG), coupled with the universal reverse primer DW519R (5′-GNTT TACCGCGGCKGCTG)[96]. Each amplification reaction (20 µL) contained PCR certified water (4 µL), SsoFast™ EvaGreen master-mix (10 µL), and 3 µM primer (2 µL). Amplification of the 16S rRNA gene was performed with an initial denaturation step (95 °C, 3 min), followed by 45 cycles of: denaturation (95 °C, 20s); annealing (55 °C, 30s (bacterial); 65 °C, 30s (archaeal)); and elongation (72 °C, 30s). This was followed by a melt curve analysis for assessing amplicon specificity (ramp-up of 0.5 °C every second, increasing from 55 °C to 95 °C). Bacterial and archaeal DNA standards, ranging from 10^2-10^8, non-template controls, and sample DNA were all

run in duplicate for both assays[96]. Given that there are variable numbers of 16S genes within a single bacterial genome, copy numbers were scaled to estimate cell abundances using an average of 2.7 16S rRNA genes per genome, based off of previous estimates on the number of 16S genes in different phyla and the topmost abundant phyla from the relative abundance data (see 2.2.7, Fig. 2.6)[97].

2.2.7 SSU rRNA gene amplification and iTag sequencing

Extracted DNA was submitted to the Joint Genome Institute (JGI) (Walnut Creek, Ca; https://jgi.doe.gov) for sequencing. Libraries for Illumina MiSeq sequencing (2x250 bp reads) were generated by amplification of the extracted genomic DNA targeting the V4-V5 region of the 16S rRNA gene, using primers 515F and 926R[98]. The presence of DNA was qualitatively confirmed by PCR amplification. The PCR master-mix contained PCR certified water, 2.5µM dNTPs, 10X PCR buffer, 50 mM $MgCl_2$, Invitrogen Taq Polymerase (5 U/µL), and 10 µM 515F/926R primers. PCR amplification was performed with an initial denaturing step (94 °C, 3 min), followed by 30 cycles of denaturation (94 °C, 45s), annealing (50° C, 1 min), and elongation (72 °C, 1 min 30s), and a final elongation step (72 °C, 10 min).

2.2.8 16S sequence data analysis & visualization

Amplicon sequence data were quality control filtered using the JGI quality control itagger pipeline (version 1.1; https://bitbucket.org/berkeleylab/jgi_itagger). Quality filtered reads were processed using USEARCH[99] and QIIME[100]. Chimeric sequences were removed using UCHIME[101], and operational taxonomic units (OTUs) were defined *de novo* using the SumaClust[102] method in QIIME at a 97% threshold (species level). Taxonomy was assigned to OTUs using the RDP classifier in QIIME and the Silva (v.1.28) QIIME release database[103]. OTUs with less than 10

occurrences were removed. Raw rRNA reads are available on the JGI Genome Portal (https://genome.jgi.doe.gov/portal/) under the project ID's 1133645 and 1154770.

Data generated here was also compared to previous data from marine sediments and this comparison was made following a reclassification of the previous data[104]. To do so, raw 454 pyrosequencing reads of the 16S rRNA gene (V6 region) were obtained from the Sequence Read Archive (accession numbers SRA009906.1, SRA009865.1, and SRA046414.1) and analyzed using Mothur (v.1.39.5) with the standard operating procedure[104,105]. Sequences were clustered using the opticlust algorithm in Mothur and singletons were removed. The representative OTUs (97% similarity) were classified using the Silva (v.1.28) database[103].

All 16S rRNA gene sequence data were plotted using RStudio (RStudio Team, 2015). Alpha-diversity was calculated using QIIME[106]. Hierarchical clustering was performed using the hclust and dist functions in RStudio. Microbial community structures were analyzed with a non-parametric multivariate analysis of variance (PERMANOVA)[107] using the adonis function in RStudio, by generating a Bray-Curtis distance matrix and Bonferroni p-value correction. Indicator species were identified using the multipatt function implemented in the R package Indicspecies. Taxa with less than 100 occurrences were removed. Indicator species were selected with a minimum threshold of 0.75.

2.2.9 Correlation Network Analysis

Correlation networks were constructed using SparCC[108] from the Spieceasi package in R. Pearson correlation coefficients were calculated between all taxa that were represented by 100 reads or more across all samples (7,806 OTUs). Pearson correlation coefficients ≥0.84 were considered robust. The network was visualized using a non-force directed layout in Cytoscape (v.3.6.1)[109]. Network properties were calculated using the "Network Analysis" plug-in. Nodes in the network

corresponded to individual taxa, and the edges were defined by computed correlations between corresponding taxa pairs. Edge betweenness indicates how central an edge is in a network, or in other words, reflects the importance of that edge to maintaining the network structure (high edge betweenness = high importance). Degree, also known as connectivity, is the number of neighbors that a node has. Nodes that have a high number of neighbors, or a high degree, are referred to as hubs[110].

2.3 Results & Discussion

2.3.1 Geochemical features

The Canadian coastal sediments studied (Fig. 2.4) have biogeochemical properties similar to most other coastal sediments deposited globally. The deposition of C_{org} and the uptake of O_2 are biogeochemical properties linked to microbial activity. Organic carbon is the primary electron donor for microbial respiration, while O_2 uptake rates are the integrated result of this respiration[12]. Average TOC concentrations in JV, SI-1, and SI-2 sediments ranged from 3.5-3.7%, whereas TOC concentrations in the BC and CASES sediments were lower, ranging from 1.2-1.7% (Table 2.2). Higher TOC concentrations in JV, SI-1, and SI-2 than in the CASES and BC sediments are likely due to sediment focusing and lateral transport at these sites due to the steep bathymetry of the inlets[111,112]. Collectively, these TOC concentrations are within the range typical for coastal sediments globally (0.1-3.1%), implying similar overall supply of electron donor[30,33,34,113]. Dissolved O_2 penetration depths reached up to 2, 4 (± 0.3), and 7 (± 2) mm below seafloor in the SI-2, JV, and BC sediments, respectively. The overlying water in SI-1 was anoxic, thus no dissolved O_2 was present in the sediments. The shallow penetration depths in these sediments indicates high microbial respiration rates, a likely result of high sedimentation[12]. Indeed, the BC and JV sediments, exhibited total oxygen uptake (TOU) rates of 23 and 19 mmol m^{-2} d^{-2}

respectively, whereas dissolved oxygen uptake rates in BC, JV, and SI-2 sediments were 7.1 (± 0.92), 15.8 (± 1.0), and 26 mmol m^{-2} d^{-1}, respectively (Table 2.2). These O_2 uptake rates are also typical of coastal sediments globally which have TOU rates ranging from ~3-90 mmol m^{-2} d^{-1} in sediments deposited up to 600 mbsl[12]. Coastal sediments deposited at sites BC and JV in the Strait of Georgia, as well as those deposited in SI and the Beaufort Sea (CASES) thus have biogeochemical properties similar to coastal sediments studied to date. The extent that electron donor supply and overall microbial activity – as indicated by TOC concentrations and O_2 uptake rates – regulate microbial abundance and select for a particular microbial community composition, in the Canadian coastal sediments should be broadly extensible to similar sediments globally, as I explore below.

2.3.3 Microbial abundances

Microbial abundances in the Canadian coastal sediments were comparable to abundances in other coastal sediments globally. This is consistent with TOC concentrations and O_2 uptake rates, which provide a first order control on microbial abundance and reflect microbial respiration and growth, respectively. 16S rRNA gene copy numbers provide an approximation of microbial abundance in the sediment cores and these ranged from 10^7- 10^9 copies cm^{-3} (Table 2.1, Fig. A1). The Arctic sediments (CASES), on average, had lower microbial abundances (1 x 10^7–9 x 10^8 copies cm^{-3}) in comparison to the temperate sediments (JV, BC, SI-1, SI-2) (1 x 10^8-1 x 10^9 copies cm^{-3}), however, all fell within the range of abundances observed in many other coastal sediments (~10^6-10^9 cells cm^{-3})[19,52-55]. 16S rRNA gene copy numbers were mirrored in direct cell counts in the JV and BC sediments, with total cell abundances of 3 x 10^9 and 4 x 10^8 cells cm^{-3}, respectively (Table A.1, Fig. A.2). The similarity between direct cell counts and abundance estimates based on 16S rRNA gene copies gives confidence that the qPCR data accurately reflect microbial abundances across

Table 2.2: Geochemical and cell quantification measurements in coastal sediment sites. Dissolved oxygen uptake (DOU) flux values were averaged over all available profiles for the given site. Total oxygen uptake (TOU) values were from whole core incubations. TOC values were averaged over the entire sediment depth. Water depth was measured in meters below sea-level (mbsl).

Station	Water Depth (mbsl)	Depth below seafloor (cmbsf)	Vertical O_2 penetration depth (mm)	Diffusive oxygen flux (mmol m^{-2} d^{-1})	Total oxygen flux (mmol m^{-2} d^{-1})	Total organic carbon (% wt)	16S rRNA (copies/cm^2)
JV	510	40	4 (± 0.3)	15.7	18.8	3.7	1.2×10^9
BC	360	30	7 (± 2)	7.11	23.2	1.7	1.4×10^9
SI-1	220	30	-	-	-	3.6	8.7×10^8
SI-2	100	25	2	26.2	-	3.5	1.1×10^8
109	570	10	-	-	-	1.2	9.4×10^8
200	-	10	-	-	-	-	3.3×10^7
312	-	5	-	-	-	-	2.1×10^7
409	378	7	-	-	-	1.7	4.5×10^7
415	-	5	-	-	-	-	2.1×10^7
600	584	15	-	-	-	1.4	1.3×10^7

all sites. While there is some variability in the TOC concentrations between sites, for example BC and CASES sediments had lower TOC concentrations than JV, SI-1, and SI-2; 1.2-1.7% vs. 3.5-3.7%, respectively, this variability is not reflected in the microbial abundances. While TOC concentrations (or rather fluxes) are expected to exert a first order control on microbial growth rate and abundance, the vertical stratification of microbial substrates throughout the depth profile likely also plays a role (see 1.1.1, 1.2.2)[34,38].

The use of more robust and higher throughput techniques, such as cell counts and qPCR, provides better confidence in measurements of microbial community abundance than in previous work which was based on microscopy[1,51]. Using this new data, it is thus possible to reevaluate global estimates for microbial abundances in coastal sediments. The surface area of coastal sediments is 36 x 10^{12} m^2 and leveraging the cell abundances reported here, I estimate that the top 0-40 cm below seafloor (cmbsf) in coastal sediments house 4 x 10^{24}- 5 x 10^{26} cells. Previous estimates implied coastal sediments house about 1.5 x 10^{29} cells, based on a 990 cm sediment profile (100-1000 cmbsf)[1]. Extending estimates from the upper 40 cmbsf to 1000 cmbsf depth using the lowest cell abundance measurements in each core as the value for 0-1000 cmbsf, I come up with an estimate of about 2 x 10^{26}-3 x 10^{28} cells in the top 1000 cmbsf in coastal sediments globally. Notably, these numbers carry some uncertainty given that the qPCR data is scaled to cell abundances with an assumed number of 16S rRNA gene copies per cell, and this could vary across sites. I also note that the Arctic sites had lower abundances than the temperate sites implying latitudinal variation in sediment microbial community size. If this variation extended to tropical sediments, these would have higher abundances than considered here and my global estimates would be correspondingly too low. Accordingly, previous studies based on tropical and temperate coastal sediments report higher global abundances in coastal sediments, and thus this latitudinal variation may be a factor in the lower values for my new estimates[1]. Overall, however, microbial

abundances determined in the Canadian coastal sediments studied here are similar to abundances reported elsewhere implying that these Canadian coastal sediments are representative of coastal sediments more broadly.

2.3.2 Microbial diversity

The Canadian coastal sediments studied host highly diverse microbial communities that rival some of the most diverse microbial habitats on Earth in terms of overall species richness. Estimates of species richness (Chao1) in these sediments ranged from 3,500-15,000 OTUs (Fig. 2.5). Generally, the Arctic sediment sites exhibited lower species richness with estimates ranging from 3,500-10,100 OTUs, whereas the temperate sediment sites had species richness estimates ranging from 7,500-15,000 OTUs (Fig. 2.5, Table A.2). The diversity of the Canadian coastal sediments is comparable to the diversity previously reported in coastal sediments deposited globally (Table 1.1), and is as high or higher than that measured in epitomically diverse microbial habitats like soils, for which estimates range from 800-20,000 OTUs[65,66]. Generally, the Canadian sediment sites displayed trends of decreasing species richness with increasing depth below the sediment surface, however, the decrease in some sites was more pronounced than in others. JV and the Arctic sediments exhibited pronounced decreases in species richness with increasing depth, with a loss of about 11-58% in taxonomic richness over the sediment profile. Sediments at BC and SI-2 exhibited richness losses of 5 and 11% respectively, while species richness increased in concert with depth by 12% in SI-1. Previous work also found decreases in sediment microbial diversity with increasing depth, up to 6 mbsf[59]. This observed decrease in diversity generally parallels decreases in cell abundances , implying that the processes governing cell abundances may also influence diversity. Overall, the diversity observed in the Canadian coastal sediments studied here implies that coastal sediments more generally harbor enormous microbial diversity, and, when

combined with high microbial abundances, suggests that coastal sediments may harbor the majority of global microbial diversity.

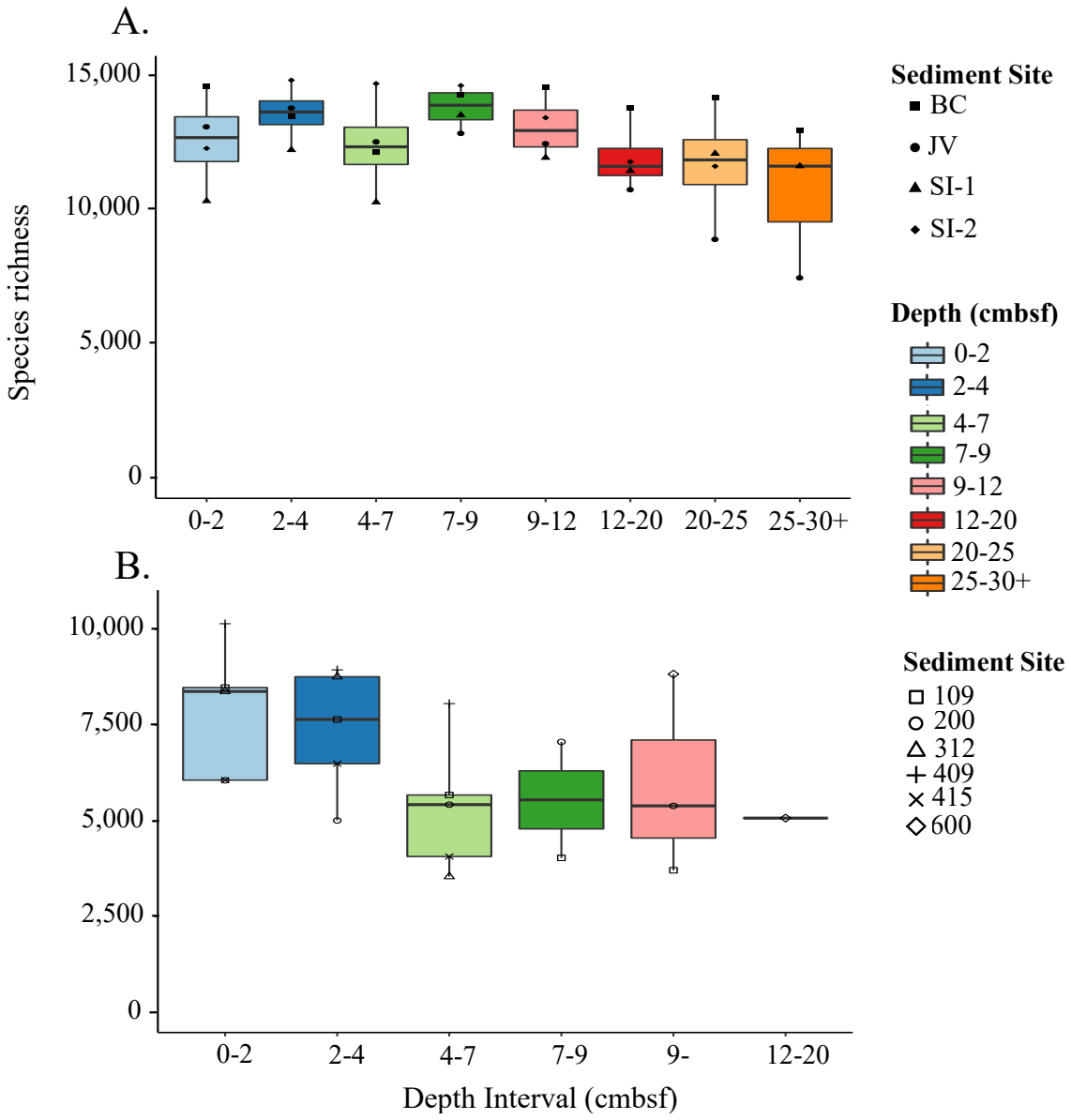

Figure 2.5: Species richness (Chao1) estimates of the Canadian coastal sediment sites A) BC coast sites; B) Arctic (CASES). Diversity estimates were averaged over the depth ranges of 0-2, 2-4, 7-9, 9-12, 12-20, 20-25, and 25-30 cmbsf. Box colour is correlated to depth interval, and point shape is correlated to sediment site.

Coastal sediments house more microbial diversity than soils. The Canadian coastal sediments studied here collectively support 41,000 taxa (35,500 Bacterial, 6,000 Archaeal),representing 81 phyla, and previous estimates had thus dramatically underestimated this species richness (6,000 – 16,000 taxa)[32,36,114,115]. In comparison, a large-scale, high-throughput analyses of soil microbial communities identified 25,000 OTUs across 237 soil sites[66]. This global analysis of soil microbial communities is comprehensive and outnumbers the number of sites that I analyze here 20-fold. Taking into consideration the limited number of sediment sites analyzed in this work, the diversity ranges that I report here are likely an underestimate of the global sediment community diversity. Much of the diversity observed in coastal sediments can be attributed to accessory microbial community members (see below) that vary between sediment sites and this suggests that as more sites are studied, the known diversity will grow.

2.3.4 Coastal sediment taxonomic composition

The Canadian coastal sediments studied had microbial community compositions with little variability in the more abundant community members with depth or location, even when compared globally against coastal sediments deposited at geographically far removed sites. The top 8 most abundant phyla comprised, on average, 78% (± 3%) of the microbial community, with the most abundant phyla including the Proteobacteria (40% ± 4.0%), Planctomycetes (9.1% ± 0.65%), and Bacteroidetes (7.8% ± 0.47%) (Fig. 2.6). These phyla are present across all sediment sites and depths, but their abundance varies somewhat between sites. Notably, with exception of the sediments from JV (12 cmbsf and deeper), microbial community compositions remained largely unchanged with increasing depth (Fig. 2.7). This was unexpected, as it has been previously thought that sediment microbial communities are strongly vertically stratified due to changing substrate availability as a function of depth, like in other vertically stratified environments[38,71,72]. With this

changing substrate availability, it is expected that a corresponding change in the microbial community composition would be observed, as differing microbial metabolisms that are able to consume the substrates would be favored under those conditions.

The availability of electron acceptors did not appear to have a dramatic effect on microbial community composition. It would be expected, for example, that the presence of O_2 in sediment

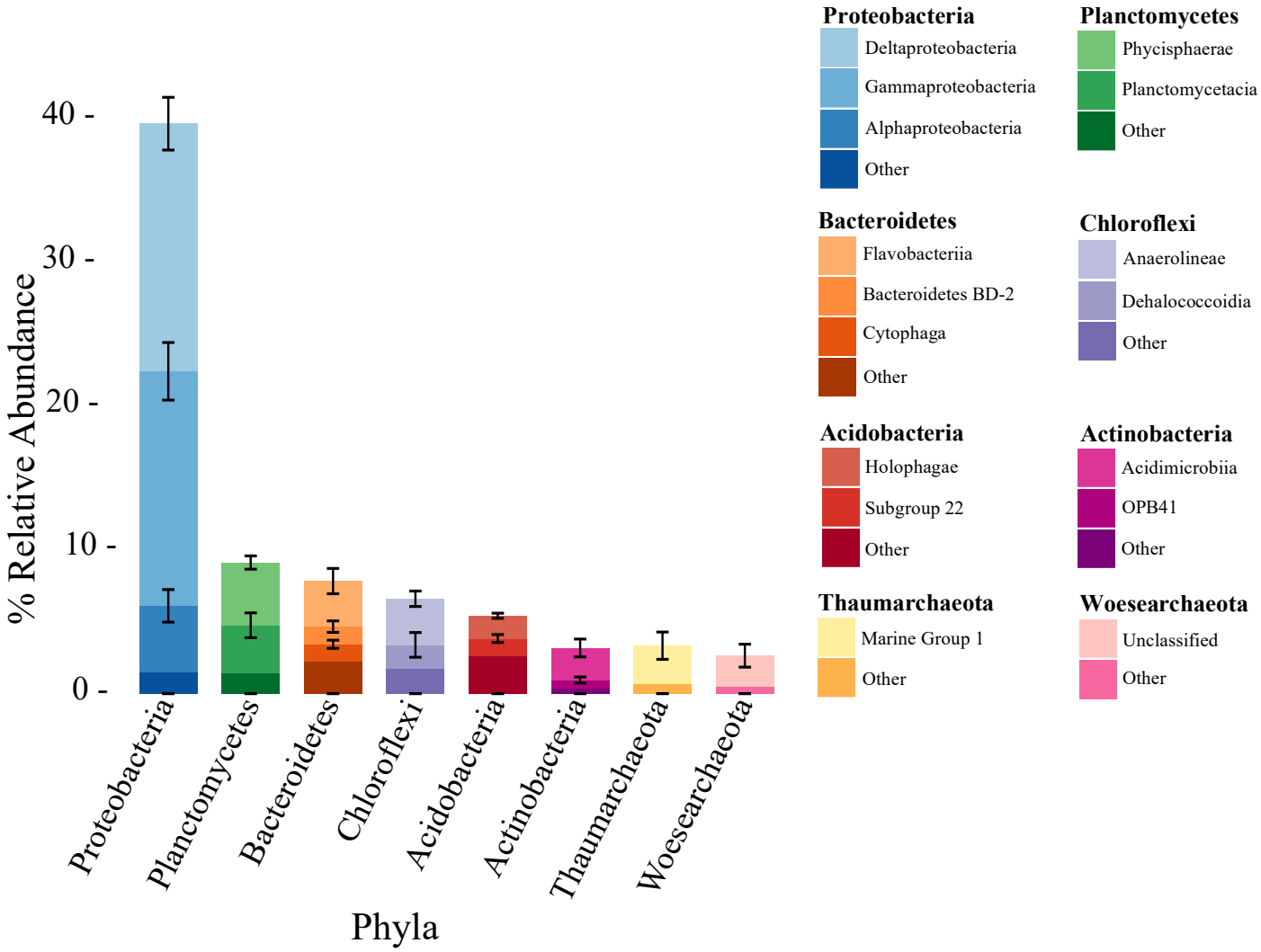

Figure 2.6: Top 8 most abundant microbial phyla and across all sediment sites. The 8 phyla were selected based on the depth-weighted average abundance across sediment sites and throughout the depths. Error bars indicate standard error. The relative abundance of reads per phylum was calculated as a percentage of the total reads for each sample.

would support aerobic metabolisms and thus alter microbial community composition. Oxygen was typically present in the upper 0-0.25 cmbsf (Table 2.2), and the microbial community composition in these uppermost sediments was very similar to that of the anoxic sediment interval immediately below, as well as deeper sediments (Fig. 2.7). The lack of strong vertical structure in the microbial community composition across most sites studied implies that the availability in respiratory electron acceptors, like O_2 and NO_3^-, is not a primary control on community composition. This may suggest instead that many of the sediment microbial community members are supported by metabolic substrates that do not vary strongly as a function of depth. While it is true that C_{org} is supplied from the overlying water as previously described, its concentration does not change dramatically as a function of depth (Fig. 2.8). In addition to respiration with terminal electron acceptors, C_{org} also supports fermentation[112]. Under anaerobic conditions fermentation plays a key role in breaking down complex C_{org} to more reactive molecules, like volatile fatty acids, that can be used in respiration[112]. Since the majority of the sediments studied are anaerobic, fermentation may be a dominant driving factor that provides the microbial community with the required electron donors for their metabolisms. Furthermore, fermentation may support much of the microbial community. If the same fermentable substrates were present throughout the sediment, this would result in the relatively constant microbial community composition, with little vertical structure, as observed here. Additionally, bioturbation has previously been implicated in homogenizing sediments and this may play some role in the apparent lack of strong vertical structure observed (as discussed further in 2.3.5). The phyla that are most abundant in the Canadian sediments studied here have also been identified in coastal sediments globally (Table 1.1), implying that they may represent a core microbial community in coastal sediments. Notably, the

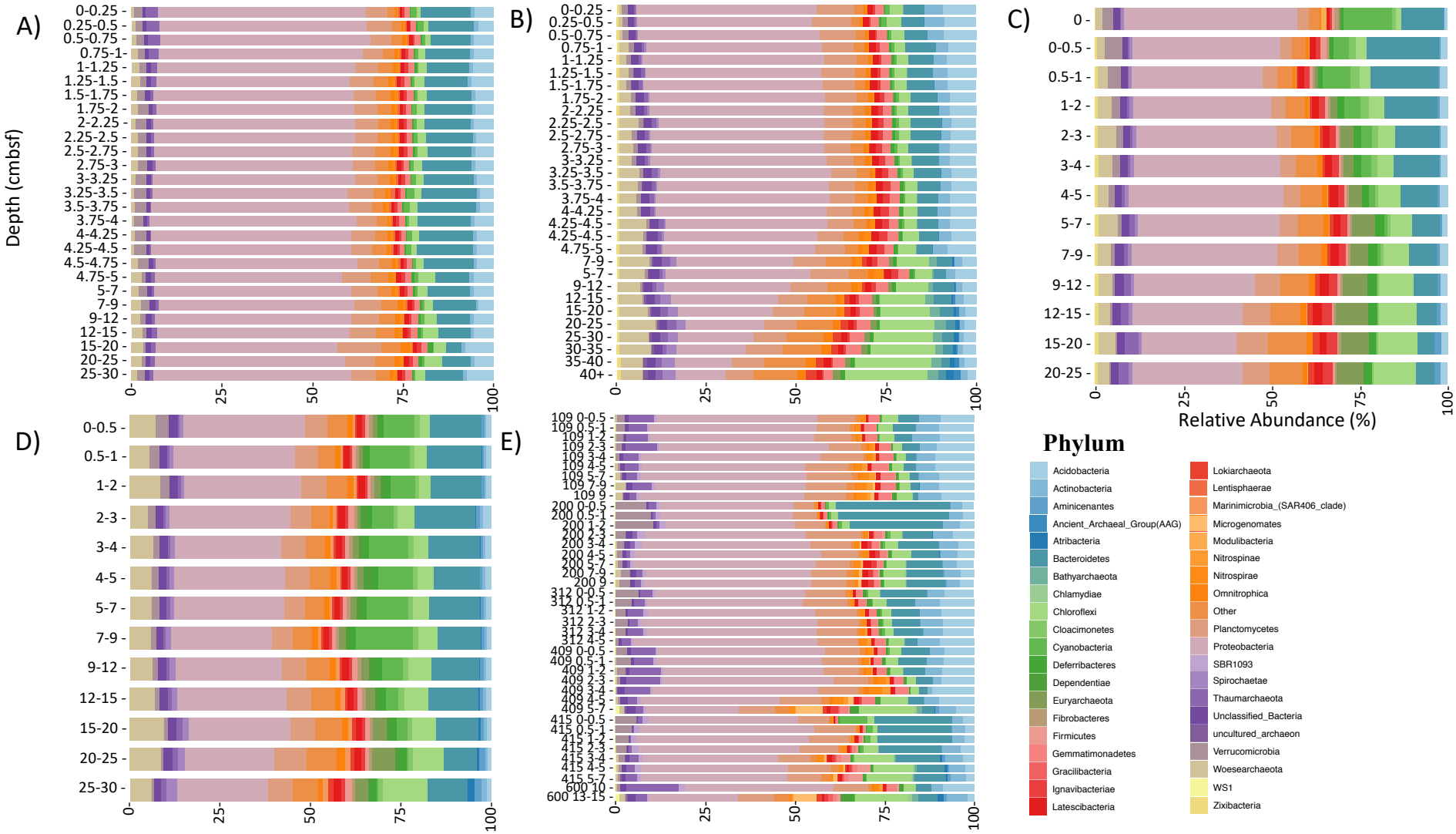

Figure 2.7: Distribution of 16S rRNA reads per phylum for the sediment sites. The relative abundance of reads per phylum is calculated as a percentage of the total reads for each sample. Other represents summed phyla that individually contributed <1% of the total number of reads per sample a) BC; b) JV; c) Saanich Oxic; d) Saanich anoxic; e) CASES.

vertical structuring of the microbial community composition in coastal sediments may not be driven by the canonical cascade of electron acceptor availability, as implied in most biogeochemical studies, and this should be explored in future work.

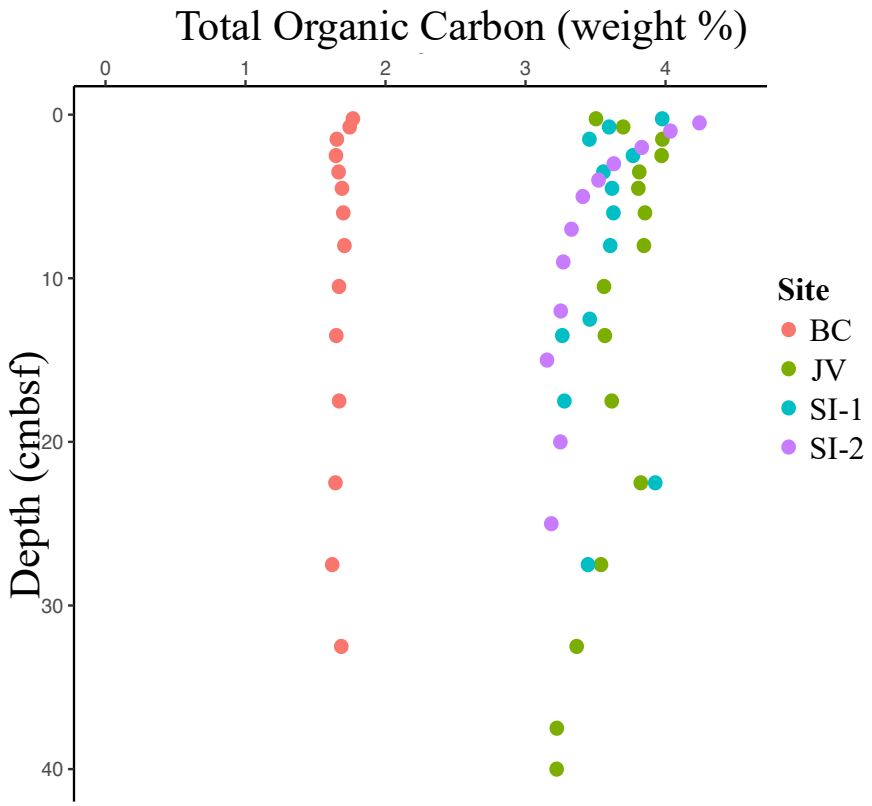

Figure 2.8: Total organic carbon (TOC) measurements (weight %) in sediments BC, JV, SI-1, and SI-2 with respect to depth.

There are cosmopolitan members of the core microbial community present across all Canadian coastal sediments, regardless of biogeographical location, indicating that these taxa comprise a portion of a core sediment community, which may be globally extensible. Of the 41,000 identified taxa, 1084 (2.6%) were shared across all Canadian coastal sediment sites. These 1084 shared taxa represented an outsized proportion of the microbial community, comprising between 51-75% of the total microbial community in the Arctic (CASES) sediments, and 33-63%of the community in the temperate sediments. At the species level (97% similarity in 16S SSU rRNA

gene sequences) the 15 most abundant microbial taxa made up 15% (± 0.10%) of the total microbial community in the sediments and mainly belonged to the Gamma-, Delta-, and Alphaproteobacteria classes (Figs. 2.6, 2.7). All 15 of the most abundant taxa reported in Fig. 2.9 are shared across all Canadian sediment sites, implying that they are cosmopolitan taxa. Of these 15, the 6 most abundant taxa each comprised ≥ 1% of the total microbial community, all of which were uncultured at the species level: *JTB255* marine benthic group species (sp.) (2.1% ± 0.41%), *Sandaracinaceae* sp. (1.4% ± 0.32%), *Desulfobulbaceae* sp. (1.2% ± 0.72%), *Flavobacteriaceae* sp. (1.2% ± 0.52%), *Desulfuromonadales* sp. (1.0% ± 0.50%), and *Hyphomicrobiaceae* (1.0% ± 0.40%). Out of these 5 species, all but the uncultured *Hyphomicrobiaceae* sp. have been previously reported in literature as being abundant (Table 1.1)[104]. The consistent abundance of these taxa, despite latitudinal differences, implies that these cosmopolitan constituents form a key component of the core microbial community in Canadian sediments and implies that these taxa may in fact be considerable contributors to a core global sediment microbial community.

Given the high global abundance of microorganisms in coastal sediments, cosmopolitan members of the core microbial community may be some of the numerically most abundant organisms on Earth. For example, based on the distribution of microbial abundances in coastal sediments from sites BC and JV (using cell counts, see 2.3.3), *JTB255* accounted for about 10^7 cells cm^{-2}. Assuming that the relative abundances of *JTB255* sediments from sites BC and JV are representative of coastal sediment microbial communities, the global population of *JTB255* in the top 40 cmbsf of coastal sediments may be as large as 2×10^{23}-4×10^{24} cells (0.8-5% of the total 4×10^{24}- 5×10^{26} microorganisms). Extrapolating to the top 1000 cmbsf of coastal sediments, the global community of *JTB255* may be as large as 2×10^{26}-1×10^{27} cells . Considering that *JTB255* is also abundant in deep ocean sediments and the oceanic deep subsurface more broadly, the global community of *JTB255* may approach 10^{28} organisms (see A.1, Fig. A.4, A.5). By comparison,

SAR11, thought to be the most abundant microorganism in the world, has an estimated global community of 2.4 x 10^{28} in the open ocean[116]. Given that sediment species *Sandaracinaceae*, *Desulfobulbaceae*, and *Flavobacteriaceae* are also cosmopolitan, and rival *JTB255* in relative abundance in Canadian coastal sediments, they too may be numerically important members of the global microbial community.

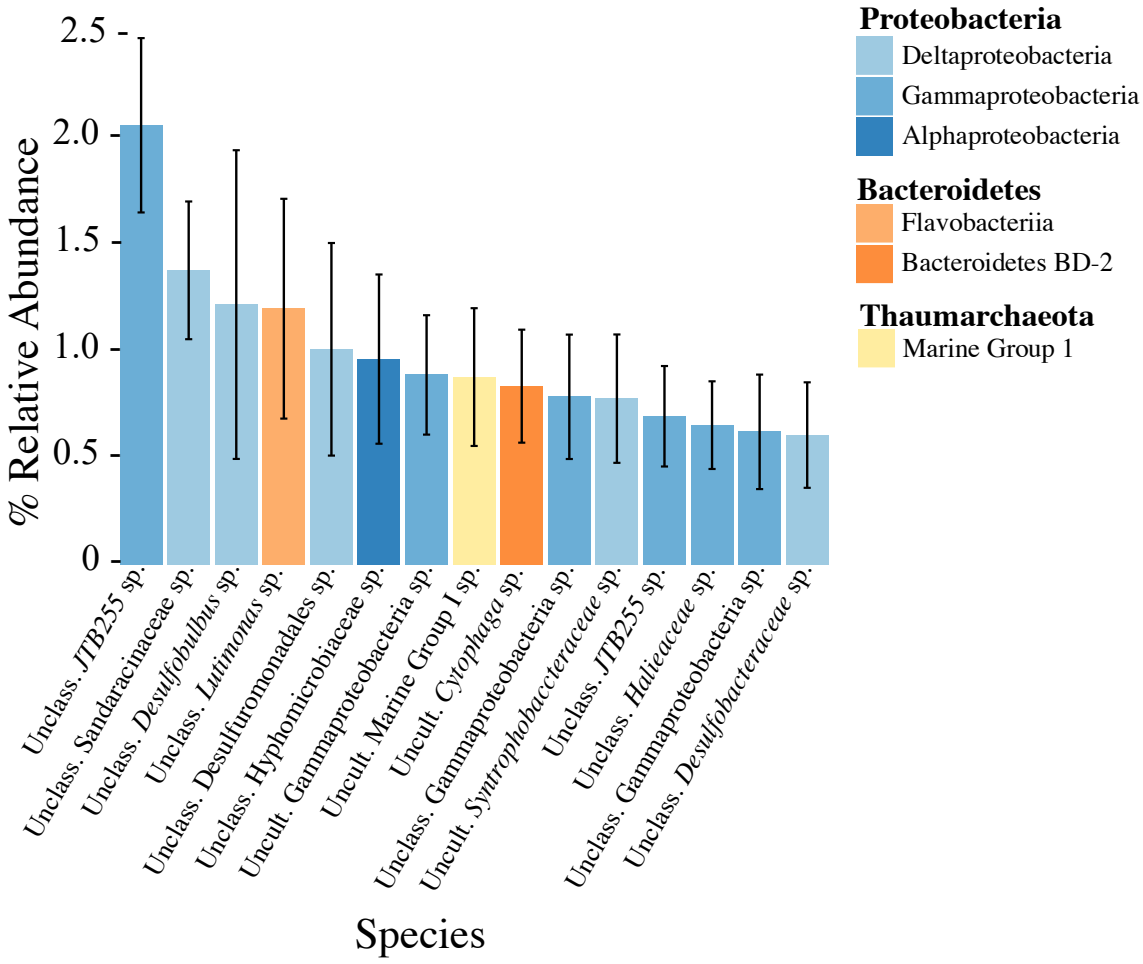

Figure 2.9: Top 15 most abundant microbial taxa (family level classification) across all sediment sites. Taxa were selected based on the depth-weighted average abundance across sediment sites and throughout the depths. Error bars indicate standard error. The relative abundance of reads per phylum was calculated as a percentage of the total reads for each sample. Abbreviations: Unclass., unclassified; Uncult., uncultured.

A core community of archaeal taxa was also present across the Canadian coastal sediments. The top 5 most abundant archaeal phyla comprised on average 8.3% (± 0.22%) of the total microbial community, the most abundant being the Thaumarchaeota (3.2% ± 0.70%) and Woesearchaeota (2.4% ± 0.60%) (Fig. 2.10, A.6). The highest relative abundances of Thaumarchaeota were present in the anoxic portion of the Canadian sediment, and the majority of the Thaumarchaeota identified belonged to either the Marine Benthic Group I (MBGI) or Group C3 classes (Fig. 2.10). Nitrosopumulis-type Thaumarchaeota have been previously reported in coastal sediments, as well as the Thaumarchaeota classes AK8, C3, Marine Benthic Group B, MBGI, pSL12, SAGMCG-1, and other unclassified classes[3,33,55]. Currently, there is only one existing cultured representative of Thaumarchaeota from coastal sediments, belonging to the Nitrosopumulis order, containing the *amoA* gene required for chemolithotrophic growth through NH_3 oxidation[117]. For the MBGI and C3 classes in coastal sediment, this chemolithotrophic potential has yet to be confirmed[118]. The Woesearchaeota in the Canadian coastal sediments belonged to unclassified and unidentified classes (Fig. 2.10, A.6). The Woesearchaeota are a recently classified phylum with no cultured representatives—so called microbial dark matter[119]—and, to date, they have not been identified in coastal sediments. Previous meta'omics studies, however, suggest that Woesearchaeota have metabolic potential for a fermentation-based lifestyle and are thought to be closely associated with methanogens[120]. Due to the lack of classification at the species level, it remains challenging with current informatics approaches to determine whether there are cosmopolitan archaeal taxa in coastal sediments, as they are grouped together as unclassified at genus and species level classifications (Fig. A.6). However, due to the global prevalence of Thaumarchaeota, I predict that Thaumarchaeotal taxa may be widely distributed in coastal sediments globally.

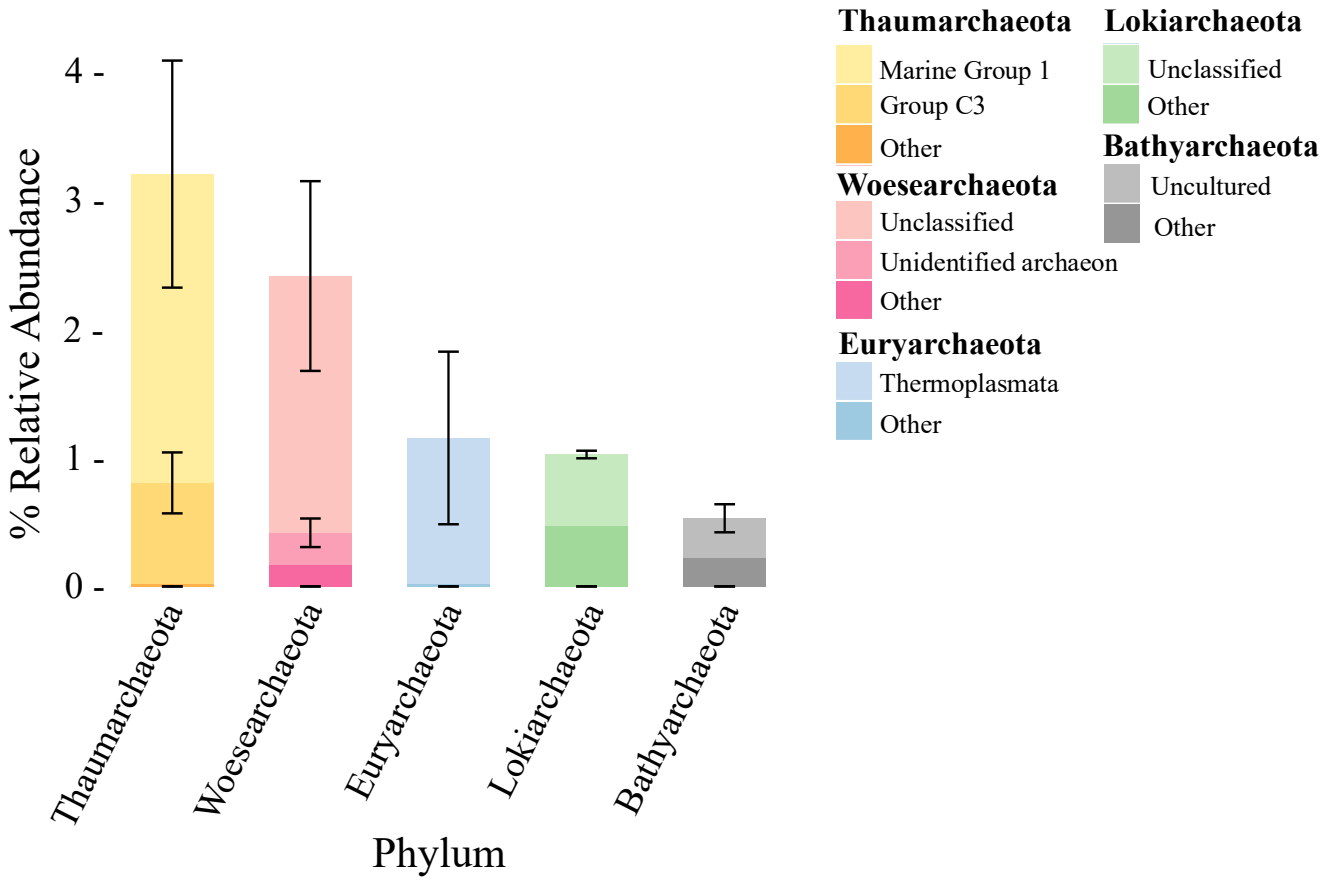

Figure 2.10: The 5 most abundant archaeal phyla across all sediment sites across all sediment sites. Phyla were selected based on the depth-weighted average abundance across sediment sites and throughout the depths. Error bars indicate standard error. The relative abundance of reads per phylum was calculated as a percentage of the total reads for each sample.

2.3.5 Sediment microbial community variation

Variability in community compositions amongst coastal sediments is primarily related to geographical location and depth below the sediment-water interface. Microbial communities within the sediments cluster primarily according to geographical location and then, to a lesser extent, with depth (Fig. 2.11). Statistical inference implies that 59% of the variability between the sediment microbial communities is related to geographical location, while another 12% of community variability is linked to sediment depth, whereas variability in this depth dependence across sites only accounted for a further 4% of community variability. 25% of the community variability was unrelated to the factors considered in my analysis and may instead be related to physical sediment properties and processes, geochemical characteristics, non-steady state

depositional conditions, or some combination of these. I predicted that the main source of variability between sites and depths is likely due to relatively low abundance organisms within the community (see 2.3.4). The taxa most clearly responsible for variability between geographical sites were generally unclassified at taxonomic resolutions higher than the family level and had low relative abundances (≤0.31%) (Table 2.4). Additionally, while these taxa largely belong to the accessory community, the SUP05 and unclassified Proteobacteria sp. from Clusters 1 and 5, respectively, were identified as cosmopolitan taxa. This is due to their relatively increased abundance in these clusters in comparison to their abundance across the sediment sites and depths. I thus conclude that the variance observed in coastal sediments is largely biogeographical in nature and is principally manifested by low-abundance community members specific to a particular site. Factors like substrate abundance and availability, that are strong functions of depth below the sediment-water interface, appear to play a secondary role in shaping community composition.

2.3.6 Comparison between water and sediment microbial communities

The historical assumption that bioturbation effectively mixes microbial communities in the uppermost sediment layers with that of the overlying water did not hold for the coastal sediments studied. Bioturbation in coastal sediments commonly mixes the uppermost layers of sediment, can reach as deep as 80 cmbsf, and the upper sediment was thus previously inferred to have a community composition similar to overlying water[75,121]. The microbial community composition in the uppermost layers of the Canadian sediments, however, was clearly different than the composition of the overlying bottom water at both the JV and BC sites (Fig. 2.12). In sediment 0-0.25 cmbsf, the *Flavobacteraceae* family is the only relatively abundant taxa shared between the sediment and overlying water at BC. The 0-0.25 cmbsf sediment from JV, however, did not share

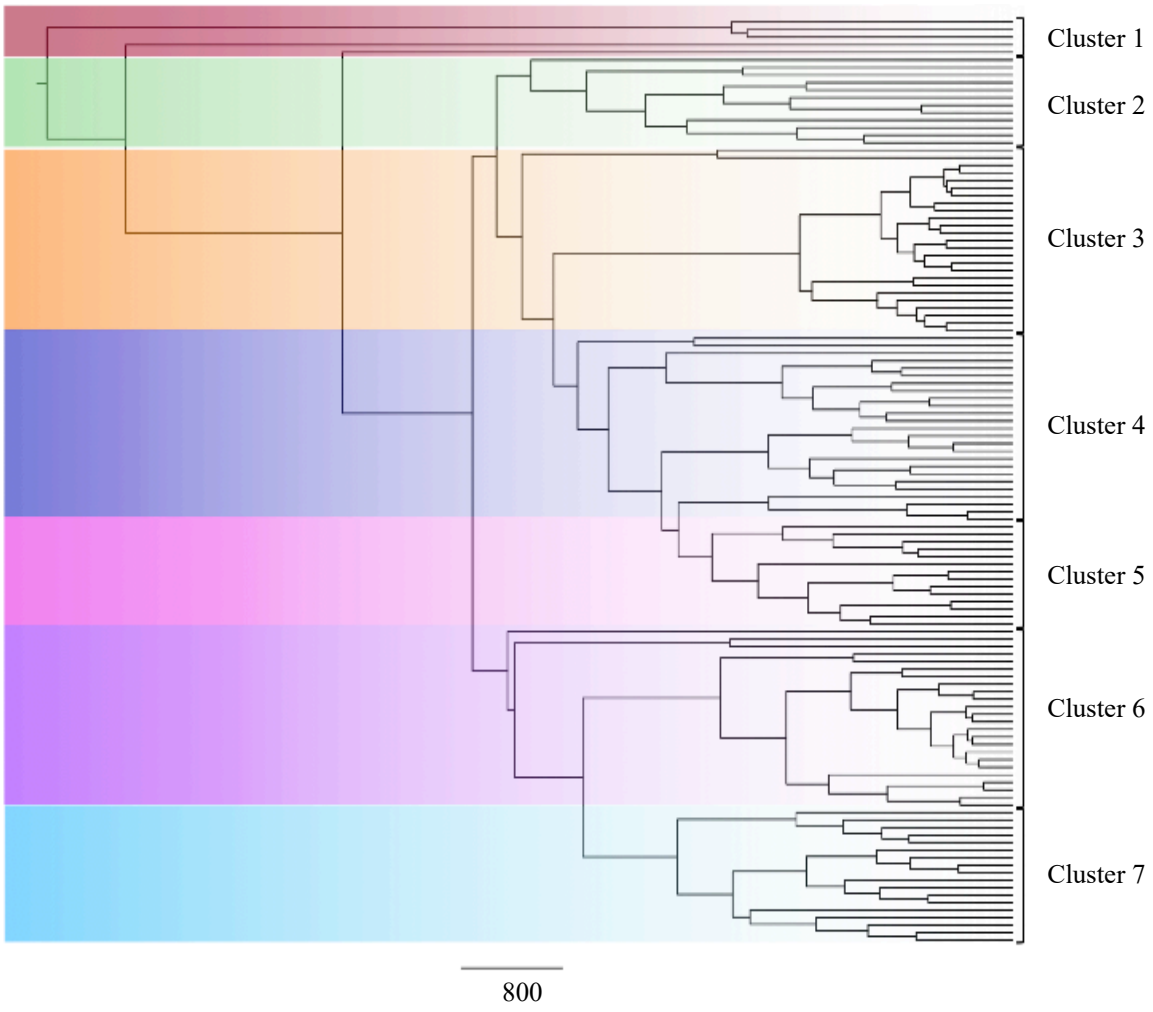

Figure 2.11: Hierarchical clustering of the sediment samples and depths. The clustering was performed by first generating a Euclidean distance matrix, then clustered using an "Average" clustering method. Colours indicate the distinct clusters used for the mISA (Table 2.4). Nodes correspond to the microbial community from the sediment site and depth interval sampled (Fig. A.7).

Table 2.4: Indicator species for hierarchical clustering groups identified in Figure 2.11. Indicator value threshold was 0.7. Average relative abundance for each taxon was calculated within the entire sample set and within the relevant cluster.

Taxonomy	Stat	P-value	% Avg. relative abundance in community	% Avg. relative abundance in cluster
Cluster 1 – CASES, SI-1				
SUP05	0.865	0.01	0.3052 (+/- 0.1889)	0.5946 (+/- 0.2828)
Rubritalea	0.857	0.005	0.0374 (+/- 0.0238)	0.2292 (+/- 0.1156)
Nannocystaceae	0.842	0.005	0.0057 (+/- 0.0035)	0.0360 (+/- 0.0201)
Cluster 2 - CASES				
Unknown Gammaproteobacteria Incertae Sedis	0.926	0.005	0.0165 (+/- 0.0120)	0.1445 (+/- 0.0428)
Ambiguous PAUC43f (Gemmatimonadetes)	0.918	0.005	0.0027 (+/- 0.0016)	0.0359 (+/- 0.0109)
Anaerolineaceae	0.915	0.005	0.0058 (+/- 0.0041)	0.0756 (+/- 0.0205)
Cluster 3 – BC				
Ambiguous Nitrospinae	0.979	0.005	0.0011 (+/- 0.001)	0. 0353 (+/- 0. 0049)
Uncultured Subgroup 21 (Acidobacteria)	0.968	0.005	0.0020 (+/- 0.0020)	0. 0638 (+/- 0. 0.0087)
Uncultured Subgroup 21 (Acidobacteria)	0.968	0.005	0.0020 (+/- 0.0020)	0. 0213 (+/- 0.0036)

Taxonomy	Stat	P-value	% Avg. relative abundance in community	% Avg. relative abundance in cluster
Cluster 4 – JV, BC, SI-1, SI-2				
Unclassified Parcubacteria	0.788	0.005	0.0004 (+/- 0.0002)	0.1234 (+/- 0.0667)
Uncultured Gemmatimonadetes	0.785	0.005	0.0399 (+/- 0.0312)	0.4923 (+/-0.1551)
Unclassed TM6 (Dependentiae)	0.783	0.005	0.0098 (+/- 0.0201)	0.0486 (+/- 0.0151)
Cluster 5 – SI-1, SI-2				
Unclassified Proteobacteria	0.996	0.005	0.0463 (+/- 0.0433)	0.4250 (+/- 0.1222)
Uncultured Marine Benthic Group D and DHVEG-1	0.983	0.005	0.0259 (+/- 0.0213)	0.2402 (+/- 0.0772)
Unclassified Coxiellaceae	0.981	0.005	0.0043 (+/- 0.0022)	0.2617 (+/- 0.0802)
Cluster 6 – JV, CASES				
Uncultured Gammaproteobacteria	0.995	0.005	0.0035 (+/- 0.0033)	0.0561 (+/- 0.0047)
Desulfarculaceae	0.995	0.005	0.0003 (+/- 0.0002)	0.0041 (+/- 0.0012)
Desulfurellaceae	0.987	0.005	0.0011 (+/- 0.0010)	0.0151 (+/- 0.0029)
Cluster 7 - CASES				
Uncultured SVA0725 (Acidobacteria)	0.959	0.005	0.0013 (+/- 0.0006)	0.0053 (+/- 0.0014)
Unclassified Latescibacteria	0.954	0.005	0.0024 (+/- 0.0012)	0.0081 (+/- 0.0009)
JTB255 marine benthic group	0.943	0.005	0.0040 (+/- 0.0020)	0.0139 (+/- 0.0033)

any relatively abundant taxa with the bottom water. In the 0-12 cmbsf depth interval the most abundant shared families between the bottom water and sediments belonged to *Planctomycetaceae*, unclassified Bacteria, and unclassified *Gammaproteobacteria* families at site JV, and the *Flavobacteraceae* family at site BC. The presence of shared taxa in the 0-12 cmbsf JV profile, and the absence of shared taxa in the 0-0.25 cmbsf sediment profile, is likely due to the presence of other lineages belonging to these families in the sediments that are not shared at the species level. Upon verification, I confirmed that only a portion of the families shared between communities were also shared at the taxa level. For example, the 0-12 cmbsf JV microbial community was comprised of 1.7% of the *Planctomycetaceae* family, however at the species level only 0.25% of these taxa were shared with the bottom water. While bioturbation is important for mixing coastal sediments, these sediments maintain microbial communities with compositions largely distinct from the overlying water.

Even though bioturbation did not appear to appreciably impact the sediment microbial community composition, it likely affected the vertical structure of communities and the lack of strong vertical stratification in the coastal sediment communities studied may be attributed to bioturbation. If the rate of bioturbation in coastal sediments outpaces cellular turnover, it is expected that the microbial community will be homogenized, whereas if the rate of bioturbation in sediments is slower than cellular turnover, the microbial community would be vertically stratified. The qPCR and cell counts indicate that this may be the case, as the microbial communities are present at a relatively consistent abundance throughout the top 12 cmbsf of the sediment core. Additionally, while there was a minor decrease in diversity with respect to depth, species richness in the sediments remained relatively consistent overall, with the exception of JV as previously discussed. This is mirrored in the microbial community composition (Fig. 2.6, 2.7), where there were no appreciable changes in the microbial communities in the upper 12 cmbsf of

the sediment core. These findings indicate that bioturbation may play an equally important role in the vertical sediment community structure as geochemical zonation.

2.3.7 Structure of coastal sediment communities

The Canadian coastal sediments share a microbial community network that is highly interconnected. A strong correlation between taxa results from the simultaneous increase or decrease in the relative abundance of both taxa in response to a variable, or suite of variables. The strongly correlated taxa that emerge in the sediment network are primarily cosmopolitan taxa (76 out of 82 nodes), and while 14 out of the 15 most abundant taxa (Fig. 2.9) are present within the network, the majority of the remaining 62 nodes are lesser abundant taxa present across most sediment sites (Fig. 2.32a). While the abundant cosmopolitan taxa are integral to the network structure, the lesser abundant cosmopolitan taxa more often act as network hubs (Fig. 2.13b). The high degree of connection between these lesser-abundant taxa implies that they likely respond to the same combined set of variables as their neighbors. Given that sediment microbial communities are supported through the breakdown of organic matter, community members that are highly connected likely play a concerted role in organic matter mineralization, and the associated reactions that contribute to early diagenesis. For example, the *JTB255* taxa that is located at the center of the network is relatively low-abundance, however its high degree suggests that it responds to the same suite of variables as its neighbors (Fig. 2.13a,b). As previously mentioned, the *JTB255* family is predicted to play a role in C_{org} remineralization, and the taxa that it is correlated with are likely dependent, along with *JTB255*, on the variables that result in increased or decreased rates of C_{org} remineralization, such as sedimentation rate. Notably, the majority of the dominant cosmopolitan taxa are located along the periphery of the network and interact with fewer neighbors, and while edge distance between taxa doesn't correlate with a measured feature of the

Figure 2.12: Comparison of the most abundant microbial families in the JV and BC bottom water and sediment (sediment sites separated by the dashed line). Bottom water was collected at water depths of 350 and 500 m for BC and JV respectively. Sediment was collected from 360 and 510 mbsl for BC and JV respectively. The relative abundance is denoted by the bubble size, and sediment depth profiles of 0-0.25 and 0-12 cmbsf for each site were analyzed.

network, the relatively low number of neighbors of these taxa implies that they are less dependent on these variables than the lesser abundant taxa. The independence of the dominant cosmopolitan taxa from these variables may indicate why they are found at higher abundances across the sediments. Overall, while this network does not provide information on the type of interaction occurring between the co-occurring taxa – for example, whether they co-occur through metabolite exchange or through the response to an external variable, such as TOC concentration – it has begun to establish a framework of hypothesis that can be the focus of future work. Specifically, since this network was generated based on microbial community compositions sourced from geographically different sediment sites, this microbial network likely serves as a foundation that shapes the microbial community at each sediment site. For example, now that it has been determined that

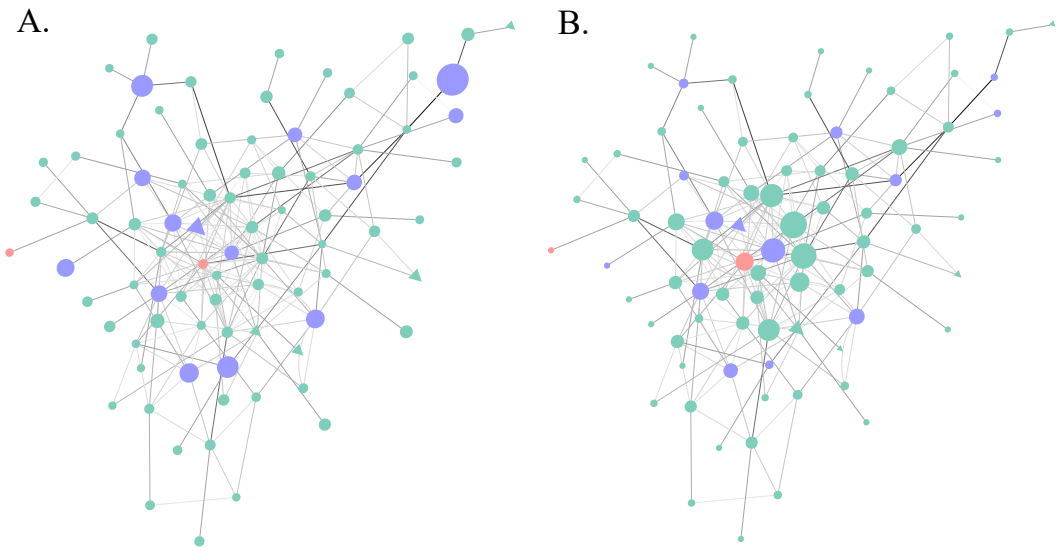

Figure 2.13: Correlation network analysis of taxa from all sediment sites and depth intervals. Edge darkness is correlated to high edge betweenness, node shape—circle or triangle—is correlated to either bacterial or archaeal taxa respectively, purple nodes indicate the most abundant taxa identified in Figure 4 and pink nodes indicate taxa belonging to the JTB255 family. Nodes correspond to individual taxa, and edges indicate a co-occurrence between the taxa. A) Node size is directly proportional to taxon relative abundance; B) node size is directly proportional to node degree. Pearson correlation coefficient threshold was set to 0.84.

that cosmopolitan taxa, such as *JTB255*, likely play an integral role in regulating network interactions, future work can now be framed to specifically investigate hypotheses framed by these observations. Given that the most abundant taxa in the sediments have been previously identified in global studies, the network properties identified here may extend to coastal sediments more broadly, thus allowing for the extrapolation of new knowledge on the functions, composition, and structure of the coastal sediment microbial community on the global scale.

2.4 Conclusion

Through the integration of microbiological data with microbial respiration rates, I have begun to link the sediment microbial community in global biogeochemical cycles. Specifically, the global abundance of cosmopolitan taxa in coastal sediments strongly implicates them in global biogeochemical cycling. Despite their hypothesized importance within coastal sediments, these cosmopolitan taxa remain largely uncharacterized, and moving forward the focus should be on better constraining their metabolic potential and taxonomic classification. Additionally, the major source of variance in the coastal sediment microbial community is primarily associated with geographical location. The portion of the microbial community that is affected, however, is mostly an accessory microbial community that is not shared amongst all sites. While this accessory microbial community generally comprises a lesser portion of the microbial community, with the lowest abundances generally in the less diverse environments, it likely still plays a role in the in global biogeochemical cycling. That being said, further work needs to be performed to better constrain both the extent of diversity within coastal sediment communities, their community composition, and the roles their respective accessory community plays in relation to global biogeochemical cycling. Overall, the new knowledge generated through this work can be used to further refine global estimates of sediment diversity, composition, and structure, allowing us to

more accurately highlight the role that the sediment microbial community plays in global biogeochemical cycles.

Chapter 3: Conclusions

The work conducted in this thesis presents the microbial community diversity, composition, and structure in northern and western Canadian coastal sediments. Through this work, I identified a shared microbial community comprised of cosmopolitan taxa in across the sediments, accompanied by an accessory community that varied largely between sediment sites due to geographical effects. Through this work we have generated new knowledge that can be utilized to better inform researchers that are studying microbially facilitated biogeochemical cycling in Canadian sediments. Additionally, the work presented here is the first step towards identifying the microorganisms in the coastal sediment microbial community that may influence global biogeochemical cycles through their underlying metabolisms.

3.1 Summary

This thesis described the microbial community diversity, composition, and structure of the Canadian coastal sediment sites JV, BC, SI-1, SI-2, and CASES. A range of diversities were identified across the sediments, which is consistent with previous literature on coastal sediments. When compared to soil microbial studies performed globally with similar sequencing technologies, I reported a species richness that rivals that of soil. Through the use of 16S rRNA sequencing, I identified a core microbial community comprised of cosmopolitan taxa present in the Canadian sediment sites that are likely significant contributors to global biogeochemical cycling. While the relative abundances of these cosmopolitan taxa varied between sediment sites, in general they comprised an appreciable portion of the total microbial community. Notably, the most abundant taxa identified in the Canadian coastal sediments were cosmopolitan, and taking into account absolute cell abundance estimates, we predict that they may be numerically important in the global sediment microbial community. The global abundance of these cosmopolitan taxa

partnered with prior predicted rates of biogeochemical activity strongly implicates them in global biogeochemical cycles. For example, as discussed above, I predict that *JTB255* comprises an appreciable portion of the global sediment microbial community, and by extension the global microbial community. This relatively high abundance, partnered with the capacity for a broad range of energy-yielding metabolisms, including chemolithoautotrophy, heterotrophy, aerobic, and anaerobic respiration, likely has an impact on global biogeochemical cycling[122]. Additionally, the prevalence of the cosmopolitan taxa in the sediment community network highlights their capacity to be key players in the sediment community structure, either through putative interactions with other taxa, or with their surrounding environment. Overall, the core microbial community identified in the Canadian sediment sites, partnered with previous reports of the same taxa identified globally, implies that the microbial community identified through this work may be more broadly extensible to coastal sediments formed under differing depositional environments.

3.3 Looking ahead

The extent to which coastal sediments harbour a globally ubiquitous core microbial community needs to be tested in a suite of sediments recovered from sites with a broader geographical distribution. Indeed, while I identified a core community comprised of cosmopolitan taxa in Canadian sediments and was able to link these taxa to their presence in other sites reported in existing literature, a systematic comparative analyses would provide a more robust test of my findings from the Canadian sites studied. Furthermore, an integrated approach using both geochemical and microbiological analyses is the critical next step that would better link the metabolic capacity of the microorganisms in the sediment to their role in global biogeochemical cycling. Here, metagenomic techniques would help with the reconstruction of the enzymatic repertoire of key members of coastal sediment microbial communities and it is this repertoire that

directly catalyzes biogeochemical cycling. Additionally, coupled process rate measurements and metagenomic analyses holds considerable power for connecting genotypic variability in sediment community composition to its phenotypic expression relevant to biogeochemistry. The work presented in this thesis is thus a first step towards linking microbial communities in coastal sediments to their role in biogeochemical cycling and further identifies key organisms to target, in enrichment-cultivation and possibly single-cell sequencing efforts.

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Appendix

Table A.1: Cell count values for JV and BC sediments. Cell counts are presented in the log scale over the sediment depth intervals.

Site	Community size (cells)	Community size, 0-1000 cmbsf (cells)
JV	4.4×10^{26}	2.00×10^{28}
BC	5.0×10^{26}	1.18×10^{27}
SI-1	3.1×10^{26}	7.82×10^{27}
SI-2	4.1×10^{26}	7.76×10^{26}
CASES 109	3.4×10^{26}	1.69×10^{26}
CASES 200	1.2×10^{25}	6.30×10^{26}
CASES 312	7.5×10^{25}	2.37×10^{26}
CASES 409	1.6×10^{25}	6.87×10^{26}
CASES 415	7.4×10^{24}	8.03×10^{26}
CASES 600	4.6×10^{24}	1.23×10^{27}

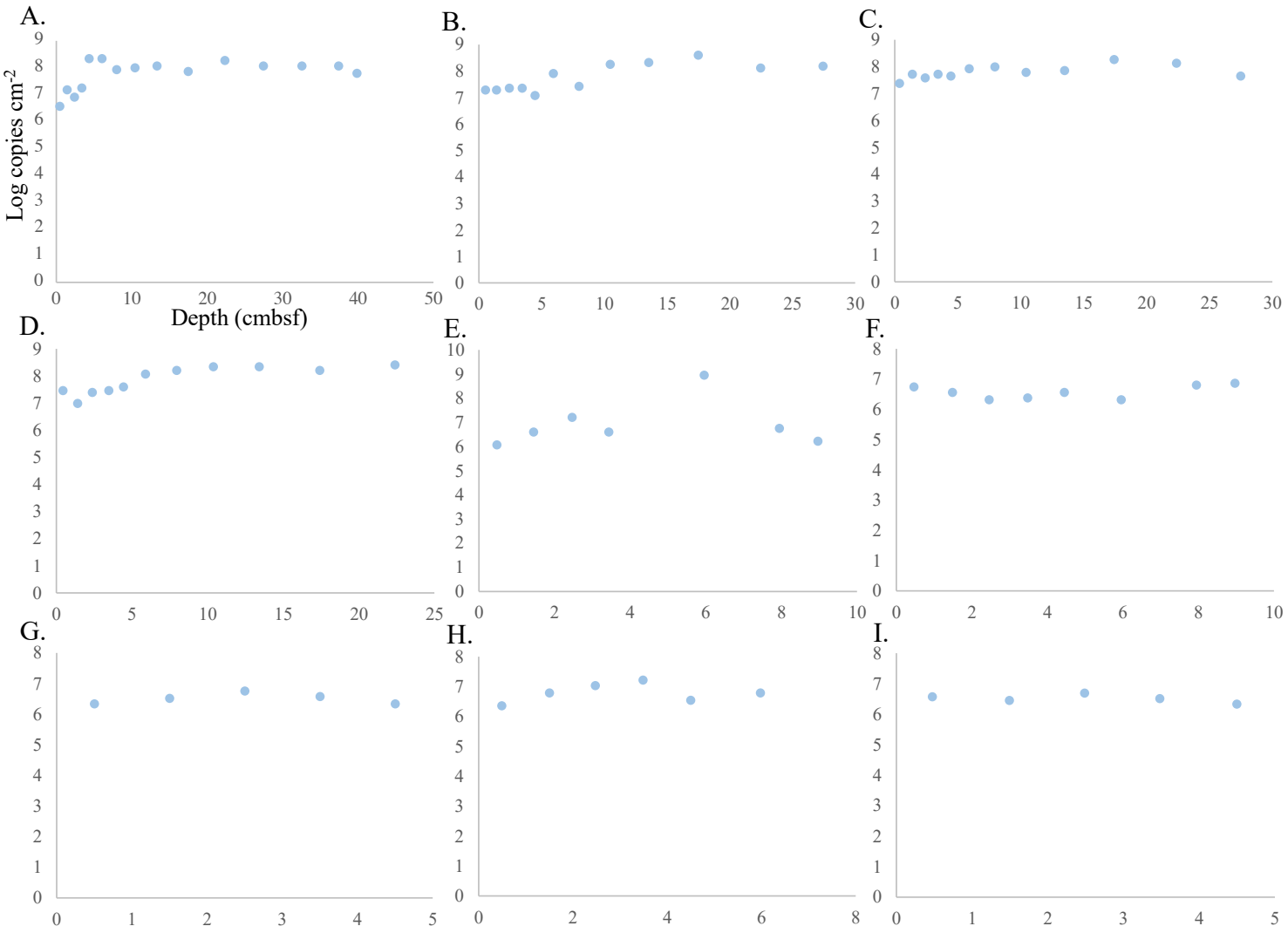

Figure A.1: qPCR quantification of the sediment microbial communities in A) JV; B) BC; C) SI-1; D) SI-2; E) Cases 109; F) Cases 200; G) Cases 312; H) Cases 409; J) Cases 415. qPCR measurements are presented in the log scale.

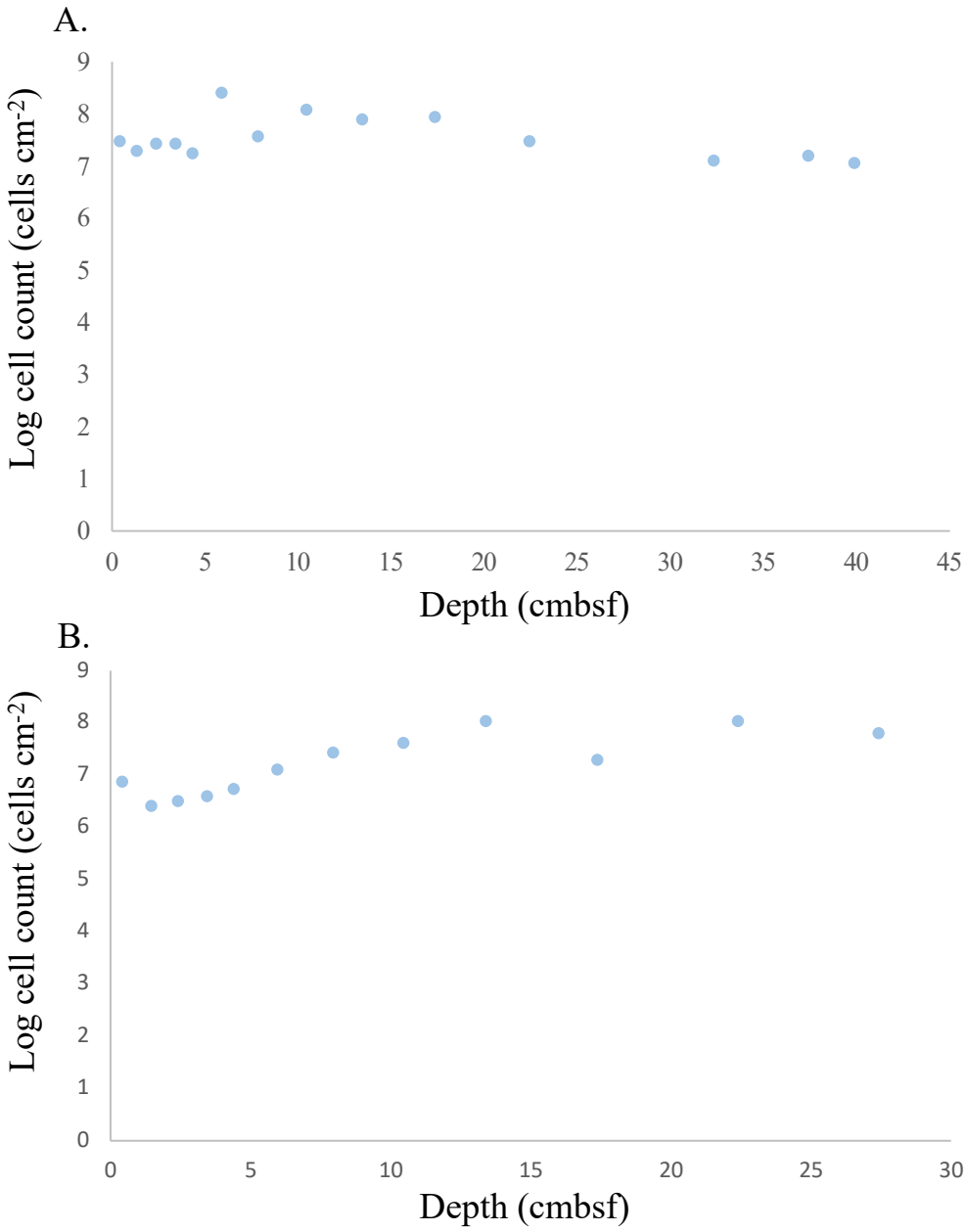

Figure A.2: Cell count quantification of the sediment microbial communities in A) JV; B) BC. Cell count measurements are presented in the log scale.

Table A.2: Chao1 richness estimates of Canadian sediment sites. Diversity estimates were averaged over the depth ranges of 0-2, 2-4, 7-9, 9-12, 12-20, 20-25, and 25-30 cmbsf

Site	Chao (OTUs)
BC	12,171-14,609
JV	7,482-13,812
SI-1	10293-13,538
SI-2	11,635-14,847
Cases 109	3,710-8,481
Cases 200	5,392-7,063
Cases 312	3,544-8,763
Cases 409	8,062-10,147
Cases 415	8,062-10,147
Cases 600	5,072-8,831

A.1 JTB Abundances globally

Members of the *JTB255* family have been reported from coastal and deep sea and sediments, globally [31,35,104](Fig. A.4), where they are present at similar relative abundances to what have been found in the Canadian coastal sediments[31]. The *JTB255* family represented 519 (1.3%) of the 41,000 OTUs in the Canadian sediments. The average relative abundance of all 519 taxa combined was 4.8% (± 0.89%) of the total microbial community, accounting for on average 39% (± 4.4) of *gammaproteobacterial* sequences. Leveraging my estimates (see 2.3.5) with data from previous work identifying *JTB255* marine benthic group as the most abundant taxa in deep sea sediments, I predict that the relative abundance of *JTB255* marine benthic group in oceanic subsurface sediments (coastal and deep-sea) is about 3.8% (± 0.38%)[104].

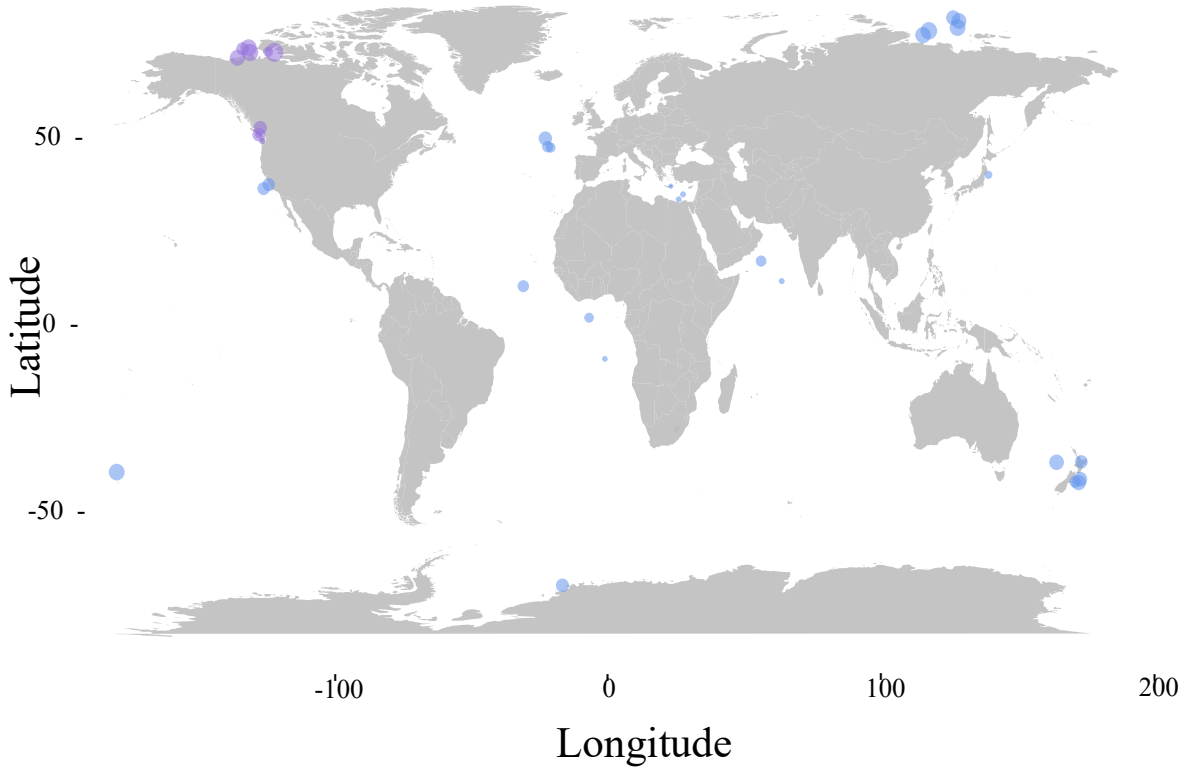

Figure A.3: Global abundances of *JTB255* using JV, BC, SI-1, SI-2, CASES and deep-sea sediments[50] (see 2.2.8). Purple bubbles correspond to estimates from this work; blue nodes correspond to deep sea sediment data, re-processed and analyzed in this work (see 2.2.8).

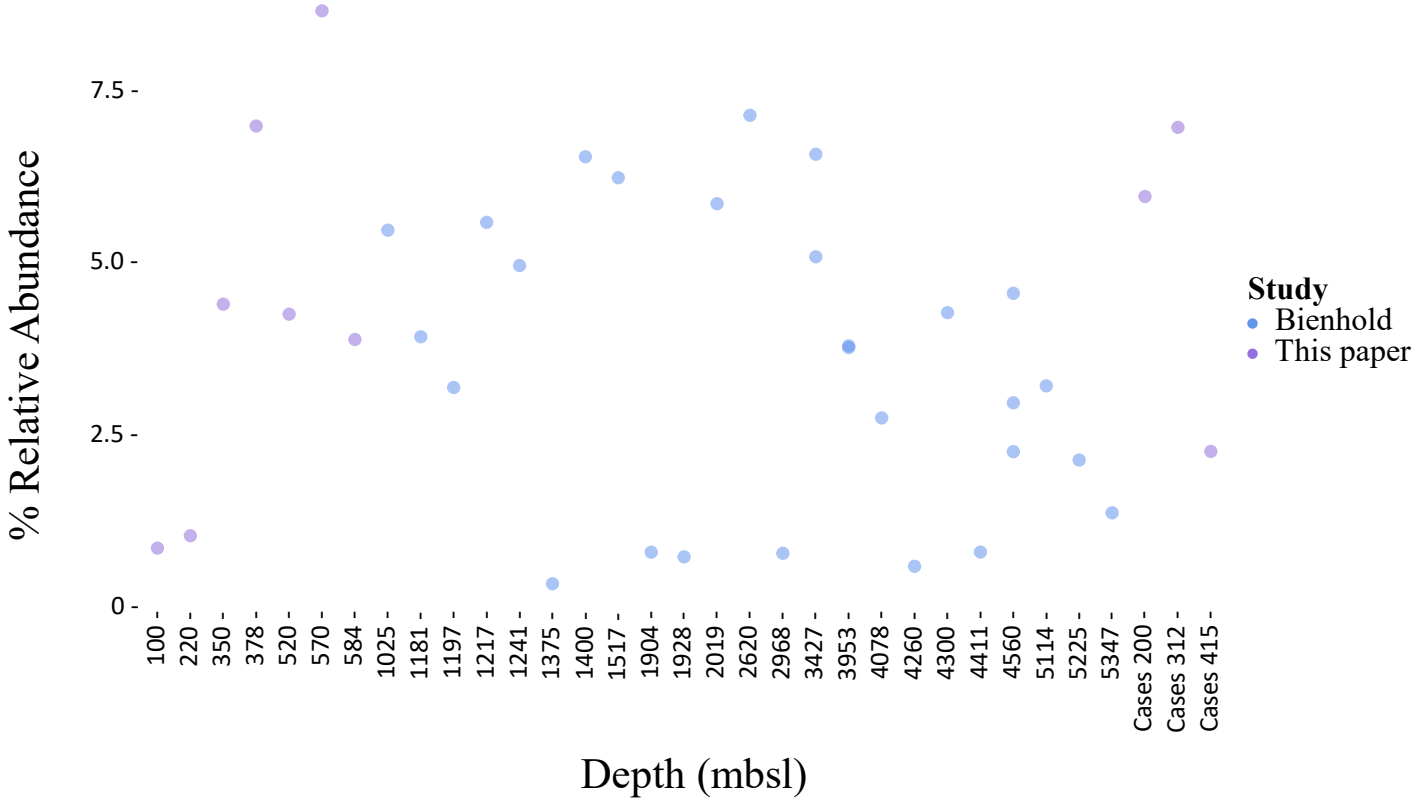

Figure A.4: The relationship between *JTB255* relative abundance and depth of sediment CASES 200, 312, and 415 did not have depths assigned to them. Purple bubbles correspond to estimates from this work; blue nodes correspond to deep sea sediment data, re-processed and analyzed in this work (see 2.2.8).

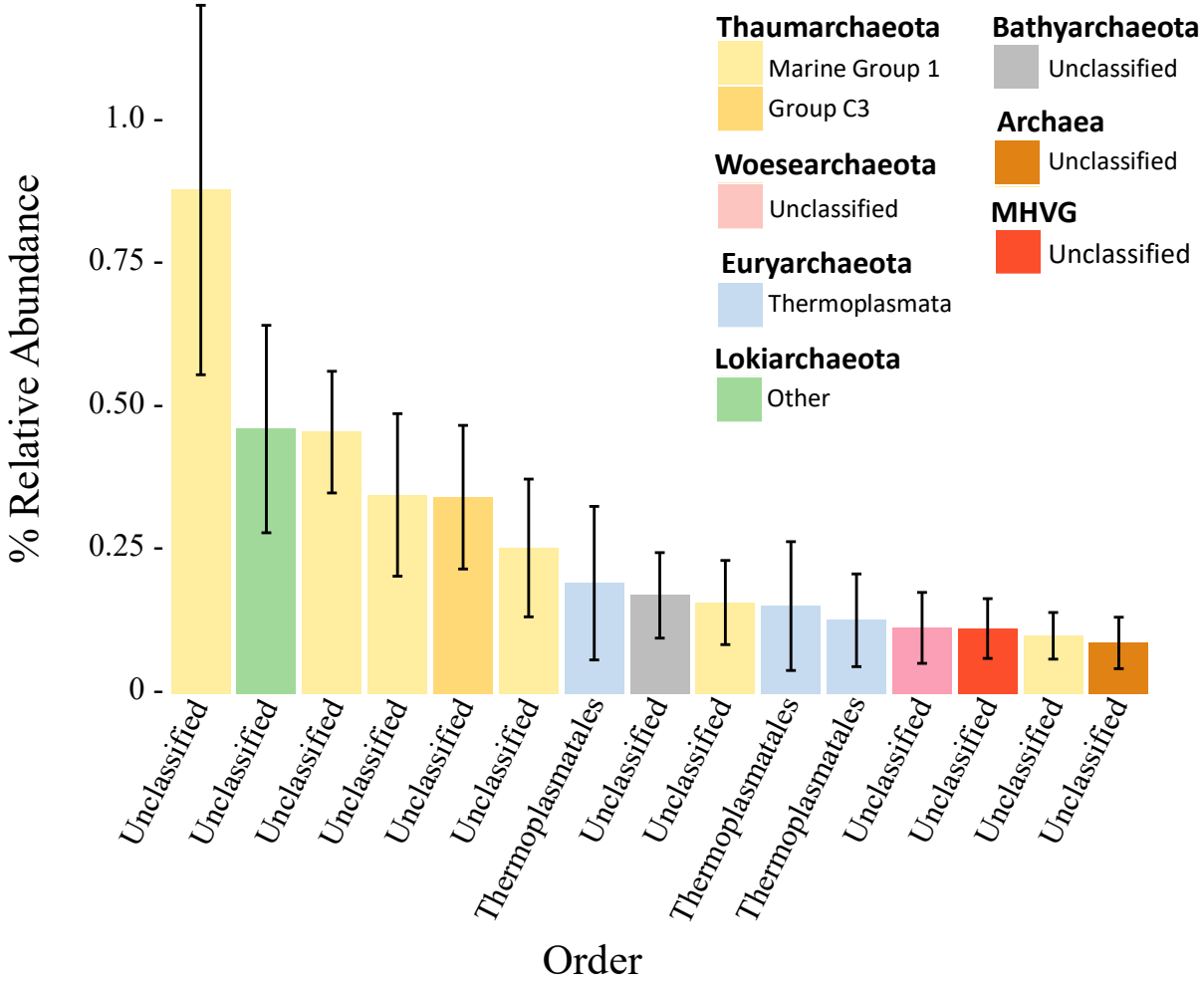

Figure A.5: Top 15 most dominant archaeal taxa at the species level (order level classification) across all sediment sites. Taxa were selected based on the depth-weighted average abundance across sediment sites and throughout the depths. Error bars indicate standard error. The relative abundance of reads per phylum was calculated as a percentage of the total reads for each sample.

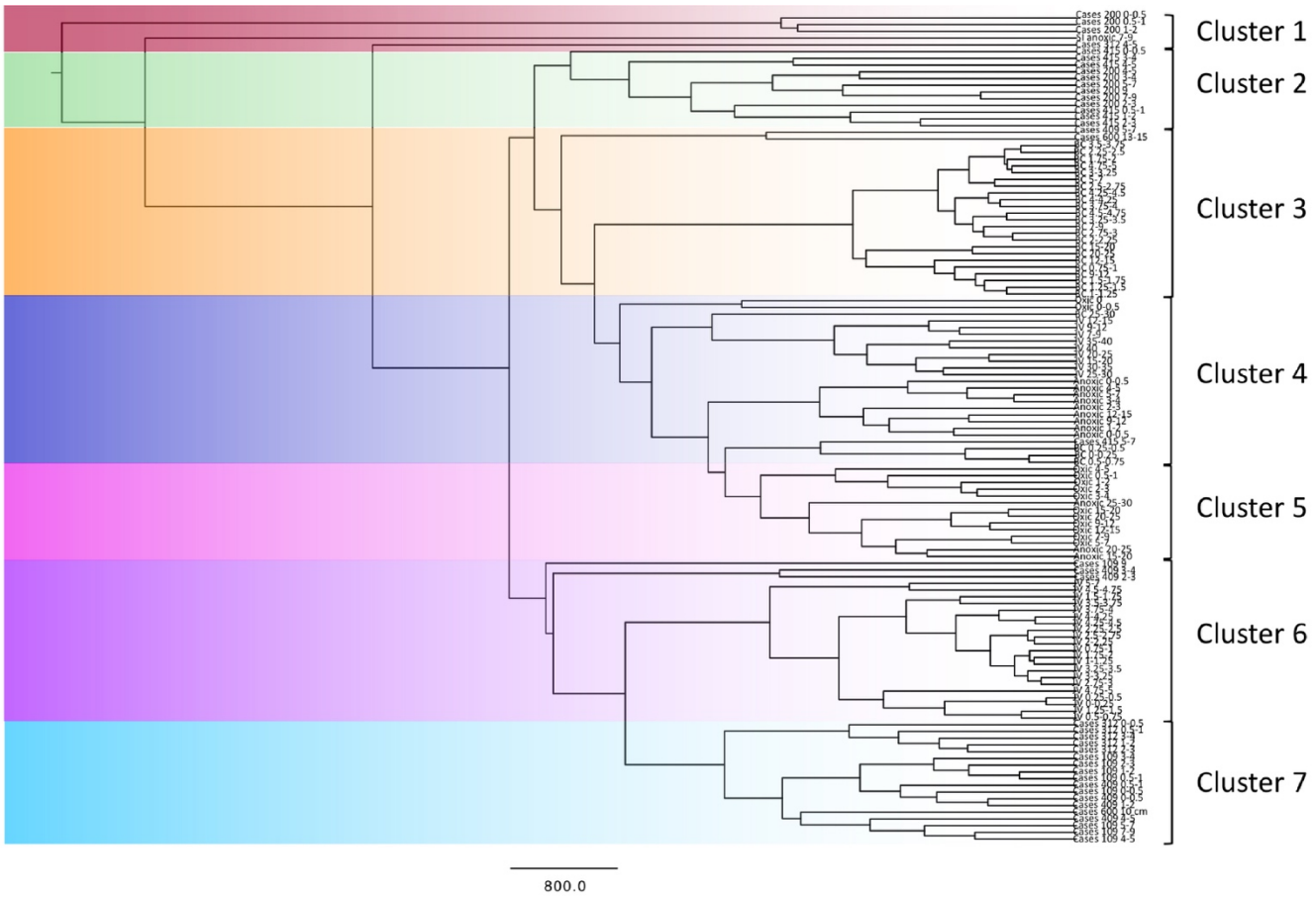

Figure A.6: Hierarchical clustering of all sediment samples and depths. The clustering was performed by first generating a Euclidean distance matrix, then clustered using an "Average" clustering method. Colours indicate the distinct clusters used for the mISA (Table 2.4). Node labels correspond to the microbial community sediment site and depth.