

Microbial Communities Transforming Dissolved Organic Matter

In a Large Estuarine Ecosystem

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ABSTRACT

Heterotrophic bacteria are responsible for degrading dissolved organic matter (DOM), and processes 50% or more of Earth's net primary production. Although integral to global nutrient cycling, the complexity of bacterial communities makes it difficult to resolve the mechanisms by which they degrade DOM. Adding to the complexity of this interaction is the compositional diversity of DOM. The St. Lawrence Estuary (SLE) is an important repository for DOM, produced both internally by phytoplankton and externally by terrestrial plants. I aim to identify the bacterial taxa that respond to differential DOM inputs using 16S rRNA abundance as a proxy for metabolic activity. A microcosm experiment was conducted in the SLE in which marine DOM and terrestrial DOM were extracted by ultrafiltration and solid-phase extraction. DOM extracts were amended to microcosms of raw SLE water and incubated at 7°C and 25°C for 32 hours. The Gammaproteobacterial lineage *Pseudoalteromonas* experienced a 70% increase in metabolic activity in response to HMW marine DOM at both 7°C and 25°C, which was not observed in any other DOM treatment. Terrestrial DOM treatments resulted in a significant increase in alpha-diversity within the bacterial community at 25°C, indicating a relative increase in the activity of rare bacteria in response to freshwater DOM.

Microcosm experiments such as this aim to provide a better understanding of how DOM composition can influence bacterial community structure and metabolism. Considerations for future experiments include transcriptomics analysis to describe the metabolic pathways involved in DOM degradation.

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1. Introduction

1.1 Microbial diversity

Microbial life on Earth comprises the most abundant and diverse groups of organisms, spanning all three domains of life (Fox et al. 1977), although the abundance and diversity of the prokaryotic Bacteria and Archaea is greater than that found within the Eukarya domain, as can be illustrated in the metabolic capacity of these groups. Where the Eukarya are able to utilize two basic types of metabolism; autotrophy and aerobic heterotrophy, Prokaryotes are able to utilize novel metabolic strategies allowing them to survive in environments with or without oxygen, light, or organic molecules (Johnson & Sieburth 1979; Waterbury et al. 1979; Staley & Konopka 1985; Chisholm et al. 1988; Beja 2000; Bèjà et al. 2002; Bremer & Dennis 1996; Button 1998; CA Carlson & Giovannoni 2002; Craig A Carlson et al. 2004; Azam 1998). This metabolic diversity may be responsible for the global distribution of prokaryotes and their ability to thrive in almost every ecosystem on our planet.

1.2 Studying bacteria

With bacteria playing such a large role in the biogeochemical cycling of nutrients throughout our planet (Gruber & Sarmiento 1997; Ganeshram et al. 2002) and maintaining nutrient concentrations within aquatic ecosystems, it is understandable that aquatic ecologists and biogeochemists alike would want a comprehensive understanding of the microbes and metabolic processes at play. While it has been known for decades that bacteria play an integral role in nutrient cycling (Waksman et al. 1933) it has only recently been possible to study bacterial species composition and metabolism in situ, thanks to advances in molecular technologies such as 16S ribosomal RNA sequencing (Fox et al. 1977).

1.3 The uncultivated majority

Historically, the field of microbiology depended on laboratory cultivation and microscopy to identify new species and to study microbial ecology, which provided a misleading view of bacterial composition and abundance in environmental samples, since many groups of bacteria resist conventional cultivation techniques. The drawbacks of cultivation-dependent methods were illustrated in the 1970s with advances in epifluorescence microscopy and DNA-staining technologies, revealing the abundance of bacterial cells in seawater to be orders of magnitude above previous counts (Staley & Konopka 1985). Even with a new approximation for the abundance of marine bacteria, without a cultivation method, it was impossible to determine which species are present in a particular environment. It wasn't until 1977 when a method for quantifying evolutionary relationships between bacterial species using differences in gene sequences was introduced; the first cultivation-independent method of studying bacteria. Carl Woese and George Fox used the 16S portion of the ribosome to study evolutionary relationships between the bacteria, archaea, and eukarya (Fox et al. 1977).

1.4 How is microbial diversity measured?

1.4.1 16S ribosomal RNA

There are some genes that have been well conserved throughout all domains of life, to the point that we are able to determine the approximate time that has elapsed since species have diverged from a common ancestor. Sometimes referred to as the 'universal gene marker', the ribosomal gene was the first and remains one of the most popular genes to

utilize when studying evolutionary relationships between organisms. The 16S portion of the bacterial ribosome is a favorite among microbiologists, with 97% sequence similarity being the usual standard for identifying an operational taxonomic unit (OTU) (Stackebrandt & Goebel 1994).

1.4.2 rDNA and rRNA

The 16S ribosome can be observed in both the DNA and the RNA fraction of the bacterial genome, and has been used as a genetic marker for the past 30 years. Looking at the 16S rDNA and rRNA can tell you very different things about the bacterial communities being examined. The 16S rDNA is found within the bacterial genome, and provides information about the taxonomic identity of the bacterial species present in the community and their relative abundance. The 16S rRNA is the product of active transcription of the ribosomal gene, and is used as an index of metabolic activity and potential growth rate of specific taxa (Kramer & Singleton 1992; Nilsson et al. 1997; Fegatella et al. 1998). There has been an increase in the coupling of both rDNA and rRNA to observe the ratio between cell abundance and the metabolic activity of those cells (BJ Campbell et al. 2009; Barbara J Campbell et al. 2011). The ratio of rRNA:rDNA abundance can reveal some characteristics of the metabolic strategy utilized by a bacterial taxa, such as whether ribosomal RNA content (rRNA) is a function of cell abundance (rDNA), or if rare taxa can have disproportionately higher rates of growth (Barbara J Campbell et al. 2011; Barbara J Campbell & Kirchman 2012).

1.4.3 Advantages/disadvantages of 16S analysis

The 16S gene has been an invaluable tool in the field of microbial ecology, allowing researchers to study organisms that have evaded laboratory cultivation. Despite its importance in the advancement of microbial ecology, there are many drawbacks that must be considered when utilizing this methodology. One of the greatest drawbacks is that 16S sequencing is dependent on pre-existing sequence databases, meaning that any bacterial species that has not previously had its 16S gene sequenced must remain unassigned or be grouped in with the taxonomic group it most closely resembles (Mande et al. 2012). Another drawback is that 16S sequencing is dependent on PCR amplification of a microbial community. This step can introduce a bias towards species with a higher copy-number of the 16S gene, giving the impression that these groups are more abundant than they actually are (Kembel et al. 2012). Current approaches to overcoming this bias include metagenomics analysis and use of mathematical modeling to normalize the observed number of ribosomes with the ribosomal copy-number of a microbial species. Despite these disadvantages, 16S sequencing is still considered one of the most valuable tools for studying microbial ecology, and it is becoming more valuable as technology is improved upon.

1.5 What controls microbial diversity?

In aquatic environments, microbial diversity is influenced by a variety of physical, chemical, and biological factors including salinity, nutrient concentrations, turbidity, and organic compound concentration (Dolan et al. 1995; Craig A Carlson et al. 2004; Bernhard et al. 2005; BJ Campbell et al. 2009; Caron et al. 2000; Azam 1998; Kan et al. 2006; Barbara J Campbell et al. 2011; Bratbak & Thingstad 1985; Sohm et al. 2011; Nogales et al. 2007; Kuypers et al. 2003; Vieira et al. 2008).

1.5.1 Physical factors

In aquatic ecosystems, thermal stratification separates bacterial habitats into warm surface layers and cold deep layers, resulting in differential microbial communities in each of these layers (Jones 1977). Stratified water columns are dynamic, exhibiting seasonal changes, which are often predictable. These patterns are a result of changes in water temperature during seasonal changes in temperate latitudes.

While temperature plays a large role in metabolism and abundance of aquatic bacteria, the general consensus is that temperature and substrate availability work synergistically to shape the microbial community (White et al. 1991; Field et al. 1998; Shiah & Ducklow 1994), with there being an apparent relationship between temperature and nutrient concentration (Jones 1977; Wiebe et al. 1993).

1.5.2 Chemical factors:

Bacterial community structure is influenced by the pH and salinity of an aquatic ecosystem. Estuarine ecosystems are ideal for studying the effect of salinity on a microbial community, due to the gradients encountered there. Bacterial communities in these salinity gradients experience physiological changes at the community level (del Giorgio & Corinne Bouvier 2002), which result in a unique community being found in these transitional zones, composed of a mixture of freshwater and marine bacteria (Troussellier et al. 2002; Thingstad 2000; Kirchman et al. 2005). Bacterioplankton abundance has been observed to be inversely related to salinity, with higher abundance values being recorded in low-salinity environments (Painchaud et al. 1995). The influence of salinity gradients on a bacterial community is

likely due to the inability to maintain osmotic regulation and protein conformation (Zwart et al. 2002; Oren 2001). There are some bacterial taxa that have developed adaptations to overcome salinity gradients, and are found in both freshwater and marine ecosystem, including the SAR11/LD12 group and the Caulobacter group (González et al. 2000; Stahl et al. 1992).

1.5.3 Biological factors

Biological factors influencing microbial community structure include the rate and source of primary production, the composition and abundance of organic matter available for consumption, and the intensity of predation on the bacterial community.

1.5.3.1 Primary production

Primary production by phototrophic organisms is responsible for the production of organic matter, which sustains all heterotrophic life on Earth. Primary production limits heterotrophic bacterial growth, since the rate of respiration cannot exceed the rate of primary production (Cole et al. 1988; Kirchman 1990). The main primary producers within aquatic ecosystems are phytoplankton, which through photosynthesis are able to fix atmospheric carbon into complex organic compounds, which are then released into the ecosystem via extracellular release or through cell lysis (Ducklow & Craig A Carlson 1992). The composition of organic compounds produced by phytoplankton is largely dependent on the composition of phytoplankton species present in an ecosystem, as the DOM produced by phytoplankton varies between species (Sarmiento & Gasol 2012). Thus, phytoplankton

community structure can directly influence heterotrophic bacterial community structure by means of influencing organic matter composition.

Phytoplankton are not the only primary producers influencing bacterial community structure, as organic matter derived from terrestrial plants makes its way into aquatic ecosystems via runoff, amounting to approximately 0.25 Pg/year (Hedges & Oades 1997).

1.5.3.2 Grazing

Bacterivorous predators sometimes exhibit preference when grazing on bacterial communities, consuming some bacterial taxa over others, and having an influence on bacterial community composition (Simek et al. 1999; Hahn & Höfle 2001). Selectivity by grazers can be influenced by factors such as prey size or chemotaxis. Grazing can also indirectly influence bacterial community composition, due to the large amount of DOM being released through the ingestion and digestion of phytoplankton and bacteria by zooplankton (Jumars et al. 1989).

1.5.3.3 Viral lysis

Viral infection can also influence structure of bacterial communities, and are responsible for 10-50% of bacterial mortality (Proctor & Fuhrman 1990; Fuhrman 1999). Viral infection is often density-dependent and species-specific, which has led to the development of the “Kill the Winner” hypothesis, where the bacterial species to become most abundant in a community becomes an easy target for viral infection (Thingstad & Lignell 1997; Thingstad 2000). Similar to the byproduct of grazing being the release of DOM compounds, viral lysis of bacterial cells results in the release of DOM compounds that can

then be utilized to sustain the rest of the bacterial community (Hornák et al. 2006; Fuhrman 1999).

1.5.3.4 Dissolved organic matter

Dissolved organic matter (DOM) is biologically-derived carbon compounds, and is the main source of energy for heterotrophic bacteria. The amount of carbon contained within marine ecosystems is almost equivalent to the carbon in atmospheric CO₂ (Hedges & Oades 1997) making it incredibly important during carbon cycling. The composition of DOM in aquatic ecosystems is difficult to obtain due to the diversity and dynamics of the compounds (Azam 1998), which are derived from a variety of sources and are constantly being transformed by physical, photochemical, and biological processes. The structure of bacterial communities is sensitive to both the concentration and composition of DOM, with bacterial taxa having developed metabolic strategies to cope with specific DOM concentrations and to utilize specific DOM compounds. DOM composition also varies with its source, which can be derived from either phytoplankton or terrestrial plants (Benner et al. 1995; Hedges & Oades 1997), which may further structure bacterial communities. Terrestrially derived organic matter (TOM) has a chemical composition that is distinct from phytoplankton-derived DOM, generally making it more resistant to biological degradation (Benner 2004). Estuarine ecosystems experience gradients in terrestrial and phytoplankton-derived DOM as salinity increases, which make these excellent ecosystems to study the effects of DOM source on a microbial community (McCallister et al. 2006).

1.6 DOM in estuarine ecosystems

Estuaries are often referred to as 'transition zones', where gradients in salinity, biodiversity, and nutrient concentrations are observed. One of the most dynamic and biologically relevant components in these transitional zones is dissolved organic matter, which sustains heterotrophic bacteria metabolism and influences bacterial diversity (Covert & Moran 2001). Over the course of an estuarine transition zone, there is a gradual mixing of DOM originating from freshwater and marine sources, which vary considerably in composition and reactivity. Freshwater DOM is typically higher in TOM, which is composed of the breakdown products of lignin and cellulose (Benner & Opsahl 2001) and has been highly photo-oxidized by the time it reaches the estuary (Valentine & Zepp 1993; Blough & Zepp 1990). In the estuarine transitional zone, this body of water containing TOM is gradually mixing with marine water, containing a very different DOM composition. Marine-derived DOM is largely composed of phytoplankton-derived compounds that have been assimilated into biomass and recycled into the ecosystem, and can be found in a variety of states varying in biological availability. These states range from labile to recalcitrant, which are highly available for degradation and highly resistant to degradation respectively (Amon & Benner 1996). Freshwater and marine DOM is also distinct in the relative size distribution found in each ecosystem, with freshwater DOM being composed of approximately 70% high molecular weight (HMW) DOM, while marine DOM is composed of approximately 30% HMW DOM (Hedges et al. 1994). Bacterial community structure can be influenced by the composition, lability, and size of organic compounds, which makes these mixing zones interesting when studying microbial ecology of estuarine ecosystems.

1.7 Estuarine bacteria

Estuarine ecosystems are sites of intense salinity gradients, changing from a completely freshwater ecosystem to completely marine in a relatively short distance. Two contrasting hypotheses have been generated concerning the composition of bacteria in estuaries, which are that (1) estuarine bacterial communities are composed of a mixture of freshwater and marine bacterial taxa, and that (2) there is a bacterial community specifically adapted to brackish waters, composed of taxa not found in either freshwater or marine environments.

There are many studies supporting the first hypothesis, which describe estuaries as transitional zones of bacterial community structure, where the salinity gradient correlates with a gradual reduction in freshwater bacteria and an increase in marine bacteria (Cottrell & Kirchman 2003). This observation has been attributed to bottom up factors affecting bacterial growth such as physiological stress on bacteria unable to cope with the change in salinity, and changes in the concentration of inorganic and organic nutrients (Thierry C Bouvier & del Giorgio 2002).

The second hypothesis is that a unique bacterial community inhabits the estuarine transition zone, which has adapted to this intermediate salinity. One important caveat to this hypothesis is that the residence time in the estuarine transition zone must be long enough for a resident microbial community to be established (Crump et al. 2004). A preliminary analysis of the distribution of OTUs in a large estuarine ecosystem has encountered a bacterial community inhabiting brackish waters that is distinct from the community inhabiting the freshwater and marine portions of the estuary (Fox et al. 1977; Herlemann et al. 2011). The retention time of is influenced by both the size of the estuary and the presence of an estuarine turbidity maxima (ETM) (Lapierre & Frenette 2008).

The change in bacterial community structure along an estuarine gradient is typically characterized by a shift from Betaproteobacteria and Actinobacteria in freshwater ecosystems (Salcher et al. 2008; Simek et al. 2005; Zwart et al. 2002) to Alphaproteobacteria, Gammaproteobacteria, and Bacteroidetes in marine ecosystems (Cottrell & Kirchman 2000a; Thierry C Bouvier & del Giorgio 2002; Barberán & Casamayor 2010).

1.8 Utilization of DOM by heterotrophic bacteria

Heterotrophic bacteria are able to uptake and assimilate a wide variety of DOM compounds found in aquatic ecosystems, which are often classified as either HMW or low molecular weight (LMW) DOM when describing the mechanisms by which they are utilized.

HMW-DOM compounds are between 1-30 kDa in size, and include polymers such as proteins, starches, and peptidoglycan. HMW-DOM requires degradation by extracellular enzymes to first break down the HMW material into smaller fragments that can be taken into the cell by transport proteins.

LMW-DOM compounds are under 1 kDa, and are composed of monomeric compounds such as glucose, amino acids, dimethylsulfopropionate (DMSP), adenosine triphosphate (ATP), glycine betaine, and vanillic acid (Kujawinski 2011). These compounds are taken into the cell by transport-proteins, many of which are only found in specific clades or phylum of bacteria (Poretsky et al. 2010), suggesting the importance of DOM composition in the structuring of bacterial community structure.

This concept of DOM composition influencing bacterial community structure is reinforced by recent experimental and transcriptomic studies suggesting resource partitioning between bacterial taxa (McCarren et al. 2010; Rinta-Kanto et al. 2012; Teeling et al. 2012),

providing further evidence to the hypothesis that bacterial groups exhibit metabolic preference to specific substrates (Cottrell & Kirchman 2000b; Elifantz et al. 2005; Alonso-Sáez & Gasol 2007). Such studies include that conducted by Mou et al. (Mou et al. 2008) which observed a coastal bacterial community exhibiting differential consumption of lignin-derived DOM compounds vs. phytoplankton-derived DOM compounds by a few specialist species, while the majority of species exhibited a generalist lifestyle, responding to both lignin-derived and phytoplankton-derived DOM. This study provides insight into the metabolic complexity of bacterial communities, and that different taxa may exhibit widely different responses to novel DOM compounds. Another study conducted by McCarren et al. (McCarren et al. 2010) observed the response of a surface bacterial community in the Pacific Ocean to HMW-DOM. They observed a succession event within the bacterial community, in which through the consumption of HMW-DOM by one taxa, new DOM compounds were produced and made available for consumption by subsequent taxa. This result illustrates that bacterial community and DOM composition is highly dynamic in aquatic ecosystems, and has the potential to influence one another. Considering the diversity of DOM compounds found in estuarine ecosystems and the ability of unique bacterial communities to form along estuarine gradients (Crump et al. 2004), it is expected that there would be a similar resource partitioning of DOM among estuarine bacterial communities.

1.9 The St. Lawrence Estuary

The St. Lawrence Estuary (SLE) is the second largest river system in North America, with an are of 10,800 km² and a drainage basin of 1.3 million km², and is responsible for discharging approximately 1.52x10⁶ t yr⁻¹ of dissolved organic carbon into the ocean (El-

Sabh & Silverberg 1990). The SLE experiences a gradient in salinity from freshwater (0) to marine (30) over an approximately 400 km transect between Quebec City and Pointe-des-Monts. There is an estuarine turbidity maximum (ETM) located in the freshwater portion of the estuary between salinity 0.06 and 1.10 (Lapierre & Frenette 2008), which has a residence time of 15 days for passive particles (Simons et al. 2006), which is long compared to the 7-day residence time of surface waters (Saucier & Chassé 2000). There are many freshwater inputs located throughout the SLE, with a higher concentration located in the upper freshwater portion of the estuary. These freshwater inputs produce a higher concentration of TOM in the upper estuary, providing a natural gradient in DOM composition. This observation makes the SLE an ideal ecosystem to study the effects of salinity and DOM composition on bacterial community structure.

1.10 Objective

This study aims to address the question of whether DOM isolated along an estuarine gradient will differentially effect the metabolic activity and community composition of the estuarine bacterial community inhabiting the high-brackish region of the SLE. Two extraction methods are utilized to isolate unique DOM compounds and determine the effect DOM composed solely of HMW compounds has on a bacterial community as opposed to DOM composed of both LMW and HMW compounds. The source of DOM originates from both the upper and lower SLE, allowing us to determine the effect a DOM isolate high and low in terrestrially-derived DOM has on a bacterial community. The microcosm communities were incubated in equal DOM concentrations over 32-hours over which time chemical and taxonomic composition of the microcosms were examined. 16S rRNA

transcript and gene sequencing were utilized to obtain data on how the metabolism and composition of the bacterial community responds to DOM isolated along an estuarine gradient. Previous studies have observed natural estuarine bacterial community composition (Crump et al. 2004) and how a coastal bacterial community responds to model compounds derived from marine and terrestrial sources (Mou et al. 2008), but this may be the first study to observe the effect of incubation natural DOM along an estuarine gradient on an estuarine bacterial community.

2. Materials and Methods

2.1 Study Location and Biomass Sampling

Water for microcosm incubation experiments was collected on the St. Lawrence Estuary (Quebec, Canada) from sampled stations seen in **Figure 1**. The water for microcosm incubations was collected from 3 m at Station 21 (49°25.40'N/66°19.50'W) using a winch-operated conductivity-temperature-depth (CTD) rosette aboard the research vessel (RV) Coriolis II on May 19th 2011 at dusk. The CTD rosette can retrieve 12 separate 12-liter water samples during its ascent (total 144 liters), which was collected to perform two separate microcosm experiments designed to isolate RNA and DNA specifically. The two experiments were identical in everything except for the volume of microcosm, the storage method of biomass samples, and 8 mL 100x Bromodeoxyuridine (BrdU) was added to the small-volume microcosm in order to measure the amount of DOM being incorporated into biomass over the course of the incubation period. At the beginning of the experiment, 84-liters of water from station 21 was distributed evenly among 12 acid-washed 5-liter polypropylene carboys (VWR), into which the specific DOM isolates were added to increase

the DOM concentration by 4x. 700 mL of the DOM-incubated water was subsequently transferred from each carboy to 12 acid-washed 1-liter polypropylene bottles (Nalgene). The RNA-isolation experiment was conducted in the 5-liter carboys, while the DNA-isolation experiment was conducted in the small-volume bottles.

2.2 DOM Preparation

Water for DOM extraction was collected from two sampling stations within the SLE using the CTD rosette. The sites were chosen based on salinity values and location within the SLE in order to isolate DOM of variable TOM concentration. DOM high in TOM was collected from Station B in the upper SLE (46°54.80'N/70°52.50'W) at a depth of 3 meters. A total of 288 liters of water was collected from Station B for DOM isolation.

DOM low in TOM was collected from Station 23 in the lower SLE (48°42.08'N/68°39.00'W) at a depth of 5 meters. A total of 228 liters of water was collected from Station 23 for DOM isolation.

The environmental variables of each station at the time samples were collected can be seen in **Table 1**.

2.2.1 Ultrafiltration

Station B and Station 23 DOM were extracted by means of tangential-flow ultrafiltration, which is a method capable of concentrating HMW DOM compounds (>1,000 Daltons) (Benner et al. 1997). While aboard the R/V Coriolis II, 50 liters of seawater from Station B and 100 liters from Station 23 were passed through a 0.7 µm filter to remove large particles. The filtrate was tangentially circulated over a 1,000 Dalton regenerated cellulose

membrane at a pressure of about 20 KPa. The HMW DOM compounds that do not pass through the regenerated cellulose membrane were retained in the concentrate, while salts and smaller organic compounds were collected in the filtrate. The concentrated DOM was passed through a 30,000 Dalton cartridge to remove viral particles. Between samples, the membrane was washed with 0.1 M NaOH. Total volumes of 90 mL (DOC concentration of 114.4 mg/L) and 325 mL (94.6 mg/L) of DOM were isolated from Stations B and 23 respectively.

2.2.2 Solid-phase extraction

Station B and Station 23 DOM were extracted by means of solid-phase extraction, in which water is passed through a cartridge filled with a styrene divinyl benzene polymer to isolate highly polar to nonpolar substances from large volumes of water (Dittmar et al. 2008). While aboard the Coriolis II, 50 liters of seawater from Station B and 100 liters from Station 23 were passed through styrene divinyl benzene polymer (PPL)-based sorbent cartridges. The resin was washed with 2 cartridge volumes of 0.01 HCL to remove salts, and the DOM was subsequently eluted from the resin by washing with 1 cartridge volume of methanol. Eluted samples were then dried under vacuum at 40°C to remove methanol, and re-dissolved in deionized water.

2.2.3 Phytoplankton DOM extraction

Phytoplankton-derived DOM was isolated from a *Nannochloropsis* phytoplankton culture (Reed Mariculture) by flash-freezing the culture with liquid nitrogen and passing the lysate through a 0.22 µm filter to isolate the DOM. *Nannochloropsis* are commonly found in marine environments, but have more recently been found in fresh and brackish waters (K P

Fawley & M W Fawley 2007). Considering its presence throughout freshwater, estuarine, and marine environments, it is a good candidate species to utilize in this estuarine microcosm experiment.

2.3 Microcosm setup and filtration

Microcosm experiments were conducted with 5 L acid-washed polypropylene carboys containing raw water from Station 21 (**Figure 1**), each spiked with a unique DOM extract from the SLE. The target spike in DOM concentration to the microcosms was 4x the natural levels of organic matter, which was estimated to be between 1.4-2.3 mg/L based on previous measurements in the SLE. Volume of DOM spikes depended on the concentration of the DOM isolated from each source, which was determined based on the estimated concentration of DOM at Station B (6.0 mg/L) and Station 23 (2.5 mg/L) and the estimated yield of DOM extracted from each station by ultrafiltration and solid phase extraction. Recovery of DOM by ultrafiltration was expected to be approximately 70% from Station B and approximately 30% from Station 23 (Amon & Benner 1996; Benner et al. 1997), and for solid-phase extraction was expected to be approximately 65% from Station B and approximately 43% from Station 23 (Dittmar et al. 2008). A total of 23.8 mL of Station 23; solid phase extracted DOM, 325 mL Station 23; ultrafiltered DOM, 14.4 mL Station B; solid phase extracted DOM, 90 mL Station B; ultrafiltered DOM, and 25 mL phytoplankton-derived DOM was added to spike the volume of the microcosm to 4x the natural DOM levels. DOM treated microcosms and the negative controls were incubated at 7°C and 25°C. Both incubations were conducted simultaneously in temperature-controlled rooms aboard the R/V Coriolis II. The 7°C incubation was the in situ temperature of the SLE, while the 25°C

incubation was conducted to ensure a metabolic response in resident microbial community. Bacterial biomass samples were taken from the microcosms at 12 hours, 22 hours, and 32 hours. A peristaltic pump was used to remove water from the carboys and pass it through the filters. Free-living bacterial biomass was collected on a 0.22 μm Sterivex filter after an initial pre-filtration through a 2.7 μm glass-fiber (GF/D) filter was used to remove particles and larger eukaryotic organisms. At each time point, 1.5-2 L of water was filtered from the microcosms for RNA analysis. Sterivex filters were sealed with paraffin film after the addition of RNAlater (Invitrogen), a storage solution that permeates cells to protect RNA and deactivate RNase enzymes. All filters were processed and stored at -80°C within 5 minutes of filtration to avoid degradation of RNA.

The microcosm experiment described above was replicated at a smaller scale, within 1 L acid-washed polypropylene bottles, from which raw unfiltered samples were taken at the same time intervals as the other microcosm experiment (12 hours, 22 hours, and 32 hours). 40 mL of water were taken from each microcosm and biomass was fixed with 3 mL of 37% formaldehyde, resulting in the final sample containing 2.8% formaldehyde, which was then incubated at room temperature for 1 hour, and stored at -80°C .

2.4 RNA extraction and cDNA synthesis

Bacterial biomass was stored at -80°C until they were ready to be processed. Total RNA was extracted from Sterivex filters with a modified protocol (Shi et al. 2009; Stewart et al. 2010) which employs both the mirVana miRNA isolation kit (Invitrogen) and the RNeasy RNA cleanup kit (Qiagen). Samples were thawed and had the RNA Later surrounding the Sterivex filter removed (approximately 1700 μl) and discarded. 1700 μl of mirVana lysis

buffer was added to the Sterivex filter and vortexed to lyse bacterial cells attached to the filter. Total RNA was then extracted from the lysate according to the mirVana protocol. Purified sample (100 ul) was treated with 2 ul DNase (New England Biotech) incubated at 65°C for 1-2 hours to remove genomic DNA, and concentrated using the RNeasy RNA cleanup kit (Qiagen). The RNA extracted samples were PCR amplified in the 16S rRNA gene region and run on a 1% Agarose gel in order to detect any DNA contamination after DNase treatment, in which case the DNase treatment and RNA cleanup was repeated on those samples. The clean RNA samples were quantified on a spectrophotometer. 1 ng of RNA was used in a reverse transcription reaction using M-MLV reverse transcriptase (Invitrogen), transcribing from the 3' end of the region to be sequenced via the reverse primer 926R (5'-CCGTCAATTCMTTTRAGT-3'). Reverse transcription reactions require heat denaturation of RNA at 65°C for 5 minutes and subsequent incubation at 37°C for 50 minutes for reverse transcription to take place. The reverse transcription reaction produces cDNA that is ready for PCR amplification. The V5 region of the 16S cDNA was selectively amplified using a reverse primer 926R and a forward primer 786F (5'-GATTAGATACCCTSGTAG-3'). Each sample was amplified with uniquely barcoded reverse primers in order to separate samples computationally after sequencing. PCR of the cDNA took place in a thermal cycler (Bio-Rad) and programmed as follows: initial denaturation at 98°C for 3 minutes, 30 cycles of denaturation at 98°C for 5 seconds, annealing at 49°C for 5 seconds, and chain extension at 72°C for 10 seconds with a final extension time of 1 minute after the final cycle.

2.5 Genomic DNA amplification

40 mL sample were collected from the 1 L microcosms for genomic DNA analysis. Biomass was collected from each sample by vacuum filtration of 1 mL microcosm water through a 0.2 µm GE polycarbonate filter (AMD Manufacturing inc.), which had first been filtered through a 2.7 µm GF/D filter (Whatman) to remove particles and eukaryotes. The 0.2 µm filter was then rinsed with 10 mL of autoclaved distilled water. Filters were cut into 1/8ths with a sterilized scalpel, and filter segments were stored at -80°C in 100 µl PCR tubes. The V5 region of the 16S rDNA was selectively amplified directly from the filter segments using the reverse primer 926R and the forward primer 786F. Each sample was amplified with uniquely barcoded reverse primers in order to separate samples computationally after sequencing. PCR of the rDNA took place in a thermal cycler (Bio-Rad) and programmed as follows: initial denaturation at 98°C for 3 minutes, 30 cycles of denaturation at 98°C for 5 seconds, annealing at 49°C for 5 seconds, and chain extension at 72°C for 10 seconds with a final extension time of 1 minute after the final cycle.

2.6 Amplicon isolation

Amplicons from both RNA and DNA samples were isolated post-PCR via gel extraction. The full volume of the PCR (25 µl for RNA and 50 µl for DNA) was run on a 2% Agarose gel at 65 volts for 2 hours. The amplicon was then excised from the gel under UV light with a sterile scalpel and purified using the QIAquick gel extraction kit (Qiagen) to a final volume of 37 µl. The gel-extracted samples were visualized on a 1% gel electrophoresis before quantification.

2.7 DNA/cDNA sequencing

Barcoded amplicons were quantified on a VICTOR2 fluorometer (PerkinElmer) using the Quantifluor ds DNA System (Promega) and pooled together in equimolar concentration of 16 pM. Each pool contains amplicons belonging to a separate sequencing run, which do not contain any overlapping barcodes. Pooled amplicons were then sequenced using the IonTorrent semiconductor sequencer at Concordia University Genomics center following the 316 Chip kit, the Ion OneTouch 200bp v2 kit, and the Ion PGM 200bp kit protocols (Life Technologies). Sequencing specifications for each sample can be seen in **Table 2**.

2.8 Bioinformatics analysis

Raw sequence data (.fastq) generated by the IonTorrent was downloaded from the IonTorrent server for bioinformatics analysis. Downstream analysis of this sequence data was conducted on the open-source software Mothur (v. 1.30.0) (Schloss et al. 2009). The first step in processing the sequence data was to use the command trim.seqs, which removed sequences that had quality scores below 25, did not match the IonXpress sequence or the PCR reverse and forward primer sequence, or were shorter than 100bp in length. Unique sequences were isolated using the unique.seq command in order to reduce the size of the dataset being analyzed, and were then aligned and clustered into operational taxonomic units (OTUs). Trimmed sequences were aligned to the reference SILVA database from which a distance matrix was generated and clustered using the furthest neighbour algorithm. The number of reads generated during sequencing is displayed in **Table 3**.

Alpha diversity was measured with the Chao1 index (Chao 1984) using the Mothur software. Samples were rarefied before analysis to maintain a consistent number of sequences (~8000). The Chao1 index estimates species richness using the equation $S_{\text{chao1}} =$

$S_{\text{obs}} + n_1(n_1 - 1) / 2(n_2 + 1)$, in which S_{chao1} is the estimated richness, S_{obs} is the observed number of species, n_1 is the number of OTUs with only one sequence, and n_2 is the number of OTUs with only two sequences. OTUs were defined by a 97% cutoff.

The dissimilarity between each sample community was calculated with the thetacyc calculator (Yue et al. 2001) using Mothur. Thetacyc measures the dissimilarity between the structures of two communities using the equation

$D_{\theta\text{YC}} = 1 - (\sum_{i=1}^{\text{ST}} a_i b_i) / (\sum_{i=1}^{\text{ST}} (a_i - b_i)^2 + \sum_{i=1}^{\text{ST}} a_i b_i)$ where S_T is to total number of OTUs in communities A and B, a_i is the relative abundance of OTU i in community A, and b_i is the relative abundance of OTU i in community B. OTUs were defined by a 97% cutoff. A matrix of pairwise thetacyc distances was created which included all samples, and was illustrated as a dendrogram.

OTUs were assigned to taxonomic groups using the Wang approach using the Mothur software, by aligning trimmed sequence data to the GreenGenes reference database with a bootstrap cutoff of >60.

2.9 Dissolved organic carbon (DOC) loss (*The following analysis was conducted in Dr. Yves Gelin's lab at Concordia University*)

The total amount of DOC lost from the microcosms over the 32-hour incubation period was measured via carbon combustion analysis. Any loss of DOC over the course of the incubation period is considered to be the result of heterotrophic bacterial respiration, and can thus be used as a proxy for the amount of carbon consumed by bacteria. 1.5 to 2-liters was taken from each DOM-incubated 7-liter microcosm during bacterial biomass filtration, in which the filtrate was deposited into a 3-liter acid-washed amber glass jug. Samples were

stored at room temperature for approximately 1-2 days, and were stored at +4°C once they arrived at Concordia University, at which time they were also acidified with 1.6 ml/L of 12M HCl to ensure preservation of DOM. Measurement of DOM concentration was measured for all zero-hour and 32-hour samples using a modified high-temperature catalytic oxidation TOC analyzer (OI Analytical Model 1010, College Station, TX), where the PTFE tubing was replaced with PEEK tubing to reduce contamination from atmospheric CO₂ background. Atmospheric CO₂ was purged from the combustion column by repeated blank injections at 680°C under ultra-high purity O₂ (Praxair) 12 hours prior to sample analysis. Exactly 500 µL of each sample was injected for combustion analysis. The percentage of total DOC consumed over the course of each incubation was calculated by using the formula:

$$100 * (([\text{DOC @ } t=0\text{h}] - [\text{DOC @ } t=32\text{h}]) / [\text{DOC @ } t=0\text{h}])$$

2.10 Fourier transform infrared (FTIR) spectroscopy *(The following analysis was conducted in Dr. Yves Gelin's lab at Concordia University)*

FTIR spectroscopy was used to provide information on the relative abundance of particular functional groups in the raw DOM amendments and in each microcosm after the 32-hour 25°C incubation period. This analysis was used to determine how the chemical composition differs between DOM source and extraction method, and which functional groups the bacterial community is preferentially consuming over the incubation period. Before FTIR spectroscopy, the microcosm samples were concentrated by solid-phase extraction to remove the salts and to concentrate DOM. After solid-phase extraction, both the raw DOM samples and the microcosm DOM samples were treated the same way. DOM

was dried by evaporation onto a calcium-fluoride window and then directly analyzed using a FTIR spectrometer (Caron & Goldman 1988; Simjouw et al. 2005).

2.11 Bacterial production *(The following analysis was conducted in Dr. Roxane Maranger's lab at Universite du Montreal)*

Water samples were taken from the 7-liter microcosms at 0, 12, 22, and 32-hours after DOM incubation, and cells were fixed with 3 ml 37% formaldehyde (final concentration = 2.8%). Bacterial production was measured for each 7°C incubated microcosm using the ³H-leucine incorporation method (Smith & Azam 1992).

Each sample had 1.2 mL dispensed in triplicate into 2-mL microcentrifuge tubes containing 50 ul ³H-leucine (115.4 Ci mmol⁻¹, Amersham) bringing the final leucine concentration to 10 nM (Garneau et al. 2008). Samples were incubated in the dark at simulated in-situ temperature (7°C) for approximately 4 hours. Leucine incorporated into cell protein was collected after precipitation by trichloroacetic acid (TCA) and centrifugation. Tubes were filled with 1.25 mL liquid scintillation cocktail (ScintiVerse, Fisher Scientific), and radioactivity was measured using a Tri-Carb 2900 TR Packard Liquid Scintillation Analyzer. Rates of leucine incorporation were corrected for radioactivity adsorption using TCA-killed controls and converted to bacterial C production (BP) using a conservative conversion factor of 1.5 kg C per mol⁻¹ ³H-leucine (Nguyen & Maranger 2011).

2.12 Bacterial abundance *(The following analysis was conducted in Dr. Paul del Giorgio's lab at the Universite de Quebec a Montreal)*

Bacterial abundance was measured using a FACScan (Becton Dickinson, Mountain View, Calif.) flow cytometer, equipped with a 15-mW, 488-nm, air-cooled argon-ion laser, and a 70-um nozzle (del Giorgio et al. 1997). Cell abundance was measured for microcosm samples taken at each time point during the incubation period.

3. Results

3.1 The environmental and biotic setting of the SLE

In May 2011, we performed a transect of the SLE that extended from the upper SLE station B to the lower SLE station 20 (**Figure 1**). There is a strong salinity gradient along the estuarine transect, which ranges from zero in the lower estuary to 27.14 at station 20.

3.2 Natural conditions in the SLE

Bacterial cell abundance and production were measured along the salinity gradient of the SLE and are illustrated in **Figure 2**. The microcosm experiment was performed in highly brackish waters of the LSLE (Station 21, salinity 27). At the time of sampling, some of the lowest values for bacterial abundance (3.5×10^5 cells/mL) and bacterial production (26.0 ug C/L/d) were observed at Station 21, suggesting the bacterial community was characterized by a relatively low level of activity. At Station 23, which served as a brackish (salinity 24) source site for DOM, both bacterial abundance (5.7×10^5 cells/mL) and production (42.4 ug C/L/d) was higher than that observed at Station 21. The higher bacterial production at Station 23 is likely a response to the higher phytoplankton abundance (measured as fluorescence; **Figure 3**) and corresponding primary production observed at Station 23. Moreover, we can

infer that DOM collected at Station 23 should, at least in part, be derived from phytoplankton, based on the high fluorescence detected in the surface water. In contrast to Station 23, the freshwater source site of DOM (Station B, salinity 0.08) was characterized by relatively high bacterial abundance (6.5×10^5 cells/mL), yet low bacterial production (21.6 ugC/L/d). This observation fits well with the recalcitrant and highly processed nature of the terrestrial DOM present in freshwaters.

3.3 Summary of free-living bacterial communities inhabiting Station 21, 23, and B surface waters

Just as we observe changes in bacterial production and cell abundance between the different stations in the SLE, we have also observed changes in the taxonomic abundance (rDNA) and metabolic activity (rRNA) of bacterial taxa. As seen in **Figure 4**, there is a distinct shift in bacterial phyla over the salinity gradient between Station B (salinity 0.08) and Station 21 (salinity 27.29). Changes in both taxonomic composition and metabolic activity include a pronounced decrease in Beta-proteobacteria and Actinobacteria, and a proportional increase in Alphaproteobacteria, Gammaproteobacteria, and Bacteroidetes as salinity increases. It is also interesting to note that taxonomic abundance and metabolic activity of phyla is not always equal, as can be seen in **Figure 4**. This change in the taxonomic distribution and activity of taxa is likely not only due to changes in salinity, but also due to other environmental parameters such as DOM composition.

It is important to note that while Alphaproteobacteria, Gammaproteobacteria, and Bacteroidetes are present in the low-salinity Station B, the taxa that comprise these phyla differ between low and high salinity stations. Alphaproteobacteria inhabiting Station B were

composed of the taxa Consistiales group HTH6, a close relative of SAR11 previously isolated from freshwater (Field et al. 1998; Stein et al. 2002), while the Alphaproteobacteria inhabiting Station 23 and Station 21 were composed of the typically marine Consistiales group SAR11, and the Rhodobacterales groups Arctic96A-1 and OM42. Gammaproteobacteria inhabiting Station B were composed of the taxa CCD24; a soil bacteria (Eilers et al. 2010), while Gammaproteobacteria inhabiting Station 23 and Station 21 were composed of the taxa SAR92, ZA2333c, SAR86, and GSO. Bacteroidetes inhabiting Station B were composed of the taxa Saprospirales and the Flavobacteriales group Sporocytophaga, while the Bacteroidetes inhabiting Station 23 and Station 21 were composed of the taxa Flavobacteriales groups Cytophaga and Polaribacter.

3.4 Composition of DOM isolated from the upper and lower SLE

Samples were taken from the raw DOM utilized in the microcosm experiment and quantified by Fourier transform infrared (FTIR) spectroscopy, which provides insight into the molecular composition of the DOM being added to each microcosm, and how it differs based on source and extraction method. FTIR spectra of raw DOM isolated from the small-volume microcosm incubations show peaks in functional groups at 3000-3500 cm^{-1} (amines, amides, phenols), 2850-3000 cm^{-1} (aldehydes and methyls), 1600-1780 cm^{-1} (alkenes, proteins), 1400-1460 cm^{-1} (aromatics), and 1000-1170 cm^{-1} (tertiary, secondary, and primary alcohols) (**Table 3**). There were some major differences in FTIR spectra between the major DOM extraction methods, most notably the Station 23 extracted DOM (**Figure 5**) containing higher peaks at 1000-1170 cm^{-1} , and Station B extracted DOM (**Figure 6**) containing higher peaks at 1400-1460 cm^{-1} , corresponding to alcohol groups and aromatic compounds, respectively. These

spectra may illustrate that the DOM extracted from Station 23 contain a higher amount of sugar compounds, and the DOM extracted from Station B contain a higher amount of terrestrially derived lignin breakdown compounds.

3.5 Response in bacterial community to DOM amendment

3.5.1 Bacterial abundance

The change in cell abundance over the course of the 32-hour incubation period was measured in each small-volume microcosm and is illustrated in **Figure 7** and **Table 3**. One trend common between all cell abundance values is that the initial cell abundance values (time = 0) are much lower than would be expected based on the cell abundance of Station 21. The natural level of cell abundance at Station 21 was 3.5×10^5 cells/mL at the time the microcosms were collected, and the initial abundance levels in our microcosms range from 1.0×10^4 to 1.0×10^5 cells/mL. The only treatment that does not experience this initial decrease in cell abundance is the 25°C phytoplankton-derived DOM incubation, which has a cell abundance of 2.7×10^5 cells/mL at time=0, but drops below 1.0×10^5 cells/mL after 22-hours. While there was an initial decrease in cell abundance, all microcosm communities make some increase in abundance over the 32-hour incubation period, excluding the UF incubations and the Station B-SPE 25°C incubation. Of all the bacterial communities that were able to increase in abundance over the incubation period, the 7°C negative-control microcosm, the 25°C phytoplankton-derived DOM microcosm, the 25°C Station 23-UF microcosm, and the 25°C Station 23-UF microcosms were able to reach the level of abundance found in the SLE at Station 21.

3.5.2 Bacterial production

The change in bacterial production over the course of the 32-hour incubation period can be seen for each small-volume microcosm in **Figure 7(a-e)** and in **Table 3**. As was observed in the cell abundance values, there is a pronounced decrease in bacterial production at the initial time point (time = 0) of our microcosms compared to what would be expected based on the bacterial production at Station 21. The levels of bacterial production at Station 21 were 26.0 ug C/L/d, and the abundance at the initial time point in the microcosms were between 1.0-7.0 ug C/L/d. Each treatment experiences this initial decrease in bacterial production, with the negative-control (**Figure 7a**) and 7°C Station 23-SPE (**Figure 7c**) DOM microcosms being the only treatments able to recover to the natural levels of bacterial production within 32-hours. The 7°C Station B-SPE (**Figure 7d**) DOM microcosm experienced an increase in bacterial production to 20 ugC/L/d after 22-hours, but begins to decrease after 32-hours. The UF-extracted DOM microcosms (Station 23 and Station B) and the phytoplankton-derived (**Figure 7b**) DOM expressed little to no change in bacterial production over the course of the time-series.

3.6 Carbon consumption

According to the carbon combustion analysis, the percent of dissolved organic carbon (DOC) consumed over the course of the small-volume incubations was not temperature-dependent. The negative-control microcosms experience a 22.3% and 28.7% loss in DOC at 7°C and 25°C respectively, indicating the bacterial communities are not starved for DOC before the incubation period. This observation supports the increase in cell abundance and production in the negative control observed in **Figure 7a**. The Station B-SPE and Station B-UF

incubations experienced a similar amount of carbon loss as the negative control, with the Station B-SPE incubation resulting in 21.7% and 28.0% loss at 7°C and 25°C respectively, and the Station B-UF incubation resulting in 19.1% and 19.7% loss at 7°C and 25°C respectively. The similar % loss in DOC between the negative control and Station B DOM may suggest that the bacteria in these microcosms are not consuming the Station B DOM but are instead consuming the same DOM that is being consumed in the negative control microcosms. The Station 23-UF DOM incubation resulted in a greater proportion of DOC lost from the system, at 37.7% and 38.7% for the 7°C and 25°C incubations, respectively. This is expected due to our hypothesis that Station 23 DOM is similar to DOM found at Station 21, owing to their similar salinity values (24 and 27) and close proximity within the estuary. The DOM incubation resulting in the highest amount of DOC lost from the system was the phytoplankton-DOM incubation. This was expected to be the most labile and biologically available DOM utilized in this experiment, and resulted in a 73.5% and 73.1% loss in DOC at 7°C and 25°C respectively, suggesting that this DOM is composed mostly of labile DOM that was rapidly utilized by the microcosm community.

3.7 Change in DOM composition post-incubation

By comparing FTIR spectrum produced from our raw DOM extract to that obtained from analyzing the microcosm water after the 32-hour incubation period, we can hypothesize which DOM compounds the bacterial community is consuming, and how DOM origin and extraction method influences this. **Figure 8** illustrates the FTIR spectra of the microcosm DOM after the 32-hour incubation period. There are many changes that are conserved throughout each DOM treatment, such as decrease in functional groups identified at bands

3000-3500 cm^{-1} (amines and amides), 1600-1700 cm^{-1} (alkenes, aromatics, proteins), and 1000-1170 cm^{-1} (tertiary, secondary, and primary alcohols). There are some compounds that are retained throughout the 32-hour incubation that are conserved between all DOM treated microcosms, including the functional groups found at 2850-3000 cm^{-1} (aldehydes and methyls; lipids), 1400-1460 cm^{-1} (aromatics), and 850-880 cm^{-1} (inorganic material). While many of these changes are conserved between all treatments, the Station B DOM incubations appear to have retained a higher proportion of functional groups at 1400-1460 cm^{-1} which likely correspond to the higher amount of terrestrially-derived material found in freshwater DOM.

3.8 Shift in 16S rRNA transcript diversity

Bacterial species richness was measured for the natural community inhabiting the SLE Station 21 surface waters, as well as each sample for which 16S rRNA transcript data was available (**Figure 9**). The levels of bacterial richness in the negative-control microcosms do not differ significantly from what is observed at Station 21. Comparing the richness found in the negative-control microcosms to each of the DOM treated microcosms, there are only four samples that do experience a change in richness over the course of the incubation period. A significant drop in richness is observed in Station 23-UF DOM incubated samples, specifically at 32-hour in the 7°C incubation and at 22-hour in the 25°C incubation. This is the only treatment in which a significant drop in richness was observed. A significant increase in richness was observed in response to Station B DOM incubated at 25°C in both UF and SPE extracted DOM, suggesting a temperature-dependent response in the bacterial community.

3.9 Shift in the taxonomic composition of 16S rRNA transcripts

Taxonomic changes in response to differential DOM inputs in our microcosms was measured by 16S rRNA sequencing, which provides information on the ribosome content of a bacterial cell, often used as a proxy for metabolic activity.

In order to visualize the dissimilarities in the 16S rRNA abundance of bacterial taxa between microcosms, we have calculated the dissimilarity of OTUs between each sample using Thetayc calculator at 97% OTU identity, and constructed a dendrogram that illustrates dissimilarity of samples as a function of branch-length (**Figure 10**). In the dissimilarity dendrogram we can see that all of the negative control samples are found within a single cluster, indicating the OTUs between these samples are highly similar (thetayc values do not exceed 0.205 indicating low dissimilarity). The dendrogram also illustrates that the dissimilarity of negative-control samples is more dependent on the time since DOM incubation than the incubation temperature, since samples taken at 12, 22, and 32-hours cluster together independent of whether the samples were incubated at 7°C or 25°C.

The Station 21 rRNA sample is highly clustered with the negative-control samples in the dissimilarity dendrogram, indicating that the OTU distribution between the source-community and the negative control is very similar (thetayc values do not exceed 0.109). The bacterial taxa exhibiting the highest rRNA abundance in the negative control microcosms can be observed in **Figure 11** (inner circle). The bacterial taxa exhibiting highest abundance of rRNA transcripts at time-zero are the Alphaproteobacteria taxa OM42, the Gammaproteobacteria taxa HTCC2207, and the Bacteroidetes group Flavobacteriales. Although there is some change in the relative abundance of 16S rRNA transcripts over the

course of the 32-hour time series in the negative control microcosms, there is less change in the control compared to each of the DOM-treated microcosms, as illustrated in the dissimilarity dendrogram (**Figure 10**).

3.9.1 Station 23 DOM

The Station 23 UF DOM utilized in this experiment is comprised of HMW compounds between 1-30 kDa, so we hypothesize any bacteria utilizing these DOM compounds will possess extracellular enzymes capable of degrading these large molecules. The dissimilarity dendrogram in **Figure 10** illustrates that the samples that experience the greatest shift in community composition from the negative control are incubated with Station 23 UF DOM. Two of these samples (Station 23-UF-32h-7°C; $\text{thetayc} = 0.971$ and Station 23-UF-22h-25°C; $\text{thetayc} = 0.974$) were shown to experience a significant decrease in species richness compared to the negative control, which upon inspection of the change in 16S rRNA transcripts in these samples, corresponds to a relative increase in the Gammaproteobacteria group *Pseudoalteromonas*, as seen in **Figure 12** (inner circle). The drops in richness observed in **Figure 9** (inner circle) coincide with the maxima in relative abundance of the *Pseudoalteromonas*, occurring at 32-hours in the 7°C microcosm and at 22-hours in the 25°C microcosm. The staggered nature of this bloom suggests the rate of this shift is temperature dependent, with the bacterial response occurring more quickly at 25°C than at 7°C. In the 25°C incubation, we are able to observe the post-bloom microcosm at the 32-hour time-point, where the relative abundance of *Pseudoalteromonas* transcripts began to decrease. It appears that the next most abundant groups after the *Pseudoalteromonas* are in similar proportions to the pre-bloom community, with the OM42, *Polaribacter*, and HTCC2207 taxa being most

prevalent. Perhaps given a longer incubation period we would observe a succession of bacterial taxa responding to the DOM made available through the *Pseudoalteromonas* bloom cleaving HMW-DOM into smaller compounds.

The Station 23-SPE DOM isolate differs from the Station 23-UF DOM in that the SPE DOM is not extracted based on size, isolating both LMW and HMW compounds. This DOM incubation resulted in a taxonomic response unique from that observed in the Station 23-UF DOM incubation, most notably in the lack of response of the *Pseudoalteromonas* taxa. In the dissimilarity dendrogram, we can see that the 25°C incubated sample exhibits a higher dissimilarity from the negative-control after 32-hours than the 7°C incubated sample (Station 23-SPE-32h-25°C; $\text{thetayc} = 0.564$ and Station 23-SPE-32h-7°C; $\text{thetayc} = 0.335$, suggesting a temperature-dependent response. **Figure 12** (inner circle) suggests the departure from the negative-control samples is due to an increase in the Bacteroidetes taxa *Polaribacter*, which increases by 20.4% at 7°C and 35% at 25°C over the 32-hour incubation period. The bacterial response to Station 23-SPE DOM did not have a significant impact on the richness of the community at either incubation temperature, as illustrated in **Figure 9**.

3.9.2 Station B DOM

The salinity at Station B is very low (PSU = 0.08) and is located in the upper SLE, far removed from the source of the microcosm community at Station 21, and is expected to contain a higher proportion of terrestrially-derived DOM, which is compositionally unique from the DOM typically encountered in the lower SLE. The ability of the high-brackish bacterial community at Station 21 to utilize Station B-DOM depends on the phenotypic plasticity of the taxa and their ability to adapt to novel DOM substrates.

The response of the microcosm community to Station B DOM appeared to be more influenced by incubation temperature than by the extraction method of DOM. The Station B DOM treatments incubated at 25°C experienced a significant increase in richness after 32-hours independent of extraction-method, and constituted the only two DOM treatments that resulted in an increase in richness (**Figure 9**). Although both the Station B-UF and Station B-SPE DOM incubated microcosms experienced this increase in richness at 25°C, the dissimilarity dendrogram suggests that the taxonomic response was unique in each microcosm. The Station B-UF 25°C DOM incubation did not result in a significant shift in OTU distribution after 32-hours, indicated by that sample being found clustered with the negative-control microcosms (thetayc = 0.093). The Station B-SPE 25°C DOM incubation, while exhibiting a similar increase in richness, also exhibited a significant shift in OTU distribution, as indicated by that sample being located far from the negative-control microcosms after the 32-hour incubation period (thetayc = 0.343). The taxonomy of the Station B DOM incubated microcosms is illustrated in **Figure 13** (inner circle) and the departure from the negative control after 32 hours is better illustrated in **Figure 15**, where we can see there is little change in the taxonomic composition of the microcosm after incubation with Station B-UF DOM at 25°C, but the Station B-SPE DOM incubation causes an increase in the Gammaproteobacteria taxa ZA2333c when incubated at 25°C. This is the only treatment in which the ZA2333c taxa exhibit a positive response.

Station B DOM incubated microcosm did not experience a significant change in richness after 32-hours when incubated at 7°C, and did not appear to be influenced by the method of DOM extraction, as they exhibited an almost identical taxonomic response over the course of the incubation period, independent of the method of DOM extraction. This is

illustrated by these samples' close proximity to each other in the dissimilarity dendrogram in **Figure 10** (thetayc = 0.017), and by the similar taxonomic response observed in both **Figure 13** (inner circle) and **Figure 15**, in which we can see both Station B-UF and Station B-SPE DOM result in an increase in Polaribacter rRNA when incubated at 7°C. It is worth noting that there is no difference in effect on the microcosm community between Station B DOM extraction methods when incubated at 7°C, but there is a distinct differential response when incubated at 25°C. It is possible that the bacterial community is not responding to the novel components of each DOM extract at 7°C, but are instead responding to the common compounds found in both UF and SPE extracted DOM. This hypothesis gains further support when we consider the common response of Polaribacter when incubated with Station 23-SPE, Station B-UF, Station B-SPE, and phytoplankton-derived DOM at 7°C (**Figure 15**), while the 25°C incubations facilitate diverse responses in the bacterial community.

3.9.3 Phytoplankton DOM

The phytoplankton-derived DOM incubations appear to have a temperature-dependent response, as illustrated on the dissimilarity dendrogram (**Figure 10**), with the 25°C incubated sample being more dissimilar from the negative-control (thetayc = 0.437) than the 7°C incubated sample (thetayc = 0.101). Incubation with phytoplankton-derived DOM did not have a significant influence on the richness of the bacterial community at either incubation temperature, as illustrated in **Figure 9**. The taxonomic response to DOM incubation at 7°C exhibits an increase in Polaribacter rRNA after 32-hours that is also observed with Station 23-SPE, Station B-UF, and Station B-SPE DOM at the same incubation temperature. The differential response of the 25°C incubation is illustrated in

Figures 14 (inner circle) and **15**, in which the Alphaproteobacteria taxa *Sulfitobacter* appears to be responsible for the higher dissimilarity in the 25°C-incubated microcosm, making it the the only microcosm in which a positive *Sulfitobacter* metabolic response is observed. While temperature has been shown to regulate the rate of response in a bacterial community to DOM input, the *Sulfitobacter* does not experience any change in relative abundance from the negative-control microcosm when incubated at 7°C, suggesting that this taxa may be unable to utilize the phytoplankton-derived DOM at this incubation temperature, or that the lower incubation temperature is inhibiting the taxa from exhibiting a rapid metabolic response.

3.10 Shift in the taxonomic composition of 16S rRNA gene

16S rRNA genes were amplified and sequenced in order to determine the change in the relative abundance of taxa present in the community in response to DOM input. Our expectation is that as bacteria respond to DOM incubations, we will first see a change in the rRNA transcript, corresponding to their metabolic response, and will then observe a change in rRNA gene content, indicating a response in cell abundance of the taxa.

The response in the bacterial community to the negative-control incubation can be seen in **Figure 11**, in which the first observation is that the taxa exhibiting the highest relative abundance of 16S rRNA transcripts (inner circle) do not necessarily define the relative abundance of 16S rRNA genes (outer circle). This does not come as a complete surprise, considering the wide array of life-history strategies employed by heterotrophic bacteria. In this case, it appears as though despite the *Polaribacter* taxa comprising a relatively small amount of the total rRNA transcripts at the zero-timepoint (12.3%), the cell

abundance of this taxa is comparatively large (55.0%), suggesting this taxa is able to maintain high levels of abundance at a relatively low level of metabolic activity.

The negative-control incubation of the microcosms appears to have a temperature-dependent response on the relative abundance of 16S rRNA genes within the bacterial community. Both temperatures appear to have a negative affect on the abundance of the Polaribacter taxa, which decreases by 18.6% at 7°C and by 37% at 25°C during the 32-hour incubation period. Interestingly, the Polaribacter increase by 7.2% after 12-hours when incubated at 25°C, suggesting that the Polaribacter initially exhibit a positive response to incubation. While this net decrease in Polaribacter is observed at both incubation temperatures, the taxa responding positively appears to be unique to each incubation temperature, with the Gammaproteobacteria taxa SAR92 increasing by 10.4% in the 7°C incubation, while the taxa Cytophaga and OM42 increasing by 19.4% and 21.8% respectively in the 25°C incubation. The observation that the Polaribacter taxa initially increase in relative abundance in the 25°C incubation suggests that bacterial succession may be taking place over the incubation period. The response in the relative abundance of 16S rRNA genes in the negative-control microcosm appears to be more dramatic than the response in 16S rRNA transcripts, which did not exhibit much of a response over the 32-hour incubation period. This observation conflicts with my hypothesis that a response in 16S rRNA transcripts would precede a response in 16S rRNA genes, and may be a result of the 16S rRNA transcripts and 16S rRNA genes being isolated from separate microcosms.

3.10.1 Station 23 DOM

The taxonomic response to Station 23 DOM extracted by UF was one of the most dramatic observations in the 16S rRNA transcript sequence data, characterized by a bloom in the Gammaproteobacteria taxa *Pseudoalteromonas*. This response was also observed in the 16S rRNA gene sequence data, as illustrated in **Figure 12** (outer circles). Similar to the 16S rRNA transcript data, the bacterial community exhibits a temperature-dependent response to Station 23-UF DOM, with a more rapid response being observed in the 25°C incubated microcosm, which experiences a 58% increase in *Pseudoalteromonadales* over 32-hours. In contrast to this observation is the 7°C incubated microcosm, in which the *Pseudoalteromonas* only increase by 1% after 32-hours, despite experiencing an 88% increase in 16S rRNA transcript abundance. This observation suggests that while the *Pseudoalteromonas* are actively metabolizing the Station 23-UF DOM at both 7°C and 25°C, the taxa is only able to utilize that energy towards cell-division at a higher incubation temperature. One interesting feature of the 25°C *Pseudoalteromonas* bloom is that the response in rRNA genes appears to lag behind the response in rRNA transcripts. This observation aligns well with my hypothesis that changes in 16S rRNA transcript abundance should precede changes in 16S rRNA gene abundance.

Similar to the observations in the 16S rRNA transcript data, the *Pseudoalteromonas* bloom appears to be specific to the HMW fraction of Station 23 DOM, and does not experience significant growth in response to S23-SPE DOM. Instead, this DOM incubation appears to influence a temperature-dependent response on the bacterial community, with greater change occurring at 25°C than at 7°C. The 7°C incubated microcosm experienced a 9.2% increase in *Polaribacter*, which contrasts with the 18.6% decrease observed in the negative-control microcosm. The 25°C incubation experienced a decrease in *Polaribacter*

similar to that observed in the negative-control, although the taxa that increase are different from the negative. While the negative-control microcosm experienced a 37.2% decrease in *Polaribacter* and subsequent 19.4% and 21.8% increase in *Cytophaga* and OM42, the Station 23-SPE DOM 25°C incubated microcosm experienced a 39.6% decrease in *Polaribacter* and subsequent 21.2% and 12.9% increase in *Sulfitobacter* and SAR92. This differential response from the negative-control suggests that these taxa are specifically responding to the Station 23-SPE DOM compounds utilized in this microcosm. While there was no obvious time-lag between the 16S rRNA transcript and gene abundance in the 25°C microcosm, there may be a relationship between the 20% increase in *Polaribacter* transcripts and 9.2% increase in *Polaribacter* genes after 32 hours in the 7°C incubated microcosm.

3.10.2 Station B DOM

The 16S rRNA transcript response in the bacterial community to Station B-DOM was temperature dependent, with a similar response observed in both Station B-UF and Station B-SPE treated microcosms when incubated at 7°C, but a differential response observed at 25°C (**Figure 13; outer circles**). Interestingly, the 16S rRNA gene data also suggests a temperature-dependent response is occurring, but in this case it is the 25°C incubated microcosms that experience a small change in taxonomy, while the 7°C incubated microcosms experience differential responses based on the extraction procedure. Considering the differential response observed in the transcript data, the 16S rRNA gene response is very similar between Station B-UF and Station B-SPE DOM in the 25°C incubated microcosms, and also experience very little deviation from the zero-hour

microcosm. However, considering the negative-control incubated samples experienced notable change in the relative abundance of taxa over the 32-hour incubation period, specifically an increase in the Cytophaga and OM42 taxa, it is possible that the Station B-derived DOM is inhibiting growth of the major taxa present at Station 21 at the start of the experiment.

Unlike the 25°C incubated microcosms, the 7°C incubations facilitated a slightly differential response between the Station B-UF and Station B-SPE DOM incubated bacterial communities. The Station B-UF incubated sample was characterized by a 8.5% increase in Cytophaga and a 6.6% decrease in OM42 over the 32-hour incubation period, while the Station B-SPE incubated sample did not deviate from the negative-control microcosm. The lack of taxonomic response in $\frac{3}{4}$ of the Station B-DOM incubations, despite the observed response in the negative-control incubations suggests that this DOM amendment may be inhibiting the growth of the resident bacterial community.

3.10.3 Phytoplankton-derived DOM

The response in the relative abundance of 16S rRNA genes among taxa after incubation with phytoplankton-derived DOM possesses some similarities to the response in the 16S rRNA transcripts. **Figure 14** (inner circle) illustrates that there is a temperature-dependent response occurring in the microcosms, characterized by differential responses between the 7°C and 25°C incubated microcosms. The 7°C incubation results in the increase in the Gammaproteobacteria taxa *Pseudoalteromonas* and SAR92 by 4.3% and 6.2% respectively, which corresponds to a decrease in *Polaribacter*.

The 25°C incubation experiences a differential response, with the Sulfitobacter increasing by 42% over the 32-hour incubation period. This increase in Sulfitobacter coincides with a 25.2% increase in 16S rRNA transcripts, suggesting a positive relationship between rRNA transcript and rRNA gene responses. Similar to the 7°C incubation, there is an increase in the SAR92 taxa by 10.1% after the 32-hour incubation period.

An increase in Sulfitobacter 16S rRNA genes was observed in only one other DOM treatment, the Station 23-SPE DOM incubated at 25°C. We hypothesize that due to a phytoplankton bloom near Station 23, this DOM isolate was high in phytoplankton-derived DOM, suggesting the composition may be similar to that found in our phytoplankton-DOM incubation.

4. Discussion

The approach of our experimental design was to monitor the changes in taxonomic composition and metabolic activity of an estuarine bacterial assemblage to DOM of variable composition. The DOM was isolated from the upper and lower SLE a short period of time before the bacterial microcosms were collected, and represent DOM composition typical of a freshwater and marine environment, respectively. Bacterial community within the microcosms were sampled over a 32-hour incubation period in 10-12 hour increments, allowing us to observe the change in taxonomic composition and metabolic activity over time, through DNA and RNA analysis. During the course of the incubation period, samples were also taken to conduct cell abundance and bacterial production analysis, allowing us to develop a clearer picture of how the bacterial community responds to changes in DOM composition.

For the remainder of this discussion, I will interpret the results of the microcosm experiment in the context of the natural state of the SLE and how our results conform or deviate from what was expected based on the available literature.

4.1 DOM composition before and after microcosm incubation

Each DOM isolate used in the microcosm experiment was analyzed using FTIR spectrometry, and these spectra were compared to spectra obtained from the microcosms after the 32-hour incubation period in order to determine which functional groups, if any, are being preferentially degraded by the bacterial community.

Some conclusions that can be drawn from the FTIR analysis of the post-incubation DOM samples are that the compounds responsible for bands at $1000\text{-}1170\text{ cm}^{-1}$ become completely lost from every microcosm over the course of the 32-hour incubation period. Previous literature has found that this spectra is comprised of tertiary, secondary, and primary alcohols, which are derived from carbohydrates (Landry & Tremblay 2012). Because we see such a notable decrease in the abundance of these compounds after the incubation period, these are likely highly labile compounds being rapidly consumed by heterotrophic bacteria. We can consider a few taxa that may be responsible for the consumption of these highly-labile compounds, based on the resident bacterial community and the response after the incubation period. Considering that estuarine and riverine ecosystems are characterized by annually recurring spring blooms which sustain heterotrophic bacterial communities, it is likely that a portion of the DOM is phytoplankton-derived, which often promotes succession of taxa within the bacterial community. Such succession events have been characterized by initial degradation of HMW compounds by

Gammaproteobacteria and Bacteroidetes, which produce LMW compounds that are subsequently degraded by other taxa in the Alphaproteobacteria phyla (Teeling et al. 2012). Previous studies have found some taxa exhibiting rapid responses to phytoplankton-derived DOM include the Gammaproteobacteria taxa SAR92 and the Bacteroidetes taxa Polaribacter (West et al. 2008), both of which can be found in the original bacterial community (**Figure 4**), and exhibit a positive metabolic response to DOM isolated from both Station B and Station 23, specifically when incubated at 7°C (**Figure 15**). Another taxa with a reputation of degrading phytoplankton-derived DOM is the Alphaproteobacteria clade Rhodobacterales (Mou et al. 2008), which generally respond to LMW DOM made available after degradation by Gammaproteobacteria and Bacteroidetes species. Members of the Rhodobacterales clade can be found in our initial bacterial community, specifically the OM42 and Sulfitobacter taxa. The Rhodobacterales clade is considered a bacterial generalist, being able to utilize a wide variety of LMW DOM compounds found in coastal ecosystems (Moran et al. 2007), although its place in the successional degradation of phytoplankton-derived DOM may explain the limited response observed in our 32-hour incubation period.

Although a similar FTIR spectra was observed in each of the DOM incubations, with the –OH groups being completely depleted from the microcosms, there are some key differences in the DOM isolated from Station B and Station 23 that add some power to the hypotheses that we can generate. The spectra obtained from Station B and Station 23 derived DOM suggest that there is a higher proportion of these –OH groups at Station 23, adding to our hypothesis that these are phytoplankton-derived DOM compounds, which typically comprise a higher proportion of the DOM found in high brackish estuaries and coastal ecosystems. Similarly, there appears to be a higher abundance of aromatics found in DOM

derived from Station B, which is expected considering the amount of terrestrial inputs to riverine and upper-estuarine ecosystems. Going forward, we will consider that Station B-DOM is higher in terrestrially-derived DOM that is likely less labile than that isolated from Station 23, which contains a higher proportion of phytoplankton-derived DOM.

4.2 Processing of estuarine DOM by Gamma-Proteobacteria

The most drastic change to the bacterial community occurred in response to DOM isolated from Station 23 by means of UF, which specifically isolates HMW compounds between 1-30 kDa. This DOM amendment facilitated the bloom of the Gammaproteobacteria taxa *Pseudoalteromonas*, which was specific to this DOM source and extraction method. The *Pseudoalteromonas* is a diverse taxa found throughout the world's oceans from deep-sea sediments to surface water (Evans et al. 2008), and are capable of producing large quantities of extracellular enzymes in order to utilize particulate and HMW organic matter (Chen et al. 2003; Zhou et al. 2009; Vera et al. 1998; Ivanova & Kiprianova 1998)

Although commonly found in marine and coastal sediments, the *Pseudoalteromonas* have been observed in Antarctic surface waters and still retain their HMW DOM degrading characteristics (Bozal et al. 1997), and are capable of growth in salinities between 1-9‰ (Ivanova & Mikhailov 2001), which fits well with our estuarine ecosystem. Additionally, the *Pseudoalteromonas* and closely related members of the Alteromonadales have been recorded dominating heterotrophic blooms in mesocosm experiments, which is exactly what was observed in this experiment (Schafer et al. 2000; McCarren et al. 2010).

Previous microcosm experiments have observed other Gammaproteobacteria taxa responding to HMW DOM from marine surface waters, including the Idiomarinaceae, and Thiotrichales (McCarren et al. 2010). Our observation of the Alteromonadaceae taxa *Pseudoalteromonas* responding to HMW-DOM derived from a high-brackish estuarine ecosystem, along with other observations of *Pseudoalteromonas* in coastal environments (Imai et al. 2006; Bozal et al. 1997) suggest that *Pseudoalteromonas* may be the dominant taxa utilizing HMW-DOM in high-brackish estuarine and coastal environments.

One question that remains and may encourage further research projects is whether the degradation of HMW DOM by *Pseudoalteromonas*, which likely produced a variety of labile LMW DOM compounds, would have facilitated a succession-event if the microcosms were incubated for a longer period of time.

4.3 Processing of phytoplankton-derived DOM by Alphaproteobacteria

The taxonomic response in our bacterial community to phytoplankton-derived DOM, as seen in **Figure 14**, exhibits a temperature-dependent response wherein the Alphaproteobacteria *Sulfitobacter* exhibit a stronger response in both 16S rRNA transcripts and genes when incubated at 25°C. The observation of this taxon belonging to the Rhodobacterales clade, responding positively to phytoplankton-derived DOM is not unexpected, considering the Rhodobacterales are characterized in coastal ecosystems to have a close relationship to phytoplankton blooms (Pinhassi et al. 2004; West et al. 2008; Alavi et al. 2001; González et al. 2000; Riemann et al. 2000). *Sulfitobacter* specifically have been shown to contain genes associated with DMSP utilization, which is a sulfurous compound produced by phytoplankton (Ledyard:1993wc González et al. 2000; Zubkov et al. 2002).

Previous studies have observed a positive-response in the Sulfitobacter taxa to incubation with Nannochloropsis phytoplankton, and have also suggested that extracellular excretions of DMSP and amino acids are responsible for this specific response (Sharifah & Eguchi 2011).

4.4 Processing of diverse DOM by Flavobacteria

The relative abundance of 16S rRNA transcripts belonging to the Flavobacteria taxa Polaribacter was observed in response to all three of the DOM sources utilized in this experiment. This taxa was found to experience a positive-response to more DOM isolates than any other taxa, suggesting there are either common compounds shared between the Station B, Station 23, and phytoplankton-derived DOM extracts, or that this taxa exhibits a particularly broad metabolic capacity.

The Polaribacter are part of the Bacteroidetes phylum, and can be found in a wide variety of aquatic ecosystems including Arctic and Antarctic euphotic zones (Abell & Bowman 2005b; Gosink et al. 1998) and sea-ice (M V Brown & Bowman 2001; Brinkmeyer et al. 2003), marine (Schattenhofer et al. 2009), and estuarine surface waters.

(Barbara J Campbell & Kirchman 2012; Crump et al. 2004). Flavobacteria are often found associated with phytoplankton-blooms, utilizing the HMW DOM compounds abundant during the bloom (DeLong et al. 1993; Glockner et al. 1999; Pinhassi et al. 2004; West et al. 2008; Teeling et al. 2012), and are often found in high nutrient ecosystems where phytoplankton blooms are prevalent (Abell & Bowman 2005a).

A microcosm study conducted by Cottrell and Kirchman (Cottrell & Kirchman 2000b) in the Delaware Bay estuary determined that estuarine Flavobacteria preferentially utilized HMW-DOM compounds, but were also able to utilize the LMW-DOM compound N-

acetylglucosamine. This observation provides evidence that while Flavobacteria may preferentially utilize HMW-DOM, they can exhibit metabolic plasticity to consume LMW DOM compounds as well.

The result of the microcosm experiment conducted in the SLE illustrate that Polaribacter have the capacity to utilize DOM derived from diverse ecosystems, but was mostly observed at 7°C, reinforcing the Polaribacter's reputation as a psychrophilic bacterial taxa (Gosink et al. 1998). Despite its namesake, Polaribacter did exhibit a positive response to high-temperature incubation with Station 23-SPE DOM, which was observed in no other 25°C incubation. This is not the first time Polaribacter was observed at high temperatures (Nedashkovskaya et al. 2013), but the question remains why no other DOM incubation resulted in a similar increase in Polaribacter at 25°C.

The Polaribacter was the only taxa to increase in relative abundance of rRNA transcripts in response to Station B-DOM, which may suggest it is able to utilize terrestrially-derived DOM compounds. This hypothesis is supported by the high abundance of terrestrially-derived DOM in the Arctic ocean (Opsahl et al. 1999; Cory et al. 2007), where Polaribacter experience regular high abundances. Given the high abundance of terrestrially-derived DOM in an ecosystem characterized by high abundance of Polaribacter, it is entirely possible this taxa is routinely exposed to and can even utilize terrestrially-derived DOM under the correct circumstances, which we may have observed in this experiment.

4.5 Elevated diversity caused by river DOM and temperature

Despite experiencing a differential response in 16S rRNA transcripts (**Figure 15**), bacterial communities incubated with Station B-derived DOM at 25°C experienced a

significant increase in species richness that was not observed at 7°C. Despite the perception that the number of species present in the microcosm is increasing over the incubation period, the actual mechanism responsible for this observation based on 16S rRNA data being reported as relative abundance instead of absolute abundance. There are three possibilities explaining the observed increase in richness: 1) the relative abundance of taxa previously below the levels of detection increases above the levels of detection, causing them to be counted in the alpha-diversity of the community at the final timepoint but not at the initial timepoint, 2) the relative abundance of the most abundant taxa decreases over the incubation period, causing the relative abundance of rare taxa previously below the levels of detection to increase, or 3) a combination of the two. Based on the cell abundance data presented in **Figure 7**, the bacterial communities incubated with Station B-SPE and Station B-UF DOM do not experience a significant increase in cell abundance over the incubation period. This observation suggests that since cell abundance doesn't drastically decrease, it is likely the rare taxa that are becoming more abundant in response to Station B-derived DOM.

Temperature has been shown to facilitate increase in species richness (Rohde 1992; Allen et al. 2002; James H Brown et al. 2004), which may explain the increased species richness observed in the 25°C microcosms incubated with Station B-DOM but not in the 7°C incubations. However, the question remains why an increase in richness was not observed in response to any other DOM amendment, despite the increase in incubation temperature. It would appear that temperature is not the only factor influencing species richness in this system. Previous studies have reported species richness increasing in response to a greater range of resources becoming available (Chapin et al. 2000; Petchey 2000). I hypothesize that this is a potential mechanism by which species richness increases in response to Station B-

DOM. The DOM found at station B contains compounds that will not be found in high abundance in the microcosms derived from Station 21, resulting in a greater range of resources available to the microcosm community.

Another hypothesis to explain the increase in richness observed in response to Station B-DOM incubation is that the addition of DOM from the upper estuary (Station B) to a microcosm isolated from the lower estuary (Station 21) may have destabilized the bacterial community due to the introduction of highly dissimilar DOM. Environmental perturbation has reportedly caused species richness to increase, specifically within the “rare biosphere” (Kim et al. 2011), which may be the same mechanism causing an increase in species richness in the SB-DOM incubated microcosms.

5. Conclusions

This study illustrates the ability of an estuarine bacterial community to utilize diverse DOM compounds isolated from an estuarine environment. The variety of DOM isolates utilized in this experiment, and the incubation at both 7°C and 25°C, has allowed for multiple conclusions to be drawn from this experiment.

In the 16S taxonomic data generated from the experiment, there are a few taxa that stand out as exhibiting particularly strong responses to the DOM additions. The *Pseudoalteromonas* experienced a bloom that dominated the bacterial community 16S rRNA transcript and gene data in response to HMW-DOM isolated from the high-brackish Station 23. This response was observed at both 7°C and 25°C, and illustrates the Gammaproteobacteria taxa *Pseudoalteromonas* ability to rapidly utilize HMW-DOM compounds. We hypothesize that given a longer incubation period, a succession event would

be observed in which subsequent blooms of bacteria would occur in response to the LMW-DOM made available after enzymatic cleavage by *Pseudoalteromonadas*.

The *Polaribacter* taxa exhibited particular tenacity, exhibiting a metabolic response to a diverse complement of DOM isolates, including those isolated from the high-brackish Station 23, the low-salinity Station B, and the phytoplankton-derived DOM. This observation also reinforces the *Polaribacter* psychrophilic lifestyle, considering it most often exhibited a response when incubated at 7°C.

Finally, the microcosms incubated with low-salinity DOM at 25°C resulted in a significant increase in species richness within the bacterial community, suggesting a response in the rare-taxa to this unique DOM amendment. This observation may provide the most insight into how terrestrial DOM would influence a high-brackish bacterial community, although future research would benefit from obtaining transcriptomics data to determine which genes are responsible for this increase in taxa from the rare-biosphere, and if they are indeed responding specifically to terrestrially-derived DOM.

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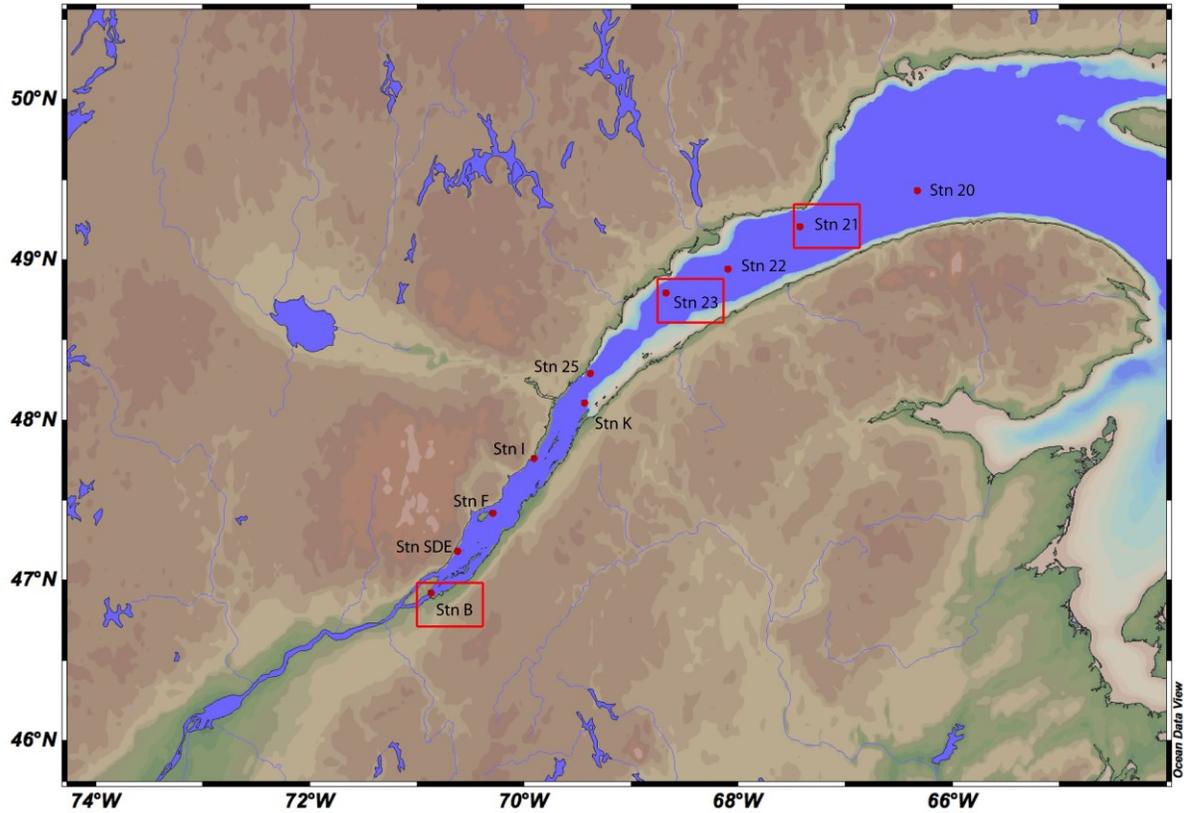


Figure 1: Map of the St. Lawrence Estuary (SLE) generated in Ocean Dataview (ODV) which includes the established sampling stations that extend from the low-salinity upper estuary (Station B) to the high-salinity lower estuary (Station 20) at the mouth of the Gulf of St. Lawrence. The stations most relevant to this experiment are outlined on the map: dissolved organic matter was isolated from Station B and Station 23, while the bacterial community utilized in the microcosms was isolated from Station 21.

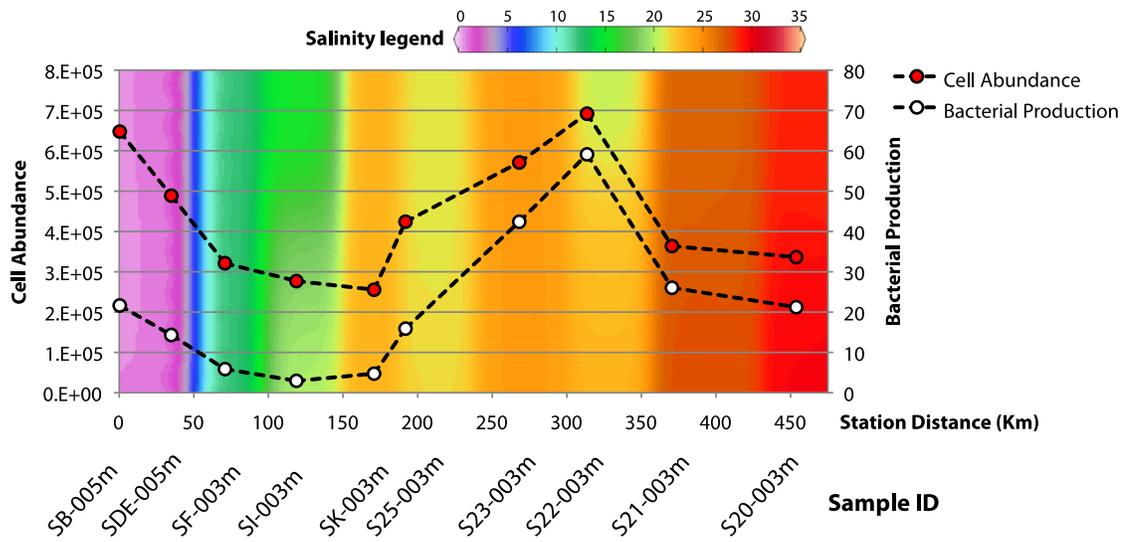


Figure 2: Cell abundance and bacterial production values were measured at the surface of each sampling station at the time water samples were collected. Cell abundance was measured by flow cytometry in Paul Del Giorgio’s lab at Universite du Quebec a Montreal (UQAM), and bacterial production was measured by the rate of leucine incorporation by the bacterial cells, and was conducted in Roxane Maranger’s lab at Universite de Montreal.

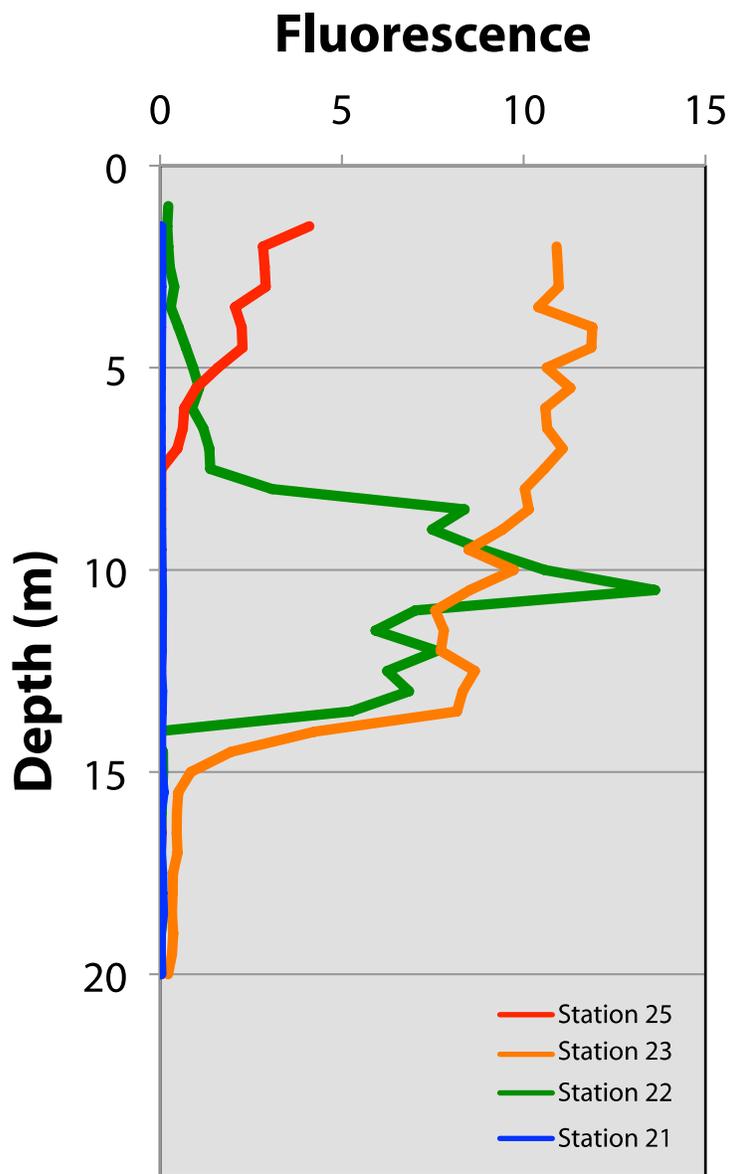


Figure 3: Fluorescence data was measured during water sampling on-board the RV Coriolis II. Fluorescence is used as a proxy for chlorophyll concentration in aquatic ecosystems, and is often used to measure phytoplankton abundance. At the time of sampling, it would appear that a phytoplankton-bloom was occurring in the surface-waters of station 23, from which one of our DOM isolates was obtained.

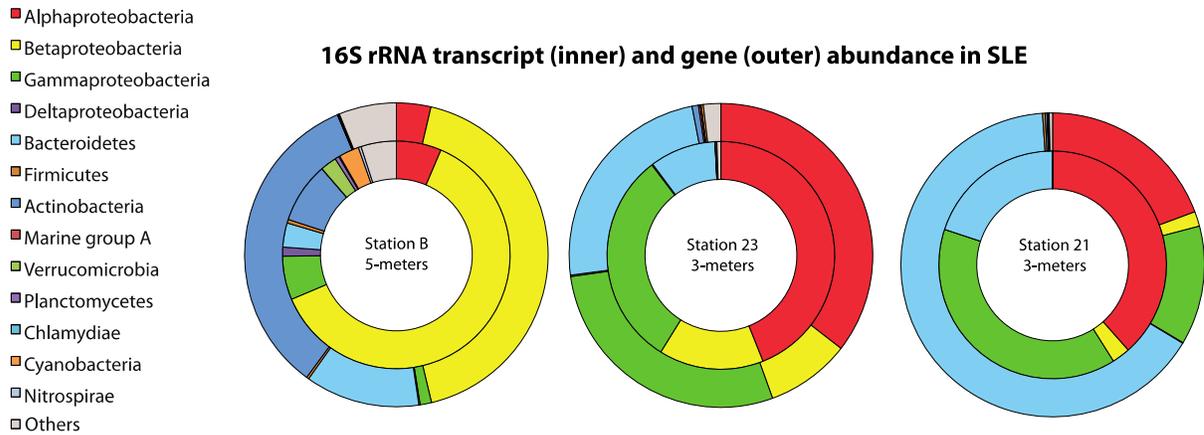


Figure 4: Bacterial biomass was collected from surface water along the salinity-gradient in the St. Lawrence Estuary, at stations specific to the microcosm experiment that was conducted. Station B and station 23 are the sites from which DOM was isolated, and station 21 was the source of the microcosm community. 16S rRNA transcripts and genes were amplified and sequenced from each of these samples and the major phyla comprising each community are illustrated in donut plots, with 16S rRNA transcript data plotted on the inside of the 16S rRNA gene data. The bacterial communities inhabiting the SLE appear to undergo a shift along the salinity gradient from SB (PSU = 0.08) to S21 (PSU = 27). 16S rRNA transcripts represent the metabolic activity of the bacterial community, while 16S rRNA genes represent the relative cell abundance of taxa.

Fourier Infrared Spectrophotometry (FTIR) values for DOM extracted from Station 23

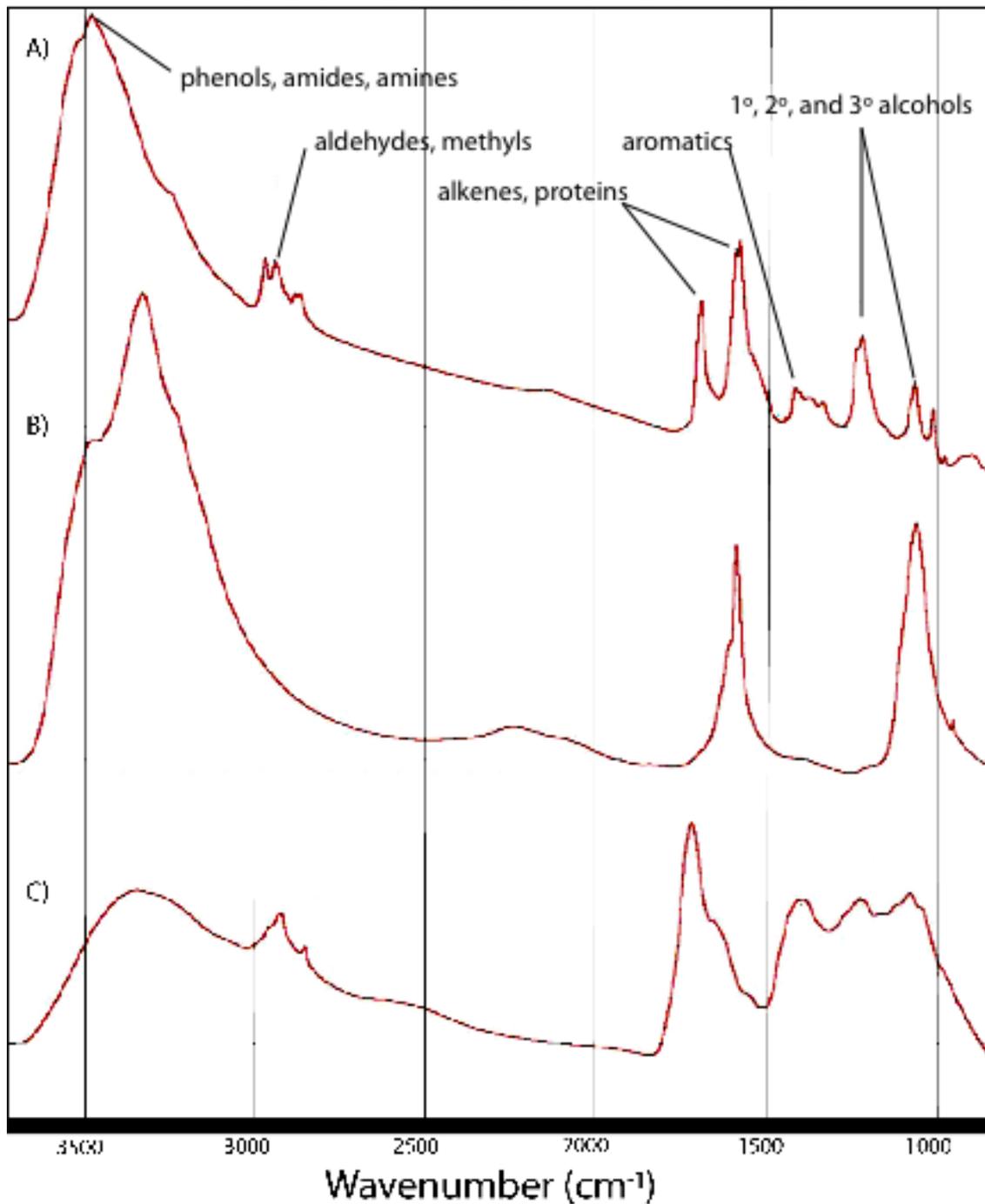


Figure 5: FTIR spectra for DOM derived from Station 23 by means of **A)** solid-phase extraction, **B)** ultrafiltration, and **C)** ultrafiltration and subsequent solid-phase extraction. FTIR spectra were generated in the Yves Gelinas lab at Concordia University.

Fourier Infrared Spectrophotometry (FTIR) values for DOM extracted from Station B

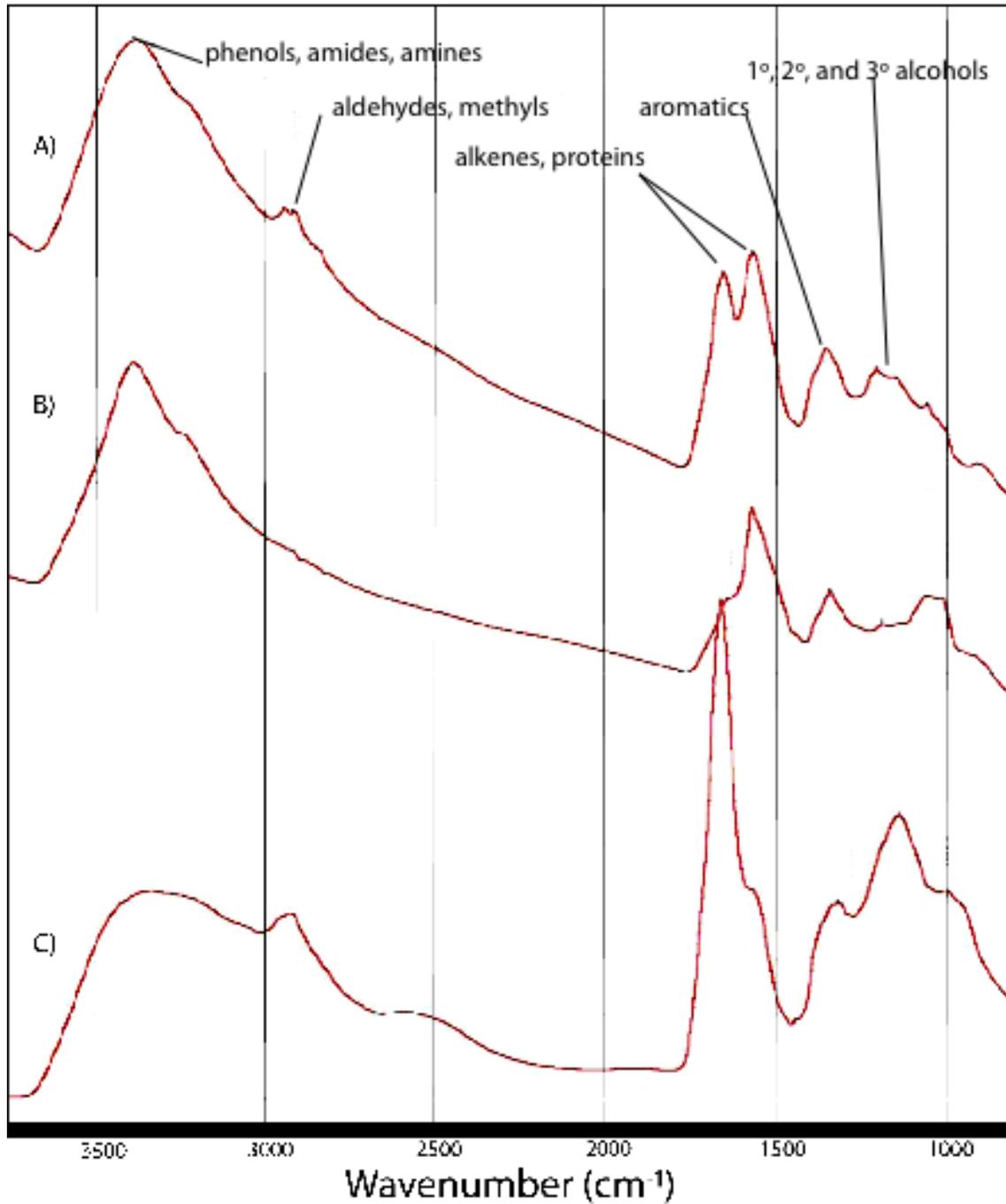


Figure 6: FTIR spectra of DOM derived from station B by means of A) solid-phase extraction, B) ultrafiltration, and C) ultrafiltration and subsequent solid-phase extraction. FTIR spectra were generated in the Yves Gelinat lab at Concordia University.

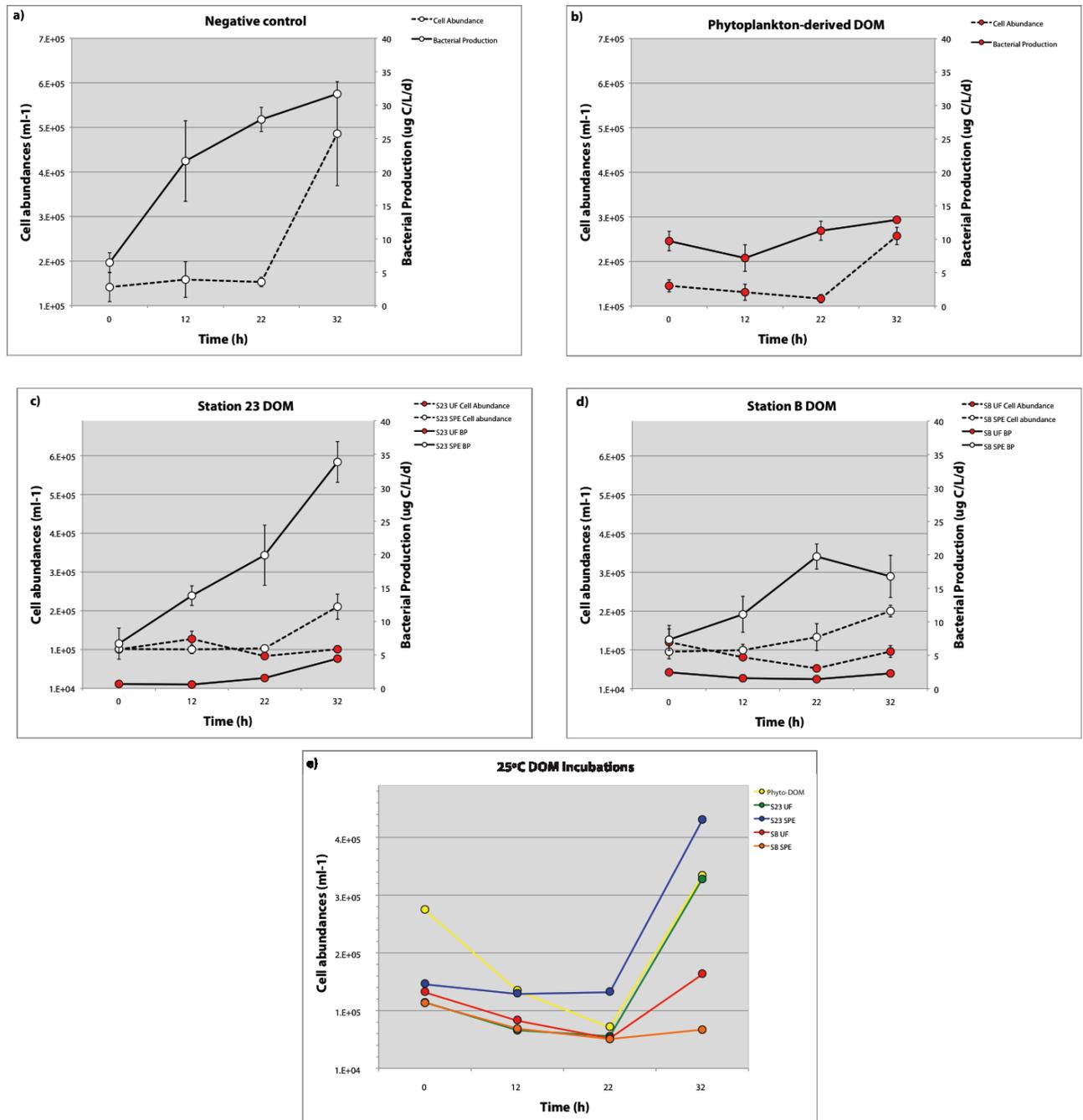


Figure 7a-e: Cell abundance and bacterial production values were measured over the course of the microcosm experiment, at the same time intervals that bacterial biomass was isolated for sequencing (0 hours, 12 hours, 22 hours, and 32 hours). Both cell abundance and bacterial production values are available for each time-point in the 7°C incubated microcosms (**a: negative-control**, **b: phytoplankton-derived DOM**, **c: station 23-derived DOM**, **d: station B-derived DOM**), but only cell abundance values are available for the time-points in the 25°C incubated microcosms (**e**). Cell abundance was measured by flow cytometry in Paul Del Giorgio’s lab at Université du Québec a Montreal (UQAM), and

bacterial production was measured by the rate of leucine incorporation by the bacterial cells, and was conducted in Roxane Maranger's lab at Universite de Montreal.

Fourier Infrared Spectrophotometry (FTIR) values for DOM extracted from post-incubation (32-hours) microcosms

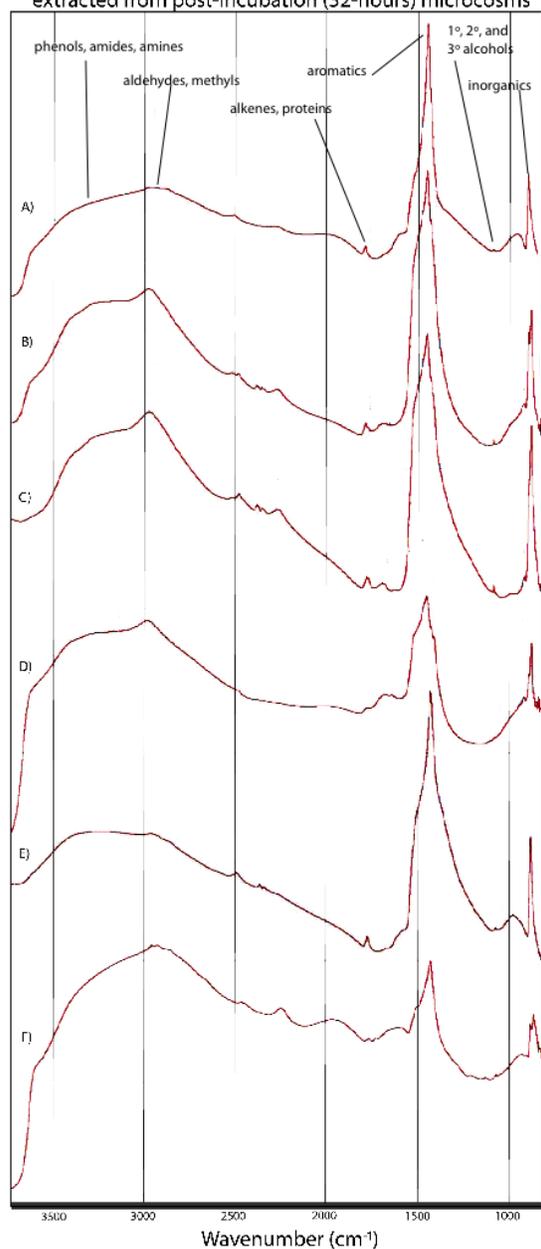


Figure 8: FTIR spectra of DOM isolated from each microcosm after 32-hours, meant to be compared to the FTIR spectra in Figures 5 and 6 to determine which DOM compounds are being utilized by the bacterial community during the incubation period. DOM was concentrated by solid-phase extraction before measured by FTIR in order to obtain an adequate concentration for the analysis. The FTIR spectra generated correspond to microcosms incubated with the following DOM isolates: A) Station-B; solid-phase extraction, B) Station-B; ultrafiltration, C) Station-23; solid-phase extraction, D) Station-23; ultrafiltration, E) phytoplankton-derived, D) negative-control. FTIR analysis was only conducted for the 25°C-incubated microcosms. FTIR spectra were generated in the Yves Gelinas lab at Concordia University.

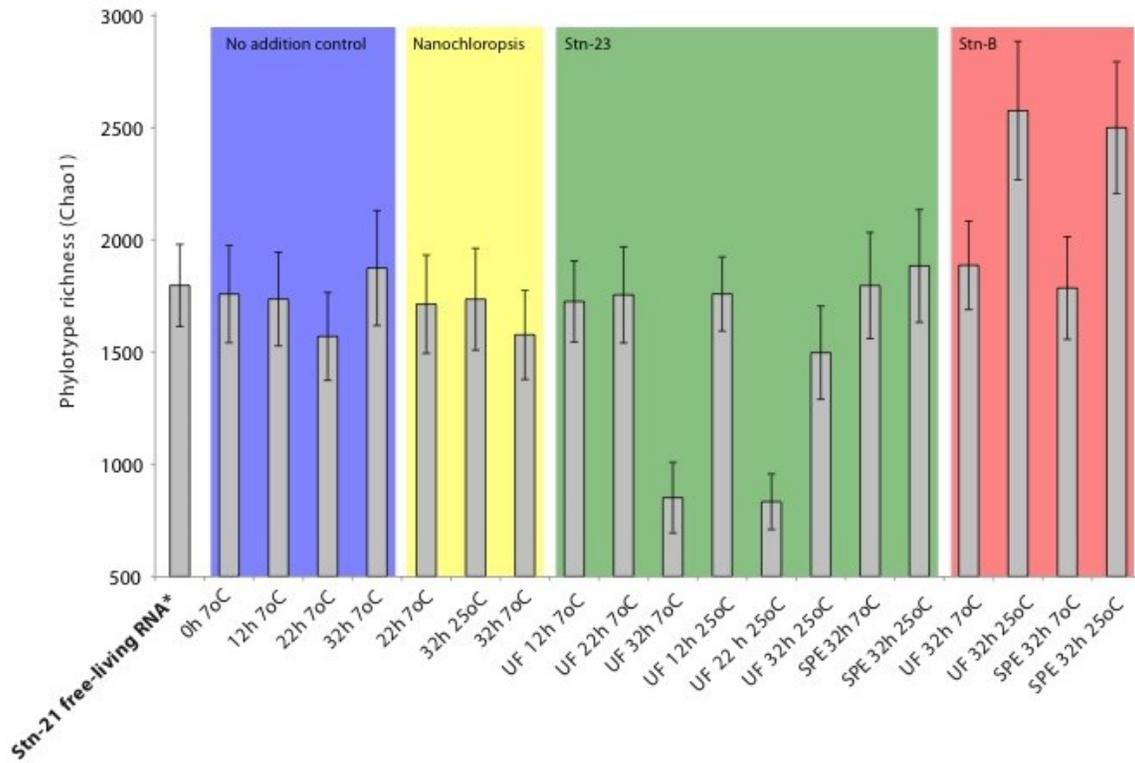


Figure 9: Alpha-diversity was measured for each microcosm sample for which 16S rRNA transcript sequence data was available, as well as the surface of Station 21 for use as a reference of natural bacterial species richness in the SLE at the time of sampling. Alpha-diversity was calculated on the Mothur software suite using the Chao1 calculator, in which a 97% cutoff was utilized to define an OTU.

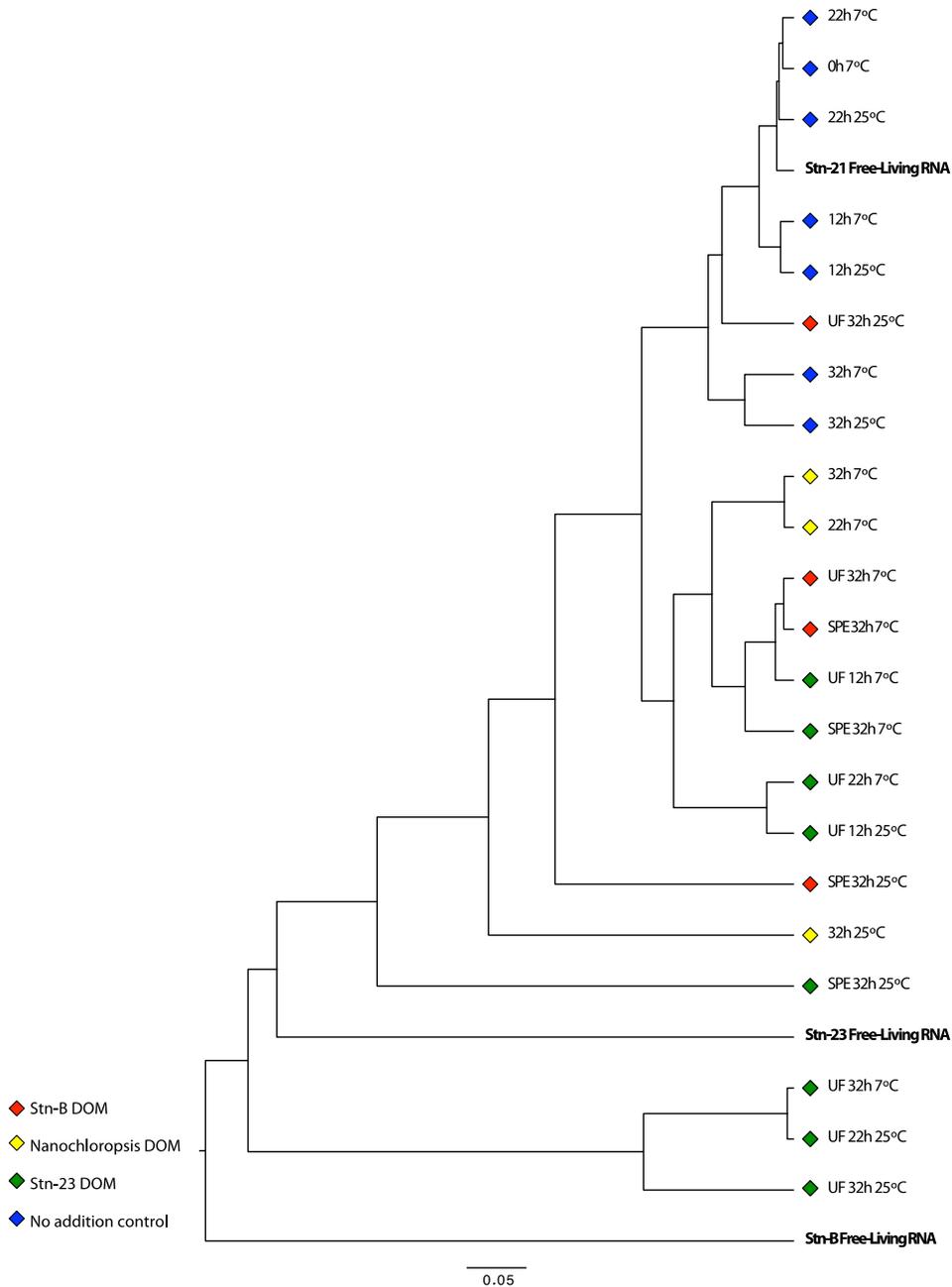


Figure 10: A dissimilarity dendrogram was constructed to illustrate the change in dissimilarity of 16S rRNA transcripts in the microcosm bacterial community in response to incubation with diverse DOM isolates. Dissimilarity of samples was defined as how dissimilar the distribution of operational taxonomic units (OTUs) was between samples. In addition to the DOM-incubated microcosm samples, the 16S rRNA transcript data for station 21, station 23, and station B surface samples were included in the dendrogram, to illustrate how dissimilar the samples are to the source community (S21) and the community associated with the DOM source (S23 and SB). Dissimilarity values were calculated in the Mothur software suite using the Thetayc calculator, in which a 97% cutoff was utilized to define an OTU.

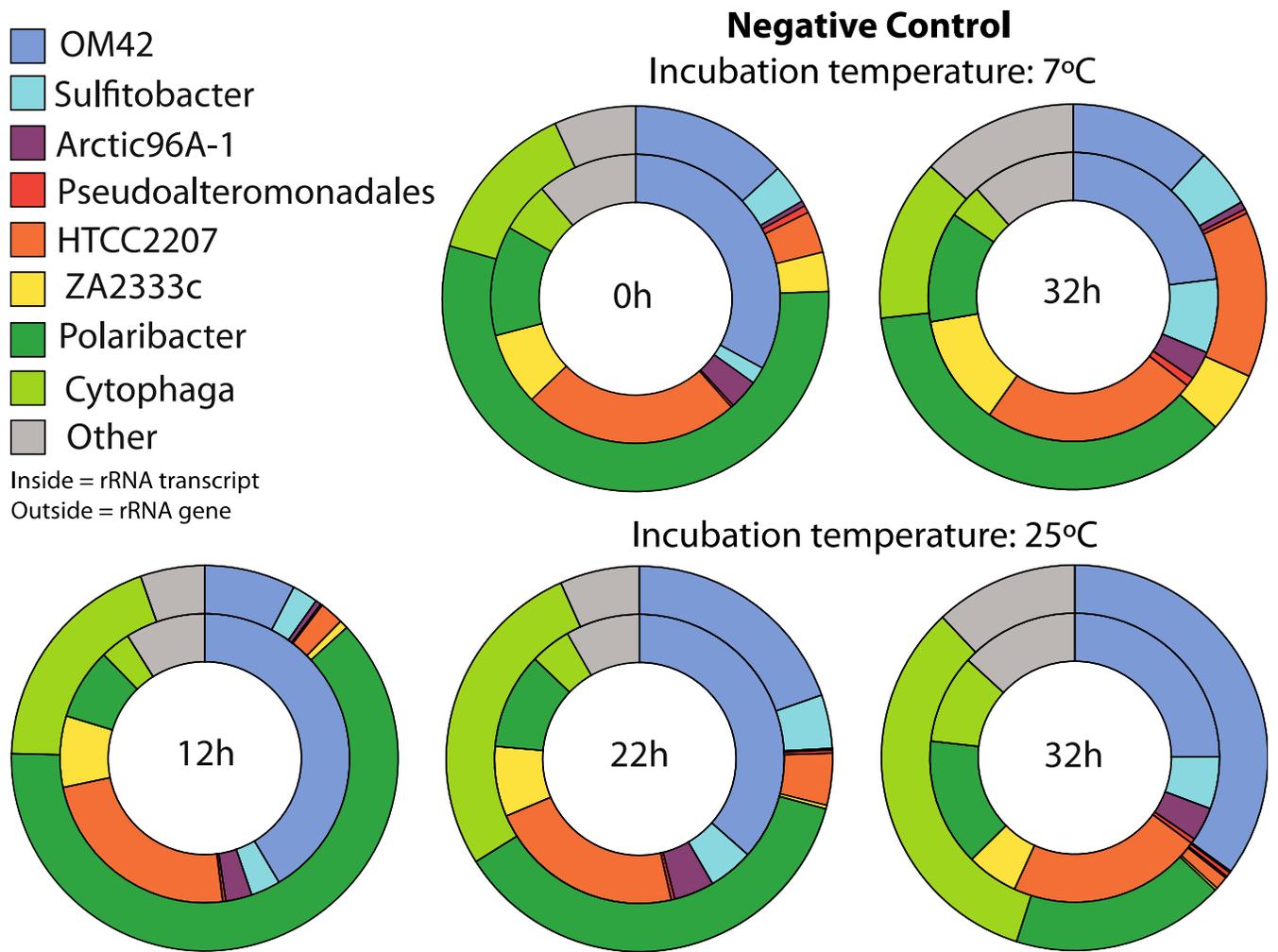


Figure 11: Bacterial biomass was collected from the negative-control microcosm during the 32-hour incubation period at both 7°C and 25°C, from which 16S rRNA transcripts and genes were amplified and sequenced. The major taxa comprising the community at each time-point are illustrated in donut plots, with 16S rRNA transcript data plotted on the inside of 16S rRNA gene data. 16S rRNA transcripts represent the metabolic activity of the bacterial community, while 16S rRNA genes represent the relative cell abundance of taxa.

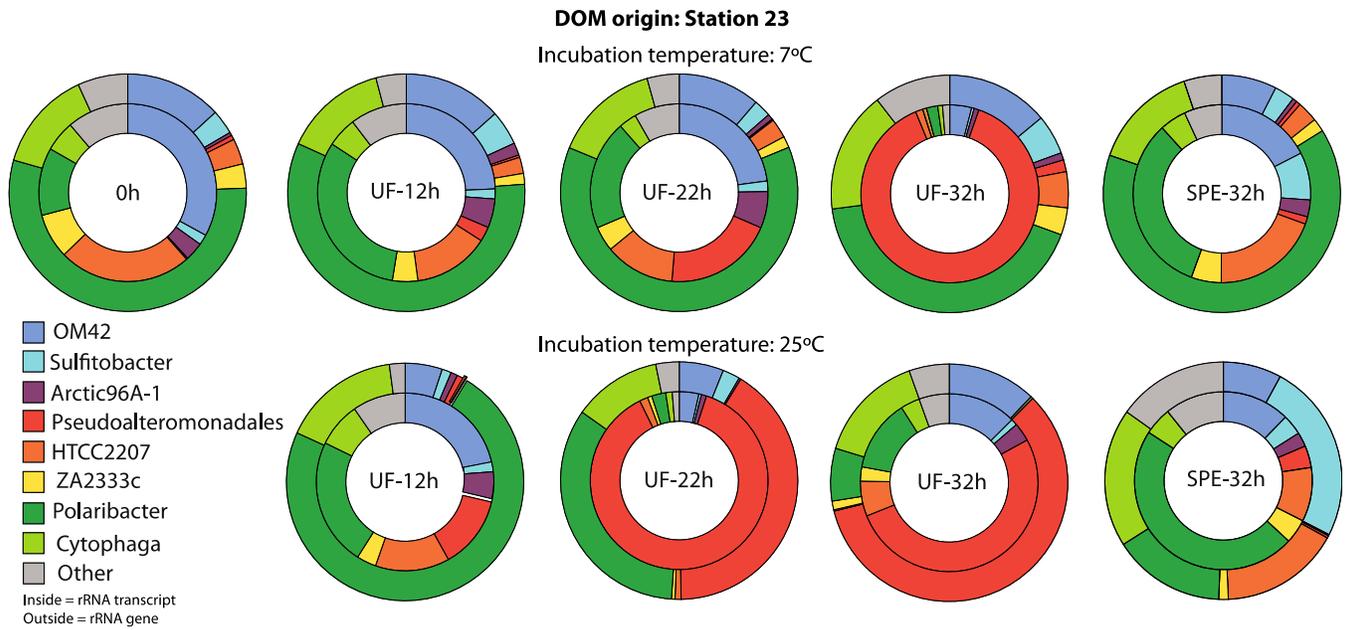


Figure 12: Bacterial biomass was collected from the microcosms incubated with DOM isolated from station 23 at both 7°C and 25°C, from which 16S rRNA transcripts and genes were amplified and sequenced. The major taxa comprising the community at each time-point are illustrated in donut plots, with 16S rRNA transcript data plotted on the inside of 16S rRNA gene data. 16S rRNA transcripts represent the metabolic activity of the bacterial community, while 16S rRNA genes represent the relative cell abundance of taxa.

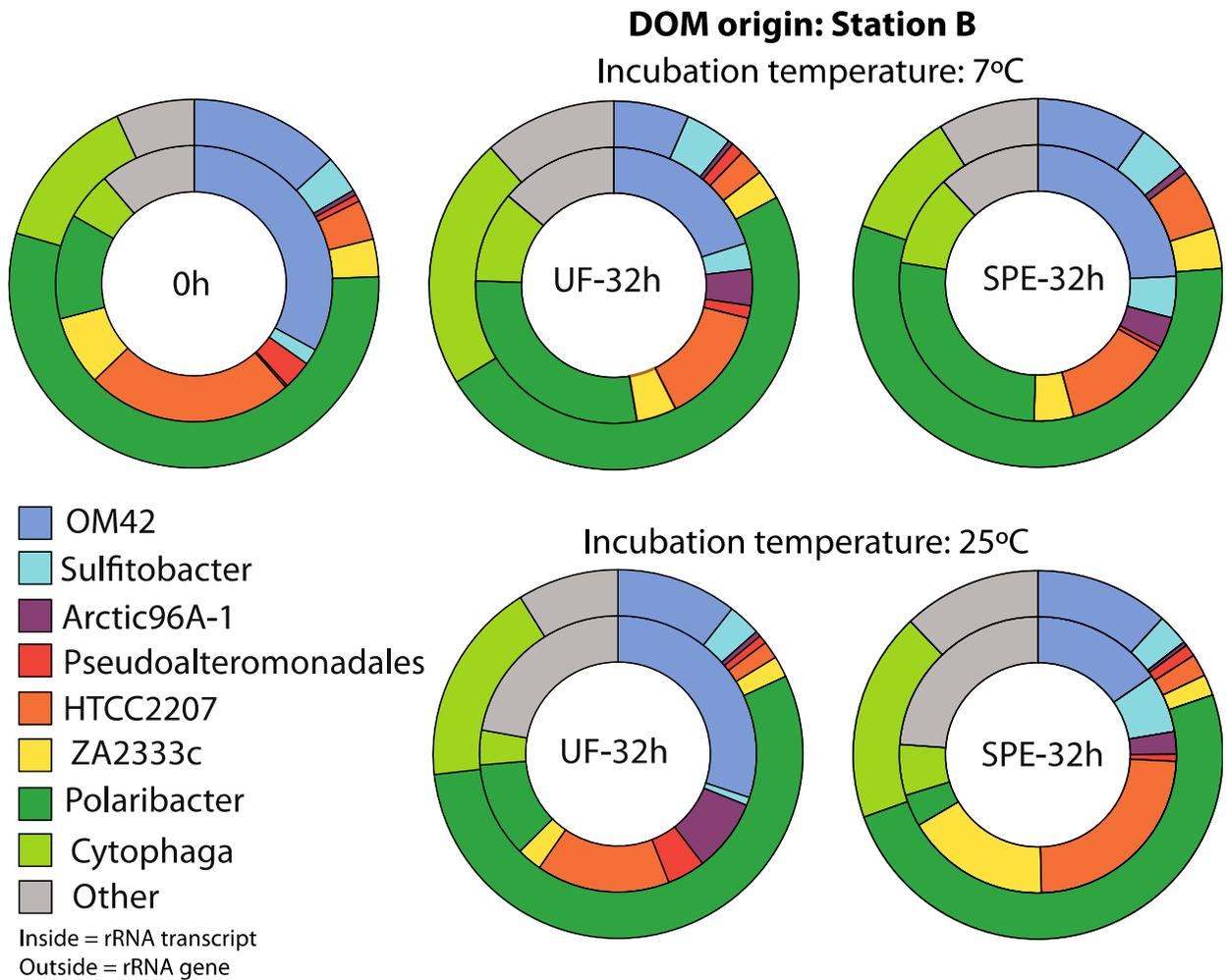
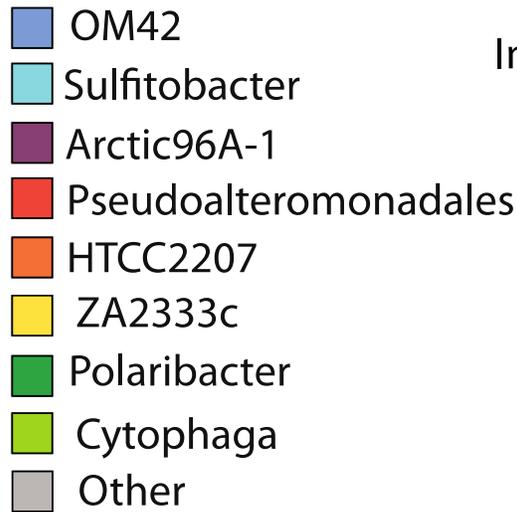
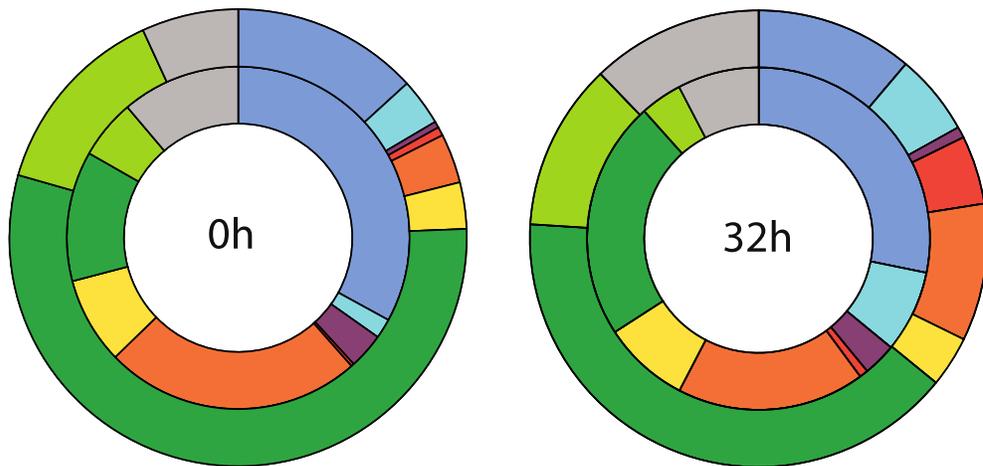


Figure 13: Bacterial biomass was collected from the microcosms incubated with DOM isolated from station B at both 7°C and 25°C, from which 16S rRNA transcripts and genes were amplified and sequenced. The major taxa comprising the community at each time-point are illustrated in donut plots, with 16S rRNA transcript data plotted on the inside of 16S rRNA gene data. 16S rRNA transcripts represent the metabolic activity of the bacterial community, while 16S rRNA genes represent the relative cell abundance of taxa.

DOM origin: Nannochloropsis

Incubation temperature: 7°C



Inside = rRNA transcript
Outside = rRNA gene

Incubation temperature: 25°C

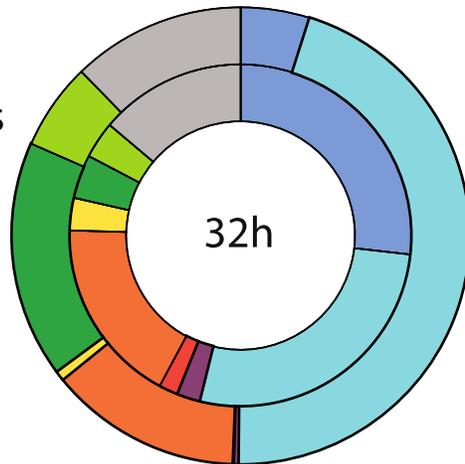


Figure 14: Bacterial biomass was collected from the microcosms incubated with DOM isolated from *Nannochloropsis* phytoplankton at both 7°C and 25°C, from which 16S rRNA transcripts and genes were amplified and sequenced. The major taxa comprising the community at each time-point are illustrated in donut plots, with 16S rRNA transcript data plotted on the inside of 16S rRNA gene data. 16S rRNA transcripts represent the metabolic activity of the bacterial community, while 16S rRNA genes represent the relative cell abundance of taxa.

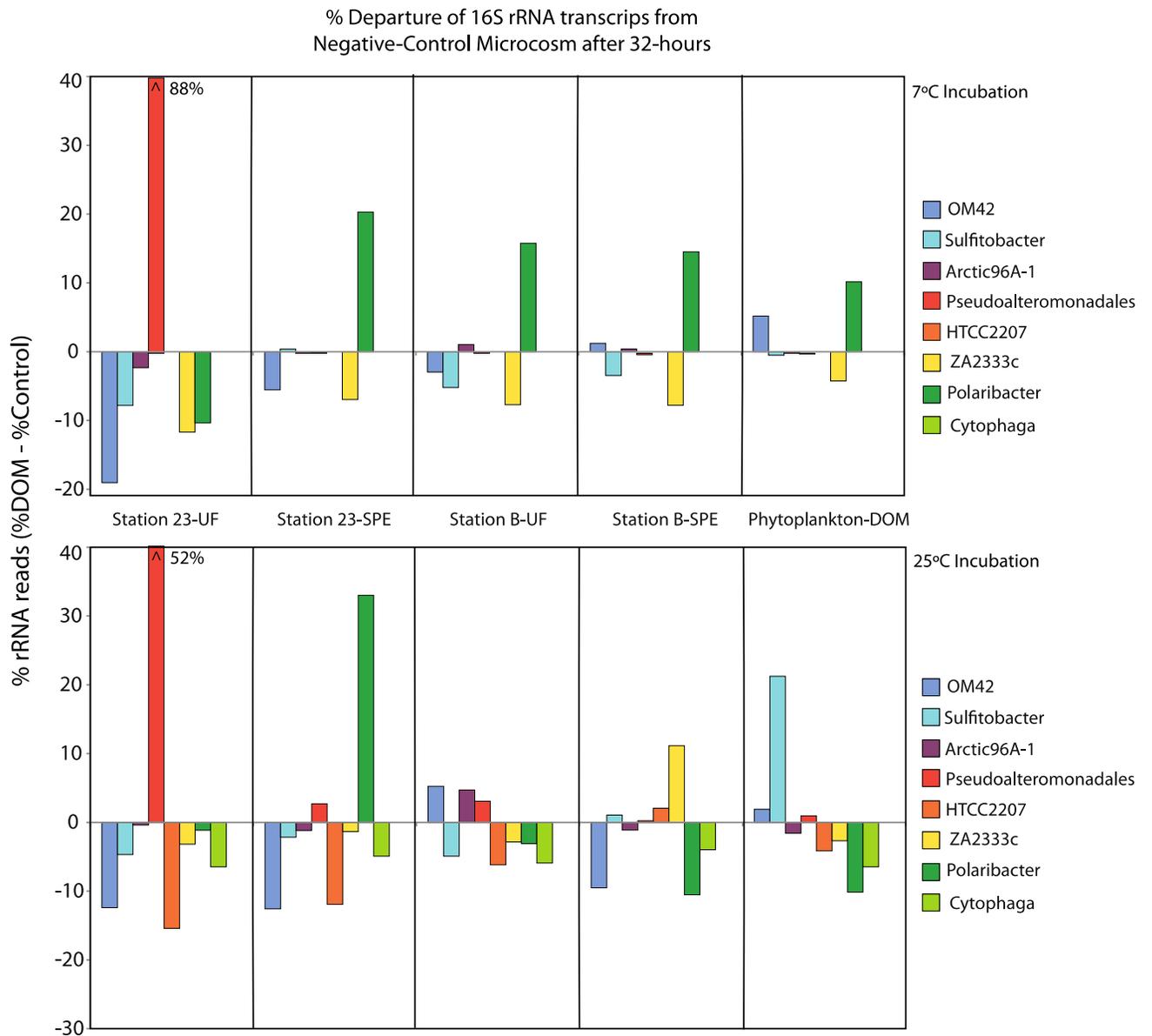


Figure 15: 16S rRNA transcript data is plotted as a departure from the negative-control for each DOM-incubated microcosm community at the 32-hour time-point. This plot illustrates how each DOM treated bacterial community deviates from the negative-control bacterial community by subtracting the relative-abundance value of each taxa from the 32-hour negative control microcosm. Ideally it is meant to illustrate the absolute change in the bacterial community in response to the DOM input, taking into account the small changes that occurred in the negative-control microcosm.

Station	Depth (meters)	Temperature (°C)	Salinity
Station 21	2.97	5.98	27.14
Station B	5.93	10.59	0.08
Station 23	2.83	4.74	24.25

Table1: The data retrieved from the CTD rosette from the SLE stations 21, B, and 23 at the time the microcosm water was collected (Station 21) and the water for DOM extraction was collected (Station B and Station 23).

RNA Sequence Summary

Sample ID	DOM Treatment	Incubation Time (hrs)	Incubation Temp (°C)	PGM Kit	Chip	Avg Read Length	Date Sequenced	Number of Seq	Number of Sub-sampled Seq	Number of OTUs (0.97)	Number of OTUs (0.90)
RNG_07	Control	0	7	200bp	316	114	04/10/2013	15002	N/A	8959	2394
RNG_127	Control	12	7	200bp	316	114	04/10/2013	15632	N/A	8959	2394
RNG_227	Control	22	7	200bp	316	114	04/10/2013	15286	N/A	8959	2394
RNG_327	Control	32	7	200bp	316	114	04/10/2013	18662	N/A	8959	2394
RNG1225	Control	12	25	200bp	316	120	04/10/2013	18066	N/A	8959	2394
RNG2225	Control	22	25	200bp	316	120	04/10/2013	20409	N/A	8959	2394
RNG3225	Control	32	25	200bp	316	120	04/10/2013	19193	N/A	8959	2394
RNJ_127	Phyto	12	7	200bp	316	119	05/24/2013	25505	N/A	8959	2394
RNJ_227	Phyto	22	7	200bp	316	114	04/10/2013	17849	N/A	8959	2394
RNJ_327	Phyto	32	7	200bp	316	114	04/10/2013	16908	N/A	8959	2394
RNJ3225	Phyto	32	25	200bp	316	114	04/10/2013	15632	N/A	8959	2394
R23U127	Marine-HMW	12	7	200bp	316	120	04/10/2013	13875	N/A	8959	2394
R23U227	Marine-HMW	22	7	200bp	316	120	04/10/2013	19423	N/A	8959	2394
R23_U327	Marine-HMW	32	7	200bp	316	114	04/10/2013	16161	N/A	8959	2394
R23U1225	Marine-HMW	12	25	200bp	316	120	04/10/2013	11531	N/A	8959	2394
R23U2225	Marine-HMW	22	25	200bp	316	120	04/10/2013	12544	N/A	8959	2394
R23U3225	Marine-HMW	32	25	200bp	316	120	04/10/2013	19167	N/A	8959	2394
R23_S327	Marine-SPE	32	7	200bp	316	114	04/10/2013	17395	N/A	8959	2394
R23S3225	Marine-SPE	32	25	200bp	316	120	04/10/2013	19866	N/A	8959	2394
RB_U327	Fresh-HMW	32	7	200bp	316	114	04/10/2013	21288	N/A	8959	2394
RB_U3225	Fresh-HMW	32	25	200bp	316	120	04/10/2013	18606	N/A	8959	2394
RB_S327	Fresh-SPE	32	7	200bp	316	114	04/10/2013	19912	N/A	8959	2394
RBS3225	Fresh-SPE	32	25	200bp	316	114	04/10/2013	18812	N/A	8959	2394

DNA Sequence Summary

Sample ID	DOM Treatment	Incubation Time (hrs)	Incubation Temp (°C)	PGM Kit	Chip	Avg Read Length	Date Sequenced	Number of Seq.	Number of Subsampled Seq.	Number of OTUs (0.97)	Number of OTUs (0.90)
DNG07	Control	0	7	200bp	316	152	09/24/12	2447	1728	236	181
DNG_327	Control	32	7	100bp	314	124	07/27/12	2840	1728	436	262
DNG1225	Control	12	25	200bp	316	152	09/24/12	3257	1728	247	169
DNG2225	Control	22	25	200bp	316	152	09/24/12	2947	1728	267	182
DNG_3225	Control	32	25	100bp	316	139	07/27/12	2956	1728	439	242
DNJ_327	Phyto	32	7	100bp	314	124	07/27/12	2799	1728	412	255
DNJ1225	Phyto	12	25	200bp	316	152	09/24/12	2139	1728	237	168
DNJ2225	Phyto	22	25	200bp	316	152	09/24/12	2580	1728	281	184
DNJ_3225	Phyto	32	25	100bp	316	139	07/27/12	2927	1728	483	284
D23U127	Marine-HMW	12	7	200bp	316	152	09/24/12	2453	1728	239	164
D23U227	Marine-HMW	22	7	200bp	316	152	09/24/12	2395	1728	220	154
D23_U327	Marine-HMW	32	7	100bp	314	124	07/27/12	2814	1728	523	318
D23U1225	Marine-HMW	12	25	200bp	316	152	09/24/12	3223	1728	177	131
D23U2225	Marine-HMW	22	25	200bp	316	152	09/24/12	2251	1728	214	141
D23_U025	Marine-HMW	0	25	100bp	316	139	07/27/12	2887	1728	496	302
D23_U3225	Marine-HMW	32	25	100bp	316	139	07/27/12	2949	1728	473	228
D23S327	Marine-SPE	32	7	200bp	316	152	09/24/12	2491	1728	228	147
D23_S025	Marine-SPE	0	25	100bp	316	139	07/27/12	2880	1728	464	300
D23S2225	Marine-SPE	22	25	200bp	316	152	09/24/12	4372	1728	242	170
D23_S3225	Marine-SPE	32	25	100bp	314	124	07/27/12	2869	1728	442	268
DB_U327	Fresh-HMW	32	7	100bp	314	124	07/27/12	2814	1728	481	280
DB_U025	Fresh-HMW	0	25	100bp	316	139	07/27/12	2885	1728	521	314
DB_U3225	Fresh-HMW	32	25	100bp	314	124	07/27/12	2837	1728	445	276
DB_S327	Fresh-SPE	32	7	100bp	314	124	07/27/12	2829	1728	492	295
DB_S3225	Fresh-SPE	32	25	100bp	314	124	07/27/12	2841	1728	438	261

Table 2: Summary of all sequence data obtained from the DOM microcosm experiment. Table 1a shows all RNA sequence specifications, while table 1b shows all DNA sequence specifications.

DOM Source	DOM Treatment	Time (hours)	Incubation Temp °C	Bacterial Production		Flow Cytometry		DOC Consumption		
				Bacterial Production (gC/L/d)	Standard Deviation	FC Cell Counts (cells/mL)	Standard Deviation	Volume of spike (mL)	Concentration of Spike (mg/L)	DOC Consumption (%)
Control	n/a	0	7	6.45667E-06	1.46303E-06	141711.06	32508.35	n/a	n/a	n/a
Control	n/a	12	7	2.16417E-05	6.01869E-06	158645.37	40015.14	n/a	n/a	n/a
Control	n/a	22	7	2.7859E-05	1.8182E-06	153182.45	10413.85	n/a	n/a	n/a
Control	n/a	32	7	3.16808E-05	4.89438E-07	486009.81	116623.26	0	0	22.3
Control	n/a	0	25	n/a	n/a	168771.75	16595.98	n/a	n/a	n/a
Control	n/a	12	25	n/a	n/a	196225.70	25029.87	n/a	n/a	n/a
Control	n/a	22	25	n/a	n/a	145981.23	66071.50	n/a	n/a	n/a
Control	n/a	32	25	n/a	n/a	292103.86	6525.84	0	0	28.7
Phyto	n/a	0	7	9.7305E-06	1.45625E-06	145586.88	13460.65	n/a	n/a	n/a
Phyto	n/a	12	7	7.186E-06	1.98452E-06	131192.68	17965.03	n/a	n/a	n/a
Phyto	n/a	22	7	1.12673E-05	1.41796E-06	116834.60	9337.46	n/a	n/a	n/a
Phyto	n/a	32	7	1.29034E-05	4.83944E-07	257375.44	19359.08	25	1500	73.5
Phyto	n/a	0	25	n/a	n/a	285266.79	134911.97	n/a	n/a	n/a
Phyto	n/a	12	25	n/a	n/a	145482.07	26858.65	n/a	n/a	n/a
Phyto	n/a	22	25	n/a	n/a	82318.59	5655.78	n/a	n/a	n/a
Phyto	n/a	32	25	n/a	n/a	344381.30	14047.05	25	1500	73.1
S23	UF	0	7	6.60468E-07	1.11001E-07	110659.48	8737.93	n/a	n/a	n/a
S23	UF	12	7	5.72092E-07	1.18061E-07	137342.18	20069.07	n/a	n/a	n/a
S23	UF	22	7	1.54546E-06	1.92722E-07	93213.18	6872.70	n/a	n/a	n/a
S23	UF	32	7	4.44518E-06	1.8263E-07	110903.45	5972.09	325	94.6	37.7
S23	UF	0	25	n/a	n/a	124363.21	40438.34	n/a	n/a	n/a
S23	UF	12	25	n/a	n/a	75962.78	14349.77	n/a	n/a	n/a
S23	UF	22	25	n/a	n/a	65784.30	2813.70	n/a	n/a	n/a
S23	UF	32	25	n/a	n/a	337922.46	10694.34	325	94.6	38.7
S23	SPE	0	7	6.69012E-06	2.33258E-06	111566.46	5723.41	n/a	n/a	n/a
S23	SPE	12	7	1.38607E-05	1.46462E-06	110445.64	11108.07	n/a	n/a	n/a
S23	SPE	22	7	1.99073E-05	4.49868E-06	113035.85	3441.79	n/a	n/a	n/a
S23	SPE	32	7	3.38447E-05	3.05075E-06	220658.35	32338.10	23.8	971.08	-8.1
S23	SPE	0	25	n/a	n/a	156863.37	17477.35	n/a	n/a	n/a
S23	SPE	12	25	n/a	n/a	140166.50	9098.29	n/a	n/a	n/a

S23	SPE	22	25	n/a	n/a	143143.04	17769.22	n/a	n/a	n/a
S23	SPE	32	25	n/a	n/a	441040.18	61040.29	23.8	971.08	-6.3
SB	UF	0	7	2.44246E-06	3.895E-07	129976.56	43021.20	n/a	n/a	n/a
SB	UF	12	7	1.55465E-06	1.37788E-07	90960.12	1774.46	n/a	n/a	n/a
SB	UF	22	7	1.41395E-06	3.96995E-07	62267.10	6057.38	n/a	n/a	n/a
SB	UF	32	7	2.27846E-06	5.49437E-10	105805.46	15243.33	90	114.41	19.1
SB	UF	0	25	n/a	n/a	142922.78	39235.69	n/a	n/a	n/a
SB	UF	12	25	n/a	n/a	93850.99	21444.38	n/a	n/a	n/a
SB	UF	22	25	n/a	n/a	63199.63	958.76	n/a	n/a	n/a
SB	UF	32	25	n/a	n/a	174032.80	340.18	90	114.41	19.7
SB	SPE	0	7	7.32233E-06	1.60336E-06	105289.32	2546.299	n/a	n/a	n/a
SB	SPE	12	7	1.11087E-05	2.68497E-06	109298.74	14893.89	n/a	n/a	n/a
SB	SPE	22	7	1.97479E-05	1.8752E-06	142872.00	34644.99	n/a	n/a	n/a
SB	SPE	32	7	1.67824E-05	3.15272E-06	210256.63	15110.23	14.4	7014.4	21.7
SB	SPE	0	25	n/a	n/a	123469.74	10588.09	n/a	n/a	n/a
SB	SPE	12	25	n/a	n/a	78830.95	19812.81	n/a	n/a	n/a
SB	SPE	22	25	n/a	n/a	60773.74	2489.19	n/a	n/a	n/a
SB	SPE	32	25	n/a	n/a	77062.67	7884.03	14.4	7014.4	28

Table 3: Summary of all non-sequence data obtained from the DOM microcosm experiment including bacterial production values with standard deviation, flow cytometry cell counts with standard deviation, volume of DOM added to each microcosm, concentration of the DOM addition, and the % DOC consumed by each microcosm after 32 hours.

Absorbance (cm⁻¹)	Functional Group	Potential composition
3000-3500	O-H/N-H stretching	Alcohols/Phenols/Amines and amides
2850-3000	C-H stretching	Aldehydes and Methyls
1600-1700	C=O and C=C bonds	Alkenes, Proteins
1400-1460	C-H bending	Aromatics
1000-1170	C-O stretching	1°, 2°, and 3° Alcohols
850-880	Inorganic material	Silica, colloids, inorganic material

Table4: Description of functional groups and chemical composition commonly associated with FTIR spectra between absorbance levels 1000-3500 cm⁻¹. Table interpreted from (Ouellet et al. 2008) and personal communication with Yves Gelinas.

DOM Origin	Concentration Method	Incubation Temperature	Measured Concentration of Microcosm DOM (Station 21) (mg/L)	Measured Concentration of DOM extract (mg/L)	Volume of DOM spike (mL)	Increase in DOM Concentration (Target: 4x)	Time = 0hr Incubation Concentration	Time = 32hr Incubation Concentration	DOC Consumed (%)
Station 23	SPE	7	1.57	971.1	23.8	3.1x	4.9	N/A	N/A
Station 23	SPE	25	1.57	971.1	23.8	3.1x	4.9	N/A	N/A
Station 23	Ultrafiltration	7	1.57	94.6	325	3.6x	5.7	3.5	37.7
Station 23	Ultrafiltration	25	1.57	94.6	325	3.6x	5.7	3.5	38.7
Station B	SPE	7	1.57	1400.7	14.4	2.8x	4.4	3.5	21.7
Station B	SPE	25	1.57	1400.7	14.4	2.8x	4.4	3.2	28.0
Station B	Ultrafiltration	7	1.57	114.4	90	1.8x	3.0	2.4	19.1
Station B	Ultrafiltration	25	1.57	114.4	90	1.8x	3.0	2.4	19.7
Phytoplankton	N/A	7	1.57	1500.0	25	4.3x	6.9	1.8	73.5
Phytoplankton	N/A	25	1.57	1500.0	25	4.3x	6.9	1.9	73.1
Control	N/A	7	1.57	N/A	N/A	N/A	1.6	1.2	22.3
Control	N/A	25	1.57	N/A	N/A	N/A	1.6	1.1	28.7

Table 5: The amount of carbon lost from the microcosms after the 32-hour incubation period was measured by carbon combustion analysis. The carbon concentration was measured at the beginning and end of the incubation period, and the amount of carbon lost is displayed as a %. Also presented are the concentration of each raw DOM isolate and the enrichment factor in each microcosm.