Composition of aquatic microbial communities and their relation to water-column methane cycling among Mackenzie Delta lakes, western Canadian Arctic

by

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Abstract

Seasonal dynamics of water-column microbial communities and methanotrophs were monitored in six lakes of the Mackenzie River Delta using gene sequencing of 16S rRNA gene and qPCR of the 16S rRNA and methane monooxygenase (*pmoA*) genes. Selected lakes varied in biogeochemistry based on annual river-to-lake connection times, which we hypothesized would impact bacterial community composition and methanotroph relative abundance. River-to-lake and seasonal influences on carbon bioavailability and quantity, nutrients, temperature and flooding correlated with seasonal changes in microbial composition. Methanotroph groups including *Methylobacter* and methylotrophs *Candidatus* methylopumilus and *Candidatus* methylophilaceae were detected in all lakes but at higher relative abundance in the winter and spring when lake-water methane concentrations were highest. Open-water methanotroph abundance was highest in spring. In experimental enclosures, methanogenesis was detected in oxygenated lake-water and rates varied by lake type. Nutrient enhancements altered microbial composition and increased rates of methane oxidation with increasing lake isolation.

Keywords: Biogeochemistry; Mackenzie River Delta lakes; water-column methane oxidation; 16S rRNA-gene; *pmoA* gene; quantitative PCR

Dedication

To Louise, The Store, and everyone that's been around to lend a helping hand or an ear to listen. This project wouldn't have been possible without the passion, shared knowledge, expertise, and experience of our research team and partners in the North. You've helped and contributed more than you will ever know to the success of this project and the maintenance of my mental health and sanity throughout this process! Thank you.

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List of Acronyms

BCC	Bacterial community composition
BP	Bacterial Production
CBL	Chesapeake Biological Laboratory
CDOM	Chromophoric Dissolved Organic Matter
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
ENA	European Nucleotide Archive
GC	Gas Chromatography
MBARI	Monterey Bay Aquarium Research Institute
MGA	Methanogenic Archaea
MOB	Methane oxidizing bacteria
MOX	Methane Oxidation
PCR	Polymerase Chain Reaction
PCR-RFLP	PCR-Restriction Fragment Length Polymerization

Glossary

Bacterial Production	BP. The rate of increase in biomass (growth rate) in a population of bacteria.
Chromophoric Dissolved Organic Matter (CDOM)	CDOM. Chromophoric or coloured dissolved organic matter is organic matter dissolved in water that interacts with light, primarily from complex humic molecules from the decay of organic matter and detritus in aquatic systems.
Chironomid	Sediment-dwelling invertebrate, the larval stage of a fly, that consumes bacteria, including MOB, in many ecosystems.
Dissolved Inorganic Carbon (DIC)	Total amount of dissolved inorganic carbon in a water sample, including carbon dioxide, carbonic acid, bicarbonate and carbonate species.
Dissolved Organic Carbon (DOC)	DOC. The soluble form of carbon available as a substrate for microbial activity in aquatic ecosystems that can pass through a 0.2 um filter membrane.
Dissolved Organic Matter (DOM)	DOM. generalized form of organic matter in aquatic systems. Contains DOC and other particles capable of passing through a ~0.7-0.22 um filter membrane.
Heterotrophic Bacteria	Bacteria that use organic molecules, e.g. from DOM or DOC, as a principal source of carbon.
Heterotrophic Bacterial Production	HBP. Rate of biomass production from heterotrophic bacteria. HBP is a function of DOC content and tends to increase asymptotically with increasing DOC, depending on DOC quality (see Section 2.2).
Methanotrophs / Methane Oxidizing Bacteria (MOB):	Bacteria that metabolize, via oxidation, methane as their primary source of carbon and energy. Typically requires oxygen as an electron acceptor in the oxidation process but occurs under anaerobic conditions.
Methanogen	An anaerobic (with few exceptions) Archaea that produces methane from carbon dioxide (CO_2), formate, acetate or other organic compounds.
Methane Oxidation	MOX. A chemical reaction involving electron transfer in which the molecule being oxidized, methane, loses electrons that are then transferred to an electron acceptor molecule (oxygen under oxic conditions or sulfate, nitrate, or nitrite under anoxic conditions).
Methanotrophic Bacterial Production	MBP. Rate of biomass production by methanotrophic bacteria. Isolates the specific contribution of MOB to the overall biomass of a given system or bacterial community.

Methylotrophy	Bacterial process where 1-carbon compounds other than methane such as carbon monoxide, carbon dioxide, and methanol are utilized
Multivariate analysis	Statistical technique used when multiple variables affect the response of other variables of interest.
Non-metric multidimensional scaling	Statistical technique used to determine relationship among factors in a large data-set where normal distributions are not present, based on their ranked dis-similarities of factors to one another.
Ordination	Method of data clustering; orders objects characterized by values on multiple variables
pmoA gene	Particulate methane monooxygenase gene, specific to MOB as part of process of MOX typically under aerobic conditions.
Polymerase Chain Reaction (PCR)	Gene amplification process using cycles of heating, annealing, and copying of genes based on specific primer-sets.
PCR-Restriction Fragment Length Polymerisation	Restriction Fragment Length Polymerization. A process whereby PCR-amplified DNA samples are enzymatically digested to create distinct banding patterns based on differences in DNA composition
Primary Production	Organisms that produce biomass from inorganic nutrients and inorganic carbon sources (i.e. CO_2).
Primer	Short segment of DNA used in PCR amplification to target specific gene regions,
Photosynthesis	Process where organisms use sunlight as energy to synthesize sugars, starches, and other energy molecules from CO_2 and water.
16S rRNA gene	Fundamental gene present in all prokaryotic organisms, partially responsible for forming a portion of the ribosome. Contains the V4V5 hypervariable region targeted by gene sequencing.
Shannon index	Alpha-diversity index of bacterial communities based on relative abundance of different organisms and the total number of different types of organisms found within a community.
Simpson index	Alpha-diversity index of bacterial communities based on relative abundance of different organisms within a community.
Species Richness	Alpha-diversity index of bacterial communities based on total number of different organisms present in a community. Does not account for relative abundance of each species.

V4V5 hypervariable region	Region of the 16S r-RNA gene, which is semi-conserved and allows for identification of organisms to the phylum level with similar accuracy to sequencing the entire 16S rRNA gene.
Vectors	Euclidian vector or geometric / special vector = geometric object used for plotting coordinates and in statistics for showing the magnitude of correlations among variables in multivariate analysis.
Quantitative PCR (qPCR)	Quantitative polymerase chain reaction; also known as real-time PCR. A form of genetic analysis used to actively measure gene expression in real-time and make estimates of species abundance based on the rate of gene expression over time.

Chapter 1.

Review of Microbial Methane Cycling in the Arctic

The Arctic is of critical importance as an ecosystem and for observing the early effects of ongoing global climate change. The Arctic contains vast stores of organic carbon in the form of permafrost that is being increasingly degraded by rising global temperatures, with permafrost thaw having substantial impacts on the complex aquatic and terrestrial ecosystems in the region. Permafrost carbon is converted to greenhouse gases - gases in the atmosphere that absorb infrared radiation - at varying rates depending on the properties of the permafrost, landscape, and watershed that influence carbon cycling and microbial processes (McGuire et al. 2009, Schuur et al. 2015). Methane (CH_4) is a greenhouse gas of importance due to its high potency as a warming agent and the substantial output of CH_4 from Arctic ecosystems (McGuire et al. 2009 and references therein, Walter Anthony et al. 2010). This review summarizes what is known about Arctic CH_4 production and release (flux) to the atmosphere from aquatic ecosystems, highlighting uncertainties and the microbial communities that drive these CH_4 dynamics.

An often-overlooked aspect of CH_4 dynamics in many aquatic ecosystems is the role of methane oxidizing bacteria (MOB) in aquatic food webs and the effect that CH_4 oxidation (MOX) has on net flux of CH_4 into the atmosphere. Biogenic CH_4 is produced in substantial quantities by methanogenic archaea (MGA) in the sediment and anoxic parts of the water column in many lake systems worldwide, particularly where DOM-rich substrate is available (Pipke 1996, Kankaala et al. 2006, Jones et al. 2008, Schubert et al. 2010, Lofton et al. 2014). Thermogenic CH_4 is released from underground stores of CH_4, which can reach the atmosphere through lakes in the form of bubble seeps or flowing through sediments in many environments (Walter Anthony et al. 2010, Wik et al. 2011). Thermogenic emissions will not be considered in detail in this review since the large bubbles from these seeps flux rapidly into the atmosphere (ebullition) with limited intervention from MOB in the water column (Walter Anthony et al. 2010, Wik et al. 2011). MOB abundance positively correlates with methanogen abundance and CH_4 concentration (Sundh et al. 2005, Rahalkar et al. 2009, Gentzel et al. 2012). MOB can

have a substantial impact on the net flux of CH_4 to the atmosphere but rates of MOX are limited by nutrient availability, temperature, and dissolved oxygen (Rahalkar et al. 2009, Schubert et al. 2010, Lofton et al., 2014).

1.1. Uncertainty in Arctic CH_4 Emission Estimates and its Consequences in Estimating Global CH_4 Flux

CH_4 emissions from lakes in the Arctic and sub-Arctic constitute a substantial portion of global CH_4 emissions and these regions have the potential to emit much more methane as global warming trends continue. Recent estimates posit upwards of 10-16 Tg CH_4 per year (yr^{-1}) from North American Arctic lakes and 15-35 Tg CH_4 yr^{-1} from all Arctic lakes of the total of approximately 15-50 Tg CH_4 yr^{-1} released from the Arctic (McGuire et al. 2009 and references therein; Walter-Anthony et al. 2010). These estimates constitute 3-9% of the net land and ocean sources of annual CH_4 emissions. These estimates have wide ranges and exclude key dynamics of methane cycling in their underlying assumptions; hence, accurate estimates of global CH_4 flux is difficult.

Uncertainty regarding the biogeochemical processes driving Arctic CH_4 production hampers our ability to accurately predict the effects that CH_4 emissions will have on the global climate and vice versa. A potential positive feedback loop could result whereby rising global temperatures accelerate permafrost thaw and degradation that in turn, release more CH_4 to the atmosphere (Schuur et al. 2015). Biogeochemical processes such as the carbon cycle, which influence CH_4 dynamics, are more sensitive to environmental changes in the circumpolar Arctic than in sub-polar regions (McGuire et al. 2009, Schuur et al. 2015). The impact that climate change will have on these biogeochemical processes, however, is unclear (McGuire et al. 2009). Increasing our understanding of the complex biogeochemical factors that influence CH_4 flux from the Arctic is an important component of enhancing the resolution and accuracy of global CH_4 flux estimates and climate change simulations.

1.2. CH_4 in Arctic Food Webs

The flow of CH_4 through aquatic food webs is an important but poorly understood determinant of net CH_4 flux from the Arctic. Stable isotope fractionation analysis and nutrient uptake experiments using radio-labelled carbon tracers are techniques used to

track the flow of carbon through aquatic and terrestrial food webs (Fry, 2006 p.120-134, Hershey et al. 2006, Jones et al. 2008, Taipale et al. 2008). CH_4-derived carbon and energy flow from MGA and MOB into higher trophic levels has been observed in many aquatic systems (Bastviken et al. 2003, 2008, Kankaala et al. 2006, Jones et al. 2008) including the Mackenzie River Delta (Tank 2009). Anaerobic microorganisms that oxidize CH_4 in the sediment of an Arctic lake were comprised of 16-33% CH_4-derived carbon (He et al. 2015). Methanotrophic bacteria are also a potentially important food source for macroinvertebrates such as fly (chironomid) larvae that are known to feed on MOB (Jones et al. 2008).

The flow of CH_4 through microbes and into higher trophic levels diverts CH_4 that would otherwise be released into the atmosphere. Research on the significance of this diversion in the Arctic is sparse and often considered only for soils (McGuire et al. 2009). Because of the substantial contribution of Arctic aquatic ecosystems to the global CH_4 budget these CH_4 dynamics, and the microorganisms responsible for driving them, deserve further investigation.

1.3. Carbon Quality and the role of CH_4 in the Carbon Cycle

Dissolved organic carbon (DOC) is the main form of organic carbon available to aquatic organisms, but the quality of DOC will vary depending on its source of origin. One way to evaluate carbon quality is through the ratio of carbon to other nutrients (nitrogen and phosphorous) and the ease with which the DOC can be broken down and processed by microorganisms (Sigee, 2005 p. 40). Variation in carbon quality means the amount of DOC entering a system can not be used to measure DOC that is available for local food webs (“bioavailable”) (Tank et al. 2011; Sigee, 2005 p.40).

DOC can be classified based on its relative stability, optical properties and relative N and P (nutrient) content (from Sigee, 2005 p. 40-41):

1. Labile DOC (unstable DOC): Labile DOC has a structure amenable to rapid breakdown by bacteria where over 50% complete breakdown is thought possible. Labile DOC has high nutrient content (lower carbon-to-nutrient ratio) and has a low refractive index.

2. Non-Labile DOC (highly stable DOC): Non-labile DOC has high molecular weight and contains complex humic acid compounds that are refractive compounds resistant to microbial decomposition: less than 1% breakdown by bacteria is thought possible. Non-labile DOC has low nutrient content (high carbon-to-nutrient ratio), is more refractive and gives lakes a characteristic dark brown or 'steeped tea' colour.

The quality, refractivity, and lability of DOC in an aquatic system exists on a spectrum and depends on its origin, e.g., terrestrially-derived DOC, DOC from macrophytes, or from photodegradation of chromophoric dissolved organic matter (CDOM) in the river. Photo-degradation by ultra-violet irradiation can convert CDOM, organic matter with a high humic acid content and low lability, into labile DOC by breaking bonds in complex humic molecules and increasing surface area for degradation by microbial enzymes (Tank et al. 2011). Labile DOC can be utilized by microorganisms and converted to bacterial biomass more easily than non-labile DOC that tends to be respired by bacteria as carbon dioxide (CO_2). In addition to carbon, bacteria require N and P to form biomass. Non-labile DOC can be processed by microbes but, because it lacks the nutrients needed for cell replication, is largely respired as CO_2 rather than creating more bacterial biomass (Tank 2009, Moquin and Wrona 2015). Allochthonous (externally derived) DOC from terrestrial sources - such as soil runoff or decaying leaves - or from thermokarst - thawing of DOM-rich permafrost along lake margins - or lakes with substantial DOM inputs from thawing permafrost along their margins, is generally non-labile while authochthonous (internally derived) DOC from macrophyte and algal biomass is highly labile and consumed extremely rapidly (Tank et al. 2011).

The MacKenzie Delta in the Canadian Arctic is a likely 'hotspot' of CH_4 production due to the high proportion of small, shallow lakes and the ample stores of carbon- and ice-rich permafrost. Thermokarst-affected lakes receive substantial inputs of permafrost-derived DOM that makes up an average of 93% of total DOM compared to lakes in bedrock (36%) or non-thermokarst Tundra soil (42%) (Wauthy et al., 2018). Small lakes and those affected by thermokarst contain carbon of varying quality and quantities, and these lake types are known to have much higher CH_4 emissions than others in the same environment (McGuire 2009 and references therein, Cunada 2018). In the Arctic, substantial quantities of carbon are converted to CH_4 (McGuire, 2009), which is likely originating from the organic-rich sediments and multiple lakes in the Delta.

1.4. Seasonal Variations in CH_4 Dynamics and Lake Properties; Introduction to the Mackenzie River Delta

Substantial flux of CH_4 from Arctic lakes occurs during spring ice-thaw events, as CH_4 built up during winter ice-cover can escape to the atmosphere. Biomass that remains from summertime primary production will become a rich source of labile DOC and nutrients for microbes under the ice (Human et al. 2015). This DOC is rapidly consumed by heterotrophic bacteria and converted to bacterial biomass (Tank 2009). This microbial activity will deplete oxygen supplies and cause a build-up of CH_4 as rates of MEG out-pace MOX, which can lead to a substantial release of CH_4 to the atmosphere at ice-out in spring. Delta CH_4 flux in spring comprised 24% of all CH_4 released from Delta lakes (Cunada et al., 2018).

Arctic lake systems such as the Mackenzie River Delta (hereafter referred to as the Delta) release substantial quantities of CH_4 to the atmosphere each Spring, with lakes more spatially isolated from the river releasing greater quantities of CH_4. The Delta is the second largest river delta in the circumpolar Arctic region and the largest river system in Canada, with up to 35.8 Gg of CH_4 released to the atmosphere per year (Cunada, 2018). The Delta contains approximately 45,000 lakes with an area greater than 0.14 ha and another 5,000 water bodies with an area less than 0.14 ha within an extensive floodplain spanning 13,000 km^2 (Marsh and Hey 1989, Lesack and Marsh 2010). The Mackenzie River is rich in silt and sediment due to its various connections to sediment-rich river systems (Pipke 1996, Marsh et al. 1999) such as the Peel, Rat, and Laird rivers.

Lakes in the Delta are grouped based on their flooding regimes into “closure classes” that capture differences in their biogeochemistry and productivity. Spring flooding occurs each year in late May or early June and raises water levels to an average peak elevation of 5.63 m above sea-level (asl) (Lesack and Marsh 2010). Peak water levels during Spring breakup are influenced by snowmelt runoff from southerly parts of the Mackenzie Basin and ice-jamming effects that cause extensive flooding by blocking normal river flow (Lesack and Marsh 2010). Secondary water-level peaks occur in response to storm events in the Beaufort Sea around August that raise water levels by up to a meter (Lesack and Marsh 2010). The relative vertical elevation of lakes in the flood-plain determines the duration that they are connected to the river following spring flooding

events and throughout the open-water period from June to November (Lesack and Marsh 2010).

There are three main “closure classes” for lakes in the Delta as described by Marsh and Hey (1989), where the term ‘isolation’ will be used in place of ‘closure’ for this study:

1) No-isolation lakes: Low elevation lakes (<1.5 m asl) that are connected almost continually throughout the open-water period. These lakes tend to mirror the productivity and biogeochemical properties of the Mackenzie River itself (Lesack and Marsh 2010). Approximately 12% of the lakes in the Delta are no-isolation.

2) Low-isolation lakes: Medium elevation lakes (1.5 m - 4 m asl) that flood at least once per year but have limited connection times with the Mackenzie during the open-water period. Approximately 55% of lakes in the Delta are low-isolation.

3) High-isolation lakes: High elevation lakes (>4 m asl) that flood infrequently (>1 year between flooding events) and have distinct biogeochemical properties due to their relative isolation from the main river channel (Lesack and Marsh 2010). These lakes are never connected during secondary flooding in August. These lakes contain approximately 40% floodwater and 60% legacy water -- the accumulation of residual waters from the multiple infrequent flooding events separated by ten or more years (Lesack and Marsh 2010). Approximately 33% of lakes in the delta are high-isolation lakes.

Lake transparency and depth strongly influence productivity and macrophyte density in Delta lakes. Benthic (sediment-dwelling) microbial communities and macrophytes are likely to be the dominant primary producers in water bodies that are clear, shallow and uniformly mixed (Sigee, 2005, p.57) due to increased light penetration and oxygen availability throughout the water column. Macrophytes outcompete algae due to their ability to mine nutrients from the sediment and dominate primary production in lakes with sufficient light availability for photosynthesis (Squires et al. 2002, Squires and Lesack 2003). Transparent low- and high-isolation Delta lakes have high macrophyte density that have higher primary productivity relative to turbid no- and low-isolation lakes (Squires et al. 2002).

1.5. Bacterial Community Composition, Bacterial Production and CH_4-Related Microbial Activity

Bacterial community composition (BCC) is a measure of the relative abundance of a given bacterial species within a community of bacteria. Bacterial communities are complex assemblages of different species with varying metabolic requirements that respond to environmental changes with shifts in dominance within the community (Adams et al. 2015). How these communities shift in response to environmental changes give researchers clues as to which species are better suited to certain environmental conditions.

Bacterial production is the rate at which a given bacterial population or bacterial community increases in biomass through growth and reproduction. Changes in environmental conditions such as nutrient concentrations (Adams et al. 2015), temperature (Adams et al. 2010), substrate availability, and carbon quality (Kritzberg et al. 2006, Moquin and Wrona 2015) can rapidly increase bacterial production for opportunistic species that are best suited to specific environmental conditions. CH_4 concentration is an important factor for influencing MOB production and abundance within bacterial communities in systems where substantial methanogenesis occurs.

Methanogenesis (MEG) is the process by which MGA convert methyl compounds such as methylamine, methanol, methyl sulfide, acetate (CH_3COOH) or hydrogen (H_2) and CO_2 into CH_4 under a specific set of environmental conditions. Archaea are the dominant MGA organisms in aquatic and terrestrial systems around the world (Billard et al. 2015 and references therein). MEG typically requires anoxic conditions and occurs in concert with microbial breakdown of DOM and DOC into H_2 and CO_2 for the hydrogenotrophic MEG pathway or acetate (CH_3COOH) for the acetoclastic MEG pathway (Hedderich and Whitman 2013 and references therein, Hershey et al. 2014). MEG in freshwater aquatic systems is largely acetoclastic (Hershey et al. 2014) and most prominent in anaerobic parts of the water column and sediment (Gentzel et al. 2012, Lofton et al. 2014). The net flux of CH_4 from Arctic lake systems is largely driven by the activity and abundance of MGA and MOB in the sediment and water column (Sundh et al. 2005, Rahalkar et al. 2009, Gentzel et al. 2012).

1.6. CH_4 Oxidation and CH_4-Linked Groups

CH_4 production and concentration in the water column has a strong influence on MOB production and abundance in aquatic ecosystems. Methanotrophic Bacterial Production (MBP) describes the specific contribution of methanotrophic organisms to the overall biomass of a given system (Pepper, 2015 p. 381), i.e., when MOB convert CH_4 to bacterial biomass. This MOB-specific biomass provides an important source of carbon and energy to higher trophic levels, e.g., macroinvertebrates such as fly larvae (Jones et al. 2008), particularly in shallow, CH_4-rich lakes (Bastviken et al. 2003, Tank 2009, He et al. 2015).

There are two main types of MOB that are distinguished based on their differing metabolic requirements. Type I MOB (e.g., species within the genera *Methylobacter*, *Methylosoma*, *Methylomonas*) preferentially use oxygen to oxidize CH_4 and are typically most abundant near the sediment-water or oxic-anoxic interface in lakes (Gentzel et al. 2012[8], Hedderich and Whitman 2013). Type II MOB (e.g., from the family *Methylocystaceae*) are typically more abundant in deeper sediment layers where dissolved oxygen is very low, CH_4 concentrations are high, and nitrogen or copper concentrations are limited (Hanson and Hanson 1996, Gentzel et al. 2012, Billard et al. 2015).

MBP reduces net CH_4 flux to the atmosphere and contributes substantially to aquatic food webs and overall lake productivity. MOB can comprise anywhere from 10-90% of total bacterial production and consume as much as 80-90% of CH_4 produced over the course of a year in CH_4-rich temperate lake systems (Kankaala et al. 2006, Bastviken et al. 2008, Trimmer et al. 2009). MOB comprised up to 27% of the total bacterial gene sequences and an average of 23-47% of total bacterial production in a survey of CH_4-rich lakes in Northern Quebec (Crevecoeur et al. 2015) and 16-33% of the total CH_4 produced in a series of oligotrophic thermokarst lakes in Alaska was converted to MOB biomass (He et al. 2015). Similar detailed analyses of BCC, and the relative abundance of methanotrophs within those communities, has yet to be carried out in the Delta lake system.

Recent studies in Arctic lakes in Alaska and sub-Arctic thermokarst lakes in Northern Quebec found that type I and type II methanotrophs had similar patterns of

distribution compared to other ice-affected systems worldwide: Type I methanotrophs were most active throughout the water column and in the anoxic-oxic boundary layer in the first 0-1 cm of the sediment while type II methanotrophs were most active in the deeper sediments (He et al. 2012, 2015, Crevecoeur et al. 2015). Some type I (*Methylobacter*) and type II (*Methylocystis*) MOB were found throughout both the water column and sediment layers in shallow Alaskan thermokarst lakes (He et al. 2012). These studies were the first to utilize advanced genetic fingerprinting techniques to accurately characterize BCC and functionally active methanotrophs in the Arctic to the family and genus level.

Most MOB activity and MOX occurs at the sediment-water interface but MOX is influenced by a variety of factors and occurs in deep sediments, in the water column, and under anoxic conditions. Temperature, dissolved oxygen, pH and the concentration of dissolved CH_4 affect Mox rates (Bédard and Knowles 1997, Kankaala et al. 2006, Hershey et al. 2014, Lofton et al. 2014). CH_4 concentration was found to be the only limiting factor for MOX rates at concentrations less than 0.4 umol CH_4 / L, assuming oxygen is present (Trimmer et al. 2009). At higher CH_4 concentrations, or under oxygen limitation, enzyme activity will be a limiting factor following Michaelis-Menten kinetics (Lofton et al. 2014). Optimal pH levels for CH_4 oxidation is in the 4-8 range, but this varies depending on MOB species and environmental conditions (Lofton et al. 2014).

Methanogenesis under oxygen-saturated conditions has recently been observed in freshwater systems, although this process is not often thought to occur under aerobic conditions. MEG was indirectly observed in the oxygenated waters of a small oligotrophic (nutrient poor) lake in Germany (Grossart et al. 2011, Tang et al. 2014). Direct evidence for aerobic MEG was recently observed in a temperate lake in the Canadian Shield (Bogard et al. 2014). Aerobic MEG occurs predominately through the acetoclastic pathway according to $\delta_{13}C$ values (Bogard et al. 2014) in the few systems studied so far. Aerobic MEG could represent a substantial but vastly unrecognized source of CH_4 in many lake systems worldwide (Bogard et al. 2014, Tang et al. 2014).

1.7. Research Gaps and Thesis Proposal

Understanding CH_4 dynamics in the Arctic, and in the Delta, is of particular importance as rising global temperatures impact ecological processes in regions with

vast stores of permafrost carbon. Ongoing climate change threatens to alter the carbon cycle and CH_4 dynamics in the Arctic but the implications of these changes are unknown (McGuire et al. 2009, Schuur et al. 2015). Profiles of sub-Arctic thermokarst systems like those in Northern Quebec (Crevecoeur et al. 2015), and Arctic deltas such as the Mackenzie system could function as proxies for future conditions in the Arctic if warming trends continue.

The overall goal of this study was to provide a set of base-line measurements of microbial community structure and activity in Delta lakes against which future changes can be compared. The main objectives of this thesis were to identify the major microbial taxa present among Mackenzie Delta lakes and determine the influence of lake biogeochemistry and seasonal changes in weather, light, and temperature regimes on the composition of microbial communities and their cycling of methane in the water column. More specifically, this research addressed the following six questions:

Chapter 2 presents the results of my studies relating to Q1-3:

Q1. What is the structure of microbial communities, and the relative abundance of methanogens and MOB in Delta lakes during winter with prevailing ice-cover and anoxic conditions in the water column?

Q2. How do these abundances and compositions change following ice break-up and through the open-water period?

Q3. What major environmental variables most strongly correlate with methane-linked organisms, and which organisms most strongly correlate with CH_4 and MOX among the Delta lakes?

Chapter 3 presents the results of my work relating to Q4-6:

Q4. Are rates of water-column methanogenesis sufficient to affect apparent MOX rates among the lakes?

Q5. Are rates of water-column MOX among the lakes limited by nutrient availability?

Q6. What are the relative contributions of methane-consuming and producing organisms to open-water CH_4 concentrations and the microbial communities among these study lakes?

1.8. Tables and Figures

Table 1.1 Summary of 43-lakes, their GPS coordinates and historical data from past studies. Lakes in bold represent a subset of 6 frequently studied lakes in the system. Adapted from Cunada, 2016.

Lake	Coordinates		Lake Area (ha)	Spring Sill (m)	Isolation Type
	North	West			
L302a	68° 21.012'	133° 47.368'	853	1.5	No
L15a	68° 20.513'	133° 48.437'	437.6	2.177	No
L4	68° 20.015'	133° 53.978'	330.5	2.363	No
L129	68° 18.238'	133° 51.145'	37.8	2.363	No
L85a	68° 18.982'	133° 51.552'	50.6	2.363	No
L80	68° 19.428'	133° 52.160'	19.3	2.631	Low
L501	68° 20.254'	133° 43.529'	126.9	2.631	Low
L148a	68° 16.928'	133° 50.517'	28.4	2.631	Low
L302b	68° 19.492'	133° 48.707'	18.9	2.631	Low
L79a	68° 19.393'	133° 53.078'	34.6	2.631	Low
L107	68° 18.041'	133° 52.404	16.7	2.99	Low
L272	68° 18.772'	133° 47.680'	27.3	2.99	Low
L300	68° 18.900'	133° 49.630'	34.4	2.99	Low
L301a	68° 19.487'	133° 47.755'	36.6	2.99	Low
L85b	68° 19.289'	133° 51.747	1.7	2.99	Low
L58	68° 19.784'	133° 52.049'	13.7	3.389	Low
L87	68° 19.015'	133° 52.460'	3.9	3.389	Low
L141	68° 17.878'	133° 50.090'	17.2	3.389	Low
L511	68° 19.763'	133° 43.617'	1.6	3.389	Low
L148b	68° 17.098'	133° 52.062'	94.8	3.389	Low
L111	68° 17.964'	133° 53.095'	5.3	3.671	Low
L184	68° 17.773'	133° 53.662'	17.7	3.671	Low
L272b	68° 18.747'	133° 46.492'	2.1	3.671	Low
L11	68° 20.612'	133° 52.864'	105.4	3.838	Low
L280	68° 19.248'	133° 50.375	2.4	3.838	Low
L538	68° 18.568'	133° 45.843'	37.9	3.838	Low
L131	68° 18.065'	133° 51.065'	1.2	4.077	High
L278	68° 18.706'	133° 50.114'	9.8	4.077	High
L287	68° 19.145'	133° 46.632'	9.8	4.077	High
L517	68° 19.377'	133° 43.662'	72.9	4.077	High
L148f	68° 16.747'	133° 51.307'	12.4	4.077	High
L56	68° 19.394'	133° 50.817'	3.1	4.623	High
L115	68° 18.673'	133° 53.980'	2.3	4.623	High
L134	68° 18.218'	133° 48.047'	3.4	4.623	High
L261	68° 17.922'	133° 47.145'	48.5	4.623	High
L275	68° 18.672'	133° 49.112'	5.9	4.768	High
L520	68° 18.816'	133° 42.854	0.2	4.913	High
L522	68° 19.257'	133° 41.518'	22.5	4.913	High
L143	68° 17.425'	133° 50.205'	2.1	5.169	High
L181	68° 17.298'	133° 53.688'	0.8	5.169	High
L186	68° 18.418'	133° 53.840'	1	5.169	High
L521	68° 19.033'	133° 41.802'	0.1	5.169	High
L527a	68° 18.957'	133° 43.530'	8.5	5.169	High

Figure 1.1 **Google Earth satellite map of Mackenzie River Delta near Inuvik, NT with zoomed-in view of set of 43 lakes for our study system in Canada's Western Arctic. 6-lake subset highlighted in yellow (b). Images adapted from Cunada et al., 2018a.**

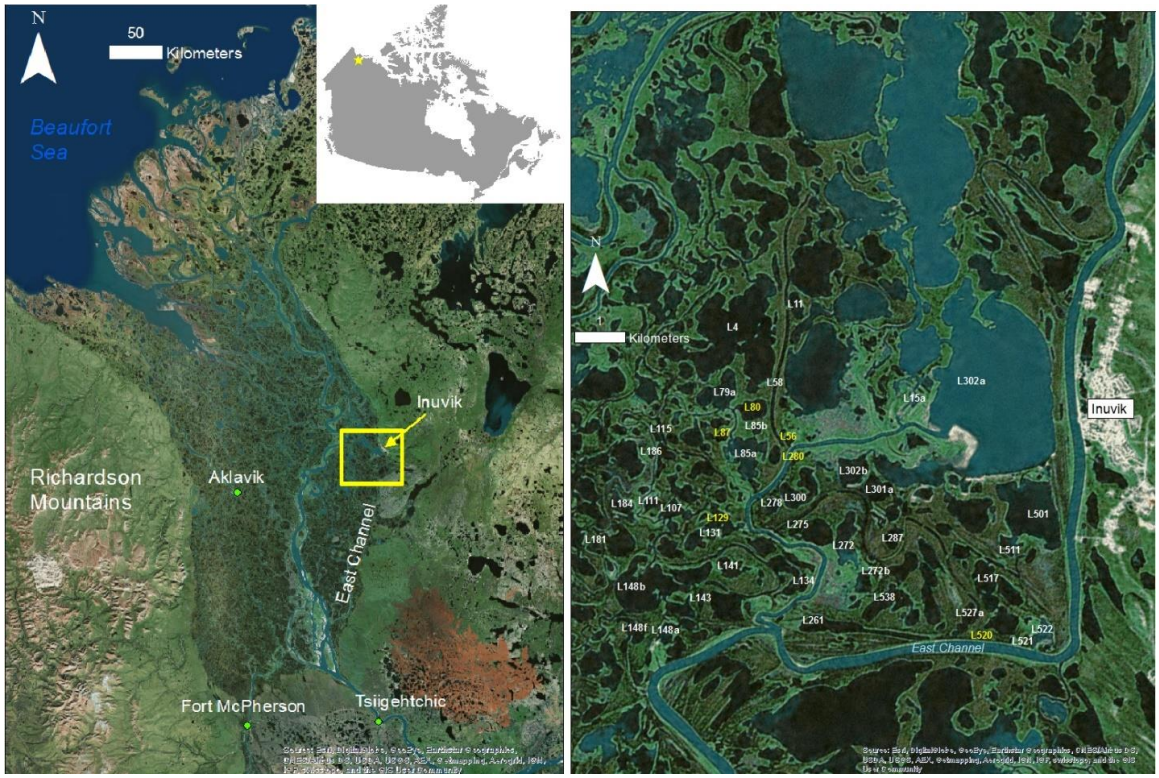
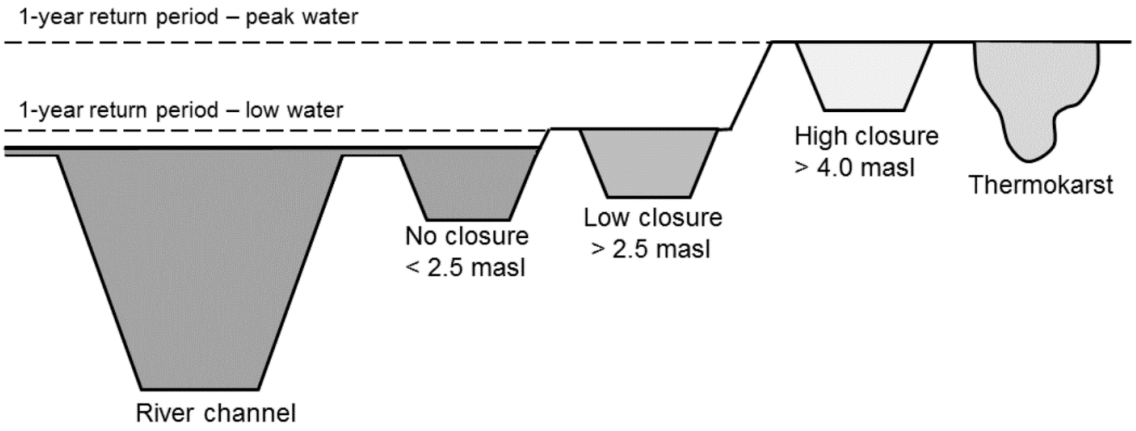


Figure 1.2 Summary of groupings of lakes into different isolation classes or "closure class" based on the total days of annual river-to-lake connection time, where more isolated lakes have fewer inputs of Mackenzie River water during spring flooding. Adapted from Tank, 2009.



Chapter 2.

Seasonal dynamics of methane and microbial community composition in Mackenzie Delta Lakes

Abstract

The influence of microbial communities on methane dynamics and carbon-cycling processes in the Mackenzie River Delta (Delta) remains poorly understood. We chose a set of lakes representative of the range of biogeochemical properties found throughout the Delta and determined the community composition and the abundance of planktonic bacteria using 16S rRNA gene sequencing and quantitative PCR (qPCR) of the 16S rRNA gene, respectively. Methane-oxidizing bacteria (MOB) were also quantified by qPCR of the methane monooxygenase (*pmoA*) gene. Major seasonal shifts in microbial community composition correlated with shifts in lake-water methane concentration and carbon substrate availability: relative abundance of MOB was highest in winter (10-40% of total population) and spring (8-20%), and during the open-water period MOB gene abundance was highest in spring. MOB generally declined throughout the summer following overall declines in methane concentration and observed increases in overall bacterial abundance, although a spike in CH_4 and MOB abundance occurred in some lakes in late summer. Delta microbial communities were dominated by globally-dispersed freshwater taxa including *Actinobacteriaceae, Burkholderiales, Comamonadaceae,* and MOB including *Methylophilaceae* and *Verrucomicrobia.*

2.1. Introduction

Methane, a potent greenhouse gas, is being released in substantial quantities from Arctic lakes and soils. The Arctic contains many large and complex lake systems but research on CH_4 dynamics and the microbial communities that influence the carbon cycle in the region has been limited. Substantive prior investigation has been done on bacterial and archaeal communities in Arctic lake systems of Alaska (Larouche et al. 2012, He et al. 2012, 2015) and northern Quebec (Crevecoeur et al. 2015), along with bacterio-plankton communities at the outflows of the great Arctic rivers (Crump et al. 2003), including the Mackenzie River (Galand et al. 2008, Vallieres et al. 2008). This has provided some information about the bacterial community composition and the variety of methane oxidizing bacteria (MOB) and methanogens associated with methane-rich permafrost landscapes; however, extensive genetic screening of microbes in Arctic permafrost lake systems has thus far been limited.

Within lakes, methanogenic archaea (MGA) are primarily active in lake sediments where dissolved oxygen is low, though significant methanogenic activity within aerobic lake-waters has been recently observed (Bogard et al. 2014, Tang et al. 2014). MOB have been shown to play a crucial role in mitigating CH_4 flux to the atmosphere in many systems world-wide. There are two main types of MOB that are distinguished based on their differing metabolic requirements. Type I MOB (e.g., from the genera, *Methylobacter*, *Methylosoma* and *Methylomonas*) preferentially use oxygen to oxidize CH_4 and are typically most abundant near the sediment-water or oxic-anoxic interface in lakes (Gentzel et al. 2012, Hedderich and Whitman 2013). Type II MOB (e.g., from the family *Methylocystaceae*) are typically more abundant in deeper sediment layers where dissolved oxygen is very low, CH_4 concentrations are high, and nitrogen or copper concentrations are limited (Hanson and Hanson 1996, Gentzel et al. 2012, Billard et al. 2015). CH_4 oxidation occurs more efficiently in oxic waters, although MOB are found in both oxic and anoxic waters in various systems, but the majority of MOX occurs at the oxic-anoxic boundary layer (Milucka et al., 2015).

Deltas of the great Arctic rivers are biological ‘hotspots’ relative to the adjacent Arctic landscape (Squires et al. 2009, Tank et al. 2009) and differ substantially from the other Arctic lake systems where methane dynamics have thus far been investigated. Specifically, lakes in the Mackenzie River Delta (hereafter referred to as the Delta) are

numerous (Emmerton et al. 2008), biodiverse, biologically productive, and many are strongly affected by thermokarst processes in which dissolved organic matter (DOM) is released into the lake when permafrost thaws along the lake margins (Lesack and Marsh 2010, Tank et al. 2011). Delta lakes are generally methane-rich but have a broad range of methane concentrations among the lake-waters and undergo strong seasonal changes in CH_4 and biogeochemistry progressing from ice-covered conditions through the open-water period (Cunada et al. 2018). Lakes with the highest autotrophic productivity, e.g., macrophyte-rich lakes, and more thermokarst-affected lakes generally produce more methane and have higher methane oxidation (MOX) rates in the water column than lakes of lower productivity. CH_4 concentration among Delta lake-waters was related to carbon substrate quantity and quality, particularly dissolved organic carbon (DOC) in the lake-waters during winter ice cover (Cunada et al. 2018). MOX rates are highest after spring ice-thaw and flooding, when CH_4 concentrations are highest, and rates are positively correlated with lake water CH_4 concentrations (Cunada 2018). However, the composition of the microbial communities and the factors that drive their activities as well as the influence of these communities on CH_4 dynamics of this system have been limited. The complex flooding regime in Delta lakes may also be a significant driver of diverse microbial communities in such systems (Lesack and Marsh 2010), but to date, this relationship has not been examined.

Based on the previous studies, we hypothesized that microbial communities in Delta lakes would resemble methane and permafrost-rich ecosystems in other parts of the world and that that MOB would make up a substantial proportion of the community based on previous observations of substantial CH_4 availability and substantial MOX, particularly in the spring when nutrients, oxygen and CH_4 substrate are readily available. Furthermore, we expected that communities would change most from winter to summer with more type II MOB in winter and more type I MOB through the open-water period following major seasonal shifts in lake biogeochemistry. Finally, we hypothesized that the structure of microbial communities would be more strongly influenced by river-to-lake connection times throughout the year because of differences in lake-water biogeochemistry.

Therefore, our specific objective were: 1) To compare the structures of microbial communities in Delta lake water, including the relative abundance of methanogens and MOB, during winter, following ice break-up and through the open-water period; 2) To

determine the environmental variables that most strongly correlate with abundance of MOB and MGA; in particular, which organisms most strongly correlate with CH_4 concentration and MOX among the Delta lakes.

2.1.1. Study System

The Mackenzie River is one of the great rivers of the circumpolar Arctic and the largest river system in Canada. The Mackenzie Delta is located where the Mackenzie River discharges into the Beaufort Sea (68-69ºN 134-137ºW; Lesack and Marsh 2010). Within this extensive 13,000 km^2 Delta, there are over 45,000 lakes (Emmerton et al. 2007) with mean depths from about 0.5 m to 4.5 m and that may be higher or lower depending on time of year, whether the year is wet or dry locally, and whether delta water levels are higher or lower than average (Lesack and Marsh 2010). The open-water period in the central Delta is from June to November. Peak annual water levels in the river are partially controlled by the amount of water contained within the winter snowpack, but they are primarily controlled by ice breakup effects (Andres and Doyle 1984; Prowse 1986). Delta lakes are perched at a range of sill elevations above distributary river channels (Marsh and Hey 1989) that govern river-to-lake connection times. During connection, lakes are replenished in varying degrees with river-water which provides nutrients, DOM, and sediments (Marsh and Lesack 1996, Lesack and Marsh 2010). Lakes with shorter connection times generally have lower particulate loads and higher average levels of underwater irradiance, thus supporting higher autotrophic production dominated by macrophytes (Squires et al. 2002; Squires and Lesack 2003; Squires et al. 2009). Delta lakes have been classified into a series of isolation levels based on their river-to-lake connectivity (Marsh and Hey 1989) as in Mackay (1963): no-isolation lakes remain in connection with river channels throughout open-water, low-isolation lakes are connected to the river during flooding each spring but are cut off from the river for some portion of the summer, and high-isolation lakes are not necessarily flooded every spring and never reconnected to river channels during the summer.

The Delta lakes chosen for our study have been examined extensively in prior work and cover the full gradient of riverine, thermokarst and autotrophic influences. Study lakes consist of 43 lakes to the west of Inuvik, including 6 lakes that were tracked at weekly intervals during prior studies (Table 2.1-2; Fig. 2.1). Among the set of 6 lakes,

connection times range from continuous open-water river-connection (no-isolation; stronger river influence and lower autotrophic production - Lakes 129 and 80) to an average river-connection of only 6.5 days per year (d^{-1} yr^{-1}) (high-isolation; weaker river influence and higher autotrophic production - Lake 520). Lakes 87 and 280 are low-isolation class, with average river connection times of 44.2 and 22.0 d^{-1} yr^{-1} respectively. Lake 56 is high-isolation with an average river connection time of 9.3 d^{-1} yr^{-1} (similar to Lake 520) and high autotrophic production, but Lake 520 by comparison is a deeper lake due to substantial thermokarst activity (Tank et al. 2009) that adds modest inputs of permafrost DOM (Tank et al. 2011). The macrophyte biomasses and communities in these lakes are an inverse function of the average river connection times, though some variation can occur in years with higher or lower than normal water levels (Squires et al. 2002, Squires and Lesack 2003).

2.2. Methods

2.2.1. Sample Collection

Three sets of surface water samples were collected via helicopter survey from a set of 43 lakes across the Winter to Late Summer season in 2016. Sampling was done before and after peak water levels which occurred on May 22nd, 2016: pre-ice-out (May 9 and 11, 2016) when 23 lakes were sampled, post-ice-out (June 5, 2016) and Late Summer (August 5, 2016) when 43 lakes were sampled (Figure 2.1). Higher resolution bi-weekly sampling throughout the open-water period was done at a subset of these lakes, including two lakes from each of the three isolation classes (Table 2.1), to represent the range of carbon quality, flooding frequency, and CH_4 concentration among lakes in the Delta.

Pre-ice out samples were collected by drilling through the ice with a 10” gas-powered ice auger and pumping sample water into acid-washed and sterilized 1L bottles using a Rule 360 Gph 12V submersible bilge-pump connected to sterile Nalgene 1/4” tubing. Water was collected just below the bottom surface of the ice and run through the pump for 3-5 minutes prior to sampling at each site to ensure the pump and tubing was thoroughly rinsed with sample water. During open-water, surface water was collected for DNA analysis were collected by hand in acid-washed, sterilized 1L polyethylene bottles, transported to the lab in coolers then refrigerated for no more than 12 hours for

the high-resolution 6-lake study and no more than 24 hours for the low-resolution 43-lake study, before filtration through sterile 0.2 µM Whatman polycarbonate filters. Filters were then frozen at -20 °C until ready for DNA extraction. This filtrate was collected into new or acid-washed vials for dissolved organic carbon (DOC) and chromophoric dissolved organic matter (CDOM) analysis and stored at 4-8 °C in the dark until analysis at Simon Fraser University, following methods outlined by Cunada et al. (2018).

Open-water samples for surface-water CH_4 measurements were collected into glass serum bottles that were pre-flushed (bottom-filled until over-flowing) with sample water for 30-90 seconds using a submersible bilge pump and Nalgene 1/4" tubing, then capped without head-space using rubber stoppers. Bottles were then injected with 0.5 mL of 5 M potassium hydroxide (KOH) to halt all microbial activity and 10 mL of sample water was replaced with 10 mL of ultra-high-purity (UHP) air to create head-space for subsequent gas chromatography (GC) analysis at the Chesapeake Biological Laboratory (CBL), following methods outlined by Magen et al. (2014).

Bulk lake surface-water was also collected for various measurements of biogeochemistry into acid-washed or new 1L polycarbonate bottles and filtered for analysis and storage of backups. Surface waters were hand-filtered through 0.45 µM Luer-lock filters and filtrate was stored in new or hot-acid-washed polyethylene (HDPE) bottles in darkness until being shipped for analysis at the Monterey Bay Aquarium Research Institute (MBARI). Ions and trace metals, e.g. sulfate (SO_4^{2-}), manganese (Mn^{2+}), iron(III) (Fe^{3+}), potassium (K^+), were chosen based on their known importance to ecological processes and analyzed following established protocols (Wheat et al., 2000, 2002, 2010).

For the sediments sampled during winter and for the 6-lake set in spring, sediment samples were collected using a gravity corer (Uwitec, Austria) with sterilized and sample-rinsed PVC tubes (86 mm inner diameter, 90 cm outer diameter, 60 cm length). Under-ice cores were sectioned on-site in 2 cm sections for the first 0-10 cm then 4 cm sections for the remaining core, bagged, and transported in coolers back to the ARI. During open-water, cores were capped at both ends with rubber stoppers as per manufacturer's instructions and brought back to the ARI for processing as during the under-ice survey. Samples were stored at -20 °C until DNA extraction. Detailed analysis of the sediment was not performed as part of this thesis project but DNA

extracts from the sediment were processed following methods in section 2.2.4 and may be examined in more detail in future work.

For the sediments sampled during winter and for the 6-lake set in spring, sediment samples were collected using a gravity corer (Uwitec, Austria) with sterilized and sample-rinsed PVC tubes (86 mm inner diameter, 90 cm outer diameter, 60 cm length). Under-ice cores were sectioned on-site in 2 cm sections for the first 0-10 cm then 4 cm sections for the remaining core, bagged, and transported in coolers back to the ARI. During open-water, cores were capped at both ends with rubber stoppers as per manufacturer's instructions and brought back to the ARI for processing as during the under-ice survey. Samples were frozen until DNA extraction following methods outlined in section 2.2.4 using approximately 0.5 g of sediment per extract. Detailed analysis of the sediment was not performed as part of this thesis project but DNA extracts from the sediment will be examined in future work.

2.2.2. Chemical Analysis

CH_4 concentration was analyzed at the Chesapeake Biological Laboratory, using gas chromatographic methods outlined by Magen et al. (2014) while pCO_2 and dissolved inorganic carbon (DIC) measurements were determined by gas chromatography at Simon Fraser University following methods of Cunada et al (2018). These results, combined with the DOC measurements, were used to infer the pH of the samples based on methods outlined by Cunada et al. (2018).

Measures of carbon quality and quantity were determined at SFU using methods developed from prior work. CDOM data was determined from spectral scans of sample water from 250 to 750 nm using a GENESYS 10S UV-Vis spectrophotometer with a 5-cm path length UV cell as described by Cunada et al. (2018). DOC was analyzed using the Shimadzu TOC-V analyzer configured according to protocol developed by Gareis et al. (2007) and Cunada et al. (2018). Briefly, sample water was acidified and sparged at 600º C. A 5-point DOC standard curve was made using potassium hydrogen phthalate (KHP), then replicate measurements taken of each sample until they matched within 5%.

2.2.3. DNA extraction and 16S rRNA gene quantification

DNA was extracted from the filters using the MOBIO PowerSoil® DNA Isolation Kit (Qiagen) following manufacturer's instructions. Bulk DNA concentrations (ng DNA per µL extract) were quantified with a Qubit® 3.0 fluorometer and the dsDNA HS Assay Kit (Life Technologies Corporation) following manufacturer's instructions with 10 µL samples. To screen the diversity in the samples and select samples for DNA sequencing, restriction fragment length polymorphism (RFLP) analysis was performed using the procedure of Orcutt et al. (2009) as follows. The 16S ribosomal RNA (rRNA) gene was amplified via polymerase chain reaction (PCR) in the extracts using 1 µL of template sample, 1 µL S-*-Univ-0341-b-S-17: 5'-CCT ACG GGR SGC AGC AG and S-*-Univ-1392-a-A-15: 5'-ACG GGC GGT GTG TRC forward and reverse primers at 200 nM final concentration, 25 uL Redtaq 2x Master Mix, and 22 µL of PCR-grade water to a final volume of 50 µL per tube. Amplification was performed on an miniPCR mini8 thermocycler with the following conditions: Initial denaturation at 95 °C for two minutes, followed by 30 cycles of 30 second denaturing, annealing and extension at 95, 55, and 72 °C and a final extension phase of 72 °C for 5 minutes. Next, the 16S rRNA genes were fragmented by digestion with BsuRI FastDigest® (Thermo Fisher) restriction enzyme following manufacturer's directions using the miniPCR thermocycler at 37 °C for 20 minutes. The 16S rRNA gene fragments were then separated on a 1.5% agarose gel in 1X TBE buffer using a BlueGel electrophoresis system (miniPCR). Band fragmentation patterns were captured by Nikon D7000 digital camera with the back-light and lens filter supplied from the BlueGel system.

2.2.4. 16S rRNA gene sequencing and sequence analysis

Selected environmental DNA extracts were sent to the Integrated Microbiome Resource (IMR) at Dalhousie University for Illumina MiSeq amplicon sequencing of the V4-V5 hypervariable region of the 16S rRNA gene using "universal" Earth Microbiome Project primers (forward primer sequence GTGYCAGCMGCCGCGGTAA; reverse primer sequence CCGYCAATTYMTTTRAGTTT). The samples correspond to the winter under-ice sampling period on May 9th-11th, the early open-water period on May 30th - 31st (spring), June 20th (early summer), July 19th (mid-summer), and August 6th (late summer). Sequencing data was validated and processed for online storage through the European Nucleotide Archive (ENA), accession number PRJEB25188.

16S rRNA gene amplicon data was processed following the MiSeq Standard Operating Procedure for *mothur* software package version 1.39.4 (Schloss et al., 2009) in September of 2017 using the WestGrid / Compute Canada supercomputer cluster. Forward and reverse reads were paired into contigs of no more than 205 base pairs in length. Contigs were trimmed to a length between 176 and 205 base pairs, zero maximum ambiguous bases, and a maximum of 8 homopolymers.

Chimeric sequences were removed using the *chimera.uchime* function within *mothur*, with sequences removed only from the sample which contained the chimeric sequences but not removed from the entire data-set (dereplicate = t enabled).

Sequences other than Bacteria and Archaea were removed, i.e., those identified as chloroplast, mitochondria, or *Eukaryota* sequences, using the ribosomal database project (RDP) classifier during *mothur* analysis. Sequences were binned into operational taxonomic units (OTUs) of 97% or greater sequence similarity, then classified using the SILVA v128 database, the most current version as of September 2017.

To compare accuracy of OTU classifications and find matching sequences in other ecosystems, BLAST database analysis was used to determine the consensus between the SILVA and NCBI OTU classification, and to determine the closest environmental and/or cultured matches to the sequence data from this study. Sequences with strong correlation to methane concentration or from known methane-cycling groups, along with representatives of the most abundant phyla from the data, were run through MegaBLAST analysis for highly-similar sequences from the NCBI nucleotide collection (nr/nt) database. Data from FASTA files was rarified to the sample with the lowest total OTU count among the chosen grouping of samples. Methane-cycling organisms from families of known methano- and methylo-troph groups from the SILVA classification were selected based on recent literature of their methane and carbon-cycling activity and separated from the rest of the data-set using the Phyloseq and Vegan package in R-studio version 3.4.3.

Alpha-diversity and relative abundance of lake surface-water communities were determined from OTU count data rarified to the sample with the lowest OTU count within sample groupings using the Phyloseq and Vegan packages. Plots and were made using R-Studio version 3.4.3, visualization package ggplots, with OTU count data for each

sample rarified to the lowest count within the sample groupings using the Phyloseq and Vegan packages.

2.2.5. Quantification of 16S rRNA and particulate methane monoxygenase (*pmoA*) genes

To assess the abundance of the microbial community in the lakes, and the relative abundance of methane-oxidizing microorganisms within the communities, we quantified the abundance of the 16S rRNA gene and the particulate methane monoxygenase subunit A (*pmoA*) gene in the DNA extracts using quantitative PCR analysis (qPCR). While all Bacteria and Archaea contained the 16S rRNA gene in variable copy numbers (Ribosomal Database Project), only microorganisms involved in methane oxidation will contain the *pmoA* gene (McDonald and Murrell 199763, Holmes et al, 1999). qPCR was performed using a BioRad IQ5 multicolor Real Time qPCR detection system. The reactions were composed of 5 µl of 10x diluted DNA template, and iCycler Quanta Perfecta SYBR Green Master Mix to a final reaction volume of 20 µl. Sample reactions were done in triplicate while reactions for the standard curve were done in duplicate. Primers A189F/MB661R at a final concentration of 667 nM were used for *pmoA* gene analysis (Kolb et al. 2003), and Bac8F Mod / Bac338Rabc primers were used for 16S rRNA gene analysis (Juretschko et al. 1998; Daims et al. 1999).

Standards for *pmoA* genes and 16S rRNA genes were prepared using *Methylomicrobium album* ATCC 33003 DNA, which was cloned into the pCR-4-TOPO vector (Invitrogen) and inserted into Invitrogen One Shot TOP10 chemically competent cells. The vector was isolated using a Qiagen qiaPREP Spin MiniPrep kit and linearized using the *NotI* enzyme. For standards, the *pmoA* gene region covered by the A198F/MB661R was inserted into the vector, while the full-length 16S rRNA-gene was inserted into the vector for the 16S rRNA-gene standard (Kolb 2003). Methanotroph abundance was inferred by assuming a copy number of two *pmoA* genes per cell while general microbial abundance was inferred from assuming 4.08 16S rRNA gene copies per cell (Stoddard et al., 2015).

Conditions for 16S rRNA-gene qPCR cycling consisted of 3 minutes of initial denaturation at 95 ºC, then 40 denaturation cycles at 95 ºC for 5 seconds, annealing at 55 ºC for 15 seconds, and extension at 68 ºC for 15 seconds. For the *pmoA* gene

analysis, an initial denaturation at 95 ºC was performed followed by 40 cycles of denaturation at 95 ºC for 30 seconds, annealing at 55 ºC for 30 seconds, and extension at 72 ºC for 15 seconds following (Kolb et al 2003). Fluorescence data was acquired following the final extension cycle with an additional 10 seconds at the extension temperature. No melt curve analysis was performed. Standard curves were determined from a 1:10 - $1:10^7$-fold dilution series of template plasmid.

2.2.6. Statistical analysis of sequence and biogeochemical data

Multivariate analysis as well as measures of alpha- and beta-diversity were used to determine the relationships among lake-water bacterial communities and how these communities changed in response to biogeochemical changes secondary to major seasonal shifts. To analyze changes in bacterial community composition among samples and over time, we used non-metric multidimensional scaling (NMDS) of OTU abundance data and principal component analysis (PCA) of log-transformed meta-variables of biogeochemical data from the 6-lake data set. NMDS utilizes a Bray-Curtis dissimilarity distance matrix of normalized and rarified taxonomic data from all samples are 'ranked' based on their similarity or dissimilarity to one another. These rankings are translated to coordinates in Euclidian space and clustered according to their ranked similarity to one another. Groupings of sample data based on NMDS results were compared to biogeochemical groupings from the PCA of lake biogeochemistry utilizing the Env-Fit function. This function fitted the vectors of a PCA with the NMDS ordination. The Mantel test, based on Spearman's rank correlation coefficient, was used to determine the significance of the correlation between the NMDS and PCA data.

Rank-sum tests such as the Kruskall-Wallis rank sum test and Pearson product-moment correlation coefficient, measure correlation among variables in non-linear or non-normally-distributed data. These tests were used to measure correlation between the biogeochemical meta-variables and 16S rRNA gene abundance data that did not have linear relationships or normal distributions. The correlations among environmental variables and specific OTU's were determined using the Rhea 16S rRNA gene amplicon analysis pipeline which is designed for these types of datasets.

To determine which OTU's had significant correlations to CH_4, the Serial Group Comparison and Correlation scripts in the Rhea package in R were utilized. These

scripts utilize a pair-wise Fisher Exact test and Kruskall-Wallis rank-sum test that compares log(x+1) transformed meta-variables from biogeochemical measurements and normalized OTU count data. The Rhea script normalizes the rank-sum matrix to a -1 to 1 scale. Visualization of OTU data was performed using the R-package Phyloseq and Vegan to sub-set and visualize the data.

Supplementary statistics for CH_4 and biogeochemical data were analyzed in JMP Version 13.1.0, the most current version as of November 2018 (JMP®, SAS Institute Inc., Cary, NC, 2018).

2.3. Results

2.3.1. Seasonal dynamics of methane among and within the lakes

Lake-water CH_4 concentrations (and other biogeochemical measures not shown) in the 43-lake set (Figure 2.2) and 6-lake subset (Figure 2.3) matched trends of river-to-lake connection times and seasonal effects observed in prior work (Cunada et al. 2018). The lakes with shorter river connection times and concomitant higher autotrophic production had higher CH_4 concentrations than lakes with longer river connection times (Figure 2.2 and Cunada et al. 2018) and the effect of river connection time was strongest during the winter. CH_4 concentrations in all lakes were highest at the end of winter prior to-ice thaw and decreased over the open-water period (Figure 2.2). In contrast, among lakes with the longest river connection times, as open water progressed, lake-water CH_4 concentrations generally increased (Figure 2.4).

2.3.2. Seasonal dynamics of bacterial community composition

Bacterial community composition was determined using DNA samples of unfrozen lake-water taken beneath winter ice-cover at the end of “winter” from a subset of 6 lakes 11-13 days before peak water levels (May 9th and 11th’) and surface-water samples throughout the open-water period (May 30-31 “spring”, June 20 “summer” July 19 “mid-summer”, and Aug 6 “late summer”). These lakes were chosen as best representing the range of river-to-lake connection times and carbon quantity/qualities of microbial substrate. General microbial abundance (based on apparent DNA concentrations) was lower in winter than during open-water (Table 2.3). Indeed, two of

the winter lake samples had DNA concentrations too low to produce adequate reads from 16S rRNA-gene Illumina sequencing, or failed quality control tests in subsequent analyses of the data. Winter genetic data for lake 80 and lake 280 were thus excluded from further analysis. Based on banding patterns of gels from RFLP analyses, microbial community composition showed stronger changes over monthly, rather than bi-weekly, intervals (results not shown). Thus, monthly sampling intervals were chosen for 16S rRNA gene sequencing and qPCR analysis of the 6-lake sample set.

DNA concentrations ranged from 0.1 - 1.3 ng/mL lake water in Winter, and 0.3 - 4.6 ng/mL through the open-water period. Winter and open-water samples contained 41,566 ± 24,382 raw reads, with 22 ± 3% of reads removed based on QA/QC Quality scores from the sequencing center (Table 2.3). Samples were normalized by rarefaction to the same sampling depth as the sample with the fewest number of reads. The lake 87 mid-summer sample had the fewest reads, so all samples were rarified to a library size of 3,576. Rarified OTU counts ranged from 181 to 778 with the highest alpha-diversity in Winter and Spring, based on measures of Species Richness and Shannon Diversity from the Rhea Serial Group Comparisons analysis (Table 2.3, Figure 2.6)

The abundance of microbial communities among the 6 lakes varied between seasons by around a factor of 100; from 1800 to 190,000 copies per mL of lake water based on quantification of the 16S rRNA gene from lake water DNA extracts (Figure 2.5a). The abundance of methanotrophs varied by a factor of 50, ranging from 28 to 1300 *pmoA* gene copies per mL of lake-water and their total and relative abundance to the general community was highest in spring (Figure 2.5c).

The dominant phyla and overall composition of lake-water bacterial communities changed from winter to spring and at monthly intervals over the open-water period following major shifts in lake biogeochemistry (Figure 2.7). All time points were dominated by the bacterial phyla *Proteobacteria*, *Bacteriodetes*, and *Actinobacteria.* Despite using universal primers, extremely few Archaea sequences were present (~0.05% of all OTU's in the entire open-water data set; between 0-2 OTU's per sample and only found in the Winter and Spring for lakes 129, 56, 520, 280, and one in Lake 520 in late summer). The under-ice communities had the highest alpha-diversity based on the Shannon diversity index (Figure 2.6) and the highest proportion of unclassified bacteria and various low-abundance phyla (phyla comprising less than 1% of the total

abundance were grouped together into the 'other' category; Figure 2.7). The relative abundance of *Actinobacteria*, *Cyanobacteria* and *Bacteroidetes* increased from winter to spring and from mid to late summer (Figure 2.7). The most abundant genera among the 6 lakes belonged to globally dispersed Betaproteobacteria including *Comamonadaceae* (one unclassified genus), *Polynucleobacter*, *Burkholderiales*, *Methylophilaceae* (OTU 10, 17); the Actinobacteria family *Sporichthyaceae* (unclassified genus; OTU 3); and the hgcI clade (*Actinobacteria*) (OTU 8, 22, 26; Figure 2.8, Table 2.5).

The most significant changes to the bacterial community composition of Delta lakes correlated with time of year, with within-group differences at each time point. The differences among lake bacterial communities, based on season and lake type, correlated with seasonal shifts in lake biogeochemistry (Figure 2.9). The main biogeochemical indices that correlated with these seasonal shift in BCC were CH_4 concentration, dissolved oxygen, temperature, major ions (K^+, Mn^{4+}, SO_4^{2-}, Fe^{3+}), pH, and ratio of carbon aromaticity ($SUVA_{254}$) to molecular weight (a_{250}:a_{365} ratio; Figure 2.9). Seasonal groupings showed within-group variance among samples, with the greatest differences among lake-water communities (beta-diversity) occurring in winter and spring, and all lake communities were more similar as open-water progressed (Figure 2.9).

2.3.3. Methane-linked groups and correlation with environmental variables

MOB comprised a substantial proportion of the total microbial community in Winter and Spring but decreased in relative abundance across all six lakes through the open-water period following spring ice-out. The relative abundance of MOB ranged from 10-40% of the total community in winter to 6-20% in spring, although mid-summer saw a slight increase (as high as 14% in lake 87) in the relative abundance of MOB in most lakes (Fig 2.10). MOB of the *Methylophilaceae* (OTU 41) and *Methylococcaceae* (OTU 43) families were present in all winter and most spring samples but relative abundance declined for both groups throughout the open-water period (Figure 2.10). The methylotroph genus *Candidatus* methylopumilus (OTU 10; Figure 2.10) had high relative abundance in winter for lakes 520 and 129 and was the most prominent group throughout the open-water period. Type II methanotroph genera including *Methylocaldum* and *Methyloparacoccus*, had high relative abundance in winter and

spring and declined through the open-water period (Fig 2.8, Fig. 2.10). Based on NCBI BLAST database comparisons, these CH_4-associated OTU sequences matched 100% to globally dispersed methanotroph genera, both cultured and uncultured, that have been isolated from various environments including Arctic, pelagic, marine systems or attached to aquatic plant-roots (Table 2.5). Several OTU's matched with soil- or sediment-associated species including *Methylophilus quayli* (OTU 17), and the gammaproteobacterial methanotroph, *Methylobacter tundripaludum* (OTU 43). Other groups matched MOB from various temperate lake or river systems worldwide but few matches to other Arctic lake-systems were found except for *Methylobacter* (OTU 43), which had identical sequence similarity to a cultured taxon from Arctic lake sediment (Table 2.5).

The most prominent Type I MOB detected in our samples were *Methylobacter* (OTU 43, 45) and *Crenothrix* (OTU 133, 150) from the order *Methylococcales*, and *Candidatus* methyloacidpiphilum from the order *Verrucomicrobia*. Trace metals involved in anaerobic methane oxidation (AOM) such as manganese, iron, and sulfate also correlated with shifts in microbial community structure, particularly in the winter.

2.4. Discussion

2.4.1. Microbial abundance and community composition among Arctic Delta lakes

We expected microbial abundance and MOB to be highest during the spring when water-column CH_4, carbon substrates, dissolved oxygen and nutrients are readily available following peak water levels in spring (Cunada et al. 2018). Surprisingly, our results showed that overall abundance in all lakes was lower in winter (based on lake-water DNA concentration, and spring (based on qPCR of the 16S rRNA gene), then increased as open-water progressed (Figure 2.5). However, MOB abundance followed our predictions: abundance was highest in spring in some lakes, particularly low-isolation lakes 280, and highest in high-isolation lakes 56 and 520 during late summer, when there was also a spike in ambient CH_4. In contrast to overall bacterial abundance, the average abundance of MOB did not change significantly over the open-water period, although their relative abundance was significantly higher in winter and spring. MOX rates in the Delta are highest in spring and correlate with indicators of carbon bio-

availability more strongly in the winter and spring than later in the open-water period (Cunada et al., 2018).

Furthermore, changes in the relative abundance of MOB among lakes of varying isolation classes were less pronounced than the major shifts we observed in community composition, e.g., decreases in the relative abundance of proteobacteria and increases in relative abundance of *Actinobacteria*, *Verrucomicrobia* and *Cyanobacteria* phyla (Fig 2.7). Species diversity changed substantially from winter to spring and through the open-water period in all lakes, which matched our expectations of major community shifts predicted from winter to spring. The proportion and abundance of Delta MOB was highest in spring, with a drop in early summer and sudden increase in some lakes in mid-summer (Fig 2.5, 2.10), which corresponds with historical rates of elevated MOX in the Delta (Cunada, 2018). Low-isolation lakes had the highest proportion of MOB in the winter and spring, while lake 280 had the highest abundance of MOB of all lakes measured (Fig 2.5). This finding was contrary to our expectations of increased MOB abundance and proportion in more isolated lakes and lakes with higher average CH_4 substrate availability. The combined effect of high macrophyte density, a potential niche for MOB (Yoshida, 2012), and moderate influence from the Mackenzie river (Squires et al. 2002) could create optimal conditions for MOB in lakes with medium river-to-lake connection times that could be investigated in future work.

Cell densities of bacteria and archaea as well as MOB derived from qPCR data were lower than other Arctic aquatic systems, including prior analysis of these lakes, where epifluorescence microscopy was used to quantify cell density (Table 2.4; Tank et al., 2009). Because DNA samples were thawed and re-frozen several times before qPCR analysis was performed, the potential under-representation of cell densities is possible through some DNA or cell degradation, although differences in methodology could also account for our low counts compared to other studies (Table 2.4).

2.4.2. Methanotrophs and methanogens in the Delta lake study system

MOB relative abundance and overall species diversity was highest in winter although lake-water DNA concentrations were too low and insufficient for qPCR analysis of bacterial and methanotroph cell abundance. Although this higher relative abundance

correlates with higher CH_4 concentration, winter is a period when CH_4 oxidation rates should be extremely limited by low oxygen availability and low temperatures and competing for alternative electron acceptors for MOX and MEG processes (Lofton et al., 2012 and references therein). Strong correlations with MOB and trace metals involved in MOX suggest that under-ice MOX could be an important process despite net build-up of under-ice CH_4 during winter (Figure 2.9). Type I MOB including *Methylomonas*, *Methylobacter* and *Methylococcaceae* are more commonly found associated with the water column and surface sediment in other systems such as Lake Killarney, a sub-arctic Taiga lake with high ambient methane and DOM-rich sediment and Lake Qalluuraq, an Arctic lake, during open-water (He et al., 2012). High proportions of methylotrophs, particularly *Methylobacter* (OTU 43 and 45) in the water column in spring that remained in some lakes through the open-water period. *Methylobacter* is known to metabolize CH_4 below the oxycline of a eutrophic Arctic lake (Schutte et al., 2016) or in the sediment-water interface of Arctic lakes where these MOB perform a significant proportion of all MOX (He et al., 2012), so these groups could be important to carbon cycling in water and sediment of Delta lakes.

MOB from the genus *Methylophilaceae* (OTU's 10, 17 and 41) comprised a significant proportion of the total community in all lakes throughout the year. These organisms are methylotrophs that feed primarily on single-carbon compounds such as methanol (Murrell et al. 1998). Methylotrophs can form a mutual 'cross-feeding' relationship whereby a non-MOB taxon consumes substantial quantities of CH_4-derived carbon such as extracellular methanol (Krause et al., 2017). *Methylophilaceae* and other methylotrophs also comprised a high proportion of the community of Lake Killarney (He et al., 2012) so these groups could play an important role in the carbon cycle of Arctic lake-systems complex community inter-relationships such as cross-feeding.

Members of the Type I MOB genus, *Crenothrix* were also present, although in low relative abundance compared to other groups and mostly in the spring or early summer. *Crenothrix* species have been found to consume substantial quantities of CH_4 despite low cell abundance (Oswald, 2017). *Crenothrix*, despite their low apparent relative abundance in the Delta, could be contributing substantially to rapid lake-water MOX that occurs in spring and help sequester CH_4 that would otherwise flux to the atmosphere (Cunada, 2018).

The decrease in total proportion of MOB to other organisms as the open-water period progressed is likely due to the observed increases in overall microbial abundance, as MOB cell abundance did not change appreciably over the open-water period. Increases in populations of common freshwater taxa such as *Comamonadaceae, Sporichthyaceae, Burkholderiaceae, Chitinophagaceae, Flavobacteriaceae* and *Cyanobacteria* families would increase competition with MOB for nutrients and resources (Zwart et al., 2002). *Verrucomicrobia Opitutae vadin*_Ha64_ge (OTU 25) which, although not known to be a methano- or methylotrophic genus, had a strong inverse correlation to water-column CH_4 concentration and had the highest relative abundance in winter and spring, as well as a significant correlation to CH_4 from our experimental results (see Chapter 3). *Verrucomicrobia,* which are prominent in northern and temperate lakes and contains many particle-associated phyla, typically have higher abundance in spring (Chiang et al. 2017). Thus, their presence in Delta lakes, and their correlation with CH_4 and higher relative abundance in the spring, suggests that they may be important in carbon cycling in Delta lakes.

Methanogenic archaea (MGA) were very difficult to detect in our 16S rRNA-gene analysis which limited the scope of this study to MOB and general microbial abundance. We did detect MGA such as *Methanosaeta* in DNA extracts from surface sediment samples (0-2 cm) taken during winter and in early spring (results not shown). This genus has been observed in the sediment of a temperate river (Buriankova et al., 2013), as the dominant methanogen in organic-rich Arctic marine sediments (Carr et al., 2017), in the water column of an oligotrophic lake (Grossart et al., 2011), and in Lake Rostee (Schubert et al., 2010), where they produced up to 90% of the CH_4 entering the water column from the sediment. *Methanosaeta* and other MGA likely have a substantial impact on carbon flow in the Delta but determining their abundance and activity would require more specific methods of detection such as those used in Carr et al., 2017. Our methods of rarification and the high abundance of general taxa, which could obscure or mask the typically low-abundance Archaea, was a likely source of bias against detection of methanogenic and other Archaea.

Future studies should utilize Archaea-specific primers and consider the low relative abundance of Archaea species relative to general microbial communities in Delta lakes. These organisms are likely more abundant in the sediment and substantial

production (and oxidation) of methane occurring in this system could be indicative of activity in other permafrost-rich arctic floodplain systems.

2.4.3. Environmental drivers of methanotroph populations

In the Delta, the relative abundance of MOB and the species diversity of microbial communities was much higher in winter and spring (Figure 2.10) when ambient methane concentrations and nutrient availability was highest. Significant correlations were found between globally dispersed MOB and methane concentration, as well as between measures of carbon bio-availability, DOC, pH, temperature and dissolved oxygen regardless of lake type or season (Table 2.4, Figure 2.9). CH_4 concentration has historically correlated with lake isolation more strongly in winter and spring, following patterns of availability of nutrients and amount of DOM in the form of DOC and pCO_2 (Cunada et al. 2018). Differences in carbon quality and varying inputs of carbon from macrophyte, thermokarst or riverine sources could have a strong influence on the dominant members of communities in Delta lake-water and sediment communities, as in other systems (He et al. 2012). Seasonal changes in the overall microbial community composition changed with major seasonal shifts to these carbon-linked variables.

2.5. Conclusions

Our results demonstrate that the communities of lake-water MOB and general microbial abundance are strongly affected by seasonal changes to Mackenzie Delta lakes, and that differences in overall abundance and composition of microbial communities among lakes are more pronounced in winter and spring than throughout the open-water period. Bacterial abundance increases through the open-water period, and differences among the lake-water communities become less pronounced as open-water progresses.

Delta MOB are comprised of a significant proportion of methylotroph OTU's and are present throughout the year in all lakes with higher relative abundance in winter, changes in MOB when lake-water concentrations of CH_4 and historical rates of MOX are highest. The prominence of methylotroph OTU's throughout the year was unexpected, and we expected to see more Type I methanotrophs throughout the 6-lake set during the open-water period, but this trend was not observed. The prominence of methylotroph

groups (OTU 10, 17, 41), and their persistence throughout the year in all samples and seasons indicates that these methylotroph groups play an important role in water-column carbon cycling and highlight the potential for complex community interactions to influence rates of lake-water MOX and other nutrient and carbon cycling processes in the Delta.

The effect of river-to-lake connection time and the associated changes to lake-water biogeochemistry on community composition of microbes in Delta lakes was less prominent than the broad-scale changes that occurred at monthly intervals from winter and through the open-water period. Differences among lake types appear greatest during winter and spring, when measures of species diversity are highest, but these differences became less pronounced as open-water progressed.

2.6. Tables and Figures

Table 2.1 **Summary of 43-lake set and their isolation type, determined by lake sill as elevation above sea-level (asl) in meters. Winter = 22 lakes sampled May 9th and 11th, 2016. Summer and Late Summer = full 43-lake set. Samples in bold are from 6-lake subset were sampled bi-weekly from May 30th to mid-August 2016.**

				Lake surveyed? Y = yes, blank = no.		
Lake	Lake Area (ha)	Spring Sill (m)	Isolation Type	Winter	Spring	Late Summer
L302a	853	1.5	No	Y	Y	Y
L15a	437.6	2.177	No	Y	Y	Y
L4	330.5	2.363	No	Y	Y	Y
L129	**37.8**	**2.363**	**No**	**Y**	**Y**	**Y**
L85a	50.6	2.363	No	Y	Y	Y
L80	**19.3**	**2.631**	**Low**	**Y**	**Y**	**Y**
L501	126.9	2.631	Low		Y	Y
L148a	28.4	2.631	Low		Y	Y
L302b	18.9	2.631	Low		Y	Y
L79a	34.6	2.631	Low		Y	Y
L107	16.7	2.99	Low	Y	Y	Y
L272	27.3	2.99	Low		Y	Y
L300	34.4	2.99	Low		Y	Y
L301a	36.6	2.99	Low		Y	Y
L85b	1.7	2.99	Low		Y	Y
L58	13.7	3.389	Low		Y	Y
L87	**3.9**	**3.389**	**Low**	**Y**	**Y**	**Y**
L141	17.2	3.389	Low		Y	Y
L511	1.6	3.389	Low	Y	Y	Y
L148b	94.8	3.389	Low		Y	Y
L111	5.3	3.671	Low	Y	Y	Y
L184	17.7	3.671	Low		Y	Y
L272b	2.1	3.671	Low		Y	Y
L11	105.4	3.838	Low		Y	Y
L280	**2.4**	**3.838**	**Low**	**Y**	**Y**	**Y**
L538	37.9	3.838	Low	Y	Y	Y
L131	1.2	4.077	High		Y	Y
L278	9.8	4.077	High	Y	Y	Y
L287	9.8	4.077	High	Y	Y	Y
L517	72.9	4.077	High		Y	Y
L148f	12.4	4.077	High		Y	Y
L56	**3.1**	**4.623**	**High**	**Y**	**Y**	**Y**
L115	2.3	4.623	High		Y	Y
L134	3.4	4.623	High	Y	Y	Y
L261	48.5	4.623	High		Y	Y
L275	5.9	4.768	High	Y	Y	Y
L520	**0.2**	**4.913**	**High**	**Y**	**Y**	**Y**
L522	22.5	4.913	High	Y	Y	Y
L143	2.1	5.169	High	Y	Y	Y
L181	0.8	5.169	High	Y	Y	Y
L186	1	5.169	High	Y	Y	Y
L521	0.1	5.169	High		Y	Y
L527a	8.5	5.169	High	Y	Y	Y

Table 2.2 Summary of depth, ice composition and thickness, snow conditions, and methane concentration from samples taken at the bottom of ice hole and, where unfrozen water depth was greater than 1 m, methane concentration at depth was within 50 cm of lake bottom sediment. 6-lake sub-set highlighted in bold text. Standard deviation of CH_4 duplicates, where available, is shown. TK = thermokarst-affected lake within the given No - Low - High isolation classification of lakes based on their degree of isolation relative to the Mackenzie River

Lake	Isolation Type	Unfrozen water depth (cm)	Ice Thickness (cm)	Snow Depth (cm)	Under-ice CH_4 (µM)		
					Bottom-ice surface water	Deep	Average
L302a	No	167	69	14	5.4 ± 0.3	5.3 ± 0.1	5.3 ± 0.3
L15a	No	73	53	4	16.4 ± 2.4	--	16.4 ± 2.4
L4	No	161	74	3	3.4 ± 2.2	5.5 ± 0.9	4.4 ± 2.4
L129	**No**	124	57	0	2.4 ± 0.2	--	2.4 ± 0.2
L85a	No	78	62	3	8.5 ± 0.2	--	8.5 ± 0.2
L80	**Low**	137	59	7	0.1	--	0.1
L107	Low	58	45	5	91.7 ± 20.1	--	91.7 ± 20.1
L87	Low	55	49	5	279.8 ± 4.8	--	279.8 ± 4.8
L511	Low	189	43	0	35.0 ± 0.5	--	35.0 ± 0.5
L111	Low	27	51	5	293.5 ± 4.1	--	293.5 ± 4.1
L280	TK	187	51	3	301.6 ± 1.0	162.3 ± 32.1	231.9 ± 32.1
L538	Low	73	42	6	259.7 ± 2.6	--	259.7 ± 2.6
L278	Low	78	44	4	1174.7 ± 105.3	--	1174.7 ± 105.3
L287	Low	278	43	5	347.0 ± 17.0	744.2 ± 8.5	545.6 ± 19.0
L56	High	88	43	2	1250.6 ± 1.4	--	1250.6 ± 1.4
L134	High	108	52	4	191.6 ± 3.9	--	191.6 ± 3.9
L275	High	157	42	5	992.8	n/a	992.8
L520	**TK**	222	48	2	224.0 ± 4.0	268.9	246.5 ± 4.0
L522	High	412	46	4	130.5 ± 19.2	91.2 ± 30.0	110.8 ± 35.7
L143	TK	215	43	2	896.5 ± 3.9	889.3 ± 1.2	892.9 ± 4.1
L181	TK	174	42	3	1149.5 ± 68.3	1134.4	1142.0 ± 68.3
L186	High	455	47	3	303.5 ± 65.5	942.7 ± 51.3	623.1 ± 83.2
L527a	High	90	41	3	818.0 ± 7.6	--	818.0 ± 7.6

Table 2.3 Summary of DNA concentration and from the 6-lake priority samples in ng / mL of sample water from a set of 6 Delta lakes, from Winter (early May) under-ice, to Spring (end of May) and through the open-water period (June - August). DNA concentration in ng / mL lake water. Raw reads obtained from 16S rRNA-gene Illumina sequencing data and rarified to the sample with the fewest reads. Species richness and Shannon (effective) diversity determined from normalized OTU count data

Lake	Season	[DNA] ng / mL	SampleVolume (mL)	RawReads (IMR)	QCReads (IMR)	%remov	Richness	Shannon effective	OTU Count Rarified
129	Winter	0.1	71				334	62.2	549
87	Winter	0.5	20				181	27.6	181
56	Winter	1.3	10				423	130.9	555
520	Winter	0.8	10				238	72.5	238
129	Spring	0.3	35	13906	10518	24	253	61.1	253
87	Spring	0.5	33	8968	6565	27	191	39.1	191
280	Spring	1.1	25	71281	56822	20	199	41.8	526
56	Spring	1.7	24	68591	51211	25	249	40.8	405
520	Spring	0.7	25	25393	20154	21	170	29.8	217
129	Summer	2.4	30	76950	60790	21	166	36.3	299
80	Summer	0.7	46	20998	16318	22	145	29.3	192
87	Summer	1.4	33	113957	93900	18	113	23.4	420
280	Summer	1.5	40	50205	38973	22	227	49.7	317
56	Summer	1.6	30	104329	84062	19	125	32.7	511
520	Summer	1.3	40	56820	45573	20	140	33.6	281
129	Mid-Summer	0.7	35	14212	10850	24	180	55.7	219
80	Mid-Summer	1.2	45	91297	71687	21	271	49.6	556
87	Mid-Summer	0.4	40	5702	4088	28	132	28.8	132
280	Mid-Summer	0.8	30	66860	53780	20	191	33.6	308
56	Mid-Summer	4.6	30	107321	86784	19	147	33.6	778
520	Mid-Summer	1.2	30	81874	66995	18	192	36.2	611
129	Late-Summer	1.6	45	101795	83358	18	164	37.6	541
80	Late-Summer	2.4	46	93203	75145	19	197	44.6	717
87	Late-Summer	1.9	33	50725	41102	19	127	28.7	279
280	Late-Summer	1.0	30	21528	17172	20	145	35.2	193
56	Late-Summer	2.8	30	46669	32405	31	178	30.8	293
520	Late-Summer	1.2	30	71017	57206	19	163	34.5	337

Table 2.4 Summary of average or range of cell abundances from current study and comparison with other systems and methods. Typical cell counts determined by direct counts (epifluorescence microscopy utilizing 4'6-diamidino-2-phynylindole (DAPI) stain), or by quantitative techniques (catalyzed reporter deposition fluorescence in-situ hybridization (CARD-FISH) or quantitative PCR, as in the current study (qPCR)). All extracts are from total suspended bacteria

* = Type I methanotroph ** = Type II methanotroph *** = mcrA gene / Type I and II methanotrophs

System	Methods	Specific location	Bacterial abundance cells mL $^{-1}$	Methanotroph Abundance cells mL $^{-1}$	Reference
Mackenzie Delta East Channel (Experimental set)	qPCR of 16S rRNA gene and pmoA gene	Lake # 129 56 520	8.9 (±4.2) x 10^4 3.5 (±0.9) x 10^4 3.6 (±0.5) x 10^3	1.6 x 10^3 ± 200 0.2 x 10^3 ± 44 0.4 x 10^3 ± 4	This study
Mackenzie Delta East Channel (Experimental set; 2006)	Epifluorescence (DAPI)	Lake # 129 56 520	4.5-5 x 10^6 5.7-6x 10^6 --	-- -- --	Tank et al 2009.
"	"	Lake # 129 56 520	9 (±3) x 10^6 9.2 (±2.5) x 10^6 6.6 (±3.3) x 10^6	--	Chateauvert et al (2012)
Lago di Coadagno	Epifluorescence (DAPI)	Varying depths	--	0.8-6 x 10^3 *	Oswald et al., (2015)
Set of Five Swedish Temperate lakes	Flow cytometry	Surface and Bottom-water	1-10 x 10^6	--	Samad and Bertilsson, (2017)
Toolik Lake (Oligotrophic arctic lake)	Epifluorescence (acridine orange)	Surface and Bottom-water	0.8-3 x 10^5	--	Adams et al., 2015
Toolik Lake Inlet/outlet streams	Epifluorescence (DAPI)	Surface water	0.4 - 2.9 x 10^6	--	Hobbie et al., 1983
Kuparuk river (Near Toolik lake)	Epifluorescence (DAPI)	Surface water	0.3-2.7 x 10^6	--	Hobbie et al.,
Lago di Cadagno (Anoxic waters)	CARD-FISH analysis (pmoA and mcrA gene)	Anoxic waters	1 x 10^7	2.4 x 10^5 ***	Milucka et al., 2015
Mono Lake (Large, shallow, saline lake)	DAPI-stained cells / FISH	Surface and bottom-water	2.1-2.5 x 10^7	5 - 9 x 10^5 * 1.5 - 3.5 x 10^5 **	Carini et al (2005)

Table 2.5 Taxonomy and BLAST database comparison of dominant microbial taxa with correlation to methane and other carbon-cycling properties from 16S-rRNA gene analysis of Mackenzie Delta lake-water sampled in winter, and through the open-water period of 2016. Uncultured / environmental representatives were all general species without specific classification to genus level; isolation locations from uncultured/environmental isolates matched those for cultured taxa. All OTU sequences were 186 base pairs and 100% identical to their BLAST counterparts unless otherwise indicated.

* = 99% identical ** = 98% identical.

SILVA Taxonomy **Phylum** Class *Genus*	OTU #	Corr. CH_4	P-Value	Cultured Genus (BLAST)	Location	Accession #
Actinobacteria						
Actinobacteria						
*Candidatus*_Planktophila	3	0.72	0.00	*Candidatus* Planktophila *Var. Actinobacterium*	FW Lakes (Zurich, Soyang) FW Pond (Japan)	LC106292.1, CP015603.1
hgcI_clade	8	0.66	0.01	*Candidatus* Nanopilagicus, Rhodococcus	FW Lake (Zurich)	CP016768.2, CP016779.1
Sporichthyaceae(UC)++	12	-0.51	0.05	*Candidatus* Planktophila lactus	FW Lake (Zurich, Soyang, Damariscotta)	CP016783.1, CP016780.1
Unclassified Microbacteriaceae	13	0.60	0.02	*Rhodoluna spp*	FW Lakes (Egelsee, Mondsee), Ichino River, East China Sea	KU173555.1, LC094690.1
Unclassified Microbacteriaceae	18	0.71	0.01	*Candidatus* Limnoluna rubra, *Aquiluna rubra**	FW Lake (Lake Grossegelsee), Ichino River*, Stream (China)	NR_125497.1
Sphingobacteria						
Sediminibacterium	7	0.80	0.00	*Bacteroidetes / Sediminibacterium**	FW Lake, (Damariscotta Lake) (Sediment* + Fishing port)	JF488143.1, HQ663413.1
Dinghuibacter	23	-0.81	0.00	*Chitinophagaceae***, *Terimonas***, *Cnuella****	Grassland***, Desert soil***	LN876538.1, KT350459.1
Dinghuibacter	24	0.69	0.04	*Bacteria***, *Flavitalea***, *Niabella Terrae***,	Paddy soil**, agricultural soil***, Aquatic plant root***, soil*** (S. Korea)	LC106253.1**, KX762320.1***
Chitinophagaceae(unclassified)	85	-0.79	0.03	*Ferruginibacter yonginensis**, *Chitinophaga sp.**, *Flavitalea**	FW Lake (Damariscotta*, Iron-Seep*, Artificial(China)*, Finnish lake),	JF488165.1*, AM988943.1*

SILVA Taxonomy

Phylum Class *Genus*	OTU #	Corr. CH_4	P-Value	Cultured Genus (BLAST)	Location	Accession #
Cytophagia						
Pseudarcicella	11	0.74	0.00	*Arciella sp. Bacterium, Pseudarcicella**	FW Lake (Artificial, Maarsseveen), FW (Korea)*	JN408290.2, AJ964899.1
Flavobacteriia						
Fluviicola	16	0.52	0.05	*Arciella sp., Bacterium****, *Pseudarcicella****, *Flavobacteria*	FW Lake (Mendota), Porphyra	HQ663127.1
Fluviicola++	16	0.68	0.01	-	-	-
Proteobacteria						
Alphaproteobacteria						
LD12_freshwater_group_ge	6	0.84	0.00	*Candidatus* Fonsibacter / Pelagibacter,	FW Lake (Damariscotta Lake)	CP024034.1, KY290650.1
Caulobacter(OTU84)	84	-0.95	0.00	*Caulobacter profundus***, *sp. WW137****,	FW Sediment (Korea), FW Lake (Kasumigaura)	
Betaproteobacteria						
Comamonadaceae	1	-0.73	0.00	*Acidovorax*	FW Lake (Lugano)	MF077115.1, MH046753
Comamonadaceae	9	-0.54	0.04	*Leptothrix*	Wetlands (2),	JQ946028.1, JQ946023.1,
Methylophilaceae	10	0.76	0.00	*Candidatus* methylopumilus	Seawater, (East China Sea) Freshwater	KU173564.1 , LN827929.1
Methylophilaceae	17	-0.73	0.00	*Methylophilus (quayli) / Methylotenera*	Soil (Japan; rice field), Russia (unspecified)	LC368136.1, AY772089.2
Polynucleobacter	31	0.84	0.00	*Polynucleobacter cosmipolitanus*	FW Lake (Mondsee, Bled, Biwa)	AJ550652.2, AJ550649.2
Verrucomicrobia						
Opitutae						
*Opitutae*_vadinHA64_ge	25	-0.86	0.00	*Verrumicrobia*	FW Lake Mendota	HQ663236.1, HQ663179.1

Figure 2.1 Satellite imagery of Mackenzie Delta East Channel near Inuvik, NT, and the 43-lake and 6-lake (highlighted in yellow) study area for the 2016 field season. Image courtesy of Cunada et al.(2018).

Figure 2.2 **Plot of log-transformed methane (CH_4) concentration vs. sill elevation from broad-scale survey of Mackenzie Delta lakes, performed pre-iceout (May 9-11, 2016; 22 lakes), then during the open-water period immediately post-iceout and in late summer (June 5, 2016 and August 6th, 2016; 43-lakes) Sill height = elevation of lakes relative to the Mackenzie River that determines river-to-lake connection times. 6-lake set highlighted in orange.**

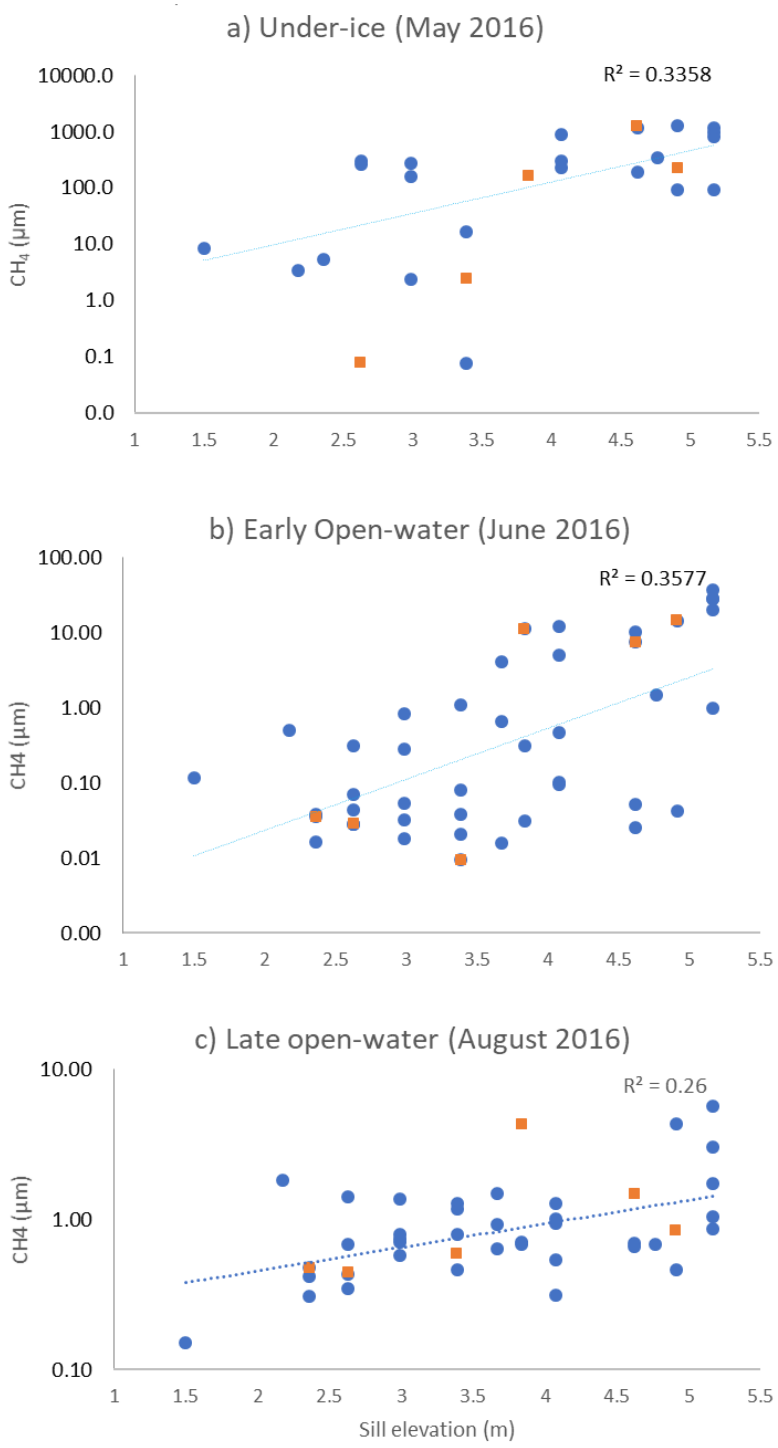


Figure 2.3 **Summary of under-ice methane concentrations for the 6-lake set, including both under-ice surface and deep samples from the two lakes with under-ice depth greater than 2 m (L280 and L520 only).**

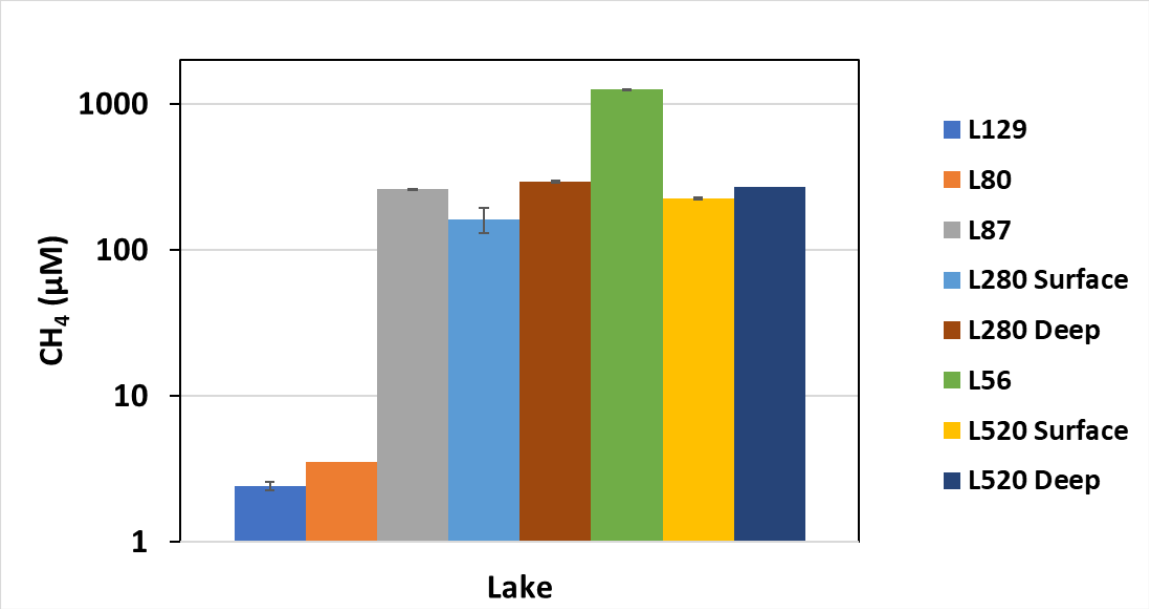


Figure 2.4 **Time-series of log-transformed concentration of methane (CH_4) from a set of 6 Mackenzie Delta lakes sampled bi-weekly during 2016 open-water period. Peak river-water levels occur during flooding in spring, with 2016 water levels reaching their peak on May 22nd. Legend arranged in order of increasing lake isolation, with L129 having nearly continuous river-to-lake connection.**

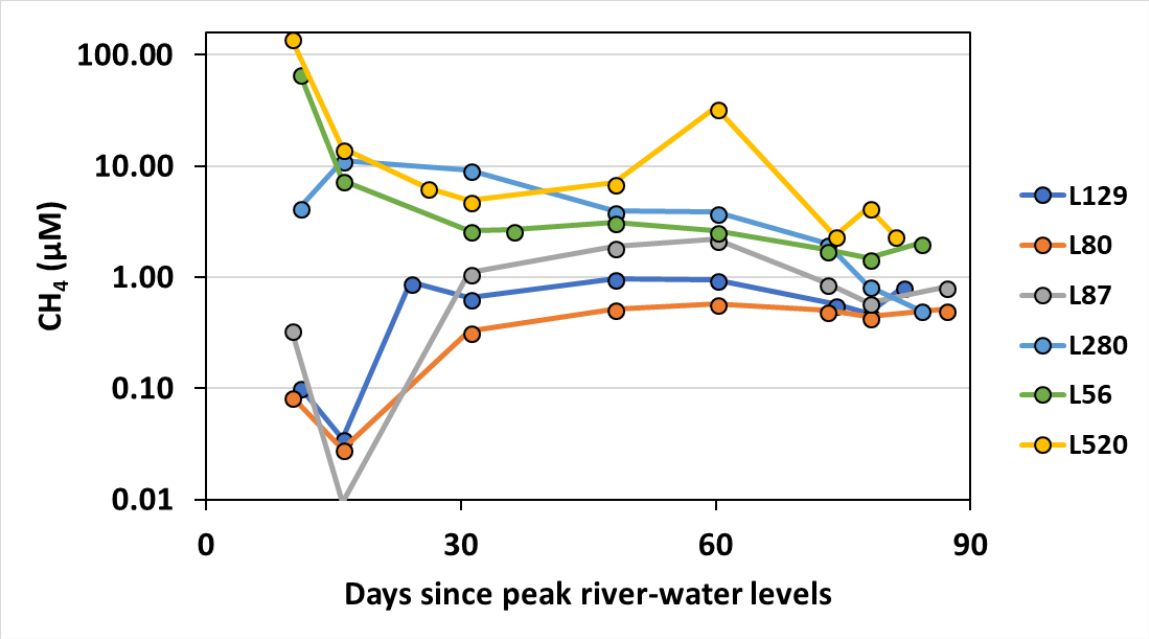


Figure 2.5 **Community cell abundances and methanotroph abundance at seasonal time-points for the 6-lake set, arranged in order of increasing sill elevation. Seasonally averaged cell abundances of general community (16S rRNA gene, a), methanotrophs (*pmoA* gene, b), and the proportion of methanotrophs to general population (c) determined from qPCR analysis of 6-lake set in Spring (Late May), Summer (Late June), Mid-Summer (Late July) and Late-Summer (Early August). Lakes listed in order of increasing lake isolation from L 129 to L 520.**

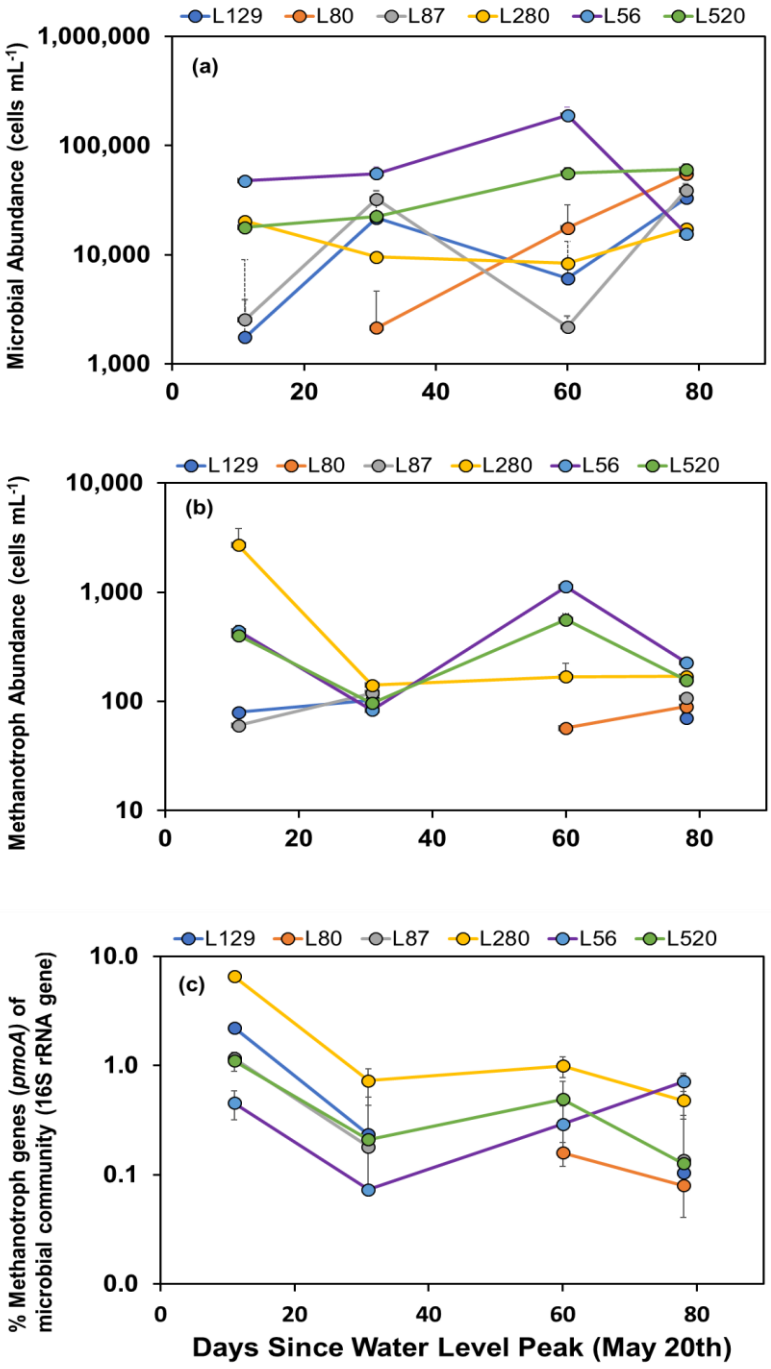


Figure 2.6 **Alpha-diversity measurement of 6-lake data set averaged by season (left) and Simpson diversity by lake. Species richness was highest in Winter and Spring, and lower overall through the rest of summer (left panel). Alpha-diversity measurement based on Simpson diversity index of 6 Delta lake samples spanning winter under-ice and through the open-water samples. No-isolation lake 129 had the highest overall abundance (right panel; Simpson / effective alpha-diversity), although differences among lake types and isolation classes not apparent. Correlation strength of per-lake comparison of diversity P-value and adjusted p-value of 0.0431. Kruskall-Wallis rank sum test of lake OTU alpha diversity showed P-values of 0.018 and adjusted P-values of 0.108.**

(A) **(B)**

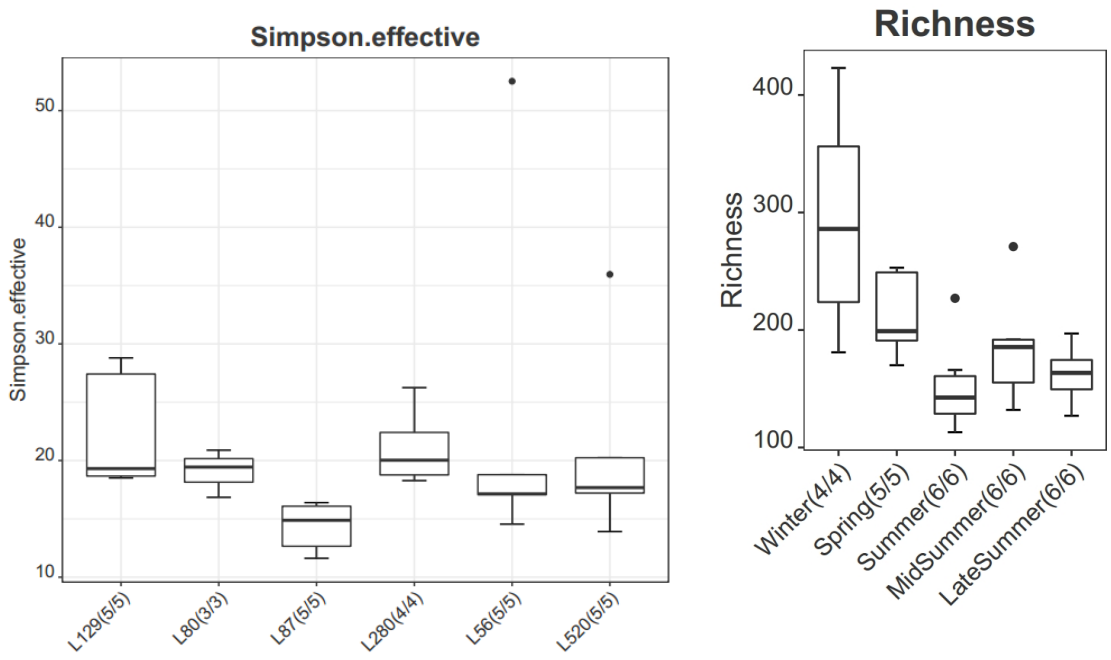


Figure 2.7 **Relative abundance of major phyla present during monthly sampling periods for a set of 6 lakes in the Mackenzie River Delta. Winter (May 9th) = under-ice survey; Spring (May 30th-31st) = early open-water survey; Summer = June 20th survey; Mid Summer = July 19th survey; Late Summer (August 6th survey. Highest proportion of 'other' groups was found in May, where phyla with abundances lower than 1% of the total community were grouped together.**

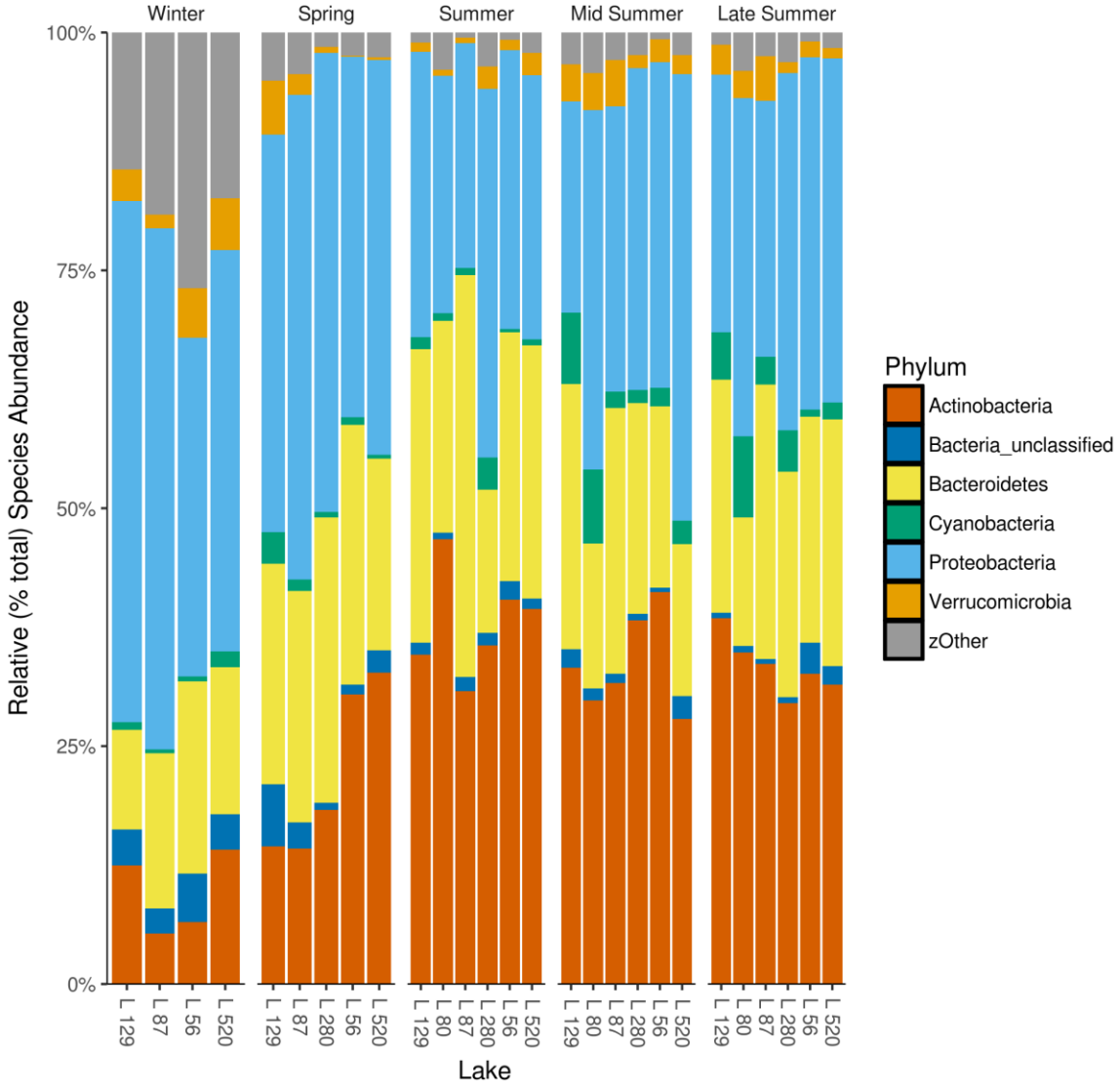


Figure 2.8 **Heat-map of the 25 most abundant OTU's among Delta lake samples, based on rarified 16S rRNA-gene data from a set of 6 lakes sampled during the Winter and open-water period of 2016.**

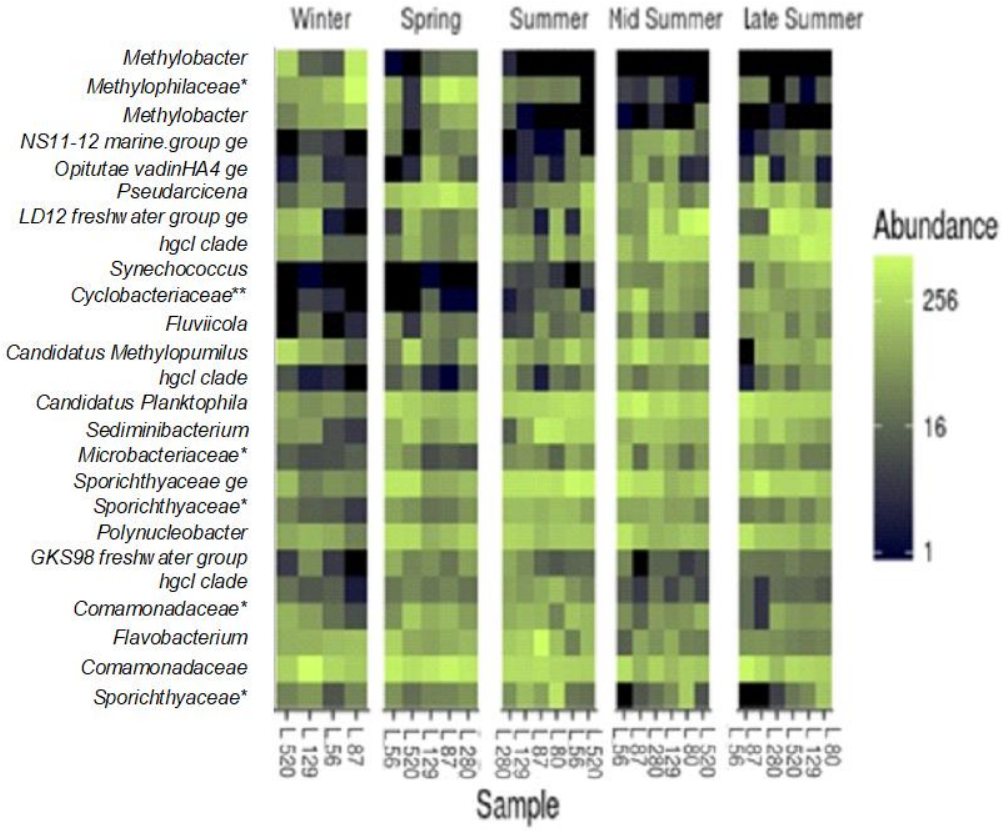


Figure 2.9 NMDS (non-metric multi-dimensional scaling) of 6-lake samples separated into seasonal periods (ellipses) based on the Bray Curtis dissimilarity among samples calculated from 16S rRNA-gene sequencing data. Gene sequences were clustered into groups that share 97% sequence similarity, known as operational taxonomic units or OTU's.

The relative distance between points indicates the similarity among samples based on shared OTU composition and abundance for each sample. Red arrows represent the correlation between environmental variables and microbial community structure as calculated by NMDS ordination of the Bray Curtis dissimilarity matrix, with the direction and magnitude of the arrow indicating the significance of the correlation.
Mantel statistic based on Pearson's product-moment correlation and Spearman's rank correlation rho showed significant correlation between OTU abundance data and principal components analysis of environmental data.
Pearson product moment: Mantel's r = 0.4168, significance = 0.001

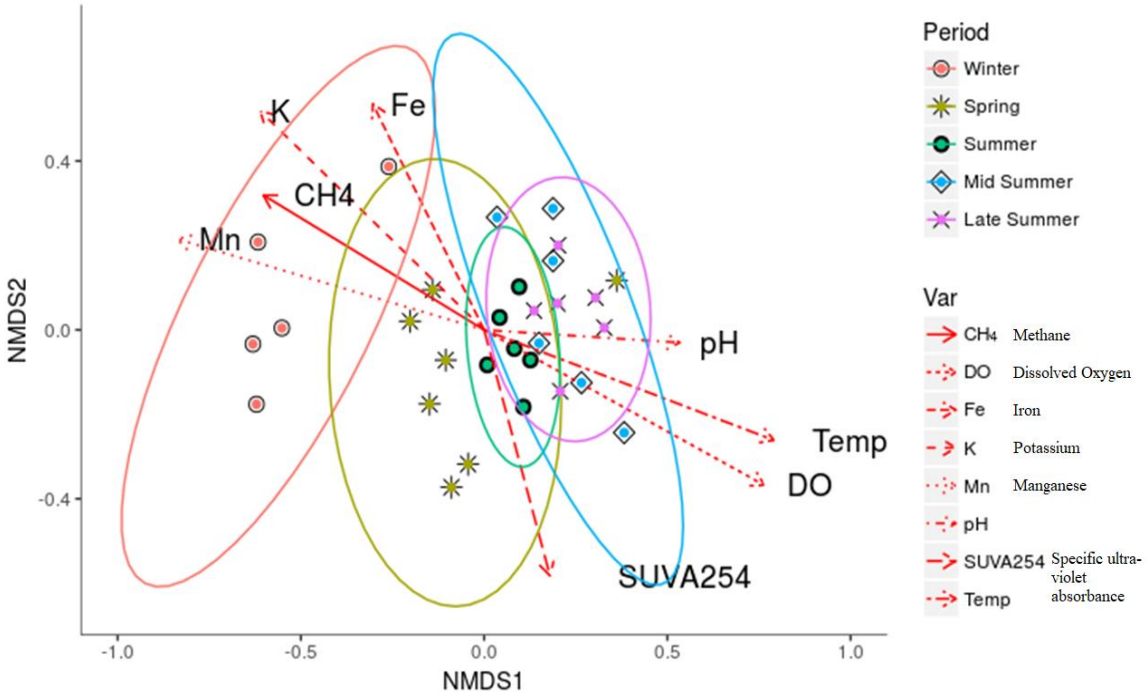


Figure 2.10 Bar-plot of the relative abundance (% of total abundance) of methano- and methylotroph genus from the 6-Lake set, organized by season.

* = methylotroph groups, or organisms that feed primarily on methanol.

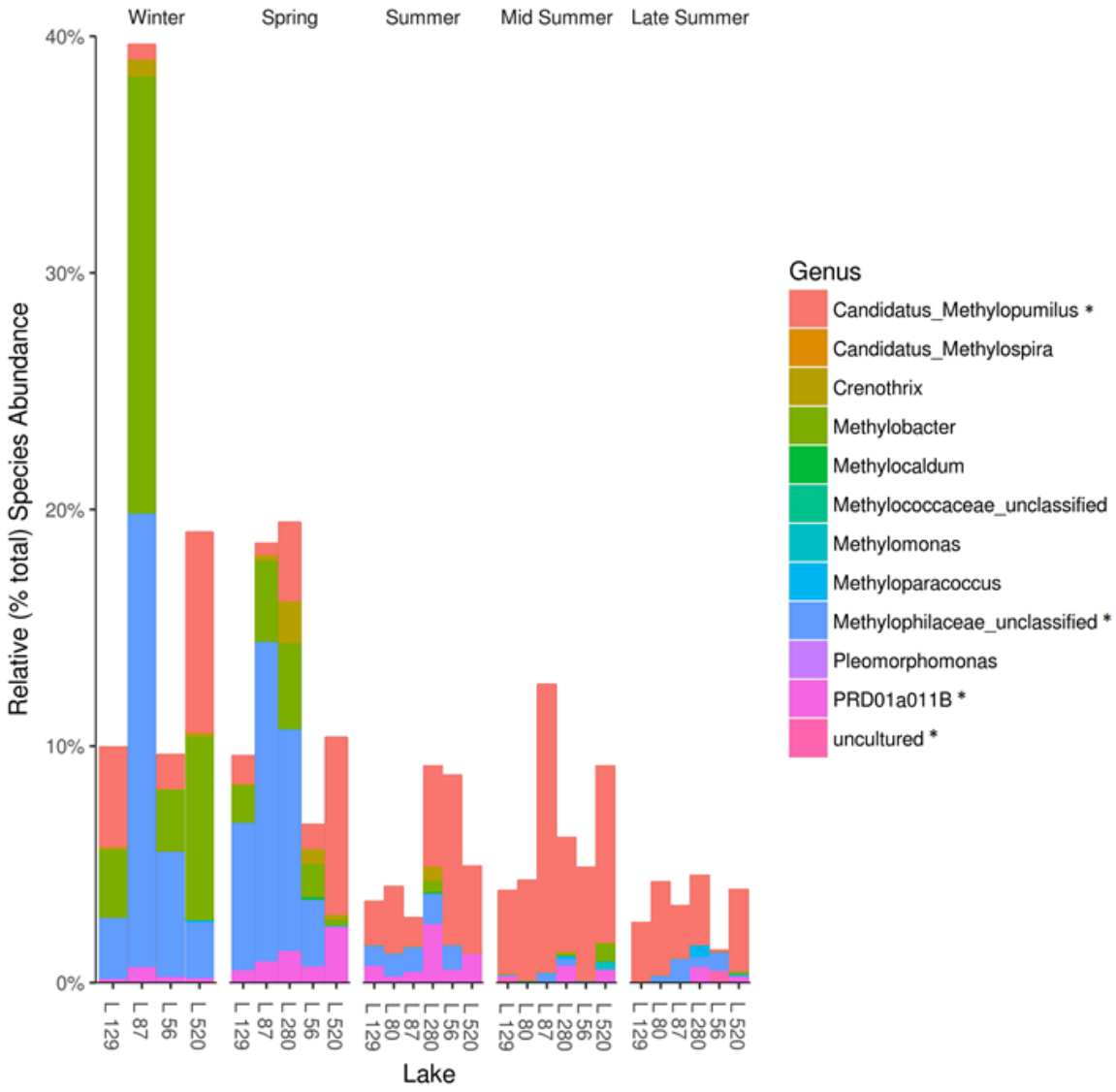
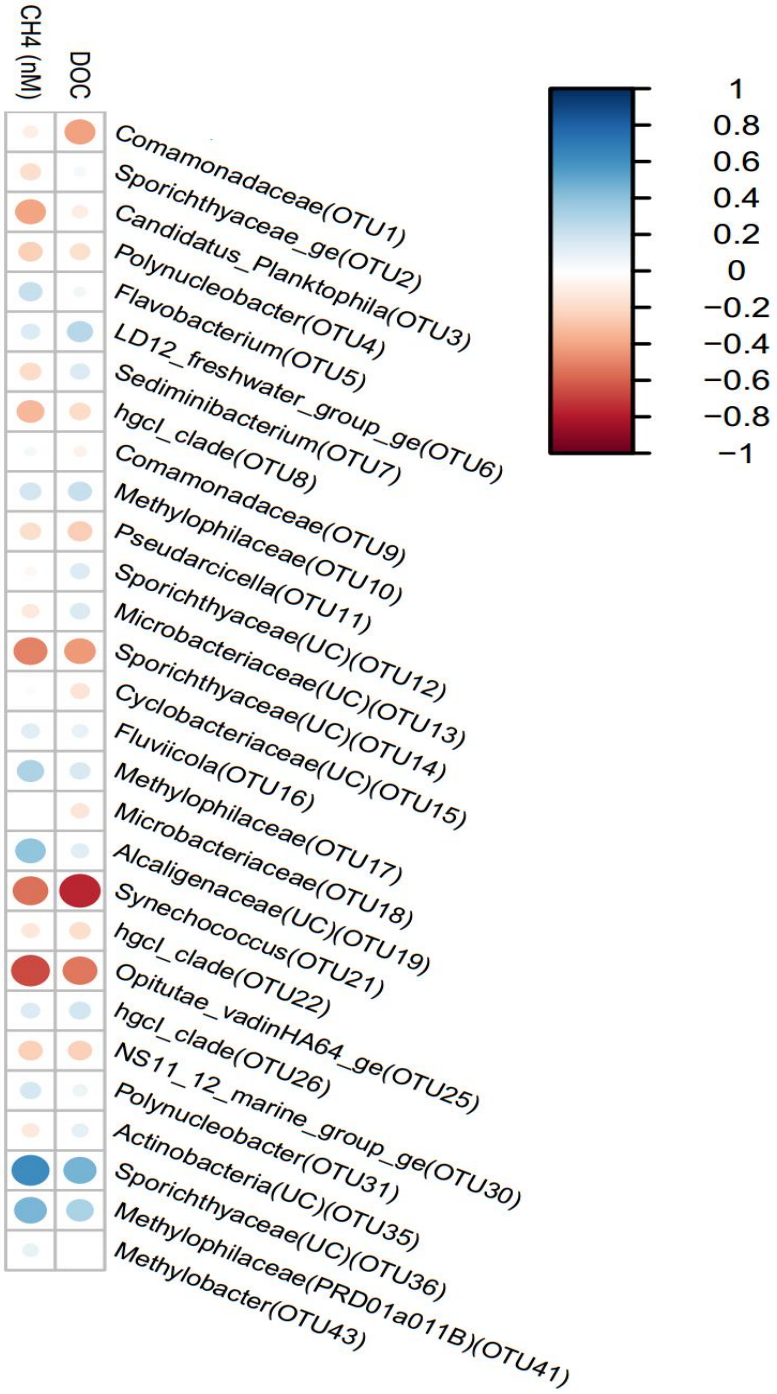


Figure 2.11 **Correlation plot showing top OTU''s linked to methane concentration and carbon quantity. Correlation determined from Rhea correlation analysis of log-transformed environmental variables and OTU data from 6-lake set at major seasonal time-points (May, June, July and August). Correlation scores are normalized to a -1 to 1 scale, with +/-1 as the maximum correlation value and colored according to the scale bar (top right). Correlation scores for taxa with strongest correlation to methane are shown in data-table 2-4.**



Chapter 3.

Effects of nutrient limitation and methanogenesis on methane oxidizing bacteria in Mackenzie Delta lakes

Abstract

Arctic lakes are hotbeds of methane production (MGA) and oxidation (MOX), but the factors that influence the structure of lake-water microbial communities and their impact on these processes remains poorly understood. We used experimental manipulation of surface waters from a set of Arctic floodplain Mackenzie Delta lakes with different end-points of carbon origin, quality and quantity to detect rates of lake-water methane oxidation (MOX), changes in the composition of microbial communities, and the potential for methanogenesis under aerobic conditions (MEG) in the water column of Delta lakes. MOX increased with increasing lake isolation and in lakes with greater inputs of carbon derived from permafrost and aquatic plants. MOX ranged from 122 to to 1529 nmols L^{-1} d^{-1} and was enhanced in the presence of added nutrients by 43 to 600 nmols L^{-1} d^{-1}. Aerobic water-column methanogenesis was detected for the first time in an Arctic lake. The most predominant MOB matched cultured *Candidatus* methylopumilus and *Methylophilus quayli* and their abundance correlated significantly with varying CH_4 substrate availability.

3.1. Introduction

Methane (CH_4) is a greenhouse gas of particular importance in Arctic ecosystems and the global biosphere due to its high potency as a climatic warming agent and the substantial output of CH_4 from the lake-rich and permafrost-affected landscape of the Arctic (McGuire et al. 2009, Walter Anthony et al. 2010). Fluxes of methane from lake-waters to the atmosphere have been documented for a range of study systems around the circumpolar Arctic, but few studies examine the compositions, abundances, and activities of microbial communities that drive these CH_4 fluxes. An often-overlooked aspect of CH_4 dynamics in many aquatic ecosystems is the role of methane oxidizing bacteria (MOB) and the effect that CH_4 oxidation has on mitigating the flux of CH_4 from lake waters to the atmosphere. Substantial CH_4 production by methanogenic archaea (MGA) occurs in the sediment and anoxic parts of the water column in many lake systems worldwide (Kankaala et al. 2006 b, Jones et al. 2008, Schubert et al. 2010, Lofton et al. 2014). MOB abundance is positively correlated with methanogen abundance and CH_4 concentration (Sundh et al. 2005, Rahalkar et al. 2009, Gentzel et al. 2012) but the rate of CH_4 oxidation (MOX) varies with differing lake-water conditions (Rahalkar et al. 2009, Schubert et al. 2010).

Dissolved oxygen, nutrient availability, temperature, pH, and the availability of dissolved CH_4 substrate are primary factors that influence rates of MOX (Bédard & Knowles 1997, Kankaala et al. 2006, Hershey et al. 2014, Lofton et al. 2014). CH_4 concentration is usually the primary limiting factor for MOX rates at concentrations less than 0.4 µmol CH_4 L^{-1} if oxygen is present (Trimmer et al. 2009) but at higher CH_4 concentrations, MOX rates will be limited following enzyme specificity, pH and temperature (Lofton et al. 2014). Key nutrients such as soluble reactive phosphorus (PO_4) have been positively correlated with MOB abundance in a series of CH_4-rich temperate lakes (Samad and Bertilsson, 2017) but studies of nutrient effects on rates of MOX and microbial community dynamics in Arctic systems has been limited.

Deltas of the great Arctic rivers contain abundant and diverse shallow lakes with complex hydrological regimes that influence productivity and carbon-cycling processes including MOX. Water-column MOX rates among lakes of the Mackenzie Delta range from below detection to as high as 2.47 nmol m^{-3} d^{-1} where lakes with shorter river-to-lake connection times had higher MOX than lakes with longer river-to-lake connection

times (Cunada et al. 2018). Rates of MOX in other systems have comparable ranges to those found in the Delta, with measured maximum rates of MOX from 2.44 nmol m^{-3} d^{-1} in a temperate lake system (Bastviken et al., 2003), 8.2 - 33.7 arctic and sub-arctic systems in Alaska (Martinez-Cruz et al. 2015, Lofton et al. 2010) and Finland (Kankaala et al 2005), and as high as 200 in lakes in Minnesota (Striegl & Michmerhuizen 1998).

These studies did not account for the possibility of methanogenesis (MEG) simultaneously occurring in the aerated parts of the water-column, a process recently observed in other freshwater systems (Tang et al. 2014, Bogard et al. 2014),.and did not include analysis of the underlying community of microbes and methanotrophs. The following specific questions will be addressed:

Are rates of water-column MEG sufficient to affect apparent MOX rates among the lakes?

Are rates of water-column MOX among the lakes limited by nutrient availability?

What are the relative contributions of the main methane-producing and consuming organisms to the CH_4 dynamics and microbial communities among these study lakes?

We hypothesized that MEG was occurring in lakes as in other freshwater systems, including small, shallow and well-oxygenated lakes. We hypothesized that rates of MOX would be enhanced with the addition of nutrients based on known history of a P- and N-debt in lakes during the open-water period, and that rates of MOX would be higher in lakes with higher ambient CH_4 concentration. We predict that the differences in potential carbon bio-availability and substrate availability in Mackenzie Delta lakes would have an influence on net rates of MOX, and that rates of MOX will be enhanced in the presence of added nutrients. We hypothesized that the potential for aerobic lake-water MEG exists in this system, as has been observed in methane-rich lake systems in other parts of the world and that rates of MOX and MEG will increase in the presence of added nutrients. Therefore, our specific objective is to assess via laboratory experiments the effects of nutrient limitation and co-occurring MEG on water column MOX rates and the microbial composition and activities of CH_4-related functional groups among three lakes with differing carbon substrate quantity, quality, and nutrient regimes in the Mackenzie Delta.

3.2. Methods

3.2.1. Study design and sample collection

The potential for water-column MEG and the accompanying microbial community composition among Delta lakes was assessed by using small replicated enclosures of surface waters from three Delta lakes representing differing carbon quality end-points and annual river-to-lake connection times. Two lakes had short river-to-lake connection times, one with high macrophyte density (Lake 56) and one with substantial inputs of DOM from thermokarst (permafrost thaw along lake margins) activity (Lake 520), and the third lake had continuous connection to the Mackenzie River (Lake 129). Enclosures from each lake were variously treated along with controls to experimentally inhibit MEG and/or enhance MOX rates by nutrient amendments. The replication setup was designed for post-experiment analysis as a two-factor ANOVA to separate the main treatment-effects, main lake-effects, and possible interaction between the two factors. The experiment was conducted during early open-water (June 15-25, 2016).

Enclosures consisted of 12 sterilized 1L Nalgene PETG gas-tight media bottles that were sample rinsed then filled inside a sterile 25L bucket with various treatments applied to bulk lake surface water then sealed without head-space using Teflon-lined rubber septa stoppers and open ring caps. Initial conditions were determined for CH_4 concentration, community analysis, and initial biogeochemical conditions as described in Chapter 2. The bottles were left to incubate in the dark in a large cooler filled with filtered Mackenzie River water (tap water) for 48 hours with the temperature maintained at 17ºC by regularly flushing the cooler with tap water (See Appendix Image B-2). Following the incubation, treatment bottles were sub-sampled for CH_4 concentration by slowly filling sterile 157 mL serum vials with sample water, capping without head-space, then processed as described in Chapter 2.

3.2.2. Experimental Treatments

The four treatments consisted of the following:

Control or Ctrl: Treatment with no amendments added, to measure net changes in CH_4 cycling potential and community dynamics over the incubation period.

BES (an inhibitor of methanogenesis): Treatment designed to inhibit potential water-column methanogenesis through the addition of 2-bromo-ethanosulfane (BES). BES is a structural analogue of methyl coenzyme M, a key enzyme involved in MEG, and has been used to block methanogenesis in sediment slurries, microbial fuel cells and other anoxic environments where substantial methanogenesis occurs (Oremland et al., 1988). We added BES to a final concentration of 10 mM, a concentration known to block the majority of MEG without disrupting lake-water ecology and microbial processes (Oremland et al. 1988, Liu et al. 2011 and references therein). See Appendix A1 for supplementary information on BES.

Spike (a nutrient spike): A treatment designed to stimulate microbial community activity through addition of nutrients (ammonium (NH_4) and soluble reactive phosphorus (PO_4^{2-})) at 10 µM above background levels, where background levels of nutrients at this time of year are typically far below 1 µM, and more isolated lakes have higher bacterial production and competition for PO_4 (Spears, 2006).

Combo (a combination of above two treatments): A treatment with both a nutrient-spike and BES inhibitor to observe the nutrient-enhancement effect of methane cycling without the effect of methanogenesis.

3.2.3. Methane cycling analysis

MOX and MEG may occur simultaneously, and both processes can be affected by nutrient limitation. We also assumed that our BES treatments shut down MEG within their experimental enclosures. These processes and the effects of our experimental treatments on them can be separated by the following conceptual equations.

Assuming that uncertainty in analytical measurement of CH_4 within each enclosure (and the initial stock of lake-waters from which enclosures were filled) is negligible relative to changes in experimental replicates, all treatment responses were converted to rates of CH_4 depletion per unit time by subtracting initial CH_4 concentrations from the final concentrations in all the enclosures. The control treatment can be defined as:

(1) Control = moxL - megL

where moxL - megL is the net balance between MOX and MEG rates occurring simultaneously under nutrient limited (L) conditions and representing MEG as a negative rate. Therefore:

(2) BES = moxL

where moxL - megL is the net MOX rate under nutrient limited conditions. Therefore:

(3) Control - BES = megL

When nutrients are added (Spike), we assumed that raising ambient concentration by 10 µM above background for ammonia and phosphate would be sufficient to eliminate potential nutrient limitation following methods outlined by Spears et al. (2006). With BES added, MEG is shut down and with nutrients also added, methane oxidation should no longer be nutrient limited. Therefore:

(4) Combo - BES = moxE

where moxE is the nutrient enhancement (E) effect on MOX, which is additive to the MOX rate under nutrient limited conditions (i.e. moxL). When nutrients are added without BES, the treatment may stimulate both MOX and MEG simultaneously, and thus:

(5) Spike - Control = moxE - megE

where moxE - megE is the effect of adding nutrients on the net balance between MOX and MEG, which is additive to the net balance between these two processes under nutrient limited conditions (i.e. moxL - megL), and thus:

(6) [Spike - Control] - [Combo - BES] = megE

To then visualize the net results of adding nutrients, in addition to the direct nutrient addition effects:

(7) moxL + moxE = moxT

which is equivalent to the overall rate of MOX with nutrient enhancement included (also equivalent to the combo treatment), and:

(8) megL + megE = megT

where T = total, or the combined effect of both processes under nutrient limited and enhanced conditions.

(9) moxT - megT = net Mox

which is equivalent to the overall net balance of MOX and MEG with the nutrient effect included. Statistical uncertainties of the above quantities are based on their standard error estimate (SE). In cases corresponding directly to an experimental treatment the SE was taken from the single-factor ANOVA for each lake. In cases where one treatment mean was subtracted from another to derive a quantity of interest, the respective ANOVA SE's for each treatment mean was propagated (Taylot 1982) to obtain an overall uncertainty as:

(10) $SE_p = (SE_1^2 + SE_2^2)^{0.5}$

3.2.4. DNA extraction and quantification, and 16S rRNA/pmoA gene quantification

Lake water was filtered through sterilized 0.2 µm millipore filters, and DNA extracted using MoBio PowerSoil DNA extraction kit. DNA concentration was determined following protocol outlined in Chapter 2. To enumerate general microbial cell abundances and abundances of methanotrophs, quantitative PCR (qPCR) was performed following protocol outlined in Chapter 2.

3.2.5. 16S rRNA gene sequencing and sequence analysis

DNA extracts from samples were sequenced by the IMR lab at Dalhousie University in Quebec City, Quebec and sequencing data was processed and cleaned along with the full set of samples from Chapter 2 using the MiSeq SOP as previously described. Data was then examined in R-studio using the phyloseq, vegan and Rhea packages as per methods outlined in Chapter 2.

3.2.6. Statistical Analysis

To determine statistical significance of relationships among the microbial communities of differing lake types and treatments, OTU count data was analyzed using multivariate techniques as described in Chapter 2.

3.3. Results

3.3.1. In situ methane and conditions among the study lakes

Initial CH_4 concentrations in lake surface-waters increased with increasing river-to-lake connection times where isolated thermokarst (Lake 520) and macrophyte-dense (Lake 56) lakes had higher CH_4 substrate availability than lake 129 (Table 3.1). PO_4 was below the detection limit of ~0.05 µM in all lakes (results not shown).

3.3.2. Effects of methanogenesis and nutrients on MOX rates among lakes

The CH_4 results from our experimental enclosures, expressed as depletion rates (nmol L^{-1} d^{-1}), were analyzed for treatment-effects and lake-effects as a two-factor ANOVA. Both main effects were significant, including a substantially significant treatment-lake interaction effect. Re-analyzing the results for each lake as a single-factor ANOVA and assessing the treatment means with the Tukey HSD test showed that all possible pairs of treatment means within each lake significantly differed from each other, with one exception in each lake (Figure 3.2). In Lakes 129 and 520, the Spike (moxT+megT) versus BES (moxL) treatments did not differ significantly, whereas in Lake 56, the nutrient-spike versus Combination (moxT = moxL + moxE) did not differ significantly. Statistical uncertainty in the CH_4 changes in the experimental enclosures (ANOVA treatment SE's of L129 = 7.52, L56 = 33.6, L520 = 77.6 nmols L^{-1} d^{-1}) were substantially larger than the analytical SE of duplicate aliquots from a given enclosure (average from all enclosures = 2.88 nmols L^{-1}) and justified the ANOVA treatment design based on CH_4 depletion rates where initial CH_4 concentrations were subtracted from final concentrations in all enclosures (Table 3.2). See Appendix C1.1-1.3 for detailed statistics tables. A key result is that BES (moxL only) versus Control (moxL + megL) treatments significantly differed in all three lakes, indicating that MEG was occurring in

their water columns, and potentially masking the rate of moxL occurring in situ in these lakes.

Further interpreting these results by partitioning the co-occurring methane oxidation (MOX) and methanogenesis (MEG) rates under nutrient limited (equated to in situ lake conditions) versus nutrient enhanced conditions according to equations 1 to 9 yields Fig. 3.3 (Table 3.3). SE bars in the Fig. were derived from the single-factor ANOVA's for each lake, or from propagating the ANOVA SE's (equation 10) in cases where one treatment mean was subtracted from another to derive a quantity of interest. The combination of both direct experimental (Fig. 3.2) and derived results (Fig. 3.3) indicate that water column MEG was occurring concomitantly with MOX, and MOX increased with added nutrients. By contrast, MEG may have increased with added nutrients in Lake 520 (not statistically significant, but could be with more statistical power), but appeared to significantly inhibit MEG in Lakes 56 and 129. Lake-water CH_4 declined by 25 to 33 % per day among the three lakes (Table 3.2), relative to initial CH_4 concentration, when MEG was experimentally inhibited, and nutrient levels were enhanced (Combination treatment). Under nutrient limited conditions, MOX rates declined by 17 to 20% per day with MEG shut down (BES treatment), and CH_4 declined only 1 to 14% per day when MOX and MEG were simultaneously occurring (Control treatment).

3.3.3. Microbial community dynamics among treatments and lake types

The abundance of microbial communities within each of the three lakes generally increased in the treatments where nutrients were added or methanogenesis was inhibited based on quantification of the 16S rRNA gene from lake water DNA extracts of the experimental treatments (Figure 3.4). Microbial abundance increased relative to initial microbial abundance by a factor of 18 to 43-fold when methanogenesis was experimentally inhibited and nutrient levels were enhanced (combination treatment) and 13 to 23-fold among the lakes with methanogenesis shut down but nutrient limitation present (BES treatment) and only 2 to 7-fold when MOX and MEG were simultaneously occurring with nutrient limitation present (control) but increased 3 to 22 fold when nutrient levels were enhanced (gross MoxE).

The abundance of methanotrophs also increased where either nutrients were added or MEG was inhibited, based on quantification of the *pmoA* gene (Figure 3.4). When methanogenesis was experimentally inhibited and nutrient levels were enhanced (i.e. Net MoxE), there were substantial increases in methanotroph abundance in lakes 56, 520, and 129 by 180 ± 12 to 1092 ± 30 to 1465 ± 24 % respectively. By comparison, methanotroph abundance with MEG shut down and nutrient limitation present (BES treatment) increased 245 ± 13 to 610 ± 29% in lakes 520 and 56 and by 1488 ± 31% in lake 129. Methanotroph abundance increased 20 ± 10 to 177 ± 28 to 514 ± 33 % in lakes 520, 56 and 129 respectively when MOX and methanogenesis were simultaneously occurring with nutrient limitation present (Control treatment), but by 514 ± 33% in lake 129. When nutrient levels were enhanced methanotroph abundance increased 64 ±18, 190 ± 39 and 1200 ± 29% in lakes 520, 56 and 129. The increases in methanotroph abundance were similar in lake 129, although not as pronounced as in lakes 56 and 520 and there was no statistically significant correlation based on lake type or treatment.

Major shifts in bacterial community composition and dominant methanotroph groups were influenced by lake type rather than by treatment type. NMDS and PCA of OTU abundance data and biogeochemical data showed grouping of samples by lake type, but no significant correlation based solely on treatment type (results not shown). Overall bacterial community composition showed no obvious pattern of change over the course of the 48-hour incubation. Dominant phyla in the 3-lake set were *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, and *Verrucomicrobia* as well as a small portion of low-abundance phyla grouped together as 'other' (Figure 3.5). Archaea were only detected in three of the experimental samples (L520 / L56 BES and L56 spike) where a total of 2 Archaea OTU's were present.

Methylotrophs were the most dominant CH_4-related taxa, where *Methylophilaceae* genus *Candidatus* methylopumilus (OTU 10) and an unclassified *Methylophilaceae* (OTU 17) were present in all lakes and treatments. These OTU's had a significant correlation with CH_4, of + 0.76 and - 0.73 respectively based on the Pearson correlation coefficient (Figure 3.6; Table 3.5). *Methylophilaceae PRD01a011B* (OTU 41) was not found to have a significant correlation with CH_4 concentration or oxidation rate but comprised a substantial proportion of the overall community for lakes and was present in all samples. Sequence data for OTU 10 was identical to cultured *Candidatus*

methylopumilus sequences from various freshwater and saline systems, whereas OTU 17 matched a cultured isolate of *Methylotenera* from soil in a Japanese rice field based (Table 3.5; Figure 3.6). The relative abundance of all methano- and methylotrophs ranged from 0.3 - 0.8% of the total population for all treatments in lake 129, 0.3 - 1.4 % in lake 520, and less than 0.2% of the total population of lake 56 (Fig. 3.5). Relative abundance of all known methanotrophs ranged from ~1 to 4.8% of the total population with the highest average proportion in lakes 56 and 520 (Fig 3.6). Type I methanotroph OTU 43 was the only group detected from the 16S rRNA data and present at very low relative abundance in lakes 129 and 56 only. *Verrucomicrobia* MOB *Candidatus* methylacidphilum (OTU 82, 492) was present in lakes 129 and 56, but not detected in lake 520. Macrophyte-rich lake 56 had the highest relative abundance of *Candidatus* methylopumilus. Other low-abundance MOB were detected, with Type I *methanotroph Methylobacter* found in lakes 56 and 129 and *Verrucomicrobia Candidatus* methylacidiphilum taxa (OTU 82, 492) and *Methylococcales* CABC2E06_ge (OTU 154) were found in all lakes. *Verrucomicrobia* genus *Opitutae vadin*_HA64_GE (OTU 25), a taxon not currently known to be involved in methane cycling, was present in all lakes and had significant inverse correlation with CH_4 concentration.

3.4. Discussion

3.4.1. Nutrient effect and rates of MOX and MEG among Delta Lakes

Our results demonstrate the potential for MOB and MGA to be affected by competition for a limited supply of nutrients and that both MOX and MEG are influenced by differences in lake biogeochemical properties including CH_4 concentration and nutrient effects. CH_4 oxidation rate is generally accelerated in lakes with higher CH_4 substrate availability in the Delta (Cunada et al. 2018) as in other systems (Veraart et al. 2015), which corresponds with the differences in CH_4 substrate availability and rates of MOX among our lakes (Table 3.2). Lake type and nutrient availability had a strong influence on rates of MOX, MGA and the abundance of general bacteria and MOB (Figure 3.4). Rates of MOX were generally enhanced by nutrient enrichment, although this effect was more pronounced in the lakes with shorter river-to-lake connection times as predicted for lakes 56 and 520 (Table 3.2, Figure 3.3). Bacterial abundance and production are known to increase with increasing lake isolation and when lakes are

amended with nutrients (Spears et al. 2006), which indicates the potential importance of competitive effects among members of lake-water microbial communities for nutrients, and the likelihood that MOX rates are limited by competitive effects with other freshwater taxa. The two isolated lakes had higher rates of gross and net MOX (Figure 3.2) but both responded differently to the nutrient spike treatment, where rates of MOX were highest in thermokarst-affected lake 520 as predicted, but rates of MEG was significantly higher in Lake 520 than the other lakes (Figure 3.3) Rates of MEG were actually lower in lakes 129 and 56 in the presence of nutrients (Table 3.3) which was surprising as we expected that MEG would generally be enhanced with added nutrients and that MEG may be more evident in the continually connected, lower MOX lake 129 as part of particle-attached communities from river-water or suspended sediment.

Very few studies to date have measured for aerobic MEG in the water column of freshwater lakes, and to our knowledge none have observed or accounted for this process in studies of other Arctic and sub-arctic lake systems. To our knowledge, this study was the first to detect MEG in lakes in the Mackenzie River Delta and corroborates findings of aerobic MEG with similar rates as those seen in small and shallow lakes (Bogard et al. 2014) and other temperate (Tang et al., 2014, Grossart et al., 2011) and tropical freshwater systems (Roland et al., 2018; Table 3.6). Aerobic MEG contributed approximately 4 % of the total greenhouse gas footprint of Lac Cromwell (Bogard et al. 2014), but the exact contribution of aerobic MEG to CH_4 flux in the Delta and other systems and the factors that influence rates of aerobic MEG require further investigation.

3.4.2. Microbial Community Composition and prominent MOB and MGA

Methylotrophs appear to comprise the majority of MOB and have significant correlation to lake-water CH_4 concentration independent of lake or treatment type. Various low-abundance MOB and methylotroph taxa were also found, but only *Methylophilaceae* (OTU's 10 ,17 and 41) were found in substantial relative abundance and with significant correlation with CH_4 (Table 3.5). These groups were prominent in a set of Arctic and sub-Arctic Tundra lakes in Alaska (He et al., 2012) and in temperate Lake Zurich, where *Candidatus* methylopumilus was also detected (Salcher, 2015), and their presence could indicate the potential for complex community behaviours such as

cross-feeding, a process whereby CH_4 is processed by extracellular digestion of methanol (Krause et al., 2017).

Substantial populations of *Verrucomicrobia* are present in many lake systems, and their relative abundance correlates with various seasonal and carbon-cycling processes. The significant correlation between *Verrucomicrobia* genus *Opitutae vadin* HA64_GE (OTU 25) relative abundance and CH_4 concentration in this study, as well as the prominence of other *Verrucomicrobia* taxa highlight their potential importance in this system, as in others, to carbon-cycling processes in Delta lakes.

Future work to determine the community structure and activity of Archaea both in the sediment and water column would give us further insight into the role of methanogens in Delta lake carbon cycling. Particle-attached bacteria, a substantial component of Delta productivity (Tank, 2011), or other potential anaerobic niches within the water-column could be locations for MEG to occur in the aerobic water column. Methanogenic Archaea such as *Methanosaeta* require specialized methods of detection (Carr et al. 2018, Plasencia et al. 2013) but could have an important effect on net rates of MOX in the delta. Future measurements of CH_4 cycling in other ecosystems in the Arctic should consider the potential influence of water-column MEG on rates of MOX and CH_4 flux, and utilize specialized means of detection and data processing to detect low-abundance Archaea that may be obscured by more abundant taxa with 16S rRNA-gene analysis.

It is possible that delta lake MOB are not necessarily getting sufficient nutrients to meet their needs throughout the open-water period and also have to compete with a much larger community of other microbes, including some methanogens within the aerated water column, for a limited share of the available nutrients. The overall community of other nutrient limited microbes may also help explain the potential reduction in the rates of methanogenesis that were inferred from the nutrient addition treatment for Lakes 129 and 56 in Figure 3.3. Nutrient effects on elevated rates of MOX were more pronounced in lakes with higher CH_4 substrate availability, which corresponds with the more isolated lakes that have a stronger influence of macrophtye-derived or permafrost carbon, and rates of MOX in each lake were significantly impacted by most of the various treatments that were applied.

3.5. Conclusion

To our knowledge, this study was the first to incorporate measurements of 16S rRNA community data, biogeochemical measurements, and correlation analysis of these variables with the combined effects of rates of water-column MOX and MEG in Arctic lakes. Our experimental results demonstrate that MEG occurs within aerobic Mackenzie Delta lake waters, that this process is affected by competition for nutrients, and that lake properties such as carbon bio-availability and CH_4-substrate could also potentially influence rates of MEG. Future experiments of Delta lake-water MEG should expand the testing to other lakes and time-points and target functional genes specific to MGA to determine dynamics of aerobic MEG that were beyond the scope of this study.

Our results show that aerobic water-column methanogenesis is likely occurring at different rates throughout the Delta, but more work should be done to understand the implications of future climate warming and community dynamics on the activity of MGA, and how much this process contributes to MOX rate sin the Delta and the global CH_4 budget. Communities of MOB were dominated by *Methylophilaceae* taxa, but many low-abundance MOB were also present and the potential for MOB which are difficult to detect, such as *Crenothrix*, or groups not yet identified as MOB, such as taxa from the prominent *Verrucomicrobia* phyla, could also affect Delta CH_4 dynamics. Rates of MOX were highest in lakes that are rich in DOM from macrophytes and permafrost, but our results suggest that MOX is limited by competition with more abundant freshwater taxa during the open-water period. Although lake-water MEG was detected, all lakes were net methanotrophic whether nutrient limited or not. Variance in MEG rates based on lake properties could influence total rates of MOX and explain some of the variation in rates of MOX found in a previous study of Delta lakes.

3.6. Tables and Figures

Table 3.1 Summary of genetic information for samples from pre- and post-incubation for 3-lake set. Shannon diversity measurements of normalized OTU data.

Lake	Treatment Type	DNA Lake (ng / mL)	Sample Volume (mL)	Raw Reads	Quality reads (# fastq '+')	% removed	Rarified OTU's	# OTU's	Shannon Diversity
129	Initial	2.35	30	50536	25404	-0.5%	328	280	3.59
129	BES	3.43	30	4897	1859	24.1%	122	119	3.24
129	Control	2.43	45	29384	12770	13.1%	268	250	3.61
129	Combo	8.95	40	44083	17409	21.0%	412	340	3.39
129	Spike	1.28	30	4976	1972	20.7%	137	127	3.24
56	BES	3.63	30	39618	15874	19.9%	434	357	3.70
56	Control	2.08	30	12077	4838	19.9%	215	193	3.82
56	Initial	0.85	40	13615	5850	14.1%	536	409	3.80
56	Combo	5.92	29	37695	16255	13.8%	399	347	3.94
56	Spike	3.60	30	36860	14570	20.9%	217	198	3.73
520	BES	4.50	30	36476	14906	18.3%	407	313	3.39
520	Combo	4.34	28	11064	4361	21.2%	176	156	3.26
520	Spike	1.40	30	4867	2063	15.2%	127	121	3.61
520	Initial	1.07	30	11429	4592	19.6%	172	153	3.58
520	Control	1.21	30	25662	9124	28.9%	216	184	3.28

Table 3.2 **Average rates of methane depletion in response to co-occurring methane oxidation (MOX) and methanogenesis (MEG) in three Mackenzie Delta lakes of varying annual river-to-lake connection times and biogeochemical properties. Rates are based on experimental incubation of triplicate enclosures from each lake variously treated along with controls (co-occurring MOX and MEG) to experimentally inhibit MEG (Inhibitor) and/or enhance MOX and MEG rates by nutrient amendments (Spike)**

Parameter	Initial	Control	Spike	BES	Combo
Lake 129:					
CH_4 concentration (nmol / L)	656				
CH_4 depletion (nmol / L / d)					
Replicate 1		21.9	115	107	140
Replicate 2		24.5	129	119	164
Replicate 3		20.3	123	116	189
Average rate (nmol / L / d)		22.2	122	114	164
Average % depletion		3.4	18.6	17.4	25.1
pmoA:16SrRNA (%)	60.2	2.3	1.4	1.8	0.8
Lake 56:					
CH_4 (nmol / L)	2672				
CH_4 depletion (nmol / L / d)					
Replicate 1		34.8	799	542	736
Replicate 2		-3.99	660	380	741
Replicate 3		60	693	429	772
Average rate (nmol / L /d)		30.3	717	450	750
Average % depletion		1.1	26.8	16.9	28.1
pmoA:16SrRNA (%)	1.4	2.3	1.3	0.8	0.8
Lake 520:					
CH_4 (nmol / L)	6398				
CH_4 depletion (nmol / L / d)					
Replicate 1		1022	1514	1100	2112
Replicate 2		844	1511	1566	2100
Replicate 3		829	1562	1222	2174
Average rate (nmol / L /d)		899	1529	1296	2128
Average % depletion		14	23.9	20.3	33.3
pmoA:16SrRNA (%)	20	2	11	4	2

Table 3.3 Rates of gross and net MOX (methane oxidation), and MEG (methanogenesis), during experimental enrichment of lake-water (derived from equations 1 to 9) from three lakes in the Mackenzie River Delta. Values in parentheses = standard error (SE) of ANOVA treatments, or equation 10 when a parameter is based on a difference between two values.

Parameter	Nutrient Limited	Nutrient Enhanced	Nutrient Effect
Lake 129 (continuous river-to-lake connection - strong influence of riverine carbon):			
ANOVA treatments SE = 7.52			
Gross MOX (nmol / L / d)	114 ± 7.5	164 ± 7.52	50.4 ± 10.6
MEG (nmol / L / d)	-91.8 ± 10.6	-42.2 ± 10.6	49.6 ± 15.0
Net MOX (nmol / L / d)	22.2 ± 7.5	122 ± 7.5	100 ± 10.6
Lake 56 (brief river-to-lake connection time - dense macrophytes and autochthonous carbon):			
ANOVA treatments SE = 33.6			
Gross MOX (nmol / L / d)	450 ± 33.6	750 ± 33.6	299 ± 47.5
MEG (nmol / L / d)	-420 ± 47.5	-32.6 ± 47.5	387 ± 67.2
Net MOX (nmol / L / d)	30.3 ± 33.6	717 ± 33.6	687 ± 47.5
Lake 520 (brief river-to-lake connection time - both macrophytic and thermokarst carbon):			
ANOVA treatments SE = 77.6			
Gross MOX (nmol / L / d)	1296 ± 77.6	2128 ± 77.6	832 ± 110
MEG (nmol / L / d)	-397 ± 110	-599 ± 110	-202 ± 155
Net MOX (nmol / L / d)	899 ± 77.6	1528 ± 77.6	630 ± 110

Table 3.4 Cell abundances for general microbial communities and methanotrophs in various freshwater environments including this study.

Cell counts for other systems were determined by direct counts (epifluorescence microscopy utilizing 4'6-diamidino-2-phynylindole (DAPI) or Acridine Orange stain), or by quantitative techniques (catalyzed reporter deposition fluorescence in-situ hybridization (CARD-FISH) or quantitative PCR (qPCR), as in present study) to identify general bacterial abundance from the 16S-rRNA gene, or utilizing the pmoA or other genes specific to methane oxidizing bacteria (MOB).
* = Type I MOB ** = Type II MOB *** = abundance from pmoA, mcrA and mmoX genes.

System	Methods	Specific location	Bacterial abundance cells mL $^{-1}$	Methanotroph Abundance cells mL $^{-1}$	Reference
Mackenzie Delta East Channel (Experimental set)	qPCR of 16S rRNA gene and pmoA gene	Lake # 129 56 520	8.9 (±4.2) x 10^4 3.5 (±0.9) x 10^4 3.6 (±0.5) x 10^3	1.6 x 10^3 ± 200 0.2 x 10^3 ± 44 0.4 x 10^3 ± 4	This study
Mackenzie Delta East Channel (Experimental set; 2006)	DAPI	Lake # 129 56 520	4.5-5 x 10^6 5.7-6x 10^6 --	-- -- --	Tank et al. (2009)
"	"	Lake # 129 56 520	9 (±3) x 10^6 9.2 (±2.5) x 10^6 6.6 (±3.3) x 10^6	--	Chateauvert et al (2012)
Lago di Coadagno	DAPI	Varying depths	--	0.8-6 x 10^3 *	Oswald et al., (2015)
Set of Five Swedish Temperate lakes	Flow cytometry	Surface and Bottom-water	1-10 x 10^6	--	Samad and Bertilsson (2017)
Toolik Lake (Oligotrophic arctic lake)	Acridine Orange	Surface and Bottom-water	0.8-3 x 10^5	--	Adams et al. (2015)
Toolik Lake Inlet/outlet streams	DAPI	Surface water	0.4 - 2.9 x 10^6	--	Hobbie et al. (1983)
Kuparuk river (Near Toolik lake)	DAPI	Surface water	0.3-2.7 x 10^6	--	Hobbie et al. (1983)
Lago di Cadagno (Anoxic waters)	CARD-FISH analysis (pmoA and mcrA gene)	Anoxic waters	1 x 10^7	2.4 x 10^5 ***	Milucka et al. (2015)
Mono Lake (Large, shallow, saline lake)	DAPI / FISH	Surface and bottom-water	2.1-2.5 x 10^7	5 - 9 x 10^5 * 1.5 - 3.5 x 10^5 **	Carini et al (2005)

Table 3.5 BLAST results of taxa with significant correlations to methane concentration and methane oxidation rates during a nutrient enrichment experiment of a set of 3 Mackenzie Delta lakes with varying CH_4 substrate availability, carbon quality and carbon quantity. OTU 16 was the only group to have a significant correlation with methane oxidation rate (indicated with a ++).

UC = uncultured, FW = freshwater, * = 99% match, ** = 98% match, *** = 97% match, **** = 96% match. Taxa without * are 100% matches to the OTU.

SILVA Taxonomy **Phylum** Class *Genus*	OTU #	CH_4 Correlation	Cultured Genus (BLAST)	Location	Accession #'s
Actinobacteria					
Actinobacteria					
*Candidatus*_Planktophila	3	0.72	*Candidatus* Planktophila *Var. Actinobacterium*	FW Lakes (Zurich, Soyang) FW Pond (Japan)	LC106292.1, CP015603.1
hgcI_clade	8	0.66	*Candidatus* Nanopilagicus, *Rhodococcus*	FW Lake (Zurich)	CP016768.2, CP016779.1
Sporichthyaceae(UC)++	12	-0.51	*Candidatus* Planktophila lactus	FW Lake (Zurich, Soyang, Damariscotta)	CP016783.1, CP016780.1
Unclassified Microbacteriaceae	13	0.60	*Rhodoluna spp*	FW Lakes (Egelsee, Mondsee), Ichino River, East China Sea	KU173555.1, LC094690.1
Unclassified Microbacteriaceae	18	0.71	*Candidatus* Limnoluna rubra, Aquiluna rubra*	FW Lake (Lake Grossegelsee), Ichino River*, Stream (China)	NR_125497.1
Sphingobacteria					
Sediminibacterium	7	0.80	*Bacteroidetes / Sediminibacterium**	FW Lake, (Damariscotta Lake) (Sediment* + Fishing port)	JF488143.1, HQ663413.1
Dinghuibacter	23	-0.81	*Chitinophagaceae***, *Terimonas***, *Cnuella***	Grassland***, Desert soil***	LN876538.1, KT350459.1

SILVA Taxonomy

Class / *Genus*	OTU #	CH_4 Correlation	Cultured Genus (BLAST)	Location	Accession #'s
Phylum					
Dinghuibacter	24	0.69	*Bacteria***, *Flavitalea****, *Niabella Terrae****,	Paddy soil**, agricultural soil***, Aquatic plant root***, soil*** (S. Korea)	LC106253.1**, KX762320.1***
Chitinophagaceae(unclassified)	85	-0.79	*Ferruginibacter yonginensis**, *Chitinophaga sp.**, *Flavitalea**	FW Lake (Damariscotta*, Iron-Seep*, Artificial (China)*	JF488165.1*, AM988943.1*
Cytophagia					
Pseudarcicella	11	0.74	*Arciella sp. Bacterium, Pseudarcicella**	FW Lake (Artificial, Maarsseveen), FW (Korea)*	JN408290.2, AJ964899.1
Flavobacteriia					
Fluviicola	16	0.52	*Arciella sp., Bacterium*****, *Pseudarcicella*****, *Flavobacteria*	FW Lake (Mendota), Porphyra	HQ663127.1
Fluviicola++	16	0.68	-	-	-
Proteobacteria					
Alphaproteobacteria					
LD12_freshwater_group_ge	6	0.84	*Candidatus* Fonsibacter / Pelagibacter	FW Lake (Damariscotta Lake)	CP024034.1, KY290650.1
Caulobacter(OTU84)	84	-0.95	*Caulobacter profundus***, *sp. WW137****,	FW Sediment (Korea), FW Lake (Kasumigaura)	
Betaproteobacteria					
Comamonadaceae	1	-0.73	*Acidovorax*	FW Lake (Lugano)	MF077115.1, MH046753
Comamonadaceae	9	-0.54	*Leptothrix*	Wetlands (2)	JQ946028.1, JQ946023.1,
Methylophilaceae	10	0.76	*Candidatus* methylopumilus	Seawater, (East China Sea) Freshwater	KU173564.1 , LN827929.1

SILVA Taxonomy

Phylum Class *Genus*	OTU #	CH_4 Correlation	Cultured Genus (BLAST)	Location	Accession #'s
Methylophilaceae	17	-0.73	*Methylophilus (quayli) / Methylotenera*	Soil (Japan; rice field), Russia (unspecified)	LC368136.1, AY772089.2
Polynucleobacter	31	0.84	*Polynucleobacter cosmipolitanus*	FW Lake (Mondsee, Bled, Biwa)	AJ550652.2, AJ550649.2
Verrucomicrobia Opitutae					
Opitutae_vadin HA64_ge	25	-0.86	*Verrumicrobia*	FW Lake Mendota	HQ663236.1, HQ663179.1

Table 3.6 Comparison of rates of aerobic methanogenesis (MEG) in various freshwater lakes. MEG = methanogenesis in nmol L^{-1} d^{-1} in the oxygenated part of the water-column of various other freshwater systems.

Lake	Trophic level	Timeframe	Lake Properties	MEG nmol L^{-1} d^{-1}	Source
Mackenzie Delta Lakes	Eutrophic	2016	Small, Shallow, varying carbon bio-availability	383 ± 267	Present Study
Lake Stechlin	Oligotrophic	2010-2014	Large, deep	906 ± 0.1	Tang et al 2014, Grossart et al., 2011
Lac Cromwell	Oligomeso-trophic	2012	Small, shallow	230 ± 0.01	Bogard et al 2014
Lake Kivu	Tropical	2011-2014	Very large (great lake)	370	Roland et al. 2018

Table 3.7 Comparison of rates of MEG in various systems and whether various other measurements related to Archaea, water-column methanogenesis, or community and abundance data for MOB was determined.

Study system	Water-column MEG (Aerobic)	Detection of Archaea?	Detection of MOB?	Dominance of Methylotrophs?	Methods	Lake Properties	Reference
Mackenzie Delta lakes (2016)	Yes	n/a	Yes	Yes	16S rRNA gene	Arctic, organic-rich permafrost lakes	Present study
Lake Killarney, Lake Qalluuraq	n/a	No	Yes	Yes	16S rRNA gene	Tundra and Subarctic Taiga Lakes (2011 - 2014)	He et al 2012 Tang et al 2014
Lake Stechlin	Yes	Yes	No	No	CARD-FISH	Oligotrophic (2010 - 2014)	Grossart et al. 2011
Lac Cromwell	Yes	No	No	No	Rates only	Oligomesotrophic (2012)	Bogard et al 2014
Lake Kivu	Yes	No	No	No	Rates only	Tropical (2011 - 2014)	Roland et al. 2018

Figure 3.1 **Map of Mackenzie Delta lakes, full sub-set, and highlighted subset of 3 lakes used for this chapter are highlighted in yellow (Lakes 129, 56 and 520).**

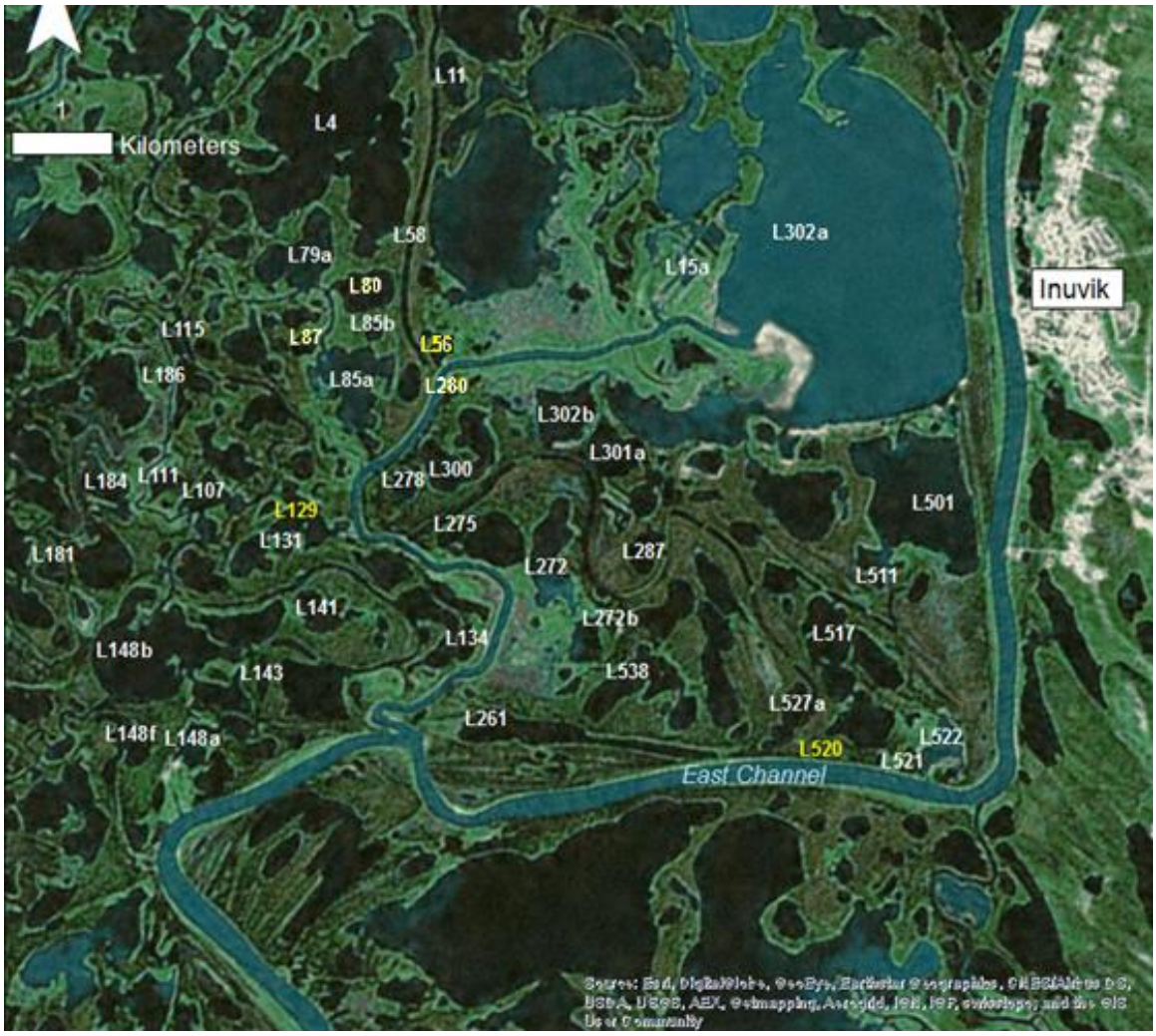


Figure 3.2 Rates of CH_4 depletion as nmols L^{-1} d^{-1} from experimental manipulation of Mackenzie Delta lake-waters.

nd = no statistically significant difference between indicated treatments.

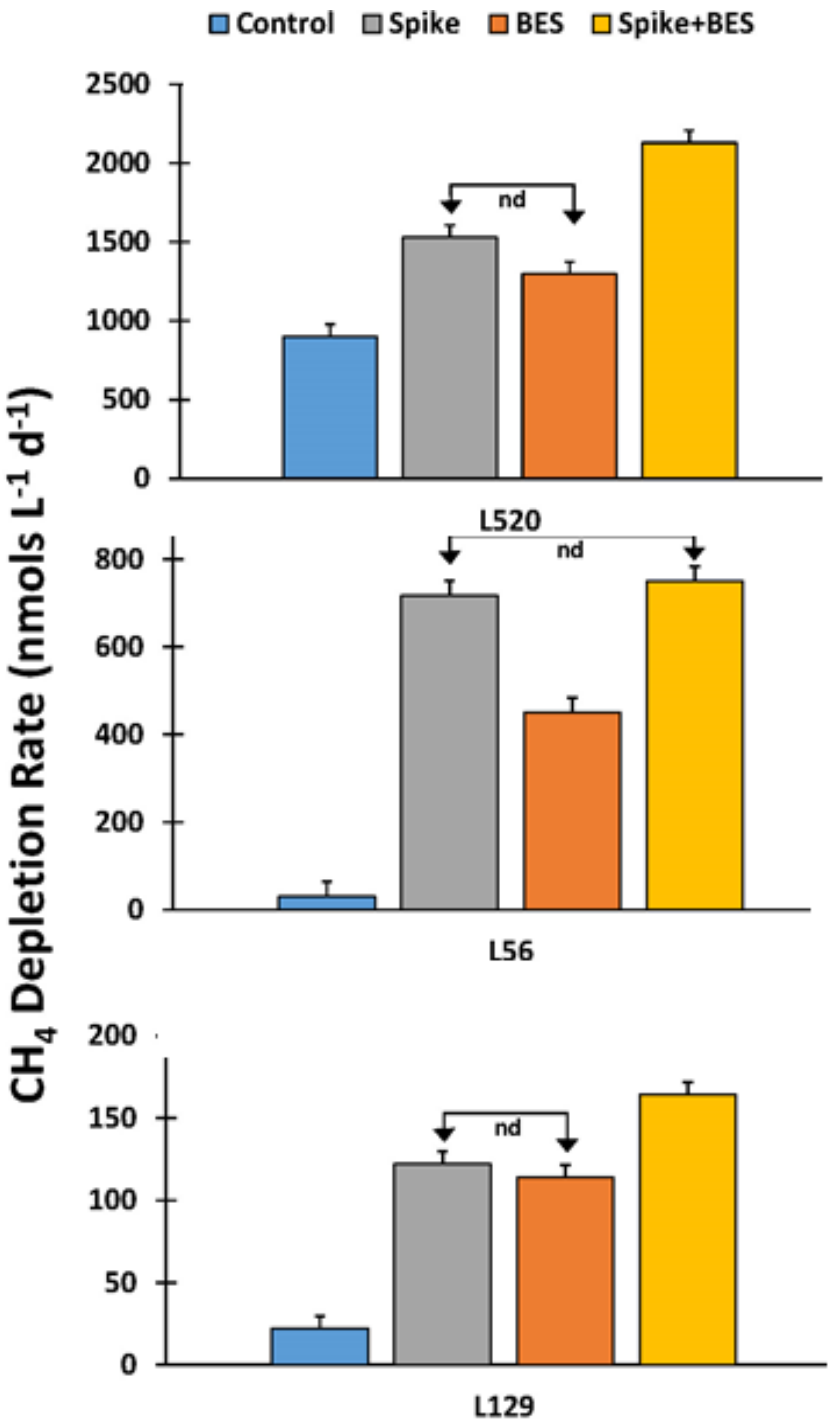


Figure 3.3 **Methane oxidation (MOX) and methanogenesis (MEG) rates from nutrient enrichment experiment. MOX - MEG = net methane oxidation after removing MEG rate. Rates as nmols L^{-1} d^{-1}. Positive values correspond to amount of CH_4 consumed per litre per day, while negative values correspond to amount of CH_4 produced**

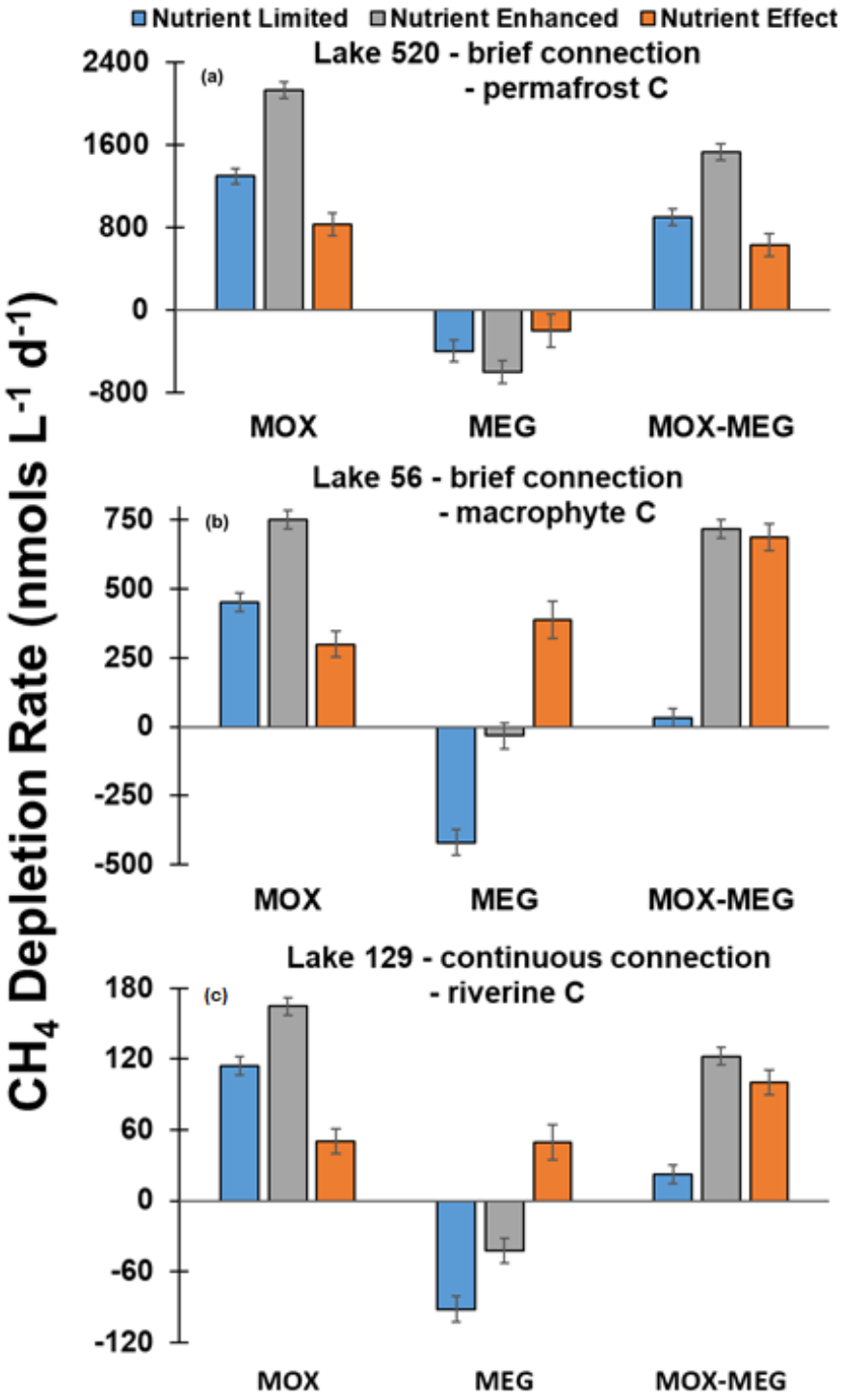


Figure 3.4 **Abundance of methanotrophs and methanogens lake water from set of Delta lakes with varying biogeochemistry and carbon-quality endpoints. Microbial community abundance and methanotroph abundance were determined from abundance of the 16S rRNA gene and *pmoA* gene mL^{-1} of lake-water.**

Treatments: Ctrl = no-treatment control of unamended lake surface-water; N+P = nutrient spike; Inhib = methanogenesis inhibitor.

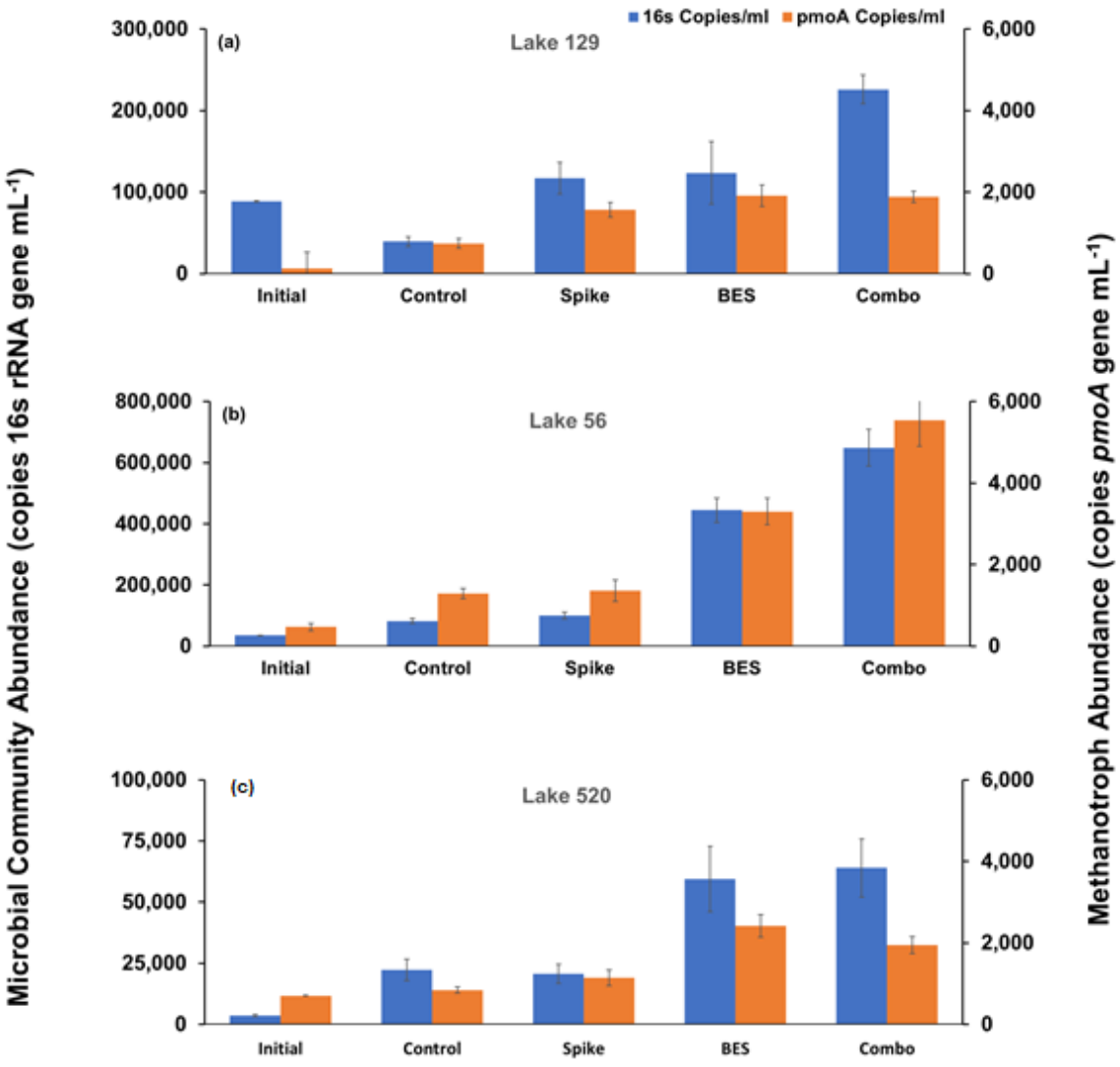


Figure 3.5 Bar plot of dominant phyla in the set of Mackenzie Delta lake surface waters from lakes of varying carbon quantity and quality and amended with various treatments over a 48-hour incubation period. Phyla determined from 16S-rRNA gene extract data. Phyla with less than 1% of overall abundance were grouped together into the "Other" category.

Treatments: Control = raw lake surface-water; Spike = nutrient addition; BES = methanogenesis inhibitor; Combo = nutrients + BES.

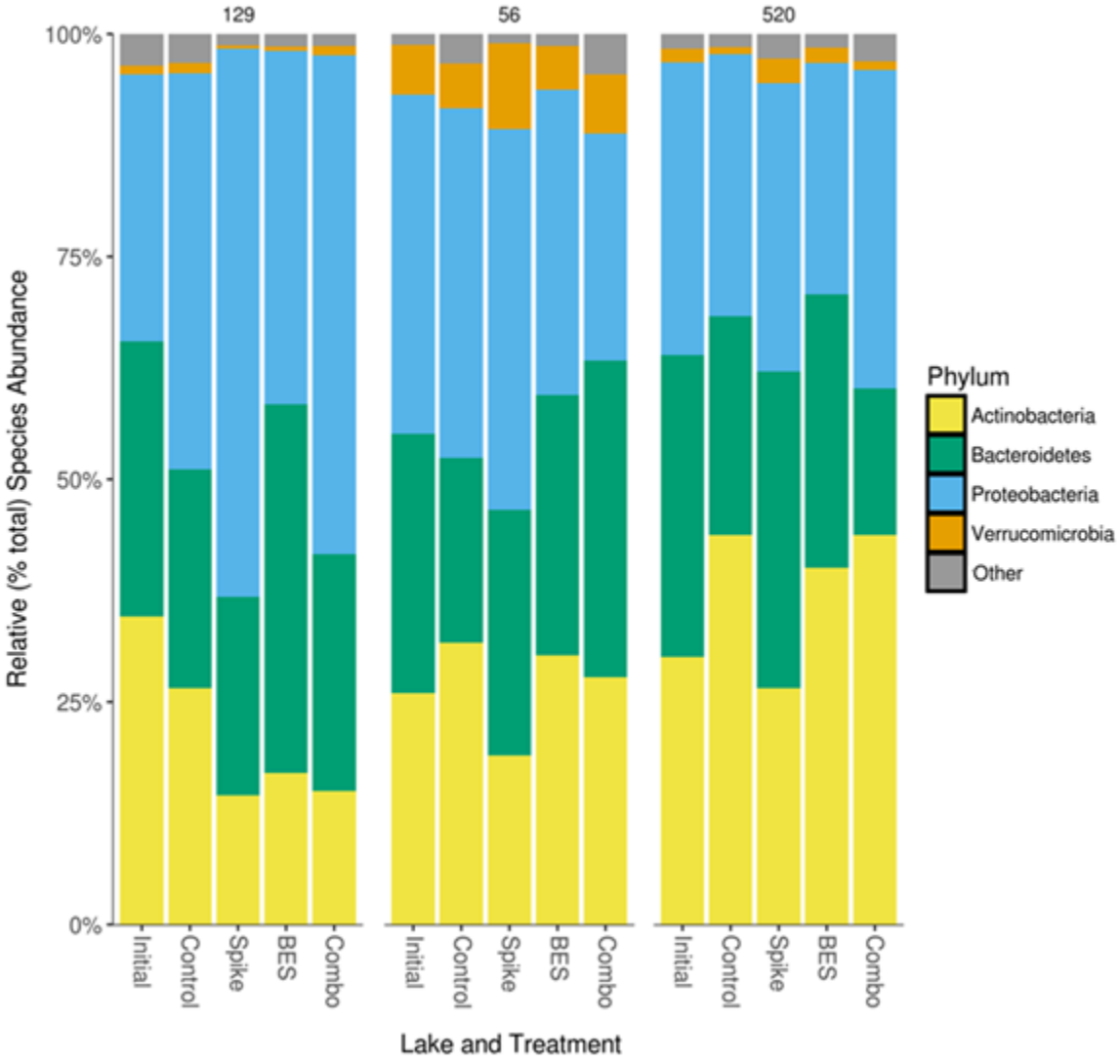


Figure 3.6 Relative abundance of major methane-linked groups based on 16S rRNA-gene analysis of Mackenzie Delta experimental lake-set surface water, amended with various treatments to observe the effects of nutrient enrichment on rates of methane oxidation and methanogenesis.

Treatments: Control = raw lake surface-water; Spike = nutrient addition; BES = methanogenesis inhibitor; Combo = nutrients + BES.

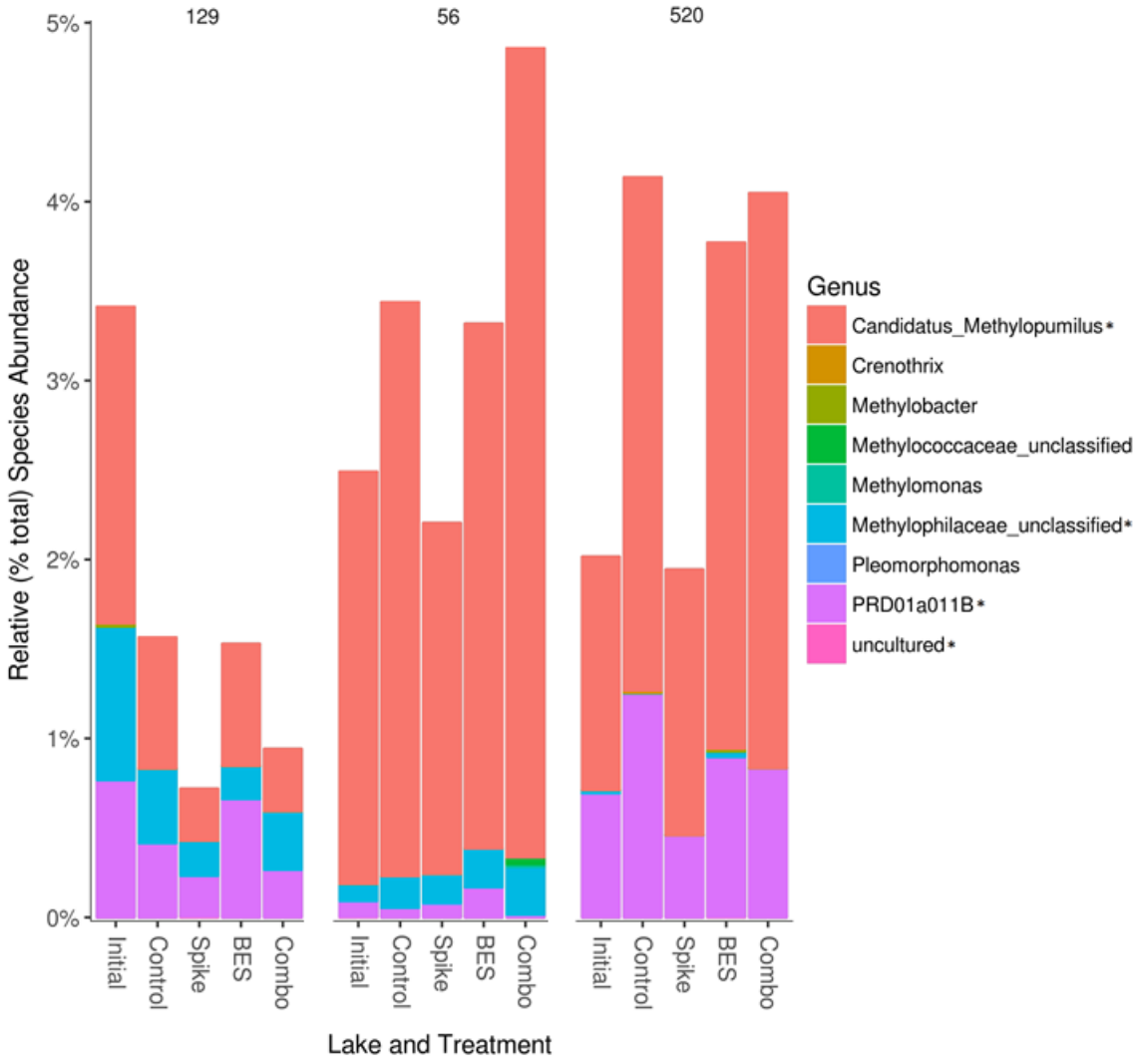
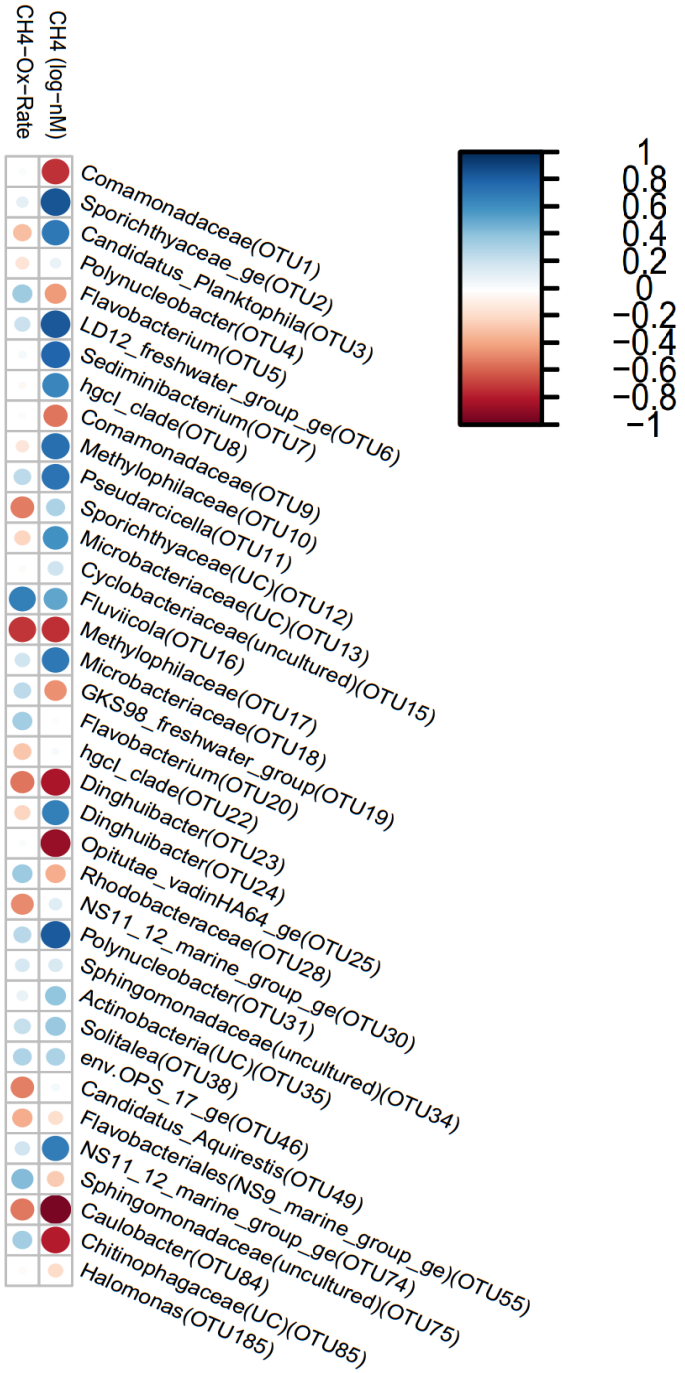


Figure 3.7 **Correlation plot showing correlation between taxa from an incubation/enrichment experiment of Mackenzie Delta lake water and the water-column methane concentration and oxidation rate. Coloured circles correspond to positive (blue) or negative (red) correlation (Pearson correlation coefficient) among measured variables and normalized OTU abundance data from all samples. OTU classification based on SILVA and BLAST taxonomy data.**



Chapter 4.

General Summary and Significance of Results

The main objectives of this study were to identify the major taxa present in Mackenzie Delta lakes and determine the influence of lake biogeochemistry and seasonal effects on the composition of microbial communities and carbon cycling in the water column. Our research was designed to address the following questions:

Q1) What is the structure of microbial communities, and the relative abundance of methanogens and MOB in Delta lakes during winter with prevailing ice-cover and anoxic conditions in the water column?

Q2) How do these abundances and compositions change following ice break-up and through the open-water period?

Q3) What major environmental variables most strongly correlate with methane-linked organisms, and which organisms most strongly correlate with CH_4 and MOX among the Delta lakes?

For Q1 and Q2, we expected that Delta lakes would likely be comprised of globally dispersed freshwater taxa with composition similar to other permafrost- and lake-rich Arctic and sub-arctic ecosystems, with a high proportion of MOB due to the high substrate availability and high potentially bio-available carbon sources. Our results matched these expectations, although more could be done to look at under-ice communities and type II MOB that likely dominate the sediment.

For Q3, we hypothesized that MOB would make up a substantial proportion of the microbial community based on previous observations of substantial CH_4 availability and known MOX in the water column of Delta lakes, particularly in the spring when nutrients, oxygen and CH_4 substrate are readily available. We hypothesized that microbial community composition would change most from winter to spring and to a lesser extent through the open-water period following major seasonal shifts in lake biogeochemistry, but that the structure of microbial communities would be more strongly influenced by annual river-to-lake connection times throughout the year. Our results

show that microbial community composition changes substantially from winter to spring and that communities are most diverse and distinct during these time-periods, but that the lake-water communities become more similar to one another as open-water progresses.

We also hypothesized that MOB abundance would correlate with lake river-to-lake connection time, where lakes with shorter connection times would have the highest proportion of MOB throughout the year due to their elevated CH_4 substrate availability and carbon bio-availability, and that environmental factors related to carbon quality, quantity and CH_4 substrate availability would have the strongest influence on MOB and microbial community structure. We detected MOB and methylotrophic organisms in all samples from all lake types throughout the year and found globally dispersed freshwater taxa with significant correlations to lake-water CH_4 and other measurements related to carbon bio-availability (pH, DOC, pCO_2, temperature, dissolved oxygen, nutrients).

Effects of nutrient limitation and methanogenesis on methane oxidizing bacteria (Chapter 3)

Q4) Are rates of water-column methanogenesis sufficient to affect apparent MOX rates among the lakes?

Q5) Are rates of water-column MOX among the lakes limited by nutrient availability?

Q6) What are the relative contributions of the main methane-producing and consuming organisms to the CH_4 dynamics and microbial communities among these study lakes?

For Q4, we hypothesized that methanogenesis was occurring in Delta lakes due to the presence of particle-attached bacteria in the water column and the potential for aerobic methanogenesis detected in other study systems. For Q5, we hypothesized that rates of MOX would be enhanced with the addition of nutrients based on known history of a P- and N-debt in the latter part of the summer and found a positive effect of increased nutrients on rates of MOX. Our expectation that MOX rates were nutrient limited was experimentally supported, but we were surprised by the negative effect of nutrients on rates of MEG in some lakes. The small scale of the experiment limits

potential conclusions we can make about the factors that influence MEG. For Q6, the lack of Archaea detected in the water-column was also surprising, as we expected to find potential methanogens to account for MEG, but the functional genes and more in-depth techniques needed to determine Archaeal abundance was beyond the scope of this study.

To our knowledge, this study was the first to compare measures of MOX, MEG, lake biogeochemistry, and microbial community diversity and abundance data in Arctic Delta lakes. We detected aerobic lake-water methanogenesis, and suspect that this process, along with lake-water MOX, is affected by competitive interactions with other microbes for nutrients. Our results were not able to properly address the relative contributions of major MGA taxa to the overall balance of MOX and MEG in our study lakes. However, we detected multiple MOB and freshwater taxa similar to species found in other freshwater, sediment and soil environments and found that the diversity and community composition of these microbial communities shifts according to major seasonal changes to lake-water biogeochemistry including CH_4 concentration, dissolved oxygen, temperature, major nutrients and indicators of carbon bio-availability.

Significance of results and questions for further research

This study sought to build upon previous work in the Mackenzie River delta to determine the influence of lake biogeochemical properties on carbon and methane-cycling in the water column and sediment of Delta lakes. Our results provide the first assessment of the composition of water-column bacterial communities in the lakes of the Mackenzie River delta and identified several prominent taxa involved in methane cycling and carbon processing. We also identified abundant methylotroph taxa that could be influencing these methane cycling processes based on their contribution to carbon cycling in other methane-rich systems. Our results show the potential for water-column methanogenesis, an anaerobic process, to occur in oxygenated parts of the water column, but that rates of CH_4 consumption were much higher, so lakes are net methanotrophic.

Rates of water-column MOX and MEG appear to be affected by nutrient availability, as well as lake biogeochemical differences in carbon inputs, CH_4 substrate availability and flooding frequency. Rates of MOX increased with increasing lake

isolation, as did the strength of the nutrient effect. Future genetic analysis of the Delta should monitor expression of other genes related to methane oxidation, such as mmoX and mxaF, and consider the potential for macrophyte-associated MOB and novel methane-cycling species, particularly *Verrucomicrobia Opitutae vadin*_HA64_ge which was found to have significant correlations to lake-water CH_4 in our system.

Results for rates of MEG and the abundance and activity of Archaea in Delta lakes remain uncertain due to difficulty of detection of Archaea and low statistical strength of our results. Further studies of methane dynamics in the Delta should consider the potential impact of water-column methanogenesis on rates of MOX and CH_4 flux, and the potential presence of Archaea such as *Methanosaeta* which were detected in the sediment of these lakes. Future work in the Delta should consider that methanogens in the oxygenated water column could be producing substantial quantities of CH_4 through the open-water period, and what role lake and sediment biogeochemistry has on this process both locally and globally.

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Appendix A

Notes and supplementary information

Note A1

Supplementary Information on BES Inhibitor used during enrichment experiment of Delta lake-water and detection of water-column methanogenesis. 2-bromoethanosulfane (BES) is a "classic structural analog of Coenzyme M (Nollet et al. 1997)," (CoM; $HSCH_2CH_2SO_3$), a cofactor which is found in all methanogens but not in other bacteria or archaea (Liu and Whitman 2008). CoM is involved in the terminal step of methane biosynthesis, where the methyl group carried by CoM is reduced to methane by methyl- CoM reductase (Liu et al., 2011 and references therein[13])". BES "competitively inhibit the methyl transfer reaction at the terminal reductive step during methane formation in methanogens using H_2 and CO_2. Normally, [BES, and other similar compounds] can specifically inhibit all the groups of methanogens at a relatively low concentration. Therefore, they are usually regarded as "specific" methanogenic inhibitors" (Liu et al., 2011 and references therein[13]). We used a final concentration of 10 mM BES to ensure complete shut-down of both hydrogenotrophic and acetoclastic MEG, that would be occurring in the water column, without causing undue impact on water chemistry and influence on microbial community (Oremland et al., 1988, Liu et al., 2011 and references therein[13]).

Note A2 - Extra samples, backup data, etc.

Backup samples and GF/C filtrate for trace ions, total dissolved nitrogen, and total dissolved phosphate was also collected, and duplicate samples for each lake are under refrigeration or frozen at SFU if needed for future analysis. Backup filters with the exception of the winter under-ice DNA samples for the 6-lake set, and DNA extracts for all samples not sent to the sequencing center or to Bigelow Laboratory for Ocean Sciences for qPCR analysis, are being stored at SFU at -80 °C.

Appendix B

Images

Image B1. Sediment core examined by author (Mitchell Bergstresser) during winter under-ice survey. Auger, Uwitec sediment corer, and cooler used to collect and store samples in background.

Included with permission of photographer Kimberley Geeves, 2016.

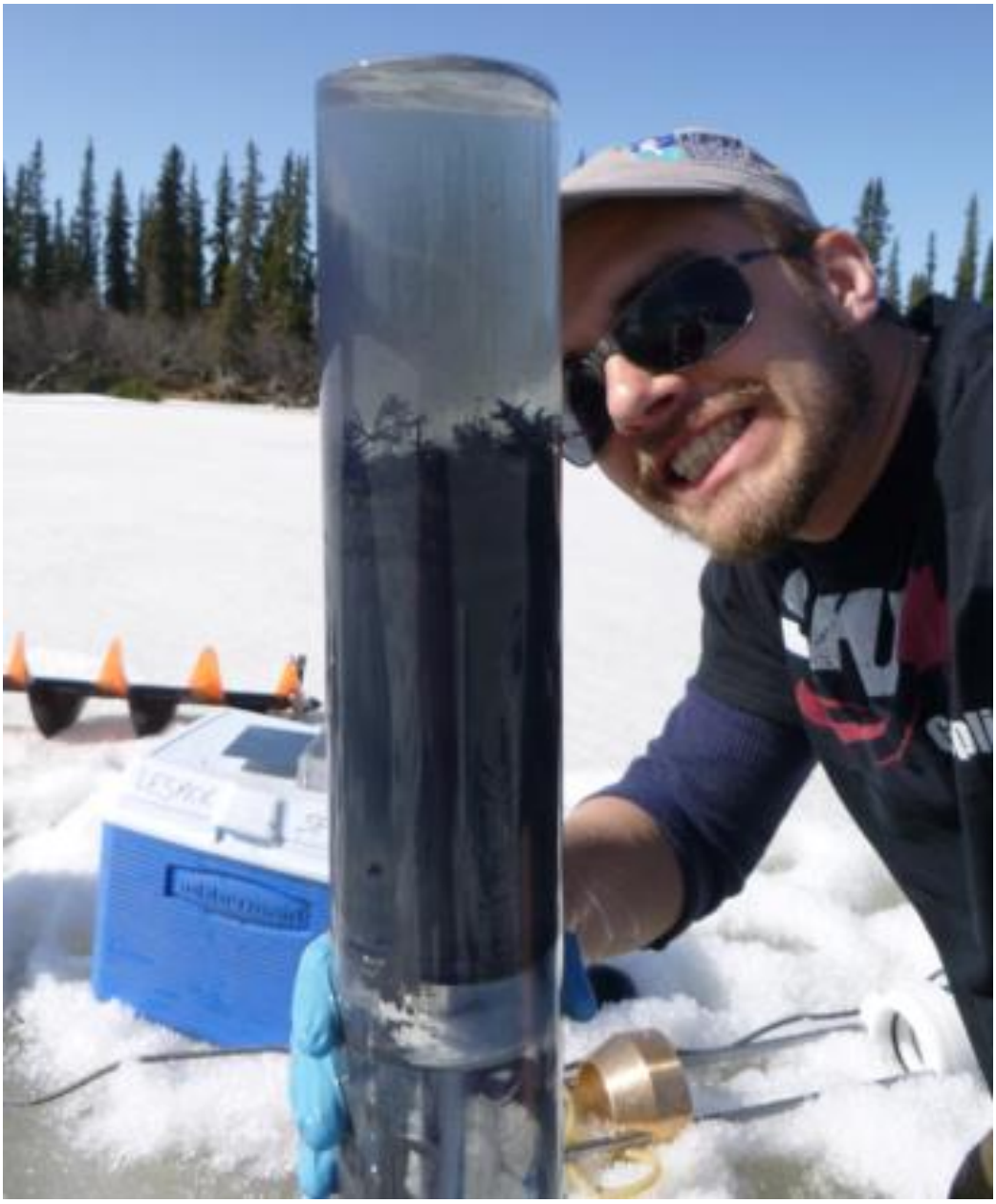


Image B2. Incubation experiment, where bottles, filled with Delta lake-water and various treatments added, are immersed in filtered Mackenzie River water (tap water) and regularly flushed throughout the 48-hour incubation period to maintain temperature at 17*C.

Photo credit: Mitchell Bergstresser, 2016

Appendix C

Supplementary Figures and Tables

C1.1 Summary of two-factor ANOVA (with replication) for MOX data from Chapter 3 experimental incubation

SUMMARY	Control	Spike	BES	BES+Spike	Total
L129					
Count	3	3	3	3	12
Sum	66.68798	366.5954	342.2053	493.2576	1268.746
Average	22.22933	122.1985	114.0684	164.4192	105.7289
Variance	4.488224	47.04399	39.09247	588.257	3057.303
L56					
Count	3	3	3	3	12
Sum	90.76126	2151.438	1351.165	2249.358	5842.722
Average	30.25375	717.146	450.3884	749.7859	486.8935
Variance	1038.167	5227.623	6915.918	379.2299	93005.92
L520					
Count	3	3	3	3	12
Sum	2695.933	4586.675	3888.138	6385.166	17555.91
Average	898.6442	1528.892	1296.046	2128.389	1462.993
Variance	11485.37	802.6812	58472.07	1567.038	229547.2
Total					
Count	9	9	9	9	
Sum	2853.382	7104.708	5581.508	9127.782	
Average	317.0424	789.412	620.1676	1014.198	
Variance	193415.7	375479.3	294521.6	763180.7	

C1.2 ANOVA of MOX data from experimental incubation results from chapter 3, summarized from table C1.1

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	11760884	2	5880442	815.1527	8.69E-23	3.402826
Columns	2329821	3	776607	107.654	4.68E-14	3.008787
Interaction	1078760	6	179793.3	24.92313	3.39E-09	2.508189
Within	173134	24	7213.915			
Total	15342599	35				

C1.3 Summarized single-factor ANOVA data from MOX experiment for 3 lakes (L129, L56, L520), Chapter 3.

Anova: Single Factor - L129

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Control	3	66.68798	22.22933	4.488224
Spike	3	366.5954	122.1985	47.04399
BES	3	342.2053	114.0684	39.09247
BES+Spike	3	493.2576	164.4192	588.257

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	32272.57	3	10757.52	63.38379	6.43E-06	4.066181
Within Groups	1357.763	8	169.7204			
Total	33630.33	11				

Anova: Single Factor - L56

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Control	3	90.76126	30.25375	1038.167
Spike	3	2151.438	717.146	5227.623
BES	3	1351.165	450.3884	6915.918
BES+Spike	3	2249.358	749.7859	379.2299

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	995943.2	3	331981.1	97.92275	1.2E-06	4.066181
Within Groups	27121.88	8	3390.234			
Total	1023065	11				

Anova: Single Factor - L520

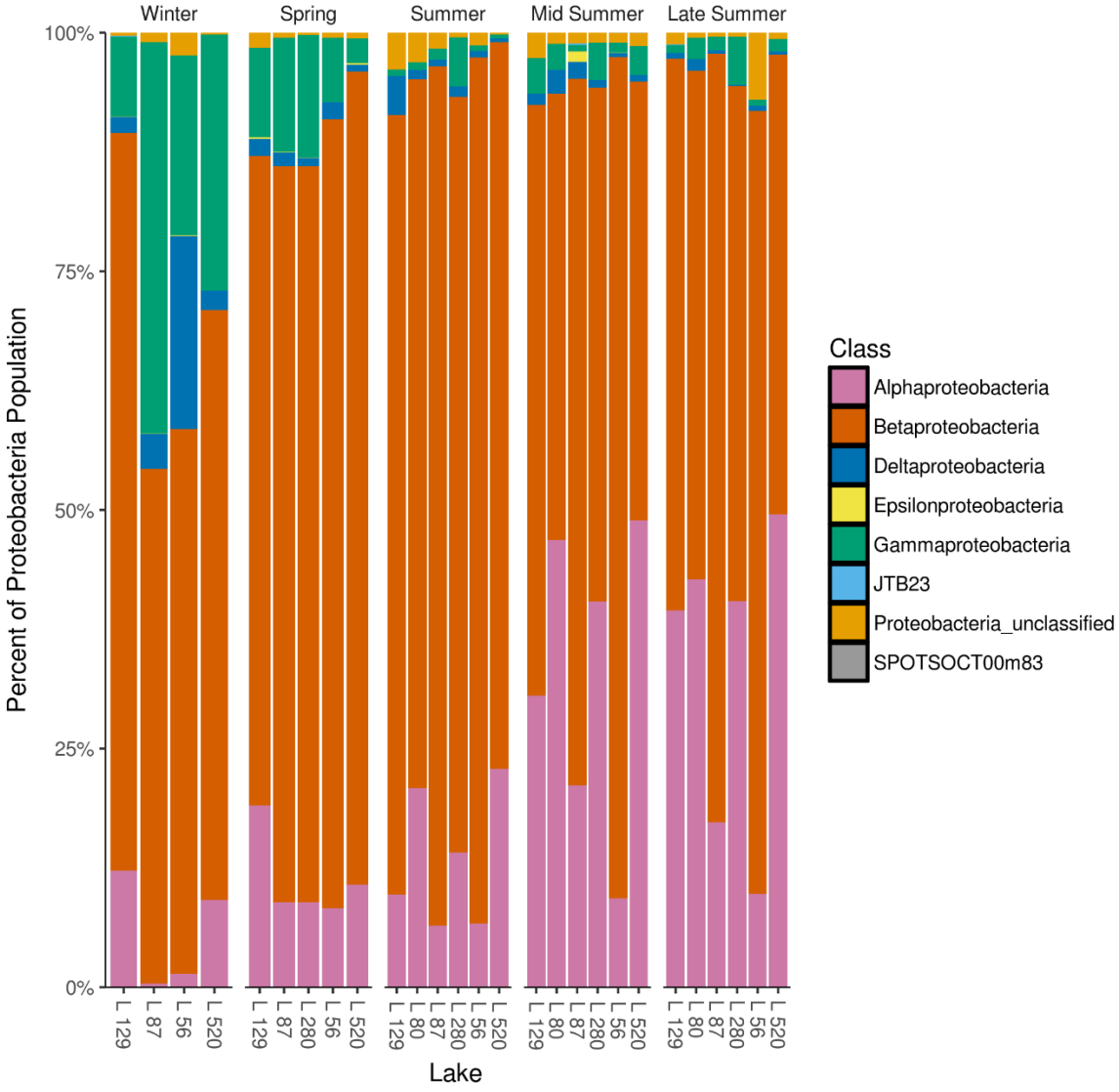
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Control	3	2695.933	898.6442	11485.37
Spike	3	4586.675	1528.892	802.6812
BES	3	3888.138	1296.046	58472.07
BES+Spike	3	6385.166	2128.389	1567.038

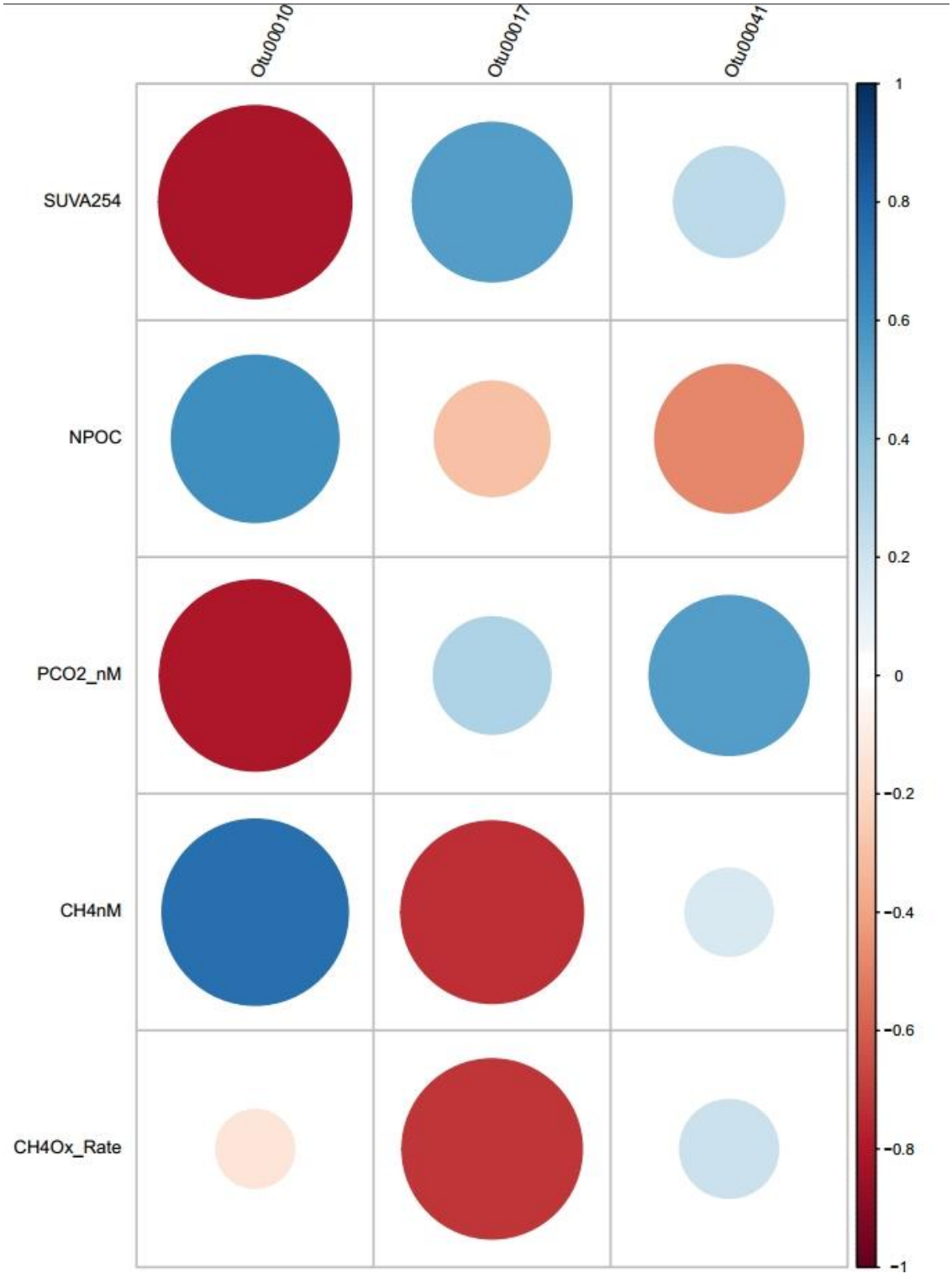
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2380365	3	793455.1	43.88145	2.59E-05	4.066181
Within Groups	144654.3	8	18081.79			
Total	2525020	11				

C-2.1 **Bar-plot of the relative abundance of classes of proteobacteria from set of 6 Mackenzie Delta lakes sampled during the Winter and open-water period of 2016.**



C-2.2 **Correlation between major *Methylotroph* OTU's and environmental variables from experimental manipulation of water from lakes of varying sill elevations, based on data Chapter 2 of this thesis.**



Appendix D

Supplementary Data Files

Description:

The accompanying Excel spreadsheet contains meta-data used to generate results for R-studio analysis and supplementary taxonomy and sequencing information, where available. Data includes sediment samples where all but the specified ion/trace metal information and the DNA/taxonomy data is from the overlying water column. Separate tabs summarize data from the experiment, and a summary of diversity indices from Rhea analysis of the 6-lake set.

Filename:

Meta-data-summaryFullMB2018.xlsx