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A Lipid Biomarker Investigation of Organic Matter Sources and Methane Cycling in Alaskan Thaw Lake Sediments

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Author
Williams, Mark

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A Lipid Biomarker Investigation of Organic Matter Sources and Methane Cycling in Alaskan Thaw Lake Sediments

A Thesis submitted in partial satisfaction of the requirements for the degree of

Master of Science

in

Geological Sciences

by

Mark Richard Williams

December 2012

Thesis Committee:
Dr. Gordon Love - Chairperson
Dr. Richard Minnich
Dr. Robert Allen
The Thesis of Mark Richard Williams is approved:

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committee Chairperson

University of California, Riverside
ABSTRACT OF THE THESIS

A Lipid Biomarker Investigation of Organic Matter Sources and Methane Cycling in Alaskan Thaw Lake Sediments

by

Mark Richard Williams

Master of Science, Graduate Program in Geological Sciences
University of California, Riverside, December 2012
Dr. Gordon Love, Chairperson

Barrow Peninsula is located on the North Slope of Alaska with much of it consisting of high latitude wetlands. Thaw lakes, an important feature of these wetlands, have been shown to be significant sources of methane to the atmosphere; however the origins of the methane remain poorly constrained. We have used lipid biomarkers to better understand biogenic methane production in lake sediments by characterizing microbial communities that may be involved in methane cycling as well as the organic matter that is available as substrates for methanogens.

Surprisingly, we found a relatively low amount of lipids associated with methane cycling, with no detection of lipids derived from anaerobic methane oxidizing archaea, low but detectable amounts of compounds produced by Type I methanotrophic bacteria, as well as low but detectable amounts of lipids likely derived from methanogens. However, archaeol concentration, and hence methanogen abundance, did appear to correlate with TOC amounts. We also found a strong signal of in situ primary production in the form of a high 2-methylhopane index (> 10 %), reflecting cyanobacterial inputs, coupled with abundant nC22-nC26 n-alkanes and high sterane/hopane ratios which are characteristic of green algae.

The elevated archaeol levels in the lake cores that displayed the greatest TOC amounts (and by extension greatest primary production) may show that future methane emission rates of these lakes may ultimately be tied to the type of organic matter deposition and preservation that occurs within the sediment beneath the lakes.
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1. Introduction

The humane impact on climate has become increasingly well-established within the last two decades through refinements in climate models and more accurate measurements of greenhouse gas emissions from both anthropogenic and natural sources. Methane is a potent greenhouse gas that has several natural sources, the largest of which is from global wetlands (~72%), but also includes sources such as coal and methane clathrates (Wuebbles & Hayhoe, 2002). Methane sources are temperature and redox sensitive, such as methane clathrates and anaerobic microbes, will be more affected by the increases in temperature and precipitation than thermogenic methane from deep reservoirs, which is relatively immune to changes in climate. Warming is expected to be greatest at high latitudes (Figure 1), which makes discriminating different methane sources and the amount of methane they produce within those cold ecosystems particularly important.

Arctic lakes have been recognized as a methane source within high latitude wetlands with methane being produced from microbes, methane clathrates and natural gas (Walter et al. 2007). Knowing the taxonomic affinity and physiology of microbial communities (e.g. methanotrophic and methanogenic) and the size of the microbial community present as well as the organic substrates available to them to function will allow for a better understanding of potential changes in the amount of methane produced.
and consumed. This project aims to use lipid biomarkers to help identify the types as well relative abundances microbes living in the lake sediment, characterize the sources and composition of the sedimentary organic matter present in the lake cores and place some constraints on past methane cycling.

![Figure 1. Atmosphere-Ocean General Circulation Model projections of surface warming.](image)

1.1 Global Warming and GHGs

Modern Global Warming or the anthropogenically mediated warming of the climate system is now a largely accepted phenomenon within the scientific community. Global warming and its consequences has the potential to radically affect world populations and economies. According to the IPCC AR4 report, global warming can be defined as a 100-year (1906-2005) linear trend of 0.74°C averaged warming, with the
warming trend of the last 50 years being nearly twice that of the warming over the last 100 (Figure 2, AR4 IPCC, 2007). Additional evidence of warming can be seen in the observed decreases in mountain glaciers, snow cover, sea ice and rising sea level. The rapid rise of global temperatures since the 19th century is now believed to be very likely due to increases in anthropogenic greenhouse gas (GHG) concentrations, temperature changes that natural CO₂ variations alone cannot explain. The source of the anthropogenic GHG has been attributed largely to the burning of fossil fuels and changes in land management.

Projected total warming at the end of the 21st century based on different emissions scenarios has a likely range from 1.1 to 6.4°C and a best estimate range of 1.8 – 4.0°C with at least 0.6°C of that warming occurring due to CO₂ that is already present in the atmosphere (Figure 1). The warming is expected to be greatest over land and at high latitudes (Southern Ocean near Antarctica and northern North Atlantic) co-occurring with an increase in precipitation. Numerous environmental effects are predicted to result from the warming that has already occurred as well as warming that is likely to occur in the future.
1.2 Methane Emissions and Wetlands

Total methane emission to the atmosphere is currently estimated at 520-625 Tg/yr with human activities producing ~375 Tg/yr and total natural sources ~160 Tg/yr. (Figure 3, Bartlett & Harriss, 1993; Lelieveld et al., 1993; Lelieveld et al., 1998; Judd et al., 2002; Wuebbles & Hayhoe, 2002). Of the 160 Tg/yr produced by nature sources, the vast majority comes from wetlands, making them a significant contributor to the global methane atmospheric budget (Figure 3, Wuebbles 2002).
Controls on methane emissions within wetlands are influenced by a variety of factors such as nutrient abundance, vegetation type, organic content and temperature within soils as well as water table position. Studies have shown the sensitivity of wetland methane emissions to changes in water table position and soil/sediment temperature, coupled with the predicted changes that will occur at high latitudes, makes these high latitude wetlands areas of importance if we are to understand the effects of global warming (Dunfield et al., 1993; Bellisario et al., 1999; Wuebbles & Hayhoe, 2002).
1.3 Methane Cycling Microorganisms/Environment

It has been estimated that 5 to 20% of the world’s global flux of methane to the atmosphere is caused by microbial action in sediments, which makes the production of methane by microbes a significant factor in wetland methane emissions (Kotelnikova, 2002). Biogenic methane production is conducted by a group of archaeabacteria (methanogens) that typically live below the zone of sulfate reduction in modern sediments. Methanogenesis is the final process of organic matter breakdown after all other electron acceptors (O\textsubscript{2}, Fe\textsuperscript{3+}, Mg\textsuperscript{4+}, SO\textsubscript{4} etc.) have been used up. The amount of methane produced is largely based on substrate availability, temperature, pore-water conditions and organic matter abundance (Sundh et al., 1994; Kotelnikova, 2002). The two dominant types of methanogens are acetotrophic methanogens that dismutate acetate to CO\textsubscript{2} and methane (Whalen, 2005, Equation 1.)

\[
\text{CH}_3\text{COOH} \rightarrow \text{CO}_2 + \text{CH}_4. \quad \text{(Equation 1.)}
\]

and hydrogenotrophic methanogens (Whalen, 2005, Equation 2.) which use hydrogen gas as an electron donor for reduction of CO\textsubscript{2}

\[
4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}. \quad \text{(Equation 2.)}
\]

Methanotrophs conversely can act as a significant microbial sink of methane, consuming the upward defusing methane in both marine and non-marine systems by anaerobic or aerobic oxidation reactions. Methanotrophs can be either archaea or bacteria that use methane as a carbon substrate utilizing oxygen in the case of aerobic bacteria, or using an alternative electron acceptor in anoxic conditions in the case of anaerobic
archaea working in symbiosis with bacteria. Anaerobic oxidation of methane (AOM) is thought to account for 25-94 Tg/yr or 5-20% loss of global marine methane to the atmosphere (Thiel, 2001; Orphan, 2002).

Much of the work spent on understanding microorganisms involved in methane cycling has taken place at modern methane seep sites where hydrocarbon rich fluids escape from marine sediments. These sites are populated by local communities of both macrofauna (mussels, clams and tubeworms) and microorganisms (bacteria and archaea) that utilize the carbon as an energy source, often working syntrophically together (Cavagna, 1999). Microbial communities can be very diverse preforming anaerobic methane oxidation, methanogenesis (using both \( \text{H}_2/\text{CO}_2 \) and acetate substrates) and sulphate reduction. The oxidation of methane coupled to the reduction of sulphate is responsible for the high levels of alkalinity and Dissolved Inorganic Carbon (DIC) at seep sites, leading to the precipitation of calcium carbonate. The maximum rate of methane oxidation typically occurs at the zone between sulfate reducers and methanogens, due to methane being oxidized by the sulfate reducers which is the dominant sulfate consuming process in marine sediments (Dunfield, 1993; Bellisario, 1999; Kotelnikova, 2002). Three archaeal phylogenetic groups (ANME-1, ANME-2 and ANME 3) were found in abundance at modern seep sites and are thought to be involved with AOM (Boetius et al., 2000; Orphan et al., 2002). ANME-2 and ANME-3 archaea belong to the order Methanosarcinales, while ANME-1 archaea are more distantly related to the orders Methanosarcinales and Methanomicrobiales. Researchers have not yet been able to isolate microorganisms capable of oxidizing methane under anoxic conditions.
Thus, no pure cultures are currently available for physiological and biochemical characterization and present knowledge about microbes mediating the anaerobic oxidation of methane is therefore mostly derived from cultivation-independent techniques. Microscopic fluorescence in situ hybridization (FISH) evidence has indicated a symbiotic relationship between ANME-2 archaea and sulfate reducing bacteria. ANME-2 is located at core of each microbial aggregate and is surrounded by its sulfate reducing partner, with the archaea preforming reverse methanogenesis while the bacteria use the products to reduce sulfate. ANME-2 has also been found to exist as monospecific aggregates in some environments.

In lake sediments, up to 60% of the upward methane flux is oxidized in a narrow aerobic depth band and 45-55% of the total carbon input is regenerated as methane (Kotelnikova, 2002). In lake environments sulfate is often limiting, lowering the abundance of the sulfate reducers present in the sediment, and leaving more substrate available for methanogens, which may result in higher methane production in the fresh water sediments vs. marine sediments. In wetlands, methane produced in the soils escapes along a molecular diffusion gradient through the soil to water or atmosphere. Due to methane generation being confined to anoxic substrates, water table position in non-marine environments is particularly important (Whalen, 2005; Turetsky et al., 2008). This is due to a vertically stratified soil systems being essential for anaerobic processes to occur, while soils that are not flooded are typically fully oxygenated, inhibiting methanogenesis (Whalen, 2005). The temperature optimum for methanogen growth exists in the range of 25-30°C, potentially as high as 40°C, and rapidly drops off below
20°C (Dunfield, 1993), thus any increase in temperature in the wetland will likely result in an increase in methanogen growth. However, the effect of temperature on methane production is less constrained due to an observed higher variability in optimum temperatures in lab and field experiments that may be related to different substrate availability and changes in methanogen species (Dunfield, 1993; Whalen, 2005; Turetsky et al., 2008). All other factors being equal, water table position appears to be more important than temperature in affecting methane production in soils (Dunfield, 1993).

1.4 Lipid Biomarkers

The breakdown of organic matter (OM) begins in the water column where organisms both macro- and micro- as well as abiogenic chemical reactions, remineralize OM beginning with the more labile and ending with the most recalcitrant. The diagenesis of organic matter is characterized by a low temperature transformation of organic compounds through microbial, chemical and thermal processes. Proteins, carbohydrates and nucleic acids constitute the more labile fraction of organic matter and therefore the easiest to break down with >99% being remineralized into compounds like CO₂ and NH₃. Nucleic acids in particular are easily susceptible to degradation with the phosphate heads being easily lost. With increasing burial, compounds become increasingly aromatized to form more stable compounds along with the loss of their heteroatoms (N, S, O, P) (Killops & Killops, 1994).

Organic matter present in sediment can be separated into two groups of compounds based on their ease of extraction with organic solvents. Bitumen, the fraction
of organic compounds that are easily extracted by organic solvents, only comprises a small fraction of the total organic matter. Kerogen, which comprises a much larger fraction of the total organic matter (>70-95 wt%), consists of structurally complex insoluble organic macromolecules comprised of covalently cross-linked repolymerized and vulcanized proteins, carbohydrates and lipids. Due to the insoluble nature of kerogen, it can be a particularly useful repository of information on primary organic input and can aid in preservation of the more easily degraded compounds. Kerogen bound compounds also are less isomerized than their free lipid counterparts due to the protection the geomacromolecular cage provides (Killops & Killops, 1994).

Biomarkers, or molecular fossils are typically derived from the lipid components of cell membranes of living organisms and, during diagenesis, are reduced in chemical complexity (through the loss of more reactive functional groups such as alcohols and more reactive heteroatoms such as N and O) into more geologically stable forms although the hydrocarbon core is very resistant. The stability of the lipid compound is due to the strong C-C bonded core of lipids which allows the majority of the hydrocarbon skeleton to survive through diagenesis and catagenesis while other biological compounds that lack a high number of C-C bonds cannot. Though lacking the specificity of other organic compounds such as DNA and proteins, lipids retain enough information post-burial to be linked to biological precursor compounds (steranes to eukaryotes (Figure 4), hopanes to bacteria (Figure 4; Figure 5). Along with the loss of certain elements and functional groups, the configuration of compounds changes from a form that life preferentially produces to one that is more geologically stable (e.g. S to R) (Killops & Killops, 1994).
Figure 4. Biological Hopane and sterane compound structure. The hopane is in ββ(22R) configuration with the 17 and 21 position carbon-hydrogen bonds in the beta orientation and the chiral center at the 22 carbon position is in the R configuration. The sterane is in the ααα(20R) configuration with the 5, 14 and 17 carbon-hydrogen bonds in the alpha orientation and the chiral center at the 20 position in the R configuration.

Figure 5. The structural differences between a sterane with 27 carbon atoms and a sterane with 29 carbon atoms. Steranes with 27 carbon atoms are produced in abundance by animals and red algae while steranes with 29 carbon atoms are produced in abundance by green algae and land plants.

Lipids often serve as membrane stabilizers, controlling the permeability within a cell and the fluidity of the membrane. Two common types of hydrocarbon chains that
make up lipids are those made up of polymethylenic units (chains made up of C₂ acetate units) or polyisoprenoid units (chains constructed of C₅ isoprene units, Figure 7). Acyclic isoprenoids can be connected either head to tail (e.g. phytol), tail to tail (e.g. crocetane, Figure 8) or head to head (e.g. farnesane, Figure 7). All archaea and some bacteria produce glycerol ethers (made of isoprenoidal units) in contrast to glycerol esters (acetate units) which are produced by bacteria and eukaryotes. The lipid precursors for the archaenal acyclic isoprenoids include: archaeal, caldarchaeol and crenarchaeol, the first two of which are precursors for phytane and biphytane, respectively (Figure 7). The combination of isoprenoidal skeletons and ether bonds are unique to archaea who use them as structural components in their membranes. Triterpenoids (constructed from 6 isoprene units) are a particularly important group of compounds that are derived from the isoprenoid squalene and are either pentacyclic (5 rings) or tetracyclic (4 rings). The pentacyclic triterpenoids are typically found in higher land plants chiefly as resin compounds, like oleanane, a compound found in flowering (angiosperm) plants.

Hopanoids are a series of pentacyclic triterpanes with a C₅ E ring and C₅ side chain (Figure 4). The hydroxyl groups in the side chain make hopanoids too polar to analyze through conventional gas chromatography without derivitization or catalytic hydropyrolysis. Hopanoids have been hypothesized to have a role in controlling fluidity in bacterial cell membranes and are often called the “bacteriohopanoids”; however their full range of functions remains a topic of extended debate. The bacterial hopanoid precursor compounds are compounds like the bacteriohopanetetrols (BHPs) in living organisms. They are present initially in sediments as hopanoidal alcohols and acids, with
defunctionalization occurring rapidly, and diagenesis and aromatization occurring later, eventually leading to the production of fully saturated hopanoidal hydrocarbon compounds, the hopanes and unsaturated hopanoidal hydrocarbon compounds, the hopenes (Killops & Killops, 1994). In general, the unsaturated hopenes are more abundant in more recent sediments and over time, with increasing aromatization, there is a reduction in the number of unsaturated compounds. The hopane compounds are initially present in their “biological configuration” of 22R 17β(H),21 β(H) (Figure 4). However, this biological configuration is not stable over geologic periods of time and there is a switch in both the orientation and configuration of the hopanoidal compounds. The switch is from the biological configuration, 22R 17β(H),21 β(H) to the more thermodynamically stable 22R + 22S 17α(H),21β(H) and 22R + S 17β(H),21α(H) configuration with increasing isomerization leading to an increase in the hopanoidal compounds of the stable “S” configuration and either of the carbon-hydrogen bonds at the 17 and 21 position being the “alpha” orientation. Hopanes and hopanoids can provide information about source organisms relevant to this study.
Methylated hopanoids, hopanes with an extra methyl group attached to either A or B ring can be diagnostic of certain types of bacteria. 2-methylhopanoids are specific to cyanobacteria and are useful for tracking the primary producer input into the sediment (Figure 6). 3-methylhopanoids are produced by a variety of organisms of which several are methanotrophic bacteria; and are therefore useful for tracking methane oxidation in sediments (Figure 6, Neunlist and Rohmer, 1985; Summons, et al., 1999).

Another common and informative group of tetracyclic triterpenoids are the steroid group of compounds. They are usually C\textsubscript{27} to C\textsubscript{30} molecules with an alcohol group, 1 double bound in the A ring, and chains off the D-ring (Figure 4; Figure 5). Steroids are
present almost exclusively in eukaryotes, typically as sterols or steroidal alcohols, like cholesterol in animals, which serve as membrane modifiers, similar in role to the hopanoids. They can either be present as saturated steroidal alcohols, stanols or unsaturated steroidal alcohols, stenols. Through a complex diagenesis pathway and the loss of functional groups, the sterols initially present become saturated and unsaturated steroidal hydrocarbons, the steranes and sterenes, respectively. However, sterenes may also undergo a structural rearrangement through the interaction with acidic clay minerals and produces diasteranes and diasterenes. Diasterenes are more resistant to structural changes than their regular counter-parts and can persist much longer in the geologic record though they do become less abundant in comparison to the regular steranes with time. The initial biological configuration of these steroid compounds is $\alpha\alpha\alpha (20R)$ with the carbon-hydrogen bonds at the 5, 14, and 17 positions being in the alpha orientation and the chiral center at the 20 position being in the R configuration (Figure 4). There is a similar process of change from the biological configuration to the thermodynamically stable configuration. The maturation process for the steroids is more complicated than the hopanoids, but can be summarized by the switching of the chiral center at the 20 position from the R configuration to the S configuration and the change in the orientation of the carbon-hydrogen bonds at the 14 and 17 position from alpha to beta. Different groups of organisms produce steroids of different carbon numbers as their most abundant steroids (Figure 4; Figure 5). For example, animals and red algae both produce the $C_{27}$ steroid, cholesterol, as their most abundant steroid, whereas green algae and land plants predominantly produce $C_{29}$ steroids (Figure 5.).
Due to the strong C-C bond, lack of attached functional groups and large number of precursor compounds, \( n \)-alkanes are geologically abundant in organic matter and can be informative regarding the relative input of plant material vs. microbial compounds into the sediments (Figure 7). Algae and bacteria are characterized by short-chain \( n \)-alkanes from \( n \)-acid precursors of low carbon numbers (\( C_{16} - C_{24} \)) though some can produce \( n \)-alkanes with higher carbon numbers and possess a low carbon preference index. Conversely, higher plants are characterized by \( n \)-alkanes with high carbon numbers (\( C_{27} - C_{33} \)) with a strong odd-over even preference due to the decarboxylation of fatty-acid waxy components in leaves during diagenesis. The relative amounts of long- and short-chain \( n \)-alkanes can be calculated as a carbon preference index (CPI; \( \frac{C_x + C_y + C_z}{C_a + C_b + C_c} \)). Shorter-chain compounds like \( C_{16} \) and \( C_{18} \) acids and alcohols are relatively labile, preferentially being decomposed during early diagenesis, leading to a dominance of higher carbon numbered plant components with a preference for odd-over-even numbered alkanes in modern surficial sediments (Killops & Killops, 1994). Over time there is a general shift in the CPI and chain length from a
dominance of longer-chained odd numbered \( n \)-alkanes in the surface sediments to shorter chained alkanes with a lower CPI during catagenesis as the longer chained odd-numbered compounds are preferentially fragmented (Killops & Killops, 1994).

**Figure 8.** Common Acyclic Isoprenoids, compound structures composed of C5 isoprene units. Archaea can produce all the displayed compounds (with the exception of Pristane) with crocetane, PMI and biphytane/phytane being produced by methane cycling organisms.

Pristane (Pr) and Phytane (Ph) (Figure 8) isoprenoidal hydrocarbon biomarkers, are thought to both be derived from chlorophyll phytol side chain, with the former derived from an oxidative pathway and the latter a reductive pathway (Figure 9):
Figure 9. Oxidation and reductive diagenesis pathways of phytol to pristane and phytane. Pristane is produced from the oxidation of phytol to phytenic acid which is then decarboxylated to pristane. Phytane is produced from the reduction of phytol to dihydrophytol and then hydrogenated to phytane (Kellops).

As a result, the ratio of pristane to phytane has been used as a means to assess the redox state of the environment of deposition in the water column and around the sediment/water interface (Figure 9). Pr/Ph ratios of less than 1 have been associated with marine carbonates deposited under oxic conditions, values between 1 and 3, marine shales and values over 3 with non-marine shales and coals during anaerobic deposition. However, caution must be used as archaeal lipids, tocopherols, vitamin-E and methyltrimethyltridecylchromans are also sources for these compounds and the ratio is best used in conjunction with other compounds that reflect redox state.

Archaeol and caldarchaeol, head-head linked isoprenoid glycerol-diethers (Figure 8), are compounds produced by a wide range of archaea which can be degraded into several acyclic isoprenoid compounds, such as phytane and biphytane (Figure 8) which can be associated with methane cycling but can also be present in halophilic archaea. In contrast to archaeol, which is only specific to the Archaea domain generally, crocetane
and pentamethylicosane (PMI), both tail-tail linked acyclic isoprenoids (Figure 8), appear to be more selective to certain types of archaea associated with methane cycling. $^{13}$C depleted archaeal compounds (di/tetra ethers), particularly crocetane and PMI, are very indicative of an anaerobic methane oxidation signature; high concentrations of these hydrocarbons are typically associated with cold seep environments. Compound specific carbon isotopic analysis (CSIA) is a useful technique in determining if a molecule is associated with methane cycling and is frequently the "silver bullet" for demonstrating that the source organisms were involved in methane cycling, particularly methane oxidation.

Lipid biomarkers associated with methane cycling have been used to characterize both modern and ancient environments. Wakeham and co-authors (2008) used biomarkers in conjunction with water chemistry and microprobe analysis in the Black Sea to illustrate how lipids can be used in modern settings to characterize microbial communities including what substrates they may utilize and which groups may be in competition with each other for these substrates. They used lipids thought to be characteristic of certain bacterial microorganism such as ladderanes (produced by anammox bacteria) and aminobacteriohopanetriols (products of Type I and Type II aerobic methane oxidizing bacteria) as well as cyanobacteria and purple non-sulfur bacteria) in conjunction with more general bacteriohopanopolyols to describe the type of bacteria living above and within the chemocline. Similarly, archaeal lipids, such as archaeol, crenarchaeol and glycerol diphytanyl glycerol tetraethers (GDGT’s),
differentiated from bacterial and eukaryotic lipids by their sn-2,3 stereochemistry and ether linkages, allow for the characterization of archaeal populations.

The incorporation of methane-derived carbon into methanotroph biomass can be seen by their extremely depleted $\delta^{13}C$ values. This is due to autotrophic carbon fixation pathways having a strong isotopic fractionation effect causing a depletion of between -20 to -30‰ between source carbon and biomass. Since methanotrophs often oxidize very $^{13}C$ depleted biogenic methane (<-40‰), this can lead to highly diagnostic lipid biosignature of -80 to -130‰ in the case of lipids associated with the methanotrophs. The $^{13}C$ depleted lipids (as low as -130‰) of microbes associated with methane cycling are isoprenoids such as PMI, its C$_{20}$ counterpart crocetane, and archaeal compounds such as archaeol, hydroxyarchaeols and (GDGT’s, Thiel, 2001; Orphan, 2002; Birgel, 2006). These biomarkers have been found in both ancient and modern environments and are widely accepted to be associated with methane cycling microbes (Birgel, 2006). Jones and Grey (2011) using stable carbon isotopic analysis found that chironomids, benthic organisms that graze on methane oxidizing bacteria, were isotopically depleted due to the incorporation of depleted bacterial biomass. They found that redox conditions had a large control on the isotopic composition of benthic grazers due to the influence of redox boundary location on the methanotroph population location and density; chironomids in relatively oxic bottom waters had heavier isotopic compositions than those in anoxic, methanotroph-rich waters (Jones & Grey, 2011).
Compounds such as archaeol, hydroxyarchaeol and the acyclic GDGTs are observed to be in low abundance within most of the Black Sea sediments but can have elevated abundance in certain modern benthic microbial mats (Blumenberg et al., 2002) where anaerobic oxidation of methane is localized around seafloor seeps. The scarcity of these compounds is attributed to the high sulfate concentrations which favors sulfate reduction by bacteria (Wakeham et al., 2007). However, methane oxidizing archaea like the ANME group, which were found to live near the bottom of the water column and feed on hydrocarbons from cold seeps and mud volcanoes, produce similar compounds, with ANME-1 archaea thought to have high archaeol/GDGT concentrations while ANME-2 has low archaeol/GDGT concentrations. In addition to biomarkers being useful in characterizing modern microbial communities, they have also been used in deep time to understand microbial structure and paleoenvironment.

Biomarker hydrocarbons associated with methane cycling can be found as far back as the Paleoproterozoic in thermally well preserved sedimentary strata. The 1.64 Ga Barney Creek Formation was deposited in a restricted marine basin characterized by euxinic bottom water conditions and aromatic carotenoid biomarkers, derived from accessory pigments from phototrophic bacteria including okenane (purple sulfur bacteria) as well as chlorobactane and isorenieratane (green sulfur bacteria) were abundant and excellently preserved in carbonaceous, dolomitic mudstones and siltstones (Brocks et al. 2005). Crocetane present in Barney Creek samples, indicative of methane cycling archaea, likely reflected low marine sulfate conditions, which would favor methanogenesis. High 3-methylhopane indices (5-7%), indicative of significant extent of
aerobic methanotrophs were also detected in the rock bitumens (Brocks et al. 2005). The oldest evidence of anaerobic methane oxidation is present is Late Pennsylvanian seep limestones found in southern Namibia (Birgel et al. 2008). The authors found $^{13}$C depleted PMI (-113‰) and crocetane/phytane (-112‰) characteristic of methanotrophic archaea oxidizing carbon depleted methane.

1.5 Study Area: Barrow Peninsula

![Figure 10. Distribution of continuous, discontinuous, sporadic, and isolated permafrost relative to high latitude wetlands in the Northern Hemisphere (Walter et al., 2007).](image)

The High Arctic wetlands are characterized by annual precipitation less than 250 mm, with snowfall being the principal form of precipitation (Figure 9, Woo, 2006). Arctic wetlands are frozen during the long winters. Vegetation growth is limited to the
short summer growing season. Arctic wetlands are typically underlain by frozen soils and sediment (permafrost), with only top centimeters to meters thawing in the summer (Woo, 2006). Permafrost covers roughly 25% of earth’s land surface and is defined as ground composed of soil and rock that remains below 0°C for at least two consecutive years (Wagner, 2010). The layer that lies above the permafrost and is subject to annual freeze/thaw cycles is called the “active layer” (Woo, 2006; Wagner, 2010). Active layer depth ranges from a few centimeters in the high arctic to more than 2 m in some subarctic regions. The active layer defines the extent of the plant root system of wetland plants (Wagner, 2010). The permafrost, rich in ice content, serves as an effectively impermeable layer, preventing the downward movement of water (Woo, 2006; Wagner, 2010).

Arctic lakes have been typically thought of as cold, nutrient poor ecosystems, unable to support significant primary production (Bonilla et al 2005). Though these ecosystems are usually nutrient limited (phosphorus and nitrogen), they have been shown to support a diverse and abundant primary food web consisting of both planktonic and benthic photoautotrophic macro- and microorganisms. These primary produces are dominated by algae and cyanobacteria, both having being found in large abundance in shallow water Arctic and Antarctic environments. Studies on benthic and planktonic algae in particular, have found them to be a significant source of organic carbon to higher trophic levels (Hecky & Hesslein, 1995;Bonilla et al., 2005). This is in contrast to the low nutrient and high indigestible matter content that typifies terrestrial and higher aquatic plant material, limiting their usefulness an important substrate source for consumers. The primary producer community of Ward Hunt Lake, a lake located in the
Canadian High Arctic, were characterized using absorption spectra and High Pressure Liquid Chromatography (HPLC). The authors found the photosynthetic community dominated by benthic cyanobacteria, a result in keeping with other studies that have found benthic cyanobacteria in lakes, ponds and streams in the Arctic and Antarctica (Bonilla et al., 2005). The large benthic photoautotrophic biomass they found relative to the phytoplankton in the overlying water column implies that nutrient availability may actually be greater in the benthos. It appears that food webs dependent on benthic and phytoplankton primary produces are the rule rather than the exception, this is based on stable carbon isotopic studies that compare the carbon values of primary producers, consumers and terrestrial material (Hecky & Hesslein, 1995).

In the hydrological cycle, snow melt accumulates over 9 to 10 cold winter months and is released during the 2 – 3 week melt period. Due to the impenetrable nature of permafrost, runoff easily collects in lowlands and depressions, with wetlands often flooded and the ground saturated with water (Woo, 2006). Wetlands are typically found in topographic depressions due to preferred sites of water runoff input and water retention. Saturated arctic wetland soils provides an excellent environment for anaerobic processes. Microorganisms residing in tundra soils inhabit an environment characterized by low temperatures, long frozen periods, water saturated soils, and restricted nutrient availability. Despite these hostile conditions, cold ecosystems can host a wide range of microorganisms (Yavitt, 2011).
The Barrow peninsula is the northern most region in the United States, extending from the Naru river where it empties into Yakutat Bay in the Arctic Ocean (Figure 10, Hinkel, 2003). The area is underlain by continuous permafrost to a thickness > 400 m with an active layer of 30-90 cm. The peninsula is characterized by low relief with elevations ranging from 0 -22 m above sea level with the land surface having roughly 65% coverage of polygonal ground (Hinkel, 2003). Soil cores taken around Barrow indicate a near surface (< 2 m) environment of 50 – 75% ice volume. Barrow has a cold maritime climate with long, dry, cold winters and short, moist, cool summers. Mean annual precipitation is 106 mm, 63% of which falls as rain through the months of July to September. The thaw lake cycle has been active on the Barrow peninsula since the emergence of the Gubik formation, a Pleistocene aged glacial formation, around 25,000 yr BP and has continued off and on to the present (Hinkel, 2003).

The thaw lake cycle begins with the formation of cold contractions in the permafrost (Figure 11.) (Billings, 1980). Snow blowing into these cracks during the winter and melt water flowing into them during the summer allow for the formation of patterned grounds due to ice formation within the active layer and permafrost (B). This marks the beginning of the primary ice wedge polygon network and once in existence primary wedges are near permanent features (Billings, 1980). Marine transgressions, warmer climates or thaw lakes may melt tops of ice wedges, but the wedges persist in deeper parts of the permafrost.
The growth of primary ice wedge results in formation of large, rectangular low center polygons, as well as the creation of secondary and tertiary wedges due to increased drainage patterns with polygons dividing into smaller polygonal units (C + D, Billings, 1980). These low areas preferentially collect water, and whipped by winds, erode the rims between polygons producing a small thaw pond (E). Thaw ponds eventually enlarge to form lakes which orient in a northwest-southeast direction due to prevailing winds (F). As the lake grows, sediment and peat are washed in from surrounding area, insulating the primary wedge (J+G). Eventually, the mature thawed lake is captured by a small stream which erodes through low shorelines and drains it (H). After drainage, barren lake sediment appears as relatively low, high center polygons with wide troughs above the ice.
wedge (I). Ice wedges begin to grow back after the removal of the water and the basin become increasingly polygonized with centers of polygons preferentially collecting water and repeating the thaw lake cycle (B). Pleistocene ice-rich erosional remains of polygonal landscapes form ice complexes or “Yedomas” which are characterized by being organic rich and are thought to be a significant source of carbon to the atmosphere as they thaw (Wagner, 2008).

The Barrow Peninsula, a wetland type environment dominated by the presence of thaw lakes, is an ideal environment to investigate methane cycling in a freshwater system at high latitudes due to the water logged, sulfate poor environment being conducive towards methanogenesis. Lipid biomarker analysis will not only allow for the characterization of the microbial community that is currently present in the lakes, as well as past changes in community structure, but also allow for the characterization of substrate that microbes feed on. Due to the sensitivity of high latitude environments to climate change, the wetland present on the barrow should respond relatively quickly to any global changes in temperature which would then be recorded in the thaw lakes. These changes will have a variety of impacts on the microbial communities living in the lake sediments and permafrost, such as metabolic rate, as well as larger changes on water table position and organic matter input into the lakes.
1.6 Primary Objectives

The primary objectives of this project were as follows to:

- Perform a detailed lipid biomarker study of selected Alaskan sediment cores to characterize changing microbial communities and organic inputs into thaw-lakes on Barrow Peninsula
- Characterize the sources and composition of the organic matter (OM) in the lake sediments
- Investigate the history of methane cycling in the lakes
Figure 12. Map of the Barrow Peninsula highlighting thaw-lake topography and sampling sites.
2. Methods

Prior to sampling, all of the lake sediment samples were freeze dried for 12-24 hours (differences based on the amount of water content). Total organic carbon (TOC) measurements were performed on all lake samples used in this study in parallel with lipid biomarker analysis. Solvent extraction was performed on 23 lake samples: - 15 Fall (NW, IK and SS, October 2010) and 8 Spring samples (S3 and LQ3, April 2010) (Appendix A:). Hydropyrolysis (HyPy) was performed on the 15 fall (NW, IK and SS, October 2010) lake samples (Appendix A:). Column chromatography separates the saturated hydrocarbons from the Total Lipid Extract (TLE). GC-MS was used to obtain n-alkane distribution patterns and short/long chain length ratios, while multiple reaction monitoring (MRM)-GC-MS was used to obtain accurate sterane/hopane ratios, various hopane and sterane maturity ratios, 2–and 3- methylhopane indices and absolute yields of individual biomarker hydrocarbons. Total lipid extract derivitization utilizing BSTFA was performed on 23 lake samples: 15 Fall (NW, IK and SS, October 2010) and 8 Spring samples (S3 and LQ3, April 2010) (Appendix A:Table II). For a more detailed description of the methods used in this study see Appendix B: Methods.
<table>
<thead>
<tr>
<th>Ratio</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterane/Hopane</td>
<td>Measure of Eukaryotic (Sterane) to Bacterial (Hopane) abundance. Low ( &lt; 1) = more bacteria, ( &gt; 1) = more eukaryotes.</td>
</tr>
<tr>
<td>Short/Long Chain Length</td>
<td>Measure of Plant/Algae vs. Bacteria abundance, High ( &gt; 1) = more plant/algae, Low ( &lt; 1 ) = more bacteria.</td>
</tr>
<tr>
<td>TIC n-alkane distribution</td>
<td>Measure of relative Plant, Algae and Bacteria abundance, Bacteria produce (C14-C18), Algae produce (C16-C26 ) and plants produce (C28-C36 ) numbered n-alkanes. C+ # = number of carbon atoms.</td>
</tr>
<tr>
<td>2-Methylhopane Index</td>
<td>Measure of Cyanobacteria abundance (only useful when gammacerane is in low abundance), High values = &gt;5 %.</td>
</tr>
<tr>
<td>3-Methylhopane Index</td>
<td>Measure of Type I Methanotrophic Proteobacteria (though not all type I's produce 3-methylhopanes), High values &gt; 2%.</td>
</tr>
<tr>
<td>Archaeol</td>
<td>Produced by all archaea, can be used as a proxy for methanogen abundance.</td>
</tr>
<tr>
<td>Archaeol/nC3 1 alkane</td>
<td>Measure of archaea/methanogen abundance to terrestrial input.</td>
</tr>
</tbody>
</table>

Table 1 Biomarker interpretation table. Table of key lipid biomarkers and biomarker ratios used in this thesis and how they are interpreted (Killops & Killops, 1994; Peters et al., 2004; Love et al., 2005).
3. Results/Discussion

3.1 Lake Cores

3.1.1 Spring Cores

Two sediment cores were sampled in April of 2010 and will be referred to as the “Spring” cores for the purpose of thesis research (Figure 12).

S3 was a 38 cm core taken from the shore margin of Lake Sukok, away from any active methane bubbling prominent and towards the lake center. S3 was a mud dominated core that was distinguished only by the presence some organic “pellets” present in the muddy – silty top 4 cm. The core was cut into 4 sections: 0 – 4 cm, 4 – 12 cm, 12 – 28 cm, 28 – 38 cm.

LQ3 was a 70 cm core taken from Lake Qalluuraq, which was the southernmost lake sampled. LQ3 contained the coarsest grain sizes of the lake sediments sampled and was dominated by largely featureless sand. The major things of note were the presence of fine dark grained horizons in the 5 – 14 cm interval and the presence of some gas bubbles in the 14 – 70 cm interval. The bottom 14 – 70 cm interval had trace amounts of silt present. The core was cut into 7 sections: 0 – 6 cm, 6 – 12 cm, 16 – 24 cm, 34 – 44 cm, 44 – 54 cm, 54 – 60 cm, and the sediment was bulked and homogenized.

3.1.2 Fall Cores

Three cores that were sampled in October 2010 and will be described as the “Fall” cores (Figure 12).
SS was a 109 cm core taken near a seep site in Lake Sukok. The top 56 cm of the core was dominated by “light” colored silt and the presence of “fibrous” linear features that was likely due to movement of fluids within the core casing as ice melted. The top 56 cm was also dominated by a sandy – silty texture. The bottom 56- 109 cm was dominated by a darker colored mud/silt and the same fibrous textural feature seen the in the top 56 cm. Intervals containing abundant pebble sized clasts were present at 10 – 20 cm, 30 – 40 cm and 60 – 70 cm. The core was cut into 10 sections: 0 – 10 cm, 10 – 20 cm, 20 – 30 cm, 30 – 40 cm, 40 – 50 cm, 50 – 60 cm, 60 – 70 cm, 70 – 80 cm, 80 – 90 cm and 90 – 109 cm, and the sediment was bulked and homogenized.

NW was a 96/46 cm core taken from Lake North of Walakpa. The lake core was originally 96 cm long but upon sampling it was discovered that the top 50 cm was ice dominated and when the core was thawed for sampling all the sediment that was present in that interval became homogenized and was not suitable for sampling. It was thought that the core sampling apparatus only reached halfway into the sediment such that the sediment water interface starts at ~30 cm depth into the core. The 30 – 50 cm interval may have been composed of very loosely compacted sediment that had high water content. Sampling was conducted over the interval from 50 – 96 cm with 50 cm being re-considered the “top” of the core. This interval was dominated by silt with high levels of water at the top of the sediment that decreased with depth. The bottom 80- 96 cm interval was also characterized by an abundance of darker colored silt. The core was cut into three sections: 50 – 63 cm, 63 – 80 cm and 80 - 96 cm, with the following corrected depths assigned, 0-13 cm, 13-30 cm and 30 – 46 cm respectively.
IK was a 48/28 cm core taken from Lake Ikroavik. Similar to the NW core, the IK core was originally 48 cm long but the top 20 cm was ice dominated and unsuited for sampling. The core is characterized by a high silt content and a noticeable color difference was observed between the top (light) and bottom (dark) of the core. There were also plant root traces present in the top 10 cm of the 20 – 48 cm interval. The remaining core was cut into two sections: 20 – 35 cm and 35 – 48 cm.
Figure 13. Alaskan lake sediment cores used in this thesis which were collected in April (Spring) and October (Fall) 2010. Included here are: description of major textural features observed, sampling interval and core length for each sediment core.

3.2 Lipid Biomarkers

3.2.1 Free Extract Hydrocarbons

Solvent extraction was performed on 23 lake sediment samples: 15 Fall and 8 Spring samples (Table 2). While column chromatography separates the total lipid extract
(TLE) into three fractions: saturated hydrocarbons, aromatic hydrocarbons and polar hydrocarbons, only the saturated hydrocarbons obtained from solvent extraction were analyzed in for this thesis. Gas Chromatography-Mass Spectrometry (GC-MS) was used to obtained the carbon preference index (CPI) and short-long chain length ratio for n-alkane components while multiple reaction monitoring (MRM)-GC-MS was used to obtain accurate sterane/hopane ratios, various hopane and sterane maturity ratios, 2 –and 3- methylhopane indices and absolute yields of individual biomarker hydrocarbons.

<table>
<thead>
<tr>
<th>Season</th>
<th>Sample</th>
<th>TOC (wt%)</th>
<th>Depth (cm)</th>
<th>CPI</th>
<th>Chain Length</th>
<th>Sterane/Hopane</th>
<th>% C27 Sterane</th>
<th>% C28 Sterane</th>
<th>% C29 Sterane</th>
<th>C31 2-Methyl Hopane Index</th>
<th>C31 3-Methyl Hopane Index</th>
<th>C31 2-Methyl Hopane Yields (ng/g TOC)</th>
<th>C31 3-Methyl Hopane Yields (ng/g TOC)</th>
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</thead>
<tbody>
<tr>
<td>Fall</td>
<td>SS 0-10</td>
<td>1.03</td>
<td>5</td>
<td>9.94</td>
<td>0.01</td>
<td>0.29</td>
<td>16.7</td>
<td>26.0</td>
<td>57.3</td>
<td>2.45</td>
<td>0.19</td>
<td>0.138</td>
<td>0.011</td>
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<td>0.27</td>
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<td>25.8</td>
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<td>24.5</td>
<td>58.6</td>
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<td>25.2</td>
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<td>25.3</td>
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<td>0.13</td>
<td>0.01</td>
<td>20.3</td>
<td>19.3</td>
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<td>0.37</td>
<td>0.046</td>
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<td>0.02</td>
<td>19.9</td>
<td>16.7</td>
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<td>X</td>
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<td>29.6</td>
<td>29.3</td>
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<td>X</td>
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<td>13.8</td>
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<td>LQ3 11-15</td>
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<td>13</td>
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<td>X</td>
<td>0.07</td>
<td>11.2</td>
<td>16.6</td>
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<td>X</td>
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<td>22.2</td>
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<td>X</td>
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<td>10.60</td>
<td>0.77</td>
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</table>

Table 2. Free extractable saturated hydrocarbons biomarker data obtained for lake cores collected in the spring and fall 2010. The lake abbreviations are as follows SS = Sukok Seep, NW = North Walakpa, IK = Ikorvik, LQ3 = Lake Qalluuraq, S3 = Sukok Center Site. See Appendix B for explanation of each of the biomarker parameters.

3.2.2. Hydropyrolysis (HyPy) of whole (unextracted) sediments

HyPy was performed on the 15 Fall lake samples (Table 3). While column chromatography separates the total hydropyrolysate into three fractions, saturated hydrocarbons, aromatic hydrocarbons and polar hydrocarbons (as for TLE), again only
the saturated hydrocarbons were analyzed in detail for this thesis. The same full scan GC-MS and MRM-GC-MS methods were used as for TLE hydrocarbons.

Table 3. HyPy product saturated hydrocarbons biomarker data obtained for lake cores collected in the spring and fall 2010. The lake abbreviations are as follows SS = Sukok Seep, NW = North Walakpa, IK = Ikroavik, LQ3 = Lake Qalluuraq, S3 = Sukok Center Site. See Appendix B for explanation of each of the biomarker parameters.

<table>
<thead>
<tr>
<th>Season</th>
<th>Sample</th>
<th>TOC (wt%)</th>
<th>Depth (cm)</th>
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<th>Chain Length</th>
<th>Sterane/Hopane</th>
<th>% C27 Sterane</th>
<th>% C28 Sterane</th>
<th>% C29 Sterane</th>
<th>C31-2-Methyl Hopane Index (%)</th>
<th>C31-3-Methyl Hopane Index</th>
<th>C31-2-Methyl Hopane Yields</th>
<th>C31-3-Methyl Hopane Yields</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall</td>
<td>SS 0-10</td>
<td>1.03</td>
<td>5</td>
<td>0.20</td>
<td>9.75</td>
<td>3.42</td>
<td>17.88</td>
<td>5.4</td>
<td>17.8</td>
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<td>0.09</td>
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<td>0.32</td>
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<td>17.86</td>
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<td>7.2</td>
<td>19.5</td>
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<td>0.36</td>
<td>3.95</td>
<td>31.04</td>
<td>5.1</td>
<td>19.4</td>
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<td>SS 50-60</td>
<td>0.77</td>
<td>55</td>
<td>0.30</td>
<td>13.12</td>
<td>18.62</td>
<td>10.6</td>
<td>23.2</td>
<td>86.2</td>
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<td>65</td>
<td>0.40</td>
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<td>3.6</td>
<td>17.9</td>
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<td>75</td>
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<td>18.83</td>
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<td>0.41</td>
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<td>56.23</td>
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<td>18.3</td>
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<td>0.42</td>
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<td>38</td>
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<td>3.15</td>
<td>11.9</td>
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<td>11.2</td>
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<td>8.97</td>
<td>0.89</td>
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3.3 Total Organic Carbon (TOC) content of sediments

The Total Organic Carbon (TOC) values (Figure 14) for the Spring and Fall lake sediments display a wide range of values from ~0.5 wt. % to 14 wt. % on a dry sediment basis. The majority of the lake cores, particularly SS, LQ3 and IK, contain sediments with TOC content below 5 wt.%. Only one core, S3, yielded sediments across all sampled depth horizons with TOC 5 wt.% in all cases while NW yielded bulk sediment spanning this range from 14 wt.% to 1 wt.% with TOC contents which decreased dramatically with depth.

The amount of organic carbon in lake sediments is important in the respect that it ultimately provides the organic substrates needed for methanogenesis (following other anaerobic decomposition reactions which yield carbon dioxide or acetate), with the
lability or “freshness” of the organic matter also being an important factor. TOC content is affected by a number of factors including: modes of organic matter deposition; (both allochthonous and autochthonous); diagenesis, which electively removes organic matter through microbially mediated remineralization; mineral matter flux which dilutes organic matter content; and/or some combination of all of them. Since TOC source and composition are important factors influencing the potential for sedimentary towards methanogenesis, subsequent sections will examine the evidence for allochthonous (transported and less labile) vs. autochthonous (in situ produced, and generally more labile) sedimentary organic matter contributions.

![Figure 14: TOC content (wt. % of dry sediments) versus core depth for Spring (LQ3, S3) and Fall (SS, NW, IK cores). The lake abbreviations are as follows SS = Sukok Seep, NW = North Walakpa, IK = Ikroavik, LQ3 = Lake Qalluuraq, S3 = Sukok Center Site.](image-url)
3.4 Bacterial / Eukaryotic Inputs

3.4.1 Sterane/hopane ratio

The sterane/hopane ratio (a measure of eukaryotic vs. bacterial input), for both the free extract hydrocarbons and the catalytic hydropyrolysis (HyPy) product hydrocarbons is a useful but broad way of characterizing the major sources of sedimentary organic matter. It should be noted that the free hydrocarbons that can be extracted with organic solvents are more susceptible to organic source biases than the HyPy product fractions due to the small overall quantitative contribution free extracts hydrocarbons make to the total organic matter. Saturated hydrocarbons in HyPy products generated from whole sediments will contain contribution from free hydrocarbons (minor) as well as from hydropyrolysis of both free and macromolecule-bound polar lipids generated by reductive removal of functional groups and conversion into hydrocarbons (Appendix A: Table II).

All of the free hydrocarbon fractions from each core generated sterane/hopane ratios of less than 0.50, with a majority; S3, IK, and NW plotting less than 0.10 and only SS plotting completely above 0.10 (Figure 15). Low sterane/hopane ratios are often found for freshwater lacustrine environments, and more generally this indicates either a significant amount of soil bacteria washing into the lake or a large aquatic bacterial biomass growing within the lake, or some combination of both (Peters et al., 2004).
Figure 15. Sterane/hopane ratios calculated for saturated hydrocarbon fractions from different cores obtained by solvent extraction (top left) and HyPy (bottom left) of whole sediments. Since HyPy of SS core generated such high sterane/hopane ratios, the HyPy-derived sterane/hopane are plotted alone for IK and NW (bottom right panel). A TOC content figure is also included (top right) for comparison. See Methods for how sterane/hopane ratio was calculated. The lake abbreviations are as follows SS = Sukok Seep, NW = North Walakpa, IK = Ikroavik, LQ3 = Lake Qalluuraq, S3 = Sukok Center Site.

Hopanes in the free extract of the SS core display a more thermally mature signal, in terms of the diastereoisomeric patterns throughout the entire core, than NW, IK, S3 and LQ3. This is most likely due to anthropogenic inputs from fuel oils resulting in a slight contamination effect from ancient mature hopanes in the case of the SS core, although other compound classes such as the n-alkanes and HyPy products are only marginally affected (Appendix A: Table III & IV). Though not as abundant as the n-alkanes, the free
hopane signal easily swamps out any (free) eukaryotic sterane signal deposited in the lakes (Figure 15). The SS core shows the highest sterane input across all the lake cores compared to the rest of the free lipid samples. Some SS sterane/hopane values approach 0.50. The absolute difference between SS and the rest of the cores is still reasonably small compared to the range of HyPy-generated sterane/hopane values from whole sediment (Figure 15).

The sterane/hopane ratios generated from the saturated hydrocarbons of the whole sediment HyPy products should theoretically yield more accurate representation of the total eukaryotic vs. bacterial input to sediments. Bound and polar lipids, which cannot be analyzed using a standard GC and constitute a much larger fraction of the total organic matter compared to the free saturated hydrocarbons, are converted into saturated hydrocarbons during HyPy procedure. Care must be taken during the HyPy method to insure no loss of whole sediment before and perhaps more importantly during the HyPy process, which will result in inaccurate HyPy product yield calculations.

The HyPy generated sterane/hopane values are consistently higher for all samples when compared to the free extracts (Table 2 & 3), with sterane/hopane ratios generally very high (>>1, with some values approaching 60), particularly for the SS core (Figure 15). The strong bacterial hopane signal that was typically found in the free saturated hydrocarbons fraction has become completely swamped in HyPy products by a eukaryotic signal from the polar and bound lipid fractions, particularly from the SS core (Figure 15). SS core also shows large scatter superimposed on a general increase in
sterane/hopane ratio down core, possibly representing changing conditions that favor green algae and/or higher plant matter input against bacterial input. IK and NW samples show no strong trend down-core and generate values from HyPy between 1.0 and 3.0, characteristic of moderate eukaryotic to bacterial input, ratios comparable to values typically reported from the ancient rock record (between 0.5 and 2.0 for ancient Phanerozoic marine rocks). The highest (>>5) sterane/hopane values for SS core are most likely the result of unintentional selection of eukaryotic material (benthic green algal or plant fragments for HyPy), perhaps as a result of particle size sieving, which would result in an anomalously strong sterane signal. The consistently lower sterane/hopane ratio values seen in NW and IK compared to SS in the free lipids is also observed in the HyPy products (Figure 15; Table 2 & Table 3). The high sterane/hopane signal seen in the HyPy products may be due to two separate, but possible factors: from a relatively large input of terrestrial plant material, or elevated algal growth in the lake (benthic or planktonic) at the time of sediment deposition, or a combination of both. The potential sources will be explored in the coming section.

All the sediment samples subjected to HyPy generated a C29 sterane predominance as revealed in the C27,C28 and C29 ternary diagram (Figure 16) indicating that the majority (>60%) of all steranes analyzed from hydropyrolysis are coming from either land plants or green algae. Only a moderate input (~20%) of C28 steranes was produced by HyPy, with the most likely sources of these compounds coming from fresh water diatoms living in the lakes (Figure 16).
The high sustained levels of eukaryotic input (particularly transported plant material) may lead to higher TOC levels over time due to accumulated plant material preferentially being preserved due to resistance to diagenesis. However, the higher sterane/hopane ratios observed in the SS core HyPy products did not correspond to the highest TOC content levels measured. In fact, the opposite trend is observed; with the highest TOC levels found (S3 and NW in particular) are those with the lowest sterane/hopane signal (Figure 15; Table 2 & 3). The highest TOC sediments also generated high 2-methylhopane indices (see section 3.5), consistent with a higher microbial contribution to preserved sedimentary organic matter. Due to the elevated sterane signal seen in the lake sediments, it becomes important to discriminate between likely sterane source(s) as this will help to resolve whether the organic matter present in the lakes is predominantly
allochthonous (terrestrial plant/soil transported into the lake) or, autochthonous (from microbial primary production within the lake).

3.4.2 \textit{n}-Alkane distribution patterns

Figure 17. Total Ion Chromatograms (TICs) for saturated hydrocarbons from HyPy products (left) and free extracts (right) for selected samples from NW (0-13) and IK (109-90) sediment cores. The dots and numbers denote the carbon chain length of \textit{n}-alkanes and a predominance of even numbered \textit{n}-alkanes for the HyPy products and odd numbered \textit{n}-alkanes for the free extracts is apparent, as expected. Pristane (Pr) and Phytane (Ph) are peaks which elute immediately after the C\textsubscript{17} and C\textsubscript{18} \textit{n}-alkanes respectively and are labeled in the HyPy products with solid triangles.

Discriminating between the major C\textsubscript{29} sterane sources (green algae vs. plant) can be achieved by comparing the \textit{n}-alkane chain-length pattern generated from both HyPy products and free lipid extracts (Figure 17).

Land plants are characterized by typically longer chain-lengths than green algae, (C\textsubscript{28}-C\textsubscript{36} for plants \textit{versus} C\textsubscript{16}-C\textsubscript{26} for algae), which have more abundant mid- and short ranged
length straight chained hydrocarbons (Love et al., 2005). There is overlap for some species of freshwater green microalgae which produce linear chain lipid hydrocarbon cores extending up to C$_{30}$ and beyond, but still with a shorter average chain length and broader range (C$_{14}$-C$_{30}$) than for plant waxes. Using the total ion chromatogram (TIC) for the HyPy products, we see abundant mid-chain length n-alkanes, suggestive of algae sources for both NW and SS but with NW displaying a greater proportion of possible land plant signal (abundant n-alkanes > C$_{28}$) compared to SS, which displays a greater microbial signal (C$_{14}$-C$_{20}$) n-alkanes (Figure 17). Even if we assume that very long chain n-alkanes in HyPy products (>C$_{28}$) are produced exclusively by terrestrial plant waxes/soils, we can still conservatively estimate that >50% of the alkanes produced are derived microbial sources (algae plus bacteria) which can be used as a rough estimate for organic source input. Given the high sterane/hopane ratios generated from HyPy and the C$_{29}$ sterane dominance observed for all samples, then green algae probably make a major contribution to this microbial organic matter which would have been produced in-situ in the lake (planktonic or benthic). This is a major finding of this study.

The HyPy product hydrocarbons have a high (>>1) short/long n-alkane chain ratio particularly those from SS which display values >15, corresponding to a high abundance of short chain n-alkanes. NW and IK display the lowest values of ~0.6 – 1.0 or a high abundance of longer chain n-alkanes (Figure 18 and Table 3). The lower short/long chain length values seen for NW and IK may represent a mixed signal of plant and green algae. This can also be observed in the more abundant longer chain-length n-alkanes in the NW HyPy product TIC (Figure 18). The mixed signal may be a result of lake margin
proximity, with NW and IK cores being spatially closer to the lake edge, which should display a greater land plant signal than SS core. The free extract short/long ratio values are much lower than those from the HyPy products, (all < .015) for all free lipid samples, consistent with an abundance of longer chained \( n \)-alkanes and terrestrial input in this fraction (Figure 18). This likely due to the source bias of the free hydrocarbons for immature sediments, with recalcitrant plant-derived material being particularly abundant compared to the HyPy products.

![Graph](image)

Figure 18. Short/long chain length \( n \)-alkane ratios (\( nC_{16-20}/nC_{27-31} \)) for SS, NW and IK sediment cores from TIC’s of the saturated hydrocarbon fractions generated from free extracts (left) and hydropyrolysis of whole sediments (right).

3.5 2-Methylhopanes (Cyanobacteria or Proteobacteria)

Along with an abundant \textit{in situ} green algal signal, evidence for an abundant cyanobacterial organic input signal was found for several of the lakes. This is based on high values (>5 %) 2-methylhopane index found for these samples (Figure 19 & Table 2) and calculated for C\(_{31}\) 2-methylhopane versus the summed C\(_{30}\) (\( \alpha \beta + \beta \alpha \) and \( \beta \beta \)) hopanes.
2-Methylhopanoids can be produced as major lipids by cyanobacteria and some soil proteobacteria. The finding of extremely low gammacerane abundance relative to hopanes (<1%) in all samples, gammacerane being the basic hydrocarbon skeleton of tetrahymanol which is abundant in the soil proteobacteria that also produce 2-methylhopanoids, indicates that the 2-methylhopanes present in our lake sediments were most likely derived from cyanobacteria.

A wide distribution of C₃₁ 2-methylhopane index values are observed among all the lake cores sampled, particularly for the free lipids which range from 0.6% to >12% (Figure 18). The free 2-methylhopane index values can be separated into two groups; those with a “high” C₃₁ value (>5%) and those with a “low” or “average” C₃₁ value (<5%). In general, TOC levels corresponded with higher 2-methylhopane values, (particularly S3 and top of NW), though a subset of low TOC sediments (samples from LQ3, IK and the bottom of NW) also generated high values for 2-methylhopane index Figure 19).

Figure 19. 2-Methylhopane index (%) for the free extracts, vs. core depth (left) and vs. TOC (wt. %) (right). See Methods for how 2-methylhopane index is calculated. The lake abbreviations are as follows SS = Sukok Seep, NW = North Walakpa, IK = Ikravik, LQ3 = Lake Qalluuraq, S3 = Sukok Center Site.
The HyPy generated 2-methylhopane indices on a smaller set of samples than for free extract show a similar trend, with NW and IK core sediments having consistently higher values compared to SS. There is also a similar attenuated relationship between TOC and 2-methylhopane indices, with higher ratio values coinciding with higher TOC amounts (Figure 20 and Table 3). The absolute values and range of values for the HyPy-generated 2-methylhopane indices is lower (9% is highest for HyPy vs. 12% for extracts). Overall, the 2-methylhopane indices found for free extracts and HyPy products suggest that cyanobacteria appear to have made a significant contribution to several of the lake sediment cores (S3, NW, IK and LQ3), adding to the evidence that in situ lake production of organic matter was/is important.

**Figure 20.** 2-methylhopane index (%) for the HyPy products from whole sediments saturated hydrocarbon fraction vs. depth (left) and vs. TOC (wt. %) (right). See Methods for how C$_{31}$ 2-methylhopane index is calculated. The lake abbreviations are as follows SS = Sukok Seep, NW = North Walakpa, IK = Ikroavik, LQ3 = Lake Qalluuraq, S3 = Sukok Center Site.

There are a variety of factors, however, that can artificially raise or lower the 2-methylhopane index, such as the C$_{30}$ hopanoid and C$_{31}$ 2-methylhopane biomarkers that
are used in the calculation having multiple potential sources which could feasibly originate both inside and outside of the lake. By normalizing the absolute 2-methylhopanoids abundances to the TOC content of sediment analyzed, it is possible to have a measure of 2-methylhopane significance that should operate independently of, but is complementary to, the 2-methylhopane index. The free C$_{31}$ 2-methylhopane absolute yields (ng/g TOC sediment), show similar trends as for the free lipid C$_{31}$ 2-methylhopane index (comparing Figure 19 & Figure 21). The difference between the highest (NW, IK and S3) and the lowest (SS and LQ3) yields is almost a factor of 1000, with the highest absolute amounts detected in the highest TOC samples.

![Figure 21. C$_{31}$ 2-methylhopane yields (ng/g TOC) for the free saturated hydrocarbon fraction. See Methods for how C$_{31}$ 2-methylhopane yields are calculated. The lake abbreviations are as follows SS = Sukok Seep, NW = North Walakpa, IK = Ikroavik, LQ3 = Lake Qalluuraq, S3 = Sukok Center Site.](image)

The two dominant sources of organic matter into the lake appear to be allochthonous material transported in at the margins (predominantly terrestrial plant and soil), and in situ primary production from algae and bacteria. Evidence for significant lake primary production is evidence by a number of molecular biomarker parameters, particularly high
2-methylhopane indexes/yields (cyanobacteria) in several of the lakes (S3, NW, IK, parts of LQ3), abundant low mid-chain \( n \)-alkanes seen in HyPy products (most likely coming from bacteria and algae, respectively), and high sterane/hopane ratios coupled with high short/long chain \( n \)-alkane ratios generated from HyPy of whole sediments. This level of lake productivity is in keeping with other work on microbes associated with primary production in small lakes in the Arctic and Antarctic (Hecky and Hesslein, 1995; Bonilla et al. 2005). Abundant sedimentary autochthonous organic matter should have a noticeable effect on the methane cycling occurring within the lakes, as the higher lability (and hence reactivity) of organic matter available for anaerobic decomposition and production of carbon dioxide and acetate substrates derived should result in higher levels of methanogenesis in freshwater settings (Hecky and Hesslein, 1995).

### 3.5 Methane Cycling

There are three main components of the microbial methane cycle: aerobic methane oxidation, anaerobic methane oxidation and methanogenesis.

#### 3.5.1 Aerobic Methane Oxidation

\( C_{31} \) 3-methylhopanoids are produced almost exclusively by restricted groups of Type I methanotrophic proteobacteria and acetic acid bacteria (Farrimond et al., 2004). The presence of 3-methylhopane and their abundance relative to generic \( C_{30} \) hopane (the \( C_{31} \) 3-methylhopane index) is therefore a proxy for the extent of past aerobic methanotrophic activity by these bacterial clades.
The total range of the free lipid C\textsubscript{31} 3-methylhopanones values is low (<1%) with a majority of data under 0.5% (Figure 22 and Table 2), indicative of low to modest levels of aerobic methane oxidation in the lakes by Type I methanotrophic bacteria. Similarly, the 3-methylhopane index from the HyPy products also display low range of values (<1%) although IK and NW have higher values compared to their free lipid counterparts. This is in contrast to typical 3methylhopane indices reported for rock bitumens from the 1.64 Ga Barney Creek Formation (Brock et al., 2005), thought to be a Proterozoic restricted basin, that preserves values from 3-5%, indicative of high levels of aerobic methane oxidation. While Barney Creek is a Precambrian rock formation, the 3-methylhopane values obtained should represent “elevated” methane oxidation levels most likely in the water column during sediment deposition. Despite the high ebullition rates seen at the seep sites SS and LQ3, these lakes do not show higher 3-methylhopane
indices than sites not near an (obviously) active seep (IK, NW, and S3). However, not all groups of Type I methanotrophic bacteria produce 3-methylhopanoids and Type II methanotrophic bacteria don’t produce any 3-methyls at all. Hence, the 3-methylhopane index may not reflect the total aerobic methanotrophic bacterial biomass deposited in the lake sediments, that the parameter is under-representing their abundance and activity.

3.5.2 Anaerobic Methane Oxidation (AOM)

There are several acyclic isoprenoids that conventionally indicate archaea: PMI, crocetane and biphytane. These are often sourced by archaea involved in anaerobic methane oxidation, particularly when these lipids are abundant relative to all other hydrocarbon components (Birgel et al, 2008). These compounds are confirmed as being sources, at least partially, from anaerobic methane oxidizing archaea when these have extremely $^{13}$C-depleted stable carbon isotopic signatures ($<-40\%$).

PMI, crocetane and biphytane were not positively identified in the lake sediments. Using two TOC end members; NW and SS, we note the absence of these compounds by comparing our TICs we obtained with published literature (Figure 23). We believe that anaerobic methane oxidation can still be occurring in the lake sediment; but could simply be occurring deeper than the lake cores and/or that microbes preforming anaerobic methane oxidation are present in such small quantities below the level of detection. Due to the low sulfate levels present in these fresh water systems, a required oxidant for anaerobic methanotrophy, the more likely explanation is that they were in low abundance rather than thriving deeper in the sediment core than the intervals which were sampled.
Figure 23. Total Ion Chromatograms (TIC) of free saturated hydrocarbons for selected samples from NW (0-13) and SS (109-90) sediment core. TICs highlight absence of compounds associated with anaerobic methane oxidation, including crocetane, PMI and biphytane in both TOC-rich (NW) and TOC-poor (SS) samples. There is a prominent odd over even \( n \)-alkane abundance (consistent with \( n \)-alkanes extracted from free extracts). Numbers refer to carbon chain lengths of some of the first of the most prominent \( n \)-alkanes detected.

3.5.3 Deciphering a Possible Biomarker for Methanogenesis

Archaeol is a compound produced by a diverse range of archaea, while not all archaea are methanogens, it has been used as a proxy for tracking methanogen abundance through time (Pancost et al., 2011). Genomic work conducted on these lake sediments by our collaborators at UNLV has shown a diverse range of methanogenic archaea in the lake.
sediments, even at shallow depths of 5 cm and below (Matheus, 2012). Because genomic techniques used are not quantitative, the archaeal DNA signals detected diversity does not necessarily equate to the relative abundance of different archaeal clades. Fortunately, we can use archaeol lipid abundance in lake sediments to assess changes in the standing biomass of archaea with depth in each lake core. In general, archaeol abundance was low, ranging from 0.01 to 0.18 % relative to $n$-C$_{31}$ alkane (a proxy for continental plant waxes as a ubiquitous background signal in each lake), reflecting a small archaeal, and by extension, past and/or present methanogenic activity in the lake sediments (Figure 24 and Figure 25). Across the whole sample set, there also appears to be a broad TOC control on archaeol content as seen by higher archaeol abundance corresponding to elevated sedimentary TOC contents (Figure 24). All the samples obtained from the NW core displayed high archaeol abundance, whereas TOC quickly decreases down core, with lowest sample having TOC levels close to that of SS (~1%) (Figure 24). This decrease in TOC, coupled with high archaeol abundance, may reflect a heavy microbe presence quickly remineralizing the available labile organic matter deposited in shallow sediments.
Figure 24. Ratio of Archaeol / $C_{31}$-alkane from total lipids extracts (TLEs) for all sediments analyzed as a function of TOC content (left) and sediment core depth (right). See methods for specifics of the ratio, but it approximates the archaea biomass abundance (archaeol) with respect to terrestrial plant wax input ($n$-$C_{31}$ alkane). The lake abbreviations are as follows SS = Sukok Seep, NW = North Walakpa, IK = Iakoavik, S3 = Sukok Center Site.

Figure 25. Total ion chromatograms from full scan GC-MS analysis of BSTFA-derivatized total lipid extracts highlighting archaeol location and abundance from selected samples from NW (13-30) and SS (109-90). The abundance of archaeol from a TOC rich sample (NW 13-30) and a TOC poor sample (SS 109-90) is highlighted (right box). Archaeol elutes at ~97 minutes and is prominent in the NW sample (A) while it is only found in trace amounts near detection limits for SS sample (B).
3.6 Interpretation and Wider Implications

3.6.1 Assessment of the Efficacy of the Combined HyPy and Free Lipid Molecular Biomarker Approach.

Utilizing more than one analytical technique for accessing lipid biomarker molecules allows for a more complete and synergistic view of the lipid biomarker record in thaw lakes. Analyzing the saturated hydrocarbons from the free lipid fraction involves a relatively simple technique and these compounds can contain a lot of useful information about the source organism input into the sediment. Care must be taken in the use of these lipids as they only represent a small fraction of total organic matter and can easily be influenced by outside sources of contamination, and free hydrocarbon fractions are most susceptible to these biases. Hydropyrolysis is a very powerful technique that allows for the analysis of bound kerogen material and highly polar lipids, which allows a more complete picture of relative lipid abundance in sediment to be determined from saturated hydrocarbon product profiles and, therefore a more accurate measure of the source organisms contributing organic matter to the sedimentary organic matter. However, hydropyrolysis is more methodically challenging and required a specialized high pressure apparatus to carry out the procedure and can occasionally give anomalous information if the sample is heterogeneous with respect to large fragments of lipid-rich organic matter (e.g. algal fragments/plant litter) as found for SS core.

Chemical treatment of TLE using silylation-derivatization is an important technique that allows for the analysis of a portion of the polar compounds that would normally be non-
amenable to run on a standard GC-MS; although highly polar molecules are still not observed and require liquid chromatography techniques to be detected. Archaeol is a compound that cannot be detected directly with a normal GC-MS with prior silylation, the core phytane (hydrocarbon)-skeleton can be detected via hydropyrolysis. However, by derivatizing it with BSTFA, it can be detected relatively intact and is GC- amenable. The benefit of derivatization is that it allows for the rapid screening of some polar compounds as well as looking at a higher proportion of TLE constituents as opposed to just a small fraction. The major logistical issue with performing large batches of derivatized TLE analyses is that the BSTFA reagent degrades the stationary phase and hence shortens the lifespan of the GC column. There is large diversity of molecular information present in the TLE which allows for a broader interpretation of the free lipid biomarkers than by just considering hydrocarbons, however due to the complex abundance of compounds present in the TLE, effects of co-elution and bias against the more polar forms of lipid compound classes can make interpretation complicated.

3.6.2 Integration of Lipid Biomarker Results with Microbial Ecology and Previous Studies.

The results from this study, including the trend we observed whereby sediments with higher TOC content generally yielded higher archaeol abundances, are broadly consistent with the methane flux microcosm experiments carried out by our collaborators at UNLV. In situ stimulation of methanogenic activity in different core horizons in experiments at UNLV, found that the greatest fluxes of methane were produced from NW core, which had the greatest TOC content and the highest archaeol abundances found in this study.
Furthermore, they found that SS core yielded the lowest methane flux emission rates, also consistent with these having the lowest archaeol and TOC results found (Matheus, pers. comm., 9 July 2012).

Though genomic work found a high diversity of archaea living in the lake sediments, 21 (Matheus et al., 2011): archaeol, and thus by inferences the amount of archaeal biomass was only found in low abundance in this study. Elvert et al. (2011) used lipid biomarkers to investigate methane cycling on the North Slope of Alaska (Elvert et al., 2011). They used radiocarbon dating, compound-specific $\delta^{13}$C isotopic analysis and a lipid biomarker proxy for estimating soil temperatures to characterize paleoclimate and methane cycling rates on the North Slope of Alaska. From analysis of an extended Lake Qalluuraq (Lake Q) core, they found a period of elevated (>2°C) temperatures coinciding with abundant and $\delta^{13}$C depleted hop-17(21)-enes (produced, at least partially by aerobic methanotrophic bacteria), indicative of increased methane flux and turnover. Unfortunately, radiocarbon dates have not yet been obtained for our samples so it is difficult to compare our results to those of Elvert et al. (2011) at this stage, but this will be done in due course.

3.6.3 Sources of Methane in the Lakes

The only lipid biomarker detected in this study which could address the magnitude of past or present methanogenic activity, and by inference the methane flux generated in each lake sediment sample was archaeol (C$_{43}$ diether compound produced by diverse archaea, including methanogens) relative abundance ratio. Since archaeol abundance
relative to C_{31} n-alkane (a proxy for terrestrial plant background organic signal) is used as a rough proxy for methanogenic archaeal biomass abundance, it is then reasonable to assume that methane from lake sites with higher archaeol abundance are likely associated with higher fluxes of biogenic methane in the sediment column. Conversely, lake sites that display high methane flux rates (as measured, or as gauged from bubble counts through the water column) but a low archaeol abundance, likely have a greater contribution of thermogenic methane. Based on this rationale, then NW and S3 core sites likely coincide with significant represent sources of biogenic methane due to their high archaeol abundance, IK represents a more mixed biogenic/thermogenic methane source or a lower biogenic methane flux while methane emanating as bubbles through the water column at SS site is most likely to be thermogenic in nature. Certainly, radiocarbon dates for methane emitted above SS core site supports a predominantly thermogenic source for the gas flux here (Katey Walter, pers. Comm.), consistent with only finding trace amounts of archaeol in SS sediment extracts. SS sediment core also displays a slight increase in archaeol abundance ~80 cm into the core (Figure 24). Whether this corresponds to the same period of increased temperatures and methane cycling as proposed by Elvert et al. (2011) for Lake Q cannot be determined at this stage without radiocarbon dates for correlation for our lake cores. Stable carbon isotope data (δ^{13}C) on the methane emanating from the sediments and through the water column in the lakes would be the best way to test the biogenic/thermogenic origin of the methane. Archaeol abundance should represent some first order approximation of biogenic methane emission flux as proposed here.
3.6.4 Methane Cycling in Alaskan Thermokarst Lakes and Environmental Change

The heterogeneities observed between the lakes, particularly the varying TOC levels and primary production signals; suggests that methane emission rates differ in response to the predicted warming and increases in precipitation at high latitudes. This is related to observed relationships between archaeal/methanogen abundance, primary production and TOC levels affecting lake nutrient levels on biogenic methane production. Thus, any change to the environment that increases the lake nutrient levels, will likely lead to an increase in methanogen abundance and biogenic methane production. However, the relationship between lake nutrients and changing climate is poorly understood so it is difficult to determine how nutrient levels, will also change with the climate.

3.6.5 Suggestions for Future Investigations

Future work on Alaskan thaw lakes that would expand on the findings of this thesis include:

i) A nutrient profile for all of the lakes using inorganic geochemistry, looking at major and trace element composition of the lakes and sediment pore waters using ICP-MS techniques. This may help explain TOC differences seen between lakes sediments as those with higher concentrations of key bioessential elements nutrients might reflect higher past lake productivity and thus be responsible for higher TOC contents.
ii) Analysis of the organic matter composition of soils in the lake catchment area to identify diagnostic soil lipid compounds for quantifying soil contributions to preserved sedimentary organic matter in lake cores. For example, soil analyses could be used to gauge if soil hopanoids made a significant contribution to the biomarkers in the lake sediments or if the hopanoids found in the free lipid fraction of sediment cores were derived mainly from *in-situ* lake production.

iii) The use of compounds specific stable carbon ($\delta^{13}C$) isotopic analysis could determine if key individual biomarker lipid compounds are sufficiently $\delta^{13}C$ depleted ($<-40\%o$) to have been produced, at least in part, by methane-oxidizing microbes utilizing biogenic methane.

iv) Performing radiocarbon dating on the sediments to help constrain absolute ages of organic matter deposition and sedimentary depositional rates.

v) Increasing sampling density and core depth for all future lakes cores garnered would be advisable in order to have a more complete understanding of how TOC content and lipid biomarker composition varies with depth, as well as allowing intra and inter-lake comparisons.

vi) Metagenomics investigations of the lake waters, sediment cores and the surrounding soils would better characterize the microbial communities involved in the ecosystems in the lake-catchment area.
4. Conclusions

This project characterizes changing microbial communities and sedimentary organic matter composition in sediment cores from several Alaskan thaw lakes, with a particular focus on analyzing lipid compounds derived from microbes involved in methane cycling. Organic matter composition is not uniform across the lakes sampled with contributions from both \textit{in situ} production and terrestrial material being present. The high levels of primary productivity in the lakes are consistent with other studies on arctic lake productivity. HyPy of whole (unextracted) sediments was a novel approach used in this study that showed that a significant proportion of the sedimentary organic matter was produced by microbial biomass (algae and bacteria) within the lake, and circumvents source biases (particularly with respect to over-estimating terrestrial plant contributions) associated with only looking at the small amounts of free hydrocarbons in the sediments.

Even assuming that the very long chain n-alkanes in HyPy products (>C\textsubscript{28}) from whole sediments were generated exclusively by terrestrial plant waxes/soils, then a conservative estimate of >50% of the alkanes produced by HyPy were derived from microbial sources (algae plus bacteria). Thus, >50% is a rough but conservative estimate of the fraction of the preserved sedimentary organic matter which was sources by in-situ microbial primary production. Given the high sterane/hopane ratios generated from HyPy and the C\textsubscript{29} sterane dominance observed for all samples, then green algae probably make a major contribution to this microbial organic matter which would have been produced \textit{in-situ} in the lake (either as planktonic or benthic biomass. Autochtonous sedimentary organic
matter provides a labile organic substrate which is more reactive to anaerobic diagenetic degradation by sedimentary microbes, compared with transported land plan biomass, and hence is important in producing feedstocks for fueling later sedimentary methanogenesis.

Lipids produced by microorganisms involved in methane cycling were detected, but were generally found in low relative and absolute abundance. The very low amounts of methane oxidation biomarkers found in the active seep sediments (SS and LQ3) were surprising, though it is possible that the methanotrophs present may not actually produce 3-methylhopanoids and thus their abundance is being underrepresented. The lack of biomarkers associated anaerobic methane oxidation, below detection limits, may be due to the low sulfate levels that are generally associated freshwater systems. The higher relative abundance of the C_{43} archaea biomarker, archaeol in total lipids extracts in high TOC sediments is consistent with the hypothesis idea that greater labile organic substrate availability leads to higher rates of methanogenesis. The future methane emission rates of these lakes is ultimately tied to the type and amount of organic matter deposition and preservation that occurs within the sediment beneath the lakes. Environmental factors, including precipitation rates, ambient temperatures increases and lake chemistry (controlling nutrient balance) will help control the amounts of in-situ lake primary production, which will change seasonally and over longer timescales. If any changes in these parameters leads to a net increase in organic matter deposition then it is likely possible that the methane emissions rates will also increase, although environmental effects on methane oxidation rates (the main microbial process which consumes methane)
must also be taken into account when predicting regional greenhouse gas emission budgets.
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### Appendix A - Tables

#### Table I – Chromatography Amounts for Free Lipids, Hypy products and Derivatized TLEs (BSTFA)

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<tr>
<th>Season</th>
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<td></td>
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<td>Total TLE Amount (mg)</td>
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#### Table II – Chromatography Yields for Free Lipids and Hypy products

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<th>Aliphatic Hydrocarbon Fraction (mg/g dry lake sediment)</th>
<th>Aromatic Fraction (mg/g dry lake sediment)</th>
<th>Total TLE (mg/g dry lake sediment)</th>
<th>Aromatic Hydrocarbon Fraction (mg/g dry lake sediment)</th>
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Table III - Maturity Parameters for Free saturated hydrocarbons for cores collected in October (Fall) 2011

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<td>0.27</td>
<td>0.35</td>
<td>0.22</td>
</tr>
<tr>
<td>Fall</td>
<td>IK 0-15</td>
<td>0.30</td>
<td>0.10</td>
<td>0.17</td>
<td>0.28</td>
<td>0.24</td>
</tr>
<tr>
<td>Fall</td>
<td>IK 15-28</td>
<td>0.31</td>
<td>0.13</td>
<td>0.19</td>
<td>0.25</td>
<td>0.23</td>
</tr>
<tr>
<td>Spring</td>
<td>LQ3 1 - 4</td>
<td>0.46</td>
<td>0.08</td>
<td>0.22</td>
<td>0.40</td>
<td>0.43</td>
</tr>
<tr>
<td>Spring</td>
<td>LQ3 6-10</td>
<td>0.30</td>
<td>0.20</td>
<td>0.19</td>
<td>0.25</td>
<td>0.24</td>
</tr>
<tr>
<td>Spring</td>
<td>LQ3 11-15</td>
<td>0.40</td>
<td>0.26</td>
<td>0.24</td>
<td>0.25</td>
<td>0.19</td>
</tr>
<tr>
<td>Spring</td>
<td>LQ3 60 - 64</td>
<td>0.21</td>
<td>0.43</td>
<td>0.06</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Spring</td>
<td>S3 0-4</td>
<td>0.28</td>
<td>0.07</td>
<td>0.16</td>
<td>0.29</td>
<td>0.27</td>
</tr>
<tr>
<td>Spring</td>
<td>S3 4-12</td>
<td>0.27</td>
<td>0.06</td>
<td>0.16</td>
<td>0.27</td>
<td>0.25</td>
</tr>
<tr>
<td>Spring</td>
<td>S3 12-28</td>
<td>0.38</td>
<td>0.08</td>
<td>0.15</td>
<td>0.32</td>
<td>0.37</td>
</tr>
<tr>
<td>Spring</td>
<td>S3 28-38</td>
<td>0.28</td>
<td>0.05</td>
<td>0.10</td>
<td>0.28</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Maturity Parameters for Free Extract hydrocarbons for cores collected in April, 2010 and October 2010. See Table 1 for Season and Sample column explanation. Columns 3-7 are maturity parameters calculated using the Free Extract Saturated Hydrocarbons, see Appendix B: Methods for explanation of each parameter.

Table IV - Maturity Parameters for Hydropyrolysis saturated hydrocarbons for cores collected in October (Fall) 2010

<table>
<thead>
<tr>
<th>Season</th>
<th>Sample</th>
<th>Sterane S/S+R</th>
<th>Hopane S/S+R</th>
<th>Sterane aaa R/S</th>
<th>Sterane abb/aaa</th>
<th>Sterane dia/reg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall</td>
<td>SS 0-10</td>
<td>0.49</td>
<td>0.33</td>
<td>0.09</td>
<td>0.45</td>
<td>0.40</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 10-20</td>
<td>0.43</td>
<td>0.26</td>
<td>0.07</td>
<td>0.43</td>
<td>0.19</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 20-30</td>
<td>0.47</td>
<td>0.32</td>
<td>0.08</td>
<td>0.45</td>
<td>0.36</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 30-40</td>
<td>0.44</td>
<td>0.26</td>
<td>0.09</td>
<td>0.41</td>
<td>0.24</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 40-50</td>
<td>0.46</td>
<td>0.28</td>
<td>0.10</td>
<td>0.38</td>
<td>0.46</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 50-60</td>
<td>0.30</td>
<td>0.34</td>
<td>0.15</td>
<td>0.41</td>
<td>0.38</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 60-70</td>
<td>0.43</td>
<td>0.24</td>
<td>0.07</td>
<td>0.41</td>
<td>0.34</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 70-80</td>
<td>0.23</td>
<td>0.40</td>
<td>0.10</td>
<td>0.43</td>
<td>0.31</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 80-90</td>
<td>0.29</td>
<td>0.32</td>
<td>0.12</td>
<td>0.43</td>
<td>0.40</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 90-109</td>
<td>0.39</td>
<td>0.27</td>
<td>0.20</td>
<td>0.36</td>
<td>0.55</td>
</tr>
<tr>
<td>Fall</td>
<td>NW 0-13</td>
<td>0.04</td>
<td>0.09</td>
<td>0.05</td>
<td>0.27</td>
<td>0.01</td>
</tr>
<tr>
<td>Fall</td>
<td>NW 13-30</td>
<td>0.04</td>
<td>0.11</td>
<td>0.05</td>
<td>0.31</td>
<td>0.02</td>
</tr>
<tr>
<td>Fall</td>
<td>NW 30 -46</td>
<td>0.12</td>
<td>0.18</td>
<td>0.08</td>
<td>0.32</td>
<td>0.14</td>
</tr>
<tr>
<td>Fall</td>
<td>IK 0-15</td>
<td>0.13</td>
<td>0.13</td>
<td>0.07</td>
<td>0.28</td>
<td>0.15</td>
</tr>
<tr>
<td>Fall</td>
<td>IK 15-28</td>
<td>0.33</td>
<td>0.11</td>
<td>0.07</td>
<td>0.30</td>
<td>0.12</td>
</tr>
</tbody>
</table>
See Table 1 for Season and Sample column explanation. Columns 3-7 are maturity parameters calculated using the Hypy product saturated hydrocarbons, see Appendix B: Methods for explanation of each parameter.

Table V - Archaeol/ nC31 Alkane Ratio

<table>
<thead>
<tr>
<th>Season</th>
<th>Sample</th>
<th>Arch/n-C31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall</td>
<td>SS 0-10</td>
<td>0.013</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 10-20</td>
<td>0.006</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 20-30</td>
<td>0.009</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 30-40</td>
<td>0.004</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 40-50</td>
<td>0.004</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 50-60</td>
<td>0.006</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 60-70</td>
<td>X</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 70-80</td>
<td>0.022</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 80-90</td>
<td>0.013</td>
</tr>
<tr>
<td>Fall</td>
<td>SS 90-109</td>
<td>0.004</td>
</tr>
<tr>
<td>Fall</td>
<td>NW 0-13</td>
<td>0.098</td>
</tr>
<tr>
<td>Fall</td>
<td>NW 13-30</td>
<td>0.174</td>
</tr>
<tr>
<td>Fall</td>
<td>NW 30-46</td>
<td>0.143</td>
</tr>
<tr>
<td>Fall</td>
<td>K 0-15</td>
<td>0.066</td>
</tr>
<tr>
<td>Fall</td>
<td>K 15-28</td>
<td>0.032</td>
</tr>
<tr>
<td>Spring</td>
<td>S3 0-4</td>
<td>0.083</td>
</tr>
<tr>
<td>Spring</td>
<td>S3 4-12</td>
<td>0.111</td>
</tr>
<tr>
<td>Spring</td>
<td>S3 12-28</td>
<td>0.104</td>
</tr>
<tr>
<td>Spring</td>
<td>S3 28-38</td>
<td>0.073</td>
</tr>
</tbody>
</table>

See Table 1 for explanation of season and sample column. X denotes a sample with no data.
Appendix B – Methods

Analytical Methods

I - Core Sampling

Lake samples were cored using a piston push apparatus and then kept frozen at (-20°C) until sampling. The three fall samples North of Walapka (NW), Ilkrovi (IK) and Sukok Seep (SS) were sampled in Reno, NV at the Desert Research Institute (DRI) over the course of 3 days. Prior to the sectioning of cores, a quick sketch of the intact core was taken to note general trends in color and sediment type. Sectioning of the core was based on core length with any icy sections removed and not sampled. The core casing was then cut open with a metal core slicer, and a hand saw if core slicer was unable to penetrate the casing completely. Upon sectioning of the core, the top ~1 cm was removed from each section to reduce contamination. Sediment was extracted from the center of the section and homogenized on fired aluminum foil for 2-3 minutes. After homogenization, each sediment sample was placed in a pre-baked 40 ml tube for biomarker analysis while another sample was taken for genomic and pore water methane analysis. The amount of lake sediment recovered from each interval varied based on composition and volume of water averaging 10 – 15 g.

II - Freeze Drier

After the core was sampled, each homogenized section was placed in a freeze drier to remove any residual water before performing solvent extraction and lipid biomarker analysis. Samples in the freeze drier were left for up to 24 hours at -40°C and 170 x 10^-3 millibars.

III - TOC Eltra Method for Total Carbon (TC), Total Inorganic Carbon (TIC) and Total Organic Carbon (TOC)
Total organic carbon (TOC) and total inorganic carbon (TIC) measurements were made using an Eltra CS-500 Infrared Carbon Sulfur Determinator. TC concentrations are measured by the combustion of ~0.100 g of sample in an Eltra CS-580 high temperature (~1400°C) resistance furnace which is flushed with pure (99.98%) oxygen gas. Inorganic carbon values are determined via a separate measurement using an Eltra TIC module wherein a ~0.100 g sample is reacted with 8 mL of a 12% hydrochloric acid solution at ~50°C while being mixed with a magnetic stirrer.

IV - Solvent Extraction

Lipid biomarkers were extracted from typically 1.5 g of freeze dried lake sediment in a Microwave Accelerated Reaction System (MARS) with a dichloromethane and methanol mixture of 9:1(v/v) at 100°C for 15 minutes. Total extracts were fractionated with hexane, hexane: dichloromethane (1:1 v/v) and dichloromethane: methanol (3:1 v/v) on a silica gel column to elute saturated hydrocarbons, aromatic hydrocarbons and polar compounds. Elemental sulfur was removed from the total extract with HCL-activated, solvent washed copper pellets prior to silica gel column chromatography.

V - TLE Derivatization (BSTFA)

TLE and SPE extracts were silylated as a means of allowing for functional groups of acidic hydrogen to be replaced with a trialkylsily (TS) group. 25uL of BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) + 1%TMCS (trimethylchlorosilane) and 25uL of pyridine was added via syringe to 2 mL vial containing up to 5 ug of sample. After adding the derivatization reagents, the sample was mixed in a vortex for 1 min. It was then heated at 70C in Reacti-vap for minimum of 30 minutes. After heating, derivatization is complete and is ready to be run on the GC-MS.

VI - Catalytic Hydropyrolysis (HyPy)
Dry lake sediment was subjected to open-system catalytic hydropyrolysis (Hypy) as a means of (i) converting functionalized free lipids into hydrocarbons, and (ii) releasing bound lipid biomarkers from organic geomacromolecules in order to gauge a representative view of the lipid skeletons distributions and relative abundances. Before Hypy treatment samples were impregnated with an aqueous solution of the catalyst ammonium dioxydithomolybdate [(NH₄)₂MoO₂S₂] which decomposes under Hypy conditions (above 250°C) to form a catalytically-active molybdenum sulfide (MoS₂) phase. Lake samples were impregnated with a 5% w/w of Mo to dry biomass solution. Catalyst loaded samples were heated in a stainless steel reactor from ambient temperature to 250°C at 100°C a min then to 480°C at 8°C a min. Operational hydrogen pressure was between 145 and 150 bars with a constant flow of 6 dm³/min of sweep gas through the reactor bed. Hypy products were collected on a column of silica gel in a specially designed dry ice-cooled product trap.

VII - Full Scan GC-MS

Gas chromatography-mass spectrometry (GC-MS) analysis of saturated hydrocarbons and BSTFA-derivatized TLEs was conducted in full scan mode with an Agilent 7890A GC interfaced to an Agilent 5975C MSD mass spectrometer. The GC was equipped with a 60 m DB-1MS capillary column and run with He as a carrier gas. The temperature program for GC-MS full scan and selected ion monitoring was 60°C (2 min), ramp to 250°C at 20°C/min, to 325°C at 2°C/min, and hold at 325°C for 20 min.

VIII - Multiple Reaction Monitoring (MRM)

Multiple reaction monitoring (MRM) GC-MS saturated hydrocarbons was carried out with a Waters Autospec Premier mass spectrometer equipped with an Agilent 7890A gas chromatograph and DB-1MS coated capillary column (60m x 0.25 mm, 0.25μ film thickness) using HE for carrier gas. MRM GC-MS measurements were conducted with a
temperature program of 60°C (held for 2 min), heated to 315°C at 4°C/min with a final hold at 315°C 25 min.

Biomarker compounds were identified based on retention time and published mass spectra and quantified in MRM GC-MS by comparison with a deuterated C_{29} sterane internal standard (d_{4}-ααα-24-ethylcholestane (20R), Chiron Laboratories, AS), assuming equal response factors between sample compounds and the internal standard. Individual yields of hopanes and steranes diasteroisomers found in laboratory procedural blanks were typically <0.1 ng of individual compounds. Analytical errors for individual hopanes and steranes absolute concentrations are estimated at ±30%.

**Ratio Calculations**

**I - Short/Long N-alkane Chain Length Ratio**

The short/long n-alkane ratio was calculated using the following formula:

\[
\frac{\sum nC_{16} - 20}{\sum nC_{27} - 31}
\]

This is also used to determine the relative plant, algal and bacterial input into the sediments as land plants tend to have more abundant high carbon numbered n-alkanes (nC_{28}-nC_{36}), algae have abundant mid-length numbered n-alkanes (C_{16}-C_{26}) while the most abundant bacterial derived n-alkanes are shorter in length (nC_{14}-nC_{18}).

**II - Sterane/ Hopane Ratio**

The sterane/hopane ratio was calculated using the following formula:

\[
\frac{C_{27} - C_{29} \text{ Steranes (all major isomers)}}{C_{29} - C_{35} \text{ Hopanes (all major isomers)}}
\]

This ratio is used to calculated the relative bacterial vs. eukaryotic input within sediment with low values indicating the bacterially derived lipids constitute a high percentage of the hydrocarbon fraction analyzed and similarly for high values which indicate a high
percentage of eukaryotic lipids in the hydrocarbon fraction. The steranes and hopanes used in this formula include both the dia- and regular forms of the compounds as well as both S and R configurations.

III - Sterane Carbon Number Patterns –

The relative percentage of each of the three major steranes (%C27, %C28, %C29) measured as a function of the total sterane abundance was calculated using the following formula:

\[
\frac{C27/C28/C29 \text{ Steranes (all major isomers)}}{C27 + C28 + C29 \text{ Steranes (all major isomers)}}
\]

This ratio is used to calculate the relative input each of the organisms that produce one dominate type of sterane into the sediments. C27 steranes are produced is large abundance in animals and red algae, C28 steranes are produced in large abundance by haptophytes (a group of phytoplankton that include coccolithophores) and Diatoms and C29 steranes are produced by green algae and plants.

IV - 2-Methylhopane Index –

The 2 methylhopane index (in %) was calculated using the following formula using multiple reaction monitoring (MRM)-GC-MS methods on the Water Autospec Premier instrument.

\[
\left( \frac{C31 \text{ 2MethylHopane}}{C31 \text{ 2MethylHopane} + C30 \text{ Reg. Hopane(ab, ba, bb)}} \right) \times 100
\]

The C31 2-methylhopane abundance was calculated by integrating the peak area in the m/z 426 ->205 MRM mass chromatogram while C30 hopane peak areas were calculated in the m/z 412->191 MRM ion chromatogram. 2-methylhopanes are believed to be mainly produced by cyanobacteria and some soil bacteria and the ratio is a useful measure in determining the relative abundance of cyanobacteria (when it can be confirmed) to total bacterial biomass present in the sediment. The 17α, 21β(H)-, 17β,21β(H)- and 17β,21α(H)-diasteroisomers were all detected.
V - 3-Methylhopane Index –

The 3 methylhopane index (in %) was calculated using the following formula using multiple reaction monitoring (MRM)-GC-MS methods on the Water Autospec Premier instrument.

\[
\left( \frac{C_{31} \text{3}-\text{methylhopane}}{C_{31} \text{3}-\text{methylhopane} + C_{30} \text{hopane}} \right) \times 100
\]

The \( C_{31} \) 3-methylhopane abundance was calculated by integrating the peak area in the m/z 426 -> 205 MRM ion chromatogram while \( C_{30} \) hopane peak areas were calculated in the m/z 412->191 MRM ion chromatogram. 3-methylhopanes are believed to be mainly produced by Type I methanotrophic proteobacteria and the ratio is a useful measure in determining the relative abundance of methanotrophic bacteria to total bacterial biomass present in the sediment. The 17\( \alpha \), 21\( \beta \)(H)-, 17\( \beta \),21\( \beta \)(H)- and 17\( \beta \),21\( \alpha \)(H)-diastereoisomers were all detected.

VI - Archaeol /n-C\(_{31}\) alkane ratio –

Archaeol abundance was calculated relative to \( n-C_{31} \) alkane by comparing base peak areas from m/z 130 and m/z 57 respectively. This ratio was used as a measure of in situ archaeal biomass in the sediment (more likely) and/or water column versus continental plant/soil wax input. Archaeol eluted at 97.6 minutes and \( n-C_{31} \) at 73.4 minutes using our standard in-house full scan GC-MS method ran on the Agilent MSD instrument.

VII- Carbon Preference Index (CPI) for long-chain n-alkanes -

The CPI was calculated using the following formula, which is usually applied to free hydrocarbon fraction:

\[
0.5 \times \left( \frac{n_{25} + n_{27} + n_{29} + n_{31} + n_{33}}{n_{24} + n_{26} + n_{28} + n_{30} + n_{32}} \right) + \left( \frac{n_{25} + n_{27} + n_{29} + n_{31} + n_{33}}{n_{26} + n_{28} + n_{30} + n_{32} + n_{34}} \right)
\]
Where $n$ = normal alkane and # indicates the total number of carbon atoms in the linear chain per molecule. This naming system will also be used in the chain length calculation. This is due to the decarboxylation that occurs during diagenesis of n-alkanes present in plants. CPI is a maturity indicator and measures the odd/even predominance waxy n-alkanes.

**VIII - Sterane S/R** –

The Sterane S/S+R ratio was calculated using the following formula:

$$\frac{C_{27} + C_{28} + C_{29} \text{ Steranes (20S)}}{C_{27} + C_{28} + C_{29} \text{ Steranes (20S + 20R)}}$$

This ratio is used a maturity parameter as “life” preferentially makes the “R” form of biological compounds and with increasing maturity compounds switch to the more stable “S” form. This means that relatively immature sediment should have a relatively “low” value that increases with increasing maturity.

**IX - Hopane S/R** –

The Hopane S/S+R ratio was calculated using the following formula:

$$\frac{C_{31} + C_{32} + C_{33} + C_{34} + C_{35} \text{ Hopanes(22S)}}{C_{31} + C_{32} + C_{33} + C_{34} + C_{35} \text{ Hopanes(22S + 22R)}}$$

Similar to the sterane S/R formula, this is a maturity parameter that can be applied to the hopanes present in the lipid fraction with the values also meaning the same thing (low values = immature).

**X - Sterane aaa S/R** –

The Sterane aaa S/S+R ratio was calculated using the following formula:

$$\frac{(C_{27} + C_{28} + C_{29})\text{aaaS}}{(C_{27} + C_{28} + C_{29})\text{aaaS + aaaR}}$$

This maturity parameter looks just at the aaa configuration of the steranes and whether the S or the R form dominates.

**XI - Sterane abb/aaa** –
The Sterane abb/aaa ratio was calculated using the following formula:

\[
\frac{(C27 + C28 + C29)_{abb}}{(C27 + C28 + C29)_{abb} + (C27 + C28 + C29)_{aaa}}
\]

This maturity parameter looks at the relative abundance of the more mature abb configuration vs. the less mature aaa configuration.

**XII - Diasterane/Regular Sterane-**

The diasterane/regular sterane ratio was calculated using the following formula:

\[
\frac{C27 + C28 + C29 \text{ Dia}}{C27 + C28 + C29 \text{ Dia} + \text{Reg}}
\]

This maturity parameter looks at the relative abundance of diasteranes, a common product of diagenesis through acid reactions with clay minerals vs. regular steranes. There is an increasing amount of dia vs. reg. with increasing maturity.