Climate Change Impacts on the Molecular-Level Carbon Biogeochemistry in Arctic Ecosystems

by

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A thesis submitted in conformity with the requirements for the degree of Masters of Science, Graduate Department of Chemistry, in the University of Toronto

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Abstract

“Climate Change Impacts on Molecular-Level Carbon Biogeochemistry in Arctic Ecosystems” by Brent Gregory Pautler (2010), for the degree of Master of Science, Graduate Department of Chemistry in the University of Toronto.

The goal of this thesis was to characterize and quantify changes to Canadian Arctic organic matter (OM) induced by a physical disruption to the permafrost active layer by employing molecular-level techniques such as biomarker extraction and NMR to help elucidate its contribution to carbon turnover and global climate change. The initial biomarker characterization study determined that the extractable plant lipids were unaltered originating from the deposition of new vascular material or permafrost melt where a high alteration of lignin-derived OM was observed suggesting a long residence time in the ecosystem. Analysis of samples where there was a new and historical physical disruption to the permafrost landscape showed an initial increase in bacterial biomass biomarkers, and was corroborated with increased bacterial protein contributions and peptidoglycan signals in the NMR spectra. It is hypothesized that this increase in bacterial biomass resulted in a faster rate of degradation, possibly leading to OM priming.
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Chapter 1: Introduction

1.1. Introduction to natural organic matter

Naturally occurring organic matter (OM) which is ubiquitous in the environment, is a complex, heterogeneous mixture of organic molecules derived from microbes, fungi, bacteria, plant litter and their degradation products (1, 2). This large carbon pool is nearly three times the mass of all aboveground life and is the basis of terrestrial ecology (1). OM present in soil and sediment often consists of identifiable molecules, organized through interactions with its surroundings and surfaces and are able to persist when physically isolated from microbes by incorporation into aggregates or by sorption onto minerals or other organic surfaces by cation bridging or intercalation between layers (1, 3-7).

Humic substances (HS) are the operationally defined component of natural OM, were once thought to be naturally abundant organic macromolecules with high structural complexity resulting in multiple chemical and physical properties (8, 9). Previous literature suggested that humic substances were produced by cross-linking of degradation products to form high molecular weight macromolecules that are microbially and chemically recalcitrant based on classical structural chemistry, chemical degradation, traditional spectroscopic techniques and apparent high molecular weights (10-13). Recent research using advanced structural tools such as nuclear magnetic resonance spectroscopy (NMR) have proposed that HS are not chemically distinct, rather consist mainly of a mixture of plant and microbial constituents and include the partial decomposition products that are associated in aqueous solutions with
macromolecular-like properties (14-17). This hypothesis was confirmed by diffusion-ordered nuclear magnetic resonance spectroscopy (DOSY-NMR) (18-20).

The large OM pool of carbon is considered to be an open, steady-state system that can undergo a wide variety of chemical and biological reactions (21). For example, labile constituents in the OM pool can be transformed and respired (as CO₂ into the atmosphere) by microorganisms within the ecosystem which often leads to the accumulation of more recalcitrant material in soil and sediment (4, 22-24). There are many factors that influence the biogeochemical cycling of carbon, however there is limited knowledge linking the degradation and transformation mechanisms to the chemical constituents and molecular-level structures present in HS and natural OM (23).

1.2. Factors contributing to the decomposition of OM

Understanding the decomposition dynamic of OM has been a challenging task for scientists and climate modellers. Stocks of OM in the environment result from the balance of inputs primarily from leaf and root detritus and output of carbon (CO₂ from the surface, root respirations, and microbial decomposition and CH₄ from anaerobic degradation) in the below ground environments (4, 25). Microbes are key contributors to many soil and sedimentary processes but due to the structural complexity of OM, accurate quantification and contribution of microbial biomass to the OM pool is challenging with a large variability ranging anywhere from 1-5% (26-28), to greater than 50% (29). This discrepancy in the literature is an indication that microbes likely play an
underestimated role in several natural OM processes, including the rate of degradation (29).

OM decomposition has been found to be highly correlated with temperature and water availability, however since OM is a large complex mixture, each structural component has its own set of inherent kinetic properties (25). Often, OM decomposition can be hindered by physical protection; OM may become physically protected in the interior of aggregates where microorganisms and their enzymes have limited access and O2 concentrations are low, or chemically protected by sorption to mineral surfaces through covalent or electrostatic bonds (30). The existence of a protection barrier from water-soluble enzymes is also possible if the OM has inherent low water solubility or if it is present in hydrophobic domains (31).

Environmental conditions have also been shown to impact decomposition of OM by the availability and physical state of water. For example, drought reduces the thickness of water films in soil, which inhibits the diffusion of extracellular enzymes and organic carbon (OC) substrates (25). Flooding or constant exposure to water slows O2 diffusion to decomposition reaction sites eventually only allowing anaerobic decomposition which involves fewer and slower pathways (25). Freezing also plays a major role in governing decomposition because even though enzymatic reactions can occur below 0°C, the diffusion of substrates and extracellular enzymes within the soil and sediment is extremely slow (32, 33). Each of these environmental constraints has the ability to affect the decomposition rates of OM either directly or indirectly by decreasing the substrate concentrations at the enzymatic reaction sites. In addition, recent observations suggest that the temperature dependence to OM concentration is
closely coupled to substrate concentration and that the affinity of particular enzymes for the substrates are critical properties to fully incorporate in carbon flux degradation models (34).

1.3. OM – atmospheric interactions

OM from soil and surficial sediment is in active exchange with the atmosphere and constitutes approximately two-thirds of the terrestrial carbon storage in the world (23). The large size and long residence time of carbon in this pool makes it a potentially important sink for carbon residing in the atmosphere as well as a potential input source (35, 36). In recent years, the total global emission of CO$_2$ from below ground has been recognized as one of the largest fluxes in the global carbon cycle with small changes in magnitude of respiration having strong implications on the overall CO$_2$ concentration in the atmosphere (36, 37). The amount of carbon in the OM pool is a balance between inputs of OM from biota and losses through heterotrophic respiration (35).

Accurate accounting of labile and refractory carbon pools is a challenging task but is considered imperative for determination of potential future increase in CO$_2$ fluxes by anthropogenic disturbances while understanding OM and carbon cycling on a global scale (24, 37-39). OM turnover rates are a function of the environment and are primarily influenced by climatic, vegetation, parent geologic material, topography and time (23, 37). The estimation of the world OM pool is important for establishing a relationship between geographical distribution of all OM with climate and vegetation for assessing the influence of changes on the global carbon cycle (35).
Temperature and moisture content appear to be the major contributor governing OM turnover rates, especially with respect to fast cycling labile constituents (39). It has been determined that soil carbon density generally increases with increasing precipitation, increases with decreasing temperature for any level of precipitation both of which indicate that OM density is strongly correlated with climatic variables (35). The potential for OM to release elevated amounts of CO\(_2\) from increased rates of OM decomposition brought on by global warming is a concern with OM reserves and it is expected to alter the total storage of carbon in natural OM (37, 39). Knowledge of the structural components is vital in the understanding for the potential response of OM to environmental change due to its heterogeneity. For example, Feng et al. (40) found that the lignin degradation was enhanced under soil warming conditions where carbon originating from plant cuticles was sequestered which challenged previous hypotheses. Feng et al. (41) also detected an increase in cuticle carbon sequestration in HS under an enriched CO\(_2\) forest site. These two examples highlight the importance in understanding the difference in OM composition to better predict response to environmental change.

1.4. The Arctic ecosystem

Arctic landscapes are characterized by a diversity of ecosystems that differ in plant species composition, litter biochemistry, and biogeochemical cycling rates (42). As a result, Arctic tundra ecosystems differing in plant composition should contain functionally distinct microbial communities that differentially alter OM as it travels through the ecosystem. Arctic regions have not been studied to the same extent of
those in temperate regions yet the northern ecosystems are estimated to contain 25-33% of the world’s stored carbon (43), 12% of which is estimated to be stored in the tundra regions alone (44). Furthermore, it has been estimated that as much as 39% of the mass of carbon in Canada is locked in permafrost in the Arctic region (45).

Arctic permafrost regions have been identified as the major sink for atmospheric CO₂ in recent geologic time (46, 47). In the global general circulation model of atmospheric CO₂ for specific source-sink distributions, there was an observed north-south atmospheric concentration gradient that could only be maintained if sinks for CO₂ are greater in the northern than southern hemisphere (46). In addition, the observed differences in partial pressures and isotopic ¹³C/¹²C ratios indicate that northern oceans are not a major sink of CO₂ indicating that a large amount of CO₂ is absorbed on the continents in northern terrestrial ecosystems (46). Therefore large quantities of carbon are stored in northern ecosystems in the permanently frozen and/or poorly drained landscapes limiting rapid OM decomposition (32, 48). Approximately 50 Gt of carbon in Arctic tundra exists as dead OM below ground historically accumulating 0.1-0.3 Gt/y worldwide (48). Over the past four decades, an unprecedented warming has occurred in the western North American Arctic, particularly during the summer months, leading to increased thaw depth, melting of permafrost and thermokarst or land subsidence (49). Arctic warming may result in the change of this ecosystems from a CO₂ sink to a CO₂ source by altering the water table and deepening the permafrost active layer thereby potentially accelerating the rate of OM decomposition which could potentially dominate over photosynthesis (48). Some studies have suggested that the limiting conditions have resulted in accumulation of OM that is in an early stage of degradation when
compared to OM characterized in temperate regions and could be more susceptible to enhanced decomposition brought on by climate warming (50-52). In addition, lignin decomposition has also been observed to be much slower in cooler climates suggesting a potential accumulation and increased amounts of unaltered lignin in the Arctic OM pool (53). Therefore, the structural molecular-level analysis of Arctic OM is vital for assessing the abundance of OM present and its availability for chemical reactions and release to the atmosphere.

1.5. Climate change impacts on Arctic ecosystems and the increased permafrost active layer

Polar ecosystems are experiencing some of the fastest rates of warming brought on by anthropogenic global climate change (54). Changes in global climate can be expected to alter total ground storage of carbon with higher latitude regions becoming substantially warmer and drier because evapotranspiration increases under warmer conditions (45, 55). Climate projections based on current trends expect warming in the Arctic to exceed the global average by a factor of two or three with the potential to stimulate decomposition and release OM from permafrost due to increased microbial activity (48, 54). Surface temperature increases of 4°C in summer and 17°C in winter have been projected which would drastically alter permafrost, ice and snow cover; all of which control Arctic ecosystem processes increasing its sensitivity to warming (48). Predictions of how rapidly carbon stored in natural OM in the Arctic will respond to warming are uncertain and little is known about its potential to turnover (4, 54). Carbon stocks that have a residence time of a few decades are the most vulnerable with
respect to climate change because physical disturbances could lead to further destabilization thereby increasing the rate of decomposition and turnover (4). The composition of OM, namely the proportion of “labile” versus “recalcitrant” carbon is important for establishing the sensitivity of OM to warming, which in northern ecosystems, will accelerate aeration through drainage and result in an increase/deepening of the permafrost active layer (39, 48). However, it has recently been shown that “recalcitrant carbon” also degrades under experimental warming conditions therefore these components must also be considered to contribute to carbon turnover (40).

Approximately one fifth of the world’s land area is in the high latitude areas of the Earth where the permafrost reaches depths of hundreds of metres (56). During the summer thaw season, the upper layer of the permafrost thaws to more than one metre altering the physical properties of the overlying soil and sediment (56). Once plant material is buried after several freeze-thaw cycles, unfavourable conditions restrict microbial activity further preventing OM decomposition (57). Projected Arctic warming can potentially lead to the thawing of the upper layer of the permafrost, thereby increasing the depth of the biologically active layer likely leading to the eventual release of previously frozen carbon as CO$_2$ and CH$_4$ (56). This increase in carbon efflux creates a positive feedback loop of warming leading to the potential shift to an atmospheric carbon source by the increase in aeration, drainage and bioavailability of the carbon (32, 48-52, 54-56).

The instantaneous temperature responses of decomposition of the current Arctic carbon stocks reflects the relative abundances of OC substrates different kinetic
properties imposed by environmental constraints (25). The constraints to decomposition are the result of chemical and biological processes that are also dependent on temperature and other climatic variables. Climate alters aggregate formation that physically protects OM; the breakdown of these aggregates is typically enzymatic, which can be directly influenced by temperature and climate (58). Temperature also alters the chemical processes of OM adsorption and desorption onto mineral surfaces altering its bioavailability (25). In addition both precipitation and evapotranspiration are likely to change as a result of a change in temperature, potentially increasing aerobic decomposition (59) or destabilizing the landscape leading to landslides and permafrost active layer detachments (ALDs) that could play a critical role in OM availability and decomposition (60, 61). The predicted increase in precipitation may lead to flooding of the landscape, which would lead to anaerobic decomposition of OM, releasing CH₄ that would result in stronger climate feedback results (62). It is hypothesized that previously constrained carbon in Arctic OM will decompose; however its magnitude and long term contribution to global climate change are still uncertain.

1.6. Major OM structural components and biomarkers

The classical methods for extraction and analysis OM were by the bulk parameters of the major compound categories including HS, lipids, carbohydrates and lignin (63-65). Since OM is a complex heterogeneous mixture with numerous aggregates/associations, often its molecular-level composition may only be examined after breakdown into smaller units by chemical or thermolytical degradation, known as biomarkers (65-67). Biomarkers are organic indicator compounds that can be used as
tracers for geological and environmental processes because their carbon skeleton is representative of its natural product precursor compound (68).

Plant biomarkers are derived from biosynthesis in higher order flora and are composed primarily of lipids and terpenoids (68). Lipid compounds are primarily \( n \)-alkanes \((C_{27}, C_{29}, C_{31}, C_{33})\), \( n \)-alkanols, and \( n \)-alkanoic acids and carry a strong carbon-number predominance inherited from their biosynthesis reflecting its biogenic origin (68-70). Labile lipids from plant waxes and biopolymers such as suberin and cutin have been proposed to be the major source of aliphatic constituents of OM and the composition is dependent on the type of overlying vegetation along with physical and chemical conditions (71, 72). Although the solvent-extractable lipids usually comprise less than 10% of the total OM, the characteristic biomarkers still provide useful information about the source and overall degradation of OM (72). Terpenoids are compounds synthesized by higher plants and many variations exist depending on the environment and system including steroids, diterpenoids, triterpenoids and tetraterpenoids (68). Common microbial biomarkers are compounds that originate from the bacterial membranes such as hopanoids (68, 73), amino sugars (74, 75) and phospholipid fatty acids (PLFAs) (76, 77). The most common biomarker present as the result of fungal input is ergosterol (38, 78, 79). Lignin is the most abundant biomolecule of vascular plants and one of the most widely quantified biomarkers of terrestrial OM are the lignin-derived phenols (53, 80, 81). Lignin is composed of derivatives of three structural classes: vanillyls, syringyls, and cinnamyls; the relative abundances of the phenols are characteristic for major plant groups (angiosperms, gymnosperms) and tissues (woody, non-woody) (53, 81).
1.7. Quantification of OM components: extraction of biomarkers

Quantification of OM biomarkers has been well established in temperate soils and completed sequentially to characterize the carbon stocks and decomposition trends at the molecular-level (38, 53, 65, 66, 69, 70, 80, 82). Labile lipids are extracted by organic solvents (38, 70), bound lipids by base hydrolysis (66, 69), lignin derived phenols by CuO chemolysis (53, 80), and living microbial biomass is quantified by PLFA extraction (76, 77, 83). This sequential biomarker analysis isolates compounds of known plant or microbial origin to assess OM sources and decomposition. The stage of lignin OM degradation and oxidation can be probed by comparing the total composition of phenol acids and aldehydes (Ad/Al) present from the lignin derived phenols after extraction (53, 80, 84). Other ratios have also been employed such as cutin and suberin-derived compounds along with sterols to assess the relative stage of decomposition (85-87). The most common quantification methods for biomarker analysis include gas chromatography-mass spectrometry (GC-MS) and tandem MS techniques (68).

1.8. Quantification of OM components: nuclear magnetic resonance spectroscopy (NMR)

The application of non-destructive spectroscopic techniques such as NMR to organic geochemistry in both the solution and solid phase has revolutionized the knowledge of OM structural features. Several novel NMR techniques have been applied to help elucidate the structural components of natural occurring OM to understand its bulk and molecular-level composition.
1.8.1. Application of 1-dimensional liquid-state $^1$H NMR for OM characterization

Liquid-state $^1$H NMR is the most common NMR technique applied in organic chemistry and has been applied to organic geochemistry for estimating the relative abundance of various functional groups of HS by detecting the broad signals and characterizing the fine splitting related to methyl, methylene, methyne and/or aromatic protons (13). $^1$H nuclei resonances are divided into different categories: 0-2ppm for methyl and methylene protons, 2-5ppm for methyl and methylene protons linked to heteroatoms, and 7-10 ppm for aromatic protons (88). In addition to the relative abundance of various functional groups, $^1$H spin-lattice ($T_1$) relaxation times are examined to probe the molecular conformation of OM which often requires deuterated solvents and water suppression techniques (13, 89). Despite the knowledge gained from the $^1$H NMR, its poor resolution for complex mixtures limits the amount of structural information that can be obtained by these experiments, and is often used in conjunction with other more sophisticated multidimensional techniques.

1.8.2. Solid-state $^{13}$C cross polarization magic angle spinning NMR for OM characterization

Solution-state $^{13}$C NMR of HS is often the preferred technique for structural characterization of samples when sufficient sample quantities are available because the greater chemical shift dispersion reduces the spectral overlap and minor structural alterations can be easily monitored (13). The disadvantages of solution-state $^{13}$C NMR is the insensitivity as only 1.1% of carbon nuclei in naturally occurring carbon
compounds are $^{13}$C nuclei, the very long relaxation times limiting the signal to noise, and that the compounds in the sample must be soluble which is not always possible (13, 63, 90, 91).

The application of solid-state $^{13}$C NMR (in particular cross-polarization magic angle spinning NMR) circumvents the low solubility of OM and allows analysis of the whole sample, rather than just the soluble HS components. Pre-treatment of the sample with HF has been shown to increase the quality of solid-state data from low $^{13}$C content soil fractions by concentrating the OM in the sample and removing natural paramagnetic ions that broaden the desired nuclei signals (63, 91). If not removed, the paramagnetic nuclei lead to spectra with overlapping resonance lines and selective quenching of signal intensity by shortening relaxation time of carbon compounds bound to the paramagnetic centres (91). To compensate for the low natural abundance of $^{13}$C nuclei, cross-polarization (CP) is employed which enhances the magnitude for $^{13}$C signal by producing a theoretical gain of 4 in signal amplitude because it relies on $^1$H relaxation allowing for faster pulsing (13).

The NMR lines generated by $^{13}$C nuclei in static, crystalline, or amorphous samples are broadened in the kHz range (or more) by heteronuclear and homonuclear dipolar interactions along with chemical shift anisotropy (90). Heteronuclear dipolar interactions are eliminated by irradiation of $^1$H nuclei while observing the $^{13}$C nuclei in a process known as proton decoupling; this however does not remove the homonuclear interactions and chemical shift anisotropy (90). Fast spinning of the samples around an axis which makes an angle with the applied magnetic field direction of 54°44’ (magic-angle spinning, MAS) eliminates the last two limitations (92). When used in combination
with proton decoupling, CP-MAS has the capability to produce higher resolved solid-state NMR spectra with higher spinning rates increasing line narrowing (92). In addition, the Hartmann-Hahn condition for the mechanism of magnetization (CP), allows for enhanced polarization in less abundant nuclei (i.e. $^{13}\text{C}$) having a low gyromagnetic ratio by exploiting a much larger polarization associated with more abundant nuclei with a higher gyromagnetic ratio such as $^1\text{H}$ (90). CP between $^1\text{H}$ nuclei and $^{13}\text{C}$ nuclei is achieved when an initial $90^\circ$ pulse is used to create a $^1\text{H}$ magnetization along the $-y$ axis followed by a contact pulse applied on the $^1\text{H}$ along $-y$ in order to spin lock, keeping the magnetic moment constant along this axis (90). Magnetization transfer from $^1\text{H}$ to $^{13}\text{C}$ nuclei arises when an analogous contact pulse is applied on the $^{13}\text{C}$ arising mainly from the dipolar interactions, the stronger the interactions (shorter H–C bond length) the faster the CP and higher the spectral sensitivity (90). Dipolar interactions between $^1\text{H}$ nuclei themselves may also occur (spin diffusion) however fast rotor spinning has been shown to weaken this effect (91).

$^{13}\text{C}$ CP-MAS NMR determines relative quantities of carbon functionalities present in OM and traditionally was the most powerful experimental approach to collect direct information on structural and conformation characteristics of its carbon backbone structure (91). This method has been successfully applied to describe the composition of OM to better understand its transformation and decomposition (91). It has been shown that structural components vary and the relative degree of decomposition can be estimated from the alkyl/O-alkyl ratio in the NMR spectrum (52, 93-95). The alkyl/O-alkyl ratio has been used to establish degradation trends because it has been observed that this ratio increases with degradation (52). Limitations to $^{13}\text{C}$ CP-MAS NMR involve
species containing quaternary or low protonated $^{13}$C nuclei which can be overcome by the direct excitation of the $^{13}$C nuclei however, this requires long acquisition times and is insensitive (90).

1.8.3. Structural characterization of HS by multidimensional NMR

The use of multidimensional NMR spectroscopy has allowed scientists to probe homonuclear $^1$H correlations, spatial interactions, exchange dynamics and heteronuclear linkages of OM by its HS fractions (13). With the advancement of NMR technology, a wide variety of multidimensional liquid-state NMR techniques have been developed which enable the ability to resolve signals that overlap in the traditional 1D spectra (96-98).

Several multidimensional NMR techniques used to study OM structure have been described by Simpson et al. (97) and are summarized briefly below. The simplest of the multidimensional techniques is correlation spectroscopy (COSY) that can detect heteronuclear or homonuclear couplings between $^1$H nuclei and allows the signals on adjacent $^{13}$C nuclei to be identified. $^1$H nuclei shifts are on both axes and coupled $^1$H nuclei are seen as cross peaks in positions reflected across the diagonal. The cross peak describes in one dimension its own chemical shift and in the other dimension the chemical shift of the $^1$H to which it is coupled. Heteronuclear multiple bond quantum correlation spectroscopy (HMQC) detects $^1$H–$^{13}$C coupling over one bond and provides chemical shift data for both nuclei in a $^1$H–$^{13}$C unit. $^1$H is displayed on the horizontal axis and the $^{13}$C along the vertical axis and a cross peak occurs at the coordinates of the chemical shifts of the $^1$H–$^{13}$C unit. Heteronuclear single quantum coherence (HSQC)
spectroscopy provides identical structural information as HMQC however $^{1}$H-$^{1}$H coupling information is filtered out resulting in sharper cross-peaks and less overlap (98). Heteronuclear multiple quantum correlation spectroscopy – total correlation spectroscopy experiments (HMQC-TOCSY) allow for the identification of all $^{13}$C nuclei that are linked to one another and to a chosen $^{1}$H nuclei. $^{1}$H chemical shifts are displayed along the horizontal and $^{13}$C chemical shifts along the vertical. Heteronuclear multiple bond connectivity (HMBC) examines the coupling between a chosen $^{1}$H nuclei, and $^{13}$C nuclei that are linked by two or three bonds ($^{1}$H–$^{13}$C–$^{13}$C or $^{1}$H–$^{13}$C–$^{13}$C–$^{13}$C). $^{1}$H chemical shifts are displayed along the horizontal axis and $^{13}$C along the vertical and note that cross peaks are not observed for single $^{1}$H–$^{13}$C bonds.

1.9. Study objectives

The composition of Arctic OM at the molecular-level and its potential decomposition, destabilization and carbon turnover in response to environmental disturbance brought on by global climate change are still unknown. This thesis aims to further the understanding of the molecular-level carbon biogeochemistry in Arctic ecosystems by the examination of sedimentary OM from the Canadian Arctic. The objectives were to:

1) Investigate and characterize the current input sources and overall degradation of Arctic littoral sedimentary OM

2) Characterize molecular-level changes in Arctic fluvial sedimentary OM induced by physical disruption of the permafrost active layer brought on by climate change
3) Hypothesize and relate Arctic OM contributions to climate change

In order to meet these objectives, two analytical techniques were employed in this study; namely biomarker extraction and quantification by GC-MS and NMR. Samples from the Cape Bounty Arctic Watershed Observatory on the south central coast of Melville Island Nunavut, in the western Canadian High Arctic (74°55'N, 109°35'W) were sampled for initial characterization in summer 2006, and again in summer 2008 after the occurrence of permafrost ALDs.

Plant biomarkers, such as solvent-extractable lipids, waxes, and terpenoids along with lignin derived phenols, and the fungal biomarker ergosterol (solvent-extracted) were used for the initial characterization of sources and degradation of Arctic littoral sedimentary OM from Cape Bounty. Identification and quantification of individual biomarkers were carried out by GC-MS and the results are presented in Chapter 2. A version of this chapter has been submitted to *Biogeochemistry*.

Changes in microbial biomass and decomposition of Arctic fluvial sedimentary OM from Cape Bounty induced by ALDs were investigated by PLFA biomarker extraction that target living microorganisms (76, 77), quantified by GC-MS and by examination of the microbial contributions in the base soluble HS by one-dimensional (1-D) and two-dimensional (2-D) solution-state NMR techniques (29, 99). The overall microbial contributions to OM degradation were also investigated by employing solid-state NMR on the whole-sediment (22, 52, 93, 94). These results are presented in Chapter 3. A version of this chapter has been submitted to *Environmental Science & Technology*. 
The research compiled in this thesis provides a better understanding of Arctic OM characterization and turnover and as well as provides a direct link between physical disruptions and climate change to destabilization and mineralization of OM. Specifically, this study will help to better understand the labile and recalcitrant components of OM stored in the Canadian Arctic, and overall enhance the understanding of the potential decomposition of preserved OM in Arctic regions brought on by global climate change.
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Chapter 2: Biomarker assessment of organic matter sources and degradation in Canadian High Arctic littoral sediments

2.1. Introduction

Arctic landscapes are characterized by highly diverse ecosystems with a large variation in plant species, litter biochemistry and biogeochemical cycling rates due to the functionally distinct microbial communities that differentially alter naturally occurring organic matter (OM) as it resides or is transported throughout the ecosystem (1). To date, Arctic regions have not been studied to the same extent as those in temperate regions, yet the northern permafrost ecosystems are estimated to contain 25–33% of the world’s stored carbon (2), 12% of which is stored in the tundra regions alone (3). Recent finding estimate as much as 192 Pg of carbon is stored in the top 30 cm of Arctic soil, 496 Pg in the top 100 cm, and as much as 1024 Pg in the top 300 cm (4). There is growing concern that climate warming could release this vast amount of carbon as greenhouse gases to the atmosphere (5, 6), however the molecular-level composition of Arctic OM has yet to be analyzed which would aid in the understanding of OM turnover mechanisms. Assessment of the decomposition of OM is a challenging task because it is highly heterogeneous and consists of numerous chemical components, from simple molecules, such as small solvent-extractable compounds, to extremely complex aggregates and associated compounds humic substances (7). Previous studies emphasize the need for accurate quantification of labile OM, such as carbohydrates and proteins, because they have been thought to be more sensitive to degradation at elevated temperatures than refractory components such as the alkyl and
lignin-derived compounds and can be a major source for increased CO₂ emissions to the atmosphere (8). However, Feng et al. (9) found that the degradation of lignin, a major component of soil OM, derived from terrestrial plants, also accelerates under soil warming conditions. This highlights the need for assessing the response of both labile and refractory OM fractions to future global warming. Such research is of particular interest for OM in Arctic regions where the carbon balance is more sensitive to environmental change than in lower latitude areas (10-12).

In this study, solvent extractable lipid and lignin-derived phenol OM biomarker composition and concentrations were measured and quantified using solvent extraction, chemolytic methods and gas chromatography–mass spectrometry (GC–MS) to investigate the input source and degradation stage of littoral sedimentary OM along the edges of an Arctic lake. The objective of this study is to examine the nature of the current OM composition of Arctic littoral sediments because they are representative of recent inputs and molecular-level OM alterations. An understanding of the nature and composition of Arctic OM will assist researchers to understand and predict the potential responses to future disturbances, vulnerability, and biogeochemistry of OM in the Canadian High Arctic.

2.2. Materials and methods

2.2.1. Littoral sediment samples

Four Arctic littoral sediment sample locations were chosen from the Cape Bounty Arctic Watershed Observatory (http://geog.queensu.ca/cbawo/index.htm) on the south central coast of Melville Island Nunavut, in the western Canadian High Arctic (74°55′N,
109°35'W, Figure 2.1). The landscape is characterized by simple drainage patterns, sparse tundra vegetation and continuous permafrost (13). The active layer depth varies between 0.5 and 1 m, with surface detachments and gullies along the river channels (14). Vegetation cover is heterogeneous and varies from sparse polar desert to dense, localized sedge meadows consisting mostly of patchy dwarf prostate shrub tundra where water sources are sustained during the summer, such as below perennial snow banks (15). Mean summer (June, July, August) and winter (December, January, February) temperatures at Rea Point (105 km northeast, 1969–1985) are 1.9 and –32.2 °C respectively (13).

Cape Bounty contains two adjacent watersheds – referred to as West (8.0 km²) and East (11.6 km²) watersheds, which drain into similar small lakes. Flow into the East Lake is dominated by the East River that drains the 11.6 km² catchment. Discharge is typical of Arctic nival systems, with a short period of high flow during the snow melt period, followed by low flow during the summer recession. In most years, discharge is limited to a 2-3 month period, and flow ceases during the remainder of the year (13). Water tracks from the south drain the slopes around the lake and are highly ephemeral, with limited flow typically only during the snow melt period. Littoral sediment samples were collected at the East Lake littoral margin and East River channel. Several subsamples that were free of vegetation were collected by hand at each site and placed in a single Whirl-pak to generate one homogenized sample from each location. Samples were kept dark and frozen for the remainder of the field season (ca 2 weeks). After sampling, the four East Lake littoral sediment samples were freeze dried and stored at -20 °C prior to analysis. Four locations were selected for sampling (Figure 2.1). N and S
were chosen to compare the OM along the north (N) and south (S) shores while NW and NE were chosen for the evaluation of the impact of fluvial delivery on the OM composition in littoral sediments.

2.2.2. Carbon content

Carbon contents were determined using an Analyzer Vario EL III (Hanau, Germany) C, H, O, N, S elemental analyzer. Samples were ground to a fine powder and milligram quantities were analyzed in duplicate for C. Inorganic carbon, such as carbonate, was measured with the method of Bundy & Bremner (16) and was not detected in any of the littoral sediments. Consequently, elemental carbon values represent the amount of organic carbon (OC) in the sediments.

2.2.3. Sequential extraction of OM biomarkers

Sequential chemical extractions (solvent extraction, CuO oxidation) were conducted to analyze the extractable compounds and lignin-derived phenols respectively (17-19). Briefly, sediments (~20 g) were sequentially extracted by sonication for 10 min with 40 ml CH₂Cl₂, CH₂Cl₂:MeOH (1:1 v/v) and MeOH. The combined extracts were transferred to a flask by gravity filtration through pre-extracted cellulose filters (Fisher Scientific P8) and then filtered through glass fibre filters (Whatman GF/A), concentrated by rotary evaporation and completely dried in 2 ml glass vials under a N₂ stream. Yields were determined by weighing the dry residue. The remaining samples were air–dried and stored at -20 °C. Total solvent extracts were re-dissolved in 300 μl hexane and separated using silica column chromatography into
alkane, aromatic and polar fractions by elution with 5 ml hexane, CH₂Cl₂, and MeOH respectively. The fractions were dried in 2 ml glass vials under N₂ and yields determined by weighing the dry residues.

The air-dried solvent-extracted sediment was then subjected to CuO oxidation to release lignin-derived phenols (17-19). Solvent-extracted sediment (~4 g) were extracted with 2 g CuO, 200 mg ammonium iron (II) sulfate hexahydrate [Fe(NH₄)₂(SO₄)₂ 6H₂O] and 16 ml 2 M NaOH in Teflon-lined bombs at 170 °C for 2.5 h. The extracts were acidified to pH 1 with 6 M HCl, and kept for 1 h at room temperature in the dark to prevent polymerization of cinnamic acids. After centrifugation (2500 rpm, 30 min), the supernatants were liquid-liquid extracted (3×) with 50 ml of diethyl ether. The extracts were dried with anhydrous Na₂SO₄ to remove any remaining water, concentrated by rotary evaporation, transferred to 2 ml glass vials and dried under N₂.

A comprehensive selection of native plants from around Cape Bounty was collected and extracted using the previously described procedure to determine potential plant-derived sources for a series of iso- and anteiso-alkanes observed in the solvent extracts. A total of 30 species (whole plant tissues, excluding roots) comprising the majority of the High Arctic tundra biomes present (15) were sampled and dried at 40 °C. The plants were ground using a mortar and pestle, weighed into 125 ml amber glass bottles and stored until extraction at room temperature.

### 2.2.4. Derivatization and biomarker analysis by GC–MS

The solvent and CuO oxidation extracts were re-dissolved in 500 μl of CH₂Cl₂:MeOH (1:1 v/v). Aliquots of the extracts (50 μl) were transferred to 2 ml vials.
and dried in a stream of N₂ and then converted to trimethylsilyl (TMS) derivatives by reaction with 90 µl N,O-bis-(trimethylsilyl)trifluoracetamide (BSTFA) and 10 µl anhydrous pyridine for 3 h at 70 °C. After cooling, 50 µl of hexane was added to dilute the extracts.

GC–MS analysis of derivatized extracts was performed with an Agilent model 6890N chromatograph coupled to an Agilent model 5973N quadrupole mass selective detector. Separation was achieved with a HP-5MS fused silica column (30 m x 0.25 mm i.d., 0.25 μm film thickness) with He as the carrier gas. GC operating conditions were: 65 °C (2 min), to 300 °C (held 20 min); the sample (2 µL) was injected in splitless mode with an injector port temperature of 280 °C using an Agilent 7683 autosampler. The spectrometer was operated in the electron ionization mode (EI) at 70 eV and scanned from m/z 50 to 650. Data were acquired and processed with Agilent Chemstation G1701DA software. Compounds were identified by comparisons of the mass spectra to a MS library (Wiley275 MS library), comparison with authentic standards and with published data. The trimethylsilyl (TMS) derivatives of behenyl alcohol (1-docosanol) and vanillic acid (as TMS ester) were used as external quantification standards for solvent extracts and CuO oxidation products respectively.

2.3. Results

2.3.1. Composition of solvent-extractable compounds

The extracts were dominated by similar compound distributions (Tables 1 and 2; Figures. 2.2 and 2.3; note only one sample is shown for brevity) that varied in concentration (Figures 2.4 and 2.5). The dominant compound included: aliphatic lipids
(\(n\)-alkanols, \(n\)-alkanoic acids, \(n\)-alkanes, \(\alpha\)-hydroxyalkanoic acids, \(\omega\)-hydroxyalkanoic acids, hopanes, \textit{iso}-alkanes, \textit{anteiso}-alkanes), with lesser contributions from steroids, terpenoids, monoacylglycerols, carbohydrates, wax esters. The OC content was observed to be 4.1% for sample NW, 3.8% for sample S, 0.6% for sample N, and 0.5% for sample NE, however the biomarker extraction yields were highest at the sample locations in close proximity to the East River (N and NE; Figure 2.4a). Flow into the East Lake is dominated by the East River that transports large sediment plumes of inorganic material that is deposited closer to the river inlet, diluting the overall OC% at these locations (13). Normalizing all biomarker concentration to OC% correct for any dilution and shows the relative contribution of identified biomarkers with respect to the total OC. \(n\)-Alkanols from \(C_{10}\) to \(C_{32}\) with an even preference and \(C_{\text{max}}\) at \(C_{26}\) are the predominant lipids in all the sediment extracts (262–1380 \(\mu g/g\) OC). The concentration of \(n\)-alkanols is highest along the north shore of the East Lake at site N (Table 1; Figure 2.4b). The \(n\)-alkanoic acids were present at concentrations of 55.9–1,350 \(\mu g/g\) OC, ranging from \(C_{11}\) to \(C_{28}\) and with an even preference and \(C_{\text{max}}\) at \(C_{16}\) and \(C_{24}\). Like the \(n\)-alkanols, the highest contents of alkanoic acids were along the north shore of the East Lake (sample N; Figure 2.4b). \(\alpha\)-Hydroxyalkanoic acids (\(C_{22–C_{25}}\)) with an even preference and \(C_{\text{max}}\) at \(C_{24}\) and \(\omega\)-hydroxyalkanoic acids (\(C_{22}, C_{24}, C_{26}\)) were found only in the west and south banks of the East Lake (NW and S) with yields ranging from 10.2–27.7 \(\mu g/g\) OC and 26.3–48.4 \(\mu g/g\) OC respectively. A series of wax esters derived from short-chain alkanoic acids and alkanols with total chain length \(C_{27}\) to \(C_{33}\) were detected in NW, with a total concentration of 66.0 \(\mu g/g\) OC.
Silica column chromatography was performed to further separate the solvent extractable compounds into non-polar alkane, aromatic, and polar compounds. The alkane fraction contained \( n \)-alkanes, methyl branched alkanes and a series of hopanes (Figure 2.3a). \( n \)-Alkanes ranged from \( C_{17} \) to \( C_{33} \) with an odd preference and highest abundance between \( C_{25} \) and \( C_{31} \). Higher concentrations were found closer to the river inlet (N and E4) decreasing with increasing distance from the East River (NW and S; Figure 2.4b). The methyl branched hydrocarbons include \textit{iso}-alkanes (\( C_{23} \text{–} C_{30} \), 1.2-4.5 \( \mu \text{g/g OC} \)) and \textit{anteiso}-alkanes (\( C_{22} \text{–} C_{31} \), 3.4–10.9 \( \mu \text{g/g OC} \); Figure 2.3). Hopanes (\( C_{27} \text{–} C_{31} \)) were in low abundance in all the East Lake littoral sediments (1.2–9.5 \( \mu \text{g/g OC} \); Figure 2.4c).

Five steroids and one triterpenoid were detected (203–1140 \( \mu \text{g/g OC} \); Table 2.1; Figure 2.2). The sterols include cholesterol (st1), campesterol (st2), stigmasterol (st3), \( \beta \)-sitosterol (st4), and stigmastanol (st5) with \( \beta \)-sitosterol as the most abundant in all the sediments. \( \alpha \)-Amyrin (olean-12-en-3\( \alpha \)-ol) was the only triterpenoid (t1). \( C_{14} \) to \( C_{26} \) (even, absence of \( C_{20} \)) monoacylglycerols (MAGs) were also detected in East Lake sediments (51.4–390 \( \mu \text{g/g OC} \)). The carbohydrates detected were glucose (gl), mannose (ma), sucrose (su), and mycose (my) with mycose as the dominant compound.

2.3.2. Lignin-derived phenols

Major products after CuO oxidation included: benzenes, phenols and short-chain \( n \)-alkanedioic acids and hydroxy acids (Table 2.3). All but one of the sediment samples (E4) contained the characteristic eight major lignin-derived biomarker compounds [vanillin, acetovanillone, vanillic acid, syringaldehyde, acetosyringone, syringic acid, \( p \)-
coumaric acid and ferulic acid; (17, 20)]. The total concentrations of benzene compounds and phenols (VSC) were high from the East Lake sediments ranged from 438.8–3,766.81 μg/g OC (Figure 2.5b). Similarly, the concentrations of diacids and hydroxyl acids ranged from 273.7–2,460.0 μg/g OC (Figure 2.5b). In addition to the eight major lignin-derived phenols, the observed benzene and phenol compounds were: benzoic acid, \( p \)-hydroxybenzaldehyde, \( m \)-hydroxybenzoic acid (3-OH), \( p \) hydroxybenzoic acid (4-OH) and 3,5-dihydroxybenzoic acid. The short-chain acids include the \( n \)-alkane dioic acids: fumaric acid (\( C_{4:1} \)), suberic acid (\( C_8 \)), azelaic acid (\( C_9 \)) and the hydroxy acids malic acid (2-hydroxybutanedioic acid) and 2-hydroxypentanedioic acid. Additionally, pyrrole-2-carboxylic acid was identified in all the sediments. Ratios of the vanillyl (vanillin, acetovanillone, vanillic acid; V), syringyl (syringaldehyde, acetosyringone, syringic acid; S) and cinnamyl units (\( p \)-coumaric acid, ferulic acid; C) were about 1:1:1.

2.4. Discussion

2.4.1. Sources of solvent-extractable OM biomarkers

Long–chain (>\( C_{20} \)) \( n \)-alkanols, \( n \)-alkanoic acids and \( n \)-alkanes are typical constituents of epicuticular and associated waxes of higher plants (21-24) while short-chain (<\( C_{20} \)) homologues are derived from algae and microorganisms (25). The predominance of long-chain \( n \)-alkanols, \( n \)-alkanoic acids, and \( n \)-alkanes in East Lake littoral sediments suggests major OM inputs from vascular plant waxes and the absence of algal activity. The occurrence of \( \alpha \)-amyrin, a specific biomarker for angiosperms (26, 27) also indicates plant inputs. Biomarkers from algae, bacteria and fungi were only
minor contributors to the extractable portion of the littoral OM, reflected by a low abundance of short-chain \( n \)-alkanols, \( n \)-alkanoic acids and \( n \)-alkanes (Tables 1 and 2; Figures 2.2 and 2.3). \( \alpha \)-Hydroxyalkanoic acids and \( \omega \)-hydroxyalkanoic acids are only minor constituents of most plant waxes (28) and were present in the Arctic littoral sediments in low concentration. In addition, \( \alpha \)-hydroxyalkanoic acids are generally found in phospholipid membranes of animals and plants (18), whereas \( \omega \)-hydroxyalkanoic acids are found mainly in cyanobacteria and mosses (29) and in the suberin biopolymer in plant roots (18). Higher concentrations of \( n \)-alkanes and \( n \)-alkanols were found in sample sites near river inlets (particularly N and E4) vs. the south edge site (S; Figure 2.5b), suggesting that the East River may be an important pathway for delivering OM into littoral sediments.

Methyl branched hydrocarbons (\( iso \)-alkanes and \( anteiso \)-alkanes) are rarely reported in higher plants, although short-chain branched alkanes (\(< C_{20} \)) have been identified in cyanobacteria (30). The branched alkanes in Arctic littoral sediments comprise predominantly homologues between \( C_{22} \) to \( C_{31} \) and are not derived from cyanobacteria. Rogge et al. (31) reported the occurrence of these particular compounds in leaf surface waxes of tobacco plants and in much smaller concentration in typical urban herbaceous vegetation but their contribution to Canadian Arctic littoral sediments is very unlikely given the remote location and local plant distributions. Recently, Fukushima et al. (32) detected long-chain \( anteiso \)-alkanes in some Japanese acidic freshwater lakes and assigned their source to planktonic microbes within lakes rather than external sources based on their presence in lake water and absence from particulate matter from inflowing river water. However, the occurrence of long-chain
methyl-branched iso- and anteiso-alkanes in Canadian Arctic littoral sediments is distinctly different from those reported for Japanese sediments, although a contribution from planktonic microorganisms may contribute to some extent to the higher abundance of anteiso-alkanes in this case. The analysis of Cerastium arcticum (Arctic mouse-ear chickweed) a native angiosperm found sporadically throughout the watershed, shows that these methyl-branched alkanes constitute the majority of the extractable compounds (Table 2.2; Figure 2.3b) and strongly suggests that this plant is the source of the iso- and anteiso-alkanes observed in the littoral sedimentary OM. Spatial patterns show a decreasing trend in iso- and anteiso-alkanes with increasing distance from the East River inlet (Figure 2.4c), further suggesting that these compounds in the from fluvial transport and not synthesized in situ.

β-Sitosterol, stigmasterol and campesterol are common steroids in higher plants (22, 24, 33). Stigmasta-3,5-diene-7-one and sitosterone, degradation products of β-sitosterol and stigmasterol (34), were not detected in any of the sediment samples. The absence of ergosterol, a common biomarker for fungi suggests a lack of recent fungal activity in the sediments (35). Cholesterol is a major sterol in animals but is also found in fungi and algae (33, 35, 36). As a result, the exact source is unclear but can be considered to be related to microbial activity as well as from vegetative input. α-Amyrin is a triterpenoid reported to be present in leaf waxes of angiosperms (22, 27, 37). A series of even MAGs ranging from C_{14} to C_{26} and a C_{16} monounsaturated MAG were detected in the sediments. MAGs are major constituents of cell membranes and fats, resulting in a contribution from all organisms (21, 33). The carbohydrates detected in the extracts are ubiquitous to all organisms and are not suitable as specific biomarkers.
with the exception of mycose, which originates from fungi, microalgae, bacteria and some plants at very low abundance (38); however, the absence of ergosterol from the littoral sediments suggests that mycose is more likely derived from plants rather than fungi.

2.4.2. Sources of lignin-derived phenol OM biomarkers

The lignin-derived phenol composition is characteristic of non-woody tissues from vascular plants (17, 39-41). The plotted C/V vs. S/V ratios from East Lake littoral sediments indicates that the lignin-derived OM and source vegetation are similar [likely angiosperms; Table 2.4; Figure 2.5a; (17, 20)]. Similar to the long-chain \textit{n}-alkanes, \textit{n}-alkanols and \textit{n}-alkanoic acids, lignin-derived phenols show higher abundance for N and E4 than S and NW, again suggesting that the East River has an important role in delivery of lignin-derived OM to littoral sediments (Figs. 4a and 5b). Our results are consistent with those of Requejo et al. (42) who found that the lignin-derived phenol concentrations were highest in North American continental shelf sediments at their most northern location, the Yukon River delta. The proposed high inputs of vascular plant material from rivers during spring and summer run-off and enhanced preservation of this material in sediments as a result of low temperatures and seasonal ice cover for much of the year may contribute to the high lignin concentration and is supported by the results of this study.

In addition to lignin-derived phenols, CuO oxidation yielded benzoic acid, hydroxybenzoic acids, hydroxybenzaldehyde and pyrrole-2-carboxylic acids which are believed to be oxidation products of cutin, polysaccharides or proteins (43).
Dihydroxybenzoic acids are thought to originate from tannins and other flavonoids (44, 45). Further evidence suggesting that some of the littoral OM components are in an advanced stage of oxidation was the detection of a number of dialkanoic acids (Table 2.4).

2.4.3. Biomarker assessment of OM degradation

Lipids in soils have been described as highly resistant to biodegradation (46, 47) whereas studies of settling particles in lakes intercepted by sediment traps indicate that lipids may undergo substantial degradation when sinking to the bottom (48). Some biodegradation may occur along the lake edge, depending on microbial activity and physico-chemical conditions (18) and the preservation and accumulation of lipids has been observed in environments with inhibited microbial activity (46, 49). Fatty acid decomposition rates show that \( n-C_{16} \) homologues typically degrade faster than \( n-C_{30} \) (50) and a depletion of the algal biomarker \( n-C_{17} \) alkane vs. long-chain hydrocarbons has been reported (51). Aquatic OM is known to degrade during sinking to the bottom due to the freshness of the material (48), however we postulate that the land–derived lipid Arctic OM components have remained unaltered and are preserved in the littoral sediments (52).

The yields of extractable compounds ranged from 6.7–31.7 mg/g OC (Fig. 2.4a). Lower yields for NW and S were observed in comparison to N and E4 (Figure 2.4a–c), indicating either a larger input source and/or higher degree of preservation for the latter. The carbon preference index (CPI) is a proxy that is applied to provide insight into the degree of diagenetic OM alteration, acting as a numerical representation of the degree
of the original biological chain length preserved in the extractable lipids (48, 53). In fresh material, odd-numbered chains dominate the hydrocarbons whereas even homologues dominate in fatty acids and alkanols (48). Values that are greater than two are an indication of fresh inputs while values less than two are the indication of a high degree of preferential degradation (54). In the East Lake littoral sediments, values were between 2.6–3.6, 1.2–19.2 and 4.0–14.9 for \( n \)-alkanes, \( n \)-alkanols and \( n \)-alkanoic acids respectively (Tables 1 and 2). In all cases (with the exception of alkanols detected at S), the values are indicative of “fresh” inputs in an early stage of decomposition (48, 54). This “fresh” OM may result from new plant biomass, transport of relatively fresh or preserved OM from up-stream, possibly from melted permafrost released as a result of climatic warming in the Arctic (55, 56), which is known to contain OM in early stages of decomposition (57-59). The CPI values suggest that overall; the lipid OM is currently preserved in a relatively fresh, unaltered state, which is consistent with other studies (52, 57, 58). These lipid biomarkers are the result of a recent deposition or deposition of OM which was only recently released into active surface processes through permafrost melting and fluvial delivery.

CuO oxidation of OM in sediments generates products from lignin, cutin, proteins and tannins (43, 47). Phenolic compounds derived from lignin have been employed as a valuable parameter for the degradation of OM in soils and fluvial sediments based on the composition of phenols and benzenes (20). Lignin biodegradation is governed by white–rot and brown–rot fungi by oxidative cleavage (60, 61). The fungal degradation of lignin is greatest in subaerial terrestrial environments but reported to be inhibited in water–logged wood (61). Increased levels of lignin degradation are reflected in elevated
acid/aldehyde (Ad/Al) values for both vanillyl and syringyl units (61-64). Ad/Al values for both vanillyls and syringyls of 0.1-0.2 have been reported for woody plant material (61), whereas non-woody tissues such as leaves and grasses have been reported to have higher values (65, 66). Preferential degradation of syringyl monomers compared to vanillyl units leads to a net decrease in S/V (61, 64).

The yields of VSC were much lower for NW and S (0.209 and 0.282 mg/g C) compared to N and E4 (2.7 and 1.2 mg/g C) suggesting a higher degree of lignin alteration in N and E4 (Table 2.4; Figure 2.5b). High values of [(Ad/Al)_v, 0.9–3.6] and [(Ad/Al)_s, 1.1–2.3] also suggest that lignin-derived OM in the littoral sediments is in an oxidized state, but no apparent trend was observed with respect to fluvial transport (Figure 2.5c). Ad/Al values for littoral sediments in this study are two to three times higher than those reported for littoral sediments in both temperate and Arctic regions (67-70). The tannin-derived 3,5-dihydroxybenzoic acid (3,5-DHBA) in the CuO oxidation products has been reported to accumulate in decaying cells exhibiting higher ratios of 3,5-DHBA over vanillyls (3,5-DHBA/V) with increasing degradation (45, 71). Furthermore, positive correlations between 3,5-DHBA/V and (Ad/Al)_v in soil and sedimentary OM have been reported (45, 71, 72). The 3,5-DHBA/V values for our experiments increased in a similar fashion to (Ad/Al)_v but the value was below detection limits for the final sample S (Figure 2.5c).

According to the degradation parameters for CuO oxidation products, the OM in these Arctic littoral sediments is at a relatively high degradation stage. This is in contrast to the lipid component, which is considered to be fresh on the basis of the CPI values. These results agree with a recent study that showed extensive degradation of lignin and
enhanced preservation of extractable lipids in cold, wet soils (52). The trend of increasing Ad/Al values with decreasing mean annual temperature has been observed for grassland soils in different climatic zones because of the readily metabolizable carbohydrates in warmer climates (73). The high Ad/Al values observed for littoral sediments and the absence of ergosterol (fungal biomarker) from the solvent extracts suggests that the lignin-derived OM may not be the result of active biodegradation but may be “old” lignin recently released from permafrost due to Arctic warming or old lignin transported through the watershed and to the littoral sediments. Another driving force for lignin degradation in the Arctic could be abiotic processes including photochemical alteration or cross-linking (64, 74, 75). The role of abiotic mechanisms in lignin alteration needs to be investigated as they may play an increasingly important role in lignin transformation in cooler climates (20).

2.5. Conclusions

The solvent-extractable and CuO oxidation biomarkers from Arctic sediments from the East Lake littoral zone on Melville Island, Nunavut was used as a case study for OM sources and degradation in this unique and previously unstudied ecosystem. The extractable compounds exhibited high CPI values indicative of “fresh” plant inputs and/or lipid preservation in littoral sediments with a spatial distribution pattern suggestive of inputs from newly deposited plant derived material or from melted permafrost soil via fluvial transport. Comparison of sediment biomarkers with those of a native Arctic plant shows that the observed series of C_{24} to C_{34} iso- and anteiso-alkanes are likely from a previously undocumented source for these biomarkers (Cerastium
arcticum; Arctic mouse-eared chickweed), which may now be used to monitor terrestrial OM input in this Arctic ecosystem. The littoral sedimentary OM is a mixture of fresh recently deposited and/or preserved lipids and oxidized lignin-derived OM that may originate from accumulated permafrost that has recently become bioavailable via melting and fluvial transport to littoral sediments. Interpretation of potential OM degradation in response to climate change based on bulk measurements is very complex due to the mixture of OM constituents, ages, and different reaction rates (76). Analysis of Arctic OM by this molecular-level biomarker methodology helps alleviate some of the complexity because not only does it provide insight into the sources, but may facilitate the prediction of the potential vulnerability of this carbon pool to environmental disturbances. For example, this Arctic OM may become more susceptible to degradation and release of carbon induced by climate change due to the lower metabolic activation energies of the large accumulation of preserved lipids (77). This hypothesis will be the basis of future research involving the potential sensitivity and vulnerability of Arctic OM in the changing environment.
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2.7. Tables

Table 2.1: Occurrence and quantities of compounds in extracts of Arctic littoral sediments and CPI values

<table>
<thead>
<tr>
<th>Symbol Compound</th>
<th>MW</th>
<th>Composition</th>
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<td></td>
<td></td>
<td></td>
<td>NW</td>
</tr>
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<td>(n)-Pentacosanoic acid</td>
<td>382</td>
<td>(C_{25}H_{50}O_2)</td>
<td>n.d.</td>
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<tr>
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<td>(C_{26}H_{52}O_2)</td>
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<td>(C_{27}H_{54}O_2)</td>
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<td>(n)-Octacosanoic acid</td>
<td>424</td>
<td>(C_{28}H_{56}O_2)</td>
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<tr>
<td>Total</td>
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<td>55.9</td>
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<td>(C_{22}H_{44}O_3)</td>
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<td>(C_{24}H_{48}O_3)</td>
<td>8.8</td>
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<td>(C_{25}H_{50}O_3)</td>
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<td>(\omega) Hydroxyalkanoic acids</td>
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<td>(C_{22}H_{44}O_3)</td>
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<td>15.9 n.d. n.d.</td>
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<td>(\omega)-Hydroxytetracosanoic acid</td>
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<td>(C_{24}H_{48}O_3)</td>
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<td>19.1 n.d. n.d.</td>
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<td>(C_{26}H_{52}O_3)</td>
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<td>({\bigstar}) Wax esters</td>
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<td>C24 wax esters</td>
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<td>(C_{27}H_{54}O_2)</td>
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<td>(C_{28}H_{56}O_2)</td>
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<td>C29 wax esters</td>
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<td>(C_{29}H_{58}O_2)</td>
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<td>C30 wax esters</td>
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<td>C31 wax esters</td>
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<td>(C_{31}H_{62}O_2)</td>
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<td>C33 wax esters</td>
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<td>Total</td>
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<td>66.0</td>
<td>n.d. n.d. n.d.</td>
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<td>MAG Monacylglyceride</td>
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<td>(C_{14}) Monacylglyceride</td>
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<td>(C_{17}H_{34}O_4)</td>
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<td>3.0 12.6 n.d.</td>
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<td>(C_{16}) Monacylglyceride</td>
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<td>7.7 34.6 24.5</td>
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<td>(C_{21}H_{42}O_4)</td>
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<td>C27H54O4</td>
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<td>C26 Monacylglyceride</td>
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<td>55.4</td>
<td>106</td>
<td>68.0</td>
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<td>Campesterol (Ergost-5-en-3-ol)</td>
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<td>Stigmasterol (Stigmasta-5,22-412 dien-3β-ol)</td>
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<td>Sitosterol (Stigmast-5-en-3β-ol)</td>
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<td>α-Amyrin (Olean-12-en-3α-ol)</td>
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<td>15.4</td>
<td>57.9</td>
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<td>Glucose</td>
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<td>Mannose</td>
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<td>Sucrose</td>
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^aAll polar compounds identified as TMS derivatives; ^bNot detected
### Table 2.2: Occurrence and quantities of components in alkane fraction of Arctic littoral sediment extracts

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<thead>
<tr>
<th>Symbol Compound</th>
<th>MW</th>
<th>Composition</th>
<th>Concentrations [μg/g OC]</th>
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<tr>
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<td>NW</td>
<td>S</td>
<td>N</td>
</tr>
<tr>
<td>n-Alkanes</td>
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<tr>
<td>n-Heptadecane</td>
<td>240</td>
<td>C\textsubscript{17}H\textsubscript{36}</td>
<td>0.7</td>
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<td>n-Octadecane</td>
<td>254</td>
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<td>C\textsubscript{20}H\textsubscript{42}</td>
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<td>n-Uncosane</td>
<td>296</td>
<td>C\textsubscript{21}H\textsubscript{44}</td>
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</tr>
<tr>
<td>n-Docosane</td>
<td>310</td>
<td>C\textsubscript{22}H\textsubscript{46}</td>
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<tr>
<td>n-Tricosane</td>
<td>324</td>
<td>C\textsubscript{23}H\textsubscript{48}</td>
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<tr>
<td>n-Tetracosane</td>
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<td>C\textsubscript{24}H\textsubscript{50}</td>
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<tr>
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<td>C\textsubscript{25}H\textsubscript{52}</td>
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</tr>
<tr>
<td>n-Hexacosane</td>
<td>366</td>
<td>C\textsubscript{26}H\textsubscript{54}</td>
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<td>C\textsubscript{27}H\textsubscript{56}</td>
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<td>n-Nonacosane</td>
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<tr>
<td>n-Hentriacontane</td>
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<td>n-Pentatriacontane</td>
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<td>C\textsubscript{35}H\textsubscript{72}</td>
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<td>Total</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>80.5</td>
<td>133</td>
<td>261</td>
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</table>

CPI alkane

2.6 | 3.5 | 3.4 | 3.6

Hopanes

h1 C\textsubscript{27} trisnorhopane | 370 | C\textsubscript{27}H\textsubscript{46} | n.d. | 0.5 | n.d. | n.d. |

h2 Trisnorhopane | 370 | C\textsubscript{27}H\textsubscript{46} | n.d. | 0.5 | n.d. | n.d. |

C\textsubscript{29} norhopane | 398 | C\textsubscript{29}H\textsubscript{50} | n.d. | 1.4 | n.d. | 1.3 |

C\textsubscript{29} norhopane | 398 | C\textsubscript{29}H\textsubscript{50} | n.d. | 2.0 | n.d. | n.d. |

h3 C\textsubscript{30} hopane | 412 | C\textsubscript{30}H\textsubscript{52} | n.d. | 1.0 | 1.3 | 1.5 |

h4 Hopene b 22S-17α(H),21α(H)-homohopane | 426 | C\textsubscript{31}H\textsubscript{54} | n.d. | 0.5 | n.d. | n.d. |

h5 22R-17α(H),21β(H)-homohopane | 426 | C\textsubscript{31}H\textsubscript{54} | n.d. | 0.3 | n.d. | n.d. |
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<th>Compounds</th>
<th>Cn</th>
<th>Hydr</th>
<th>n. d.</th>
<th>2.0</th>
<th>n. d.</th>
<th>n. d.</th>
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<td>Homohopane 17β(H), 21α(H)-</td>
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<tr>
<td>Homohopane 22R-17β(H), 21T(H)-</td>
<td>426</td>
<td>C31H54</td>
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<td>0.5</td>
<td>1.4</td>
<td>0.8</td>
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<td>Hopene</td>
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<td>1.2</td>
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<td>C30 hopane</td>
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<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
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<td>338</td>
<td>C24H50</td>
<td>n.d.</td>
<td>0.1</td>
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<td>n.d.</td>
</tr>
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<td>352</td>
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<td>0.4</td>
<td>n.d.</td>
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<td>0.4</td>
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<tr>
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<tr>
<td>2-Methyloctacosane</td>
<td>394</td>
<td>C28H58</td>
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<td>0.2</td>
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<td>0.7</td>
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<tr>
<td>3-Methyltricosane</td>
<td>408</td>
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<td>n.d.</td>
<td>0.53</td>
<td>n.d.</td>
</tr>
<tr>
<td>3-Methyltricosane</td>
<td>408</td>
<td>C29H60</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.53</td>
<td>n.d.</td>
</tr>
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<td>3-Methylpentacosane</td>
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<td>6.1</td>
<td>5.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**a**Not detected.
Table 2.3: Occurrence and quantities of major compounds in CuO oxidation products of Arctic littoral sediments

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>Composition</th>
<th>Concentration [μg/g OC]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>NW</td>
</tr>
<tr>
<td>Benzenes and phenols</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>122</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>16.6</td>
</tr>
<tr>
<td>p-Hydroxybenzaldehyde</td>
<td>122</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>33.3</td>
</tr>
<tr>
<td>m-Hydroxybenzoic acid (3-OH)</td>
<td>138</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;</td>
<td>27.8</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid (4-OH)</td>
<td>138</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;</td>
<td>77.8</td>
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<tr>
<td>Vanillin</td>
<td>152</td>
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<td>20.5</td>
</tr>
<tr>
<td>Acetovanillone</td>
<td>154</td>
<td>C&lt;sub&gt;8&lt;/sub&gt;H&lt;sub&gt;10&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;</td>
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<td>Vanillic acid</td>
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<td>Syringaldehyde</td>
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<tr>
<td>Acetosyringone</td>
<td>196</td>
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<td>Syringic acid</td>
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<td>p-Coumaric acid</td>
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<td>Ferulic acid</td>
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<td>3,5-Dihydroxybenzoic acid</td>
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<td>439</td>
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<td>Diacids and hydroxy acids</td>
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<td>Fumaric acid (but-2-enedioic acid)</td>
<td>116</td>
<td>C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
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<td>Suberic acid (n-octanedioic acid)</td>
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<td>n.d.</td>
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<tr>
<td>Azelaic acid (n-nonanedioic acid)</td>
<td>188</td>
<td>C&lt;sub&gt;9&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>123</td>
</tr>
<tr>
<td>Malic acid (2-hydroxybutanedioic acid)</td>
<td>134</td>
<td>C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt;</td>
<td>21.9</td>
</tr>
<tr>
<td>2-hydroxypentanedioic acid</td>
<td>148</td>
<td>C&lt;sub&gt;5&lt;/sub&gt;H&lt;sub&gt;8&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt;</td>
<td>n.d.</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>413</td>
</tr>
<tr>
<td>Pyrrole-2-carboxylic acid</td>
<td>110</td>
<td>C&lt;sub&gt;5&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;N</td>
<td>92.3</td>
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<sup>a</sup> Not detected
Table 2.4: Degradation parameters calculated from CuO lignin oxidation products from Arctic littoral sediments

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<th>S</th>
<th>N</th>
<th>NE</th>
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<td>VSC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>209</td>
<td>282</td>
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<td>1200</td>
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<tr>
<td>S/V&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.7</td>
<td>0.6</td>
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<tr>
<td>C/V&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.7</td>
<td>1.1</td>
<td>1.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Vanillic acid/vanillin (Ad/Al)&lt;sub&gt;v&lt;/sub&gt;</td>
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<td>1.5</td>
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<tr>
<td>Syringic acid/syringaldehyde (Ad/Al)&lt;sub&gt;s&lt;/sub&gt;</td>
<td>2.1</td>
<td>1.1</td>
<td>2.3</td>
<td>1.9</td>
</tr>
<tr>
<td>3,5-DHBA/V&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.7</td>
<td>n.d.&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Major 8 lignin-derived phenols after CuO oxidation: VSC (μg/g OC) = vanillin + acetovanillone + vanillic acid + syringaldehyde + acetosyringone + syringic acid + p-coumaric acid + ferulic acid.

<sup>b</sup> Ratio of S (syringyls: syringaldehyde, syringic acid, acetosyringone) to V (vanillyls: vanillin, vanillic acid, acetovanillone).

<sup>c</sup> Ratio of C (cinnamyls: p-coumaric acid, ferulic acid) to V (vanillyls: vanillin, vanillic acid, acetovanillone).

<sup>d</sup> 3,5-Dihydroxybenzoic acid (3,5-DHBA/V) / V (vanillyls: vanillin + vanillic acid + acetovanillone).

<sup>e</sup> Not detected.
2.8. Figures

Figure 2.1: East Lake littoral sample locations, Cape Bounty Arctic Watershed Observatory, Melville Island, Nunavut, Canada. Note that the indicated watershed boundary of the East River is based on the location of a long term hydrometric gauging site (data not used in this study). Lake input is dominated by the East River with a small ephemeral water track on the south edge with limited flow from the slopes around the lake only during the snow melt period, and the output is indicated by an arrow.
Figure 2.2: GC-MS chromatograms (total ion current, TIC) of TMS solvent-extract of the Arctic littoral sediment S showing: (a) dominance of mycoses and (b) all identified compounds (+, n-alkanoic acids; ∇, n-alkanols; st#, sterols; MAG, monoacylglycerols; *, contaminants). Numbers refer to total carbon numbers in aliphatic lipid series.
Figure 2.3: GC-MS chromatogram (TIC) of (a) alkane fraction of solvent extract of S Arctic littoral sediment (•, n-alkane; i, iso-alkane; ai, anteiso-alkane; h#, hopane); (b) solvent extract of *Cerastium arcticum* (Arctic mouse-ear chickweed angiosperm •,n-alkane; i, iso-alkane, ai, anteiso-alkane. Numbers refer to total carbon numbers in aliphatic lipid series.
Figure 2.4: Carbon-normalized extract yields and concentrations of major biomarker classes in Arctic littoral sediment samples from Cape Bounty, Melville Island, Nunavut, Canada: (a) Total yield of extractable compounds and CuO oxidation products; (b) aliphatic and cyclic lipids; (c) alkane fraction of hopanes, iso-alkanes and anteiso-alkanes (sample locations plotted from N to S).
**Figure 2.5:** Lignin-derived OM sources and degradation parameters: (a) Lignin source parameters for sediment samples (C/V, cinnamyl/vanillyl phenols; S/V, syringyl/vanillyl phenols); (b) carbon normalized extraction yields of benzene compounds, lignin-derived phenols (VSC), short-chain acids; (c) degradation parameters for CuO products: VSC, lignin-derived phenols; 3,5-DHBA, 3,5-dihydrobenzoic acids (sample locations plotted from N to S.)
Chapter 3: Permafrost active layer detachments result in increased microbial activity in Arctic organic matter

3.1. Introduction

Arctic ecosystems have been accumulating organic carbon in large permafrost zones that have grown to hold almost twice as much carbon than that present in the atmosphere (1-3). Soil organic matter (SOM) in these zones is a complex mixture of natural organic molecules that include labile constituents such as fresh vascular plant material and microbial biomass to more complex refractory components that have accumulated over time (2, 4). SOM composition in Arctic regions has not been studied to the same extent as temperate regions, however it has been suggested that due to the permanently frozen/wet ground and a short melt season, there is a large accumulation of labile SOM constituents in an early stage of degradation that may be susceptible to climate change due to their lower metabolic activation energies (5, 6). Recent studies have focused on quantification of the climatic controls and turnover of SOM by measuring CO$_2$ respiration (3, 6-9). Heating during the short melt season in the Arctic results in the thawing of the uppermost layer of soil giving microbes the opportunity to turnover some of the accumulated SOM in permafrost regions although the decomposition mechanisms and feedbacks to climate change are very complex (10). Interpretation of SOM respiration to warming temperatures is complicated by the mixture of SOM ages, the different reaction rates of recalcitrant vs. labile materials and limited biomass in Arctic ecosystems (7). A recent study using radiocarbon dating has identified that “old carbon” was respired from tundra ecosystems over the past 15 years.
and determined that the loss of SOM from permafrost thaw in Arctic regions will act as a large source of atmospheric CO₂ with warmer temperatures (3).

In addition to traditional respiration, physical disruption to the permafrost due to Arctic warming may amplify or perhaps become an even greater contributor to the flux and degradation of older, accumulated SOM (10). Permafrost disturbance that results in the mass movements of soil and surface sediment along the base of the seasonally-thawed active layer are known as active layer detachments (ALDs) and occur when saturated overburdened material (resulting from warm temperatures and large precipitation events) slides over a frozen substrate and results in the rapid down slope movement of that material up to hundreds of meters over low slope angles (11). These ALDs may result in the improved availability of nutrient sources for native microbes, promoting SOM degradation. Such an event would augment the transitioning of Arctic ecosystems from an atmospheric CO₂ sink to a source through the degradation of previously unavailable SOM.

The objective of this study is to characterize changes in Arctic SOM composition induced by ALDs by employing organic geochemical biomarkers with modern NMR spectroscopy. Arctic ecosystems are currently experiencing some of the fastest rates of warming (12), particularly during the summer months (13), therefore understanding SOM at the molecular-level will aid in understanding of carbon turnover and microbial activity in a changing climate. Unprecedented warm temperatures during July 2007 in the Canadian High Arctic resulted in ALDs (14) and provided a unique opportunity to measure alterations of freshly disrupted Arctic SOM. Biomarkers are organic compounds that can be used as environmental tracers because their carbon skeleton is
indicative of their natural product precursor (15). Changes in microbial activity as the result of ALDs are measured by phospholipid fatty acids (PLFAs) biomarkers that are characteristic of living microorganisms (16, 17) and by identification of distinct microbial protein/peptide signals in SOM humic extracts by multidimensional solution-state NMR techniques (18, 19). In addition, solid-state $^{13}$C NMR is employed for a total assessment of the overall composition and degradation of Arctic SOM. This study attempts to relate SOM structural changes to an environmental disruption by the combination of several complementary molecular-level techniques to provide insight into Arctic SOM in a changing environment.

3.2. Materials and Methods

3.2.1. Study Site

Arctic soil samples were collected from the Cape Bounty Arctic Watershed Observatory located on the south-central coast of Melville Island, Nunavut, Canada (74°54′N, 109°35′W; Figure 3.1) in August 2008. The site contains two adjacent watersheds – unofficially referred to as West (8 km$^2$) and East (11.6 km$^2$) which drain into similar small lakes. The landscape is covered with sparse tundra vegetation and the seasonal active layer varies between 0.5 and 1 m depth. Persistent warm temperatures during July 2007 at Cape Bounty together with a heavy rainfall event (10.8 mm) in late July deepened the active layer and resulted in widespread ALDs across the West watershed (14). Four study locations were selected for sampling in the West watershed, and two samples from the East (Figure 3.1). W3 represents an area of the watershed, recently affected by a 2007 ALD, and was chosen to measure the impact the ALDs had
on SOM composition whereas W2 represents an area where there were clear signs of a historic ALD, which happened prior to 2003 when the comprehensive watershed monitoring network was established (14). Two undisturbed samples were collected from the West catchment in close proximity to these sites to represent SOM of natural, undisturbed locations (W1 and W4) along with two intact East watershed samples (E1 and E2) to test for variations between the watersheds. Soil samples (top 5 cm) were collected from the West and East watershed by hand and placed in single Whirl-paks to generate one homogenized sample from each location. Samples were kept dark and frozen for the remainder of the field season (ca 2 weeks). After sampling, the samples were freeze dried and stored at -20 °C prior to analysis.

3.2.2. Biomarker extraction

Organic carbon (OC) contents were determined by combustion with a LECO analyzer by the University of Guelph Laboratory Services (Guelph, Ontario, Canada). Lipid biomarkers were extracted by sonication of soil (~20g) sequentially in 30 ml of DCM, DCM:MeOH (1:1 v/v) and MeOH followed by filtration, concentration by rotary-evaporation and drying under N2 (20). Total solvent extracts were re-dissolved in 300 µl of hexane and separated using silica column chromatography into alkane, aromatic and polar fractions by elution with 5 ml of hexane, DCM and MeOH respectively. Fungi- and bacterial derived compounds (PLFAs) were extracted by a modified Bligh-Dyer method (16); the total lipid extracts were fractionated into neutral lipids, glycolipids, and polar lipids with 10 ml chloroform, 20 ml acetone, and 10 ml methanol by silica column chromatography respectively. The polar lipid fraction containing the phospholipids was
evaporated to dryness under N₂, and converted to fatty acid methyl esters (FAMEs) by a mild alkaline methanolsynthesis reaction. The FAMEs were recovered with a hexane:chloroform mixture (4:1 v/v). The solvents were evaporated under a stream of N₂. PLFAs are named based on the number of carbon atoms and number of double bonds followed by the position of the double bond from the methyl end of the molecule. The symbol ω indicated that the first carbon-carbon double bond starts on the nᵗʰ carbon from CH₃ end while the prefixes i- and a- referred to iso-branched and anteiso-branched, respectively. The designation cy indicated cyclopropane fatty acids.

Biomarkers were quantified by gas chromatography/mass spectrometry (GC/MS) and normalized to OC content. The aromatic and polar fractions of the solvent extracts were re-dissolved in 500 µl DCM:MeOH (1:1 v/v). Aliquots of the extracts (100 µl) were transferred to 2 ml vials and dried under a stream of N₂ and converted to trimethylsilyl (TMS) derivatives by reaction with 90 µl N, O-bis-(trimethylsilyl)trifluoracetamide (BSTFA) and 10 µl anhydrous pyridine for 3 h at 70 °C. After cooling, 400 µl of hexane was added to dilute the extracts. 200 µl of hexane was added to re-dissolve both the non-polar alkane fraction and FAMEs for gas chromatography/mass spectrometric analysis. Tetracosaine (C₂₄ n-alkane), TMS derivatized behenyl alcohol (1-docosanol-TMS), and oleic acid (C₁₈:1 alkanoic acid) methyl ester were used as external standards for GC/MS quantification of the alkane fraction, aromatic/polar fractions, and PLFA extracts respectively.

GC/MS analysis was performed on an Agilent model 6890N chromatograph coupled to an Agilent model 5973N quadrupole mass selective detector. Separation was achieved with a HP-5MS fused silica column (30 m × 0.25mm i.d., 0.25 µm
thickness) and with He as the carrier gas. GC operating conditions were: 65 °C (2 min) to 300 °C (at 6 °C/min, held 20 min). Solvent extract samples (1 µl) were injected with a 2:1 split ratio and PLFA extracts were injected in splitless mode with an injector port temperature of 280 °C using and Agilent 7683 autosampler. The spectrometer was operated in electron ionization mode (EI) at 70 eV and scanned from m/z 50 to 650. Data were acquired and processed with Agilent Chemstation G17001DA software. Compounds were identified by comparisons of the mass spectra patterns to a MS library (Wiley275 MS library), comparison with authentic standards and with published data.

3.2.3. NMR Spectroscopy

Soil samples (~20g) were repeatedly treated with HF (0.3M) to concentrate the amount of OM and remove paramagnetic minerals that are problematic during NMR acquisition. Samples were rinsed with deionized water several times to remove excess salts and freeze dried. HF treated soil (~100 mg) was packed into a 4-mm zirconium rotor with a Kel-F cap. The $^{13}$C Cross Polarization with Magic Angle Spinning (CP-MAS) NMR spectra were acquired on a Bruker Avance III 500 MHz NMR spectrometer, equipped with a 4-mm H-X MAS probe, using a ramp-CP pulse program. The data was acquired with a spinning rate of 13 kHz, a ramp-CP contact time of 1 ms, a 1 s recycle delay, and line broadening of 50 Hz.

The remaining HF treated soil was subjected to five additional treatments with 10% HF before humic extraction. Soil humic materials were exhaustively extracted from the remainder of the HF-treated soil with NaOH (0.1 M) under nitrogen, filtered through
0.2µm Teflon filter, cation exchanged with Amberjet 1200H ion exchange resin, freeze-dried and then dried over P₂O₅. Humic extract samples (100mg) were dissolved in D₂O/NaOD (1 mL) and transferred to 5 mm NMR tubes for analysis. The ¹H PURGE NMR technique was employed for solvent suppression (21) and diffusion edited (DE) ¹H NMR was employed to emphasize signals from macromolecules and/or rigid domains (22). Heteronuclear Multiple Quantum Coherence (HMQC) NMR spectroscopy experiments were completed to provide ¹H-¹³C bond correlations to resolve overlapping signals (23).

NMR data was acquired on a Bruker Avance III 500 MHz spectrometer using a QXI probe. 1-D solution state ¹H NMR experiments were performed with 256 scans, a recycled delay of 3 s and 32 K time domain points. Spectra were apodized through the multiplication with an exponential decay corresponding to 1 Hz line broadening in the transformed spectrum, and a zero filling factor of 2. Diffusion Edited (DE) experiments were employed by a bipolar pulse longitudinal encode-decode sequence (22). 1024 scans were collected using 1.25 ms, 53.5 gauss/cm, sine shaped gradient pulse, a diffusion time of 50 ms, 16 384 time domain points and a sample temperature of 298K. Heteronuclear Multiple Quantum Coherence (HMQC) spectra were collected in phase sensitive mode using Echo/Antiecho gradient selection. 2048 scans were collected for each of the 128 increments in the F1 dimension. 2 K data points were collected in F2, a 1 J ¹H-¹³C (145 Hz). The F2 planes were multiplied by an exponential function corresponding to a 5 Hz line broadening, while the F1 dimension was processed using sine-squared functions with a π/2 phase shift and a zero-filling factor of 2.
3.3. Results and Discussion

3.3.1. Biomarkers

Differences in the concentration of soil OC and biomarkers were observed at the selected sampling locations (Figure 3.2). A high concentration of PLFAs was observed at W3 where the ALDs occurred in 2007 (Figure 3.2B). More than double the concentration of PLFAs were observed for bacteria: gram-negative bacteria: 16:1ω7, cy17:0, 18:1ω7, and cy19:0; + gram positive bacteria: i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, i18:0, and a18:0), fungi (18:2ω6), and actinomycetes (10Me18:0; Figure 3.2B) than the undisturbed sites (W1, W4, E1, and E2; Figure 3.3). In addition, a low PLFA concentration was observed at W2 where historic ALDs have taken place. PLFAs are only detected in viable cells because they decay within 2-4 days after cell death (24) and are useful for assessing changes in microbial activity (17). An increase in total PLFA biomarkers at W3 suggests that the ALDs introduced a microbial SOM nutrient source for the new population to flourish, whereas SOM introduced at W2 has been depleted years after ALDs. This hypothesis is tested by calculating PLFA stress proxies (9, 25). An increase in cyclopropane PLFA-to-monoenoic precursor (ratios of cy17:0/16:1ω7 and cy19:0/18:1ω7) has been observed to with declining substrate availability (9, 25). The ratio of cy17:0/16:1ω7 was four times higher in W2 than the remaining samples indicating that microbes at this location have limited OM substrates available as nutrients, whereas this ratio is the lowest at W3 (Figure 3.2C). The differences in cy19:0/18:1ω7 ratios between the samples were less prevalent, but still indicate higher microbial substrate constraints at W2. Concentrations of solvent-extractable labile plant lipids (C20-C32: n-alkanes, n-alkanols, n-alkanoic acids) for both
W3 and W2 are lower than the undisturbed samples which may reflect microbial activity and preferential mineralization of labile constituents at W3 (Figure 3.2D). Plant lipids from W2 are most depleted, which suggests a previous surge in microbial activity that depleted some of the labile components, leading to a lower OC% overall.

3.3.2. Solid-state $^{13}$C NMR spectroscopy

$^{13}$C CP-MAS NMR analysis of SOM provides general structural information for the large number of compounds present in the SOM mixtures from the broad signals resulting from strong dipolar coupling in solid-state (26). It provides information obtained about the OC structure distribution complements other molecular-level techniques (27). Chemical shifts regions arising from specific functional groups have been well established and are labelled accordingly in Figure 3.4 and integration values are reported in Table 3.1 (26). The relative degree of SOM degradation is estimated from the alkyl/O-alkyl ratio because this ratio has been observed to increase with degradation (5, 26, 27). The calculated alkyl/O-alkyl ratios (Table 3.1) range from 0.59-0.88, indicating that OM at W2 is the most degraded, whereas W3 is the least degraded. O-alkyl and acetal carbons (anomeric carbon constituents) are considered to serve as substrates for a large number of bacteria and fungi in SOM (5). In addition, increased microbial activity in soils results in new carbohydrates, initially increasing the labile component of SOM (28). Overtime, the microbes catabolize O-alkyl carbon, synthesizing alkyl carbon structures and results in net alkyl carbon accumulation and increased degradation (28). Note that alkyl lipids produced from microbes are distinctly different from the detected lipid biomarkers in this study because microbial lipids contain
chains $< C_{20}$ whereas the lipid data presented in this study specifically targeted labile lipids originating from plants (20).

The $^{13}$C NMR data suggests a depletion of carbohydrates in W2 in comparison to the undisturbed sites (W1, W4) and W3 with the lowest alkyl/O-alkyl ratio. The increased levels of carbohydrates at W3 are likely a combination of newly synthesized carbohydrates (28), along with “old” preserved carbohydrates released from permafrost (3, 5, 10) by ALDs that contribute to the overall “fresh” nature of this SOM. In contrast, the carbohydrate contribution at W2 is substantially lower (highest alkyl/O-alkyl ratio), likely from labile structure catabolism into the more stable alkyl fraction (28) further suggesting that increased microbial activity from ALDs results in an overall degradation of Arctic SOM which is also apparent by the enrichment of more recalcitrant aromatic carbon (Table 3.1). This generalization, though helpful, does not ascertain the degree of alteration of specific SOM components (27), therefore higher-resolution solution-state NMR techniques are also employed to confirm these results.

3.3.3. Solution-state NMR spectroscopy

The study of microbial signatures by $^1$H NMR spectroscopy has recently been used to assess the contributions of microbial populations in soil extracts in comparison to cultured microbes (18). The $^1$H NMR spectrum of the Arctic SOM extract from W1 is shown in Figure 3.5A with the major chemical contributors assigned to the regions (spectra for the remaining samples is in Figure 3.6). DE $^1$H NMR was also employed to remove NMR signals from compounds that exhibit significant mobility during NMR analyses, leaving signals originating from macromolecules or large rigid domains (22).
Comparison of the DE (Figure 3.5B) with the unedited spectrum (Figure 3.5A) indicates that aliphatic signals are mainly attenuated suggesting that this component of the SOM is comprised mainly of smaller aliphatic compounds such as smaller cuticular-derived materials and/or lipids (29, 30). Contributions from carbohydrates and protein/peptide side chain residues are observed in the DE spectrum, the protein especially likely originates from microbial species (18). While some contributions from carbohydrates may be from the microbes (18), the contribution from carbohydrates is considerably larger than seen in microbial cells suggesting the additional inputs from SOM. The broad peak at 2.03 ppm (N-Acetyl functional group) remains in the DE spectrum, suggesting the presence of peptidoglycan (19). Any information beyond broad peak characterization is inhibited by the complexity of this mixture; therefore more sophisticated 2-dimensional (2-D) NMR techniques were also employed for more detailed information regarding microbial peptide/protein contribution (and therefore microbial biomass) to this Arctic OM.

HMQC NMR spectroscopy provides $^{1}$H-$^{13}$C bond correlations which helps resolve overlapping signals observed in $^{1}$H NMR data (23). Assignments are based on previous studies (18, 19, 31-33) and have been confirmed by spectral prediction and pattern matching (19, 34). Resonances associated with microbial contributions and carbohydrates are assigned for W1 in Figure 3.7 (spectra for the remaining samples can be found in Figure 3.8). HMQC spectra include resonances originating from CH and CH$_{2}$ functionalities from carbohydrates, possibly originating plant cellulose or hemicellulose (18, 19, 33), α-CH groups likely from microbial peptides/proteins (18), and the N-acetyl functional group from peptidoglycan, an additional indicator of microbial
contributions (19). A semi-quantitative approach was taken where the integration of specific cross peaks in each sample HMQC spectrum was normalized to the total intensity of the entire spectrum to compare samples (Table 3.2). Note that absolute quantification from 2D HMQC NMR is complicated by relaxation processes and use of a single average $^1$J C-H coupling constant during acquisition; however relative quantification between similar samples acquired under identical conditions is valid. Due to peak overlap of CH$_3$ functional groups from plant lipids and proteins/peptides, region 6 in Figure 3.7 was not included in the integration analysis of the 2-D NMR (34). The increases/decreases in the normalized area ratios between the samples are used as a proxy for assessment of changes in microbial populations from ALDs. $\alpha$-CH groups from microbial peptides/proteins have the largest normalized ratio in W3, corroborating observations made from biomarkers (Figure 3.2) and solid-state $^{13}$C NMR (Table 3.1; Figure 3.4). In addition, $\alpha$-CH groups have the smallest value in W2, also consistent with previous measurements and the undisturbed samples (W1 and W4) lie between W3 and W2 (Table 3.2). The same relationship was observed for the normalized ratios pertaining to carbohydrates, however an inverse trend was observed for the signal of the $N$-acetyl group of peptidoglycan. Peptidoglycan is a major component of bacterial cell walls, therefore can be used as an additional measure of bacterial biomass (19, 35). W2 contained the largest normalized ratio of $N$-acetyl functional groups suggesting that a large amount of bacterial cell walls are present. However, experimental data has shown that peptidoglycan can be protected from microbial degradation after cell death in soils by copolymerization reactions and transformation, substantially adding to the refractory nitrogen pool in SOM (36-38). Therefore it is plausible that the observed
peptidoglycan originates from both living and dead bacteria. This knowledge in conjunction with the low concentration of extracted PLFA biomarkers at W2 suggests that the majority of the detected peptidoglycan is from dead cells that have been protected, and further indicates a potentially historical higher microbial population at W2. The peptidoglycan normalized ratio is lower in W3 than the controls (W1, W4) despite having the highest living biomass based on PLFA measurements (Figure 3.2B). This suggests a possible dilution effect from the release of SOM from ALDs. As time progresses, the microbial biomass at W3 may continue to degrade the available labile SOM, and may result in increased peptidoglycan relative to the remaining SOM area (similar to W2).

3.4. Conclusions

This molecular-level approach for measuring permafrost ALDs to changes in microbial activity suggests that the biogeochemical cycling rates of SOM previously stabilized under the harsh Arctic climatic conditions are altered, perhaps leading to an overall SOM priming depleting the overall SOM concentration (Figure 3.9). ALDs brought on by warmer temperatures provide the available labile nutrients and optimal living conditions that may allow microbes to flourish and accelerate the decomposition of SOM. SOM priming effects have been documented experimentally by the alteration of CO₂ levels and plant biomass (39, 40). The precise mechanism by which ALDs could release previously locked carbon from permafrost is not known and there are multiple complicating factors pertaining to the mineralization of this carbon (10). Some plausible explanations are that ALDs in conjunction with rising temperatures result in the
destabilization of frozen SOM, SOM-mineral associations that form naturally in the environment (41), both of which could have been contributing to the “protection” of this SOM thereby increasing SOM exposure to oxygen. The increase in microbial biomass activity observed by PLFA biomarkers, and both solid- and solution-state NMR spectroscopy suggests that ALDs induce microbial activity, thereby enhancing the mineralization of Arctic SOM from permafrost. This in turn may release more CO$_2$ into the atmosphere, which will further amplify the climate change impact on the Arctic, potentially leading to more ALDs, microbial decomposition, and SOM priming. The net result is a positive feedback loop to climate change, leading to a depletion of the SOM stored in the Arctic making permafrost in the Arctic an important carbon source in the future.
3.5. References


3.6. Tables

Table 3.1: $^{13}$C NMR Integration Results and Calculated Alkyl/O-Alkyl Ratios (26, 27)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Alkyl C (0-50 ppm)</th>
<th>O-Alkyl C (50-110 ppm)</th>
<th>Aromatic C (110-165 ppm)</th>
<th>Carboxylic C (165-210 ppm)</th>
<th>Alkyl/O-Alkyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1</td>
<td>34</td>
<td>48</td>
<td>11</td>
<td>7</td>
<td>0.70</td>
</tr>
<tr>
<td>W2</td>
<td>33</td>
<td>34</td>
<td>20</td>
<td>9</td>
<td>0.88</td>
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<tr>
<td>W3</td>
<td>30</td>
<td>50</td>
<td>12</td>
<td>7</td>
<td>0.58</td>
</tr>
<tr>
<td>W4</td>
<td>30</td>
<td>51</td>
<td>11</td>
<td>8</td>
<td>0.60</td>
</tr>
</tbody>
</table>
Table 3.2: Summary Comparison of Normalized HMQC Ratios of Carbohydrates and Microbial Associated Functional Groups (18, 19, 31-34)

<table>
<thead>
<tr>
<th>Sample</th>
<th>α^1^H-^1^3^C from proteins (4.0-5.0, 48-58 ppm)</th>
<th>Carbohydrates (CH + CH₂) (4.1-5.6, 96-106 ppm + 3.1-4.5, 58-90 ppm)</th>
<th>Peptidoglycan (N-acetyl) (2.0-2.1, 18-22 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1</td>
<td>2.07</td>
<td>3.32</td>
<td>1.01</td>
</tr>
<tr>
<td>W2</td>
<td>1.86</td>
<td>3.27</td>
<td>1.16</td>
</tr>
<tr>
<td>W3</td>
<td>2.09</td>
<td>4.51</td>
<td>0.96</td>
</tr>
<tr>
<td>W4</td>
<td>1.96</td>
<td>3.75</td>
<td>0.98</td>
</tr>
</tbody>
</table>
3.7. Figures

Figure 3.1: SOM sample locations from Cape Bounty Arctic Watershed Observatory, Melville Island, Nunavut, Canada.
Figure 3.2: Biomarker concentrations for the West soil OM (A) OC content (by % Dry Weight), (B) Total PLFA (bacterial, fungal, actinomycetes), C) PLFA substrate availability stress factors, D) Labile plant lipids (C\textsubscript{20}-C\textsubscript{32}).
Figure 3.3: Concentration of individual PLFA biomarkers for each location.
Figure 3.4: $^{13}$C CP-MAS NMR spectra of whole HF-treated soil. Major integration areas and peaks are identified. An increase in carbohydrate derived carbon is observed in W3 with an increased intensity of identified peaks in the O-Alkyl region (smallest Alkyl/O-Alkyl, Table 3.1) where there is significant degradation observed in W2 (highest Alkyl/O-Alkyl, Table 3.1).
Figure 3.5: $^1$H NMR spectra for the humic extract from W1. A) Conventional $^1$H NMR spectrum acquired with PURGE NMR for solvent suppression (21) and B) The analogous diffusion-edited (DE) $^1$H NMR. The application of DE $^1$H NMR allows for the identification of major indicators of microbial peptide/protein contributions to OM (18).
Figure 3.6: $^1$H NMR spectra of humic extracts for sites W2, W3, and W4. Top spectra are conventional HMR spectra acquired with PURGE NMR with the bottom spectra are the analogous DE $^1$H NMR spectra.
Figure 3.7: HMQC spectrum of the W1 humic extract. Major assignments pertaining to microbial proteins and carbohydrates are as follows: 1, anomeric protons (carbohydrates); 2, other CH in carbohydrates; 3, CH₂ in carbohydrates; 4, α-protons in peptides and proteins; 5, N-Acetyl functionality in peptidoglycan; 5, CH₃ (majority from peptides with a small contribution from terminal CH₃ from lipids) (19, 34). Normalized peak area integrations for each sample are presented in Table 2. Region 6 is not integrated due to overlapping resonances from several different classes of compounds.
Figure 3.8: HMQC spectra of the humic extracts from W2, W3, and W4. Major assignments pertaining to microbial proteins and carbohydrates are as follows: 1, anomeric protons (carbohydrates); 2, other CH in carbohydrates; 3, CH$_2$ in carbohydrates; 4, $\alpha$-protons in peptides and proteins; 5, N-Acetyl functionality in peptidoglycan; 5, CH$_3$ (majority from peptides with a small contribution from terminal CH$_3$ from lipids). Normalized peak area integrations for each sample are presented in Table 2. Region 6 is not integrated due to overlapping resonances from several different classes of compounds.
Figure 3.9: Illustration of possible SOM priming induced by ALDs at Cape Bounty.
Chapter 4: Summary and synthesis

Molecular-level biomarker techniques (plant and microbial) and various nuclear magnetic resonance spectroscopy (NMR) methodologies have been carried out to characterize Arctic organic matter (OM), and quantify changes to OM caused by physical land disruption induced by unprecedented climate warming in the Canadian High Arctic. The biomarker characterization of littoral sedimentary OM for the East Lake at the Cape Bounty Arctic Watershed Observatory indicated that the OM is a mixture of fresh recently deposited and/or preserved lipids from plants and highly altered/oxidized lignin-derived phenols. In addition comparison of sediment biomarkers in the alkane solvent extract fraction with those of a native Arctic plant showed that the observed series of C\textsubscript{24} to C\textsubscript{34} iso- and anteiso-alkanes are likely from a previously undocumented source (Cerastium arcticum; Arctic mouse-ear chickweed). These compounds may be useful for monitoring terrestrial OM input as tracers in this Arctic ecosystem now that they have a documented source. Overall, it is postulated that the OM may have in fact originated from accumulated permafrost that has recently become bioavailable via melting and fluvial transport to the littoral sediments.

Persistent warm temperatures during July 2007 at Cape Bounty together with 10.8 mm of rainfall in late July resulted in rapid and deep active layer formation and widespread permafrost active layer detachments (ALDs) across the West watershed. Changes in the Arctic soil organic matter (SOM) composition brought on by the ALDs were documented by microbial biomarkers (PLFAs), multidimensional solution-state NMR and solid-state NMR. Analysis of permafrost ALDs locations in comparison to undisturbed locations in the watershed determined noticeable changes in microbial
activity which suggests that the biogeochemical cycling rates of SOM previously stabilized under the harsh Arctic climate conditions were altered, perhaps leading to an overall SOM priming effect. Furthermore ALDs brought on by warmer temperatures could in fact release available labile nutrients and oxygen availability, allowing for optimal living conditions that may allow microbes to flourish and further accelerate the decomposition of Arctic SOM. The increase in microbial activity suggests that ALDs induce microbial activity, thereby enhancing the mineralization of Arctic SOM from permafrost. This in turn may release more CO$_2$ into the atmosphere, which will further amplify the climate change impact on the Arctic, potentially leading to more ALDs, microbial decomposition, further SOM priming and a positive feedback to climate change.

Natural OM characterization by the biomarker methodology is a “targeted” chemical approach due to the complexity of the OM mixture. Major contributors to naturally occurring OM are plants and microbes (1, 2). There are a wide variety of analytical approaches to determine molecular-level structural information by targeted extractions. Chemolytic techniques employed in for this project are common techniques to determine amino acids/proteins, polysaccharides, lipids and aromatic oxidation products from lignin (3). Microbial markers (amino sugars, muramic acid, phospholipid fatty acids) can be analyzed after hydrolysis and separation. Alternatively, pyrolysis techniques can be employed and the fragments can be used to reconstruct the original OM structure however little is known about how the OM changes when it is subjected to these conditions (3).
In this work, solvent-extraction is performed to release chemically unbound lipids (4) which comprise approximately 10% of the total OM (5). The lipid composition does however contain characteristic biomarkers that contain information about source and degradation residues present in the OM (6). Additional extraction techniques could be carried out to release the chemically bound lipids that such as base and acid hydrolysis that target compounds originating from cutin/suberin and carbohydrates, amino acids, and peptides (7). Lignin is the second most abundant molecule in vascular plants and represents a major input of plant OM to soils (1, 8). The transformation of the extractable lignin-derived phenol biomarkers from soil, sediment and water in a wide variety of environments has been related to carbon cycling and degradation of natural OM (9-15) which was the rationale for extracting it in this study.

It has been shown experimentally that these targeted extraction techniques are complementary of one another and are a good representation to extrapolate trends regarding OM composition and degradation (7). When directly compared to the general functional group characterization by nuclear magnetic resonance spectroscopy (NMR) to the different biomarkers, it was found that the biomarker trends were in agreement with the NMR results (16). Therefore the variety of extraction techniques may be viewed as complementary and targeted OM extractions are representative of OM sources and degradation overall.

Very recently it has been shown experimentally that permafrost thaw from warmer temperature results in “old” carbon release and net carbon exchange from Arctic regions by respiration (17). However, the change from a carbon accumulation zone to a potential carbon source may not be directly coupled to a change in
temperature; temperature changes may result in enhanced drainage, soil-tundra aeration, a decrease in the water table (18), or as discussed in chapter 3, a physical disruption. Respiration can represent a significant fraction from the annual carbon cycle, however the temperature response of microbial CO₂ production from these frozen regions are still not fully understood (19).

There are still many questions to consider for the enhancement of the understanding of how environmental change will alter OM storage in Arctic regions. Assuming that all other variables are held constant, a rise in atmospheric CO₂ concentration should increase plant growth resulting in the greater delivery of plant debris to soil/sediment where a small fraction will persist, eventually leading to an overall increase in natural OM (20). Also, an increase in average Earth atmospheric temperature should increase soil/sediment temperature at higher latitudes, leading to an increase in respiration (21). In addition, the initial response to warming may be the net loss of carbon from this large OM pool by respiration and mineralization, however over the long term, the Arctic ecosystem may alter and the invasion of shrub and tree species may result in an increase of an above ground carbon storage (18). The simultaneous increase in CO₂ in the atmosphere in conjunction with an average rise in Earth atmospheric temperature provides a high level of uncertainty for prediction of carbon turnover response from climate change (21). Fresh plant material will decompose faster under elevated temperatures however plant residues are deposited at a variety of ages and quality, which contribute to the rate of decomposition (22). Natural OM in soil and sediment is not well mixed resulting in a spatial distribution of
To attempt to predict the future response of Arctic OM destabilization and its contribution to rising concentrations of atmospheric CO₂ further experiments should be conducted. The knowledge gained from the studies presented in this thesis could be used to carry out subsequent studies to determine/quantify the carbon dynamics from OM in Arctic ecosystems. Some proposed studies include:

1) A long term monitoring program of the ALDs at the Cape Bounty Arctic Watershed Observatory where samples are collected every field season to determine the long term effects on Arctic OM to monitor for OM priming. The study in Chapter 3 suggested that ALDs lead to an increase in microbial activity, ultimately depleting the OM pool. Therefore, applying those techniques to monitor the changes in OM composition induced by the ALDs on a yearly basis will precisely monitor the long-term changes/decomposition of OM released from permafrost.

2) The isolation of specific biomarkers from Arctic sedimentary OM to determine the age of the material by compound specific radiocarbon isotopic analysis (24). In Chapter 2, the carbon preference index (CPI) predicted that the labile plant lipids were relatively “fresh” indicating the recent deposition of unaltered material. However, it was not possible to determine whether this resulted from the deposition and transport of recent plant debris, or is from permafrost release that has been suggested to contain preserved, unaltered material (19, 25). Therefore the extraction and isolation of lipid biomarkers by gas chromatography –
preparative fraction collection (GC-PFC) could be used to distinguish the age of this material. In particular, the \( n \)-alkane biomarkers would be ideal candidates because carbon impurities would not be added during derivitization.

3) The isolation of specific lignin-derived phenols after CuO extraction for compound specific radiocarbon isotopic analysis (24) would provide insight into the cycling and residence time of lignin in the Arctic. The results in Chapter 2 indicated a highly altered lignin that was suggestive a very long residence time in the Arctic. Dating the extracted lignin-derived phenols would confirm these results. This could be done using GC-PFC as previously described, however that would be complicated by the derivitization procedure where TMS is added to replace the OH functional groups to volatilize the compounds. Separation of the extraction lignin-derived phenols has been achieved using high performance liquid chromatography (HPLC), which would be an optimal alternative for isolation of specific phenols (26). This method could be applied, and the phenols collected by an HPLC-fraction collector and the isolated compounds could then be \(^{14}\)C dated. This would be advantageous because the lignin-derived phenols would not have to be derivitized, limiting that source of error.

4) The determination of microbial degradation of old permafrost carbon could be determined by radiocarbon analysis. A recent study has indicated that “old carbon” is being released from Arctic ecosystems by respiration (17), however the contribution of microbial degradation was not investigated. Little is known about how microbial degradation dynamics in the Arctic will change in a warming climate or by the release of “new” permafrost OM (27). Chapter 3 has shown that
ALDs result in the increase of microbial biomass, however whether the release is just stimulates the activity, or whether there is a microbial preference for “old” or “new” carbon is not known. Therefore, these ALDs provide a unique opportunity to monitor the microbial degradation process. PLFA biomarkers could be extracted and isolated by GC-PFC, then dated by compound specific radiocarbon analysis (24). If there is a preference of “old” or “new” carbon, this could be shown by its incorporation into the microbial membrane. Care must be taken in radiocarbon analysis, and an isotopic mass balance approach must be done to account for the derivitization of the PLFAs to the fatty acid methyl esters (FAMEs). Low concentrations may also be a limiting factor; therefore bulk radiocarbon analysis on the entire lipid phosphate extract may serve as an optimal starting point.

These future studies will enhance the knowledge regarding the carbon cycling, turnover and biogeochemistry in Arctic ecosystems ultimately determining whether the Arctic will in fact transition from a net carbon sink to a carbon source in a warmer climate.
4.1. References


