Holocene Perspectives on Hydrogen Isotope Ratios of Boreal Plant Waxes in Northwestern Canada

by

Aleesha Mary Pokrant Bakkelund

A thesis submitted in conformity with the requirements for the degree of Master of Science
Department of Geography and Planning
University of Toronto

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Abstract

This thesis provides novel insights into plant wax characteristics and fractionation in high latitude boreal forests in the Holocene. The isotopic composition of precipitation is a tracer for climatic changes. The hydrogen isotopic composition of plant waxes ($\delta D_{\text{wax}}$) in sediments is a proxy for precipitation $\delta D$ and paleoclimate but is offset from $\delta D_{\text{precip}}$ due to a large net fractionation from several biotic and abiotic factors. The net fractionation is constrained for the northern boreal forest based on topsoils from a 13-site network in NW Canada. This will help future studies quantitatively reconstruct $\delta D_{\text{precip}}$ from plant waxes. This thesis also presents the first $\delta D_{\text{wax}}$ record that extends through the full Holocene in Eastern Beringia from a lake sediment core in SW Yukon. Good coherence with other isotope proxies in the region suggests that this record accurately reconstructs variability in $\delta D_{\text{precip}}$ and is likely driven by changes in atmospheric circulation and aridity.
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Chapter 1
Introduction

1.1 Introduction

Understanding climate change in the recent past is critical for preparing for future climate change. Fossil plant waxes are ubiquitous and are emerging as an exciting proxy for paleoclimate reconstructions. However, plant waxes have been underutilized in eastern Beringia (the North American portion of the landmass spanning northeastern Siberia to northwestern Canada), and northern boreal regions generally. Holocene climate reconstructions from northern boreal regions are of particular interest to the paleoclimate community because they provide evidence for how this rapidly changing region responds to forcings when the boundary conditions are similar to present (Kaufman et al., 2016). My research aims to, first, constrain the offset between the isotopic composition of hydrogen in plant waxes and the isotopic composition of hydrogen in precipitation in northwestern North America, in order to make more accurate inferences about climatic change in the Holocene, and second, create a stable hydrogen isotope plant wax record spanning the full Holocene in southwestern Yukon. The modern soils calibration dataset will be invaluable to the paleoclimate community and allow researchers to better interpret sedimentary archives in boreal regions. Proxy climate datasets are necessary for improving knowledge of Holocene climate dynamics, and ultimately will lead to improved predictive models for future climate change.

1.2 Holocene climate reconstructions for Eastern Beringia

The transition from the Pleistocene to the Holocene was a time of pronounced climatic change, with rapidly warming temperatures and large volumes of meltwater flowing from retreating glaciers, raising sea levels. Most quantitative temperature reconstructions of the Holocene have been based on midge and pollen assemblages; however, these two proxies have not yet reached a consensus. Overall, midge records in eastern Beringia show warmer than modern temperatures in the early (10 to 9.5 ka; Kaufman et al., 2016). Whereas, Viau et al. (2008) produced quantitative temperature estimates from 45 sites with pollen assemblages using the Modern Analogue Technique and found evidence that eastern Beringia experienced less seasonal variation in temperature than present (warmer winters, cooler summers) in the early Holocene than today,
however mean annual temperatures were similar to present. Pollen and midge records both show a Holocene Thermal Maximum (HTM) in the mid Holocene (7 to 5 ka). However, midges show a general warming over the last thousand years, whereas pollen shows a slight cooling of both summer and mean annual temperatures over that period (Kaufman et al., 2016). Midge-based temperature reconstructions are generally thought to be reliable; however, midge assemblages can also be effected by other variables including water depth, vegetation, water chemistry and nutrient availability (Kurek and Cwynar, 2009; Kaufman et al., 2016). Quantitative temperature reconstructions from pollen assemblages using the Modern Analogue Technique may be impacted by shifts in soil conditions, disturbance or other factors that may also vary with climatic changes. The effectiveness of this technique may also be limited when they rely on taxa that thrive in a wide variety of climatic conditions (Birks and Birks, 2000). This may be particularly problematic in the Holocene, given that most reconstructions estimate less than 5°C change over the past 12 ka (Kaufman et al., 2016). Therefore, our understanding of Holocene climatic change in eastern Beringia may benefit from perspectives from other proxies. Paleoclimatic trends that are corroborated by more than one independent proxy can be viewed with a higher degree of confidence.

1.3 Paleoclimate reconstructions using hydrogen isotopes

Stable hydrogen and oxygen isotopes are commonly used in paleoclimate studies because the isotopic composition of precipitation is strongly correlated with temperature (Dansgaard, 1964; Porter et al., 2016). Hydrogen has two stable isotopes, protium or Hydrogen-1 (H) and deuterium or Hydrogen-2 (²H or D). δD is used to represent the ratio of D/H in permille relative to a standard (Vienna Standard Mean Ocean Water (VSMOW); δD_{VSMOW} = 0‰). δD is calculated using δD = (R_{sample} − R_{standard})/R_{standard}, where R represents D/H isotopic ratios.

Proxy estimates of δD can be used to estimate paleotemperature with an appropriate transfer function (Porter et al., 2016). Temperature is the dominant control on the isotopic composition of precipitation; however, this relationship is not spatially uniform because it is affected by the moisture source and rainout history of air masses (Fisher et al., 2004; Anderson et al., 2005; Birks and Edwards, 2009). Therefore, it is important to use a regionally appropriate transfer function for paleotemperature estimates. Porter et al. (2016) developed a transfer function
$\left( \delta D_{\text{precip}} = (3.1 \pm 0.1) \text{‰} \times T - (155 \pm 1) \text{‰} : 1 \sigma_{\text{residuals}} \right)$ for Canada, based on data from twenty-two Global Network of Isotopic Precipitation (GNIP) stations. This calibration is similar to one used in northeastern Russia (Wilkie et al., 2013).

## 1.4 Plant waxes as a paleoclimate proxy

It is impossible to study climate in the past directly, so an array of proxies have been developed to measure paleotemperatures. The carbon-bound hydrogen isotopes in long (>C$_{20}$) straight chain $n$-alkanoic acids and $n$-alkanes in plant waxes are derived from hydrogen atoms in precipitation. Plant waxes are primarily found in leaf cuticles and are designed to protect the leaf tissues from the atmosphere, reduce water loss from the leaf, and limit transfer of water and chemicals into the leaf (Diefendorf et al., 2011; Yeats and Rose, 2013). Studies have shown $\delta D$ of plant waxes reflect the $\delta D$ of meteoric waters (Hou et al., 2008; Tierney et al., 2008; Sachse et al., 2012; Wilkie et al., 2013). This proxy is relatively new because it was not possible to measure the $\delta D$ of individual organic compounds until improvements in isotope-ratio mass spectrometry were made in the 1990s (Eglinton and Eglinton, 2008; Sachse et al., 2012).

There are several advantages of plant waxes as a paleoclimate proxy. Plant waxes are well preserved in sedimentary archives and widespread in continental regions. Stable oxygen and hydrogen isotopes in ice cores are a ‘gold standard’ proxy in paleoclimate research and are unparalleled in their contribution to knowledge of Quaternary climate change. However, ice core archives are limited to glaciated locales, which leaves a spatial gap in the understanding of paleoclimate change in continental regions (Hou et al., 2008). Plant wax archives can be used to fill this spatial gap. Plant waxes can be preserved over geologic timescales because they are hydrophobic, chemically inert, resistant to biodegradation, and have negligible volatility (for compounds with more than 20 carbon atoms) (e.g. Yang and Huang, 2003; Tierney et al., 2008; Vonk et al., 2017). Long chain (>20 carbon atoms) $n$-alkyls with even over odd chain length predominance are biomarkers for terrestrial vegetation, and are more straight-forward to interpret than bulk organic material (Eglinton and Eglinton, 2008). Additionally, lipid hydrogen atoms are covalently bound to carbon atoms and are not readily exchanged below 150°C (Sessions et al., 2004).
1.5 Fractionation factors

Studies have shown that while the δD of plant waxes ($\delta^{18}D_{\text{wax}}$) reflect the δD of precipitation ($\delta^{18}D_{\text{precip}}$), $\delta^{18}D_{\text{wax}}$ is offset due to the combined effects of soil evaporation, leaf transpiration, and biosynthetic processes. This offset is referred to as the net (or apparent) fractionation or enrichment factor and is represented as $\varepsilon_{\text{wax/precip}}$. It is important to constrain the net fractionation to estimate $\delta^{18}D_{\text{precip}}$ accurately.

Most plants absorb water from soil moisture, which is ultimately derived from precipitation. Lighter water molecules evaporate first, causing the soil water to become enriched in D, however this fractionation is generally minor (Feakins and Sessions, 2010). Hydrogen isotopes are not fractionated during uptake of source water by roots (White et al., 1985; Ehleringer and Dawson, 1992; Roden et al., 2000); however, leaf transpiration can result in D enrichment of plant water. Evaporative fractionation effects are controlled by the relative humidity, temperature, and isotopic composition of the water vapour surrounding the leaf (Kahmen et al., 2008; Sachse et al., 2012). Aridity can increase δD enrichment of soil and plant water, and it has been hypothesized that a 24-hour daylight in high latitude regions can have a similar effect (Yang et al., 2009, 2011; Shanahan et al., 2013). Yang et al. (2009) conducted a greenhouse experiment on woody plants which demonstrated that plants grown in low humidity had up to 40‰ heavier δD values for $n$-alkanes than plants grown in high humidity (Yang et al., 2009). McInerney et al. (2011) found that soil evaporation has a greater impact than leaf transpiration on C3 and C4 grasses due to their rooting depth (McInerney et al., 2011); however, Brader et al. (2010) found that evaporation has a minimal effect on Sphagnum (Brader et al., 2010). This indicates that the relative fractionation effects of source water evaporation and leaf water transpiration are largely dependent on plant species and their rooting depths.

Biosynthetic hydrogen-transfer reactions result in substantial isotopic fractionations that result in D-depletion. Hydrogen isotope fractionations are a result of four factors: different biosynthetic pathways, secondary hydrogen exchange reactions, different isotopic composition of NADPH, and extrinsic secondary factors.

There are three primary biosynthetic pathways that each produce different types of lipids. The acetogenic pathway synthesizes $n$-alkyl lipids such as the $n$-alkanes, $n$-alkanols and $n$-alkanoic acids ($n$-acids), which are used as terrestrial biomarkers (Diefendorf et al., 2011). The acetogenic
pathway results in the least D-depletion of the biosynthetic pathways (Chikaraishi et al., 2004b; Sachse et al., 2012). Acetogenic biosynthesis begins in chloroplasts and produces a butyryl chain with seven hydrogen atoms derived from different sources: three from acetate, two from NADPH, and two from water. The cycle is repeated, adding additional acyl-CoA units to lengthen the hydrocarbon until it is about 16 carbons long. The hydrocarbon is then transferred to the endoplasmic reticulum for further elongation (Sachse et al., 2012; Yeats and Rose, 2013).

Homologous molecules with different degrees of desaturation show varied δD values because of hydrogenation and dehydrogenation reactions (Chikaraishi et al., 2004b; Schwab and Sachs, 2009; Sachse et al., 2012). Decarboxylation of n-alkanoic acids to form n-alkanes also results in a D depletion (Sachse et al. 2012). Hydrogen derived from NADPH is strongly D depleted relative to water. NADPH produced through photosynthesis is more depleted in D than NADPH produced through sugar metabolism (Chikaraishi et al., 2004b; Sachse et al., 2012). Generally, plant lipid biosynthesis primarily uses NADPH created through photosynthesis (Sachse et al., 2012). External factors such as salinity, temperature, growth rate, growth stage, and light intensity may affect biosynthetic fractionations; however, the relative effects of these are not known and are difficult to isolate from other fractionation effects (Sachse et al., 2012).

Net fractionation values (εwax-precip) are calculated to capture the total effects of the fractionation factors discussed above. Variation in εwax/precip has been noted between different lifeforms and photosynthetic pathways. Large variability in net fractionation values have been recorded between plant lifeforms. Shrubs are, on average, the most enriched in deuterium, followed by trees, forbs, and graminoids (Sachse et al., 2012). D-depletion in graminoids is primarily related to physiological differences. Graminoids are monocotyledonous, whereas shrubs, trees, and forbs are dicotyledonous. Monocots differ from dicots in leaf structure and location and timing of lipid biosynthesis. Monocots show consistent patterns in δD within individual leaves (Helliker and Ehleringer, 2002; Sachse et al., 2012). Changes in species composition affect the average εwax-precip values, therefore it is important to supplement plant wax data with independent vegetation data such as pollen assemblages when using plant waxes as paleoenvironmental proxies (Feakins, 2013).

Plant waxes have proven to be a robust paleoclimate proxy and have provided insights into past climatic conditions despite the complications discussed above (Tierney et al., 2008; Shanahan et
al., 2013; Wilkie et al., 2013; Feakins et al., 2014). The research into fractionation factors demonstrates that it is vital to constrain the net fractionation before making interpretations about paleoclimates. For example, Feakins (2013) was able to pair plant wax analysis with pollen data to correct for changes in net fractionation that were the result of vegetation changes to reconstruct δDprecip from 11.4 to 3.8 Ma from marine sediments from the Gulf of Aden (Deep Sea Drilling Program Site 231). The effects of latitude, relative humidity, plant lifeform and species composition on δDwax indicate that it is important to use a net fractionation factor that is appropriate to the vegetation community and region of study. Limited studies (Shanahan et al., 2013; Wilkie et al., 2013) have been done in high latitude regions; however, empirical data on plant waxes from boreal forests are absent from the literature. This is a significant source of uncertainty that prevents precise interpretation of fossil boreal plant waxes in sedimentary archives.

1.6 Research Gaps

High latitude regions have largely been neglected in plant wax calibrations and paleoclimate reconstructions. Only four studies have attempted to calibrate the net fractionation, only three of which analyzed n-alkanoic acids at high latitudes (>60°N) (n-alkanes: Sachse et al., 2004; n-acids: Shanahan et al., 2013; Wilkie et al., 2013; Daniels et al., 2017). These calibration studies have a wide range of net fractionation values (-126‰ to -61‰, Sachse et al., 2004 and Shanahan et al., 2013 respectively); therefore, more work is needed to understand this diversity in high-latitude environments. One high-latitude ecosystem that remains largely unstudied in the n-alkyl literature is the northern boreal forest. The boreal forest covers an immense portion of the Earth’s surface, especially in North America and Eurasia (Soja et al., 2007). Furthermore, the boreal ecosystem was present in the Western Subarctic during the Holocene and past interglacials (Schweger et al., 2011; Kaufman et al., 2012), and at higher latitudes during the Pliocene (Csank et al., 2011, 2013), making it an important target for modern calibration studies to exploit fossil boreal δDwax as a paleoenvironmental proxy. However, to date, no systematic attempt has been made to constrain net fractionation for boreal forests.
Only one study has systematically compared lipid source and degradation patterns in different depositional environments (e.g. soils vs. lake sediments) (Nguyen Tu et al., 2017). Therefore, large uncertainties exist regarding the applicability of net fractionation values constrained for one depositional environment to another.

Only a few studies have utilized the plant wax δD in Beringia (Holland et al., 2013; Wilkie et al., 2013; Nichols et al., 2014), and no full Holocene plant wax records have been developed in this region. Furthermore, only three full Holocene stable isotope records have been constructed in Beringia (Fisher et al., 2008; Wooller et al., 2012; Jones et al., 2014). Long-term stable isotope records have the potential to quantitatively reconstruct paleoclimate change and provide a valuable supplement to the existing pollen and midge-based climate reconstructions.

1.7 Thesis Objectives

The objective of this thesis is to develop a better understanding of plant wax characteristics and fractionation in high latitude boreal forests in the Holocene. In order meet this broad objective, each chapter has its own secondary objectives. The second chapter aims to (1) to characterize the n-acid chain length distributions of common boreal vegetation types in order to better understand the major contributor(s) to sedimentary lipid pools; and (2) to quantify the net fractionation of n-acids in northern boreal soils by sampling soils from a transect of sites in NW Canada (Fig. 1.7.1). These boreal forest calibrations will inform future work on paleoclimate reconstructions from boreal forest ecosystems. The third chapter will present a full Holocene δD record using n-alkanoic acids from plant waxes recovered from a sediment core from a small kettle lake in southwestern Yukon referred to as Spindly Pine Lake (unofficial name; Fig. 1.7.1) and (1) determine an appropriate net fractionation value to reconstruct δD_precip for this lake sediment core record, and (2) determine the likely driver(s) of the isotopic composition of precipitation and compare to previous stable isotope records in the region. These chapters both investigate plant wax net fractionation in high latitude boreal forests, however they study n-acids in different depositional environments. The third chapter provides an example of the way calibrated net fractionation values can be applied to assess paleoclimate change in Eastern Beringia using plant waxes as a proxy.
Figure 1.7.1 Map of study sites, grey circles represent soil sampling sites discussed in Chapter 2, green cross represent lake sediment core discussed in Chapter 3.

1.8 Thesis Structure

This thesis is divided into 4 chapters. Chapter 1 introduces plant waxes as reliable archives of stable hydrogen isotope and paleoclimate proxies and the current understanding of Holocene climate changes in Eastern Beringia. Chapter 2 describes the development and applicability of a net fractionation value calibrated to boreal forest soils based on a regional transect of soil and modern vegetation samples in the Yukon, Alaska, and Northwest Territories. This is the first systematic attempt to calibrate a net fractionation value for northern boreal forests. Chapter 3 looks at the downcore variability in the full Holocene plant wax record from Spindly Pine Lake in southwestern Yukon and assesses possible drivers. Chapter 3 also compares plant wax samples from lake sediments and soils to determine if the regional net fractionation value calibrated in chapter 2 is applicable to plant waxes from different depositional environments.
Chapter 4 summarizes the key findings of this research thesis and highlights the limitations to the study, and avenues for future research.

1.9 References


Sachse, D., Dawson, T.E., Kahmen, A., 2015. Seasonal variation of leaf wax n-alkane production and δ2H values from the evergreen oak tree, Quercus agrifolia. Isotopes in Environmental and Health Studies 51, 124–142.

Sachse, D., Radke, J., Gleixner, G., 2006. dD values of individual n-alkanes from terrestrial plants along a climatic gradient – Implications for the sedimentary biomarker record. Organic Geochemistry 37, 469–483.


Chapter 2
Net fractionation of hydrogen isotopes in \( n \)-alkanoic acids from soils in the northern boreal forest

Aleesha Bakkelund, Trevor J. Porter, Duane G. Froese, Sarah J. Feakins

Abstract

Plant-derived \( n \)-alkyl lipids are well-preserved in sedimentary archives, and their stable hydrogen isotope ratio (\( \delta D_{\text{wax}} \)) is a proxy for precipitation \( \delta D \) and climate. Net fractionation of H isotopes between source water and \( n \)-alkyl lipids (\( \varepsilon_{\text{wax/precip}} \)) is the largest uncertainty for interpreting this proxy and depends on plant type and environment. Although popular proxies, \( n \)-alkanoic acids (\( n \)-acids) are less frequently calibrated in modern environments than \( n \)-alkanes. We constrain \( \varepsilon_{\text{wax/precip}} \) for the northern boreal forest based on \( n \)-C\(_{24-28} \) acids in topsoils from a 13-site network in Yukon, Alaska and Northwest Territories, encompassing a range of latitudes (60-68°N) and climates (mean annual temperature, MAT: -8.4 to -1.7°C; mean annual precipitation, MAP: 238 to 387 mm). \( n \)-Acid homologue distributions for common boreal plants (gymnosperm trees, shrubs, forbs, C3 grasses and mosses) reveal that soil \( n \)-acids are dominated by mosses, but with a reduced carbon preference index compared to fresh mosses, possibly owing to post-depositional degradation. Net-fractionation values for \( n \)-C\(_{24} \) and \( n \)-C\(_{28} \) acids are not correlated with any geographic or environmental variables; however, \( n \)-C\(_{26} \) is significantly correlated with latitude (\( r = 0.60 \)) and elevation (\( r = 0.61 \)), which covary. The regional mean net fractionation values relative to MAP are -93 ± 10‰ for C\(_{24} \), -102 ± 11‰ for C\(_{26} \) and -96 ± 11‰ for C\(_{28} \) acids, which is consistent with published \( \varepsilon_{\text{wax/precip}} \) values from low to high latitudes. These soil-derived values are relevant for reconstructions of \( \delta D_{\text{precip}} \) from fossil \( n \)-acids in paleosols derived from a comparable paleovegetation and latitude.

2.1 Introduction

Stable isotope ratios of hydrogen (D/H) and oxygen (\( ^{18}\text{O}/^{16}\text{O} \)) in precipitation are well-established tracers for hydroclimatic variables, including precipitation amount and air temperature (Dansgaard, 1964; Craig and Gordon, 1965). Hydrogen isotopes from meteoric waters are preserved in the long straight-chain hydrocarbons (\( n \)-alkanoic acids, \( n \)-alkanes, and \( n \)-alcohols) that make up the cuticular waxes of plants (Hou et al., 2008; Sachse et al., 2012; Wilkie et al., 2013), which has made fossil plant waxes a popular proxy in paleo-hydroclimate studies. Fossil plant waxes are well-preserved in sedimentary archives (e.g., relict permafrost, paleosols, lacustrine sediment cores, etc.) over geologic timescales (e.g. Yang and Huang, 2003; Tierney et al., 2008; Vonk et al., 2017) owing to their hydrophobicity, inertness, and resistance to biodegradation (Huang et al., 1997; Nguyen Tu et al., 2017). Long-chain (>20 carbon atoms) \( n \)-alkanoic acids (hereafter \( n \)-acids) with a characteristic predominance of even-number C-
chains, and \(n\)-alkanes with a predominance of odd-number C-chains, are both derived from a common precursor (Zhou et al., 2010). Together these compounds form major constituents of the waxy coating within and on top of the cuticle of plant leaves, thus both are used as biomarkers for terrestrial plants (Eglinton and Hamilton, 1963). These alkyl hydrogens do not exchange readily at temperatures less than 150°C (Sessions et al., 2004) allowing isotopic signals of synthesis to be retained in sedimentary storage. The hydrogen isotopic composition of plant waxes (\(\delta D_{\text{wax}}\)) is offset from the hydrogen isotopic composition of precipitation (\(\delta D_{\text{precip}}\)) by a large negative fractionation. This offset is referred to as the net (or apparent) fractionation (\(\epsilon_{\text{wax/precip}}\)) and varies regionally with climate and vegetation type (Sachse et al., 2012).

Terrestrial plants absorb water from soil moisture, which is ultimately derived from precipitation. Evaporation can enrich deuterium (D) in soil water, however many plants do not use evaporatively-enriched soil water (Feakins and Sessions, 2010), although it may be a factor in some shallow rooted plants, e.g. grasses (Smith and Freeman, 2006). Hydrogen isotopes are generally not fractionated during uptake of source water by roots (White et al., 1985; Ehleringer and Dawson, 1992; Roden et al., 2000a); however, transpiration results in D-enrichment of leaf waters (Feakins and Sessions, 2010) and the magnitude of this enrichment depends on relative humidity, isotopic composition of the ambient water vapour, and leaf geometry (Barbour et al., 2004; Kahmen et al., 2008; Sachse et al., 2012). \(n\)-Acid precursors inherit H isotopes from leaf water, but a series of biosynthetic fractionations then results in D-depletion of the resulting \(n\)-alkyl lipids relative to leaf water (Chikaraishi et al., 2004a; Zhou et al., 2010; Sachse et al., 2012).

Large variability in \(\epsilon_{\text{wax/precip}}\) has been noted between different groups of plants and photosynthetic pathways. On average, \(n\)-C\(_{29}\) alkanes produced by shrubs are the most enriched in deuterium, followed by trees, forbs, and graminoids (Sachse et al., 2012). Therefore, changes in vegetation type and abundance within a catchment can result in variability in sedimentary \(\delta D_{\text{wax}}\) that is unrelated to \(\delta D_{\text{precip}}\) (Fornace et al., 2014). For \(\delta D_{\text{wax}}\)-based paleoclimate reconstructions, this underscores the need to use \(\epsilon_{\text{wax/precip}}\) values that are appropriate to the plant community and climate, to enable robust estimates of \(\delta D_{\text{precip}}\) (Feakins, 2013; Nichols et al., 2014). The \(\epsilon_{\text{wax/precip}}\) has been calibrated extensively for low and mid latitude ecosystems, but there are only a few high-latitude examples, limiting our ability to interpret high-latitude \(\delta D_{\text{wax}}\) records.
Previous calibration studies have constrained $\varepsilon_{\text{wax/precip}}$ in various ways. Growth-chamber experiments have tested select species, under known conditions where irrigation water (w) and other factors (e.g., light, humidity) are controlled, and the $\delta D_{\text{wax}}$ of new foliage is measured and used to determine $\varepsilon_{\text{wax/w}}$ (e.g., Yang et al., 2009). Natural ecosystem surveys have compared the $\delta D_{\text{wax}}$ of leaves and $\delta D$ of plant water (e.g., xylem water) to calculate $\varepsilon_{\text{wax/w}}$ (e.g., Feakins and Sessions, 2010; Daniels et al., 2017). Other sedimentary approaches have compared the $\delta D_{\text{wax}}$ of integrated waxes in surface (ca. modern) sediments with a best estimate of local $\delta D_{\text{precip}}$ in order to estimate $\varepsilon_{\text{wax/precip}}$ for the plant community as represented in sediments (e.g., Shanahan et al., 2013; Wilkie et al., 2013). All are valid approaches with different strengths and limitations. For example, $\varepsilon_{\text{wax/w}}$ can be directly determined for specific plants in growth-chamber experiments and ecosystem surveys, but fossil waxes in the geologic record are subject to other uncertainties related to post-depositional processes and wax inputs from other vegetation. Conversely, $\varepsilon_{\text{wax/precip}}$ estimates from sedimentary waxes (e.g., soil, lake sediment) integrate all wax inputs and pre-depositional processes, but source water $\delta D$ cannot be directly measured and, thus, must be estimated to constrain $\varepsilon_{\text{wax/precip}}$. The $\delta D$ value of amount-weighted Mean Annual Precipitation (MAP) is commonly used as an approximation for source water $\delta D$ (e.g., meta-analysis by Sachse et al., 2012). For future reference, we use ‘$\varepsilon_{\text{wax/MAP}}$’ when referring to net fractionation estimates expressed relative to $\delta D_{\text{MAP}}$, and ‘$\varepsilon_{\text{wax/precip}}$’ when a more generic term is required.

In a survey of lake sediment cores in dwarf shrub-tundra catchments on Baffin Island (62-74°N), Canadian High Arctic, Shanahan et al. (2013) found a mean $\varepsilon_{\text{wax/MAP}}$ of -61‰, based on the mean $\delta D_{\text{wax}}$ of $n$-$C_{26}$ and $C_{28}$ acids. This value is smaller than what is typical for herbaceous plants in low and middle latitudes (Sachse et al., 2012). Shanahan et al. (2013) linked this observation to an evapotranspiration effect on leaf water that was first observed in growth chamber experiments by Yang et al. (2009) who found $n$-alkanes from deciduous conifers under 24-hour light (simulating High Arctic conditions) were up to 40‰ enriched compared to foliage grown in diurnal light conditions.

At a comparable latitude in northeastern Siberia (67.5°N), Wilkie et al. (2013) found a $\varepsilon_{\text{wax/MAP}}$ of -95‰ for $n$-$C_{30}$ acids for ca. modern lake sediments of Lake El’gygytgyn, surrounded by tundra plants including lichen and herbaceous taxa. From lacustrine core top sediments of tundra lakes in northern Alaska (68.5°N), Daniels et al. (2017) observed larger $\varepsilon_{\text{wax/MAP}}$ values of -118‰ for $n$-$C_{25-33}$ alkanes, and -114‰ for $n$-$C_{22-30}$ acids. Sachse et al. (2004) report the largest $\varepsilon_{\text{wax/MAP}}$
values from lacustrine sediments from taiga and tundra catchments in Fennoscandia (61-69°N) with a mean of -126‰ for \( n-C_{29} \) alkanes. With the exception of the study on Baffin Island (Shanahan et al., 2013), net fractionation estimates from most high-latitude sites are comparable in value to observations from mid to low latitude sites (< 60°) with mean values ranging from -90 to -130‰ for both \( n \)-alkanes (Chikaraishi et al., 2004b; Pagani et al., 2006; Sachse et al., 2006; Smith and Freeman, 2006; Hou et al., 2007; Kahmen et al., 2013; Gao et al., 2014; Feakins et al., 2016b; Freimuth et al., 2017) and \( n \)-acids (Hou et al., 2007; Feakins et al., 2014, 2016b; Gao et al., 2014; Freimuth et al., 2017). Additionally, studies spanning large latitudinal gradients do not appear to support a strong relationship between latitude and net fractionation (Sachse et al., 2006; Liu et al., 2016), which suggests that light-dependent controls on net fractionation may be unique to certain high-latitude environments. More work is needed to understand this diversity in high-latitude environments.

One high-latitude ecosystem that remains largely unstudied in the \( n \)-alkyl literature is the northern boreal forest. The boreal forest covers an estimated 1.2 billion hectares of the Earth, spanning large portions of North America and Eurasia (Soja et al., 2007). Furthermore, the boreal ecosystem was present in the Western Subarctic during the Holocene and past interglacials (Schweger et al., 2011; Kaufman et al., 2012), and at higher latitudes during the Pliocene (Csank et al., 2011, 2013), making it an important target for modern calibration studies to exploit fossil boreal \( \delta D_{\text{wax}} \) as a paleoenvironmental proxy.

We address this knowledge gap with a study of \( n \)-acids from modern vegetation and top-soils from southern Yukon to the northernmost extent of boreal treeline in Northwest Territories. We focus specifically on \( n \)-acids because Pinaceae gymnosperm trees (e.g., \textit{Picea}, \textit{Pinus} and \textit{Larix}), which dominate the boreal canopy, are known to produce negligible amounts of \( n \)-alkanes (Diefendorf et al., 2011) and, thus, are likely to be unrepresented in soil \( n \)-alkanes (Schäfer et al., 2016). Soil surveys have been widely used for calibration of \( \delta D_{\text{wax}} \) elsewhere (Chikaraishi and Naraoka, 2006; Jia et al., 2008; Peterse et al., 2009; Rao et al., 2009; Bai et al., 2011; Ponton et al., 2014; Schwab et al., 2015) to survey the signals of plant wax as incorporated into soils. Our research objectives are two-fold: (1) to characterize the \( n \)-acid chain length distributions of common boreal vegetation types in order to better understand the major contributor(s) to sedimentary lipid pools; and (2) to quantify the net fractionation of \( n \)-acids (\( \varepsilon_{\text{wax/precip}} \)) in northern boreal soils. We chose to focus on
the latitudinal variability in net fractionation over intra-site variability in order to assess the need for a specific high latitude net fractionation value and to characterize boreal forest fractionation over a wide range of climatic conditions to calibrate a robust regional net fractionation value. These boreal forest calibrations will inform future work on paleoclimate reconstructions from boreal forest ecosystems.

2.2 Methods

2.2.1 Study area

Soil and vegetation samples were collected from thirteen locations in the Yukon, Northwest Territories, and Alaska (Fig. 2.2.1), spanning 60.3 to 68.3°N. The climate of the study region is cold and semi-arid, characterized by short, cool summers and long, cold winters (Fig. 2.2.2). Mean annual temperature (MAT) ranges from -8.4 in the north to -1.7°C in the south, with average daily temperatures above freezing from May to September based on ERA-Interim data (Table 2.1.1, Dee et al., 2011). Average growing season temperatures range from 4 to 12°C. Daylight hours at the summer solstice range from 19 hours in southern Yukon to 24 hours in the north (Wahl, 2004). The northern half of the site network (>65°N) falls within the continuous permafrost zone, and the more southern sites are in the discontinuous or sporadic permafrost zones (Brown et al., 1997).

As our study region encompasses a large range of latitudes and mean temperatures, the start and end points of the growing season (timing and duration of leaf wax synthesis) also vary regionally. The growing season is primarily limited by minimum temperatures above the freezing mark. In the southern end of our transect (e.g., Whitehorse) minimum daily temperatures are above freezing from May 18 to September 24 (duration = 129 days), and in the northern end (e.g., Inuvik) from May 27 to September 23 (duration = 119 days) (Dee et al., 2011). However, other abiotic factors such as sunlight and ground temperatures (influencing unfrozen water availability) also influence the timing of biosynthesis during the growing season.

The Alaska Range and St. Elias Mountains represent a significant barrier to the advection of Pacific moisture into continental Yukon. Areas of low elevation receive 250 to 300 mm of precipitation annually, while higher elevations receive more, up to 400 to 500 mm, based on WorldClim 2 data (Fick and Hijmans, 2017). Over 75% of precipitation falls between May and
September (Wahl, 2004). Peak summer (JJA) average humidity differs by 13% (62 to 74%) between study sites, and varies by ~20% at each site annually based on CliMond data (Table 2.1.1, Fig. 2.2.2; Kriticos et al., 2012).

The study sites are mature subarctic woodlands, which are broadly representative of the northern boreal forest ecology (Fig. 2.2.3) and span a range of latitudes and climate conditions which allows us to examine the range of variability in net fractionation within this ecotype. The sites are primarily spruce (Picea glauca and Picea mariana) and moss dominated woodlands characterized by moderately open canopies. Pinus contorta and Larix laricina trees are dominant or co-dominant at two of our sites. The sub-canopy typically includes a thick (5 – 15 cm) moss ground cover with patches of Poaceae grass and Ericaceae forbs, and sparse shrub cover including alder (Alnus sp.), willow (Salix sp.) and dwarf birch (Betula nana). The mosses associated with subarctic woodlands are diverse and include Spahgnum hummock varieties (e.g., Sphagnum fuscum and S. balticum) and feathermoss varieties (e.g., Pleurozium schreberi, Ptilium crista-castrensis, Tomentypnum nitens, and Dicranum polysetum) with the assemblage being influenced by soil moisture and woodland successional stage (Black and Bliss, 1988; Turetsky et al., 2012). Most sites are relatively flat with moist but unsaturated, organic-rich soils, and underlain by clastic parent material (clay, silts).
Figure 2.2.1 Regional maps of (a) annual precipitation (mm/year) (WorldClim 2) and (b) mean annual temperature (°C) (WorldClim 2), with locations of the study sites (black dots), local GNIP sites (white triangles), and the Arctic circle (thin black line), north of which sites experience 24-hr daylight at least once per year.
Figure 2.2.2 Average monthly $\delta D_{\text{precip}}$ (OIPC), relative humidity (CliMond), air temperature (ERA-Int), and precipitation (WorldClim 2). Error bars represent range of values for sampling sites.
Figure 2.2.3 Site photos from (a) CL, (b) DHP 174, (c) INU, and (d) MYO, showing typical boreal forest ecology
Table 2.2.1 Sampling site locations and climate

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<th>MJJAS</th>
<th>Annual</th>
<th>JJA</th>
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*aERA-Interim surface temperature data (1981-2010), (~80 km spatial resolution) mean annual temperature and mean temperature May-September (Dee et al., 2011)

*bWorldClim2 precipitation data, annual (30° spatial resolution) and total May-September (Fick and Hijmans, 2017)

*c CliMond humidity data (10° spatial resolution), annual and average May-September (Kriticos et al., 2012)
2.2.2 Sampling methods

Soil samples were collected from most sites in July 2014, and from the LC and SH sites in July 2016 and SP site in July 2017. Soil samples were collected to examine the homologue distribution and hydrogen isotope ratios of long chain (C_{24}, C_{26}, and C_{28}) n-acids. Soils were collected from ~30 cm soil pits in 3-10 cm intervals, depending on the thickness of the O-horizon; the O- and A- horizons were collected separately. Soil pits were dug in open areas within 2 m of the canopy dominant tree, and in close proximity to shrubs and forbs and these soil pit criteria were held constant across our site network. A single pit was sampled at most sites, in order to compare the O and A horizons at a representative location. We do not describe within-site heterogeneity such as may vary with plant cover, plant growth-limiting factors, or microtopography. Instead, we prioritize a large number of sites across a long transect to characterise latitudinal variability in net fractionation across the northern boreal forest.

Vegetation samples were collected at the same sites to examine the distribution of n-acid homologues and to determine which plant types are contributing most to soils. Fresh leaves or needles with minimal evidence of damage or decomposition were collected from up to three individual dominant or co-dominant shrubs, trees, mosses, grasses and forbs, typically within ~5 m of the soil pit. The isotopic composition of vegetation samples was not measured. All vegetation and soil samples were collected using nitrile gloves and stored in Whirlpak™ bags, chilled over ice in a field cooler, and shipped frozen to the laboratory for processing and analysis.

2.2.3 Vegetation sample classification

Vegetation samples were classified as tree, shrub, moss, grass or forb. An inventory of plant taxa sampled at each site is provided in the supplement (Appendix B). All trees were gymnosperms. White spruce (Picea glauca, n = 33; here, ‘n’ refers to the number of individual P. glauca trees that were sampled across the regional network) was dominant at all sites except TSI, MYO, DHP174 and SP. Larch (Larix Laricina, n = 1) trees are dominant at the TSI site. Black spruce (Picea mariana, n = 3) was co-dominant with white spruce at MYO and DHP174. Lodgepole pine (Pinus contorta, n = 1) was co-dominant with white spruce at SP. Shrub cover was sparse and variable, and included willow (Salix sp., n = 20), alder (Alnus sp., n = 5) or dwarf birch (Betula nana, n = 7). Mosses common in northern boreal woodlands are diverse, and an
inventory of all moss taxa at each site was not attempted. Fresh mosses (n = 12) were sampled from the top of the O horizon of our soil pits, and mostly the red-stemmed feathermoss *Pleurozium schreberi* (Hylocomiaceae); minor co-mingled fractions of *Tomentypnum nitens, Ptilium crista-castrensis* and *Dicranum polysetum* were also observed. Grasses in this region are Poaceae and use the C3 metabolic pathway (n = 13). All forbs were Ericaceae, including crowberry (*Empetrum nigrum, n = 1*), lingonberry (*Vaccinium vitis-idaea, n = 2*), blueberry (*Vaccinium uliginosum, n = 1*), and Labrador tea (*Rhododendron sp., n = 3*).

### 2.2.4 Lipid extraction

Soil samples were freeze dried in a Labconco freezone 2.5 unit. n-Acid concentrations varied between soil samples. Initially, 5 g of dry, homogenized sediment was subsampled for lipid extraction. Following the initial quantification of n-acids by GC-FID (described below), additional sediment (up to 60 g) was sometimes required to yield sufficient n-acid amounts for the compound-specific isotope analysis.

Soil bound-lipids were extracted with 9:1 (v/v) Dicholoromethane (DCM):Methanol (MeOH) by microwave-assisted heating (Milestone Ethos Up unit) at 70°C for 20 minutes, and continuous magnetic stirring, a method selected for optimal recovery (Chávez-Lara et al., 2018). Samples were then centrifuged at 1800 RPM for 10 minutes to settle the sediment, and the supernatant was removed and retained. The sediment sample was rinsed three to five additional times with DCM-MeOH, centrifuged and transferred to the collection vial. Excess solvent was evaporated under a nitrogen blow down system.

Foliage samples were dried in paper bags in an oven at 60°C for 48 hours. Chopped, dry foliage (1 g) was submerged in DCM:MeOH (9:1, v/v) and agitated by Pasteur pipette pumping and the extract removed (repeated three times) to remove the epicuticular and intracuticular waxes (similar to the methods of Feakins and Sessions, 2010; Feakins et al., 2016a). Leaves were not powdered as this method releases short chain lipids from internal leaf cells but does not alter the leaf wax yield.

The total lipid extract was separated by column chromatography in a 5.75” borosilicate glass Pasteur pipette packed with Phenomenex 60 Å NH2 sepra stationary phase. The neutral fraction was eluted with 2:1 DCM:Isopropanol, followed by the acid fraction with 4% formic acid in
diethyl ether. The acid fraction was methylated to Fatty Acid Methyl Esters (FAMEs) in 5% Hydrochloric acid and 95% Methanol ($\delta_{\text{MeOH}} = -246.6\%$, following methodology in Lee et al., 2017) at 60°C for 12 hours. The mixture was cooled and ~1 mL MilliQ water was added. Then, 1 mL hexanes was added and shaken vigorously for 30 s, to partition the FAMEs into the hexanes and this liquid-liquid extraction was repeated (three times) to ensure recovery. The extract was passed through a column of anhydrous sodium sulfate to remove any water. The extract was purified by column chromatography with 5% water-deactivated 100-200 mesh silica gel, and the FAMEs were eluted with 3 column rinses of hexanes and then DCM. The saturated FAMEs were further purified by column chromatography with silver nitrate on silica gel (+230 mesh), also eluted with hexanes and DCM.

2.2.5  $n$-Acid abundance and isotope analysis

FAMEs were analysed at University of Toronto Mississauga with a Gas Chromatograph (Thermo Trace 1310) Flame Ionization Detector (GC-FID), equipped with a Programmable Temperature Vaporizing (PTV) injector and a Rxi-5 ms column (30 m x 0.25 mm, film thickness 1 μm). The FID results were normalised to known quantities of an in-house FAME standard ($n$-C$_{17}$, -C$_{21}$, -C$_{25}$ and -C$_{29}$ FAMEs). For all samples, we calculated the average chain length (ACL = $\Sigma(C_n \times n)/\Sigma C_n$, where C$_n$ is the abundance for chain-lengths from $n = 20$-34), modal chain length (C$_{\text{max}}$), and the carbon preference index (CPI = ([C$_{20}$, 22, 24, 26, 28, 30] + [C$_{22}$, 24, 26, 28, 30, 32])/(2 $\times$ [C$_{21}$, 23, 25, 27, 29, 31])), the latter a measure of degradation (Bray and Evans, 1961).

Compound-specific hydrogen isotope ratios were measured at the University of Southern California using a Thermo Scientific Trace GC equipped with a PTV inlet operated in solvent split mode and a Rxi-5 ms column (30 m x 0.25 mm x 0.25 μm), connected via a GC Isolink with pyrolysis (1400°C), via a Conflo IV to an Isotope Ratio Mass Spectrometer (Delta V IRMS). The H$_3^+$ factor was measured daily to check for linearity and remained close to 8 ppm mV$^{-1}$. H$_2$ reference peaks were injected at the beginning and end of each sample run on the GC-IRMS, with two used for standardization between sample and standard runs. Hydrogen isotope ratios are reported in delta notation ($\delta D = (R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}$ where R is the ratio of deuterium to protium, $^2$H/$^1$H). Precision of replicate sample injections was typically better than 1‰. Data were normalized to the VSMOW-SLAP hydrogen isotopic scale using the A3 mix
alkane standard with δD values ranging from -233.7 to -46.3‰ (supplied by A. Schimmelmann, Indiana University, Bloomington). The RMS error of replicate analyses of the A3 mix was typically better than 4‰ over the month of analysis. Hydrogen atoms added by methylation were corrected for by mass balance ($\delta D_{\text{wax}} = \delta D_{\text{meas}} (2i + 2-3 \times \delta D_{\text{MeOH}})/2i-1$), where $\delta D_{\text{meas}}$ is the normalized δD value of the FAME and $i$ is the number of carbon atoms the fatty acid molecule of interest).

### 2.2.6 Net fractionation

Net (or apparent) fractionation was calculated for each soil sample based on the general formula: $\varepsilon_{\text{wax/precip}} = (\delta D_{\text{wax}} + 1)/(\delta D_{\text{precip}} + 1) - 1$, where $\delta D_{\text{wax}}$ is a measured value from the soils and $\delta D_{\text{precip}}$ is an estimate of average meteoric waters integrated in soil (and available to plants) during the growing season. We assume amount-weighted mean annual of precipitation $\delta D_{\text{precip}}$ ($\delta D_{\text{MAP}}$) is a reasonable source water approximation for our sites. Compared to unweighted mean annual precipitation, $\delta D_{\text{MAP}}$ is biased to warm-season values, reflecting higher precipitation totals in summer (Fig. 2.2.2), and accounting for inputs of D-depleted snowmelt to the ground in springtime (Mackay, 1983). Further rationale for the $\delta D_{\text{MAP}}$ assumption is discussed in Section 2.4.2. We used the Online Isotopes in Precipitation Calculator (OIPC; Bowen and Wilkinson, 2002; Bowen and Revenaugh, 2003) to estimate monthly $\delta D_{\text{precip}}$ (Fig. 2.2.2) and $\delta D_{\text{MAP}}$ for our sites, which is common practice in other $\delta D_{\text{wax}}$ calibration studies (e.g. Sachse et al., 2004; Shanahan et al., 2013; Freimuth et al., 2017). The OIPC provides interpolated $\delta D_{\text{precip}}$ estimates constrained by empirical GNIP (Global Network for Isotopes in Precipitation), including local stations at Whitehorse, Mayo, and Inuvik (Fig. 2.2.1). $\delta D_{\text{MAP}}$ ranges from -160 ± 2‰ at Lost Chicken, to -176‰ at Inuvik, with a regional, all-site average of -168‰ (1σ = 6) (Table 2.2.2). This region is characterized by a large seasonal range in monthly $\delta D_{\text{precip}}$ of 113‰. Minimum $\delta D_{\text{precip}}$ occurs in January and ranges from -203 to -252‰ between sites, and maximum $\delta D_{\text{precip}}$ occurs in either July and ranges from -123 to -132‰ between sites (Fig. 2.2.2).
Table 2.2.2 Site-averaged hydrogen isotope ratios and net fractionations of \( \text{n-alkanoic acids} \) in modern soil (0-30 cm) samples

<table>
<thead>
<tr>
<th>Site</th>
<th>( \delta\text{D}_{\text{MAP}} )(‰)(^a)</th>
<th>( \delta\text{D}_{\text{wax}} )(‰) ± 1σ (( n ))</th>
<th>( \epsilon_{\text{wax/MAP}} )(‰) ± 1σ (( n ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP</td>
<td>-163</td>
<td>-237 ± 6.9 (9)</td>
<td>-99 ± 9.9 (9)</td>
</tr>
<tr>
<td>WH</td>
<td>-165</td>
<td>-252 ± 2.6 (3)</td>
<td>-101 ± 9.9 (9)</td>
</tr>
<tr>
<td>TKR</td>
<td>-164</td>
<td>-240 ± 3.9 (5)</td>
<td>-102 ± 11.8 (5)</td>
</tr>
<tr>
<td>KL</td>
<td>-167</td>
<td>-243 ± 1.1 (1)</td>
<td>-105 ± 11.8 (5)</td>
</tr>
<tr>
<td>MYO</td>
<td>-161</td>
<td>-253 ± 1.8 (2)</td>
<td>-111 ± 11.8 (5)</td>
</tr>
<tr>
<td>BRM</td>
<td>-164</td>
<td>-251 ± 1.2 (2)</td>
<td>-100 ± 7.5 (5)</td>
</tr>
<tr>
<td>LC</td>
<td>-160</td>
<td>-251 ± 1.2 (2)</td>
<td>-96 ± 7.5 (5)</td>
</tr>
<tr>
<td>SH</td>
<td>-174</td>
<td>-260 ± 2.1 (2)</td>
<td>-111 ± 7.5 (5)</td>
</tr>
<tr>
<td>DHP174</td>
<td>-174</td>
<td>-263 ± 2.1 (2)</td>
<td>-104 ± 7.5 (5)</td>
</tr>
<tr>
<td>EAG</td>
<td>-171</td>
<td>-251 ± 1.2 (2)</td>
<td>-103 ± 7.5 (5)</td>
</tr>
<tr>
<td>TSI</td>
<td>-175</td>
<td>-244 ± 1.2 (2)</td>
<td>-99 ± 7.5 (5)</td>
</tr>
<tr>
<td>CL</td>
<td>-176</td>
<td>-251 ± 1.2 (2)</td>
<td>-96 ± 7.5 (5)</td>
</tr>
<tr>
<td>INU</td>
<td>-176</td>
<td>-244 ± 1.2 (2)</td>
<td>-96 ± 7.5 (5)</td>
</tr>
<tr>
<td>Mean</td>
<td>-168</td>
<td>-246 ± 5.2 (2)</td>
<td>-96 ± 7.5 (5)</td>
</tr>
<tr>
<td>1σ</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

\(^a\)OIPC \( \delta\text{D}_{\text{MAP}} \) (Bowen and Revenaugh, 2003)
2.3 Results

2.3.1 Vegetation

Vegetation samples yielded long chain \(n\)-C\(_{20}\) to \(-C_{34}\) acids with notable differences in chain length distribution between vegetation types (Fig. 2.3.1). All vegetation types demonstrate the expected predominance of even-over-odd C-chain \(n\)-acid homologues with mean CPI values ranging from 10.5 to 14.2. Mosses and shrubs have the shortest modal chain lengths \((C_{\text{max}} = 24\) and 26 for mosses and shrubs, respectively); grasses \((C_{\text{max}} = 28\) and forbs and trees have longer dominant chain length \((C_{\text{max}} = 30)\). Mosses and shrubs have the lowest ACL of 24.8 and forbs had the highest ACL of 29.3 (Fig. 2.3.1, Appendix B). Shrubs yielded the greatest average concentration of total long-chain \(n\)-acids \((\sum_{C_{20}}C_{34})\) per gram of dry vegetation \((716 \mu g \, g^{-1})\), followed by forbs \((398.1 \mu g \, g^{-1})\), grasses \((156.6 \mu g \, g^{-1})\), trees \((132.2 \mu g \, g^{-1})\), and mosses \((60.2 \mu g \, g^{-1})\) (Appendix A for inventory of sampled plants, and Appendix B for \(n\)-acid data averaged by vegetation type and site). Site-averaged ACL and CPI for most plant types are not significantly correlated with site-specific climatic (temperature, relative humidity, precipitation amount) or geographic (latitude, longitude, elevation) variables, except for the ACL of shrubs which is significantly correlated \((p \leq 0.05)\) with latitude, longitude, elevation, MAT and annual RH (see Appendix C for all correlations).
Figure 2.3.1 Type-averaged vegetation (a-e) Relative abundance of n-acids; and (f) site-averaged soil relative abundance of n-acids, compounds are normalized to total yield of n-acids. Error bars represent 1σ.

2.3.2 Modern soils

n-Acids with carbon chain-lengths between C_20 and C_30 are the most abundant in the average soil sample (averaged across all sites, equal weight given to all sites) with a modal chain length of 24, and an ACL of 24.2 ± 0.5 (Fig. 2.3.1; see Appendix D for site averages, and Appendix E for all samples). Most of this distribution is centered between C_22 and C_26, and relative abundances are especially low (3% or less) for chain-lengths greater than C_30. The all-site average CPI is 2.8 ± 0.9 indicating even over odd chain length predominance, but is notably smaller than the CPI...
values observed for fresh vegetation which were greater than 10. The same homologue
distribution is also observed for all-site averages calculated separately for the O and A soil
horizons (Appendix F). There is no significant (p < 0.05) differences between ACL and CPI
values for O- and A-horizon site averages (determined by a 2-sample t-test). As was true for
most plants, site-averaged soil ACL and CPI values are not significantly correlated with any of
the climatic or geographic variables (see Appendix C).

In terms of absolute abundance, total long-chain n-acid (ΣC20-C34) concentrations were highly
variable between sites, ranging from 17.2 to 267.4 μg g⁻¹ (Appendix D). Likewise, absolute
concentrations for individual n-acids also varied between sites, for example, ranging from 6-94
μg g⁻¹ for n-C24, 3-25 μg g⁻¹ for n-C26, and 2-21 μg g⁻¹ for n-C28.

Site-averaged n-acid δDwax values ranged from -255 to -234‰ (mean = -246‰, 1σ = 7‰) for n-
C24, -264 to -241‰ (mean = -253‰, 1σ = 7‰) for n-C26, and -260 to -238‰ (mean = -248‰, 1σ
= 7‰) for n-C28 acids (see Appendix D for site-averages, and Appendix E for individual soil
samples from each site). Site-averaged n-C24, C26 and C28 acid δDwax values are not significantly
(p ≤ 0.05) different between the O- and A- horizon (Appendix F), as determined by a 2-sample t-
test. Site-averaged δDwax values are not significantly correlated with climate or geographic
variables, with the exception of amount of annual precipitation for n--C26 acids (Appendix C).

2.3.3 Calculation of net fractionation

Net fractionations of δDwax relative to δDMAP (εwax/MAP) were calculated for soil-derived n-C24, -
C26 and -C28 acids for each soil sample (O and A horizons), which were then used to calculate
site averages. The site averages were used to calculate regional averages (each site contributing
equally to the regional average).

Site-averaged (including O and A horizon soils) εwax/MAP values range from -110 to -71‰ for n-
C24, -122 to -80‰ for n-C26, and -111 to -77‰ for n-C28 acids (Table 2.2.2). The regional ‘all-
site’ average εwax/MAP is -93‰ (1σ = 10), -102‰ (1σ = 11) and -96‰ (1σ = 11) for n-C24, C26 and
C28 acids, respectively (Table 2.2.2). We find no significant differences between the regional
εwax/MAP values (t-tests, p ≤ 0.05) for the three major n-acids. Furthermore, we find no significant
differences (t-tests, p ≤ 0.05) between regional mean εwax/MAP values when calculated for O and A
horizons separately (see regional O and A horizon εwax/MAP values in Appendix F).
However, we find trends in $\varepsilon_{\text{wax/MAP}}$ across the N-S transect through the boreal forest biome. Site-averaged $\varepsilon_{\text{wax/MAP}}$ for $n$-$C_{26}$ acids (but not $C_{24}$ or $C_{28}$ acids) is significantly ($p \leq 0.05$) correlated with latitude ($r = 0.60$) and elevation ($r = -0.61$), but is not significantly correlated with annual precipitation, summer relative humidity, or mean growing season temperature (Fig. 2.3.2).

These variables are also highly correlated with MAT, however the relationship between MAT and $C_{24}$, $C_{26}$, and $C_{28}$ are not significant. $\varepsilon_{\text{wax/MAP}}$ for $n$-$C_{26}$ acids decreases with increasing latitude (Fig. 2.3.2a) and increasing elevation (Fig. 2.3.2b) – factors which generally covary along the latitudinal transect. If we parse the data based on latitude, a factor that is known for most geological applications, we find sites north of 65°N have significantly smaller (t-test, $p \leq 0.05$) net fractionations than more southern sites (Fig. 2.3.2g) for $n$-$C_{26}$ acids but not $n$-$C_{24}$ or $C_{28}$ acids. For sites < 65°N, we calculate mean $\varepsilon_{\text{wax/MAP}}$ values of $-97\%$ ($1\sigma = 8\%$), $-107\%$ ($1\sigma = 8\%$) and $-101\%$ ($1\sigma = 12\%$) for $n$-$C_{24}$, $C_{26}$ and $C_{28}$ acids, respectively. For sites > 65°N, we calculate mean $\varepsilon_{\text{wax/MAP}}$ values of $-88\%$ ($1\sigma = 11\%$), $-95\%$ ($1\sigma = 11\%$) and $-90\%$ ($1\sigma = 12\%$) for $n$-$C_{24}$, $C_{26}$ and $C_{28}$ acids, respectively.

**Figure 2.3.2** Site-averaged $\varepsilon_{\text{wax/MAP}}$ for $n$-$C_{24}$, $-C_{26}$, and $-C_{28}$ acids (red, yellow and blue circles, respectively) versus (a) latitude, (b) elevation (m a.s.l.), (c) annual precipitation (mm; WorldClim 2), (d) mean annual air temperature (°C; ERA-Int), (e) mean growing season air temperature (May-September), and (f) Summer (JJA) relative humidity (%; CliMond); all correlations significant at $p \leq 0.05$ are indicated. Box plots (g) of site-averaged $\varepsilon_{\text{wax/MAP}}$ for $n$-$C_{24}$, $-C_{26}$, and $-C_{28}$ acids grouped by latitude (< 65°N and > 65°N); the group means are significantly different ($p \leq 0.05$; student’s 2-sample t-test) for $n$-$C_{26}$ acids (indicated by *).
2.4 Discussion

2.4.1 n-Acid molecular abundance in vegetation and soils

Although \( n \)-alkyl homologue distributions aren’t taxonomically diagnostic (Eglinton and Hamilton, 1963), some generalisations can be made between plant groups. For example, grasses, herbs and forbs produce a high proportion of high molecular weight (>C\(_{30}\)) \( n \)-alkanes and -acids compared to other plants (Eglinton and Hamilton, 1963; Diefendorf et al., 2011; Bush and McInerney, 2013), while mosses produce abundant lower molecular weight \( n \)-alkanes (C\(_{23}\) and C\(_{25}\)) and \( n \)-acids (C\(_{22}\) and C\(_{24}\)) (Pancost et al., 2002; Bush and McInerney, 2013; Vonk et al., 2017). These tendencies are also reflected in our modern vegetation and soil samples (Fig. 2.3.1).

A comparison of \( n \)-acid relative abundances from our type-averaged vegetation and average soil (0-30 cm below surface, including A and O layers) shows that the distribution of \( n \)-acids in soils (Fig. 2.3.1f) closely resembles that of mosses (Fig. 2.3.1d), with a C\(_{24}\) modal chain-length, followed by C\(_{22}\) and then C\(_{26}\) in relative abundance. This distribution is unique to mosses (Pancost et al., 2002). We find this pattern even when the O and A horizons are examined separately (see Appendix F) and, persists when we average the data from the two horizons, suggesting that mosses dominate soils throughout the profile.

Shrubs have a modal chain-length of C\(_{26}\), high proportions of C\(_{24}\) and C\(_{28}\), (Fig. 2.3.1b) which is inconsistent with the homologue pattern in soils. We do find a spatial variation in the distribution of homologues, but only in the shrubs, which have a longer ACL at higher latitudes (\( r = 0.8, p < 0.01 \)) or colder MAT (\( r = -0.77, p < 0.01 \)) (see Appendix C for all correlations). Other studies have noted significant correlations between plant ACL and hydroclimate gradients (e.g., Bush and McInerney, 2015; Feakins et al., 2016b), which is thought to reflect a functional role long \( n \)-alkyl chains have in mitigating leaf-water loss. However, soil ACL across our network of sites is poorly correlated (n.s.) with the climate and geographic variables, which is further evidence that shrubs are not a major \( n \)-acid contributor to boreal soils at the average site.

Grasses produce mainly \( n \)-C\(_{26}\) and -C\(_{28}\) acids (Fig. 2.3.1a), which is inconsistent with the soil \( n \)-acid pattern, and suggests grasses are not a major \( n \)-acid source. This is not surprising, as grasses prefer dry, raised tussocks which represent a negligible area of the boreal forest floor.
Overall, we note that \( n \)-acids with chain-lengths \( >C_{30} \) are negligible in boreal soils, with no detectable \( n \)-C\(_{34} \) acids at all sites, no detectable \( n \)-C\(_{32} \) acids at five sites, and no detectable \( n \)-C\(_{30} \) acids at three sites (Appendix B). These higher molecular weight \( n \)-acids feature prominently in forbs and trees (Figs. 2.3.1c and e) but are largely absent in boreal soils (Fig. 2.3.1f). Thus, it is unlikely forbs or trees are major \( n \)-acid contributors to soils.

The evidence points strongly to mosses as a primary \( n \)-acid contributor to boreal soils. Although mosses have the lowest average \( n \)-acid (\( \Sigma C_{20-C_{34}} \)) concentration per dry mass (60.2 \( \mu g \) g\(^{-1} \)), they are spatially ubiquitous and dominate the O horizon in terms of raw biomass, which likely overcompensates for its lower absolute \( n \)-acid concentrations compared to other plants. The low stature and physical connection of mosses at the surface of the O-layer may also favour greater transfer (and representation) of \( n \)-acids in boreal soils.

Substantial inter- and intra-site variability is noted in soil \( n \)-acid (\( \Sigma C_{20-C_{34}} \)) concentrations and chain length distributions, consistent with the heterogeneous nature of soils observed elsewhere (Lehmann et al., 2008; Schäfer et al., 2016), which we attempt to correct for by averaging soils from various depths from the O and A soil horizons (1 to 9 soil samples per site). Site-averaged soil \( n \)-acid concentrations vary from 17.2 to 267.4.0 \( \mu g \) g\(^{-1} \) between sites (Appendix D), which likely reflects differences in rates of lipid production, deposition, and mineralization and degradation, including the effects of bacterial activity for example. The \( \Sigma C_{20-C_{34}} \) concentrations are on average slightly higher for the O horizon compared to the A horizon – 133.0 \( \mu g \) g\(^{-1} \) versus 74.8 \( \mu g \) g\(^{-1} \), respectively – which may indicate a higher ratio of \( n \)-acid accumulation to degradation in the O horizon; however, this difference is not statistically significant (\( p = 0.31 \)). The overall range of soil \( \Sigma C_{20-C_{34}} \) \( n \)-acid concentrations by site (17.2 to 267.4 \( \mu g \) g\(^{-1} \)) is in line with total soil \( n \)-acid concentrations from the litter and Ah soil horizons of grasslands, and conifer and deciduous forests in Western Europe which can range from ~10-200 \( \mu g \) g\(^{-1} \) (Schäfer et al., 2016).

Previous studies have demonstrated that microbial lipid degradation can result in substantial decreases in \( n \)-acid concentrations between vegetation and soil (Chikaraishi and Naraoka, 2006; Schäfer et al., 2016; Nguyen Tu et al., 2017). Preferential degradation of long chain \( n \)-acids (\( >C_{28} \)) has been suggested by previous studies (Chikaraishi and Naraoka, 2006; Nguyen Tu et al., 2017). However, due to the low concentrations of high molecular weight (\( >C_{28} \)) \( n \)-acids observed in fresh moss samples (\( \Sigma C_{29-C_{34}} \): 8.3 \( \mu g \) g\(^{-1} \)), the low concentrations of \( n \)-C\(_{28} \) to -C\(_{34} \) acids in soils
(Σ_{C_{28-34}}: 8.8 μg g^{-1}) can be explained without preferential microbial degradation. However, the site-averaged CPI of soils (2.8 ± 0.9) is significantly lower (t-test, p < 0.05) than the CPI we observe in fresh mosses (10.5 ± 3.3), which has the lowest CPI of all major vegetation types (Fig. 2.3.1). This suggests that some microbial degradation or other pedogenic factors may be affecting n-acid chain length distributions, with a disproportionate loss of the relatively abundant even chain lengths, resulting in a reduced CPI. The same effect was observed for soil n-acids along a transect from northern Croatia to southern Sweden (Schäfer et al., 2016). However, the process by which this degradation occurs in soils has not been fully explored in the literature.

2.4.2 Source water δD and timing of lipid synthesis

Before discussing our ε_{wax/MAP} results, we briefly discuss the relevance of δD_{MAP} as a source water δD approximation for sub-Arctic regions, and related uncertainties. ε_{wax/precip} is commonly calculated relative to δD_{MAP} (e.g. Sachse et al., 2012), which is reasonable for many mid- to low-latitude regions where the growing season is long and plants use a blend of year-round precipitation. Furthermore, δD_{MAP} is reasonably well constrained for continental areas of the Northern Hemisphere where the historical GNIP network has coverage, and can be estimated for any given point based on the interpolation method of Bowen and Revenaugh (2003). However, owing to the large seasonal changes in δD_{precip} and restricted growing season at high latitude sites, questions remain regarding whether δD_{MAP} is a reasonable approximation for the δD of plant source water in northern boreal regions. The relevance of this approximation will depend mainly on two variables: the phenology (timing and duration) of leaf wax synthesis for the major plants that contribute n-alkyls to soils; and mean soil water δD available to those plants; both of which are largely unknown for northern boreal woodlands. We provide some discussion of these uncertainties below.

The growing season in our study region is thermally limited to mid-May to late-September (Section 2.2.1). However, the phenology of n-acid synthesis for boreal plants is unknown. Studies on the timing of plant wax synthesis have focused primarily on n-alkanes from mid-latitude sites and have not yet reached a consensus. Sachse et al. (2015) found that n-alkanes in angiosperm trees are synthesized over the growing season. Conversely, Freimuth et al. (2017) found n-alkanes were synthesized rapidly for two to three weeks after leaf emergence before ceasing. Similarly, Gamarra and Kahmen (2017) and Tipple et al. (2013) found the majority of alkanes in C3 grasses and angiosperm trees, respectively, are synthesized during leaf
development. To our knowledge, only one study has examined \( n \)-acid synthesis, which found that \( n \)-acids were synthesized continuously throughout the growing season in angiosperm trees in temperate forests (Freimuth et al., 2017). More systematic investigations on the timing of \( n \)-acid synthesis in boreal regions would help to better understand the soil water seasonality that is most relevant to constraining net fractionation of \( \delta D_{\text{wax}} \).

The value of soil water \( \delta D \) as it changes through the growing season is also a major uncertainty, but is likely influenced by several factors including: (1) \( \delta D \) of remnant soil water from the previous fall; (2) water-ice fractionation (see Lacelle, 2011) of remnant pore waters that occurs during fall freezeback period, which results in isotopic stratification in soils with D-enriched ice near the surface and D-depleted ice in the middle active layer; (3) ‘cryosuction’ of D-depleted ice from the mid-active layer toward the upper freezing front during winter (Mackay, 1983), which applies to fine-grained soils, and would counter some of the near-surface D-enrichment in factor 2; (4) infiltration of D-depleted snowmelt in spring, which depends in part on the volume of dry pore space in the middle active layer and presence of impermeable ice lenses near the surface (Mackay, 1983); (5) gradual soil water enrichment as D-enriched summer rains infiltrate; and (6) summer evaporative D-enrichment of near-surface soil water. Many of these fundamental processes have not been adequately characterised at natural field sites and, therefore, explicit modelling of soil water \( \delta D \) across a regional network is not a viable option for simulating seasonal changes in soil water \( \delta D \) and assessing the \( \delta D_{\text{MAP}} \) approximation.

However, field studies that have sampled soil water \( \delta D \) through the growing season can provide insights. There are a few examples in northern sub-Arctic regions, but we draw on one example from a shrub-tundra environment in N. Alaska. Daniels et al. (2017) sampled soil water \( \delta D \) at various depths in the active layer in mid-July (2014) and the first week of August (2013, 2014); n.b., the first half of the growing season (June 1-July 17) was not sampled. The July and August soil water \( \delta D \) profiles by Daniels et al. (2017) are consistent with our understanding of seasonal dynamics of the isotopic composition of soil water discussed above. In mid-July, mid-active layer soil water (20 cm) is D-depleted by \( \sim 12\% \) relative to \( \delta D_{\text{MAP}} \) (due to snowmelt inputs according to Daniels et al., 2017), while near-surface waters are only slightly D-enriched (\( \sim 5\% \)) relative to \( \delta D_{\text{MAP}} \) (owing to D-enriched July \( \delta D_{\text{precip}} \) inputs – Daniels et al., 2017). By August, soil water \( \delta D \) in the middle active layer (\( >20 \text{ cm} \)) is comparable to \( \delta D_{\text{MAP}} \) due to sustained D-enriched summer precipitation, and near-surface soil water is also D-enriched by \( \sim 20\% \) relative
to July. While data are not available for the first half of the growing season, it is reasonable to assume that soil waters were D-depleted relative to mid-July and δD_MAP (i.e., if early summer D-enriched precipitation were subtracted) and, therefore, δD_MAP may be a reasonable approximation for mean growing season soil waters at this site.

This example is not meant to serve as a ubiquitous analogue. Rather, we use it simply to demonstrate that soil water δD varies in a predictable manner in response to snowmelt and rainwater inputs, and that mean soil waters at the mid-point of the summer are reasonably well approximated by δD_MAP. We assume that average growing season soil waters for our boreal sites may also be well approximated by δD_MAP, which has the benefit of direct comparison with many other modern calibration studies for which reported ε_wax/MAP (e.g., Shanahan et al., Wilkie et al., 2013), but acknowledge that monitoring studies similar to Daniels et al. (2017) are needed in the boreal region to verify this assumption.

2.4.3 Net fractionation

Our regional ε_wax/MAP averages are -93 ± 10‰ for n-C_{24} acids, -102 ± 11‰ for n-C_{26} acids and -96 ± 11‰ for n-C_{28} acids, which are similar to the mean ε_wax/MAP of -96 ± 8‰ (n-C_{30} acids) reported by Wilkie et al. (2012) from N.E. Siberia, and a mean ε_wax/MAP of -114 ± 13‰ (n-C_{22-30} acids) by Daniels et al. (2017) from N. Alaska, all of which are far more negative than the mean ε_wax/MAP of -61 ± 20‰ (n-C_{26,28} acids) reported by Shanahan et al. (2013) from Baffin Island (Fig. 2.4.1). However, it is important to note that all previous high latitude net fractionation calibrations were done using lake sediments rather than soils; therefore, the different depositional environments may have an impact on the derived net fractionation value; for example, it is possible that lake sediments and soils have different primary lipid sources due to differing lipid deposition and taphonomy. Our regional ε_{C28/MAP} mean is also within range of ε_wax/MAP estimates for n-C_{28} or longer acids from tropical and temperate sites ranging from -90 to -121‰ (mean: -105‰) (Hou et al., 2008; Feakins et al., 2014, 2016a; Gao et al., 2014). Collectively, this argues for a relatively similar net fractionation in n-acids across a wide range of latitudes and ecosystem types.
There is a correlation between \(n\)-C26 acids and latitude (\(r = 0.60, p < 0.05\)) and elevation, (\(r = -0.61, p < 0.05\)); however, the correlation is not significant for C24 or C28 acids (\(p > 0.05\)). It is unclear which of elevation or latitude is the driving variable since they are strongly correlated with each other (\(r = -0.82, p < 0.01\)). However, previous literature has suggested sunlight availability may be linked to \(\varepsilon_{\text{wax}/\text{MAP}}\), and therefore latitude may be a more plausible mechanism for this trend. All major chain lengths show a smaller average fractionation for northern sites (> 65°N) than southern sites (< 65°N); however, this difference is also only significant for \(n\)-C26 acids (\(p < 0.05\); Fig. 2.3.2).

Both empirical studies (Shanahan et al., 2013) and growth-chamber experiments (Yang et al., 2009) have demonstrated smaller fractionations in plants exposed to increased daylight hours (a condition that is proportional to latitude) which is thought to enhance transpiration and D-enrichment of leaf waters, ultimately leading to higher \(\delta D_{\text{wax}}\) values, and a smaller overall offset between \(\delta D_{\text{wax}}\) and the \(\delta D\) of source water. This work tentatively supports the assertion made by previous authors that, \(\varepsilon_{\text{wax}/\text{precip}}\) is smaller at high latitudes (Shanahan et al., 2013, Yang et al., 2009, Sessions, 2006; Cormier et al., 2018). However, as this trend was only significant for \(n\)-C26 acids, we recommend the use of regional average \(\varepsilon_{\text{wax}/\text{MAP}}\) for paleo-applications rather than a latitude specific \(\varepsilon_{\text{wax}/\text{MAP}}\).
2.4.4 Additional uncertainties in net fractionation

While regional trends in $\varepsilon_{\text{wax/MAP}}$ are partially explained by latitude ($r = 0.60$; which equates to 36% of explained variance for C$_{26}$), a significant fraction of inter-site variability in $\varepsilon_{\text{wax/MAP}}$ must be related to other factors. We discuss these other possible factors below.

Differences in relative wax contributions from different vegetation types may contribute to soil $\varepsilon_{\text{wax/precip}}$ variability. For example, globally, $n$-alkane $\varepsilon_{\text{C29/MAP}}$ values for C3 grasses, forbs, trees and shrubs are -149‰, -128‰, -121‰ and -99‰, respectively, or a 50‰ range between C3 grasses and shrubs (Sachse et al., 2012). These differences in $\varepsilon_{\text{wax/precip}}$ owe to a variety of factors, including differences in biochemical fractionation and fraction of enriched leaf water that imprints on $n$-alkyl precursors (Gamarra et al., 2012), as well as rooting depth which can lead to major differences between shallow versus deep-rooted plants in environments where near surface source waters are evaporatively D-enriched (Nichols et al., 2010). For a tundra site in N Alaska, Daniels et al. (2017) report a 64‰ range in $\varepsilon_{\text{wax/xylem}}$ values between C3 grasses (Eriophorum vaginatum; $\varepsilon_{\text{C28/xylem}} = -160‰$) and shrubs (Betula nana; $\varepsilon_{\text{C28/xylem}} = -96‰$). These Alaskan $n$-acid $\varepsilon_{\text{C28/xylem}}$ values are similar to global $n$-alkane $\varepsilon_{\text{C29/MAP}}$ values for C3 grasses and shrubs (Sachse et al., 2012); therefore, a similarly large range in $\varepsilon_{\text{C28/xylem}}$ may be assumed for the boreal forest. It is possible that some inter-site $\varepsilon_{\text{C28/MAP}}$ variance in our network owes to variable wax contributions from the minor plant types (e.g., C3 grasses and shrubs). However, C3 grasses occupy a small fraction of the boreal forest understorey, restricted to micro-topographic high points that are well drained, and likely do not contribute much biomass to soils. Conversely, shrubs occupy a larger area of the boreal understory and have the highest $n$-acid concentrations in fresh foliage of all vegetation types. Therefore, it seems more probable that shrubs would have a greater influence on the soil $n$-acid pool then other minor vegetation types such as C3 grasses.

Site slope, aspect and soil character (texture, compaction, permafrost) may also contribute to soil $\varepsilon_{\text{wax/MAP}}$ variability, since these variables have some implications for drainage (Christensen et al., 2013) and, therefore, soil water $\delta^D$. During the spring melt, winter precipitation runs off sloped, impermeable sites more rapidly, whereas sites in flat areas or depressions with permeable soils are likely to retain more winter precipitation in soil water. In turn, plants from such sites may thus have larger apparent net fractionations relative to $\delta^D_{\text{MAP}}$ because of a greater proportional uptake of winter precipitation. Future studies could consider
systematically examining site specific and topographic effects on δD of soil water and net fractionation.

Inaccurate δD_{MAP} estimates for our sites may also contribute to the variability in calculated net fractionations. We used δD_{MAP} estimates from the OIPC (Bowen and Revenaugh, 2003), which is dependent on available data from local GNIP stations. There are three GNIP stations in proximity to our network of sites, including Inuvik, Mayo, and Whitehorse (Fig. 2.2.1). However, the number of monthly records, continuity and temporal coverage of records for these GNIP stations is highly variable. For example, the Inuvik GNIP record holds 14 monthly observations spanning the years 1986-1989, but most (n = 8) of which are from 1988. Conversely, Whitehorse has the best coverage with 95 monthly records with relatively continuous coverage from 1961-1965 and 1985-1989. The spatial and temporal inequities in GNIP record density leaves open the possibility that there may be some regional bias in the δD_{MAP} isoscape, with implications for ε_{wax/MAP}. However, similar estimates of δD_{MAP} are predicted for each site based on the regional temperature-δD_{precip} transfer function by Porter et al. (2016), and we find no significant difference in mean ε_{wax/MAP} when using these values instead. This result lends confidence to the use of OIPC-estimates of δD_{MAP} in this region.

Within-site variability of δD_{wax} is also substantial (4-24‰) and increasing the number of soil samples per site would allow for more robust mean estimate from each site. However, considering the range of latitude and climatic conditions covered by this transect as well as the documented heterogeneity of soils at even nanometer scales (Lehmann et al., 2008), the inter- and intra-site variability we observe is not unexpected.

2.4.5 Potential for paleoenvironmental applications
Continental Yukon and Alaska, collectively known as Eastern Beringia, were largely unglaciated during the last glacial maximum and previous glacial periods. Eastern Beringia hosts an abundance of fossil-rich sediments spanning the last ca. 2.9 Ma compared to most other sub-Arctic regions which lack terrestrial Pleistocene deposits due to glacial scouring, (Elias, 2000; Matheus et al., 2003; Matthews et al., 2003; Zazula et al., 2003; Péwé et al., 2009; Schweger et al., 2011). The pre-50 ka chronology of these deposits is also exceptional, owing to datable ash beds sourced from volcanoes in Alaska and Yukon (Froese et al., 2009; Preece et al., 2011). Recent studies in central Yukon have documented well-preserved n-alkanes and -acids from steppe-tundra paleoenvironments in relict permafrost deposits dating to MIS 4, 3/2 and 2 (Pautler et al., 2014;
Porter et al., 2016). However, this proxy has not yet been applied to Pleistocene interglacial paleosols, which have been reported at numerous locales (Schweger et al., 2011). Pollen and macrofossil evidence reveals that *Picea sp.* dominated woodlands, consistent with the majority of our study sites, were typical during most interglacials in this region (Schweger et al., 2011). The net fractionation constrained in our study represents an important first step toward interpreting $\delta D_{\text{wax}}$ records from interglacial paleosol deposits in Eastern Beringia.

To date, most quantitative paleoclimate estimates from this region are based on the Modern Analogue Technique using fossil pollen (Viau et al., 2008) or the Mutual Climate Range approach using fossil insects (Elias, 2000; Zazula et al., 2011). However, pollen and midge reconstructions, like all paleoclimate proxies, have uncertainties and do not always produce coherent paleoclimate estimates in this region (see discussion by Porter et al., 2016). Fossil $n$-acids provide an independent proxy that can be readily applied in this region, in concert with other more traditional proxies (e.g., pollen and insect assemblages), to better resolve mean climate changes in this region.

### 2.5 Conclusions

This study analyzed $n$-acids from modern vegetation and soils from a network of 13 sampling sites in Yukon, Alaska and Northwest Territories to better constrain the hydrogen isotope net fractionation of $n$-acids deposited in northern boreal forest soils, an understudied ecosystem in modern plant wax calibrations, toward developing understanding of the proxy for paleoenvironmental applications. This is the first study to constrain net fractionation from a regional soil survey within the boreal forest ecotype. Soil-based calibrations represent a powerful approach as soils integrate the variability associated with individual plants, relative contributions of vegetation types, microtopography and soil conditions, as well as a wide range of climatic conditions (mean annual temperatures, annual precipitation, summer daylight hours, relative humidity, elevation) to produce net fractionation values that are widely applicable in northern boreal forests. The net fractionation values presented here are most directly applicable for paleoclimate reconstructions based on paleosols, as wax transport, sources and degradation may vary between soils and other depositional systems (e.g. lake sediments; Nguyen Tu et al., 2017).

We find the overall $n$-acid homologue pattern in boreal soils (O and A horizons) is closely associated with the pattern observed in fresh mosses, which suggests mosses are a primary $n$-acid contributor to the average boreal soil. The overall net fractionation of soil $\delta D_{\text{wax}}$ relative to
δD_MAP \left( \varepsilon_{\text{wax/MAP}} \right) \text{ is } -93 \pm 10\%o, -102 \pm 11\%o, \text{ and } -96 \pm 11 \%o \text{ for } n-C_{24}, C_{26} \text{ and } C_{28} \text{ acids, respectively. These findings will help to inform } \delta D \text{ wax interpretations based on fossil boreal } n\text{-acids, especially in Eastern Beringia where interglacial paleosols are associated with } Picea \text{ dominated woodlands that are compositionally similar to the majority of sites included in our study.}

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2.7 References


Sachse, D., Billault, I., Bowen, G.J., Chikaraishi, Y., Dawson, T.E., Feakins, S.J., Freeman, K.H., Magill, C.R., McInerney, F. a., van der Meer, M.T.J.J., Polissar, P., Robins, R.J.,

Sachse, D., Dawson, T.E., Kahmen, A., 2015. Seasonal variation of leaf wax n-alkane production and δ2H values from the evergreen oak tree, Quercus agrifolia. Isotopes in Environmental and Health Studies 51, 124–142.


Sachse, D., Radke, J., Gleixner, G., 2006. dD values of individual n-alkanes from terrestrial plants along a climatic gradient – Implications for the sedimentary biomarker record. Organic Geochemistry 37, 469–483.


Tipple, B.J., Berke, M.A., Doman, C.E., Khachatryan, S., Ehleringer, J.R., 2013. Leaf-wax n-
alkanes record the plant-water environment at leaf flush. Proceedings of the National Academy of Sciences 110, 2659–2664.


Chapter 3
Holocene variability in plant wax D/H from Spindly Pine Lake, Southwestern Yukon

The isotopic composition of precipitation is a tracer for hydrological change, and is sensitive to temperature, seasonality and amount of precipitation, and moisture trajectory among other processes. The hydrogen isotopic composition of fossil plant waxes (δD$_{\text{wax}}$) in sediments is a proxy for precipitation δD and, thus, paleoclimate, but is offset from δD$_{\text{precip}}$ due to a large net fractionation from several biotic and abiotic factors. We collected a ~5 m sediment core from Spindly Pine Lake in southwest Yukon Territory (60.30°N, 134.99°W) that spans the full Holocene with a basal date of 12.1 ka cal. BP, and measured the δD$_{\text{wax}}$ of $n$-alkanoic acids (C$_{24}$, C$_{26}$ an C$_{28}$) at ~400-yr resolution. The amount-weighted mean $n$-C$_{26}$ and -C$_{28}$ acid δD$_{\text{wax}}$ record has a mean of -220‰ (1σ = 10‰, n = 28) and a 37‰ range. The net fractionation value for core top (0-6 cm) δD$_{\text{wax}}$ relative to mean annual precipitation is -59 ± 2‰. This is significantly smaller than the net fractionation calculated for soil samples surrounding the lake. ACL, CPI, and $n$-alkanoic acid distributions are also significantly different between the sediment core and soil samples suggesting that differing plant wax sources or degradation patterns occur in these different depositional environments. This suggests that not only regional ecology and climate, but also sedimentary environment should be considered when selecting an appropriate net fractionation value for reconstructions of δD$_{\text{precip}}$ from δD$_{\text{wax}}$. The Spindly Pine δD$_{\text{precip}}$ record shows good coherence with the nearby Mt. Logan PR Col ice core δD and δ$^{18}$O record and the Jellybean Lake δ$^{18}$O lake carbonate record in the mid Holocene, suggesting that it faithfully tracks the regional isotopic composition of precipitation of this region which is likely driven by shifts in atmospheric circulation.

3.1 Introduction

Beringia, the region spanning northeastern Siberia to northwestern Canada, has been the focus of Holocene (11.7 ka BP to present) paleoclimate reconstructions in order to better understand climatic responses to forcing under similar to modern boundary conditions (Kaufman et al., 2016), as well as to better understand Beringia’s role in the migration of humans, plants and animals into North America (Fladmark, 1979; Reich et al., 2012; Pedersen et al., 2016). This region experienced major paleoecological changes from shrub-tundra to boreal woodlands following deglaciation of the marginal region (Cwynar and Spear, 1995). While much has been done to reconstruct climate in this region, the majority of quantitative climate reconstructions are based on fossil pollen or midge assemblages, both of which are associated with their own set of uncertainties, and do not always agree (Kaufman et al., 2016). Some of the discrepancies between midge and pollen reconstructions are related to secondary environmental uncertainties
that are unrelated to the target variable of interest (e.g. lake chemistry) (Kaufman et al., 2016). This underscores the need for developing reconstructions from other proxy types, which have their own unique uncertainties. Paleoclimate trends that are corroborated by more than one independent proxy can be viewed with a higher degree of confidence.

Stable isotope ratios of hydrogen (2H/1H or δD) and oxygen (18O/16O) in precipitation are well-established tracers of climate and can be preserved in numerous archives including ice cores (Zdanowicz et al., 2014) and relict permafrost (Porter et al., 2016), as well as in proxies including lake sediment carbonates (Anderson et al., 2005), plant-derived cellulose (Anderson et al., 2001), and bulk organic matter (Jones et al., 2014). The δD of fossil plant waxes (δD_wax) are widely applied as a proxy for the δD of precipitation (δD_precip) (e.g. Hou et al., 2007; Tierney et al., 2008; Garcin et al., 2014); however, only a few studies have utilized the δD_wax proxy in Beringia (Holland et al., 2013; Wilkie et al., 2013; Nichols et al., 2014). The δD_wax of the long straight-chain hydrocarbons (n-alkanes, n-alkanoic acids, and n-alcohols) that make up cuticular waxes of plants (Eglinton and Hamilton, 1967) record the isotopic composition of cellular plant waters, which are ultimately derived from meteoric waters (Sachse et al., 2012). Plant waxes are robust proxies because they are resistant to degradation in anoxic environments leading to good preservation in sedimentary archives including in lake sediments spanning the Holocene (Nelson et al., 2013; Aichner et al., 2015).

Several environmental and biosynthetic fractionations need to be accounted for in order to reconstruct δD_precip from δD_wax. Collectively, these fractionations are referred to as the net (or apparent) fractionation (ε_wax/precip), and result in δD_wax values that are depleted relative to δD_precip (Chikaraishi et al., 2004b; Feakins and Sessions, 2010; Zhou et al., 2010). Estimating ε_wax/precip remains the largest uncertainty in the application of δD_wax as a proxy for δD_precip because it varies by region and vegetation community. Soil water, charged by precipitation, can be enriched in deuterium (D) in soils through evaporation; however, this fractionation primarily effects shallow rooted plants e.g. grasses (Smith and Freeman, 2006; Feakins and Sessions, 2010). Hydrogen isotopes are generally not fractionated during uptake of source water by roots (White et al., 1985; Ehleringer and Dawson, 1992; Roden et al., 2000); however, transpiration results in D-enrichment of leaf waters (Feakins and Sessions, 2010). n-Alkyl synthesis results in a series of biosynthetic fractionations that result in significant D-depletion of the resulting lipids relative to leaf water δD. As a result of different biosynthetic fractionations, large variability in ε_wax/precip has
been noted between different groups of plants and photosynthetic pathways. A meta-analysis by Sachse et al. (2012) revealed that, on average, $n$-C$_{29}$ alkanes produced by shrubs are the most enriched in deuterium, followed by trees, forbs, and graminoids. Therefore, changes in vegetation type and abundance within a catchment can result in variability in sedimentary $\delta$D$_{\text{wax}}$ that is unrelated to $\delta$D$_{\text{precip}}$ (Fornace et al., 2014). For $\delta$D$_{\text{wax}}$-based paleoclimate reconstructions, this underscores the need to use $\varepsilon_{\text{wax/precip}}$ values that are appropriate to the plant community and climate, to enable robust estimates of $\delta$D$_{\text{precip}}$ (Feakins, 2013; Nichols et al., 2014).

Four previous studies (Shanahan et al., 2013; Wilkie et al., 2013; Daniels et al., 2017, Bakkelund et al., in review) have calibrated $\varepsilon_{\text{wax/precip}}$ values for long chain $n$-alkanoic acids relative to mean annual precipitation in high latitude regions. Three of these calibrations are based on lake sediments from tundra ecosystems in Baffin Island (Shanahan et al., 2013), N. Alaska (Daniels et al., 2017), and N.E. Siberia (Wilkie et al., 2013), and one is based on boreal forest soils in the Yukon, Alaska and Northwest territories (Bakkelund et al., in review). The lake sediment calibrations found $\varepsilon_{\text{wax/precip}}$ values ranging from -61 ± 20‰ ($n$-C$_{26,28}$ acids; Shanahan et al., 2013) to -114 ± 13‰ ($n$-C$_{22-30}$ acids; Daniels et al., 2017), and the boreal forest soils calibration yielded a mean $\varepsilon_{\text{wax/precip}}$ of 96 ± 11‰ ($n$-C$_{28}$ acids; Bakkelund et al., in review).

High-latitude $\delta$D$_{\text{wax}}$ records from sedimentary archives, which commonly span many thousands of years, have great potential to be used for quantitative temperature reconstruction because of the strong relation between temperatures and $\delta$D$_{\text{precip}}$ in sub-Arctic and Arctic regions (Dansgaard, 1964; Porter et al., 2016). However, $\delta$D$_{\text{precip}}$ is also influenced by other factors such as shifts in oceanic moisture source, air mass trajectory and the seasonality of precipitation, which can dominate variability in the proxy record (Anderson et al., 2016a). Studies have found the sensitivity of $\delta$D$_{\text{precip}}$ to these other factors is spatially dependent (Field et al., 2010; Porter et al., 2014) and there remains debate on the precise drivers of the isotopic composition of precipitation in southwest Yukon (Anderson et al., 2016a; Kaufman et al., 2016).

Here, we present a full Holocene $\delta$D record using $n$-acids from plant waxes recovered from a sediment core from a small kettle lake in southwestern Yukon referred to as Spindly Pine Lake (unofficial name). Our objectives are to (1) determine an appropriate net fractionation value to reconstruct $\delta$D$_{\text{precip}}$ for this lake sediment core record, and (2) determine the likely driver(s) of the isotopic composition of precipitation.
3.2 Methods

3.2.1 Study location

Spindly Pine Lake is located approximately 60 km south of Whitehorse, Yukon (60°18'6.03"N, 134°59'40.33"W, 792 m a.s.l.; Fig. 3.2.1). It is a small (0.66 ha), closed basin, kettle lake (formed when an isolated block of ice left by a retreating glacier melts) with a maximum depth of 8 m that was formed by retreating glaciers from the Cordilleran ice sheet (Prince et al., 2018). Spindly Pine Lake is located in the Yukon Southern Lakes ecoregion of the Boreal Cordillera ecozone (Yukon Ecoregions Working Group, 2004) and experiences long cold winters and short cool summers, with mean annual temperatures around 0°C. The growing season in this region is primarily limited by minimum temperatures above the freezing mark. In Whitehorse, Yukon, minimum daily temperatures are above freezing from mid May to late September (average duration = 129 days). The rain shadow effect from the St. Elias Mountains results in a semi-arid climate in interior Yukon. Relative humidity is greatest in winter reaching 75% in November but drops to 56% in May and June. The region receives about 260 mm of precipitation annually, distributed throughout the year, with peak precipitation from June to September (Fig. 3.2.2; Environment Canada, 2018).

The Online Isotopes of Precipitation Calculator (OIPC) is an interpolated data product that estimates \( \delta D_{\text{precip}} \) from Global Network of Isotopes in Precipitation (GNIP) stations. OIPC estimates \( \delta D \) of monthly precipitation varies by 77‰ over the course of a year with the most depleted precipitation (-201‰) in January, and the most enriched precipitation falling in June (-124‰). The amount weighted annual average \( \delta D \) of precipitation (\( \delta D_{\text{MAP}} \)) is -163 ± 2‰ (Bowen and Revenaugh, 2003; IAEA/WMO, 2016; Bowen, 2018). \( \delta D_{\text{precip}} \) is relatively well constrained in this region compared to other northern regions with 95 monthly records with relatively continuous coverage from 1961-1965 and 1985-1989.

The Yukon Southern Lakes ecoregion was glaciated from 26 ka to approximately 13 to 10 ka (depending on elevation), and the landscape is marked by glacial till, glaciofluvial gravels, and glaciolacustrine clay and silt deposits. The White River Ash east (WRAe) tephra originating from Mount Churchill, which dates to 1147 cal yr BP (Before Present, Present = 1950 CE) (Clague et al., 1995), is a widely distributed visible age marker in this region.
Spindly Pine Lake is located within the northern boreal forest and is surrounded by a mixed stand (open- to semi-closed canopy) of white spruce (*Picea glauca*) and lodgepole pine (*Pinus contorta*). The understory includes shrubs (alder, *Alnus sp.*, willow, *Salix sp.*, and dwarf birch, *Betula nana*) and thick feathermoss ground cover (e.g. *Pleurozium schreberi*, *Ptilium crista-castrensis*, *Tomentypnum nitens*, and *Dicranum polysetum*). A small grove of trembling aspen trees (*Populus tremuloides*) are located on a steep south-facing slope on the north side of the lake.

**Figure 3.2.1** (a) study region, detail of red square shown in panel b; (b) detailed view of study region showing locations of Mt. Logan Prospector Russel Col ice core (Mt. L), Jellybean Lake (JB), Marcella Lake (ML) and Spindly Pine Lake (SP); (c) detail of Spindly Pine Lake showing small size and closed basin; (d) oblique aerial photograph of Spindly Pine Lake (Photo by Tyler Prince).
Figure 3.2.2 Monthly OIPC $\delta D_{\text{precip}}$ from Spindly Pine Lake (SP), and average monthly $\delta D_{\text{precip}}$ from Whitehorse GNIP station, and average monthly relative humidity, air temperature, and precipitation from Whitehorse (Environment Canada, 2018). Dashed line represents amount-weighted mean annual $\delta D_{\text{precip}}$.

3.2.2 Lake sediment core and chronology

Two sediment cores were collected from the centre of Spindly Pine Lake: a surface core was collected in April 2006 using a Glew gravity coring system (Glew et al., 2001) with internal diameter of 7.6 cm and deeper sediments were collected in August 2016 using a modified Livingstone piston core with an internal diameter of 5 cm (Prince, Tyler et al., 2018). The cores were transported to the Brock University Water and Environmental Sciences Laboratory where they were subsampled for macroscopic charcoal, loss-on-ignition (LOI), magnetic susceptibility (MS) and pollen (Prince, Tyler et al., 2018). The overlap between the Glew gravity core and the Livingstone piston cores was determined using LOI, MS and the macroscopic charcoal profiles. The top (0 cm) of the piston core aligns with 27.5 cm of the gravity core, resulting in a total adjusted core length of 539.5 cm. All downcore depth measurements are reported in terms of adjusted core length.
3.2.3 Dating

Macrosfossils and bulk sediment were analyzed for $^{14}$C by accelerator mass spectrometry (AMS) at the A.E. Lalonde AMS Laboratory at the University of Ottawa, Canada. Bulk sediment was used to date sections of the core that did not contain appropriate macrosfossils. Ten $^{14}$C dates (4 macrosfossils and 6 bulk sediment), calibrated using the IntCal13 (Reimer et al., 2013) and the White River ash (WRA) tephra, were used to develop the age depth model, using the bayesian age model software BACON (Fig. 3.2.3; R Studio package Bacon V 2.2; Blaauw and Andrés Christen, 2011). In order to account for old carbon offsets between bulk sediment and macrosfossils, bulk sediments were collected directly below the WRA and the offset between bulk sediment age and WRA age was applied to subsequent bulk sediment samples assuming a constant offset (Patterson et al., 2017).

Radiocarbon dating yielded a basal date for the sediment core of ~12 450 BP, indicating that the core spans the full Holocene. Sedimentation rates were slowest in the early Holocene (0.16 mm year$^{-1}$) and more rapid in the late Holocene (1.35 mm year$^{-1}$; Prince, Tyler et al., 2018).
Figure 3.2.3 Age-depth model of Spindly Pine Lake sediment core created by Tyler Prince (reproduced with permission). (a) Markov chain Monte Carlo iterations of the BACON output; (b) Distribution of accumulation rates; (c) memory/autocorrelation, demonstrating how much the accumulation rate at a certain depth in the core is dependent on nearby depths. Low memory indicates changing sedimentation rates throughout the core, while high memory indicates a more smooth, and consistent depositional history; (d) Age depth model created with BACON V 2.2 in R Studio. The vertical green lines represent known dates, blue lines represent the 14C dates and the red line is the model of best fit. (Prince et al., 2018); WRA: White River Ash deposit.

3.2.4 Plant wax analysis

Sampling

For leaf wax analysis, the sediment core was sub-sampled at mean sampling resolution of ~400-years (20 cm) between samples. Four pilot samples were analyzed and yielded a mean concentration of 70 μg g⁻¹, indicating ~30 g (wet-weight) of sediment was needed to ensure high enough concentrations of $n$-C₂₄ to C₂₈ acids for isotope analysis. This corresponded to a mean of
5 cm of core and 120 years integrated in each sample. Thirty-three (33) samples were collected from the sediment core, and 28 samples yielded viable n-acid concentrations for isotope analysis.

**Lipid extraction**

Sediment samples were freeze dried in a Labconco freezezone 2.5 unit and homogenized. Soil bound-lipids were extracted with 9:1 (v/v) Dichloromethane (DCM):Methanol (MeOH) by microwave-assisted heating (Milestone Ethos Up unit) at 70°C for 20 minutes, and continuous magnetic stirring, a method selected for optimal recovery (Chávez-Lara et al., 2018). Samples were then centrifuged at 1800 RPM for 10 minutes to settle the sediment, and the supernatant was removed and retained. The sediment sample was rinsed three to five additional times with DCM-MeOH, centrifuged and transferred to the collection vial. Excess solvent was evaporated under a nitrogen blow down system.

The total lipid extract was separated by column chromatography in a 5.75” borosilicate glass Pasteur pipette packed with Phenomenex 60 Å NH2 septra stationary phase. The neutral fraction was eluted with 2:1 DCM:Isopropanol, followed by the acid fraction with 4% formic acid in diethyl ether. The acid fraction was methylated to Fatty Acid Methyl Esters (FAMEs) in 5% Hydrochloric acid and 95% Methanol (δDMeOH = -187.7‰ ± 0.5, following methodology in Lee et al., 2017) at 60°C for 12 hours. This step is necessary because the compounds must be non-polar for GC analysis. The mixture was cooled and ~1 mL MilliQ water was added. Then, 1 mL hexanes was added and shaken vigorously for 30 seconds, to partition the FAMEs into the hexanes and this liquid-liquid extraction was repeated (three to five times) to ensure recovery. The extract was passed through a column of anhydrous sodium sulfate to remove any water. The extract was purified by column chromatography with 5% water-deactivated 100-200 mesh silica gel, and the FAMEs were eluted with 3 column rinses of hexanes and then DCM. The saturated FAMEs were further purified by column chromatography with silver nitrate on silica gel (+230 mesh), also eluted with hexanes and DCM.

**n-Acid abundance and isotope analysis**

FAMEs were analysed at University of Toronto Mississauga with a Gas Chromatograph (Thermo Trace 1310) Flame Ionization Detector (GC-FID), equipped with a Programmable Temperature Vaporizing (PTV) injector and a Rxi-5 ms column (30 m x 0.25 mm, film thickness 1 μm). The FID results were normalised to known quantities of an in-house FAME standard (n-
C_{17}, -C_{21}, -C_{25} and -C_{29} FAMEs). For all samples, we calculated the average chain length (ACL = Σ(C_n × n)/ΣC_n, where C_n is the abundance for chain-lengths from n = 20-34), modal chain length (C_{max}), and the carbon preference index (CPI = ([C_{20}, 22, 24, 26, 28, 30] + [C_{22}, 24, 26, 28, 30, 32])/(2 × [C_{21}, 23, 25, 27, 29, 31])), the latter a measure of degradation (Bray and Evans, 1961).

Compound-specific hydrogen isotope ratios were measured at the University of Southern California using a Thermo Scientific Trace GC equipped with a PTV inlet operated in solvent split mode and a Rxi-5 ms column (30 m x 0.25 mm x 0.25 μm), connected via a GC Isolink with pyrolysis (1400°C), via a Conflo IV to an Isotope Ratio Mass Spectrometer (Delta V IRMS). The H\textsuperscript{3+} factor was measured daily to check for linearity and remained close to 8 ppm mV\textsuperscript{-1}. H\textsubscript{2} reference peaks were injected at the beginning and end of each sample run on the GC-IRMS, with two used for standardization between sample and standard runs. Hydrogen isotope ratios are reported in delta notation (δD = (R_{sample} − R_{standard})/R_{standard} where R is the ratio of deuterium to protium, D/H). Precision of replicate sample injections was typically better than 1‰. Data were normalized to the VSMOW-SLAP hydrogen isotopic scale using the A3 mix alkane standard with δD values ranging from -233.7 to -46.3‰ (supplied by A. Schimmelmann, Indiana University, Bloomington). The RMS error of replicate analyses of the A3 mix was typically better than 4‰ over the month of analysis. The hydrogen atoms added by methylation were corrected for by mass balance to determine the original δD of n-acids (δD_{acid} = δD_{FAME} (2i + 2-3 × δD_{MeOH})/2i-1), where δD_{FAME} is the normalized δD value of the FAME and i is the number of carbon atoms the fatty acid molecule of interest).

### Net fractionation

Net (or apparent) fractionation was calculated for core top sediment sample (0-6 cm) based on the general formula: ε_{wax/MAP} = (δD_{wax} + 1)/(δD_{MAP} + 1) − 1, where δD_{wax} is a measured value from the core top sediments and δD_{MAP} is an estimate of average meteoric waters integrated in soil (and available to plants) during the growing season. We assume amount-weighted mean annual precipitation δD (δD_{MAP}) is a reasonable source water approximation for Spindly Pine Lake because, in comparison to unweighted mean annual, δD_{MAP} is biased to warm-season values, reflecting higher precipitation totals in summer (Fig. 3.2.2), and accounting for inputs of D-depleted snowmelt to the ground in springtime (Mackay, 1989; see discussion in
Bakkelund et al., in review). We used the OIPC (Bowen and Wilkinson, 2002; Bowen and Revenaugh, 2003) to estimate $\delta D_{\text{MAP}}$.

### 3.3 Results

#### 3.3.1 Lake core molecular abundance and isotopic composition

Spindly Pine Lake core samples produced long chain $n$-$C_{20}$ to $C_{34}$ acids. The distribution of $n$-acids remained relatively consistent throughout the core, with a unimodal distribution and a modal chain length of $C_{26}$ ($n = 24$) or $C_{28}$ ($n = 4$) and low abundances of high molecular weight $n$-acids ($>C_{30}$; Fig. 3.3.1). We find an average ACL of $25.0 \pm 0.5$ ($n = 28$) with no downcore trend (Fig. 3.3.2; Table 3.3.1). We find the expected even over odd predominance of $n$-acid chain lengths with average CPI of $6.1 \pm 1.6$. Concentrations range from 2.7 to 33.1 $\mu g \, g^{-1}$ for $C_{24}$, 2.5 to 61.8 $\mu g \, g^{-1}$ for $C_{26}$, and 3.5 to 46.6 $\mu g \, g^{-1}$ for $C_{28}$. The total concentration of long-chain $n$-acids ($\Sigma_{C_{20}-C_{34}}$) ranges from 66.9 to 1050.3 $\mu g \, g^{-1}$ ($\bar{\chi} = 577, n = 28$).

![Figure 3.3.1](image-url) **Figure 3.3.1** Average relative abundance of $n$-$C_{20}$ to $C_{34}$ acids from Spindly Pine lake sediment samples (blue; average of 28 samples from 5.4 m sediment core), Spindly Pine Lake upland soil samples (light orange, Bakkelund et al., in review, 9 samples) and boreal soil transect samples (dark orange, thirteen sites, including SP, in the Yukon, Northwest Territories, and Alaska, spanning 60.3 to 68.3°N., Bakkelund et al. in review). Error bars represent 1σ.
Figure 3.3.2 Downcore variation in (a) δD values and (b) concentrations for n-C24, -C26, -C28 acids, (c) Total concentration (Σ20-34) of n-acids, (d) ACL, and (e) CPI, and relative abundances of pollen from three key taxa – Pinus (green), Picea (purple), and Betula (yellow; replotted from Prince, Tyler et al., 2018).
Twenty-eight sediment samples yielded sufficient concentrations for isotopic analysis. Mean δD<sub>Wax</sub> values were -226 ± 16‰ for n-C<sub>24</sub>, -221 ± 11‰ for -C<sub>26</sub>, and -219 ± 9‰ for -C<sub>28</sub>. δD values for various chain lengths are highly correlated (C<sub>24</sub> an C<sub>26</sub>: r = 0.85, p < 0.001; C<sub>24</sub> an C<sub>28</sub>: r = 0.87 p < 0.001; C<sub>26</sub> and C<sub>28</sub>: r = 0.90, p > 0.001; Fig. 3.3.3; Table 3.3.1), however C<sub>24</sub> is generally slightly more depleted than C<sub>26</sub> or C<sub>28</sub>. For the remainder of this paper an amount-weighted mean value of δD<sub>C26</sub> and δD<sub>C28</sub> wax will be used and referred to as δD<sub>Wax</sub>, as higher molecular weight n-acids (C<sub>26</sub> or longer) are considered unambiguous terrestrial plant biomarkers and commonly the focus of other n-acid studies (e.g., Hou et al., 2008; Tierney et al., 2008; Wilkie et al., 2013).

**Figure 3.3.3** Correlation between hydrogen isotopic composition of (a) n-C<sub>24</sub> and C<sub>28</sub> and (b) C<sub>26</sub> and C<sub>28</sub> acids of Spindly Pine Lake sediment core samples (n = 28).

### 3.3.2 Reconstructed δD<sub>precip</sub>

An ε<sub>Wax/MAP</sub> of -59 ± 2‰ based on the core top (0-6 cm) sediment sample was used to reconstruct downcore δD<sub>precip</sub> for all lake core samples. δD<sub>precip</sub> ranges from 40‰ (-189 to -149‰) over the downcore record. δD<sub>precip</sub> values decrease from the maximum value (-149‰) at 11.9 ka to the minimum value at 8.6 ka (-189‰), before increasing to ~165‰ in the mid Holocene between 6.5 and 5 ka. δD<sub>Wax</sub> values decrease from 5 ka to 4 ka reaching a secondary minimum at -186‰, before increasing again to -156‰ at ~0.3 ka. δD<sub>Wax</sub> values then decrease to present, with values similar to the mid Holocene (-163‰).
Table 3.3.1 n-acid characteristics and isotopic composition of Spindly Pine Lake sediment core samples

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<th>Mean Age (ka)</th>
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<th>CPI&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Σ acids&lt;sup&gt;c&lt;/sup&gt;</th>
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<th>C&lt;sub&gt;26&lt;/sub&gt; ± 1σ</th>
<th>C&lt;sub&gt;28&lt;/sub&gt; ± 1σ</th>
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a Average Chain Length
b Carbon Preference Index
c Total \( n \)-acid (C\(_{20}\)-C\(_{34}\)) concentration
d Amount-weighted average of \( n \)-C\(_{26}\) and C\(_{28}\) acids
e \( \delta^{13}D_{\text{precip}} \) reconstructed from \( \delta^{13}D_{\text{wax}} \)
3.4 Discussion

3.4.1 n-Acid molecular abundances in lake sediment core

n-Acid relative abundances from Spindly Pine Lake sediment samples show distributions that resemble those from shrub leaves (Bakkelund et al., in review), with a modal chain length of C\textsubscript{26} with large proportions of C\textsubscript{28} and C\textsubscript{24} homologues, with very low abundances of high molecular weight n-acids (>C\textsubscript{30}; Fig. 3.3.1). This distribution is not mirrored by other vegetation types in this region (Bakkelund et al., in review). Grasses also produce high abundances of n-C\textsubscript{26} and C\textsubscript{28} acids; however, they produce less n-C\textsubscript{24} acids and more n-C\textsubscript{30} acids. Forbs and trees also produce relative abundances of n-C\textsubscript{30} acids that are much higher than those in lake sediments. Mosses produce lower abundances of n-C\textsubscript{26} and C\textsubscript{28} acids than lake sediments. Although n-alkyl homologue distributions aren’t taxonomically diagnostic (Eglinton and Hamilton, 1963), some generalisations can be made between plant groups. The evidence suggests that shrubs are the primary contributor to Spindly Pine Lake sediment samples, with secondary contributions from grasses which grow in large abundance around the edge of the lake (Appendix G). This pattern differs from the n-acid homologue distributions seen in boreal soils sampled at Spindly Pine Lake (n=9) and along a transect in the Yukon, Alaska, and Northwest Territories (sites = 13 including Spindly Pine, samples = 42; Fig. 3.3.1). The Spindly Pine soil sample data are presented in Supplemental Appendix B. Conversely, boreal soils have a modal chain length of C\textsubscript{24} with high abundances of C\textsubscript{22} acids and are likely dominated by contributions from mosses (Bakkelund et al., in review). The ACL is also significantly (p < 0.01) higher in lake core sediments (ACL = of 25.0 ± 0.5) than boreal soils (ACL = 24.2 +0.5). The differences in n-acid characteristics between the lake core and soils are likely the result of differing sources of waxes or differing degradation patterns.

The large contribution of n-acids from shrubs to lake sediments and n-acids from mosses to soils is likely a result of their differing morphologies. The structure of plants and morphology of their leaves effects how susceptible vegetation is to being transported by wind and run off (Rich, 1989). It is likely that the large surface area of leaves from shrubs and their height make their leaves susceptible to being transported into the lake by wind, and may account for the significant contributions of shrubs to lake sediments. Additionally, the abundance of shrubs and grasses growing around the perimeter of the lake likely also led to increased deposition in lake sediments.
Mosses grow low to the ground in tightly intertwined networks rooted in the soil increasing the likelihood of them decomposing *in situ*, rather than be blown or washed into the lake leading to their over representation in the soils (Bakkelund et al., in review).

Besides different vegetation sources for waxes in lake sediments and soils, it is likely that degradation also occurs differently in these two depositional environments. Low CPI has been associated with degradation of waxes (*n*-acids: Chikaraishi and Naraoka, 2006; Schäfer et al., 2016; *n*-alkanes: Zech et al., 2011; Wang et al., 2016; Zhang et al., 2017). CPI values for lake sediments (6.1 ± 1.6) are significantly (*p* < 0.05) lower than those for average vegetation (forbs, grasses, trees, shrubs and mosses; 12.2 ± 1.4) as well as lower than CPI values from shrubs (13.1 ± 3.9) and mosses (10.5 ± 3.3) specifically. This suggests that some microbial degradation or other factors may be affecting *n*-acid chain length distributions in lake sediments, with a disproportionate loss of the relatively abundant even chain lengths, resulting in a reduced CPI. However, the processes leading to this degradation are not yet fully understood. Spindly Pine Lake sediment CPI values are similar to CPI values from lake sediments in Northern Alaska (5.2 ± 1.0; Daniels et al., 2017); however, they are significantly higher (*p* < 0.05) than those from boreal soils (2.8 ± 0.9; Fig. 3.4.1).

Waxes in lake sediments may be less prone to microbial degradation than those in soils because they are cold, anaerobic environments, whereas soils are seasonally warm, aerobic environments that are subject to freeze-thaw cycles. Therefore, the higher CPI in lake sediments than soils may be a result of less degradation of *n*-acids in lake sediments than soils. Comparative studies examining degradation of biomarkers in different environments are rare. The only study that has done a controlled comparison of leaf wax degradation in soils and pond sediments found no significant difference in degradation pattern over two years. However, this study investigated a pond that was well oxygenated which the authors suggest may be the reason no significant differences were found (Nguyen Tu et al., 2017). Therefore, the interpretations presented here are preliminary, and further work should be done on *n*-acid sources and degradation in different environments to determine if the patterns seen at Spindly Pine Lake are local or representative of systematic differences in depositional environments.
Figure 3.4.1 Box plots comparing (a) ACL, (b) CPI, and (c) δD_{wax} between the Spindly Pine Lake sediment core, Spindly Pine soil samples and regional soil transect samples. Blue diamonds represent 20th Century (core top) Spindly Pine Lake sediment core samples.

3.4.2 Net fractionation of \textit{n}-acids in Spindly Pine Lake sediments

The net fractionation value derived from the core top sediments from Spindly Pine Lake is -59 ± 2‰, which is significantly smaller (p < 0.01) than the mean boreal soil net fractionation value (-99 ± 11‰) and the mean net fractionation value for soils sampled from the forest surrounding Spindly Pine Lake (-112 ± 11‰). Correspondingly, the mean δD_{wax} value (-220 ± 10‰) from the lake sediment core is significantly more enriched than the mean δD_{wax} value from boreal soils (mean = -257 ± 6‰) and Spindly Pine Lake soil samples (-251 ± 6‰). Differences in \textit{n}-acid sources or differences in the δD of source water used in \textit{n}-acid synthesis, may each contribute to the smaller ε_{wax/MAP} in lake sediments than soils at Spindly Pine Lake.

It is likely that lake sediments are dominated by \textit{n}-acid contributions from shrubs, and soils are dominated by \textit{n}-acid contributions from mosses (as discussed in the previous section). Mosses, such as \textit{Sphagnum sp.}, have been shown to have more negative δD_{wax} values and, therefore, larger net fractionations than shrubs and other vascular plants (\textit{n}-acids: Wilkie et al., 2013; \textit{n}-alkanes: Sachse et al., 2006, 2012; Brader et al., 2010; Nichols et al., 2010). Brader et al. (2010) and Nichols et al. (2010) found \textit{n}-alkane mean net fractionations for \textit{Sphagnum} were approximately -150 to -200‰, and Sachse et al. (2012) in their review found \textit{n}-alkane shrub fractionations averaged around -90‰. Therefore, it is possible that \textit{n}-acids from mosses are more depleted than \textit{n}-acids from vascular plants, contributing to the different δD_{wax} values between soils and lake sediment core samples.
Another possible explanation for the difference in net fractionation between lake sediments and soils is that lipid inputs to the lake are dominated by \( n \)-acids from vegetation utilizing source water from the lake that is enriched relative to \( \delta D_{\text{MAP}} \). There is a perimeter of vegetation around the margin of Spindly Pine Lake that are rooted directly in the lake (Appendix G), and therefore are using lake water for biosynthesis of lipids. Spindly Pine Lake is a small closed-basin lake, with surface inflow limited to overland runoff from a very small watershed (<1 ha), and outflow limited to evaporation. Such closed-basin lakes in this region are highly sensitive to evaporation and tend to have D-enriched lake waters (Anderson et al., 2007, 2011). However, because summer evaporative enrichment has not been directly measured at Spindly Pine Lake, and it is unknown whether it is primarily fed by precipitation or groundwater, it is unclear how sensitive Spindly Pine lake water is to evaporation. However, evaporative enrichment was measured for two nearby lakes: Marcella Lake and Seven Mile Lake which provide good constraints on the degree of evaporative enrichment that might be expected in a small, closed-basin kettle lakes such as Spindly Pine Lake.

Marcella Lake (60.07°N, 133.81°W, 697 m a.s.l.), located ~70 km southwest of Spindly Pine Lake, has a surface area of 0.4 km\(^2\), a maximum depth of 9.7 m and only inflow is a small sub-aquatic spring. Evaporative enrichment of \( ^{18}O \) and D in Marcella Lake relative to mean annual precipitation were ~14‰ and 59‰, respectively (Anderson et al., 2007). Seven Mile Lake (62.18°N, 136.38°W, 520 m a.s.l.), located ~220 km northeast of Spindly Pine Lake, has a surface area of 0.1 km\(^2\), and a maximum depth of 14 m, and is fed primarily by groundwater with no surface inflow or outflow. Lake levels, and lake water \( ^{18}O \) were recorded at Seven Mile Lake beginning in 1999. These observations indicate that lake water \( ^{18}O \) is enriched by evaporation by up to 6 to 10‰ (corresponding to ~14 to 40‰ D) above the groundwater inflow, which is very similar to \( ^{18}O_{\text{MAP}} \). Years with more precipitation and increased groundwater inflow rates to the lake resulted in less enriched \( ^{18}O \) (Anderson et al., 2011). Based on these two analogous lakes, it is likely that Spindly Pine Lake experiences summer evaporative enrichment of D between 10 and 60‰.

Assuming plant wax inputs to lake sediments are dominated by vegetation growing near the shoreline that are able to access enriched lake waters through the rhizosphere’s interface with the shallow water table, \( \delta D \) of source water used in \( n \)-acid synthesis may be enriched relative to MAP, resulting in a smaller \( \varepsilon_{\text{wax/MAP}} \) in lake sediments. Conversely, soils farther from the margin
of the lake are likely dominated by plant wax inputs from mosses, which are unlikely to access evaporatively enriched lake waters due to distance from and elevation above the lake, and instead rely on soil moisture that is mainly fed by precipitation. This results in more depleted $\delta D_{\text{wax}}$ values and a larger $\epsilon_{\text{wax/MAP}}$ in upland soils. However, this conceptual model remains speculative in the absence of lake water data and more targeted analyses of $n$-acid inputs from vegetation growing along the perimeter of the lake. Future field sampling is needed to test these hypotheses more rigorously.

Regardless of whether the small $\epsilon_{\text{wax/MAP}}$ in lake sediments is attributable to differences in $n$-acid sources or differences in the $\delta D$ of source water used in $n$-acid synthesis, the core top $\epsilon_{\text{wax/MAP}}$ is most applicable for reconstructing long-term (downcore) changes in $\delta D_{\text{MAP}}$, as the mechanisms by which plants absorb source water and $n$-acids are deposited into lake sediments are unlikely to have fundamentally changed over the Holocene.

### 3.4.3 Holocene vegetation changes at Spindly Pine Lake

Pollen analysis shows there were three main vegetation changes in the Holocene at Spindly Pine Lake (Prince, Tyler et al., 2018). The early Holocene (12.45 to 10 ka) was dominated by *Betula* and *Alnus*, around 10 ka *Picea* migrated into the region suggesting a transition from a shrub tundra to boreal forest. *Picea* was dominant until around 3.85 ka when it began to decline, and *Pinus* became the dominant conifer.

Two data points fall within the “shrub tundra” period at Spindly Pine Lake which is a very different ecosystem comparable to the modern boreal vegetation assemblage around the lake. The core top-derived net fractionation value is potentially inappropriate for interpreting this period because plant type has a large effect on net fractionation. There are not enough data to test if these points are statistically different than the boreal forest period data; however, it is worth noting that the earliest point (11.86 ka) is the maximum $\delta D_{\text{wax}}$ value (-199‰) in the core.

Previous studies of $n$-alkanes have found shrubs to have the smallest net fractionation of all plant types (Sachse et al., 2012); therefore, a potentially smaller (but unknown) net fractionation value is needed to accurately constrain $\delta D_{\text{precip}}$ from the Betula zone $\delta D_{\text{wax}}$ values. The following interpretation of downcore $\delta D_{\text{wax}}$ focusses on the boreal section of the core for which a single net fractionation is thought to be appropriate. However, the fractionations of $n$-acids from *Betula* and *Alnus* shrubs and *Pinus* and *Picea* trees have not been measured for this region so the effect of
this transition is unknown. Regardless, these two data points represent an entirely different ecotype and are not directly comparable to data from the boreal forest period.

There is no significant (p < 0.05) difference in ACL or CPI between periods when Pinus or Picea were the dominant conifers. The total concentration of n-acids (C_{20}-C_{34}) as well as the concentrations of n-C_{26} and -C_{28} acids specifically, were significantly different between the periods when Pinus and Picea were dominant (p < 0.05); however, when the concentrations were normalized to the number of years integrated by the sample the differences were not significant. Therefore, this ‘apparent’ difference in the raw δD_{wax} dataset is likely a result of changing sedimentation rates rather than a result of vegetation change. There is also no significant (p < 0.05) difference in δD_{wax} values between periods when Pinus (-219 ± 10‰) or Picea (-224 ± 7‰) were the dominant tree species around the lake. Therefore, it is unlikely that the change from a Picea to Pinus dominated forest resulted in a substantial change in the biosynthetic fractionation of n-acids incorporated into the bulk lipid pool in the lake sediment core.

3.4.4 Downcore variability in δD_{precip}

Temperature is often the dominant control on the isotopic composition of precipitation in high latitude regions (Dansgaard, 1964; Porter et al., 2016). The Spindly Pine Lake δD_{precip} record shows similarities to previous temperature reconstructions (Fig. 3.4.2), generally following the trends seen in midge-based July air temperature and pollen-based mean annual temperature from ~8 ka to present. These records show particularly good coherence from 7 to 5 ka, a period generally referred to as the Holocene Thermal Maximum (HTM), a period which is uniformly warmer than the early Holocene across Eastern Beringia (Kaufman et al., 2016). A composite of midge records (n = 7) from Eastern Beringia show July air temperatures in the HTM reaching 0.2°C warmer than the average for last 1 ka. Similarly, composite pollen records (n = 9) show a warming of 0.3 and 0.2°C above summer and annual values from the last millennium, respectively.
Figure 3.4.2 Comparison of Holocene records averaged to 500-year bins. (a) Spindly Pine Lake $\delta_{\text{D}}$\text{precip}$ (green; dashed line represents un-binned $\delta_{\text{D}}$\text{precip})$; (b) Marcella Lake $\delta^{18}$O carbonate record (orange; Anderson et al., 2007); (c) Mt. Logan PR Col $\delta$ ice core (blue; Fisher et al., 2004); (d) Jellybean Lake $\delta^{18}$O carbonate records (yellow; Anderson et al., 2005); (e) temperature reconstructions: midge-inferred July air temperature (light blue; $n = 7$) and pollen-inferred mean-annual temperature (purple; $n = 9$) (Kaufman et al., 2016); and (f) June Insolation ($60^\circ$N) data (black; Berger and Loutre, 1991). Holocene Thermal Maximum (HTM) is highlighted by a grey bar. Vegetation changes at Spindly Pine Lake inferred from pollen data (Prince et al. 2018) are represented by color shading (green - Pinus; purple - Picea; yellow - Betula; shown top).
The observed range in $\delta D_{\text{precip}}$ values from the Spindly Pine Lake record is 40‰, which corresponds to a temperature change of 12°C, using the temperature-$\delta D_{\text{precip}}$ transfer function reported in Porter et al. (2016). The interquartile range (which excludes extreme values) provides a more conservative estimate of variability in $\delta D_{\text{precip}}$ across the Holocene of 16‰, or approximately 6°C. This is larger than previous Holocene paleotemperature reconstructions in Eastern Beringia which find a total change in mean annual temperature of 1 to 5°C (Heusser et al., 1985; Viau et al., 2008; Kaufman et al., 2016). Additionally, several large and abrupt shifts are recorded in the Spindly Pine Lake core that are unrealistic if interpreted purely as a temperature signal. For example, a change of 18‰ between 11.1 and 11.5 ka would correspond to a 6°C change over ~400 years, or a difference of 10‰ between 0.01 and 0.28 ka would correspond to a 3°C over ~300 years, which is an unrealistic temperature change over such a short time period. No previous studies have suggested such large and abrupt temperature changes during the Holocene (Kaufman et al., 2016). Therefore, it is highly unlikely that the $\delta D_{\text{precip}}$ signal at Spindly Pine Lake is driven by temperature alone.

Shifts in atmospheric circulation can result in changes in moisture source, storm track, or seasonality of precipitation, which can account for large magnitude changes in isotopic composition (Fisher et al., 2004; Anderson et al., 2005, 2016a). This is especially true in Southern Yukon where changes in storm track orientation relative to the St. Elias Mountains can result in different degrees of rainout and fractionation as air masses move inland from the Gulf of Alaska. Shifts in the synoptic scale circulation patterns is therefore a plausible mechanism to account for the large and sudden shifts in the $\delta D_{\text{precip}}$ signal at Spindly Pine Lake.

Previous modelling and proxy studies provide strong evidence to support the hypothesis that the isotopic composition of precipitation in southwestern Yukon is strongly influenced by changes in atmospheric circulation (Anderson et al., 2016a). Isotope-enabled general circulation models (Field et al., 2010; Porter et al., 2014; Bailey et al., 2015) have implicated changes in the strength and position of the Aleutian Low, a semi-permanent low-pressure system over the Gulf of Alaska (Mock et al., 1998; Anderson et al., 2005), as the primary driver of circulation changes in this region which is supported by proxy records in the region (e.g. Fisher et al., 2004; Anderson et al., 2005, 2007, Jones et al., 2014). However, the effects of changes in the strength and position of Aleutian Low remain open to debate. Fisher et al. (2004) suggested strong Aleutian Lows brought more southerly moisture and the longer transport distance resulted in enhanced isotopic
depletion; however, several isotope-enabled General Circulation Models have suggested that strong Aleutian Lows produce isotopically enriched precipitation (Field et al., 2010; Berkelhammer et al., 2012; Liu et al., 2014; Porter et al., 2014). Assessing these theories is beyond the scope of this study, and regardless, there is a clear consensus that the isotopic composition of precipitation in this region is driven by changes in atmospheric circulation (Anderson et al., 2016a).

Comparing the Spindly Pine Lake core record to existing stable isotope records in the region can help elucidate drivers of the δD_{\text{precip}} signal. Three high resolution stable isotope records have been developed in southwest Yukon, near Spindly Pine Lake, and largely inform our understanding of regional paleohydroclimates. Two of these records – Mt. Logan Prospector Russel Col (PR Col) δD and δ^{18}O ice core record (60.59°N 140.50°W, 5240 m a.s.l.) and the Jellybean Lake δ^{18}O carbonate record (60.35°N 134.80°W, 730 m a.s.l.) – are interpreted as reflecting the isotopic composition of precipitation driven by changes in atmospheric circulation, and one – Marcella Lake δ^{18}O carbonate record (60.07°N, 133.81°W, 697 m a.s.l.) – is interpreted as reflecting effective moisture. The PR Col ice core records annual precipitation δD and δ^{18}O at decadal to multi-decadal resolution and spans 16.2 ka (Fisher et al., 2008). The Jellybean Lake core is a sedimentary calcite record at decadal to centennial resolution spanning the last 7.5 ka, and reflects the mean annual δ^{18}O of precipitation in the catchment area (mean elevation of catchment area: 1650 m a.s.l.; Anderson et al., 2005). The Marcella Lake core is an endogenic carbonate record at century scale resolution, spanning 4.5 ka (Anderson et al., 2005).

Spindly Pine Lake δD_{\text{precip}} record shows broad similarities to each of these records over the Holocene (Fig. 3.4.2). Spindly Pine and Mt. Logan records show increasingly depleted values from 10 to 7 ka before both increasing over the HTM but this enrichment over the HTM is not reflected in the Jellybean Lake record. However, all three records show more enriched δD_{\text{precip}} before 7 ka than after 5 ka. Values become more depleted in all three records between 6 and 4 ka. After 4 ka, values generally increase in all records including Marcella Lake until around 2 to 1 ka. After 1 ka, Mt. Logan and Jellybean Lake records trend towards more depleted values, however Spindly Pine and Marcella Lake records continue to be more enriched to present.

General agreement between the Spindly Pine δD_{\text{precip}} record, Jellybean lake and Mt. Logan records suggest that downcore δD_{\text{precip}} variability at Spindly Pine Lake broadly reflects the
regional isotopic composition of precipitation for this region (Fig. 3.4.2) and is likely driven by changes in atmospheric circulation. However, the Spindly Pine Lake record and the evaporatively-sensitive Marcella Lake and Seven Mile Lake records (not shown in Fig. 3.4.2 because it only spans 1 ka) are in good agreement over the last 1 ka and show a divergence with the evaporatively-insensitive Jellybean Lake and Mt. Logan records during this period. This suggests that the Spindly Pine record is evaporatively sensitive to some degree and provides some support for the conceptual model that n-acids deposited in Spindly Pine Lake accessed enriched lake water (outlined in section 3.4.2).

3.4.5 Discrepancies between isotope records

There are several discrepancies between the Mt. Logan, Jellybean, Marcella and Spindly Pine isotope records which are attributable to multiple factors including elevation differences, proxy-specific differences, and differing temporal resolutions. A discontinuity in the isotopic composition of precipitation was identified on Mt. Logan through snowpack studies (Holdsworth et al., 1991; Holdsworth and Krouse, 2002). These data suggested the presence of three atmospheric layers: (1) a planetary boundary layer (< 3 km), (2) a mixed layer (typically 1 to 2 km thick) characterized by fairly constant isotopic composition of water vapour regardless of altitude, and (3) a “quasi geostrophic flow” layer (> 5.3 km) with a separate fractionation sequence than the planetary boundary layer (Holdsworth and Krouse, 2002; Fisher et al., 2004; Anderson et al., 2016a). The vapour in the upper and lower layers have different sources and isotopic signatures, with the lower layer likely receiving more local moisture and the upper layers receiving more distant moisture (Holdsworth and Krouse, 2002; Fisher et al., 2004). Therefore, changes in the boundary layer height may result in changes in the isotopic composition of precipitation at different elevations, leading to discrepancies between the Mt. Logan (5240 m a.s.l.), Jellybean Lake (mean elevation of catchment area: 1650 m a.s.l., lake elevation: 730 m a.s.l.), Marcella Lake (697 m a.s.l.) and Spindly Pine (792 m a.s.l.) records (Anderson et al., 2016a). This complexity in high altitude regions emphasizes the need for more long term studies, such as the Rocky Mountain snowpack survey (Anderson et al., 2016b), measuring spatial and temporal heterogeneity of the isotopic composition of precipitation in these isoscapes.
Proxy-specific differences may also contribute to some of the discrepancies between the isotope records in southwestern Yukon. The Jellybean Lake and Marcella Lake δ^{18}O carbonate records reflect an average of the precipitation that falls within its catchment area throughout the year. The Spindly Pine δD_{precip} record reflects the local precipitation that is absorbed by plants during plant wax synthesis, which is also thought to reflect annual average precipitation. The Spindly Pine record may therefore be more influenced by years with anomalous amounts of precipitation in winter or early spring than the Jellybean Lake record which is smoothed by groundwater effects (Anderson et al., 2005). However, given the time integrated in each Spindly Pine sample it is unlikely that any of these anomalous events had a substantial effect on the record. The Mt. Logan ice core recorded both summer and winter precipitation; however, Rupper et al. (2004) found that only winters with high accumulation had a robust connection to atmospheric circulation. These differences may also contribute to the discrepancies between the records.

Changes in aridity over the Holocene may contribute to some of the discrepancies between records because the proxies have different sensitivities to evaporation. Lake water in Jellybean Lake is insensitive to evaporation due to the short residence time, and instead accurately reflects the isotopic composition of precipitation, which is, then recorded in the carbonate record, whereas, lake water in Marcella Lake is evaporatively enriched relative to MAP. The degree of evaporative enrichment at Marcella Lake (and similar closed basin lakes in the region) is dependent on aridity, therefore, when coupled with the Jellybean Lake record (as a control for the isotopic composition of precipitation), Marcella Lake serves as a proxy for aridity and moisture balance (Anderson et al., 2007). The Spindly Pine Lake δD_{wax} record is also likely also influenced by aridity due to plants accessing D-enriched lake water (as hypothesized in section 3.4.2), or transpiration leading to D-enrichment of leaf water (Feakins and Sessions, 2010). Plants around Spindly Pine Lake are likely absorbing soil water that is charged by a mixture of precipitation and D-enriched lake water, therefore, the aridity signal in Spindly Pine Lake is likely dampened relative to Marcella Lake. The sensitivity of different isotope proxies to seasonality of precipitation and aridity is a complicating factor in their interpretation (as Kauffman et al. pointed out in their 2016 review); however, it is also a strength of the proxies because, with appropriate constraints, multiple proxies used in conjunction have the potential to produce more nuanced pictures of hydroclimatic changes over the Holocene.
The differing temporal resolutions of the records also contribute to their apparent differences. For example, Mt. Logan and Jellybean Lake both record a shift to more depleted values at ~1850 AD (Fisher et al., 2004; Anderson et al., 2005, 2016a); though the resolution is not sufficient to observe such a feature in the Spindly Pine Lake record. However, the records show good coherence over low frequency features of Holocene.

3.5 Conclusions

The Spindly Pine record presented here is the fourth stable isotope record, and the first δD\text{wax} record, that extends through the full Holocene in Eastern Beringia (Kaufman et al., 2016). We interpret the downcore δD\text{wax} record in terms of δD\text{precip} after applying the ε\text{wax/precip} of -59 ± 2‰ determined from the lake core top (0-6 cm) sediments. The δD\text{precip} record preserved in the Spindly Pine Lake sediment core provides evidence of the isotopic composition of precipitation in southwestern Yukon spanning the entire Holocene.

Analysis of the plant wax n-alkanoic acid molecular distribution and isotopic composition in the surface lake sediments reveal that they differ from that of the surrounding soils. The lake sediments have a distribution suggestive of shrub and grass-dominated inputs (modal chain length C\text{26}). Whereas, distributions are shifted to shorter chain lengths (modal chain length is C\text{24}) in the surrounding soils suggesting mosses likely dominate the soil wax signal. Additionally, δD\text{wax} values were significantly more enriched in lake sediments than soils which may be due to plants around the margins of the lake accessing D-enriched lake water; however, additional field work is needed to test this theory. The contrasting dominance of plant inputs and differing δD\text{wax} values in this boreal system mean that not only regional ecology and climate, but also sedimentary environment should be considered when selecting an appropriate net fractionation value. The effect of depositional environment on plant wax sources and isotopic signatures has not been systematically explored in the literature; however, the findings of this study suggests that differing depositional environments can have a large impact on the characteristics and isotopic signature of plant waxes, and therefore is an important avenue for future research.

We find δD values decrease from ~10 to 7 ka before increasing to the mid Holocene (5-6 ka), these trends match carbonate and ice core records (Fisher et al., 2004; Anderson et al., 2005). δD values drop to a local minimum at 4.6 ka, before increasing from 4.2 to ~2 ka. This increase is
mirrored in the Mt. Logan, Jellybean Lake, and Marcella Lake records. However, from 2 ka to present Marcella Lake and Spindly Pine Lake records continue to increase, while the evaporatively-insensitive Mt. Logan and Jellybean Lake records decrease. The agreement between these different isotope proxy archives (ice, carbonate and wax) from southwestern Yukon provide strong evidence that they represent the regional isotopic composition of precipitation. However, while the Spindle Pine record primarily reflects broad regional trends in the isotopic composition of precipitation, it also appears to be sensitive to changes in aridity. Our results contribute a new plant wax record of precipitation isotopes spanning the Holocene in eastern Beringia and confirm the long term regional trends in the isotopic composition of precipitation.

3.6 Acknowledgements

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3.7 References


Environment Canada, 2018. Canadian Climate Normals 1981-2010 Station Data: Whitehorse,


58, 337.


Sachse, D., Dawson, T.E., Kahmen, A., 2015. Seasonal variation of leaf wax n-alkane production and δ2H values from the evergreen oak tree, Quercus agrifolia. Isotopes in Environmental and Health Studies 51, 124–142.


Sachse, D., Radke, J., Gleixner, G., 2006. dD values of individual n-alkanes from terrestrial plants along a climatic gradient – Implications for the sedimentary biomarker record. Organic Geochemistry 37, 469–483.


Chapter 4
Conclusions and Future Directions

4.1 Overview
The objective of this thesis was to develop a better understanding of plant wax characteristics and fractionation in high latitude boreal forests in the Holocene. In order to meet this broad objective, each chapter has its own secondary objectives. The second chapter characterized the n-acid chain length distributions of common boreal vegetation types and determined that mosses were the primary contributor to boreal soils, and quantified the regional net fractionation in boreal soils, finding an average net fractionation for n-C_{28} acids of $-96 \pm 11\%$ relative to mean annual precipitation. The third chapter presented a full Holocene δD record using n-alkanoic acids from plant waxes recovered from a sediment core from Spindly Pine Lake in southwestern Yukon and determined that there are large differences in lipid source and isotopic composition of n-alkanoic acids between boreal soils and lake sediments, therefore the most appropriate net fractionation value to reconstruct δD_{precip} for this lake sediment core record is $-59 \pm 2\%$ based on the core top sediments relative to mean annual precipitation. This record is in good agreement with existing stable isotope records in the region and therefore accurately records changes in regional δD_{precip}, which are likely driven by changes in atmospheric circulation (Anderson et al., 2016a).

4.2 Chapter 2 concluding remarks and future directions
This study analyzed n-acids from modern vegetation and soils from a network of 13 sampling sites in Yukon, Alaska and Northwest Territories to better constrain the hydrogen isotope net fractionation of n-acids deposited in northern boreal forest soils, an understudied ecosystem in modern plant wax calibrations. The overall net fractionation of soil δD_{wax} relative to δD_{MAP} ($\varepsilon_{wax/MAP}$) is $-93 \pm 10\%$, $-102 \pm 11\%$, and $-96 \pm 11\%$ for n-C_{24}, C_{26} and C_{28} acids, respectively. n-Acid distributions indicate that boreal soils are dominated by contributions from mosses.

The net fractionation values presented here are calibrated to high latitude boreal forests and should be applied with a high level of caution to different depositional environments or vegetation communities as it may not be appropriate. Uncertainties remain about the correct estimation of source water in this region and future systematic studies measuring the seasonal
progression of the isotopic composition of precipitation, down-profile soil water, leaf water and plant wax values would help constrain the appropriate value. Until such systematic studies are done, mean annual precipitation remains a good estimation of plant source water over the growing season. Future studies examining the $\delta D_{\text{wax}}$ values from vegetation samples from this region would be a valuable addition to global datasets, as well as greatly increase our understanding of high latitude fractionation and would help provide some constraints on the effect of changing vegetation patterns in paleo-wax records.

The net fractionation value constrained here is applicable for paleoclimate reconstructions based on plant wax $\delta D$ from high latitude boreal soils. There is an abundance of well-dated paleosols in Eastern Beringia, which preliminary studies indicate represent a boreal ecotype and have high organic content making them prime deposits to exploit for plant wax analysis (Elias, 2000; Matheus et al., 2003; Matthews et al., 2003; Zazula et al., 2003; Péwé et al., 2009; Schweger et al., 2011). This study is the first to systematically examine $n$-acid distributions in boreal soils and constrain a boreal net fractionation value and therefore provides a novel and valuable contribution to the plant wax and paleoclimate community.

### 4.3 Chapter 3 concluding remarks and future directions

The Spindly Pine record presented here is the fourth stable isotope record, and the first $\delta D_{\text{wax}}$ record, that extends through the full Holocene in Eastern Beringia (Kaufman et al., 2016). $\delta D_{\text{precip}}$ is reconstructed after applying the $\varepsilon_{\text{wax/precip}}$ of $-59 \pm 2\%$ determined from the lake core top (0-6 cm) sediments. The variability in $\delta D_{\text{precip}}$ was shown to be too large to represent a temperature signal alone. However, the record shows good coherence with other stable isotope proxies from the region suggesting the Spindly Pine record accurately records the regional isotopic composition of precipitation, which are driven by changes in atmospheric circulation. A limitation of this study is that due to its ~400-year resolution it is only able to capture long term trends in $\delta D_{\text{wax}}$ and is unable to capture sudden isotopic excursions such as the 4.2 ka or 8.2 ka events that are reflected in the Mt. Logan record and globally (Menounos et al., 2008). However, similarities between the Spindly Pine record and the Marcella Lake and Seven Mile Lake records over the last 1-2 ka suggest that the $\delta D_{\text{wax}}$ record at Spindly Pine lake may also be affected by changes in aridity.
The second key finding from this study is that, significant differences between the $n$-acid characteristics and $\delta D_{\text{wax}}$ values exist between soils sampled around Spindly Pine Lake and lake sediments. It appears the lake sediments are dominated by contributions from shrubs and grasses growing around the margins of the lake, and soils are dominated by contributions from mosses. This indicates that depositional environment as well as vegetation community and geography should be considered when choosing an appropriate net fractionation value for plant wax studies. The more enriched $\delta D_{\text{wax}}$ values seen in lake sediments than soils may be the result of increased shrub inputs (which generally have a smaller fractionation) or due to plants that contribute to the lake utilizing D-enriched lake water for biosynthesis of waxes.

An intriguing avenue for future research would be to test the hypothesis that plants are utilizing D-enriched lake water for biosynthesis of waxes. To do this, field studies measuring lake water $\delta D$ over summer to constrain the local evaporation line, down-profile soil water $\delta D$, and soil $\delta D_{\text{wax}}$ in a transect moving away from the lake shore to constrain the amount of infiltration of D-enriched water into the soils and determine if proximity to the lake impacts soil $\delta D_{\text{wax}}$ values. If the hypothesis is supported this would have important implications for plant wax studies near evaporatively-sensitive lakes.
Appendices

Appendix A: Vegetation Taxa

Vegetation samples collected from each site.
* The dominant moss was *Pleurozium schreberi*, but minor (co-mingled) fractions of *Tomentypnum nitens*, *Ptilium crista-castrensis* and *Dicranum polysetum* were also observed.

<table>
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<th>Site</th>
<th>Plant type</th>
<th>Common name</th>
<th>Order</th>
<th>Family</th>
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<th>Sample ID</th>
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<td>Pinaceae</td>
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Appendix B: Vegetation Concentration

Average n-acid concentrations by vegetation type and site.

\[ (C_{20} - C_{34}) = \frac{\sum C_s \times n}{\sum C_n} \]

\[ (C_{20} - C_{32}) = \frac{[(C_{20,22,24,26,28,30}) + (C_{22,24,26,30,32})]}{2 \times [C_{21,23,25,27,29,31})]} \]

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\(^a\) Average Chain Length  \( (C_{20} - C_{34}) = \frac{\sum C_s \times n}{\sum C_n} \)

\(^b\) Carbon Preference Index  \( (C_{20} - C_{32}) = \frac{[(C_{20,22,24,26,28,30}) + (C_{22,24,26,30,32})]}{2 \times [C_{21,23,25,27,29,31})]} \)
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Appendix C: n-acid Correlation

Correlations between site-averaged n-acid variables (soil and vegetation) and geographic/climate variables.

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<th>δDwax n-C24</th>
<th>δDwax n-C26</th>
<th>δDwax n-C28</th>
<th>εC24/MAP</th>
<th>εC26/MAP</th>
<th>εC28/MAP</th>
<th>ACL_soil</th>
<th>CPI_soil</th>
<th>ACL_grass</th>
<th>CPI_grass</th>
<th>ACL_moss</th>
<th>CPI_moss</th>
<th>ACL_shrub</th>
<th>CPI_shrub</th>
<th>ACL_tree</th>
<th>CPI_tree</th>
<th>ACL_forb</th>
<th>CPI_forb</th>
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<th>δDwax n-C28</th>
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<th>εC26/MAP</th>
<th>εC28/MAP</th>
<th>ACL_soil</th>
<th>CPI_soil</th>
<th>ACL_grass</th>
<th>CPI_grass</th>
<th>ACL_moss</th>
<th>CPI_moss</th>
<th>ACL_shrub</th>
<th>CPI_shrub</th>
<th>ACL_tree</th>
<th>CPI_tree</th>
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* correlation is significant at p < 0.05 (two-tailed)

* significance of correlations for forb n-acid variables is not assessed due to small sample size (N = 5)
Appendix D: Soils by Site

Soil n-acid hydrogen isotope composition, net fractionation, and molecular abundance, averaged by site (includes O and A horizons, 0-20 cm).

\(^a\) OIPC weighted annual and average May to September δD of precipitation (Bowen and Revenaugh, 2003)

\(^b\) Average Chain Length (C\(_{20}\)-C\(_{34}\)); arithmetic mean for all values from site

\(^c\) Carbon Preference Index (C\(_{20}\)-C\(_{32}\)); arithmetic mean for all values from site

\(^d\) \(wx\): amount-weighted average of \(n\)-C\(_{26}\) and \(C\(_{28}\) acids

### Appendix D-1: Soil n-acid hydrogen isotope composition and net fractionation

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<th>Site</th>
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<th>(\varepsilon_{\text{wax/MAP}}(%o))</th>
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<td>SH</td>
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<td>DHP174</td>
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## Appendix D-2: Soil $n$-acid concentrations

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$^a$ Concentrations in μmol/L.

$^b$ Σ acids = sum of all acids.
## Appendix E: Soil by Sample

Soil n-acid hydrogen isotope composition, net fractionation, and molecular abundance for individual soil samples.

<table>
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<tr>
<th>Site</th>
<th>Sample</th>
<th>δD&lt;sub&gt;wax&lt;/sub&gt; (%)</th>
<th>ε&lt;sub&gt;wax/MAP&lt;/sub&gt; (%)</th>
<th>Concentration (μg g&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>ACL (&lt;sup&gt;13&lt;/sup&gt;C&lt;sub&gt;29&lt;/sub&gt;-&lt;sup&gt;13&lt;/sup&gt;C&lt;sub&gt;31&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CPI (&lt;sup&gt;13&lt;/sup&gt;C&lt;sub&gt;29&lt;/sub&gt;-&lt;sup&gt;13&lt;/sup&gt;C&lt;sub&gt;33&lt;/sub&gt;)&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>-93 -92 -106</td>
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<td>-105 -108 -99</td>
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<td>Concentration (µg g&lt;sup&gt;-1&lt;/sup&gt;)</td>
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<td>-83 -107 -111</td>
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<td>-105 -100</td>
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<sup>a</sup> OIPC weighted annual and average May to September δD of precipitation (Bowen and Revenaugh, 2003)

<sup>b</sup> Average Chain Length  \( C_{20} - C_{34} = \frac{\sum C_n \times n}{\sum C_n} \)

<sup>c</sup> Carbon Preference Index  \( (C_{20} - C_{32}) = \frac{(C_{20,22,24,26,28,30} + C_{22,24,26,30,32})}{2 \times (C_{21,23,25,27,29,31})} \)
# Appendix F: Soil by Horizon

Soil n-acid hydrogen isotope composition, net fractionation, and molecular abundance, averaged by site and soil horizon (O and A).

<table>
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<tr>
<th>Site</th>
<th>Horizon</th>
<th>n</th>
<th>$^\delta D_{\text{wax}}$ (%)</th>
<th>$\varepsilon_{\text{wax/MAP}}$ (%)</th>
<th>Concentration (µg g$^{-1}$)</th>
<th>ACL (C$^{13}$C/C$^{12}$C)$^a$</th>
<th>CPI (C$^{13}$C/C$^{13}$C)$^b$</th>
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<td>$\varepsilon_{wax/MAP}$ (%)</td>
<td>Concentration (µg g⁻¹)</td>
<td>ACL (C&lt;sub&gt;20&lt;/sub&gt;-C&lt;sub&gt;34&lt;/sub&gt;)</td>
<td>CPI (C&lt;sub&gt;20&lt;/sub&gt;-C&lt;sub&gt;33&lt;/sub&gt;)</td>
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<sup>a</sup> OIPC weighted annual and average May to September δD of precipitation (Bowen and Revenaugh, 2003)

<sup>b</sup> Average Chain Length  
$$C_{20} - C_{34} = \frac{\sum C_n \times n}{\sum C_n}$$ 

<sup>c</sup> Carbon Preference Index  
$$C_{20} - C_{32} = \frac{([C_{20,22,24,26,28,30}] + [C_{22,24,26,30,32}])}{(2 \times [C_{21,23,25,27,29,31}])}$$
**Appendix G**: Spindly Pine Lake Photos

Photos of Spindly Pine Lake (Photo credit: Trevor Porter) showing large abundances (a) topography around lake (b) shrubs and grasses growing in abundance around the margins of the lake (c) shrubs and grasses rooted in the lake water, and (d) absence of mosses on the banks of the lake.
Appendix H: Statement of Coauthorship

I am the primary author of this thesis and any errors or omissions are my own. However, several authors were involved in the production of this thesis and their contributions to the research chapters are outlined below.

Chapter 2 was submitted to Organic Geochemistry on March 14, 2018 and is currently under minor revisions as of July 11, 2017 under the reference ID: OG-3751R1. The co-authors of this chapter are Trevor Porter (University of Toronto, ON, Canada), Duane Froese (University of Alberta, AB, Canada) and Sarah Feakins (University of Southern California, CA, USA). I conducted all primary research, laboratory analysis, and wrote the first draft. Trevor Porter contributed to research design, field data collection, methodology, and result interpretation. Duane Froese contributed samples. Sarah Feakins assisted with laboratory analysis. All of the co-authors revised the manuscript critically for important intellectual content, and two anonymous reviewers suggested revisions for the manuscript.