Seasonal and spatial variation in the chemical character of dissolved organic matter within a small boreal forest watershed.

Alexander W Newman

Supervisor: Dr. Susan Ziegler

A thesis presented to the Department of Earth Science and the School of Graduate Studies at Memorial University of Newfoundland in partial fulfillment of the requirements for the degree of Master of Science.

Department of Earth Science

Memorial University of Newfoundland

October 2018

Abstract:

Dissolved organic matter (DOM) is a significant carbon reservoir and component of the terrestrial-to-aquatic flux (Qualls et al., 1991). The terrestrial-to-aquatic carbon flux, a relatively new addition to global carbon models, is currently estimated to transfer a total of 1.7 petagrams (Pg) carbon (C) yr^{-1} globally (IPCC, 2013). Terrestrially derived DOM has been identified as a significant pool of organic matter in the aquatic environment. However, the quantity and chemical composition of DOM transferred, as well as the mechanisms driving its transfer, are less understood. This thesis focuses on expanding our knowledge of the processing DOM undergoes as it is transferred from terrestrial-to-aquatic environments by: 1) developing a standardized extraction methodology that can yield representative eluates when applied to sourced samples from throughout the terrestrial-to-aquatic interface and 2) applying the designed methodology to conduct a year long study of DOM quantity and composition in the terrestrial-to-aquatic interface in a boreal forest watershed. Experimental results suggest that although solid phase extraction with a divinyl benzene sorbent (SPE-PPL) yields high extraction efficiencies when applied to DOM, it is subject to selectivity. Extractions performed at high loading volumes were found to select against O-alkyl DOM hydrogen constituents, additionally all SPE-PPL experiments were found to select against nitrogenous DOM components. However, by considering proper extraction parameters, SPE-PPL can produce bulk representative eluates for nuclear magnetic resonance (NMR) analysis from land positions spanning the

terrestrial-to-aquatic interface. Results from the field study revealed that DOM transferred from terrestrial-to-aquatic land positions in a boreal forest watershed is both temporally and regionally variable, however, proximity immediately downstream of ponds appeared to be a major hydrologic control, while seasonal variation in hydrologic flow paths may represent another control in boreal forest watersheds. Dissolved organic matter chemical composition and quantity in traditional boreal forest streams related to shifts in the hydraulic flow path of the watershed, indicated by changes in riverine DOM chemical composition that correlated to seasonal wet and dry periods. Increases in both dissolved organic carbon (DOC) concentration and the presence of O-alkyl DOM hydrogen functionalities in the stream indicated a shift from groundwater sources during the dry period to soil water sources during the wet period. Conversely streams downslope of ponds seemed to be buffered against shifts in DOM chemical composition associated with changes in hydrologic flow paths. Dissolved organic matter chemical composition of streams downslope of ponds were relatively constant throughout the year resembling the characterization of pond outflows, even during periods of high hydraulic conductivity, via additions of autochthonous DOM produced in the pond. These additions of autochthonous DOM are negligible in streams not downslope of ponds. Further application of this approach during key periods of DOM export, such as spring snowmelt and fall rain periods may prove help to reveal the processes controlling the terrestrial-to-aquatic carbon flux in boreal forest landscapes.

ACKNOWLEDGMENTS:

I would like to first thank my supervisor, Dr. Susan Ziegler, for her patience, insight, guidance, and motivation these past three years. This research project represents an immense amount of work that would not have been possible without contributions from many different colleagues and friends. I would like to extend my sincere appreciation to Jamie Warren, Keri Bowering, Allison Meyers-Pigg, Matthew Northwood, Mackenzie Patrick, Dr. Michael Philbin, Dr. Celine Schneider, Dr. Penny Morrill, and all other colleagues and friends in the biogeochemistry laboratory group. I would also like to thank my colleagues at Canadian forest service: Dr. Kate Edwards, Darrell Harris and Andrea Skinner. Without the help of the Canadian forest service the many trips out Pynn's Brook Experimental Watershed would no doubt have been much less efficient and effective. I would also like to thank Michelle Miskell, Keir Hiscock, Diane Guzzwell, and the Department of Earth Science main office staff for the help and advice throughout this experience. Special thanks are also due to my friends and family that supported me throughout: Amanda Vansteensel, Robert Chaytor, Susan Wells, Dr. Gregory Newman.

Table of Contents

List of tables:

Chapter 1: Proposed parameters for solid phase extraction of low-concentration freshwater dissolved organic matter

Chapter 2: Spatial variation in the chemical composition of dissolved organic matter within the Pynn's Brook Experimental Watershed

List of Figures:

Chapter 1: Proposed parameters for solid phase extraction of low-concentration freshwater dissolved organic matter.

Co-authorship statement

The research questions and experimental procedures for this thesis were designed and outlined by me and my supervisor Dr. Susan Ziegler. The research proposal was reviewed by Dr. Penny Morrill, Memorial University of Newfoundland. All samples for both research chapters were collected by me with assistance from Jamie Warren, Dr. Susan Ziegler and the Canadian Forest Service in Corner Brook, Newfoundland. All sample analysis, except for nuclear magnetic resonance, were performed by me with assistance from Jamie Warren. Nuclear magnetic resonance analysis was performed by Jamie Warren under the guidance of Dr. Celine Schneider. Laboratory protocols were designed by me with assistance from Dr. Susan Ziegler, Jamie Warren, and Dr. Celine Schneider. Statistical analysis and data interpretation was completed by me with the assistance of Dr. Susan Ziegler. Two reviewers provided very helpful comments to this thesis. Each chapter was written by me and revised by my supervisor Dr. Susan Ziegler and Dr. Penny Morrill.

Thesis introduction and overview: dissolved organic matter dynamics in boreal headwater streams

I.1 The role of dissolved organic matter in the terrestrial-to-aquatic carbon flux

Dissolved organic matter (DOM), a colloidal suspension of molecules, plays an important role in the carbon balance of watersheds as it is highly mobile and the dominant form of total organic carbon (C) in aquatic ecosystems (Mattsson et al., 2005). Terrestrial DOM exported into aquatic C pools represents a poorly constrained flux that connects marine and terrestrial pools that are typically studied in isolation (Tranvik et al., 2009, Cole et al.,2007). The first estimates of the terrestrial-to-aquatic carbon flux, based on indirect estimates of freshwater sedimentation, burial and gas evasion, ranged from 0.37-0.41 Pg C y^{-1} and were largely equivalent to the estimates of the annual discharge of dissolved organic carbon (DOC), a quantitative measure of DOM, from the world's largest rivers (Schlesinger et al., 1981). Currently the International Panel on Climate Change (IPCC) reports the terrestrial-to-aquatic carbon flux to be 1.7 Pg C yr^{-1} (IPCC, 2013). The terrestrial-to-aquatic carbon flux will likely continue to grow as climate change amplifies the hydrologic cycle and threatens to export large quantities of carbon from terrestrial systems (Evans et al., 2005). Furthermore, DOM exported by this flux has associated temporal and spatial variability that directly effect its bioavailability, mobility and thus the magnitude of the flux (Freeman et al., 2004). Empirical measurements of the chemical character of DOM across the terrestrial-to-aquatic interface will help to reveal not

only the source of DOM exported, but also the processes that result in losses of DOM, and thereby controls associated with its transport (Tranvik et al., 2009).

I.2 Extraction methodologies suitable for dissolved organic matter

Empirical measurements of DOM across the terrestrial-to-aquatic interface requires a combination of both an isolation and analysis methodology. Dissolved organic matter occurs in low concentrations with a myriad of other reactive chemical species that interfere with subsequent chemical analysis (Li et al., 2016, Hertkorn et al,. 2007). Application of an isolation method both concentrates and purifies the analyte, providing an analyzable sample when applied to a DOM (Li et al., 2016). Multiple methodologies exist that can extract DOM from bulk water samples, however each method selects for certain fractions of DOM based on both the chemical and physical properties of the DOM applied, as well as the parameters under which the extraction was performed. Three popular extraction methodologies that have been applied to isolate DOM are: ultrafiltration (UF), reverse osmosis (RO) and solid phase extraction (SPE).

Ultrafiltration is a physical extraction methodology in which membranes with different pore sizes (usually about 0.01 microns) are used to isolate analytes of a certain size from the bulk sample. However, because UF separates the analyte by molecular size it is highly selective (Dittmar et al., 2008, Kaiser et al., 2003, Simjouw et al., 2005). Ultrafiltration isolates large, colloidal and polymeric molecules such as peptides, proteins and aliphatic/fatty acids (Kaiser et al. 2003, Benner et al., 2001). Lower molecular weight components such as dissolved

salts remain in solution. Ultrafiltration cannot effectively remove the matrix from a bulk DOM sample, which will result in much lower signal to noise ratios in subsequent analysis steps (Kaiser et al., 2003). Researchers who have used UF as a method to isolate DOM have also found that yields were low compared to other methods and highly variable (Simjouw et al., 2005). An experiment conducted on DOM in the Mississippi river found that UF extracted 49% of what was present in the bulk sample, and only 22% from samples sourced from the Gulf of Mexico (Benner et. all 2001). The low and variable yield suggests that a large fraction of DOM is unable to be extracted by UF and furthermore the amount of this DOM is regionally variable.

Reverse osmosis utilizes pressure to push a solvent, usually water, across a semi-permeable membrane. This membrane allows the solvent to pass through while larger particles are isolated. Reverse osmosis has been a useful and practical tool for scientists seeking to isolate freshwater DOM as it enables large volumes of water to be processed quickly and has yields as high as 80% (Gurtler et al. 2008, Perdue and Ritchie, 2003). Reverse osmosis utilizes smaller pore sizes than UF, usually ~0.0001 microns, to separate the matrix, or permeate, from the analyte, or retentate. However, due the small size of the pores, RO also extracts reactive aqueous salts from solution. Recently scientists have combined the process of RO and UF with electro-dialysis to purify the retentate. This new method successfully purges the sample of salts while maintaining high yields of DOM (Gurtler et al. 2008). However, studies have

found that molecule on molecule interactions occur after the sample has been processed (Maurice et al. 2002). This is unfavorable because these interactions change the chemical nature of the DOM from its natural state and impose selectivity on the retentate.

Unlike RO or UF, SPE is a chemical extraction methodology that utilizes a reactive solid phase sorbent to bind to analytes and extract them from solution. The analyte can then be extracted from the sorbent by a solvent, which is known as the eluate. Solid phase extraction has become a widely used method to extract DOM for analytical purposes due to high yields, and matrix-free eluates (Li et al., 2016, Minor et al., 2014). Despite SPE's popularity, many aspects of the method that could introduce selectivity into the process remain understudied. Different solid phases, or sorbents, are chemically tailored to extract certain analytes from a bulk sample, however, DOM refers to such a broad range of chemical species that no one sorbent can extract it exhaustively. Recent studies have attempted to quantify the yields of different commercially available sorbents on extractions of DOM. These sorbents included silica structures bonded with hydrocarbon chains: C18, C18EWP, C18OH, and C8, and divinyl benzene copolymers: PPL, and ENV. After testing, researchers found that PPL sorbents extracted on average 15% more DOM then other sorbents and recovered both polar and nonpolar DOM constituents (Dittmar et al. 2008). However, more recent studies have found that the PPL sorbents have poor recoveries of nitrogenous DOM species (Raeke et al., 2016). Although SPE sorbent dynamics

have been the subject of many recent studies, some aspects of the SPE method, such as flow rate and loading volume, have been hypothesized to introduce selectivity into the extraction (Li et al., 2016). Furthermore, the variety of DOM compounds present in DOM sampled from throughout the terrestrial-to-aquatic interface presents another unique challenge as these compounds may partition into the solid phase at different rates. To ensure extractions performed on different land positions are comparable, different amounts of sample must be extracted to achieve the optimum sample loading. An experiment to assess the recovery and selectivity of the SPE-PPL method over different flow rates, and loading volumes is needed to truly affirm that SPE-PPL is a suitable method to extract DOM from across the terrestrial-to-aquatic interface.

I.3 Analysis methodologies suitable for dissolved organic matter

Dissolved organic matter is an operationally defined categorization of carbon due to the spatial and temporal variation in the composition of DOM pools, and limitations in analytical techniques (Lin et al., 2015, Hertkorn et al., 2007). Recent advances in analytical methods have allowed for a more detailed characterization of DOM, however like isolation methodologies, each analytical method has associated selectivity and limitations. Two popular analytical methods that have been used for DOM characterization are: Fourier transform ion cyclotron resonance (FTICR) mass spectrometry, and nuclear magnetic resonance (NMR) (Hertkorn et al., 2013, Feng et al., 2011). Both methods potentially provide detailed insights into the chemical composition of DOM.

However, due to analytical costs and time restraints it is not always possible to apply both analytical methods. It becomes necessary to weigh the advantages and disadvantages of different analytical methods to find the one best suited to the goals the of the experiment. Fourier transform ion cyclotron resonance can yield high molecular mass resolution, but this method is unable to compile a complete chemical structure without being subject to extensive selectivity; this is particularly the important to consider when applying this method to complex mixtures such as DOM (Hertkorn et al., 2008, Stenson et al., 2003). Furthermore, Fourier transform ion cyclotron resonance relies on ionization techniques to analyze compounds, however, not all compounds in a sample are converted to ions.

Opposite of the highly resolved molecular view provided by FTICR, modern methods of NMR approach DOM characterization by analyzing the proportion of broad chemical functionalities making up a sample (Feng et al., 2011, Hertkorn et al., 2013, Kaiser et al., 2003). This presents a unique non-destructive view of DOM that other modern methods cannot offer. However, NMR has low specificity requiring large concentrations of the analyte to be present in a sample to obtain adequate data. This has been addressed by concentration and clean up steps such as SPE-PPL prior to NMR analysis (Minor et al., 2014, Hertkorn and Kettrup, 2005).

Nuclear magnetic resonance uses a magnetic field to manipulate nuclei's quantum properties. Nuclei have two quantum states known as the excited and

relaxed states. By generating electromagnetic waves at certain frequencies specific nuclei can be raised from their relaxed state to their excited state. Then, by either recording the energy used to excite the nuclei, or the energy released by the nuclei as it returns to its relaxed state, researchers can identify the nuclei and characterize the compound. (Richards et al., 2010). Modern NMR instruments use a method called pulsed Fourier transform (FT) to record the energy released by excited nuclei. The pulsed FT technique releases short pulses of multiple resonant frequencies. These pulses excite analytes simultaneously, but also, because they are low energy, allow for short relaxation times. This allows the NMR instrument to generate multiple spectra of the same sample in relatively short experimental times. The spectra are then averaged together to give a final spectrum (Richards et al., 2010). Researchers are able to obtain information in proton H-NMR by interpreting specific nuclei's chemical shifts, or the extent to which some nuclei absorb their resonant frequency (Richards et al., 2010).

Two other features of H-NMR spectrum that can be used in characterization are splitting and integration. Splitting occurs when protons magnetically interact with one another during NMR analysis. These interactions result in multiple chemical shifts (Sharma et al., 2000). Each of these multiple shifts, signified by peaks on the NMR spectrum, represents neighboring hydrogens according to the N-1 rule. The N-1 rule states that for N number of peaks you will have (N-1) number of adjacent protons. Integration, or the relative

area under the curves, also reveals structural information about the molecule being analyzed. This value correlates the number of protons present at each chemical shift. (Richards et al., 2010). Nuclear magnetic resonance analysis is a unique and powerful approach to characterize DOM.

A standardized isolation methodology must be designated before empirical databases that characterize the composition of DOM subjected to the terrestrial-to-aquatic flux can be compiled. To designate such a method, researchers must first test the selectivity, precision and accuracy of the isolation and subsequent analysis techniques used to characterize DOM from across the terrestrial-to-aquatic interface. This is the focus of the first chapter of this thesis, which is to identify a suitable combination of methodologies to isolate and analyze freshwater DOM from throughout the terrestrial-to-aquatic interface with minimal selectivity and high precision.

I.4 Dissolved organic matter dynamics in a boreal forest ecosystem

Newly discovered fluxes of carbon, such as the terrestrial-to-aquatic carbon flux, are already being amplified by climate change (Haei et al. 2013, Solomon et al., 2015). Even more troubling is the extent to which the mechanisms controlling this flux are understudied. To effectively collect empirical measures of DOM, the current understanding of its dynamics throughout the terrestrial-to-aquatic interface must be reviewed. To fully understand the spatial and temporal variability in DOM detected using the SPE-PPL H-NMR approach this section focuses on the chemical nature of DOM present in land positions

across the terrestrial-to-aquatic interface in a boreal forest watershed including: precipitation, streams, soil water and groundwater reservoirs.

Precipitation contributes a total of 0.43 Pg C yr^{-1} to the global flux of dissolved carbon, 80% of which is organic, while the other 20% is inorganic (Likens et al., 1983). Dissolved organic matter present in precipitation contains amino acids derived from bacterial activity, and the by-products of the incomplete combustion of fossil fuels and biomass (Fonselius, 1954, Likens et al., 1983). Many studies have found that DOM introduced via precipitation is quickly cycled due to its labile nature and thus does not contribute to the terrestrial-to-aquatic carbon flux (Qualls et al., 1992). Precipitation can fall uninterrupted to the ground, however more than often, precipitation is intercepted by vegetation, which is known as throughfall. Often throughfall will contain low molecular weight DOM molecules leached from vegetation (Thurman, 1985, Moore et al., 2003). This results in larger amounts of variability in the chemical nature and concentration of DOM in throughfall as opposed to precipitation. The ratio of enrichment of total organic carbon concentrations ranged from two to seven in an experiment that measured the difference between carbon concentrations in precipitation versus throughfall in Finland (Starr et al., 2004). Other studies found that precipitation in a boreal zone averaged 9 mg L^{-1} in an open plot, 14 mg L^{-1} in an aspen dominated plot and 19 mg L^{-1} in a pine dominated plots (Ukonmaanaho et al., 2002). Although precipitation is hypothesised not contribute directly to the carbon exported from watersheds, research has shown that large precipitation

events can mobilize new pools of carbon from soils to streams due to changes in the hydraulic flow path of the watershed (O'Donnell et al., 2010).

Soil is formally defined as a heterogeneous mixture of minerals, air, water and organic matter. Soil acts as a medium for plant, animal, and microbial life, as well as a reservoir for carbon, storing 200 Pg C globally (IPCC report 2013). Dissolved organic matter originating from soil pools contains a large variety of both labile and recalcitrant DOM constituents introduced via inputs from litter, microbial production, root exudates and inputs from precipitation (Qualls et al., 1992, Kaiser et al., 2003). Soil DOM is subject to convoluted seasonal controls that help to determine its composition (Marchner et al., 2002, Lützow et al., 2006). Variation in DOM composition within soil pools is well correlated with shifts in season. Oxygen functional groups, associated with carbohydrates and low weight compounds tend to be more prominent during winter and spring, while during the summer and autumn higher weight molecules dominate (Kaiser et. al 2002). Studies suggests that this is the case due to greater rates of mineralization in the winter, and higher rates of production during the spring (Kaiser et. al 2002, Kalibitz et al., 2000). It is vital that researchers understand the mechanisms driving this variation within soil reservoirs as these pools contain the largest concentrations of DOM of any of the land positions within the terrestrial-to-aquatic interface, and thus are extremely sensitive to climate change (Kalibitz et al., 2000). Soil DOM is transferred to stream systems via lateral flow where it is exported from the watershed, or percolates to groundwater reservoirs.

Soil water that percolates to groundwater pools is subjected to a unique combination of biotic and abiotic reactions that can significantly alter its characterization (Shen et al., 2015, Hedges et al., 1994).

Groundwater, or water that has percolated to water reservoirs in the mineral soil, is a vital component of the water budget of a watershed. As DOM from the surface percolates to groundwater reservoirs it undergoes several biotic and abiotic processes that allow it a slower turnover time and a more hydrophilic molecular nature (Hedges et al., 1994, Qualls et al., 1991, Shen et al., 2015). These processes include: microbial respiration, and sorption to mineral surfaces. The extent to which these mechanisms alter soil DOM characterization depends largely on the water residence time of the groundwater reservoirs; this process is conceptualized in the regional chromatography model (Shen et al., 2015, Hedges et al., 1994). Groundwater eventually drains into stream sites, where it has been found to greatly contribute to the DOM signature of stream water during dry periods (Cai et al., 2008, Walvoord and Striegl, 2007). However, some studies have found that groundwater dominates throughout the year. (Wallis et al., 1981). Conflicting findings on the role of groundwater DOM in streams suggest that contributions are regionally variable, and dependent on several variables such as the bioavailability of soil solution DOM, soil porosity, and hydraulic conductivity (Shen et al., 2015, Wallis et al., 1981). This demonstrates the importance of monitoring groundwater DOM composition temporally, especially during intense hydrologic periods.

Streams ultimately receive all DOM exported by terrestrial systems which has prompted many studies on the controls and variability of the DOM they export. Analysis of stream DOM chemistry has found that on average DOM is comprised of: 75% humic substances, 13% carbohydrates and 2% amino acids, with the remainder being too small to identify (Volk et al., 1997). However, this composition is both regionally and temporally variable. Researchers have found that controls on the composition and concentration of DOM exported from streams include: watershed hydrology, topography and climate. Studies on stream DOM dynamics have found that watershed hydrology plays a significant role in connecting aquatic systems to previously immobile terrestrial pools (Sanderman et. al 2009, Raymond et al., 2007). Large quantities of bioavailable DOM observed in streams after large hydraulic events is indicative of transfers from soil pools. However, during the dry period the composition of DOM in streams has been found to be comprised of more recalcitrant DOM constituents resembling contributions from groundwater (Neff et al., 2006, Raymond et al., 2007, Claire et al., 1996).

Studies on the controls of DOM composition in streams has found conflicting results on the importance of seasonal shifts in watershed hydrology and watershed topography. Regional watershed features such as lakes and ponds can alter the DOM composition in downslope stream outflows. These water bodies have higher water residence times than streams and have been found to increase the importance of biological controls and reduce the effect of

shifts in watershed hydrology on the composition of DOM exported from downslope streams (Kaste et al., 2003, Goodman et al., 2011). Longer residence times allows for microbial production to take place providing autochthonous DOM, which represents a much smaller source within streams where water residence time is much lower (Hedges and Oades 1997, del Giorgio and Peters 1993). The relationship that watershed hydrology and topography have on the export of stream DOM is convoluted with some studies reporting that hydrology plays a dominant role, while others designate topography as the dominant control. This gap in scientific knowledge highlights the importance of conducting field studies that utilize synoptic sampling throughout the year to truly understand the controls on DOM exported from streams within natural watersheds.

The second chapter of this thesis utilizes the designated isolation and analysis methodologies to both quantitatively and qualitatively catalog the annual variation of DOM's composition and quantity throughout the terrestrial-to-aquatic interface in Pynn's Brook Experimental Watershed Area (PBEWA) located in Western Newfoundland.

I.5 Research focus:

This thesis is focused on distinguishing suitable analysis and isolation methodologies capable of producing comparable results when applied across the terrestrial-to-aquatic interface. Additionally, these designated methods are applied to catalog the spatial and temporal variability in the DOM transported by

the terrestrial-to-aquatic carbon flux in a small boreal forest watershed. This research has resulted in two chapters entitled:

1) Determining best practices for the solid phase extraction of dissolved organic matter from the terrestrial-to-aquatic continuum

2) Annual spatial variation of dissolved organic matter chemical composition along Pynn's Brook Experimental Watershed

The first chapter of this thesis describes the suitability of SPE-PPL to produce comparable DOM eluates from across the terrestrial-to-aquatic interface. In this work, I looked at what parameters of SPE-PPL process could be optimized to provide the least selective and most complete analysis of each bulk DOM pool from along the terrestrial-to-aquatic interface.

The research addressed in the second chapter of this thesis catalogued both the spatial and temporal variability of DOM across the terrestrial-to-aquatic interface in a boreal forest watershed. I specifically investigated a) precipitation and throughfall's role in the terrestrial-to-aquatic carbon flux in boreal forest watersheds and b) the seasonal and regional drivers of variability in the export of DOM from boreal forest streams.

The research addressed in this thesis will contribute to the understanding of terrestrial carbon's role in aquatic systems in a boreal forest watershed and help to constrain the terrestrial-to-aquatic carbon flux in boreal areas. Results from this research are also relevant to areas around the world where climatic

change threatens to increase terrestrial carbon export and thus its role in aquatic systems.

I.6 References:

Benner R and Opsahl, S. (2001) Molecular indicators of the sources and transformation of dissolved organic matter in the Mississippi River Plume. Organic Geochemistry 32(4): 597–611.

Cai Y, Guo L and Douglas T A. (2008). Temporal variations in organic carbon species and fluxes from the Chena River, Alaska. Limnology and Oceanography 53(4): 1408–1419.

Claire T A, Sayer B G, Kramer J R and Eaton D R. (1996). Seasonal variation in the composition of aquatic organic matter in some Nova Scotian brown waters: a nuclear magnetic resonance approach. Hydrobiologia 317(2): 141-150.

Cole J J, Prairie Y T, Caraco N F, McDowell W H, Tranvik L J, Striegl R G and Melack, J. (2007). Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. Ecosystems 10(1): 172-185.

Del Giorgio P A and Peters R H. (1993). The influence of DOC on the bacteria-chlorophyll relationship in lakes. Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen, 25(1): 359-362.

Dittmar T, Koch B, Hertkorn N and Kattner G. (2008). A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. Limnology and Oceanography: Methods 6(6): 230-235.

Evans C D, Monteith D T and Cooper D M. (2005). Long-term increases in surface water dissolved organic carbon: Observations, possible causes and environmental impacts. Environmental Pollution. 137(1): 55-71.

Freeman C, Fenner N, Ostle J, Kang H, Dowrick J, Reynolds B, and Hudson J. (2004). Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels. *Nature*, *430*(6996), 195.

Fellman J B, Hood E, Edwards R T and D'Amore, D V. (2009). Changes in the concentration, biodegradability, and fluorescent properties of dissolved organic matter during stormflows in coastal temperate watersheds. Journal of Geophysical Research: Biogeosciences, 114(G1): G01021

Feng X and Simpson M J. (2011). Molecular-level methods for monitoring soil organic matter responses to global climate change. Journal of Environmental Monitoring. 13(5): 1246-1254.

Fonselius S. (1954). Amino acids in rainwater. Tellus. 6(1): 90-90.

Freeman C, Fenner N, Ostle J, Kang H, Dowrick J, Reynolds B, and Hudson J. (2004). Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels. *Nature*, *430*(6996), 195.

Goodman K J, Baker M A and Wurtsbaugh W A. (2011). Lakes as buffers of stream dissolved organic matter (DOM) variability: Temporal patterns of DOM characteristics in mountain stream-lake systems. Journal of Geophysical Research: Biogeosciences. 116(G4): G00N02

Gurtler B K, Vetter T A, Perdue E M, Ingall E, Koprivnjak J F and Pfromm P H. (2008). Combining reverse osmosis and pulsed electrical current electrodialysis for improved recovery of dissolved organic matter from seawater. Journal of Membrane Science. 323(2): 328-336.

Haei M, Öquist M G, Buffam I, Ågren A, Blomkvist P, Bishop K, and Laudon H. (2010). Cold winter soils enhance dissolved organic carbon concentrations in soil and stream water. Geophysical Research Letters. 37(8): L08501

Hedges J I and Oades J M. (1997). Comparative organic geochemistries of soils and marine sediments. Organic geochemistry. 27(7-8): 319-361.

Hedges J I, Cowie G L, Richey J E, Quay P D, Benner R, Strom M and Forsberg B R. (1994). Origins and processing of organic matter in the Amazon River as indicated by carbohydrates and amino acids. Limnology and oceanography. 39(4): 743-761.

Hernes P J and Benner R. (2006). Terrigenous organic matter sources and reactivity in the North Atlantic Ocean and a comparison to the Arctic and Pacific oceans. Marine Chemistry. 100(1-2): 66-79.

Hertkorn, N., & Kettrup, A. (2005). Molecular level structural analysis of natural organic matter and of humic substances by multinuclear and higher dimensional NMR spectroscopy. In: Use of Humic Substances to Remediate Polluted Environments: From Theory to Practice, Springer, Dordrecht, p 391-435.

Hertkorn N, Ruecker C, Meringer M, Gugisch R, Frommberger M, Perdue E M and Schmitt-Kopplin P. (2007). High-precision frequency measurements: indispensable tools at the core of the molecular-level analysis of complex systems. Analytical and bioanalytical chemistry. 389(5): 1311-1327.

Hertkorn N, Frommberger M, Witt M, Koch B P, Schmitt-Kopplin P and Perdue E M. (2008). Natural organic matter and the event horizon of mass spectrometry. Analytical chemistry. 80(23): 8908-8919.

Hertkorn, N., Harir, M., Koch, B. P., Michalke, B., Grill, P., & Schmitt-Kopplin, P. (2013). High field NMR spectroscopy and FTICR mass spectrometry: powerful discovery tools for the molecular level characterization of marine dissolved organic matter from the South Atlantic Ocean. Biogeosciences Discussions, 9(1): 745-833.

Hood E W, Williams M W and Caine N. (2003). Landscape controls on organic and inorganic nitrogen leaching across an alpine/subalpine ecotone, Green Lakes Valley, Colorado Front Range. Ecosystems. 6(1): 0031-0045.

IPCC (Intergovernmental Panel on Climate Change). (2013). Climate change 20013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change

Kaiser E, Simpson A J, Dria K J, Sulzberger B and Hatcher P G. (2003). Solid-state and multidimensional solution-state NMR of solid phase extracted and ultrafiltered riverine dissolved organic matter. Environmental science and technology. 37(13): 2929-2935.

Kaiser K, Guggenberger G, Haumaier L and Zech W. (2002). The composition of dissolved organic matter in forest soil solutions: changes induced by seasons and passage through the mineral soil. Organic Geochemistry. 33(3): 307-318.

Kalbitz K, Solinger S, Park J H, Michalzik B and Matzner E. (2000). Controls on the dynamics of dissolved organic matter in soils: a review. Soil science. 165(4): 277-304.

Kaste, Ø, Stoddard J L and Henriksen A. (2003). Implication of lake water residence time on the classification of Norwegian surface water sites into progressive stages of nitrogen saturation. Water, air, and soil pollution. 142(1-4): 409-424.

Kellerman A M, Dittmar T, Kothawala D N and Tranvik L J. (2014). Chemodiversity of dissolved organic matter in lakes driven by climate and hydrology. Nature communications, 5: 1-8.

Li Y, Harir M, Lucio M, Kanawati B, Smirnov K, Flerus R and Hertkorn N. (2016). Proposed guidelines for solid phase extraction of Suwannee River dissolved organic matter. Analytical chemistry. 88(13): 6680-6688.

Likens G E, Edgerton E S and Galloway J N. (1983). The composition and deposition of organic carbon in precipitation. Tellus. 35(1): 16-24

Lin V S. (2015). Research highlights: challenges in the characterization, storage, and isolation of natural organic matter. Environmental Science: Processes & Impacts. 17(12): 2002-2005.

Lützow M V, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner, B and Flessa H. (2006). Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions–a review. European Journal of Soil Science. 57(4): 426-445.

Mattsson T, Kortelainen P and Räike A. (2005). Export of DOM from boreal catchments: impacts of land use cover and climate. Biogeochemistry. 76(2): 373-394.

Maurice P A, Pullin M J, Cabaniss S E, Zhou Q, Namjesnik-Dejanovic K and Aiken G R. (2002). A comparison of surface water natural organic matter in raw filtered water samples, XAD, and reverse osmosis isolates. Water Research. 36(9): 2357-2371.

Minor E C, Swenson M M, Mattson B M and Oyler A R. (2014). Structural characterization of dissolved organic matter: a review of current techniques for isolation and analysis. Environmental Science: Processes & Impacts. 16(9): 2064-2079.

Moore T R. (2003). Dissolved organic carbon in a northern boreal landscape. Global Biogeochemical Cycles. 17(4).

Neff J C, Finlay J C, Zimov S A, Davydov S P, Carrasco J J, Schuur E A G and Davydova A I. (2006). Seasonal changes in the age and structure of dissolved organic carbon in Siberian rivers and streams. Geophysical Research Letters. 33(23): L23401.

O'Donnell J A, Aiken G R, Kane E S and Jones J B. (2010). Source water controls on the character and origin of dissolved organic matter in streams of the Yukon River basin, Alaska. Journal of Geophysical Research: Biogeosciences. 115(G3): G03025

Perdue E M and Ritchie J D. (2003). Dissolved organic matter in freshwaters. Treatise on geochemistry. 5: 605.

Qualls R G and Haines B L. (1992). Biodegradability of dissolved organic matter in forest throughfall, soil solution, and stream water. Soil Science Society of America Journal. 56(2): 578-586.

Raeke J, Lechtenfeld O J, Wagner M, Herzsprung P and Reemtsma T. (2016). Selectivity of solid phase extraction of freshwater dissolved organic matter and its effect on ultrahigh resolution mass spectra. Environmental Science: Processes & Impacts. 18(7): 918-927.

Raymond P A, McClelland J W, Holmes R M, Zhulidov A V, Mull K, Peterson B J and Gurtovaya T Y. (2007). Flux and age of dissolved organic carbon exported to the Arctic Ocean: A carbon isotopic study of the five largest arctic rivers. Global Biogeochemical Cycles. 21(4).

Richards S A and Hollerton, J C. (2010). Essential practical NMR for organic chemistry. John Wiley & Sons.

Sanderman J, Lohse K A, Baldock J A and Amundson R. (2009). Linking soils and streams: Sources and chemistry of dissolved organic matter in a small coastal watershed. Water Resources Research, 45(3): W03418.

Schlesinger W H and Melack J M. (1981). Transport of organic carbon in the world's rivers. Tellus. 33(2): 172-187.

Schumacher M, Christl I, Vogt R D, Barmettler K, Jacobsen C and Kretzschmar R. (2006). Chemical composition of aquatic dissolved organic matter in five boreal forest catchments sampled in spring and fall seasons. Biogeochemistry. 80(3): 263-275.

Sharma, B. K. (2000). Instrumental methods of chemical analysis. Krishna Prakashan Media.

Shen Y, Chapelle F H, Strom E W and Benner R. (2015). Origins and bioavailability of dissolved organic matter in groundwater. Biogeochemistry. 122(1): 61-78.

Simjouw J P, Minor E C and Mopper K. (2005). Isolation and characterization of estuarine dissolved organic matter: comparison of ultrafiltration and C18 solid-phase extraction techniques. Marine Chemistry. 96(3-4): 219-235.

Solomon C T, Jones S E, Weidel B C, Buffam I, Fork M L, Karlsson J and Saros J E. (2015). Ecosystem consequences of changing inputs of terrestrial dissolved organic matter to lakes: current knowledge and future challenges. Ecosystems. 18(3): 376-389.

Starr M and Ukonmaanaho L. (2004). Levels and characteristics of TOC in throughfall, forest floor leachate and soil solution in undisturbed boreal forest ecosystems. Water, Air and Soil Pollution: Focus. 4(2-3): 715-729.

Stenson A C, Marshall A G and Cooper W T. (2003). Exact masses and chemical formulas of individual Suwannee River fulvic acids from ultrahigh resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectra. Analytical Chemistry, 75(6): 1275-1284.

Tranvik L J, Downing J A, Cotner J B, Loiselle S A, Striegl R G, Ballatore T J and Kortelainen P L. (2009). Lakes and reservoirs as regulators of carbon cycling and climate. Limnology and Oceanography. 54(6 part 2): 2298-2314.

Ukonmaanaho L and Starr M. (2002). Major nutrients and acidity: budgets and trends at four remote boreal stands in Finland during the 1990s. Science of the Total Environment. 297(1-3): 21-41.

Volk C J, Volk C B and Kaplan L A. (1997). Chemical composition of biodegradable dissolved organic matter in stream water. Limnology and Oceanography. 42(1): 39-44.

Wallis P M, Hynes H B N and Telang S A. (1981). The importance of groundwater in the transportation of allochthonous dissolved organic matter to the streams draining a small mountain basin. Hydrobiologia. 79(1): 77-90.

Walvoord M A and Striegl R G. (2007). Increased groundwater to stream discharge from permafrost thawing in the Yukon River basin: Potential impacts on lateral export of carbon and nitrogen. Geophysical Research Letters, 34(12): L12402.

Chapter 1:

Determining best practices for the solid phase extraction of dissolved organic matter from the terrestrial-to-aquatic continuum

Keywords:

Dissolved organic matter (DOM), solid phase extraction (SPE), Nuclear magnetic resonance (NMR), terrestrial-to-aquatic interface

Abstract:

Solid phase extraction via a styrene-divinylbenzene copolymer sorbent (SPE-PPL) is a chemical isolation method that is commonly used to prepare dissolved organic matter (DOM) samples for solution-state nuclear magnetic resonance (NMR) analysis. Despite its growing popularity, certain parameters of the SPE-PPL method have been hypothesized to select against major constituents of DOM. This selectivity is troublesome as many researchers are seeking to characterize the chemical heterogeneity of DOM originating from different land positions along the terrestrial-to-aquatic interface. If the SPE-PPL isolation method minimizes these differences, scientific efforts to define DOM's spatial and temporal chemical heterogeneity using SPE-PPL could be compromised. This study investigates how the methodological parameters used in SPE-PPL may affect extraction yields and selectivity of DOM sourced from different land positions along the terrestrial-to-aquatic interface (stream, soil and groundwater). Quantitative analysis of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) was used to assess the relationship between SPE-PPL yields and flow rate, sample volume and sample type. Solution-state proton H-NMR was performed to investigate if and how chemical selectivity is observed with any changes in DOM yields associated with different SPE-PPL

parameters such as flow rate, sample volume and sample type. Average SPE-PPL DOC yields ranged from 50-80% of the original sample concentration, while DON yields ranged from: 15-40%. SPE-PPL yields and selectivity were independent of sample flow rates. However, higher sample loading volumes displaced O-alkyl hydrogen functionalities relative to aliphatic moieties. Due to the difference in chemical composition of DOM originating from across the terrestrial-to-aquatic interface, chemical selectivity in response to sample volume was dependent on the source of the DOM applied. Soil water was found to be the most likely to be subjected to this selectivity due to its higher relative O-alkyl content. High loading volumes, however, are not required for soil water samples, due to higher concentrations of DOM. Although groundwater samples required larger loading volumes to achieve sample masses required for analysis, selectivity was not observed in groundwater samples likely due to the larger contribution of aliphatic hydrogen moieties in groundwater DOM samples. By considering the proper parameters and the possible composition of DOM applied, SPE-PPL eluates were comparable across the land positions sampled in this study. However, if proper SPE-PPL parameters are not considered, the large range in sample volumes used isolate DOM via SPE-PPL required in the study of the terrestrial-to-aquatic interface can elicit chemical selectivity which must be assessed.

1.1 Introduction:

Dissolved organic matter (DOM) refers to a colloidal suspension of molecules distinguished from other organic matter categories by its size classification of <0.45 µm. Dissolved organic matter is ecologically important as it is highly mobile, acts as an energy and nutrient source to heterotrophic microbes and is the dominant form of total organic carbon (C) in aquatic ecosystems (Mattsson et al., 2005, Kalbitz et al., 2000). Terrestrial DOM inputs to aquatic systems, known as allochthonous inputs, make up a dominant proportion of natural organic matter (NOM) in lakes (Karlsson et al., 2012). Once in aquatic systems, terrestrial DOM is either stored or exported into marine or atmospheric C pools globally depending on both biotic and abiotic variables. This transfer represents a poorly constrained flux that connects marine, terrestrial and atmospheric C pools that are typically studied in isolation (Tranvik et al., 2009). The flux of terrestrial C to aquatic systems, now coined the "terrestrial-to-aquatic carbon flux", was first conceptualized in the 1980's as a passive transport system delivering terrestrially organic matter to the ocean via river transport and was termed the "riverine pipe". The first estimates of this flux ranged from 0.37- 0.41 petagrams (Pg) C yr^{-1} and were largely equivalent to the estimates of the annual discharge of DOC from the world's largest rivers (Schlesinger et al., 1981, Cole and Caraco 2001). The "riverine pipe" concept was called into question as measurements of respiration from the world's rivers started to exceed autochthonous gross primary production. Rivers were no longer considered to be

passive systems and it became clear that the terrestrial-to-aquatic carbon flux had to be better defined. In 2007 the Intergovernmental Panel on Climate Change (IPCC) included the terrestrial-to-aquatic carbon flux in the global C cycle for the first time where it was reported as 0.8 Pg C yr^{-1}. In 2013 the IPCC reported the terrestrial-to-aquatic carbon flux as 1.7 Pg C yr^{-1} based upon freshwater burial, sedimentation and degassing (IPCC 2007,2013). Efforts to constrain the global C budget continues with estimates of the terrestrial-to-aquatic carbon flux increasing but remain difficult to quantify since the flux is determined indirectly through poorly resolved freshwater burial and degassing fluxes worldwide. Assessing the chemical characterization of DOM across the terrestrial-to-aquatic interface can enable a better understanding of the source, transformations and ultimately fate of this critical C flux and its role in C-climate feedbacks (Tranvik et al., 2009, Jaffé et al. 2008).

The chemical characterization of DOM has been challenging due to its chemical complexity that prevents it from being fully characterized via traditional analysis techniques, which require simple and recognizable components to be generated prior to chemical analyses by common analytical methods such as gas chromatography-mass spectrometry and high-pressure liquid chromatography. The chemical complexity of DOM sourced from across the terrestrial-to-aquatic continuum is largely a result of the varied sources and transformations in the aquatic environment. Streams are considered one end member of the watershed receiving allochthonous DOM inputs from soil water and precipitation, however,

they also receive more altered and microbially-derived sources of DOM via groundwater (Qualls et al., 1992). Not only can each of these pools of DOM have relatively unique chemical compositions, but the relative proportions of DOM exported from each pool typically varies temporally in response to hydrology and landscape flow paths (Sanderman et al., 2009). Determining source of DOM contributing to and transported by freshwater systems will help constrain the terrestrial-to-aquatic flux by uncovering which C pools are more sensitive to climate change.

Analytical characterization of DOM has been approached in four ways: 1) characterization of individual compounds, 2) characterization of chemical classes through the identification of carbon, hydrogen, nitrogen or phosphorus types, 3) characterization by size classification, 4) characterization of acid/base soluble portions of DOM (Leenheer et al., 1981). The most appropriate analytical method is determined by the research goals of the experiment. Advances in non-destructive detection methods, such as NMR, have allowed for a holistic view of bulk DOM and established proportions of compound classes indicative of DOM source. One-dimensional NMR can be applied qualitatively or semi-quantitatively with proper sample handling, however, with the development of two-dimensional NMR entire networks of heteroatoms and their functional groups are revealed (Hedges et al., 2000, Buddrus et al., 1989). A suite of studies has established compound specific tables based on chemical shift areas that reveal greater detail on DOM moieties relative to previous approaches (Cook et al., 2004, Hertkorn et

al., 2013, Li et al., 2016, Soucémarianadin et al., 2017, Clemente et al., 2009). Despite advances in this analytical technique, NMR often remains a method with low analytical specificity when applied to DOM. This is because NMR requires a relatively pure analyte to achieve proper resolution. Therefore, to analyze the chemical character of DOM via NMR, DOM must be isolated and concentrated.

Multiple isolation methodologies have been employed to prepare DOM samples for NMR analysis. Extraction methodologies range from chemical separation techniques such as solid phase extraction (SPE), to physical separation methods such as ultrafiltration (UF) or reverse osmosis (RO). Reverse osmosis (RO) and UF are viable isolation methods, however, without introduction of additional clean up steps, matrix components are also concentrated. The presence of these matrix components during NMR analysis results in magnetic field homogeneity which ultimately makes the spectra uninterpretable (Kaiser et al., 2003, Simpson et al., 2003, Minor et al. 2014). Solid phase extraction is favorable as it isolates a fraction of the DOM from matrix components during wash steps carried out prior to eluting the DOM fraction in solvent (Figure 1.1) (Kim et al., 2003). Additionally, SPE eluates need only be dried down and reconstituted in a deuterated solvent before they are ready for liquid state NMR analysis. Improvements in sorbent characteristics has increased the range of DOM species that can be extracted from a bulk sample to include hydrophobic and polar compounds and has allowed the sorbent to be stable at lower pHs (Aiken et al.,1979). Certain analyte, matrix, sorbent

interactions that lead to selectivity during the extraction, however, still exist. Studies that investigated the selectivity and yields of available sorbents, including C18, C18EWP, C18OH, and C8, and PPL, found that PPL extracted on average 15% more DOM then other sorbents, and could extract both polar, nonpolar and aliphatic constituents of DOM (Dittmar et al., 2008, Perminova et al. 2014). Further investigations into the selectivity of these new sorbents has found that even the PPL sorbent has only an average recovery of 30% of nitrogenous species, much lower than the 70% average recovery of C (Raeke et al., 2016). A bulk DOM sample contains many nitrogenous species, which suggests that SPE-PPL extraction eluates may be not representative of bulk DOM.

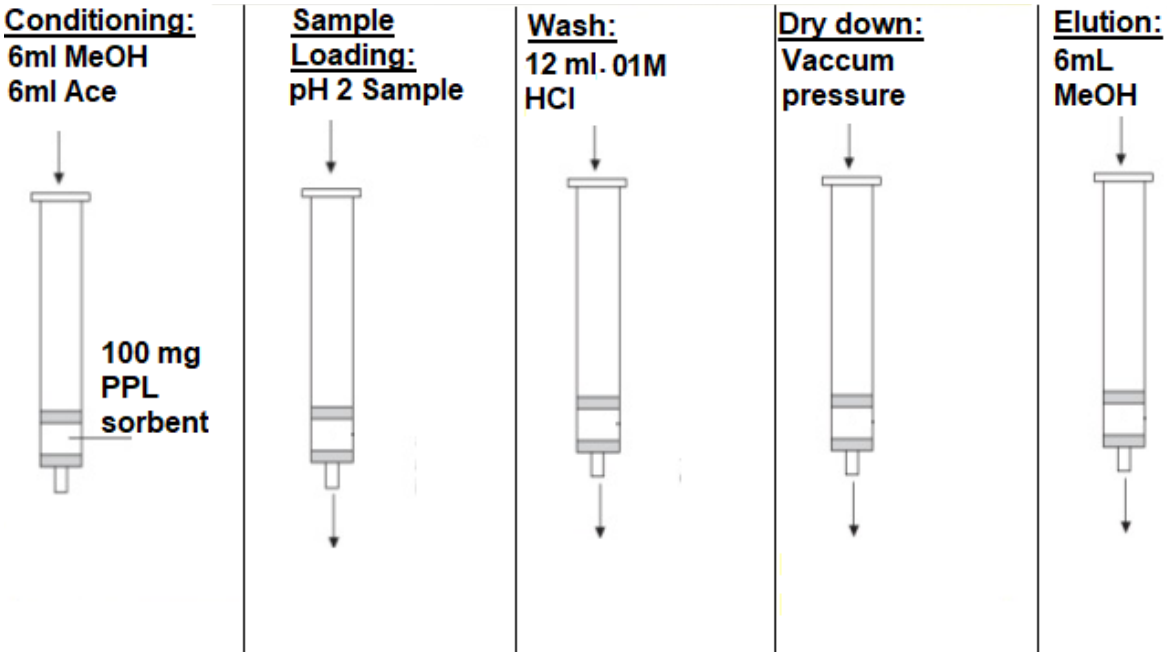
The yield of DOM from any SPE procedure is not solely based on sorbent selection, proper sample handling and extraction parameters are required to achieve maximum extraction efficiency. A more recent study has investigated parameters such as loading volume, flow rate relative to SPE-PPL yields. This study found that flow rate had a minimal effect on the recovery of SPE-PPL eluate yields from natural waters, however, SPE-PPL performed at high loading volumes selected against carboxylic-rich aromatic molecules (CRAM), an important constituent of DOM (Li et al., 2016). In this study loading volume and flow rates applied were on the low end (1.25-125 mL, and 0.5-5 mL min^{-1}), this is concerning as often DOM from natural sources requires a higher loading volume and faster flow rate than what was applied here.

Regional differences in DOM concentration and characterization, as well as differences in the composition of the matrix between bulk samples may lead to unexpected selectivity and may require different extraction parameters to achieve optimum yields. This selectivity is troublesome when investigating DOM from across the terrestrial-to-aquatic continuum where both quality and quantity of DOM varies and may require specialized SPE isolation procedures to achieve an analyzable sample. Carboxylic-rich aromatic molecules (CRAM) is distributed throughout the terrestrial-to-aquatic interface. However, these compounds are progressively removed as soil water percolates down to groundwater reservoirs by both biotic and abiotic processes (Hedges et al., 1994). As a result, soil water DOM samples contain a higher proportion of CRAM relative to groundwater DOM. The implications of this selectivity suggest that SPE-PPL eluates may misrepresent DOM character across the terrestrial-to-aquatic interface if proper extraction parameters are not considered. Therefore, SPE performance must be optimized for the range of sample types encountered across this continuum.

The objective of this study was to determine the optimal SPE parameters required to obtain bulk representative eluates with comparable recovery of C and N from samples representative of the terrestrial-to-aquatic continuum in a boreal forest watershed. Here, sample loading volume and the rate of sample introduction during the SPE isolation process using the PPL sorbent were investigated on DOM acquired from stream water, groundwater and soil water samples. Extraction yields of DOC and DON were investigated quantitively,

additionally molecular characterization of DOM and selectivity associated with each parameter and with source was investigated qualitatively via solution state H-NMR. By assessing selectivity and the yields of the SPE-PPL method when applied to samples collected from across the terrestrial-to-aquatic interface, the extraction parameters produced comparable DOM eluates for solution state H-NMR analysis in a boreal forest watershed.

Figure 1.1. Solid phase extraction (SPE) protocol applied to prepare samples for structural analysis of freshwater dissolved organic matter (DOM) via solution state hydrogen nuclear magnetic resonance (H-NMR). A 100mg PPL column used in each case.



1.2 Methods:

1.2.1 Site descriptions:

Experiments were conducted on samples collected from two sites on the island of Newfoundland, which lies in the boreal forest biome. Samples were collected from sites in both eastern, and western Newfoundland. Both areas are within the boreal forest, with eastern sites dominated by *Abies balsamea* and sites in the west dominated by *Picea mariana* and both underlain by podzolic soils. The mean daily temperature of the eastern and western regions is 5 and 4˚C respectively (Environment Canada normals 1980-2010, eastern St. John's A and western Deer Lake weather stations). Three sample types were collected to be representative of the terrestrial-to-aquatic continuum in boreal watersheds; (1) water samples taken from passive pan lysimeters installed under the organic horizon of forest soils (soil water), (2) water samples taken from a forested headwater stream (stream water), and (3) groundwater collected from a natural seep (groundwater) (Table 1.1).

Table 1.1: Location, description of site, dissolved organic carbon (DOC) (mg C L^{-1}), total dissolved nitrogen (TDN) (mg N L^{-1}), and dissolved inorganic and organic nitrogen (DIN/DON) (mg N L^{-1}) are reported. All numerical parameters are reported as the mean ± one standard deviation where n=3.

Location:	Site/sample description:	DOC:	TDN:	DIN:	DON:
Soil water	Passive pan lysimeters sampled from black spruce forest plots	40.0 (±1.2)	0.60 (±0.20)	0.2 (±0.01)	0.4 (±0.2)
Concentrated Stream water	First order clear-water stream entering Long Pond in eastern Newfoundland	17.4 (±0.5)	1.70 (±0.4)	0.3 (±0.01)	1.4 (±0.40)
Concentrated Ground water	Groundwater spring sampled in eastern Newfoundland	0.20 (±0.02)	-	-	-

1.2.2 Sample collection:

Soil water: Samples were collected from passive pan lysimeters installed under the organic horizon (approximately 8.17 cm in depth) within the forest stand of the Pynn's Brook experimental forest, Newfoundland. A battery-operated pump was used to empty a 25 L high-density polyethylene (HDPE) carboy buried further downslope and plumbed to the lysimeters (Ziegler et al., 2016, Bowering et al., 2017). Twenty liters of sample was pooled from two lysimeters within the same forested plot.

Stream water: On the 16th of June 2016 100 L of stream water was collected from a clear-water stream just upslope of Long Pond on Rennie's River in St. John's, Newfoundland. The stream sample was collected into acid washed HDPE carboys and transported immediately back to the laboratory where it was stored at 4˚C.

Groundwater: 450 L of groundwater was collected from a seep located on Pitt's Memorial highway in St. John's eastern Newfoundland during June 2017 using acid-washed HDPE carboys. Collection of the groundwater sample took place over the course of a week, in intervals of 100-liters. The sample was immediately transported to the laboratory and stored at 4˚C for one evening until further processing.

1.2.3 Sample preparation prior to solid phase extraction:

Samples collected were prepared in accordance to the requirements of testing the variation in DOC recovery due to two parameters that can be varied in the SPE method; (1) sample loading volume and (2) flow rate of sample application (Table 1.2).

All samples, except those requiring pre-concentration, underwent filtration via pre-combusted GF/F Whatman Filters (6 h, 500 C°) immediately after sample collection. Saturated mercuric chloride ($HgCl_2$) solution was then added to the filtered samples at a ratio of 10 µl $HgCl_2$:10 mL sample to halt any biological degradation of DOM during the dark 4˚C storage prior to SPE. Fifteen mL subsamples were taken for initial DOC and total dissolved nitrogen (TDN) analysis after $HgCl_2$ was added to sample. Subsamples were collected in individual 24 mL glass vials (Fisher Scientific, NH). Remaining bulk samples were then either directly used for SPE, diluted or concentrated depending upon the sample type and experimental treatment (Figure 1.2).

Samples with low DOC concentration, such as groundwater and stream water, were concentrated via RO prior to SPE to enable similar loading volumes to be tested on sample types with vastly different concentrations of DOC. For example, the soil water and the groundwater sample would normally require an average loading volume of 0.5 and 10 L respectively to achieve the mass of C required for NMR analysis. Concentration was required to perform SPE on the

stream and groundwater samples at the same volumes as the soil water samples. A Realsoft RO system (Atlanta, GA, USA) was set up with two membranes each with an 800 Dalton cut-off (Kent, WA, USA). Prior to RO, the sample was pumped through an inline 0.1 µm polycarbonate cartridge filter (Kent, WA, USA). After concentration, samples were filtered through pre-combusted (6 h, 500C°) Whatman GF/F filter (Kent, WA, USA), fixed with $HgCl_2$, subsampled again for DOC/TDN to determine concentration factor, and stored in the dark at 4˚C until extraction (Figure 1.2).

To conduct loading volume experiments, the soil water and RO concentrated stream and groundwater samples were diluted to the volumes to be tested by adding Nano-UV water immediately before SPE. The purpose of this dilution was to test whether SPE would yield similar extraction recoveries from samples with equal masses of DOC but different loading volumes (Table 1.2).

Blanks were run in triplicate with all experiments except the flow rate experiment conducted on soil water. Blanks were generated from Nano-UV water just prior to each experiment and were run through the SPE-PPL process.

Figure 1.2: Flowchart depicting sample treatment prior to solid phase extraction (SPE). Once sampled, samples were processed differently depending on experiment parameters. The three methods of sample preparation were: direct analysis, pre-concentration, and dilution.

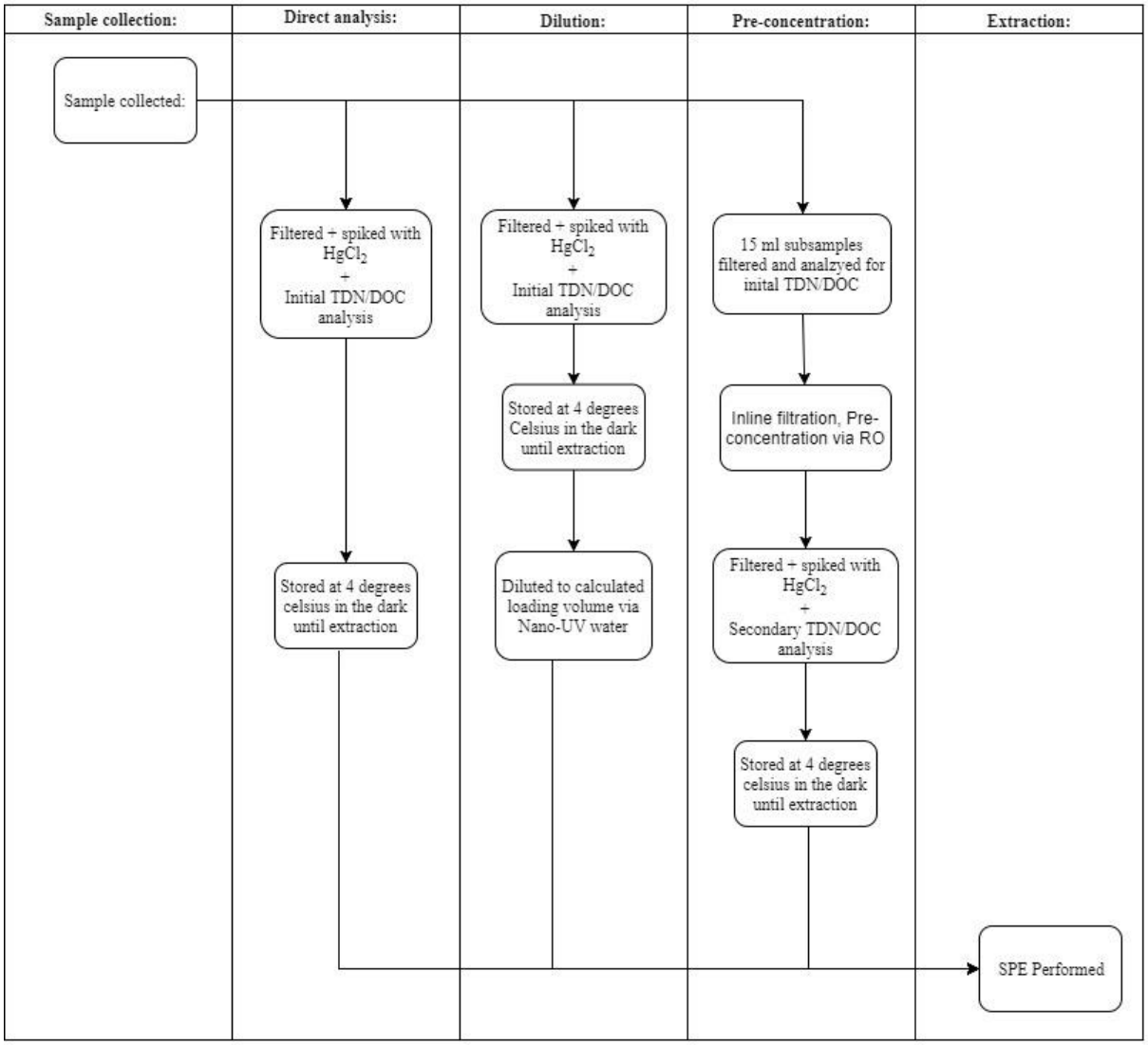


Table 1.2: Solid phase extraction parameters for each experiment. Loading volumes, flow rates, sample applied, and loading mass of carbon and nitrogen are reported. Five flow rates were tested with two different sample types. Four loading volumes were tested with three different sample types

Experiment type:	Flow rate (mL min^{-1}):	Loading Volume (L):	Carbon applied (mg):	Nitrogen applied (mg):	Sample:
Flow rate	10-50	0.5	18	0.6	Soil water
	20-50	1.0	13	1.7	Stream water[a]
Loading Volume	30	0.5-10	18	0.6	Soil water
	30	1.15-10	20	1.7	Stream water[a]

1.2.4 Solid phase extraction:

All SPE experiments were carried out with Agilent Varian Bond Elute 100-mg PPL cartridges (Santa Clara, CA, USA) using the manufacturer recommended loading limit of 24 mg of C. Experiments were designed to be below this 24 mg C limit but large enough to reflect C quantities required for solution state H-NMR analysis. Prior to SPE all samples, including blanks, were acidified to a pH of 2 using stock solution of hydrochloric acid (32% ACS grade HCl; Sigma Aldrich) to increase recovery of organic acids and phenols (Dittmar et al., 2008). Cartridges were rinsed with a stock solution of acetone (99.5% HPLC Millipore) and then methanol (Millipore HPLC 99.9%) to ensure complete removal of any remaining C applied to the column from previous extractions, and to prime the cartridges for sample loading. Extractions were performed in triplicate according to Dittmar et al., 2008. Extractions were then adjusted to test for the effects of sample volume applied and flow rate on SPE recovery and repeated. Flow rates were monitored via stopwatch and measuring against a graduated syringe. Initially a 15 mL reservoir attached to the SPE cartridge was manually refilled, however following the first flow rate experiment, the SPE process was automated. Sample was drawn into the SPE cartridge via 1/8" silicon tubing via head pressure from a 500 mL HDPE bottle, which was manually refilled. After loading, cartridges were washed with two cartridge volumes (~12 mL) of 0.01 M HCl to remove any matrix components bonded to the solid phase. Cartridges were then dried for 15 minutes by vacuum pressure and eluted with 6 mL of

methanol into individual 24 mL glass vials. 500 µL subsamples for DOC, TDN, dissolved inorganic nitrogen and dissolved organic nitrogen (DIN/DON) analysis were taken from sample eluates to determine SPE recovery. The parameters used in each SPE experiments are given in Table 1.2.

Sample eluates were then dried at 20˚C using a Pierce Reacti-vap (Dallas, Texas, USA) for 10 minutes, sealed with polytetrafluoroethylene-lined caps and stored in the dark until analysis.

1.2.5 Carbon and nitrogen measurements:

Samples were analyzed for total C and N using a Shimadzu TOC-V high temperature combustion total carbon analyzer (Shimadzu, Japan) at Memorial University of Newfoundland. Detection limit for DOC was 0.07 mg L^{-1} with a coefficient of variation of 1.1% of the 5 mg L^{-1} check standards. Detection limits for TDN was 0.001 mg L^{-1}, with a coefficient of variation of 1.8% of 0.1 mg L^{-1} check standards. Carbon and nitrogen analysis were performed on both the original samples and extracts to calculate the percent recovery of the SPE method.

To calculate the percent recovery of either carbon or nitrogen in the total elution rather than the 500 µL subsample that was analyzed, the following formula was employed:

$$\%\text{C recovery} = \frac{(DOC_{(measured)}\left(\frac{mg}{L}\right) * 0.015L)}{C_{(applied\ to\ column)}(mg)} * 100\%$$

Where $DOC_{measured}$ was the concentration of DOC in mg L^{-1} measured in the SPE eluate, and where $C_{(applied\ to\ column)}$ was the mass of DOC in mg applied to the column. The eluate was re-dissolved in 15 mL of Millipore water prior to chemical analysis.

Analysis of DIN was completed through the individual colorimetric analysis of dissolved ammonium and nitrate using a QuikChem 8500 Series 2 FIA System (Hach, Colorado, USA). Dissolved inorganic nitrogen measurements were subtracted from TDN measurements to calculate DON. Nitrate, and ammonia were both processed using a colorimetric method discussed in Pritzlaff, 2003 to determine quantity via absorbance measurements. Detection limits for the ammonia method was 0.003 mg L^{-1} with a coefficient of variation of 7.21% of the 0.2 mg L^{-1} check standards. Detection limits for the nitrate method was 0.007 mg L^{-1} with a coefficient of variation of 8.26% of the 0.2 mg/L check standards. Total DIN was subtracted from TDN to calculate DON.

1.2.6. Solution state hydrogen nuclear magnetic resonance analysis:

Solution state NMR data was acquired using Bruker AVANCE 500 spectrometer with a 5mm TXI 1H/D-13C/0154 probe, NMR 64 scans were carried out with a 3 second delay time. Peak analysis, and integration areas were obtained using MRestnova software (Bajo, Spain). Assignments of compound classes was based upon Clemente et al. (2009).

To test the precision of the processing of NMR spectra, the NMR results of two samples were each processed five separate times. The standard deviation and coefficient of variation of relative abundance of compound classes among these five interpretations provided an assessment of the error associated with the processing used to identify the relative proportion of each compound class (Table 1.3). Analytical precision was determined by analyzing one sample three times and determining the standard deviation for each of the integral areas. The average analytical precision was 0.4% (Table 1.3).

Table 1.3: Chemical shift regions (ppm), hydrogen functionality, average contribution from each functional group to total percent hydrogen (%), standard deviation (%) and coefficient of variation (%), for compound classes relating to dissolved organic matter (DOM) analyzed via solution state hydrogen nuclear magnetic resonance (H-NMR) analysis. Integration areas were compiled from studies that utilized the same deuterated solvent (Clemente et al. 2012). Precision and relative standard deviation for analysis of solution state H-NMR spectra are reported for chemical shift regions (ppm) based on solution state H-NMR where n=5 (Clemente et al. 2012).

Chemical Shift region:	Hydrogen functionality:	Percent contribution:	Standard Deviation:	Coefficient of variation:
8.60 - 7.80	amides from peptides	3.7	0.4	9.8
7.80 - 6.20	aromatic from lignin and proteins	11.3	0.2	1.6
4.80 - 4.00	Peptides	15.2	0.2	1.6
4.00 - 2.90	O alkyl mainly from carbohydrates and lignin	36.4	0.2	0.6
2.90 - 1.30	aliphatic methyl and methylene near O and N	22.7	0.6	2.8
1.30 - 0.60	aliphatic methyl and methylene	10.7	0.3	3

1.2.7 Statistical analysis:

A one-way analysis of variance (ANOVA) was applied to assess effect of flow rate and loading volume on the recovery of C and N of SPE-PPL eluates within samples tested as described in Table 1.2. Assumptions required to conduct the ANOVA test include: equal population variations across groups, adherence of the response variable to a normal distribution, and each sample in an experiment must be independent and random. Shapiro tests were conducted to test for normality of residuals, while Leven's Test was performed to test the distribution of error variances (O`Brien et al., 1979). Lag plots were constructed in R studio for each experiment to ensure samples were independent and random. Tukey honesty post-hoc tests were performed to investigate significance differences between means for experiments whose data passed the necessary assumptions. For experiments whose data did not pass the necessary assumptions, non-parametric statistical tests were performed using the Kruskal-Wallace test, followed by post-hoc Nemenyi's test to determine significant differences between the means. The results of all statistical tests performed are present in Table A.1.

1.3 Results:

1.3.1 Initial sample characteristics:

Samples were measured for DOC, DIN and TDN prior to any processing to determine amount of C and N that was to be applied to the SPE-PPL column. Quantitative assessments of DON and DOC demonstrated the vast speciation of DOM between different land positions present in the terrestrial-to-aquatic interface (Table 1.1).

Solid phase extraction performance under varying flow rates was investigated in two series of experiments conducted on soil and stream water DOM. Soil and stream water samples were used to assess any differences in SPE flow rate performance when applying sample with dissimilar DOM composition from across the terrestrial-to-aquatic interface. 18 and 13 mg of C was applied to the SPE column during experiments on soil water, and stream water respectively. Both series of experiments were performed with equal loading volume (1-liter) but the rate at which sample was applied varied; 10-50 mL min^{-1} in the case of the soil water and 20-50 mL min^{-1} in the case of the stream water mL min^{-1} (Table 1.2).

The effect of flow rate on DON recovery during SPE-PPL extractions of soil water and stream water was also investigated (Table 1.2). 0.6-mg and 1.7-mg of TDN were applied to PPL columns in the soil water and stream water experiments, respectively. 31 percent of the total nitrogen applied during the soil

water flow rate experiment was inorganic, and 69 percent was organic. 21 percent of the applied nitrogen in the pre-concentrated stream water experiments was inorganic, and 79 percent was organic.

Solid phase extraction loading volume performance was also investigated in three series of experiments conducted using soil, stream and groundwater DOM samples (Table 1.2). The relationship between sample loading volumes and percent DOC recovered was examined using equal masses of carbon (13.5 - 20 mg C depending upon sample) applied to 100mg PPL cartridges using an extraction flow rate of 30 mL min^{-1} across a range of loading volumes (0.5 - 10 liters) (Table 1.2). Due to the low concentration of DOC in stream water (3.4 mg L^{-1}) and groundwater samples (0.19 mg C L^{-1}), concentration via RO was necessary prior to SPE to achieve similar loading volumes. Concentrated stream water samples had a DOM concentration of 17.4 mg C L^{-1} while concentrated groundwater sample had a DOM concentration of 9 mg C L^{-1} resulting in 20 and 13.5 mg C applied to 100 mg PPL cartridges, respectively (Table 1.2).

The effect of loading volume on DON recovery for SPE performed across the terrestrial-to-aquatic interface was also investigated. Soil water applied contained 0.6 mg TDN, 31 percent of which was inorganic, while 69 percent was organic. Pre-concentrated stream water contained 1.7 mg of TDN. 17 percent of the total nitrogen applied was inorganic, the remaining 83 percent was organic. 0.8 mg of total nitrogen was applied during groundwater loading volume

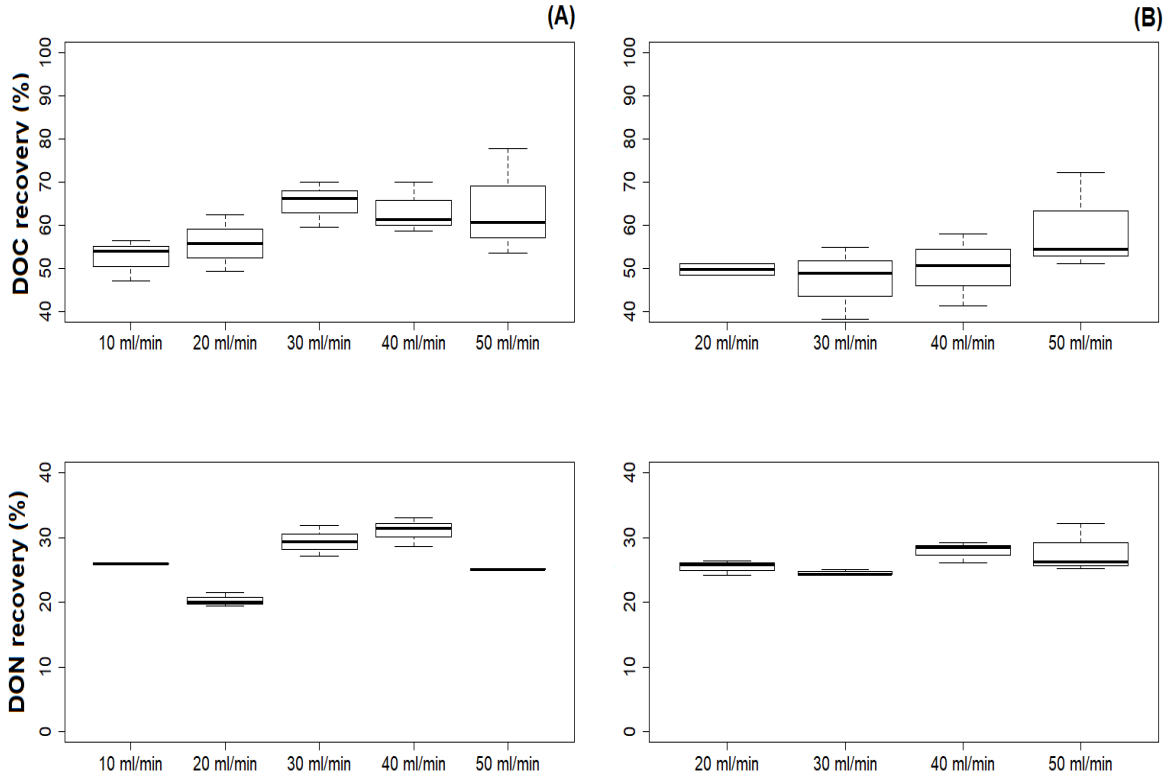
experiments. 20 percent of this total nitrogen was inorganic, while 80 percent was organic (Table 1.2).

1.3.2 Effects of flow rate of solid phase extraction eluate yields:

Statistical investigation revealed that no significant relationship between DOC recovery of extractions performed on soil water and flow rate existed ($P > 0.27$, Kruskal Wallace; Figure 1.3a). This was also the case in experiments conducted on stream water ($P > 0.4$, Kruskal Wallace; Figure 1.3b). Dissolved organic carbon recoveries ranged from 50 to 80 percent and from 50 to 70 percent for the experiments conducted on soil water and stream water respectively (Figure 1.3a).

Like DOC recovery, statistical investigation revealed no significant relationship between DON recoveries and SPE flow rates for extractions performed on both soil water ($P > 0.14$, Kruskal Wallace; Figure 1.3a) and stream water ($P > 0.15$, Kruskal Wallace; Figure 1.3b). Maximum DON recoveries were achieved at 40 mL min^{-1} for both experiments, after which recoveries decreased slightly. Dissolved organic nitrogen recoveries ranged from 33 to 43 percent for soil water experiments and 28 to 35 percent for stream water.

Figure 1.3: Solid phase extraction (SPE) carbon and nitrogen yields reported as % recovery versus flow rate applied. **A)** Percent dissolved organic carbon (DOC) recovery for soil water flow rate experiment. One liter with 18 mg DOC applied. Percent nitrogen recovery for soil water flow rate experiment. One liter with 0.6 mg total dissolved nitrogen (TDN) applied. **B)** Percent DOC recovery for stream water flow rate experiment. Half liter with 13 mg DOC applied. Percent nitrogen recovery for stream water flow rate experiment.



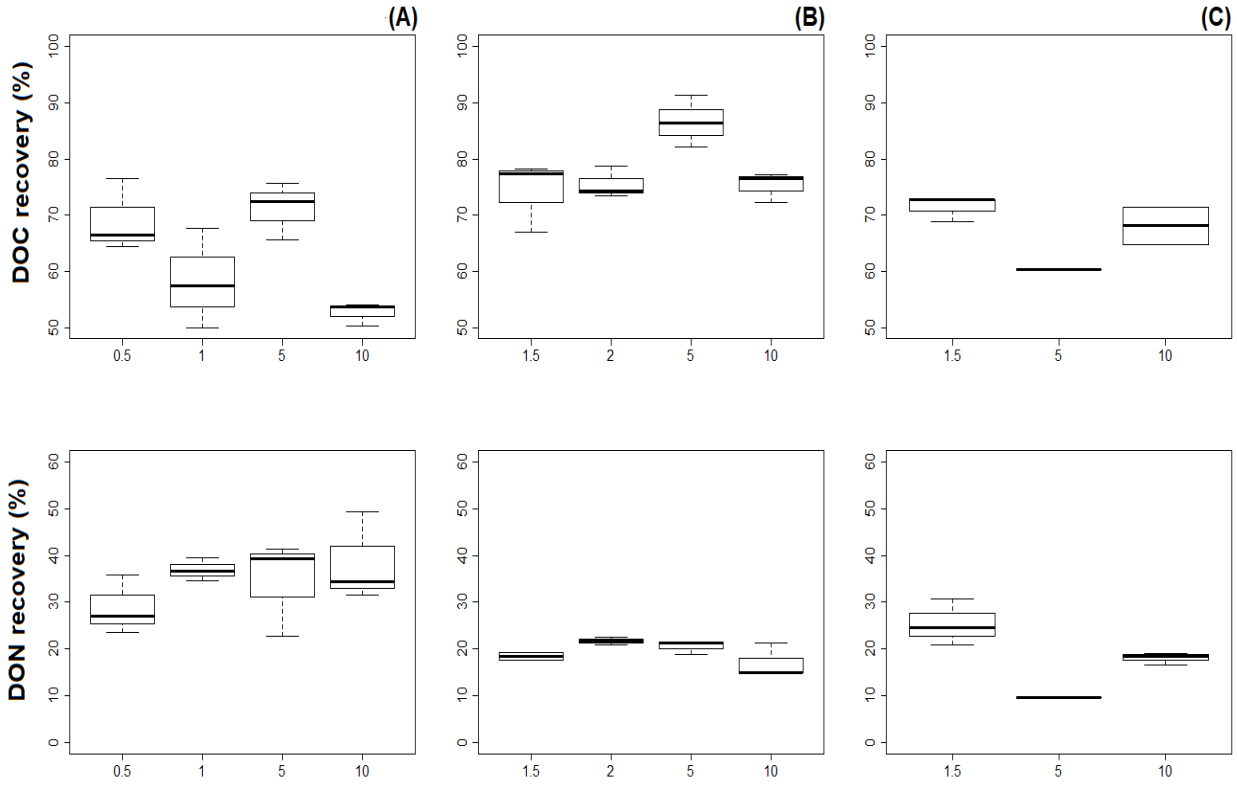
1.3.3. Effect of loading volume on solid phase extraction eluate yields:

Dissolved organic carbon recoveries were found to decrease significantly with increasing loading volume in experiments conducted on soil water. Soil water experiments had a minimum of 55 percent recovery of carbon and a maximum of 84 percent. The large difference in DOC recoveries between 1 liter and 10 liters extractions was revealed to be significant upon statistical investigation ($P > 0.02$ ANOVA; Figure 1.4a). Experiments performed on stream water had similar recoveries, ranging from 60 to 82 percent of carbon applied. However, no significant differences were observed ($P > 0.17$, Kruskal Wallace; Figure 1.4b). Similar ranges in recoveries were also observed in loading volume experiments performed on groundwater. Recoveries ranged from 55 to 80 percent of total carbon applied. Extractions performed at 5 liters loading volumes had a significantly lower DOC yield than extractions performed at 1.5 liters but not 10 liters ($P > 0.004$, ANOVA; Figure 1.4c).

Percent DON recovered from extractions of soil water exhibited an inverse relationship with loading volume in the soil water experiment (Figure 1.4a). Statistical investigation of this trend revealed that loading volumes of 10 liters had significantly lower DON recovery than extractions completed at lower loading volumes (0.5, 2, and 5L) ($P > 0.03$, Kruskal-Wallace; Figure 1.4a). Percent of DON recovered from pre-concentrated stream water had a similar range as soil water recovering 10 to 22 percent, however unlike the soil water experiment, the

relationship between loading volume and DON recovery exhibited was not significant ($P > 0.51$, ANOVA, Figure 1.4b). Extractions of pre-concentrated groundwater also exhibited similar DON recoveries yielding 10 to 29 percent recovery, however extractions completed at 5 liters had significantly lower DON recovery than extractions completed at either 1.5 or 10 liters ($P > 0.0007$, Kruskal-Wallace; Figure 1.4c).

Figure 1.4: Solid phase extraction (SPE) carbon and nitrogen yields reported as percent recovery versus loading volume applied. **A)** Percent carbon recovery for soil water loading volume experiment completed at flow rates of 30 mL min^{-1} with 18 mg dissolved organic carbon (DOC) applied. Percent nitrogen recovery for soil water loading volume experiment completed at flow rates of 30 mL min^{-1} with 0.6 mg total dissolved nitrogen (TDN) applied. **B)** Percent carbon recovery for stream water loading volume experiment completed at flow rates of 30 mL min^{-1} with 20 mg DOC applied. Percent nitrogen recovery for stream water loading volume experiment completed at flow rates of 30 mL min^{-1} with 1.7 mg TDN applied. **C)** Percent carbon recovery for groundwater loading volume experiment at flow rates of 30 mL min^{-1} with 13.6 mg DOC applied. Percent nitrogen recovery for groundwater loading volume experiment completed at flow rates of 30 mL min^{-1} with 0.8 mg TDN applied.



1.3.4 Nuclear magnetic resonance analysis:

SPE-PPL eluates selected for NMR analysis were end members of the tested parameters and had the largest differences in their DOC recoveries. It was hypothesized that end members of the treatments (i.e. 50 mL min^{-1} vs 10 mL min^{-1}) with large differences in recoveries of DOC would reveal selectivity specifically associated with the tested parameter. Six SPE-PPL eluates were selected for solution state H-NMR analysis: two samples from the flow rate experiment testing stream water, two from the loading volume experiment testing soil water, and two from the loading volume experiment testing groundwater (Table 1.4). Stream water samples selected from the flow rate experiment had a difference of 30% in DOC recoveries, while the soil water and groundwater samples selected from the loading volume experiments had a difference of 22, and 7% respectively.

Solution state H-NMR analysis of eluates selected from the experiment testing flow rate revealed little differences in the relative contribution of hydrogen moieties to total percent hydrogen across the applied flow rates. The largest contribution to total % hydrogen came from O-alkyl functionalities at 2.9 to 4.1 ppm which occupied 36% of the 50 mL min^{-1} treatment and 37% of the 20 mL min^{-1} treatment. Despite a difference of 30% in DOC recovery (77% versus 47%), there was little difference observed within any of the six hydrogen compound classes in either flow rate treatment (Figure 1.5).

Solution H-NMR analysis of the pair of samples selected from the soil water loading volume experiment revealed that the one-liter (77% DOC recovery) extraction had a higher proportion of O-alkyl (41%), aromatic (12%) and amide (3%) hydrogen functionalities compared to the ten-liter (55% DOC recovery) extractions which had 24, 7 and 2% respectively (Figure 1.6). The ten-liter sample had a higher proportion of alkyl functionalities (57%) compared to the one-liter extractions (32%) (Figure 1.6). In contrast to the soil water experiment, a similar proportion of all compound classes were observed for both loading volume treatments of the groundwater experiment. The largest contribution to the one-and-a-half-liter extraction came from alkyl functionalities (73%), similarly alkyl functionalities also dominated total % hydrogen of the ten-liter extraction (68%) (Figure 1.7). Although there was no repeated analysis of the samples, assessment of the analytical precision of the instruments allowed us confidence in our analysis (Table 1.3).

Table 1.4: Samples selected from across terrestrial-to-aquatic interface for solution state hydrogen nuclear magnetic resonance (H-NMR) analysis based on quantitative differences in dissolved organic carbon (DOC) concentration (mg L^{-1}). Stream water samples selected had a difference of thirty percent in DOC recoveries. Soil water and groundwater samples selected from the loading volume experiments had a difference of twenty-two, and seven percent respectively.

Experiment/Sample:	Sample replicate:
Flow rate (Stream water)	50 mL min^{-1}
	20 mL min^{-1}
Loading Volume (Soil water)	5-liter
	1-liter
Loading volume (Groundwater)	1.5-liter
	10-liter

Figure 1.5: Contribution to percent total hydrogen of varied compound classes as derived by solution-state hydrogen nuclear magnetic resonance (H-NMR) spectra (500 MHz, dimethyl sulfoxide (DMSO)) of solid phase extractions (SPE) of stream water during flow rate experiments. Classification of broad compound classes was based on chemical shift regions: 0.3–1.3 ppm: aliphatic methyl and methylene; 1.3–2.9 ppm: aliphatic methyl and methylene near oxygen and nitrogen; 2.9– 4.1 ppm: O-alkyl, mainly from carbohydrates and lignin; 4.1–4.8 ppm: α^1H from proteins; 6.2–7.8 ppm: aromatic, from lignin and proteins; 7.8–8.4 ppm: amide from proteins.

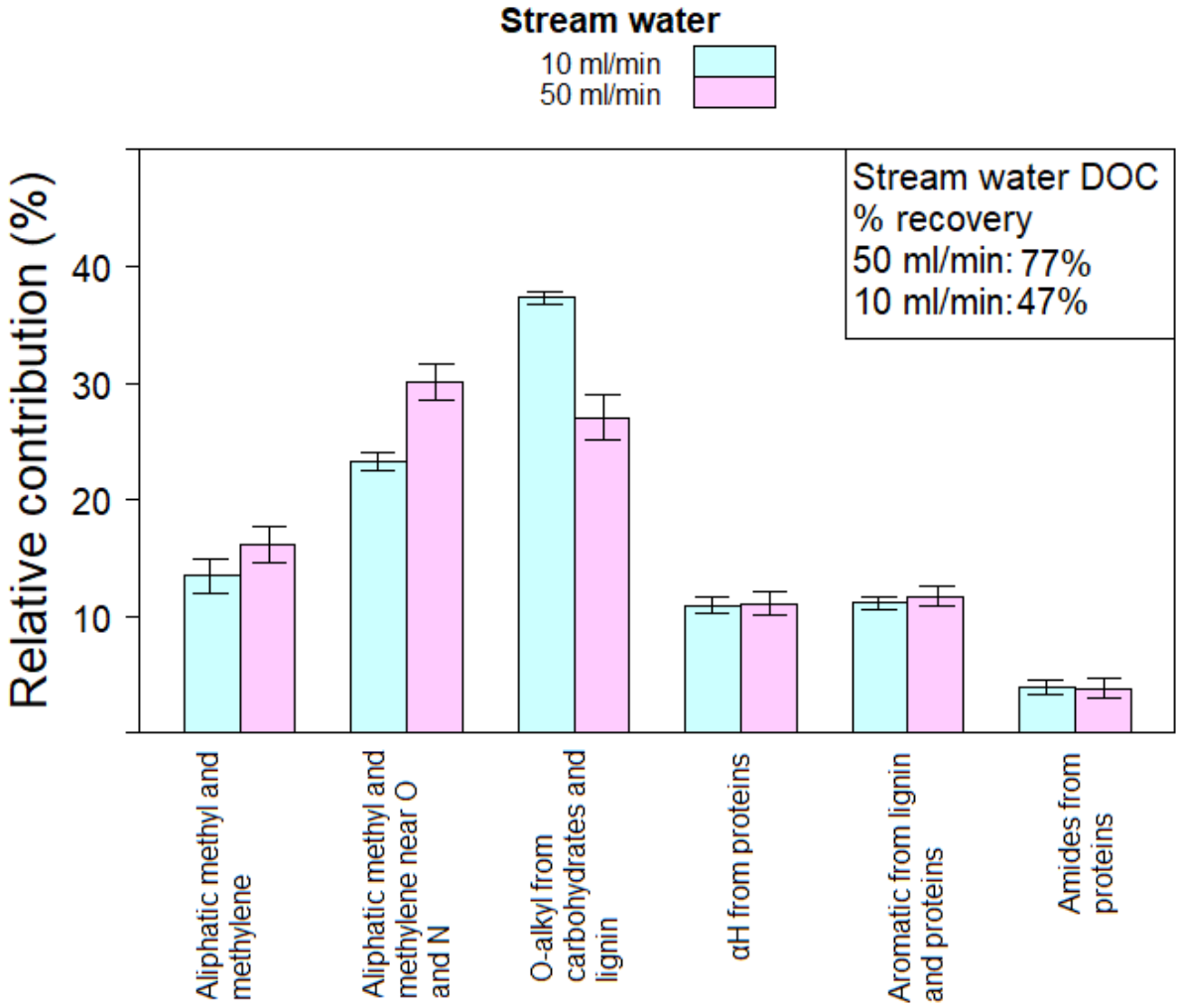


Figure 1.6: Contribution to percent total hydrogen of varied compound classes as derived by solution-state hydrogen nuclear magnetic resonance (H-NMR) spectra (500 MHz, dimethyl sulfoxide (DMSO)) of solid phase extractions (SPE) of soil water during loading volume experiments. Classification of broad compound classes was based on chemical shift regions: 0.3–1.3 ppm: aliphatic methyl and methylene; 1.3–2.9 ppm: aliphatic methyl and methylene near O and N; 2.9– 4.1 ppm: O-alkyl, mainly from carbohydrates and lignin; 4.1–4.8 ppm: α^1H from proteins; 6.2–7.8 ppm: aromatic, from lignin and proteins; 7.8–8.4 ppm: amide from proteins.

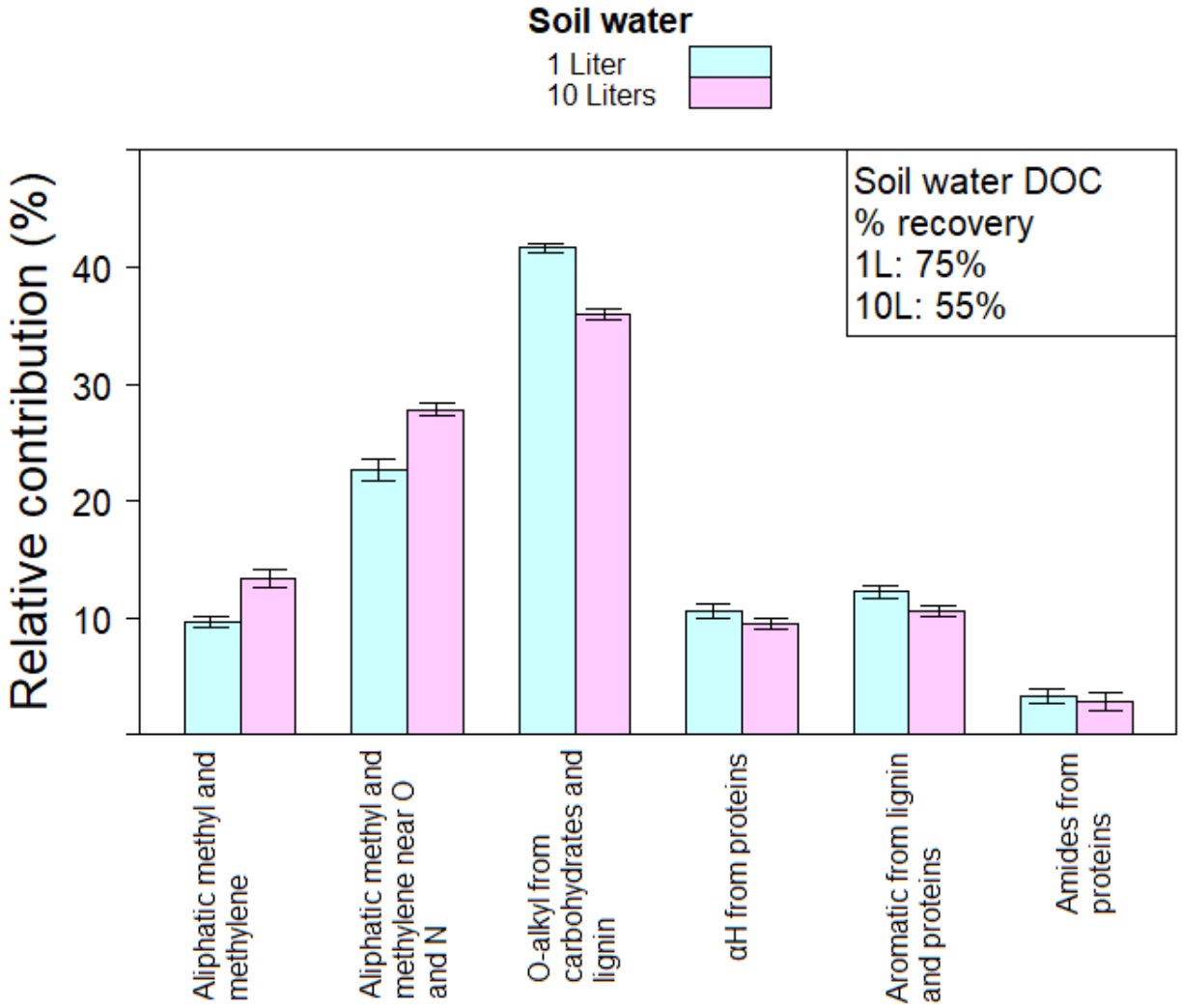
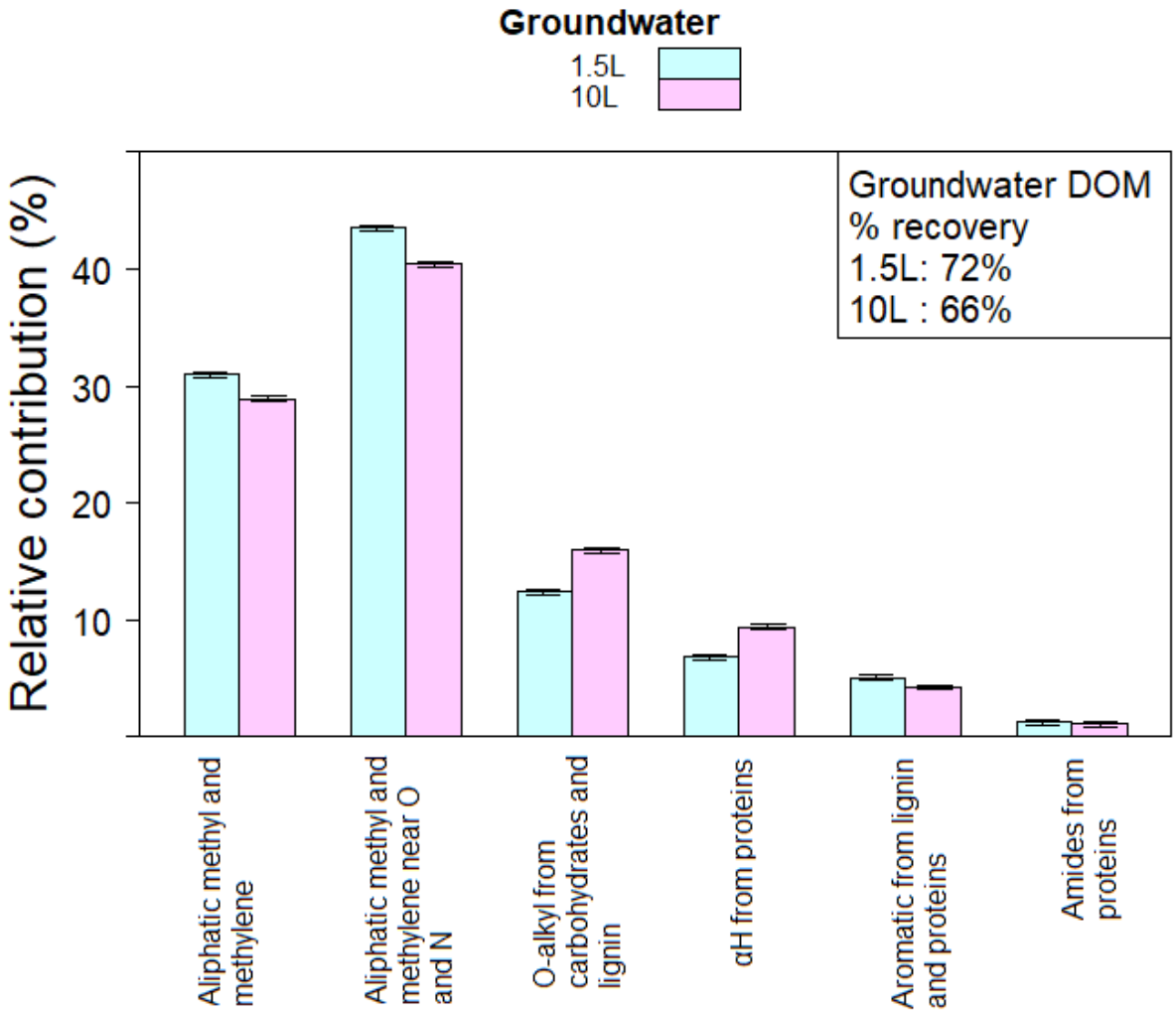


Figure 1.7: Contribution to percent total hydrogen of varied compound classes as derived by solution-state Hydrogen nuclear magnetic resonance (H-NMR) spectra (500 MHz, dimethyl sulfoxide (DMSO)) of solid phase extractions (SPE) of groundwater during loading volume experiments. Classification of broad compound classes was based on chemical shift regions: 0.3–1.3 ppm: aliphatic methyl and methylene; 1.3–2.9 ppm: aliphatic methyl and methylene near O and N; 2.9– 4.1 ppm: O-alkyl, mainly from carbohydrates and lignin; 4.1–4.8 ppm: α^1H from proteins; 6.2–7.8 ppm: aromatic, from lignin and proteins; 7.8–8.4 ppm: amide from proteins.



1.4 Discussion:

1.4.1 Flow rate effects on yields solid phase extraction eluates:

Although studies that have examined flow rates have found no significant differences in SPE-PPL DOC yields between slower rates (0.5 - 5 mL min^{-1}), it has been hypothesized that faster flow rates (i.e. > 5 mL min^{-1}) could reduce recovery of extractions due to insufficient time for the analyte to bind to the solid phase (Li et al., 2016). Freshwater sources often contain low concentrations of Dissolved organic matter. If researchers were restricted to slow flow rates, it would feasibly take days to extract enough DOC to achieve an analyzable sample. In this investigation of flow rate, I similarly found no significant quantitative difference in DOC or DON recoveries across tested flow rates (10 mL min^{-1} – 50 mL min^{-1}), furthermore, experiments testing both soil and stream water had similar yields of DOC and DON. These findings suggest that high flow rates (50 mL min^{-1}) can be employed to practically prepare DOM samples for analysis via SPE-PPL. This investigation into flow rate was limited to soil and stream water samples, however, qualitative analysis gives us reason to believe that SPE-PPL recovery of DOC and DON is independent of flow rate even in the case of groundwater.

When the effect of flow rate on DOM yields during SPE-PPL extraction was examined qualitatively, via solution state H-NMR, selectivity was not observed. Spectra produced were like other published H-NMR spectra of stream water with major contributions from alkyl and O-alkyl hydrogen functionalities (Zhang et al.,

2007, Kaiser et al., 2003). Our analysis is confined to soil and stream water sampled, however, the chemical characterization of DOM inherent to groundwater reservoirs makes it likely chemical selectivity will not be observed in extractions of groundwater performed with high flow rates. Groundwater DOM has a larger contribution of aliphatic functionalities that strongly bind to the PPL solid phase, while more weakly binding O-alkyl functionalities only occupy 10 to 20% of total hydrogen, thus it is unlikely that selectivity would be observed in extractions of groundwater (Hedges et al. 1986, Hedges et al., 1994, Jardine et al., 1989, Kaiser et al., 2004; Shen et al. 2014).

1.4.2 Loading volume effects on yields of solid phase extraction eluates:

Investigation of loading volume effects on SPE-PPL recovery of freshwater DOM are limited. Studies have typically focused on samples sourced from one location and exclude DON dynamics in their investigation of SPE loading volume. Loading volume, however, is a critical parameter of SPE as every sample requires a distinct loading volume to achieve optimal carbon load. Optimal carbon load in SPE depends on the sensitivity of subsequent analysis methodologies as well as column breakthrough point. Researchers that have systematically investigated the effect of loading volumes on SPE-PPL extractions have found that loading volume does have associated selectivity, and yet, no observable reduction of bulk C recovery. (Li et al., 2016). In their study on Suwanee river DOM, Li et al., (2016) found that high loading volume selected against CRAM and carbohydrate DOM constituents, while selecting for more

aliphatic compounds. My investigation into loading volume supports the findings obtained from the Suwanee River study and extend these findings to lower DOC waters that span a range of source and degree of transformation. This experiment is relevant to boreal forest watersheds, however, the loading volumes tested (0.5 to 10L) are typical of many terrestrial and aquatic ecosystems.

Quantitative investigation into SPE-PPL dynamics revealed DOC and DON recoveries were not entirely independent of loading volume. Experiments on soil water samples revealed that ten-liter samples had a lower recovery of both DON and DOC compared to samples extracted at lower volumes. This, however, was not the case for groundwater or stream water samples, which did not experience any effects on DOC recovery associated with loading volumes. Groundwater DON recovery at loading volumes of five liters, however, was significantly lower than extractions completed at ten liters, but not one liter. The difference in the dynamics of loading volume across sample types representative of the terrestrial-to-aquatic interface is most likely due to the distinct chemical composition of DOM inherent to each sample type. Furthermore, these results suggest that SPE-PPL can yield representative eluates from across the terrestrial-to-aquatic interface when carefully considering the sample type and volume to be applied.

Selectivity present in the soil water loading volume experiment was not present in the groundwater experiment likely due in part to the lower relative proportion of O-alkyl hydrogen functionalities present in the groundwater sample

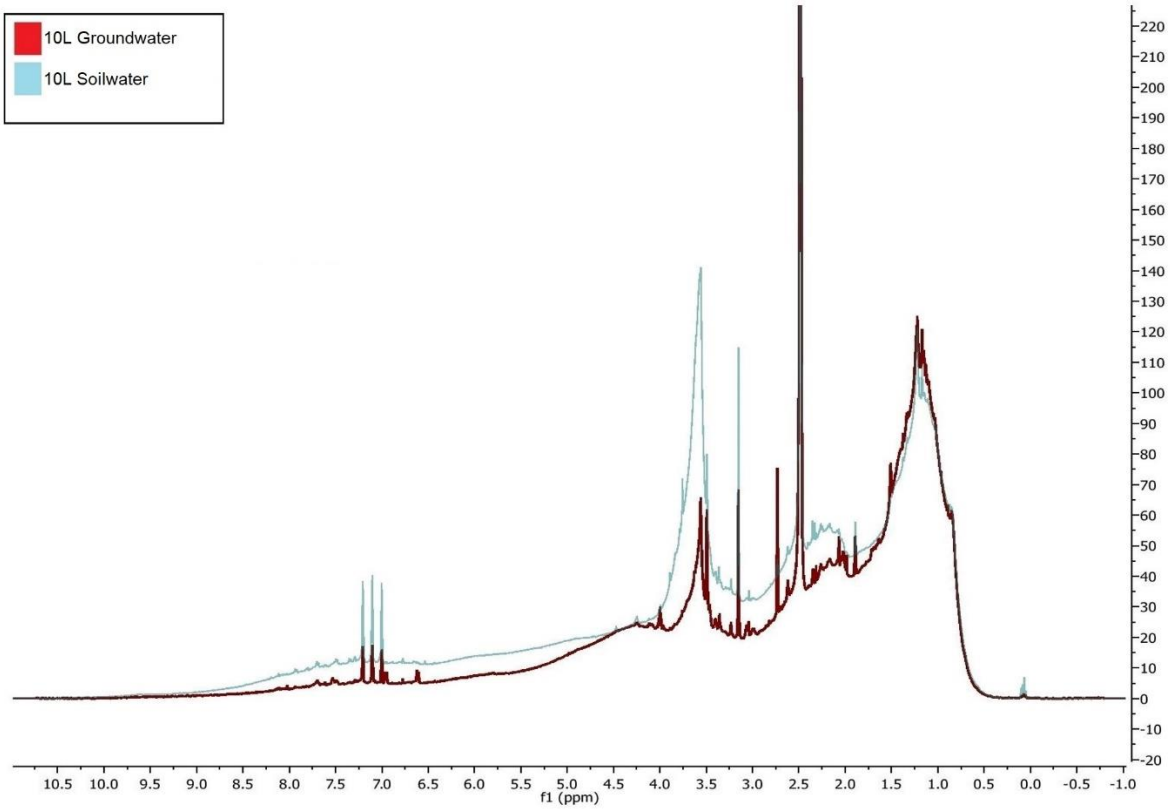
(Figure 1.8). DOM from soil water sources have much higher proportions of bioavailable DOM, such as sugars and proteins, than groundwater sources (Shen et al., 2014). This is evident when comparing the spectra of groundwater with spectra of soil, or stream water (Figure 1.8). The O-alkyl functionalities in groundwater samples spectra have much less detail specifically in the region of 3.5 - 3.8 ppm. This region contains many methoxy and ethoxy compounds originating from lignin, carbohydrates and peptides (Clemente et al., 2012). These are relatively bioavailable compounds that are removed as water percolates through the soil and regolith, resulting in fewer bioavailable compounds and higher quantities of slower turnover, recalcitrant, aliphatic moieties, via a process described as regional chromatography (Hedges et al. 1986, Hedges et al., 1994, Jardine et al., 1989, Kaiser et al., 2004; Shen et al. 2014). Results from this study suggest that either higher loading volumes displace DOM hydrogen components that are weakly bonded to the PPL sorbent, such as O-alkyl moieties associated with carbohydrates and lignin, for compounds with a higher affinity to the PPL-solid phase such as aliphatic functionalities (Li et al., 2016). During soil water experiments higher loading volume extractions selected against O-alkyl structures and CRAM, and instead preferentially extracted aliphatic compounds. This suggests that components weakly bonded to solid phase, such as O-alkyl compounds, partition back into the mobile phase as more volume is passed over them, while aliphatic compounds that bind to the solid phase are retained. This results in higher aliphatic contribution to total percent hydrogen of the soil water eluate. Additionally,

aromatic and amide structures were also selected against in the same manner as O-alkyl compounds at higher loading volumes for soil water. This selectivity is troublesome, however by limiting loading volumes or utilizing a larger SPE cartridge, it can be minimized and eliminated. It is especially important to use low loading volumes in SPE of soil water as DOM present in soil water has large amounts of these weakly bonding O-alkyl functionalities.

After my experimentation, SPE-PPL still seems a preferable method to prepare DOM samples from across the terrestrial-to-aquatic interface for solution state H-NMR analysis. Although high loading volume was found to select against components of DOM, by considering proper extraction loading volume and source of DOM applied, researchers can optimize SPE-PPL procedure to better interpret compositional data of DOM. For example, due to larger proportions of O-alkyl hydrogen functionalities, soil water eluates were found to exhibit selectivity associated with increased loading volume unlike groundwater eluates. Fortunately, soil water DOM in our region has high DOC concentration and do not require loading volumes of higher than one liter (Bowering et al., in preparation). Groundwater, on the other hand, tends to have a very low concentration of DOC, requiring loading volumes on the order of ten liters to achieve analyzable samples. However, groundwater has much lower contributions of weakly bonding hydrogen functionalities, which makes it less likely to experience this selectivity (Figure 1.8). Stream water presents the most variable source of DOM both in terms of concentration and characterization. Allochthonous DOM in streams is a

combination of groundwater and soil water, determined by many seasonal and regional mechanisms. Stream water DOM samples may resemble soil water DOM signature during periods of when the water table is elevated, and more resemble groundwater during dryer periods (Sanderman et. al 2009). Therefore, it is best to tailor SPE parameters, specifically loading volume, to the DOC concentration to avoid selectivity. By considering the chemical composition of DOM in the sample applied researchers can predict how selectivity may affect SPE-PPL extractions, and how representative the SPE-PPL eluate will be of the bulk sample.

Figure 1.8: Superimposed solution-state hydrogen nuclear magnetic resonance (H-NMR) spectra (500 MHz, dimethyl sulfoxide (DMSO)) for 10-liter testing groundwater water depicted in red, and 10-liter soil water sample depicted in cyan. Lower contributions in groundwater samples around 3.5-4 (ppm) is indicative of a loss of methyl and methoxy compounds originating from lignin.



1.7 References:

Aiken G R, Thurman E M, Malcolm R L and Walton H F. (1979). Comparison of XAD macroporous resins for the concentration of fulvic acid from aqueous solution. Analytical Chemistry. 51(11): 1799-1803.

Bianchi T S, Filley T, Dria K and Hatcher P G. (2004). Temporal variability in sources of dissolved organic carbon in the lower Mississippi River. Geochimica et Cosmochimica Acta. 68(5): 959-967.

Buddrus J, Burba P, Lambert J and Herzog H. (1989). Quantitation of partial structures of aquatic humic substances by one-and two-dimensional solution 13C nuclear magnetic resonance spectroscopy. Analytical Chemistry. 61(6): 628-631.

Clemente J S, Gregorich E G, Simpson A J and Simpson M J. (2009). Molecular-level analysis of organic matter structure and composition from different soil mineral fractions. In: EGU General Assembly Conference Abstracts. 11: 2064.

Cole J J and Caraco N F. (2001). Carbon in catchments: connecting terrestrial carbon losses with aquatic metabolism. Marine and Freshwater Research. 52(1): 101-110.

Cole J J, Prairie Y T, Caraco N F, McDowell W H, Tranvik L J, Striegl R G and Melack J. (2007). Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. Ecosystems. 10(1): 172-185.

Cook R L. (2004). Coupling NMR to NOM. Analytical and bioanalytical chemistry. 378(6): 1484-1503.

Dittmar T and Paeng J. (2009). A heat-induced molecular signature in marine dissolved organic matter. Nature Geoscience. 2(3): 175.

Dittmar T, Koch B, Hertkorn N and Kattner G. (2008). A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. Limnology and Oceanography: Methods. 6(6): 230-235.

Hedges J I, Cowie G L, Richey J E, Quay P D, Benner R, Strom M and Forsberg B R. (1994). Origins and processing of organic matter in the Amazon River as indicated by carbohydrates and amino acids. Limnology and oceanography, 39(4): 743-761.

Hedges J I, Eglinton G, Hatcher P G, Kirchman D L, Arnosti C, Derenne S and Michaelis W. (2000). The molecularly-uncharacterized component of nonliving organic matter in natural environments. Organic geochemistry. 31(10): 945-958.

Hedges J I, Ertel J R, Quay P D, Grootes P M, Richey J E, Devol A H and Salati, E. (1986). Organic carbon-14 in the Amazon River system. Science, 231(4742): 1129-1131.

Hertkorn N, Harir M, Koch B, Michalke B and Schmitt-Kopplin P. (2013). High-field NMR spectroscopy and FTICR mass spectrometry: powerful discovery tools for the molecular level characterization of marine dissolved organic matter. Biogeosciences, 10(3): 1583-1624.

IPCC (Intergovernmental Panel on Climate Change). (2007). Climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change.

IPCC (Intergovernmental Panel on Climate Change). (2013). Climate change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Jaffé R, McKnight D, Maie N, Cory R, McDowell W H and Campbell J L. (2008). Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties. Journal of Geophysical Research: Biogeosciences. 113(G4): G04032.

Jaffé R, Yamashita Y, Maie N, Cooper W T, Dittmar T, Dodds W K and Watanabe A. (2012). Dissolved organic matter in headwater streams: compositional variability across climatic regions of North America. Geochimica et Cosmochimica Acta. 94: 95-108.

Jardine P M, McCarthy J F and Weber N L. (1989). Mechanisms of dissolved organic carbon adsorption on soil. Soil Science Society of America Journal. 53(5): 1378-1385.

Jonsson A, Meili M, Bergström A K and Jansson M. (2001). Whole-lake mineralization of allochthonous and autochthonous organic carbon in a large humic lake (Örträsket, N. Sweden). Limnology and oceanography. 46(7): 1691-1700.

Kaiser E, Simpson A J, Dria K J, Sulzberger B and Hatcher P G. (2003). Solid-state and multidimensional solution-state NMR of solid phase extracted and ultrafiltered riverine dissolved organic matter. Environmental science & technology. 37(13): 2929-2935.

Kalbitz K, Solinger S, Park J H, Michalzik B and Matzner E. (2000). Controls on the dynamics of dissolved organic matter in soils: a review. Soil science, 165(4): 277-304.

Kaiser K, Guggenberger G and Haumaier L. (2004). Changes in dissolved lignin-derived phenols, neutral sugars, uronic acids, and amino sugars with depth in forested Haplic Arenosols and Rendzic Leptosols. Biogeochemistry. 70(1): 135-151.

Kim S, Simpson A J, Kujawinski E B, Freitas, M. A and Hatcher P G. (2003). High resolution electrospray ionization mass spectrometry and 2D solution NMR for

the analysis of DOM extracted by C18 solid phase disk. Organic Geochemistry. 34(9): 1325-1335.

Kim S, Simpson A J, Kujawinski E B, Freitas M A and Hatcher P G. (2003). High resolution electrospray ionization mass spectrometry and 2D solution NMR for the analysis of DOM extracted by C18 solid phase disk. Organic Geochemistry. 34(9): 1325-1335.

Lam B, Baer A, Alaee M, Lefebvre B, Moser A, Williams A and Simpson A J. (2007). Major structural components in freshwater dissolved organic matter. Environmental science & technology. 41(24): 8240-8247.

Leenheer J A. (1981). Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. Environmental science & technology. 15(5): 578-587.

Li Y, Harir M, Lucio M, Kanawati B, Smirnov K, Flerus R, and Hertkorn N. (2016). Proposed guidelines for solid phase extraction of Suwannee River dissolved organic matter. Analytical chemistry. 88(13): 6680-6688.

Lin V S. (2015). Research highlights: challenges in the characterization, storage, and isolation of natural organic matter. Environmental Science: Processes & Impacts. 17(12) 2002-2005.

Mattsson T, Kortelainen P and Räike A. (2005). Export of DOM from boreal catchments: impacts of land use cover and climate. Biogeochemistry. 76(2): 373-394.

Minor E C, Swenson M M, Mattson B M and Oyler A R. (2014). Structural characterization of dissolved organic matter: a review of current techniques for isolation and analysis. Environmental Science: Processes & Impacts. 16(9): 2064-2079.

O'brien R G. (1979). A general ANOVA method for robust tests of additive models for variances. Journal of the American Statistical Association. 74(368): 877-880.

Perminova I V, Dubinenkov I V, Kononikhin A S, Konstantinov A I, Zherebker A Y, Andzhushev M A and Popov I A. (2014). Molecular mapping of sorbent selectivities with respect to isolation of arctic dissolved organic matter as measured by fourier transform mass spectrometry. Environmental science & technology. 48(13): 7461-7468.

Pritzlaff, D. (2003) Determination of nitrate/nitrite in surface and wastewaters by flow injection analysis. QuikChem ® Method 10-107-04-1-C. Lachat Instruments, Loveland, CO.

Raeke J, Lechtenfeld O J, Wagner M, Herzsprung P and Reemtsma T. (2016). Selectivity of solid phase extraction of freshwater dissolved organic matter and its

effect on ultrahigh resolution mass spectra. Environmental Science: Processes & Impacts. 18(7): 918-927.

Schlesinger W H and Melack J M. (1981). Transport of organic carbon in the world's rivers. Tellus. 33(2): 172-187.

Shen Y, Chapelle F H, Strom E W and Benner R. (2015). Origins and bioavailability of dissolved organic matter in groundwater. Biogeochemistry. 122(1): 61-78.

Simpson A J, Kingery W L and Hatcher P G. (2003). The identification of plant derived structures in humic materials using three-dimensional NMR spectroscopy. Environmental science & technology: 37(2): 337-342.

Soucémarianadin L N, Erhagen B, Nilsson M B, Öquist M G, Immerzeel P and Schleucher J. (2017). Two-dimensional NMR spectroscopy for molecular characterization of soil organic matter: Application to boreal soils and litter. Organic geochemistry, 113: 184-195.

Thurman E M and Mills M S. (1998). Solid-phase extraction: principles and practice (Vol. 16).

Tranvik L J, Downing J A, Cotner J B, Loiselle S A, Striegl R G, Ballatore T J and Kortelainen P L. (2009). Lakes and reservoirs as regulators of carbon cycling and climate. Limnology and Oceanography: 54(6 part 2): 2298-2314.

Woods G C, Simpson M J and Simpson A J. (2012). Oxidized sterols as a significant component of dissolved organic matter: evidence from 2D HPLC in combination with 2D and 3D NMR spectroscopy. Water research. 46(10): 3398-3408.

Zhang Y, Huang W, Ran Y and Mao J. (2014). Compositions and constituents of freshwater dissolved organic matter isolated by reverse osmosis. Marine pollution bulletin. 85(1): 60-66.

Chapter 2:

Spatial variation in the chemical composition of dissolved organic matter within the Pynn's Brook Experimental Watershed

Keywords:

Dissolved organic matter (DOM), solid phase extraction (SPE), terrestrial-to-aquatic carbon flux, boreal forest watershed, nuclear magnetic resonance (NMR).

Abstract:

The terrestrial-to-aquatic carbon flux is a transfer of carbon that is not well constrained in current models of the global carbon cycle. Efforts to constrain this flux have been hampered by the temporal and spatial chemical heterogeneity of the flux in both small and large catchment scales. Constraining the chemical composition of dissolved organic matter (DOM), a significant form of carbon in inland waters, in conjunction with its flux should provide insights into the controls on the terrestrial-to-aquatic carbon flux. In this study I: 1) captured the chemical heterogeneity of DOM reservoirs across the terrestrial-to-aquatic interface over the course of a year in a small boreal forest watershed; 2) compare quantitative and qualitative measures to reveal potential controls on DOM composition; and 3) consider how these results better inform controls on the terrestrial-to-aquatic flux of DOM in this boreal forest watershed. To capture the wet and dry periods, samples were collected for four months: May, June, August, and October. Solution state hydrogen nuclear magnetic resonance (H-NMR) analyses were performed on samples collected during June and October, the respective dry and wet periods for the sampled watershed, to capture seasonal variation that may alter the hydrological connectivity of the watershed. Increases in both dissolved organic carbon (DOC) concentration and the presence of O-alkyl hydrogen functionalities in the lower stream site indicated DOM contributions from forest

soils during the fall wet period, while DOM at the same site was compositionally similar to groundwater DOM during the summer base flow. A shift in DOM composition, however, was not observed in the up-stream site, which drains a small headwater pond, where DOM chemical signature remained similar throughout the year based on similar DOM composition determined via H-NMR analysis, and DOC concentration. This lack of temporal variability in the upper site was likely due to greater water residence time caused by the up-stream pond. Increased water residence time may serve to reduce shifts in DOM composition initiated by hydraulic events by increasing the presence of autochthonous carbon. The findings of this study suggest that seasonal variation in the hydrologic connectivity of a watershed can impact the composition of DOM representing the terrestrial-to-aquatic flux even under base flow conditions in this boreal forest watershed. However, other localized watershed features, in this case a small headwater pond, can modify the chemical character of DOM masking this apparent connectivity between terrestrial and aquatic systems within this boreal landscape.

2.1 Introduction:

The transfer of terrestrial carbon to aquatic areas, known as the terrestrial-to-aquatic carbon flux, is a poorly constrained transfer of global carbon (Cole et al., 2007; Raymond et al., 2013; Tranvik et al., 2009). Current estimations of this flux, determined using mass balance calculations based on recent studies of lake carbon burial and degassing, are approximately double in magnitude compared to the flux's first estimations in the 1980s (Evans et al., 2005, Larsen et al., 2011, Lapierre et al., 2013). Researchers studying the terrestrial-to-aquatic carbon flux are especially interested in dissolved organic matter (DOM) which is highly mobile and the dominant form of total organic carbon (C) in aquatic ecosystems (Mattsson et al., 2005, Kalbitz et al., 2000). High latitude regions such as the boreal forest have the highest proportion of surface water coverage globally and typically exhibit high dissolved organic carbon (DOC) concentration, a quantitative measure of DOM (Houghton et al., 1995). Understanding the controls on the terrestrial-to-aquatic flux of DOM in high latitudes is critical given that this region is especially sensitive to climatic change (Goulden et al., 1998). Climate change is expected to increase both precipitation and production in boreal forests (IPCC 2013), that will facilitate the transfer of more DOC; a phenomenon that has been observed in recent studies (Evans et al., 2005, Tranvik and Jansson, 2002). To predict the potential climatic feedbacks associated with terrestrial carbon pools, the mechanisms that drive the terrestrial-to-aquatic carbon flux must be understood (Cole et al., 2007, Battin et al., 2009).

Modern techniques to characterize DOM require a combination of an isolation and analytical methodology. Solid phase extraction with a di-vinyl benzene copolymer sorbent (SPE-PPL) followed by solution state hydrogen nuclear magnetic resonance (H-NMR) has become a popular combination of methodologies to isolate and analyze DOM (Mopper et al., 2007, Hertkorn et al., 2013, Kalbitz et al., 2003, Li et al., 2017). Solid phase extraction with a di-vinyl benzene copolymer sorbent allows for a matrix-free, bulk representative DOM eluate (Li et al., 2017, Chapter 1), which, when analyzed via NMR, can provide the composition of DOM in terms of broad chemical functionalities (Mopper et al., 2007, Sanderman et al., 2009, Clemente et al., 2009). By interpreting DOM composition in terms of these broad chemical functionalities, supplemented by quantitative measurements of carbon and nitrogen, DOM source and transformations across the terrestrial-to-aquatic interface may be revealed. Different DOM reservoirs in the terrestrial-to-aquatic interface are subjected to diverse biotic and abiotic controls that influence the chemical characterization of DOM in that reservoir. These controls lead to a semi-unique DOM chemical characterization among potential terrestrial sources of DOM including precipitation, soil water and groundwater that may all contribute to stream water DOM composition.

Dissolved organic matter enters terrestrial systems via litter inputs, root exudates, microbial production, and inputs from precipitation (Kalibitz et al., 2000). Although precipitation samples have been found to contain DOM, studies

show that it is bioavailable and low in concentration which results in rapid losses once introduced to the terrestrial environment, and therefore does not significantly contribute to downstream locations (Qualls and Hanes 1992, Fellman et al., 2009). Fresh inputs from litter, microbial production, and root exudates contribute to relatively large concentrations of bioavailable DOM constituents, such as carbohydrates and amino acids, in surface soils relative to deeper soils and groundwater. These fresh inputs result in soil DOM reservoirs having a higher concentration of bioavailable DOM than any other reservoir within the terrestrial-to-aquatic interface (Kalibitz et al., 2000). The chemical composition of soil DOM changes as it percolates down through soil horizons and into groundwater pools because of combined biological and physiochemical processes occurring within the soil and groundwater matrices (Shen et al., 2015). Labile DOM hydrogen functionalities, such as O-alkyl or proteinaceous moieties, are biologically degraded, while lignin DOM constituents are sorbed to mineral surfaces as water percolates into deeper soil reservoirs (Hedges et al., 1994, Volk et al., 1997, Amon et al., 2001, Kalbitz et al., 2003). The magnitude of these changes is dependent the rate at which soil water percolates to groundwater reservoirs. Aliphatic functionalities, that are relatively recalcitrant compared to O-alkyl or proteinaceous moieties, are selectively retrained en route to the aquatic environment (Baldock et al., 1992, Kögel-Knabner et al., 1992, Baldock and Preston, 1995). Controls such as mineral sorption and microbial respiration are ultimately responsible for giving groundwater DOM its slow turnover time and low molecular weight (Kalbitz et al. 2000, Shen et al., 2015).

Dissolved organic matter from both soil and groundwater reservoirs contribute to the flux of terrestrial DOM ultimately exported to streams. The relative contribution of soil and groundwater pools to streams, along with other climatic and topological variables then determine DOM characterization and concentration in streams.

Dissolved organic matter in boreal stream systems is almost all allochthonous in origin because it is hydraulically connected to the terrestrial landscape and has low rates of autochthonous productions compared to ponds and lakes (Hedges and Oades 1997, del Giorgio and Peters 1993, Jansson et al. 2000). Relative inputs from terrestrial land positions, such as both soil water and groundwater, depend primarily on hydrologic flow paths and watershed topography (Hongve et al., 2004). Dissolved organic matter character has been reported to shift greatly during periods of heavy rainfall or snowmelt. After such hydrological events, larger quantities of bioavailable DOM functionalities including O-alkyl and proteinaceous moieties can be observed in stream water (Raymond et al., 2007, Spencer et al., 2008) suggesting that the elevated water table helps to mobilize new pools of soil derived DOM into stream systems (West et al., 1996, Hood et al., 2005). On the other hand, during the dry base flow period, the water table drops inhibiting contributions from soil DOM. The outflow from stream resembles groundwater DOM character (Cai et al., 2008, Walvoord and Striegl 2007). To understand the dynamics of stream DOM, researchers must characterize DOM derived from both soil and groundwater reservoirs within a

catchment and assess how these terrestrial land positions relate to stream DOM composition during different periods of transport throughout the year.

Although DOM pools across the terrestrial-to-aquatic interface have been studied in isolation, studies that have systematically investigated DOM composition across related terrestrial and aquatic positions and at different hydrologic periods are rare. Furthermore, studies on the seasonal variability of the composition of the DOM exported yield variable results with some studies relating changes in hydrological flow-path to major shifts in DOM composition (Hood et al., 2005, Kellerman et al., 2014), and some reporting non-significant changes in DOM composition throughout wet and dry periods (Schumacher et al., 2006).

In this study, I investigated the seasonal and regional variability of DOM composition and concentration across the terrestrial-to-aquatic interface in a small boreal forest watershed. Here the investigation of DOM composition was considered in terms of potential changes in the source and processing occurring across samples originating from precipitation, groundwater, soil water, and stream water in the Pynn's Brook Watershed. The objectives of this study were to: 1) capture the chemical composition of DOM systematically along the terrestrial-to-aquatic interface in a small boreal watershed over the course of a year; 2) compare quantitative and qualitative measures to reveal potential controls on DOM composition; and 3) consider how these results better inform controls on the terrestrial-to-aquatic flux of DOM in this boreal forest watershed.

2.2 Methods:

2.2.1 Site description:

This study was conducted in the Pynn's Brook experimental watershed area (PBEWA) located ~50 km away from the town of Corner Brook in western Newfoundland and Labrador, Canada (lat. 48° 53'14", 105 long. 63° 24' 8") from May 2016 to October 2016 (Figure 2.1). The watershed receives an average of ~1096 mm of precipitation annually and has a mean annual temperature of 3.6°C (Environment Canada climate normals, Deer Lake airport 1981-2010). Average rain and snow during the study period are visible in Figure 2.2. Pynn's Brook experimental watershed area is an experimental watershed consisting of first and second order stream sites and plots of both mature black spruce and harvested black spruce. Pynn's Brook experimental watershed area is a part of the larger Pynn's Brook watershed which consists of 68% boreal forest, 21% wetlands and 9% disturbed areas, such as roads and quarries. Soils in PBEWA are humo-ferric podozols.

Figure 2.1: Topographical map of Pynn's Brook Experimental Watershed Area (PBEWA) obtained from geographic information system (GIS) measurement. Stream sites are shown in black, the lower and upper sites are denoted with a 1 and 2 respectively. Soil water collection sites are shown in red, and precipitation collection sites are show in green.

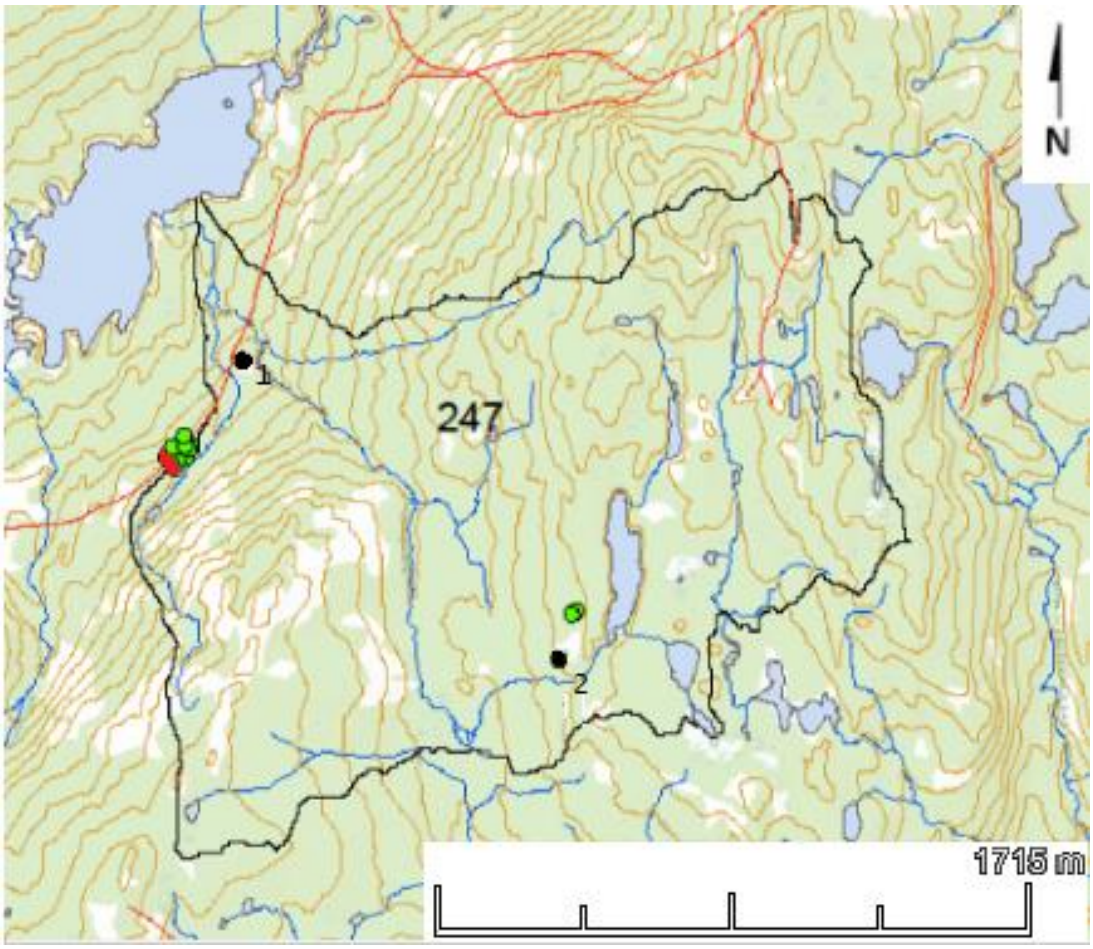
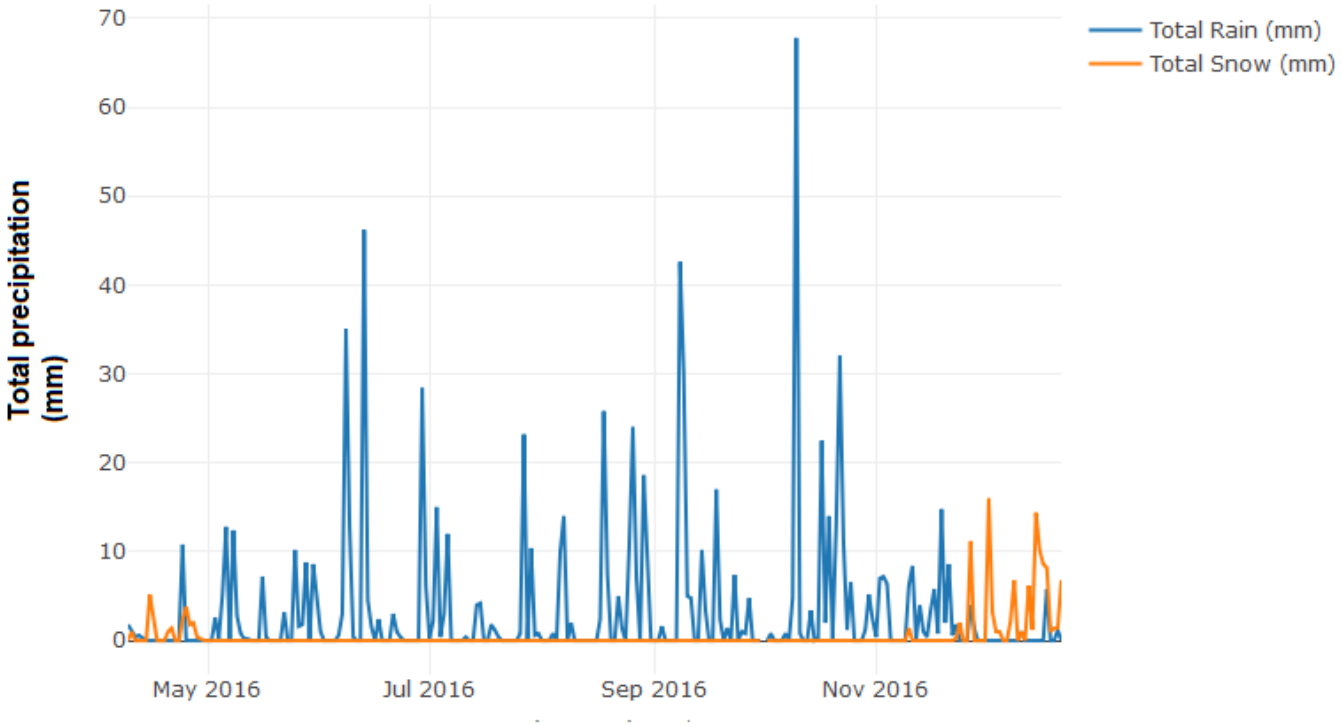


Figure 2.2: Total precipitation (mm) versus date for Deer Lake A (ID: 8401501, Environmental Canada). Total rain (mm) in blue, while total snow is portrayed in orange.



2.2.2 Sample collection:

Samples were collected during four weeks in 2016 to capture the seasonal variation in DOM chemical characterization. Samples were collected on multiple days during the weeks of: May 20th, 2016, June 16th, 2016, August 24th, 2016, and October 24th, 2016. Within the PBEWA two specific pairs of experimental stands were designated for the sampling of soil solution and precipitation. These areas were separated by 176 m of elevation. The lower elevation site was located within the Pynn's Brook experimental forest (PBEF) which consisted of eight 50X50-meter plots, while the upper elevation site consisted of two similar sized plots. Half of the PBEF plots were harvested in 2003; while the others were left unharvested. Further details on the PBEF can be found in Moroni et al., 2009. Harvested plots will be referred to as regenerating, while unharvested plots will be referred to as mature. To capture the regional variability of the terrestrial-to-aquatic carbon flux multiple land positions within the watershed were sampled during each of the selected weeks; these included: ground water, soil water, stream water, and precipitation.

Precipitation and throughfall samples were collected from both upper and lower PBEF stands. Precipitation was collected in the regenerating plots, while throughfall was collected in the mature plots. Precipitation/throughfall samples were collected in individual acid-washed and deionized water rinsed 20-gallon high-density polyethylene (HDPE) buckets. Three two-foot-long stakes were used to elevate the buckets off the ground and to establish a fixed sampling

position that was replicated throughout the year. Prior to the first sampling date a preliminary variability experiment was conducted in October 2015 in the PBEF to determine the most practical number of gauges required to capture the variability within mature and regenerating plots. Twenty gauges were installed in the mature plot and ten were deployed in the regenerating plot and left out for one rain fall event. Each bucket was then sampled, filtered and analyzed for DOC concentration. From this data a Monte Carlo simulation was used to predict the relationship between number of precipitation gauges deployed and the variability of DOC concentration captured. It was found that installing ten gauges while collecting throughfall, and five whole collecting precipitation captured a similar amount of variation in DOC concentration as deploying twenty gauges while collecting throughfall and ten in while collecting precipitation (Table A.1). Considering these results, ten gauges were installed in the mature plot while five were installed in the regenerating plot during each sampling week.

Soil water samples were collected from passive pan lysimeters installed under the O-horizon (approximately 8 cm in depth). Two lysimeters from two different regenerating plots and two from different mature plots in the lower elevation PBEF stand had one-liter of soil water sampled into a one-liter acid-washed, deionized water and sample rinsed HDPE plastic bottle for DOM characterization. Sampling was conducted using a battery-operated pump through HDPE tubing connected to a 25 L HDPE carboy buried downslope and plumbed into each lysimeter. Prior to each sampling, all lysimeters were

completely emptied. Details on the lysimeter design and installation are described in Bowering et al., in preparation.

A bulk sample of ten liters was collected during each sampling date from a groundwater seep located ~5 km outside the Horseshoe Brook watershed. Groundwater was collected using acid-washed, deionized water and sample rinsed 10L HDPE carboys. This seep was the closest groundwater source available near PBEWA sites.

Stream water was collected from two sites within the Horseshoe Brook stream located in PBEWA. Although the sites were in the same stream the regional topography surrounding the sites differed. The upper elevation stream, referred to as the upper stream site, was located 50 meters downstream of Horseshoe pond. A sizeable pond that represented one percent of the total catchment area drained into the lower stream site, but 24% of the catchment area drained to the upper site. During each sampling date 5 L of stream water was collected in 5 L acid-washed, DI rinsed, and sample rinsed HDPE carboys.

2.2.3 Environmental monitoring:

Soil moisture was recorded via two soil moisture probes (Decagon ECH$_2$O -TM) installed at 5 cm depth in the O horizon in the upper and lower PBEF stands. Data were downloaded seasonally.

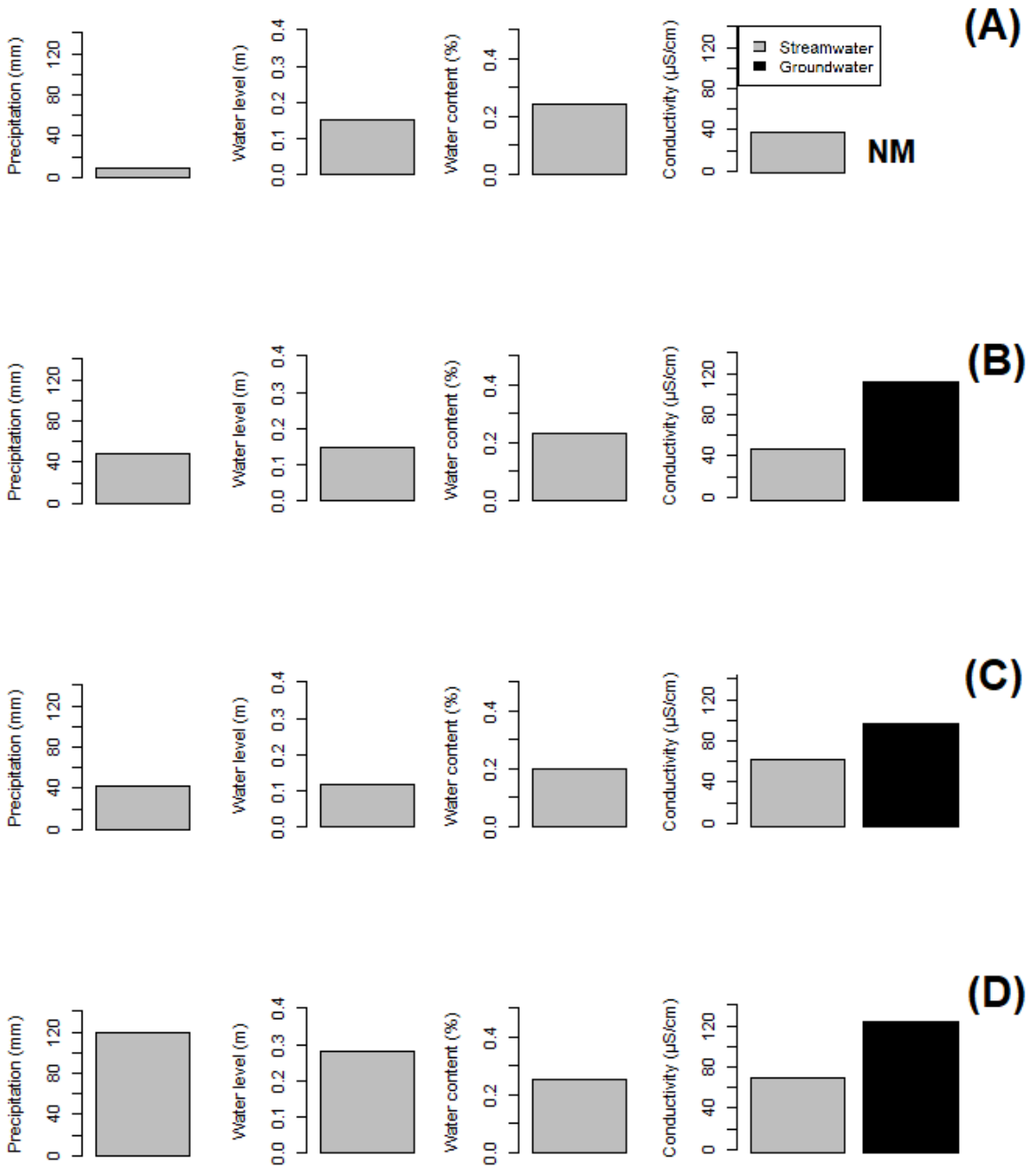
Precipitation data were acquired from Environmental Canada at the Deer Lake Airport (lat. 49°13'00" N, long. 57°24'00" W) located ~40km from the study

area. These data were compared to data collected from tipping bucket (RST Instruments Model TR-525) precipitation logger installed in the lower stand of the PBEF. These site-specific data were found to be well correlated to the Deer Lake Airport precipitation record (Bowering et al., in preparation).

Continuous stream water level data were collected using a pressure transducer probe (ONSET, MA) installed in the lower stream site. The probe provided water pressure data which were corrected using atmospheric barometric pressure collected from an additional probe installed in a protective housing bolted to a tree adjacent to the lower stream site. Measurements of stream depth were acquired every 15 min and averaged to obtain mean daily values (Figure 2.3).

Handheld measurements of conductivity were taken for precipitation, groundwater and stream water via YSI Professional Plus (YSI, OH) and YSI 60530-1 conductivity probe. The YSI probe was calibrated the night prior to the sampling period for conductivity. The YSI instrument was calibrated using the YSI conductivity calibration solution.

Figure 2.3: Precipitation (mm), water level (m), volumetric soil water content (%), and conductivity (µs/cm) of ground water in black and stream water in grey. Sampling dates are shown sequential order going by (A) May, (B) June, (C) August, and (D) October. NM = Not measured.



2.2.4 Sample preparation prior to solid phase extraction:

After collection, samples were transported back to the Canadian Forest Service field station located in Pasadena, Newfoundland. Bulk water samples from every land position underwent filtration through pre-combusted Glass Fiber (GF/F) Whatman Filters (6h, 500 F°). After filtration, 15 mL sub-samples were taken for dissolved organic nitrogen (DON), DOC, and total dissolved nitrogen (TDN) analysis, while 10 mL sub samples were taken for inductively coupled plasma - optical emission spectrometry (ICP-OES) and dissolved inorganic nitrogen (DIN) analysis. Twenty-four mL glass vials (Fisher Scientific, NH) were used for DON, DOC and TDN subsamples, while 15 mL falcon tubes (Fisher Scientific, NH) were used for ICP-OES and DIN samples. 0.2 mL of metal free nitric acid (70% ACS grade HCl; Sigma Aldrich) was added to ICP-OES subsamples, while, saturated mercuric chloride ($HgCl_2$) solution was added to the bulk filtered samples and TDN, DIN and DOC subsamples at a ratio of 10 µl $HgCl_2$:10 mL sample to impede any biological degradation of DOM during transport to Memorial University of Newfoundland and subsequent storage at 4°C until analysis.

2.2.5 Carbon and nitrogen measurements:

Sub-samples were analyzed for DOC and TDN using a Shimadzu TOC-V high temperature combustion total carbon analyzer (Shimadzu, Japan) at Memorial University of Newfoundland. The detection limit for DOC was 0.07 mg C L^{-1} with a coefficient of variation of 1.1% of the 5 mg L^{-1} check standards. The

detection limit for TDN was 0.001 mg N L^{-1}, with a coefficient of variation of 1.8% of 0.1 mg L^{-1} check standards.

2.2.6 Inorganic nitrogen analysis:

Analysis of dissolved inorganic nitrogen (DIN) was completed through the individual colorimetric analyses of dissolved ammonium and nitrate using a QuikChem 8500 Series 2 FIA System (Hach, Colorado, USA). Nitrate was reduced to nitrate then, in the presence of sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride was analyzed via absorbance measurements. Ammonia concentration was determined via absorbance measurements after heating the sample in the presence of salicylate hypochlorite in a solution of alkaline phosphate buffer (Pritzlaff, 2003). Detection limits for the ammonia method was 0.003 mg N L^{-1} with a coefficient of variation of 7.21% of the 0.2 mg L^{-1} check standards. Detection limits for the nitrate method was 0.007 mg N L^{-1} with a coefficient of variation of 8.26% of the 0.2 mg L^{-1} check standards. Total DIN was subtracted from TDN to calculate Dissolved Organic Nitrogen (DON). Calculated DON values had detection limits of 0.002 mg N L^{-1}.

2.2.7 Metal analysis:

Analysis of metals was completed using an iCap 6500 Series ICP-OES. Analysis was performed by Dr. Chris Finch at the Howley building in St. John's Newfoundland. Limits of detection for calcium and iron were 0.01 mg Ca L^{-1} and 2

µg Fe L^{-1} respectively. All other analytes are shown in tables 4.7, 4.8, 4.9, 4.10 and 4.11, and their detections limits are shown in Table A.7.

2.2.8 Solid phase extraction:

All SPE-PPL experiments were carried out with Varian Bond Elute 100 mg PPL cartridges. Extraction volumes were applied to obtain loadings of ~20 mg C as DOC, large enough for the resulting solution state H-NMR spectrum to have adequate resolution, but below the manufacturers loading limit. Prior to SPE, all samples were acidified to a pH of 2 using stock solution of hydrochloric acid (32% ACS grade HCl; Sigma Aldrich) to increase the recovery of organic acids and phenols (Dittmar et al., 2008). The SPE-PPL cartridges were rinsed with a stock solution of acetone (99.5% HPLC Millipore) and then methanol (Millipore HPLC 99.9%) to ensure complete removal of any remaining C applied to the column from previous extractions, and to prime the cartridges for sample loading. Sample was drawn into the SPE cartridges via 1/8" silicon tubing (Fisher Scientific, NH) via vacuum pressure from a 500 mL HDPE bottle, that was manually refilled. After loading, cartridges were washed with two cartridge volumes (~12 mL) of 0.01 M HCl to remove any matrix components that had bonded to the solid phase. Cartridges were then dried for 15 minutes by vacuum pressure and eluted with 6 mL of methanol into individual 24 mL glass vials. Samples were then dried down and placed in a desiccator until they could be analyzed by NMR.

2.2.9 Solution state hydrogen nuclear magnetic resonance analysis:

Solution state H-NMR analysis was performed on all samples collected during the October and June sampling periods. Dried samples were reconstituted in deuterated dimethyl sulfoxide (DMSO) before solution state H-NMR analysis. Data were acquired using Bruker AVANCE 500 spectrometer with a 5 mm TXI 1H/D-13C/ 15N Z-GRD Z8161/ 0154 probe, 64 scans were carried out with a 3 second delay time. Processing of NMR spectra was completed via Mrestnova software. Assignments of compound classes were verified from other solution state H-NMR studies of natural organic matter that utilized DMSO as the NMR solvent (Clemente et al., 2009, Table 2.1). Analytical precision of NMR was assessed by analyzing one SPE-PPL soil water DOM eluate three times and determining the standard deviation for each of the integral areas. The average analytical precision of all functional groups was found to be 0.4%.

Table 2.1: Integration areas of hydrogen functional groups relating to the elucidation of DOM for solution state H-NMR. Integration areas were compiled from studies that utilized the same deuterated solvent (Clemente et al. 2012).

Chemical Shift region:	Hydrogen functionality:
8.60 .. 7.80	amides from peptides
7.80 .. 6.20	aromatic from lignin and proteins
4.80 .. 4.00	Peptides
4.00 .. 2.90	O alkyl mainly from carbohydrates and lignin
2.90 .. 1.30	aliphatic methyl and methylene near O and N
1.30 .. 0.60	aliphatic methyl and methylene

2.2.10 Statistical analysis:

Due to differences among land positions, it was necessary to use two statistical approaches to help interpret the data**.** A repeated measures linear mixed effects model was used to assess the effect of time by elevation interactions on DOC, DON and metal concentrations, in the case of stream samples. The same analysis was applied to assess the effect of time by harvesting treatments in the case of soil water and precipitation, and their effect on DOC, DON, and metal concentrations (Table A.2, Table A.3, Table A.4, Table A.5). Post-hoc t-tests were used to determine significant differences in DOC, DON, and metal concentrations in sampling periods and treatment types.

A one-way analysis of variance (ANOVA) was applied to assess the effect of time on measures of DOC, DON, and metal concentrations for groundwater. Assumptions required to conduct the ANOVA tests include: equal population variations across groups, adherence of the residuals to a normal distribution, and the independence and randomness of all variables. The following tests were performed to determine whether data passed all necessary assumptions. Shapiro tests were conducted to test for normality of distribution in the response variables, while Leven's Test was performed to test the distribution of population variances (O`Brien et al., 1979). Lag plots were constructed in R studio to ensure samples were independent and random. Tukey honesty post-hoc tests were performed to investigate significance differences between means where significant effects were found, and the necessary assumptions were validated. In

cases where the data did not meet the necessary assumptions, the non-parametric statistical tests were performed using the Kruskal-Wallace test, followed by post-hoc Nemenyi's test to determine significant differences between the means. All statistics and tests used are reported in Table A.6.

2.3 Results:

2.3.1 Site characteristics:

In the lower elevation PBEF stand soil volumetric water content was highest during May and October periods, and fell during the June and August periods (Figure 2.3). The stream water level also followed a similar pattern with highest levels during the May and October periods and lowest during the June and August periods. When considering the patterns of soil moisture and stream level in addition with measures of precipitation, May and October periods were found to be the wettest periods, while June and August were drier periods in this catchment. These assignments were consistent with the average climate data reported from Environmental Canada climate normals for the Deer Lake Airport during 1981-2010 located ~40km from the study area. Assignments of seasonal wet and dry periods was essential to this study, as the differences in hydraulic flow path between these periods was hypothesized to greatly contribute to the variability in the composition of DOM exported from these systems.

2.3.2 Precipitation/throughfall:

The range of DOC concentrations in precipitation/throughfall samples collected from the upper elevation PBEF stand was 1-18 mg L^{-1}, like DOC concentrations in the lower PBEF stand which exhibited a range of 2-18 mg L^{-1}. The DOC concentration in precipitation collected from the upper elevation PBEF plot varied with both sampling period and plot treatment, exhibiting a significant

interaction of sampling period by plot treatment ($P < 0.0001$, Figure 2.4A, Table A.3). Dissolved organic carbon concentrations were highest in May but then remained relatively constant during other sampling periods with throughfall exhibiting higher concentrations of DOC than precipitation (Figure 2.4A). Like the upper PBEF stands, the lower PBEF stands exhibited a higher DOC concentration than precipitation, exhibiting a significant interaction of sampling period by plot treatment ($P < 0.0001$, Figure 2.4B, Table A.2). Average carbon fluxes for both upper and lower PBEF stands were calculated to be on average 0.14 and 0.48 mg C $m^{2,-1}$ yr^{-1} in throughfall and precipitation respectively based upon DOC concentration and volume of rainwater collected during each sampling trip.

The range of DON concentrations in the upper elevation PBEF stand was 0.015-0.19 mg L^{-1}, while the DON concentrations in the lower PBEF stand exhibited a range of 0.4-0.17 mg L^{-1}. Precipitation and throughfall DON concentrations collected from upper PBEF stands were similar during the June sampling dates but exhibited large differences during other sampling periods. Rainwater DON concentrations collected from the upper PBEF stands exhibited a significant interaction of sampling period by plot treatment ($P < 0.0001$, Figure 2.4A, Table A.3). Dissolved organic nitrogen concentration in throughfall collected from lower PBEF stand reached its maximum and minimum value during the October and August sampling periods respectively. Precipitation exhibited a different pattern reaching its maximum and minimum value during the

June and August sampling periods respectively (Figure 2.4B). Variation in DON concentration in lower elevation PBEF stand was due to an interaction of sampling period by plot treatment ($P < 0.0001$, Figure 2.4B, Table A.2).

The range of molar C:N ratios in the upper elevation PBEF stand was 150-30, while the molar C:N ratio in the lower PBEF stand exhibited a range of 30-80 in precipitation and a range of 150-100 in throughfall (Figure 2.4). Molar C:N ratios of throughfall collected from the upper elevation PBEF stands were higher than C:N ratios collected from precipitation on all sampling periods excluding August. The molar C:N ratio in upper elevation PBEF stands exhibited a significant interaction of sampling period and plot treatment ($P < 0.0001$ Figure 2.4A, Table A.3). The molar C:N ratios in rainwater collected from the lower elevation PBEF stand exhibited a similar pattern as upper elevation stands and again this variation was due to an interaction of sampling period by plot treatment ($P < 0.0001$ Figure 2.4B, Table A.2).

The range of iron concentration in rainwater samples collected from the upper elevation PBEF stand was 9 to 44 µg L^{-1}, while the iron concentration collected from rainwater in the lower PBEF stand exhibited a range of 7 to 44 µg L^{-1}. (Figure 2.4). Iron concentrations in rainwater collected from both upper PBEF stands were higher during the May sampling period and lower during the June, August and October sampling periods. Rainwater collected from the upper PBEF stand exhibiting an interaction of sampling period and plot type ($P = 0.0004$, Figure 2.4A, Table A.3). Rain water collected from the lower PBEF stand also exhibited

an interaction of sampling period by plot treatment (P = 0.0126, Figure 2.4B, Table A.2).

The range of calcium concentration in rain water samples collected from the upper PBEF stand was 0.08 to 0.5 mg L^{-1}, while calcium concentration collected from rain water samples in the lower PBEF stands was 0.06 to 0.6 mg L^{-1}. (Figure 2.4A). Calcium concentrations in rain water collected from upper PBEF stands reached their maximum concentration in May and October sampling periods, with lower concentrations observed in June exhibiting a significant interaction of sampling period and plot treatment (P = 0.0025, Figure 2.4A, Table A.3). Calcium concentration within rain water collected from the lower elevation PBEF plots increased significantly during the wet period exhibiting an interactive effect of sampling period by plot treatment (P = 0.0084, Figure 2.4B, Table A.2).

Solution state H-NMR revealed that both throughfall and precipitation DOM samples consisted mostly of aliphatic and O-alkyl hydrogen moieties. Throughfall DOM had higher contributions from aliphatic functionalities than precipitation DOM during both wet and dry periods but had lower contributions from O-alkyl functionalities to total percent hydrogen (Table 2.2). The October sampling period exhibited the highest amounts of aliphatic and amide hydrogen functionalities within both precipitation and throughfall samples.

Figure 2.4: Average measures of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), molar DOC:DON ratio (± 0.02 in precipitation, and ± 0.1 in throughfall), iron, and calcium for upper and lower elevation PBEP precipitation sites where n=3. Mature sites are presented in green, while Regenerating sites are shown in red. NM = Not measured, BD= below detection. Figure 4A represents upper PBEF stands while Figure 4B represents lower PBEF stands. Error bars are show standard error of average measurements. Bars with dissimilar letters are significantly different.

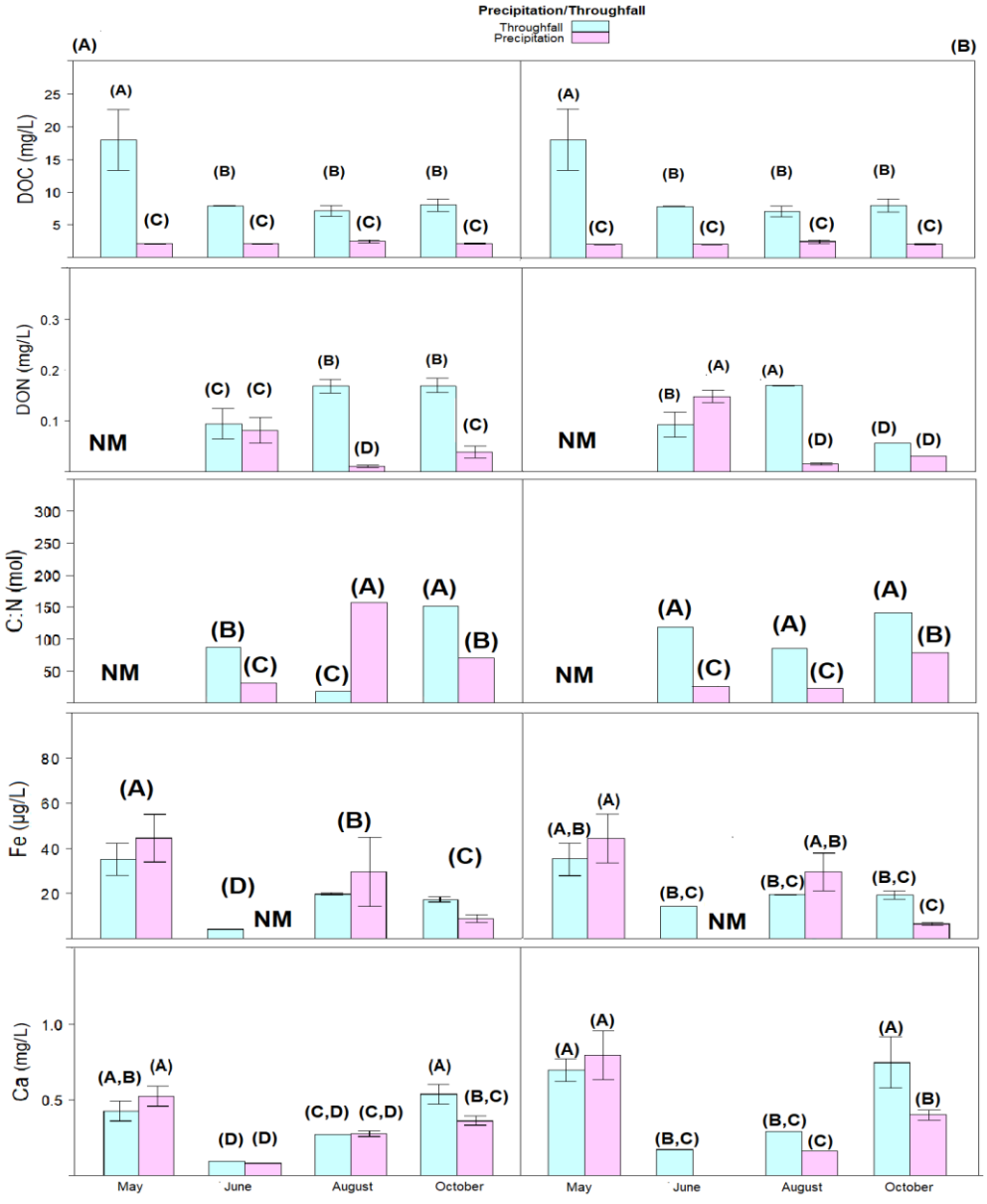


Table 2.2: Distribution of hydrogen functionalities as percent of total hydrogen as determined by hydrogen nuclear magnetic resonance (H-NMR) (500 Mhz, DMSO) for dissolved organic matter (DOM) isolated via solid phase extraction (SPE-PPL) from all precipitation samples collected in both wet and dry periods. Classification of hydrogen functional groups was based on chemical shift regions: 0.6–1.3 ppm: aliphatic methyl and methylene; 1.3–2.9 ppm: aliphatic methyl and methylene near O and N; 2.9– 4.0 ppm: O-alkyl, mainly from carbohydrates and lignin; 4.0–4.8 ppm: α^1H from proteins; 6.2–7.8 ppm: aromatic, from lignin and proteins; 7.8–8.6 ppm: amide from proteins.

Precipitation				
Functional Group	June		October	
	Mature plot	Regenerating plot	Mature plot	Regenerating plot
Amides from proteins	1.02	3.41	2.15	2.89
Aromatic from lignin and proteins	9.26	13.43	8.88	12.38
Proteins	10.61	12.48	8.27	8.49
O-alkyl mainly from carbohydrates and lignin	20.30	25.54	18.23	22.59
Aliphatic methyl and methylene near O and N	39.21	31.83	36.66	32.60
Aliphatic methyl and methylene	19.60	13.32	25.76	21.05

2.3.3 Soil water:

The range in DOC concentration in soil solution DOM collected from mature and regenerating plots in the lower PBEF stand was 18-27 mg L^{-1} and 24-38 mg L^{-1}, respectively (Figure 2.5) and was on average 200% greater than what was observed in precipitation. Dissolved organic carbon concentration increased slightly after the May sampling period and remained relatively constant during the subsequent sampling periods. Dissolved organic carbon concentration did not exhibit a significant interaction of sampling period by plot treatment ($P = 0.2612$ Figure 2.5, Table A.4), or between plot treatments ($P = 0.2342$). However, a significant difference among the DOC concentration was observed at different sampling periods ($P < 0.0001$).

The range of DON concentration in soil solution DOM samples was 0.62–0.13 mg L^{-1} in mature plots, and 0.48-0.2 mg L^{-1} in regenerating plots (Figure 2.5). Dissolved organic nitrogen concentrations were elevated during the May, June and August sampling periods and lower in the October sampling period. Dissolved organic nitrogen concentration exhibited a significant interaction of sampling period by plot treatment ($P < 0.0001$, Figure 2.5, Table A.4).

The range in molar C:N ratio of soil solution DOM samples was 69-120 in mature plots and from 55-103 in regenerating plots (Figure 2.5) and exhibited a significant sampling period by plot treatment interaction ($P < 0.0001$ Figure 2.5, Table A.4). Mature plots had higher soil molar C:N ratios than regenerating plots at all sampling periods except October.

The range in iron concentrations in soil solution DOM samples was 112-193 μg L^{-1} in regenerating plots, and 39-276 μg L^{-1} in mature plots in soil solution DOM samples (Figure 2.5). Iron concentrations varied with both time and treatment type, exhibiting a significant interaction of sampling period by plot treatment ($P < 0.0001$, Figure 2.5, Table A.4).

The range in calcium concentrations in soil solution DOM samples was 0.53-1.0 mg L^{-1} in regenerating plots, and 1.6-3.3 mg L^{-1} in mature plots (Figure 2.5). Calcium concentration exhibited a significant difference between regenerating and mature stands ($P = 0.0002$, Figure 2.5, Table A.4) with mature stands consistently containing higher concentrations of calcium.

Solution state H-NMR analysis of the SPE-PPL fraction of soil DOM solution samples collected from both regenerating and mature plots during the dry periods revealed only slight compositional differences between the plot treatments. DOM from both plot types contained major contributions of aliphatic and O-alkyl hydrogen functionalities and contained substantially smaller contributions from protein, aromatic and amide functionalities (Table 2.3). When comparing the June and October sampling periods the composition of DOM from both plots differed slightly. June sampling periods exhibited elevated contributions of amide hydrogen functionalities, while October sampling periods exhibited elevated O-alkyl functionalities (Table 2.3). All other functionalities remained relatively constant between these two periods.

Figure 2.5: Average measures of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), molar DOC:DON ratio (±0.01 in mature plots, and ±0.02 in regenerating plots), iron and calcium for PBEW lysimeters. Mature sites are presented in green, while regenerating sites are shown in red. Error bars are show standard error of average measurements. Bars with dissimilar letters are significantly different. NM = not measured, BD= below detection

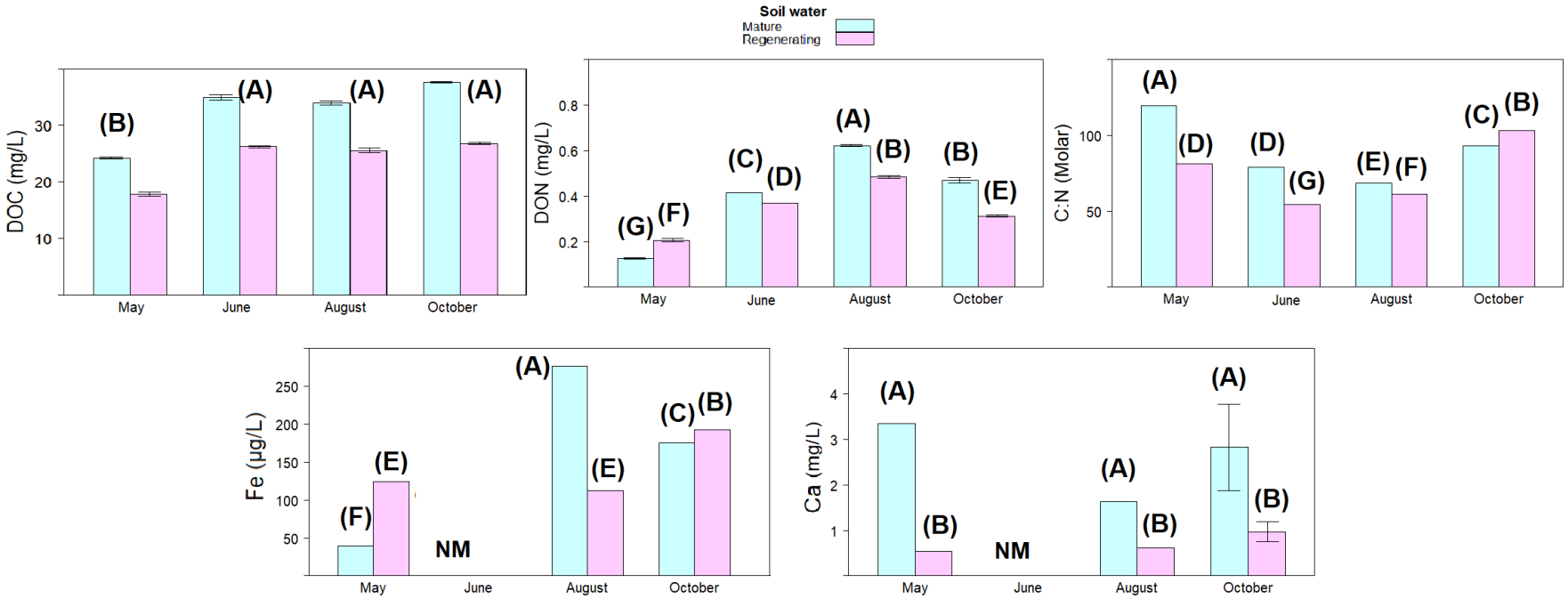


Table 2.3: Distribution of hydrogen functionalities as percent of total hydrogen as determined by hydrogen nuclear magnetic resonance (H-NMR) (500 Mhz, DMSO) for dissolved organic matter (DOM) isolated via solid phase extraction (SPE-PPL) from all soil water samples collected in both wet and dry periods. Classification of hydrogen functional groups was based on chemical shift regions: 0.6–1.3 ppm: aliphatic methyl and methylene; 1.3–2.9 ppm: aliphatic methyl and methylene near O and N; 2.9– 4.0 ppm: O-alkyl, mainly from carbohydrates and lignin; 4.0–4.8 ppm: α^1H from proteins; 6.2–7.8 ppm: aromatic, from lignin and proteins; 7.8–8.6 ppm: amide from proteins.

Soil water

Functional Group	June		October	
	Mature plot	Regenerating plot	Mature plot	Regenerating plot
Amides from proteins	6.52	6.51	4.94	3.78
Aromatic from lignin and proteins	19.14	14.2	14.19	15.12
Proteins	10.90	10.04	9.88	10.84
O-alkyl mainly from carbohydrates and lignin	27.27	30.42	31.47	32.97
Aliphatic methyl and methylene near oxygen and nitrogen	27.22	27.32	27.65	28.55

2.3.4 Groundwater:

The range of DOC and DON concentrations from groundwater DOM samples was 4-5 mg L^{-1}, and 0.09-0.14 mg L^{-1}, respectively (Figure 2.6). Many of the chemical species analyzed from the groundwater seep did not change over the course of the study period suggesting that the groundwater seep sampled was disconnected from the rest of samples collected where temporal variation was observed. Dissolved organic carbon concentrations in the groundwater seep were found to be significantly different across sampling dates ($P < 0.0001$, ANOVA, Table A.6). However, no significant difference was detected among DON concentrations sampled throughout the study ($P = 0.0710$, Kruskal Wallace, Table A.6) Iron and calcium concentrations ranged from 5-23 µg L^{-1}, and 23-25 mg L^{-1} (Figure 2.6) respectively, no significant difference was detected among any of the sampling periods.

Molar C:N ratio of groundwater sampled over the course of the experiment ranged from 39 to 62 (Figure 2.6) and significantly differed across the sampled dates ($P < 0.0001$, Kruskal Wallace, Table A.6). Molar C:N ratios in groundwater were higher during the August sampling period than the June period. The October sampling period had higher C:N ratios than either the June or August sampling period.

Despite changes in molar C:N ratio from June to October no composition changes were revealed during H-NMR analysis. Groundwater DOM H-NMR spectra in June was dominated by aliphatic hydrogen moieties occupying 68%, a

larger proportion than any other land position sampled. Other functionalities of groundwater DOM in the June period were O-alkyl (16%), proteins (10%), aromatic (5%), and amides (1%). In the October sampling period, aliphatic functionalities still dominated the H-NMR spectrum contributing a total of 69% of the total hydrogen in the DOM sample. Other functionalities decreased slightly or remained constant with O-alkyl, protein, aromatic and amide hydrogen functionalities comprising of 16%, 9%, 4%, and 1%, respectively (Table 2.4).

Figure 2.6: Average measures of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), DOC:DON ratio (molar) (± 0.4), iron, and calcium for PBEW groundwater seep where n =3. Error bars are show standard error of average measurements. Bars with dissimilar letters are significantly different. NM = not measured, BD= below detection.

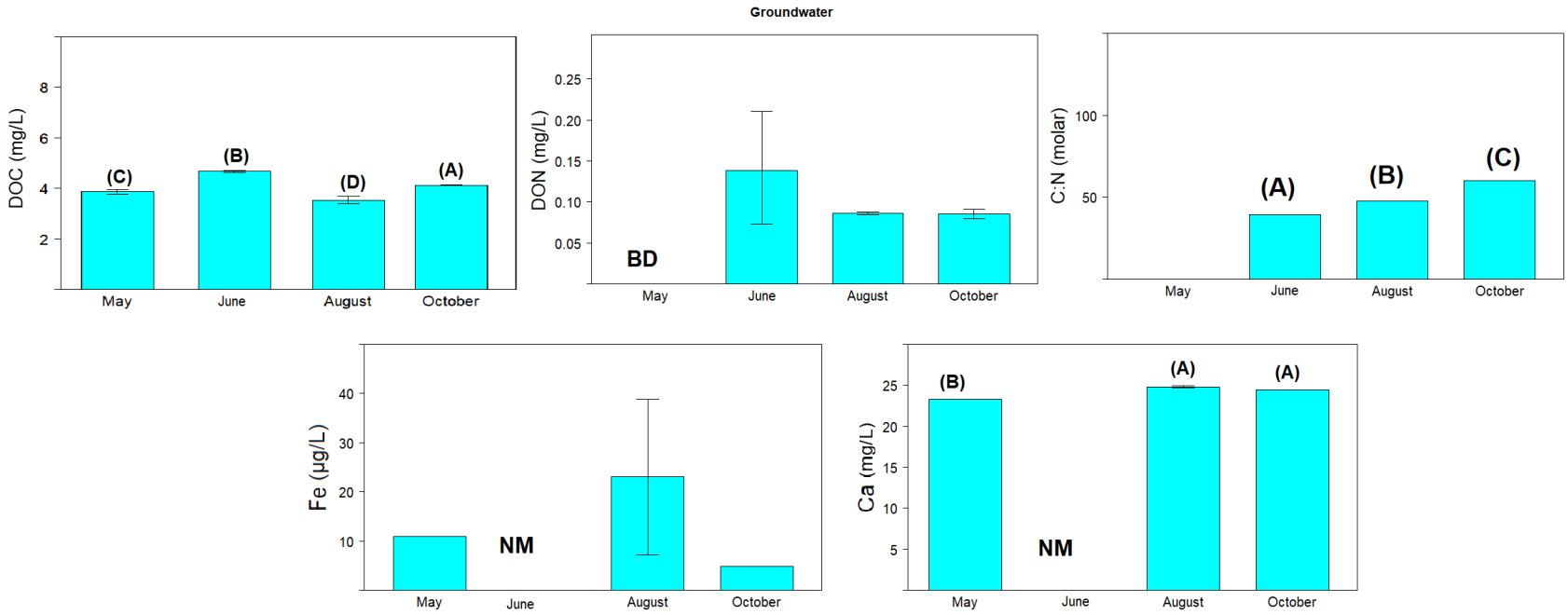


Table 2.4: Distribution of hydrogen functionalities as percent of total hydrogen as determined by hydrogen nuclear magnetic resonance (H-NMR) (500 Mhz, DMSO) for dissolved organic matter (DOM) isolated via solid phase extraction (SPE-PPL) from all groundwater samples collected in both wet and dry periods. Classification of hydrogen functional groups was based on chemical shift regions: 0.6–1.3 ppm: aliphatic methyl and methylene; 1.3–2.9 ppm: aliphatic methyl and methylene near O and N; 2.9– 4.0 ppm: O-alkyl, mainly from carbohydrates and lignin; 4.0–4.8 ppm: α^1H from proteins; 6.2–7.8 ppm: aromatic, from lignin and proteins; 7.8–8.6 ppm: amide from proteins.

Groundwater		
	June	October
Amides from proteins	0.92	1.09
Aromatic from lignin and proteins	5.22	4.28
Proteins	10.17	9.39
O-alkyl mainly from carbohydrates and lignin	16.19	15.78
Aliphatic methyl and methylene near oxygen and nitrogen	39.68	40.99
Aliphatic methyl and methylene	27.82	28.48

2.3.5 Stream water:

The range of DOC concentrations in both the upper and lower stream site was 6-10 mg L^{-1} in both lower and upper stream sites across all four sampling periods (Figure 2.7). The upper stream site exhibited highest DOC concentrations during the May sampling period, while the lower stream sites exhibited elevated DOC concentrations during the June and October sampling periods. Dissolved organic carbon concentration in the stream sites changed slightly across sampling periods and exhibited a significant interaction of sampling period by stream site elevation ($P < 0.0001$, Figure 2.7, Table A.5).

The range of DON concentration in upper stream sites was 0.08–0.28 mg L^{-1} in the upper elevation streams and exhibited a range of 0.16-0.28 mg L^{-1} in lower elevation streams (Figure 2.7). The lower stream site exhibited the lowest DON concentration during the May and June sampling periods, while the upper site exhibited the lowest concentration of DON during the August and October sampling periods suggesting that different DOM contributions in each stream. The variation in DON concentration exhibited a sampling period by stream elevation interaction ($P < 0.0001$, Figure 2.7, Table A.5).

The range of molar C:N ratio of DOM sampled from lower stream sites was 36-101 while the upper sites exhibited a range of 28–58. Molar C:N ratios of DOM in lower and upper stream sites were similar in the May sampling period, however, they had pronounced differences during the October sampling period. Variation in

molar C:N ratio stream sites exhibited a significant interaction of sampling period by stream elevation ($P < 0.0001$ Figure 2.7, Table A.5).

The range in iron concentrations in stream water collected from the lower site was 37-68 µg L^{-1}, while iron concentration in the upper site exhibited a range of 49-108 µg L^{-1}. The upper stream site was found to contain more iron the than lower site; however, both sites increased in iron concentration from the May sampling period to the October sampling period. Variation in iron concentration exhibited a significant interaction of sampling period by stream elevation ($P = 0.0126$, Figure 2.7, Table A.5).

The range in calcium concentrations in stream water collected from the lower site was 4-9 mg L^{-1}, similarly the calcium concentration in the upper elevation stream site exhibited a range of 4-8 mg L^{-1}. The upper stream site progressively increased in calcium concentration during the May, June, August and October sampling periods, while the concentration in lower site remained relatively constant during all sampling periods, except during the August date where it was elevated (Figure 2.7). The variation in calcium concentration exhibited a significant interaction of sampling period by stream elevation ($P = 0.0085$, Figure 2.7, Table A.5).

Stream DOM chemical composition, assessed via solution state H-NMR analysis of the SPE-PPL fraction, varied extensively this study. In June the lower stream DOM sample was dominated by aliphatic functionalities which comprised

66% of the total hydrogen. O-alkyl, proteins, aromatic and amide moieties contributed 19%, 4%, 9, and 2% to total hydrogen, respectively. During October DOM composition from the lower stream sample differed from what was observed in June with a lower proportion of aliphatic functionalities occupying 51% of total hydrogen. All other functionalities (O-alkyl, proteins, aromatic, and amide) increased in the relative contribution to total hydrogen during October, with O-alkyl, protein, aromatic and amide functionalities contributing 26%, 9%, 11% and 3%, respectively (Table 2.5). Dissolved organic matter isolated from the upper stream site was mainly comprised of contributions from aliphatic (57%), and O-alkyl (23%) functionalities, with minor contributions from protein (8%) aromatic (8%) and amide (2%) functionalities to total hydrogen. The upper stream DOM sample changes little between these time points in contrast with the lower stream DOM sample. Aliphatic functionalities were still the dominant functionality occupying 56% of total hydrogen present. However, O-alkyl functionalities increased to occupy 24%, while amide functionalities decreased to 3% of total hydrogen, respectively (Table 2.5).

Figure 2.7: Average measures of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), molar DOC:DON ratio (± 0.03 at the upper site, and ± 0.04 is lower sites), iron and calcium for PBEW stream sites where n=3. Upper sites are presented in green, while lower sites are shown in red. Error bars are show standard error of average measurements. Bars with dissimilar letters are significantly different. NM = not measured, BD= below detection.

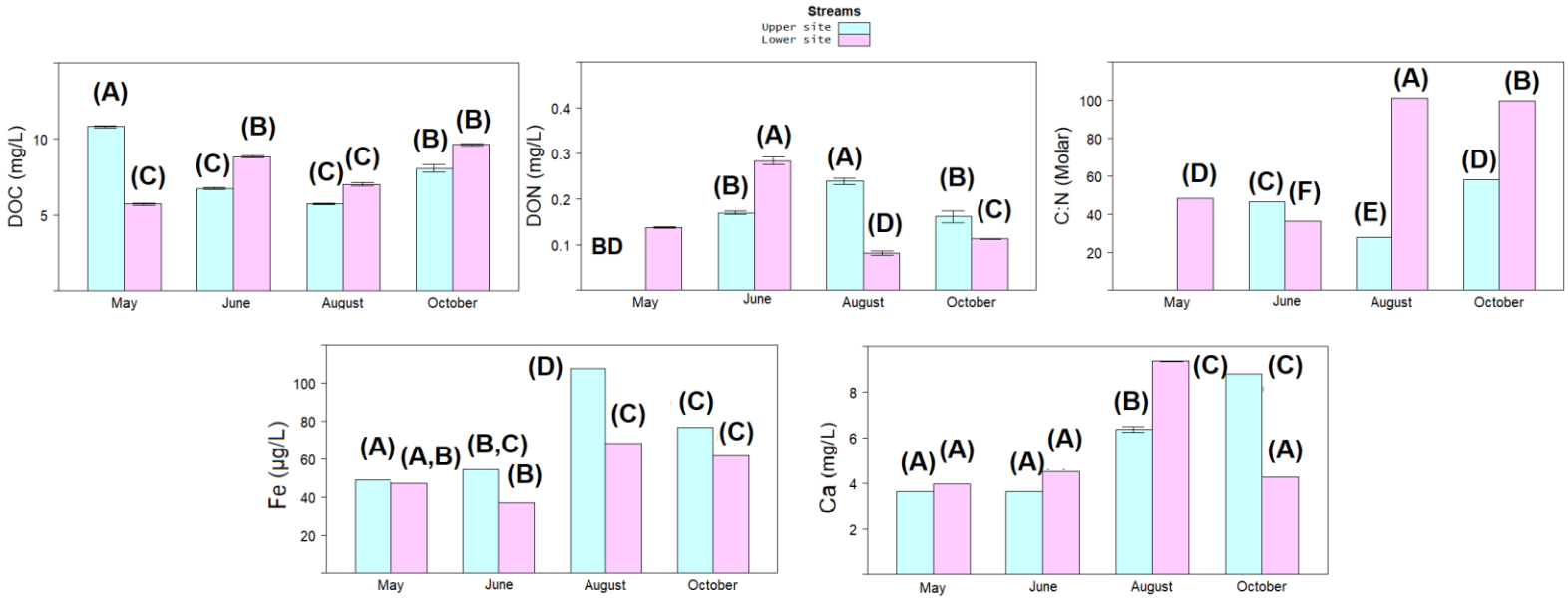


Table 2.5: Distribution of hydrogen functionalities as percent of total hydrogen as determined by hydrogen nuclear magnetic resonance (H-NMR) (500 Mhz, DMSO) for dissolved organic matter (DOM) isolated via solid phase extraction (SPE-PPL) from all stream water samples collected in both wet and dry periods. Classification of hydrogen functional groups was based on chemical shift regions: 0.6–1.3 ppm: aliphatic methyl and methylene; 1.3–2.9 ppm: aliphatic methyl and methylene near O and N; 2.9– 4.0 ppm: O-alkyl, mainly from carbohydrates and lignin; 4.0–4.8 ppm: α^{1}H from proteins; 6.2–7.8 ppm: aromatic, from lignin and proteins; 7.8–8.6 ppm: amide from proteins.

Stream water

	June		October	
	Lower site	Upper site	Lower site	Upper site
Amides from proteins	1.74	2.75	2.87	3.30
Aromatic from Lignin and proteins	9.35	8.15	11.24	7.56
Proteins	3.83	8.44	8.91	7.77
O-alkyl mainly from carbohydrates and lignin	19.32	22.55	25.85	23.81
Aliphatic methyl and methylene near oxygen and nitrogen	39.85	39.23	35.97	38.93
Aliphatic methyl and methylene	26.24	18.89	15.16	18.62

2.4 Discussion:

2.4.1 Overview

Direct contributions to the terrestrial-to-aquatic carbon flux include allochthonous inputs of carbon from precipitation, throughfall, soil and groundwater reservoirs as well as autochthonous contributions from streams, lakes and rivers (O'Donnell et al., 2010). Dissolved organic carbon composition in land positions throughout the terrestrial-to-aquatic continuum varies both temporally and regionally demonstrating variation in both source, pathway and processing of DOM en route to the aquatic environment (Boyer et al., 1997, McClain et al., 2003). Streams are an endmember of the terrestrial-to-aquatic carbon flux and thus the composition of DOM exported by streams can aid our understanding of both source and degree to which terrestrial DOM has been processed en route to the aquatic environment. It is especially important that the dynamics of DOM transferred by boreal streams is understood as boreal systems are perfused by water and are currently undergoing climatic change. To capture both the regional and temporal variability associated with contributions from each potential source along the terrestrial-to-aquatic interface, this study sampled precipitation, throughfall, soil water, groundwater and stream water in multiple positions in a watershed during multiple time points throughout the year. May and October sampling periods were collected during the wet period in the watershed, while June and August were collected during the dry period in this catchment. Quantitative and qualitative results suggest that A) precipitation and

throughfall DOM is likely an ephemeral source of carbon in boreal landscapes and B) the temporal and spatial variation in stream DOM composition observed in this study relate to the impact of flow path and hydrology that likely determines DOM source and controls the terrestrial-to-aquatic carbon flux.

2.4.2 Precipitation and throughfall DOM has a unique chemical composition but is likely labile and ephemeral source in boreal forest landscapes

Measurements of precipitation and throughfall DOM allowed for an assessment of their impact as potential sources of DOM to the terrestrial-to-aquatic carbon flux. Results produced by this study suggest that precipitation and throughfall contributes little to soil DOM fluxes in this boreal system. Dissolved organic carbon fluxes in throughfall was on average 4% of the soil carbon flux in mature plots, while DOC fluxes in precipitation was only 2% of soil carbon fluxes recorded in regenerating plots. Our regions transfer 0.14 and 0.48 mg C $(m^2\ yr)^{-1}$ in precipitation and throughfall plots respective to soil organic horizons, similar to the values reported in other studies (Mcdowell et al., 1988). Precipitation and throughfall carbon fluxes were based on DOC concentration and volume of rainwater collected during each sampling trip while soil carbon fluxes were reported in Bowering et al., in preparation. Precipitation and throughfall carbon flux data suggests that additions of DOM from precipitation and throughfall is not a major contributor to soil water DOM pools and cannot drive soil carbon fluxes in this boreal landscape. These findings are supported by solution state H-NMR analysis which found vast compositional differences between soil and

precipitation and throughfall DOM. Total percent hydrogen in DOM samples collected from precipitation is highly aliphatic in nature, opposed to soil water DOM, which exhibited elevated O-alkyl and amide functionalities (Table 2.1). Selectivity of the methodology used must also be considered. Solid phase extractions do select against O-alkyl functionalities when performed at high loading volumes (Chapter 1). As these extractions were performed at loading volumes of 7 L, it is possible that SPE-PPL DOM eluates of throughfall and precipitation were subject to this selectivity. This selectivity would result in an underestimation of O-alkyl hydrogen moieties relative to other functionalities. However, as DOM from throughfall and precipitation have relatively low contributions from O-alkyl functionalities, the resulting SPE eluate would still be representative of the bulk precipitation/throughfall sample. These findings are consistent with other comparisons of throughfall, precipitation, and soil water DOM which exhibited higher proportions of aliphatic functionalities present in throughfall and precipitation than that found in soil DOM (Bischoff et al., 2015, Feng et al., 2011).

2.4.3 Terrestrial sources of DOM within a boreal forest watershed are seasonally variable

Research on the controls on DOM composition in soil pools is vital if the terrestrial-to-aquatic carbon flux is to be constrained as these reservoirs introduce large quantities of DOM into the aquatic environment via lateral flow, or contributions from groundwater. Our results indicate that seasonal variation, as well as plot treatment, can drive variation in DOM composition and quantity in surface soils. Higher concentrations of DOC and higher C:N ratios present in mature plots compared to regenerating plots throughout the year suggest higher inputs from root exudates and litter. Hydrogen nuclear magnetic resonance analysis of the SPE-PPL fraction revealed that both mature and regenerating plots had similar DOM composition, despite differences in molar C:N ratio and DOC concentration (Table 2.2). It is possible that differences exist between the DOM composition of mature and regenerating plots, however, application of the SPE-PPL method removed these differences. Solid phase extraction has been found to select against DON rich DOM constituents, leading to an underestimation of proteinaceous and O-alkyl functionalities (Li et al., 2016, Chapter 1). As mature plots have higher C:N ratios and concentrations of DOC it is possible they also have higher contributions of those moieties, however the SPE process selectively removed proteinaceous and labile functionalities from soil solution eluates, thus making the composition of soil solution DOM collected from mature and regenerating plots similar.

Although only minor shifts in DOM composition were observed in extractions of soil solution sampled from mature and regenerating plots, large shifts in DOM composition were observed when comparing plots sampled during wet and dry periods. Larger contributions of O-alkyl and amide functionalities to total percent hydrogen in the wet period suggest that new pools of DOM were mobilized by increased litterfall and precipitation into soil DOM reservoirs. Large contributions from O-alkyl and amide functionalities present in soil water are consistent with the findings of many studies that have suggested that greater hydraulic connectivity mobilizes large quantities of fresh, bioavailable DOM present in soil solution (Qualls et al., 1991, D'amore et al., 2010, Finlay et al., 2006). This is most prominent after prolonged dry periods that allow for pools of soluble DOM to become concentrated, which is subject to mobilization upon the first storm event (Palmer et al., 2001). Our results support these findings but also suggest that temporal changes in soil DOM characterization detected with the SPE-PPL method can supersede those observed among plots of different age or disturbance. These temporal changes in soil DOM composition could have ramifications for DOM delivered to aquatic systems, as soil water is laterally transferred into stream systems, or percolates down to groundwater DOM reservoirs.

As surface soil derived DOM percolates through soil and subsurface media (i.e. till and mineral soil) it undergoes a series of biotic and abiotic reactions that give groundwater DOM a unique chemical signature; this process

has been conceptualized in the regional chromatography model (Hedges et al., 1994, Shen et al., 2015). The extent of these transformations depends on factors such as the hydraulic connectivity of the watershed and DOM residence time. My results contradict the findings of previous studies that state the groundwater DOC concentrations are better correlated to soil DOC concentrations during periods of high hydraulic connectivity (Shen et al., 2015). The similar differences between soil and groundwater DOM composition and carbon and nitrogen concentration observed in this study throughout the year either suggest that these reservoirs are disconnected, or that hydraulic variation among seasons is not variable enough to drive changes in regional chromatography within this small watershed area. Groundwater DOM samples had on average ten times less DOC and three times less DON than soil water samples, resulting in a lower C:N ratio, which is indicative of greater microbial processing relative to surface soil DOM throughout the year (Figure 2.5, Figure 2.6).

Solution state H-NMR analysis of the SPE-PPL DOM fraction of groundwater provided evidence of significant alteration of DOM as compared to surface soil DOM. Contributions from O-alkyl and amide functionalities present in soil DOM samples were replaced with larger contributions from aliphatic functionalities to total percent hydrogen in groundwater DOM samples. However, the characterization of the SPE-PPL fraction of DOM did not change over either sampling point providing additional evidence that DOM inputs to groundwater were chemically similar year-round in contrast to soil DOM. When performing

SPE-PPL it is necessary to consider how chemical selectivity may affect the resulting eluate. Groundwater DOM reservoirs had the lowest DOC concentration of many of the positions in the terrestrial-to-aquatic interface, which necessitates high loading volumes during SPE. SPE-PPL extractions performed at high loading volumes select against O-alkyl hydrogen functionalities, however, it is likely that extractions performed on groundwater are not subjected to selectivity due to low contributions from O-alkyl functionalities to total percent hydrogen (Chapter 1). Temporal variability in groundwater DOM composition was not observed in this experiment as groundwater was sourced from a natural seep that may not be hydrologically connected to the landscape studied here.

Although the groundwater seep sampled may not directly represent the seasonal effects experienced by groundwater DOM from the watershed, it may contribute to the terrestrial-to-aquatic carbon flux during periods of baseflow. Groundwater sources have been found to greatly contribute to the DOM exported from streams during the dry periods due to the low water table that inhibits lateral flow and thus contributions from soil reservoirs (Qualls et al., 1992). If this is the case, then stream water DOM should resemble a groundwater signature during the dry season, exhibiting large proportions of aliphatic hydrogen functionalities. During the wet period stream DOM may resemble a soil water signature with large contributions from protein O-alkyl and amide moieties.

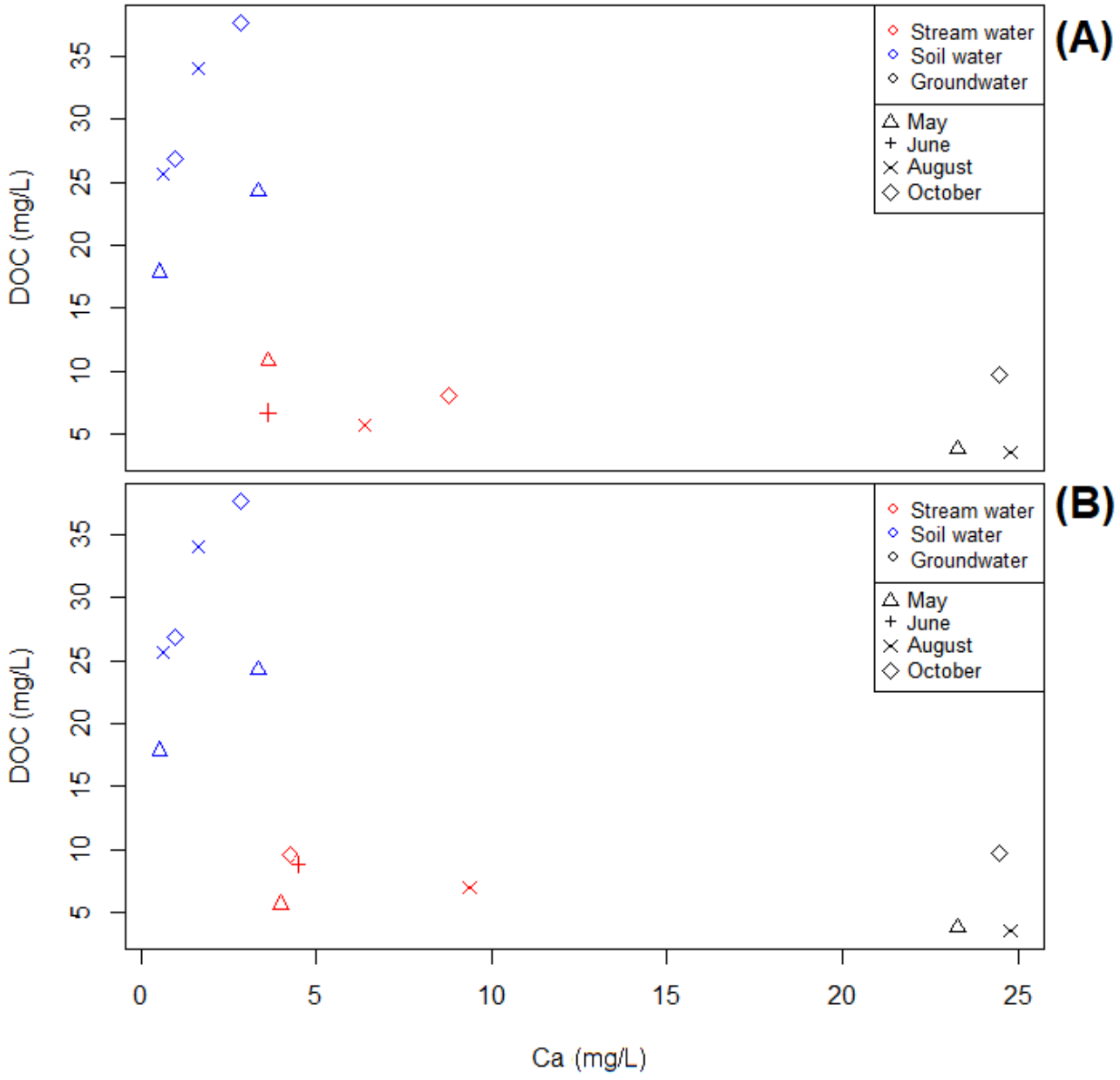
2.4.4 Variability in export of DOM from boreal forest streams is driven by regional topography and seasonal variation

Previous research has found that the composition of DOM exported from boreal forest streams reflects a mix of DOM mainly from surficial soils and deeper flow paths, however autochthonous DOM inputs were found to contribute in lower quantities (O'Donnell et al 2010). Contrary to streams, ponds and lakes receive contributions from autochthonous sources due to longer water residence times (Ito et al., 2007, Kaste et al., 2003, Lepistö et al., 2006). Additionally, the presence of these bodies of water can greatly influence the DOM characterization and quantity in the outflow of streams downslope (Hood et al., 2003). To study the DOM source contributions and dynamics of boreal forest streams as well as streams downslope of ponds this study monitored two stream sampling sites separated by ~10 km of distance. Our results support the findings of other studies that have found that more labile DOM character, indicative of soil sourced DOM, is exported from boreal streams during periods of high hydraulic connectivity (Petrone et al., 2006, Striegl et al., 2005, O'Donnell et al., 2010). High contributions of aromatic and aliphatic functionalities to total percent hydrogen present in the lower elevation stream site during the dry period shifted during the wet period to include higher proportions of O-alkyl, proteinaceous, and amide functionalities indicating a shift in DOM source from groundwater to soil water.

To support these findings and to help identify shifts in the sources of DOM contributing to stream site outflows; DOC was plotted against more conservative

species (calcium). Calcium concentration in the lower stream site remained similar year-round; this is likely due to the sampling periods which occurred during baseflow periods and calcium inputs from groundwater reservoirs being quickly cycled in the hyporheic zone before it can be measured downstream (Ford et al., 1989). Comparing DOC concentration over time, however, showed an increase in DOC concentrations that indicate that the lower stream site shifted from a groundwater to a soil water source over the periods sampled (Figure 2.8B). During the summer months, the lower stream site receives more contributions from groundwater DOM sources as soil DOM cannot be mobilized into streams via lateral flow due to the lower water table. However, during the wet period increases in precipitation and lower rates of evapotranspiration drive soil derived DOM into streams.

Figure 2.8: Concentration of calcium and dissolved organic carbon for the upper (A) and lower (B) stream samples, as well as soil and groundwater samples across the four sampling dates. Triangles represent May sampling dates, + represents June samples, x represents August sampling dates, and diamonds represent October sampling dates.



Unlike the lower stream site, the upper site's molar C:N ratio as well as the H-NMR characterization of the SPE-PPL DOM fraction stayed relatively constant over the course of the sampling periods, indicative of stable inputs to the stream throughout the year (Figure 2.7, Table 2.5). The shift in DOM composition present in the lower stream site, as opposed to the lack there of in the upper site is due to topological differences between the sites that affect the delivery of terrestrial carbon to aquatic systems. The upper site is located downstream of a sizeable pond, which occupies 1% of the total catchment area but represents a larger ~24% of the catchment areas draining to the upper elevation stream site. The DOM characterization of the upper stream site reflected high contributions from aliphatic and O-alkyl hydrogen functionalities and minor contributions from proteinaceous, aromatic and amide functionalities. No major shift in the composition of DOM was detected over the sampling periods which providing evidence that DOM inputs to the upper site were relatively constant throughout the year (Table 2.5).

To help identify differing source contributions between the two stream sites the same DOC versus calcium concentration plot was constructed for the upper site (Figure 2.8A). The two stream sites had similar behavior in the June sampling period which is consistent with greater contributions from soil water. However, during the October sampling period, the shift in source contribution that was observed in the lower site was not apparent in the upper site. Water bodies, such as the pond upstream from the upper stream site, increase water residence

time in the landscapes and therefore, have been found to increase the importance of biological controls on exported DOM and reduce the effect of hydrologic events (Kaste et al., 2003, Goodman et al., 2011). The upper sites proximity to this pond makes the streams outflow more closely represent the DOM signature of the pond, rather than a boreal forest stream.

The results of this study suggest that the character of DOM exported from boreal forest stream systems and thus its source depends on several variables including hydraulic connectivity as well as watershed topography. These features can vary regionally as demonstrated by the upper and lower stream sites. While seasonal shifts in DOM mobility in soil reservoirs can greatly affect stream DOM signatures, regional topological differences such as the presence of large bodies of water, can supersede seasonal effects by contributing autochthonous DOM inputs that would otherwise be present in negligible amounts.

2.5 Conclusion:

Recent research on DOM composition that relates to its source as well as the transformations experienced within the terrestrial-to-aquatic interface has greatly contributed to our understanding of the terrestrial-to-aquatic carbon flux. It is vital that researchers continue to constrain the terrestrial-to-aquatic carbon flux as the changing climate threatens to amplify precipitation amounts and intensity as well as plant productivity, all of which are important controls on DOM export (Evans et al., 2005, Tranvik and Jansson, 2002). By applying qualitative solution state H-NMR analysis as well as quantitative analyses I was able to track shifts in DOM composition across the terrestrial-to-aquatic interface suggesting that both watershed topography and seasonal hydraulic variation are important controls on DOM composition. This emphasizes the need for higher temporal resolution sampling to better resolve watershed connectivity and carbon fluxes. In-situ probes offer views of stream parameters at a temporal resolution impossible to achieve by discrete sampling. Combining such monitoring with methodologies such as two-dimensional NMR will enable the identification and tracking of hot spots and moments of DOM transport and transformation which is crucial to understanding the controls on the terrestrial-to-aquatic carbon flux.

2.8 References:

Aitkenhead-Peterson J A, Alexander J E and Clair T A. (2005). Dissolved organic carbon and dissolved organic nitrogen export from forested watersheds in Nova Scotia: Identifying controlling factors. Global Biogeochemical Cycle. 19(4): GB4016.

Aitkenhead-Peterson J A, McDowell W H and Neff J C. (2003). Sources, production, and regulation of allochthonous dissolved organic matter inputs to surface waters. In: Aquatic Ecosystems. 2: 25-70.

Amon R M W, Fritznar H P and Benner R. (2001). Linkages among the bioreactivity, chemical com- position, and diagenetic state of marine dissolved organic matter. Limnology Oceanography. 46(2): 287-297.

Baldock J A and Preston C M. (1995). Chemistry of carbon decomposition processes in forests as revealed by solid-state carbon-13 nuclear magnetic resonance. In: Carbon forms and functions in forest soils. 6: 89-117.

Baldock J A, Oades J M, Waters A G, Peng X, Vassallo A M, Wilson M A. (1992) Aspects of the chemical structure of soil organic materials as revealed by solid-state 13C NMR spectroscopy. Biogeochemistry. 16(1): 1–42.

Battin T J, Luyssaert S, Kaplan L A, Aufdenkampe A K, Richter A and Tranvik L J. (2009). The boundless carbon cycle. Natural Geoscience. 2: 598–600.

Bischoff S, Schwarz M, Siemens J, Thieme L, Wilcke W and Michalzik B. (2015). Properties of dissolved and total organic matter in throughfall, stemflow and forest floor leachate of central European forests. Biogeosciences. 12(9): 2695-2706.

Boyer EW, Hornberger GM, Bencala KE, McKnight DM (1997) Response characteristics of DOC flushing in an alpine catchment. Hydrological Processes 11(16): 35–47.

Cai Y, Guo L and Douglas T A. (2008). Temporal variations in organic carbon species and fluxes from the Chena River, Alaska. Limnology and Oceanography. 53(4): 1408-1419.

Clemente J S, Gregorich E G, Simpson A J, Kumar R, Courtier-Murias D and Simpson M J. (2012). Comparison of nuclear magnetic resonance methods for the analysis of organic matter composition from soil density and particle fractions. Environmental Chemistry. 9(1): 97-107.

Cole J, Prairie Y T, Caraco N F, McDowell W H, Tranvik L J, Striegl R G and Melack J. (2007). Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. Ecosystems.10(1): 172-185.

D'amore D V, Fellman J B, Edwards R T and Hood E. (2010). Controls on dissolved organic matter concentrations in soils and streams from a forested wetland and sloping bog in southeast Alaska. Ecohydrology, 3(3): 249-261.

De Troyer I, Merckx R, Amery F and Smolders, E. (2014). Factors controlling the dissolved organic matter concentration in pore waters of agricultural soils. Vadose Zone Journal, 13(7).

Decagon. (2015) ECH$_2$O-TM: Instrumental Manual. Washington

Degens ET, Kempe S, Richey JE. (1991) Chapter 15, summary: biogeochemistry of major world rivers. In: Degens ET, Kempe S, Richey JE, Eds. Biogeochemistry of major world river. Scope 42, New York: Wiley:323-444.

Del Giorgio P A and Peters R H. (1993). The influence of DOC on the bacteria-chlorophyll relationship in lakes. Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen. 25(1): 359-362.

Dittmar T, Koch B, Hertkorn N and Kattner G. (2008). A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. Limnology and Oceanography: Methods. 6(6): 230-235.

Evans C D, Monteith D T and Cooper D M. (2005). Long-term increases in surface water dissolved organic carbon: Observations, possible causes and environmental impacts. Environmental Pollution. 137(1): 55-71.

Fellman, J. B., Hood, E., Edwards, R. T., & D'Amore, D. V. (2009). Changes in the concentration, biodegradability, and fluorescent properties of dissolved organic matter during stormflows in coastal temperate watersheds. Journal of Geophysical Research: Biogeosciences, 114(G1): G01021.

Feng X and Simpson M J. (2011). Molecular-level methods for monitoring soil organic matter responses to global climate change. Journal of Environmental Monitoring. 13(5): 1246-1254.

Finlay, J., Neff, J., Zimov, S., Davydova, A., & Davydov, S. (2006). Snowmelt dominance of dissolved organic carbon in high-latitude watersheds: Implications for characterization and flux of river DOC. Geophysical Research Letters, 33(10): L10401.

Ford T E and Naiman R J. (1989). Groundwater–surface water relationships in boreal forest watersheds: dissolved organic carbon and inorganic nutrient dynamics. Canadian Journal of Fisheries and Aquatic Sciences. 46(1): 41-49.

Goodman K J, Baker M A and Wurtsbaugh W A. (2011). Lakes as buffers of stream dissolved organic matter (DOM) variability: Temporal patterns of DOM characteristics in mountain stream-lake systems. Journal of Geophysical Research: Biogeosciences, 116(G4): G00N02.

Goulden M L, Wofsy S C, Harden J W, Trumbore S E, Crill P M, Gower S T and Bazzaz A. (1998). Sensitivity of boreal forest carbon balance to soil thaw. Science. 279(5348): 214-217.

Hedges J I, Cowie G L, Richey J E, Quay P D, Benner R, Strom M and Forsberg B R. (1994) Origins and processing of organic matter in the Amazon River as indicated by carbohydrates and amino acids. Limnology Oceanography. 39(4): 743-761.

Hedges J I and Oades J M. (1997). Comparative organic geochemistries of soils and marine sediments. Organic geochemistry. 27(7-8): 319-361.

Hongve D, Riise G and Kristiansen J F. (2004). Increased colour and organic acid concentrations in Norwegian forest lakes and drinking water–a result of increased precipitation? Aquatic sciences. 66(2): 231-238.

Hood E, Williams M W and McKnight D M. (2005). Sources of dissolved organic matter (DOM) in a Rocky Mountain stream using chemical fractionation and stable isotopes. Biogeochemistry. 74(2): 231-255.

Hood E W, Williams M W and Caine N. (2003). Landscape controls on organic and inorganic nitrogen leaching across an alpine/subalpine ecotone, Green Lakes Valley. Colorado Front Range. Ecosystems. 6(1): 31-45.

Houghton, J. T. (Ed.). (1996). Climate change 1995: The science of climate change: contribution of working group I to the second assessment report of the Intergovernmental Panel on Climate Change (Vol. 2). Cambridge University Press.

IPCC (Intergovernmental Panel on Climate Change). (2007). Climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change.

IPCC (Intergovernmental Panel on Climate Change). (2013). Climate change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Ito M, Mitchell M J, Driscoll C T, Newton R M, Johnson C E and Roy K M. (2007). Controls on surface water chemistry in two lake-watersheds in the Adirondack region of New York: differences in nitrogen solute sources and sinks. Hydrological Processes: An International Journal. 21(10): 1249-1264.

Jansson M, Bergström A K, Blomqvist P and Drakare S. (2000). Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. Ecology. 81(11): 3250-3255.

Kalbitz K, Schwesig D, Schmerwitz J, Kaiser K, Haumaier L, Glaser B, Ellerbrock R and Leinweber. (2003). Changes in properties of soil-derived dissolved organic matter induced by biodegradation. Soil Biology Biochemistry. 35(8): 1129-1142

Kalbitz K, Solinger S, Park J H, Michalzik B and Matzner E. (2000). Controls on the dynamics of dissolved organic matter in soils: a review. Soil Science. 165(4): 277-304.

Kaste Ø, Stoddard J L and Henriksen A. (2003). Implication of lake water residence time on the classification of Norwegian surface water sites into progressive stages of nitrogen saturation. Water, air, and soil pollution. 142(1-4): 409-424.

Kellerman A M, Dittmar T, Kothawala D N and Tranvik L J. (2014). Chemodiversity of dissolved organic matter in lakes driven by climate and hydrology. Nature communications. 5: 3804.

Bowering K, Edwards K and Ziegler S. (2016). Fluxes of dissolved organic carbon from soil organic horizons of mature and regenerating black spruce stands. Manuscript submitted for publication.

Kling G W, Kipphut G W, Miller M and O'Brien W J. (2000). Integration of lakes and streams in a landscape perspective: the importance of material processing on spatial patterns and temporal coherence. Freshwater Biology. 43(3): 477-497.

Kögel-Knabner, I. (1997). 13C and 15N NMR spectroscopy as a tool in soil organic matter studies. Geoderma, 80(3-4), 243-270.

Lapierre J F, Guillemette F, Berggren M and Del Giorgio P A. (2013). Increases in terrestrially derived carbon stimulate organic carbon processing and CO 2 emissions in boreal aquatic ecosystems. Nature communications. 4: 2972.

Larsen S, Andersen T O M and Hessen D O. (2011). Climate change predicted to cause severe increase of organic carbon in lakes. Global Change Biology. 17(2): 1186-1192.

Lepistö A, Granlund K, Kortelainen P and Räike A. (2006). Nitrogen in river basins: Sources, retention in the surface waters and peatlands, and fluxes to estuaries in Finland. Science of the total environment. 365(1-3): 238-259.

Li Y, Harir M, Lucio M, Kanawati B, Smirnov K, Flerus R and Hertkorn N. (2016). Proposed guidelines for solid phase extraction of Suwannee River dissolved organic matter. Analytical chemistry. 88(13): 6680-6688.

Lockwood R S. (2009), Nitrogen transport, transformation and cycling through a mountain lake, Bull Trout Lake, Idaho, USA, M.S. thesis, 45 pp., Utah State Univ., Logan

Mattsson T, Kortelainen P and Räike A. (2005). Export of DOM from boreal catchments: impacts of land use cover and climate. Biogeochemistry. 76(2): 373-394.

McClain, M. E., Boyer, E. W., Dent, C. L., Gergel, S. E., Grimm, N. B., Groffman, P. M., and McDowell, W. H. (2003). Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. *Ecosystems*, *6*(4): 301-312.

McKnight D M, Harnish R, Wershaw R L, Baron J S and Schiff S. (1997). Chemical characteristics of particulate, colloidal, and dissolved organic material in Loch Vale Watershed, Rocky Mountain National Park. Biogeochemistry. 36(1): 99-124.

Mopper K, Stubbins A, Ritchie J D, Bialk H M and Hatcher P G. (2007). Advanced instrumental approaches for characterization of marine dissolved organic matter: extraction techniques, mass spectrometry, and nuclear magnetic resonance spectroscopy. Chemical Reviews. 107(2): 419-442.d

O'Brien P C and Fleming T R. (1979). A multiple testing procedure for clinical trials. Biometrics. 35(3): 549-556.

O'Donnell J A, Aiken G R, Kane E S and Jones J B. (2010). Source water controls on the character and origin of dissolved organic matter in streams of the Yukon River basin, Alaska. Journal of Geophysical Research: Biogeosciences, 115(G3): G03025.

Petrone K, Jones J, Hinzman L, and Boone R. (2006). Seasonal export of carbon, nitrogen, and major solutes from Alaskan catchments with discontinuous permafrost. Journal of Geophysical Research: Biogeosciences, 111(G2): G02020.

Pumpanen J, Lindén A, Miettinen H, Kolari P, Ilvesniemi H, Mammarella I and Ojala A. (2014). Precipitation and net ecosystem exchange are the most important drivers of DOC flux in upland boreal catchments. Journal of Geophysical Research: Biogeosciences, 119(9): 1861-1878.

Qualls R and Haines B. (1992). Biodegradability of dissolved organic matter in forest throughfall, soil solution, and stream water. Soil Science Society of America Journal. 56(2): 578-586.

Raymond, A, McClelland W, Holmes M, Zhulidov V, Mull K, Peterson J and Gurtovaya Y (2007). Flux and age of dissolved organic carbon exported to the Arctic Ocean: A carbon isotopic study of the five largest arctic rivers. Global Biogeochemical Cycles, 21(4).

Raymond P A, Hartmann J, Lauerwald R, Sobek S, McDonald C, Hoover M and Kortelainen P. (2013). Global carbon dioxide emissions from inland waters. Nature. 503(7476): 355.

Sanderman J, Lohse K A, Baldock J A and Amundson R. (2009). Linking soils and streams: Sources and chemistry of dissolved organic matter in a small coastal watershed. Water Resources Research. 45(3): W03418.

Santos P S, Otero M, Duarte R M and Duarte A C. (2009). Spectroscopic characterization of dissolved organic matter isolated from rainwater. Chemosphere. 74(8): 1053-1061.

Schlesinger W H and Melack J M. (1981). Transport of organic carbon in the world's rivers. Tellus, 33(2): 172-187.

Schumacher M, Christl I, Vogt R D, Barmettler K, Jacobsen C and Kretzschmar R. (2006). Chemical composition of aquatic dissolved organic matter in five boreal forest catchments sampled in spring and fall seasons. Biogeochemistry. 80(3): 263-275.

Shen Y, Chapelle F H, Strom E W and Benner R. (2015). Origins and bioavailability of dissolved organic matter in groundwater. Biogeochemistry. 122(1): 61-78.

Smith C Y, Moroni M T and Warkentin I G. (2009). Snag dynamics in post-harvest landscapes of western Newfoundland balsam fir-dominated boreal forests. Forest ecology and management. 258(5): 832-839.

Spencer R, Aiken G, Wickland, K, Striegl R and Hernes P. (2008). Seasonal and spatial variability in dissolved organic matter quantity and composition from the Yukon River basin, Alaska. Global Biogeochemical Cycles, 22(4).

Striegl R, Aiken G, Dornblaser M, Raymond A and Wickland K P (2005) A decrease in discharge-normalized DOC export by the Yukon River during summer through autumn. Geophysical Research Letters. 32(21): L21413.

Tranvik L J and Jansson M. (2002). Climate change (Communication arising): Terrestrial export of organic carbon. Nature. 415(6874): 861.

Tranvik L J, Downing J A, Cotner J B, Loiselle S A, Striegl R G, Ballatore T J, Dillon P, Finlay K, Fortino K and Knoll L B. (2009). Lakes and reservoirs as regulators of carbon cycling and climate. Limnology and Oceanography. 54(6): 2298-2314.

Volk C J, Volk C B and Kaplan L A. (1997). Chemical composition of biodegradable dissolved organic matter in streamwater. Limnology and Oceanography. 42(1): 39-44.

Walvoord M A and Striegl R G. (2007). Increased groundwater to stream discharge from permafrost thawing in the Yukon River basin: Potential impacts on lateral export of carbon and nitrogen. Geophysical Research Letters. 34(12): L12402.

West S G, Aiken L S and Krull J L. (1996). Experimental personality designs: Analyzing categorical by continuous variable interactions. Journal of personality. 64(1): 1-48.

Sobek S, Tranvik L J, Prairie Y T, Kortelainen P and Cole J J. (2007). Patterns and regulation of dissolved organic carbon: An analysis of 7,500 widely distributed lakes. Limnology and Oceanography. 52(3): 1208-1219.

YSI. (2009). YSI professional Plus: Instruction manual. Ohio

Ziegler S E, Benner R, Billings S A, Edwards K A, Philben M, Zhu X and Laganière J. (2017). Climate warming can accelerate carbon fluxes without changing soil carbon stocks. Frontiers in Earth Science. 5(2): 1-12.

Chapter 3: Summary and general conclusions:

Recognition of terrestrial DOM's role in aquatic and marine systems has spurred research into the controls on its export, and cycling (Hernes and Benner, 2006, Hertkorn et al., 2013). However, before researchers can elucidate the dynamics of DOM, extraction methodologies need to be evaluated for their reproducibility and recoveries for the environments they are applied too. The first chapter of this thesis addresses questions concerning DOM extraction methodologies and the differences in the composition of DOM from across the terrestrial-to-aquatic interface.

Researchers studying DOM often must apply an extraction step prior to analysis due to low concentrations of the analyte and the presence of an interfering matrix composition. Recently SPE-PPL has become a popular method to prepare DOM for analysis, however, some parameters of the SPE-PPL process such as volume of sample applied, as well as the rate at which it is applied have been hypothesized to select against major DOM constituents during extractions (Li et al., 2016). My investigation into dynamics of SPE-PPL during extractions of DOM sourced from throughout the terrestrial-to-aquatic interface in a boreal forest watershed revealed no selectivity or difference in DOC yields for extractions performed at slower versus faster flow rates. Selectivity was observed, however, in the eluates of SPE-PPL extractions performed at larger loading volumes. At loading volumes of ten liters SPE-PPL extractions of soil water selected against O-alkyl hydrogen moieties of DOM, and instead

preferentially extracted aliphatic hydrogen functionalities. This selectivity is troublesome as O-alkyl hydrogen functionalities are a major component of total percent hydrogen in many land positions across the terrestrial-to-aquatic interface. Luckily, DOM that has the largest contribution from O-alkyl hydrogen moieties, soil water, also has the largest concentration of DOM. Extractions performed on soil water often do not require high loading volumes, and thus are less susceptible to selectivity. These results suggest that SPE-PPL may be a suitable method to extract DOM from across the terrestrial-to-aquatic interface in a boreal forest watershed when applied with caution.

Categorization of DOM compounds subjected to the terrestrial-to-aquatic interface is critical in boreal ecosystems as they contain large quantities of carbon and are sensitive to climate change (Haei et al. 2010). Land positions along the terrestrial-to-aquatic interface have distinct DOM compositions due to different biotic and abiotic controls present at every land position (Fellman et al., 2009). Researchers that have attempted to constrain the export of DOM from headwater catchments, which receive inputs from across the terrestrial to aquatic interface, have reported that watershed hydrology and topography play large roles in controlling the composition and quantity of DOM exported from watersheds (Schumacher et al., 2006, Hood et al., 2003, Kellerman et al., 2014). Results from my field study supported these findings as both the quantity and composition of DOM from all land positions monitored was related to either watershed hydrology or topography. DOM export and character from soils and

streams located in boreal forests was correlated to shifts in the watershed's hydraulic flow paths. Large quantities of bioavailable O-alkyl hydrogen DOM constituents were observed in both pools during the wet period, suggesting that the elevated water table had mobilized previously immobile pools of DOM. Streams located near ponds had different DOM dynamics than streams in traditional boreal forests. This is likely because these water bodies provide a buffering effect that reduces the impact of hydraulic events on the composition of stream outflows (Kaste et al., 2003, Goodman et al., 2011). Longer residence times provided by such ponds allows for increased photochemical and biological processing of terrestrial DOM as well as in-situ production. Due to this, DOM characterization of streams located near pond areas are more likely to resemble pond outflows than traditional boreal forest streams which resemble terrestrial DOM sources throughout the year. This study revealed that hydrology plays an important role in transferring terrestrial DOM to aquatic systems, but also suggests that the concept of regional chromatography likely also applies to the lateral transport of DOM. Evidence produced by this study revealed that water residence times at each system within the terrestrial to aquatic interface in this study site directly effected the chemical composition of DOM observed at subsequent land positions.

To fully understand DOM dynamics in boreal watersheds, as well as the terrestrial-to-aquatic carbon flux, future studies should:

1) Apply similar approaches as this study and include higher resolution sampling that focus on periods of increased terrestrial and aquatic connectivity such as snowmelt. Higher resolution sampling during these periods will help to better identify controls on the degree of terrestrial processing and therefore help constrain the terrestrial to aquatic carbon flux.
2) Better inform earth system models of DOM by conducting these studies across a variety of boreal zones, as hydrologic functionating varies greatly with water availability across the boreal.
3) Trace terrestrial DOM out to marine systems. This will allow for a fully integrated view of the terrestrial-to-aquatic carbon flux and thus help to predict climate related feedbacks.

3.1 References:

Fellman J B, Hood E, Edwards R T and D'Amore D V. (2009). Changes in the concentration, biodegradability, and fluorescent properties of dissolved organic matter during stormflows in coastal temperate watersheds. Journal of Geophysical Research: Biogeosciences. 114(G1): G01021.

Goodman K J, Baker M A and Wurtsbaugh W A. (2011). Lakes as buffers of stream dissolved organic matter (DOM) variability: Temporal patterns of DOM characteristics in mountain stream-lake systems. Journal of Geophysical Research: Biogeosciences. 116(G4): G00N02.

Haei M, Öquist M G, Buffam I, Ågren A, Blomkvist P, Bishop K and Laudon H. (2010). Cold winter soils enhance dissolved organic carbon concentrations in soil and stream water. Geophysical Research Letters, 37(8): L08501.

Hernes P J and Benner R. (2006). Terrigenous organic matter sources and reactivity in the North Atlantic Ocean and a comparison to the Arctic and Pacific oceans. Marine Chemistry. 100(1-2): 66-79.

Hertkorn N, Harir M, Koch B, Michalke B and Schmitt-Kopplin P. (2013). High-field NMR spectroscopy and FTICR mass spectrometry: powerful discovery tools for the molecular level characterization of marine dissolved organic matter. Biogeosciences, 10(3): 1583-1624.

Hood E W, Williams M W and Caine N. (2003). Landscape controls on organic and inorganic nitrogen leaching across an alpine/subalpine ecotone, Green Lakes Valley, Colorado Front Range. Ecosystems. 6(1): 31-45.

Kaste Ø, Stoddard J L and Henriksen A. (2003). Implication of lake water residence time on the classification of Norwegian surface water sites into progressive stages of nitrogen saturation. Water, air, and soil pollution, 142(1-4): 409-424.

Kellerman A M, Dittmar T, Kothawala D N and Tranvik L J. (2014). Chemodiversity of dissolved organic matter in lakes driven by climate and hydrology. Nature communications. 5: 3804.

Li Y, Harir M, Lucio M, Kanawati B, Smirnov K, Flerus R and Hertkorn N. (2016). Proposed guidelines for solid phase extraction of Suwannee River dissolved organic matter. Analytical chemistry. 88(13): 6680-6688.

Schumacher M, Christl I, Vogt R D, Barmettler K, Jacobsen C and Kretzschmar R. (2006). Chemical composition of aquatic dissolved organic matter in five boreal forest catchments sampled in spring and fall seasons. Biogeochemistry, 80(3): 263-275.

Appendix for Chapter 1:

Table A.1: Results of ANOVA and Kruskal Wallace statistical tests assessing the effect of flow rate and loading volume on SPE-PPL DOC and DON recovery. (alpha = 0.05).

Experiment	Analysis:	Statistical test:	P value
Flow rate 1	DOC	Kruskal Wallace	0.27
	DON	Kruskal Wallace	0.137
Flow rate 2	DOC	Kruskal Wallace	0.4
	DON	Kruskal Wallace	0.1572
Loading volume 1	DOC	ANOVA	0.02
	DON	Kruskal Wallace	0.03
Loading volume 2	DOC	Kruskal Wallace	0.17
	DON	ANOVA	0.5072
Loading volume 3	DOC	ANOVA	0.004
	DON	Kruskal Wallace	0.0007

Appendix for Chapter 2:

Table A.2: Results of repeated measure linear mixed model assessing the effects of collection day and a treatment effect and their interaction on DOC DON calcium and iron concentration as well as molar C:N ratio.(alpha=0.05).

Experiment	Analysis	Effect test	P value
Upper Pynn's Brook Experiment Forest stand	DOC	Month	<0.0001
		Treatment	<0.0001
		Month*treatment	<0.0001
	DON	Month	<0.0001
		Treatment	<0.0001
		Month*treatment	<0.0001
	C:N ratio	Month	<0.0001
		Treatment	<0.0001
		Month*treatment	<0.0001
	Calcium	Month	<0.0001
		Treatment	0.3627
		Month*treatment	0.0025
	Iron	Month	0.0004
		Treatment	0.1645
		Month*treatment	0.1287

Table A.3: Results of repeated measure linear mixed model assessing the effects of collection day and a treatment effect and their interaction on DOC DON calcium and iron concentration as well as molar C:N ratio.(alpha=0.05).

Experiment	Analysis	Effect test	P value
Lower Pynn's Brook Experiment Forest stand	DOC	Month	<0.0001
		Treatment	<0.0001
		Month*treatment	<0.0001
	DON	Month	<0.0001
		Treatment	<0.0001
		Month*treatment	<0.0001
	C:N ratio	Month	<0.0001
		Treatment	<0.0001
		Month*treatment	<0.0001
	Calcium	Month	<0.0001
		Treatment	0.1221
		Month*treatment	0.0041
	Iron	Month	<0.0001
		Treatment	0.0554
		Month*treatment	0.0126

Table A.4: Results of repeated measure linear mixed model assessing the effects of collection day and a treatment effect and their interaction on DOC DON calcium and iron concentration as well as molar C:N ratio.(alpha=0.05).

Experiment	Analysis	Effect test	P value
Soil water	DOC	Month	<0.0001
		Treatment	0.23
		Month*treatment	0.26
	DON	Month	<0.0001
		Treatment	<0.0001
		Month*treatment	<0.0001
	C:N ratio	Month	<0.0001
		Treatment	<0.0001
		Month*treatment	<0.0001
	Calcium	Month	0.05
		Treatment	0.0002
		Month*treatment	0.12
	Iron	Month	<0.0001
		Treatment	<0.0001
		Month*treatment	<0.0001

Table A.5: Results of repeated measure linear mixed model assessing the effects of collection day and a treatment effect and their interaction on DOC DON calcium and iron concentration as well as molar C:N ratio.(alpha=0.05).

Experiment	Analysis	Effect test	P value
Stream	DOC	Month	<0.0001
		Stream elevation	<0.0001
		Month* Stream elevation	<0.0001
	DON	Month	<0.0001
		Stream elevation	<0.0001
		Month* Stream elevation	<0.0001
	C:N ratio	Month	<0.0001
		Stream elevation	<0.0001
		Month* Stream elevation	<0.0001
	Calcium	Month	0.0478
		Stream elevation	0.0009
		Month* Stream elevation	0.0085
	Iron	Month	<0.0001
		Stream elevation	<0.0001
		Month* Stream elevation	0.0126

Table A.6: Results of ANOVA and Kruskal Wallace test assessing the effect of collection day on DOC DON calcium and iron concentration as well as C:N ratio (alpha = 0.05).

Experiment	Analysis	Effect test	P value
Groundwater	DOC	ANOVA	<0.0001
	DON	Kruskal Wallace	0.0710
	C:N ratio	Kruskal Wallace	<0.0001
	Calcium	ANOVA	0.0125
	Iron	Kruskal Wallace	0.0594

Table A.8: Detection levels and concentrations for all metals excluding calcium for all terrestrial and aquatic land positions for the May sampling period.

Site:	Treatment:	Month: May								
		Al (µg L^{-1})	Fe (µg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Mn (µg L^{-1})	Na (mg L^{-1})	P (µg L^{-1})	S (mg L^{-1})	Si (mg L^{-1})
	Detection Levels:	2	2	0.02	0.001	0.4	0.01	3	0.01	0.01
Upper precipitation	Mature	12.7 (2.1)	20.1 (19.5)	0.5 (0.4)	0.3 (0.4)	49.2 (26.1)	1.8 (0.3)	15.9 (7)	0.3 (0.08)	0.04 (0.04)
	Regenerating	30.5 (15.2)	33.9 (29.9)	0.8 (0.4)	0.2 (0.07)	217.4 (236)	1.4 (0.6)	32.4 (39.7)	0.3 (0.06)	0.06 (0.04)
Lower precipitation	Mature	45.6 (28.3)	35.3 (31.6)	1.9 (1.9)	0.4 (0.3)	578.8 (943.3)	3.0 (2.3)	185.2 (242.9)	0.5 (0.3)	0.07 (0.04)
	Regenerating	49.8 (24.3)	44.5 (33.2)	1.9 (1.2)	0.6 (0.6)	657 (383.1)	32.4 (39.7)	180.2 (171.1)	0.6 (0.4)	0.08 (0.03)
Stream	Upper site	24.7	48.9	1.1	0.9	8.6	2.7	BD	0.4	0.6
	Lower site	76.7	47.1	0.9	0.9	2.9	3.1	5.7	0.4	1.3
Soil waiter	Mature	160.6	39.3	0.5	1.3	96.8	5.3	18.8	0.8	1.4
	Regenerating	129.7	124.2	0.2	0.4	112.8	1.4	35.9	0.2	0.5
Groundwater spring	N/A	8.3	10.9	0.6	2.3	0.5	3.7	4.6	0.9	3.6

Table A.9: Concentrations for all metals excluding calcium for all terrestrial and aquatic land positions for the June sampling period.

Site:	Treatment:	Month: June								
		Al (µg L^{-1})	Fe (µg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Mn (µg L^{-1})	Na (mg L^{-1})	P (µg L^{-1})	S (mg L^{-1})	Si (mg L^{-1})
Upper precipitation	Mature	6	4.2	0.07	0.01	1.7	0.2	3.9	0.09	BD
	Regenerating	BD	BD	0.02	0.01	BD	0.1	BD	0.06	BD
Lower precipitation	Mature	16.3	14.7	0.5	0.08	46.74	1.2	25.3	0.2	0.01
	Regenerating	NM	NM	NM	NM	NM	NM	NM	NM	NM
Stream	Upper site	50.9	54.3	0.2	0.9	5	2.9	4.1	0.2	0.6
	Lower site	21.3	37	0.3	1.1	9	2.9	11.1	0.3	0.4
Soil water	Mature	NM	NM	NM	NM	NM	NM	NM	NM	NM
	Regenerating	NM	NM	NM	NM	NM	NM	NM	NM	NM
Groundwater spring	N/A	NM	NM	NM	NM	NM	NM	NM	NM	NM

Table A.10: Concentrations for all metals excluding calcium for all terrestrial and aquatic land positions for the August sampling period.

Site:	Treatment:	Month: August								
		Al (µg L^{-1})	Fe (µg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Mn (µg L^{-1})	Na (mg L^{-1})	P (µg L^{-1})	S (mg L^{-1})	Si (mg L^{-1})
Upper precipitation	Mature	16.9 (6.2)	11.6 (0.4)	0.3 (0.017)	0.08 (0.005)	41.2 (0.2)	0.4 (0.01)	15.4 (1.1)	0.3 (0.08)	0.06 (0.04)
	Regenerating	10.4 (3.1)	8 (0.09)	0.1 (0.05)	0.02 (0.05)	2.2 (0.3)	0.08 (0.01)	8.8 (4.3)	0.37 (0.06)	0.01 (0.01)
Lower precipitation	Mature	22.6 (4.4)	19.8 (0.9)	0.5 (0.006)	0.1 (0.001)	64.4 (360)	0.6 (0.001)	32.6 (2.4)	0.1 (0.001)	0.07 (0.03)
	Regenerating	16.2 (1.9)	29.6 (26.5)	0.27 (0.04)	0.026 (0.002)	8 (0.1)	0.09 (0.003)	19.2 (7.6)	0.2 (0.1)	0.02 (0.003)
Stream	Upper site	16.1 (6.2)	68.2 (17.6)	0.2 (0.02)	1.5 (0.04)	17.8 (0.3)	3.17 (0.09)	16.7 (7.9)	0.3 (0.03)	0.5 (0.03)
	Lower site	7.8 (5.8)	107.7 (1.5)	0.3 (0.007)	1.9 (0.004)	3.3 (0.3)	3.8 (0.02)	1.4 (6.2)	0.5 (0.002)	1.7 (0.9)
Soil water	Mature	479.7 (667.6)	170.8 (89.2)	1.4 (1.6)	1.1 (1)	115.5 (158.7)	2.7 (1.7)	56 (80)	0.5 (0.08)	1.4 (1)
	Regenerating	323 (192.2)	216.2 (146.2)	0.5 (0.2)	0.6 (0.4)	149.9 (4.6)	2.9 (2.4)	76.2 (29)	0.3 (1.2)	1.3 (0.2)
Ground water spring	N/A	7.4 (0.5)	23 (27.4)	0.5 (0.02)	2.5 (0.02)	BD	3.7 (0.02)	9.2 (17.2)	1 (0.01)	1.2 (0.09)

Table A.11: Concentrations for all metals excluding calcium for all terrestrial and aquatic land positions for the October sampling period.

Site:	Treatment:	Month: October Al (µg L^{-1})	Fe (µg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Mn (µg L^{-1})	Na (mg L^{-1})	P (µg L^{-1})	S (mg L^{-1})	Si (mg L^{-1})
Upper precipitation	Mature	4.1 (7.2)	17.3 (5.4)	0.47 (0.32)	0.29 (0.19)	107.49 (161.80)	1.77 (0.60)	14.21 (10.98)	0.23 (0.06)	0.01 (0.03)
	Regenerating	1.5 (7)	8.9 (7.5)	0.26 (0.15)	0.18 (0.02)	25.73 (38.69)	1.41 (0.10)	4.39 (5.78)	0.18 (0.02)	0.01 (0.02)
Lower precipitation	Mature	14.4 (30)	19.6 (7.9)	0.70 (0.47)	0.37 (0.17)	203.87 (172.63)	1.90 (0.76)	76.59 (112.07)	0.30 (0.20)	0.07 (0.04)
	Regenerating	BD	6.8 (1.5)	0.18 (0.03)	0.19 (0.55)	7.46 (1.96)	1.19 (0.08)	8.36 (2.62)	0.16 (N/A)	0.05 (0.03)
Stream	Upper site	28.19	61.60	0.44	1.49	7.08	2.69	BD	0.41	1.22
	Lower site	34.29	76.67	0.31	1.01	4.31	2.54	BD	0.28	0.90
Soil water	Mature	640.02 (161.35)	175.70 (67.94)	1.09 (0.75)	0.92 (0.62)	92.71 (110.04)	3.24 (2.99)	50.90 (53.15)	0.46 (0.28)	1.39 (0.67)
	Regenerating	375.58 (280.54)	193.01 (116.84)	0.20 (0.06)	0.52 (0.19)	95.95 (42.22)	1.4 (0.92)	21.99 (11.76)	0.19 (0.08)	1.36 (0.90)
Groundwater spring	N/A	BD	4.84	0.52	2.49	BD	3.77	3.63	0.90	3.43

Table A.1: Averages of Monte Carlo simulations ran on DOC (mg/L) in both mature and regenerating plots where alpha = .05. 5000 simulations were ran estimating the amount of variability captured by deploying 1- 25 precipitation gauges in mature plots and 1-10 in forested plots. Points are the amount of variability in DON concentration (mg/L) reduced as more gauges are deployed.