# Biogeochemical Transformation in Lake Superior

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## *A Radiocarbon and Stable Isotope Study*

By

Prosper Kojo Zigah

Cambridge Scholars Publishing

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This book first published 2025

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library



ISBN: 978-1-0364-1768-0
ISBN (Ebook): 978-1-0364-1769-7

I dedicate this book to my children Ian Landon Zigah and Ivan Carter Zigah. I also dedicate this book to my parents John Zigah (deceased) and Rejoice Adetsi, and to my siblings Patience Zigah and Godwin Zigah.

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# ACKNOWLEDGEMENTS

I thank my advisors Dr. Elizabeth C. Minor and Dr. Josef P. Werne for giving me the opportunity to work in their laboratories for my doctorate degree. I also thank all my Ph.D committee members, James Cotner, Erik Brown, Richard Axler, and Steve Colman for their excellent guidance, encouragement, and direction. I thank Daniel Repeta and Ann McNichol of Woods Hole Oceanographic Institution for hosting and allowing me to use their laboratories for some of my work. My gratitude goes to the faculty at the Large Lakes Observatory for all their encouragements and support during my doctoral studies. Robert Hecky and S. Leigh McCallister were very resourceful to me throughout this work.

I thank Sarah Grosshuesch and Koushik Dutta and the staff at National Ocean Sciences Accelerator Mass Spectrometry Facility for helping with some of my laboratory analyses. Finally, I thank all my friends from University of Minnesota, University of California Irvine and Woods Hole Oceanographic Institution for their support and fun times during my doctorate studies.

The United States National Science Foundation, National Ocean Sciences Accelerator Mass Spectrometry Facility, Woods Hole Oceanographic Institution and University of Minnesota provided funding for my doctorate research.

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# PREFACE

The primary goal of the work presented in this book is to use the distributions of radiocarbon (and also stable carbon and nitrogen isotopes) to determine the sources, ages, and bioreactivity of dissolved organic matter (DOM), particulate organic matter (POM), and dissolved inorganic carbon (DIC) (in this case only sources and ages) in Lake Superior. Specific objectives include:

1. To determine the provenance and biogeochemical cycling of organic matter in the water column of the western arm of Lake Superior using higher temporal sampling resolution. The idea was to capture seasonal and annual dynamics in sources, ages, and turnover of the various carbon pools using their radiocarbon and stable isotopic compositions at the surface productive waters, deep chlorophyll layer, and the deeper hypolimnetic waters.
2. To assess the large-scale (that is the lake-wide) radiocarbon and stable carbon isotopic compositions and the carbon concentrations within DIC, DOC, and POC, and the stable nitrogen isotopic concentrations for DOM and POM, during both stratified and isothermal lake conditions. The focus here was to examine the lateral heterogeneity of the sources and biogeochemistry of organic matter in the lake using carbon concentration and isotopic distributions, and the effect of thermal stratification on the dynamics of mean ages, bioreactivity and origins of organic carbon across the lake.
3. To examine the radiocarbon and stable isotopic distributions within specific physical size-fractions of DOM, and the Nuclear magnetic resonance (NMR) spectra of high molecular weight (HMW) DOM, to more robustly constrain the sources and cycling of DOM in Lake Superior. DOM is the largest organic carbon pool in the lake, and its dynamics have implications for biogeochemistry of the lake ecosystem as well as regional climate. Finer evaluation of the sources and turnover rates of the constituent size fractions of DOM is therefore fundamental to our understanding of OM dynamics in the lake.
4. To investigate the radiocarbon and stable carbon isotopic composition of organic fractions of high molecular DOM in the lake. As with multiple sizes, DOM also contains biochemical fractions with numerous sources, reactivity, and different formation and diagenetic pathways. A

better constraint on this considerably enhances the understanding DOM biogeochemistry and roles in the lake.

5. To examine the basal food sources sustaining zooplankton in Lake Superior using natural abundance radiocarbon and stable carbon and nitrogen distributions. This ultimately will help in understanding the fate of terrestrial and old (sediment-derived) OM in the lake, that is whether it is channeled up the food web, or not, and will also provide insights into which food options are potentially critical in sustaining economically important fish in the lake.

The organization of the book is presented below.

CHAPTER ONE gives introductory overview of the sources and transformation of total or bulk organic matter and its constituent biochemical fractions, and size-fractionated organic matter. It presents the use of radiocarbon as a time and source tracer. And also, spectroscopic insights into the sources and recycling of organic matter in aquatic systems.

CHAPTER TWO addresses the first objective by evaluating the sources and cycling of DIC, DOC, and POC in the western water column of Lake Superior using multiyear distributions of radiocarbon and stable carbon isotopic compositions. Samples were taken during spring mixing and late summer thermal stratification over a two-year period (2007-2009).

CHAPTER THREE presents lake-wide distributions of the radiocarbon and stable carbon and nitrogen isotopes to gain a large-scale picture of the sources and transformations of OM in the lake. Data was collected from 8 stations across the lake sampled during the isothermal and thermally stratified lake water column.

CHAPTER FOUR examines the carbon isotopes ($\Delta^{14}C$ and $\delta^{13}C$) and concentrations of four size fractions of DOM, and the proton and $^{13}C$-NMR of HMW DOM to further investigate DOM dynamics and sources.

CHAPTER FIVE presents the natural abundance carbon isotopes ($\Delta^{14}C$ and $\delta^{13}C$) of bulk and thermal fractions of solid phase extracted (SPE) DOC and high molecular weight (HMW) DOC. It also examines the organic fractions within HMW DOC including extractable lipids, hydrolyzable carbohydrates, amino acids, and acid insoluble organic fraction (which is part of molecularly uncharacterized fraction) in order to investigate the sources, turnover times, and cycling of DOC in Lake Superior.

CHAPTER SIX presents radiocarbon ($\Delta^{14}C$) and stable isotope ($\delta^{13}C$, $\delta^{15}N$) analyses to show that mesozooplankton in Lake Superior selectively feed on within-lake produced OM even though allochthonous carbon sources represented a considerable portion of the available food resource.

CHAPTER SEVEN is a summary of the conclusions of the studies presented and recommendations for future studies.

CHAPTER EIGHT is the list of references.

# ABOUT THE AUTHOR

Dr. Zigah is the President and CEO of PKZ Climate Institute. Before then, Dr. Zigah was a visiting Assistant Professor of Chemistry in the Department of Chemistry, Biochemistry and Physics at Georgia Southern University. Professor Dr. Prosper Zigah is a scientist and a teacher. He holds professional certificates from Harvard University and University of Cambridge. He completed his Doctor of Philosophy degree (PhD) in Water Resources Science at the University of Minnesota. He holds two master's degrees in Environmental Science from Wageningen University and the University of Nottingham. He got his bachelor's degree in Agricultural Science from the University of Cape Coast.

Dr Zigah has held research scientist positions at Swiss Federal Institute of Aquatic Science and Technology, Woods Hole Oceanographic Institution and the University of Pittsburgh.

Dr. Zigah has been a visiting researcher at Harvard University, University of Massachusetts Amherst, National Ocean Sciences Accelerator Mass Spectrometry Facility, University of Florida and the International Water Management Institute.

Dr. Zigah has two lovely boys Ian Landon Zigah and Ivan Carter Zigah. He has lived and worked in several US states including Florida, Maryland, Minnesota, Massachusetts, Pennsylvania and Georgia. He has also lived and worked in the Netherlands, United Kingdom and Switzerland. In his free time, Dr. Zigah enjoys hiking, jogging, traveling, cooking, watching soccer, watching football, and writing.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Sources and biogeochemical transformations of organic matter in aquatic systems

Organic matter (OM) is a major reactive component in aquatic systems and is ultimately derived from in situ primary production (autochthonous) or from terrestrial plant debris (allochthonous). The relative importance of these sources varies, and is related to surface area, size of drainage basin, nutrient availability, and trophic status.

Measurements of organic carbon are usually used to determine the relative amounts of organic matter present in a system. In most aquatic systems, especially the oceans and large lakes (surface area > 10, 000 $m^2$), suspended particulate organic carbon (POC), usually measured on particulate organic matter collected on filters (POM) is usually a much smaller pool than dissolved organic carbon (DOC), albeit a more dynamic one and a major conduit for coupling surface water and deep water biogeochemical processes such as photosynthesis and respirations, and surface-to-sediment transport of OM (Mcknight et al. 1997; Druffel and Bauer 2000; Volkman and Tanoue 2002; Verdugo et al. 2004; McNichol and Aluwihare 2007). However, in riverine and estuarine systems, the POC pool can be comparable in magnitude to DOC (Richey et al. 1990; Fisher et al. 1998). Most studies of POM over the past several decades have been conducted to understand its fluxes, biogeochemical transformations, and fate in aquatic systems. These studies, most of which were in oceanic systems, have shed light on the transit times of sinking POM from the surface to deep ocean, and also helped in quantifying what fraction of POC produced in the surface waters is able to evade oxidation/remineralization and get sequestered in the sediments (Bacon and Anderson 1982; Lee et al. 2004; McNichol and Aluwihare 2007). Sequestration of organic matter in sediments is an important control of atmospheric carbon dioxide ($CO_2$) levels. A major fraction of POM, which becomes a larger proportion of total remaining POM in deeper waters and

surface sediments, remains molecularly uncharacterized (MUC); understanding this MUC continues to be an active field of research (Wakeham et al. 1997; Hedges et al. 2000; Hwang and Druffel 2003; Lee et al. 2004; Hwang and Druffel 2006).

Dissolved organic matter (DOM), operationally defined as the fraction of OM that passes through the 0.1-1.0 μm filter, consists of a heterogeneous mixture of biomolecular and geochemical compounds. The importance of DOM in the global carbon cycle is reflected in the fact that DOC is the largest OC pool (~90%) in the oceans and large freshwater systems, and one of the Earth's largest reactive carbon pools. The magnitude of DOC in the world's oceans (~685 Gt C) is similar to the magnitude of atmospheric $CO_2$ (Hansell and Carlson 1998; Hedges 1992). Accordingly, perturbations of DOC in terms of varying pool sizes could have considerable implications for the global biogeochemical systems. Therefore DOC fate in aquatic systems has potential implications for local, regional, and global climate dynamics if there are shifts in the rate or extent of DOC mineralization to atmospheric $CO_2$. For example, additional mineralization of just 1% of oceanic DOC would generate more atmospheric $CO_2$ than annual fossil fuel emissions (Hedges 2002; Mopper et al. 2007). DOC also plays key roles in nutrient recycling and aquatic chemistry: serving as food resource for heterotrophic bacteria and zooplankton, attenuating harmful UV-B radiation and protecting DNA and pigments from damage, pH buffering, regulating toxicity and bioavailability of pollutants and trace metals (Hedges 1992; Balakrishna et al. 2006; Klaminder et al. 2006).

Despite being one of the largest reservoirs of carbon on earth with key physicochemical and biogeochemical roles, the fate of DOC in aquatic systems is poorly constrained. Our understanding of the sources and cycling of DOC is complicated by the numerous sources, heterogeneous size and molecular distributions, and varying diagenetic transformations and biogeochemical sinks (Hedges 1992; Guo et al. 1996; McCallister et al. 2006; Beaupre and Druffel 2009). For instance, while several studies have reported the transport of terrestrial OM to oceans (~0.25 Gt DOC per year) (Opsahl and Benner 1997; Raymond and Bauer 2001; Cauwet 2002), to rivers (Saliot et al. 2001; Kaiser et al. 2004), and to lakes (Cole et al. 1994), the fate of the terrigenous OM in these aquatic systems are not well known. In the oceans, while Hedges et al. (1997) suggested that estuarine mixing processes account for the rapid degradation of terrigenous OM en route to the oceans, other studies indicate a lesser role for this process in removing terrigenous OM (Doering et al. 1994). Also, whereas Moran et

al. (1991) noted that microbial degradation in the ocean margins was the main mechanism that degrades terrigenous OM, Benner and Opsahl (2001) in their study in the Mississippi river plume indicated that photochemical oxidation was rather the main sink for terrigenous OM. In lakes, although the terrigenous OM subsidy accounts for a generally net heterotrophic nature (del Giorgio and Peters 1994; Kritzberg et al. 2005; Karlsson et al. 2007), the terrestrial OM flux and transformation pathways are not well constrained.

OM diagenetic transformation and cycling in aquatic systems is mainly mediated by microbial degradation and/or photochemical oxidation as noted by studies in rivers (Benner et al. 1995; Opsahl and Benner 1998), estuaries (Benner and Opsahl 2001; Wang et al. 2004), oceans (Miller and Zepp 1995; Miller and Moran 1997; Opsahl and Benner 1998) and lakes (McCallister et al. 2004). At surface-to-deep water integrated level, microbial mineralization is generally thought to be the dominant OM oxidation process (Cotner et al. 2004), and its extent is related to oxygen level, ionic strength, pH and temperature (Benner 2004). Photochemical oxidation of DOM occurs in the mixed surface waters where the DOM absorbs ultraviolet radiation, degrading the humic substances and other large colored dissolved organic compounds into smaller molecules (Moran and Zepp 1997). Studies show that photo-oxidation makes OM either more biolabile (through nutrient release or alteration of structure to a more edible form) or less biolabile (due to photochemical crosslinking and other OM alterations as well as production of radical oxygen species such as singlet oxygen, superoxide, peroxides and hydroxyl radical (Cotner et al. 2004)). Other processes such as enzymatic hydrolysis (Arnosti and Holmer 2003), flocculation (Fox 1983; Wang et al. 2004), sedimentation and solubilization (Hedges 1992; Prahl et al. 1994; Azam and Long 2001) and physical mixing and transport (Findlay et al. 1998; Cloern et al. 2002) could modify and/or recycle OM in aquatic systems.

Due to the numerous sources and fates of OM as noted earlier, constraining its distributions and processing is challenging, albeit fundamental to understanding the carbon sources supporting the aquatic food web. The fate of the autochthonous and/or terrestrially-derived OM in aquatic systems, and whether these OM components are permanently sequestered in the sediment, channeled to higher trophic levels in the food web or remineralized to $CO_2$, is critical to understanding the timescales of the atmospheric $CO_2$ sink, and/or generation of atmospheric $CO_2$ and to evaluating the food sources for economically important fishes. Therefore, the fate of OM in aquatic systems could potentially have implications for

regional and global climate dynamics through heat exchange and effects on radiative forcing and could impact ecosystem function (including the global fishery) as OM is the source of energy and biomass for heterotrophs.

Natural abundance stable isotopes have proven useful for identifying sources and biogeochemical processes occurring in aquatic environments (Hedges et al. 1988; Prahl et al. 1994). Stable carbon isotope ($\delta^{13}C$) values have been utilized as a tool to estimate relative OM from autochthonous and allochthonous sources based on the general $^{13}C$ depletion in terrestrial organic materials. However because of the smaller dynamic range of OM $\delta^{13}C$ values (-32 to -12‰, McCallister et al. 2004; Wakeham et al. 2006), and the considerable overlapping of $\delta^{13}C$ values of numerous OM sources, its use for inferring OM sources in aquatic systems is usually difficult. The natural abundance radiocarbon ($\Delta^{14}C$) signature in OM samples can act as a robust time, source and process tracer because of its larger dynamic range (-1000 to ~+200‰) (Petsch et al. 2001; McCallister et al. 2004; Wakeham et al. 2006). The isotopic, biomolecular, and chemical compositions of OM have been successfully utilized as proxies for identifying sources and biogeochemical processes occurring in aquatic environments (Hedges et al. 1986; Raymond and Bauer 2001; Hwang et al. 2004; Williams et al. 1992; Druffel et al. 1992). Each of these is described in detail in the subsequent sections (see sections 1.2 to 1.5). The main focus of this work is to use natural abundance radiocarbon, coupled to an additional suite of parameters ($\delta^{13}C$, $\delta^{15}N$, C:N ratio) within bulk, and size and compound fractions of OM to assess the sources, relative ages, and the potential bio-reactivity of POC and DOC (and to compare with the dissolved inorganic carbon or DIC pool) in Lake Superior, the Earth's largest freshwater lake by surface area.

## 1.2 Radiocarbon production, and its use as a time, source and process tracer

Radiocarbon is naturally generated in the upper stratosphere and lower troposphere by highly energetic charged particles (cosmic rays) colliding with molecules of the earth's atmosphere generating neutrons, which then interact with nitrogen to produce $^{14}C$ (McNichol and Aluwihare 2007). The $^{14}C$ quickly combines with oxygen into $CO_2$ and becomes incorporated into the terrestrial biosphere through photosynthesis. Radiocarbon declines with a half-life of 5730 years making it amenable for studying surficial environmental processes over the past 60,000 years

(Petsch et al. 2001). The use of $^{14}C$ for determining the age of organic materials is constrained by fluctuations in the flux of cosmic rays, dilution effect from combustion of fossil fuels and the $^{14}C$ spike in the atmosphere caused by above-ground thermonuclear weapons testing during the 1950-1960s. The bomb spike artificially elevated the $\Delta^{14}C$ values of $CO_2$ from ~ -20‰ in the pre-1950 atmospheric air to ~+1000‰ in 1964 at the peak of the above-ground bomb testing. Similarly, $\Delta^{14}C$ of oceanic surface dissolved inorganic carbon (DIC) was elevated from -50‰ before 1950 to approximately +110‰ in 1964 (Nydal 1963; Nydal et al. 1980; William et al. 1992; Bauer et al. 2001; McNichol and Aluwihare 2007).

As a result of the bomb spike, organic matter synthesized from inorganic carbon in equilibrium with atmospheric $CO_2$ has elevated $^{14}C$ content. This $^{14}C$ elevation has provided opportunity for determining the importance of modern (post 1950s or post bomb) and pre-aged (pre-1950 or pre-bomb) organic carbon in the biogeochemistry of aquatic systems, including Lake Superior. However, the atmospheric $^{14}C$ spike has been steadily declining after the 1964 (McNichol and Aluwihare 2007) due to cessation of the above-ground testing of nuclear devices and the equilibration of the excess atmospheric $^{14}C$ into the carbon cycle. This can lead to the potentially confusing situation where material photosynthesized 20 to 30 years ago will have a more enriched signature (i.e., reflect a more "modern" signal) than material being photosynthesized today. However, on decadal time-scales the approach can be applied to study ancient (pre-bomb) versus contemporary (post-bomb) material.

The natural abundance of $^{14}C$ within the DIC, POC and DOC reservoirs allows for the determination of their relative ages and the various processes that control their dynamics in the lake ecosystem. The $\Delta^{14}C$ (per mil deviation of $^{14}C/^{12}C$ ratio relative to nineteenth century wood standard) contents of carbon pools have been used by several researchers for studying carbon sources and cycling in rivers (Hedges et al. 1986; Raymond and Bauer 2001) and oceanic systems (Druffel et al. 1992; Hwang et al. 2004; Williams et al. 1992; Nagao et al. 2005). Despite the successes of these studies, $\Delta^{14}C$ has not been used to comprehensively examine the sources and biogeochemical sinks of OM in any of the great lakes of the world, including the Laurentian Great Lakes of North America.

## 1.3 Sources and cycling of size-fractionated and compound-class-fractionated DOC based on natural abundance radiocarbon composition

The $^{14}C$ signals of fraction-specific DOC such as colloidal or high molecular weight (HMW) DOC (> 1000 Da) and low molecular weight (LMW) DOC (< 1000 Da) have gained currency as a tracer of OC source and fate in aquatic systems (Guo et al. 1996; McNichol and Aluwihare 2007). These measurements, however, allow only a small fraction (< 20-40%) of the DOC reservoir to be studied as compared to the total (bulk) DOC pool (Benner et al. 1992; Guo et al. 1994; McNichol and Aluwihare 2007). Several studies have used the cross-flow ultrafiltration technique for isolating and concentrating large amounts of HMW DOC for radiocarbon and biomolecular analyses in order to investigate the provenance and reactivity of DOC (Bianchi et al. 1995; Guo et al. 1996; Loh et al. 2004). HMW DOC is usually enriched in contemporary ($^{14}C$-enriched) labile biochemicals relative to the concurrent bulk DOC and recycles rapidly. The LMW DOC on the other hand is typically depleted in $^{14}C$ as compared to the bulk DOC and recycles on a much longer time scale (Guo et al. 1995; Santschi et al. 1995; Guo et al. 1996). In their study in the Mid-Atlantic Bight (MAB), Aluwihare et al. (2002) noted that in the surface and deep waters, HMW DOC was more $^{14}C$-enriched than the bulk DOC by 22‰ and 150‰, respectively. Accordingly, HWM DOC represents the modern bio-reactive fraction of the bulk DOC whereas refractory organic molecules tend to concentrate in the LMW DOC. Different DOC size fractions could have unique origins, and be subject to varying diagenetic transformations. Therefore, it is a critical first step to characterize the amount, age, and biochemical nature of various DOC size classes in addition to the bulk DOC.

In addition to size fractions, the bulk DOC in aquatic systems can be split into different organic fractions that could have unique provenances and diagenetic transformations as well. The $^{14}C$ signatures of organic compound classes such as carbohydrates, lipids and proteins are important in teasing apart specific sources and the turnover times of molecules and compounds within the bulk DOC. Typically, however, a large amount of organic material has to be gleaned and concentrated in order for their constituent biochemicals to be isolated. Because size fractionation into HMW DOC and LWM DOC does not alter the chemical composition of the isolates and has been shown to have a low carbon blank, most compound class radiocarbon investigations are carried out on HMW DOC

(Santschi et al. 1995; Aluwihare et al. 2002; Loh et al. 2004; Wang et al. 2004; Guo and Macdonald 2006; Guo et al. 2009; Walker et al. 2011). Previous studies in various aquatic systems show that carbohydrates are the most abundant biochemicals within the HMW DOM, and are, accordingly, the most common macromolecules that have been $^{14}C$ characterized (Wang et al. 1998; Santschi et al. 1998; Loh et al. 2004; Aluwihare et al. 2002; Repeta and Aluwihare 2006). Studies in oceanic systems show that carbohydrates are typically more $^{14}C$-enriched than concurrent HMW DOM. For instance, the $\Delta^{14}C$ of carbohydrates in the surface waters of Mid-Atlantic Bight (Santschi et al. 1998), North Atlantic, and North Pacific oceans (Loh et al. 2004) were 138‰, 18‰, and 99‰ more $^{14}C$-enriched than the co-occurring HMW DOM. Repeta and Aluwihare (2006) used radiocarbon to study neutral sugars in the North Pacific Ocean, and noted that carbohydrates cycles faster with a residence time of $\leq$ 3 years in the surface ocean. Similarly, proteins recycle faster and are typically $^{14}C$-enriched relative to concurrent HMW DOC at all depths. In their study in the open Pacific and Atlantic Oceans, Loh et al. (2004) reported that protein-like substances in the surface waters were respectively 7‰ and 71‰ more enriched in $\Delta^{14}C$ than concurrent HMW DOM.

Lipids usually make up only a small fraction of HMW DOM as compared to carbohydrates (Loh et al. 2004; Wang et al. 2004). Refractory lipid compounds such as n-alkanes, alkenones, and alcohols tend to be well-preserved in aquatic systems and their distributions have been used successfully as source and/or process-specific identifiers (Meyers 1997; Pearson et al. 2000; Pearson et al. 2001; Druffel et al. 2010). In contrast to carbohydrates and proteins, lipids are usually more $^{14}C$-depleted relative to concurrent HMW DOM and total DOC due to their general refractory nature and long residence times in aquatic systems. In the North Atlantic Ocean for instance, the $\Delta^{14}C$ of solvent extractable lipids was -637‰ (compared to $\Delta^{14}C$ of HMW DOC of -5‰) in the surface waters, and -730‰ (compared to $\Delta^{14}C$ of HMW DOC of -270‰) in the deep waters (Loh et al. 2004).

## 1.4 Spectroscopic insights into the provenance and cycling of organic matter in aquatic systems

Dissolved organic matters have distinct chemical compositions, which reflect their origin and diagenetic state in an aquatic system. In order to obtain enough organic material to study chemical and molecular

characteristics and/or properties of OM via compound-class isotopic and nuclear magnetic resonance (NMR) analyses, isolation is generally necessary for concentrating material. There are several methods used for isolating and concentrating DOM depending on the desired isolate needed as some of these do alter and/or isolate specific chemical constituents of the bulk DOM. Some of the common DOM isolation approaches are briefly described here.

Ultrafiltration isolates DOM based on physical size distributions into HMW DOM and LMW DOM (see also Section 1.3), and has been used to characterize the chemical compositions and sources and processing of DOM from several aquatic environments (Minor et al. 2002; Benner et al. 1992; Hernes and Benner 2002; Helms et al. 2008). Also, ultrafiltration has been shown to have negligible blank or contamination issues (Guo and Santschi 1996). Other techniques that are used for isolating and concentrating OM are solid phase extraction techniques such as the use of $C_{18}$ membranes, alkyl chains with 18 carbons that are covalently bonded to a silicate surface (Kim et al. 2003; Simjouw et al. 2005, Minor and Stephens 2008), and XAD resins, stationary phase is made of polystyrene resin (Hedges et al. 1992). In contrast to ultrafiltration, these techniques often employ large pH changes within the sample to maximize separation and isolation of the DOM, and also chemically fractionate the bulk DOM into polar and non-polar fractions, which could be shifted in terms of sources and origins relative to the bulk material (Simjouw et al. 2005; Schwede-Thomas et al. 2005; Kruger et al. 2011).

Ultraviolet (UV)-visible spectroscopy is an analytical approach for studying DOM properties, and it is applicable to whole water samples as well as extracts and HMW DOM and can thus give an idea of the bulk chromophoric DOM as well as how isolation techniques might fractionate samples. The proportion of DOM that absorbs UV-visible light is termed chromophoric or colored dissolved organic matter (CDOM). The UV-visible absorption spectra of CDOM have been used successfully to distinguish between terrigenous and aquatic OM. The ratio of absorption at 250 nm to 365 nm (termed the $E_2/E_3$ ratio) is inversely related to molecular size, and has been used by some researchers to trace the changes in the size of DOM in aquatic systems (Peuravouri and Pihlaja 1997; Minor and Stephens 2008). The UV absorption at 254 nm normalized to DOC concentration (called $SUVA_{254}$) has been shown to correlate strongly with the aromaticity of DOM (Weishaar et al. 2003; Minor et al. 2006), and is therefore a good proxy for terrestrial input. The spectral slope (S, $nm^{-1}$) is computed from the absorption data from the

equation: $\alpha_\lambda = \alpha\lambda_{ref}\, e^{-s\,(\lambda - \lambda ref)}$, where $\alpha$ = Napierian absorption coefficient in $m^{-1}$, $\lambda$ = wavelength in nm, and $\lambda_{ref}$ = reference wavelength in nm (Helms et al. 2008). This index is inversely related to molecular weight, and provides insights into the photochemistry, source and cycling of CDOM (Helms et al. 2008).

Nuclear magnetic resonance (NMR) spectroscopy is another powerful analytical approach for studying the broad overall chemical composition of DOM. NMR is non-destructive and allows measurement of the distributions of the major functional groups in DOM. The NMR technique works by determining the chemical environments around nuclei with magnetic moments by looking at the magnetic properties of these nuclei (e.g., $^{13}C$, $^{1}H$, $^{15}N$, $^{31}P$) when placed in strong magnetic fields (Mopper et al. 2007). The peaks or resonances in specific chemical shifts in the NMR spectra could indicate the presence of specific chemical groups or biochemical classes such as carbohydrates, proteins, and lignin (Repeta et al. 2002; Baldock et al. 2004; Abdulla et al. 2010; Gogou and Repeta 2010). NMR spectroscopy has been used to study DOM sources and cycling in several aquatic systems (Malcolm 1990; Benner et al. 1992, Hedges et al. 1992; Aluwihare et al. 2002; Repeta et al. 2002; Bianchi et al. 2004; Abdulla et al. 2010; Gogou and Repeta 2010) based on relative composition and variations in carbohydrates, proteins, aliphatic and aromatic components.

# CHAPTER TWO

# RADIOCARBON AND STABLE CARBON ISOTOPIC INSIGHTS INTO PROVENANCE AND CYCLING OF CARBON IN LAKE SUPERIOR

## 2.1 Introduction

The biogeochemical cycling of carbon in aquatic ecosystems has gained increased attention in the past few decades due to global climate changes encompassing increases in atmospheric $CO_2$, potential hydrologic accelerations and additional attendant ecosystem changes. Due to their proximity to terrestrial environments and the associated delivery of allochthonous organic carbon, inland aquatic systems can function simultaneously as both sinks and sources of atmospheric $CO_2$ (Cole et al. 2007). Multiple biogeochemical processes such as carbonate buffering, heterotrophic respiration and sedimentary sequestration of organic carbon (OC) allow inland aquatic systems such as Lake Superior to outgas $CO_2$ to the atmosphere while concurrently burying OC at depth (Kritzberg et al. 2005; Urban et al. 2005). The bioavailability and cycling of carbon is also important in within-ecosystem processes as inorganic carbon is a basic building block for autotrophy and labile OC provides necessary carbon and energy sources for heterotrophy.

The in situ biological and chemical transformations of OC in aquatic systems are largely dependent on its source, physical packaging (e.g., mineral associations) and prior diagenetic alterations in both the water column and surface sediments (Kaiser et al. 2004). However, the sources and processing of OC are poorly constrained in many ocean, river and lake systems even though delineating these is critical in understanding their transformation pathways and ultimate fate. For instance, even though considerable evidence exists for transport of terrestrial OC to the oceans (Opsahl and Benner 1997), rivers (Raymond and Cole 2003), and lakes (Cole and Caraco 2001), the fate of terrestrial OC in these ecosystems is not well known.

Natural abundance stable and/or radiocarbon isotopes have been useful for studying organic carbon sources and carbon cycling in the oceans (Druffel et al. 1992; Bauer et al. 2002), estuaries (Peterson et al. 1994), rivers (Kaiser et al. 2004; Raymond and Bauer 2001), and lakes (Karlsson et al. 2007; McCallister et al. 2008). The decadal scale resolution added by the spike in $\Delta^{14}C$ resulting from 1950s above-ground nuclear testing has been employed to study C cycling and turnover in both terrestrial and aquatic environments (McCallister et al. 2004; Trumbore 2009) and has provided an additional timescale to that offered by radioactive decay methods. While such studies of 'modern' processes in aquatic ecosystems have yielded insight into C transformation and cycling, to date there has not been a radiocarbon study investigating carbon cycling in any of the great lakes of the world, including the Laurentian Great Lakes of North America (Cotner et al. 2004). Therefore this study (a time series from an open-lake station and a nearshore site in Lake Superior) was designed to assess sources, ages and relative reactivity of particulate and dissolved OC (POC and DOC, respectively) in a temperate and relatively pristine large lake and to compare the stable- and radio- carbon isotopic composition of lake OC with that of co-occurring dissolved inorganic carbon (DIC).

Lake Superior is the Earth's largest freshwater lake by surface area (8.2 x $10^{10}$ $m^2$) with maximum and mean depths of, respectively, 406 m and 150 m (Urban et al. 2005). The lake is biogeochemically similar to open-ocean locations due to its oligotrophic nature, low terrestrial nutrient loading and the dominance of its microbial food web on carbon cycling (Cotner et al. 2004). However, unlike the oceans, Lake Superior is dimictic, thus there is density-driven complete vertical mixing of the water column in spring and early winter each year which homogenizes the water column. The dimictic nature of Lake Superior provides a unique opportunity to study how organic matter (OM) from different allochthonous and autochthonous sources can vary in processing and reactivity on short time scales (annual to decadal), an aspect of OM dynamics that is not amenable to in situ study in the oceans since exchange of OM between surface and deep waters generally occurs over very long time scales (McNichol and Aluwihare 2007).

## 2.2 Methods

### 2.2.1 Sampling

Multiple cruises were undertaken on the R/V *Blue Heron* to sample the thermally stratified water column in August or September 2007, 2008,

2009 and the isothermal (mixed) water column in May or June 2008, 2009. Water samples were collected from an open-lake (OL) site (47º19.20'N, 89º49.49'W), chosen to take advantage of data from a National Oceanic and Atmospheric Administration (NOAA) buoy (National Data Buoy Center (NDBC) station 45006) and sediment trap moorings within 1-2 km of the site, and a nearshore site (site Baptism River (BR), 47º19.95'N, 91º11.51'W**)** just offshore of the Baptism River in the western arm of Lake Superior (Fig. 2-1). At the offshore site (total water depth approximately 165 m), three water depths were sampled each time: the surface (5 m), the depth of the summer deep chlorophyll maximum (DCM) (30 m), and the deep hypolimnion (127 m). The nearshore site (total water depth ~21 m) was sampled only at 5 m.

Water samples were collected via Niskin bottles mounted on a Seabird model 911 plus conductivity, temperature, and depth (CTD) rosette equipped with fluorometer, transmissometer, dissolved oxygen sensor, photosynthetically active radiation (PAR) sensor, pH meter, and altimeter. DIC samples were taken directly from the Niskin bottles by rinsing three times with sample and then overflowing two volumes of the unfiltered water into previously acid-cleaned and combusted (450ºC for 4 hours) 500 mL amber Pyrex bottles. Note that these are technically total inorganic carbon samples but Lake Superior water does not contain measurable particulate inorganic carbon. After removing a known and consistent headspace, the samples were immediately preserved with saturated mercuric chloride solution, sealed air-tight with glass stoppers coated with Apiezon grease, and stored at room temperature in the dark.

Water samples for POC and DOC were filtered through pre-combusted Whatman GF/F glass fiber filters (450ºC for 4 hours; 0.7 µm nominal pore size) via nitrogen- pressurized stainless-steel canisters. For each DOC sample for radiocarbon analysis, approximately 1 L of the resulting filtrate was collected into an acid-leached and combusted glass bottle. For each DOC concentration measurement, approximately 40 mL of the filtrate was collected into an amber glass vial (previously acid-leached and combusted). Both types of DOC samples were preserved by acidifying to pH 2 (using 6 mol $L^{-1}$ HCl, American Chemical Society (ACS) Plus grade) and stored refrigerated. After ~10 L of lake water had passed through a GF/F filter, the filter and retained particulate matter was removed from the stainless steel holder, folded, placed in previously-combusted aluminum foil and stored frozen until analysis.

To ensure that our sampling techniques are appropriately clean, a large-volume ultra pure water (Millipore Milli-Q Plus) blank was processed by filtering through the canister set-up. This large volume blank consisted of > 100 L of Milli-Q water (note that this is ten times the volume of lake water filtered for POC samples) and was designed to provide enough carbon for a radiocarbon measurement.

Sediment cores from the open-lake site were taken in October 2008, and June and August 2009 using an Ocean Instruments multi-corer. Upon recovery, the overlying water was collected via acid-cleaned syringe to just above the flocculant layer and sediments (with the flocculant layer included in the first section) were sectioned at 2-cm resolution with the depth 'slices' placed in pre-combusted glass jars and stored refrigerated for the duration of the cruise. Upon return to shore, within 9 days of coring, the overlying water DOC and sedimentary pore-water DOC from each depth slice were obtained by centrifuging and filtering the supernatant through pre-combusted glass fiber filters. These pore-water DOC samples were then acidified to pH 2 and stored refrigerated until analysis.

Mesozooplankton samples were collected at site OL via vertical net tows (using a 300 μm mesh net) from 50 m to the water surface. Sampling was done at night, thus taking advantage of zooplankton diurnal migration patterns and maximizing biomass collection for radiocarbon analysis. The biomass was rinsed with lake water into the cod end of the net and concentrated onto glass fiber filters (pre-combusted GF/F filters, 0.7 μm pore size), which were placed in combusted aluminum foil and stored frozen.

Amity Creek was sampled just above its confluence with the Lester River in June and September 2008 during stormflow and baseflow conditions, respectively. Amity Creek drains a small, primarily forested watershed in the western arm of Lake Superior. It flows into the Lester River less than 0.5 km before that river enters western Lake Superior, and is conveniently located near the Large Lakes Observatory. Baseflow samples were taken as grab samples using acid-cleaned carboys while storm flow samples were taken using a Sigma 900 autosampler holding 24 acid-cleaned sample containers, with sampling triggered by stage height changes as determined by a pressure sensor. Whole water samples were removed from the autosampler within 24 hours of the storm event. Both baseflow and stormflow samples were filtered through previously-combusted GF/F filters to isolate POC vs. DOC. The baseflow DIC sample was collected by

overflowing two volumes of the unfiltered water into previously acid-cleaned and combusted amber Pyrex bottles. After removal of a known headspace, the sample was preserved with saturated mercuric chloride solution, air-tight sealed and stored at room temperature in the dark until analysis.

In September 2009, corn leaves (*Zea mays*) were collected from the watershed of western Lake Superior in order to determine the radiocarbon content of atmospheric $CO_2$. The sampling site was chosen to minimize fossil contamination (i.e., avoiding highways). The collected leaves were stored in perforated paper envelopes and refrigerated.

All sampling activities were carried out carefully to avoid $^{14}C$ contamination. Powder-free nitrile gloves were used during sampling. Plastic tubing (silicone, Teflon, and polypropylene) was rigorously cleaned with dilute HCl, and rinsed with ~10 L of distilled water. All other plastic ware was cleaned with soap and distilled water, leached with 10% (by volume) HCl in water, and then rinsed with distilled water. Glassware was cleaned in the same manner, followed by combustion at 450ºC for > 4 hours. The R/V Blue Heron was free of $^{14}C$ contamination during a radiocarbon swipe test at the beginning of the project. Subsequent $^{14}C$ tracer work has been limited to a radiation van loaded and unloaded from the boat specifically for tracer work.

### 2.2.2 Concentration measurements

DOC concentrations were measured via high temperature catalytic combustion on a Shimadzu total organic carbon (TOC)-Vcsh analyzer, except for 2007 samples, which were analyzed by heated persulfate oxidation on an Oceanography International Corporation (OI) Analytical 1030 W TOC analyzer. Aliquots of the acidified DOC were bubbled with high grade $CO_2$-free air for 3.3 minutes to remove all inorganic carbon, 50 μL was then combusted at 680ºC, and the evolved $CO_2$ was measured by a non-dispersive infrared (NDIR) detector. The TOC analyzer was calibrated using potassium hydrogen phthalate (KHP) and additional KHP standards were interspersed and analyzed along with the samples to assess instrumental performance. For each sample, three injections were performed. If the standard deviation was not ≤ 2.5%, two more injections were performed and the closest three of the five injections were averaged to yield sample concentration (Minor and Stephens 2008).

DIC samples were also analyzed on the Shimadzu TOC-Vcsh Analyzer. In this case, the analyzer was calibrated using primary standard grade sodium carbonate and ACS reagent grade sodium bicarbonate. The inorganic carbon in the sample was volatilized with 25% ACS grade $H_3PO_4$ (by weight) in a $CO_2$-free closed reaction vessel, and the $CO_2$ evolved was measured by the NDIR gas detector.

Suspended particulate organic matter (POM) samples were either freeze-dried or oven dried to constant weight at 60°C, and homogenized, fumigated with 12 mol $L^{-1}$ HCl (ACS Plus grade) overnight to remove carbonates, dried again, and cooled in a dessicator. They were then loaded into tin capsules and analyzed for particulate organic carbon (POC) and particulate organic nitrogen (PON) concentrations on a Costech ESC 4010 elemental analyzer.

### 2.2.3 Radiocarbon and stable carbon isotope measurements

All isotope measurements were performed at the National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS) at the Woods Hole Oceanographic Institution (WHOI), with the exception of the corn leaves sample, which was measured at the Keck Carbon Cycle AMS Facility (KCCAMS) at University of California, Irvine. Most water-column and porewater DOC samples were processed for radiocarbon measurement at NOSAMS by ultraviolet (UV) oxidation using a protocol based upon Beaupre et al. (2007) but oxidizing for three hours rather than four. DOC samples from September 2007 were freeze-dried before combustion to $CO_2$. In both approaches, the evolved $CO_2$ was trapped in a vacuum line, purified cryogenically and reduced to graphite with $H_2$ over Fe catalyst. A subsample of the purified $CO_2$ was collected for $\delta^{13}$C-DOC measurement.

Suspended particulate organic carbon and mesozooplankton biomass were oven-dried to constant weight at 60°C, fumigated with 12 mol $L^{-1}$ HCl (ACS Plus grade) for 24 hours to remove carbonates, re-dried, and combusted to $CO_2$ in a modified Carlo Erba NA1500 elemental analyzer. The evolved $CO_2$ was separated from the carrier gas, cryogenically trapped, and stored in a modular manifold, and then reduced to graphite. A subsample of the purified $CO_2$ was taken for $\delta^{13}$C-POC measurement.

DIC samples were directly hydrolyzed with $H_3PO_4$, and the resulting $CO_2$ was ‘stripped’ with nitrogen gas and trapped. The evolved $CO_2$ was cleaned cryogenically and reduced to graphite. A portion of the cleaned $CO_2$ was taken for $\delta^{13}$C-DIC measurement.

The radiocarbon content of atmospheric $CO_2$ was determined at KCCAMS using the protocol of Hsueh et al. (2007). The corn leaves sample was cleaned with ~5 L (in 5 separate rinses) of Milli-Q water to remove extraneous particles on the leaves, and dried to a constant weight for ~30 hours at 60ºC. The dried sample was homogenized, combusted to $CO_2$ and graphitized as outlined in Santos et al. (2004). A subsample of the $CO_2$ from the corn leaves was taken for $\delta^{13}C$ analysis at KCCAMS using a gas bench coupled to a Finnigan Delta Plus Isotope Ratio Mass Spectrometer (IRMS).

In all cases, the graphite produced was compacted onto an aluminum cartridge target and analyzed by accelerator mass spectrometry (AMS) along with primary and secondary standards, and combustion and graphitization process blanks. Radiocarbon values are reported as $\Delta^{14}C$, the part per thousand deviation of the sample's $^{14}C$:$^{12}C$ ratio relative to a nineteenth century wood standard that has been corrected to the activity it would have had in 1950 and a $\delta^{13}C$ of -25‰. $\Delta^{14}C$ was corrected for fractionation using $\delta^{13}C$ of samples according to the convention of Stuiver and Polach (1977). Instrumental precision of $\Delta^{14}C$ analysis based on error of standards or multiple analyses on a target are explicitly given in Table 3, and ranged from 2‰ to 10‰. Total measurement uncertainties for $\Delta^{14}C$ analyses based on measurement of duplicate natural samples were 6‰ for $\Delta^{14}C$-DIC, and 16‰ for $\Delta^{14}C$-POC.

Combusting our large-volume blank 'POC' sample generated 94.75 μmol of C (or <0.95 μmol C $L^{-1}$), enough carbon for a radiocarbon analysis although this carbon would also include contributions from the MilliQ water as well as the filtration apparatus. The $\Delta^{14}C$ and $\delta^{13}C$ values of the large volume blank POC sample were -95 ± 3‰ and -27.3‰, respectively. For additional comparison, combusting a 'clean' GF/F filter generated 2.19 μmol of C. While this GF/F blank did not provide sufficient carbon for a radiocarbon analysis, it may be more representative of the amount of blank carbon in our samples, as it is not subject to the addition of carbon from 100 liters of MilliQ water. Using this GF/F filter blank and our lowest concentration POC sample, our processing techniques contribute 9% or less to the carbon identified in POC samples. Estimates of blank-carbon in our samples based upon the large-volume blank ranged from 6.0% to 37.7% of the carbon identified in our POC samples and the resulting radiocarbon correction would shift samples an average of 23‰ (with a standard deviation of 15‰) toward more $^{14}C$-enriched values. Basic depth and locational trends remained similar in the blank-adjusted and measured $\Delta^{14}C$ values but the extent of the depth variations is less