



An investigation of size-fractionated organic matter from Lake Superior and a tributary stream using radiocarbon, stable isotopes and NMR

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Abstract

This study investigated the concentration and isotopic composition of different size fractions of organic matter (OM) in Lake Superior and in one of its many tributary streams and rivers (Amity Creek, Duluth, Minnesota, USA). Structural compositional drivers of the $\Delta^{14}\text{C}$ of high molecular weight (HMW, >1 kDa) dissolved organic carbon (DOC) in the lake were also evaluated. Low molecular weight (LMW, <1 kDa) DOC was the fraction containing the largest proportion (68–88%) of organic carbon (OC) in the lake. Particulate organic carbon (POC, >0.7 μm) was generally ^{13}C -depleted ($-29 \pm 1.2\text{‰}$) relative to “bulk” (<0.7 μm) DOC ($-26.4 \pm 0.7\text{‰}$), “init” (<0.2 μm) DOC ($-26.6 \pm 0.8\text{‰}$), HMW DOC ($-26.9 \pm 0.3\text{‰}$) and LMW DOC ($-26.5 \pm 0.9\text{‰}$), and had more variable ^{14}C content ($\Delta^{14}\text{C}$ of -94‰ to 53‰ ; 735 years BP to modern) than the other size fractions. Init DOC ($\Delta^{14}\text{C}$ of 17–59‰), HMW DOC ($\Delta^{14}\text{C}$ of 23–64‰) and LMW DOC ($\Delta^{14}\text{C}$ of 16–62‰) all reflected contemporary (modern) radiocarbon signatures. Bulk DOC ($\Delta^{14}\text{C}$ of -19‰ to 57‰ ; 90 years BP to modern) had modern radiocarbon values in the offshore sites ($\Delta^{14}\text{C}$ of 2–57‰) with pre-aged samples ($\Delta^{14}\text{C}$ of -8‰ to -19‰) seen at the nearshore site. HMW DOM was relatively N-poor (C:N of 12–19) compared to particulate organic matter (POM, C:N of 8–10) revealing either a more diagenetically altered state or contrasting sources. ^{13}C NMR data showed that biochemical composition of HMW DOC in Lake Superior was dominated by carbohydrates (53–65%) with only trace aromatic components (2–4%). Structurally complex components such as heteropolysaccharides (HPS), amide/peptides and amino sugars (AMS) constitute 75–84% of HMW DOC whereas carboxylic-rich alicyclic molecules (CRAM) made up 16–25% of HMW DOC in the lake. Combined HPS and AMS, O-alkyl carbohydrate carbon, and total carbohydrate carbon contents were significantly positively correlated to the $\Delta^{14}\text{C}$ of HMW DOC suggesting they contribute a contemporary ^{14}C -enriched component to the HMW DOC in the lake. In contrast, CRAM and aliphatic carbons were significantly inversely correlated with $\Delta^{14}\text{C}$ of HMW DOC implying that these represent ^{14}C -depleted (older) components of HMW DOC in the lake. At Amity

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Creek, storm events led to larger loads of bulk DOC and POC, which were both ^{14}C -enriched (modern). However, in baseflow conditions ^{14}C -depleted bulk DOC and POC were exported. LMW DOC from the creek was considerably ^{14}C -depleted compared to concurrent HMW DOC.

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1. INTRODUCTION

Organic matter in aquatic systems exists as a continuum of sizes, although, in practice, our view of it is split into discrete, operationally defined pools, based upon isolation and characterization approaches: particulate OM ($\geq 0.7\ \mu\text{m}$ in this study) and dissolved OM ($< 0.7\ \mu\text{m}$ or $< 0.2\ \mu\text{m}$ in this study) fractions, with the dissolved OM often further subdivided based on ultrafiltration into HMW DOM ($> 1\ \text{kDa}$) and LMW DOM ($< 1\ \text{kDa}$) (Amon and Benner, 1996; Guo et al., 1996; Mannino and Harvey, 2000; Minor et al., 2002; Loh et al., 2004; Verdugo et al., 2004). Each OM size class (relative to others from the same aquatic system) may have distinct source, chemical and molecular composition, reactivity, and biogeochemical functions (Guo et al., 1995; Skoog and Benner, 1997; Mannino and Harvey, 2000; Loh et al., 2006; Guo and MacDonald, 2006).

The influence of molecular size (or weight) on the microbially-mediated and photochemical fate(s) of OM has received much attention in the past few decades, but is not yet well constrained. While some studies reported that LMW DOM is more bioreactive and preferentially used by microbes (Wright and Hobbie, 1965; Barber, 1968; Ogura, 1975, 1977; Saunders, 1976; Munster and Chrost, 1990; Sundh, 1992), others have reported that bioreactivity decreases with OM size, and that HMW DOM is, therefore, more bioreactive and rapidly degraded by microbes than LMW DOM (Benner et al., 1992; Amon and Benner, 1994; Skoog and Benner, 1997; Mannino and Harvey, 2000; Hama et al., 2004; Loh et al., 2004). In their study of various freshwater and marine environments, Amon and Benner (1996) incubated bacteria with different size fractions of OM and reported greater bacterial utilization (growth and respiration) of HMW DOM compared to LMW DOM (although bacterial growth efficiency was higher in LMW DOM), implying a size-reactivity continuum model which states that bioreactivity increases with increasing OM molecular size. In contrast (or perhaps in refinement) to this size-reactivity model, however, others have reported temporal variability in the relative utilization of HMW DOM vs. LMW DOM by microbes such that a portion of LMW DOM is utilized first (during the initial 20 days of the dark incubation experiment) and rapidly, but that the proportion of HMW DOM that is mineralized increases over time (after 20 days in the 10-month experiment) (Ogura, 1977). Other observations show that only a portion of HMW DOM is rapidly mineralized (in hours to few days), and by extension, some portion persists over longer timescales (Meyer et al., 1987; Tranvik, 1990; Arnosti et al., 1994). The combination of these observations shows that additional factors, such as intrinsic chemical composition, reactivity as an enzymatic substrate, or

physical availability, rather than simply size, are involved in determining OM fate.

The challenge in OM studies is that many measurements, including compound class or molecular-level characterization and many isotopic analyses, require or are strongly facilitated by isolation (often filtration, and thus size-fractionation) approaches to concentrate and desalt aquatic samples. However, natural abundance radiocarbon ($\Delta^{14}\text{C}$) signals of isolated fractions of DOC such as HMW DOC only give insight into a proportion ($< 20\text{--}40\%$) of the DOC reservoir (Benner et al., 1992; Guo et al., 1995; McNichol and Aluwihare, 2007). But when coupled to the radiocarbon composition of concurrent bulk DOC, LMW DOC and POC, useful insights into the source and fate of OC can be gained (Guo et al., 1996; McNichol and Aluwihare, 2007). The radiocarbon (^{14}C) age of OM size fractions has traditionally been thought to predict/reflect relative biological reactivity; and the observed relationship between molecular size and ^{14}C age in the open ocean, coupled with the idea of the size-reactivity continuum model, lends support to this hypothesis. Loh et al. (2004) in their study in the open ocean reported that ^{14}C age is inversely related to OM size; the age of OM increased in the sequence from sinking POM (youngest, rapidly cycling) $>$ suspended POM $>$ HMW DOM $>$ LMW DOM (the oldest, cycling slowly). Similar trends in $\Delta^{14}\text{C}$ of size-fractionated OM have been seen in other oceanic sites (Guo et al., 1996, 2009; Aluwihare et al., 2002).

Observed size-age relationships in freshwater systems provide an interesting contrast to marine systems. Most of these studies have focused primarily upon river systems and in these systems radiocarbon trends do not conform to the OM size-age sequence seen in the oceans. In the Amazon, Hudson, York, Susquehanna, Delaware, and Parker Rivers in the USA (Raymond and Bauer, 2001; Raymond et al., 2004; Caraco et al., 2010), Strickland and Fly Rivers in Papua New Guinea (Alin et al., 2008), and Congo River in DR Congo (Spencer et al., 2012), POC was more ^{14}C -depleted (much older) than concurrent bulk DOC. In their study in Yukon River in Alaska, Guo and Macdonald (2006) examined the radiocarbon contents of POC, colloidal OC (1 kDa – 0.45 μm), and LMW DOC, and observed that POC was considerably ^{14}C -depleted relative to concurrent colloidal OC and LMW DOC, and also that colloidal OC was modern whereas the LMW DOC had pre-aged radiocarbon signatures. The few published radiocarbon measurements of organic matter in freshwater lakes have, until now, been limited to bulk DOC and POC, and mostly show ^{14}C -enriched (modern) POC relative to concurrent DOC in smaller lakes (McCallister and Giorgio, 2008; McCallister and del Giorgio, 2012), and ^{14}C -depleted (old) POC relative to co-occurring DOC in larger lakes

(Zigah et al., 2011, 2012a,b). Extension of radiocarbon measurements to microbially respired carbon (McCallister and del Giorgio, 2012) and to carbon incorporated into zooplankton biomass (Zigah et al., 2012b) has provided additional insights into radiocarbon trends in lacustrine organic matter pools. It appears that in lakes which are more influenced by surrounding terrestrial systems and have shorter water residence times, there is the ability to incorporate more old terrestrially-derived organic carbon into modern biogeochemical cycles (Zigah et al., 2012b). This may be due to the disturbance of “refugia” during cross-system (land-to-lake) transport of the old organic matter (McCallister and del Giorgio, 2012). For example, sorbed or aggregated material may be released into the dissolved phase (Keil et al., 1994), altered into a more reactive form by photochemistry (Biddanda and Cotner, 2003) or made more bioavailable for the lake ecosystem by mixing with fresher labile organic matter from the lake (priming mechanism) (Bianchi, 2011).

Lakes are a key component of the global carbon cycle with estimated global CO₂ efflux of 140 Tg C y⁻¹ (Cole et al., 1994) and annual carbon burial per surface area exceeding that of the oceans (Dean and Gorham, 1998; Cole et al., 2007) and, as described above, lakes may also play a role in introducing old terrestrial carbon into modern biogeochemical cycles. Therefore, information on the size-age relationships in lakes would enhance our understanding of the dynamics of global carbon cycling, and attendant ecosystem and climate change implications.

In this study, we examined the natural abundance radiocarbon and stable carbon (and nitrogen) isotope composition of size-fractionated OM from the water column of Lake Superior, the world’s largest freshwater lake by surface area, and one of its more than 300 tributary streams and rivers (Amity Creek, Duluth, Minnesota, USA) to provide insights into the size-age relationships and potential fate of OM in lacustrine systems. By analyzing the ¹⁴C composition of POC, bulk DOC, HMW DOC and LMW DOC, we provide the first comprehensive information on the mean ¹⁴C ages, sources and fate of OM size fractions in a lacustrine system. Because ultrafiltration enables the isolation of large amounts of organic material, and has been shown to have negligible blank or contamination issues (Guo and Santschi, 1996) samples obtained by this technique can be analyzed for their radiocarbon and biochemical compositions to assess HMW-DOC provenance and reactivity in aquatic systems. As a non-destructive analytical technique for studying the chemical composition of DOM, nuclear magnetic resonance (NMR) affords examination of the influence of biochemical constituents on the radiocarbon composition of HMW DOC. Hence, we used ¹³C NMR and ¹H NMR to examine the structural constituents of HMW DOC, and coupled this information to the Δ¹⁴C of HMW DOC to provide insights into the structural compositional drivers of the ¹⁴C age of HMW DOC.

2. MATERIALS AND METHODS

2.1. Study sites

Lake Superior is the Earth’s largest freshwater lake by surface area with maximum and mean depths of, respec-

tively, 406 m and 150 m (Urban et al., 2005). It is the coldest and deepest of the Laurentian Great Lakes of North America. The topography of the eastern province of Lake Superior is characterized by north–south trending troughs covered by ~13–15 cm of soft silts and mud overlying stiff post-glacial clays; the western province is marked by very low relief (although two deep troughs have been spotted at the northwestern shore) (Boyer and Hedrick, 1989; Watrus and Rausch, 2001). The offshore region of 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). Lake Superior is, however, dimictic, thus there is density-driven complete vertical mixing of the water column in spring and early winter each year that homogenizes the water column.

Amity Creek (in Duluth, Minnesota, USA) drains a small, primarily forested basin. The creek flows into the Lester River less than 0.5 km before that river enters western Lake Superior. The area of Amity Creek watershed is 42.6 km². The watershed of the Creek is covered mostly by forest (71%) and grassland (19%) with small contributions from wetland (3%) and urbanized areas (2%) (www.lakesuperiorstreams.org).

2.2. Sampling

Samples were collected from three Lake Superior sites (Fig. 1 and Table 1) during spring mixing in late May/early June (henceforth “June” in labelling), and during thermal stratification in August of the year 2010. Both surface (5 m) and deep water (127 m at WM, and 210 m at EM) were sampled at the offshore sites; the nearshore site (ONT) was sampled at only 5 m. Lake water samples were collected using twelve 8 L Niskin bottles mounted on a rosette along with a Seabird Model 911 Plus Conductivity, Temperature, and Depth (CTD) package. Because the lake is undersaturated with respect to calcium carbonate, dissolved inorganic carbon (DIC) samples were taken directly from the Niskin bottles via pre-cleaned silicone tubing (10% v/v HCl, ACS Plus grade and then ultra-pure water [Millipore Milli-Q Plus]) into previously acid-cleaned and combusted (450 °C for ≥4 h) 0.5 L amber Pyrex bottles. 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 until analysis. Bulk DOC and POC samples were obtained by filtering lake water through pre-combusted Whatman GF/F glass fiber filters (450 °C for 4 h; 0.7 μm nominal pore size) via nitrogen-pressurized stainless-steel canisters. Approximately 40 mL of the bulk DOC was collected into an acid-cleaned and combusted vial and acidified to pH 2 using 6 M HCl (ACS Plus grade) for DOC concentration analysis. Also, 1.0 L of the bulk DOC was collected into a pre-combusted glass bottle and acidified to pH 2 for radiocarbon analysis.

In September 2010, corn leaves (*Zea mays*) were collected from the watershed of western Lake Superior with care taken to avoid contamination (e.g. avoiding highways) in order to determine the radiocarbon content of

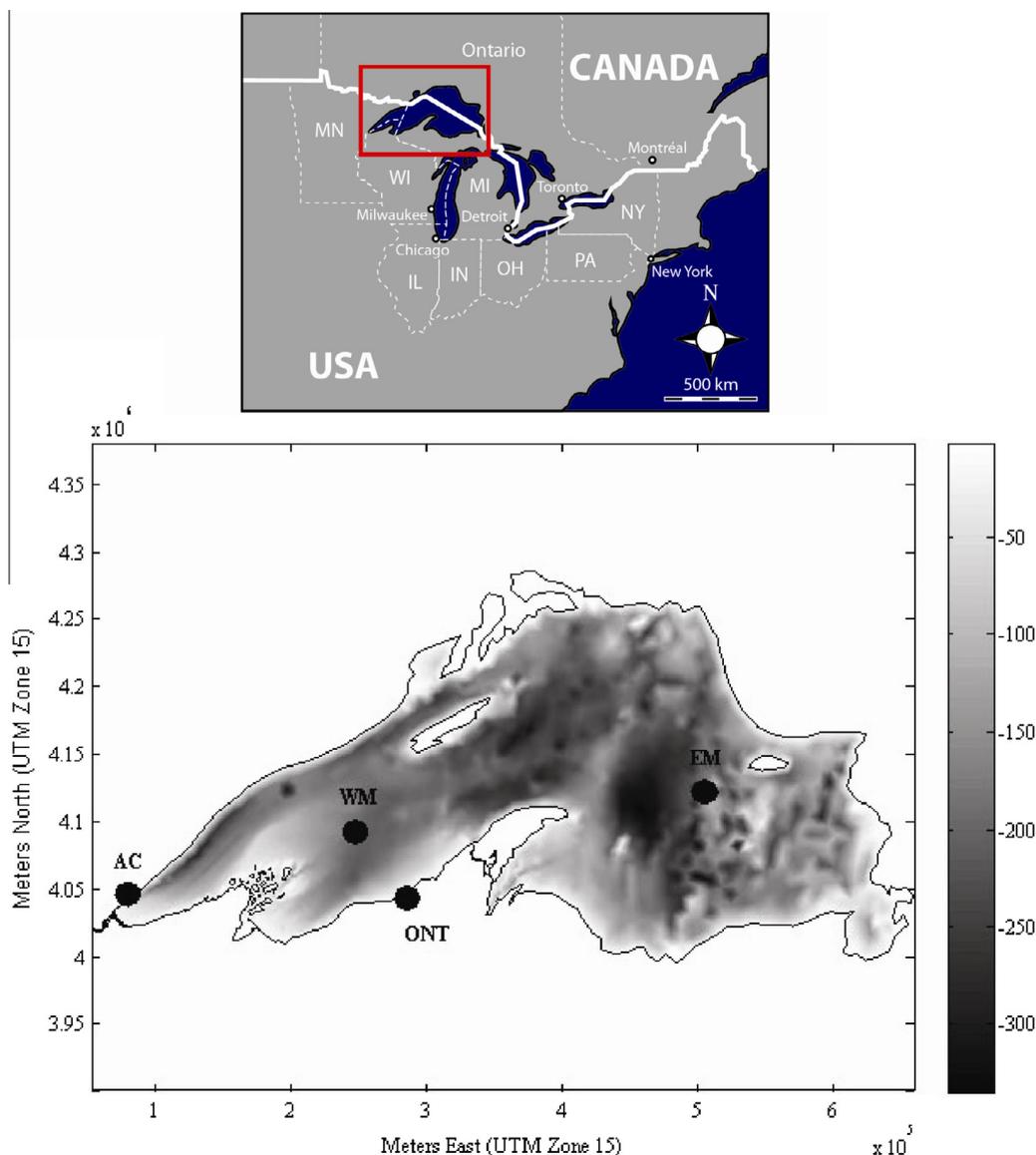


Fig. 1. Location of Lake Superior in North America (upper figure), and a map showing Amity Creek (AC), and nearshore and offshore sites in Lake Superior. The offshore lake sites include western station/mooring (WM, 47°19'N, 89°51'W) and eastern mooring (EM, 47°34'N, 86°39'W), and the nearshore site is off Ontonagon River (ONT, 46°54'N, 89°20'W). The contours are total water depth in meters.

atmospheric CO₂ as integrated in these annual-plant leaves (Zigah et al., 2011). The leaves were rinsed several times with ultra clean water (MQ water), dried, homogenized, acid fumigated, and redried before radiocarbon analysis.

Amity Creek (AC; 46°84'N, 92°01'W) was sampled just above its confluence with the Lester River (which enters western Lake Superior) in September 2010 during baseflow conditions. Storm-flow samples were collected in June 2008 using acid-cleaned sample containers in a Sigma 900 auto-sampler, and the storm samples were retrieved within a day after the storm event (Zigah et al., 2011). Creek water samples (both storm flow and baseflow) were filtered through previously combusted Whatman GF/F glass fiber filters (450 °C for ≥4 h; 0.7 μm nominal pore size) to obtain DOC vs. POC. For DIC samples, the creek water

was collected directly into previously acid-cleaned (10% HCl by volume, ACS Plus grade) and combusted 0.5 L amber Pyrex bottles after three rinses with sample. After creating 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.

2.3. Ultrafiltration

Large-volume samples of 122–131 L and 168–223 L were drawn at the nearshore and offshore sites respectively, using an oil-free diaphragm pump and polypropylene tubing and fittings. At Amity Creek 80 L of creek water was drawn by hand with four 20-L stainless-steel canisters that had been previously cleaned with soap and rinsed several

Table 1

Sampling depths, ultrafiltration details, and the stable nitrogen isotopic compositions and C:N ratios of HMW DOM and bulk POM in the water column of Lake Superior and Amity Creek. The values in parenthesis are the average deviations from analyses of duplicate natural samples.

Site description	Depth sampled (m)	Water depth (m)	Ultrafiltration					HMW DOM		POM	
			Total vol. (L)	Retentate vol. (L)	CF	HMW DOC recovery (%)	Mass balance (%)	Molar C:N	$\delta^{15}\text{N}$ (‰)	Molar C:N	$\delta^{15}\text{N}$ (‰)
<i>June 2010</i>											
Isothermal											
ONT	5	20	131	2.2	60	12.0	86	15.1(0.2)	1.5(0.2)	9.1	−1.3
WM	5	171	203	2.0	102	8.4	101	13.9(0.4)	2.5(0.1)	7.8	0.9
WM	127	171	214	2.0	107	8.5	88	12.46(0.02)	2.9(0.0)	8.4	1.1
EM	5	242	213	1.5	142	12.0	92	17.04(0.04)	2.4(0.1)	8.5	1.7
EM	210	242	192	2.3	83	10.3	89	12.4(0.1)	3.0(0.1)	8.2	2.1
<i>August 2010</i>											
Stratified											
ONT	5	20	122	2.8	44	24.3	108	19.1(0.2)	0.3(0.2)	9.6	−2.6
WM	5	171	175	2.1	83	19.4	104	17.0(0.2)	1.7(0.0)	nd	nd
WM	127	171	168	1.6	105	14.3	93	16.2(0.2)	2.6(0.2)	9.5	2.8 (0.1)
EM	5	242	223	1.2	186	12.3	82	14.7(0.0)	0.4(0.1)	10.0	−4.06
EM	210	242	205	2.5	82	13.2	105	15.5(0.2)	3.1(0.2)	nd	nd
<i>June 2008</i>											
AC – Stormflow	<1	nd	nd	nd	nd	nd	nd	nd	nd	10.5	1.6
<i>September 2010</i>											
AC – Baseflow	<1	nd	80	1.6	50	21.2	94	28.7(0.1)	1.5(0.0)	11.2	2.6 (0.2)

CF is concentration factor and is calculated as total volume divided by retentate volume.

HMW DOC recovery = DOC concentration of retentate multiplied by the retentate volume, all divided by the concentration of Init DOC multiplied by the init volume.

nd = Not measured or lost during sampling and/or processing in laboratory.

times with ultra-pure water. In each case, the samples were filtered to remove bacteria and small particles using a cleaned (forward-and-back flushing with distilled water for ~4 h, and rinsing with 7 L sample) dual layer (0.8 μm prefilter and 0.2 μm filter) polyether sulfone filter with polypropylene housing (Whatman Polycap 75 TC). In this study, we refer to the DOC obtained by sterile filtration (at 0.2 μm) to remove most bacteria and other POC as init DOC. Filter blank DOC, and total organic carbon (TOC) vs. init DOC (sterile filtered <0.2 μm DOC) were monitored to ensure that the cartridge filters were not contributing measurable DOC to the samples. Portions (1 L) of init DOC were collected in acid-cleaned and combusted 1 L bottles for radiocarbon and stable carbon isotope analysis. A stainless-steel and Teflon cross-flow ultrafiltration system was used with a gear pump (Cole-Parmer Instrument Company, IL, USA) and an Amicon spiral-wound cellulose cartridge membrane (Amicon S10N1) with a nominal molecular weight cut-off of 1000 Da (based on >99% rejection of Vitamin B₁₂; Repeta et al., 2002; Repeta and Aluwihare, 2006) to isolate HMW DOC (>1000 Da) vs. LMW DOC (<1000 Da). The ultrafiltration membrane was cleaned by sequential rinsing with isopropanol, detergent (0.01% Micro-90), HCl (0.01 M), NaOH (0.01 M),

and Milli-Q water, and then pre-conditioned with ~20 L of <0.2 μm filtered sample water before processing the samples. LMW DOC was collected at the beginning, midway, and end of processing and the average concentration of these three was used in DOC mass balance analysis to assess ultrafiltration performance. The sample retentates were concentrated to 2–3 L and diafiltered with Milli-Q water; the resulting HMW DOC fractions were then frozen, and later freeze-dried in the lab for chemical and isotopic measurements.

2.4. Measurement of concentrations of DOC, DIC, POC, and PON

Concentrations of DOC and DIC were measured on a Shimadzu V_{CSH} analyzer. The analyzer was calibrated with potassium hydrogen phthalate (KHP) for DOC measurements, and primary standard grade sodium carbonate and ACS reagent grade sodium bicarbonate for DIC measurements (Zigah et al., 2011). As an additional reference check, deep seawater from Florida Strait at 700 m (Batch-8 2008) was obtained from the DOC Consensus Reference Program (Hansell laboratory, University of Miami, USA) and our measurements were $44 \pm 4 \mu\text{M}$ in agreement with the

consensus value of 41–43 μM . Particulate organic matter (POM) samples were freeze-dried and homogenized, fumigated with 12 N HCl (ACS Plus grade) in silver cups overnight to remove carbonates, dried again at 60 °C, and cooled in a desiccator. They were then loaded into tin capsules and analyzed for particulate organic carbon (POC) and particulate organic nitrogen (PON) concentrations on a Costech ECS 4010 elemental analyzer (EA) coupled to Finnigan Delta Plus XP isotope ratio mass spectrometer (IRMS). The EA was calibrated with acetanilide (Costech Analytical Technologies Inc., Valencia, CA, USA) and caffeine (Wheaton Science Products, NJ, USA) reference standard materials. Typical instrumental precisions for POC and PON measurements were 0.2% and 0.4% of the measured concentrations, respectively.

2.5. Radiocarbon ($\Delta^{14}\text{C}$) and stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) measurements

Radiocarbon and stable carbon isotope measurements were performed at the National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS) at the Woods Hole Oceanographic Institution, with the exception of the corn leaves sample, which was measured at the Keck Carbon Cycle AMS Facility (KCCAMS) at University of California, Irvine. DIC samples were treated with H_3PO_4 , and the resulting CO_2 was reduced to graphite with H_2 over Fe catalyst. Wet DOC, including bulk DOC and init DOC, was converted to CO_2 by ultraviolet (UV) oxidation using a protocol based upon [Beaupre et al. \(2007\)](#) but oxidizing for three hours rather than four ([Zigah et al., 2011](#)). The evolved CO_2 was reduced to graphite. POC and freeze-dried HMW DOC samples were fumigated with 12 M HCl (ACS Plus grade) for 24 h to remove carbonates, re-dried at 60 °C, and combusted to CO_2 in a modified Carlo Erba NA1500 elemental analyzer, and then reduced to graphite. In all cases, a subsample of the CO_2 was taken for $\delta^{13}\text{C}$ measurement, and 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. The radiocarbon content of LMW DOC was calculated using a dual (HMW DOC and LMW DOC) isotopic mixing model as follows:

$$\Delta^{14}\text{C}_{\text{init DOC}} = f \times \Delta^{14}\text{C}_{\text{HMW DOC}} + (1 - f) \times \Delta^{14}\text{C}_{\text{LMW DOC}} \quad (1)$$

where f and $(1 - f)$ are the proportions of HMW DOC and LMW DOC, respectively, in the init DOC. All radiocarbon values are reported as $\Delta^{14}\text{C}$, the part per thousand deviation of the sample's $^{14}\text{C}:^{12}\text{C}$ ratio relative to a nineteenth century wood standard that has been corrected to the activity it would have had in 1950 and a $\delta^{13}\text{C}$ of -25‰ . $\Delta^{14}\text{C}$ was corrected for fractionation using $\delta^{13}\text{C}$ of samples according to the convention of [Stuiver and Polach \(1977\)](#). Precision of $\Delta^{14}\text{C}$ analysis is based on analysis of multiple external standards or multiple analyses on a graphite target.

Stable carbon isotope ratios ($\delta^{13}\text{C}$) of samples were measured at NOSAMS using an Optima stable isotope ratio

mass spectrometer. Stable nitrogen isotope ratios ($\delta^{15}\text{N}$) were measured at the Large Lakes Observatory (LLO) using a Finnigan Delta Plus XP IRMS with ConFlo III interface (Thermo Fisher Scientific Inc., Waltham, MA) coupled to a Costech ECS 4010 EA. The $\delta^{13}\text{C}$ of LMW DOC was also calculated from a binary isotopic mixing model as follows:

$$\delta^{13}\text{C}_{\text{init DOC}} = f \times \delta^{13}\text{C}_{\text{HMW DOC}} + (1 - f) \times \delta^{13}\text{C}_{\text{LMW DOC}} \quad (2)$$

Typical instrumental precision of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ based on analyses of multiple external standards were 0.17‰ and 0.15‰, respectively. The stable isotope ratios ($^{13}\text{C}:^{12}\text{C}$ and $^{15}\text{N}:^{14}\text{N}$) are reported as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively, which are the per mil difference relative to Pee Dee Belemnite carbon and atmospheric air standards.

2.6. ^1H NMR spectroscopy

All ^1H NMR spectra of HMW DOM were acquired using a Bruker Avance III 400 spectrometer. About 5 mg of each freeze-dried HMW DOM sample was dissolved in 1 ml of D_2O (>99.9%, Aldrich Chemical Company, Milwaukee, WI) and analyzed in a 5 mm glass NMR tube (Wilmad Glass Co., NJ). The solution-state ^1H NMR spectrum was acquired using a modified water suppression technique described by [Lam and Simpson \(2008\)](#), with a recycle delay of 2 s and 119 ms acquisition time.

2.7. Solid-state ^{13}C NMR spectroscopy

Solid-state NMR spectra were acquired by cross polarization/magic angle spinning (CP/MAS) using a Bruker Avance II 400 spectrometer operating at resonance frequency of 100 MHz for ^{13}C . Rotor spin speed was 14 kHz and 4569 acquisitions were averaged for each spectrum. The optimum relaxation delay was 1 s, and the contact time was 1.5 ms. A 4 mm triple resonance probe was used. Chemical shifts are reported in ‘ δ ’ notation (in part per million, ppm) relative to tetramethylsilane (TMS). We computed the relative contribution of the various regions of the spectra as a percent of the total integrated area, excluding the area (~ 167 – 169 ppm) attributed to inorganic carbonate ([Bianchi et al., 2004](#)). For compound class information, the spectra were integrated over the regions $^1\text{CH}_n$ (0–29 ppm), $^2\text{CH}_n$ (29–40 ppm), $^1\text{CH}_n\text{-O}$ (40–55 ppm), $^2\text{CH}_n\text{-O}$ (55–62 ppm), HC–OH (62–90 ppm), O–C–O (90–115 ppm), C=C/Ar (115–140 ppm), Ar–O (140–160 ppm), COO/CON (160–190 ppm) and the aldehyde and ketone region (190–220 ppm) ([Abdulla et al., 2010b](#)). Our estimation of carboxylic-rich compounds (CRC) is based on [Abdulla et al. \(2010b\)](#), and is associated with the NMR regions: $^2\text{CH}_n$ (29–40 ppm), $^1\text{CH}_n\text{-O}$ (40–55 ppm), C=C/Ar (115–140 ppm), Ar–O (140–160 ppm), and C=O (190–220 ppm). CRC has similar spectral qualities as carboxylic-rich alicyclic molecules (CRAM) ([Abdulla et al., 2010b](#)), and is referred throughout the rest of the paper as CRAM. Because we could not deconvolute the carboxylic, amide, and ester components of the COO/CON (160–190 ppm) region based on the NMR data alone, we could not partition

the full respective components for heteropolysaccharide (HPS) and amide/amino sugars (AMS), and therefore combined them as (HPS + AMS) representing both the complex structural carbohydrates, peptides, and amino sugars. We estimated HPS + AMS component based on [Abdulla et al. \(2010b\)](#) as the NMR regions $^1\text{CH}_n$ (0–29 ppm), $^2\text{CH}_n\text{-O}$ (55–62 ppm), HC–OH (62–90 ppm), O–C–O (90–115 ppm), and amide and ester carbons. Precision of ^{13}C NMR integrated areas of each compound class was based on analyses of multiple external DOC standard and was $\leq 1\%$ of the reported values.

3. RESULTS

3.1. Concentrations of OC size fractions in Lake Superior and Amity Creek

Spring-mixing (June) concentrations of OC in the lake (average \pm standard deviation) were $94 \pm 9 \mu\text{M}$ and $87 \pm 10 \mu\text{M}$ for bulk DOC and init DOC, respectively ([Table 2](#)). The difference in bulk DOC ($< \text{GF/F}$) and init DOC ($< 0.2 \mu\text{m}$), which represents colloidal OC (including bacterial biomass and smaller particles) constitutes an average of 7% of OC in the mixed-lake. In the stratified lake (August), the concentrations of bulk DOC and init DOC were $97 \pm 13 \mu\text{M}$ and $95 \pm 10 \mu\text{M}$, respectively ([Table 3](#)) leading to a very small colloidal OC estimation (average 2% of OC). Within the dissolved OC pool ($< 0.2 \mu\text{m}$), LMW DOC was the largest fraction (76–92%) with concentrations of 63–85 μM in the mixed lake, and 74–85 μM during stratification ([Tables 2 and 3](#)). HMW DOC concentrations were 6–11 μM in the mixed lake and exhibited higher concentrations at each sampling site and depth (range 11–27 μM) during stratification ([Tables 2 and 3](#)). POC was the least abundant OC fraction for all samples (6–10%) with lake concentrations of 4–19 μM . The highest lake POC concentrations appeared at site ONT ([Tables 2 and 3](#)). DIC concentrations were 820–830 μM in the mixed lake and showed little variation with depth. In the stratified lake, DIC concentrations were lower in the surface (775–780 μM) and higher in the deep waters (805–806 μM , [Table 3](#)).

Baseflow concentration of DIC in Amity Creek was $1869 \pm 6 \mu\text{M}$ ([Table 3](#)). Bulk DOC was the largest OC pool in Amity Creek during both baseflow and stormflow conditions with concentrations of 815 ± 8 and $885 \pm 8 \mu\text{M}$, respectively, compared with baseflow and stormflow POC concentrations of 25 ± 1 and $532 \pm 1 \mu\text{M}$, respectively ([Table 3](#)). Init DOC concentration during baseflow conditions was $776 \pm 2 \mu\text{M}$ ([Table 3](#)), and the colloidal OC estimation was $\sim 5\%$ of OC. Within the dissolved OC ($< 0.2 \mu\text{m}$) during baseflow conditions in the Creek, LMW DOC with concentration of 755 μM was a larger fraction ($\sim 79\%$) relative to HMW DOC ($\sim 21\%$) with concentration of 22 μM .

The C:N value of HMW DOM (12.4–19.1) was consistently higher than that of concurrent POM (7.8–10.0) in the lake ([Table 1](#)). C:N of HMW DOM was higher in the stratified lake relative to the mixed lake (except at EM 5 m, [Table 1](#)). In Amity Creek during baseflow condition, the C:N values in HMW DOM (C:N of 28.7) and POM (C:N of 11.2) were higher than the corresponding lake

HMW DOM and POM, respectively ([Table 1](#)). C:N values of POM during stormflow (C:N of 11) conditions were similar to POM C:N during baseflow conditions.

3.2. Stable isotopic distributions in size-fractionated OC in Lake Superior and Amity Creek

In general, POC in the lake was more ^{13}C -depleted ($-29.0 \pm 1.2 \text{‰}$) than concurrent bulk DOC ($-26.4 \pm 0.7 \text{‰}$), init DOC ($-26.6 \pm 0.8 \text{‰}$), HMW DOC ($-26.9 \pm 0.3 \text{‰}$) and LMW DOC ($-26.5 \pm 0.9 \text{‰}$) ([Tables 2 and 3](#)). In the productive stratified lake in August, HMW DOC was more ^{13}C -depleted than the other dissolved OC size fractions ([Table 3](#)). $\delta^{15}\text{N}$ of POM in the lake was -1.3‰ to 2.1‰ during spring mixing, and -4.1‰ to 2.8‰ in the stratified lake whereas $\delta^{15}\text{N}$ of HMW DOM was $2.5 \pm 0.6 \text{‰}$ (range 1.5–3.0 ‰ , $n = 5$) in the mixed lake, and $1.6 \pm 1.3 \text{‰}$ (range 0.3–3.1 ‰ , $n = 5$) in the stratified lake ([Table 1](#)). HMW DOM was in general ^{15}N -enriched relative to concurrent POM in Lake Superior.

During baseflow conditions, POC in Amity Creek was ^{13}C -depleted ($\delta^{13}\text{C}$ of -29.6‰) relative to the bulk, init, HMW, and LMW DOC ([Table 3](#)). However, stormflow POC in the Creek was ^{13}C -enriched ($\delta^{13}\text{C}$ of -27.3‰) relative to concurrent stormflow bulk DOC ($\delta^{13}\text{C}$ of -28.6‰).

3.3. Radiocarbon distributions within OC size fractions in Lake Superior and Amity Creek

In the lake, $\Delta^{14}\text{C}$ of init DOC ($44 \pm 15 \text{‰}$, range 17–59 ‰ , $n = 10$) was similar to $\Delta^{14}\text{C}$ of HMW DOC ($48 \pm 14 \text{‰}$, range 23–64 ‰ , $n = 9$) and $\Delta^{14}\text{C}$ of LMW DOC ($43 \pm 16 \text{‰}$, range 17–62 ‰ , $n = 9$) ([Figs. 2 and 3](#)). As with DIC, init DOC and its constituents HMW DOC and LMW DOC consistently reflected modern radiocarbon signatures. Throughout sampling (with the exception of site WM in the mixed lake), the $\Delta^{14}\text{C}$ values of DIC were comparable to those for init DOC, HMW DOC and LMW DOC in the lake ([Figs. 2 and 3](#)).

The radiocarbon content of POC (relative to that of init DOC and its constituent size classes) was more variable in the lake. In the stratified lake, surface POC ($\Delta^{14}\text{C}$ of 37–53 ‰) was modern, and slightly ^{14}C -depleted relative to concurrent DIC ($\Delta^{14}\text{C}$ of 57–65 ‰). In contrast, deep POC ($\Delta^{14}\text{C}$ of 12 ‰ at WM and -94‰ at EM) in the stratified lake was considerably more ^{14}C -depleted than concurrent DIC ($\Delta^{14}\text{C}$ of 56 ‰ at WM and 69 ‰ at EM) ([Fig. 3](#)). During spring mixing, POC had a $\Delta^{14}\text{C}$ of $14 \pm 27 \text{‰}$ (range -15 to 48 ‰), and except for POC from deep water at the EM site, was consistently more ^{14}C -depleted than co-occurring DIC ($44 \pm 4 \text{‰}$) ([Fig. 2](#)). Also, except for EM 210 m, POC was consistently more ^{14}C -depleted than concurrent init, HMW, and LMW DOC during spring mixing ([Fig. 2](#)).

Contrary to the other dissolved OC fractions, the radiocarbon signature of bulk DOC (which includes colloidal material 0.2 to $\sim 0.7 \mu\text{m}$ in size as well as dissolved organic carbon) was more variable with a $\Delta^{14}\text{C}$ of $30 \pm 28 \text{‰}$ (range -19 to 57 ‰ , $n = 10$) in the lake ([Figs. 2 and 3](#)). Bulk DOC at the nearshore ONT site contained a pre-bomb radiocarbon signature (pre-aged) with $\Delta^{14}\text{C}$ values of $-19 \pm 5 \text{‰}$.

Table 2

Concentration, stable carbon, and radiocarbon isotopic compositions of size fractionated OM and DIC in isothermal Lake Superior in June 2010 and in the Lake Superior tributary Amity Creek (AC) in June 2008 (storm flow). Instrumental precision of $\delta^{13}\text{C}$ based on analyses of multiple external standards was 0.15‰ .

	Concentration (μM)	$\delta^{13}\text{C}$ (‰)	$\Delta^{14}\text{C}$ (‰)	^{14}C ages (BP yrs)
<i>AC < 1 m</i>				
Storm-flow POC ($>0.7 \mu\text{m}$) [*]	532 ± 1	-27.3	11 ± 2	Modern
Storm-flow bulk DOC ($<0.7 \mu\text{m}$) [*]	885 ± 8	-28.6	62 ± 4	Modern
<i>ONT 5 m</i>				
DIC	820 ± 2	0.7	48 ± 4	Modern
POC ($>0.7 \mu\text{m}$)	11 ± 1	-29.8	-10 ± 4	25 ± 30
Bulk DOC ($<0.7 \mu\text{m}$)	109 ± 2	-28.2	-19 ± 5	90 ± 40
Init DOC ($<0.2 \mu\text{m}$)	92 ± 1	-26.0	52 ± 4	Modern
HMW DOC ($>1 \text{ kDa}$)	11 ± 2	-27.2	34 ± 4	Modern
LMW DOC ($<1 \text{ kDa}$)	82 ± 2	-25.8	54 ± 4	Modern
<i>WM 5 m</i>				
DIC	830 ± 4	0.4	46 ± 4	Modern
POC ($>0.7 \mu\text{m}$)	5 ± 0.2	-29.7	-15 ± 2	65 ± 15
Bulk DOC ($<0.7 \mu\text{m}$)	88 ± 1	-26.0	57 ± 4	Modern
Init DOC ($<0.2 \mu\text{m}$)	69 ± 2	-28.3	17 ± 4	Modern
HMW DOC ($>1 \text{ kDa}$)	6 ± 1	-27.3	23 ± 5	Modern
LMW DOC ($<1 \text{ kDa}$)	63 ± 2	-28.4	16 ± 5	Modern
<i>WM 127 m</i>				
DIC	829 ± 3	0.3	45 ± 4	Modern
POC ($>0.7 \mu\text{m}$)	6 ± 0.4	-29.1	12 ± 4	Modern
Bulk DOC ($<0.7 \mu\text{m}$)	94 ± 1	-26.4	51 ± 4	Modern
Init DOC ($<0.2 \mu\text{m}$)	93 ± 1	-26.9	20 ± 3	Modern
HMW DOC ($>1 \text{ kDa}$)	8 ± 1	-27.0	49 ± 4	Modern
LMW DOC ($<1 \text{ kDa}$)	85 ± 1	-26.9	17 ± 4	Modern
<i>EM 5 m</i>				
DIC	827 ± 2	0.3	39 ± 4	Modern
POC ($>0.7 \mu\text{m}$)	4 ± 0.2	-29.8	33 ± 5	Modern
Bulk DOC ($<0.7 \mu\text{m}$)	90 ± 2	-26.1	45 ± 4	Modern
Init DOC ($<0.2 \mu\text{m}$)	90 ± 1	-27.5	47 ± 5	Modern
HMW DOC ($>1 \text{ kDa}$)	11 ± 1	-26.4	60 ± 4	Modern
LMW DOC ($<1 \text{ kDa}$)	80 ± 1	-27.7	45 ± 5	Modern
<i>EM 210 m</i>				
DIC	830 ± 3	0.4	40 ± 4	Modern
POC ($>0.7 \mu\text{m}$)	5 ± 0.2	-29.7	48 ± 4	Modern
Bulk DOC ($<0.7 \mu\text{m}$)	88 ± 2	-25.9	50 ± 3	Modern
Init DOC ($<0.2 \mu\text{m}$)	90 ± 2	-26.0	42 ± 4	Modern
HMW DOC ($>1 \text{ kDa}$)	9 ± 1	-27.0	38 ± 4	Modern
LMW DOC ($<1 \text{ kDa}$)	81 ± 2	-25.9	42 ± 4	Modern

^{*} Data from Zigah et al. (2011).

(90 ± 40 years BP) and $-8 \pm 5\text{‰}$ (40 ± 5 years BP), respectively during mixed and stratified lake conditions (Figs. 2 and 3). Offshore bulk DOC was comparable to concurrent DIC and the other dissolved OC fractions, except at the EM site during stratification, where the surface and deep water bulk DOC (as with the POC at this site) was ^{14}C -depleted relative to concurrent DIC and other dissolved OC fractions (Figs. 2 and 3).

DIC in Amity Creek during baseflow conditions (September 2010) had $\Delta^{14}\text{C}$ of $42 \pm 3\text{‰}$, similar to the $\Delta^{14}\text{C}$ of atmospheric CO_2 ($37 \pm 2\text{‰}$) as determined from the leaves of a corn plant taken in the same year (Fig. 3). During stormflow, the creek POC ($\Delta^{14}\text{C}$ of $11 \pm 2\text{‰}$) and bulk DOC ($62 \pm 4\text{‰}$) were more ^{14}C -enriched than during baseflow, which exhibited POC $\Delta^{14}\text{C}$ of $-127 \pm 4\text{‰}$ and bulk DOC $\Delta^{14}\text{C}$ of $29 \pm 4\text{‰}$ (Figs. 2 and 3). In both base-

flow and stormflow conditions, POC was consistently more ^{14}C -depleted than the corresponding DOC from the creek (Figs. 2 and 3). $\Delta^{14}\text{C}$ values of HMW DOC ($36 \pm 4\text{‰}$) in the creek during baseflow condition were similar to concurrent DIC (and atmospheric CO_2), but more ^{14}C -enriched than init and LMW DOC, and POC (Fig. 3). The oldest OC size fraction in the creek (and the oldest in this study of lake and creek OM) was baseflow POC from Amity Creek ($\Delta^{14}\text{C}$ of $-127 \pm 4\text{‰}$, 1040 ± 35 BP years) (Fig. 3).

3.4. ^{13}C NMR data

The ^{13}C NMR spectra of the HMW DOC from the various lake sites displayed similar chemical shifts and resonances and were characterized by four major resonance regions at 160–190 ppm, 100–120 ppm, 55–90 ppm, and

Table 3

Concentration, stable carbon, and radiocarbon isotopic compositions of size fractionated OM and DIC in thermally stratified Lake Superior in August 2010 and in the Lake Superior tributary Amity Creek (AC) in September 2010 (base flow). Instrumental precision of $\delta^{13}\text{C}$ based on analyses of multiple external standards was 0.15‰.

	Concentration (μM)	$\delta^{13}\text{C}$ (‰)	$\Delta^{14}\text{C}$ (‰)	^{14}C ages (BP yrs)
<i>AC < 1 m</i>				
Base-flow DIC	1869 ± 6	−11.1	42 ± 3	Modern
Base-flow POC (>0.7 μm)	25 ± 1	−29.6	−127 ± 4	1040 ± 35
Base-flow bulk DOC (<0.7 μm)	815 ± 8	−28.3	29 ± 4	Modern
Base-flow Init DOC (<0.2 μm)	776 ± 2	−28.3	−4 ± 3	Modern
Base-flow HMW DOC (>1 kDa)	22 ± 1	−28.0	36 ± 4	Modern
Base-flow LMW DOC (<1 kDa)	755 ± 2	−28.3	−5 ± 4	Modern
<i>ONT 5 m</i>				
DIC	780 ± 2	1.1	65 ± 5	Modern
POC (>0.7 μm)	19 ± 1	−26.4	49 ± 3	Modern
Bulk DOC (<0.7 μm)	118 ± 1	−26.5	−8 ± 5	40 ± 5
Init DOC (<0.2 μm)	112 ± 1	−26.2	58 ± 4	Modern
HMW DOC (>1 kDa)	27 ± 1	−26.7	59 ± 3	Modern
LMW DOC (<1 kDa)	85 ± 1	−26.0	58 ± 5	Modern
<i>WM 5 m</i>				
DIC	780 ± 2	1.4	57 ± 2	Modern
POC (>0.7 μm)	nd	−28.5	53 ± 4	Modern
Bulk DOC (<0.7 μm)	97 ± 1	−26.3	40 ± 4	Modern
Init DOC (<0.2 μm)	96 ± 1	−26.4	59 ± 4	Modern
HMW DOC (>1 kDa)	19 ± 1	−27.1	48 ± 3	Modern
LMW DOC (<1 kDa)	77 ± 1	−26.2	62 ± 4	Modern
<i>WM 127 m</i>				
DIC	806 ± 2	0.1	56 ± 3	Modern
POC (>0.7 μm)	4 ± 0.1	−29.3	12 ± 4	Modern
Bulk DOC (<0.7 μm)	96 ± 1	−26.1	56 ± 3	Modern
Init DOC (<0.2 μm)	90 ± 1	−26.3	53 ± 4	Modern
HMW DOC (>1 kDa)	13 ± 0.3	−26.6	nd	nd
LMW DOC (<1 kDa)	77 ± 1	−26.3	nd	nd
<i>EM 5 m</i>				
DIC	775 ± 4	1.5	58 ± 3	Modern
POC (>0.7 μm)	9 ± 0.2	−27.3	37 ± 4	Modern
Bulk DOC (<0.7 μm)	91 ± 1	−26.3	2 ± 3	Modern
Init DOC (<0.2 μm)	91 ± 2	−26.1	44 ± 3	Modern
HMW DOC (>1 kDa)	12 ± 0.2	−27.3	57 ± 4	Modern
LMW DOC (<1 kDa)	79 ± 2	−25.9	42 ± 4	Modern
<i>EM 210 m</i>				
DIC	805 ± 2	0.2	69 ± 3	Modern
POC (>0.7 μm)	nd	−29.2	−94 ± 5	735 ± 40
Bulk DOC (<0.7 μm)	83 ± 1	−26.3	28 ± 4	Modern
Init DOC (<0.2 μm)	86 ± 1	−26.1	52 ± 3	Modern
HMW DOC (>1 kDa)	11 ± 1	−26.6	64 ± 4	Modern
LMW DOC (<1 kDa)	74 ± 1	−26.0	50 ± 4	Modern

20–40 ppm (Fig. 4). O-alkyl carbohydrate carbon (HCOH) was the dominant component of HMW DOC, and constitutes $45 \pm 4\%$ of the HMW DOC in Lake Superior (Table 4). Substituted aromatic carbon or double bonded carbon (C=C/Ar-C), oxygen substituted aromatic carbon (Ar-O), and carbonyl carbon were each only trace components ($\leq 3\%$) of the HMW DOC in the water column of Lake Superior (Table 4).

With respect to compound classes, the NMR data show that the total carbohydrate carbon (HC-OH and O-C-O) was 52–63% of HMW DOC, total aliphatic carbon ($^1\text{CH}_n$, $^2\text{CH}_n$, $^1\text{CH}_n\text{-O}$, $^2\text{CH}_n\text{-O}$) was 27–36% of HMW DOC, and total aromatic carbon (C=C/Ar and Ar-O)

was just 2–4% of HMW DOC in the lake (Table 4). Of the structural components, our model indicates that CRAM constitutes 16–25% of HMW DOC, whereas the HPS + AMS component makes up 75–84% of HMW DOC in the lake (Table 4).

There was a significant positive relationship between HPS + AMS and $\Delta^{14}\text{C}$ of HMW DOC ($R = 0.84$, $P = 0.037$, Fig. 5A) and O-alkyl carbohydrate carbon and $\Delta^{14}\text{C}$ of HMW DOC ($R = 0.87$, $P = 0.024$) (Fig. 5B), and total carbohydrate carbon and $\Delta^{14}\text{C}$ of HMW DOC ($R = 0.97$, $P = 0.001$) (Fig. 5D). In contrast, there was significant inverse relationship between CRAM and $\Delta^{14}\text{C}$ of HMW DOC ($R = 0.84$, $P = 0.037$) (Fig. 6A), aliphatic

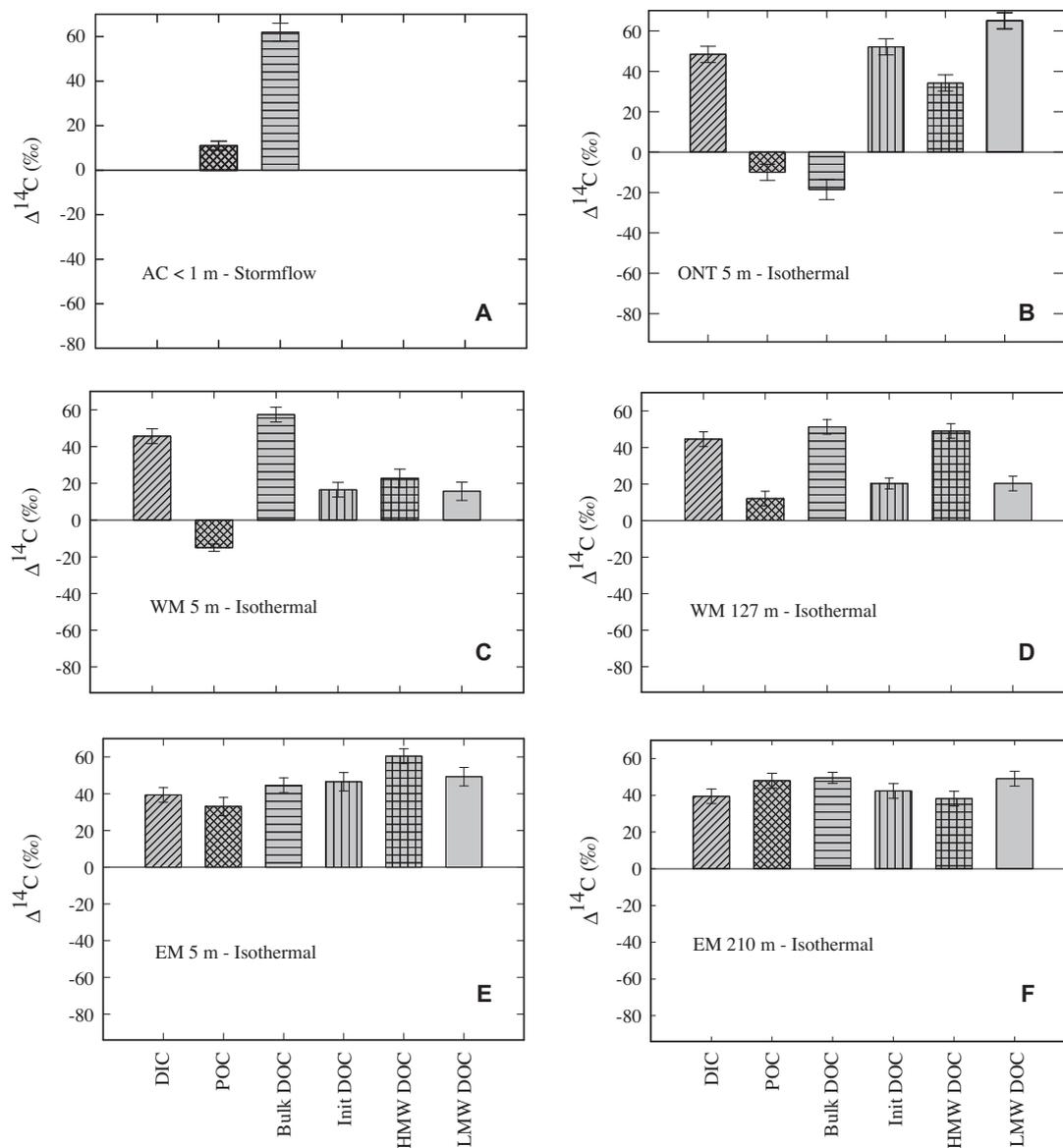


Fig. 2. The radiocarbon distributions of DIC and various organic carbon size fractions including POC ($>0.7 \mu\text{m}$), bulk DOC ($<0.7 \mu\text{m}$), Init DOC ($<0.2 \mu\text{m}$), LMW DOC ($<1 \text{ kDa}$), and HMW DOC ($1 \text{ kDa} - 0.2 \mu\text{m}$) in the mixed Lake Superior water column (June 2010) and Amity Creek during stormflow condition (June 2008).

alkyl carbon and $\Delta^{14}\text{C}$ of HMW DOC (Fig. 6B), aliphatic O-alkyl carbon and $\Delta^{14}\text{C}$ of HMW DOC (Fig. 6C) and total aliphatic carbon and $\Delta^{14}\text{C}$ of HMW DOC ($R = 0.99$, $P < 0.0003$) (Fig. 6D).

3.5. ^1H NMR data

The ^1H NMR spectra of all lake samples and AC baseflow (Fig. 7A–J) show characteristic major resonances centered at 3.3 ppm from carbohydrate protons (CHOH), 2.0 ppm from methyl protons of acetate (CH_3CO), and an intense peak at 1.1 ppm from methyl and methylene protons (CH_3 and CH_2) (Aluwihare et al., 1997, 2002; Repeta et al., 2002). In contrast to open-water Lake Superior, HMW DOC from Amity Creek shows a broad peak at 6–9 ppm from aromatic protons

(Fig. 7A). To obtain the relative compositions of bound acetates (1.76–2.03 ppm from protons of CH_3CO) in the HMW DOC, the ^1H NMR spectra were integrated (Repeta et al., 2002; Koprivnjak et al., 2009). These integrations show that acetates account for 9% of HMW DOC in Amity Creek, 10–12% of HMW DOC at near-shore ONT site, and 11–14% of HMW DOC in open Lake Superior.

4. DISCUSSION

4.1. Abundances of size-fractionated OC in Lake Superior and Amity Creek

Surface-water POC and DOC phases increased in concentration in the stratified (relative to mixed) surface waters

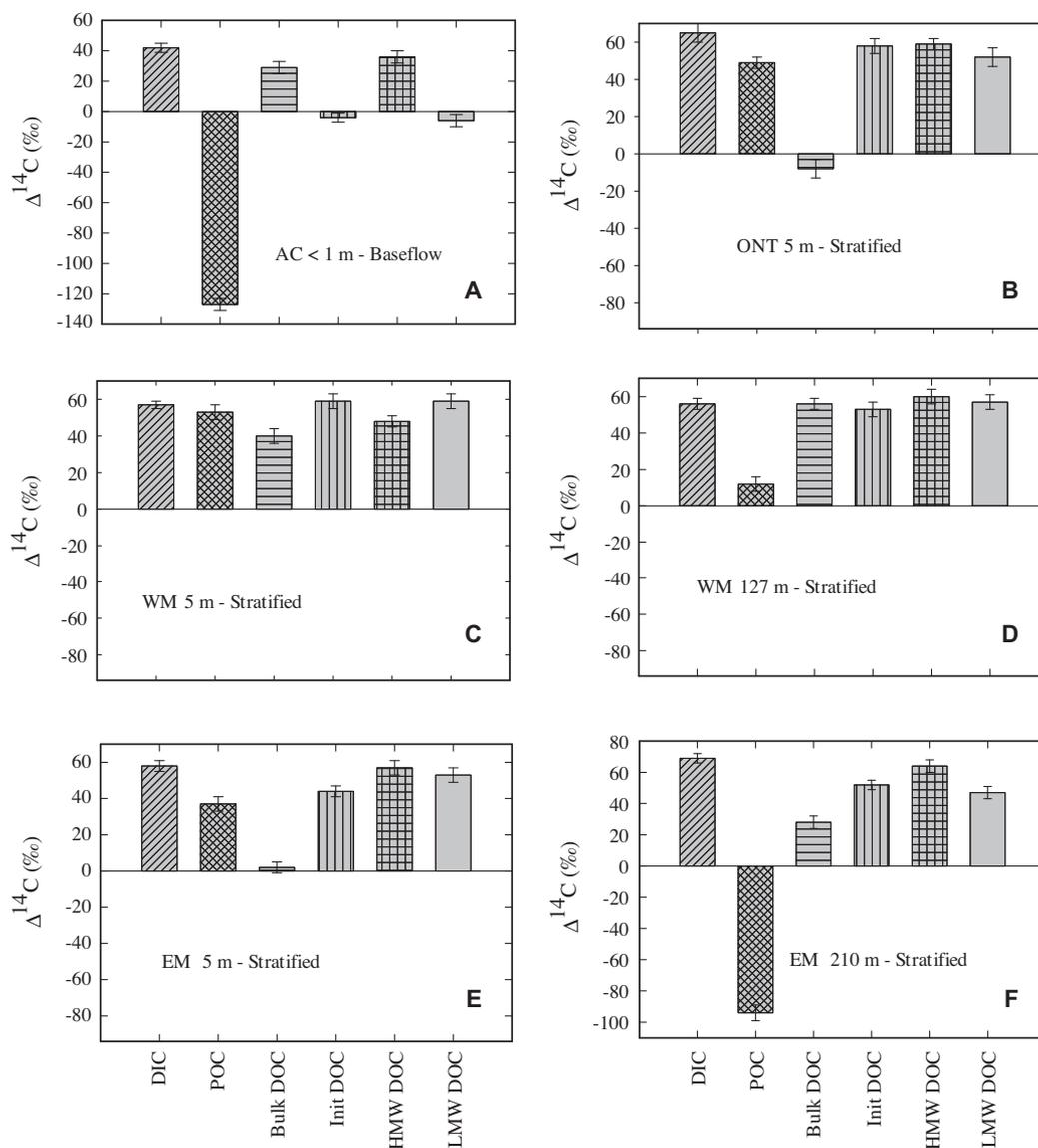


Fig. 3. The radiocarbon distributions of various organic carbon size fractions and DIC in the stratified Lake Superior water column (August 2010) and Amity Creek during baseflow condition (September 2010).

(Table 3), as would be expected from the addition of freshly synthesized materials from photosynthesis. The observed increase in DOC implies that some portion of the lake's primary production is routed through the dissolved pool (not just the POC), as seen in the world's oceans (Azam et al., 1983; Hedges, 1992; Mannino and Harvey, 2000; Kaiser and Benner, 2009). The higher amounts of bulk DOC, HMW DOC and LMW DOC at the nearshore (ONT) site relative to open lake sites appears due to terrestrial inputs in June (based upon radiocarbon and stable C isotope data) and a combination of terrestrial inputs and higher primary production in the summer (as the POC and bulk DOC isotopes shift to more modern radiocarbon values and less depleted $\delta^{13}\text{C}$ values). In the open lake, the observed larger increases in bulk DOC, HMW DOC, and LMW DOC upon stratification at the WM site (Tables 2 and 3), as compared to the EM site, are likely due to higher primary

productivity in the western region of the lake, as has previously been reported (Zigah et al., 2012a).

Throughout the lake and in the baseflow stream sample, most of the init DOC was in the LMW fraction (76–92%), consistent with previous size-class characterization of DOC in the far western arm and just off the Keweenaw Peninsula in Lake Superior (Repeta et al., 2002; Urban et al., 2005). The molecular weight distribution in Lake Superior is similar to that seen in open oceans; most oceanic DOC (60–80%) consists of LMW compounds (Guo et al., 1995; Amon and Benner, 1996; McCarthy et al., 1996; Hansell and Carlson, 2002; Repeta et al., 2002; Walker et al., 2011). In contrast to open Lake Superior and the open oceans, estuarine DOC (13–48% of HMW DOC; Sigleo, 1996; Guo et al., 1996; Wang et al., 2004) and large-river DOC (24–87% HMW DOC; Benner and Hedges, 1993; Hedges et al., 1994; Benner and Opsahl, 2001; Repeta

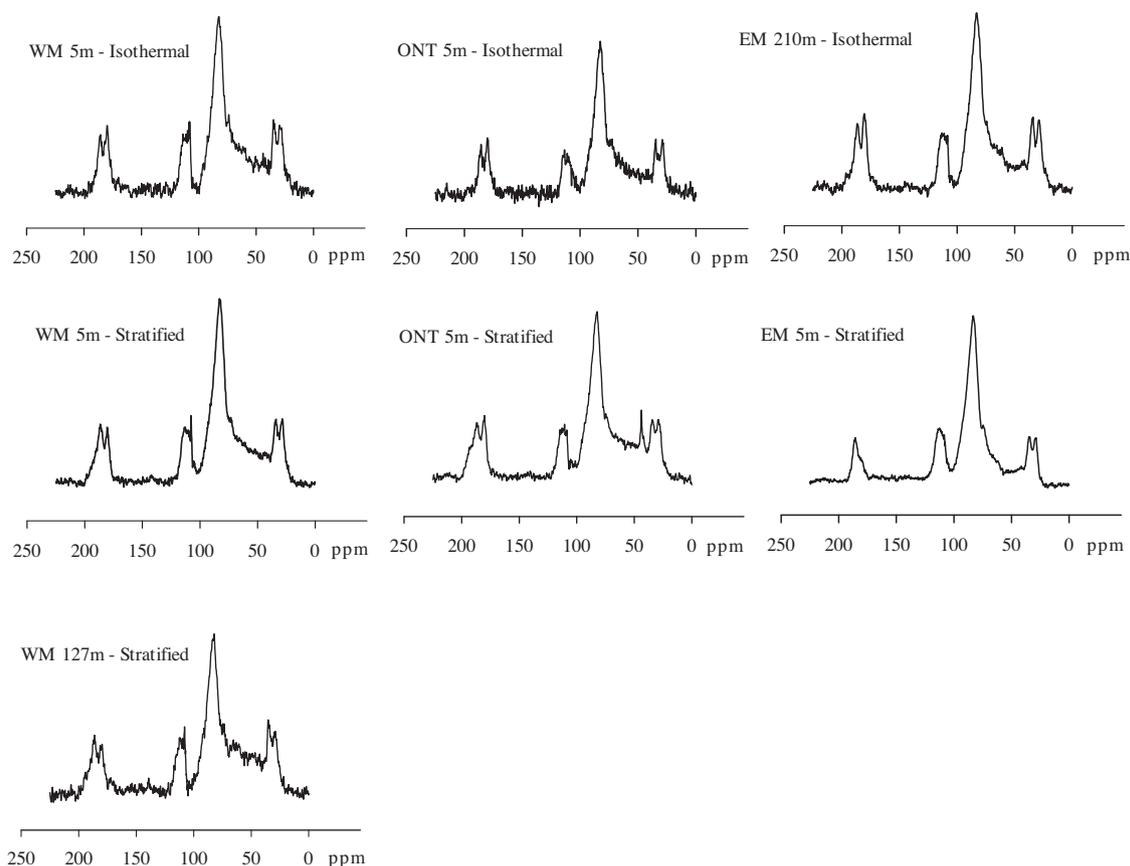


Fig. 4. The CPMAS ^{13}C NMR spectra of HMW DOC from the nearshore site (ONT) and open Lake Superior locations covering the western (WM) and eastern (EM) regions.

et al., 2002) contain a higher proportion of HMW molecules because of relatively greater terrestrial influence and higher aquatic primary production.

C:N values in HMW DOM from Lake Superior (C:N of 12–19) are within the range seen at various oceanic sites (C:N range 15–23; Amon and Benner, 1994; McCarthy et al., 1996; Benner et al., 1997) and estuaries (C:N of 10–25; Guo et al., 1996; Engelhaupt and Bianchi, 2001; Wang et al., 2004; McCallister et al., 2006), but lower than the range reported for riverine HMW DOM (C:N of 34–87; Amon and Benner, 1996; Repeta et al., 2002). Higher riverine HMW-DOM C:N values most likely reflect higher terrestrial influence. Consistent with this, we see relatively higher HMW-DOM C:N values at Amity Creek (~29; Table 1), our most terrestrially impacted site. The slight shift to higher C:N values within HMW DOM (with the exception of the surface EM samples) and POM during stratification in Lake Superior (Table 1) may reflect higher fresh lipid or polysaccharide content from increased primary production or an enhancement of removal mechanisms (via photochemical and microbial processes) that preferentially target nitrogen containing species (Bushaw et al., 1996). The consistently higher C:N values of HMW DOM (12–19) relative to concurrent POM (8–10) in Lake Superior (Table 1) possibly reflect higher microbial rework-

ing, and/or other diagenetic alteration of the HMW DOM (Abell et al., 2000; McCallister et al., 2006; Guo and Macdonald, 2006), or relatively higher terrestrial influence of HMW DOM vs. POM (Hedges et al., 1986; McCallister et al., 2006).

4.2. Cycling and sources of OC size fractions in Lake Superior and Amity Creek

The modern radiocarbon signature of LMW DOC in the lake indicates that most of it is derived from organic material synthesized ≤ 60 years ago, implying a semi-labile nature. This turnover time is similar to ^{14}C based turnover times of HMW DOC and bulk DOC in the lake, but is much shorter than the hydrologic residence time (170 years) of the lake. The lake DOC radiocarbon values contrast with those seen in oceanic systems, where the LMW DOC is typically pre-aged and recycles on a longer time scale than the bulk DOC and HMW DOC (Amon and Benner, 1994; Guo et al., 1995; Santschi et al., 1995; Skoog and Benner, 1997; Mannino and Harvey, 2000; Loh et al., 2004). The faster turnover of LMW DOC in Lake Superior relative to the oceans (and relative to the hydrologic residence time of the lake) suggests that microbial and/or photochemically-mediated remineralization is more effective at removing

Table 4

The relative percent distributions of functional groups and the relative carbon percentage of carboxylic-rich alicyclic molecules (CRAM) and combined heteropolysaccharides and amide/amino sugars (HPS + AMS) within HMW DOC based on CPMAS ^{13}C NMR spectra of HMW DOC from isothermal (in June) and stratified (in August) Lake Superior. Precision of each compound class was $\leq 1\%$ of the reported value, based on analyses of multiple external DOC standards.

NMR region/site	$^1\text{CH}_n$	$^2\text{CH}_n$	$^1\text{CH}_n\text{-O}$	$^2\text{CH}_n\text{-O}$	HCOH	O-C-O	C=C/Ar-C	Ar-O	$\frac{\ddagger\text{COO/CON}}{\text{Acid Amide/ Ester}}$		C=O	CRAM	HPS and AMS
	0–29	29–40	40–55	55–62	62–90	90–115	115–140	140–160	160–190	190–220			
ONT 5 m – Isothermal	11	7	9	5	40	13	2	2	3	6	2	25	75
ONT 5 m – Stratified	11	4	7	5	49	13	1	1	2	6	1	16	84
WM 5 m – Isothermal	14	6	11	5	42	10	1	1	2	7	1	22	78
WM 5 m – Stratified	11	5	8	5	44	14	1	1	3	6	2	20	80
WM 127 m – Stratified	11	5	8	5	43	14	3	1	2	6	2	21	79
EM 210 m – Isothermal	13	5	8	5	44	12	2	2	2	6	1	20	80
EM 5 m – Stratified	12	4	6	5	50	13	2	2	1	4	1	16	84

lake LMW DOC. The dimictic nature (complete vertical mixing twice per year) of the lake brings deep water DOC to the more photochemically and microbially reactive surface waters at a regular and faster rate than can occur in the open ocean. Such mixing could also act as a priming mechanism (Bianchi, 2011), mixing “refractory” material with “labile” material and leading to more efficient remineralization of this “refractory” organic carbon. In addition, Lake Superior also has a shorter water residence time (170 years) than seen for the deep ocean (1000–1500 years), which could prevent the build-up of a pre-aged LMW DOC component in the lake as compared to the open oceans.

The general ^{15}N -enrichment of HMW DOM (relative to POM) could be due to preferential release of ^{14}N during mineralization, and together with the elevated C:N values, and modern radiocarbon signatures suggests that the lake HMW DOM is both rapidly altered and rapidly recycled. That HMW DOC is of contemporary origin and recycles rapidly has also been observed in several oceanic systems based on radiocarbon (Santschi et al., 1995; Guo et al., 1996; Aluwihare et al., 2002; Loh et al., 2004; Repeta and Aluwihare, 2006), molecular and/or biochemical composition (McCarthy et al., 1996; Skoog and Benner, 1997; Mannino and Harvey, 2000; Aluwihare et al., 2002; Loh et al., 2004) and bacterial utilization of size-fractionated DOC (Amon and Benner, 1994, 1996).

At the nearshore site (ONT) in both mixed and stratified lake conditions, and in the stratified open-lake waters (except deep WM), bulk DOC ($<0.7\ \mu\text{m}$) was more ^{14}C -depleted than init DOC ($<0.2\ \mu\text{m}$) (Tables 2 and 3), indicating that the colloidal fraction ($0.2\text{--}0.7\ \mu\text{m}$) which includes both bacterial biomass and small particles (e.g. resuspended silt and/or clay materials) represents a more ^{14}C -depleted (older) component of the bulk DOC. In contrast, the colloidal fraction represented a more contemporary, ^{14}C -enriched, component of bulk DOC in the open lake sites in the mixed lake (Table 2). The ^{14}C -depletion of bulk DOC relative to DIC in the stratified waters at site EM (both surface and deep waters) and at the ONT site during both mixed and stratified seasons suggests that most of the bulk DOC at these sites and seasons could be from a different (non-algal) source or could be the residual material after preferential

remineralization of younger DOC. The ^{13}C -depletion of POC relative to bulk DOC seen in most of the surface and deep samples in the mixed and stratified lake (Fig. 8), and the ^{14}C -depletion of POC relative to DIC (except at deep EM site during mixed condition) is consistent with a significant lipid-enriched and variably-aged POC source (i.e., resuspended sediments, Hawley, 2000; Urban et al., 2004; Zigah et al., 2012a,b).

In Amity Creek, the baseflow bulk DOC, init DOC, and POC $\Delta^{14}\text{C}$ show ^{14}C -depleted values and stormflow bulk DOC and POC were relatively ^{14}C -enriched. This implies that the majority of DOC and POC delivered from the creek during flow events is not pre-aged (very old) but modern (post-bomb). The ^{14}C -depletion of bulk DOC ($<0.7\ \mu\text{m}$) relative to init DOC ($<0.2\ \mu\text{m}$) in the stream (Table 3) during baseflow condition shows that the colloidal fraction was an older component of the bulk DOC. POC was pre-aged, and LMW DOC was near pre-aged (Fig. 3A) during baseflow conditions indicating older sources or longer residence time in the creek and/or watershed. The similarity between $\Delta^{14}\text{C}$ of HMW DOC and $\Delta^{14}\text{C}$ of DIC (Fig 3A) in the creek suggests most of the creek HMW DOC is biolabile with a rapid turnover time.

4.3. Structural composition of HMW DOC in Lake Superior

The major structural components in the HMW DOC from Lake Superior as revealed by ^{13}C NMR spectra were carbohydrates, aliphatic compounds and carboxylic-rich alicyclic molecules (CRAM) with little contribution from aromatic compounds; such HMW-DOC compositions have previously been reported for open ocean and Lake Ontario samples (Hertkorn et al., 2006; Lam et al., 2007). That carbohydrate is a major biochemical constituent within HMW DOC (52–63%) in Lake Superior (Table 4) is consistent with previous observations in the lake (Repeta et al., 2002) and in oceanic HMW DOC (where carbohydrate is 40–70%; Benner et al., 1992; McCarthy et al., 1993; Aluwihare et al., 2002; Repeta et al., 2002; Gogou and Repeta, 2010) and could be linked to carbohydrates' roles as structural (e.g. chitin and peptidoglycan), and energy storage (e.g. glycogen) components of aquatic living

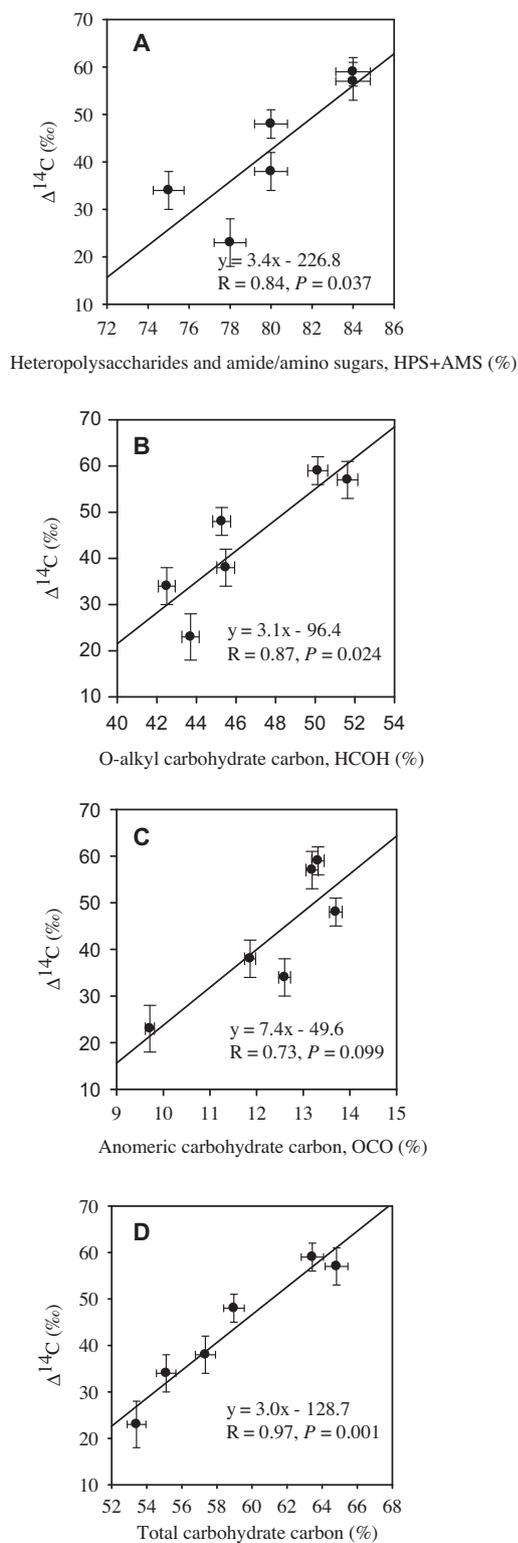


Fig. 5. Relationships between radiocarbon composition and carbohydrate and amide/peptide components of HMW DOC in Lake Superior showing positive correlation between the radiocarbon composition and each of the following: combined heteropolysaccharides, peptide, and amino sugars (A), O-alkyl carbohydrate carbon (B), and total carbohydrate carbon (D).

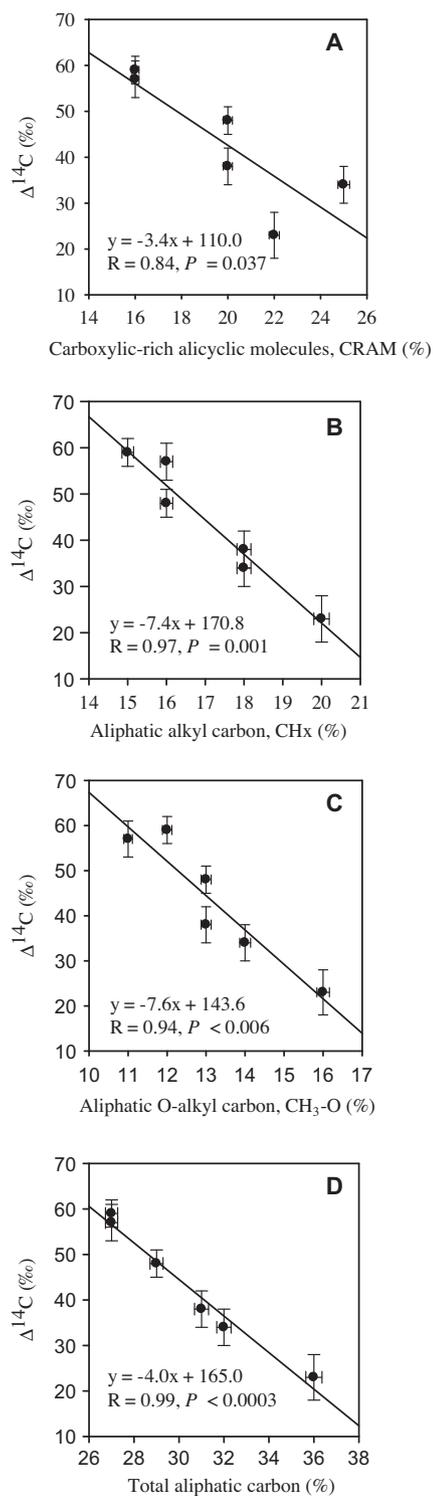


Fig. 6. Relationships between radiocarbon composition and carboxylic-rich alicyclic molecules and various aliphatic carbon components of HMW DOC in Lake Superior showing inverse correlation between radiocarbon composition and each of carboxylic-rich alicyclic molecules (A), alkyl aliphatic carbon (B), O-alkyl aliphatic carbon (C), and total aliphatic carbon (D).

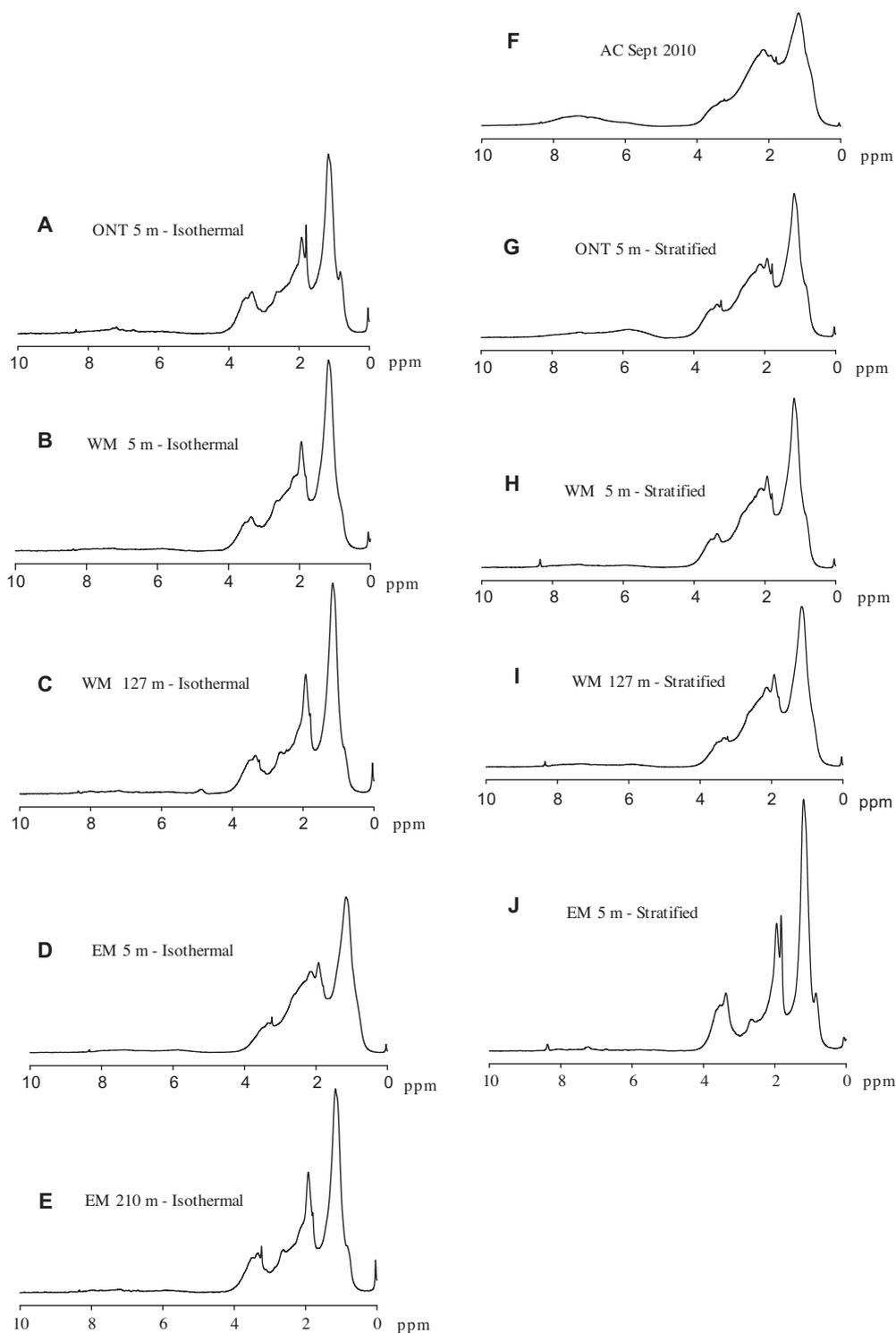


Fig. 7. ^1H NMR spectra of HMW DOC from a tributary stream (AC), nearshore site (ONT) and open Lake Superior locations covering the western (WM) and eastern (EM) regions. (A)–(E) are lake samples from the isothermal water column; (F) is an Amity Creek surface sample, and (G)–(J) are surface and deep lake samples from the stratified period.

organisms including algae (Romankevich, 1984), bacteria (Boon et al., 1998; McCarthy et al., 1998), fungi (Wershaw et al., 2005), and terrestrial plants (Mannino and Harvey, 2000; Wershaw et al., 2005). In oceanic HMW DOC, most

of the carbohydrates are found to consist of complex polymeric structures known as heteroglycans or heteropolysaccharides (HPS) (Benner et al., 1992; Aluwihare et al., 1997, 2002); these HPS include both structural and storage

polysaccharides. Amino sugars are carbohydrates with an amino group (NH_2) replacing a hydroxyl group (OH). They often have structural roles in biota (e.g. peptidoglycans are important components of prokaryotic cell walls), and are an important component of freshwater and marine organic matter (Benner and Kaiser, 2003; Tremblay and Benner, 2009). Chitin, a biopolymer of amino sugar N-acetyl-D-glucosamine present in arthropod exoskeletons and fungal cell walls, has estimated production rates of 1.3×10^{12} kg chitin per year and 2.8×10^{10} kg chitin per year in marine and freshwater environments, respectively, and supports about 10% of bacterial production in the oceans (Kirchman and White, 1999). Complicating studies of organic matter composition, N-acetyl amino sugars, peptides and proteins all contribute amide groups to organic matter. Most oceanic HMW dissolved organic nitrogen has been shown to exist as amide, and this amide is most likely from a combination of sources, as some fraction of peptide/proteins is thought to be resistant to microbial degradation and to persist or accumulate in the ocean (Tanoue et al., 1995; McCarthy et al., 1997).

That heteropolysaccharides, amides/peptides, and amino sugars (HPS + AMS) in HMW DOC constitute a considerable fraction (75–84%) of HMW DOC in Lake Superior (as well as in the open oceans; Benner et al., 1992; Aluwihare et al., 1997), could be due to the fact that the simple carbohydrate and protein/peptide compounds are labile and are mineralized much faster, leaving behind the more complex structural heteropolysaccharide, amino sugar, and protein forms. For instance, Repeta and Aluwihare (2006), based on radiocarbon composition, noted rapid cycling of neutral sugars (≤ 3 years) in the surface North Pacific Ocean. Alternatively, significant contribution of bacterial biomass (from in situ aquatic or soil OM) to the HMW DOC in the lake could result in the higher proportion of structural carbohydrate and protein components such as HPS and AMS (Benner et al., 1992; Aluwihare et al., 1997; McCarthy et al., 1997, 1998; Ogawa et al., 2001).

The aliphatic carbon region of the ^{13}C NMR spectra broadly includes carbon contributions from multiple chemical structures such as short chain aliphatic compounds (e.g. volatile fatty acids), long chain aliphatic compounds (e.g. plant aliphatic biopolymers) and proteins (Tambone et al., 2009), and other lipid compounds such as n-alkanes, alkenones, and alcohols. The aliphatic carbon content of HMW DOC seen in Lake Superior is consistent with that seen in oceanic systems (Benner et al., 1992; Hedges et al., 1992; Bianchi et al., 1995, 2004) and could be reflective of the numerous sources (aquatic and terrigenous) of these compounds. McKnight et al. (1997) and Repeta et al. (2002) observed a high degree of similarity of NMR spectra of HMW DOC from more terrestrially influenced (creeks, rivers, some lakes) and less terrestrially influenced aquatic (open oceanic) systems.

Carboxylic-rich alicyclic molecules (CRAM) are a recently identified major component of aquatic HMW DOC with a carboxyl-carbon to aliphatic-carbon ratio of 1:2 – 1:7 and structural characteristics similar to hopanoids and sterols (Hertkorn et al., 2006). Our estimates of the CRAM content of HMW DOC (17–25%) extend to earth's largest

lake (by area), observations that CRAM is a major structural component of isolated dissolved OM in freshwater and oceanic systems (Hertkorn et al., 2006; Lam et al., 2007; Abdulla et al., 2010a,b; McCaul et al., 2011).

The low aromatic signature of lake HMW DOC based on ^{13}C NMR spectra (Fig. 4) suggests little terrigenous input, and is most likely due to photochemical alterations which lead to losses of dissolved phenolic compounds from lignin and tannins, thereby leading to an underestimation of terrestrial influence (Opsahl and Benner, 1998; Opsahl and Zepp, 2001; Minor et al., 2007). In Lake Superior, although significant amounts of algal-derived DOC are added to the lake each year (Cotner et al., 2004; Urban et al., 2005; Sterner, 2010), the algal-derived DOC is thought to be mineralized rapidly (Maier and Swain, 1978). Thus most of the semi-labile bulk DOC in the lake appears to be derived from terrestrial sources (Urban et al., 2005). It is likely that the HMW DOC is derived from both algal and terrestrial sources, with the algal portion being the most labile portion of HMW DOC.

^1H NMR data show that HMW DOC from open-water Lake Superior is relatively rich in acetate; oceanic HMW DOC is also relatively rich in acetate functional groups, believed to be from amino sugars (Repeta et al., 2002). If the acetate signature in Lake Superior is also from amino sugars, lake HMW DOC is then strongly impacted by microbial input (from either aquatic or soil bacterial cells). This is not surprising as several studies in oceans and estuaries show that bacteria could provide substantial carbon sources to the HMW DOC (McCarthy et al., 1998; Minor et al., 2001; Ogawa et al., 2001; Benner and Kaiser, 2003; Zou et al., 2004).

4.4. Structural compositional drivers of the radiocarbon composition of HMW DOC

4.4.1. Contribution of carbohydrate carbon to $\Delta^{14}\text{C}$ of HMW DOC

The significant linear correlations observed between heteropolysaccharides and amide/amino sugars and O-alkyl carbohydrate carbon with $\Delta^{14}\text{C}$ of HMW DOC (Fig. 5A and B) suggest that these components are derived from contemporary (modern) sources. The absence of significant linear correlation between anomeric carbohydrate carbon and $\Delta^{14}\text{C}$ of HMW DOC (Fig. 5C) could be reflective of varying extents of degradation and/or multiple sources (terrestrial, soil and aquatic) of these carbohydrate components. Irrespective of the source, the significant linear correlation between total carbohydrate carbon and $\Delta^{14}\text{C}$ of HMW DOC indicates that, in general, carbohydrate carbon contributes ^{14}C -enriched signal to the HMW DOC; its biolability may be a function of both inherent reactivity and priming effects. It is worth noting that our study relates the entire HMW carbohydrate carbon pool to the $\Delta^{14}\text{C}$ of HMW DOC as opposed to previous studies based on only the extractable carbohydrate within the HMW DOC. In oceanic systems, extractable carbohydrates are typically more ^{14}C -enriched than concurrent HMW DOC (Loh et al., 2004), consistent with our observation in Lake Superior. For instance, the $\Delta^{14}\text{C}$ of extractable carbohydrates in

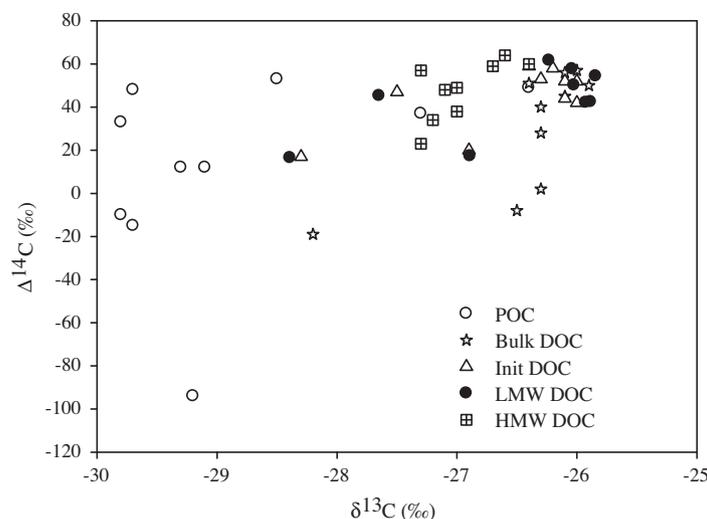


Fig. 8. Cross plot of $\Delta^{14}\text{C}$ vs. $\delta^{13}\text{C}$ of OC size fractions including POC ($>0.7\ \mu\text{m}$), bulk DOC ($<0.7\ \mu\text{m}$), Init DOC ($<0.2\ \mu\text{m}$), LMW DOC ($<1\ \text{kDa}$), and HMW DOC ($1\ \text{kDa} - 0.2\ \mu\text{m}$) in Lake Superior water column covering both stratified and mixed lake conditions. POC shows wider variability in $\Delta^{14}\text{C}$ values, and is more ^{13}C -depleted than the other OC size fractions.

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 DOC. In Lake Superior, an extrapolation of the observed linear relationship between $\Delta^{14}\text{C}$ of HMW DOC and total carbohydrate carbon (Fig. 5D) to 100% total carbohydrate carbon content gives a putative $\Delta^{14}\text{C}$ of total carbohydrate carbon of 171‰, which is 112–148‰ (mean 128‰) more ^{14}C -enriched than the concurrent HMW DOC ($\Delta^{14}\text{C}$ of 23–59‰) in the lake.

4.4.2. Contribution of aliphatic carbon to $\Delta^{14}\text{C}$ of HMW DOC

The possible sources of aliphatic carbon in HMW DOC include a multitude of chemical structures, including CH , CH_2 and CH_3 groups within proteins, alcohols, fatty acids, methyl sugars, and alkanes and alkenes, that can vary widely in age both within and between compound classes. However, the observed significant inverse relationship between aliphatic carbons and $\Delta^{14}\text{C}$ of HMW DOC (Fig. 6B–D) implies that most of the aliphatic carbon in HMW DOC is derived from aged (^{14}C -depleted) compounds, potentially representative of refractory compounds. The sources of the aged components of the aliphatic carbon are not clear, but could be from older deep horizon soil materials.

4.4.3. Contribution of CRAM carbon to $\Delta^{14}\text{C}$ of HMW DOC

Although CRAM has been identified as a major structural component of DOC in freshwater and oceanic systems, its radiocarbon signature is not known. To our knowledge, no study has directly or indirectly estimated the radiocarbon content of CRAM. The significant inverse correlation observed between CRAM and $\Delta^{14}\text{C}$ of HMW DOC (Fig. 6A) suggests that CRAM constitutes a ^{14}C -de-

pleted component of the lake HMW DOC. Extrapolation of the observed relationship between CRAM and $\Delta^{14}\text{C}$ of HMW DOC to 100% CRAM content gives a putative $\Delta^{14}\text{C}$ -CRAM of -230‰ (2043 BP years). The estimated old age of CRAM, and CRAM's inverse relationship with $\Delta^{14}\text{C}$ of HMW DOC is consistent with the refractory nature of CRAM reported for oceanic (Hertkorn et al., 2006) and freshwater (Stubbins et al., 2010) systems. CRAM is thought to be derived mostly from cyclic and linear terpenoids (Hertkorn et al., 2006; Lam et al., 2007), although partitioning terrigenous vs. aquatic sources is difficult because of the ubiquitous nature of terpenoids in natural environments (Leenheer et al., 2003; Lam et al., 2007). In this study, however, coupling CRAM carbon and radiocarbon composition helps to constrain the potential source of the CRAM in Lake Superior. If most of the CRAM was derived from a recent within-lake source, it would be more ^{14}C -enriched (similar to $\Delta^{14}\text{C}$ of DIC) and reflect a direct relationship with $\Delta^{14}\text{C}$ of HMW DOC. In addition, the short water residence time (175 years) and twice-a-year mixing nature of the lake makes it unlikely for aquatic-derived CRAM to age considerably within the lake. Therefore, most of the CRAM in HMW DOC from Lake Superior is likely to be terrigenous (e.g. from deeper soil horizons) and/or from solubilized sedimentary OC released via pore water diffusion and sediment resuspension events. As with the aromatic phenolic signal, CRAM has been reported to be photolabile (Stubbins et al., 2010), possibly explaining why CRAM is not as dominant in Lake Superior and the surface ocean relative to the deep oceans (Hertkorn et al., 2006). Alternatively, CRAM could be a larger proportion of deep-ocean organic matter due to a deep water source. The NMR spectra of the HMW DOC from the deep ocean indicate significant CRAM content (Hertkorn et al., 2006). Also, the radiocarbon age of DOC in the deep ocean is, intriguingly, very old (4000–6000 years; Druffel et al.,

1992). We note that our dataset is limited, and the oceans are a different system, although NMR spectra of HMW DOC of the oceans and Lake Superior are remarkably similar (Repeta et al., 2002). If the results of our study in Lake Superior are applicable to the oceans, then the CRAM content could be a key driver of the observed old age of deep ocean DOM.

5. CONCLUSIONS

POC is the least abundant OC size fraction in the water column of Lake Superior, and is variably influenced by older resuspended sedimentary OC, resulting in the ^{14}C -depleted (older) POC seen at some of the sites in the lake. Colloidal DOC is composed of ^{14}C -enriched (modern) constituents (bacteria and small particles) in the offshore regions of the lake. However, contributions from older terrigenous and/or resuspended particles are reflected in ^{14}C -depleted (older) colloidal DOC at the nearshore site. LMW DOC is the dominant OM fraction in Lake Superior and bears a contemporary radiocarbon signature. Coupled lake mixing, photochemical and/or microbial mineralization, and priming are effective in recycling LMW DOC, and preventing long-term (century to millennium) storage (residence time) of low molecular weight molecules in the lake, thus differentiating open-lake from open-ocean DOC cycling. HMW DOC is more abundant than POC in the lake, and the coupled isotopic ($\Delta^{14}\text{C}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$) and elemental (C:N ratio) signatures robustly constrain HMW DOC in Lake Superior as derived mostly from contemporary (modern) OM sources with a larger component being labile (on annual to decadal timescales).

HMW DOC in Lake Superior is largely composed of carbohydrates, especially complex forms such as heteropolysaccharides (HPS). HPS and amide/amino sugars (AMS), and other carbohydrate compounds are ^{14}C -enriched (contemporary) structural components of HMW DOC in the lake. In contrast, carboxylic-rich alicyclic molecules (CRAM) and aliphatic compounds are older (^{14}C -depleted) structural components of HMW DOC in the lake. If the ^{14}C -depletion of CRAM seen in Lake Superior is also applicable to (and amplified within) the deep oceans, the dominance of CRAM in deep oceanic DOC could explain the age of deep-ocean HMW DOC (2440–4570 years BP, Loh et al., 2004) and if CRAM is also present in LMW DOC, why bulk deep ocean DOC has radiocarbon ages of 4000–6000 years BP (Druffel et al., 1992).

Larger amounts of bulk DOC and POC with contemporary (modern) radiocarbon signatures are exported from the watershed by a representative tributary stream (Amity Creek) during peak (storm) flows. In contrast, during base-flow conditions, the stream exports smaller amounts of relatively ^{14}C -depleted bulk DOC and pre-aged POC (1040 years BP). Also, during baseflows, HMW DOC and colloidal DOC from the stream reflect modern radiocarbon signatures, thus, HMW DOC in the stream cycles rapidly and the stream colloidal DOC is derived from contemporary sources (particles and bacterial biomass).

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