Constraining the sources and cycling of dissolved organic carbon in a large oligotrophic lake using radiocarbon analyses

Prosper K. Zigah,^{a*} Elizabeth C. Minor,^b Ann P. McNichol,^a Li Xu,^a Josef P. Werne,^c

^aWoods Hole Oceanographic Institution, Woods Hole, MA

^bLarge Lakes Observatory and Dept. of Chemistry and Biochemistry, University of Minnesota, Duluth, MN

^cDepartment of Geology and Environmental Sciences, University of Pittsburgh, Pittsburgh, PA

*Corresponding author: pzigah@whoi.edu

243 McLean Laboratory
Woods Hole Oceanographic Institution
266 Woods Hole Road, Woods Hole, MA 02543.

Tel. 508 289 3235

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Abstract

We measured the concentrations and isotopic compositions of solid phase extracted (SPE) dissolved organic carbon (DOC) and high molecular weight (HMW) DOC and their constituent organic components in order to better constrain the sources and cycling of DOC in a large oligotrophic lacustrine system (Lake Superior, North America). SPE DOC constituted a significant proportion (41-71 %) of the lake DOC relative to HMW DOC (10-13%). Substantial contribution of ^{14}C -depleted components to both SPE DOC ($\Delta^{14}\text{C} = 25$ to 43‰) and HMW DOC ($\Delta^{14}\text{C} = 22$ to 32‰) was evident during spring mixing, and depressed their radiocarbon values relative to the lake dissolved inorganic carbon (DIC; $\Delta^{14}\text{C} \sim 59\text{‰}$). There was preferential removal of ^{14}C -depleted (older) and thermally recalcitrant components from HMW DOC and SPE DOC in the summer. Contemporary photoautotrophic addition to HMW DOC was observed during summer stratification in contrast to SPE DOC, which decreased in concentration during stratification. Serial thermal oxidation radiocarbon analysis revealed a diversity of sources (both contemporary and older) within the SPE DOC, and also showed distinct components within the HMW DOC. The thermally labile components of HMW DOC were ^{14}C -enriched and are attributed to heteropolysaccharides (HPS), peptides/amide and amino sugars (AMS) relative to the thermally recalcitrant components reflecting the presence of older material, perhaps carboxylic-rich alicyclic molecules (CRAM). The solvent extractable lipid-like fraction of HMW DOC was very ^{14}C -depleted (as old as 1270-2320 ^{14}C years) relative to the substances isolated by acid hydrolysis of HMW DOC. Our data constrain relative influences of contemporary DOC and old DOC, and DOC cycling in a modern freshwater ecosystem.

1. Introduction

Dissolved organic carbon (DOC) is ubiquitous in aquatic ecosystems and is important in carbon and energy flow in food webs (Mann et al. 2015; Hitchcock et al. 2016), and in the cycling of nutrients, organic pollutants and trace metals (Riedel et al. 2013). Lakes play important roles in the global carbon cycle with an estimated CO₂ emission of 140 Tg C y⁻¹ (Cole et al. 1994) and annual carbon burial of up to 58% more than the oceans (Dean and Gorham, 1998). However, the sources and cycling of DOC in freshwater lacustrine systems, especially large lakes, are not well understood. This is primarily because DOC consists of a complex mixture of organic functional groups present in various higher order structures and coming from multiple sources (Hedges 1992; Miller and Zepp 1995). Consequently, DOC has multiple fates. DOC may be routed up to higher organisms in the food web via the microbial loop (Cole et al. 2006; Tanentzap et al. 2014), become sequestered in sedimentary organic matter through particle association (Masiello and Druffel 1998; Coppola et al. 2014) or be mineralized to carbon dioxide via photochemical and microbial oxidation (Obernosterer and Benner 2004; Cory et al. 2007; Amado et al. 2015).

Constraining the sources and cycling of DOC is a necessary step in understanding functioning of aquatic ecosystems and the global carbon cycle, but the molecular diversity of DOC means that a full separation, identification and characterization of all the organic molecules within it is currently not feasible. These compounds, however, can be grouped into operationally-defined discrete fractions based on their molecular weight (e.g. high molecular weight DOC; > 1000 daltons) and polarity (e.g. more hydrophobic DOC fractions) (Zigah et al. 2017).

In most freshwater systems, a substantial fraction (~30 to 80%) of DOC is comprised of components that can be isolated by adsorption onto a hydrophobic resin (e.g. PPL, C18, XAD-8,

SDB-XC) at acidic pH, commonly referred to as SPE DOC (Thurman and Malcolm 1981; Dittmar et al. 2008; Sickman et al. 2010). Nuclear magnetic resonance (NMR) analyses of SPE DOC from freshwater lakes and streams show that it is comprised significantly of hydrophobic humic substances, aromatic proteins, and lipids/fatty acids (Harvey et al. 1984; Kaiser et al. 2003; Lam et al. 2007; Zhang et al. 2014; Goldberg et al. 2015). The relative contributions of autochthonous versus allochthonous materials to the SPE DOC fraction are widely variable and are a function of initial chemical composition of the source materials, the humification/degradation state, the matrix of the water sample, and the structure of the SPE resin used for the extraction (e.g. Kruger et al. 2011; Li and Minor 2015). In clear-water systems such as the offshore regions of stratified oligotrophic large freshwaters, SPE DOC concentrations would be relatively low as should be expected for more dilute and less terrestrially-influenced systems with a lower contribution from humic substances (Dittmar et al. 2008; Kruger et al. 2011); input of humic substances from the sediments during mixing conditions could increase the SPE DOC amounts in these freshwaters (Goldberg et al. 2016). The concentration of SPE DOC is expected to be higher in freshwaters with abundant humic substances derived from terrestrial sources or microbial sources (Goldberg et al. 2016; Cawley et al. 2016; McKnight et al. 2001; Schwede-Thomas et al. 2005).

Up to 87% of freshwater DOC, especially in stream, river, or river-impacted coastal systems can be recovered as HMW DOC (nominal molecular weight of >1000 Daltons) using ultrafiltration (Benner and Opsahl 2001; Aluwihare et al. 2002; Repeta et al 2002; Stephens and Minor 2010; Zigah et al. 2014), although freshwater lakes with more autochthonous DOC appear to have less HMW DOC than these other freshwater systems (e.g., Kruger et al., 2011; Zigah et al. 2014, Repeta et al 2002). NMR analyses of HMW DOC in large freshwater lakes and in

marine systems show that it is rich in complex polymeric structures known as heteropolysaccharides (HPS), peptides/amide and amino sugars (AMS), and carboxylic-rich alicyclic molecules (CRAM) (Hertkorn et al. 2006; Abdulla et al. 2010; Zigah et al. 2014). In freshwater systems with little terrestrial influence, autochthonous microbial sources are the dominant source of the carbohydrate and protein fractions within HMW DOC (Zigah et al. 2014) whereas degraded higher plant materials and soils (allochthonous sources) will be dominant in more terrestrially-influenced smaller lakes or rivers (Kaiser et al. 2004).

The mechanisms of removal of DOC from freshwaters include microbial respiration, and photochemical oxidation to carbon dioxide and adsorption onto sinking particles (Moran et al. 2000; Xie et al. 2004; Cory et al. 2007). Photochemical oxidation is possibly the dominant sink for SPE-DOC; light-absorbing components appear to be preferentially concentrated in SPE extracts (Moran et al. 2000; Ma and Green 2004; Cory et al. 2007; Li and Minor, 2015). Pathways of removal of freshwater HMW DOC are not well constrained (Kaiser et al. 2004). Microbial and photochemical oxidations can mineralize both autochthonous and allochthonous material within HMW DOC in freshwater systems (Kaiser and Sulzberger 2004; Amon and Benner 1996). Microbial mineralization is likely dominant in the recycling of the autochthonous, carbohydrate and protein-rich, fraction of HMW DOC because of its relative bioreactivity and relative lack of light-absorbing components. In a similar vein, direct photochemical oxidation is likely the predominant pathway in the recycling of allochthonous material within HMW DOC because of its enrichment in light-absorbing components and relative bio-recalcitrance (Amon and Benner 1996, Moran et al. 2000; Cory et al. 2007). However, light-absorbing DOC (from isolated fractions and whole water) can also participate as a photo-sensitizer promoting indirect photochemical reactions which can then also affect molecules that do not absorb light themselves

(Sultzberger and Durisch-Kaiser, 2009). In addition, the non-linear coupling of microbial and photochemical processes in altering DOC pools has been shown to occur in many aquatic systems. Photochemical preprocessing of DOC has been reported to have both negative and positive effects upon microbial oxidation (Kaiser et al. 2004; Amado et al. 2015), perhaps because photochemical and microbial reactions are competing for key substrates or because there are competing interactions where reactive oxygen species suppress the microbial response while photochemical reactions make parts of the DOC pool more bioavailable (Anesio et al., 2005; Amado et al 2015).

In the past few decades, radiocarbon (^{14}C) has been useful in studying the ages, cycling and sources of total DOC in freshwater lakes and streams (Trumbore et al. 1992; Alberic et al. 2013; McCallister and del Giorgio 2012; Zigah et al. 2011; Butman et al. 2014; Keaveney et al. 2015). Natural abundance radiocarbon ($\Delta^{14}\text{C}$) and stable isotope ($\delta^{13}\text{C}$) compositions of the major fractions of DOC provide additional constraints on the multiple sources and cycling of the DOC. $\Delta^{14}\text{C}$ calculation corrects for biochemical fractionations so differences in $\Delta^{14}\text{C}$ values reflect variable sources and/or cycling. For instance organic material produced from recent within-lake photoautotrophy bears a $\Delta^{14}\text{C}$ value of the contemporaneous lake dissolved inorganic carbon (DIC). The few studies in freshwater systems that do partition DOC show that it consists of a mixture of contemporary and older components (Abbott and Stafford 1996; Guo et al. 2003, Sickman et al. 2009). More radiocarbon measurements of DOC fractions from various freshwater systems will help constrain the roles of contemporary and aged carbon in modern biogeochemical cycles and improve our understanding of the global carbon cycle.

This study investigated the isotopic compositions of various dissolved organic fractions from Lake Superior, an oligotrophic freshwater lake in North America. We measured the

radiocarbon and stable isotope compositions of SPE DOC and HMW DOC from the eastern basin of the lake. We also used serial thermal oxidation and compound class analyses to examine the isotopic diversity of organic matter classes within these two fractions. This is the first study to apply serial thermal oxidation to water-column dissolved organic matter (in this case, both ultrafiltered samples and SPE-extracted material) in a freshwater system. To the best of our knowledge, it is also the first study to compare the natural-abundance radiocarbon composition of both freshwater ultrafiltered and SPE-extracted DOM isolated from the same sampling stations. This unique data set improves our understanding of the multiple sources and dynamic cycling of DOC in the lake. Lake Superior is an important system for this study because the DOC cycling is representative not only of large temperate oligotrophic freshwater/lacustrine systems, but also provides insights for comparison with the open ocean. Both Lake Superior and the open ocean have similar concentrations of DOC (Cotner et al. 2004; Ma and Green 2004), apparent low inputs of allochthonous organic matter and nutrients, a pH range controlled by bicarbonate buffering (Lake Superior's pH range is 7.61-8.03, slightly lower than the average ocean pH, Tennant 2016), and a primarily microbial food web (Cotner et al. 2004).

2. Methods

2.1 Study site

Lake Superior (Fig. 1) is the Earth's largest freshwater lake by surface area and the deepest of the Laurentian Great Lakes of North America, with a maximum depth of 406 m. The lake is dimictic, with complete vertical mixing of the water column in spring and early winter each year. The hydraulic residence time of the lake (lake volume divided by outflows, including diversions) is 173 years (Quin et al. 1992). DOC is the largest organic carbon pool with an average lakewide surface and deep water concentration of ~ 90 μM during spring mixing. During

summer stratification, lakewide surface DOC is ~ 100-120 μM and is about 10 μM larger than the deep DOC (Zigah et al. 2012). The sources and cycling of DOC in the lake are not well constrained. Annual autochthonous DOC input is estimated at 0.9 Tg C (~ 9% of the primary production; Urban et al. 2005, Sterner 2010), similar to the annual terrestrial DOC loading of 0.4-0.9 Tg C (Cotner et al. 2004; Urban et al. 2005). In spite of the substantial terrestrial input, spectroscopic studies of the DOC, including ultraviolet-visible spectroscopy of total DOC, NMR of HMW DOC, and FTIR of total and HMW DOC have shown fairly low contributions from aromatic constituents that indicate terrigenous sources (Minor and Stephens, 2008, Zigah et al. 2014). Hence, the presence, amount and fate of terrigenous DOC in the lake remains poorly understood. To provide greater insights into the composition, cycling and sources of DOC in the lake, we previously investigated the radiocarbon and stable isotope composition of total DOC (Zigah et al. 2011; 2012), and size-fractionated DOC (Zigah et al. 2014). HMW DOC constitutes 8-20% of the total DOC in the western arm and 10-13% of the total DOC in the eastern arm of the lake based on isolation via cross-flow ultrafiltration. NMR spectra of the HMW DOC from the lake show that heteropolysaccharides, peptides/amide and amino sugars together constitute 75-84% of the carbon, with carbohydrate carbon alone representing 53-65% of the HMW DOC (Zigah et al. 2014).

2.2 Sampling

Surface (5 m) and deep (210 m) samples were collected from eastern Lake Superior (47°34'N, 86°39'W; Fig. 1) during spring mixing in June 2009, and thermal stratification in August 2009 (Fig. 2). Lake water was collected using twelve 8-L Niskin bottles mounted on a rosette equipped with a Seabird Model 911 Plus Conductivity, Temperature, and Depth (CTD) profiler. Dissolved inorganic carbon (DIC) samples were taken directly from the Niskin bottles

via pre-cleaned silicone tubing into previously acid-cleaned (10% v/v HCl followed by rinsing with milli-Q water) and combusted (450°C for ≥ 4 hours) 0.5 L amber Pyrex bottles. The samples were immediately preserved with 100 μ L of saturated mercuric chloride solution, sealed air-tight with glass stoppers coated with Apiezon grease, and stored at room temperature in the dark until analysis (McNichol et al. 1994). DOC ($< 0.7 \mu\text{m}$) samples were obtained by filtering lake water through pre-combusted Whatman GF/F glass fiber filters (450°C for 4 hours) using stainless-steel canisters pressurized with nitrogen. Approximately 40 mL of lake water was collected into an acid-cleaned and combusted vial and acidified to pH 2 using 6M HCl for DOC analysis by high temperature catalytic oxidation. For DOC radiocarbon analysis, 1 L of the GF/F filtered water was collected into a pre-combusted glass bottle, acidified with 6M HCl to pH 2, and stored in a refrigerator at 4°C until analysis.

2.3 Ultrafiltration and solid phase extraction

Large-volume samples (200-400 L) (Table 1) were drawn from the lake using an air-driven diaphragm pump, 0.2 μm filtered (Whatman Polycap 75 TC), and ultrafiltered as described previously (Zigah et al. 2014). Previously frozen 0.7 μm -filtered water samples stored in precombusted 1L glass bottles or acid-cleaned 1L high density polyethylene (Nalgene) bottles were thawed, filtered (0.2 μm), and solid phase extracted using the protocol of Dittmar et al. (2008). Briefly, the filtered water was acidified to pH 2 with 6M HCl and extracted using methanol-activated styrene divinylbenzene polymer resin (PPL; 1g, Varian Mega Bond Elut) at a flow rate of 10 mL/ min. The DOC extract was desalted with acidified Milli-Q water (pH 2), dried with ultrahigh purity (UHP) N₂ and eluted with methanol. The SPE DOC sample was transferred into quartz combustion tubes and completely dried; Ag powder and precombusted CuO were added; the tubes were evacuated and then flame-sealed.

2.4 Measurement of DOC concentrations

DOC and TOC samples were analyzed on a Shimadzu V_{CSH} TOC analyzer (Zigah et al. 2011). Lyophilized HMW DOC samples were analyzed on a Costech ECS 4010 elemental analyzer (EA) coupled to Finnigan Delta Plus XP isotope ratio mass spectrometer (IRMS) after fumigation with 12 N HCl (ACS Plus grade) and drying (Zigah et al. 2014). Typical instrumental precision for OC measurements was 0.2% of the measured concentration. SPE DOC samples were combusted to CO₂ in sealed quartz tubes and quantified manometrically.

2.5 Extraction of organic fractions from HMW DOC

All frozen HMW DOC samples were freeze-dried and homogenized before analysis. Approximately 75-100 mg of each freeze-dried sample was used for the extractions. The lipid-like, carbohydrate-like and protein-like fractions were extracted from the HMW DOC as described previously (Wang et al. 1998; Loh et al. 2004). The solid residual material after the HCl hydrolysis (unhydrolysable fraction) was also isotopically characterized. In all cases, the extracted organic fractions were transferred into quartz combustion tubes and completely dried, precombusted CuO and Ag were added, and the tubes were evacuated and sealed on a vacuum line.

2.6 Serial thermal oxidation of HMW DOC and SPE DOC from Lake Superior

Serial thermal oxidation was performed at the National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS) at the Woods Hole Oceanographic Institution (WHOI) using the Ramped PyrOx system in oxidation mode (Rosenheim et al. 2008, Plante et al. 2013). A dried sample was weighed and transferred to a quartz reaction vessel. The vessel was then placed in the top of the thermal analyzer (two coupled ovens with the bottom oven set at a constant temperature of 800 °C and the top oven holding the sample reactor). With an oxygen (8%) and

helium (92%) mixed gas solution flowing through the reactor at a rate of 35 mL per minute, the sample was thermally oxidized to CO₂ by gradually raising the top oven temperature from ambient to 1000 °C at a rate of 5 °C per minute. The evolving gas was passed over a Pt/Ni/Cu twisted wire in the 800 °C oven to ensure complete oxidation to CO₂. The CO₂ was first quantified downstream with an in-line CO₂ analyzer, and then collected in successive fractions using flow-through glass traps (Rosenheim et al. 2008; Plante et al. 2013).

2.7 Radiocarbon and stable isotope measurements

Radiocarbon ($\Delta^{14}\text{C}$) and stable carbon isotope ($\delta^{13}\text{C}$) measurements were performed at NOSAMS using standard protocols (McNichol et al. 1994). Typical instrumental precision of $\delta^{13}\text{C}$ based on multiple analyses of standards was 0.15‰. The graphite produced in all cases was analyzed using either a 3MV tandemron accelerator mass spectrometer (USAMS) or 500kV pelletron continuous flow accelerator mass spectrometer (CFAMS) along with primary and secondary standards, and combustion and graphitization process blanks (Reden et al. 2004; Roberts et al. 2010; Brett et al. 2015). Radiocarbon values are reported as $\Delta^{14}\text{C}$ according to the convention of Stuiver and Polach (1977). Instrumental precision of the $\Delta^{14}\text{C}$ analysis is based on the error of standards or multiple analyses on a target and was 3-6‰. The radiocarbon composition of lake-water DIC was collated from Zigah 2012, Zigah et al. 2011, 2012 and 2014. The atmospheric CO₂ $\Delta^{14}\text{C}$ data was based on the radiocarbon composition of corn leaves (*Zea mays*) from the lake watershed (Hseuh et al. 2007; Zigah 2012; Kruger 2014) and from atmospheric CO₂ in the Northern Hemisphere (Graven et al. 2012). The atmospheric CO₂ data covering 2004-2007 is from Hseuh et al. (2007) and Graven et al. (2012), and the data covering 2009-2012 are from Zigah 2012 and Kruger 2014.

2.8 Blank assessment

To assess the process blanks and potential fractionation associated with the extractions of organic fractions from HMW DOC for isotopic measurements, we measured the isotopic values of known standard materials before and after extraction. In all cases, the amount of carbon extracted from the standard material was comparable to or smaller than the amount extracted from the samples. For the lipid standard (stearic acid), the $\Delta^{14}\text{C}$ values of non-extracted and extracted stearic acid were $48 \pm 4\text{‰}$ and $51 \pm 4\text{‰}$, respectively. The $\Delta^{14}\text{C}$ values of non-extracted and extracted D-glucose (carbohydrate standard) were $35 \pm 4\text{‰}$ and $41 \pm 4\text{‰}$ and those of bovine serum albumin (protein standard) were $184 \pm 3\text{‰}$ and $179 \pm 3\text{‰}$ respectively. These results indicate that there is a negligible amount of non-modern carbon added during the extraction processes for the organic fractions. Also, non-extracted and extracted $\delta^{13}\text{C}$ values of stearic acid ($-30.4 \pm 0.15\text{‰}$ vs. $-30.4 \pm 0.15\text{‰}$), D-glucose ($-11.2 \pm 0.15\text{‰}$ vs. $-11.2 \pm 0.15\text{‰}$) and bovine serum albumin ($-10.2 \pm 0.15\text{‰}$ vs. $-10.6 \pm 0.15\text{‰}$) indicate no fractionation and/or contamination from the extraction processes. The lipid standard we used was modern but the samples we analyzed were pre-aged, so we must evaluate the potential for contamination by modern carbon. If we assume the process blank for the total lipid extraction contains $1 \mu\text{g C}$ (Loh et al. 2004) with a $\Delta^{14}\text{C}$ value of 48‰ , similar to the modern stearic acid standard, addition of this will increase the $\Delta^{14}\text{C}$ values of the lipid samples by $\leq 1\text{‰}$. If the process blank contained as much as $12 \mu\text{g C}$ with a $\Delta^{14}\text{C}$ value of 48‰ , the lipid samples' $\Delta^{14}\text{C}$ values would decrease from the range of -153‰ to -256‰ reported here to -159‰ to -268‰ . This change, while significant, is too small to alter the discussion or conclusions of this paper.

To assess the process blank associated with the SPE DOC extraction, acidified Milli-Q water was extracted using methanol-activated PPL resin, eluted with methanol, dried and combusted to CO_2 in sealed quartz tubes at 850°C for 5 hours. Less than $1 \mu\text{g C}$ was obtained,

small relative to the SPE sample size (421-774 $\mu\text{g C}$), and there was not enough for isotopic analysis. If we assume this carbon has a fraction modern value of 1 ($\Delta^{14}\text{C} \sim -7\text{‰}$) or 0 ($\Delta^{14}\text{C} = -1000\text{‰}$), the SPE sample $\Delta^{14}\text{C}$ values only decrease by $\leq 2\text{‰}$. To assess the process blank associated with serial thermal oxidation on the Ramped PyrOx (RPO), we cycled the thermal analyzer without any sample in the reactor and quantified the blank carbon to be $\sim 4 \mu\text{g C}$ from ambient to 1000°C , similar to the $< 4 \mu\text{g C}$ reported by Plante et al. (2013) and Hemingway et al. (In Press). This amount is not enough for isotopic measurement and is small ($\leq 6\%$) relative to the CO_2 amounts in the thermal fractions of the sample (63-131 $\mu\text{g C}$). Further evidence of the negligible impact of the process blank on ^{14}C results from the RPO comes from the agreement between the $\Delta^{14}\text{C}$ values of the bulk HMW and SPE samples and the values calculated for the bulk samples using a mass balance of the RPO data. Due to negligible blank carbon and/or isotopic fractionation, reported $\Delta^{14}\text{C}$ and $\delta^{13}\text{C}$ values of organic fractions of HMW DOC, SPE DOC and organic fractions from serial thermal oxidation were not corrected for process blanks or fractionation.

3. Results

3.1 Trends in the radiocarbon values of Lake DIC and atmospheric CO_2 from 2004 to 2012

The $\Delta^{14}\text{C}$ value of atmospheric CO_2 decreased from $\sim 66\text{‰}$ in 2004 to 38‰ during our sampling in 2009, and to 25‰ in 2012 (Fig. 3) corresponding to a decline of $\sim 5\text{‰/yr}$. The lake DIC $\Delta^{14}\text{C}$ value decreased from 83‰ to 44‰ between 2007 and 2012 with a decline $\sim 7\text{‰/yr}$ (Fig. 3). DIC was consistently ^{14}C -enriched by $\sim 20\text{‰}$ relative to atmospheric CO_2 from 2004-2014, indicating a carbon isotope equilibration time of ~ 4 years between the DIC and atmospheric CO_2 over this period.

3.2 Concentration and isotopic values of HMW DOC vs. SPE DOC vs. Total DOC

The concentration of HMW DOC was 9-11 μM (10-13% of total DOC) with molar C:N values of 14.5-23.6; the highest C:N ratio was for surface HMW DOC from June 2009 (Tables 1 and 2). SPE DOC concentrations were 35.5 to 48.8 μM (41 to 51% of total DOC) and 60 to 64 μM (65 to 71% of total DOC) during stratification and mixed-lake conditions, respectively (Table 2). The $\delta^{13}\text{C}$ values of SPE DOC, HMW DOC and total DOC in the lake were -26.4‰ to -26.8‰, -25.9‰ to -26.3‰ and -25.9‰ to -26.3‰, respectively (Table 2). The $\delta^{13}\text{C}$ values of total DOC, HMW DOC and SPE DOC did not exhibit considerable variation between spring mixing and summer stratification (Table 2).

The $\Delta^{14}\text{C}$ values of SPE DOC (25 to 43‰) were slightly depleted or similar to the $\Delta^{14}\text{C}$ value of atmospheric CO_2 , and by extension, recent land-plant primary production ($38 \pm 2\text{‰}$) from 2009-2010, but more depleted relative to lake DIC ($\Delta^{14}\text{C}$ of 59 to 63‰). The stratified deep and mixed-lake surface SPE DOC values (25 to 30‰) were more depleted relative to the $\Delta^{14}\text{C}$ of lake DIC (Table 2) than were the values for the other two SPE DOC samples. During spring mixing conditions in June, $\Delta^{14}\text{C}$ values of HMW DOC were 22 to 32‰ and increased to 51 to 54‰ during stratification in August (Table 2). HMW DOC, SPE DOC and total DOC were consistently more depleted in ^{14}C than the lake DIC ($\Delta^{14}\text{C}$ of 58 to 59‰ in June and 59 to 63‰ in August) (Table 2). SPE DOC was more depleted than HMW DOC and total DOC during stratification (Table 2).

3.3 $\Delta^{14}\text{C}$ and $\delta^{13}\text{C}$ values of extracted organic fractions within HMW DOC

The solvent extractable lipid-like fraction had $\Delta^{14}\text{C}$ values of -204‰ to -256‰ (corresponding to 1770 - 2320 ^{14}C years) during spring mixing in June, and $-153 \pm 13\text{‰}$ (~1270 BP years) in the stratified surface waters in August (Table 3). The lipid-like fractions were consistently more ^{14}C -depleted (older) than the concurrent carbohydrate-like ($\Delta^{14}\text{C}$ of 20 to 31‰)

and protein-like ($\Delta^{14}\text{C}$ of 34 to 39‰) fractions. The latter fractions were consistently modern, but ^{14}C -depleted relative to the DIC ($\Delta^{14}\text{C}$ of 58 to 63‰) (Tables 2 and 3). The $\delta^{13}\text{C}$ values of the carbohydrate-like and protein-like fractions (-25.0‰ to -26.9‰) were more ^{13}C -enriched than the lipid-like fraction (-28.0‰ to -29.7) (Table 3). The $\Delta^{14}\text{C}$ and $\delta^{13}\text{C}$ values of the unhydrolysable fraction were more variable with depth in the lake during the stratified period (Table 3).

3.4 Serial thermal oxidation of HMW DOC

The thermogram of the stratified surface HMW DOC shows components oxidizing over the low- temperature range $\sim 200\text{-}390\text{ }^{\circ}\text{C}$ with two discernable peaks (larger one at $\sim 270\text{ }^{\circ}\text{C}$ and smaller one at $\sim 380\text{ }^{\circ}\text{C}$). High-temperature components thermally oxidize at $\sim 420\text{-}630\text{ }^{\circ}\text{C}$ with peaks at $\sim 513\text{ }^{\circ}\text{C}$ and $\sim 575\text{ }^{\circ}\text{C}$ (Fig. 4a). The thermogram of the mixed-lake surface HMW DOC also shows components oxidizing over low-temperature and high-temperature ranges. A considerably lower percentage of the carbon appeared in the low-temperature range during mixed-lake condition as compared to stratified lake condition (Fig. 4a; Table S2). The high-temperature peak at $\sim 513\text{ }^{\circ}\text{C}$ was smaller in the mixed-lake sample but other high-temperature components were observed including a large peak at $\sim 630\text{ }^{\circ}\text{C}$ (Fig 4a). The thermograms show a strong difference in the composition of thermally refractory material during stratification and mixing conditions. For radiocarbon analysis, six thermal fractions with relative amounts of carbon (in mol %) of 5, 10, 14, 14, 13 and 44 (Fig. 4c; Table S2) were collected from the mixed-lake surface HMW DOC. The CO_2 fractions from the lower temperature range of $200\text{-}330\text{ }^{\circ}\text{C}$ were more ^{14}C - enriched ($\Delta^{14}\text{C}$ of 60-64‰) than the CO_2 fractions from the higher temperature range of $388\text{-}800\text{ }^{\circ}\text{C}$ ($\Delta^{14}\text{C}$ of -23 to 34‰) (Fig. 4c). Five thermal fractions with relative amounts of carbon (in mol %) of 22, 24, 21, 21 and 12 (Fig. 4e; Table S1) were collected from

the stratified-lake surface HMW DOC. The CO₂ fractions from the lower temperature range 200-387 °C were more ¹⁴C-enriched ($\Delta^{14}\text{C}$ of 60-75‰) than the CO₂ fractions from the higher temperature range 387-800 °C ($\Delta^{14}\text{C}$ of 27-30‰) (Fig. 4e). For both mixed-lake and stratified surface HMW DOC samples, the mass-weighted mean $\Delta^{14}\text{C}$ value was similar to the independently measured bulk $\Delta^{14}\text{C}$ value.

3.5 Serial thermal oxidation of SPE DOC

The thermogram of the stratified surface SPE DOC shows components oxidizing over a broad temperature range ~ 200-530 °C (peak at 375 °C). High-temperature components, a peak at ~ 504 °C and a shoulder at ~550 °C, were observed in the surface SPE DOC during lake mixing, but these components were absent during stratification (Fig. 4b). Five thermal fractions (F1-F5) with relative amounts of carbon (in mol %) of 13, 17, 17, 20 and 33 (Fig. 4d; Table S4) were collected from the mixed-lake surface SPE DOC for radiocarbon analysis. SPE DOC components within thermal fractions F1, F4, and F5 were more ¹⁴C-depleted ($\Delta^{14}\text{C}$ of -22 to 48‰) relative to the components within fractions F2 and F3 ($\Delta^{14}\text{C}$ of 54-58‰) (Fig. 4d). The relative amounts of carbon (in mol %) in the five thermal fractions (F1-F5) from stratified surface SPE DOC were 17, 23, 34, 18 and 8 (Fig. 4f; Table S3). SPE DOC components within fractions F1, F4, and F5 were more ¹⁴C-depleted ($\Delta^{14}\text{C}$ of -8 to 47‰) relative to the components within fractions F2 and F3 ($\Delta^{14}\text{C}$ of 60-65‰) (Fig. 4f) and in this case F1 and F5 both exhibited negative $\Delta^{14}\text{C}$ values. The mass-weighted mean $\Delta^{14}\text{C}$ values matched the independently measured bulk values of both the mixed-lake and stratified surface SPE DOC.

4. Discussion

4.1 Sources and cycling of HMW DOC and SPE DOC fractions

The $\Delta^{14}\text{C}$ values of DIC declined from a range of 76‰ to 83‰ in 2007 to a range of 59‰ to 63‰ in 2009 mirroring decreases in the atmosphere. This implies that DOC originating from planktonic productivity within the lake since the influence of atmospheric bomb ^{14}C on the lake DIC (i.e., in the past ~60 years) would bear $\Delta^{14}\text{C}$ values $\geq 59\text{‰}$ (Fig. 3).

During mixed-lake conditions in spring, the radiocarbon values of HMW DOC were more depleted than the values of DIC and recent atmospheric CO_2 . This suggests there is a significant mobilization of ^{14}C -depleted (aged) HMW DOC components. The aged DOC must derive from sources other than current primary productivity in the lake, such as the sediments and/or soils (Table 4) or productivity pre-dating the bomb spike in the 1950-60's. The observed ^{14}C -depletion may be due to a relatively smaller input (and thus smaller overprinting of the residual DOC signal) from autochthonous DOC in the lake during winter and spring when temperature and light levels limit primary production (Sternner 2010). The depletion could also result, in part or wholly, from a substantial DOC input from the sediments during lake over-turn, or input from the watershed via spring snowmelt and ice-out (Stottlemyer and Toczydlowski 1991, Zigah et al. 2012).

The ^{14}C -enrichment of HMW DOC during stratification could, in part, result from planktonic photosynthesis in the surface waters in the summer months (Urban et al. 2005). Increases in the HMW DOC $\Delta^{14}\text{C}$ values in the stratified lake could also be due to an increased contribution from terrestrial material synthesized 2-4 years prior to our sampling in the lake (52-60‰ in 2005-2007) (Fig. 2 and Table 2). However, the dramatically lower C:N values of surface-water HMW DOC in August relative to June (Table 1) coupled with the radiocarbon results suggest that an increased contribution from photoautotrophy is more likely than a substantial terrestrial input. The radiocarbon values of HMW DOC and total DOC in the

stratified deep waters were also enriched by 19‰ and 16‰ respectively, relative to the values in the mixed-lake (Table 2). This implies either rapid vertical transfer within weeks to months of more enriched surface-derived DOC to the deep waters or a preferential removal (through mineralization or conversion to POC) of more ^{14}C -depleted deep-water DOC; or a combination of the two mechanisms. The exact route of DOC delivery to the deep waters is not known, but could be from convective mixing, downwelling events (Austin 2013), and/or solubilization of sinking particles.

Although the isotopic composition shifted significantly, the concentrations of surface water HMW DOC and total DOC at both depths only changed slightly (5 to 6%) between the mixed-lake and stratified conditions (Table 2), indicating that the isotopic changes are not solely the result of changing inputs. The radiocarbon values of surface HMW DOC and surface total DOC would be 25‰ and 43‰, respectively, in the stratified lake if the additional autochthonous DOC input estimated from concentration changes was the only factor modulating the DOC $\Delta^{14}\text{C}$ values. However, the observed radiocarbon values were 54‰ for both HMW DOC and total DOC in the stratified surface waters. The observed changes in radiocarbon content relative to carbon concentration between mixed and stratified conditions indicate that a significant fraction of the ^{14}C -depleted components of HMW DOC and total DOC in the mixed lake is removed by August, by either remineralization or sorption processes. In a previous study linking NMR characteristics to radiocarbon age within HMW DOM samples, CRAM and aliphatic carbon were identified as ^{14}C -depleted within Lake Superior, with CRAM age estimated at 2040 ^{14}C years (Zigah et al, 2014). The remineralization of such aged components could be in part responsible for the observed increase in $\Delta^{14}\text{C}$. Zigah et al. (2014) used ^{13}C NMR spectra to demonstrate that 16-20% of the HMW DOC from the eastern Lake Superior consists of CRAM,

and estimated $\Delta^{14}\text{C}$ value of -230‰ for the HMW DOC CRAM. Using these values in a mass balance, we estimate that complete oxidation of Lake Superior's HMW CRAM pool would decrease the concentration of HMW DOC by 1.7-2.2 μM and increase the HMW DOC $\Delta^{14}\text{C}$ values from 22‰ in the mixed-lake to ~70‰ during stratification. HMW DOC concentration actually increased by 0.6-2 μM during stratification, and the $\Delta^{14}\text{C}$ value only increased up to 54‰ (Table 2), indicating that DOC removal likely occurs across DOC of multiple ages, and that this removal is overlaid by the autochthonous input discussed above. Further evidence for both the removal of older DOC and input of autochthonous DOC in surface waters is found in the NMR spectra of surface HMW DOC. The spectra indicates that CRAM is a relatively lower proportion of total DOC than in the mixed-lake HMW DOC (Zigah et al. 2014).

Complicating this autochthonous input and older-carbon removal story, is the fact that there may also be a considerable mobilization or input of DOC that is more ^{14}C - enriched in the surface water than the current lake DIC during stratification. For example, autochthonous DOC and terrigenous DOC from the past 5-10 years previous to our sampling in 2009 would have $\Delta^{14}\text{C}$ values as high as +120‰ (Fig. 3). By extrapolation of the linear relationship between HMW DOC $\Delta^{14}\text{C}$ values and HPS and AMS and between HMW DOC $\Delta^{14}\text{C}$ values and total carbohydrate carbon from the ^{13}C NMR spectra of lake samples, Zigah et al. (2014) estimated the $\Delta^{14}\text{C}$ values of combined HPS and AMS and total carbohydrate carbon within HMW DOC to be 113‰ and 171‰, respectively, which is ^{14}C -enriched relative to the lake DIC. However, the radiocarbon values for carbohydrate-like carbon in surface-water HMW DOC in the mixed vs stratified lake (Table 3) actually show a decrease between June and August, indicating that remobilization of such ^{14}C -enriched material does not seem to be a major factor in the mixed vs stratified-season differences.

The radiocarbon values of the SPE DOC from the surface water increased from a $\Delta^{14}\text{C}$ value of 25‰ in the mixed-lake to 41‰ during stratification while the concentration decreased by 11 μM (18%, Table 2). This suggests that the increase in the $\Delta^{14}\text{C}$ of SPE DOC results mainly from preferential remineralization or removal of older SPE DOC. Mass balance calculations indicate that ~11 μM of old SPE DOC with an average $\Delta^{14}\text{C}$ value of - 44‰ is removed from the surface during thermal stratification. The serial thermal oxidation results shown in Fig. 4 b,d, and f provide further evidence supporting preferential removal of an older and thermally recalcitrant pool. The removal of SPE-DOC during summer stratification along with a concurrent enrichment in ^{14}C has been seen in other lake systems. In their study of oligotrophic high-elevation lakes in California, Goldberg et al. (2015) reported that the total DOC concentration in Lake Tahoe increased from 35 to 44 μM between January and June, but the SPE DOC, isolated in this case with a DAX-8 resin, decreased from 12.1 to 11.7 μM . The Lake Tahoe SPE DOC was enriched in ^{14}C during summer stratification in June ($\Delta^{14}\text{C}$ of 35‰) relative to lake mixing in January ($\Delta^{14}\text{C}$ of -6‰). A similar radiocarbon enrichment was also reported in the SPE DOC from Fallen Leaf Lake in Sierra Nevada during stratification in summer (Goldberg et al. 2015). The relative depletion of ^{14}C in a significant fraction of SPE DOC relative to bulk DOC and DIC is consistent with observations by Abbott and Stafford (1995). In a study of three Arctic Lakes, they noted humic substances isolated by XAD-8 resin were depleted in ^{14}C relative to total DOC and other carbon pools, but were similar in ^{14}C values relative to soils and peat from the watershed. They concluded that these ^{14}C -depleted terrestrial sources contribute to the lake humic substances. These observations are consistent with our observations in Lake Superior and suggest that seasonal dynamics in the chemical and isotopic compositions of SPE DOC are controlled by similar processes in these lakes. It appears that ^{14}C -depleted inputs of SPE DOC

during lake mixing and snowmelt in spring and photochemical and/or microbial removal of ^{14}C -depleted components during stratification in summer may be a widespread phenomenon in temperate freshwater lakes.

The relative importance, potential competition, and synergistic relationships of photo-oxidation versus microbial oxidation in remineralization processes (especially removal of old DOC) in aquatic systems such as Lake Superior are not well understood (Amado et al. 2015). Chemically, increased microbial oxidation, and the concomitant increase in the percentage of microbial biomass in the organic matter pools, has been associated with increased nitrogen content whereas photochemical oxidation decreases aromaticity (Brooks et al. 2007; Cory et al. 2007). Light-absorbing components of DOC such as aromatic compounds and black carbon released from chars have been shown to be more susceptible to photoalteration (Cory et al. 2007; Ward et al. 2014; Ward and Cory 2016).

In Lake Superior, terrestrial sources deliver ~0.4-0.9 Tg C to the lake each year (Cotner et al. 2004; Urban et al. 2005), enough to account for the lake's DOC reservoir of 14-17 Tg C (Zigah et al. 2012) and DOC steady state cycling given the hydraulic residence time of 173 years. But evidence for substantial terrigenous DOC in the lake is lacking. Studies based on ultraviolet-visible spectroscopy of total DOC, NMR of HMW DOC, and FTIR of total and HMW DOC have shown fairly low contributions from the aromatic constituents which would indicate terrigenous sources (Chin et al. 1994; Ma and Green 2004; Minor and Stephens, 2008, Stephens and Minor, 2010; Zigah et al. 2014). It is possible that some of the aromatic terrigenous compounds are photochemically degraded or oxidized, possibly through interaction with singlet oxygen, a photochemically produced oxidant that has been reported to increase in Lake Superior relative to its tributaries (Peterson et al. 2012). Therefore, the reported low aromatic constituents

coupled with our radiocarbon data showing the removal of old ^{14}C -depleted components in the sunlit waters in summer (Table 2) suggest that photochemical oxidation plays a major role in the removal of old allochthonous DOC in Lake Superior during stratification in summer.

4.2 Radiocarbon ages, sources and cycling of organic compound classes within HMW DOC

4.2.1 HMW DOC Carbohydrate-like and protein-like fractions

Hydrolysable carbohydrate-like and protein-like substances from the HMW DOC were 24- 39‰ depleted in ^{14}C relative to the DIC, and 7 - 18‰ depleted in ^{14}C relative to the atmospheric CO_2 in the sampling year (Table 3, Fig. 5). These indicate that there must be a significant contribution from organic material that pre-dates bomb testing. HMW DOC from recent in-lake productivity may be remineralized quickly, as it does not seem to impart a signal persistently identifiable in mixed and stratified waters. An estimate of $\Delta^{14}\text{C}$ of the total carbohydrate carbon within HMW DOC based upon the correlation between NMR signals and $\Delta^{14}\text{C}$ yielded a lakewide carbohydrate $\Delta^{14}\text{C}$ value of 171‰ (Zigah et al. 2014). The relative depletion of $\Delta^{14}\text{C}$ values of the hydrolysable carbohydrate-like material observed in this study implies either that the more labile ^{14}C -enriched components of these hydrolysable substances are not recovered in the hydrolysis, that there is a significant difference in the carbohydrate structures identified by NMR compared to carbohydrate-like material recovered by hydrolysis, or that the NMR signal in several samples from Zigah et al. (2014), which come from sites spatially distributed across the lake, is preferentially influenced by the carbohydrate from materials synthesized closer to the timing of the bomb spike (perhaps terrigenous material that is a few decades old). It is also possible that some ^{14}C -depleted noncarbohydrate impurities from CRAM or humic substances in the HMW DOC were co-isolated during acid hydrolysis. Overall, the modern radiocarbon signatures of the hydrolysable substances in both stratified and mixed-lake

conditions (Fig. 5, 6) and the previous NMR-based results showing post-bomb values for the carbohydrate carbon, HPS and AMS constituents indicate that, on average, these carbohydrate-like and protein-like substances do not persist over time scales approaching a century or longer. The large differences in ^{14}C -enrichment determined by the two methods used to analyze these fractions of DOC indicate the need for further work in linking compound-class characterization and radiocarbon content.

The $\delta^{13}\text{C}$ values of DIC in the lake were 0.3-0.4‰ in all but the stratified surface water which had a value of 0.9‰ (Table 2). Aqueous CO_2 is typically ^{13}C -depleted by $\sim 10\text{‰}$ relative to the bulk DIC (de Kluijver et al. 2014). Applying a typical lake algal photosynthetic fractionation of 17‰ (de Kluijver et al. 2014), organic materials recently synthesized within the lake will have a $\delta^{13}\text{C}$ value of -26 to -27‰, similar to the reported algal $\delta^{13}\text{C}$ values of -26.0 to -27.8‰ in the lake (Keough et al. 1996). Photochemically altered terrestrially-derived vascular plant sources have similar $\delta^{13}\text{C}$ values of -25.0 to -26.6‰ (Vahatalo and Wetzel 2008). Therefore, the $\delta^{13}\text{C}$ values of -25.0 to -26.9‰ observed here for the hydrolysable carbohydrate-like and protein-like substances reflect both the values expected from photochemically altered terrigenous sources as well as autochthonous algal-derived sources.

4.2.2 HMW DOC Lipid-like fraction

Possible sources of the lipid-like fraction are cellular lipids and lipid components of humic substances or petroleum hydrocarbons that may be co-isolated with cellular-derived lipids during solvent extraction (McIntyre et al. 2002). Petroleum hydrocarbons most likely derive from anthropogenic inputs to Lake Superior. The ^{13}C -depletion of the lipid-like fraction (Fig. 6) is consistent with fractionation associated with cellular lipids biosynthesis (De Niro and Epstein 1977). $\Delta^{14}\text{C}$ values of the lipid-like fraction (-256‰ to -153‰) were by far more ^{14}C -depleted

than the acid hydrolysable substances. The millennia ages of the lipid-like fractions are surprising given the hydraulic residence time of the lake (173 years), and indicates that this fraction does not derive from recent bomb ^{14}C -influenced plankton productivity within the lake or recent terrestrial productivity. In Lake Michigan lipid-like materials from particulate organic carbon (POC) have been reported to be influenced by resuspended sediments (Meyers et al. 1984) and such influences may also occur in the POC and DOC in Lake Superior. The lipid-like fraction and CRAM ($\Delta^{14}\text{C}$ of -230‰; Zigah et al. 2014) from HMW DOC are similarly ^{14}C -depleted suggesting that ^{14}C -depleted CRAM may be included within the lipid-like fraction. Co-recovery of 70-90% of CRAM with a $\Delta^{14}\text{C}$ value of -230‰ or 20-25% of petroleum hydrocarbon with $\Delta^{14}\text{C}$ value of -1000‰ along with recently-synthesized cellular lipids would account for the ^{14}C -depletion observed in the lipid-like fraction relative to the hydrolysable substances. The relative ^{14}C -enrichment of the surface lipid-like fraction during stratification in August (Fig. 5) could be explained by the addition of ~19% of lake-DIC derived ^{14}C -enriched 'cellular' lipids from photoautotrophy, or the removal of ~20% of ^{14}C -depleted components with a radiocarbon signature similar to that of CRAM. Irrespective of the source, the ^{14}C -depletion of the HMW DOC lipid-like fraction indicates a ^{14}C -depleted origin (deep soil horizon from for instance cliff erosion along south shore near apostle islands or lake sediments or petroleum source; Abbot and Stafford 1995; Sickman et al. 2009; Zigah et al. 2014).

4.2.3 HMW DOC Unhydrolysable fraction

The isotopic depletion of the unhydrolysable fraction ($\Delta^{14}\text{C} = -57\text{‰}$, $\delta^{13}\text{C} = -29.5\text{‰}$; Fig. 6) in deep waters during summer stratification implies that a considerable proportion of this fraction may come from similar sources as the old and ^{13}C -depleted lipid-like fraction. Selective preservation of lipid-like material has been proposed as the dominant formation mechanism of

the unhydrolysable fraction within sinking particulate organic matter in estuaries and oceans (Wang et al. 1998; Hwang et al. 2006). Isotopic enrichment in the surface unhydrolysable fraction during stratification ($\Delta^{14}\text{C} = 16\text{‰}$, $\delta^{13}\text{C} = -27.5\text{‰}$; Fig 6) and mixing condition ($\Delta^{14}\text{C} = 59\text{‰}$, $\delta^{13}\text{C} = -26.6\text{‰}$; Fig 6) suggests some addition from isotopically enriched sources with protein-like and/or carbohydrate-like precursors or removal of some isotopically depleted sources, perhaps through photo-oxidation.

4.3 Composition and cycling of HMW DOC and SPE DOC based on thermal fractionation

The thermochemical fractionation data show that during spring mixing, about 15% of the surface HMW DOC was in the thermally labile/reactive components, i.e. is oxidized at temperatures $< 331^\circ\text{C}$. These thermally reactive components were ^{14}C -enriched ($\Delta^{14}\text{C}$ values of 60 to 64‰) relative to the more thermally refractory components (Fig 4c). During summer stratification, a significant component (about 46%) of the surface HMW DOC was thermally labile (at $< 326^\circ\text{C}$) and ^{14}C -enriched ($\Delta^{14}\text{C}$ values of 70 to 75‰) (Fig 4e). The enriched isotopic values are consistent with an in-lake source reflecting the past 3 years (2007-2009), a recent-past terrigenous source (2004-2005) or a mixture of sources with a substantial post-bomb component (1955-2009) (Fig. 4a). The increase in % of HMW DOC showing thermal lability and the enriched isotopic values of this pool in the stratified surface waters and the lack of these in the mixed period suggest that a substantial component of HMW DOC recycles over shorter times; this is consistent with studies that have reported rapidly recycling semi-labile components within HMW DOC (Amon and Benner 1994; Loh et al. 2004; Repeta and Aluwihare 2006).

In addition to this enriched, thermally labile component, a substantial proportion of the mixed-lake surface HMW DOC (85%) and stratified surface HMW DOC (54%) were more thermally recalcitrant (Fig. 4a, c, e). In the mixed lake HMW sample, the thermally recalcitrant

fractions ($>331^{\circ}\text{C}$) had $\Delta^{14}\text{C}$ values ranging from -23 to 34‰, lower than post-bomb atmospheric $\Delta^{14}\text{C}$. In the stratified HMW sample, the divide between $\Delta^{14}\text{C}$ values higher and lower than the contemporaneous atmospheric value occurred at a higher temperature range, with fractions $>378^{\circ}\text{C}$ showing ^{14}C -depletion relative to the atmosphere. The $\Delta^{14}\text{C}$ values of the two most thermally stable fractions in each HMW sample were similar to the $\Delta^{14}\text{C}$ values of total SPE DOC and HMW DOC in the lake during spring mixing. In a previous study, NMR data showed that HMW DOC can be modeled as consisting of two polymeric components - ^{14}C -enriched HPS and AMS, and older ^{14}C -depleted component that may include considerable CRAM and aliphatic components (Zigah et al. 2014). It is likely that the more thermally labile components likely include HPS and AMS whereas the thermally recalcitrant components may include the presence of CRAM or humic substances of varying ages. The absence of the large thermally stable components (e.g., peak at $\sim 630^{\circ}\text{C}$) in the surface HMW DOC during stratification is consistent with surface removal by photochemical and/or microbial oxidation of thermally recalcitrant DOC with a lower $\Delta^{14}\text{C}$ value. In addition, the relative increase in the thermally labile components during stratification suggests some photoautotrophic inputs during summertime. The stratified-sample enrichment in ^{14}C relative to concurrent DIC values for the most thermally labile fractions indicates that there may also be removal of more ^{14}C -depleted material from these thermally labile fractions. The combination of these processes is consistent with the relative increase in concentration and ^{14}C -enrichment in HMW DOC during stratification (Table 2).

The data from serial oxidation and acid hydrolysis of HMW DOC differ greatly; there are several possible mechanisms for this. It is possible that both ^{14}C -enriched and ^{14}C -depleted material contribute to the same “compound class” in the acid hydrolysis (as might be seen for

structural carbohydrates from older woody material as well as younger woody material, grasses and algae); or conversely that both enriched and depleted material have similar thermal labilities. These differences could also be explained by differences in recovery for the wet chemical fractions vs. thermal oxidation fractions. Also, isotopic mixing during thermal fractionation could lead to the observed disparity. For instance, the depleted lipid-like fraction could be easily mixed into one of the thermal fractions due to their small relative abundance. Coupling both acid hydrolysis and thermal oxidation appears to be better in constraining the isotopic heterogeneity, and by extension, the sources and cycling of HMW DOC in the lake. However, better distinguishing the windows of view into the DOC pool that each approach provides is an area needing further study.

The thermograms of SPE DOC show that the thermally-labile components in the mixed and stratified samples overlap considerably. The ^{14}C values for the thermal fractions show that both old and contemporary material contributes to the SPE DOC (Fig. 4d). About 57% of the stratified surface SPE DOC (mean $\Delta^{14}\text{C}$ values of 59-65‰) and 34% of the mixed-lake SPE DOC (mean $\Delta^{14}\text{C}$ values of 54-58‰) was ^{14}C -enriched, similar to the lake DIC and terrestrial productivity 2-4 years prior to our sampling in the Lake (in 2005-2007), and derived from contemporary sources, possibly hydrophobic/aromatic proteins (Goldberg et al. 2014). Also, substantial components of the SPE DOC in the mixed-lake (66% with $\Delta^{14}\text{C}$ values of -22 to 48‰) and stratified lake (43% with $\Delta^{14}\text{C}$ values of -8 to 47‰) were ^{14}C -depleted relative to the lake DIC. The most thermally refractory portion, with pre-bomb ^{14}C values, was considerably smaller during stratification (Fig. 4 b, d, and f). However, the most thermally labile SPE DOC component in each SPE sample was also ^{14}C -depleted, especially in the stratified sample (Fig 4d, f). As with the HMW DOC, thermally recalcitrant components present in the surface SPE DOC

during lake mixing in spring appear to be removed from the surface SPE DOC during stratification consistent with summertime removal of thermally recalcitrant and old DOC from the lake (Fig 4b, d, f). In contrast to HMW DOC, there does not appear to be a considerable input of recently synthesized organic material into the thermally labile portion of the stratified lake SPE sample, and, in fact, the most thermally labile fraction actually shifted to pre-bomb values (Fig 4b, f). Overall, these observations suggest there was little contribution to the SPE DOC from photoautotrophy during summertime, and are consistent with the decrease in concentration and relative ^{14}C -enrichment observed in surface SPE DOC during stratification (Table 2).

5. Summary and conclusions

We show that there is a significant mobilization of ^{14}C -depleted DOC in the water column during spring mixing. Thermochemical fractionation data indicates that this older DOC likely derives predominantly from thermally refractory components. Lake stratification in summer enhanced the removal of the older ^{14}C depleted and thermally refractory DOC in the lake, implying strong roles for enhanced photochemical and microbial remineralization in the sunlight-filled, warmer surface-layer of the lake. Climate change induced effects on the extent and duration of ice cover and summer stratification could impact DOC cycling, nutrient availability and carbon and energy transfers in the lake food web in the future.

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Table 1. Sampling depth, sample volumes, and elemental composition of HMW DOC isolated from eastern Lake Superior in June and August 2009. Total water depth at this site is 242 m.

Sample	Depth (m)	Total volume (L)	Retentate volume (L)	Molar C:N
<i>Mixed - June 2009</i>				
Surface	5	200	1.25	23.6
Deep	210	400	0.90	17.7
<i>Stratified - August 2009</i>				
Surface	5	400	1.45	14.5
Deep	210	383	1.20	17.8

12 **Table 2.** Concentrations and carbon isotopic compositions of total DOC (< ~0.7 μm), high molecular weight DOC (HMW DOC, >
13 1kDa, <0.2 μm), solid phase extracted DOC (SPE DOC, <0.2 μm) and dissolved inorganic carbon (DIC) from eastern Lake Superior
14 during isothermal condition in June and thermal stratification in August 2009.

Season / Depth	DIC			Total DOC			HMW DOC			SPE DOC		
	μM	$\delta^{13}\text{C}$ (‰)	$\Delta^{14}\text{C}$ (‰)	μM	$\delta^{13}\text{C}$ (‰)	$\Delta^{14}\text{C}$ (‰)	μM	$\delta^{13}\text{C}$ (‰)	$\Delta^{14}\text{C}$ (‰)	μM	$\delta^{13}\text{C}$ (‰)	$\Delta^{14}\text{C}$ (‰)
Mixed – June 2009												
5 m	819	0.4	59 ± 4	91.7	-26.3	42 ± 4	10.8	-26.1	22 ± 4	60.0	-26.8	25 ± 3
210 m	830	0.3	58 ± 2	90.4	-26.0	30 ± 4	9.3	-25.9	32 ± 3	64.0	-26.7	43 ± 2
Stratified – August 2009												
5 m	810	0.9	59 ± 4	95.7	-26.0	54 ± 3	11.4	-26.0	54 ± 3	48.8	-26.5	41 ± 3
210 m	824	0.3	63 ± 4	85.5	-25.9	46 ± 4	11.3	-26.3	51 ± 5	35.5	-26.4	30 ± 4

Table 3. Radiocarbon and stable carbon isotopic composition of organic fractions extracted from HMW DOC from eastern Lake Superior in 2009. Instrumental precision of $\Delta^{14}\text{C}$ and $\delta^{13}\text{C}$ analyses are 3-5‰ and 0.15‰, respectively. The values in parenthesis are the ages in years BP.

Organic fraction	Mixed-lake		Stratified-lake	
	$\delta^{13}\text{C}$ (‰)	$\Delta^{14}\text{C}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\Delta^{14}\text{C}$ (‰)
Surface (5 m)				
Lipid-like	-28.0	-204 (1770)	-29.1	-153 (1270)
Carbohydrate-like	-25.8	31	-26.9	20
Protein-like	-25.0	nd	-26.2	39
Unhydrolysable material	-26.6	59	-27.5	16
Deep (210 m)				
Lipid-like	-29.7	-256 (2320)	nd	nd
Carbohydrate-like	-26.9	28	nd	nd
Protein-like	-25.2	34	-25.7	36
Unhydrolysable material	-26.7	25	-29.5	-57

nd = sample lost during extraction, combustion to CO_2 or graphitization.

Table 4. Elemental and isotopic composition of putative sources of HMW DOC in the water column of Lake Superior.

Putative sources	C:N	$\delta^{13}\text{C}$ (‰)	$\Delta^{14}\text{C}$ (‰)	Reference
In situ primary production	8-16	-26 to -27.8	61	Keough et al. 1996; this study
Recent terrestrial vascular plant	35-48	-25 to -30	38	Lara et al 1998; Lobbes et al. 2000; Guo et al, 2003;this study
Terrestrial vascular plants ca. 30-50 yr	35-48	-25 to -30	200-500	Lara et al 1998; Lobbes et al. 2000; Guo et al, 2003; this study
Lake sediment organic carbon	10-12	-25.6 to -27.9	-36 to -180	Zigah et al. 2012; Li et al 2013
Surficial soil organic carbon	10-12	-26 to -28	ca. 50 to -50	Peterson and Fry 1987; Fry 1991; Marwick et al. 2015
Deep horizon soil organic carbon	10-12	-26 to -28	ca. -200 to -400	Peterson and Fry 1987; Fry 1991; Marwick et al. 2015

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Figure 1. Sampling site in the eastern basin of Lake Superior. EM means eastern mooring.

Figure 2. Depth profiles of (a) dissolved oxygen, temperature and chlorophyll-a florescence in the isothermal water column of Lake Superior in June 2009, and (b) dissolved oxygen, temperature and chlorophyll-a florescence (WETLabs WETStar, mg/m³) in the stratified water column of Lake Superior in August 2009. The oxycline and the thermocline were observed at ~2-40 m, and the entire column remained oxygenated during stratification in August.

Figure 3. Radiocarbon compositions of lake-water DIC from surface (5 m) and deep chlorophyll maximum (30-40m) depths and atmospheric CO₂ covering the years 2004-2012. The $\Delta^{14}\text{C}$ -DIC data was collated from Zigah 2012 and Zigah et al. 2011, 2012 and 2014. Where available, both the surface (5 m) and depth of deep chlorophyll maximum (30-40 m) were included. Data from 2009-2010 were from the eastern region of the lake (and were similar to those from the western region of the lake) and data from 2007, 2008, and 2012 were from the western region of the lake (which we assume to be same as those at the eastern region of the lake given the similarity of the 2009-2010 data in particular, and homogeneity of $\Delta^{14}\text{C}$ -DIC in the lake in general as reported by Zigah et al. 2012). The atmospheric CO₂ $\Delta^{14}\text{C}$ data was adapted from Zigah 2012, Hseuh et al. 2007, Graven et al. 2012 and Kruger 2014.

Figure 4. Thermogram of (a) surface HMW DOC during stratification and mixed-lake conditions and (b) surface SPE DOC during stratification and mixed-lake conditions from eastern Lake Superior. CO₂ values were normalized to the height of base peak. Different components were thermally oxidized at different temperature ranges. The radiocarbon values of the isolated thermal fractions are given for surface HMW DOC during mixed-lake (c) and stratified conditions (e) for surface SPE DOC during mixed lake (d) and stratified conditions (f). The values within the histograms are the carbon mole percent of each thermal fraction. The error bars are 1 σ accelerator mass spectrometer (AMS) instrumental precision.

Figure 5. Radiocarbon composition of DIC, SPE DOC, HMW DOC, and compound classes isolated from HMW DOC (carbohydrate-like, protein-like, lipid-like and unhydrolysable fractions from (a) the surface waters (5 m) during mixed-lake condition (b) the deep waters (210 m) during mixed-lake condition (c) surface waters during stratification (d) deep waters during stratification.

Figure 6. $\Delta^{14}\text{C}$ and $\delta^{13}\text{C}$ cross-plots of SPE DOC, HMW DOC and organic fractions from HMW DOC from (a) the surface waters (5 m) during mixed-lake condition (b) the deep waters (210 m) during mixed-lake condition (c) surface waters during stratification (d) deep waters during stratification. The lipid-like fraction was more depleted than the other organic fractions.

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