

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

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37 **Abstract**

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

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60 **1. Introduction**

61 Dissolved organic carbon (DOC) is ubiquitous in aquatic ecosystems and is important in
62 carbon and energy flow in food webs (Mann et al. 2015; Hitchcock et al. 2016), and in the
63 cycling of nutrients, organic pollutants and trace metals (Riedel et al. 2013). Lakes play
64 important roles in the global carbon cycle with an estimated CO₂ emission of 140 Tg C y⁻¹ (Cole
65 et al. 1994) and annual carbon burial of up to 58% more than the oceans (Dean and Gorham,
66 1998). However, the sources and cycling of DOC in freshwater lacustrine systems, especially
67 large lakes, are not well understood. This is primarily because DOC consists of a complex
68 mixture of organic functional groups present in various higher order structures and coming from
69 multiple sources (Hedges 1992; Miller and Zepp 1995). Consequently, DOC has multiple fates.
70 DOC may be routed up to higher organisms in the food web via the microbial loop (Cole et al.
71 2006; Tanentzap et al. 2014), become sequestered in sedimentary organic matter through particle
72 association (Masiello and Druffel 1998; Coppola et al. 2014) or be mineralized to carbon dioxide
73 via photochemical and microbial oxidation (Obernosterer and Benner 2004; Cory et al. 2007;
74 Amado et al. 2015).

75 Constraining the sources and cycling of DOC is a necessary step in understanding
76 functioning of aquatic ecosystems and the global carbon cycle, but the molecular diversity of
77 DOC means that a full separation, identification and characterization of all the organic molecules
78 within it is currently not feasible. These compounds, however, can be grouped into
79 operationally-defined discrete fractions based on their molecular weight (e.g. high molecular
80 weight DOC; > 1000 daltons) and polarity (e.g. more hydrophobic DOC fractions) (Zigah et al.
81 2017).

82 In most freshwater systems, a substantial fraction (~30 to 80%) of DOC is comprised of
83 components that can be isolated by adsorption onto a hydrophobic resin (e.g. PPL, C18, XAD-8,
84 SDB-XC) at acidic pH, commonly referred to as SPE DOC (Thurman and Malcolm 1981;
85 Dittmar et al. 2008; Sickman et al. 2010). Nuclear magnetic resonance (NMR) analyses of SPE
86 DOC from freshwater lakes and streams show that it is comprised significantly of hydrophobic
87 humic substances, aromatic proteins, and lipids/fatty acids (Harvey et al. 1984; Kaiser et al.
88 2003; Lam et al. 2007; Zhang et al. 2014; Goldberg et al. 2015). The relative contributions of
89 autochthonous versus allochthonous materials to the SPE DOC fraction are widely variable and
90 are a function of initial chemical composition of the source materials, the
91 humification/degradation state, the matrix of the water sample, and the structure of the SPE resin
92 used for the extraction (e.g. Kruger et al. 2011; Li and Minor 2015). In clear-water systems such
93 as the offshore regions of stratified oligotrophic large freshwaters, SPE DOC concentrations
94 would be relatively low as should be expected for more dilute and less terrestrially-influenced
95 systems with a lower contribution from humic substances (Dittmar et al. 2008; Kruger et al.
96 2011); input of humic substances from the sediments during mixing conditions could increase
97 the SPE DOC amounts in these freshwaters. The concentration of SPE DOC is expected to be
98 higher in freshwaters with abundant humic substances derived from terrestrial sources or
99 microbial sources (McKnight et al. 2001; Schwede-Thomas et al. 2005; Goldberg et al. 2015;
100 Cawley et al. 2016).

101 Up to 87% of freshwater DOC, especially in stream, river, or river-impacted coastal
102 systems can be recovered as HMW DOC (nominal molecular weight of >1000 Daltons) using
103 ultrafiltration (Benner and Opsahl 2001; Aluwihare et al. 2002; Repeta et al 2002; Stephens and
104 Minor 2010; Zigah et al. 2014), although freshwater lakes with more autochthonous DOC appear

105 to have less HMW DOC than these other freshwater systems (e.g., Repeta et al., 2002; Kruger et
106 al., 2011; Zigah et al. 2014,). NMR analyses of HMW DOC in large freshwater lakes and in
107 marine systems show that it is rich in complex polymeric structures known as
108 heteropolysaccharides (HPS), peptides/amide and amino sugars (AMS), and carboxylic-rich
109 alicyclic molecules (CRAM) (Hertkorn et al. 2006; Abdulla et al. 2010; Zigah et al. 2014). In
110 freshwater systems with little terrestrial influence, autochthonous microbial sources are the
111 dominant source of the carbohydrate and protein fractions within HMW DOC (Zigah et al. 2014)
112 whereas degraded higher plant materials and soils (allochthonous sources) will be dominant in
113 more terrestrially-influenced smaller lakes or rivers (Kaiser et al. 2004).

114 The mechanisms of removal of DOC from freshwaters include microbial respiration, and
115 photochemical oxidation to carbon dioxide and adsorption onto sinking particles (Moran et al.
116 2000; Xie et al. 2004; Cory et al. 2007). Photochemical oxidation is possibly the dominant sink
117 for SPE-DOC; light-absorbing components appear to be preferentially concentrated in SPE
118 extracts (Moran et al. 2000; Ma and Green 2004; Cory et al. 2007; Li and Minor, 2015).
119 Pathways of removal of freshwater HMW DOC are not well constrained (Kaiser et al. 2004).
120 Microbial and photochemical oxidations can mineralize both autochthonous and allochthonous
121 material within HMW DOC in freshwater systems (Amon and Benner 1996; Kaiser and
122 Sulzberger 2004). Microbial mineralization is likely dominant in the recycling of the
123 autochthonous, carbohydrate and protein-rich, fraction of HMW DOC because of its relative
124 bioreactivity and relative lack of light-absorbing components. In a similar vein, direct
125 photochemical oxidation is likely the predominant pathway in the recycling of allochthonous
126 material within HMW DOC because of its enrichment in light-absorbing components and
127 relative bio-recalcitrance (Amon and Benner 1996, Moran et al. 2000; Cory et al. 2007).

128 However, light-absorbing DOC (from isolated fractions and whole water) can also participate as
129 a photo-sensitizer promoting indirect photochemical reactions which can then also affect
130 molecules that do not absorb light themselves (Sultzberger and Durisch-Kaiser, 2009). In
131 addition, the non-linear coupling of microbial and photochemical processes in altering DOC
132 pools has been shown to occur in many aquatic systems. Photochemical preprocessing of DOC
133 has been reported to have both negative and positive effects upon microbial oxidation (Kaiser et
134 al. 2004; Amado et al. 2015), perhaps because photochemical and microbial reactions are
135 competing for key substrates or because there are competing interactions where reactive oxygen
136 species suppress the microbial response while photochemical reactions make parts of the DOC
137 pool more bioavailable (Anesio et al., 2005; Amado et al 2015).

138 In the past few decades, radiocarbon (^{14}C) has been useful in studying the ages, cycling
139 and sources of total DOC in freshwater lakes and streams (Trumbore et al. 1992; Zigah et al.
140 2011; McCallister and del Giorgio 2012; Alberic et al. 2013; Butman et al. 2015; Keaveney et al.
141 2015). Natural abundance radiocarbon ($\Delta^{14}\text{C}$) and stable isotope ($\delta^{13}\text{C}$) compositions of the
142 major fractions of DOC provide additional constraints on the multiple sources and cycling of the
143 DOC. $\Delta^{14}\text{C}$ calculation corrects for biochemical fractionations so differences in $\Delta^{14}\text{C}$ values
144 reflect variable sources and/or cycling. For instance organic material produced from recent
145 within-lake photoautotrophy bears a $\Delta^{14}\text{C}$ value of the contemporaneous lake dissolved inorganic
146 carbon (DIC). The few studies in freshwater systems that do partition DOC show that it consists
147 of a mixture of contemporary and older components (Abbott and Stafford 1996; Guo et al. 2003,
148 Sickman et al. 2010). More radiocarbon measurements of DOC fractions from various
149 freshwater systems will help constrain the roles of contemporary and aged carbon in modern
150 biogeochemical cycles and improve our understanding of the global carbon cycle.

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

168 **2. Methods**

169 **2.1 Study site**

170 Lake Superior (Fig. 1) is the Earth's largest freshwater lake by surface area and the
171 deepest of the Laurentian Great Lakes of North America, with a maximum depth of 406 m. The
172 lake is dimictic, with complete vertical mixing of the water column in spring and early winter
173 each year. The hydraulic residence time of the lake (lake volume divided by outflows, including

174 diversions) is 173 years (Quin et al. 1992). DOC is the largest organic carbon pool with an
175 average lakewide surface and deep water concentration of $\sim 90 \mu\text{M}$ during spring mixing. During
176 summer stratification, lakewide surface DOC is $\sim 100\text{-}120 \mu\text{M}$ and is about $10 \mu\text{M}$ larger than
177 the deep DOC (Zigah et al. 2012). The sources and cycling of DOC in the lake are not well
178 constrained. Annual autochthonous DOC input is estimated at 0.9 Tg C ($\sim 9\%$ of the primary
179 production; Urban et al. 2005, Sterner 2010), similar to the annual terrestrial DOC loading of
180 $0.4\text{-}0.9 \text{ Tg C}$ (Cotner et al. 2004; Urban et al. 2005). In spite of the substantial terrestrial input,
181 spectroscopic studies of the DOC, including ultraviolet-visible spectroscopy of total DOC, NMR
182 of HMW DOC, and FTIR of total and HMW DOC have shown fairly low contributions from
183 aromatic constituents that indicate terrigenous sources (Minor and Stephens, 2008, Zigah et al.
184 2014). Hence, the presence, amount and fate of terrigenous DOC in the lake remains poorly
185 understood. To provide greater insights into the composition, cycling and sources of DOC in the
186 lake, we previously investigated the radiocarbon and stable isotope composition of total DOC
187 (Zigah et al. 2011; 2012), and size-fractionated DOC (Zigah et al. 2014). HMW DOC
188 constitutes 8-20% of the total DOC in the western arm and 10-13% of the total DOC in the
189 eastern arm of the lake based on isolation via cross-flow ultrafiltration. NMR spectra of the
190 HMW DOC from the lake show that heteropolysaccharides, peptides/amide and amino sugars
191 together constitute 75-84% of the carbon, with carbohydrate carbon alone representing 53-65%
192 of the HMW DOC (Zigah et al. 2014).

193 **2.2 Sampling**

194 Surface (5 m) and deep (210 m) samples were collected from eastern Lake Superior
195 ($47^{\circ}34'\text{N}$, $86^{\circ}39'\text{W}$; Fig. 1) during spring mixing in June 2009, and thermal stratification in
196 August 2009 (Fig. 2). Lake water was collected using twelve 8-L Niskin bottles mounted on a

197 rosette equipped with a Seabird Model 911 Plus Conductivity, Temperature, and Depth (CTD)
198 profiler. Dissolved inorganic carbon (DIC) samples were taken from the Niskin into previously
199 acid-cleaned (10% v/v HCl followed by rinsing with milli-Q water) and combusted (450°C for ≥
200 4 hours) 0.5 L amber Pyrex bottles. The samples were immediately preserved with 100 µL of
201 saturated mercuric chloride solution, sealed air-tight with glass stoppers coated with Apiezon
202 grease, and stored at room temperature in the dark until analysis (McNichol et al. 1994). DOC (<
203 0.7 µm) samples were obtained by filtering lake water through pre-combusted Whatman GF/F
204 glass fiber filters (450°C for 4 hours) using stainless-steel canisters pressurized with nitrogen.
205 Approximately 40 mL of lake water was collected into an acid-cleaned and combusted vial and
206 acidified to pH 2 using 6M HCl for DOC analysis by high temperature catalytic oxidation. For
207 DOC radiocarbon analysis, 1 L of the GF/F filtered water was collected into a pre-combusted
208 glass bottle, acidified with 6M HCl to pH 2, and stored in a refrigerator at 4°C until analysis.

209 **2.3 Ultrafiltration and solid phase extraction**

210 Large-volume samples (200-400 L) (Table 1) were drawn from the lake using an air-driven
211 diaphragm pump, 0.2 µm filtered (Whatman Polycap 75 TC), and ultrafiltered as described
212 previously (Zigah et al. 2014). Previously frozen 0.7 µm-filtered water samples stored in
213 precombusted 1L glass bottles or acid-cleaned 1L high density polyethylene (Nalgene) bottles
214 were thawed, filtered (0.2 µm), and solid phase extracted using the protocol of Dittmar et al.
215 (2008). Briefly, the filtered water was acidified to pH 2 with 6M HCl and extracted using
216 methanol-activated styrene divinylbenzene polymer resin (PPL; 1g, Varian Mega Bond Elut) at a
217 flow rate of 10 mL/ min. The DOC extract was desalted with acidified Milli-Q water (pH 2),
218 dried with ultrahigh purity (UHP) N₂ and eluted with methanol. The SPE DOC sample was

219 transferred into quartz combustion tubes and completely dried; Ag powder and precombusted
220 CuO were added; the tubes were evacuated and then flame-sealed.

221 **2.4 Measurement of DOC concentrations**

222 DOC and TOC samples were analyzed on a Shimadzu V_{CSH} TOC analyzer (Zigah et al.
223 2011). Lyophilized HMW DOC samples were analyzed on a Costech ECS 4010 elemental
224 analyzer (EA) coupled to Finnigan Delta Plus XP isotope ratio mass spectrometer (IRMS) after
225 fumigation with 12 N HCl (ACS Plus grade) and drying (Zigah et al. 2014). Typical instrumental
226 precision for OC measurements was 0.2% of the measured concentration. SPE DOC samples
227 were combusted to CO₂ in sealed quartz tubes and quantified manometrically.

228 **2.5 Extraction of organic fractions from HMW DOC**

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

237 **2.6 Serial thermal oxidation of HMW DOC and SPE DOC from Lake Superior**

238 Serial thermal oxidation was performed at the National Ocean Sciences Accelerator Mass
239 Spectrometry Facility (NOSAMS) at the Woods Hole Oceanographic Institution (WHOI) using
240 the Ramped PyrOx system in oxidation mode (Rosenheim et al. 2008, Plante et al. 2013). A
241 dried sample was weighed and transferred to a quartz reaction vessel. The vessel was then placed

242 in the top of the thermal analyzer (two coupled ovens with the bottom oven set at a constant
243 temperature of 800 °C and the top oven holding the sample reactor). With an oxygen (8%) and
244 helium (92%) mixed gas solution flowing through the reactor at a rate of 35 mL per minute, the
245 sample was thermally oxidized to CO₂ by gradually raising the top oven temperature from
246 ambient to 1000 °C at a rate of 5 °C per minute. The evolving gas was passed over a Pt/Ni/Cu
247 twisted wire in the 800 °C oven to ensure complete oxidation to CO₂. The CO₂ was first
248 quantified downstream with an in-line CO₂ analyzer, and then collected in successive fractions
249 using flow-through glass traps (Rosenheim et al. 2008; Plante et al. 2013).

250 **2.7 Radiocarbon and stable isotope measurements**

251 Radiocarbon ($\Delta^{14}\text{C}$) and stable carbon isotope ($\delta^{13}\text{C}$) measurements were performed at
252 NOSAMS using standard protocols (McNichol et al. 1994). Typical instrumental precision of
253 $\delta^{13}\text{C}$ based on multiple analyses of standards was 0.15‰. The graphite produced in all cases was
254 analyzed using either a 3MV tandetron accelerator mass spectrometer (USAMS) or 500kV
255 pelletron continuous flow accelerator mass spectrometer (CFAMS) (von Reden et al. 2004;
256 Roberts et al. 2010; Longworth et al., 2015). Radiocarbon values are reported as $\Delta^{14}\text{C}$ according
257 to the convention of Stuiver and Polach (1977). Instrumental precision of the $\Delta^{14}\text{C}$ analysis was
258 3-6‰. The radiocarbon composition of lake-water DIC was collated from Zigah 2012, Zigah et
259 al. 2011, 2012 and 2014. The atmospheric CO₂ $\Delta^{14}\text{C}$ data was based on the radiocarbon
260 composition of corn leaves (*Zea mays*) from the lake watershed (Hseuh et al. 2007; Zigah 2012;
261 Kruger 2014) and from atmospheric CO₂ in the Northern Hemisphere (Graven et al. 2012). The
262 atmospheric CO₂ data covering 2004-2007 was from Hseuh et al. (2007) and Graven et al.
263 (2012), and the data covering 2009-2012 are from Zigah 2012 and Kruger 2014.

264 **2.8 Blank assessment**

265 To assess the process blanks and potential fractionation associated with the extractions of
266 organic fractions from HMW DOC for isotopic measurements, we measured the isotopic values
267 of known standard materials before and after extraction. In all cases, the amount of carbon
268 extracted from the standard material was comparable to or smaller than the amount extracted
269 from the samples. For the lipid standard (stearic acid), the $\Delta^{14}\text{C}$ values of non-extracted and
270 extracted stearic acid were $48 \pm 4\text{‰}$ and $51 \pm 4\text{‰}$, respectively. The $\Delta^{14}\text{C}$ values of non-
271 extracted and extracted D-glucose (carbohydrate standard) were $35 \pm 4\text{‰}$ and $41 \pm 4\text{‰}$ and those
272 of bovine serum albumin (protein standard) were $184 \pm 3\text{‰}$ and $179 \pm 3\text{‰}$ respectively. These
273 results indicate that there is a negligible amount of non-modern carbon added during the
274 extraction processes for the organic fractions. Also, non-extracted and extracted $\delta^{13}\text{C}$ values of
275 stearic acid ($-30.4 \pm 0.15\text{‰}$ vs. $-30.4 \pm 0.15\text{‰}$), D-glucose ($-11.2 \pm 0.15\text{‰}$ vs. $-11.2 \pm 0.15\text{‰}$)
276 and bovine serum albumin ($-10.2 \pm 0.15\text{‰}$ vs. $-10.6 \pm 0.15\text{‰}$) indicate no fractionation and/or
277 contamination from the extraction processes. The lipid standard we used was modern but the
278 samples we analyzed were pre-aged, so we must evaluate the potential for contamination by
279 modern carbon. If we assume the process blank for the total lipid extraction contains $1 \mu\text{g C}$
280 (Loh et al. 2004) with a $\Delta^{14}\text{C}$ value of 48‰ , similar to the modern stearic acid standard, addition
281 of this will increase the $\Delta^{14}\text{C}$ values of the lipid samples by $\leq 1\text{‰}$. If the process blank contained
282 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
283 from the range of -153‰ to -256‰ reported here to -159‰ to -268‰ . This change, while
284 significant, is too small to alter the discussion or conclusions of this paper.

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

288 small relative to the SPE sample size (421-774 $\mu\text{g C}$), and there was not enough for isotopic
289 analysis. If we assume this carbon has a fraction modern value of 1 ($\Delta^{14}\text{C} \sim -7\text{‰}$) or 0 ($\Delta^{14}\text{C} = -$
290 1000‰), the SPE sample $\Delta^{14}\text{C}$ values only decrease by $\leq 2\text{‰}$. To assess the process blank
291 associated with serial thermal oxidation on the Ramped PyrOx (RPO), we cycled the thermal
292 analyzer without any sample in the reactor and quantified the blank carbon to be $\sim 4 \mu\text{g C}$ from
293 ambient to 1000°C , similar to the $< 4 \mu\text{g C}$ reported by Plante et al. (2013) and Hemingway et al.
294 (2017). This amount is not enough for isotopic measurement and is small ($\leq 6\%$) relative to the
295 CO_2 amounts in the thermal fractions of the sample (63-131 $\mu\text{g C}$). Further evidence of the
296 negligible impact of the process blank on ^{14}C results from the RPO comes from the agreement
297 between the $\Delta^{14}\text{C}$ values of the bulk HMW and SPE samples and the values calculated for the
298 bulk samples using a mass balance of the RPO data. Due to negligible blank carbon and/or
299 isotopic fractionation, reported $\Delta^{14}\text{C}$ and $\delta^{13}\text{C}$ values of organic fractions of HMW DOC, SPE
300 DOC and organic fractions from serial thermal oxidation were not corrected for process blanks
301 or fractionation.

302 **3. Results**

303 **3.1 Trends in the radiocarbon values of Lake DIC and atmospheric CO_2 from 2004 to 2012**

304 The $\Delta^{14}\text{C}$ value of atmospheric CO_2 decreased from $\sim 66\text{‰}$ in 2004 to 38‰ during our
305 sampling in 2009, and to 25‰ in 2012 (Fig. 3) corresponding to a decline of $\sim 5\text{‰/yr}$. The lake
306 DIC $\Delta^{14}\text{C}$ value decreased from 83‰ to 44‰ between 2007 and 2012 with a decline $\sim 7\text{‰/yr}$
307 (Fig. 3). DIC was consistently ^{14}C -enriched by $\sim 20\text{‰}$ relative to atmospheric CO_2 from 2004-
308 2014, indicating a carbon isotope equilibration time of ~ 4 years between the DIC and
309 atmospheric CO_2 over this period.

310 **3.2 Concentration and isotopic values of HMW DOC vs. SPE DOC vs. Total DOC**

311 The concentration of HMW DOC was 9-11 μM (10-13% of total DOC) with molar C:N
312 values of 14.5-23.6; the highest C:N ratio was for surface HMW DOC from June 2009 (Tables 1
313 and 2). SPE DOC concentrations were 35.5 to 48.8 μM (41 to 51% of total DOC) and 60 to 64
314 μM (65 to 71% of total DOC) during stratification and mixed-lake conditions, respectively
315 (Table 2). The $\delta^{13}\text{C}$ values of SPE DOC, HMW DOC and total DOC in the lake were -26.4‰ to
316 -26.8‰, -25.9‰ to -26.3‰ and -25.9‰ to -26.3‰, respectively (Table 2). The $\delta^{13}\text{C}$ values of
317 total DOC, HMW DOC and SPE DOC did not exhibit considerable variation between spring
318 mixing and summer stratification (Table 2).

319 The $\Delta^{14}\text{C}$ values of SPE DOC (25 to 43‰) were slightly depleted or similar to the $\Delta^{14}\text{C}$
320 value of atmospheric CO_2 , and by extension, recent land-plant primary production ($38 \pm 2\%$)
321 from 2009-2010, but more depleted relative to lake DIC ($\Delta^{14}\text{C}$ of 59 to 63‰). The stratified deep
322 and mixed-lake surface SPE DOC values (25 to 30‰) were more depleted relative to the $\Delta^{14}\text{C}$ of
323 lake DIC (Table 2) than were the values for the other two SPE DOC samples. During spring
324 mixing conditions in June, $\Delta^{14}\text{C}$ values of HMW DOC were 22 to 32‰ and increased to 51 to
325 54‰ during stratification in August (Table 2). HMW DOC, SPE DOC and total DOC were
326 consistently more depleted in ^{14}C than the lake DIC ($\Delta^{14}\text{C}$ of 58 to 59‰ in June and 59 to 63‰
327 in August) (Table 2). SPE DOC was more depleted than HMW DOC and total DOC during
328 stratification (Table 2).

329 **3.3 $\Delta^{14}\text{C}$ and $\delta^{13}\text{C}$ values of extracted organic fractions within HMW DOC**

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

334 and protein-like ($\Delta^{14}\text{C}$ of 34 to 39‰) fractions. The latter fractions were consistently modern, but
335 ^{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
336 carbohydrate-like and protein-like fractions (-25.0‰ to -26.9‰) were more ^{13}C -enriched than
337 the lipid-like fraction (-28.0‰ to -29.7) (Table 3). The $\Delta^{14}\text{C}$ and $\delta^{13}\text{C}$ values of the
338 unhydrolysable fraction were more variable with depth in the lake during the stratified period
339 (Table 3).

340 **3.4 Serial thermal oxidation of HMW DOC**

341 The thermogram of the stratified surface HMW DOC shows components oxidizing over
342 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
343 smaller one at $\sim 380\text{ }^\circ\text{C}$). High-temperature components thermally oxidize at $\sim 420\text{-}630\text{ }^\circ\text{C}$ with
344 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
345 also shows components oxidizing over low-temperature and high-temperature ranges. A
346 considerably lower percentage of the carbon appeared in the low-temperature range during
347 mixed-lake condition as compared to stratified lake condition (Fig. 4a; Table S2). The high-
348 temperature peak at $\sim 513\text{ }^\circ\text{C}$ was smaller in the mixed-lake sample but other high-temperature
349 components were observed including a large peak at $\sim 630\text{ }^\circ\text{C}$ (Fig 4a). The thermograms show a
350 strong difference in the composition of thermally refractory material during stratification and
351 mixing conditions. For radiocarbon analysis, six thermal fractions with relative amounts of
352 carbon (in mol %) of 5, 10, 14, 14, 13 and 44 (Fig. 4c; Table S2) were collected from the mixed-
353 lake surface HMW DOC. The CO_2 fractions from the lower temperature range of $200\text{-}330\text{ }^\circ\text{C}$
354 were more ^{14}C - enriched ($\Delta^{14}\text{C}$ of 60-64‰) than the CO_2 fractions from the higher temperature
355 range of $388\text{-}800\text{ }^\circ\text{C}$ ($\Delta^{14}\text{C}$ of -23 to 34‰) (Fig. 4c). Five thermal fractions with relative
356 amounts of carbon (in mol %) of 22, 24, 21, 21 and 12 (Fig. 4e; Table S1) were collected from

357 the stratified-lake surface HMW DOC. The CO₂ fractions from the lower temperature range
358 200-387 °C were more ¹⁴C-enriched ($\Delta^{14}\text{C}$ of 60-75‰) than the CO₂ fractions from the higher
359 temperature range 387-800 °C ($\Delta^{14}\text{C}$ of 27-30‰) (Fig. 4e). For both mixed-lake and stratified
360 surface HMW DOC samples, the mass-weighted mean $\Delta^{14}\text{C}$ value was similar to the
361 independently measured bulk $\Delta^{14}\text{C}$ value.

362 **3.5 Serial thermal oxidation of SPE DOC**

363 The thermogram of the stratified surface SPE DOC shows components oxidizing over a
364 broad temperature range ~ 200-530 °C (peak at 375 °C). High-temperature components, a peak
365 at ~ 504 °C and a shoulder at ~550 °C, were observed in the surface SPE DOC during lake
366 mixing, but these components were absent during stratification (Fig. 4b). Five thermal fractions
367 (F1-F5) with relative amounts of carbon (in mol %) of 13, 17, 17, 20 and 33 (Fig. 4d; Table S4)
368 were collected from the mixed-lake surface SPE DOC for radiocarbon analysis. SPE DOC
369 components within thermal fractions F1, F4, and F5 were more ¹⁴C-depleted ($\Delta^{14}\text{C}$ of -22 to
370 48‰) relative to the components within fractions F2 and F3 ($\Delta^{14}\text{C}$ of 54-58‰) (Fig. 4d). The
371 relative amounts of carbon (in mol %) in the five thermal fractions (F1-F5) from stratified
372 surface SPE DOC were 17, 23, 34, 18 and 8 (Fig. 4f; Table S3). SPE DOC components within
373 fractions F1, F4, and F5 were more ¹⁴C-depleted ($\Delta^{14}\text{C}$ of -8 to 47‰) relative to the components
374 within fractions F2 and F3 ($\Delta^{14}\text{C}$ of 60-65‰) (Fig. 4f) and in this case F1 and F5 both exhibited
375 negative $\Delta^{14}\text{C}$ values. The mass-weighted mean $\Delta^{14}\text{C}$ values matched the independently
376 measured bulk values of both the mixed-lake and stratified surface SPE DOC.

377 **4. Discussion**

378 ***4.1 Sources and cycling of HMW DOC and SPE DOC fractions***

379 The $\Delta^{14}\text{C}$ values of DIC declined from a range of 76‰ to 83‰ in 2007 to a range of
380 59‰ to 63‰ in 2009 mirroring decreases in the atmosphere. This implies that DOC originating
381 from planktonic productivity within the lake since the influence of atmospheric bomb ^{14}C on the
382 lake DIC (i.e., in the past ~60 years) would bear $\Delta^{14}\text{C}$ values $\geq 59\text{‰}$ (Fig. 3).

383 During mixed-lake conditions in spring, the radiocarbon values of HMW DOC were
384 more depleted than the values of DIC and recent atmospheric CO_2 . This suggests there is a
385 significant mobilization of ^{14}C -depleted (aged) HMW DOC components. The aged DOC must
386 derive from sources other than current primary productivity in the lake, such as the sediments
387 and/or soils (Table 4) or productivity pre-dating the bomb spike in the 1950-60's. The observed
388 ^{14}C -depletion may be due to a relatively smaller input (and thus smaller overprinting of the
389 residual DOC signal) from autochthonous DOC in the lake during winter and spring when
390 temperature and light levels limit primary production (Sterner 2010). The depletion could also
391 result, in part or wholly, from a substantial DOC input from the sediments during lake over-turn,
392 or input from the watershed via spring snowmelt and ice-out (Stottlemyer and Toczydlowski
393 1991, Zigah et al. 2012).

394 The ^{14}C -enrichment of HMW DOC during stratification could, in part, result from
395 planktonic photosynthesis in the surface waters in the summer months (Urban et al. 2005).
396 Increases in the HMW DOC $\Delta^{14}\text{C}$ values in the stratified lake could also be due to an increased
397 contribution from terrestrial material synthesized 2-4 years prior to our sampling in the lake (52-
398 60‰ in 2005-2007) (Fig. 2 and Table 2). However, the dramatically lower C:N values of
399 surface-water HMW DOC in August relative to June (Table 1) coupled with the radiocarbon
400 results suggest that an increased contribution from photoautotrophy is more likely than a
401 substantial terrestrial input. The radiocarbon values of HMW DOC and total DOC in the

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

409 Although the isotopic composition shifted significantly, the concentrations of surface
410 water HMW DOC and total DOC at both depths only changed slightly (5 to 6%) between the
411 mixed-lake and stratified conditions (Table 2), indicating that the isotopic changes are not solely
412 the result of changing inputs. The radiocarbon values of surface HMW DOC and surface total
413 DOC would be 25‰ and 43‰, respectively, in the stratified lake if the additional autochthonous
414 DOC input estimated from concentration changes was the only factor modulating the DOC $\Delta^{14}\text{C}$
415 values. However, the observed radiocarbon values were 54‰ for both HMW DOC and total
416 DOC in the stratified surface waters. The observed changes in radiocarbon content relative to
417 carbon concentration between mixed and stratified conditions indicate that a significant fraction
418 of the ^{14}C -depleted components of HMW DOC and total DOC in the mixed lake is removed by
419 August, by either remineralization or sorption processes. In a previous study linking NMR
420 characteristics to radiocarbon age within HMW DOM samples, CRAM and aliphatic carbon
421 were identified as ^{14}C -depleted within Lake Superior, with CRAM age estimated at 2040 ^{14}C
422 years ($\Delta^{14}\text{C}$ value of -230‰) (Zigah et al, 2014). The remineralization of such aged components
423 could be in part responsible for the observed increase in $\Delta^{14}\text{C}$. Zigah et al. (2014) used ^{13}C NMR
424 spectra to demonstrate that 16-20% of the HMW DOC from the eastern Lake Superior consists

425 of CRAM ($\Delta^{14}\text{C}$ of -230‰). Using these values in a mass balance, we estimate that complete
426 oxidation of Lake Superior's HMW CRAM pool would decrease the concentration of HMW
427 DOC by 1.7-2.2 μM and increase the HMW DOC $\Delta^{14}\text{C}$ values from 22‰ in the mixed-lake to
428 ~70‰ during stratification. HMW DOC concentration actually increased by 0.6-2 μM during
429 stratification, and the $\Delta^{14}\text{C}$ value only increased up to 54‰ (Table 2), indicating that DOC
430 removal likely occurs across DOC of multiple ages, and that this removal is overlaid by the
431 autochthonous input discussed above. Further evidences for both the removal of older DOC and
432 input of autochthonous DOC in surface waters are found in the results from serial thermal
433 oxidation experiments shown in Fig. 4a, c, and e, and the NMR analyses of surface HMW DOC
434 which indicate that CRAM is a relatively lower proportion of stratified surface HMW DOC than
435 in the mixed-lake HMW DOC (Zigah et al. 2014).

436 Complicating this autochthonous input and older-carbon removal story is the fact that
437 there may also be a considerable mobilization or input of DOC that is more ^{14}C - enriched in the
438 surface water than the current lake DIC during stratification. For example, autochthonous DOC
439 and terrigenous DOC from the past 5-10 years previous to our sampling in 2009 would have
440 $\Delta^{14}\text{C}$ values as high as +120‰ (Fig. 3). By extrapolation of the linear relationship between
441 HMW DOC $\Delta^{14}\text{C}$ values and HPS and AMS and between HMW DOC $\Delta^{14}\text{C}$ values and total
442 carbohydrate carbon from the ^{13}C NMR spectra of lake samples, Zigah et al. (2014) estimated
443 the $\Delta^{14}\text{C}$ values of combined HPS and AMS and total carbohydrate carbon within HMW DOC to
444 be 113‰ and 171‰, respectively, which is ^{14}C -enriched relative to the lake DIC. However, the
445 radiocarbon values for carbohydrate-like carbon in surface-water HMW DOC in the mixed vs
446 stratified lake (Table 3) actually show a decrease between June and August, indicating that

447 remobilization of such ^{14}C -enriched material does not seem to be a major factor in the mixed vs
448 stratified-season differences.

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

470 suggest that seasonal dynamics in the chemical and isotopic compositions of SPE DOC are
471 controlled by similar processes in these lakes. It appears that ^{14}C -depleted inputs of SPE DOC
472 during lake mixing and snowmelt in spring and photochemical and/or microbial removal of ^{14}C -
473 depleted components during stratification in summer may be a widespread phenomenon in
474 temperate freshwater lakes.

475 The relative importance, potential competition, and synergistic relationships of photo-
476 oxidation versus microbial oxidation in remineralization processes (especially removal of old
477 DOC) in aquatic systems such as Lake Superior are not well understood (Amado et al. 2015).
478 Chemically, increased microbial oxidation, and the concomitant increase in the percentage of
479 microbial biomass in the organic matter pools, has been associated with increased nitrogen
480 content whereas photochemical oxidation decreases aromaticity (Brooks et al. 2007; Cory et al.
481 2007). Light-absorbing components of DOC such as aromatic compounds and black carbon
482 released from chars have been shown to be more susceptible to photoalteration (Cory et al. 2007;
483 Ward et al. 2014; Ward and Cory 2016).

484 In Lake Superior, terrestrial sources deliver $\sim 0.4\text{-}0.9$ Tg C to the lake each year (Cotner
485 et al. 2004; Urban et al. 2005), enough to account for the lake's DOC reservoir of 14-17 Tg C
486 (Zigah et al. 2012) and DOC steady state cycling given the hydraulic residence time of 173
487 years. But evidence for substantial terrigenous DOC in the lake is lacking. Studies based on
488 ultraviolet-visible spectroscopy of total DOC, NMR of HMW DOC, and FTIR of total and
489 HMW DOC have shown fairly low contributions from the aromatic constituents which would
490 indicate terrigenous sources (Chin et al. 1994; Ma and Green 2004; Minor and Stephens, 2008,
491 Stephens and Minor, 2010; Zigah et al. 2014). It is possible that some of the aromatic terrigenous
492 compounds are photochemically degraded or oxidized, possibly through interaction with singlet

493 oxygen, a photochemically produced oxidant that has been reported to increase in Lake Superior
494 relative to its tributaries (Peterson et al. 2012). Therefore, the reported low aromatic constituents
495 coupled with our radiocarbon data showing the removal of old ^{14}C -depleted components in the
496 sunlit waters in summer (Table 2) suggest that photochemical oxidation plays a major role in the
497 removal of old allochthonous DOC in Lake Superior during stratification in summer.

498 **4.2 Radiocarbon ages, sources and cycling of organic compound classes within HMW DOC**

499 ***4.2.1 HMW DOC Carbohydrate-like and protein-like fractions***

500 Hydrolysable carbohydrate-like and protein-like substances from the HMW DOC were
501 24- 39‰ depleted in ^{14}C relative to the DIC, and 7 - 18‰ depleted in ^{14}C relative to the
502 atmospheric CO_2 in the sampling year (Table 3, Fig. 5). These indicate that there must be a
503 significant contribution from organic material that pre-dates bomb testing. HMW DOC from
504 recent in-lake productivity may be remineralized quickly, as it does not seem to impart a signal
505 persistently identifiable in mixed and stratified waters. An estimate of $\Delta^{14}\text{C}$ of the total
506 carbohydrate carbon within HMW DOC based upon the correlation between NMR signals and
507 $\Delta^{14}\text{C}$ yielded a lakewide carbohydrate $\Delta^{14}\text{C}$ value of 171‰ (Zigah et al. 2014). The relative
508 depletion of $\Delta^{14}\text{C}$ values of the hydrolysable carbohydrate-like material observed in this study
509 implies either that the more labile ^{14}C -enriched components of these hydrolysable substances are
510 not recovered in the hydrolysis, that there is a significant difference in the carbohydrate
511 structures identified by NMR compared to carbohydrate-like material recovered by hydrolysis, or
512 that the NMR signal in several samples from Zigah et al. (2014), which come from sites spatially
513 distributed across the lake, is preferentially influenced by the carbohydrate from materials
514 synthesized closer to the timing of the bomb spike (perhaps terrigenous material that is a few
515 decades old). It is also possible that some ^{14}C -depleted noncarbohydrate impurities from CRAM

516 or humic substances in the HMW DOC were co-isolated during acid hydrolysis. Overall, the
517 modern radiocarbon signatures of the hydrolysable substances in both stratified and mixed-lake
518 conditions (Fig. 5, 6) and the previous NMR-based results showing post-bomb values for the
519 carbohydrate carbon, HPS and AMS constituents indicate that, on average, these carbohydrate-
520 like and protein-like substances do not persist over time scales approaching a century or longer.
521 The large differences in ^{14}C -enrichment determined by the two methods used to analyze these
522 fractions of DOC indicate the need for further work in linking compound-class characterization
523 and radiocarbon content.

524 The $\delta^{13}\text{C}$ values of DIC in the lake were 0.3-0.4‰ in all but the stratified surface water
525 which had a value of 0.9‰ (Table 2). Aqueous CO_2 is typically ^{13}C -depleted by $\sim 10\%$ relative
526 to the bulk DIC (de Kluijver et al. 2014). Applying a typical lake algal photosynthetic
527 fractionation of 17‰ (de Kluijver et al. 2014), organic materials recently synthesized within the
528 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 -
529 27.8‰ in the lake (Keough et al. 1996). Photochemically altered terrestrially-derived vascular
530 plant sources have similar $\delta^{13}\text{C}$ values of -25.0 to -26.6‰ (Vahatalo and Wetzel 2008).
531 Therefore, the $\delta^{13}\text{C}$ values of -25.0 to -26.9‰ observed here for the hydrolysable carbohydrate-
532 like and protein-like substances reflect both the values expected from photochemically altered
533 terrigenous sources as well as autochthonous algal-derived sources.

534 ***4.2.2 HMW DOC Lipid-like fraction***

535 Possible sources of the lipid-like fraction are cellular lipids and lipid components of
536 humic substances or petroleum hydrocarbons that may be co-isolated with cellular-derived lipids
537 during solvent extraction (McIntyre et al. 2002). Petroleum hydrocarbons most likely derive
538 from anthropogenic inputs to Lake Superior. The ^{13}C -depletion of the lipid-like fraction (Fig. 6)

539 is consistent with fractionation associated with cellular lipids biosynthesis (De Niro and Epstein
540 1977). $\Delta^{14}\text{C}$ values of the lipid-like fraction (-256‰ to -153‰) were by far more ^{14}C -depleted
541 than the acid hydrolysable substances. The millennia ages of the lipid-like fractions are
542 surprising given the hydraulic residence time of the lake (173 years), and indicates that this
543 fraction does not derive from recent bomb ^{14}C -influenced plankton productivity within the lake
544 or recent terrestrial productivity. In Lake Michigan lipid-like materials from particulate organic
545 carbon (POC) have been reported to be influenced by resuspended sediments (Meyers et al.
546 1984) and such influences may also occur in the POC and DOC in Lake Superior. The lipid-like
547 fraction and CRAM ($\Delta^{14}\text{C}$ of -230‰; Zigah et al. 2014) from HMW DOC are similarly ^{14}C -
548 depleted suggesting that ^{14}C -depleted CRAM may be included within the lipid-like fraction. Co-
549 recovery of 70-90% of the lipid-like fraction from CRAM with a $\Delta^{14}\text{C}$ value of -230‰ or 20-
550 25% of the lipid-like fraction from petroleum hydrocarbon with $\Delta^{14}\text{C}$ value of -1000‰ along
551 with recently-synthesized cellular lipids would account for the ^{14}C -depletion observed in the
552 lipid-like fraction relative to the hydrolysable substances. The relative ^{14}C -enrichment of the
553 surface lipid-like fraction during stratification in August (Fig. 5) could be explained by the
554 addition of ~19% of lake-DIC derived ^{14}C -enriched 'cellular' lipids from photoautotrophy, or the
555 removal of ~20% of ^{14}C -depleted components with a radiocarbon signature similar to that of
556 CRAM. Irrespective of the source, the ^{14}C -depletion of the HMW DOC lipid-like fraction
557 indicates a ^{14}C -depleted origin (deep soil horizon from for instance cliff erosion along south
558 shore near apostle islands or lake sediments or petroleum source; Abbot and Stafford 1995;
559 Sickman et al. 2009; Zigah et al. 2014).

560 ***4.2.3 HMW DOC Unhydrolysable fraction***

561 The isotopic depletion of the unhydrolysable fraction ($\Delta^{14}\text{C} = -57\text{‰}$, $\delta^{13}\text{C} = -29.5\text{‰}$; Fig.
562 6) in deep waters during summer stratification implies that a considerable proportion of this
563 fraction may come from similar sources as the old and ^{13}C -depleted lipid-like fraction. Selective
564 preservation of lipid-like material has been proposed as the dominant formation mechanism of
565 the unhydrolysable fraction within sinking particulate organic matter in estuaries and oceans
566 (Wang et al. 1998; Hwang et al. 2006). Isotopic enrichment in the surface unhydrolysable
567 fraction during stratification ($\Delta^{14}\text{C} = 16\text{‰}$, $\delta^{13}\text{C} = -27.5\text{‰}$; Fig 6) and mixing condition ($\Delta^{14}\text{C} =$
568 59‰ , $\delta^{13}\text{C} = -26.6\text{‰}$; Fig 6) suggests some addition from isotopically enriched sources with
569 protein-like and/or carbohydrate-like precursors or removal of some isotopically depleted
570 sources, perhaps through photo-oxidation.

571 **4.3 Composition and cycling of HMW DOC and SPE DOC based on thermochemical** 572 **fractionation**

573 The thermochemical fractionation data show that during spring mixing, about 15% of the
574 surface HMW DOC was in the thermally labile/reactive components, i.e. is oxidized at
575 temperatures $< 331^\circ\text{C}$. These thermally reactive components were ^{14}C -enriched ($\Delta^{14}\text{C}$ values of
576 60 to 64‰) relative to the more thermally refractory components (Fig 4c). During summer
577 stratification, a significant component (about 46%) of the surface HMW DOC was thermally
578 labile (at $< 326^\circ\text{C}$) and ^{14}C -enriched ($\Delta^{14}\text{C}$ values of 70 to 75‰) (Fig 4e). The enriched isotopic
579 values are consistent with an in-lake source reflecting the past 3 years (2007-2009), a recent-past
580 terrigenous source (2004-2005) or a mixture of sources with a substantial post-bomb component
581 (1955-2009) (Fig. 4a). The increase in % of HMW DOC showing thermal lability and the
582 enriched isotopic values of this pool in the stratified surface waters and the lack of these in the
583 mixed period suggest that a substantial component of HMW DOC recycles over shorter times;

584 this is consistent with studies that have reported rapidly recycling semi-labile components within
585 HMW DOC (Amon and Benner 1994; Loh et al. 2004; Repeta and Aluwihare 2006).

586 In addition to this enriched, thermally labile component, a substantial proportion of the
587 mixed-lake surface HMW DOC (85%) and stratified surface HMW DOC (54%) were more
588 thermally recalcitrant (Fig. 4a, c, e). In the mixed lake HMW sample, the thermally recalcitrant
589 fractions ($>331^{\circ}\text{C}$) had $\Delta^{14}\text{C}$ values ranging from -23 to 34‰, lower than post-bomb
590 atmospheric $\Delta^{14}\text{C}$. In the stratified HMW sample, the divide between $\Delta^{14}\text{C}$ values higher and
591 lower than the contemporaneous atmospheric value occurred at a higher temperature range, with
592 fractions $>378^{\circ}\text{C}$ showing ^{14}C -depletion relative to the atmosphere. The $\Delta^{14}\text{C}$ values of the two
593 most thermally stable fractions in each HMW sample were similar to the $\Delta^{14}\text{C}$ values of total
594 SPE DOC and HMW DOC in the lake during spring mixing. In a previous study, NMR data
595 showed that HMW DOC can be modeled as consisting of two polymeric components - ^{14}C -
596 enriched HPS and AMS, and older ^{14}C -depleted component that may include considerable
597 CRAM and aliphatic components (Zigah et al. 2014). It is likely that the more thermally labile
598 components likely include HPS and AMS whereas the thermally recalcitrant components may
599 include the presence of CRAM or humic substances of varying ages. The absence of the large
600 thermally stable components (e.g., peak at $\sim 630^{\circ}\text{C}$) in the surface HMW DOC during
601 stratification is consistent with surface removal by photochemical and/or microbial oxidation of
602 thermally recalcitrant DOC with a lower $\Delta^{14}\text{C}$ value. In addition, the relative increase in the
603 thermally labile components during stratification suggests some photoautotrophic inputs during
604 summertime. The stratified-sample enrichment in ^{14}C relative to concurrent DIC values for the
605 most thermally labile fractions indicates that there may also be removal of more ^{14}C -depleted
606 material from these thermally labile fractions. The combination of these processes is consistent

607 with the relative increase in concentration and ^{14}C -enrichment in HMW DOC during
608 stratification (Table 2).

609 The data from serial oxidation and acid hydrolysis of HMW DOC differ greatly; there are
610 several possible mechanisms for this. It is possible that both ^{14}C -enriched and ^{14}C -depleted
611 material contribute to the same “compound class” in the acid hydrolysis (as might be seen for
612 structural carbohydrates from older woody material as well as younger woody material, grasses
613 and algae); or conversely that both enriched and depleted material have similar thermal labilities.
614 These differences could also be explained by differences in recovery for the wet chemical
615 fractions vs. thermal oxidation fractions. Also, isotopic mixing during thermal fractionation
616 could lead to the observed disparity. For instance, the depleted lipid-like fraction could be easily
617 mixed into one of the thermal fractions due to their small relative abundance. Coupling both acid
618 hydrolysis and thermal oxidation appears to be better in constraining the isotopic heterogeneity,
619 and by extension, the sources and cycling of HMW DOC in the lake. However, better
620 distinguishing the windows of view into the DOC pool that each approach provides is an area
621 needing further study.

622 The thermograms of SPE DOC show that the thermally-labile components in the mixed and
623 stratified samples overlap considerably. The ^{14}C values for the thermal fractions show that both
624 old and contemporary material contributes to the SPE DOC (Fig. 4d). About 57% of the
625 stratified surface SPE DOC (mean $\Delta^{14}\text{C}$ values of 59-65‰) and 34% of the mixed-lake SPE
626 DOC (mean $\Delta^{14}\text{C}$ values of 54-58‰) was ^{14}C -enriched, similar to the lake DIC and terrestrial
627 productivity 2-4 years prior to our sampling in the Lake (in 2005-2007), and derived from
628 contemporary sources, possibly hydrophobic/aromatic proteins (Goldberg et al. 2014). Also,
629 substantial components of the SPE DOC in the mixed-lake (66% with $\Delta^{14}\text{C}$ values of -22 to

630 48‰) and stratified lake (43% with $\Delta^{14}\text{C}$ values of -8 to 47‰) were ^{14}C -depleted relative to the
631 lake DIC. The most thermally refractory portion, with pre-bomb ^{14}C values, was considerably
632 smaller during stratification (Fig. 4 b, d, and f). However, the most thermally labile SPE DOC
633 component in each SPE sample was also ^{14}C -depleted, especially in the stratified sample (Fig 4d,
634 f). As with the HMW DOC, thermally recalcitrant components present in the surface SPE DOC
635 during lake mixing in spring appear to be removed from the surface SPE DOC during
636 stratification consistent with summertime removal of thermally recalcitrant and old DOC from
637 the lake (Fig 4b, d, f). In contrast to HMW DOC, there does not appear to be a considerable input
638 of recently synthesized organic material into the thermally labile portion of the stratified lake
639 SPE sample, and, in fact, the most thermally labile fraction actually shifted to pre-bomb values
640 (Fig 4b, f). Overall, these observations suggest there was little contribution to the SPE DOC from
641 photoautotrophy during summertime, and are consistent with the decrease in concentration and
642 relative ^{14}C -enrichment observed in surface SPE DOC during stratification (Table 2).

643 **5. Summary and conclusions**

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

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1

2 **Table 1.** Sampling depth, sample volumes, and elemental composition of HMW DOC isolated from eastern Lake Superior in June and
3 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

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

15

16 **Table 3.** Radiocarbon and stable carbon isotopic composition of organic fractions extracted from
 17 HMW DOC from eastern Lake Superior in 2009. Instrumental precision of $\Delta^{14}\text{C}$ and $\delta^{13}\text{C}$
 18 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

19 nd = sample lost during extraction, combustion to CO₂ or graphitization.

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27 **Table 4.** Elemental and isotopic composition of putative sources of 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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1 LIST OF FIGURES

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

3 **Figure 2.** Depth profiles of (a) dissolved oxygen, temperature and chlorophyll-a florescence in
4 the isothermal water column of Lake Superior in June 2009, and (b) dissolved oxygen,
5 temperature and chlorophyll-a florescence (WETLabs WETStar, mg/m³) in the stratified water
6 column of Lake Superior in August 2009. The oxycline and the thermocline were observed at
7 ~2-40 m, and the entire column remained oxygenated during stratification in August.

8 **Figure 3.** Radiocarbon compositions of lake-water DIC from surface (5 m) and deep chlorophyll
9 maximum (30-40m) depths and atmospheric CO₂ covering the years 2004-2012. The $\Delta^{14}\text{C}$ -DIC
10 data was collated from Zigah 2012 and Zigah et al. 2011, 2012 and 2014. Where available, both
11 the surface (5 m) and depth of deep chlorophyll maximum (30-40 m) were included. Data from
12 2009-2010 were from the eastern region of the lake (and were similar to those from the western
13 region of the lake) and data from 2007, 2008, and 2012 were from the western region of the lake
14 (which we assume to be same as those at the eastern region of the lake given the similarity of the
15 2009-2010 data in particular, and homogeneity of $\Delta^{14}\text{C}$ -DIC in the lake in general as reported by
16 Zigah et al. 2012). The atmospheric CO₂ $\Delta^{14}\text{C}$ data was adapted from Zigah 2012, Hseuh et al.
17 2007, Graven et al. 2012 and Kruger 2014.

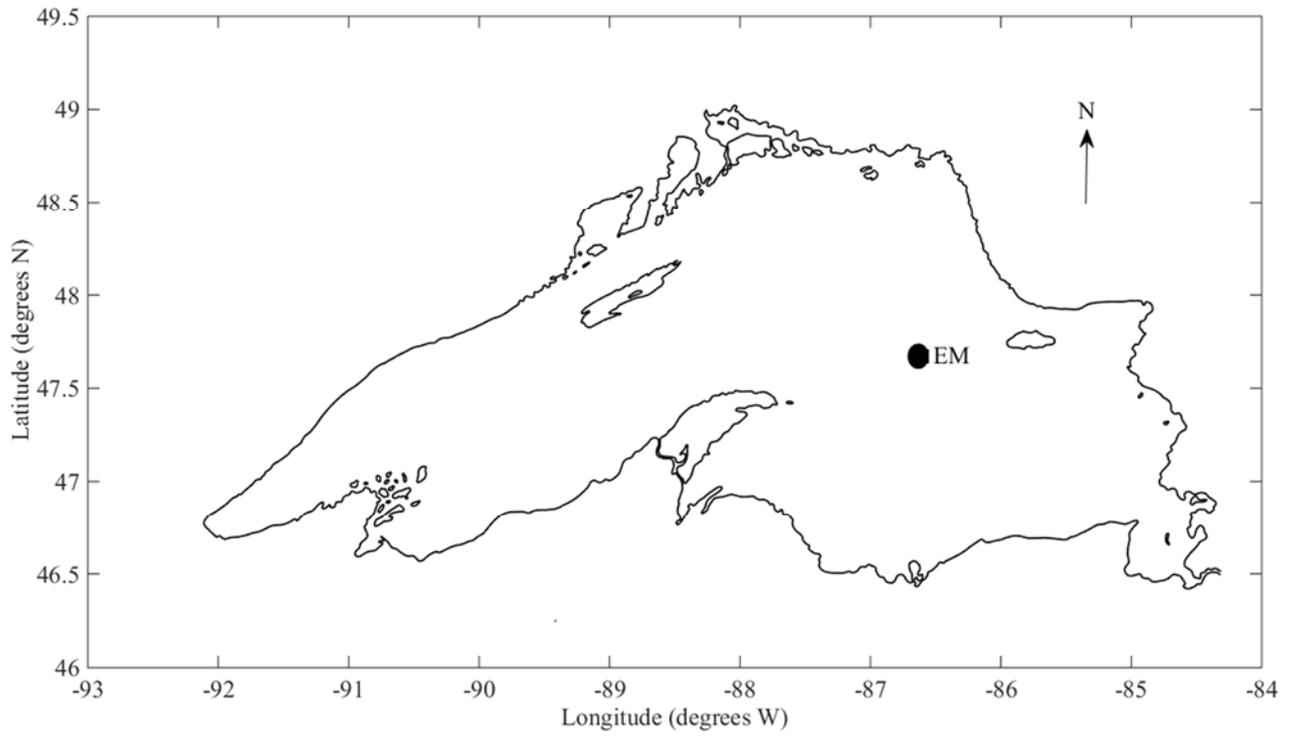
18 **Figure 4.** Thermogram of (a) surface HMW DOC during stratification and mixed-lake
19 conditions and (b) surface SPE DOC during stratification and mixed-lake conditions from
20 eastern Lake Superior. CO₂ values were normalized to the height of base peak. Different
21 components were thermally oxidized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

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

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

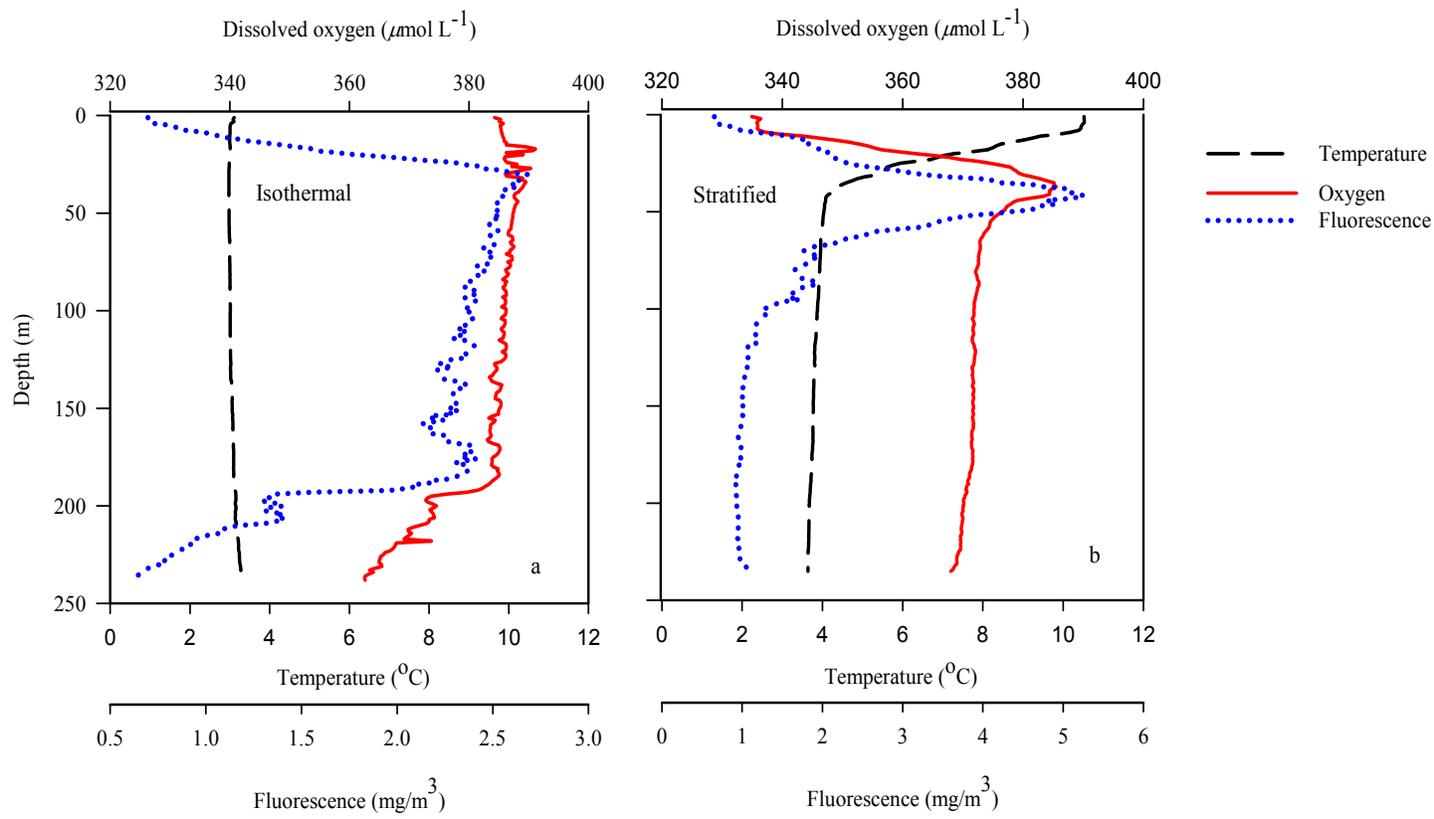
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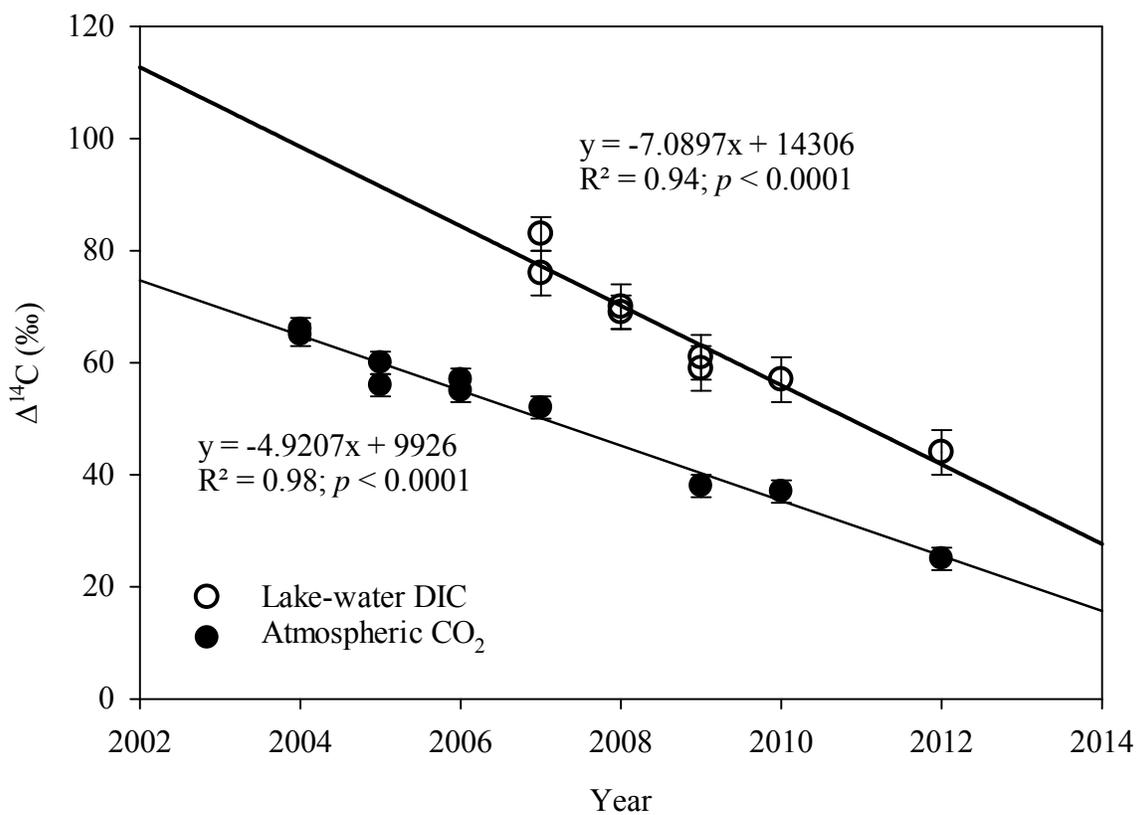
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Fig 1



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Fig 2



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Fig 3

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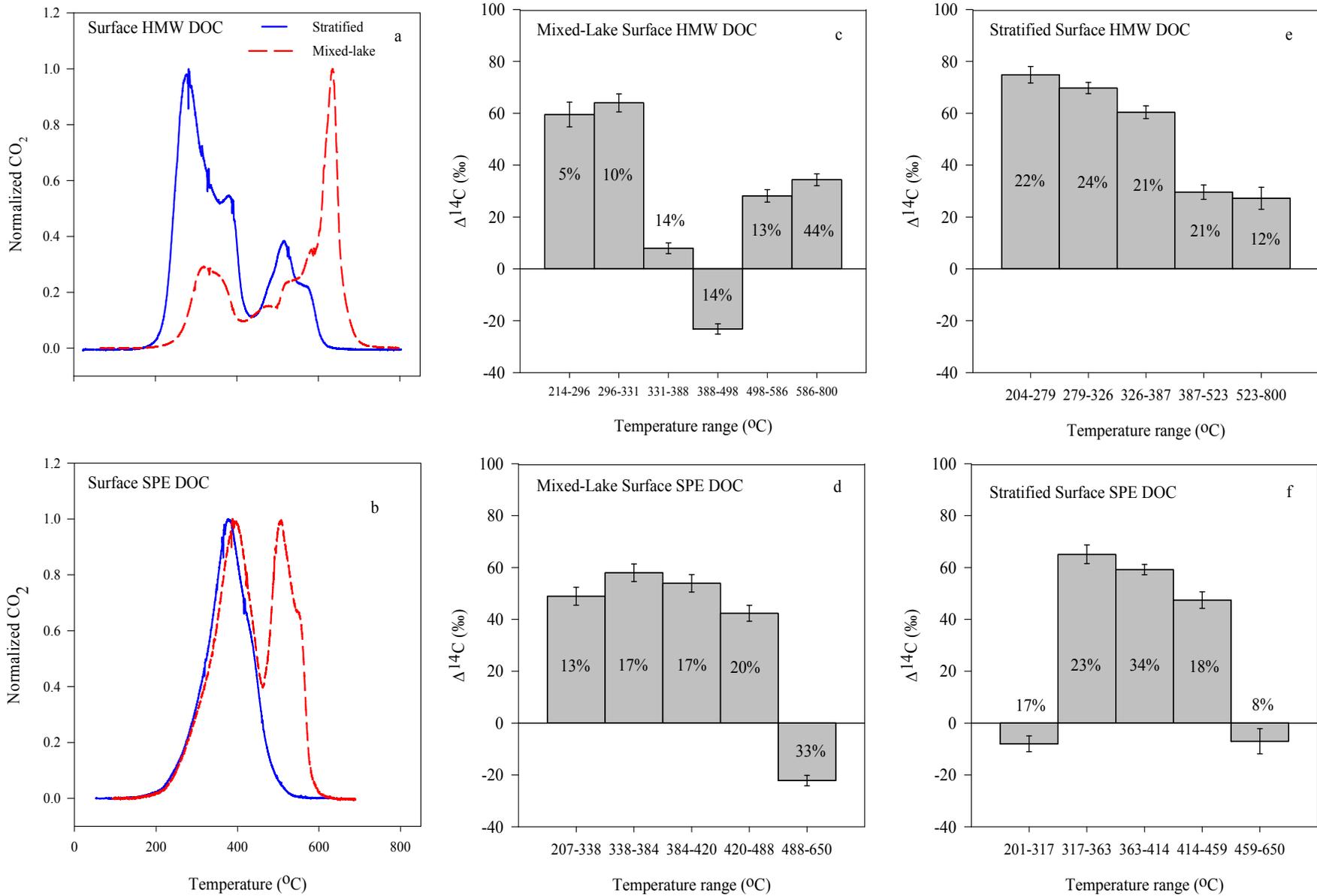
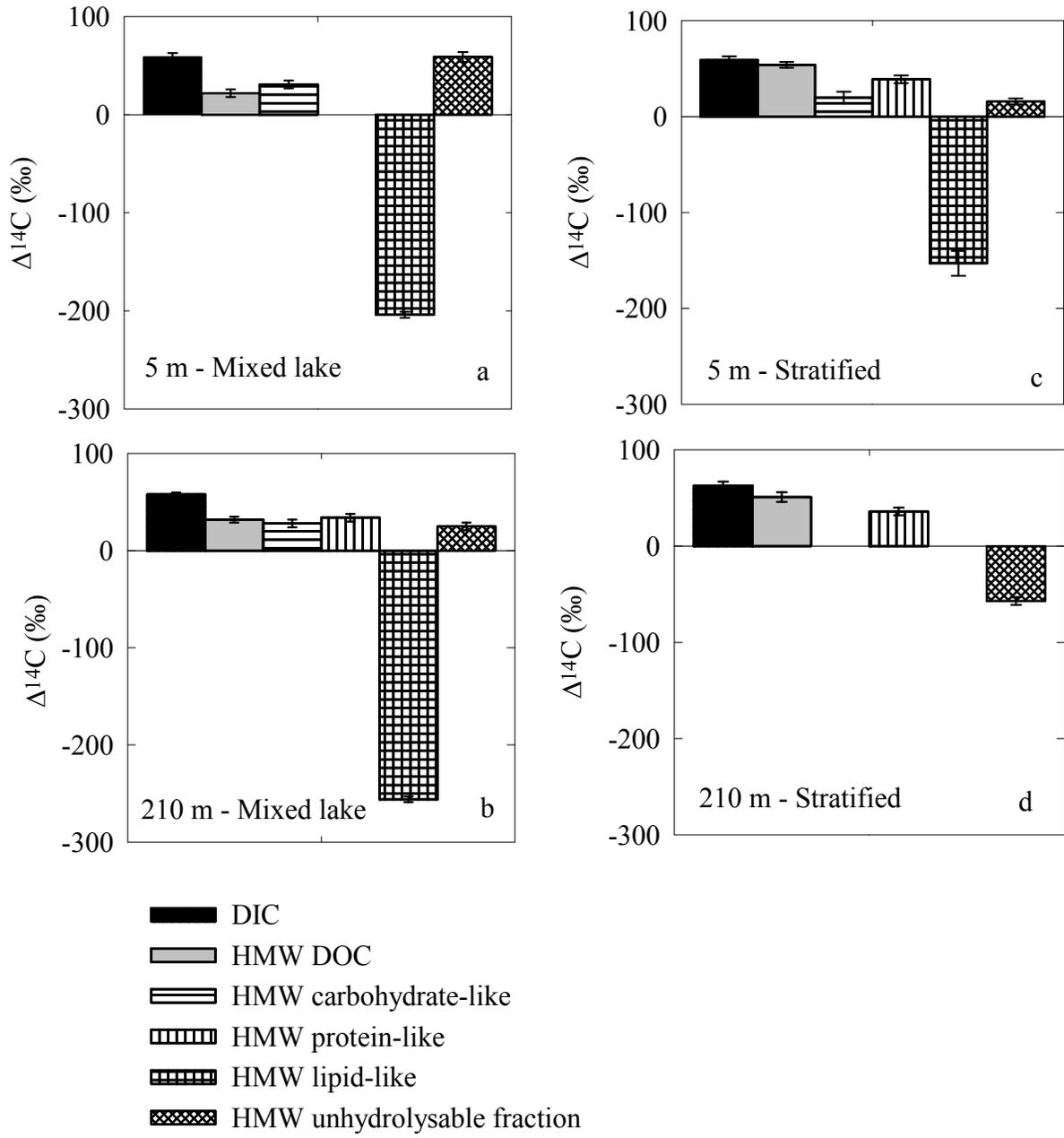


Fig 4



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Fig 5

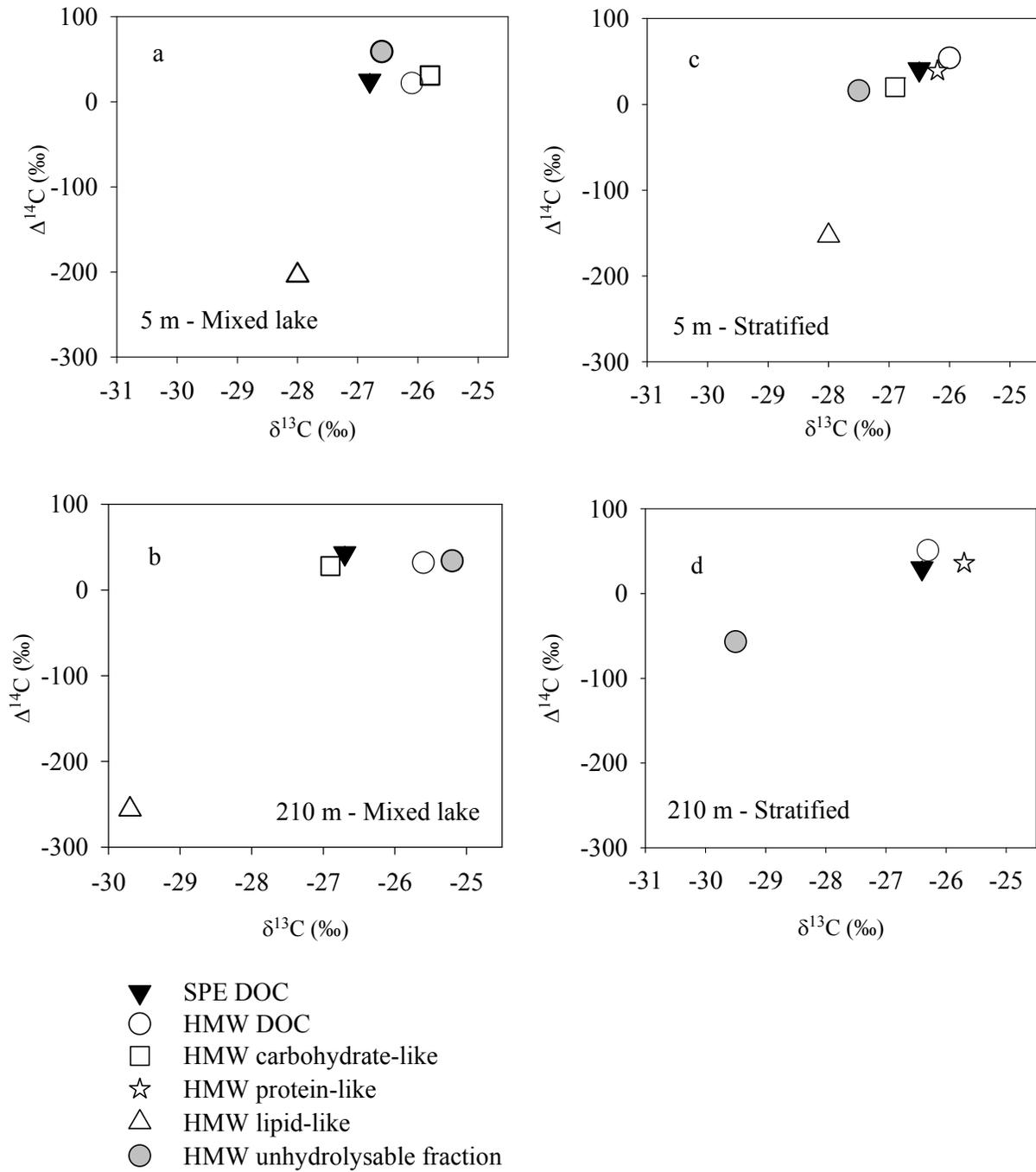


Fig 6