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

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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 (Δ^{14}C = 25 to 43‰) and HMW DOC (Δ^{14}C = 22 to 32‰) was evident during spring mixing, and depressed their radiocarbon values relative to the lake dissolved inorganic carbon (DIC; Δ^{14}C ~ 59‰). 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 carbohydrate-like and protein-like 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 CO_2 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).

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 (Δ^{14}C) and stable isotope (δ^{13}C) compositions of the

142   major fractions of DOC provide additional constraints on the multiple sources and cycling of the

143   DOC. Δ^{14}C calculation corrects for biochemical fractionations so differences in Δ^{14}C values

144   reflect variable sources and/or cycling. For instance organic material produced from recent

145   within-lake photoautotrophy bears a Δ^{14}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 ~ 90 μM during spring mixing. During

176 summer stratification, lakewide surface DOC is ~ 100-120 μM and is about 10 μM 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 (~ 9% of the primary

179 production; Urban et al. 2005, Sterner 2010), similar to the annual terrestrial DOC loading of

180 0.4-0.9 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 (47º34'N, 86º39'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 $\geq$

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) N_2 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 CO_2 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 $800\,^{\circ}C$ 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 CO_2 by gradually raising the top oven temperature from

246   ambient to $1000\,^{\circ}C$ at a rate of $5\,^{\circ}C$ per minute. The evolving gas was passed over a Pt/Ni/Cu

247   twisted wire in the $800\,^{\circ}C$ oven to ensure complete oxidation to CO_2. The CO_2 was first

248   quantified downstream with an in-line CO_2 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 ($\Delta^{14}C$) and stable carbon isotope ($\delta^{13}C$) measurements were performed at

252   NOSAMS using standard protocols (McNichol et al.1994). Typical instrumental precision of

253    $\delta^{13}C$ 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 $\Delta^{14}C$ according

257   to the convention of Stuiver and Polach (1977). Instrumental precision of the $\Delta^{14}C$ 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 CO_2 $\Delta^{14}C$ 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 CO_2 in the Northern Hemisphere (Graven et al. 2012). The

262   atmospheric CO_2 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 $\Delta^{14}C$ values of non-extracted and

270   extracted stearic acid were 48 ± 4‰ and 51 ± 4‰, respectively. The $\Delta^{14}C$ values of non-

271   extracted and extracted D-glucose (carbohydrate standard) were 35 ± 4‰ and 41 ± 4‰ and those

272   of bovine serum albumin (protein standard) were 184 ± 3‰ and 179 ± 3‰ 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 $\delta^{13}C$ values of

275   stearic acid (-30.4 ± 0.15‰ vs. -30.4 ± 0.15‰), D-glucose (-11.2 ± 0.15‰ vs. -11.2 ± 0.15‰)

276   and bovine serum albumin (-10.2 ± 0.15‰ vs. -10.6 ± 0.15‰) 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 µg C

280   (Loh et al. 2004) with a $\Delta^{14}C$ value of 48‰, similar to the modern stearic acid standard, addition

281   of this will increase the $\Delta^{14}C$ values of the lipid samples by ≤ 1‰. If the process blank contained

282   as much as 12 µg C with a $\Delta^{14}C$ value of 48‰, the lipid samples' $\Delta^{14}C$ 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 µg C was obtained,

288   small relative to the SPE sample size (421-774 µg C), and there was not enough for isotopic

289   analysis. If we assume this carbon has a fraction modern value of 1 ($\Delta^{14}C \sim$ -7‰) or 0 ($\Delta^{14}C$ = -

290   1000‰), the SPE sample $\Delta^{14}C$ values only decrease by $\leq$ 2‰. 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 µg C from

293   ambient to 1000°C, similar to the < 4 µg 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 µg 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 $\Delta^{14}C$ 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 $\Delta^{14}C$ and $\delta^{13}C$ 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 $\Delta^{14}C$ value of atmospheric CO_2 decreased from ~66‰ in 2004 to 38‰ during our

305   sampling in 2009, and to 25‰ in 2012 (Fig. 3) corresponding to a decline of $\sim$ 5‰/yr. The lake

306   DIC $\Delta^{14}C$ value decreased from 83‰ to 44‰ between 2007 and 2012 with a decline $\sim$ 7‰/yr

307   (Fig. 3). DIC was consistently ^{14}C-enriched by ~20‰ relative to atmospheric CO_2 from 2004-

308   2014, indicating a carbon isotope equilibration time of $\sim$ 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**

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 $\delta^{13}C$ 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 $\delta^{13}C$ values of

total DOC, HMW DOC and SPE DOC did not exhibit considerable variation between spring

mixing and summer stratification (Table 2).

The $\Delta^{14}C$ values of SPE DOC (25 to 43‰) were slightly depleted or similar to the $\Delta^{14}C$

value of atmospheric CO_2, and by extension, recent land-plant primary production (38 ± 2‰)

from 2009-2010, but more depleted relative to lake DIC ($\Delta^{14}C$ of 59 to 63‰). The stratified deep

and mixed-lake surface SPE DOC values (25 to 30‰) were more depleted relative to the $\Delta^{14}C$ of

lake DIC (Table 2) than were the values for the other two SPE DOC samples. During spring

mixing conditions in June, $\Delta^{14}C$ 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 ($\Delta^{14}C$ 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 $\Delta^{14}C$ and $\delta^{13}C$ values of extracted organic fractions within HMW DOC

The solvent extractable lipid-like fraction had $\Delta^{14}C$ values of -204‰ to -256‰

(corresponding to 1770 - 2320 ^{14}C years) during spring mixing in June, and -153 ± 13‰ (~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 ($\Delta^{14}C$ of 20 to31‰)

334 and protein-like ($\Delta^{14}C$ of 34 to 39‰) fractions. The latter fractions were consistently modern, but

335 ^{14}C-depleted relative to the DIC ($\Delta^{14}C$ of 58 to 63‰) (Tables 2 and 3). The $\delta^{13}C$ 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 $\Delta^{14}C$ and $\delta^{13}C$ 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 ~ 200-390 °C with two discernable peaks (larger one at ~ 270 °C and

343 smaller one at ~ 380 °C). High-temperature components thermally oxidize at ~ 420-630 °C with

344 peaks at ~513 °C and ~575 °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 ~ 513 °C was smaller in the mixed-lake sample but other high-temperature

349 components were observed including a large peak at ~630 °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 200-330 °C

354 were more ^{14}C- enriched ($\Delta^{14}C$ of 60-64‰) than the CO_2 fractions from the higher temperature

355 range of 388-800 °C ($\Delta^{14}C$ 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 CO_2 fractions from the lower temperature range

358   200-387 °C were more ^{14}C-enriched (Δ^{14}C of 60-75‰) than the CO_2 fractions from the higher

359   temperature range 387-800 °C (Δ^{14}C of 27-30‰) (Fig. 4e). For both mixed-lake and stratified

360   surface HMW DOC samples, the mass-weighted mean Δ^{14}C value was similar to the

361   independently measured bulk Δ^{14}C value.

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 ^{14}C-depleted (Δ^{14}C of -22 to

370   48‰) relative to the components within fractions F2 and F3 (Δ^{14}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 ^{14}C-depleted (Δ^{14}C of -8 to 47‰) relative to the components

374   within fractions F2 and F3 (Δ^{14}C of 60-65‰) (Fig. 4f) and in this case F1 and F5 both exhibited

375   negative Δ^{14}C values. The mass-weighted mean Δ^{14}C values matched the independently

376   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

379   The $\Delta^{14}C$ 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 $\Delta^{14}C$ values $\geq$ 59‰ (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 $\Delta^{14}C$ 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 Δ^{14}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 (Δ^{14}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 Δ^{14}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 (Δ^{14}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 Δ^{14}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 Δ^{14}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 Δ^{14}C values as high as +120‰ (Fig. 3). By extrapolation of the linear relationship between

441 HMW DOC Δ^{14}C values and HPS and AMS and between HMW DOC Δ^{14}C values and total

442 carbohydrate carbon from the ^{13}C NMR spectra of lake samples, Zigah et al. (2014) estimated

443 the Δ^{14}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 Δ^{14}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 Δ^{14}C of SPE DOC results mainly

452   from preferential remineralization or removal of older SPE DOC. Mass balance calculations

453   indicate that ~11 μM of old SPE DOC with an average Δ^{14}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 (Δ^{14}C of 35‰) relative to lake mixing

462   in January (Δ^{14}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 ~0.4-0.9 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 $\Delta^{14}C$ of the total

506 carbohydrate carbon within HMW DOC based upon the correlation between NMR signals and

507 $\Delta^{14}C$ yielded a lakewide carbohydrate $\Delta^{14}C$ value of 171‰ (Zigah et al. 2014). The relative

508 depletion of $\Delta^{14}C$ 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

22

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 δ^{13}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 ~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 δ^{13}C value of -26 to -27‰, similar to the reported algal δ^{13}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 δ^{13}C values of -25.0 to -26.6‰ (Vahatalo and Wetzel 2008).

531   Therefore, the δ^{13}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). $\Delta^{14}C$ 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 ($\Delta^{14}C$ 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 $\Delta^{14}C$ value of -230‰ or 20-

550   25% of the lipid-like fraction from petroleum hydrocarbon with $\Delta^{14}C$ 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 ($\Delta^{14}C$ = -57‰, $\delta^{13}C$ = -29.5‰; 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 ($\Delta^{14}C$ = 16‰, $\delta^{13}C$ = -27.5‰; Fig 6) and mixing condition ($\Delta^{14}C$ =

568   59‰, $\delta^{13}C$ = -26.6‰; 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°C. These thermally reactive components were ^{14}C-enriched ($\Delta^{14}C$ 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°C) and ^{14}C-enriched ($\Delta^{14}C$ 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°C) had $\Delta^{14}C$ values ranging from -23 to 34‰, lower than post-bomb

590   atmospheric $\Delta^{14}C$. In the stratified HMW sample, the divide between $\Delta^{14}C$ values higher and

591   lower than the contemporaneous atmospheric value occurred at a higher temperature range, with

592   fractions >378°C showing ^{14}C-depletion relative to the atmosphere. The $\Delta^{14}C$ values of the two

593   most thermally stable fractions in each HMW sample were similar to the $\Delta^{14}C$ 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 ~630 °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 $\Delta^{14}C$ 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 Δ^{14}C values of 59-65‰) and 34% of the mixed-lake SPE

626 DOC (mean Δ^{14}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 Δ^{14}C values of -22 to

630   48‰) and stratified lake (43% with $\Delta^{14}C$ 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.

652   **Acknowledgements**

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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
Mixed - June 2009				
Surface	5	200	1.25	23.6
Deep	210	400	0.90	17.7
Stratified - August 2009				
Surface	5	400	1.45	14.5
Deep	210	383	1.20	17.8

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

Season / Depth	DIC			Total DOC			HMW DOC			SPE DOC		
	μM	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	μM	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	μM	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	μM	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)
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

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

Organic fraction	Mixed-lake		Stratified-lake	
	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)
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.

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

Putative sources	C:N	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	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/m^3) 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 CO_2 covering the years 2004-2012. The Δ^{14}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 Δ^{14}C-DIC in the lake in general as reported by Zigah et al. 2012). The atmospheric CO_2 Δ^{14}C data was adapted from Zigah 2012, Hseuh et al. 2007, Graven et al. 2012 and Kruger 2014.
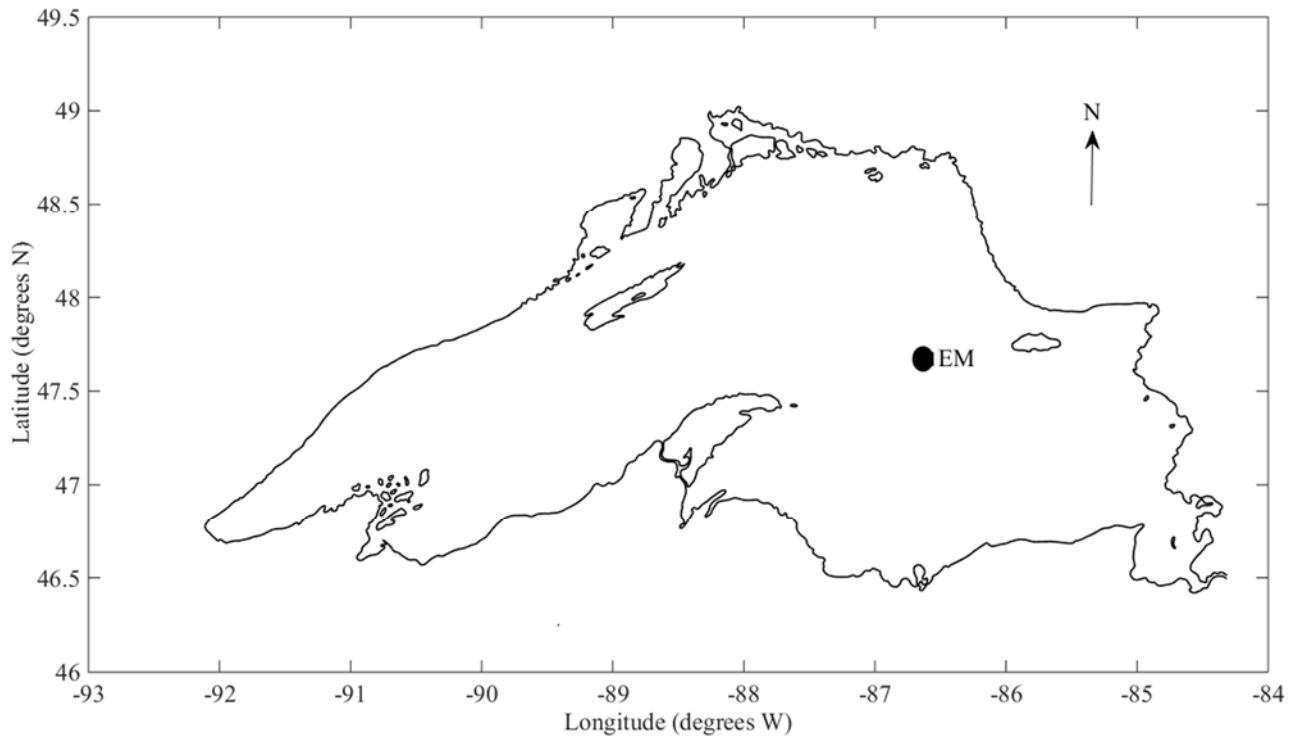
Figure 4. Thermogram of (a) surface HMW DOC during stratification and mixed-lake conditions and (b) surface SPE DOC during stratification and mixed-lake conditions from eastern Lake Superior. CO_2 values were normalized to the height of base peak. Different components were thermally oxidized at different 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 1σ 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. Δ^{14}C and δ^{13}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.
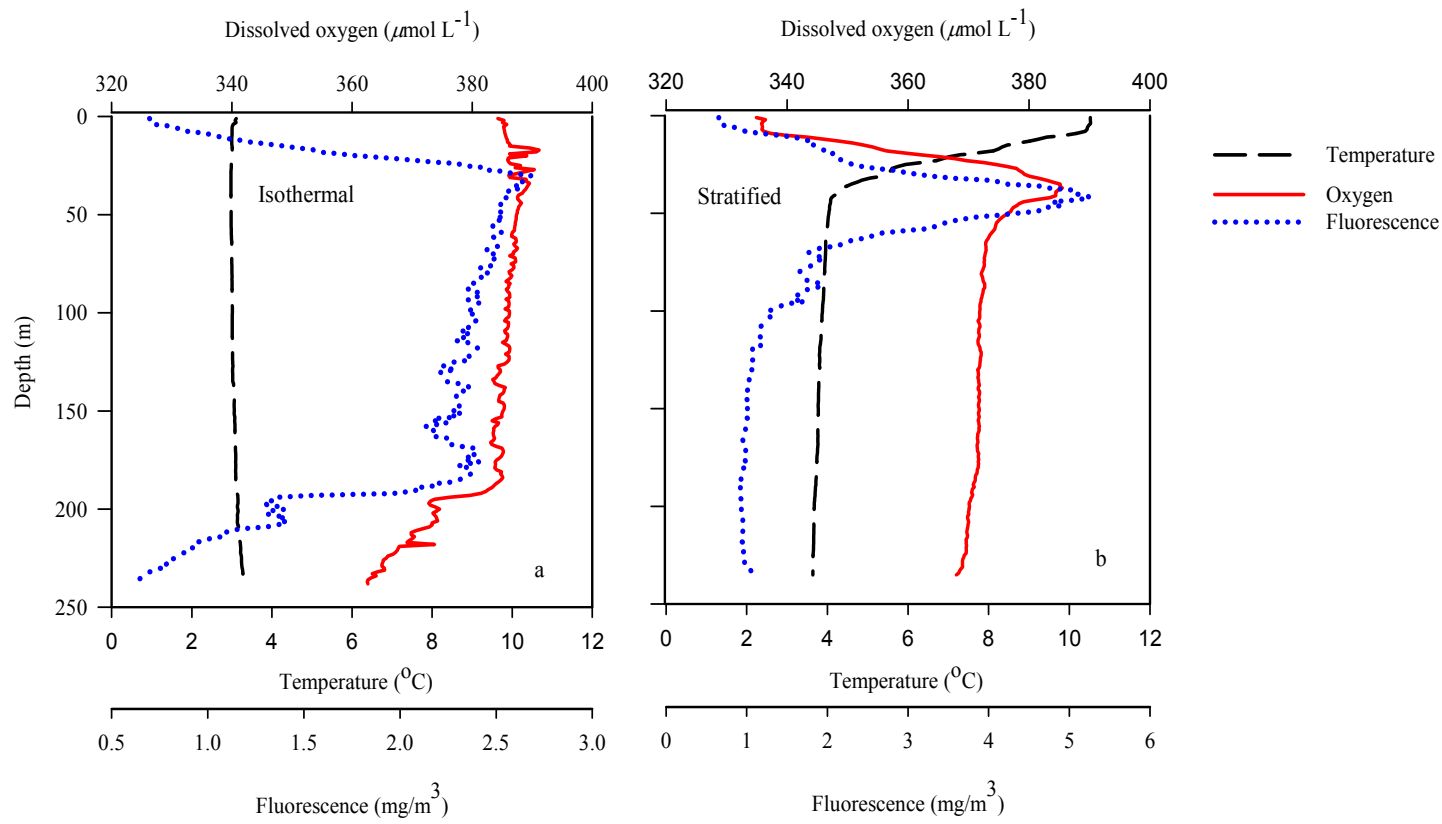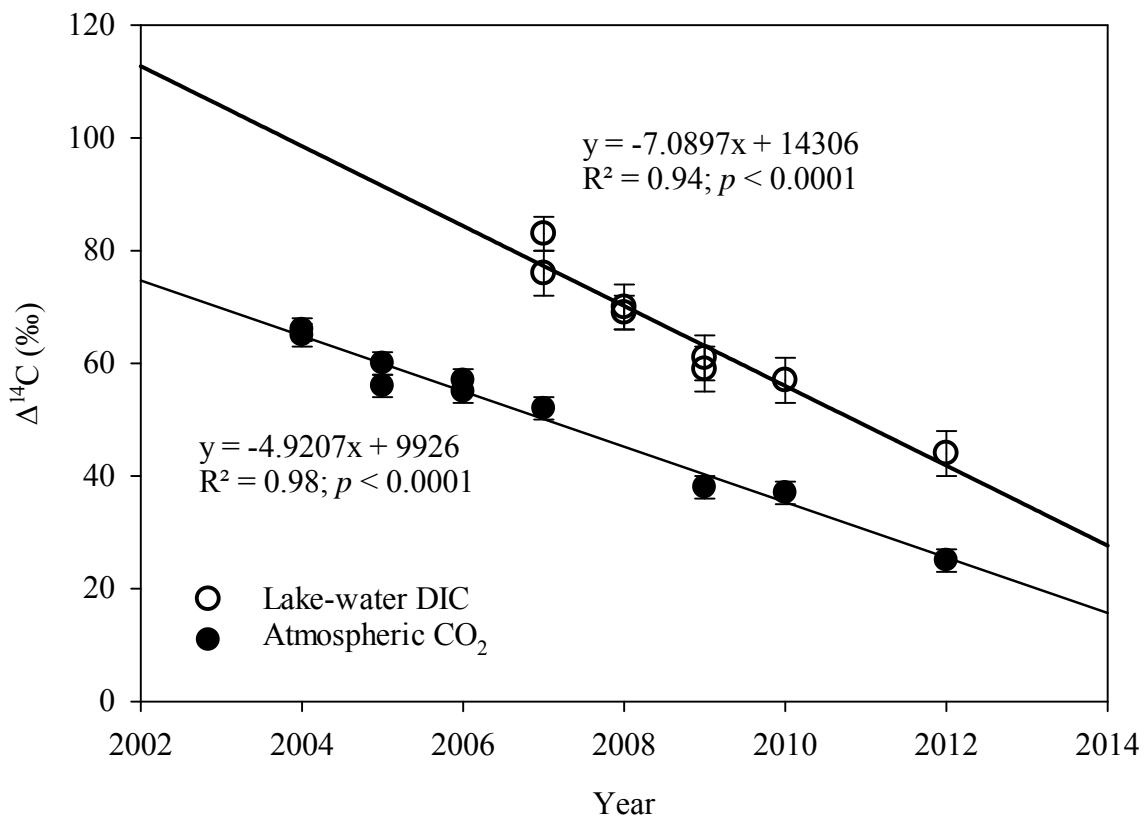
Fig 1

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Fig 2

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$y = -7.0897x + 14306$
$R^2 = 0.94$; $p < 0.0001$

$y = -4.9207x + 9926$
$R^2 = 0.98$; $p < 0.0001$

○ Lake-water DIC
● Atmospheric CO_2

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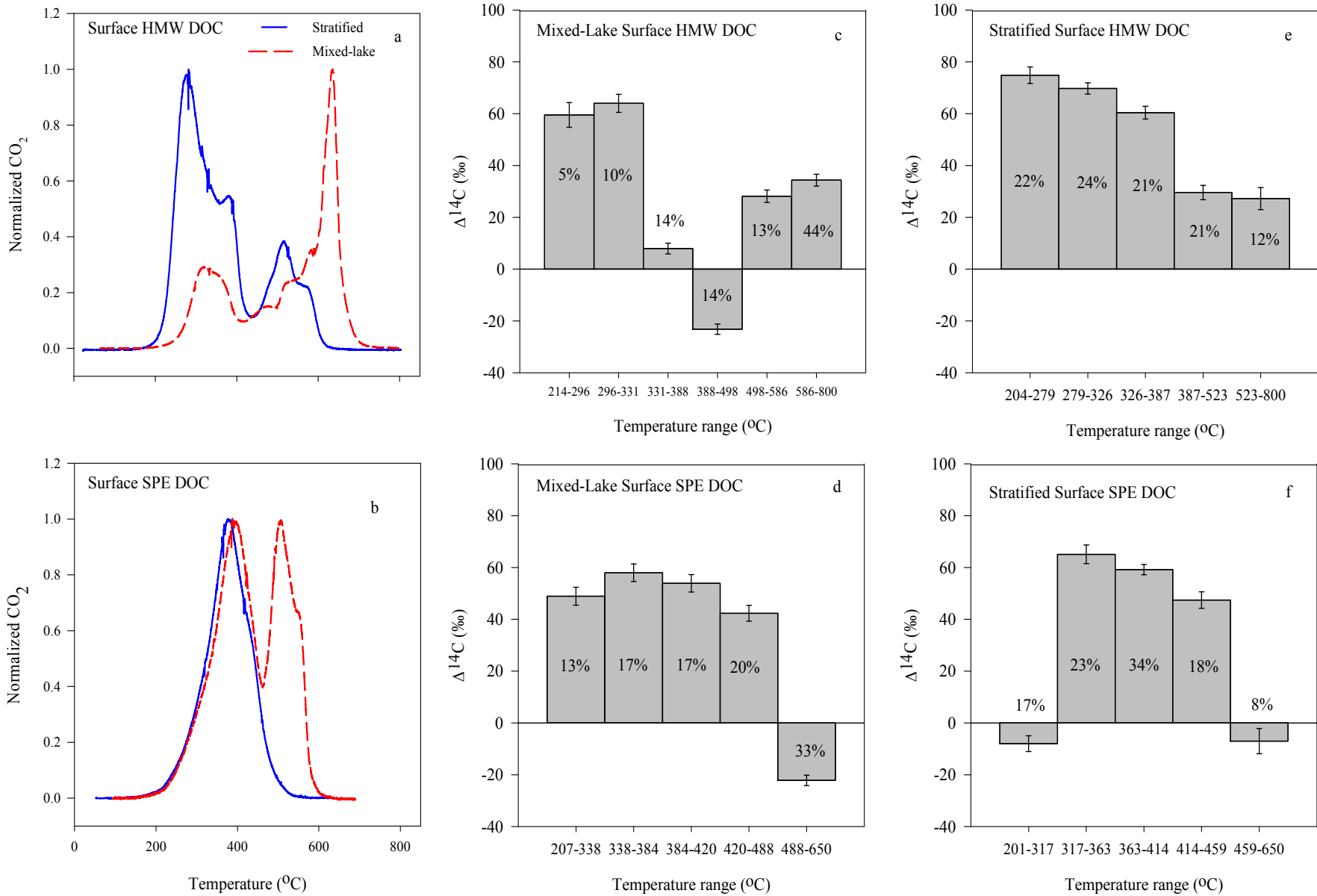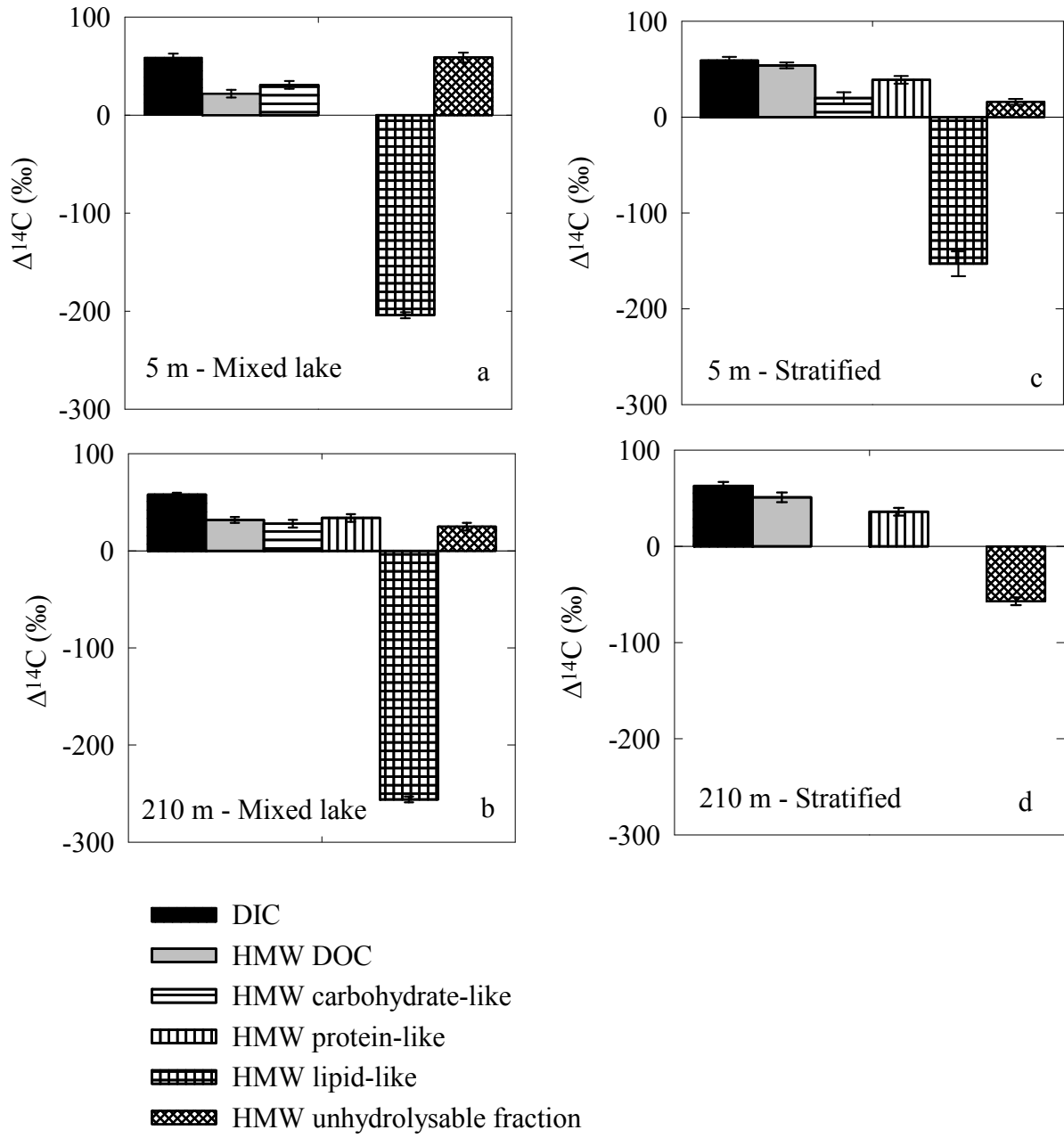60   Fig 3

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Fig 4

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Fig 5

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Fig 6