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Naphthenic acids in groundwater overlying undeveloped shale gas and tight oil reservoirs

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1 **Naphthenic acids in groundwater overlying undeveloped shale gas**
2 **and tight oil reservoirs**

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19 **Abstract**

20 The acid extractable organics (AEOs) containing naphthenic acids (NAs) in groundwater
21 overlying undeveloped shale gas (Saint-Édouard region) and tight oil (Haldimand sector, Gaspé)
22 reservoirs in Québec, Canada, were analysed using high resolution Orbitrap mass spectrometry
23 and thermal conversion/elemental analysis – isotope ratio mass spectrometry. As classically
24 defined by $C_nH_{2n+Z}O_2$, the most abundant NAs detected in the majority of groundwater samples
25 were straight-chain ($Z = 0$) or monounsaturated ($Z = -2$) C_{16} and C_{18} fatty acids. Several
26 groundwater samples from both study areas, however, contained significant proportions of
27 presumably alicyclic bicyclic NAs (i.e., $Z = -4$) in the C_{10} - C_{18} range. These compounds may
28 have originated from migrated waters containing a different distribution of NAs, or are the
29 product of *in situ* microbial alteration of shale organic matter and petroleum. In most
30 groundwater samples, intramolecular carbon isotope values generated by pyrolysis ($\delta^{13}C_{pyr}$) of
31 AEOs were on average around 2-3‰ heavier than those generated by bulk combustion ($\delta^{13}C$) of
32 AEOs, providing further support for microbial reworking of subsurface organic carbon.
33 Although concentrations of AEOs were very low (< 2.0 mg/L), the detection of potentially toxic
34 bicyclic acids in groundwater overlying unconventional hydrocarbon reservoirs points to a
35 natural background source of organic contaminants prior to any large-scale commercial
36 hydrocarbon development.

37

38 **Keywords:** Acid extractable organics; Orbitrap; Carbon isotopes; Unconventional hydrocarbons;
39 Bicyclic acids; Principal Components Analysis

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41

42 **Highlights:**

- 43 • NAs were found in groundwater overlying undeveloped shale gas and tight oil plays.
- 44 • Most abundant NAs were straight-chain or monounsaturated C₁₆ and C₁₈ fatty acids.
- 45 • Alicyclic bicyclic NAs (Z = -4) were detected in several groundwater samples.
- 46 • Natural background source of organic contaminants prior to hydrocarbon extraction.

47

48 **1. Introduction**

49 Naphthenic acids (NAs) are a complex mixture of alkyl-substituted acyclic and
50 cycloaliphatic carboxylic acids described by the general chemical formula C_nH_{2n+Z}O₂, where n
51 indicates the carbon number and Z is zero or a negative, even integer that specifies the hydrogen
52 deficiency resulting from ring formation (Clemente and Fedorak, 2005). Found naturally in crude
53 oil deposits, NAs are toxic to a wide range of aquatic organisms including microalgae (Debenest
54 et al., 2012), fish (Marentette et al., 2015; Scarlett et al., 2013), and amphibians (Melvin and
55 Trudeau, 2012), and also pose a problem during oil refining due to their corrosivity (Jayaraman
56 et al., 1986; Laredo et al., 2004; Tomczyk et al., 2001). Due to their high abundance in bitumen,
57 most recent research into the environmental behaviour and fate of NAs has been focused on
58 northern Alberta's Athabasca oil sands region, where the alkaline water hot extraction process
59 used in surface mining concentrates these compounds in oil sands process-affected water
60 (OSPW). Potential seepage of the large volumes of OSPW stored in tailings ponds (Ahad et al.,
61 2013; Frank et al., 2014; Oiffer et al., 2009; Ross et al., 2012; Savard et al., 2012) poses a risk to

62 local ecosystems and is one of the main environmental concerns associated with oil sands mining
63 operations.

64 Spurred on by the need to better understand this complex group of emerging
65 contaminants (Headley et al., 2013), the past decade has seen significant advances in the
66 characterization, identification and quantification of NAs in environment samples. High and
67 ultrahigh resolution mass spectrometry (MS) analysis carried out using Orbitrap MS and Fourier
68 Transform Ion Cyclotron Resonance (FTICR) MS has demonstrated that the acid extractable
69 organics (AEOs) fraction of most samples from the oil sands region contain not just the
70 classically defined NAs as described previously, but other compounds which incorporate
71 nitrogen and/or sulfur atoms, and have various levels of unsaturation and aromaticity (see
72 Headley et al., 2016, and references therein). Other work carried out using multidimensional
73 comprehensive gas chromatography mass spectrometry (GC × GC-MS) has identified individual
74 compounds in OSPW such as adamantane diamondoid acids (Rowland et al., 2011a),
75 monoaromatic C-ring steroidal acids (Rowland et al., 2011b) and bicyclic aromatic acids
76 (Bowman et al., 2014; West et al., 2014).

77 Microbial degradation of petroleum hydrocarbons generates carboxylic acids (Atlas,
78 1981; Barth et al., 2004; Meredith et al., 2000; Thorn and Aiken, 1998; Watson et al., 2002);
79 consequently, highly biodegraded, viscous crude oil deposits such as Athabasca oil sands
80 bitumen generally contain a significant component of AEOs. Other organic-rich sediments such
81 as coal and shale, however, may also contain NAs. Straight-chain fatty acids, which fall within
82 the definition of NAs described by $C_nH_{2n+Z}O_2$ (i.e., $Z = 0$), were detected in supercritical gas and
83 solvent extracts of lignite and sub-bituminous coals (Snape et al., 1981). Using FTICR-MS, Scott
84 et al. (2009) detected a range of NAs in distilled water leachates percolated through crush

85 samples of lignite and sub-bituminous coal. In shale from the Green River formation in the
86 western USA, normal ($C_nH_{2n}O_2$) and isoprenoid ($C_nH_{2n-2}O_4$) acids were found to be the major
87 extractable acidic constituents (Eglinton et al., 1966; Haug et al., 1971). Naphthyl-carboxylic
88 acids and cycloaromatic acids were also partly identified Haug et al. (1971). Exploiting the much
89 higher resolution afforded by FTICR-MS, Salmon et al. (2011) reported a series of polar CHO,
90 CHOS and CHON compounds between C_{12} and C_{50+} in Green River shale. The major series
91 (CHO) confirmed the abundance of normal and isoprenoid acids found in earlier studies, whereas
92 the CHOS and CHON series contained sulfoxide groups and nitrile-type compounds. FTICR-MS
93 analysis has also revealed a wide array of nitrogen-containing compounds in Chinese and
94 Russian shale oils (Bae et al., 2010; Chen et al., 2012; Tong et al., 2013).

95 Although the geochemical properties of bitumen and the processes involved with its
96 extraction can account for the environmental significance of AEOs in the Athabasca oil sands
97 region, as outlined above, the development of unconventional fossil fuels may also release
98 potentially harmful NAs into the subsurface. Recent work has reported a wide range of organic
99 compounds in hydraulic fracturing fluids, produced waters and formation waters from
100 unconventional natural gas extraction throughout the USA (Drollette et al., 2015; Hoelzer et al.,
101 2016; Orem et al., 2014; Stringfellow et al., 2017). The organic compounds detected in produced
102 waters included straight-chain fatty acids (e.g., C_{12} , C_{14} and C_{16}) thought mainly to be the
103 biodegradation products of geopolymeric substances present in the shale (Hoelzer et al., 2016;
104 Orem et al., 2014; Orem et al., 2010). Despite the potential for naturally-occurring NAs in shale
105 to be released into produced waters during unconventional hydrocarbon extraction, to the best of
106 our knowledge, no attempt to characterize these waters specifically for these compounds has
107 been reported in the literature.

108 In this study, AEOs in groundwater overlying undeveloped shale gas (Saint-Édouard) and
109 tight oil (Haldimand sector, Gaspé) reservoirs located in the province of Québec, Canada, were
110 analysed using high resolution Orbitrap MS and thermal conversion/elemental analysis – isotope
111 ratio mass spectrometry (TC/EA-IRMS). The intramolecular carbon isotope ratios generated by
112 online pyrolysis ($\delta^{13}\text{C}_{\text{pyr}}$) using TC/EA-IRMS were previously used to distinguish bitumen-
113 derived sources of AEOs in the Athabasca oil sands region (Ahad et al., 2013; Ahad et al., 2012).
114 AEOs extracted from unprocessed oil and naturally-occurring oil seeps from the Haldimand
115 sector and crushed samples of Upper Ordovician shales from the Saint-Édouard region were also
116 characterized. The main objectives of this study were to 1) confirm the presence of NAs (as
117 defined by the O_2 species class in AEOs) in groundwaters from these generally pristine aquifers,
118 2) carry out a Principal Components Analysis (PCA) using all samples' species data to compare
119 AEO distributions in groundwater with those from potential NA sources, and 3) evaluate the
120 potential of $\delta^{13}\text{C}_{\text{pyr}}$ values to discriminate sources of AEOs in unconventional hydrocarbon
121 deposits from other locations than Canada's oil sands region. We have opted to use the classical
122 definition of NAs ($\text{C}_n\text{H}_{2n+Z}\text{O}_2$) since much of the paper focuses on the O_2 species. The term
123 AEOs is used when discussing the S and N heteratomics and aromatic components in these
124 samples.

125

126 **2. Materials and methods**

127 *2.1. Study sites and samples*

128 The Haldimand sector, Gaspé, Québec, Canada (Figure 1), contains a tight sandstone
129 petroleum reservoir (York River and Battery Point formations, Middle Devonian) underlying a

130 shallow fractured rock aquifer system (Raynauld et al., 2014; Raynauld et al., 2016). The drilling
131 of a horizontal exploration well was halted in December 2012 by a municipal regulation aimed at
132 protecting the local groundwater, although several vertical exploration wells had been previously
133 drilled in the region. One unprocessed oil (Haldimand oil) and two naturally-occurring seep
134 samples (POT2 and S1) from the Haldimand sector were collected during autumn 2012, and
135 groundwater samples (12 L) from three observation wells (PH-02, POH-11-10 and POH-11-14)
136 and one municipal well (SJ/PE-02) were collected during summer 2013. Oil and seep samples
137 were collected using pre-combusted (450 °C for hours) amber glass jars with
138 polytetrafluoroethylene (PTFE)-lined lids. Groundwater samples were collected using
139 fluorinated HDPE carboys. Further information on the geology and hydrogeology of the
140 Haldimand sector and sampling methodology and well locations can be found in (Raynauld et
141 al., 2014) .

142 The Saint-Édouard region (southern Québec, Canada; Figure 1) contains at depth (> 2
143 km) units of the Upper Ordovician Utica Shale, which has been considered as an excellent source
144 rock for conventional hydrocarbon exploration and was recently evaluated for its unconventional
145 reservoir potential (Chatellier et al., 2013; Lavoie et al., 2014; Rivard et al., 2014). Recent
146 interest in the area has focused on potential risks to local aquifers due to potential future shale
147 gas development (Lavoie et al., 2014; Bordeleau et al., 2015; Lavoie et al., 2016) and recent
148 research results have documented the presence of sub-cropping hydrocarbon-loaded Utica-
149 equivalent or younger Late Ordovician shales (Lavoie et al., 2016). However, as with the
150 Haldimand sector of Gaspé, the current government of Québec moratorium on fracking has thus
151 far prevented further exploration and the assessment of the commercial shale gas production
152 potential. Groundwater from observations wells examined here were obtained from wells drilled

153 in the shallow fractured rock aquifer (< 60 m depth) in the Lotbinière (F1), Les Fonds (F2 and
154 F4) and Nicolet formations (F3, F5, F7 and F8) in 2013, 2014 and 2015 (Lavoie et al., 2016).
155 Two shale samples were collected from the Nicolet (F7 and F8), one from the Lotbinière (F20)
156 and one from the Les Fonds formations (F21) wells. Since drilling mud was not used to collect
157 these cores, the drilling process is not expected to have introduced a significant amount of
158 hydrocarbon contamination into the wells. Core samples were wiped down with 70% ethanol
159 after collection, wrapped in pre-combusted (450 °C for 4 hours) aluminum foil and stored in the
160 freezer at -20 °C until analysis. The Lotbinière and Les Fonds formations have significantly
161 higher total organic carbon (TOC), hydrogen index (HI), genetic potential and transformation
162 ratios compared to the Nicolet formation (Lavoie et al., 2016). Groundwater samples (12 L) from
163 observation wells and local residential wells (INRS 447, Zone 10R, Zone 11S, Zone 12R, Zone
164 2S, Zone 5R and Zone 9R) were collected during 2013, 2014 and 2015 using fluorinated HDPE
165 carboys. Further information on the geology and hydrogeology of the Saint-Édouard area and
166 sampling methodology and well locations can be found in Lavoie et al. (2014), Lavoie et al.
167 (2016) and Bordeleau et al. (accepted).

168 2.2. *Extraction of acid extractable organics (AEOs)*

169 Prior to crushing, shale cores were split into approximately 1 cm thick “disks” using a
170 stainless steel hammer and chisel rinsed with methanol, dichloromethane (DCM) and hexane.
171 Around 1 cm from the edges of the disks were chipped off and discarded. Samples F7 and F8
172 were homogenised using a metallic mortar and pestle, whereas samples F20 and F21 were
173 homogenised using a SPEX SamplePrep (Metuchen, NJ) 8500 Shatterbox grinding mill. The
174 stainless steel grinding container and puck were cleaned with solvents between samples.

175 The AEOs containing NAs in groundwater, seep, oil and crushed shale samples were
176 extracted following protocols similar to those described by Ahad et al. (2012). For aqueous
177 samples, approximately 0.5 L (seeps) and 12 L (groundwater) was acidified to pH 4.5 and
178 extracted using loose Strata-X-A solid phase extraction sorbent (Phenomenex, Torrance, CA,
179 USA). Crushed shale samples (~ 140 to 1000 g) were extracted in a soxhlet apparatus using a
180 mixture of 9:1 DCM to methanol. Following a subsequent re-dissolution in about 30 mL alkaline
181 solution, AEOs in shale were extracted in the same manner as aqueous samples. The Haldimand
182 oil sample (~ 200 g) with relatively high volatile hydrocarbon content was evaporated in a rotary
183 evaporator for 10 min at 40 °C to remove volatile hydrocarbons and then dissolved in 300 mL of
184 cold hexane while stirring. Asphaltene, the hexane insoluble fraction, was filtered off to recover
185 the maltene fraction. The extraction was repeated twice to remove most of the asphaltenes. The
186 maltene fraction was then dissolved in 100 mL hexane and extracted with 4 × 100 mL 1N NaOH
187 in water. The aqueous phase was further extracted with 2 × 100 mL hexane. The hexane fraction
188 was discarded. The aqueous fraction containing AEOs was acidified to pH 2 and extracted with
189 10% methanol in DCM (5 × 100 mL). Process blanks were carried out using 12 L of Mill-Q
190 water for aqueous samples and an equal amount of solvents as used for extraction of oil and
191 crushed shale samples.

192 2.3. High-resolution mass spectrometry (MS)

193 Qualitative and semi-quantitative analysis of subsamples of AEOs were completed at
194 Environment and Climate Change Canada (Saskatoon, SK, Canada) by 5 µL loop injection (flow
195 injection analysis) using a Surveyor MS pump (Thermo Fisher Scientific Inc.) and a mobile
196 phase of 50:50 acetonitrile/water containing 0.1% NH₄OH. Mass spectrometry analysis was
197 carried out using a dual pressure linear ion trap–orbitrap mass spectrometer (LTQ Orbitrap Elite,

198 Thermo Fisher Scientific, Bremen, Germany) equipped with an ESI interface operated in
199 negative ion mode. Data was acquired in full scan mode from m/z 100 to 600 at a setting of
200 240,000 resolution. The majority of ions were singly charged, and the average mass resolving
201 power ($m/\Delta m_{50\%}$) was 242,000 at m/z 400. Mass accuracies of less than 1 ppm were obtained
202 using a lock mass compound (n-butyl benzenesulfonamide) for scan-to-scan mass calibration
203 correction. Concentrations of AEOs were determined using a five point external standard
204 calibration of Athabasca oil sands OSPW-derived AEOs at known concentrations as described
205 elsewhere (Frank et al., 2014; Hughes et al., 2017).

206 2.4. Intramolecular and “bulk” stable carbon isotope ($\delta^{13}C$) analysis

207 The carbon isotope ratio of the CO_2 generated by the pyrolytic decarboxylation of AEOs
208 ($\delta^{13}C_{pyr}$) was determined by TC/EA-IRMS (Delta+ XL, Thermo-Finnigan) at the Delta-Lab of
209 the Geological Survey of Canada (Québec, QC, Canada). Full details of the protocol for these
210 analyses are found in Ahad et al. (2012). As no significant variability in intramolecular carbon
211 isotope values was observed across the entire molecular weight range of AEOs (Ahad et al.,
212 2012), here we report $\delta^{13}C_{pyr}$ values from samples not separated into different mass fractions.
213 The “bulk” or non-intramolecular carbon isotope ratios of AEOs generated by combustion were
214 analyzed by EA-IRMS using a Costech EA (Valencia, CA, USA) interfaced with the Delta+ XL
215 IRMS system. Based on replicate standard and sample analyses, the uncertainty for $\delta^{13}C_{pyr}$ and
216 bulk $\delta^{13}C$ analyses was ± 0.6 and $\pm 0.3\%$, respectively.

217

218 3. Results and Discussion

219 3.1 Distributions of AEOs

220 At 0.01% by weight of petroleum, the amount of AEOs in Haldimand oil was around 10
221 times lower than that determined in Athabasca oil sands bitumen (Ahad et al., 2012). The amount
222 of blank-corrected AEOs in the two shales from the Lotbinière (F20; 0.51 $\mu\text{g/g}$ dry weight) and
223 the Les Fonds (F21; 0.49 $\mu\text{g/g}$ dry weight) formations were greater than the Nicolet formation
224 shales (F7 and F8; 0.19 and 0.27 $\mu\text{g/g}$ dry weight). While this discrepancy may be largely
225 attributed to the higher TOC and hydrogen index values in the F20 and F21 cores (Lavoie et al.,
226 2016), the more robust homogenization technique used to prepare these two samples (i.e.,
227 electric mill versus manual grinding) may have also contributed to this difference.

228 The concentrations of total AEOs in all groundwater samples were low, ranging from 0.1
229 to 1.0 mg/L in the Haldimand sector and from 0.1 to 1.9 mg/L in the Saint-Édouard region
230 (Supplementary data, Table S1). The concentrations of total AEOs in seeps from the Haldimand
231 sector were slightly higher at 2.1 and 4.0 mg/L for samples POT2 and S1, respectively. The low
232 levels of AEOs in groundwater observed here were similar to those found in background surface
233 water and groundwaters in the Athabasca oil sands region not significantly impacted by bitumen,
234 whether mining-related or naturally occurring (Ahad et al., 2013; Frank et al., 2014; Ross et al.,
235 2012).

236 Naphthenic acids, as classically defined by $\text{C}_n\text{H}_{2n+Z}\text{O}_2$, were detected in all the samples
237 analysed here. As a percentage of the total AEOs, the O_2 species class (Table S1) was highest in
238 oil (39.4%), seep (16.7 to 59.4%) and shale samples (34.1 to 73.8%) compared to groundwater
239 samples from both Saint-Édouard (3.7 to 12.3% and 0.5 to 9.7% in observation and residential
240 wells, respectively) and Gaspé regions (0.7 to 4.8%). The most common compounds found
241 within the O_2 species class in shale and the majority of groundwater samples were straight-chain
242 ($Z = 0$) or monounsaturated ($Z = -2$) C_{16} and C_{18} fatty acids (Table 1). These ubiquitous

243 compounds are two of the most abundant biologically-produced lipids and thus typically
244 comprise a main component of naturally-occurring “background” AEOs (Headley et al., 2011;
245 Ross et al., 2012). Process blanks were also dominated by straight-chain C_{16} and C_{18} fatty acids
246 (Figure S1), however, since the blank comprised $< 1\%$ of the total AEO mass in oil, seep and
247 groundwater samples, it is considered a negligible component in these samples’ profiles. In
248 contrast, the concentrations of AEOs in shale process blanks were between 6 to 20% of the total
249 AEO masses. The higher shale process blank is attributed to trace fatty acid contamination in the
250 DCM used during extraction.

251 The distributions of NAs as a function of carbon number, Z value and percentage of the
252 total O_2 species class are plotted in Figure 2 for four representative samples from each of the
253 Gaspé and Saint-Édouard regions. Due to their predominantly high levels in many of the
254 samples, $Z = 0$ (all) and $Z = -2$ (C_{16} and C_{18}) fatty acids were excluded from most of the profiles
255 to better illustrate the distribution patterns of less abundant NAs, in a manner similar to that
256 carried out in another study examining natural background levels NAs (Ross et al., 2012). The
257 two exceptions are the oil and seep samples from the Haldimand sector, for which the
258 distributions of all NAs are presented on Figure 2.

259 Comprising almost half of the total O_2 class, straight-chain NAs in the C_8 to C_{20} range
260 centred on C_{12} dominated the Z series distribution in the Haldimand oil (Figure 2). Significant
261 abundances of NAs with $Z = -2$ (one cycloalkyl ring) and $Z = -8$, likewise centred on C_{12} , were
262 also detected. The dominance of light molecular weight straight-chain or monocyclic NAs points
263 to a low degree of degradation in the Haldimand crude oil (Hughey et al., 2008; Kim et al.,
264 2005). The representative seep sample (POT2) contains a higher component of $Z = -2$ to -12
265 NAs, and a slightly heavier distribution centred between C_{16} to C_{18} . This shift in NA distribution

266 is expected as crude oil undergoes degradation (Hughey et al., 2008; Kim et al., 2005) and thus
267 points to natural weathering in the surface oil seep over time.

268 Regardless of geological unit, the distributions in all four Saint-Édouard region shale
269 samples were similar and dominated by C₁₀ to C₁₈ NAs ranging from Z= -2 to Z= -16 (Table 1
270 and Figure 2). The compounds included a suite of NAs with Z = -8 and -10 in the C₈ to C₁₆
271 range, which given the number of carbon atoms points to compounds containing aromatic rings
272 (Figure 2). Relatively smaller proportions of monocyclic NAs or monounsaturated fatty acids
273 (i.e., Z = -2) and presumably alicyclic bicyclic NAs (Z = -4) in the C₁₁ to C₁₈ range were also
274 detected (Figure 2). With the exception of a minor component of C₈ to C₁₀ Z = -8 NAs, none of
275 the compounds shown on Figure 2 were found in shale process blanks (Figures 2 and S1).

276 Bearing in mind the dominant C₁₆ and C₁₈ fatty acid peaks found in most samples, the NA
277 distributions in the majority of groundwaters (as represented by wells F7 and PH-02 on Figure
278 2), irrespective of regional origin (Saint-Édouard or Gaspé) or well type (observation, residential,
279 municipal), could in general be characterized as containing very low amounts of a narrow range
280 of NAs. The exceptions to this pattern were three wells from the Saint-Édouard region (F2, F4
281 and Zone 10R) and one from the Gaspé region (POH-11-14); all four of which contained
282 noticeably more significant levels of presumably alicyclic bicyclic (Z = -4) C₁₀-C₁₈ NAs (Figure
283 2). Interestingly, in the Saint-Édouard region, several of these wells (Zone 10R and F2) were
284 situated near fault zones (Bordeleau et al., accepted), suggesting the migration of waters
285 containing a distribution of NAs different from that extracted from the shale samples examined
286 here. Another possibility is that bicyclic acids were generated from *in situ* microbial alteration of
287 shale organic matter (Saint-Édouard) and petroleum (Gaspé), as was inferred for the POT2 seep
288 sample from the Haldimand region (Figure 2). For instance, Wilde et al. (2015) has suggested

289 that the bicyclic acids identified in OSPW represent the biotransformation products of the
290 initially somewhat more recalcitrant bicyclanes of petroleum. Synthetic bicyclic acids in the C₁₁
291 to C₁₃ range were found to be acutely toxic (Jones et al., 2011); thus the occurrence of similar
292 NAs in groundwater from the Saint-Édouard and Gaspé regions indicates a potential background
293 source of organic contaminants.

294 3.2 Principal Components Analysis (PCA)

295 Principal Components Analysis (PCA) was carried out on log-transformed ($\log(x+1)$)
296 species percentages (N_x , N_xS_x , N_xO_x , $N_xO_xS_x$, O_xS_x , O_1 to O_{10} and S_x) in AEOs using SigmaPlot
297 13.0 (Systat Software Inc., San Jose, CA). The data was log-transformed to improve normality
298 and to avoid the inherent bias in compositional (i.e., closed) datasets consisting of proportions
299 that sum up to a constant such as 100% (Filzmoser et al., 2009). The PCA loadings (Figure 3a)
300 and scores (Figure 3b) for the first (PC1) and second (PC2) principal components accounted for
301 51.50 and 15.44% of the total explained variance, respectively. PC1 was strongly ($r > 0.5$)
302 positively correlated with N_x , N_xS_x , and O_5 to O_{10} , and strongly ($r > 0.5$) negatively correlated
303 with O_xS_x , O_2 and O_3 . PC2 was strongly ($r > 0.5$) negatively correlated with $N_xO_xS_x$, O_xS_x , O_1 ,
304 O_4 and S_x .

305 As illustrated on Figure 3b, the PCA scores plot revealed several distinct groupings for
306 the samples analysed as part of this study. The Haldimand oil, shale samples, shale process blank
307 and one of the groundwater process blanks were grouped closely together on the left side of the
308 plot and away from most of the other samples chiefly as a result of their greater component of O_2
309 and O_3 species (Figure 3a). Another groundwater process blank containing a high amount of
310 O_xS_x plotted in the lower left section of the graph away from any other samples. Groundwater

311 samples plotted to the right as a reflection of the greater components of more oxygenated, N_x and
312 N_xS_x species (Figure 3a), with the four groundwater samples from the Gaspé region grouped
313 together further to the right than most samples from the Saint-Édouard region. Amongst Saint-
314 Édouard region samples, as a result of greater components of N- and S-containing species in
315 residential wells, these samples generally plotted lower on the y-axis than groundwaters from
316 observation wells (Figure 3a). The two seep samples from the Haldimand region plotted between
317 the oil/shale and groundwater groupings, with the oil seep sample (POT2) containing a
318 naphthenic acid profile suggestive of degraded oil (Figure 2) plotting further to the left and
319 closer to the oil/shale grouping (Figure 3b).

320 The wide range of highly oxygenated and nitrogen- and sulfur-bearing compounds
321 present in both pristine and contaminated groundwaters (e.g., Ahad et al., 2013; Longnecker and
322 Kujawinski, 2011) reflects both the origin and biogeochemical processing of dissolved organic
323 matter. Given the variability in water types and well conditions found in the Saint-Édouard
324 region (Bordeleau et al., accepted), therefore, it is not surprising that groundwater samples from
325 this area were plotted across a wide spectrum on the PC2 axis (Figure 3b). An additional PCA
326 carried out using only observation and residential wells from the Saint-Édouard region, however,
327 suggested an important geological role in controlling AEO distributions (Figure S2). A greater
328 number of samples from the Lotbinière and Les Fonds formations are needed to further examine
329 this influence.

330 3.3 Stable carbon isotope analysis

331 Stable carbon isotope ratios of AEOs (Figure 4) generated by pyrolysis ($\delta^{13}C_{pyr}$) ranged
332 between -35.2 to -19.5‰ and were on average around 2-3‰ heavier compared to those

333 generated by bulk combustion ($\delta^{13}\text{C}$; -31.7 to -27.2‰). The one noticeable exception was the
334 Haldimand oil sample, whose bulk $\delta^{13}\text{C}$ value (-28.5‰) was significantly more positive than its
335 $\delta^{13}\text{C}_{\text{pyr}}$ value (-35.2‰). The first and previous application of intramolecular isotopic analysis of
336 AEOs, carried out on environmental samples from the Athabasca oil sands region, found an ~9‰
337 enrichment in samples containing predominantly bitumen-derived AEOs ($\delta^{13}\text{C}_{\text{pyr}}$ around -21‰)
338 compared to those comprised of non-bitumen-derived plant and soil organic matter,
339 demonstrating the ability of this technique to discriminate certain sources of NAs (Ahad et al.,
340 2013; Ahad et al., 2012). In contrast, bulk $\delta^{13}\text{C}$ values of AEOs showed no systematic variation
341 between sample types and thus were not considered suitable for source apportionment (Ahad et
342 al., 2012). Samples containing mostly bitumen-derived AEOs were also strongly correlated with
343 O_2 and O_2S species classes (Ahad et al., 2013).

344 In groundwater samples from the Saint-Édouard and Gaspé regions, neither systematic
345 trends in $\delta^{13}\text{C}_{\text{pyr}}$ values (Figure 4) nor significant linear relationships between $\delta^{13}\text{C}_{\text{pyr}}$ and key
346 species classes were evident (Figure S3). Other than the distinct offset between $\delta^{13}\text{C}$ and $\delta^{13}\text{C}_{\text{pyr}}$
347 values, the lack of any discernible trends in isotopic data is attributed to the overall low
348 proportions of O_2 and dominance of C_{16} and C_{18} fatty acids in most groundwater samples.
349 Although the proportion of O_2 is relatively high in shale and petroleum samples (Figure 3, Table
350 S1), the organic matter in the Saint-Édouard and Gaspé regions has not been subjected to the
351 same geological processes which has resulted in much more enriched $\delta^{13}\text{C}_{\text{pyr}}$ values (i.e., ~ -
352 21‰) in oil sands bitumen AEOs. For instance, the significantly more depleted $\delta^{13}\text{C}_{\text{pyr}}$ compared
353 to bulk $\delta^{13}\text{C}$ value in the Haldimand oil sample (Figure 4) conforms to the notion of petroleum
354 that has not undergone extensive degradation, since carboxyl group carbon is generally presumed
355 to be isotopically lighter than the rest of the carbon atoms in the molecule (Sun et al., 2004).

356 Given that biodegradation of fatty acids involves a decarboxylation step (Sun et al., 1997), the
357 enrichment in $\delta^{13}\text{C}_{\text{pyr}}$ relative to bulk $\delta^{13}\text{C}$ values found in groundwater and oil seep samples
358 from the Gaspé region and in groundwater samples from the Saint-Édouard region suggests
359 microbial reworking associated with isotopic fractionation of carboxyl group carbon in AEOs.
360 Significant isotopic fractionation was previously reported in linear fatty acids (Dai et al., 2005)
361 and could potentially explain the most enriched $\delta^{13}\text{C}_{\text{pyr}}$ value of -19.5‰ found in well F7, a
362 sample that contained high percentages of Z = 0 and -2 C₁₆ and C₁₈ (Table 1).

363

364 4. Conclusions

365 Naphthenic acids were detected in groundwater overlying two undeveloped,
366 unconventional hydrocarbon reservoirs in the province of Québec, Canada. The concentrations
367 of total AEOs were low and characterized by highly oxygenated and N- and S-containing species
368 classes. The distribution patterns of NAs in most samples were dominated by straight-chain (i.e.,
369 Z = 0) C₁₆ and C₁₈ fatty acids – ubiquitous compounds found in plant and soil organic matter.
370 However, a range of C₁₀ to C₁₈, Z = -4 to Z = -12 NAs were also found, with several samples
371 containing relatively greater proportions of potentially toxic alicyclic bicyclic acids (i.e., Z = -4).
372 The occurrence of these compounds points to a natural background source of organic
373 contaminants prior to large-scale hydrocarbon development. Moreover, their detection in wells
374 near fault zones implies a potential for migration of NAs during extraction activities such as
375 hydraulic fracturing, as has been reported for other organic contaminants in the Marcellus Shale
376 region (Llewellyn et al., 2015). In the Saint-Édouard region, however, recent work has shown
377 that faults are not likely to offer a fluid flow-path reaching all the way to the Utica Shale

378 (Ladevèze, 2017). It is uncertain whether the presumably bicyclic acids ($Z = -4$) and other NAs
379 in groundwater samples originated from mixing with a different water source or were generated
380 by *in situ* microbial transformation of shale organic matter (Saint-Édouard) or petroleum
381 (Gaspé). The latter is supported by the 2-3‰ enrichment in $\delta^{13}\text{C}_{\text{pyr}}$ compared to bulk $\delta^{13}\text{C}$ values
382 of AEOs which suggests a possible isotopic fractionation due to biodegradation.

383 In light of the findings reported here in the Saint-Édouard and Gaspé regions, routine
384 screening for NAs in environmental samples from areas undergoing shale gas or tight oil
385 development may be warranted. In addition, future investigations into the origins and
386 distributions of NAs in these regions should incorporate the use of other analytical techniques
387 such as GC \times GC-MS to identify and quantify individual compounds, in particular those which
388 are toxic and pose a risk to the environment.

389

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399

400 **Appendix A. Supplementary data**

401 Supplementary data related to this article can be found at xxxx.

402

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622 **List of Tables**

623 **Table 1.** The percentage of linear ($Z = 0$) and monounsaturated ($Z = -2$) C_{16} and C_{18} fatty acids
 624 comprising the O_2 species class for eight representative samples from the Gaspé and Saint-
 625 Édouard regions (Figure 2).

626

Sample Type	Name	C16		C18	
		Z = 0	Z = -2	Z = 0	Z = -2
<i>Gaspé Region</i>					
Reservoir oil	Haldimand Oil	2.8	1.3	1.0	0.6
Surface oil seep	POT2	6.1	6.8	5.2	2.4
Groundwater (observation well)	PH-02	18.7	14.4	10.9	0.9
Groundwater (observation well)	POH-11-14	1.2	0.4	0.0	0.0
<i>Saint-Édouard Region</i>					
Rock core	F7 Shale	25.9	2.1	12.4	1.3
Groundwater (residential well)	Zone 10R	2.3	14.0	0.6	0.8
Groundwater (observation well)	F2	18.4	0.0	8.6	0.3
Groundwater (observation well)	F7	40.2	7.7	28.7	0.0

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635 **List of Figures**

636 **Figure 1.** Map of the study area showing the locations of the Saint-Édouard region and
637 Haldimand sector (Gaspé) in the province of Québec, Canada.

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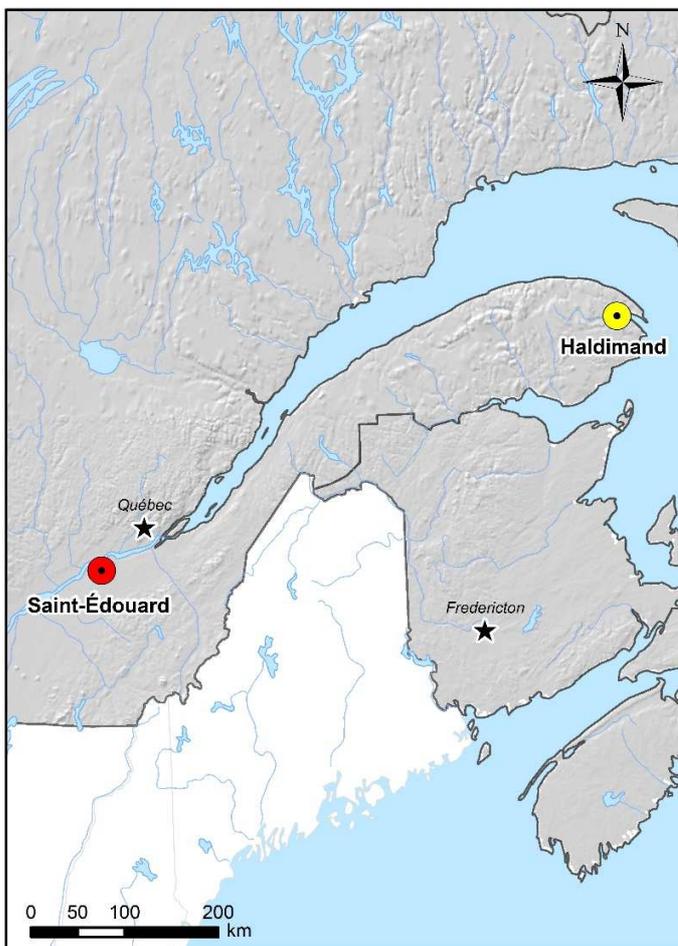
639 **Figure 2.** The distributions of naphthenic acids (NAs) in representative samples from the Gaspé
640 (Haldimand reservoir oil, natural surface oil seep POT2, groundwater from observation wells
641 PH-02 and POH-11-10) and Saint-Édouard (shale F7, groundwater from residential well Zone
642 10R and observation wells F2 and F7) regions plotted according to carbon number, the
643 percentage of O₂ species class and Z value. NAs are defined by the classical chemical formula
644 C_nH_{2n+Z}O₂, where n indicates the carbon number and Z is zero or a negative, even integer that
645 specifies the hydrogen deficiency resulting from ring formation.

646 **Figure 3.** Principal Components Analysis (PCA) loadings (a) and scores (b) carried out on log-
647 transformed ($\log(x+1)$) species percentages (N_x , N_xS_x , N_xO_x , $N_xO_xS_x$, O_xS_x , O_1 to O_{10} and S_x) in
648 acid extractable organics (AEOs) determined in Haldimand sector oil (open square), natural seep
649 (solid red squares) and groundwater samples (inverted yellow triangles), and Saint-Édouard
650 region shale (black circles), residential well groundwater (solid blue triangles) and observation
651 well groundwater samples (open triangles). The solid green diamond is the shale sample process
652 blank, and the open diamonds are the groundwater sample process blanks.

653 **Figure 4.** Stable carbon isotope ratios of acid extractable organics (AEOs) generated by
654 pyrolysis ($\delta^{13}C_{\text{pyr}}$; solid colours) and bulk combustion ($\delta^{13}C$; open symbols) in Haldimand sector
655 reservoir oil (red squares), natural surface oil seep (red diamonds) and groundwater samples (red
656 inverted triangles), and Saint-Édouard region residential well (blue circles) and observation well
657 groundwater samples (blue triangles).

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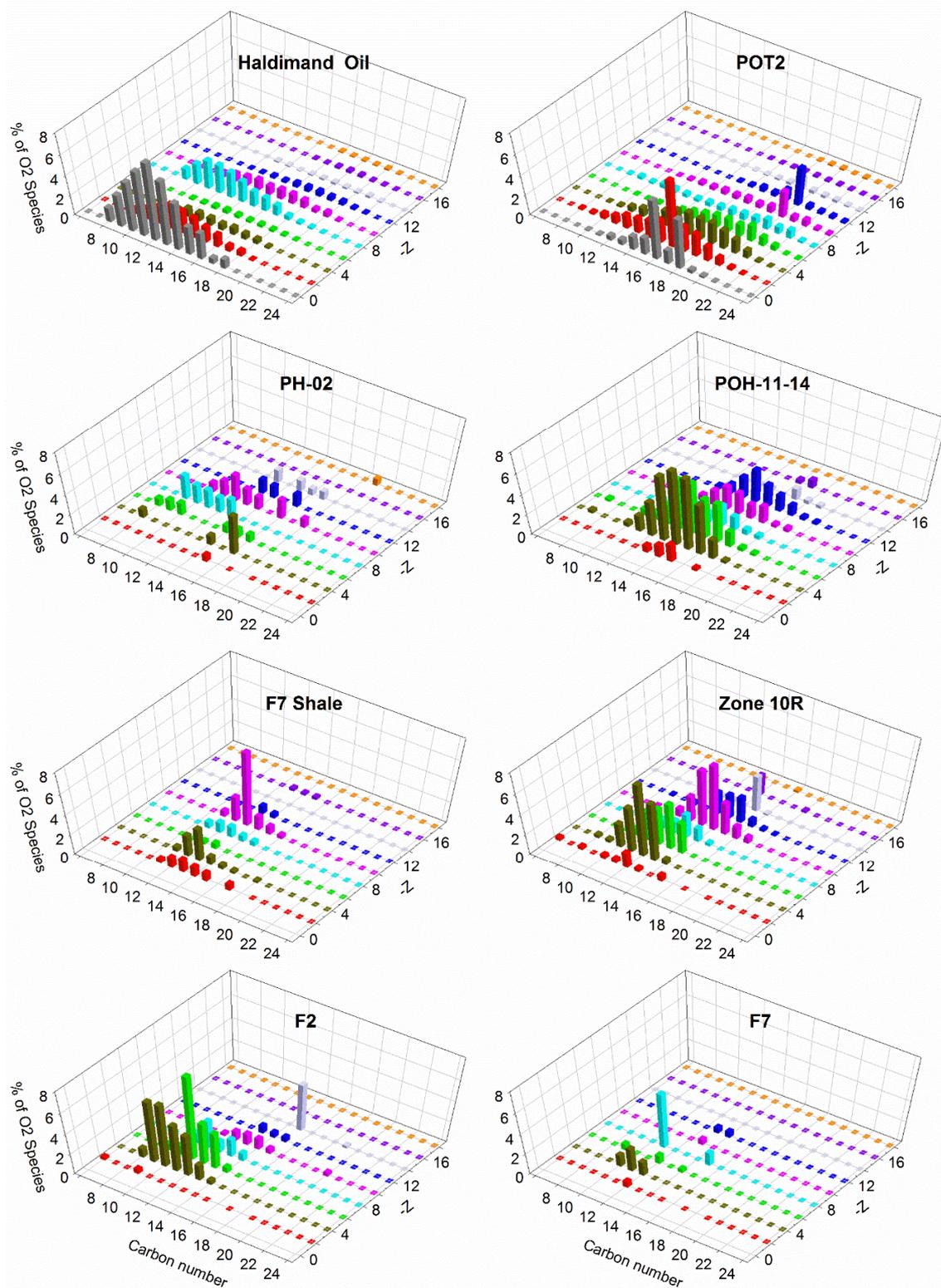
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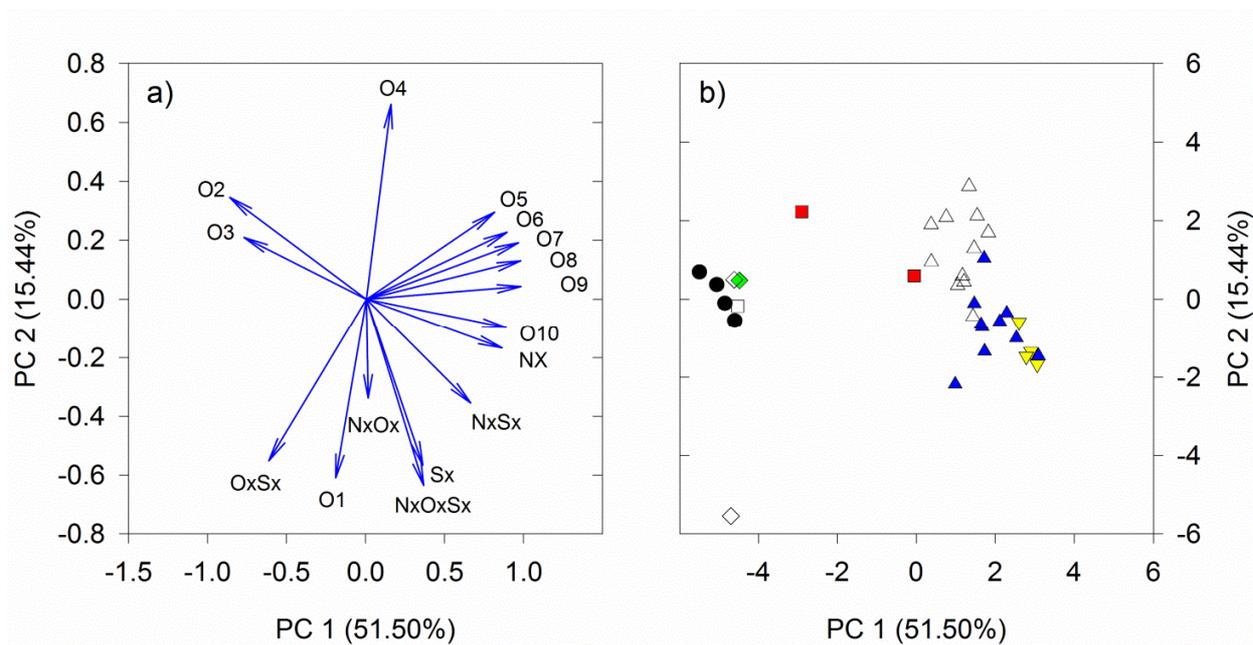
661 **Figure 1**

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664 **Figure 2**



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666 **Figure 3**

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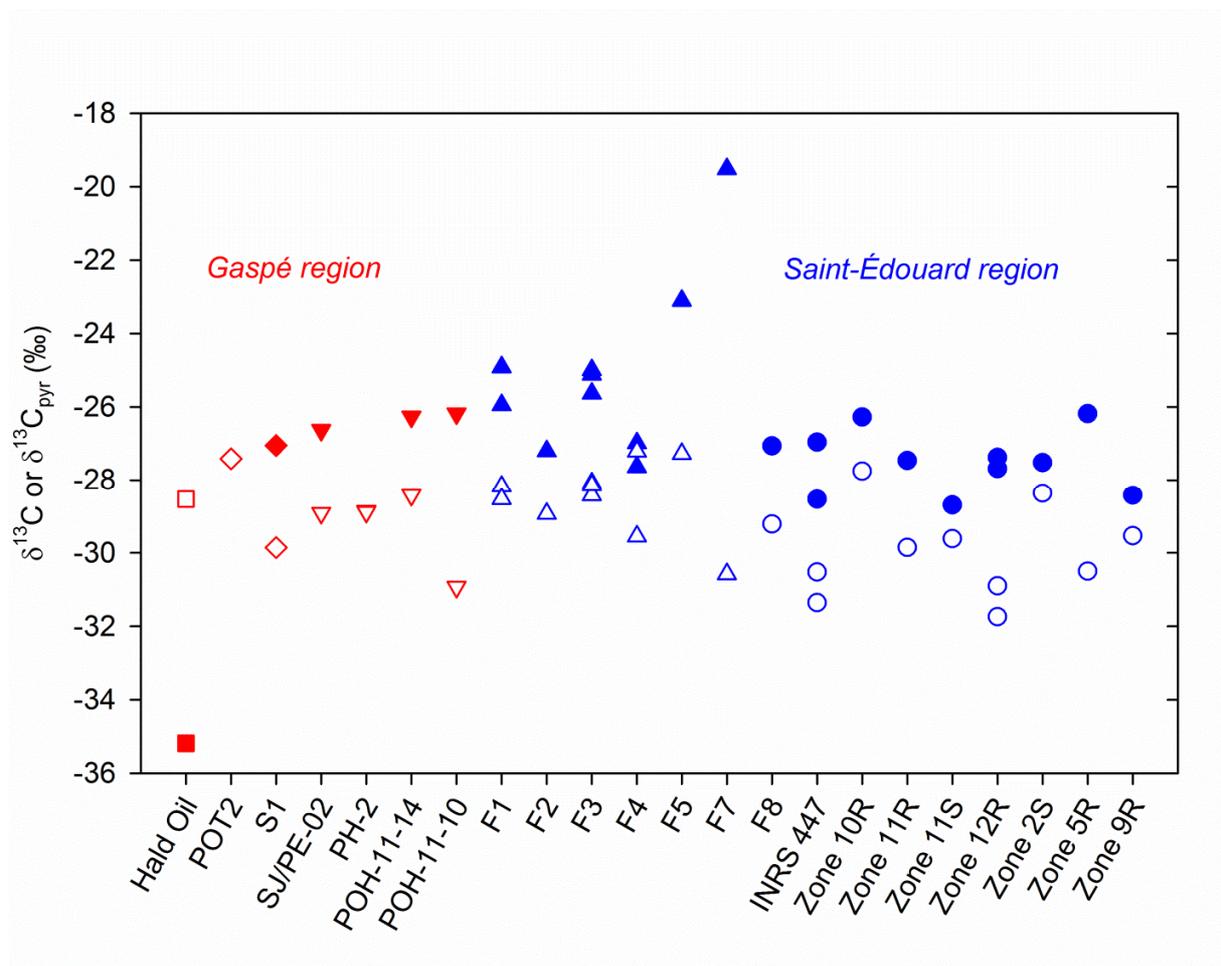
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677 **Figure 4**

Highlights:

- NAs were found in groundwater overlying undeveloped shale gas and tight oil plays.
- Most abundant NAs were straight-chain or monounsaturated C₁₆ and C₁₈ fatty acids.
- Alicyclic bicyclic NAs (Z = -4) were detected in several groundwater samples.
- Natural background source of organic contaminants prior to hydrocarbon extraction.