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

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Abstract

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

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

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.

1. Introduction

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

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

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

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

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

2. Materials and methods

2.1. Study sites and samples

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

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

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

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

2.2. Extraction of acid extractable organics (AEOs)

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

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

2.3. High-resolution mass spectrometry (MS)

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

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

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

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

3. Results and Discussion

3.1 Distributions of AEOs

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

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

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

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

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

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

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

Regardless of geological unit, the distributions in all four Saint-Édouard region shale samples were similar and dominated by C_{10} to C_{18} NAs ranging from $Z = -2$ to $Z = -16$ (Table 1 and Figure 2). The compounds included a suite of NAs with $Z = -8$ and -10 in the C_8 to C_{16} range, which given the number of carbon atoms points to compounds containing aromatic rings (Figure 2). Relatively smaller proportions of monocyclic NAs or monounsaturated fatty acids (i.e., $Z = -2$) and presumably alicyclic bicyclic NAs ($Z = -4$) in the C_{11} to C_{18} range were also detected (Figure 2). With the exception of a minor component of C_8 to C_{10} $Z = -8$ NAs, none of the compounds shown on Figure 2 were found in shale process blanks (Figures 2 and S1).

Bearing in mind the dominant C_{16} and C_{18} fatty acid peaks found in most samples, the NA distributions in the majority of groundwaters (as represented by wells F7 and PH-02 on Figure 2), irrespective of regional origin (Saint-Édouard or Gaspé) or well type (observation, residential, municipal), could in general be characterized as containing very low amounts of a narrow range of NAs. The exceptions to this pattern were three wells from the Saint-Édouard region (F2, F4 and Zone 10R) and one from the Gaspé region (POH-11-14); all four of which contained noticeably more significant levels of presumably alicyclic bicyclic ($Z = -4$) C_{10} - C_{18} NAs (Figure 2). Interestingly, in the Saint-Édouard region, several of these wells (Zone 10R and F2) were situated near fault zones (Bordeleau et al., accepted), suggesting the migration of waters containing a distribution of NAs different from that extracted from the shale samples examined here. Another possibility is that bicyclic acids were generated from *in situ* microbial alteration of shale organic matter (Saint-Édouard) and petroleum (Gaspé), as was inferred for the POT2 seep sample from the Haldimand region (Figure 2). For instance, Wilde et al. (2015) has suggested

that the bicyclic acids identified in OSPW represent the biotransformation products of the initially somewhat more recalcitrant bicyclanes of petroleum. Synthetic bicyclic acids in the C_{11} to C_{13} range were found to be acutely toxic (Jones et al., 2011); thus the occurrence of similar NAs in groundwater from the Saint-Édouard and Gaspé regions indicates a potential background source of organic contaminants.

3.2 Principal Components Analysis (PCA)

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

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

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

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

3.3 Stable carbon isotope analysis

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

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

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

Given that biodegradation of fatty acids involves a decarboxylation step (Sun et al., 1997), the enrichment in $\delta^{13}\text{C}_{\text{pyr}}$ relative to bulk $\delta^{13}\text{C}$ values found in groundwater and oil seep samples from the Gaspé region and in groundwater samples from the Saint-Édouard region suggests microbial reworking associated with isotopic fractionation of carboxyl group carbon in AEOs. Significant isotopic fractionation was previously reported in linear fatty acids (Dai et al., 2005) and could potentially explain the most enriched $\delta^{13}\text{C}_{\text{pyr}}$ value of -19.5‰ found in well F7, a sample that contained high percentages of $Z = 0$ and -2 C_{16} and C_{18} (Table 1).

4. Conclusions

Naphthenic acids were detected in groundwater overlying two undeveloped, unconventional hydrocarbon reservoirs in the province of Québec, Canada. The concentrations of total AEOs were low and characterized by highly oxygenated and N- and S-containing species classes. The distribution patterns of NAs in most samples were dominated by straight-chain (i.e., $Z = 0$) C_{16} and C_{18} fatty acids – ubiquitous compounds found in plant and soil organic matter. However, a range of C_{10} to C_{18} , $Z = -4$ to $Z = -12$ NAs were also found, with several samples containing relatively greater proportions of potentially toxic alicyclic bicyclic acids (i.e., $Z = -4$). The occurrence of these compounds points to a natural background source of organic contaminants prior to large-scale hydrocarbon development. Moreover, their detection in wells near fault zones implies a potential for migration of NAs during extraction activities such as hydraulic fracturing, as has been reported for other organic contaminants in the Marcellus Shale region (Llewellyn et al., 2015). In the Saint-Édouard region, however, recent work has shown that faults are not likely to offer a fluid flow-path reaching all the way to the Utica Shale

(Ladevèze, 2017). It is uncertain whether the presumably bicyclic acids ($Z = -4$) and other NAs in groundwater samples originated from mixing with a different water source or were generated by *in situ* microbial transformation of shale organic matter (Saint-Édouard) or petroleum (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 of AEOs which suggests a possible isotopic fractionation due to biodegradation.

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

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Appendix A. Supplementary data

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

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List of Tables

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

		C16		C18	
Sample Type	Name	Z = 0	Z = -2	Z = 0	Z = -2
Gaspé Region					
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
Saint-Édouard Region					
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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Figure 1. Map of the study area showing the locations of the Saint-Édouard region and Haldimand sector (Gaspé) in the province of Québec, Canada.

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

Figure 3. Principal Components Analysis (PCA) loadings (a) and scores (b) carried out on log-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 acid extractable organics (AEOs) determined in Haldimand sector oil (open square), natural seep (solid red squares) and groundwater samples (inverted yellow triangles), and Saint-Édouard region shale (black circles), residential well groundwater (solid blue triangles) and observation well groundwater samples (open triangles). The solid green diamond is the shale sample process blank, and the open diamonds are the groundwater sample process blanks.

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

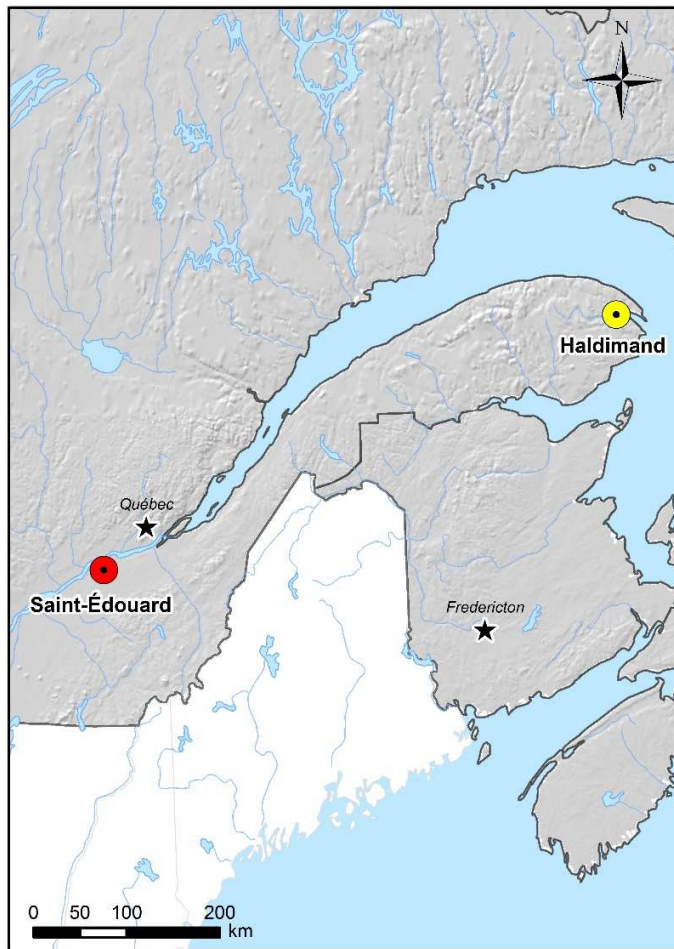


Figure 1

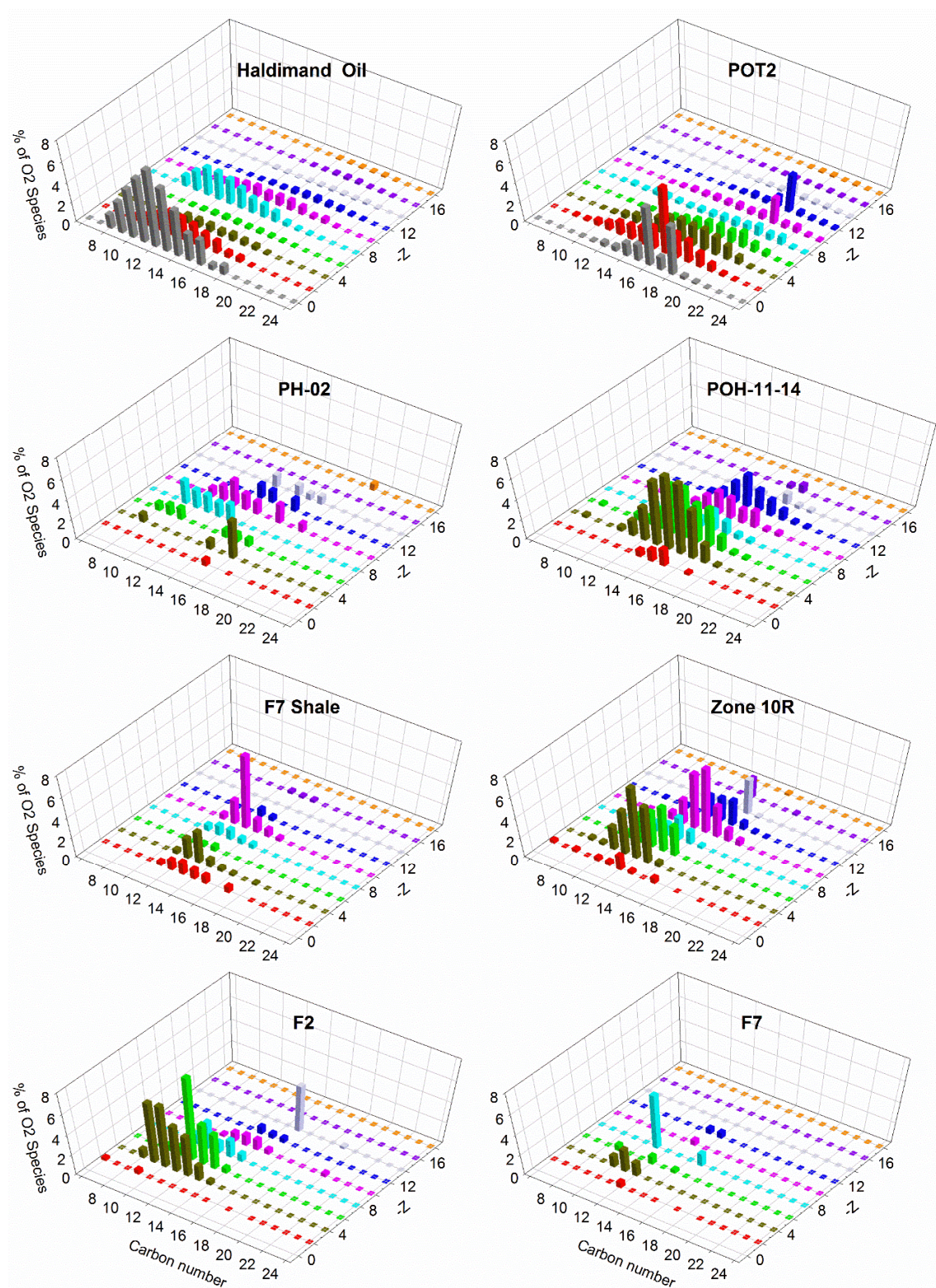
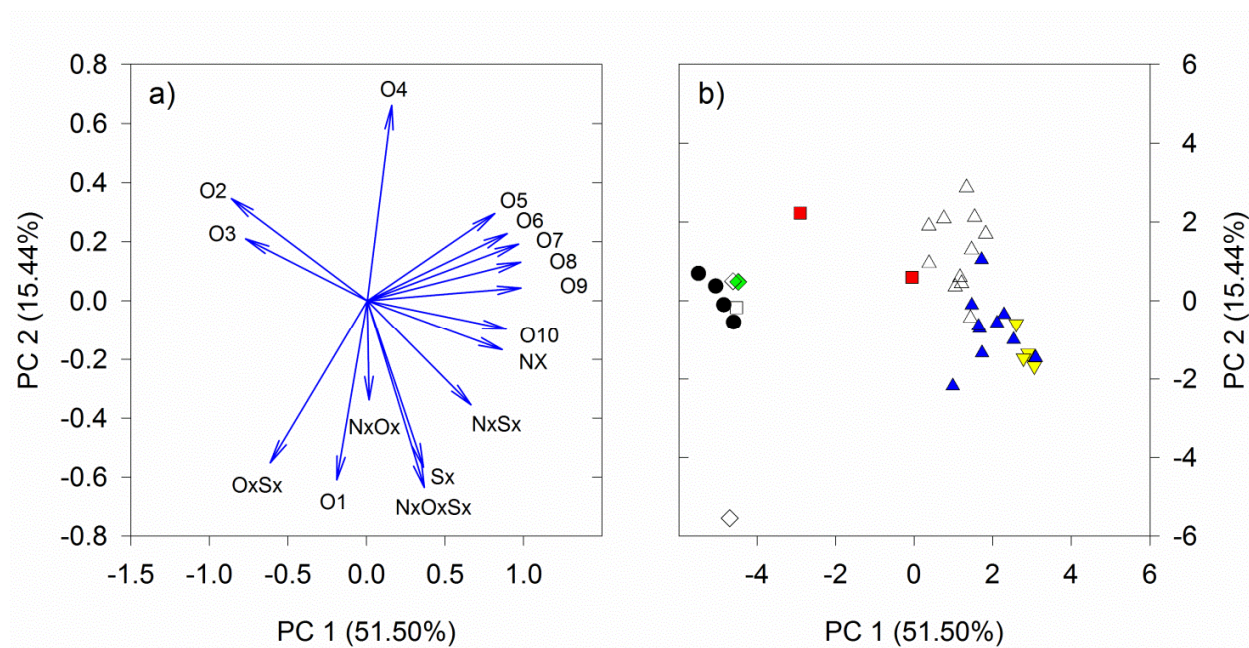


Figure 2

**Figure 3**

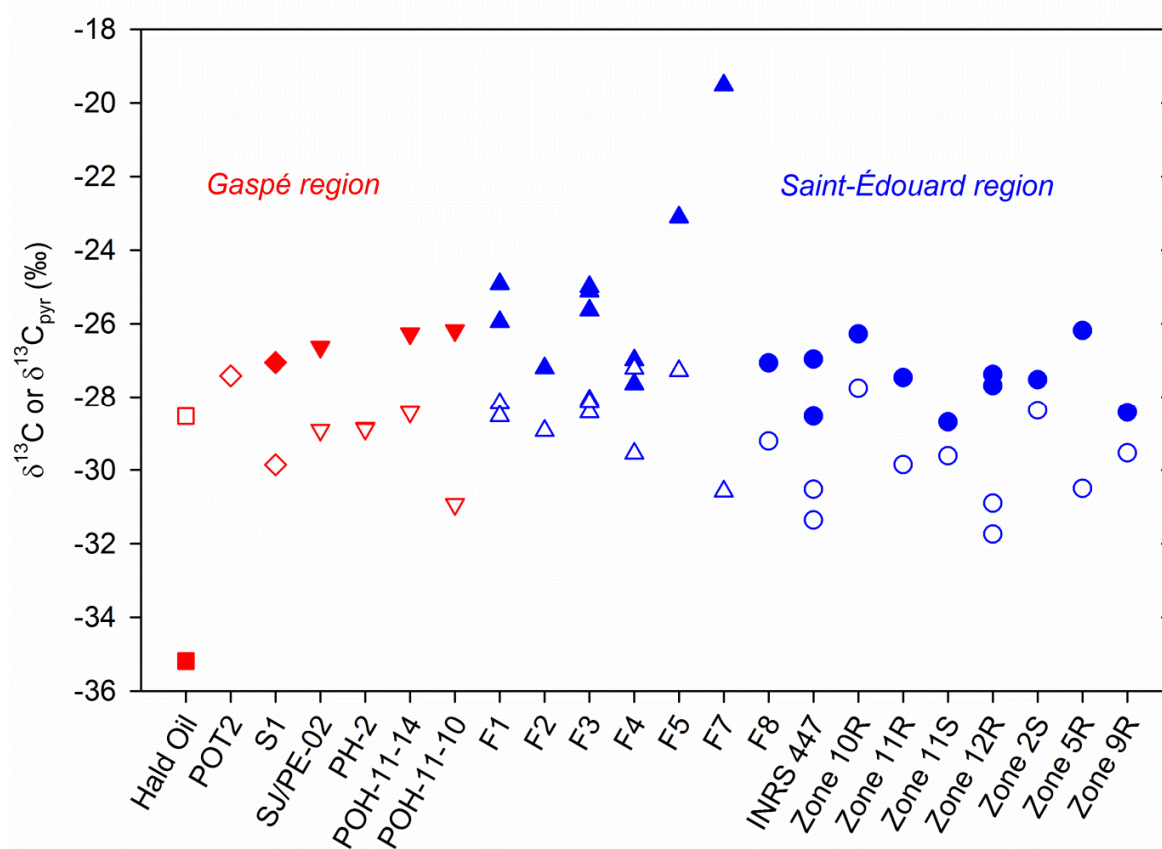


Figure 4

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.