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## Quantitative and Qualitative Analysis of Naphthenic Acids in Natural Waters Surrounding the Canadian Oil Sands Industry

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### Supporting Information



**ABSTRACT:** The Canadian oil sands industry stores toxic oil sands process-affected water (OSPW) in large tailings ponds adjacent to the Athabasca River or its tributaries, raising concerns over potential seepage. Naphthenic acids (NAs;  $C_nH_{2n-Z}O_2$ ) are toxic components of OSPW, but are also natural components of bitumen and regional groundwaters, and may enter surface waters through anthropogenic or natural sources. This study used a selective high-resolution mass spectrometry method to examine total NA concentrations and NA profiles in OSPW ( $n = 2$ ), Athabasca River pore water ( $n = 6$ , representing groundwater contributions) and surface waters ( $n = 58$ ) from the Lower Athabasca Region. NA concentrations in surface water (< 2–80.8  $\mu\text{g/L}$ ) were 100-fold lower than previously estimated. Principal components analysis (PCA) distinguished sample types based on NA profile, and correlations to water quality variables identified two sources of NAs: natural fatty acids, and bitumen-derived NAs. Analysis of NA data with water quality variables highlighted two tributaries to the Athabasca River—Beaver River and McLean Creek—as possibly receiving OSPW seepage. This study is the first comprehensive analysis of NA profiles in surface waters of the region, and demonstrates the need for highly selective analytical methods for source identification and in monitoring for potential effects of development on ambient water quality.

### INTRODUCTION

The Athabasca oil sands, in northern Alberta, Canada, are one of the largest reserves of crude oil in the world, with approximately 169 billion barrels of recoverable bitumen available.<sup>1</sup> For shallow deposits, current recovery methodology relies on surface mining (Figure 1: Surface Mineable Area) and aqueous extraction technology to facilitate the separation of bitumen from the oil sands ore. The resulting oil sands process-affected water (OSPW) is stored onsite in large tailings ponds. Recent estimates are that 720 million  $\text{m}^3$  of OSPW are currently stored,<sup>2</sup> and these volumes are expected to increase with continued bitumen production and further expansion of the surface-mining industry.

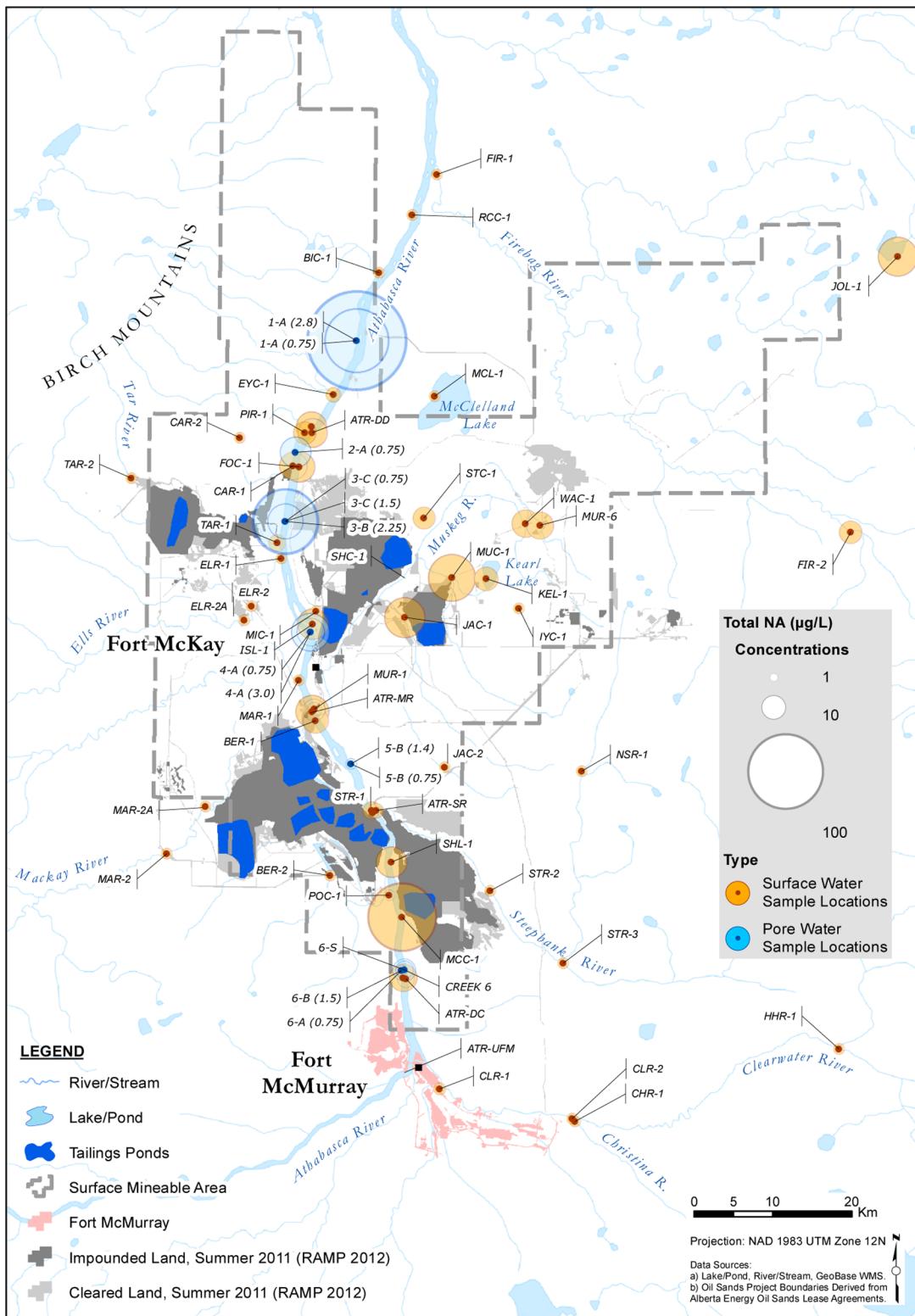
OSPW is acutely toxic to aquatic organisms,<sup>3,4</sup> and in subchronic testing can cause endocrine disruption and immunological impairments.<sup>5–7</sup> Relative to regional ambient water quality of the lower Athabasca River Basin, OSPW can be characterized by high concentrations of salts, residual hydro-

carbons, and a highly complex mixture of dissolved polar organic compounds.<sup>8,9</sup> The acute toxicity has been attributed to the extractable organic acid fraction, which includes naphthenic acids (NAs).<sup>10,11</sup> NAs are a complex class of aliphatic and cycloaliphatic monocarboxylic acids ( $C_nH_{2n-Z}O_2$ ), where  $n$  is the number of carbons and  $Z$  is either zero or a negative even integer representing the hydrogen deficiency of the molecule due to rings or double bonds.<sup>12</sup> A number of individual NAs present in OSPW have recently been identified, including tri-, tetra-, and pentacyclic monocarboxylic acids, as well as monoaromatic species.<sup>13–15</sup> NAs are natural components of bitumen and are solubilized into OSPW during the extraction process. Concentrations of total NAs in tailings ponds range

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**Figure 1.** Map of study area showing sampled surface water and groundwater seep locations. Colors of circles represent surface water or sediment pore water sample collection sites. The size of each circle is proportional to the total naphthenic acids (NA) concentration measured.

from 2.9 to 100 mg/L<sup>16,17</sup> depending on operator, the age of the pond, and quantitative analytical method used.

As the scale of oil-sands development increases in the lower Athabasca River basin, so do concerns regarding potential effects of development on the natural environment, particularly on surface water quality. NA concentrations have been

routinely monitored in surface waters in the lower Athabasca basin, and reported concentrations range from <0.1 to 10 mg/L.<sup>16,18</sup> Although potential exists for seepage or runoff of OSPW into surface and groundwaters,<sup>19,20</sup> natural erosion of exposed oil sands deposits and groundwaters naturally containing NAs may also contribute to NA loadings in surface waters. Thus, a

key challenge facing aquatic monitoring programs today is differentiating between anthropogenic and natural sources of NAs.

Environmental monitoring and NA source determination studies have so far been hampered by the complexity of NAs and co-occurring interferences. Within the organic acid fraction of OSPW, the molecular formula of more than 3000 acidic compounds, many containing nitrogen, sulfur, and multiple oxygen atoms, have been identified to co-occur with NAs.<sup>21,22</sup> However, all previous quantitative monitoring data for NAs in the Athabasca region have been collected using low-resolution mass spectrometry (MS) or infrared spectroscopy.<sup>16,18,23</sup> Both of these methods lack the specificity to accurately quantify NAs, and overestimate NA concentrations.<sup>17,24,25</sup> Owing to the many isobaric interferences in OSPW and natural waters, the use of low-resolution MS is also not advised for qualitative purposes, as it leads to misclassification of NAs by *n* and *Z*. High-resolution MS (i.e., resolving power >10 000) has been advised as a better tool for this purpose.<sup>24,25</sup> Headley et al.<sup>26</sup> used ultrahigh resolution MS (resolving power >100 000) to qualitatively analyze natural waters in the lower Athabasca region, confirming that NAs were present, as well as many isobaric interferences that might preclude accurate analysis of NAs by low-resolution MS. The past use of low-resolution MS and infrared spectroscopy methods leads to questions about the accuracy of previous monitoring data. Thus, a need exists for more selective and accurate quantification of NAs in the Athabasca River basin to determine whether NAs in surface water are from natural sources or oil sands mining activity.

In the current study, liquid chromatography was coupled to high-resolution MS for the selective detection of NAs, allowing for accurate quantitative and qualitative NA data for surface and pore waters in the lower Athabasca River region. All data and analyses are discussed with respect to distinguishing possible sources to regional surface waters.

## MATERIALS AND METHODS

**Chemicals and Reagents.** Methanol, acetonitrile, and dichloromethane (HPLC or Optima grade) were purchased from Fisher Scientific (Edmonton, AB, Canada). Acetic acid and 18.8 M sulphuric acid were purchased from Sigma-Aldrich (Oakville, ON, Canada). Water was purified using a Milli-Q apparatus. Refined Merichem was obtained from the Merichem Company (Houston, TX, USA) and was used for calibration of NA concentrations, while <sup>13</sup>C-tetradecanoic acid was purchased from Isotec for use as an internal standard.

**Field Methods and Sample Collection.** All surface water samples were collected by the Regional Aquatics Monitoring Program (RAMP) in September 2011; further details of field-sampling methods and exact sampling locations appear elsewhere.<sup>18</sup> Briefly, sampling sites along the Athabasca River mainstem were typically upstream of major tributaries, with paired samples collected along the east and west banks. Downstream sampling sites (denoted as "1" in sampling codes; Figure 1, Supporting Information Table SI 1) in tributaries were located upstream of their confluence with the Athabasca River. In some tributaries, additional samples were collected upstream of known oil sands development. All samples were collected as grab samples, except those from lakes, where composite samples from 10 locations in each lake were collected. Samples were collected in 1-L amber glass bottles, but upon arrival at the University of Alberta each sample was

quantitatively transferred to a 1-L polypropylene bottle using a 10-mL methanol rinse, and frozen until extraction.

Sediment pore water from the Athabasca River mainstem was collected to represent regional groundwater. Sediment pore water sampling locations were chosen based on previous identification of zones of high conductivity in the Athabasca River mainstem, which indicated areas of groundwater seepage into river sediments. Six seepage zones were identified, ranging from 4 km (Zone 6) to 80 km (Zone 1) downstream from the town of Fort McMurray (sample codes 6 through 1, respectively). Pore water was sampled in October 2010 using manually installed drive point piezometers (Solinist, Georgetown, ON, Canada) and collected with a peristaltic pump. At each location, samples were taken at depths of approximately 0.75, 1.5, and 3m below the sediment surface, although samples from all depths were not analyzed from each location. Samples of OSPW (*n* = 2) from active tailings ponds were obtained from two oil sands mining operators.

**Extraction and Analysis of NAs.** The organic acid fraction was isolated from river water samples (1 L), pore water (500 mL), or OSPW (100 mL) by liquid–liquid extraction.<sup>27</sup> After thawing, the initial pH was adjusted to less than 2.5 with the addition of 1 mL of 18.8 M sulphuric acid, spiked with an internal standard (<sup>13</sup>C-tetradecanoic acid), and extracted two times with 200 mL of dichloromethane. The organic phases were combined and evaporated to approximately 10 mL by rotary evaporation, transferred to a glass vial, and reduced to dryness with nitrogen. The dry extract was reconstituted in 1 mL of 50:50 water/acetonitrile for analysis.

Samples were chromatographed on a Shimadzu LC 20XR LC system using a Cosmosil C18-MS column (10 cm × 3.0 mm, 2.5 μm d.p.). Acetic acid (0.1%) in water (Solvent A) and methanol (Solvent B) were used for gradient elution. The mobile phase composition was kept at 5% B for 1 min, followed by a linear gradient to 95% B in 9 min, 95–99% B in 5 min, returned to 5% B in 1 min, and remained at this composition for 4 min prior to the next injection. An API 5600 (AB Sciex, Framingham, MA) time-of-flight high-resolution mass spectrometer (mass resolution was ~30 000 at *m/z* 250) with an electrospray source, operating in negative ionization mode, was used for detection. Acquisition was performed in scan mode from *m/z* 100 to *m/z* 700. Data was acquired using Analyst TF and chromatographic peaks were integrated with Multiquant 2.0 software (AB Sciex).

**Quality Control.** Deionized water (1 L) exposed to sample site conditions was used for field blanks. All blanks were extracted and analyzed analogously to natural water. One laboratory blank (1 L of Milli-Q water) was extracted after every 5 samples. Total NA concentrations in all blanks were below the limit of quantification (2 μg/L). Recoveries were evaluated by calculating the peak area of the internal standard in each extracted sample compared to the peak area of internal standard spiked directly to the makeup solvent and averaged 69.9% (range: 37.1–122%).

**Analysis of Total NAs and NA Profiles.** Identification of NA homologue peaks was based on both high-resolution mass measurement within ±0.02 amu of the theoretical masses of NAs corresponding to *n* = 7–22, and *Z* = 0 to *Z* = −20 homologues, and retention times corresponding to the retention time (±0.5 min) of the same homologue group in the Refined Merichem standard. Only peaks with a signal-to-noise ratio greater than or equal to 3:1 were integrated.

Total NA concentrations were calculated based on a 4-point calibration curve generated from serial dilutions of Refined Merichem commercial NAs (concentration range: 0.2–200 µg/L,  $r^2 = 0.999$ ). Total NAs were quantified by summing the peak area ratios for all detected homologues, relative to the internal standard peak area. The total area response for all  $Z = 0$  NAs, and two  $Z = -2$  NAs ( $n = 16$  and 18) were eliminated from the data sets due to high concentrations in laboratory blank samples, previous detection at high concentrations in nonoil-sands-affected river water samples (previously analyzed in our laboratory), and the fact that the high concentrations tended to obfuscate profiles of lesser-concentrated NA homologues. These homologues are likely naturally occurring saturated ( $Z = 0$ ) or monounsaturated ( $Z = -2$ ) fatty acids, and were also excluded from the calibration curve and samples prior to quantification.

**Statistical Analyses.** NA profiles were examined by Principal Component Analysis (PCA), using each sample's percentage of NA homologues (relative to the total) to provide a comprehensive comparison of sites with respect to potential NA sources. PCA can reveal the internal structure of data in a way that best explains the variance in the data.<sup>28</sup> PCA is often used for source determination in environmental forensics, particularly for complex samples such as petroleum.<sup>29,30</sup> For samples where the total concentrations of a particular NA homologue were less than the LOQ, the percentage concentrations were calculated by setting the total concentration of that particular homologue equal to the LOQ. Data were log<sub>10</sub>-transformed and autoscaled before PCA to help the data meet PCA's underlying assumptions of normal distribution<sup>31</sup> and because PCA is sensitive to the numerical scale or ranges of different variables included in the analyses.<sup>32</sup> PCA was performed using the "prcomp" function in R statistical software's *stats* package.<sup>33</sup> Correlations among individual NA homologues, and between individual NA homologues and NA PC scores, were assessed using Spearman's rank correlation analysis. Given the large number of variables analyzed, it was assumed that several data sets would contain outliers. Therefore, Spearman's rank correlation analysis was chosen for its robustness to the presence of outliers.<sup>34</sup>

To better understand the potential influence of sources on the NA profiles of surface water, correlations between NAs PC scores and 84 other water quality variables (e.g., major ions, total and dissolved nutrients, total recoverable and dissolved metals, and various organic compounds, including total and dissolved organic carbon, total phenolic compounds, and petroleum hydrocarbons) were explored in each surface water sample. Only surface water quality data from tributaries to the lower Athabasca River ( $n = 44$ ), not from the Athabasca River itself, were used in this correlation analysis to reduce the influence of water originating upstream of oil-sands deposits or related development.

The potential for OSPW or groundwater-derived origins of NAs in surface waters was examined further by plotting relative ion balance in surface water, OSPW, and pore water samples using Piper plots. Piper plots are a common graphical technique for examining the ionic character of ambient waters and used most commonly to identify groundwater types. Ion balance of surface and pore water was calculated directly, while ion balance for OSPW was taken from Piper plots of similar samples reported in Gibson et al.<sup>9</sup>

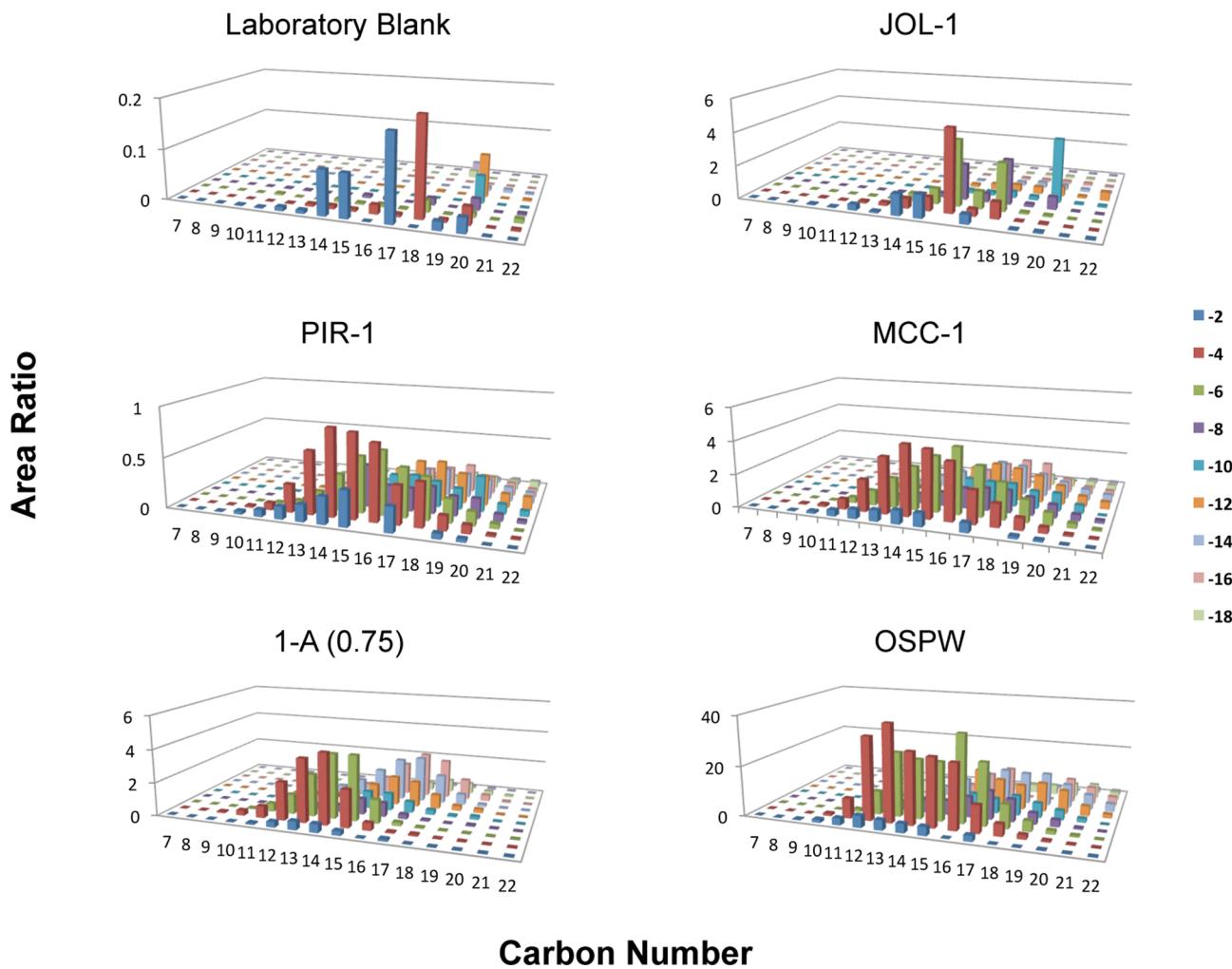
## RESULTS AND DISCUSSION

**Concentration and Spatial Trends.** Quantifiable levels of NAs were detected in 45% of surface water samples, with concentrations ranging from <2 to 80.7 µg/L, with a median value of 3.19 µg/L (Table SI 1). Total NAs were quantifiable in most (7 of 8) samples from the Athabasca River, ranging from <2 to 19.5 µg/L. No clear trend in total NA concentration was noted along the Athabasca River mainstem (Figure 1), with concentrations up to 14.1 µg/L upstream of oil-sands developments (but downstream of Fort McMurray) at station ATR-DC, and up to 15.8 µg/L downstream of surface mining (at station ATR-DD).

Among all surface waters, the highest total NA concentrations were found in tributaries and lakes, including in McLean Creek (MCC-1: 80.8 µg/L), lower Muskeg Creek (MUC-1: 39.2 µg/L), lower Jackpine Creek (JAC-1: 28.5 µg/L), and Johnson Lake (JOL-1: 27.40 µg/L) (Figure 1). McLean Creek is a small wetland-headed tributary to the Athabasca River whose upper watershed is partially impounded by tailings ponds and oil-sands development (23.4% of total watershed closed-circuited<sup>18</sup>). Muskeg Creek is a small tributary to Muskeg River with no oil-sands mining activity in its watershed beyond a small amount of land clearing at the time of sampling.<sup>18</sup> Lower Jackpine Creek is also a tributary to the Muskeg River, located adjacent to the Shell Jackpine mine, while Johnson Lake is a pristine lake that is not directly affected by oil sands mining activity except through possible atmospheric routes. Overall, the range of lake and tributary sampling sites with high NA concentrations (both impacted and nonimpacted sites) make it difficult to draw conclusions about potential sources of NAs based only on total NA concentration data.

Nonetheless, a consistent observation within several tributaries was that the total NA concentration downstream (near the confluence with the Athabasca River) was often higher than upstream on the same tributary (Figure 1). For example, concentrations in the Beaver River (BER) and Jackpine Creek (JAC) were <2 µg/L in upstream samples, but were 10.9 and 28.5 µg/L, respectively, at downstream sampling sites. Similar trends were present in other paired upstream/downstream tributary samples, including the Steepbank (STR), Tar (TAR), Clearwater (CLR), and Calumet (CAR) Rivers. This pattern was not observed in the Muskeg River, the watershed that has experienced the greatest amount of oil-sands development, where NA concentrations were higher in the headwaters (12.9 and 13.8 µg/L in the upper Muskeg River MUR-6 and Wapasu Creek WAC-1, respectively) than at its mouth (MUR-1: 3.64 µg/L).

The highest concentrations of NAs among all natural waters examined were in sediment pore water, representing groundwater intrusion into the Athabasca River (Figure 1, Table SI 1). Concentrations ranged from <2 to 173 µg/L, and generally increased with distance downstream on the Athabasca River and with sampling depth. The Athabasca River contacts at least three geological formations, including the Clearwater, McMurray, and Devonian formations, as it flows downstream through this reach.<sup>9</sup> Differences in pore water concentrations may be attributable to the different geological formations through which groundwaters flow, age of groundwater, and the amount of mixing with other surface and groundwater sources. Analysis of other water quality variables (e.g., chloride, sulfate, total dissolved solids) in pore water from this and other studies



## Carbon Number

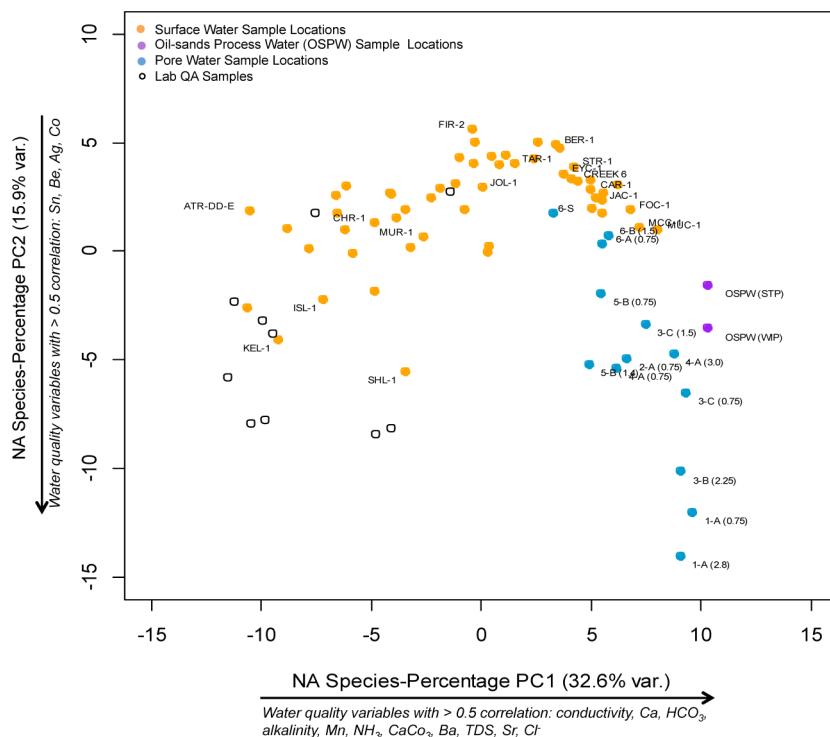
**Figure 2.** Typical NA profiles representing the following: laboratory blank, a background site with natural fatty-acid profile (JOL-1), a nonimpacted site with a strong bitumen-derived profile (PIR-1), a potentially impacted site with a strong bitumen-derived profile (MCC-1), Athabasca River sediment pore water (1-A(0.75)), and OSPW.

similarly showed changes dependent on depth and sampling location.<sup>9,35</sup> In general, samples from Zones 1 and 3 (Samples 1-A and 3-B/C), which had the highest concentrations of NAs, can be characterized as older waters with little to no influence of modern recharge or mixing with other groundwater sources, based on tritium analysis.<sup>35</sup> This finding is notable, as it indicates the presence of high concentrations (relative to surface waters) of NAs in natural groundwater that has not been impacted by anthropogenic activities, such as oil sands mining.

It is important to note that total NA concentrations in surface waters reported herein are 1–2 orders of magnitude lower than concentrations of NAs reported previously for surface waters from the lower Athabasca River region.<sup>16,18</sup> This difference is likely attributable to the higher specificity of the current method. For example, in recent monitoring campaigns, RAMP has quantified and reported NA concentrations based on a low-resolution gas-chromatography MS method, which also is used by the provincial environment ministry for ambient water quality monitoring.<sup>18,23</sup> This method misclassifies NAs in OSPW<sup>24</sup> and inherently lacks the mass resolution necessary to differentiate NAs from other organic acids (e.g., S, N, SO<sub>x</sub>, NO<sub>x</sub>, and O<sub>x</sub> containing organic acids) that are known to co-occur at similar or higher concentrations in surface water

samples.<sup>22,26</sup> Inconsistent results between previous studies and this study may also be attributable to quantification of isobaric interferences or high concentrations of natural fatty acids by low-resolution MS. It is important to point out that, although we subtracted the signal of the “natural NAs” from all samples for quantification (i.e., all Z = 0 NAs, and Z = -2, n = 16 and n = 18 NAs, discussed in detail below), these only contributed, on average, 56% of the total NA signal. For example, taking the most extreme case, in the upstream Beaver River sample (BER-2, <2 µg/L) if natural fatty acids had been included in the quantification then the concentration would have been 22.4 µg/L (an additional 20 µg/L), which is still 20-fold lower than that reported by GC-MS in a sample taken at the same location and at the same time as the current sample.<sup>18</sup> Therefore, exclusion of these natural compounds does not explain the major divergence between the current results and previous monitoring campaigns using less selective analytical methods.

**NA Profiles.** The combined use of liquid chromatography with high-resolution MS (i.e., retention time information and resolving power ~30 000 at m/z 250) resulted in homologue profiles specific for NAs. The NA profiles of two OSPW samples were similar to each other, whereby an overall Gaussian-like distribution of homologues, centered around n = 16 and Z = -6, was evident, consistent with previous reports



**Figure 3.** Principal components analysis scores plot for surface waters, pore waters, OSPW, and QA samples using only the NA homologue profiles as input data. In independent analyses, correlations were also tested between PC1 and PC2 scores for each site against various water quality parameters; the directionality of significant correlations is noted in italicics text adjacent to the PC1 or PC2 scale. Not all sample names are included for clarity.

(Figure 2).<sup>24</sup> This general NA profile will be described as “bitumen-derived” in subsequent discussion. Similar bitumen-derived signatures were evident in some surface and pore water samples, and were clearly discernible from laboratory blanks and some natural river water samples (Figure 2).

Profiles in surface water samples with no signature of bitumen-derived NAs had high proportions of even carbon chain-length carboxylic acids, particularly  $n = 14–18$  ( $Z = -2$ ),  $n = 16$  and  $18$  ( $Z = -4$  to  $Z = -8$ ), and  $n = 20$  ( $Z = -8$ ,  $-10$ , and  $-12$ ) (Figure 2). This pattern is considered exemplary of background natural fatty acids, likely originating from biological sources such as bacteria and phytoplankton. A similar pattern of predominantly even carbon chain-length saturated and polyunsaturated fatty acids, corresponding to  $Z = 0$  to  $Z = -8$ , was reported in high Arctic lakes and was attributable to cyanobacteria and algae.<sup>36</sup> Although the relative proportion of individual natural fatty acids varied among samples, the presence of predominantly  $\text{C}_{16}$ ,  $\text{C}_{18}$ , and  $\text{C}_{20}$  unsaturated acids, with no evidence of bitumen-derived acids, was diagnostic of background NA profiles. Headley et al.<sup>26</sup> previously reported differences in NA and  $\text{O}_4$  acid compositional patterns between reference sites (upstream of mining industry and outside of the oil sands region) and OSPW; a difference attributed to the lack of bitumen-derived acids and the predominance of  $\text{C}_{16}$  and  $\text{C}_{18}$  fatty acids at reference sites.<sup>26</sup>

Bitumen-derived NA profiles were detected in some samples from sites that could not have been impacted by OSPW. For instance, upstream sites on the Steepbank (STR) and Firebag (FIR) Rivers showed evidence of bitumen-derived NAs (Figure S1), as did samples from the Redclay (RCC) and Pierre Rivers (PIR) (Figure 2). These sites are located upstream of potential oil sands mining activity or are not known to be impacted by

mining. The presence of bitumen-derived NAs in these samples clearly indicates a natural (nonanthropogenic) contribution of bitumen-derived NAs to aquatic systems in the region. Such sources may include runoff and erosion of exposed oil sands deposits, and/or natural groundwater flow through oil sands deposits. Other bitumen-derived contaminants, such as PAHs and metals, detected in the Athabasca River and tributaries at unimpacted locales was attributed to erosion of exposed oil sands deposits.<sup>37</sup>

Changes in NA profile between upstream and downstream sites were evident in several tributaries. Profiles in upstream samples of the Jackpine Creek (JAC), Firebag River (FIR), and Tar River (TAR) were similar to other background surface waters, and lacked evidence of bitumen-derived acids (Figure S1). In contrast, the associated downstream profiles for these three tributaries were markedly different, and contained clear evidence of a bitumen-derived NA source. These changes in profiles corresponded to an increase in overall NA concentrations between upstream and downstream sites, as discussed above, and indicate an input of bitumen-derived NAs to the tributaries at some point between sampling sites.

Examination of NA profiles provided an important means of discerning bitumen-derived NAs from biologically derived fatty acids. For instance, Johnson Lake (JOL-1) had elevated concentrations of NAs ( $27.4 \mu\text{g/L}$ ) despite being a pristine lake located approximately 100 km northeast of the center of oil-sands developments. Similarly, with the exception of McClelland Lake (MCL-1), where NAs were  $<\text{LOQ}$ , the other three lakes sampled (SHL-1, ISL-1, KEL-1) had total NA concentrations above the median value of the overall data set, ranging from 9.80 to  $16.7 \mu\text{g/L}$ . However, inspection of the associated NA profiles revealed that the NAs did not originate

from bitumen, as the profiles were composed predominantly of C<sub>16</sub>, C<sub>18</sub>, and C<sub>20</sub> unsaturated acids, likely of biological origin (Figure 2). Similarly, the upstream site on the Firebag River (FIR) had higher concentrations of NAs than the downstream Firebag site, but also had a signature characteristic of natural unsaturated acids (Figure S1). Overall, the highly specific analysis of NA profiles resulted in the ability to discern between bitumen-derived acids and naturally occurring “background” fatty acids, which if not differentiated could lead to erroneous conclusions regarding the sources of NAs to water. This evidence demonstrates the need to incorporate highly specific analytical techniques into future routine monitoring efforts.

**Fingerprinting and Multivariate Analysis.** A primary goal of this study was to attempt source identification of NAs based on the NA profiles. The first three PCs derived from NA profiles explained 57.6% of the total variance in NA profiles among samples: PC1 = 32.6%, PC2 = 15.9, PC3 = 9.1% (Figure 3). Correlation analysis among proportions of NA homologues from all stations indicated two major intercorrelated groups of NA species, suggestive of two common sources. The first group was composed primarily of acids with an even-number of carbons, many of which are present in background samples and likely indicate a biological origin of the acids: (by *n* and *-Z* number) n8Z4, n10Z2, n11Z14, n12Z10, n13Z16, n14Z2, n15Z18, n17Z2, n18Z4, n19Z2, n20Z2, n20Z6, n20Z8, n20Z10, n22Z2, n22Z4, n22Z6, and n22Z12). The remainder of NAs, which form the bulk of the second group of intercorrelated acids, likely represent bitumen-derived NAs.

Examination of the PCA scores plot (Figure 3) revealed several generalized groupings, which may be broadly characterized as laboratory and field blanks, analytical standards (Refined Merichem), surface waters, OSPW, and Athabasca River pore water samples. Blank samples plotted most negatively on PC1, consistent with a lack of bitumen-derived acids, while OSPW from two different operators plot furthest to the right along PC1. Surface water samples plotted as a continuum across three quadrants of the scores plot, with the majority of surface water samples separated based on PC1. Surface water samples in the upper right corner were characterized by bitumen-derived profiles, and plotted more positively in PC1 (regardless of total NA concentration, which was not included in the PCA) than surface water samples with background signatures. In fact, several samples with total NA concentrations <LOQ had visibly strong bitumen-derived acid profiles and plotted similarly in PC1 to samples with higher total NA concentration. With increasingly negative PC1 scores, samples had NA profiles that increasingly resembled blanks and background sites, with a decreasing bitumen-derived NA profile and an increasing proportion of even-chained unsaturated acids. Upstream tributary sites often plotted more negatively along PC1 than the associated downstream sites, corresponding to differences in the NA profiles that were evident between upstream and downstream sites for most tributaries, as discussed above.

All pore water samples had strong bitumen-derived NA signatures and plotted far to the right on PC1, near OSPW (Figure 3). With the exception of two locations, pore water samples were well separated from surface water samples along PC2. This separation is based on increased loadings of higher Z-series compounds in pore water, such as *Z* = -14, *Z* = -16, and *Z* = -18, as can be observed in Figure 2. As with total NA concentrations in pore water, the increased proportion of higher Z-series NAs followed a general upstream to down-

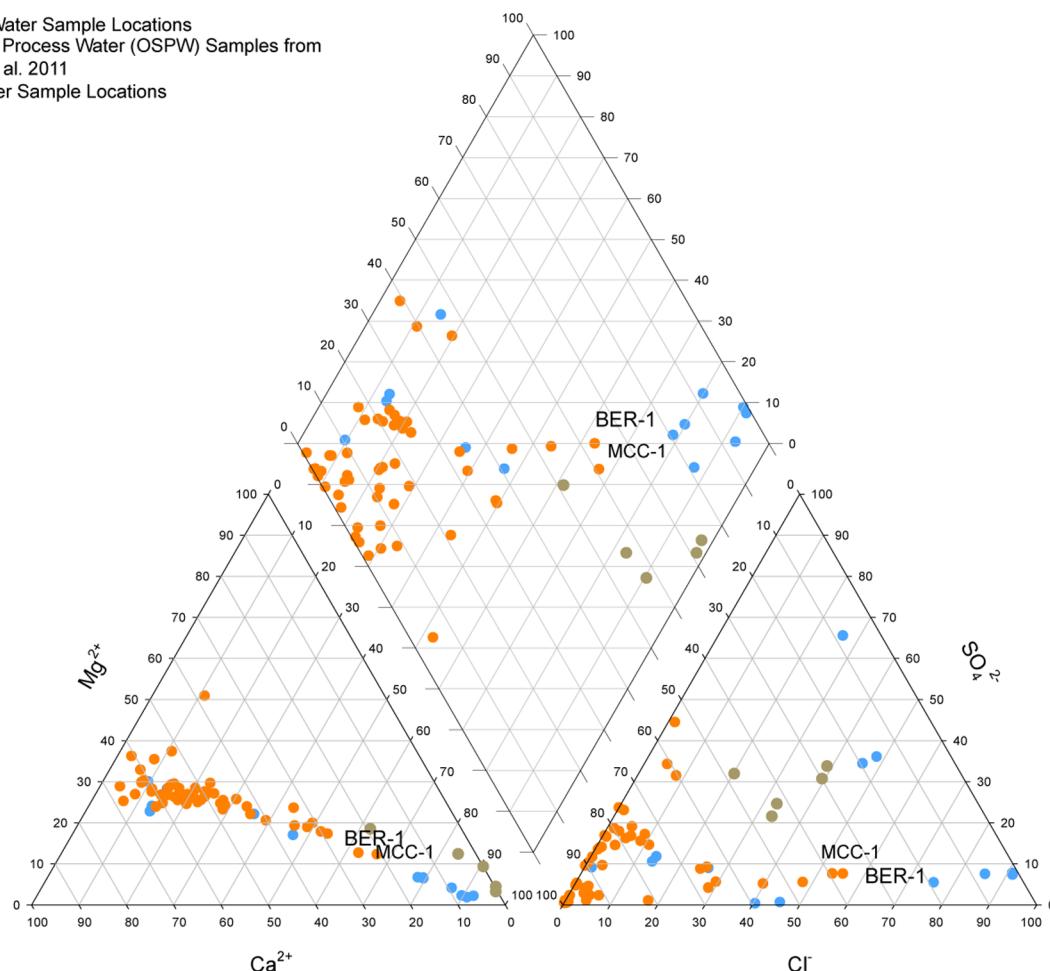
stream trend, with sample sites located further downstream plotting more negatively on PC2. Additionally, samples taken from deeper pore water at individual sites plotted more negatively along PC2. These differences are likely due to the differences in groundwater sources and the underlying geological formations,<sup>9</sup> as discussed previously. A significant correlation was found between hydrogen stable isotope ratios (<sup>3</sup>H/<sup>2</sup>H) and PC scores of the NA homologues in a separate PCA, wherein only pore water samples were examined (Figure S3), supporting our hypothesis that variation in groundwater sources contributes to observed differences in NA profiles. However, more research is needed to identify natural factors influencing NA concentrations and profiles in groundwater, not only along the mainstem Athabasca River, but also for its tributaries.

Although surface water samples were generally well separated from OSPW and most pore water samples by PCA, samples from several mining-affected areas plotted closely to OSPW and pore water, including McLean Creek, Fort Creek, and Jackpine River (Figure 3). However, the clustering of OSPW and pore water sites provided little evidence to differentiate the contribution of either natural or anthropogenic sources to surface water. Nonetheless, the separation between OSPW, pore water, and surface water samples indicates that there are subtle compositional differences that may be exploited in future source identification studies.

A lack of information on the NA profile of other potential sources, including other tailings ponds and a more spatially resolved assessment of groundwater, or the important effects that in-stream environmental fate processes may have on NA profiles, hampers a more conclusive interpretation of NA sources to the current surface waters examined. Furthermore, no data are currently available describing dissolved NA profiles that result from water washing naturally over exposed bitumen outcrops. Considerable heterogeneity may exist among tailings ponds, as OSPW organic compounds can vary depending on operator<sup>9,26,38</sup> and the age of the pond.<sup>39</sup>

Once NAs are released from their source (whether through OSPW seepage, natural runoff, or groundwater intrusion), little is known about how in-stream environmental fate processes might alter NA profiles. Some evidence suggests that profiles may be conserved through groundwater transport in the absence of biological transformation,<sup>40</sup> although this evidence is based on low resolution GC/MS analysis. Sorption of NAs to sediment is known to be dependent on the substrate, with sorption coefficients ranging from <0.5 in sand to ~2.75 in clays.<sup>16</sup> Janfada et al.,<sup>41</sup> using low-resolution MS, reported linear isotherms for OSPW-derived NAs, with *n* = 13–17 (irrespective of *Z*-series) being preferentially adsorbed to soil in simulated groundwater. Several factors, including pH, salinity, and organic content, may also influence the sorption of NAs.<sup>41</sup> Once in surface waters, aerobic transformation may also alter profiles by preferentially degrading lower carbon number NAs,<sup>19</sup> NAs with fewer rings, or NAs with the least branched molecular structures.<sup>42–44</sup> Similarly, atmospheric partitioning of lower carbon and *Z* series NAs may result in a profile enriched with higher *n* and *Z* series acids.<sup>45</sup> McMartin et al.<sup>46</sup> found that saturated model NA compounds (i.e., without double bonds or aromatic rings) and OSPW-derived acids were photodegraded slowly by natural sunlight. However, this photolysis study did not consider the full suite of NAs that may exist in groundwater, particularly those NAs with higher *Z* (which differentiated pore water NA profiles from surface water, Figure

- Surface Water Sample Locations
- Oil-sands Process Water (OSPW) Samples from Gibson et al. 2011
- Pore Water Sample Locations



**Figure 4.** Piper plot of OSPW, surface water, and sediment pore water samples. Only MCC-1 and BER-1 are listed for clarity.

2) that likely contain UV-absorbing double bonds or aromatic rings.<sup>14,38</sup> Therefore, we cannot rule out that NA profiles observed in pore water may be altered by in-stream photolysis (direct or indirect) to resemble the bitumen-derived NA profile observed in many surface waters.

#### Correlation with Other Water Quality Variables.

Correlations between NA profiles (as PC scores) and other water quality variables in tributaries were examined to better understand the potential influence of different sources to the NA profiles. Significant correlations ( $p < 0.01$ ) with PC1 and PC2 are noted adjacent the relevant PC axes in Figure 3. PC1 showed significant positive correlation with several variables, including total dissolved solids (TDS), hardness, total alkalinity, bicarbonate, calcium, barium, magnesium, manganese, chloride, and ammonia. Concentrations of these variables are generally higher in groundwaters than in surface waters, thus correlations between them and the increased proportion of bitumen-derived NAs along PC1 are together suggestive of an increasing groundwater influence moving left to right in Figure 3. PC2 scores were negatively correlated with total Kjeldahl nitrogen (which represents organic nitrogen bound to particulates and ammonium). This supports our previous assertion that NAs detected in lakes, which were primarily separated from surface waters along PC2, are derived predominantly from fatty acids of biological origin. A major contributor to summer and fall streamflow of major stream and river tributaries in this region is drainage of muskeg, along with groundwater inflow and

sporadic increases in flow due to storm events.<sup>47</sup> Thus, it is expected that sources of naturally occurring acids in monitored surface waters include contributions from groundwater and from peatland drainage or general biological activity in surface waters. Observed correlations between NA profiles and water quality variables are consistent with this etiology.

To further explore associations between NA concentrations and groundwater, the ion balance of samples was examined using Piper plots (Figure 4). Most surface waters in the lower Athabasca River region are of a calcium/sodium-bicarbonate type, whereas OSPW shows greater sodium-bicarbonate or sodium-bicarbonate/chloride type, with an influence of sulfate (Figure 4). Consistent with previous studies,<sup>9</sup> pore water samples exhibit a wide range of ionic characteristics, from those typical of surface water to those similar to OSPW. Two surface water samples fell closest to OSPW in their ionic character: McLean Creek (MCC-1), which also had the highest NA concentrations of all surface water samples, and lower Beaver River (BER-1), which is a small creek downstream of the Syncrude Mildred Lake tailings facility, whose upper watershed (including site BER-2) was diverted into the Poplar Creek drainage in the 1970s. Lower Beaver River (BER-1) typically exhibits very high TDS and ion concentrations relative to other stations,<sup>18,23</sup> suggestive of an influence of seepage from either groundwater or the upstream tailings pond. Profiles of NAs in both McLean Creek and Beaver River indicate a bitumen-derived profile; McLean Creek plotted most closely to OSPW

relative to all other surface water samples (Figure 3). Beaver River, in contrast, exhibited a midrange NA concentration (10.9 µg/L), and plotted near the middle of most surface water samples (Figure 3). A bitumen-derived NA profile was evident in the downstream sample from Beaver River while the NA profile at the upstream sampling location was characteristic of a natural source of NAs. However, in the downstream site the influence of natural fatty acids on the profile was partially evident, likely due to mixing of the bitumen-derived source with background water containing natural fatty acids. Consistent with this, a 5-fold increase in NA concentrations was detected at the downstream sampling site on Beaver River, along with an increase in the proportion of bitumen-derived NAs, and a shift in ion chemistry from bicarbonate type to chloride type between upstream and downstream sites was also noted. Unfortunately, no upstream sample was collected for McLean Creek. The similarity of bitumen-derived profiles to OSPW, elevated NA concentrations, and similarity in water ion chemistry to OSPW suggests that these two tributaries to the Athabasca River may be receiving OSPW seepage or runoff from nearby tailings ponds. Little information was available regarding seepage to McLean Creek, but Beaver River is known to receive seepage and runoff from the nearby Syncrude Mildred Lake Settling Basin and site.<sup>48</sup> This finding is consistent with previous findings of increased metals, chloride, and PAH concentrations between upstream and downstream sampling sites.<sup>18,48</sup> Alternatively, groundwater from Zones 1, 3, and 5 displayed strong Na–Cl type characteristics, and also contained high concentration of NAs and strong bitumen-derived profiles. It is therefore possible that older groundwater, originating from the Devonian limestone formation, may also be contributing to the shifts in ion chemistry and NA profiles observed in these tributaries.

This study is the first comprehensive analysis of NA profiles in surface and ground waters of the region, and highlights the complex nature of NA source determination. Furthermore, it demonstrates the need for highly selective analytical methods for source identification and in monitoring for potential effects of development on ambient water quality.

## ASSOCIATED CONTENT

### Supporting Information

List of sites and total NA concentrations (Table S1), profiles of NAs at upstream and downstream sites on the same tributary (Figure S1), PCA analysis loadings of individual NA homologues (Figure S2) and groundwater-only PCA and correlations between groundwater profiles and stable isotopes (S3). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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