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Article

Supra-Permafrost Groundwater is a Source of Highly Biodegradable Dissolved Organic Matter to the Arctic Ocean

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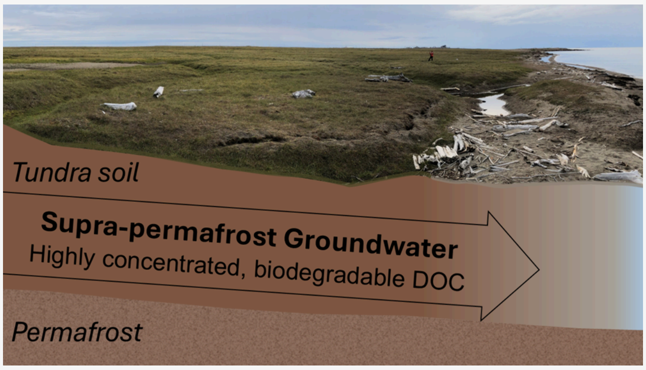

ABSTRACT: Supra-permafrost groundwater (SPGW) flowing through seasonally thawed soils is an important source of dissolved organic matter (DOM) to the Arctic Ocean, yet few studies have investigated the quality of this DOM. We sampled SPGW, runoff, and rivers near Simpson Lagoon, Alaska, during spring ice breakup, summer open water, and fall freeze-up seasons. Through incubation experiments, we compared biodegradable DOC (BDOC) across sources and seasons and linked these results with DOM composition using Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS). DOM composition was distinctly different between SPGW and rivers and shifted throughout the year. SPGW contained an order of magnitude more dissolved organic carbon (DOC) than rivers, with average concentrations increasing from breakup (22 ± 5 mg C L^{-1}) to freeze-up (110 ± 42 mg C L^{-1}). SPGW also contained three times or more BDOC than rivers, averaging 18 ± 1% during breakup and declining to 3.4 ± 0.7% during freeze-up. BDOC was positively correlated with condensed aromatic and polyphenolic DOM formula classes that are often associated with recalcitrant organic matter. Thus, SPGW DOM is highly aromatic yet biodegradable and is likely an important energy source to Arctic coastal waters in summer when river inputs are low.

KEYWORDS: *dissolved organic matter, permafrost, groundwater, surface waters, 21 T FT-ICR MS*

■ INTRODUCTION

Groundwater discharge is an important source of solutes to the ocean, impacting nearshore biogeochemical cycling and coastal ecosystems.[1−3] Despite the importance of freshwater inputs to the Arctic Ocean,[4] only a few studies have examined groundwater discharge[5] and associated constituent fluxes along Arctic coastlines.[6−8] Studies near Kaktovik and Deadhorse, Alaska estimate that supra-permafrost groundwater (i.e., subsurface flow within the seasonally thawed active layer above permafrost; SPGW) DOC fluxes to the Beaufort Sea are on the order of riverine inputs during summer,[8,9] emphasizing SPGW as an important source of DOC to the Arctic Ocean.

Depending on its lability, this DOC may be decomposed to greenhouse gases and/or impact marine production.[10] However, very little is known about the composition or biodegradability of dissolved organic matter (DOM) in SPGW. While studies of Arctic soil leachates demonstrate that DOM composition varies across regions and deposit types,[11] the biodegradability of soil DOM is generally high relative to surface waters.[12] Since SPGW flows through these organic-rich soils, we expect that coastal groundwater could be an important source of biodegradable, allochthonous DOM to the Arctic Ocean. We also hypothesize that there are seasonal changes in DOM composition and biodegradability[13,14] that accompany shifts in hydrologic connectivity[15] and thaw depth. During spring, snowmelt moves rapidly across the landscape as surface runoff and extremely shallow SPGW, leaching relatively fresh DOM from vegetation and upper soil horizons.[13] As the season progresses and soils thaw, SPGW flow paths deepen, percolating through older soils.[6] Understanding seasonal patterns in the quantity and quality of SPGW DOM is needed to predict its impact on marine food webs and how quickly DOM may be decomposed to greenhouse gases. This work is particularly relevant in a rapidly warming Arctic where ancient permafrost is vulnerable to thaw,[16] altering SPGW flow paths and geochemistry.[17,18]

To gain insight on the composition and biodegradability of fresh SPGW DOM in relation to surface waters entering the Arctic Ocean, we sampled fresh SPGW, runoff, and rivers near

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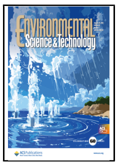

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Simpson Lagoon on the central Alaska Beaufort Sea coast during three seasons. The molecular-level composition of DOM species was determined by negative-ion electrospray ionization 21 T Fourier-transform ion cyclotron resonance mass spectrometry (21 T FT-ICR MS) and compared to biodegradable DOC (BDOC) that was assessed by measuring DOC consumption throughout a 28-day dark, aerobic, bottle incubation experiment. Although a wide range of organic moieties exist within the DOM pool, we utilize ultrahigh resolution mass spectrometry and focus on evaluation with negative-ion electrospray ionization due to the broad insights into the DOM pool this provides.[19,20] To our knowledge, this is the first study to examine biodegradable DOC in Arctic coastal groundwater across seasons, and one of relatively few studies worldwide combining BDOC experiments with FT-ICR MS analyses.

■ METHODS

Sample Collection

SPGW, surface runoff, river, and lagoon surface water samples were collected along the coast of Simpson Lagoon, located near Deadhorse, Alaska, during three field campaigns in 2022 (Supporting Information (SI) Figures S1−S4). This low-elevation tundra landscape is underlain by continuous permafrost and the surface geomorphology includes ice-wedge polygons, lakes, and drained lake basins typical of the Beaufort Sea coast. Our study area was covered with high-centered ice-wedge polygons with sedges as the dominant vegetation. On the tundra in this area, the active layer (maximum seasonal thaw depth) typically reaches 30−40 cm in polygons centers and 10−30 cm in ice-wedge troughs, and becomes deeper in the intertidal zone.[8] SPGW for this study was collected from wells within ∼ 10 m of the Simpson Lagoon shoreline, near the interface of ice-wedge polygon troughs and beach (SI Figures S2−S4). We chose sites where SPGW was fresh (salinity <1) but close to the subterranean estuary mixing zone where groundwater became brackish. Samples from the spring breakup season were collected from June 14−17 when Simpson Lagoon was partially ice-covered, but river ice was absent and there was ample surface runoff flowing across the tundra. Samples from the summer open water season were collected from July 22−27 when no lagoon ice was present, surface runoff streams at the coast had stopped flowing, and SPGW was flowing through deeper, more mineral soil horizons. At this site during summer, fresh groundwater discharge to the lagoon is estimated to be 1,450 m^3 day^{-1} km^{-1}.[8] Samples from the fall freeze-up season were collected from September 30-October 2, when the soil surface was beginning to freeze and ice was forming in Simpson Lagoon.

SPGW was collected using partially screened PVC or stainless-steel wells that were installed to the depth of the seasonally variable ice table. At the tundra-beach interface, ice table depth increased from ∼ 0.25 m during spring breakup sampling to ∼ 0.6m at fall freeze-up. Piezometers were reinstalled each season, but wells were flushed before sampling to minimize disturbance from installation. Three wells were sampled during breakup and freeze-up seasons, and five wells were sampled during the open water season.

Three rivers, capturing a range of watershed sizes draining to the coast, were sampled each season: the Kuparuk River which discharges into Simpson Lagoon and is one of the largest rivers draining the North Slope of Alaska, the Putuligayuk River which is intermediate in size, and No Point Creek, a small stream near our SPGW study area (SI Figure S1). Additionally, we sampled surface runoff during the spring breakup season (n = 5) and summer open water season (n = 3). These small, ephemeral surface water features were flowing into Simpson Lagoon during June sampling, but by July were largely absent, except for small areas of ponded water in low areas or between ice wedge polygons. Since surface runoff was not flowing when we sampled in July, open water season runoff samples were not included in BDOC incubation experiments. All runoff samples were collected within ∼ 10 m of the Simpson Lagoon shoreline. Two lagoon surface water samples were collected during the open water and freeze-up seasons (one sample each season). Lagoon samples were collected from a small boat away from the shoreline. All surface water samples were collected just below the water surface.

All water samples were collected using a peristaltic pump with Masterflex C-flex tubing. Samples were filtered using in-line, precombusted GF/F (0.7 μm) filters into 1L, preleached and acid washed HDPE bottles. Samples were stored in the dark in coolers until subsampling for the BDOC experiment.

Biodegradable Dissolved Organic Carbon Experiment

To compare the biodegradability of DOC in different water types across seasons, we completed dark, aerobic incubations of DOC over 28 days at ∼ 20 °C following a standard protocol.[12] Biodegradable DOC (BDOC) was quantified as the percentage of initial DOC lost during incubation, representing the fraction of organic carbon that was remineralized by microbial respiration.

Within 12 h of sample collection and field filtration, each water sample was subsampled into preleached, acid washed polycarbonate bottles for initial (t0) geochemical analyses and for the BDOC experiment. The BDOC incubation was performed in triplicate. Bottles for t0 DOC concentrations and t0 FT-ICR MS analyses were immediately frozen after subsampling, shipped with ice packs in coolers to the University of Texas Marine Science Institute (UTMSI), and stored at −20 °C. Bottles for the BDOC incubation remained unfrozen for the 28-day incubation. GF/F filtering removes particulate matter but allows some microbial biomass to pass through, so no additional inoculation was performed.[12] This step may remove microbial aggregates, potentially altering the communities present, but previous work indicates that 0.7 μm filtration allows a sufficient amount of bacterial to pass through.[12] Samples were kept in the dark to prevent photo-oxidation and primary production. Bottles also had large headspace volumes and were uncapped weekly to prevent hypoxic conditions. Incubation samples were stored in a cooler, with ice packs as necessary, to keep temperatures controlled while completing field work and during transport to the UTMSI. Once at UTMSI, bottles were incubated at 20 °C in an environmental chamber. This temperature was chosen to facilitate comparisons with other studies,[12] and to mitigate logistical challenges associated with controlling incubation temperatures while working at remote sites and during shipment. Incubation temperatures averaged 19.6 °C, 20.1 °C, and 19.0 °C for breakup, open water, and freeze-up samples, respectively. After the 28-day incubation period, BDOC bottles were frozen and stored at −20 °C.

Dissolved Organic Carbon

Concentrations of DOC were measured on a Shimadzu TOC-V CSH analyzer at UTMSI. Many BDOC studies refilter

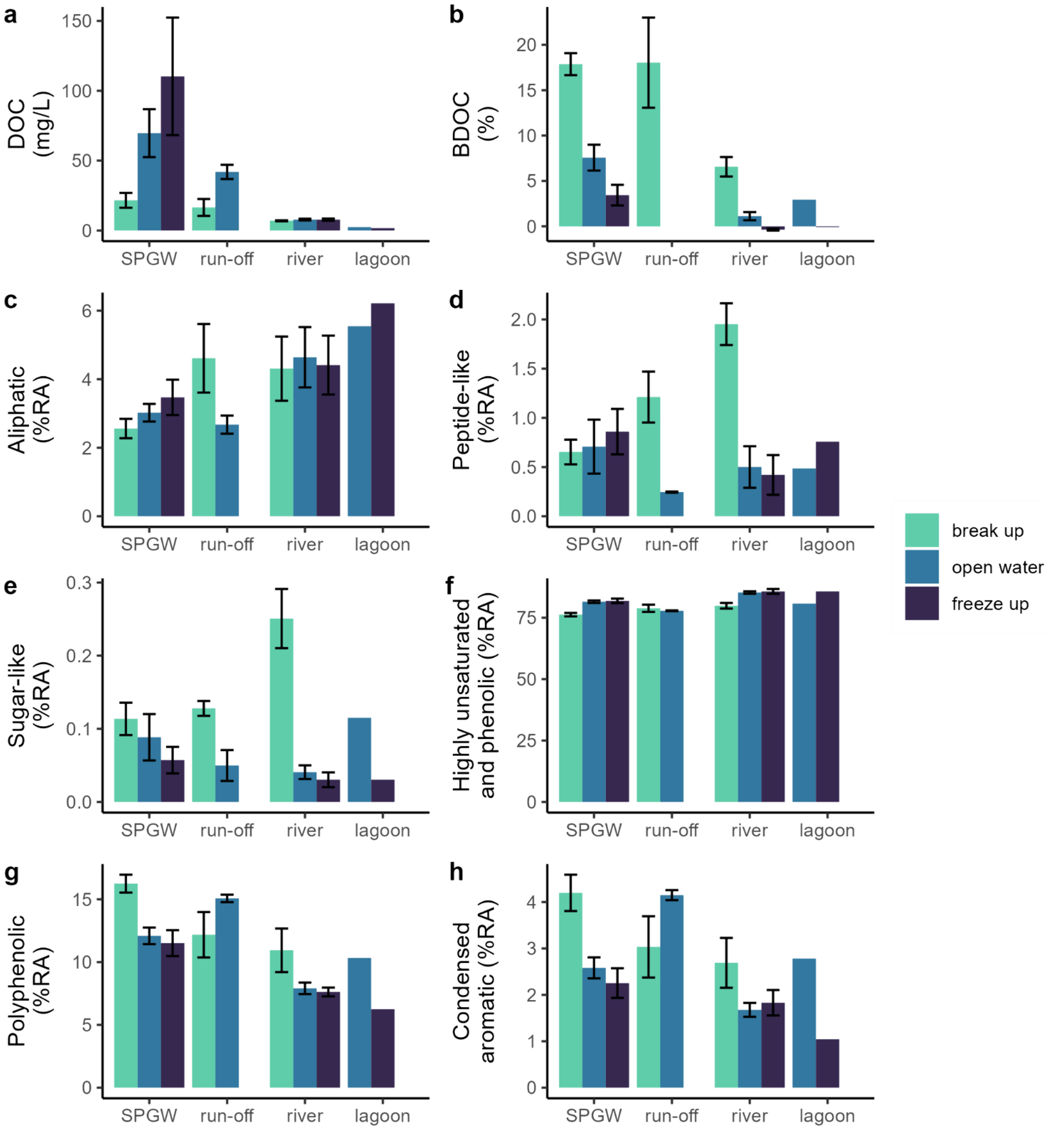

Figure 1. Mean (a) DOC concentrations, (b) biodegradable DOC, and (c−h) percent relative abundance (%RA) of compound classes in supra-permafrost groundwater (SPGW), surface runoff, river surface water, and lagoon surface waters. Colors represent the season sampled, with spring breakup in light green, summer open water in blue, and fall freeze-up in dark purple. Error bars show standard error. Each bar represents the mean of three field sites sampled each season ($n = 3$), except for summer open water SPGW ($n = 5$), spring runoff ($n = 5$), and lagoon water ($n = 1$ in summer open water and fall freeze-up). Run-off was not sampled during fall freeze-up.

incubated samples to remove aggregates of microbial biomass >0.7 μm, such that DOC loss measures remineralization plus microbial incorporation of DOC.[12] However, we observed DOC flocculation in some filtered samples within hours of collection that was more likely attributed to physical processes. Thus, we intentionally did not refilter any samples before analysis. DOC loss in our experiments therefore solely represents the organic carbon that was remineralized by microbial activity, which is a more conservative measurement of biodegradability. To break up flocs prior to analysis, samples were thawed at 4 °C, acidified to a pH of 2 using ACS reagent grade concentrated HCl (JT Baker) and placed in an ultrasonic bath. Additionally, the TOC analyzer was equipped to homogenize samples using magnetic stir bars, preventing any remaining flocculated organic matter from settling out. Thus, organic carbon quantified during this analysis represents both the organic carbon that remained truly dissolved plus any organic carbon that flocculated after field filtration or freezing. Since the organic carbon quantified and described throughout this work was dissolved (<0.7 μm) at the time of sample collection, we refer to it as "DOC" throughout.

Negative-Ion Electrospray Ionization 21 T FT-ICR MS

DOM isolates for each field sample in this study, as well as a subset of t28 bioincubation samples, were isolated through solid phase extraction (SPE) to concentrate the organic matter and remove salts prior to FT-ICR MS analysis. T28 samples from SPGW, runoff (breakup season only) and rivers were analyzed to examine the changes to DOM composition due to microbial processing. SPE was performed using reverse phase BondElut PPL cartridges (100 mg; Agilent) at UTMSI using standard methods.[21] Briefly, samples were thawed at 4 °C and acidified to a pH of 2 with HCl. If any flocs were observed, samples were placed in an ultrasonic bath. Then, acidified samples were passed through precleaned PPL cartridges for a target concentration of 40 μg C mL^{-1}, assuming an extraction efficiency of 40%.[21] Cartridges were rinsed with acidified water and eluted with 1 mL methanol into precombusted vials with acid washed, PFTE-lined caps. ACS reagent grade HCl (JT Baker) and LC/MS grade methanol and water (Fisher Chemical) were used for cartridge cleaning and rinsing, acidifying samples, and elution. Extracts were stored at −20 °C until analysis.

SPE-DOM samples were analyzed by negative-ion electrospray ionization with a custom-built hybrid linear ion trap 21 T FT-ICR MS at the National High Magnetic Field Laboratory in Tallahassee, Florida.[22,23] Thus, only molecules capable of negative ionization were assessed. For each spectrum, 100 time-domain acquisitions were conditionally coadded. Mass spectra were phase-corrected[24] and elemental compositions iteratively assigned to peaks with signal magnitude >6σ root-mean-square baseline noise[25] with PetroOrg © software.[26] Formulae were assigned using elemental constraints of $C_{1-45}H_{1-92}O_{0-35}N_{0-4}S_{0-2}$.

The modified aromaticity index (AI_{mod}) was calculated from the neutral elemental composition to provide qualitative measure of the degree of aromaticity.[27,28] Elemental ratios and AI_{mod} were used to correlate elemental compositions to compound classes defined by atomic elemental ratios of H/C and O/C: polyphenolics ($0.5 < AI_{mod} \leq 0.66$); condensed aromatics ($AI_{mod} > 0.66$); highly unsaturated and phenolics ($AI_{mod} \leq 0.5$, $H/C < 1.5$, $O/C \leq 0.9$); aliphatic ($1.5 \leq H/C \leq 2.0$, $O/C \leq 0.9$ and $N = 0$); sugar-like ($O/C > 0.9$); and peptide-like ($1.5 \leq H/C \leq 2.0$, and $N > 0$).[13] Each assigned molecular formula may contain multiple isomers, and compound structure cannot be assessed from direct infusion mass spectrometry of DOM with any mass detector. The relative abundance of each formula was calculated by normalizing each peak magnitude to the sum of all peak magnitudes assigned in each sample. The relative abundance (expressed as percentages; %RA) of each compound class and elemental composition grouping (e.g., CHO, CHON) were then calculated as the sum of all the relative abundances of all the peaks in each compound class divided by the sum of all the assigned formulae abundances in each sample.

For all mass spectra presented herein, 7,313−20,556 peaks were assigned elemental compositions with root-mean-square mass measurement accuracy of 0.18−200.07 ppb (SI Table S1). FT-ICR MS data files are publicly available via the Open Science Framework.[29] DOC concentrations, BDOC measurements, and summarized FT-ICR MS data (e.g., compound class and elemental composition relative abundance) for individual samples are publicly available via Environmental Data Initiative.[30]

Data Analyses

Triplicate DOC concentrations from the incubation experiment were averaged prior to statistical analyses. Therefore, reported standard error values represent variability between different SPGW wells or surface water samples within a season. Two-way ANOVA were used to examine the effect of freshwater type and seasonal groups on numerical variables such as DOC concentrations and BDOC; lagoon water samples were excluded from this ANOVA due to lack of replication. Pairwise comparisons were examined using Tukey's Honest Significant Difference method. Principal components analysis was used to qualitatively examine relationships between season, water type, BDOC, and bulk metrics for DOM composition measured with FT-ICR MS (e.g., %RA of compound classes); all variables were scaled to unit variance prior to analysis. Open water season runoff samples were not included in the principal components analysis since they were not included in the BDOC experiment. Spearman's rank correlation coefficients (ρ) were used to examine relationships between BDOC and FT-ICR MS metrics for DOM composition. We also calculated Spearman's rank correlation coefficients between BDOC and relative peak intensity of individual molecular formulae. Due to the large data set size for the latter analysis, p-values were adjusted using a false discovery rate correction. All reported correlations had p-values or adjusted p-values <0.05. All statistical analyses were completed using R Statistical Software[31] and tidyverse,[32] data.table,[33] ggfortify,[34] and cowplot[35] packages were used to process and visualize data.

RESULTS AND DISCUSSION

Dissolved Organic Carbon Concentrations

Concentrations of DOC in SPGW increased from spring breakup (22 ± 5 mg C L^{-1}) to summer open water (70 ± 17 mg C L^{-1}) to fall freeze-up (110 ± 42 mg C L^{-1}; Figure 1; SI Table S2). SPGW DOC concentrations were more than double river DOC concentrations during breakup (7.0 ± 0.3 mg C L^{-1}), and an order of magnitude more than rivers during open water (7.8 ± 0.6 mg C L^{-1}) and freeze-up (7.8 ± 0.8 mg C L^{-1}). Surface runoff DOC concentrations were intermediate to SPGW and rivers during both breakup (17 ± 6 mg C L^{-1}) and open water (42 ± 5 mg C L^{-1}). DOC concentrations were notably different between freshwater source categories ($F = 9.4$, $p = 0.001$) and seasons ($F = 3.6$, $p = 0.04$).

SPGW sampled along this coastline contains higher DOC concentrations than most aquatic ecosystems. In a global meta-analysis of groundwater focused on lower latitudes, most of our SPGW samples would fall within the 99th percentile of DOC concentrations.[36] While there are very few Arctic studies to compare our SPGW results to, one study near the village of Kaktovik (eastern Alaska Beaufort Sea coast) measured SPGW DOC concentrations of 33 ± 2 mg C L^{-1} during August.[6] This is somewhat lower, but within the same order of magnitude of the summer SPGW DOC concentrations we measured near Simpson Lagoon. High DOC concentrations in Arctic groundwater is likely due to shallow flowpaths (<1 m), confined by the permafrost table, that constrain groundwater flow within organic-rich tundra soils.[37−39] Previous work has demonstrated that the active layer (i.e., seasonally thawed soil) pore waters contains high concentrations of DOC,[40−42] and tundra soils rapidly leach DOC in freshwater.[6,43,44]

Increasing SPGW DOC concentrations throughout the year likely reflect seasonal changes in snowmelt, soil thaw depth, and flow. During the spring breakup period, snowmelt combined with minimal active layer thaw leads to large quantities of freshwater flowing across the landscape as surface water runoff and extremely shallow SPGW. As the year progresses, the active layer continues to thaw and SPGW flows more slowly through deeper horizons with lower porosity and hydraulic conductivity,[39,45] accumulating more DOC due to greater contact time. Moreover, if older DOC leached from deeper flowpaths is less bioavailable compared to DOC leached from fresher organic matter at the tundra surface (discussed below), later-season SPGW DOC would be remineralized more slowly.

Biodegradable Dissolved Organic Carbon

Biodegradable DOC (BDOC; measured as percent DOC loss due to microbial remineralization over a 28-day incubation at 20 °C) varied greatly across water types and seasons, with the most biodegradable DOC found in spring SPGW and runoff (Figure 1; SI Table S2). There was a notable decline in BDOC throughout the year. SPGW had 17.9 ± 1.2% BDOC during breakup, declining to 7.6 ± 1.4% during open water, and 3.4 ± 1.1% during freeze-up. Rivers had lower BDOC than SPGW, with 6.6 ± 1.1% BDOC during breakup, 1.1 ± 0.4% during open water, and no measurable BDOC during freeze-up. We observed slight DOC losses in lagoon water BDOC incubations, but these values were close to zero. BDOC was distinctly different across freshwater sources ($F = 13.1$, $p < 0.001$) and seasons ($F = 6.2$, $p < 0.01$), with all pairwise water source comparisons being significant different ($p < 0.05$) as well as differences between breakup and freeze-up seasons ($p = 0.03$).

While previous studies have leached soils in laboratory settings and/or examined small order streams to understand DOM composition and biodegradability across hydrologic continua in the Arctic, this is one of the first studies to examine SPGW directly. Like our data, past studies generally show a decline in BDOC across the continuum of groundwater or soil leachates to streams to larger rivers. One similar study measured 4.5 ± 0.2% BDOC in a composite sample of SPGW collected near Kaktovik, Alaska in August when nearby rivers had ∼ 2% BDOC.[46] A study on the Yukon Coastal Plain sampled porewaters from upper active layer soil and measured BDOC of up to 17% over 7 days, whereas streams had an average of 4% BDOC.[42] Our measurements of SPGW BDOC during spring breakup are similar to soil leachates from permafrost regions,[12] unsurprisingly, as we expect that SPGW is leaching fresh DOC from the active layer soils. In comparison to relatively high BDOC in leachates and SPGW, pan-Arctic streams in summer have a median 9% BDOC[47] and large Arctic rivers range from 0 to 10% BDOC.[14,48,49] Overall, declining DOC concentrations and biodegradability across hydrologic continua suggests that organic carbon is remineralized as it moves through the landscape.[50,51]

Across SPGW and river samples, we observed clear declines in BDOC from spring breakup to fall freeze-up. Declining biodegradable DOC from spring freshet into summer has been shown in many studies of northern high latitude rivers and streams.[12,14,48,52−54] Higher biodegradability of earlier season surface water DOM in northern high-latitude environments has been attributed to greater hydrological connectivity between soils and freshwaters,[15,55] and to the rapid flush of fresh DOM from the upper layer of vegetation and peat when the snow melts and the ground is just beginning to thaw.[14,48] High degradation rates in spring could also be fueled by a flush of bioavailable nitrogen from lysed microbial cells following freeze−thaw cycles.[56,57] As the year progresses, warmer temperatures and longer water residence times through deeper flowpaths may increase microbial processing of both SPGW and surface water DOC during transit to the coast.[15,58]

DOM Composition across Water Types and Seasons

The composition of DOM varied across both water types and seasons (Figure 1). Water type had a strong effect on the percent relative abundance (%RA) of polyphenolic ($F = 8.4$, $p < 0.01$) and condensed aromatic ($F = 4.5$, $p = 0.02$) compounds, with rivers having distinctly lower %RA of these classes than SPGW and/or runoff ($p < 0.05$). Percent relative abundance weighted molecular mass was notably different across both water types ($F = 4.0$, $p = 0.03$) and seasons ($F = 8.0$, $p < 0.01$); molecular mass was lower in rivers compared to SPGW ($p = 0.04$) and higher during breakup than open water ($p < 0.01$) or freeze-up ($p = 0.02$). We hypothesize that highly aromatic and high molecular weight formulae are derived from terrestrial plants and soils.[59,60] Sampling season had a strong effect on the %RA of sugar-like ($F = 11.7$, $p < 0.001$) and peptide-like ($F = 5.7$, $p < 0.01$) compounds; breakup samples had notably higher %RA of sugar-like formulae than open water or freeze-up ($p < 0.01$), and there were more peptide-like compounds in breakup compared to open water ($p = 0.01$). Higher %RA of peptide-like and sugar-like formulae, found in runoff and rivers in spring, may be a derived from aquatic production which is a large source of particulate organic matter in surface waters.[61] Major Arctic rivers show these same trends in compound class %RA across seasons (i.e., decreasing polyphenolic, condensed aromatic, and sugar-like formulae from spring to winter), possibly reflecting declining inputs of freshly leached terrestrial DOM.[13] Compound class %RA data from SPGW at this site was similar in composition to SPGW near Kaktovik, Alaska[46] and active layer leachates from Drew Point, Alaska,[62] but more data is needed to examine spatial variability in SPGW DOM composition.

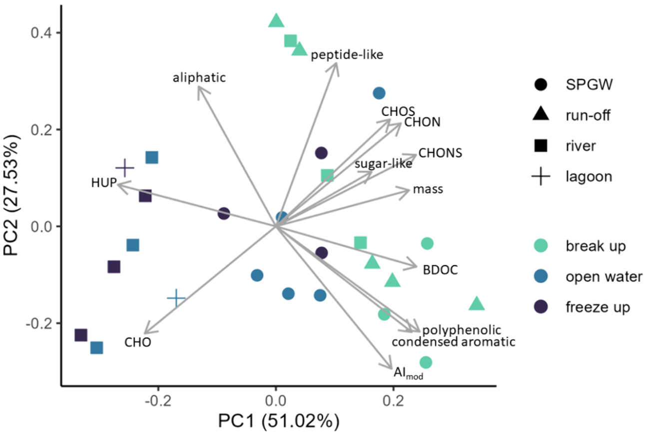

Figure 2. Principal components analysis of 32 observations of 13 variables representing DOM compound classes (sugar-like, aliphatic, peptide-like, highly unsaturated and phenolic (HUP), polyphenolic, condensed aromatic; %RA) and elemental compositions (CHO, CHON, CHOS, CHONS; %RA), relative abundance weighted molecular weight (mass), modified aromaticity index (AI_{mod}), and BDOC (%). Each point represents a unique sample with symbols indicating the water type and colors indicating the season sampled.

Principal components analysis of the %RA data provides additional insight on the differences in DOM composition between water types and seasons (Figure 2). PC1 separates samples by season and water type with nearly all breakup samples falling on the positive end of PC1 along with loadings for aromaticity (i.e., modified aromaticity index (AI_{mod}), polyphenolics, condensed aromatics), heteroatom containing molecular formulae (i.e., CHON, CHONS, CHOS) and molecular weight. All river samples from open water and freeze-up seasons fall on the negative end of PC1 with the loading for HUP compounds. Most SPGW samples from open water and freeze-up seasons fall in between breakup SPGW

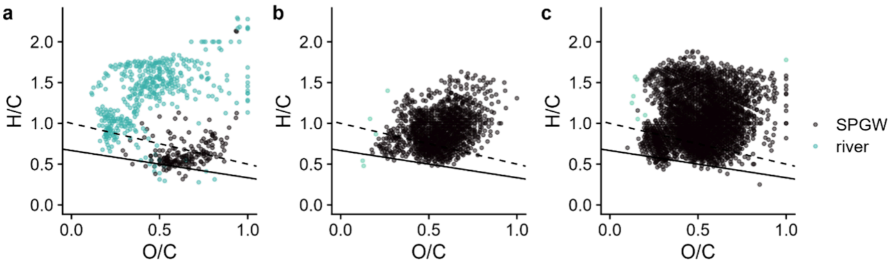

Figure 3. Van Krevelen diagrams of molecular formulae unique to supra-permafrost groundwater (SPGW) and rivers during (a) spring breakup, (b) summer open water, and (c) fall freeze-up. Black points indicate molecular formulae that were unique to SPGW, and light blue points indicate molecular formulae unique to rivers. Molecular formulae were defined as unique if they were present in all samples of one water type during a season and absent from other water types during a season. During spring, there were 179 formulae unique to SPGW and 440 formulae unique to rivers. During summer, there were 1240 formulae unique to SPGW and 6 formulae unique to rivers. During fall, there were 2959 formulae unique to SPGW and 10 formulae unique to rivers. Dashed lines represent the boundary between molecular formulae classified as highly unsaturated and phenolic and polyphenolic; solid lines represent the boundary between molecular formulae classified as polyphenolic and condensed aromatic.

samples and later season river samples on PC1. These data show that SPGW, runoff, and river water are relatively similar in breakup, reflecting a close link between runoff and SPGW when thaw depths are shallow and there is a large pulse of meltwater moving across the tundra.

Seasonal shifts in DOM composition, which are particularly dramatic within rivers, are also apparent when examining individual molecular formulae. To gain more detailed information about differences between riverine and SPGW inputs, we examined which formulae were unique to SPGW versus river samples within each season. Formulae were defined as "unique" if they were detected in all SPGW samples but none of the river samples or if detected in all river samples but none of the SPGW samples within a season (Figure 3). These analyses revealed that rivers have unique formulae during spring breakup, but groundwater contains signature formulae throughout the year with the number of unique molecular formulae relatively increasing from spring to fall. In addition, we identified roughly twice as many total molecular formulae in rivers during breakup compared to open water or freeze-up seasons, whereas the number of molecular formulae identified in SPGW was similarly high throughout the year (SI Table S2). These data indicate that there is a diverse pool of DOM molecular formulae in rivers during spring that are absent in the later seasons. Further, we analyzed unique molecular formulae within SPGW and river samples separately, identifying molecular formulae that were unique to a season to examine how the diversity of DOM within each water type shifts throughout the year (SI Figure 5). Few SPGW DOM molecular formulae were unique to a season, demonstrating that there is a considerable overlap in the molecular formulae present in SPGW throughout the year. Rivers show a much more dramatic seasonal shift: there were 4,651 molecular formulae identified in all river breakup samples that were below detection in all river samples in the later seasons, whereas only 1−2 molecular formulae were unique to open water or freeze-up seasons. Together, these analyses suggests a seasonal decline in SPGW and/or runoff inputs to rivers (i.e., declining connectivity), seasonal changes in surface water autochthonous production, and/or an increase in the processing by microbial communities and/or photo-oxidation[63,64] along the hydrologic continuum throughout the year. Thus, in spring, rivers have a distinct signal not present in later seasons, but SPGW maintains chemical diversity as the year progresses.

Relationships Between DOM Composition and Biodegradability

In addition to comparing composition of SPGW versus surface waters, FT-ICR MS analyses help explain large differences in BDOC. Principal components analysis (PCA) indicates that BDOC is associated with samples containing a higher %RA of polyphenolic and condensed aromatic compounds, higher molecular weight DOM, and DOM containing nitrogen and/or sulfur (Figure 2). Similarly, Spearman's rank correlations (SI Table S3) also indicated that BDOC was strongly positively correlated ($\rho > 0.5$, $p < 0.05$) with %RA of polyphenolic, condensed aromatic, sugar-like, and heteroatom-containing (CHON, CHONS, and CHOS) molecular formulae. In addition, we found positive correlations with molecular weight and the total number of molecular formulae identified within samples. These correlations complement above-mentioned findings showing that SPGW (all seasons) and spring river samples were the most biodegradable and contained high %RA of polyphenolic, condensed aromatic, and N-containing formulae (Figure 1-2).

Spearman correlations between BDOC and the peak intensity of individual molecular formulae identified across samples also show similar patterns (Figure 4). There are many

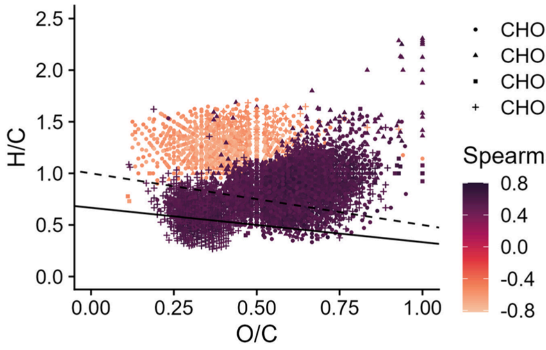

Figure 4. Van Krevelen diagram of Spearmans correlations ($p_{adj} < 0.05$) between relative peak intensity of individual molecular formulae and BDOC (%). Light colors indicate positive correlations between a molecular formulae and BDOC, and dark colors indicate negative correlations. Shape indicates the elemental composition of each molecular formulae. Dashed lines represent the boundary between molecular formulae classified as highly unsaturated and phenolic and polyphenolic; solid lines represent the boundary between molecular formulae classified as polyphenolic and condensed aromatic.

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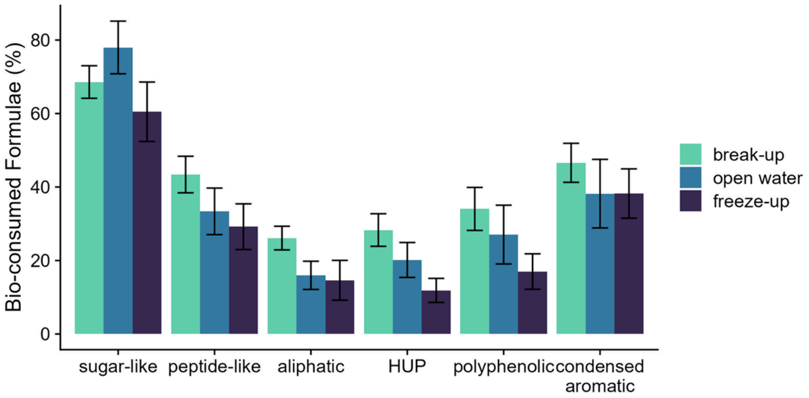

Figure 5. Mean percent of individual molecular formulae within compound classes that were detected before the BDOC incubation but below detection after the incubation within each sample. Error bars show standard error. Color indicates the season sampled.

strong positive correlations between BDOC and highly aromatic molecular formulae with low H/C. We also saw positive correlations between BDOC and sugar-like molecular formulae, which are expected to be highly biodegradable.[49,65] Negative correlations with biodegradability were primarily found in the middle of van Krevelen space (e.g., highly unsaturated and phenolic compounds), an area that encompasses the "island of stability" due to the persistence of these molecular formulae in freshwater and marine environments.[13,59,66,67] This examination of individual molecular formulae provides additional evidence that polyphenolic and condensed aromatic formulae may be utilized by microbial communities.

To further support the above-mentioned findings based on linking BDOC (%) with initial (t0) DOM composition, we also analyzed incubated (t28) DOM samples using FT-ICR MS. To gain additional insight on what DOM molecular formulae are consumed or modified by microbial communities, we examined which individual molecular formulae were detected at t0 but were below detection at t28 within samples (i.e., "bioconsumed formulae"). Similarly, we also examined molecular formulae were below detection at t0 but detected at t28 (i.e., "bioproduced formulae"). The number of bioconsumed molecular formulae (i.e., the number of molecular formulae that were detected at t0 but not at t28 within a sample) was positively correlated with BDOC (%) measurements ($\rho = 0.44$; $p = 0.03$), supporting the idea that these molecular formulae were associated with decomposition. van Krevelen diagrams of bioconsumed and bioproduced molecular formulae show marked changes in the DOM formulae present in the samples pre- and postincubation (SI Figures 6–8). There was evidence of bioconsumption and bioproduction across van Krevelen space, suggesting that microbial communities can use or alter many different types of DOM. The lack of a final filtering step in our incubations means that some of this bioproduced DOM may be derived from the lysis of microbial cells during the acidification step, and thus represents the full array of microbial metabolites and exudates that microbial populations introduce to their environment during their life, DOM consumption, and death. To summarize these results and gain insight on which categories of DOM were most biodegradable, we calculated the average percent of molecular formulae that were bioconsumed within each compound class for each season (Figure 5). Sugar-like molecular formulae had the highest bioconsumption; across seasons, an average of ~ 70% of individual sugar-like molecular formulae present in a sample at t0 were below detection at t28. Condensed aromatic molecular formulae had the next highest percentage of bioconsumption (42%), followed by peptide-like (37%), and polyphenolic (28%) formulae. Highly unsaturated and phenolic and aliphatic compound classes had the lowest percent losses of individual molecular formulae (20–22%). The sugar-like compound class, defined by high H/C and O/C, contains energy-rich compounds like cellulose and other polysaccharides that are expected to be highly biodegradable.[49,65,68] Despite high apparent utilization of sugar-like molecular formulae throughout the incubation, however, this group of molecular formulae was less strongly associated with BDOC in earlier analyses (e.g., PCA, Spearman's correlations), likely due to the low (<1%) %RA of this compound class (Figure 1). While low abundance of sugar-like formulae is also observed in large Arctic rivers,[13] hydrophilic species are discriminated against during solid phase extraction and a fraction of DOM is lost prior to FT-ICR MS analysis, likely contributing to the low %RA measured here.[21,69] Our comparison of DOM composition pre- and post- incubation provides additional evidence that a fraction of condensed aromatic molecular formulae is decomposed and not just correlated with BDOC. In addition to seasonal declines in the %RA of the more biodegradable compounds classes (i.e., sugar-like, condensed aromatic, polyphenolic), this analysis also indicates seasonal declines in the biodegradability of molecular formulae within each compound class.

Studies examining natural organic matter composition across plant litter, soils, glacial, freshwater, and marine environments globally show that aliphatic molecular formulae (H/C > 1.5) are associated with biodegradability.[44,49,53,65,70,71] Molecular formulae classified as aliphatic and protein-like (i.e., N-containing aliphatic) in freshwater include compounds that are thought to be largely derived from autochthonous sources such as microbial communities or algae.[59,65,72] Thus, it is not surprising that our results show consumption of these formulae. Our results showing consumption of highly aromatic DOM (i.e., polyphenolic and condensed aromatic compounds), on the other hand, are less expected. Polyphenolic and condensed aromatic DOM often originate from structural components in terrestrial plants or from combustion products.[59,60,73] Given the relative lack of wildfires in this area, we hypothesize the primary source of condensed aromatic molecular formulae are terrestrial, lignin-derived compounds leached from vegetation, peat, and soils, rather than combustion-derived "black carbon".[59,74,75] While highly aromatic molecular formulae are generally thought to be resistant to microbial degradation,[49,70,76,77] a few other studies provide evidence that aromatic compounds can be biodegradable. For example, polycyclic and other highly aromatic compounds have been shown to be preferentially degraded by microbes during soil passage.[78,79] Both aliphatic and aromatic molecular formulae were decomposed in dark incubations of supra-glacial DOM from Antarctica[72] as well as upland and fen soil leachates from a coastal temperate rainforest.[74] Our findings for SPGW are consistent with findings for Arctic riverine DOM, which is most bioavailable during the spring freshet[14,48] despite having a greater relative signature of terrestrially derived, aromatic compounds.[80] We hypothesize that in situ microbial communities can partially decompose large, highly aromatic biopolymers from terrestrial plants, producing lower weight microbial byproducts in the aliphatic and highly unsaturated and phenolic compound classes.[79] Higher molecular weight, biodegradable DOM are likely leached from vegetation and surface soils, and therefore we see highest BDOC in spring when runoff transports freshly leached DOM to rivers and infiltrates surface soils. As soils thaw, SPGW flows through deeper horizons containing somewhat more degraded organic matter, and allochthonous inputs to rivers decline.

Results comparing SPGW to surface water DOM across seasons suggest that a large fraction of condensed aromatic, polyphenolic, and other classes of DOM are decomposed or modified along flowpaths (i.e., along the SPGW to runoff to river continuum) and throughout the year (i.e., from spring breakup to fall freeze-up). As biodegradable molecular formulae are modified and/or consumed, however, the DOM pool becomes increasingly dominated by highly unsaturated and phenolic compounds. This class contains plant-derived lignin degradation products, as well as carboxyl rich alicyclic molecules (CRAM) and other stable compounds that persist in aquatic environments.[59,66,67,80,81] Unlike rivers, which discharge more processed, less biodegradable DOM to the Arctic Ocean, SPGW is a direct source of highly aromatic yet biodegradable terrestrial DOM.

Relative Importance of Coastal Groundwater Inputs

While our understanding of coastal groundwater in the Arctic is limited, a handful of studies show that groundwater discharge may be a significant source of water and dissolved constituents to the Beaufort Sea.[5−8,37,82,83] Based on work at this study area and near Kaktovik, AK, Demir et al. estimates that fresh supra-permafrost groundwater supplies as much DOC to the Alaska Beaufort Sea as the three largest North Slope rivers during summer (July − September).[8] If we consider our biodegradability measurements from SPGW (∼8%) and rivers (∼1%) during this time frame, we estimate that SPGW is a larger source of BDOC during the summer open water season.

Given the spatial and temporal variability in groundwater discharge and DOC concentrations, additional data is needed to refine these estimates and determine how the relative importance of river versus SPGW inputs varies seasonally. We expect that surface water inputs are a much larger source of BDOC to coastal waters during spring when river discharge and biodegradability are high,[14] but that groundwater discharge becomes an increasingly important source of BDOC over the summer and into fall until freeze-up.[5] SPGW at this field site also contained high concentrations of dissolved inorganic nitrogen relative to surface waters, potentially impacting microbial activity and the ecological significance of SPGW inputs to the coastal ocean.[84] Future work should also examine the processing of SPGW within subterranean estuaries that span the land-sea interface.[85,86] Research in lower latitudes demonstrates that mixing zones of fresh and saline groundwater can be hotspots for chemical transformations,[3] likely altering the composition and thus fate of DOM that ultimately enters coastal ocean surface waters.[87,88]

This study provides novel insight into the composition and biodegradability of SPGW, demonstrating that coastal groundwater can be a source of highly biodegradable, relatively aromatic DOM to the Arctic Ocean. While our focus here is to examine coastal groundwater that flows directly to the Beaufort Sea from a low elevation tundra landscape, this study also provides insight on how DOM is modified along hydrologic continua (i.e., SPGW and/or runoff to rivers) and across seasons in permafrost regions. Bioincubation experiments demonstrate that SPGW DOM is more biodegradable than river DOM entering Simpson Lagoon and that biodegradability declines in both SPGW and surface waters throughout the year from spring breakup to fall freeze-up. In contrast to many studies that suggest that aromatic DOM is resistant to biodegradation, FT-ICR MS analysis indicated that, in this system, samples with the highest relative abundance of polyphenolic and condensed aromatic compounds were the most biodegradable. We hypothesize that coastal SPGW is an important source of biodegradable DOM to the Beaufort Sea in summer, providing an energy subsidy to nearshore food webs when surface water fluxes and biodegradability decline. While future studies are needed to examine these processes in other regions, this work provides a baseline for understanding coastal SPGW in a warming Arctic, where intensifying hydrological cycles and thawing permafrost will likely impact regional carbon cycling.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.5c08206.

> Twenty-one tesla FT-ICR MS root-mean-square mass measurement accuracy (SI Table S1), maps of the study area (SI Figure S1), Field photos during breakup, open water, and freeze-up seasons (SI Figures S2−S4), DOC concentrations, BDOC, and DOM composition metrics across water types and seasons (SI Table S2), van Krevelen diagrams of molecular formulae unique to breakup, open water, and freeze-up seasons in supra-permafrost groundwater and rivers (SI Figure 5), Spearman's correlations between BDOC and DOM composition metrics (SI Table 3), van Krevelen diagrams of bioconsumed and bioproduced formulae in supra-permafrost groundwater (SI Figure 6), van Krevelen diagrams of bioconsumed and bioproduced formulae in rivers (SI Figure 7), van Krevelen diagrams of bioconsumed and bioproduced formulae in runoff (SI Figure 8) (PDF)

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Author Contributions

E.M.B., J.W.M., M.B.C., and M.A.C. conceived the study and participated in field work. E.M.B. and A.M.M. completed laboratory analyses. E.M.B., M.I.B., J.W.M., and R.G.M.S. analyzed the data. The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

BDOC, biodegradable dissolved organic carbon; DOC, dissolved organic carbon; DOM, dissolved organic matter; FT-ICR MS, Fourier transform ion cyclotron mass spectrometry; HUP, highlight unsaturated and phenolic; %RA, percent relative abundance; AImod, modified aromaticity index

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