PAPER

Check for updates

Assessing the prevalence, products, and pathways of dissolved organic matter partial photo-oxidation in arctic surface waters†

Collin P. Ward [ID] *[a] and Rose M. Cory [ID] *[b]

In sunlit waters, photodegradation of dissolved organic matter (DOM) yields completely oxidized carbon (*i.e.*, CO_2) as well as a suite of partially oxidized compounds formed from oxygen incorporation (*i.e.*, partial photo-oxidation). Of these two groups of DOM photo-products, more studies focus on CO_2 (a greenhouse gas) than on partially oxidized DOM, which is likely a diverse group of compounds with poorly constrained roles in aquatic carbon cycling or biogeochemistry. The objective of this study is to address knowledge gaps on the prevalence, products, and pathways of DOM partial photo-oxidation. Here we traced the photochemical incorporation of isotopically labelled $^{18}O_2$ into DOM isolated from Alaskan Arctic surface waters using high-resolution mass spectrometry. Complete and partial photo-oxidation of DOM was also quantified as CO_2 production and O_2 consumption. The majority of ^{18}O-containing partial oxidation photo-products were classified as carboxylic rich alicyclic molecules (CRAM) and overlapped in composition with previously reported photo-products known to result from the oxidation of DOM by singlet oxygen. These results support a previously proposed hypothesis that photo-oxidation by singlet oxygen may contribute to the formation of CRAM, a compound class of DOM ubiquitously observed in surface waters. The novel application of an isotopic tracer for oxygen incorporation with a mass balance approach to quantify complete and partial photo-oxidation of DOM revealed that less than one mol of O_2 is required to produce one mol of CO_2. A sensitivity analysis based on this new knowledge demonstrated that the magnitude of DOM partial photo-oxidation may be underestimated by up to four-fold. Consequently, partial photo-oxidation likely plays a more prominent role in shaping DOM composition in sunlit waters of the Arctic than previously understood. Therefore, partial photo-oxidation should be increasingly incorporated into the experimental framework of studies focused on DOM composition in surface waters.

Environmental significance

Sunlight exposure shapes the chemical composition of DOM in surface waters. By tracing the photochemical incorporation of isotopically labelled dissolved oxygen into arctic freshwater DOM using mass spectrometry, we add new knowledge of the prevalence, products, and pathways of DOM photo-oxidation. Partial photo-oxidation of DOM yields a class of oxidized molecules (CRAM) found in all sunlit waters. Rates of DOM partial photo-oxidation reported in the literature are substantially underestimated, indicating that sunlight plays a more prominent role in shaping the chemical composition of DOM in arctic surface waters than previously understood.

1. Introduction

Recent work demonstrated that nearly 75% of dissolved organic matter (DOM) degradation in sunlit waters of the Arctic is due to partial photo-oxidation,[1] a process that alters the composition of DOM due to the incorporation of oxygen.[1–6] Partial photo-oxidation of DOM is quantified as the difference between photochemical O_2 consumption and CO_2 production by DOM, on a per mol C basis.[1,2,5,6] However, this approach to quantify partial photo-oxidation of DOM on a per mol C basis requires one key assumption about the O_2 requirements for CO_2 production (*i.e.*, 1 mol O_2 per mol CO_2). Although this assumption is hypothesized to be conservative,[1] it has never been explicitly tested, raising the possibility that rates of DOM partial photo-oxidation may be systematically over or underestimated.

[a]Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA. E-mail: cward@whoi.edu; Tel: +1 508 289 2931

[b]Department of Earth and Environmental Sciences, University of Michigan, 1100 North University Avenue, Room 2354, Ann Arbor, Michigan 48109, USA. E-mail: rmcory@umich.edu; Tel: +1 734 615 3199

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c9em00504h

1214 | *Environ. Sci.: Processes Impacts*, 2020, **22**, 1214–1223

This journal is © The Royal Society of Chemistry 2020

For example, in Imnavait Creek, a small headwater stream in the Arctic, the ratio of photochemical O_2 consumption to CO_2 production is consistently less than or equal to 1, suggesting no partial photo-oxidation of DOM.[1,6,7] However, other evidence suggests substantial changes in chemical composition of DOM in Imnavait Creek remaining after photodegradation.[7,8] For example, when DOM from the Imnavait Creek basin was exposed to sunlight, microbes shifted their metabolic rates and pathways in response to changes in the oxygen content of DOM (compared to dark controls).[9] This response suggests that partial photo-oxidation of DOM is a poorly understood control on DOM lability to microbes in arctic freshwaters like Imnavait Creek.

In addition to the challenge of quantifying partial photo-oxidation of DOM, too little is known about the products to understand the impact of partial photo-oxidation on biogeochemical processes such as microbial degradation of DOM. Carboxylic-rich alicyclic molecules (CRAM) are a class of partially oxidized DOM observed ubiquitously in aquatic ecosystems and thought to be produced by photo-oxidation, among other pathways. For example, the abundance of CRAM has been reported to increase upon exposure of DOM to sunlight.[4,6,10,11] Evidence suggests that CRAM is relatively recalcitrant to microbial degradation.[12] Thus, photochemical production of CRAM could contribute to some responses of microbes to photo-oxidized DOM.[4]

Finally, too little is known about the pathways by which DOM is partially photo-oxidized to evaluate its influence on DOM composition in surface waters. Reactive oxygen species (ROS) produced photochemically by DOM, such as singlet oxygen, hydroxyl radical, or superoxide, likely oxidize DOM,[3,4,13–18] although the relative importance of each ROS-mediated oxidation pathway is poorly understood. For example, singlet oxidation of DOM has been hypothesized to account for a substantial fraction of DOM photo-oxidation.[3,4] Exposure of DOM to sunlight in the presence of chemical sensitizers for singlet oxygen ($^1O_2$) yielded partially oxidized products that were operationally defined as CRAM.[4,18] This finding raised the possibility that singlet oxygen is a critical intermediate in the formation of CRAM. However, there is no evidence in support of this hypothesis in the absence of chemical sensitizers for singlet oxygen.

Here we address knowledge gaps on the prevalence, products and pathways of DOM partial photo-oxidation. Specifically, we exposed DOM isolated from arctic surface waters to ^{18}O-labelled O_2 and sunlight, characterized the ^{18}O-containing photo-products using high-resolution mass spectrometry, and quantified complete and partial photo-oxidation of DOM. The majority of DOM photo-products classified as CRAM and were consistent with partial oxidation of DOM by singlet oxygen. Our experimental results demonstrated that less than one mol of O_2 is required to photochemically produce one mol CO_2. A sensitivity analysis based on this new knowledge suggests that rates of partial photo-oxidation of DOM have been substantially underestimated in arctic freshwaters.

2. Materials and methods

2.1 DOM sample preparation

Two freeze-dried XAD-8 extracted fulvic acid isolates were used in this study, Imnavait Creek and Toolik Lake DOM. Both water bodies are located on the North Slope of Alaska and are routinely monitored by the Toolik Lake Long-Term Ecological Research program (https://arc-lter.ecosystems.mbl.edu/). Imnavait Creek (68.61°N, 149.32°E) is a first-order headwater stream and was sampled on 23-June-2002. Toolik Lake (68.63°N, 149.59°E) is a 150 Ha, 25 m deep, oligotrophic kettle lake and was sampled on 19-June-2002. Isolation of the fulvic acid fraction of both DOM samples was previously described.[19] Elemental analysis and ^{13}C NMR of the isolates were previously reported.[19] Preparation of DOM used in the experimental work proceeded as follows: reconstitution of DOM in Milli-Q water at a target DOC concentration of 10 mg C L^{-1},[7,8] pH adjustment to 7.0 ± 0.1 using dilute sodium hydroxide, equilibration with the atmosphere overnight on a stir-plate at room temperature, and filtration (pre-combusted GF/F, nominal 0.7 μm, Whatman).

2.2 DOC concentration and optical characterization of DOM

Dissolved organic carbon concentration (DOC) was quantified as CO_2 after high-temperature combustion using a Shimadzu 5000A TOC analyzer.[20] Absorption spectra were collected using a 1 cm pathlength UV-visible spectrophotometer (Aqualog; Horiba Scientific). Naperian absorption coefficients were calculated by multiplying absorbance (A) by 2.303 and dividing by the pathlength (m) of the quartz cuvette. Specific UV-visible absorbance as 254 nm (SUVA$_{254}$; L mg C^{-1} m^{-1}) were calculated as previously described.[21] The spectral slope ratio was calculated as the ratio of the slope from 275 to 295 nm to the slope from 350 to 400 nm.[22] Protocols for collecting DOM fluorescence excitation-emission matrices are previously described.[23–25] All bulk measurements (DOC and optical spectroscopy) were conducted prior to PPL solid-phase extraction for FT-ICR MS analysis.

2.3 Photochemical O_2 consumption and CO_2 production

Air-equilibrated DOM was transferred to pre-combusted, air-tight 12 mL borosilicate vials with no headspace (Labco, Inc.; catalog # 9RK8W). Light transmission of the vials is ≥70% at 320 nm.[26] The vials were placed horizontally in an Atlas XLS + solar simulator along the horizontal profile of the long-arc lamp, which minimized irradiance variability in the chamber. During the photo-exposure the temperature of the vials was maintained at 20 °C, similar to the room temperature outside of the chamber where the foil-wrapped dark controls were kept. The exposure time was six hours, equivalent to ~15 hours of natural clear-sky, mid-day June sunlight at Toolik Field Station, AK.[9] Consistent with previous studies,[4,6] addition of catalase following light exposure (200 units per mL) did not result in detectable differences in dissolved O_2 (i.e., <1 μM O_2 production from H_2O_2 decomposition). These results indicate that H_2O_2 production had no detectable influence on photochemical O_2 consumption to CO_2 production ratios reported in this study.

This journal is © The Royal Society of Chemistry 2020

Environ. Sci.: Processes Impacts, 2020, **22**, 1214–1223 | **1215**

The short exposure time was selected to ensure the detection of a change in DOM chemical composition, photochemical O_2 consumption, and photochemical CO_2 production, while minimizing the amount of time that DOM composition is altered in the absence of microbes. Photodegradation of DOM in sunlit waters occurs in the presence of microbes, and the interactions between sunlight and microbes in arctic surface waters are well documented.[27] Moreover, there is currently no evidence for large shifts in photochemical O_2 consumption to CO_2 production ratios over long periods of sunlight exposure, upwards of 21 days.[28] Consistently, kinetic isotope fractionation of O_2 during photochemical oxidation of DOM is constant with increasing irradiation time (upwards of two weeks of natural sunlight exposure), indicating that some processes of DOM photodegradation remain the same with increasing light exposure.[29] Therefore, the results from our short experimental exposure times should apply to the relatively short residence times of DOM in sunlit surface waters of the Arctic.[7,30]

Following irradiation, photochemical CO_2 production and O_2 consumption was quantified. CO_2 production was quantified as the light minus dark difference in dissolved inorganic carbon (AS-C3 DIC analyzer; Apollo SciTech, Inc.). Oxygen consumption was quantified as the dark minus light difference in dissolved oxygen using membrane inlet mass spectrometry (MIMS; Bay Instruments, Inc.).[31]

2.4 Photochemical incorporation of $^{18}O_2$ into DOM

Six mL of DOM was placed into four pre-combusted, air-tight 12 mL borosilicate vials and purged with ultra-high purity N_2 until O_2 (mass 32) was non-detectable using MIMS. Ten cm^3 of 97 atom percent $^{18}O_2$ (Sigma Aldrich) was injected into two of the four vials (referred to as "$^{18}O_2$ treatment"). Monitoring of masses 32 ($^{16}O_2$) and 36 ($^{18}O_2$) on the MIMS revealed that 91% of total O_2 in the $^{18}O_2$ treatment experimental vial (i.e., $^{16}O_2$ + $^{18}O_2$) was isotopically labelled. Ten cm^3 of lab air was injected into the two remaining vials (referred to as "$^{16}O_2$ treatment"). One vial of each treatment was kept in the dark, while the other vial of each treatment was exposed to six hours of simulated sunlight, as described above. The $^{18}O_2$ treatments were made by addition of pure O_2 while the $^{16}O_2$ treatments were made by addition of lab air (~20% O_2). It is unlikely that differences in initial O_2 concentrations between these treatments impacted the products of photo-oxidation because O_2 was not limiting, as indicated by ~20 μM O_2 consumption for samples equilibrated with lab air (<10% depletion; Fig. 1). Following the exposure, the DOM was acidified with trace metal grade HCl, placed on ice and shipped overnight to the Environmental Molecular Sciences Laboratory (EMSL) at the Pacific Northwest National Laboratory for high resolution mass spectrometry analysis.

Prior to analysis, the DOM was loaded onto PPL solid-phase cartridges (Agilent) to minimize ionization suppression by residual salts that were incompletely removed during the XAD-8 isolation. Due to limited volume, the extraction efficiency of DOM by the PPL solid-phase was not quantified. Previous studies of nearly a dozen DOM sources from Alaskan Arctic surface and soil waters reported approximately 60% extraction

Fig. 1 Photochemical oxygen consumption and carbon dioxide production by Imnavait Creek (left) and Toolik Lake (right) DOM. Error bars represent one standard deviation from the mean ($N = 3$).

efficiency by PPL solid-phase.[6,9,25] The DOM PPL extracts were analyzed using a 12 T Bruker SolariX FT-ICR mass spectrometer. The DOM extracts were ionized using electrospray ionization conducted in negative mode. Elemental formulas were assigned to internally calibrated masses followed previously described approaches,[4–6,9,25] with one important exception. Elemental formulas including ^{16}O were initially assigned (considering C, H, O, N, and S), and then the unassigned masses were subjected to additional formula assignment including ^{18}O. Only formulas that agreed within an error of <0.5 ppm to the calculated exact mass of the formula were accepted. Formulas were classified as aromatic using the modified aromaticity index (AI_{MOD}).[32] Formulas were classified as CRAM if: DBE/C = 0.30–0.68, DBE/H = 0.20–0.95, and DBE/O = 0.77–1.75.[33] ^{18}O-containing photo-products were only detected in the light-exposed $^{18}O_2$ treatment, indicating that isotopically labelled $^{18}O_2$ and irradiation were required to yield ^{18}O-containing photo-products. Due to the cost of $^{18}O_2$ and limited FT-ICR MS instrument time, replicates of $^{18}O_2$ treatments and controls were not conducted. Previous work on FT-ICR MS of SPE-DOM demonstrated high reproducibility in peak intensity and formula assignments from terrestrially-derived DOM leached from soils within the Imnavait Creek watershed.[25]

3. Results

3.1 Chemical composition of Imnavait Creek and Toolik Lake DOM

Although both Imnavait Creek and Toolik Lake DOM are terrestrially-derived, there were notable differences in their chemical compositions. Optical spectroscopy and high-resolution mass spectrometry analyses indicated that Imnavait Creek DOM was more aromatic and had a higher average molecular weight than Toolik Lake DOM. Specific ultraviolet absorbance at 254 nm ($SUVA_{254}$), average double bond equivalents normalized to carbon number, and AI_{MOD} was higher for Imnavait Creek compared to Toolik Lake DOM indicating that Imnavait Creek DOM was more aromatic than Toolik Lake DOM (Tables 1 and 2). Slope ratio was lower for Imnavait Creek compared to Toolik Lake DOM (Table 1) consistent with

1216 | Environ. Sci.: Processes Impacts, 2020, 22, 1214–1223

This journal is © The Royal Society of Chemistry 2020

Table 1 Dissolved gas concentrations and bulk properties of dark-control and light-exposed Imnavait Creek and Toolik Lake DOM. O_2 = dissolved oxygen, DIC = dissolved inorganic carbon, $O_2 : CO_2$ = photochemical O_2 consumption to CO_2 production ratio, DOC = dissolved organic carbon, $SUVA_{254}$ = specific UV absorbance at 254 nm, INT CDOM = naperian absorbance integrated from $300-400$ nm, S_R = slope ratio, INT fluor = integrated fluorescence from $240-600$ nm (after removing first and second order Rayleigh and Raman scattering peaks), and FI = fluorescence index. $\pm$ 1SD from the mean of triplicates

Treatment	Units	Imnavait Creek		Toolik Lake	
		Dark	Light	Dark	Light
O_2	(μM)	274 $\pm$ <1	255 $\pm$ <1	272 $\pm$ <1	255 $\pm$ <1
DIC	(μM)	60 $\pm$ 1	77 $\pm$ 1	55 $\pm$ <1	64 $\pm$ 1
$O_2 : CO_2$	—		1.1 $\pm$ 0.1		2.0 $\pm$ 0.1
DOC	(μM)	839	—	1046	—
$SUVA_{254}$	(L mg C^{-1} m^{-1})	4.1 $\pm$ <0.1	—	3.7 $\pm$ <0.1	—
INT CDOM	(m^{-1})	3157 $\pm$ 33	2702 $\pm$ 244	2435 $\pm$ 35	2167 $\pm$ 4
S_R	—	0.68 $\pm$ 0.01	0.75 $\pm$ <0.01	0.79 $\pm$ 0.01	0.86 $\pm$ <0.01
INT fluor	(RU)	19 942 $\pm$ 74	13 131 $\pm$ 74	20 931 $\pm$ 503	15 657 $\pm$ 706
FI	—	1.40 $\pm$ 0.01	1.26 $\pm$ 0.01	1.37 $\pm$ <0.01	1.32 $\pm$ <0.01

Table 2 Average chemical characteristics of (i) all formulas detected within Imnavait Creek DOM, (ii) formulas within Imnavait Creek DOM that were produced by sunlight and contained ^{18}O, (iii) all formulas detected within Toolik Lake DOM, (iv) formulas within Toolik Lake DOM that were produced by sunlight and contained ^{18}O, (v) the common subset of formulas detected in both (ii) and (iv), and (vi) singlet oxidation photo-products detected within Suwanee River and Pony Lake fulvic acid.[4] Mass = daltons, C # = the number of carbon atoms, O/C = atomic oxygen to carbon ratio, H/C = atomic hydrogen to carbon ratio, DBE = double bond equivalents, DBE/C = double bond equivalents normalized to carbon number, and AI_{MOD} = modified aromaticity index. $\pm$ 1SD from the mean of all formulas

DOM sources	N	Mass	C #	O/C	H/C	DBE	DBE/C	AI_{MOD}
(i) Imnavait	3187	494 $\pm$ 126	24 $\pm$ 7	0.45 $\pm$ 0.20	1.00 $\pm$ 0.31	14 $\pm$ 6	0.55 $\pm$ 0.16	0.41 $\pm$ 0.23
(ii) Imnavait ^{18}O	379	348 $\pm$ 79	16 $\pm$ 5	0.53 $\pm$ 0.15	1.03 $\pm$ 0.27	9 $\pm$ 3	0.40 $\pm$ 0.18	0.40 $\pm$ 0.18
(iii) Toolik Lake	3032	440 $\pm$ 108	24 $\pm$ 7	0.33 $\pm$ 0.19	1.09 $\pm$ 0.38	12 $\pm$ 6	0.51 $\pm$ 0.19	0.39 $\pm$ 0.28
(iv) Toolik Lake ^{18}O	287	382 $\pm$ 111	20 $\pm$ 7	0.39 $\pm$ 0.12	1.19 $\pm$ 0.23	9 $\pm$ 4	0.47 $\pm$ 0.12	0.34 $\pm$ 0.14
(v) IMN + TL ^{18}O common	159	353 $\pm$ 62	17 $\pm$ 4	0.44 $\pm$ 0.12	1.15 $\pm$ 0.19	8 $\pm$ 2	0.49 $\pm$ 0.10	0.34 $\pm$ 0.13
(vi) Singlet oxidation products	167	442 $\pm$ 30	23 $\pm$ 2	0.41 $\pm$ 0.10	1.22 $\pm$ 0.16	10 $\pm$ 2	0.44 $\pm$ 0.08	0.29 $\pm$ 0.09

a higher average molecular weight for Imnavait Creek compared to Toolik Lake DOM as determined by high-resolution mass spectrometry (Table 2).

For both DOM sources, sunlight degraded chromophores and fluorophores within the DOM pool. Exposing Imnavait Creek DOM to sunlight decreased integrated absorbance by 14 $\pm$ 8%, integrated fluorescence by 34 $\pm$ 1%, and fluorescence index by 10 $\pm$ 1%, and increased slope ratio by 10 $\pm$ 1% (Table 1). Exposing Toolik Lake DOM to sunlight decreased integrated absorbance by 11 $\pm$ 1%, integrated fluorescence by 25 $\pm$ 4%, and fluorescence index by 4 $\pm$ 1%, and increased slope ratio by 10 $\pm$ 2% (Table 1).

3.2 Photochemical O_2 consumption and CO_2 production

Exposure of Imnavait Creek DOM to sunlight resulted in a similar amount of O_2 consumption as CO_2 production (Fig. 1, $p = 0.14$, two-tailed paired Students t-test; O_2 consumption = 18 $\pm$ <1 μM, CO_2 production = 17 $\pm$ 1 μM, $N = 3$, $\pm$SD). In contrast, Toolik Lake DOM consumed two-fold greater O_2 than CO_2 it produced (Fig. 1, $p = 0.002$, two-tailed paired Students t-test; O_2 consumption = 18 $\pm$ <1 μM, CO_2 production = 9 $\pm$ 1 μM, $N = 3$, $\pm$SD). Accordingly, the ratio of photochemical O_2 consumption to CO_2 production was significantly lower for Imnavait Creek DOM compared to Toolik Lake DOM (p-value = 0.001, two-tailed

unpaired Students t-test; Imnavait Creek = 1.1 $\pm$ 0.1, Toolik Lake = 2.0 $\pm$ 0.1, $N = 3$, $\pm$SD).

3.3 Detection of ^{18}O photo-products using high resolution mass spectrometry

No masses corresponding to ^{18}O-containing photo-products were detected in ^{16}O dark-control and light-exposed treatments. In contrast, in the ^{18}O treatments hundreds of ^{18}O-containing formulas were produced by sunlight and detected within the Imnavait Creek and Toolik Lake DOM mass spectra (Fig. 2; Table 2). On a number basis, ^{18}O photo-products accounted for $\sim$10% of total formulas detected in each DOM after exposure to sunlight. The majority of ^{18}O photo-products contained one ^{18}O atom rather than two atoms (Imnavait Creek = 69%, Toolik Lake = 80%). The majority (>75%) of ^{18}O-formulas contained only CHO for both Imnavait Creek and Toolik Lake DOM. ^{18}O was incorporated into Imnavait Creek DOM that was more oxidized (higher O/C), more conjugated (higher DBE/C), and more aromatic (higher AI_{MOD}) than Toolik Lake DOM (Fig. 3, Table 2). DOM partial photo-oxidation products primarily classified as carboxylic-rich alicyclic molecules (CRAM),[33,34] comprising 61 and 83% of ^{18}O photo-products detected in Imnavait Creek and Toolik Lake DOM, respectively.

This journal is © The Royal Society of Chemistry 2020

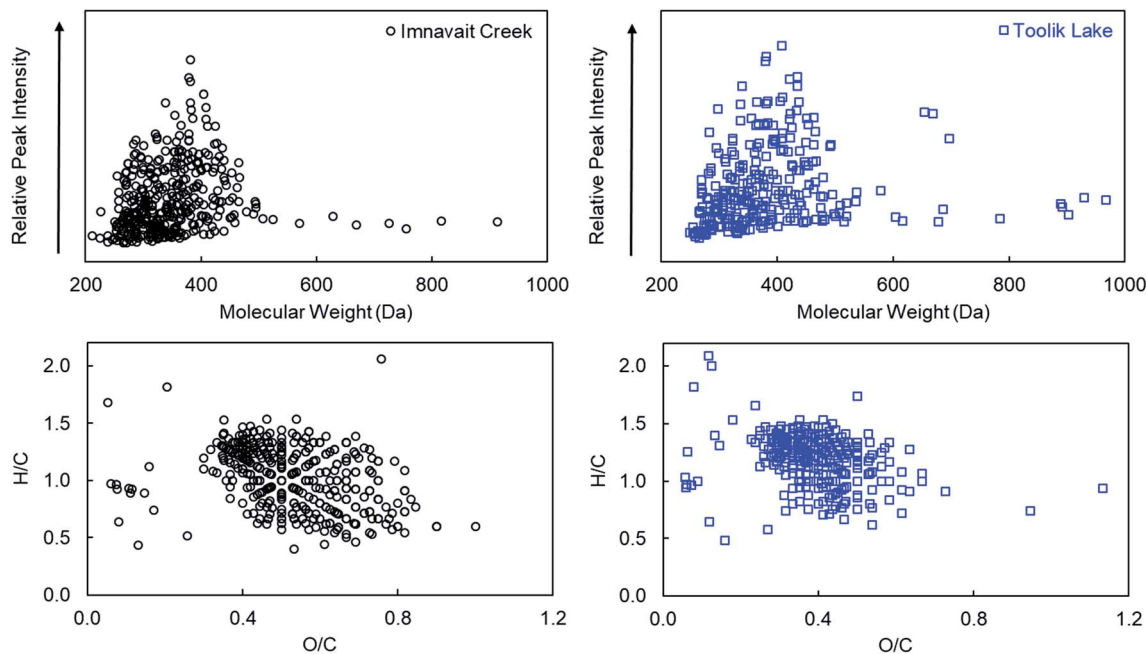Environ. Sci.: Processes Impacts, 2020, 22, 1214–1223 | 1217

Fig. 2 Molecular-level compositional characteristics of ^{18}O-containing products after exposing Imnavait Creek (black circles) and Toolik Lake DOM (blue squares) to sunlight. Top: molecular weight *vs.* relative peak intensity of ^{18}O photo-products. Bottom: van Krevelen plots of ^{18}O photo-products.

The average compositional characteristics of ^{18}O photo-products detected in both Imnavait Creek and Toolik Lake DOM spanned a narrower range of H/C and O/C ratios in van Krevelen space compared to all formulas detected in each DOM (Fig. 3; Table 2). Imnavait Creek ^{18}O photo-products generally clustered from O/C 0.4–0.8 and H/C 0.5–1.4, while all Imnavait Creek formulas spanned a wider range from O/C 0.1–0.9 and H/C 0.4–1.8. Toolik Lake ^{18}O photo-products generally clustered from O/C 0.3–0.6 and H/C 0.6–1.5, while all Toolik Lake formulas spanned a wider range from O/C 0.1–0.9 and H/C 0.2–2.0.

4. Discussion

4.1 Evidence for partial photo-oxidation of DOM

Three lines of evidence indicate that the ^{18}O-containing formulas categorized as partial oxidation products were produced by sunlight. First, there were no masses categorized as ^{18}O-containing photo-products in the ^{16}O dark-controls and light-exposed treatments, indicating that $^{18}O_2$ and sunlight exposure was required to yield ^{18}O-containing photo-products. Second, no masses corresponding to ^{18}O-containing photo-products were detected in the $^{18}O_2$ dark controls. This result is expected because isotopic exchange does not occur between O_2 and organically bound oxygen within DOM, and auto-oxidation of DOM is negligible. Third, the ^{18}O-containing photo-products had similar chemical compositions as formulas produced by the partial photo-oxidation of other sources of terrestrial DOM, specifically Suwannee River DOM[4] and Great Dismal Swamp DOM.[18] Together, these results demonstrate that the ^{18}O-containing formulas detected in this study were products of partial photo-oxidation formed through

the incorporation of $^{18}O_2$ into DOM, consistent with the partial photo-oxidation of DOM by reactive oxygen species.[3,4,18]

There are several reasons why the number of ^{18}O-containing photo-products identified by high resolution mass spectrometry is conservative. First, ^{18}O-containing photo-products were identified on DOM following SPE-PPL extraction, during which some relatively hydrophilic partially-oxidized DOM may not have been retained.[25,35] Second, a substantial fraction of high O/C compounds are likely not detected by FT-ICR MS analysis of DOM.[25,35] Third, others have estimated that about 20% of photo-oxidized DOM of terrestrial origin are low molecular weight compounds such as acetate,[36] which would not be detected by FT-ICR MS. Thus, we infer that the ^{18}O-containing photo-products identified in this study provide a conservative assessment of the number and type of compounds produced by partial photo-oxidation of DOM.

4.2 Evidence for singlet oxidation of DOM

Singlet oxygen ($^1O_2$) is hypothesized to be an important oxidant of DOM in sunlit surface waters. Using DOM derived from Sigma Aldrich humic acid, it was first reported that steady state concentrations of ($^1O_2$) are orders of magnitude higher in the hydrophobic microenvironments of DOM compared to the bulk aqueous phase.[37] These results were later corroborated using Suwannee River and Pony Lake fulvic acids (SRFA and PLFA, respectively).[38] Subsequent studies revealed that under conditions that artificially sensitize the formation of $^1O_2$, a wide range of DOM sources (including SRFA and PLFA) are susceptible to singlet oxidation,[4,18] at rates independent of DOM source.[4]

The results from this study add additional support to the hypothesis that $^1O_2$ is an important oxidant of DOM. Imnavait

1218 | *Environ. Sci.: Processes Impacts*, 2020, **22**, 1214–1223

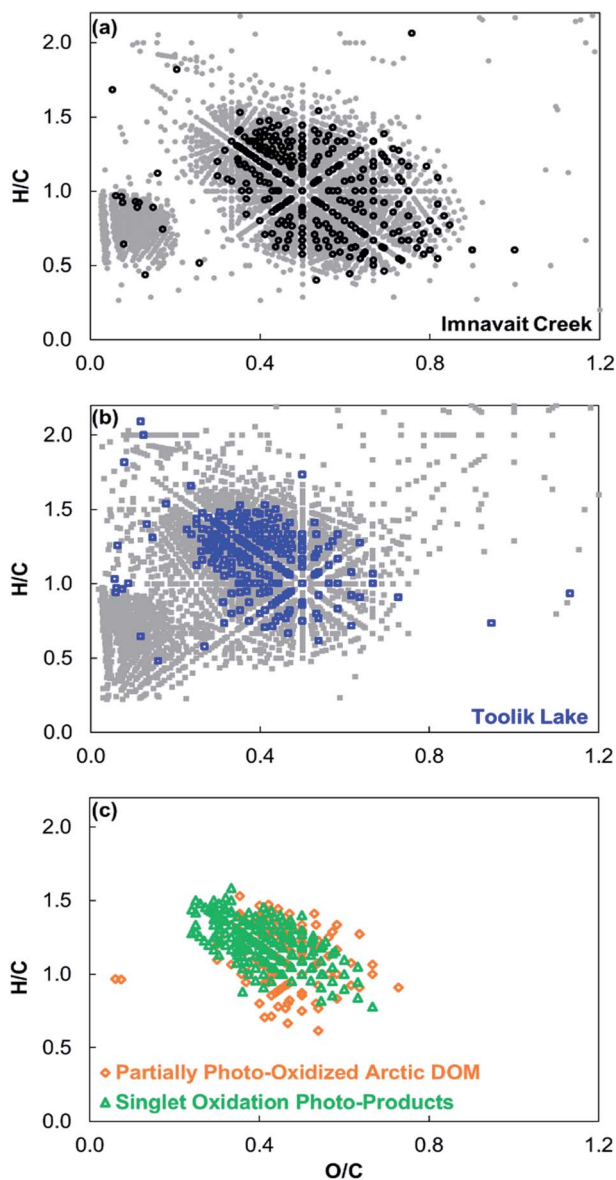This journal is © The Royal Society of Chemistry 2020

Fig. 3 (a) Comparison of ^{18}O-containing photo-products for Imnavait Creek DOM (foreground black circles) to all formulas detected in Imnavait Creek DOM (background grey circles). (b) Comparison of ^{18}O-containing photo-products for Toolik Lake DOM (foreground blue squares) to all formulas detected in Toolik Lake DOM (background grey squares). (c) Comparison of ^{18}O-containing formulas produced by sunlight in both Imnavait Creek and Toolik Lake DOM (orange diamonds) *versus* ^{18}O-containing formulas produced through the singlet oxidation of Suwannee River and Pony Lake Fulvic Acid (green triangles[4]).

Creek and Toolik Lake ^{18}O photo-products overlapped in chemical composition with singlet oxidation products of SRFA and PLFA (Fig. 3; Table 2),[4] and lignin-derived DOM from the Great Dismal Swamp.[18] In van Krevelen space (*i.e.*, O/C and H/C) there is considerable overlap in chemical composition between Imnavait Creek and Toolik Lake ^{18}O photo-products and singlet oxidation products of SRFA and PLFA (Fig. 3). Accordingly, the majority of Imnavait Creek and Toolik Lake ^{18}O photo-products

are classified as CRAM, similar to singlet oxidation products of SRFA and PLFA.[4] The overlap in products produced by photo-oxidation in this study with those known to result from $^1O_2$ (i) support prior evidence for singlet oxidation of DOM as a potential pathway to form CRAM,[4] a class of DOM detected ubiquitously in surface waters,[33,34] and (ii) contributes to the mounting body of evidence that $^1O_2$ may be an important oxidant of DOM in sunlit surface waters.

Alternative DOM partial oxidation pathways include reaction with hydroxyl radical ($^{\bullet}$OH) and superoxide ($O_2^{\bullet -}$). Photochemical production of $^{\bullet}$OH in arctic surface waters has previously been reported to account for up to 4% of total photochemical mineralization of DOM to CO_2.[15] Considering that photochemical O_2 consumption to CO_2 production ratios are ≥ 1, and conservatively assuming that 50% of hydroxyl radical production comes from an O_2 dependent pathway,[39] it is very unlikely that $^{\bullet}$OH contributes substantially to the partial photo-oxidation of DOM in arctic surface waters. The role of $O_2^{\bullet -}$ in the photo-oxidation of arctic DOM has never been tested. However, unlike the substantial overlap between ^{18}O photo-products reported in this study and $^1O_2$ products of lignin-derived DOM from the Great Dismal Swamp,[18] the overlap between ^{18}O photo-products and $O_2^{\bullet -}$ oxidation products of lignin-derived DOM is low.[18] This qualitative assessment suggests that $O_2^{\bullet -}$ likely does not play a major role in the oxidation of Imnavait Creek or Toolik Lake DOM reported in this study. However, given the well-established limitations of electrospray FT-ICR MS, such as SPE extraction and ionization efficiency, this conclusion warrants further testing with more quantitative approaches and DOM spanning a broader range of compositions than in this study.

4.3 Evaluating the potential for underestimation of DOM partial photo-oxidation

A key assumption to the current approach to quantify partial photo-oxidation of DOM[1] is that one mol of O_2 is photochemically consumed per mol of CO_2 produced. A stoichiometry of 1 mol O_2 consumed per mol of CO_2 produced for photo-oxidation of DOM has been assumed because the average oxidation state of DOM is likely close to zero[40] and the oxidation state of CO_2 is +4. In cases where photochemical O_2 consumption is greater than CO_2 production (*e.g.*, freshwaters),[1] the excess O_2 consumed is interpreted as molecular incorporation into DOM (*i.e.*, partial photo-oxidation). Therefore, in cases where O_2 consumption is equal to or less than CO_2 production (*e.g.*, headwaters streams; Fig. 1), partial photo-oxidation of DOM is assumed to be negligible. Consistent with our previous findings,[1,6,7] the stoichiometry of photochemical O_2 consumption per CO_2 production from Imnavait Creek DOM was ~ 1 (Fig. 1), suggesting that it had low or no susceptibility to partial photo-oxidation. However, hundreds of ^{18}O photo-products were detected, indicating that Imnavait Creek DOM was susceptible to partial photo-oxidation despite its low photochemical O_2 to CO_2 ratio (Fig. 2 and 3, Table 2). The only way to explain this discrepancy is that less than one mol of O_2 is photochemically consumed per mol of CO_2 produced.

Photo-decarboxylation of DOM to CO_2 is a plausible explanation for the low O_2 requirement for CO_2 production from Imnavait Creek DOM. Photo-decarboxylation (eqn (1)) is a reaction where organic acids are oxidized to CO_2 in the presence of sunlight with relatively low O_2 consumption.

$$R\text{-}COOH + (XO_2) \rightarrow R\text{-}H + CO_2 \tag{1}$$

Estimates from model compounds and experimental manipulations suggest that photo-decarboxylation reactions require 0 to 0.5 mol O_2 per mol of CO_2 produced,[28,41–43] much lower than currently assumed for DOM.[1] Photo-decarboxylation has been implicated in the photo-oxidation of terrestrial DOM to CO_2.[6,11,28,42,44] Quantitatively, photo-decarboxylation is estimated account for 40–90% of Imnavait Creek DOM mineralized to CO_2 (ref. 6) and up to 100% of temperate river DOM converted to CO_2.[28]

The low O_2 requirement of photo-decarboxylation reactions has substantial implications for quantifying the magnitude of DOM partial photo-oxidation. For example, assuming (i) a typical ratio of photochemical O_2 consumption to CO_2 production of 1.5 (ref. 1) (between the ratios observed for Imnavait Creek and Toolik Lake DOM in Fig. 1 and Table 1), (ii) that photo-decarboxylation accounts for 10 to 100% of CO_2 production, and (iii) that photo-decarboxylation requires 0.5 mol O_2 per mol of CO_2, partial photo-oxidation is underestimated by 1.3 to 4-fold, respectively (relative to a scenario where no CO_2 production comes from photo-decarboxylation; Table S1†). This underestimation of partial photo-oxidation decreases as the relative importance of partial to complete photo-oxidation increases. For example, as partial photo-oxidation increases relative to complete photo-oxidation to CO_2, as indicated by a high photochemical O_2 consumption to CO_2 production ratio of ∼5, partial photo-oxidation is underestimated only by 4 to 38% (Table S1†). In other words, when partial photo-oxidation dominates DOM photodegradation, any assumption made about the O_2 needed for complete oxidation to CO_2 has a relatively small impact on the quantification of partial photo-oxidation.

5. Environmental implications and remaining knowledge gaps

The results of this study challenge a common assumption made to estimate partial photo-oxidation of DOM in surface waters, and, in turn, reveal that the magnitude of partial photo-oxidation of DOM could be underestimated by up to 4-fold (Table S1†). The identification of ^{18}O-containing partial photo-oxidation products (Fig. 2 and 3) at a 1 : 1 ratio of photochemical O_2 consumption per CO_2 production from DOM (Fig. 1) demonstrates that less than 1 mol O_2 may be required to produce 1 mol CO_2. Therefore, quantification of partial photo-oxidation as the O_2 consumption in excess of photochemical CO_2 production (based on the assumed 1 : 1 stoichiometry) may result in underestimation of partial photo-oxidation.

This finding implies that estimates of partial photo-oxidation of DOM have been too conservative, particularly in

high iron freshwaters of the Arctic[7,15] where there is almost always less photochemical O_2 consumption than CO_2 production.[1,6,7] Iron is hypothesized[28] to catalyze the photo-decarboxylation of DOM to CO_2 (by ligand-to-metal charge transfer reactions); consistent with less O_2 consumption than CO_2 production in high iron waters of the Arctic. There was likely relatively less influence of iron on the ratio of O_2 consumption to CO_2 production for the fulvic acid fraction of DOM in this study compared to the DOM in the source waters due to loss of iron during the extraction process. Thus, in high iron waters of the Arctic,[1,6,7] when photochemical O_2 consumption is less than or equivalent to CO_2 production our results imply that partial photo-oxidation is altering the composition of the DOM.

The results from this study suggest that partial photo-oxidation of DOM is a widespread and underestimated process influencing DOM composition in arctic surface waters. For example, even when using the assumptions shown here to be markedly conservative, partial photo-oxidation dominated the water-column processing of DOM in arctic freshwaters, occurring at rates up to 4-fold faster than photomineralization of DOM and 2 to 15-fold faster than rates of bacterial respiration.[1] Consequently, partial photo-oxidation of DOM, possibly involving singlet oxygen (Fig. 3), is a more important process controlling DOM composition in sunlit surface waters than previously understood. Therefore, studies of DOM photo-degradation that quantify only complete oxidation of DOM to CO_2 (i.e., photomineralization) are potentially missing partial photo-oxidation, a major process that alters the chemical composition of DOM remaining after sunlight exposure.

Results of this study suggest additional work is needed to address another key uncertainty used to quantify partial photo-oxidation of DOM. For example, partial photo-oxidation of DOM is quantified assuming that the average O/C of partial photo-oxidation products is 0.5, which in turn is based on the average O/C of bulk DOM (i.e., ∼0.5). Results from high resolution mass spectrometry qualitatively suggest a range of O/C of partially photo-oxidized DOM. For example, the average O/C of SRFA and PLFA singlet oxidation photo-products was 0.42 and 0.32, respectively.[4] In this study, the average O/C of partial photo-oxidation products for Imnavait Creek and Toolik Lake DOM was 0.53 and 0.39, respectively (Fig. 2, Table 2). These findings suggest that the average O/C of partial photo-oxidation production could deviate substantially from the assumed 0.5 value, and this deviation could have major implications for quantifying the magnitude of DOM partial photo-oxidation. However, given the widely documented biases of FT-ICR MS, such as SPE extraction and ionization efficiency, future research should prioritize quantification of the O/C stoichiometry of partial photo-oxidation products.

Partial photo-oxidation of DOM has been hypothesized as a pathway for the formation of CRAM given that formulas characterized as CRAM increase in abundance following photo-exposure.[4,6,10,11] In this study, the majority of ^{18}O-containing partial oxidation photo-products were classified as CRAM (Fig. 2) and overlapped in composition with previously reported photo-products known to result from [1]O_2 (Fig. 3).[4] Therefore,

1220 | Environ. Sci.: Processes Impacts, 2020, 22, 1214–1223

This journal is © The Royal Society of Chemistry 2020

our results provide indirect support for the hypothesized pathway of CRAM formation: partial photo-oxidation of DOM by singlet oxygen.

Understanding how partial photo-oxidation alters the chemical composition of DOM is important because DOM composition is a major control on microbial community structure and function.[8,9,45–51] In arctic lakes[46] and rivers,[47] DOM composition is one of the strongest predictors of microbial community composition. Likewise, minor photochemical alterations to DOM draining Arctic permafrost soils (<10% of the total C) substantially shifts microbial metabolic rates and pathways.[9] The influence of photochemical alterations of DOM on microbial processing of DOM likely applies to sunlit freshwaters outside the Arctic.[52] For example, less than one hour of sunlight exposure to temperate stream water had substantial impacts on microbial respiration of DOM due to changes in the chemical composition of DOM (*i.e.*, partial photo-oxidation).[51] Therefore, partial photo-oxidation of DOM may be a widespread process that impacts the structure and function of microbial communities in inland surface waters, a hypothesis that should be explicitly tested in future work. Partial photo-oxidation of DOM should be increasingly incorporated into experimental frameworks in order to accurately estimate its role in shaping DOM composition and driving biogeochemical processes in sunlit waters.

Conflicts of interest

The authors declare no conflicts of interest

Acknowledgements

References

1 R. M. Cory, C. P. Ward, B. C. Crump and G. W. Kling, Sunlight controls water column processing of carbon in arctic fresh waters, *Science*, 2014, **345**, 925–928.

2 S. Andrews, S. Caron and O. Zafiriou, Photochemical oxygen consumption in marine waters: a major sink for colored dissolved organic matter?, *Limnol. Oceanogr.*, 2000, **45**, 267–277.

3 R. M. Cory, J. B. Cotner and K. McNeill, Quantifying interactions between singlet oxygen and aquatic fulvic acids, *Environ. Sci. Technol.*, 2009, **43**, 718–723.

4 R. M. Cory, K. McNeill, J. P. Cotner, A. Amado, J. M. Purcell and A. G. Marshall, Singlet oxygen in the coupled photochemical and biochemical oxidation of dissolved organic matter, *Environ. Sci. Technol.*, 2010, **44**, 3683–3689.

5 C. P. Ward, R. L. Sleighter, P. G. Hatcher and R. M. Cory, Insights into the complete and partial photooxidation of black carbon in surface waters, *Environ. Sci.: Processes Impacts*, 2014, **16**, 721–731.

6 C. P. Ward and R. M. Cory, Complete and partial photo-oxidation of dissolved organic matter draining permafrost soils, *Environ. Sci. Technol.*, 2016, **50**, 3545–3553.

7 R. M. Cory, K. H. Harrold, B. T. Neilson and G. W. Kling, Controls on DOM degradation in a headwater stream: the influence of DOM amount and lability, light attenuation and exposure, *Biogeosciences*, 2015, **12**, 6669–6685.

8 R. M. Cory, B. C. Crump, J. A. Dobkowski and G. W. Kling, Surface exposure to sunlight stimulates CO_2 release from permafrost soil carbon in the Arctic, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 3429–3434.

9 C. P. Ward, S. G. Nalven, B. C. Crump, G. W. Kling and R. M. Cory, Photochemical alteration of organic carbon draining permafrost soils shifts microbial metabolic pathways and stimulates respiration, *Nat. Commun.*, 2017, **8**, 1–8.

10 M. Gonsior, N. Hertkorn, M. H. Conte, W. J. Cooper, D. Bastviken, E. Druffel and P. Schmitt-Kopplin, Photochemical production of polyols arising from significant photo-transformation of dissolved organic matter in the oligotrophic surface ocean, *Mar. Chem.*, 2014, **163**, 10–18.

11 M. Gonsior, P. Schmitt-Kopplin and D. Bastviken, Depth-dependent molecular composition and photo-reactivity of dissolved organic matter in a boreal lake under winter and summer conditions, *Biogeosciences*, 2013, **10**, 6945–6956.

12 R. L. Sleighter, R. M. Cory, L. A. Kaplan, H. A. N. Abdulla and P. G. Hatcher, A coupled geochemical and biogeochemical approach to characterize the bioreactivity of dissolved organic matter from a headwater stream, *J. Geophys. Res.: Biogeosci.*, 2014, **119**, 1520–1537.

13 B. A. Southworth and B. M. Voelker, Hydroxyl radical production via the photo-fenton reaction in the presence of fulvic acid, *Environ. Sci. Technol.*, 2003, **37**, 1130–1136.

14 E. M. White, P. P. Vaughan and R. G. Zepp, Role of the photo-Fenton reaction in the production of hydroxyl radicals and photobleaching of colored dissolved organic matter in a coastal river of the southeastern United States, *Aquat. Sci.*, 2003, **65**, 402–414.

15 S. E. Page, J. R. Logan, R. M. Cory and K. McNeill, Evidence for dissolved organic matter as the primary source and sink of photochemically produced hydroxyl radical in arctic surface waters, *Environ. Sci.: Processes Impacts*, 2014, **16**, 807–822.

16 J. V. Goldstone and B. M. Voelker, Chemistry of superoxide radical in seawater: CDOM associated sink of superoxide in coastal waters, *Environ. Sci. Technol.*, 2000, **34**, 1043–1048.

17 J. Ma, H. Zhou, S. Yan and W. Song, Kinetics studies and mechanistic considerations on the reactions of superoxide

This journal is © The Royal Society of Chemistry 2020

Environ. Sci.: Processes Impacts, 2020, **22**, 1214–1223 | **1221**

radical ions with dissolved organic matter, *Water Res.*, 2019, **149**, 56–64.

18 D. C. Waggoner, A. S. Wozniak, R. M. Cory and P. G. Hatcher, The role of reactive oxygen species in the degradation of lignin derived dissolved organic matter, *Geochim. Cosmochim. Acta*, 2017, **208**, 171–184.

19 R. M. Cory, D. M. McKnight, Y.-P. Chin, P. Miller and C. L. Jaros, Chemical characteristics of fulvic acids from Arctic surface waters: microbial contributions and photochemical transformations, *J. Geophys. Res.*, 2007, **112**, 1–14.

20 G. W. Kling, G. W. Kipphut, M. M. Miller and J. W. O'Brien, Integration of lakes and streams in a landscape perspective: the importance of material processing on spatial patterns and temporal coherence, *Freshwater Biol.*, 2000, **43**, 477–497.

21 J. L. Weishaar, G. R. Aiken, B. A. Bergamaschi, M. S. Fram, R. Fujii and K. Mopper, Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon, *Environ. Sci. Technol.*, 2003, **37**, 4702–4708.

22 J. R. Helms, A. Stubbins, J. D. Ritchie, E. C. Minor, D. J. Kieber and K. Mopper, Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter, *Limnol. Oceanogr.*, 2008, **53**, 955–969.

23 R. M. Cory and D. M. McKnight, Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter, *Environ. Sci. Technol.*, 2005, **39**, 8142–8149.

24 M. P. Miller, B. E. Simone, D. M. McKnight, R. M. Cory, M. W. Williams and E. W. Boyer, New light on a dark subject: comment, *Aquat. Sci.*, 2010, **72**, 269–275.

25 C. P. Ward and R. M. Cory, Chemical composition of dissolved organic matter draining permafrost soils, *Geochim. Cosmochim. Acta*, 2015, **167**, 63–79.

26 C. P. Ward, C. M. Sharpless, D. L. Valentine, D. P. French-McCay, C. Aeppli, H. K. White, R. P. Rodgers, K. M. Gosselin, R. K. Nelson and C. M. Reddy, Partial Photochemical Oxidation Was a Dominant Fate of Deepwater Horizon Surface Oil, *Environ. Sci. Technol.*, 2018, **52**, 1797–1805.

27 R. M. Cory and G. W. Kling, Interactions between sunlight and microorganisms influence dissolved organic matter degradation along the aquatic continuum, *Limnol. Oceanogr. Lett.*, 2018, **3**, 102–116.

28 H. Xie, O. C. Zafiriou, W.-J. Cai, R. G. Zepp and Y. Wang, Photooxidation and its effects on the carboxyl content of dissolved organic matter in two coastal rivers in the southeastern United States, *Environ. Sci. Technol.*, 2004, **38**, 4113–4119.

29 C. P. Ward, C. M. Sharpless, D. L. Valentine, C. Aeppli, K. M. Sutherland, S. D. Wankel and C. M. Reddy, Oxygen Isotopes (δ18O) Trace Photochemical Hydrocarbon Oxidation at the Sea Surface, *Geophys. Res. Lett.*, 2019, **46**, 6745–6754.

30 B. T. Neilson, M. B. Cardenas, M. T. O'Connor, M. T. Rasmussen, T. V. King and G. W. Kling, Groundwater Flow and Exchange Across the Land Surface Explain Carbon Export Patterns in Continuous Permafrost Watersheds, *Geophys. Res. Lett.*, 2018, **45**, 7596–7605.

31 T. M. Kana, C. Darkangelo and M. D. Hunt, Membrane inlet mass spectrometer for rapid high-precision determination of N_2, O_2, and Ar in environmental water samples, *Anal. Chem.*, 1994, **66**, 4166–4170.

32 B. P. Koch and T. Dittmar, From mass to structure: an aromaticity index for high-resolution mass data of natural organic matter, *Rapid Commun. Mass Spectrom.*, 2016, **30**, 250.

33 N. Hertkorn, R. Benner, M. Frommberger, P. Schmitt-Kopplin, M. Witt, K. Kaiser, A. Kettrup and J. I. Hedges, Characterization of a major refractory component of marine dissolved organic matter, *Geochim. Cosmochim. Acta*, 2006, **70**, 2990–3010.

34 B. Lam, A. Baer, M. Alaee, B. Lefebvre, A. Moser, A. Williams and A. J. Simpson, Major structural components in freshwater dissolved organic matter, *Environ. Sci. Technol.*, 2007, **41**, 8240–8247.

35 W. C. Hockaday, J. M. Purcell, A. G. Marshall, J. a. Baldock and P. G. Hatcher, Electrospray and photoionization mass spectrometry for the characterization of organic matter in natural waters: a qualitative assessment, *Limnol. Oceanogr.: Methods*, 2009, **7**, 81–95.

36 R. G. Wetzel, P. G. Hatcher and T. S. Bianchi, Natural photolysis by ultraviolet irradiance of recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism, *Limnol. Oceanogr.*, 1995, **40**, 1369–1380.

37 D. E. Latch, B. L. Stender, J. L. Packer, W. A. Arnold and K. McNeill, Photochemical fate of pharmaceuticals in the environment: cimetidine and ranitidine, *Environ. Sci. Technol.*, 2003, **37**, 3342–3350.

38 M. Grandbois, D. E. Latch and K. Mcneill, Microheterogeneous concentrations of singlet oxygen in natural organic matter isolate solutions, *Environ. Sci. Technol.*, 2008, **42**, 9184–9190.

39 S. E. Page, W. a. Arnold and K. McNeill, Assessing the contribution of free hydroxyl radical in organic matter-sensitized photohydroxylation reactions, *Environ. Sci. Technol.*, 2011, **45**, 2818–2825.

40 D. E. LaRowe and P. Van Cappellen, Degradation of natural organic matter: a thermodynamic analysis, *Geochim. Cosmochim. Acta*, 2011, **75**, 2030–2042.

41 M. Xu and P. Wan, Efficient photodecarboxylation of aroyl-substituted phenylacetic acids in aqueous solution: a general photochemical reaction, *Chem. Commun.*, 2000, 2147–2148.

42 C. Miles and P. Brezonik, Oxygen consumption in humic-colored waters by a photochemical ferrous-ferric catalytic cycle, *Environ. Sci. Technol.*, 1981, **15**, 1089–1095.

43 D. Budac and P. Wan, Photodecarboxylation: mechanism and synthetic utility, *J. Photochem. Photobiol., A*, 1992, **67**, 135–166.

44 Y. Gu, A. Lensu, S. Perämäki, A. Ojala and A. V. Vähätalo, Iron and pH Regulating the Photochemical Mineralization

1222 | *Environ. Sci.: Processes Impacts*, 2020, **22**, 1214–1223

This journal is © The Royal Society of Chemistry 2020

of Dissolved Organic Carbon, *ACS Omega*, 2017, **2**, 1905–1914.

45 V. Amaral, D. Graeber, D. Calliari and C. Alonso, Strong linkages between DOM optical properties and main clades of aquatic bacteria, *Limnol. Oceanogr.*, 2016, **61**, 906–918.

46 B. Crump, G. Kling, M. Bahr and J. Hobbie, Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source, *Appl. Environ. Microbiol.*, 2003, **69**, 2253–2268.

47 B. C. Crump, B. J. Peterson, P. A. Raymond, R. M. W. Amon, A. Rinehart, J. W. McClelland and R. M. Holmes, Circumpolar synchrony in big river bacterioplankton, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 21208–21212.

48 K. E. Judd, B. C. Crump and G. W. Kling, Variation in dissolved organic matter controls bacterial production and community composition, *Ecology*, 2006, **87**, 2068–2079.

49 D. L. Kirchman, A. I. Dittel, S. E. G. Findlay and D. Fischer, Changes in bacterial activity and community structure in response to dissolved organic matter in the Hudson River, New York, *Aquat. Microb. Ecol.*, 2004, **35**, 243–257.

50 H. J. Smith, M. Dieser, D. M. McKnight, M. D. SanClements and C. M. Foreman, Relationship between dissolved organic matter quality and microbial community composition across polar glacial environments, *FEMS Microbiol. Ecol.*, 2018, **94**, fiy090.

51 J. C. Bowen, L. A. Kaplan and R. M. Cory, Photodegradation disproportionately impacts biodegradation of semi-labile DOM in streams, *Limnol. Oceanogr.*, 2019, 1–14.

52 M. A. Moran and R. G. Zepp, Role of photoreactions in the formation of biologically compounds from dissolved organic matter, *Limnol. Oceanogr.*, 1997, **42**, 1307–1316.

This journal is © The Royal Society of Chemistry 2020

Environ. Sci.: Processes Impacts, 2020, **22**, 1214–1223 | **1223**