



Cite this: *Environ. Sci.: Processes Impacts*, 2020, 22, 1214

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

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In sunlit waters, photodegradation of dissolved organic matter (DOM) yields completely oxidized carbon (i.e.,  $\text{CO}_2$ ) 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  $\text{CO}_2$  (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  $^{18}\text{O}_2$  into DOM isolated from Alaskan Arctic surface waters using high-resolution mass spectrometry. Complete and partial photo-oxidation of DOM was also quantified as  $\text{CO}_2$  production and  $\text{O}_2$  consumption. The majority of  $^{18}\text{O}$ -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  $\text{O}_2$  is required to produce one mol of  $\text{CO}_2$ . 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.

Received 1st November 2019  
Accepted 10th February 2020

DOI: 10.1039/c9em00504h  
rsc.li/espi

### 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,<sup>1</sup> a process that alters the composition of DOM due to the incorporation of oxygen.<sup>1–6</sup> Partial photo-oxidation of DOM is quantified as the difference between photochemical  $\text{O}_2$  consumption and  $\text{CO}_2$  production by DOM, on a per mol C basis.<sup>1,2,5,6</sup> However, this approach to quantify partial photo-oxidation of DOM on a per mol C basis requires one key assumption about the  $\text{O}_2$  requirements for  $\text{CO}_2$  production (i.e., 1 mol  $\text{O}_2$  per mol  $\text{CO}_2$ ). Although this assumption is hypothesized to be conservative,<sup>1</sup> it has never been explicitly tested, raising the possibility that rates of DOM partial photo-oxidation may be systematically over or underestimated.

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



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.<sup>1,6,7</sup> However, other evidence suggests substantial changes in chemical composition of DOM in Imnavait Creek remaining after photodegradation.<sup>7,8</sup> 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).<sup>9</sup> 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.<sup>4,6,10,11</sup> Evidence suggests that CRAM is relatively recalcitrant to microbial degradation.<sup>12</sup> Thus, photochemical production of CRAM could contribute to some responses of microbes to photo-oxidized DOM.<sup>4</sup>

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,<sup>3,4,13-18</sup> 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.<sup>3,4</sup> 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.<sup>4,18</sup> 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.<sup>19</sup> Elemental analysis and  $^{13}C$  NMR of the isolates were previously reported.<sup>19</sup> Preparation of DOM used in the experimental work proceeded as follows: reconstitution of DOM in Milli-Q water at a target DOC concentration of  $10\text{ mg C L}^{-1}$ ,<sup>7,8</sup> pH adjustment to  $7.0 \pm 0.1$  using dilute sodium hydroxide, equilibration with the atmosphere overnight on a stir-plate at room temperature, and filtration (pre-combusted GF/F, nominal  $0.7\text{ }\mu\text{m}$ , 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.<sup>20</sup> 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  $254\text{ nm (SUVA}_{254}\text{; L mg C}^{-1}\text{ m}^{-1}\text{)}$  were calculated as previously described.<sup>21</sup> 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.<sup>22</sup> Protocols for collecting DOM fluorescence excitation-emission matrices are previously described.<sup>23-25</sup> 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  $\geq 70\%$  at 320 nm.<sup>26</sup> 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  $20\text{ }^\circ\text{C}$ , 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  $\sim 15$  hours of natural clear-sky, mid-day June sunlight at Toolik Field Station, AK.<sup>9</sup> Consistent with previous studies,<sup>4,6</sup> addition of catalase following light exposure (200 units per mL) did not result in detectable differences in dissolved  $O_2$  (*i.e.*,  $<1\text{ }\mu\text{M}$   $O_2$  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.



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.<sup>27</sup> 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.<sup>28</sup> 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.<sup>29</sup> 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.<sup>7,30</sup>

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.).<sup>31</sup>

#### 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<sup>3</sup> 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<sup>3</sup> 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  $\mu M$   $O_2$  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

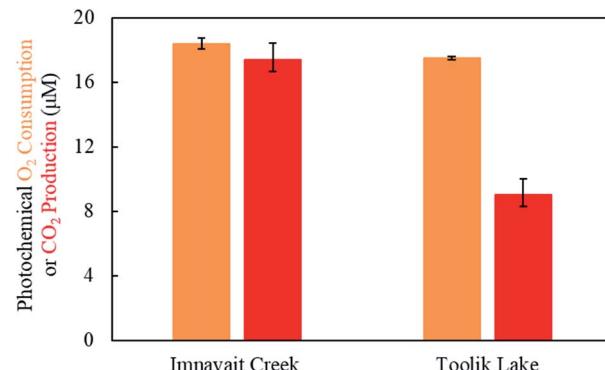


Fig. 1 Photochemical oxygen consumption and carbon dioxide production by Imnavait Creek (left) and Toolik Lake (right) DOM. Error bars represent one standard deviation from the mean ( $N = 3$ ).

efficiency by PPL solid-phase.<sup>6,9,25</sup> 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,<sup>4-6,9,25</sup> 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 ( $AI_{MOD}$ ).<sup>32</sup> Formulas were classified as CRAM if:  $DBE/C = 0.30-0.68$ ,  $DBE/H = 0.20-0.95$ , and  $DBE/O = 0.77-1.75$ .<sup>33</sup>  $^{18}O$ -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.<sup>25</sup>

## 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



**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<sub>254</sub> = 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
$O_2$	( $\mu M$ )	274 $\pm$ <1	255 $\pm$ <1	272 $\pm$ <1	255 $\pm$ <1
DIC	( $\mu M$ )	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	( $\mu M$ )	839	—	1046	—
SUVA <sub>254</sub>	( $L mg C^{-1} m^{-1}$ )	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.<sup>4</sup> 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<sub>MOD</sub> = modified aromaticity index.  $\pm 1SD$  from the mean of all formulas

DOM sources	<i>N</i>	Mass	C #	O/C	H/C	DBE	DBE/C	AI <sub>MOD</sub>
(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 =  $18 \pm <1 \mu M$ ,  $CO_2$  production =  $17 \pm 1 \mu M$ ,  $N = 3$ ,  $\pm SD$ ). 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 =  $18 \pm <1 \mu M$ ,  $CO_2$  production =  $9 \pm 1 \mu M$ ,  $N = 3$ ,  $\pm SD$ ). 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<sub>MOD</sub>) than Toolik Lake DOM (Fig. 3, Table 2). DOM partial photo-oxidation products primarily classified as carboxylic-rich alicyclic molecules (CRAM),<sup>33,34</sup> comprising 61 and 83% of  $^{18}O$  photo-products detected in Imnavait Creek and Toolik Lake DOM, respectively.



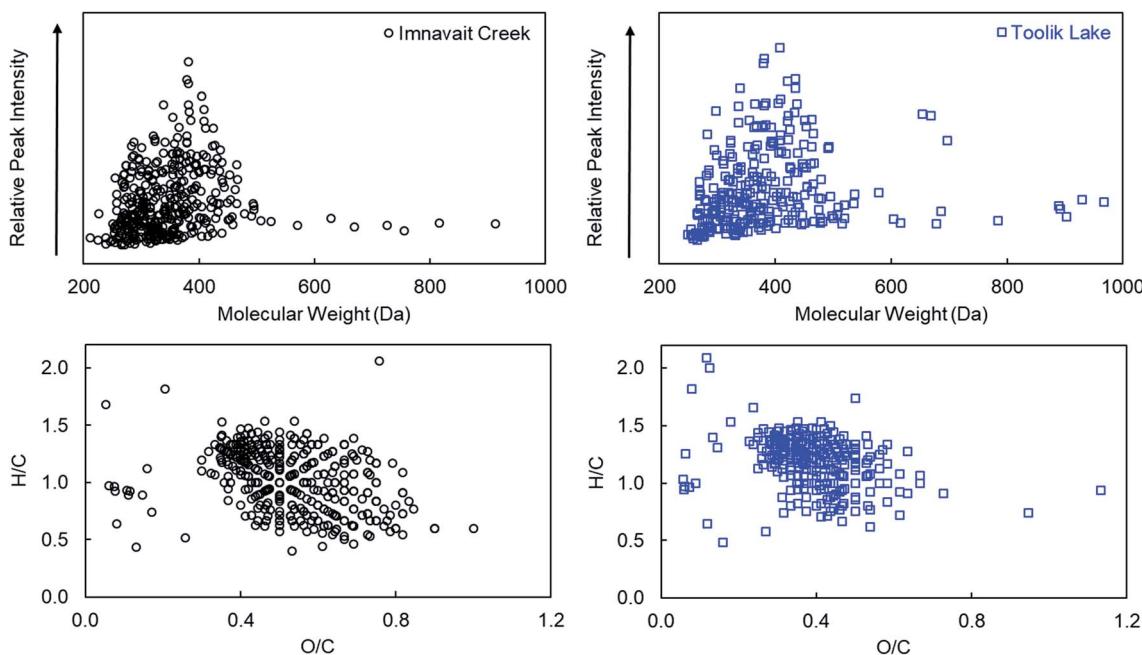


Fig. 2 Molecular-level compositional characteristics of  $^{18}\text{O}$ -containing products after exposing Imnavait Creek (black circles) and Toolik Lake DOM (blue squares) to sunlight. Top: molecular weight vs. relative peak intensity of  $^{18}\text{O}$  photo-products. Bottom: van Krevelen plots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

The average compositional characteristics of  $^{18}\text{O}$  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  $^{18}\text{O}$  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  $^{18}\text{O}$  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  $^{18}\text{O}$ -containing formulas categorized as partial oxidation products were produced by sunlight. First, there were no masses categorized as  $^{18}\text{O}$ -containing photo-products in the  $^{16}\text{O}$  dark-controls and light-exposed treatments, indicating that  $^{18}\text{O}_2$  and sunlight exposure was required to yield  $^{18}\text{O}$ -containing photo-products. Second, no masses corresponding to  $^{18}\text{O}$ -containing photo-products were detected in the  $^{18}\text{O}_2$  dark controls. This result is expected because isotopic exchange does not occur between  $\text{O}_2$  and organically bound oxygen within DOM, and auto-oxidation of DOM is negligible. Third, the  $^{18}\text{O}$ -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<sup>4</sup> and Great Dismal Swamp DOM.<sup>18</sup> Together, these results demonstrate that the  $^{18}\text{O}$ -containing formulas detected in this study were products of partial photo-oxidation formed through

the incorporation of  $^{18}\text{O}_2$  into DOM, consistent with the partial photo-oxidation of DOM by reactive oxygen species.<sup>3,4,18</sup>

There are several reasons why the number of  $^{18}\text{O}$ -containing photo-products identified by high resolution mass spectrometry is conservative. First,  $^{18}\text{O}$ -containing photo-products were identified on DOM following SPE-PPL extraction, during which some relatively hydrophilic partially-oxidized DOM may not have been retained.<sup>25,35</sup> Second, a substantial fraction of high O/C compounds are likely not detected by FT-ICR MS analysis of DOM.<sup>25,35</sup> Third, others have estimated that about 20% of photo-oxidized DOM of terrestrial origin are low molecular weight compounds such as acetate,<sup>36</sup> which would not be detected by FT-ICR MS. Thus, we infer that the  $^{18}\text{O}$ -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 ( $^1\text{O}_2$ ) 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 ( $^1\text{O}_2$ ) are orders of magnitude higher in the hydrophobic microenvironments of DOM compared to the bulk aqueous phase.<sup>37</sup> These results were later corroborated using Suwannee River and Pony Lake fulvic acids (SRFA and PLFA, respectively).<sup>38</sup> Subsequent studies revealed that under conditions that artificially sensitize the formation of  $^1\text{O}_2$ , a wide range of DOM sources (including SRFA and PLFA) are susceptible to singlet oxidation,<sup>4,18</sup> at rates independent of DOM source.<sup>4</sup>

The results from this study add additional support to the hypothesis that  $^1\text{O}_2$  is an important oxidant of DOM. Imnavait



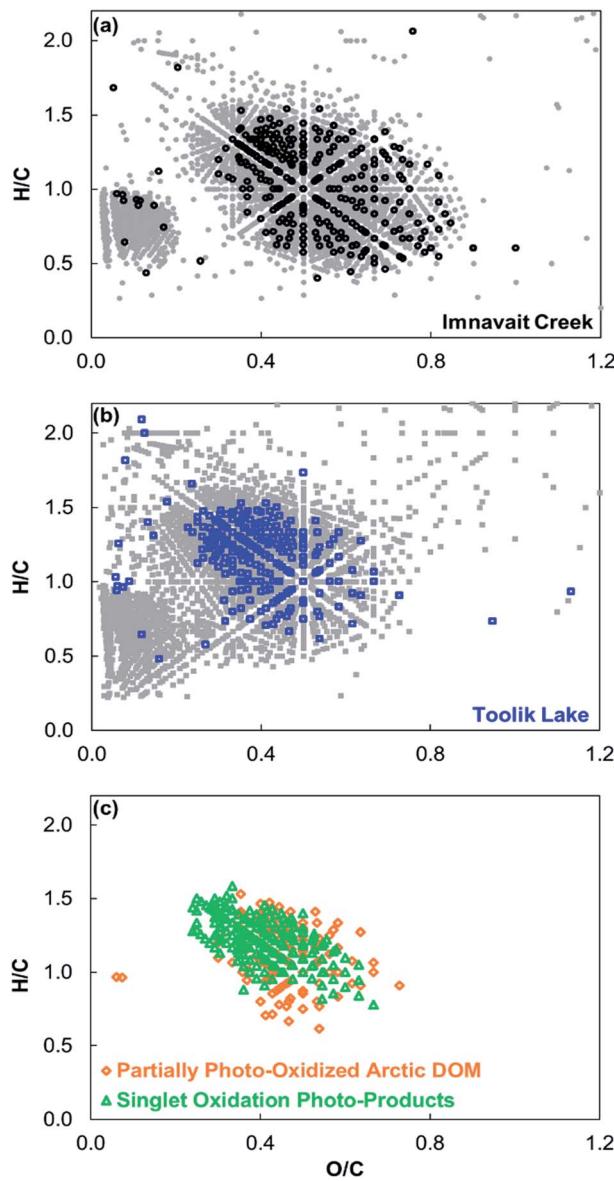


Fig. 3 (a) Comparison of  $^{18}\text{O}$ -containing photo-products for Imnavait Creek DOM (foreground black circles) to all formulas detected in Imnavait Creek DOM (background grey circles). (b) Comparison of  $^{18}\text{O}$ -containing photo-products for Toolik Lake DOM (foreground blue squares)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

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

are classified as CRAM, similar to singlet oxidation products of SRFA and PLFA.<sup>4</sup> The overlap in products produced by photo-oxidation in this study with those known to result from  $^1\text{O}_2$  (i) support prior evidence for singlet oxidation of DOM as a potential pathway to form CRAM,<sup>4</sup> a class of DOM detected ubiquitously in surface waters,<sup>33,34</sup> and (ii) contributes to the mounting body of evidence that  $^1\text{O}_2$  may be an important oxidant of DOM in sunlit surface waters.

Alternative DOM partial oxidation pathways include reaction with hydroxyl radical ( $\cdot\text{OH}$ ) and superoxide ( $\text{O}_2^{\cdot-}$ ). Photochemical production of  $\cdot\text{OH}$  in arctic surface waters has previously been reported to account for up to 4% of total photochemical mineralization of DOM to  $\text{CO}_2$ .<sup>15</sup> Considering that photochemical  $\text{O}_2$  consumption to  $\text{CO}_2$  production ratios are  $\geq 1$ , and conservatively assuming that 50% of hydroxyl radical production comes from an  $\text{O}_2$  dependent pathway,<sup>39</sup> it is very unlikely that  $\cdot\text{OH}$  contributes substantially to the partial photo-oxidation of DOM in arctic surface waters. The role of  $\text{O}_2^{\cdot-}$  in the photo-oxidation of arctic DOM has never been tested. However, unlike the substantial overlap between  $^{18}\text{O}$  photo-products reported in this study and  $^1\text{O}_2$  products of lignin-derived DOM from the Great Dismal Swamp,<sup>18</sup> the overlap between  $^{18}\text{O}$  photo-products and  $\text{O}_2^{\cdot-}$  oxidation products of lignin-derived DOM is low.<sup>18</sup> This qualitative assessment suggests that  $\text{O}_2^{\cdot-}$  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<sup>1</sup> is that one mol of  $\text{O}_2$  is photochemically consumed per mol of  $\text{CO}_2$  produced. A stoichiometry of 1 mol  $\text{O}_2$  consumed per mol of  $\text{CO}_2$  produced for photo-oxidation of DOM has been assumed because the average oxidation state of DOM is likely close to zero<sup>40</sup> and the oxidation state of  $\text{CO}_2$  is +4. In cases where photochemical  $\text{O}_2$  consumption is greater than  $\text{CO}_2$  production (*e.g.*, freshwaters),<sup>1</sup> the excess  $\text{O}_2$  consumed is interpreted as molecular incorporation into DOM (*i.e.*, partial photo-oxidation). Therefore, in cases where  $\text{O}_2$  consumption is equal to or less than  $\text{CO}_2$  production (*e.g.*, headwaters streams; Fig. 1), partial photo-oxidation of DOM is assumed to be negligible. Consistent with our previous findings,<sup>1,6,7</sup> the stoichiometry of photochemical  $\text{O}_2$  consumption per  $\text{CO}_2$  production from Imnavait Creek DOM was  $\sim 1$  (Fig. 1), suggesting that it had low or no susceptibility to partial photo-oxidation. However, hundreds of  $^{18}\text{O}$  photo-products were detected, indicating that Imnavait Creek DOM was susceptible to partial photo-oxidation despite its low photochemical  $\text{O}_2$  to  $\text{CO}_2$  ratio (Fig. 2 and 3, Table 2). The only way to explain this discrepancy is that less than one mol of  $\text{O}_2$  is photochemically consumed per mol of  $\text{CO}_2$  produced.



Photo-decarboxylation of DOM to  $\text{CO}_2$  is a plausible explanation for the low  $\text{O}_2$  requirement for  $\text{CO}_2$  production from Imnavait Creek DOM. Photo-decarboxylation (eqn (1)) is a reaction where organic acids are oxidized to  $\text{CO}_2$  in the presence of sunlight with relatively low  $\text{O}_2$  consumption.



Estimates from model compounds and experimental manipulations suggest that photo-decarboxylation reactions require 0 to 0.5 mol  $\text{O}_2$  per mol of  $\text{CO}_2$  produced,<sup>28,41–43</sup> much lower than currently assumed for DOM.<sup>1</sup> Photo-decarboxylation has been implicated in the photo-oxidation of terrestrial DOM to  $\text{CO}_2$ .<sup>6,11,28,42,44</sup> Quantitatively, photo-decarboxylation is estimated to account for 40–90% of Imnavait Creek DOM mineralized to  $\text{CO}_2$  (ref. 6) and up to 100% of temperate river DOM converted to  $\text{CO}_2$ .<sup>28</sup>

The low  $\text{O}_2$  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  $\text{O}_2$  consumption to  $\text{CO}_2$  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  $\text{CO}_2$  production, and (iii) that photo-decarboxylation requires 0.5 mol  $\text{O}_2$  per mol of  $\text{CO}_2$ , partial photo-oxidation is underestimated by 1.3 to 4-fold, respectively (relative to a scenario where no  $\text{CO}_2$  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  $\text{CO}_2$ , as indicated by a high photochemical  $\text{O}_2$  consumption to  $\text{CO}_2$  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  $\text{O}_2$  needed for complete oxidation to  $\text{CO}_2$  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  $^{18}\text{O}$ -containing partial photo-oxidation products (Fig. 2 and 3) at a 1 : 1 ratio of photochemical  $\text{O}_2$  consumption per  $\text{CO}_2$  production from DOM (Fig. 1) demonstrates that less than 1 mol  $\text{O}_2$  may be required to produce 1 mol  $\text{CO}_2$ . Therefore, quantification of partial photo-oxidation as the  $\text{O}_2$  consumption in excess of photochemical  $\text{CO}_2$  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<sup>7,15</sup> where there is almost always less photochemical  $\text{O}_2$  consumption than  $\text{CO}_2$  production.<sup>1,6,7</sup> Iron is hypothesized<sup>28</sup> to catalyze the photo-decarboxylation of DOM to  $\text{CO}_2$  (by ligand-to-metal charge transfer reactions); consistent with less  $\text{O}_2$  consumption than  $\text{CO}_2$  production in high iron waters of the Arctic. There was likely relatively less influence of iron on the ratio of  $\text{O}_2$  consumption to  $\text{CO}_2$  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,<sup>1,6,7</sup> when photochemical  $\text{O}_2$  consumption is less than or equivalent to  $\text{CO}_2$  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.<sup>1</sup> 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 photodegradation that quantify only complete oxidation of DOM to  $\text{CO}_2$  (*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.<sup>4</sup> 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 products 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.<sup>4,6,10,11</sup> In this study, the majority of  $^{18}\text{O}$ -containing partial oxidation photo-products were classified as CRAM (Fig. 2) and overlapped in composition with previously reported photo-products known to result from  $^{1}\text{O}_2$  (Fig. 3).<sup>4</sup> Therefore,

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.<sup>8,9,45–51</sup> In arctic lakes<sup>46</sup> and rivers,<sup>47</sup> 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.<sup>9</sup> The influence of photochemical alterations of DOM on microbial processing of DOM likely applies to sunlit freshwaters outside the Arctic.<sup>52</sup> 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).<sup>51</sup> 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

We thank K. Harrold, L. Treibergs, K. Yuhas, and G. Kling for assistance with the experimental design and work. Thanks to M. Tfaily for assisting with the mass spectrometry analysis, which was performed using EMSL, a DOE Office of Science User Facility sponsored by the Office of BER and located at PNNL. Funding for this work was provided by NSF CAREER 1351745, DEB 1147378, 1347042, 0639790, 1147336, 1026843, PLR 1504006, the Frank and Lisina Hoch Endowed Fund (CPW), the Andrew W. Mellon Foundation Endowed Fund for Innovative Research (CPW), and the Camille and Henry Dreyfus Foundation Postdoctoral Program in Environmental Chemistry (CPW and RMC).

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