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Relationships Between Dissolved Organic Matter Composition and Photochemistry in Lakes of Diverse Trophic Status

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Abstract

The North Temperate Lakes Long-Term Ecological Research site includes seven lakes in northern Wisconsin that vary in hydrology, trophic status, and landscape position. We examine the molecular composition of dissolved organic matter (DOM) within these lakes using Fourier transform-ion cyclotron resonance mass spectrometry (FT-ICR MS) and quantify DOM photochemical activity using probe compounds. Correlations between the relative intensity of individual molecular formulas and reactive species production demonstrate the influence of DOM composition on photochemistry. For example, highly aromatic, tannin-like formulas correlate positively with triplet formation rates, but negatively with triplet quantum yields, as waters enriched in highly aromatic formulas exhibit much higher rates of light absorption, but only slightly higher rates of triplet production. While commonly utilized optical properties also correlate with DOM composition, the ability of FT-ICR MS to characterize DOM subpopulations provides unique insight into the mechanisms through which DOM source and environmental processing determine composition and photochemical activity.

Graphical abstract

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Supporting Information

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INTRODUCTION

Dissolved organic matter (DOM) is a compositionally diverse assembly of molecules that is ubiquitous in natural waters. DOM contributes to numerous environmental processes including carbon transport,¹ redox cycling,² and reactions with environmental contaminants.³ Of special interest to lacustrine systems is the ability of DOM to degrade xenobiotic compounds through the photochemical production of reactive triplet states (^3DOM)⁴ and reactive intermediates such as singlet oxygen ($^1\text{O}_2$)⁵ and hydroxyl radicals.^{6,7}

DOM derives from dissimilar sources and, accordingly, is diverse in composition and photochemical behavior.^{8–11} Broadly, DOM is considered allochthonous when derived from degraded terrestrial plant material or autochthonous when derived from aquatic microorganisms. Allochthonous DOM is typically higher in molecular weight,¹² lower in heteroatom content (e.g., N and S),¹³ and more aromatic than autochthonous DOM.¹⁴ DOM is further differentiated in natural systems by physical,¹⁵ chemical,¹⁶ and biological^{17–19} processing. While it is challenging to apportion individual mechanisms to DOM modification in environmental systems, DOM tends to become more aliphatic, less oxidized, more diverse in elemental composition, and less chromophoric as it moves from uplands to oceans.^{20–22} Similarly, formulas that are more aliphatic, less oxidized, and N-rich are more persistent in lakes.²³

DOM photochemistry also varies with source and environmental processing. ^3DOM and $^1\text{O}_2$ quantum yields are generally higher in autochthonous DOM than allochthonous DOM.^{24,25} Similarly, $^1\text{O}_2$ quantum yields increase while ^3DOM and $^1\text{O}_2$ steady-state concentrations decrease as DOM moves from headwaters to the ocean.^{26,27} DOM photochemistry is commonly evaluated with the ^3DOM probes sorbic acid (HDA) and 2,4,6-trimethylphenol (TMP), and the $^1\text{O}_2$ probe furfuryl alcohol (FFA).²⁸ While HDA and TMP directly measure ^3DOM and $^1\text{O}_2$ is formed from the reaction of ^3DOM and O_2 , there is evidence that these probes measure distinct ^3DOM subpopulations.^{15,29} Recent reviews have proposed using a combination of multiple probes to better describe ^3DOM photoreactivity.^{30,31}

The molecular composition of DOM is increasingly assessed with Fourier transform-ion cyclotron resonance mass spectrometry (FT-ICR MS).^{32,33} The ability of FT-ICR MS to identify individual molecular formulas in DOM has increased our understanding of how physical,³⁴ chemical,³⁵ and biological processes³⁶ modify DOM and how DOM changes across environmental transects.^{20,21,37} For example, FT-ICR MS can determine the

composition of heteroatom-containing formulas and identify the source of specific DOM subpopulations.¹⁶ Additionally, FT-ICR MS can detect bimodal distributions in aromaticity and heteroatom composition that are not apparent using bulk measurements.²⁰ However, as with other methods for determining DOM composition, FT-ICR MS is subject to analytical biases, such as the preferential detection of readily ionized formulas.³⁸

There is growing interest in relating DOM photochemistry and composition, as xenobiotic compounds are increasingly identified in remote waters.^{39,40} However, ³DOM production has not been previously related to molecular composition of DOM. Therefore, we combine photochemical analyses with FT-ICR MS to investigate how DOM composition controls photochemical activity in seven diverse lakes. Samples were collected from the North Temperate Lakes Long-Term Environmental Research (NTL-LTER) site in northern Wisconsin. Little is known about the DOM composition and photoreactivity of NTL-LTER lakes, and the diversity of DOM sources and distinct UV–vis absorbance spectra make them an ideal site to relate these attributes.

MATERIALS AND METHODS

Sample Location

The NTL-LTER site includes two dystrophic (Crystal and Trout Bogs), one mesotrophic (Allequash Lake), and four oligotrophic lakes (Big Muskellunge, Crystal, Sparkling, and Trout Lakes). The NTL-LTER lakes vary in surface area and water source, as well as in DOM source and concentration (Supporting Information (SI) Table S1).^{41–44} The site is in a forested region of northern Wisconsin that is dominated by lakes.⁴⁵

Samples were collected from the center of the NTL-LTER lakes between 02/1990 and 11/2014 and analyzed within 2–3 weeks for the concentration of dissolved organic carbon ([DOC]) and by UV–visible spectroscopy (UV–vis). Additional samples (1–4 L) were collected from the center of each lake in June 2015 and the edge of each lake in August 2016 for photochemical analysis. Detailed information about sample collection, analysis, and materials is available in SI Sections S1–S2.

UV–vis Spectroscopy

Absorbance of samples collected in 2015 and 2016 was determined using a Shimadzu UV-2401 PC, relative to a Milli-Q reference. $SUVA_{254}$ is the ratio of absorbance at 254 nm (A_{254}) to [DOC].¹⁴ $E_2:E_3$ is the ratio of absorbance at 250 to 365 nm.²²

Mass Spectrometry

DOM was extracted from the NTL-LTER samples collected in August 2016 by solid phase extraction (SPE) and analyzed by FT-ICR MS. Details about the SPE protocol are included in SI Section S3.⁴⁶ Sample extracts were diluted 1:10 in 1:1 acetonitrile:Milli-Q and aspirated with 0.3 psi pressure into an electrospray source with an applied voltage of –1.4 V. Analysis was performed with a Solarix XR 12T FT-ICR MS (Bruker) coupled to a Triversa NanoMate sample delivery system (Advion), as described previously.⁴⁷ Details about instrument settings and formula identification are available in SI Section S3. Formula

relative intensities were determined by dividing the intensity of each formula by the sum of intensities of all identified formulas in a sample. Weighted average compositional values (e.g., $H:C_{w,avg}$) are the average of that compositional value in all formulas, weighted by the relative intensity of each formula. It should be noted that these average compositional values are determined only from formulas identified by FT-ICR MS, rather than bulk elemental analysis. Average compositional values exhibit similar qualitative trends to bulk analysis (e.g., elevated H:C and N:C in autochthonous fulvic acid isolates)¹³ and provide insight into compositional differences between natural DOM samples.²¹ Double bond equivalents per carbon (DBE/C) was calculated as $(C - 0.5(H) + 0.5(N+S) + 1)/C$.⁴⁸ Optical properties and photochemical measurements are correlated by Pearson's coefficients with the relative intensities of individual formulas.

Photochemistry

Samples collected in 2015 and 2016 were irradiated in a Rayonet photoreactor with UV-A bulbs (365 ± 9 nm).^{25,49} The initial photon flux of the experimental apparatus was determined to be 7.35×10^{-8} einsteins $cm^{-2} s^{-1}$ with *p*-nitroanisole (PNA)-pyridine actinometry.²⁵ Irradiation durations varied according to probe and sample (10–360 min), and all irradiations were conducted in triplicate. HDA was added at initial concentrations of 10, 100, 250, 500, and 1000 μM . 3DOM quantum yields (Φ_{3DOM}), formation rates (F_{3DOM}), steady-state concentrations ($[^3DOM]_{ss}$) and first-order loss rate constants (k_d) were calculated from the isomerization rate of *t*, *t*-HDA and previously estimated rate constants.^{25,50} Additionally, the observed loss rates of the 3DOM probe TMP (initial concentration = 10 μM) were used to calculate 3DOM quantum yield coefficients (f_{TMP}) and steady-state concentrations of 3DOM ($[^3DOM]_{ss, TMP}$) using estimated rate constants as described previously.^{51,52} The quantum yields (Φ_{1O_2}) and steady-state concentrations of 1O_2 ($[^1O_2]_{ss}$) were calculated from the observed loss rates of FFA (initial concentration = 10 μM) and previously measured rate constants.^{53,54} Quantum yields and f_{TMP} were calculated with PNA-pyridine actinometry. All calculations are described in detail in SI Section S4.⁵⁵ FFA, PNA, TMP, and HDA isomer concentrations were quantified by high-performance liquid chromatography as described previously.²⁵

RESULTS AND DISCUSSION

[DOC] and Optical Properties

[DOC] and UV–vis measurements taken from 1990 through 2014 distinguish the seven NTL-LTER lakes according to trophic status and reflect the source and transformation of DOM within each lake. [DOC] represents the concentration of bulk DOM, whereas A_{254} quantifies light absorption by chromophoric DOM. DOM composition is described by $SUVA_{254}$, which correlates with aromaticity,¹⁴ and $E_2:E_3$, which is inversely related to molecular weight.^{25,56} The bogs have the highest mean [DOC], A_{254} , and $SUVA_{254}$, and lowest mean $E_2:E_3$ (Figure 1; SI Table S2). The high $SUVA_{254}$ and low $E_2:E_3$ values are typical of terrestrially-derived DOM,^{57–59} and are reflective of the high fraction of DOM from adjacent wetlands (i.e., ~65%), high DOC loading rates, shallow photic zones, and short hydraulic residence times (HRTs).⁴⁴

In contrast, optical properties cannot be used to distinguish the source of DOM in the oligotrophic lakes. These lakes have the lowest [DOC] and A_{254} due to longer HRTs and lower carbon loading rates (SI Table S1),⁴⁴ but are highly variable in $SUVA_{254}$ and $E_2:E_3$. The major sources of DOM to the oligotrophic lakes are precipitation, aerial deposition, surface waters, and groundwater, rather than terrestrially-derived DOM.⁴⁴ Additionally, more DOM is lost to mineralization compared with export and sedimentation, indicating higher rates of DOM processing.⁴⁴ The optical properties of the oligotrophic lakes could indicate the presence of autochthonous DOM, which typically has higher $E_2:E_3$ and lower $SUVA_{254}$ than allochthonous DOM.^{14,22,59–61} However, photobleaching of terrestrially derived DOM decreases $SUVA_{254}$ and increases $E_2:E_3$,¹⁵ and the presence of measurable autochthonous DOC in these lakes is debated.^{44,62} Collectively, the lower $SUVA_{254}$ and higher $E_2:E_3$ values in oligotrophic lakes could reflect DOM from autochthonous sources or allochthonous DOM that has undergone extensive processing.

Mesotrophic Allequash Lake is unique among the seven NTL-LTER lakes. Although the DOC loading rate is similar to the bogs, Allequash Lake has the shortest HRT and, uniquely, exports more DOC than is lost to mineralization and sedimentation.⁴⁴ Additionally, it receives more DOM from adjacent wetlands than the oligotrophic lakes, but less than the bogs (i.e., ~10%).⁴⁴ These factors produce DOM that shares some properties with the bogs (high $SUVA_{254}$, low $E_2:E_3$) and some properties with the oligotrophic lakes (low [DOC] and A_{254}).

Samples were collected in 2016 for photochemistry and FT-ICR MS measurements, and have [DOC], A_{254} , and $E_2:E_3$ slightly above historical means (Figure 1; SI Table S2). The elevated [DOC] and A_{254} of samples collected in 2016 may reflect their collection from the lake edges, rather than centers. However, UV–vis compositional measurements are generally within a standard deviation of historical means, indicating that the photochemistry and FT-ICR MS results are reflective of representative DOM for NTL-LTER lakes. Long-term trends in the optical properties of the NTL-LTER lakes have been discussed previously.⁶³

Molecular Composition of DOM

[DOC] and UV–vis measurements differ between lakes according to trophic status, but the cause of these variations is unclear. Analysis of DOM by FT-ICR MS provides more detailed compositional information. 3037 unique $CHON_{0-1}P_{0-1}S_{0-1}$ formulas are identified in the NTL-LTER lakes (Table 1), with 975–1791 unique formulas in individual lakes. In each lake, 60–81% of identified formulas contain only CHO. The predominance of CHO formulas is typical of allochthonous DOM.^{13,17} Similarly, the bulk compositional measurements of CHO formulas are characteristic of lignin-like, terrestrially-derived molecules ($H:C_{w,avg} = 1.05–1.36$; $O:C_{w,avg} = 0.46–0.56$; Figure 2 and SI Figure S2).^{21,64–66} While the terms “lignin-like” and “tannin-like” are not definitive (i.e., a lignin-like formula may not necessarily be derived from lignin), these elemental ratio cutoffs are useful in visualizing the FT-ICR MS data. $H:C_{w,avg}$ increases and $DBE/C_{w,avg}$ decreases from the bogs to Allequash Lake to the oligotrophic lakes, confirming that the DOM in the bogs is more aromatic (Table 1). $O:C_{w,avg}$ is highest in the bogs and lower in Allequash Lake and the oligotrophic lakes.

CHON formulas comprise 12–31% of all identified formulas (Table 1) and occupy similar van Krevelen space as CHO formulas from the same water body (Figure 2 and SI Figure S3; Table S5). Higher fractions of formulas containing N are observed in the oligotrophic lakes (21–31%) than in the bogs (12–15%) or Allequash Lake (17%). The enrichment of CHON formulas in oligotrophic lakes could derive from the extensive irradiation of allochthonous DOM or the presence of autochthonous DOM.¹⁷ However, the compositional similarity of CHON and CHO formulas suggests a terrestrial source.

Few CHOP and CHOS formulas are detected (SI Figures S4 and S5; Table 1 and SI Table S6). Crystal Bog and Allequash Lake have the highest abundance of P-containing formulas (9.4 and 6.1%, respectively), which could indicate preferential uptake of P-containing formulas in the nutrient-poor lakes. Phospholipid formulas have been identified in offshore coastal waters, but the CHOP formulas observed here have higher O:C than expected for phospholipids.²¹ While few CHOS formulas are identified in NTL-LTER lakes, some are observed at very high intensity, likely reflecting their ease of ionization.³⁸

Molecular Composition Differences Among Lakes

Bray–Curtis dissimilarity analysis according to the log-weighted intensity of all identified formulas separates the lakes by trophic status (SI Figure S6). Crystal and Trout Bogs are highly similar, with Allequash Lake being slightly more dissimilar, while the oligotrophic lakes are highly dissimilar from the bogs. This trend reflects the differences in the 25-year record of optical properties. For example, SUVA₂₅₄ measurements were highest in the bogs and lowest in Crystal Lake (Figure 1).

DOM compositional variation with trophic status is apparent in comparisons of CHON_{0–1} formulas that are commonly identified in five or more lakes ($n = 844$; SI Figure S7). The 481 CHO and 14 CHON formulas with higher relative intensity in the bogs and Allequash Lake have lower average H:C (1.01 ± 0.24) and higher average O:C (0.54 ± 0.15) than 250 CHO and 99 CHON formulas enhanced in the oligotrophic lakes ($H:C = 1.37 \pm 0.02$; $O:C = 0.47 \pm 0.14$). 25% of formulas enhanced in oligotrophic lakes have H:C values > 1.5 , which are typical of microbially derived compounds,^{64,67} compared with $< 1\%$ of common formulas enhanced in the bogs and Allequash Lake. Interestingly, 59% of the 64 common CHON_{0–1} formulas with $H:C < 1.2$ that are enhanced in the oligotrophic lakes contain nitrogen. An enhancement of highly aromatic CHON formulas was observed during the irradiation of Congo River water.⁶⁸

DBE/C describes the sum of double bonds or ring structures per carbon atom in a molecule. Dystrophic bogs show a bimodal distribution of CHO formulas with peaks centered on DBE/C ~ 0.4 and ~ 0.6 when log-normalized intensity is plotted against DBE/C (Figure 2 and SI Figure S8). These peaks correspond to H:C of 1.3 and 0.9, respectively, assuming an average of 20 carbon atoms per formula, which is reasonable given that the intensity-weighted average C of all observed formulas ranges from 19.6 in Crystal Lake to 22.4 in Trout Bog. The two peaks have similar maximum intensities in Trout Bog, while the more aromatic peak is visibly depressed in Allequash Lake and nearly absent in Crystal Lake. Therefore, the lower relative aromaticity (i.e., lower SUVA₂₅₄ and higher $H:C_{w,avg}$) of oligotrophic lake DOM arises from the absence of a class of highly aromatic formulas. A

similar bimodal distribution of DBE-O was observed in CHO formulas in samples collected near New Zealand, but was absent in waters collected farther offshore.²⁰ The trends in intensity as a function of DBE/C are similar for CHON formulas, in agreement with the compositional similarity of CHO and CHON formulas in van Krevelen diagrams (Figure 2 and SI Figure S8). The presence of highly aromatic formulas in bogs and, to a lesser degree, Allequash Lake is attributable to the input of DOM from adjacent wetlands.⁴⁴ Since irradiation preferentially removes highly aromatic formulas from terrestrial DOM,^{34,68,69} the absence of more aromatic DOM in the oligotrophic lakes may reflect their deeper photic zone and longer HRT. These formulas are related to long-wavelength absorption and therefore affect photoreactivity, as described below.

While highly aromatic formulas are largely absent in the oligotrophic lakes, the formulas with DBE/C of ~0.4 represent a ubiquitous class of aliphatic compounds (Figure 2d–f). The 307 CHO formulas observed in all seven lakes have an average DBE/C of 0.42 (SI Figure S9; Table S7). Furthermore, their average bulk compositional values ($H:C = 1.22 \pm 0.22$; $O:C = 0.47 \pm 0.13$) are similar to average values in the oligotrophic lakes. Aliphatic character was observed to correlate with environmental persistence in a previous comparison of DOM from diverse lakes.²³ Additionally, the ubiquitous CHO formulas are compositionally similar to CHO formulas identified as universally present in terrestrial and marine samples ($H:C \sim 1.4$; $O:C \sim 0.45$) near New Zealand.²⁰

Relating Optical Properties and Composition

UV–vis and FT-ICR MS measurements strongly correlate among the NTL-LTER lakes. For example, $SUVA_{254}$ correlates with FT-ICR MS measurements of aromaticity (e.g., $H:C_{w,avg}$ and $DBE/C_{w,avg}$; SI Figure S10). A linear correlation between DBE_{avg} and $SUVA_{254}$ was previously observed among the molecular weight fractions of a terrestrially derived DOM isolate.²⁵ These correlations are noteworthy because the two techniques sample different populations of DOM; UV–vis spectroscopy only detects molecules that absorb light, while FT-ICR MS only detects molecules that ionize with the selected ionization source (i.e., negative mode electrospray).

Bulk optical properties also correlate with the relative intensity of individual molecular formulas. For this analysis, the Pearson's correlation coefficient is calculated between the relative intensity of commonly identified $CHON_{0-1}$ formulas and the optical properties in each lake. For data visualization, the formulas are divided into those enhanced in bogs and Allequash Lake and those enhanced in oligotrophic lakes (Figures 3a–d). The 477 $CHON_{0-1}$ formulas that correlate positively with $SUVA_{254}$ ($r > 0.5$) are more aromatic and oxidized than the 217 formulas that show negative correlations ($r < -0.5$). 91% of formulas that positively correlate with $SUVA_{254}$ are enhanced in the bogs and Allequash Lake, while 82% of those that negatively correlate with $SUVA_{254}$ are enhanced in the oligotrophic lakes. Conversely, 97% of the 173 formulas that correlate positively with $E_2:E_3$ have higher average relative intensity in the oligotrophic lakes; these formulas are more aliphatic and oxidized than the 452 formulas that correlate negatively with $E_2:E_3$. Negative correlations between formulas with high $H:C$ and $SUVA_{254}$ have been reported in diverse systems,

^{23,47,70} but this is the first reported correlation between $E_2:E_3$ and the relative intensity of aliphatic formulas in lakes.

Photochemistry

The photochemical activity of the NTL-LTER lakes gives further insight into DOM composition. $\Phi_{3\text{DOM}}$ (0.002–0.0081), f_{TMP} (5.4–68.3 M⁻¹), and $\Phi_{1\text{O}_2}$ (0.005–0.019) quantified in the 2016 samples are generally lower than previously reported for similar waters (Figure 4a; SI Table S8). For example, $\Phi_{3\text{DOM}}$ values are slightly below previous measurements in allochthonous DOM isolates (~0.012),^{25,50} f_{TMP} values are below previously reported values in river waters (60–100 M⁻¹),⁶⁰ and $\Phi_{1\text{O}_2}$ values are below values reported in waters from Lake Superior and its tributaries (~0.02–0.035).²⁶ However, the apparent discrepancy in f_{TMP} is partially due to a revision in the quantum yield of the actinometer.^{55,71} For example, using historical constants for the PNA-pyridine system increases f_{TMP} by 30% (SI Table S9).⁷¹ Lower $\Phi_{3\text{DOM}}$ compared to $\Phi_{1\text{O}_2}$ has been reported previously,^{25,52} and arises from differences in the concentration of ³DOM subpopulations that react with HDA and O₂.^{4,29}

$\Phi_{3\text{DOM}}$, $\Phi_{1\text{O}_2}$, and f_{TMP} increase linearly with $E_2:E_3$, and are lowest in the bogs and highest in the oligotrophic lakes (Figure 4a and SI Figure S11a). While the slopes of $\Phi_{3\text{DOM}}$ and f_{TMP} are shallower than some previous reports,^{25,53,56} the observed slope of $\Phi_{1\text{O}_2}$ vs $E_2:E_3$ is similar to one recorded in waters collected from Lake Superior and its tributaries.²⁶ As with other compositional measurements, quantum yields of ³DOM and ¹O₂ vary with DOM source and environmental processing. $\Phi_{1\text{O}_2}$ increases as terrestrial DOM undergoes photobleaching,¹⁵ whereas $\Phi_{3\text{DOM}}$ and $\Phi_{1\text{O}_2}$ are higher in autochthonous, rather than allochthonous, DOM.^{24,25}

$[\text{}^3\text{DOM}]_{\text{ss}}$, measured with HDA ($0.1320100335.0 \times 10^{-14}$ M) and TMP ($1.1\text{--}11.7 \times 10^{-14}$ M), and $[\text{}^1\text{O}_2]_{\text{ss}}$ ($2.7\text{--}100 \times 10^{-14}$ M) are highest in the bogs and lowest in the oligotrophic lakes (Figure 4b and SI Figure S11b; Table S10). While steady-state concentrations are sensitive to irradiation intensity, $[\text{}^3\text{DOM}]_{\text{ss}}$ and $[\text{}^1\text{O}_2]_{\text{ss}}$ reported here are similar to values measured in surface waters.^{26,27,72} $[\text{}^3\text{DOM}]_{\text{ss}}$, HDA and $[\text{}^1\text{O}_2]_{\text{ss}}$ increase linearly with [DOC] (Figure 4b). A similar slope of $[\text{}^1\text{O}_2]_{\text{ss}}$ with [DOC] was observed in waters from Lake Superior and its tributaries.²⁶

$[\text{}^3\text{DOM}]_{\text{ss}}$, TMP showed less variation than $[\text{}^3\text{DOM}]_{\text{ss}}$ and $[\text{}^1\text{O}_2]_{\text{ss}}$ because $[\text{}^3\text{DOM}]_{\text{ss}}$, TMP levels off in samples with [DOC] > 5 mg-C L⁻¹ (SI Figure S11b; Table S10). Similar results were also observed in NTL-LTER waters collected in 2015 (SI Figure S11c and Table S11). When Crystal and Trout Bog waters from 2015 were diluted to below 5 mg-C L⁻¹, the linear correlation between $[\text{}^3\text{DOM}]_{\text{ss}}$, TMP and [DOC] was similar to the trend in the other NTL-LTER lakes (SI Figure S11d and Table S12). The suppression of TMP loss rates at high [DOC] has been observed previously,^{52,60} and may affect $[\text{}^3\text{DOM}]_{\text{ss}}$, TMP ranges in samples with varied [DOC].

$\Phi_{3\text{DOM}}$ describes the ratio of $F_{3\text{DOM}}$ to light absorption, whereas $[\text{}^3\text{DOM}]_{\text{ss}}$ describes the ratio of $F_{3\text{DOM}}$ to the ³DOM quenching rate constant (k_d). The ³DOM probe HDA allows these parameters to be quantified separately.⁵⁰ $F_{3\text{DOM}}$ is highest in the bogs, and increases

linearly with [DOC] and SUVA₂₅₄ (Figure 4c and SI Figure S12a). Similar $F_{3\text{DOM}}$ values and correlations between $F_{3\text{DOM}}$ and [DOC] have been reported for allochthonous DOM.^{27,50} While both $F_{3\text{DOM}}$ and rates of light absorbance (R_{abs}) are higher in the bogs than the oligotrophic lakes, R_{abs} varies by much more than $F_{3\text{DOM}}$ and results in lower quantum yields in the bogs (SI Table S8). Therefore, the trend in $\Phi_{3\text{DOM}}$ across the NTL-LTER lakes is primarily driven by differences in R_{abs} , rather than $F_{3\text{DOM}}$. In contrast, the consistency of k_d among the lakes (SI Figure S13) demonstrates that ^3DOM formation rates determine the differences seen in $[\text{}^3\text{DOM}]_{\text{ss}}$.

Relationships Between Photochemistry and Molecular Composition

Photochemical reactivity correlates with bulk FT-ICR MS measurements in the NTL-LTER lakes. For example, ^3DOM and $^1\text{O}_2$ quantum yields linearly correlate positively with $\text{H:C}_{\text{w.avg}}$ and negatively with $\text{DBE/C}_{\text{w.avg}}$ (Figure 4d and SI Figure S14). While previous studies have noted correlations between ^3DOM production and UV-vis measurements,⁵⁶ correlations between quantum yields and molecular composition determined by FT-ICR MS have not been previously reported.

As with optical properties, photochemical measurements correlate with the relative intensity of individual commonly identified CHON_{0-1} formulas (Figure 3). The 237 formulas that positively correlate with $\Phi_{3\text{DOM}}$ are less aromatic ($\text{H:C} = 1.45 \pm 0.15$) and oxidized ($\text{O:C} = 0.45 \pm 0.13$) than the 466 formulas with negative $\Phi_{3\text{DOM}}$ correlations ($\text{H:C} = 0.98 \pm 0.23$; $\text{O:C} = 0.56 \pm 0.14$). Similar trends are apparent when correlating the relative intensity of commonly identified CHON_{0-1} with $\Phi_{1\text{O}_2}$ or f_{TMP} (SI Figure S15). Conversely, the 449 formulas that correlate positively with $F_{3\text{DOM}}$ are more aromatic ($\text{H:C} = 0.96 \pm 0.23$) and oxidized ($\text{O:C} = 0.56 \pm 0.14$) than 196 formulas with negative $F_{3\text{DOM}}$ correlations ($\text{H:C} = 1.45 \pm 0.14$; $\text{O:C} = 0.47 \pm 0.12$). Formulas that correlate positively with $F_{3\text{DOM}}$ are more likely to be in the lignin- or tannin-like regions of the van Krevelen diagram (395/449) than formulas that correlate positively with $\Phi_{3\text{DOM}}$ (123/237). Formulas enhanced in oligotrophic lakes are more likely to positively correlate with $\Phi_{3\text{DOM}}$ (231/273) and negatively with $F_{3\text{DOM}}$ (190/233), while formulas enriched in bogs and Allequash Lake are more likely to correlate positively with $F_{3\text{DOM}}$ (406/412) and negatively with $\Phi_{3\text{DOM}}$ (437/443).

There is extensive overlap between the commonly identified formulas that correlate positively with $\Phi_{3\text{DOM}}$ and $E_2:E_3$, as well as between those that correlate positively with $F_{3\text{DOM}}$ and SUVA₂₅₄. 97% of the formulas that positively correlate with $\Phi_{3\text{DOM}}$ also positively correlate with $E_2:E_3$. Similarly, 99% of the formulas that correlate positively with $F_{3\text{DOM}}$ also correlate positively with SUVA₂₅₄. These results agree with the linear correlations between bulk UV-vis and quantum yield measurements (Figure 4a and SI Figure S12a). Positive correlations between $E_2:E_3$ and $\Phi_{3\text{DOM}}$, and between absorbance and $F_{3\text{DOM}}$, have been observed previously.^{25,56,60} However, this is the first study using FT-ICR MS to demonstrate that the same molecular formulas correlate with both optical properties and photochemical measurements.

No common formulas correlate positively with $F_{3\text{DOM}}$ and $\Phi_{3\text{DOM}}$, which is the ratio of $F_{3\text{DOM}}$ to R_{abs} . Instead, 627 common CHON_{0-1} formulas correlate positively with one and negatively with the other. This unintuitive result derives from the higher variation in R_{abs}

than $F_{3\text{DOM}}$, as described above. While $F_{3\text{DOM}}$ is highest in the bogs (Figure 4a), R_{abs} is even higher relative to the oligotrophic lakes (SI Table S8). This results in an inverse relationship between $F_{3\text{DOM}}$ and $\Phi_{3\text{DOM}}$ in the NTL-LTER lakes (SI Figure S12b).

The charge-transfer model of DOM photochemistry describes long-wavelength absorbance as arising from intramolecular charge transfer interactions between electron-rich donor groups (e.g., hydroxy- or methoxy-aromatic moieties) and electron-poor acceptor groups (e.g., quinones or aldehydes) that are largely derived from the partial oxidation of lignins.^{9,73,74} These compounds should therefore be prevalent in highly aromatic, terrestrially derived DOM, such as the DOM collected from the bogs and Allequash Lake. Conversely, ^3DOM production is related to the presence of borohydride-reducible carbonyls,⁷⁵ which are found in compositionally diverse DOM formulas with H:C up to ~ 1.6 .⁷⁶

Although it is not possible to distinguish specific functional groups (e.g., carbonyls or phenols) using FT-ICR MS, the compositional and photochemical trends in NTL-LTER lakes support this model. For example, the increased intensity of aromatic formulas in the bogs and Allequash Lake, particularly the class of highly aromatic formulas apparent in plots of intensity versus DBE/C (Figure 2 and SI Figure S9), accompanies enhanced long-wavelength absorption (i.e., high SUVA_{254} , low $E_2:E_3$). As a result, these waters exhibit both high rates of light absorbance (SI Table S8) and high ^3DOM formation rates (Figure 4). Conversely, the lack of oxidized, aromatic formulas in oligotrophic lakes results in strongly reduced absorbance, but only moderately reduced $F_{3\text{DOM}}$. Therefore, the oligotrophic lakes have higher ^3DOM and $^1\text{O}_2$ quantum yields (Figure 4). These results suggest that previous observations of higher ^3DOM and $^1\text{O}_2$ quantum yields in autochthonous DOM^{24,25} and increases in $^1\text{O}_2$ quantum yields as DOM moves from headwaters to the ocean^{26,27} may be attributable to changes in DOM composition similar to those observed in the NTL-LTER lakes.

The correlations between relative formula intensity and photochemical measurements should be interpreted cautiously, as there are multiple possible explanations. For example, $^1\text{O}_2$ is quenched within the DOM microenvironment⁷⁷ and ^3DOM may also be susceptible to intramolecular quenching. Intramolecular quenching is not apparent to hydrophilic probes, like the ones used here.⁷⁷ Therefore, the apparently elevated $\Phi_{3\text{DOM}}$ in oligotrophic lake waters could reflect decreased intramolecular quenching. Despite this caveat, this is the first direct link between ^3DOM photochemistry and the molecular composition of DOM.

Environmental Implications

The NTL-LTER lakes represent a wide range of DOM source and hydrology. For example, Trout Bog receives DOM primarily from adjacent wetlands and water from precipitation, while Allequash Lake receives DOM and water from a mixture of surface and groundwater inputs.⁴⁴ Despite these differences, DOM composition, optical properties, and photochemical activity are tightly correlated with lake trophic status. Further, there is a high correlation between FT-ICR MS measures of composition and both optical properties and photochemical reactivity. For example, highly aromatic formulas correlate with higher ^3DOM production rates, while more aliphatic formulas correlate with higher quantum yields. These correlations suggest that straightforward models and UV-vis measurements can be

used as a first approximation of DOM composition and photochemical reactivity in related water bodies. Future work should focus on relating FT-ICR MS measurements to the formation of other photochemically produced reactive intermediates, including hydrogen peroxide and hydroxyl radical.

However, while UV–vis measurements are useful in assessing DOM composition, FT-ICR MS provides uniquely detailed analysis of DOM composition and environmental processing. For example, here FT-ICR MS is used to identify a bimodal distribution in aromaticity and compare the composition of heteroatom-containing formulas. By relating compositional information about individual molecular formulas, FT-ICR MS provides insight into the processes that modify DOM composition. For example, while UV–vis measurements in NTL-LTER lakes show compositional dissimilarity between bogs and oligotrophic lakes, the decreased SUVA₂₅₄ and increased $E_2:E_3$ in oligotrophic lakes could represent either autochthonous or photobleached allochthonous DOM. Conversely, FT-ICR MS results suggest that photoradiation, rather than biological processing, could produce the compositional distinctions between bogs and oligotrophic lakes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Measured (a) [DOC], (b) A_{254} , (c) $SUVA_{254}$, and (d) $E_2:E_3$ in NTL-LTER lakes. Values from samples collected from 1990–2014 are displayed as box-and-whisker plots; the mean is marked with a horizontal line, the edges of the box correspond to the 25th and 75th percentiles, and the whiskers mark 1.5 times the interquartile range. Outliers are marked with gray circles. Values from samples collected in 2016 are marked with triangles.

Figure 2. van Krevelen diagrams of CHO (circles) and CHON (diamonds) formulas identified in (a) Trout Bog, (b) Allequash Lake, and (c) Crystal Lake. Orange boxes indicate regions commonly defined as (1) lignin-like and (2) tannin-like.^{21,64–67} Marker color denotes the log-normalized intensity. The log-normalized intensity of CHO (blue circles) and CHON (red diamonds) formulas as a function of DBE/C in (d) Trout Bog, (e) Allequash Lake, and (f) Crystal Lake.

Figure 3.

Commonly identified CHO (circles) and CHON (diamonds) formulas that have higher average relative intensity in (a, c, e, g) bogs and Allequash Lake or (b, d, f, h) oligotrophic lakes. Formulas have Pearson's correlation coefficient -0.5 or 0.5 between relative intensity and (a, b) SUVA₂₅₄, (c, d) E₂:E₃, (e, f) Φ_{3DOM}, and (g, h) F_{3DOM}. Marker fill denotes the Pearson's coefficient for correlation between relative intensity and optical or photochemical properties.

Figure 4.

(a) Quantum yields of ^3DOM determined with HDA ($\Phi_{3\text{DOM}}$; square markers) and $^1\text{O}_2$ with FFA (round markers) plotted versus $E_2:E_3$ where $\Phi_{3\text{DOM}} = 0.00088 \times E_2:E_3 - 0.00187$ ($r^2 = 0.81$)² and $\Phi_{1\text{O}_2} = 0.00216 \times E_2:E_3 - 0.00424$ ($r^2 = 0.91$). (b) Steady-state concentrations of ^3DOM (square markers) and $^1\text{O}_2$ (round markers) plotted versus $[\text{DOC}]$, where $[^3\text{DOM}]_{ss}$ ($\times 10^{-15}$ M) = $4.31 \times [\text{DOC}] + 0.26$ ($r^2 = 0.94$) and $[^1\text{O}_2]_{ss}$ ($\times 10^{-15}$ M) = $52.9 \times [\text{DOC}] - 102.8$ ($r^2 = 0.99$). (c) ^3DOM formation rate plotted versus $[\text{DOC}]$, where $F_{3\text{DOM}}$ ($\times 10^{-9}$) = $3.82 \times [\text{DOC}] - 4.76$ ($r^2 = 0.97$). (d) $\Phi_{3\text{DOM}}$ (square markers; $r^2 = 0.97$) and $\Phi_{1\text{O}_2}$ (round markers; $r^2 = 0.94$) versus $\text{DBE} / C_{w,\text{avg}}$. Oligotrophic lakes are indicated with hollow markers, Allequash Lake is indicated with gray markers, and bogs are indicated with black markers. Error bars represent the standard deviation of triplicate analysis. Trend lines show linear, least-squares regressions.

Table 1Formulas Identified in NTL-LTER Lakes by FT-ICR MS^a

	Total (#)	CHO (#)	CHON (#)	CHOP (#)	CHOS (#)	O:C _{w,avg}	H:C _{w,avg}	DBE _{w,avg}	DBE/C _{w,avg}
Dystrophic									
Crystal Bog (CB)	1168	855	179	110	20	0.55	1.08	11.25	0.51
Trout Bog (TB)	975	793	113	44	18	0.56	1.06	11.63	0.52
Mesotrophic									
Allequash L. (AL)	1299	943	224	79	51	0.52	1.14	10.63	0.48
Oligotrophic									
Big Muskellunge L. (BM)	1581	962	404	90	109	0.50	1.30	8.26	0.41
Crystal L. (CR)	1523	915	466	55	78	0.44	1.39	7.05	0.36
Sparkling L. (SP)	1791	1174	463	54	84	0.52	1.22	9.22	0.44
Trout L. (TR)	1583	1101	334	59	81	0.50	1.24	9.01	0.43

^aCompositional information is calculated for all formulas identified in each lake.