

Understanding Dissolved Organic Matter Reactivity and Composition in Lakes and Streams Using Proton-Transfer-Reaction Mass Spectrometry (PTR-MS)

Mike Peacock,^{*,†} Dušan Materić,[‡] Dolly N. Kothawala,[§] Rupert Holzinger,[‡] and Martyn N. Futter[†]

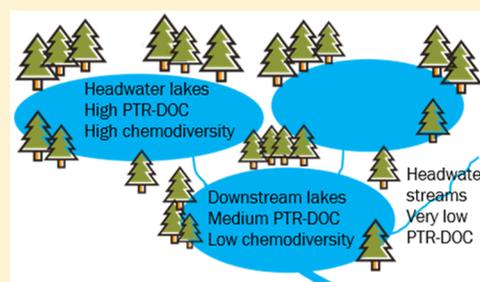
[†]Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Lennart Hjelms väg 9, 756 51 Uppsala, Sweden

[‡]Institute for Marine and Atmospheric Research, Faculty of Science, Utrecht University, Princetonplein 5, 3584 CC Utrecht, The Netherlands

[§]Department of Limnology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, 752 36 Uppsala, Sweden

Supporting Information

ABSTRACT: Here, we present a novel approach for investigating dissolved organic matter (DOM) composition using thermal desorption proton-transfer-reaction mass spectrometry (PTR-MS), a technique that provides insight into the molecular composition of DOM $< m/z$ 500 (termed “PTR-DOC”). The applicability of PTR-MS for understanding the relationship between DOM composition and reactivity has yet to be explored. We present results from a synoptic sampling campaign of streams and lakes in a Swedish forest catchment where we measured DOM composition using PTR-MS and traditional optical methods and conducted DOM biodegradability assays. PTR-DOC comprised $\leq 12\%$ of the total DOC pool. We found significant relationships between PTR-DOC and DOM degradability; reduced chemodiversity and low concentrations of PTR-DOC were both associated with the total DOM pool being more susceptible to microbial degradation. Furthermore, molecular differences were apparent among headwater lakes, headwater streams, and lakes further down the catchment. Direct linkages between PTR-DOC and optical methods were observed. Using the quantitative data that PTR-MS generates, it could become possible to identify the fluorescing components of DOM, and the method may be particularly informative in low-DOC waters such as marine environments where PTR-DOC may dominate the total DOM pool.



1. INTRODUCTION

The importance of inland waters in the global carbon cycle is well recognized.¹ Lakes process dissolved organic matter (DOM), either by complete degradation, which results in emissions of carbon dioxide and methane, or by partial degradation, which causes larger DOM molecules to be broken down into smaller molecules.² These processes therefore depend on, and can influence, the composition of DOM,³ which, in turn, is influenced by catchment land cover (e.g., forest, mire, or agriculture), climate, and water residence times.^{4,5} As a result, the age of DOM varies throughout the aquatic continuum. In general, older DOM is less reactive and contains a greater proportion of autochthonous (internally produced) material.⁶

DOM composition can be investigated using numerous methods of varying complexity. Simple methods, including absorbance and fluorescence spectroscopy, generate metrics such as specific ultraviolet absorbance (SUVA) and a suite of quality indices⁷ that act as proxies for molecular structure and the degree of autochthonous DOM production.^{8,9} More complex methods, including thermochemolysis gas chromatography and mass spectrometry (GC-MS) and ¹³C nuclear magnetic resonance spectroscopy, provide direct information

about functional group composition¹⁰ and DOM aromaticity.⁸ Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) is widely used to identify individual DOM molecular constituents and provide new insights into DOM dynamics.^{11,12} Similarly, Orbitrap MS has been suggested as a viable, more widely accessible alternative to FT-ICR MS.¹³

Proton-transfer-reaction mass spectrometry (PTR-MS) is a novel method for molecular analysis of aquatic DOM.¹⁴ PTR-MS has previously been used to measure gaseous volatile organic compounds and organic aerosol composition.^{15–17} For instance, the method was able to identify differences in DOM composition between intact and degraded tropical peatlands.¹⁴ The method is fast, and the only pretreatment required to analyze aquatic samples is evaporation/sublimation, leaving behind the residue of organic matter for analysis.¹⁴ Unlike Fourier transform-based methods, PTR-MS is quantitative (i.e., it provides actual concentrations of individual ions) and is unique in this respect among high-resolution MS methods.

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Table 1. Details of Sampling Sites Showing Water Body Type (HWL, headwater lake; DSL, downstream lake; HWS, headwater stream; S, stream draining a lake), Water pH, and Dissolved Organic Carbon (DOC) Concentration and PTR-DOC Metrics Such as Ratios of Hydrogen to Carbon Atoms (H/C), Ratios of Oxygen to Carbon Atoms (O/C), Concentrations of DOC Measured by Proton-Transfer-Reaction Mass Spectrometry (PTR-MS) Normalized against DOC Concentration (%PTR-DOC), Mean Oxidative States of Carbon (OSC), Mean Numbers of Carbon Atoms per Molecule (nC), Molecular Chemodiversity, and Numbers of Ions^a

	water body type	pH	DOC (mg L ⁻¹)	H/C	O/C	%PTR-DOC	OSC	nC	chemodiversity	no. of ions	kO_2 (day ⁻¹)
Gaffeln	HWL	6.2	4.80	1.51	0.42	11.8	-0.62	3.81	4.07	301	0.001
Gaffeln Stream	S	6.0	9.24	1.50	0.46	3.9	-0.52	3.49	3.89	300	0.012
Stora Hästevatten North	HWL	6.5	3.43	1.42	0.36	9.4	-0.64	4.52	4.08	294	0.077
Stora Hästevatten South	HWL	6.5	4.06	1.47	0.39	10.2	-0.64	4.11	4.07	301	0.037
Gårdsjön inflow	DSL	6.7	7.33	1.50	0.41	11.3	-0.63	3.94	4.06	302	0.023
F1 stream	HWS	4.6	14.56	1.39	0.51	0.8	-0.31	3.03	3.46	260	0.325
F2 stream	HWS	4.8	43.29	1.46	0.49	0.4	-0.43	3.15	3.67	289	0.277
F3 stream	HWS	4.9	16.25	1.34	0.34	1.8	-0.63	4.90	3.83	294	0.107
Gårdsjön outflow	DSL	6.9	6.19	1.47	0.40	5.5	-0.62	4.09	4.01	299	0.029
Stora Bjurevatten	DSL	7.0	9.68	1.49	0.48	3.3	-0.47	3.40	3.80	292	0.165
Västersjön	DSL	7.2	8.68	1.52	0.47	1.6	-0.53	3.43	3.78	266	0.037

^a kO_2 is the first-order oxygen decay rate. Values for all analytical replicates are listed in Table S2.

While it is optimized to target low-molecular weight (LMW) (≤ 500 m/z) DOM and targets a small fraction of the total DOM pool, how this relates to DOM optical properties and reactivity remains unknown. To address this knowledge gap, we collected samples from seven lakes and four streams in a boreal forest catchment and analyzed them using PTR-MS. We also used traditional optical metrics of DOM composition and investigated DOM degradability with an incubation study.

2. MATERIALS AND METHODS

Sampling was undertaken on August 19, 2017, in the Gårdsjön research catchment in southwest Sweden (Table S1 and Figure S1). The climate is temperate, and the annual precipitation is 700–1200 mm. The altitude is 100–170 m above sea level.¹⁸ The catchment is predominantly forest covered with *Picea abies* and *Pinus sylvestris* and small areas (10%) of mire. Soils are mainly thin podzols, with bedrock outcrops.¹⁸ The sampling included 11 sites: three headwater lakes, the inflow and outflow of Gårdsjön, three headwater streams draining wholly terrestrial catchments, and one stream draining a headwater lake. Additionally, we sampled two nearby brown-water lakes that had much larger catchments.

Samples for DOM degradability analyses were filtered in situ with prerinsed cellulose acetate 1.2 μ m filters and collected in precombusted 40 mL glass vials. Samples were stored overnight in the dark at 20 °C and transported to the laboratory (6 h journey) where they were stored in a dark incubator at 20 °C. These samples were used to measure oxygen (O_2) concentration as a proxy for bacterial respiration.¹⁹ A PreSens O_2 sensor spot (PreSens GmbH, Regensburg, Germany) placed on the inner wall of each vial allowed for non-invasive measurement of O_2 concentrations with a PreSens Fibox 3. The first O_2 reading was taken after all samples had been collected and allowed to adjust to the ambient temperature. Measurements were taken twice a day for the first 4 days and then once a day for 3 days (7 day incubation). We plotted log-transformed oxygen concentrations against time and calculated slopes for each sample, representing first-order oxygen decay rate constants (kO_2).

Samples for DOM composition using optical analysis were collected in 100 mL glass bottles, filtered in situ with prerinsed

0.45 μ m cellulose nitrate filters, and then stored in the dark at 4 °C. They were transported on ice the following day to the laboratory. Dissolved organic carbon (DOC) concentrations were measured as nonpurgeable organic carbon on a Shimadzu TOC-V_{CPH} instrument (Shimadzu, Kyoto, Japan). The fluorescence and absorbance were measured on an Aqualog spectrofluorometer (Horiba, Kyoto, Japan), and from this, we calculated the fluorescence index,²⁰ freshness index,⁹ humification index,²¹ SUVA,⁸ and fluorescence peaks²² (detailed in the Supporting Information).

To prepare samples for PTR-MS analysis,¹⁴ a low-pressure evaporation/sublimation system removed water from the samples, leaving residues of semivolatile and nonvolatile OM. The samples were then thermally desorbed at 250 °C and measured using a PTR-TOF 8000 instrument (IONICON Analytik, Innsbruck, Austria). Five of the samples were analyzed in duplicate, and the reproducibility was comparable to those of other MS methods (Bray–Curtis dissimilarity = 5.24%). Peak identification and integration were performed using PTRwid.²³ Chemical formulas were assigned using PTRwid and mMass²⁴ and classified into structural groupings (e.g., aliphatic).²⁵ We term the output of this analysis “PTR-DOC”. The PTR-DOC metrics O/C, H/C, mean oxidative state of carbon (OSC), and mean number of carbon (nC) atoms per molecule were calculated for each sample. Percent PTR-DOC was calculated as the sum of individual ion concentrations multiplied by the ion-specific C mass fraction obtained from the chemical formulas and expressed as a percentage of the DOC concentration from the Shimadzu instrument. We used Shannon’s index of diversity²⁶ as a measure of chemodiversity (see the Supporting Information for further PTR-MS information).

We conducted principal component analysis (PCA) using Canoco 5.0, with pH, DOC concentration, PTR-DOC metrics (listed above), and fluorescence and absorbance metrics to investigate differences in DOM quality. We used Pearson’s correlation coefficient to test for relationships between individual ions and DOM fluorescence and absorbance metrics.²⁷ For these tests, we included only ions that were detected in all samples. Additionally, we performed Pearson correlation analyses using the PTR-DOC metrics. Correlation

analyses were performed using SPSS Statistics 24, with a significance level set at $p < 0.05$.

3. RESULTS AND DISCUSSION

PTR-MS detected a total of 314 unique ions across the data set ranging from m/z 27.023 to 395.374 (Figures S2 and S3), which corresponded to a mean of 5.5% of the measured DOC pool (%PTR-DOC in Table 1). Aliphatic ions were most numerous (72), followed by condensed aromatics (63), aromatics (60), and peptides (63); 246 of the ions were found in all samples. Mean ion concentrations ranged over multiple orders of magnitude and generally declined with increasing m/z values. Two of the three streams had small numbers of ions and low chemodiversity, as did two downstream lakes (Bjurevatten and Västernsjön). In contrast, the three headwater lakes had the highest chemodiversity (Table 1). The percent of total DOC detected by PTR-MS (%PTR-DOC) was lowest in the headwater streams and for all samples was inversely related to the log-transformed DOC concentration (Figure S4; $r^2 = 0.62$; $p = 0.004$). PCA showed that headwater streams grouped distinctly from lakes and the stream with an upstream lake (Figure 1). Specifically, streams

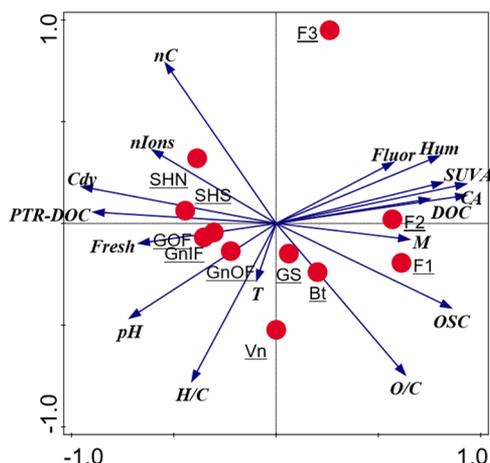


Figure 1. Principal component analysis (PCA) of pH and dissolved organic matter (DOM) composition. Circles indicate sampling sites, while DOM metrics are shown in bold. Site codes are underlined: Gaffeln (GOF), Gaffeln Stream (GS), Stora Hästevatten North (SHN), Stora Hästevatten South (SHS), Gårdsjön inflow (GnIF), F1 stream (F1), F2 stream (F2), F3 stream (F3), Gårdsjön outflow (GnOF), Stora Bjurevatten (Bt), and Västernsjön (Vn). PTR-DOC metrics: ratio of hydrogen to carbon atoms (H/C), ratio of oxygen to carbon atoms (O/C), concentration of DOC measured by proton-transfer-reaction mass spectrometry (PTR-MS) normalized against DOC concentration (PTR-DOC), mean oxidative state of carbon (OSC), mean number of carbon atoms per molecule (nC), molecular chemodiversity (Cdv), and number of ions ($nIons$). DOM metrics from fluorescence and absorbance: fluorescence index, freshness index, humification index, SUVA, and fluorescence peaks C, A, M, and T. 53% of the variation is explained on the horizontal axis, and 16% on the vertical axis.

were defined by high values for DOC, SUVA, humification and fluorescence indices, and fluorescence peaks A and M. Upstream lakes were defined by higher values for chemodiversity, %PTR-DOC, freshness index, and total number of ions detected. The two lakes farthest downstream, Bjurevatten and Västernsjön, were defined by higher values for O/C and H/C. Running a PCA using only the concentrations of ions

detected resulted in similar groupings being detected between sites (Figure S5). Notably, the headwater streams clustered separately from the lakes on both analyses, and the two downstream lakes were close to one another.

First-order oxygen consumption rate constants (kO_2) varied considerably, from 0.001 to 0.325 day^{-1} . Significant correlations were found between kO_2 and three PTR-DOC metrics: OSC ($r = 0.84$), chemodiversity ($r = 0.86$), and %PTR-DOC ($r = -0.80$) (Figure 2). Furthermore, significant correlations were detected between kO_2 and 70 individual ions, meaning 28.5% of ions significantly correlated (mean absolute $r = 0.64$; mean $p = 0.036$) (Table S3). Of these correlations, 67 were negative and only three were positive. The majority of correlated ions were peptides (21), aromatics (13), and condensed aromatics (19).

Three PTR-DOC metrics, %PTR-DOC, OSC, and chemodiversity, showed significant correlations with fluorescence and absorbance measures of DOM composition (mean absolute $r = 0.73$; mean $p = 0.017$) (Table 2). Additionally, we found 21 ions that had significant correlations with optical measures of DOM composition (mean absolute $r = 0.64$; mean $p = 0.032$) (Table S4). For this analysis, we included only ions that were detected in all samples, giving a total of 246. Thus, 8.5% of ions had nominally significant correlations.

PTR-MS detected proportionally less of the total DOC pool in headwater streams. These streams drain highly organic peat soils²⁸ that typically have humic, HMW DOM that is less likely to be detected by PTR-MS, which, in this study, principally targets DOM $< m/z$ 500. Our findings show that headwater streams (i.e., “new” water) generally have fewer ions and lower chemodiversity of PTR-DOC. Other work in boreal forests has shown that extensive degradation of LMW DOM occurs at the soil–stream interface,²⁹ which could explain the observed lack of chemodiversity in our headwater streams when compared to that of lakes. The small sample size makes robust statistical analysis unwise, but these data suggest that DOM composition in streams draining small, terrestrially dominated catchments can vary considerably across small spatial scales.

The headwater lakes had high chemodiversity and proportionally more PTR-DOC. These are clear, low-DOC lakes, so it is likely that autochthonous production and photochemical production result in more PTR-DOC in these systems.³⁰ As water moves through the catchment, highly humic autochthonous DOM enters the system from wetlands and headwater streams. In downstream, brownwater lakes, microbial processes should lead to decreases in LMW DOM.³¹ Our analysis shows some indication of this, with an increased level of DOM oxidation, a smaller number of C atoms per molecule, and less PTR-DOC as a percentage of total DOC in downstream waters. All of these can be considered signatures of DOM processing.¹⁴ Indeed, the proportion of PTR-DOC to total DOC is approximately halved between the inflow and outflow of Gårdsjön and declines again in the next lake down the catchment, presumably as a result of DOM processing and also due to the input of DOM from headwater streams. Furthermore, of all the lakes sampled, chemodiversity and the total number of ions were lowest in Bjurevatten and Västernsjön. These brownwater lakes have large catchments and will receive older water with highly processed DOM from upstream lakes and streams. This is consistent with other studies that reported molecular alterations related to the aging and reworking of DOM.^{27,32}

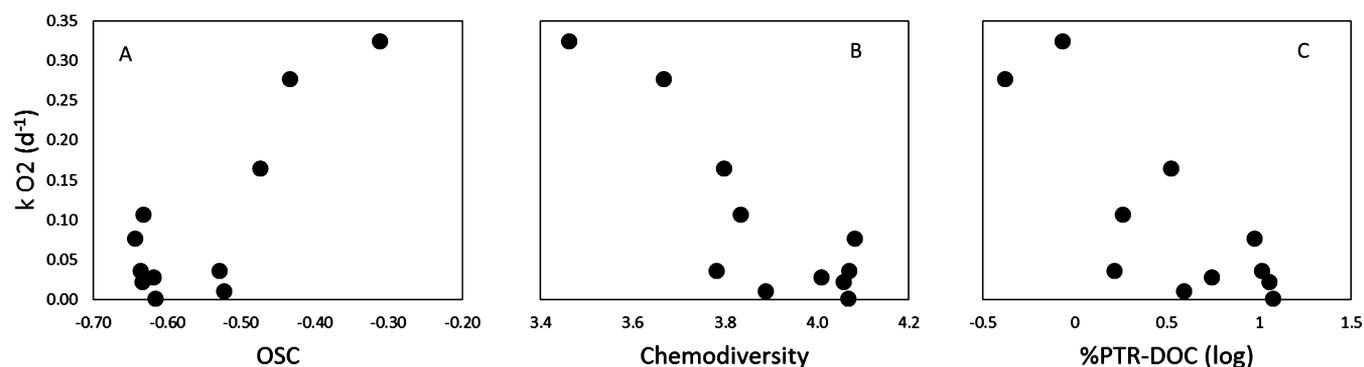


Figure 2. Scatter plots between first-order oxygen decay rates (k_{O_2}) and (A) the mean oxidative state of carbon (OSC) ($r = 0.84$; $p = 0.001$), (B) molecular chemodiversity ($r = -0.86$; $p = 0.001$), and (C) the total concentration of DOC measured by proton-transfer-reaction mass spectrometry (PTR-MS) normalized against DOC concentration (log-transformed) (%PTR-DOC) ($r = -0.80$; $p = 0.003$).

Table 2. Pearson r Values and Nominal p Values (in parentheses) for Significant ($p < 0.05$) Correlations between PTR-DOC Metrics and Fluorescence and Absorbance Metrics^a

	%PTR-DOC	OSC	chemodiversity
SUVA	-0.73 (0.01)		-0.73 (0.012)
freshness	0.62 (0.044)		
humification	-0.66 (0.028)		-0.62 (0.041)
peak A	-0.85 (0.001)	0.68 (0.02)	-0.82 (0.002)
peak C	-0.82 (0.002)	0.68 (0.021)	-0.81 (0.003)
peak M	-0.70 (0.017)		

^aPTR-DOC metrics are the total concentration of DOC measured by proton-transfer-reaction mass spectrometry (PTR-MS) normalized against DOC concentration (%PTR-DOC), the mean oxidative state of carbon (OSC), and molecular chemodiversity.

Both the bulk molecular signal and individual ions detected by PTR-MS were significantly related to DOM degradability, as measured by optode experiments. Such experiments can be informative measures of bacterial respiration and carbon processing.^{19,33,34} Correlation analyses produced statistically significant relationships between O_2 losses and both DOM chemodiversity and the oxidation state of carbon. Specifically, the lower the chemodiversity and the more oxidized the PTR-DOC, the more susceptible the total DOM pool appears to be to rapid microbial consumption. Furthermore, our findings show that as the amount of PTR-DOC increases (either as a proportion of the total DOC concentration or as individual ions), DOM degradability decreases, suggesting that a higher concentration of PTR-DOC is associated with a recalcitrant bulk DOM pool. This contrasts with research showing that LMW compounds such as dissolved free carboxylic acids can be important microbial substrates,³⁵ although we observed few of these compounds in our samples, presumably because of their high volatility. It could be that HMW compounds are more bioavailable³⁶ or that chemical structure (OSC and chemodiversity) is a more informative indicator of DOM reactivity,³⁷ but it is also important to consider that some PTR-DOC is likely to be generated during analysis, by fragmentation of larger ions (see the Supporting Information).

This is the first use of PTR-MS to characterize DOM across the aquatic continuum. Despite our relatively small sample size, we observed several statistically significant correlations between PTR-DOC and optical analyses. Furthermore, PCA found similar groupings of sample sites when it was performed

on either the optical data or the molecular data. Relationships have been found between molecular formulas generated by FTICR-MS and optical indices for DOM from rivers, wetlands, and seas,^{25,27,38} but our data provide evidence that PTR-DOC behaves like the bulk DOM pool measured by absorbance and fluorescence.

In conclusion, PTR-MS is a useful tool for understanding DOM. It provides detailed molecular information about DOM composition and appears to act as a proxy for the bulk DOM pool. Furthermore, PTR-DOC can comprise a significant portion of total DOC and significantly relates to the degradability of the total DOM pool. Future studies with PTR-MS analysis before and after incubation would elucidate whether PTR-DOC is an accessible substrate for microbial respiration. If combined with optical analyses, PTR-MS would enrich our understanding of DOM dynamics by giving detailed molecular metrics (chemodiversity and oxidation state) alongside the LMW/HMW, autochthonous/allochthonous, and humic/nonhumic data provided by fluorescence and absorbance. The fact that the method is quantitative also potentially paves the way to understanding what portions of DOM are responsible for fluorescence properties. Additionally, as PTR-DOC increases proportionally with a decrease in DOC, it could be that PTR-MS would be a particularly useful tool in understanding marine and coastal DOM.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.estlett.8b00529](https://doi.org/10.1021/acs.estlett.8b00529).

Location map of sampling sites, depths and areas of sampled lakes, several additional figures displaying PTR-MS data, two tables of statistical results, and detailed information concerning methods (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: michael.peacock@slu.se.

ORCID

Mike Peacock: [0000-0002-3086-2854](https://orcid.org/0000-0002-3086-2854)

Notes

The authors declare no competing financial interest.

The full PTR-MS data are available at Figshare: <https://figshare.com/s/53ab73e8f7f7a5084ee9>.

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