AGU PUBLICATIONS

JGR

Journal of Geophysical Research: Biogeosciences

RESEARCH ARTICLE
10.1002/2015JG003218

Key Points:
- DOM biodegradability varies synchronously among watersheds varying in size
- DOM composition was only distinct among different watersheds during storm flow
- 15.7% of annual DOC export from these northern temperate watersheds was biodegradable

Supporting Information:
- Supporting Information S1

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Citation:
Coble, A. A., A. M. Marcarelli, E. S. Kane, D. Toczydlowski, and R. Stottlemyer (2016), Temporal patterns of dissolved organic matter biodegradability are similar across three rivers of varying size, *J. Geophys. Res. Biogeosci.*, *121*, 1617–1631, doi:10.1002/2015JG003218.

Received 18 SEP 2015
Accepted 23 MAY 2016
Accepted article online 26 MAY 2016
Published online 18 JUN 2016

Temporal patterns of dissolved organic matter biodegradability are similar across three rivers of varying size

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Abstract Dissolved organic matter (DOM) composition may be an important determinant of its fate in freshwaters, but little is known about temporal variability in DOM composition and the biodegradability of DOM in northern temperate watersheds. We measured biodegradable dissolved organic carbon (BDOC) via incubation assays and DOM composition using optical indices on 11 dates in three Lake Superior tributaries. Percent BDOC (%BDOC) and BDOC concentrations were seasonally synchronous across these watersheds, despite that they vary in size by orders of magnitude (1.7 to 3400 km^2). Relative to %BDOC, BDOC concentrations were more tightly constrained among sites on any given date. BDOC also varied within seasons; for example, % BDOC on two different dates in winter were among the highest (29% and 54%) and lowest (0%) values observed for each site (overall %BDOC range: 0 to 72%). DOM composition varied the most among tributaries during a summer storm event when BDOC (both as percent and concentration) was elevated but was remarkably similar among tributaries during fall, spring, and winter. Multivariate models identified humic-like and tryptophan-like fluorophores as predictors of %BDOC, but DOM composition only described 21% of the overall variation in %BDOC. Collectively, these three rivers exported ~18 Gg C yr^{-1} as DOC and ~2 Gg C yr^{-1} as BDOC, which corresponded to 9 to 17% of annual DOC exported in biodegradable form. Our results suggest much of the C exported from these northern temperate watersheds may be biodegradable within 28 days and that large pulses of labile DOM can be exported during storm events and spring snowmelt.

1. Introduction

Dissolved organic carbon (DOC) plays an integral role in biogeochemical cycling in aquatic ecosystems because it provides an energy source for aquatic organisms, alters pH, affects light penetration, and binds elements [*Maranger and Pullen*, 2003; *Laudon et al.*, 2004]. Streams can supply a large amount of DOC to downstream ecosystems [e.g., *Schindler et al.*, 1997], and much of the spatial variability in riverine dissolved organic matter (DOM) concentration and character can be attributed to watershed land cover characteristics such as percentage of wetland cover, wetland type, and agricultural land use [*Gergel et al.*, 1999; *Frost et al.*, 2006; *Wilson and Xenopoulos*, 2008; *Yamashita et al.*, 2011]. However, streams are not simply a conduit from terrestrial environments to downstream ecosystems [*Bernhardt et al.*, 2005]. In-stream processing of terrestrial DOC can transform or mineralize terrestrially derived organic C before it reaches downstream ecosystems [*Frazier et al.*, 2005; *Hall and Meyer*, 1998; *Butman and Raymond*, 2011], and in-stream processing can vary with stream size. Previous research has shown that the greatest amount of CO_2 evasion occurs from the smallest streams, which is controlled by both gas transfer velocity and in-stream C processing [*Butman and Raymond*, 2011; *Wallin et al.*, 2012; *Hotchkiss et al.*, 2015]. Photodegradation and autochthonous production of DOM in aquatic systems can also alter the quantity and chemical composition of DOM [*Kaplan and Bott*, 1982; *Larson et al.*, 2007], further contributing to spatial variability of DOM composition.

Variation in DOM composition among watersheds is important because it may determine the biodegradability of the DOC pool that is found within or exported from rivers, and biodegradability will determine the ultimate fate of DOC—e.g., whether it is respired by microbes and released as CO_2 or stored in freshwater or marine ecosystems. DOM composition has been linked with biodegradable DOC (BDOC) across a wide range of ecosystems including temperate soils [*Marschner and Kalbitz*, 2003] and freshwater and marine ecosystems [*Benner*, 2003]. For example, studies suggest that some humic fractions of DOM may be important

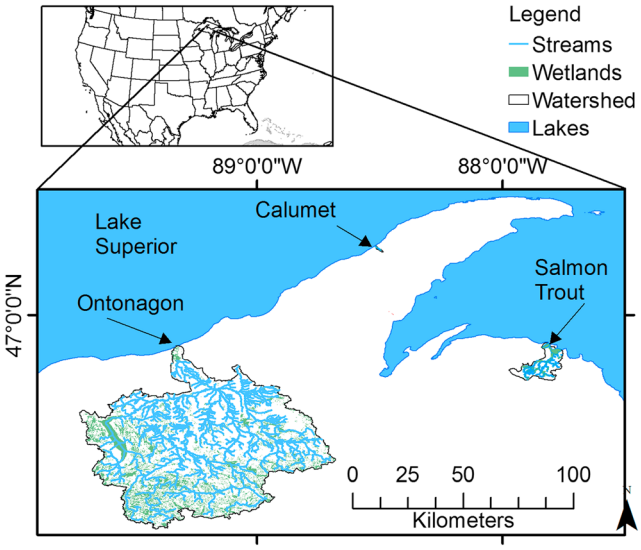

Figure 1. Map displaying the location of the study sites with wetlands, streams, and lakes within each watershed. Streams and lakes were identified from National Hydrography Dataset (http://nhd.usgs.gov) and wetlands were identified from the National Wetlands Inventory (http://www.fws.gov/wetlands/).

contributors to BDOC, and humic substances can account for as much as 75% of streamwater BDOC [*Moran and Hodson*, 1990; *Volk et al.*, 1997]. However, a wide range of low molecular weight humic substances with varying susceptibility to degradation can be found in aquatic ecosystems, and their contributions to the BDOC pool may also vary. Furthermore, proteins, carbohydrates, and organic acids have also been linked with high BDOC [*Marschner and Kalbitz*, 2003; *Balcarczyk et al.*, 2009; *Fellman et al.*, 2009a; *Wickland et al.*, 2012].

Temperate rivers alone account for 23% of global total organic carbon export from terrestrial environments, trailing only tropical rivers [*Meybeck*, 1982], yet we know little about short-term (week to month) variations in biodegradability and composition of organic carbon in these rivers. By investigating changes in bulk DOC concentrations, *Wollheim et al.* [2015] suggest that the reactivity of DOC is minimal in a temperate river network under base flow conditions. However, previous research in temperate streams has shown that %BDOC may differ depending on season [*Hosen et al.*, 2014; *Coble et al.*, 2015] or over several months [*Volk et al.*, 1997]. Recent studies in Arctic rivers demonstrated that a high fraction of labile DOC was exported during winter and the spring freshet and attributed this variability to changes in N availability, DOM chemical composition, and flow paths [*Holmes et al.*, 2008; *Wickland et al.*, 2012]. We may expect different seasonal patterns in BDOC in northern temperate rivers versus those observed in arctic rivers due to differing hydrologic flow paths, particularly in winter and spring, which may alter rates of DOM processing. In the arctic during the spring freshet, frozen soils can restrict runoff to surface or shallow soil flow paths, which allows runoff to leach the upper organic carbon rich layers [*Striegl et al.*, 2005; *Finlay et al.*, 2006]. By comparison, in northern temperate regions without permafrost, shallow flow paths also persist during snowmelt [*Kendall et al.*, 1999] and deep snowpack can insulate temperate soils during the winter [*Stottlemyer*, 1987; *Stottlemyer and Rutkowski*, 1990], preventing soil freezing and allowing winter microbial processing of organic matter beneath the snowpack.

The overall goal of our study was to examine how variability in DOM composition caused by seasonal variation in hydrology and solute inputs may alter the biodegradability of DOC in three northern temperate rivers. The study rivers are all tributaries of Lake Superior, where DOM exported from rivers can be important for fueling near-shore microbial dynamics [*Biddanda and Cotner*, 2002]. We measured DOC concentrations and conducted short (28 day) incubations approximately monthly over a full year to determine DOC biodegradability, relative concentration of BDOC, and estimate annual DOC and BDOC export. Our objectives were to (1) estimate the percent of total annual DOC exported from these three rivers that is present in biodegradable form and determine whether biodegradability varies temporally, (2) identify relationships between DOM composition and the amount and proportion of biologically available DOM in these rivers, and (3) estimate total annual DOC and BDOC export from these rivers to Lake Superior.

2. Methods

2.1. Study Sites

All three study tributaries are located on the south shore of Lake Superior in Michigan's Upper Peninsula (Figure 1). Calumet watershed is located in Houghton County, drains a 1.76 km^2 watershed area [*Stottlemyer and Toczydlowski*, 2006], and comprises 0.001% of Lake Superior's watershed area (127,700 km^2). The Salmon

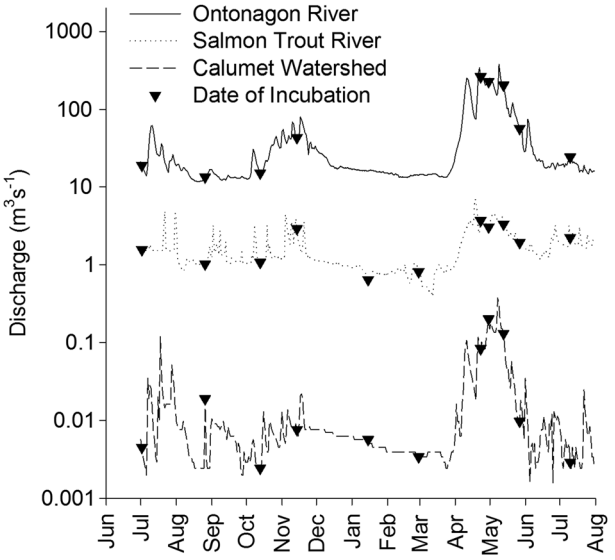

Figure 2. Annual discharge from 1 July 2013 to 31 July 2014 for Calumet Watershed, Salmon Trout River, and Ontonagon River. Downward triangles indicate sampling dates.

Trout River is located in Marquette County, drains a 127 km^2 watershed area [*Bullen,* 1988], and comprises approximately 0.1% of Lake Superior's drainage area. The Ontonagon River is located in Ontonagon County Michigan, drains a 3470 km^2 watershed area [*Thompson*, 1978], and comprises 2.6% of Lake Superior's drainage area. In this region, mean annual precipitation is ~80 cm (National Atmospheric Deposition Program, station MI99, Chassell Michigan, available from http://nadp.sws.uiuc.edu.data/) with up to 50% received as snow [*Stottlemyer and Toczydlowski*, 2006]. Bedrock within Calumet watershed is composed of Freda sandstone, Copper Harbor conglomerate, and Nonesuch formation; within the Salmon Trout watershed bedrock includes Archean granitic and gneissic, Michigamme formation, and Jacobsville sandstone; and within the Ontonagon watershed bedrock includes Jacobsville sandstone, Michigamme formation, and granite and gneissic bedrock. Land cover was determined using the 2011 National Land Cover Database (NLCD) [*Homer et al.*, 2015] and wetlands were determined using the National Wetlands Inventory [*U. S. Fish and Wildlife Service*, 2015]. Within these watersheds land cover is predominately composed of forests (63.5% at Calumet watershed, 74.5% at Salmon Trout River, and 69.0% at Ontonagon River; [*Homer et al.*, 2015] and wetlands (30% at Calumet watershed, 9.9% at Salmon Trout River, and 20% for Ontonagon River; NWI). Development, defined as open space, low-, medium-, and high-intensity developed areas, within these watersheds is minimal (0% at Calumet watershed, 2.4% at Salmon Trout River, and 2.6% at Ontonagon River) as is agricultural land cover (0% at Calumet watershed, 0.01% at Salmon Trout River, and 3.5% at Ontonagon River) [*Homer et al.*, 2015].

2.2. Sample Collection and Analysis

To determine DOC biodegradability we collected stream water twice per season at each of the three tributaries. In spring we collected stream water on two additional dates for a total of four dates because we expected that BDOC may change rapidly during spring snowmelt and runoff [e.g., *Wickland et al.*, 2012]. For these four spring dates, we targeted two collections on the rising limb or at the peak of the hydrograph and two collections on the falling limb, but actual collection captured periods of sustained peak discharge and the falling limb at the Calumet and Ontonagon watersheds and only captured the falling limb at the Salmon Trout River (Figure 2). At the Ontonagon River, we were unable to collect water during the winter because ice cover prevented safe access to the water column. The other two study rivers were also ice covered during the winter, but we were able to safely access the water beneath the ice.

On each sampling date, stream water was filtered (0.45 μm Whatman sterile nylon membrane) within four hours of collection, and then stored at 4°C until the experiment was set up, which occurred within 24 h. An additional water sample was filtered on site for analysis of DOC concentration and DOM composition. Initial stream water temperature, conductivity, pH, turbidity, and dissolved oxygen were measured by deploying a YSI 6920 multiparameter sonde (YSI Incorporated, Yellow Springs, Ohio) in the stream on each sampling date. Because inorganic nutrients have been previously linked with BDOC [e.g., *Wickland et al.*, 2012; *Coble et al.*, 2015], we also collected filtered water samples to measure dissolved inorganic nitrogen (DIN) concentrations as ammonium (NH_4^+) and nitrate (NO_3^-), total dissolved N (TDN), and phosphorus as soluble reactive P (SRP) and total dissolved P (TDP). Finally, we collected stream sediment from each site by extracting three 1 cm deep cores (2 cm diameter) from the stream channel to use along with unfiltered stream water as a microbial inoculum for the BDOC incubations as described below. Sediment cores were covered with parafilm to maintain field moisture conditions, and cores were kept cool until the experiment was initiated.

DOC and TDN concentrations were determined using a TOC-V_{CSN} with a total nitrogen module TNM-1 (Shimadzu Scientific Instruments, Columbia Maryland). NH_4^+ analyses were conducted on a Turner Trilogy fluorometer (Turner Designs, Palo Alto, California) and followed *Taylor et al.* [2007], adapted from *Holmes et al.* [1999]. NO_3^- was analyzed on a Dionex ICS-900 Ion Chromatograph (Dionex, Sunnyvale California). SRP analyses were conducted using the ascorbic acid method [*American Public Health Association (APHA)*, 2005] on a Thermo Scientific 10s UV-Vis spectrophotometer. TDP analysis followed the same analysis as SRP preceded by an ammonium persulfate digestion [*APHA*, 2005].

2.3. %BDOC

To determine DOC biodegradability, 28 day laboratory incubation experiments were conducted by placing 50 mL of stream water filtered through 0.45 μm sterile nylon membrane filters into 15 replicate 100 mL amber glass serum bottles for each site on each date. To make the inoculum, 1 g of sediment from each of the three cores was measured and added to 500 mL of unfiltered stream water. From this slurry, 1 mL of inoculum was added to each bottle to introduce a microbial assemblage from the water column and from the benthos [e.g., *Coble et al.*, 2015]. The inoculum was site and date specific to better mimic in situ stream conditions and to account for seasonal variability in the microbial assemblage. For all sites and dates, DOC concentration from stream water alone were compared to concentration in the initial incubation vials with added inocula, and no differences were observed.

The serum bottles were sealed, and then CO_2 was analyzed for all bottles in 1 mL aliquots of equilibrated headspace using gas chromatography (model SRI 8610C; TCD detector; He carrier gas; Hayesep D packed column; column temperature 62°C). Calibration curves were created with five standard dilutions of 1000 ppm CO_2 standard (Scotty analytical gas, Supelco Analytical, Bellefonte PA). To calculate CO_2 concentrations, we used CO_2 equilibrium constants and corrections for temperature and pressure [*Plummer and Busenberg*, 1982; *Striegl et al.*, 2001]. The volume of headspace removed was replaced with an equal amount of N_2.

Following the initial CO_2 measurements on day 0, three replicate bottles per stream were acidified with 2 mL 43.5% H_3PO_4 and the headspace was analyzed for dissolved inorganic carbon (DIC) by measuring CO_2 as described above. Three additional replicate bottles per stream were analyzed for DOC concentration as described above and DOM composition as described below. The nine remaining bottles per stream were then placed on a shaker table in the dark at a temperature of 21°C to control for temperature among seasons. After 28 days, all replicate bottles were analyzed for headspace CO_2 concentrations, then six replicates were acidified and analyzed for DIC, and the final three replicates were analyzed for DOC concentration and composition. For each incubation, the percent BDOC (hereafter referred to as %BDOC) was calculated as follows [*Wickland et al.*, 2012]:

$$\%\mathrm{BDOC} = \frac{\mathrm{DIC_{final}} - \mathrm{DIC_{initial}}}{\mathrm{DOC_{initial}}} \times 100 \quad (1)$$

We opted to calculate %BDOC via headspace changes in CO_2 rather than as changes in DOC concentration over time because the analytical techniques we used for CO_2 were more sensitive than those for DOC concentration, and because analysis of headspace CO_2 production allows for repeated sampling over time and was recommended by *McDowell et al.* [2006] to differentiate between refractory and labile DOC pools.

BDOC concentration values were also determined by multiplying %BDOC by DOC concentration on each sampling date. Three of the BDOC values (Salmon Trout River January 2014, Calumet Watershed July 2014, and Ontonagon River 10 July 2014) were negative, but standard error ranges of our estimates overlapped with zero, suggesting that these values of %BDOC were below our detection ability using these assays.

To determine if BDOC varied temporally, we conducted a repeated measure analysis of variance (rm ANOVA) for each stream with date as the repeated factor and %BDOC and BDOC concentrations as the response variables with SAS version 9.4 (SAS Institute, Cary, North Carolina, USA). %BDOC data were arcsine transformed, and BDOC concentrations were log transformed to meet assumptions of normality. Because we were unable to sample the Ontonagon River in winter we conducted two separate rm ANOVA analyses (1) excluding Ontonagon River samples and (2) excluding winter samples to determine whether %BDOC or BDOC concentration varied among sample dates or sites. To investigate whether DOC concentration or BDOC (as % and concentration) followed similar patterns among sites, we determined Pearson correlation coefficients between each site. For all statistical analyses alpha was set a priori at 0.05.

Table 1. Spectral Characteristics of Dissolved Organic Matter for the Six Components Identified by Parallel Factor Analysis (PARAFAC)[a]

Component	Excitation Maxima (nm)	Emission Maxima (nm)	Similar to Components Identified From Previous Studies	Description	Likely Origin
1	<250 (340)	455	*Stedmon and Markager* [2005a], C4	Fulvic-like fluorophore	Terrestrial/autochthonous
2	<250	448	*Stedmon and Markager* [2005a], C1	Humic-like fluorophore	Terrestrial
3	<250 (310)	395	*Stedmon and Markager* [2005a], C3	Humic-like fluorophore	Terrestrial
4	282 (380)	514	*Olefeldt et al*. [2013], C*x*	Fulvic-like, humic-like, high molecular weight	Terrestrial
5	396 (277)	477	*Stedmon and Markager* [2005b], C7	Humic-like fluorophore	
6	279	344	*Stedmon and Markager* [2005a], C7	Tryptophan-like fluorescence	Autochthonous

[a]Secondary excitation maxima are in parentheses.

2.4. DOC Composition and PARAFAC Modeling

We characterized DOM composition using UV-Vis spectrophotometric and fluorescence methods. Specific ultraviolet absorbance at 254 nm ($SUVA_{254}$), an indicator of C aromaticity, was measured using a GENESYS™ 10s UV-Vis spectrophotometer (Thermo Scientific, Waltham, Massachusetts) and determined by dividing the UV absorbance at 254 nm wavelength by the DOC concentration [*Weishaar et al*., 2003]. Fluorescence excitation-emission matrices (EEMs) of stream water and incubation samples were determined with a Horiba Aqualog fluorometer (Jobin Yvon Horiba, France) at 3 nm excitation wavelength intervals between 240 and 600 nm and at emission wavelength coverage between 212 and 620 nm with 3.28 nm increments. Fluorescence spectra were corrected for inner filter effects using PLS Toolbox and the flucut function (Eigenvector Research Inc., Wenatchee, WA, USA) with MATLAB software (MATLAB®, The Mathworks, Natick, USA), accounting for the absorption of both emission and excitation light by the DOM sample. To remove the Raman signal, a Raman-normalized Milli-Q water sample was removed from each fluorescence spectrum. All EEMs were then expressed in Raman Units (R.U.; nm^{-1}). Fluorescence index (FI) has been correlated to the relative contribution of microbial versus higher plant organic matter sources and also is strongly correlated with percent aromaticity and the degree of structural conjugation [*McKnight et al*., 2001]. FI was identified from corrected EEMs as the ratio of the emission intensity at 450 nm to 550 nm acquired with an excitation of 370 nm [*McKnight et al*., 2001].

In addition, fluorescence EEMs were analyzed using the multivariate modeling technique parallel factor analysis (PARAFAC), a three way decomposition method [*Stedmon et al*., 2003]. PARAFAC modeling was completed using MATLAB software (MATLAB®, The Mathworks, Natick, USA), and the PLS-toolbox (Eigenvector Research Inc., Wenatchee, WA, USA) on 374 samples collected in Michigan's Upper Peninsula comprised primarily of river samples, but also including near-shore Lake Superior samples, snow samples, and soil lysimeter samples from study sites. This analysis validated six components, and split-half analysis following *Stedmon et al.* [2003] and *Stedmon and Bro* [2008] revealed that splits were 92.5% similar. All of the six components identified by the model have been previously described for aquatic systems (Table 1). Component one (C1) was identified as a fulvic-like peak, and components two (C2), three (C3), and five (C5) were identified as humic-like peaks (Table 1). Component four (C4) was identified as humic-like and fulvic-like (Table 1). One protein-like component, component six (C6), was identified as tryptophan-like in our data set, but tyrosine, another protein-like component commonly observed in aquatic ecosystems, was not identified. The fluorescence intensity of each component is expressed as a percentage of the total intensity of all components identified.

2.5. Multivariate Analysis of %BDOC and DOM Composition

To identify which DOM characteristics covaried with and explained the greatest variation in %BDOC across sites and dates, we used a partial least squares (PLS) with %BDOC (*y* response variable) and all of the measured DOM characteristics (*x* predictor variables which included: the six fluorescence components identified by PARAFAC, FI, $SUVA_{254}$, and DOC concentration). PLS is well suited for data sets with a large number of predictors relative to a small sample size and for predictors that are correlated [e.g., *Boulesteix and Strimmer*, 2006]. For this analysis we included only stream water samples from day 0 of the incubation. The importance of each *X* variable was determined by the variable importance on the projection scores (VIP) with a value ≥ 1

considered highly influential, between 0.8 and 1 as moderately influential, and <0.8 as less influential. Cross validation of the data set was performed with 10 splits. The number of components included in the model was determined by assessing eigenvalues and the root-mean-square error of the cross validation (RMSECV). Outliers were identified using the Hotelling's T^2 analysis if they exceeded the 95% confidence limit. PLS analyses were performed using MATLAB (MATLAB®) with the PLS Toolbox (Eigenvector Research Inc., Wenatchee, WA, USA) [*Wise et al*., 2006]. Following PLS analysis, we used linear regression to test for relationships between %BDOC and the most influential components identified with the PLS model. We also explored the relationship between %BDOC and [DOC]:[DIN] using linear regression [cf., *Wickland et al*., 2012].

2.6. Load Modeling

To quantify current DOC export from streams to Lake Superior, we calculated annual DOC loads from the three study tributaries. Water samples to measure DOC concentrations were collected at least monthly across a variety of flow and environmental conditions as described above. While these samples include river water collected during some high flow events (storms or spring freshet) our sampling scheme did not capture the majority of storm events that occurred at these sites. To calculate the total annual and monthly DOC loads from these Lake Superior tributaries, we used the FORTRAN load estimator (LOADEST) program [*Runkel et al*., 2004]. LOADEST requires at least 12 measurements of DOC concentrations; thus, we used >12 measurements of DOC at each site to calibrate the LOADEST model. Additionally, we used daily discharge measurements from each site to calculate monthly and annual loads.

Discharge at Calumet watershed is continuously monitored with a Parshall flume, Stevens pressure transducer (Stevens Water Monitoring Systems Inc., Portland, Oregon), and Li-Cor data logger (Li-Cor, Lincoln, Nebraska) installed at the site [*Stottlemyer and Toczydlowski*, 2006]. Discharge of the Salmon Trout and Ontonagon Rivers is gaged by the USGS (http:/waterdata.usgs.gov); discharge records were obtained for station 04043238, located in the headwaters of the Salmon Trout River, and for station 04040000, located on the Ontonagon River near Rockland, MI. Our study location at the Salmon Trout River was downstream of the gaging station; to relate discharge at the downstream site to the upstream USGS discharge measurements, we deployed a level logger downstream and measured discharge under a variety of flow conditions. To measure discharge, we set up a transect [*Gordon et al*., 2004] and measured velocity in at least 10 locations along the transect using a Flomate flow meter and wading rod. During high flow conditions (e.g., spring runoff) discharge was measured with a StreamPro Acoustic Doppler Current Profiler (Teledyne RDI, Poway, California). A logarithmic regression of discharge measurements from 2012 to 2014 was used to estimate discharge downstream based on the upstream gage data ($Q_{downstream} = 82.484 \times \ln(Q_{upstream}) - 89.519$, $n = 20$, $r^2 = 0.49$, $p = 0.0006$, $F_{(1,18)} = 17.46$).

To estimate BDOC loads from the study rivers we multiplied the monthly loads of DOC obtained from LOADEST by the %BDOC measured within that month at each site. When BDOC was not measured in that month then the measurement from the nearest month or an average of the nearest months were applied. The August storm flow BDOC measurement was not applied to the August estimate but was applied to specific days when storm flow events occurred in summer and fall as identified by examining the hydrograph.

3. Results

3.1. Biodegradable DOC Incubations: Spatial and Temporal Variability

Temporal patterns in discharge, temperature, and conductivity were similar among the three sites (Figure 2, Table S1). Dissolved inorganic N and P concentrations were often below detection limits, and for modeling purposes we replaced values below detection with a value of ½ the detection limit (Table S2). DOC concentrations ranged from 5.63 to 18.96 mg L^{-1} at Calumet Watershed, 2.73 to 11.14 mg L^{-1} at the Salmon Trout River, and 7.85 to 14.85 mg L^{-1} at the Ontonagon River (Table S2). During the August storm event, DOC concentration increased at the two smaller watersheds but decreased at the Ontonagon River relative to base flow DOC concentrations (Figure 3a). On all other dates the Ontonagon River had the greatest DOC concentration among the rivers (Figure 3a). The greatest DOC concentrations observed at the start of each experiment was the storm event (August) for Calumet watershed, during spring snowmelt for the Salmon Trout River (April), and in fall (November) for the Ontonagon River.

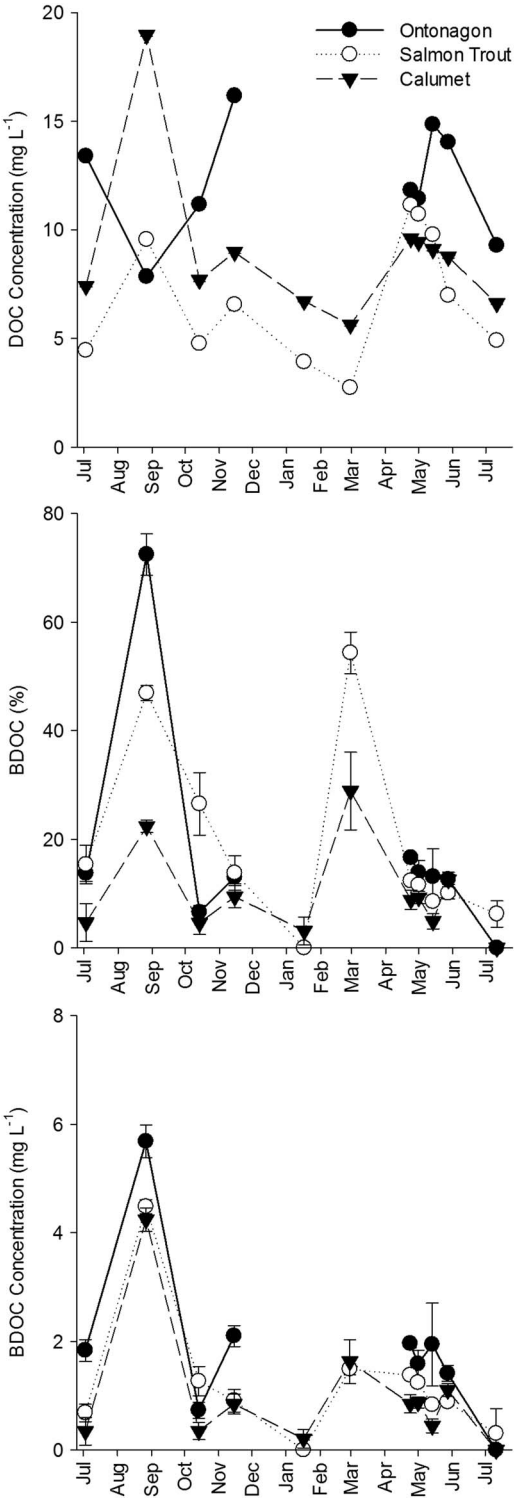

Figure 3. (a) Concentration of DOC (mg L^{-1}), (b) percent of biodegradable dissolved organic carbon within 28 days (%BDOC), and (c) concentration of BDOC (mg L^{-1}) measured in Calumet Watershed, Salmon Trout River, and Ontonagon River on all sampling dates in 2013–2014. Error bars represent standard error. Values below zero were replaced with a zero value for graphing purposes only; negative BDOC values were not altered for statistical analyses.

The %BDOC in these streams exhibited temporal variability, and some of this variability occurred within seasons (Figure 3b). Summer measurements of %BDOC ranged from negligible (Calumet Watershed and Ontonagon River, 10 July 2014) to moderate (all sites, 2 July 2013) to exceptionally high during the summer storm event (all sites, 26 August 2013). The greatest %BDOC at the Salmon Trout River and Calumet Watershed occurred in winter, on 28 February 2014, exceeding the magnitude of %BDOC during the summer storm event. However, %BDOC was low to negligible a month earlier on 15 January 2014 at both sites, in spite of continuous ice cover over both rivers from December until late April. Within-season variability in %BDOC was not as pronounced in fall and spring. We did not observe a relationship between %BDOC and position on the hydrograph during spring snowmelt; however, we were unable to capture the rising and falling limb at all sites due to variability in the timing of snowmelt and amount of snow among these three watersheds (Figure 2). Repeated measures ANOVA analysis of %BDOC among sample dates confirmed significant effects of site and date, and a significant interaction of site *x* date when the Ontonagon River was excluded (site ($F_{(1,38.7)} = 32.81$, $p < 0.001$), date ($F_{(10,37.4)} = 54.31$, $p < 0.001$), site *x* date ($F_{(10,37.4)} = 16.72$, $p < 0.001$), and when the Ontonagon River was included but winter was excluded (site ($F_{(2,47)} = 21.82$, $p < 0.001$), date ($F_{(8,51.1)} = 131.70$, $p < 0.001$), site *x* date ($F_{(16,60.7)} = 24.72$, $p < 0.001$).

There were some notable differences in temporal patterns of BDOC concentrations compared with the patterns of %BDOC described above. In contrast to the high %BDOC observed in February, BDOC concentration was much lower and of similar magnitude to concentrations during spring (Figure 3c). In November, BDOC concentration was elevated in the Ontonagon relative to the other two sites while %BDOC was similar among the three sites (Figure 3). In July 2013 BDOC concentration was also elevated in the Ontonagon relative to the other two sites. Overall, the BDOC concentrations were more tightly constrained among sites on any given date than %BDOC (Figure 3c). Repeated measures ANOVA analysis of BDOC concentration among sample dates also confirmed significant effects of site and date, and a significant interaction of site *x* date when the Ontonagon River was excluded (site ($F_{(1,34.5)} = 4.56$, $p = 0.040$), date ($F_{(10,41.4)} = 85.36$, $p < 0.001$), site *x*

Table 2. Partial Least Squares Model Performance as a Function of the Number of Latent Variables

Latent Variable	*X* Cumulative Variance Explained (%)	*Y* Cumulative Variance Explained (%)	RMSECV
1	64.01	5.92	14.22
2	78.53	13.22	14.83
3	84.69	15.25	15.45
4	94.20	16.95	15.39

date ($F_{(10,41.4)} = 5.28$, $p < 0.001$), and when the Ontonagon River was included but winter was excluded (site ($F_{(2,34)} = 4.35$, $p = 0.021$), date ($F_{(8,49.2)} = 115.81$, $p < 0.001$), site *x* date ($F_{(16,59.1)} = 2.56$, $p = 0.005$).

Both %BDOC and BDOC concentrations appear to be synchronous across the study sites, as suggested by significant Pearson correlation coefficients among all pairs of rivers for %BDOC (Calumet and Ontonagon: $r = 0.89$, $p = 0.001$; Salmon Trout and Ontonagon: $r = 0.87$, $p = 0.003$; Calumet and Salmon Trout: $r = 0.87$, $p < 0.001$) and BDOC concentration (Calumet and Ontonagon: $r = 0.94$, $p < 0.001$; Salmon Trout and Ontonagon: $r = 0.92$, $p < 0.001$; Calumet and Salmon Trout: $r = 0.97$, $p < 0.001$). This suggests that high and low values of BDOC generally occur at the same time in the three study streams. However, DOC concentrations were not correlated among all pairs of rivers (Calumet and Ontonagon: $r = 0.49$, $p = 0.184$; Salmon Trout and Ontonagon: $r = 0.08$, $p = 0.838$; Calumet and Salmon Trout: $r = 0.60$, $p = 0.05$), suggesting that DOC concentrations are not synchronous.

3.2. DOM Composition

3.2.1. Predictors of %BDOC

We used a PLS analysis and subsequent linear regression to determine the influence of DOM characteristics on riverine %BDOC across all sites and dates. The PLS model identified four latent variables (LV; Table 2) which explained 88% of the variability in the DOM composition (*X* variables) and 17% of the variability in %BDOC (*Y* variable). The model's ability to fit the data (RMSEC = 12.49) was similar to the model's ability to predict samples that were not used in model building (RMSECV = 15.39 [*Wise et al.*, 2006]). Analysis of *Q* residuals (11.8%) and Hotelling T^2 value (88.2%) indicate each sample conformed well to the model and revealed little variation in each sample within the model [*Wise et al.*, 2006].

To best illustrate the differences among DOM composition (*X* variables), we display only LV1 and LV2 (Figure 4). The first component (LV1) explained 64% of the variability in *X* variables (DOM composition), 6% of the variability in the *Y* variable (%BDOC), and represented a gradient of aromaticity; $SUVA_{254}$ and C1, (humic-like fluorophore) had negative loadings, while C3 (humic-like fluorophore) and C6 (tryptophan-like fluorophore) had positive loadings (Figure 4). The LV2 axis explained 15% of the variability in *X* variables and 7% of the variability in the Y variable and represented a gradient from a humic-like fluorophore (C3) to a tryptophan-like fluorophore (C6; Figure 4).

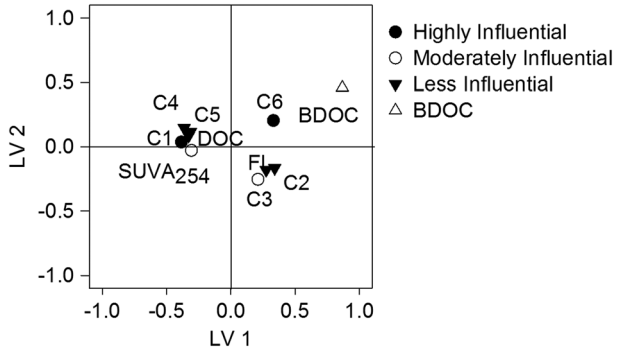

Figure 4. Partial least squares (PLS) plot predicting the variability of BDOC (response variable) with the following DOM characteristics as predictor variables: PARAFAC components (C1, C2, C3, C4, C5, and C6), specific ultraviolet absorbance at 254 nm ($SUVA_{254}$), fluorescence index (FI), and DOC concentration (DOC). Highly influential predictors were determined by having a variable importance on the projection (VIP) score > 1, and moderately influential predictors were identified by having a VIP score between 0.8 and 1; less influential predictors were identified by having a VIP score less than 0.8.

Based on VIP scores, C6 and C1 were identified as highly influential predictors of %BDOC in the model, $SUVA_{254}$ and C3 were moderately influential, and all other variables were less influential (Figure 4). Of these highly influential predictors, tryptophan-like fluorescence (C6) was positively related to %BDOC, indicated by its positioning near %BDOC on the PLS biplot and, the fulvic-like fluorophore (C1) was negatively related to %BDOC, indicated by its positioning opposite % BDOC (Figure 4). Linear regression analysis indicated that individually, none of the components (C1–C6), DOC concentration, $SUVA_{254}$, FI, nor [DOC]:[DIN] alone were significant predictors of % BDOC. The lack of significant individual regressions coupled with the low

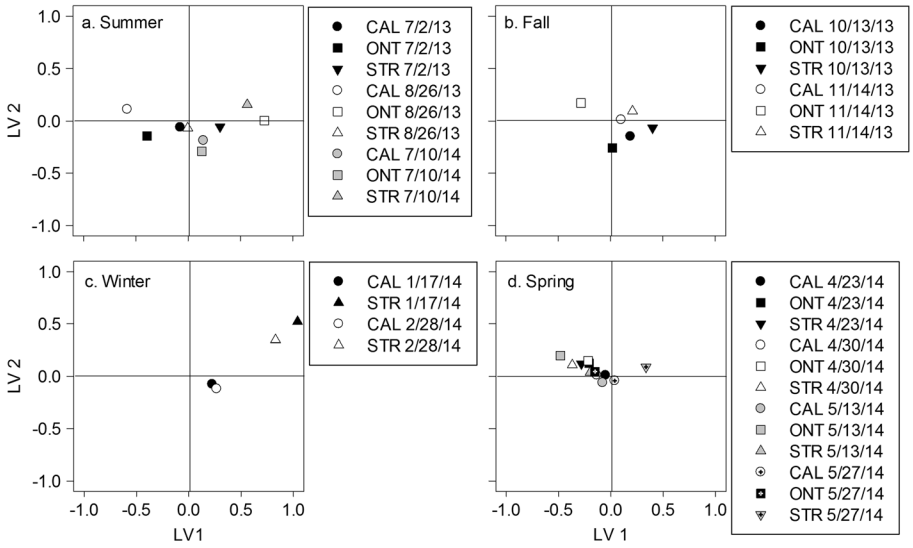

Figure 5. Partial least squares loading plots depicting the mean component scores for each date/site (Calumet Watershed = CAL; Ontonagon River = ONT; Salmon Trout River = STR) displayed and separated by season: (a) summer, (b) fall, (c) winter, and (d) spring. Axes for each of these panes are the same as in Figure 4, and positioning within the plot provides information about the DOM character for each site/date relative to the predictor variables described in that figure: sites/dates associated with a more positive score along the first axis (LV1) have a greater relative contribution of C2, C3, and C6, and a higher value of FI while sites/dates with a more negative score generally have a greater relative contribution of C1, C4, and C5; higher $SUVA_{254}$; and DOC concentration. Along the second axis (LV2) a positive score indicates a greater relative contribution of C2, C5, and C6, and a more negative score indicates a greater relative contribution of C2 and C3, and a greater FI.

explanatory power for the *Y* variable in the PLS model (17% of variation in %BDOC) suggests limited predictive ability of %BDOC by DOM composition variables.

3.2.2. Temporal Variability in DOM Concentration and Composition

The PLS analysis also provides insight into how the composition of DOM relative to %BDOC changed through time in the three study rivers. To illustrate these changes, Figure 5 depicts the DOM characteristics for each site on each study date on the same PLS axes as in Figure 4, separated into four panes by season. During summer base flow (Figure 5a), DOM composition shifted from the left side of LV1 (associated with the humic-like fluorophore C1 and aromaticity $SUVA_{254}$) in July 2013 to the right size of LV1 (associated with the humic-like fluorophore C3 and tryptophan-like fluorophore C6 in July 2014 (Figure 5a), when %BDOC was lower than in July 2013 (Figure 3). DOM composition during the summer storm flow event in August was distinct for each of the three sites, with all sites spread from smallest to largest along the first axis (LV1, Figure 5a), yet similarly high %BDOC was observed across all three sites during this event (Figure 3).

In fall, DOM composition at each site shifted negatively along LV1 toward greater contributions of the humic-like fluorophore C1 and aromaticity ($SUVA_{254}$) from October to November and also shifted positively along LV2 toward greater contributions of the tryptophan-like fluorophore C6 (Figure 5b). During this same time period, %BDOC increased at Calumet Watershed and the Ontonagon River and decreased at the Salmon Trout River, suggesting that these shifts in DOM composition were not associated with consistent changes in biodegradability. At Calumet Watershed, winter DOM composition was similar to the fall, while at the Salmon Trout River winter DOM shifted positively along LV1 and LV2 relative to fall, toward a greater contribution of the tryptophan-like fluorophore C6 (Figures 5b and 5c). Despite the similar DOM composition among winter measurements in each stream, winter %BDOC was variable with extremely low and high % BDOC observed (Figure 3b). BDOC concentration also increased more than 8 times from January to February at the two sites (Figure 3c).

In spring, all sites except for the Salmon Trout River in late May were located on the negative side of LV1, indicating that DOM was associated with the humic-like fluorophore C1 and higher aromaticity ($SUVA_{254}$). This suggests that DOM composition in the Salmon Trout River had returned to similar composition observed during summer earlier than the other rivers (Figures 5a and 5d). All other sites and dates revealed similar

Table 3. Watershed Area (km^2), Annual Discharge, Annual DOC, and BDOC Loads and Yields for Each River for the Period of Sampling (1 July 2013–30 June 2014)[d]

River	Watershed Area (km^2)	Annual Discharge ($m^3\ s^{-1}$)	DOC Load ($kg\ yr^{-1}$)	BDOC Load ($kg\ yr^{-1}$)	DOC Yield ($kg\ km^{-2}\ yr^{-1}$)	BDOC Yield ($kg\ km^{-2}\ yr^{-1}$)
Calumet Watershed	1.76[a]	7.01	4,899 (391)	422 (31)	2780	240
Salmon Trout River	127[b]	580	310,100 (45,000)	52,440 (6,500)	2,440	413
Ontonagon River	3,470[c]	15,900	17,810,000 (1,511,000)	2,788,000 (201,000)	5,130	803
Total export			18,124,999 (1,556,786)	2,840,862 (207,531)		

[a] *Stottlemyer and Toczydlowski* [2006].
[b] *Bullen* [1988].
[c] *Thompson* [1978]
[d] Standard error of DOC and BDOC loads as determined from LOADEST shown in parentheses.

positioning along LV1 across spring dates and varied mainly along the LV2 axis, suggesting that the amount of tryptophan-like (C6) versus humic-like fluorescence (C3) varied among dates. Consistency in DOM composition among spring dates concurs with the consistent %BDOC values observed for all study sites and dates (Figure 3b).

3.3. DOC, N, and BDOC Loads

As expected, magnitudes of the annual DOC and BDOC loads from these three rivers in 2013–2014 were reflective of watershed size and annual discharge (Table 3). A majority of the annual DOC loads were exported in the months of April and May, which alone comprised 62.9% of total annual DOC loads at Calumet watershed, 43.3% at the Salmon Trout River, and 62.5% at the Ontonagon River. The percent of the annual DOC load that was biodegradable within 28 days was comparable for the two larger rivers (17% and 16%) and lower for the smallest river (9%). Collectively, the three rivers exported 18.12 $Gg\ C\ yr^{-1}$ as DOC and 2.84 $Gg\ C\ yr^{-1}$ as BDOC that is decomposable within 28 days, which corresponds with 15.7% of annual export from these tributaries (Table 3).

4. Discussion

We found that BDOC varied synchronously across three northern temperate rivers despite large differences in watershed size. DOM composition, particularly compounds containing tryptophan-like and humic-like fluorophores, was a predictor of %BDOC but only explained 17% of the total variation in %BDOC. In our study the synchronous pattern of %BDOC cannot be explained by DOM composition as described by individual fluorometric and spectrophotometric indices or DOC concentration alone. For example, we observed large differences in indices of DOM composition among study sites during a summer storm event, yet on this date BDOC (% and concentration) was the highest observed for the entire year at all three sites. The greatest magnitude of difference in BDOC among sites also occurred during this storm event, indicating important differences in BDOC also existed among sites that were not reflected in our measurements of DOM composition. Our estimates suggest that on average, 15.7% of C that was exported to Lake Superior from these rivers was biodegradable within 28 days and large contributions of BDOC can be exported during much of the year, particularly during storm flow events and spring runoff. Given this apparent relationship between BDOC and hydrologic events like storm flow and spring runoff, future changes in climate that alter the timing of seasons or increase the frequency or severity of storms [e.g., *Easterling et al.*, 2000] may alter the quantity and timing of BDOC exported from northern temperate rivers, with potential consequences for productivity in downstream rivers, lakes, and oceans.

4.1. DOM Composition and Synchronous Biodegradability of DOC

Consistent with previous studies of riverine DOM [*Fellman et al.*, 2010; *Mann et al.*, 2012; *Wickland et al.*, 2012], we found that DOM composition was an influential predictor but only explained 17% of the variability in %BDOC across the study rivers. Evidence from several studies has indicated that terrestrially derived humic DOM may be an important component of the stream BDOC pool [*Moran and Hodson*, 1990; *Volk et al.*, 1997]. In our study

we did not find humic-like fluorophores to strongly influence variability in %BDOC (based on VIP scores in the PLS model), although a fulvic-like fluorophore (C1) was a highly influential predictor. Consistent with previous studies, we found that tryptophan-like fluorescence, an autochthonous DOM source, was also an influential predictor of %BDOC in the PLS model [*Fellman et al*., 2009a; *Balcarczyk et al*., 2009; *Wickland et al*., 2012]. However, it was not significantly related to %BDOC when tested using linear regression, consistent with *Lu et al.* [2013].

The apparent weak relationship observed between the potential autochthonous fluorescence component identified by our PARAFAC model (tryptophan-like fluorescence) and %BDOC may be reconciled by evidence that tryptophan-like DOM is generally recalcitrant relative to other autochthonous DOM components [*Guillamete and del Giorgio*, 2011; *Cory and Kaplan*, 2012]. For example, using stream water from White Clay Creek in Pennsylvania, *Cory and Kaplan* [2012] found that a greater fraction of tryptophan-like DOM was recalcitrant (73%) with less biodegradation observed (~30%) than tyrosine-like DOM (100% biodegradation). We further acknowledge the limitations in interpreting the source and identity of DOM strictly from PARAFAC components. C6, which is classically interpreted as tryptophan-like fluorescence, could also represent a nonautochthonously derived, low molecular weight material that fluoresces in the same region [*Stubbins et al*., 2014]. For example, molecular characterization of this component using high-resolution mass spectrometry was associated with 244 molecular formulae [*Stubbins et al*., 2014], likely with differing susceptibilities to microbial degradation. Furthermore, it should be noted that because percent tryptophan-like fluorescence is a relative measurement compared to all the compounds able to fluoresce in a sample, an increase in percent tryptophan-like fluorescence on any given date could also indicate a reduction in the relative amounts of other fluorescing compounds.

We found that DOM composition during a summer storm event was extremely variable among watersheds, and our results demonstrate that a wide range of DOC concentrations, percent protein-like fluorophores, and percent humic-like fluorophores can yield high %BDOC and BDOC concentration during storm events across a range of river sizes, which contrasts with the findings of several other studies [*Fellman et al*., 2009b; *Singh et al*., 2014]. *Singh et al.* [2014] investigated the effects of stream size on DOM composition during storm events and found that DOM composition was similar across a narrow range of stream sizes in nested watersheds (0.035 to 0.79 km^2). Perhaps a greater range in river size and spatial independence among rivers in our study led to greater variability in DOM composition during a storm event than that observed by *Singh et al.* [2014]. *McGuire et al.* [2005] found that flow path length and gradient were correlated with residence time and that these topographic characteristics influenced watershed-scale transport more than watershed size. We suggest that perhaps, storm events allow for differences in flow paths and residence times among watersheds included in our study to become more pronounced [e.g., *McGuire et al*., 2005; *Uchida et al*., 2006], leading to greater variation in DOM composition than observed on other dates. However, testing this hypothesis would require additional in-stream and flow path sampling for multiple storm events. Regardless of the mechanism controlling variability in DOM composition among rivers during storm events, biodegradability was high (but variable) in all rivers, suggesting that DOM mobilized during storm events may be rapidly decomposed and/or respired by microbes in recipient aquatic ecosystems.

DOM composition was remarkably similar among these watersheds on most sampling dates despite variation in land cover characteristics (wetland area: 10 to 30%; agriculture: 0 to 3.6%; and developed land area: 0 to 2.5%). In northern temperate regions DOM composition has been linked to variability in land cover characteristics such as percent agriculture, percent lake cover, percent wetland, and climate (periods of soil dryness) [*Frost et al*., 2006; *Wilson and Xenopoulos*, 2008]. *Wilson and Xenopoulos* [2008] found that the structural complexity of DOM decreased with increasing agricultural land cover and decreasing wetland cover and that microbially derived DOM increased with agricultural land use. Therefore, within our study watersheds we would expect the wide range in wetland coverage, rather than the narrow range in agricultural coverage, to contribute to a range in DOM structural complexity among sites. However, another study in the Upper Peninsula of Michigan found that minor differences in the amount of agriculture in a watershed can be related to DOC concentrations, ammonium concentrations, and pH [*Burtner et al*., 2011] suggesting that even minor variations in agriculture could influence chemical properties in watersheds. Given the similarity in DOM composition and differences in DOC concentrations among sites, we hypothesize that land use may have a greater influence on concentrations [e.g., *Wilson and Xenopoulos*, 2008; *Burtner et al*., 2011; *Larson et al*., 2014] while flow paths and hydrology may have a greater influence on DOM composition across these sites, although detailed field measurements of seasonal flow paths across each watershed and hydrological modeling would be needed to test this hypothesis.

One notable finding of our study is that BDOC was correlated among all of our study sites, suggesting that the temporal patterns of BDOC percent and concentration in the study streams were synchronous. For example, all sites exhibited lowest BDOC (as percent and concentration) in January and July 2014, highest BDOC in February (as percent) and during a summer storm event (as percent and concentration), and moderate BDOC (as percent and concentration) in spring. Other studies have also observed similarities in temporal patterns of %BDOC among rivers in temperate and Arctic ecosystems [*Jung et al*., 2015; *Wickland et al*., 2012]. In our study, temporal synchronicity in BDOC was likely driven by seasonal changes of biological or physical factors experienced by all rivers in the region, such as changes in microbial community composition, flow, water temperature, light availability, or elemental concentration, and interactions among these factors. For example, the site and date-specific inoculum used in our study introduced variability in microbial community composition that could explain temporal variability in BDOC and may also have contributed to the weak relationship between %BDOC and DOM composition. However, *Wickland et al.* [2012] also used date and site-specific inoculum and found a strong relationship between protein-like DOM and %BDOC. Different microbial communities present on each experimental date may also have responded differently to the drastic temperature differences between in situ and controlled laboratory temperatures.

Intriguingly, our data also suggest that similar compositions of DOM can have different biodegradability depending on the time of year. Summer base flow measurements demonstrate similarities in DOM composition but differences in biodegradability across dates (i.e., %BDOC and BDOC concentration was greater in July 2013 than in July 2014). Additionally, potentially autochthonous sources of DOM were most prevalent on both experimental dates in the winter, as indicated by higher percent of tryptophan-like components and higher FI values, yet the biodegradability of DOC varied dramatically between January and February. Alternatively, the greater contribution of C6 during the winter dates could be due to low molecular weight materials leached by groundwaters rather than due to autochthonous sources. Other studies measuring % BDOC beneath ice in rivers have found that the greatest percent tryptophan-like fluorescence occurred during winter in the Yukon River [*Wickland et al*., 2012] or the prespring freshet in the Kolyma River [*Mann et al*., 2012], but in the Yukon River the %BDOC was high (41 to 53%), while in the Kolyma River %BDOC was very low (0.1%) [*Wickland et al*., 2012; *Mann et al*., 2012]. Monthly measurements from our smallest study stream, Calumet watershed, indicate an increase in periphytic biomass in winter relative to other seasons [*Coble,* 2015], suggesting that autochthonous production of DOC may be greatest during this season. While the optical measurements used in our study provide efficient and informative characterization of DOM, more detailed chemical analysis of DOM molecular structure (e.g., using ultrahigh resolution mass spectrometry) may help elucidate the specific relationships between DOM composition and BDOC [*Stubbins et al*., 2014; *Kellerman et al*., 2015]. Furthermore, other factors such as microbial community composition, water temperature, and light availability may be important in regulating %BDOC during this season, and more intensive sampling and BDOC analysis combined with characterization of microbial community composition during the winter months is needed to understand the high amount of variability observed.

4.2. Export of Terrestrially Derived DOM

It is important to understand the fate of terrestrially derived DOM because it plays an essential role in aquatic productivity and energy budgets. We estimate that annually, 15.7% of DOC exported from these three rivers to Lake Superior is biodegradable within 28 days. However, we acknowledge that uncertainty of DOC and BDOC concentrations throughout storm events or across multiple storm events introduces additional uncertainty into our flux estimates. For example, *Fellman et al.* [2009b] found that the response of %BDOC during storm events varied with the position on the landscape, with upland streams showing decreased %BDOC and wetland streams showing increased %BDOC as storm events progressed. Despite the elevated BDOC observed in storm event or winter sampling periods, we estimate that 23 to 73% of BDOC is exported in spring across these rivers. This is not because the DOC pool is particularly susceptible to biodegradation during this time but rather because the majority of annual DOC was exported in spring (40 to 63%). During this snowmelt period, high water velocity leads to low river residence time, suggesting that much of this highly labile C is likely exported downstream to Lake Superior rather than stored or processed in tributary streams.

It is widely acknowledged that storm flow events can provide conditions conducive to rapid biogeochemical cycling when large DOC pulses occur in rivers [e.g., *Raymond and Saiers*, 2010], and some studies have shown that BDOC (expressed as percent or concentration) also increases during storm events [*Kaplan and Newbold*,

1995; *Volk et al*., 1997; *Buffam et al*., 2001; *Fellman et al*., 2009b]. Our results support the concept that these short time periods of rapid biogeochemical cycling are important in exporting not only high-quantity DOM but also high-quality DOM and further suggest that across a range in river sizes, a substantial amount of BDOC can be exported from northern temperate watersheds during storm events. However, our BDOC experiments only captured one storm flow event, and further study should examine how biodegradability and composition of DOM varies among storms within and across seasons.

Annual estimates of DOC and BDOC loads in our three study streams highlight the role of stream size in total DOC export. DOC yields were similar at the Salmon Trout (24.4×10^2 kg km^{-2} yr^{-1}) and Calumet (27.8×10^2 kg km^{-2} yr^{-1}) watersheds but were doubled at the Ontonagon watershed (51.3×10^2 kg ha^{-2} yr^{-1}). Furthermore, DOC concentrations between the Salmon Trout and Calumet were more closely correlated ($p = 0.05$) than with concentrations in the Ontonagon watershed, suggesting that there are likely different mechanisms driving DOC concentrations at the largest of these rivers. This could be due to differences in land use [e.g., *Burtner et al*., 2011; *Larson et al*., 2014], hydrological flow paths, soils, or relative contributions of groundwater. BDOC yields doubled with each increase in watershed size (Calumet 2.4×10^2 kg km^{-2} yr^{-1}, Salmon Trout 4.1×10^2 kg km^{-2} yr^{-1}, Ontonagon 8.0×10^2 kg km^{-2} yr^{-1}). Clearly, the largest of these rivers accounts for a considerably larger proportion of estimated loads exported from these rivers. DOC concentrations at our study sites (2.73 to 18.96 mg L^{-1}; Table S2) were similar to other Lake Superior tributaries (2.0 to 34.0 mg L^{-1}, Table S3) [*Back et al*., 2002; *Urban et al*., 2005; *Frost et al*., 2006; *Minor and Stephens*, 2008]. Using stream data layers from Michigan, Wisconsin, Minnesota, and Ontario (Canada), we estimate that there are approximately 1550 tributaries surrounding Lake Superior's shorelines. Of these 1550 streams, 1544 of them are smaller than the Ontonagon River, the sixth largest tributary of Lake Superior. Thus, DOC and BDOC export in the majority of these streams is likely more reflective of the smaller tributaries included in the current study.

At Calumet watershed *Stottlemyer and Toczydlowski* [2006] found that stream DOC export increased in the winter and spring seasons over a 14 year period from 1988 to 2002. This increase in DOC export suggests that the winter/spring snowmelt season may be critical for biogeochemical change in these aquatic systems. In our study, snowmelt is the time of peak BDOC export and unique DOM composition relative to base flow. Therefore, our results suggest that any future increases in spring DOC export may result in greater annual export of BDOC. Projected future changes in the amount of winter precipitation and increases in air temperature may affect the type of precipitation received in winter [*Wuebbles and Hayhoe*, 2004] and potential for soil freeze-thaw cycles [e.g., *Henry*, 2008]. Currently, substantial snowpack in the study region prevents soil freezing, in turn enabling belowground soil microbial processing to occur in winter in northern temperate ecosystems, and microbial processing ultimately influences the composition of runoff delivered to rivers [e.g., *Semkin et al*., 2002]. Alterations in the intensity or quantity of freeze thaw cycles may alter soil DOM pools [*Schmitt et al*., 2008] as well as subsurface flow paths [*Stottlemyer and Toczydlowski*, 2006]. Therefore, any alteration in quantity of snowpack or timing of snowmelt may affect seasonal and annual patterns of DOC and BDOC export from these and other northern temperate rivers.

Acknowledgments

Funding for this research was provided by NASA Michigan Space Grant Consortium, U.S.D.A. McIntire-Stennis funding to Michigan Technological University, a Research Seed Grant from Michigan Technological University's Research Excellence Fund, the Huron Mountain Wildlife Foundation, and the Michigan Tech Ecosystem Science Center. A.A.C. was also supported with a fellowship from the National Science Foundation's GK-12 Global Watershed program (award DGE-0841073). We thank E. Collins, B. Danhoff, K. Heiden, and T. Matthys for assistance in the field and lab and T. Veverica and K. Meingast for assistance with analysis of fluorescence characteristics. We also thank I. Buffam and four anonymous reviewers for helpful comments which improved the quality of this manuscript. Additional data supporting our findings are provided in the supporting information. This is contribution 37 of the Great Lakes Research Center at Michigan Tech.

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