3-11-2013

Photochemical and microbial alteration of dissolved organic matter in temperate headwater streams associated with different land use

Randolph Chambers
rmcham@wm.edu

Recommended Citation

Chambers, Randolph, Photochemical and microbial alteration of dissolved organic matter in temperate headwater streams associated with different land use (2013). *Journal of Geophysical Research - Biogeosciences*, 118(2), 566-580.
10.1002/jgrg.20048

Photochemical and microbial alteration of dissolved organic matter in temperate headwater streams associated with different land use

Yuehan Lu,[1] James E. Bauer,[2] Elizabeth A. Canuel,[3] Youhei Yamashita,[4] R. M. Chambers,[5] and Rudolf Jaffé[6]

Received 5 March 2012; revised 26 February 2013; accepted 2 March 2013; published 22 April 2013.

[1] Photochemical and microbial transformations of DOM were evaluated in headwater streams draining forested and human-modified lands (pasture, cropland, and urban development) by laboratory incubations. Changes in DOC concentrations, DOC isotopic signatures, and DOM fluorescence properties were measured to assess the amounts, sources, ages, and properties of reactive and refractory DOM under the influence of photochemistry and/or bacteria. DOC in streams draining forest-dominated watersheds was more photoreactive than in streams draining mostly human-modified watersheds, possibly due to greater contributions of terrestrial plant-derived DOC and lower amounts of prior light exposure in forested streams. Overall, the percentage of photoreactive DOC in stream waters was best predicted by the relative content of terrestrial fluorophores. The bioreactivity of DOC was similar in forested and human-modified streams, but variations were correlated with temperature and may be further controlled by the diagenetic status of organic matter. Alterations to DOC isotopes and DOM fluorescence properties during photochemical and microbial incubations were similar between forested and human-modified streams and included (1) negligible effects of microbial alteration on DOC isotopes and DOM fluorescence properties, (2) selective removal of ^{13}C-depleted and ^{14}C-enriched DOC under the combined influence of photochemical and microbial processes, and (3) photochemical alteration of DOM resulting in a preferential loss of terrestrial humic fluorescence components relative to microbial fluorescence components. This study provides a unique comparison of DOC reactivity in a regional group of streams draining forested and human-modified watersheds and indicates the importance of land use on the photoreactivity of DOC exported from upstream watersheds.

Citation: Lu, Y. H., J. E. Bauer, E. A. Canuel, Y. Yamashita, R. M. Chambers, and R. Jaffé (2013), Photochemical and microbial alteration of dissolved organic matter in temperate headwater streams associated with different land use, *J. Geophys. Res. Biogeosci.*, *118*, 566–580, doi:10.1002/jgrg.20048.

1. Introduction

[2] Human modifications of terrestrial environments may have potentially profound impacts on the transfer of organic and other biologically relevant materials to aquatic systems [*Wilson and Xenopoulos*, 2009; *Aufdenkampe et al.*, 2011]. Recent studies have shown that human land uses affect the amounts, quality, sources, and ages of dissolved organic matter (DOM) in streams and rivers. Such impacts include increases in the ^{14}C ages of organic carbon exported from watersheds [*Stern et al.*, 2007; *Sickman et al.*, 2010], changes in dissolved organic carbon (DOC) concentrations [*Stern et al.*, 2007; *Yamashita et al.*, 2010], and alterations to the structural complexity and chemical composition of DOM [*Warner et al.*, 2009; *Wilson and Xenopoulos*, 2009; *Williams et al.*, 2010; *Edmonds and Grimm*, 2011; *Yamashita et al.*, 2011a]. These changes are predicted to lead to modifications in DOM processing and metabolism in aquatic systems, of which photochemical and microbial transformations are two essential components.

[3] Photochemical processes may degrade DOM to smaller organic compounds and remineralize DOM to inorganic species such as CO_2 and CO [*Moran and Zepp*, 1997; *Obernosterer and Benner*, 2004], whereas bacteria can utilize DOM as sources of both energy and C, N, and P for cellular synthesis [*Benner*, 2003; *Kirchman*, 2003]. Both photochemical and microbial processing of terrestrially derived DOM may thus play important roles in key biogeochemical processes, such as carbon fluxes between terrestrial, aquatic and atmospheric reservoirs [e.g., *Gennings et al.*, 2001; *McCallister and del Giorgio*, 2008; *Aufdenkampe et al.*, 2011] and

Additional supporting information may be found in the online version of this article.

[1]Department of Geological Sciences, University of Alabama, Tuscaloosa, Alabama, USA.
[2]Aquatic Biogeochemistry Laboratory, Department of Evolution, Ecology and Organismal Biology, Ohio State University, Columbus, Ohio, USA.
[3]Department of Physical Sciences, Virginia Institute of Marine Sciences, Gloucester Point, Virginia, USA.
[4]Faculty of Environmental Earth Science, Hokkaido University, Sapporo, Hokkaido, Japan.
[5]Department of Biology, College of William and Mary, Williamsburg, Virginia, USA.
[6]Southeast Environmental Research Center and Department of Chemistry & Biochemistry, Florida International University, Miami, Florida, USA.

Corresponding author: Y. H. Lu, Department of Geological Sciences, University of Alabama, Tuscaloosa, AL 35485, USA. (yuehan.lu@ua.edu)

2169-8953/13/10.1002/jgrg.20048

energy/substrate transfer in aquatic food webs [*Cole and Caraco*, 2001; *del Giorgio and Davis*, 2003; *Moran and Covert*, 2003].

[4] While photochemical and microbial processing of stream and river water DOM may vary as a function of land use, relatively few studies have directly characterized these effects. *Findlay et al*. [2001] found marked changes in the fluorescence characteristics of DOM in subsurface waters from pastures after a 2 h sunlight exposure, compared to smaller changes in DOM from forested areas. They also showed that stream DOC bioavailability, as reflected by bacterial growth and respiration rates, was more closely related to the physical nature of stream flow paths (e.g., slumping of hillslope soils into the stream) than to land use types. *Williams et al*. [2010] documented that in situ microbial activity was higher in streams with watersheds modified by human land use relative to those with less anthropogenic modification. However, further work is needed to evaluate DOM reactivity across different land uses, to better constrain the effects of land use on DOM reactivity, and to assess the underlying mechanisms.

[5] Assessing how human alteration of watersheds impacts DOM reactivity is important for improving our understanding of DOM transformation along the land-fluvial-coastal ocean continuum and for assessing land-to-ocean carbon and organic matter (OM) fluxes. Terrestrial DOM, for example, has been suggested as a major but relatively unexplored factor contributing to coastal hypoxia [*Bianchi et al*., 2010]. While studies suggest that terrestrial OM is more refractory than aquatic DOM [*Benner*, 2003], the reactivity of terrestrial DOM may be altered by human activities, contributing to low dissolved oxygen concentration and poor water quality in downstream regions. In addition, DOC concentrations and fluxes in streams and rivers of Europe and North America have increased in the last decade [*Hejzlar et al*., 2003; *Evans et al*., 2005; *Skjelkvåle et al*., 2005]. Postulated mechanisms for these increases include declining acid deposition [*Krug and Frink*, 1983; *Driscoll et al*., 2003] and rising temperatures [*Freeman et al*., 2001]. Changes in land use may also play a role in these decadal-scale changes in DOC by altering its reactivity. In addition, OM inputs via streams and rivers have been extensively studied to evaluate the sources of terrestrial (i.e., allochthonous) versus aquatic (i.e., autochthonous) OM supporting estuarine and coastal metabolism [e.g., *Raymond and Bauer*, 2001a; *McCallister et al*., 2004; *Yamashita et al*., 2011b]. Alteration of terrestrial DOM by photochemical and microbial processes during its downstream transit may be an important factor regulating these terrestrial-aquatic linkages and the extent to which downstream metabolism is supported by terrestrial versus aquatic sources of DOM.

[6] The primary objective of the present study was to characterize how DOM exported from varying land use differs in its characteristics and in its photochemical and microbial reactivity and transformations. We selected seven headwater streams, three draining watersheds dominated by forests, i.e., forested-streams, and four draining watersheds dominated by human-modified land uses, i.e., human-modified streams, including pasture, cropland, and urban development, in a temperate watershed in Virginia, USA. Laboratory photochemical and microbial incubations were conducted, and changes in DOC concentrations were characterized over the incubation time course to quantify rates of DOC remineralization. Stable and radio-carbon isotopes ($\delta^{13}C$ and $\Delta^{14}C$) were used to evaluate changes in the sources and ages of DOC under the influence of photochemistry and/or microbes. Fluorescence properties of chromophoric DOM (CDOM) determined by excitation emission matrix-parallel factor analysis (EEM-PARAFAC) were further used to assess the DOM preferentially mineralized by photochemistry or/and microbes. Findings from this study provide new insights on the potential role of watershed land use on the sources and reactivity of DOM and alterations to DOM characteristics during photochemical and microbial processing.

2. Methods

2.1. Sampling Sites and Watershed Land Use Classification

[7] Seven first-order streams (Strahler scale) located within the lower Chesapeake Bay watershed in Virginia (USA) were chosen for this study (Figure 1). The watersheds of the three forested streams (F1, F2, and F3) have oak-pine forest coverage ranging between 87 and 100% (Table 1). Among the four human-modified streams, two streams drained pasture-dominated watersheds (P1 and P2), one drained a watershed dominated by cropland (C1), and one was influenced by urbanization (U1) (Table 1 and Figure 1). Pastures were annually rotated between warm-season grasses (May–October) and cool-season grasses (November–April), whereas croplands alternated between corn (May–October) and soybeans (November–April). All streams except U1 were located in rural areas (population density: 18 per km^2 as of 2000). U1 was situated in Williamsburg, Virginia (population density: 564 per km^2 as of 2008), and was located ~35–39 km from the other streams (Table 1 and Figure 1). During our sampling period (May 2009 to November 2009), monthly precipitation ranged between 7 and 21 cm and averaged 13 cm, which is typical compared to the precipitation range over the last decade (www.sercc.com).

[8] The watersheds associated with each of the study streams were delineated according to the 1 : 24,000 topographic maps (U.S. Geological Survey), which were then overlain on aerial photos (scale of either 1 : 1200 or 1 : 2400) and divided into polygons based on different land use types. The areas of the polygons were calculated in ArcGIS to determine the dominant land use in each watershed (Table 1). Assuming that stream DOM was primarily controlled by upstream land use, we only considered the watersheds upstream of each sampling location.

2.2. Sample Collection

[9] All containers and sampling equipment that were in direct contact with water samples were either combusted at 450 °C for 5 h for all glass materials, or acid soaked (10% HCl) and thoroughly rinsed with Milli-Q water for all plastic materials. Stream water samples were collected in 20 l polycarbonate carboys using a Masterflex® E/S™ portable sampler (Cole-Parmer) equipped with acid-cleaned silicone tubing. Due to the shallow nature of the sampling sites (15–30 cm), care was taken to avoid disturbing surface sediments. Sample carboys were stored in the dark on ice until filtration, which was done within ~6 h of sample collection. Parameters measured in situ included water temperature,

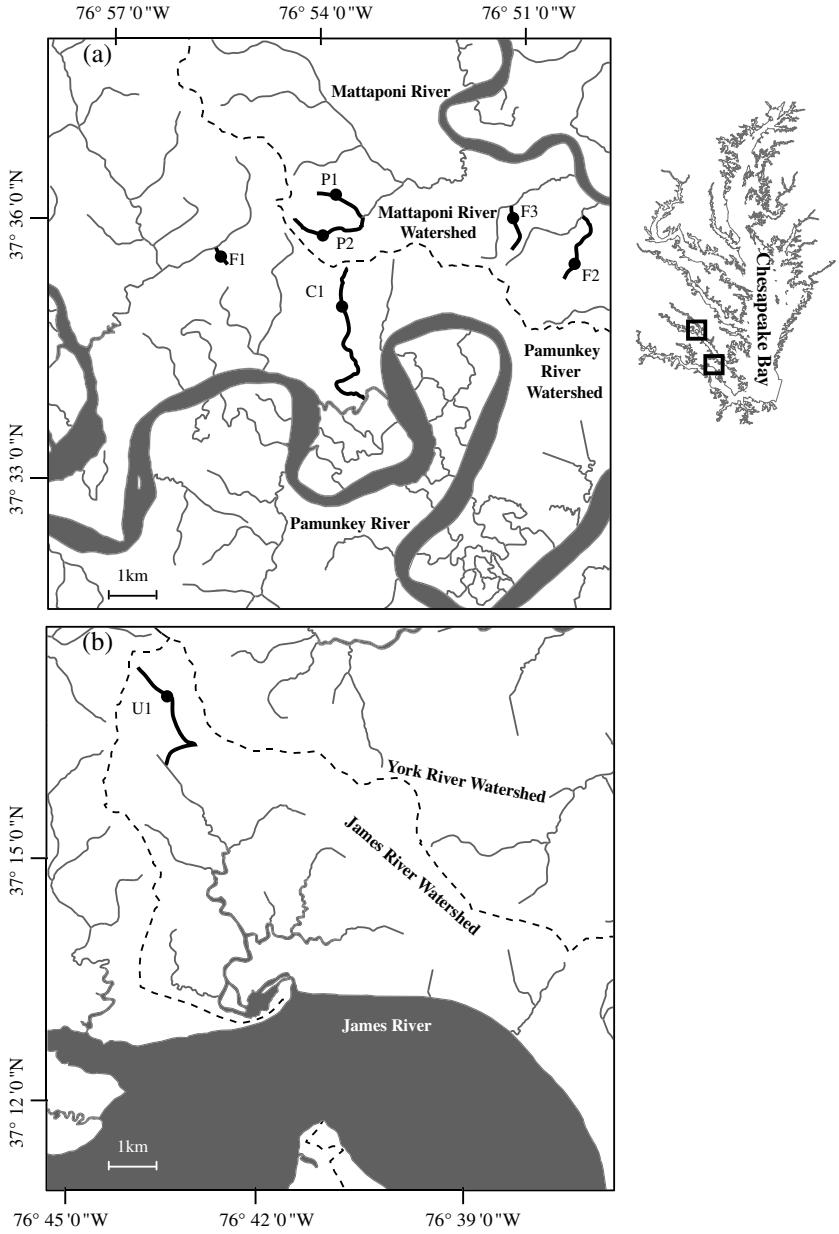

Figure 1. Locations of study sites within the (a) York River and (b) James River watersheds. Sampling streams are indicated by heavy black lines, and sampling sites are indicated by solid black dots. Other streams in this area that were not sampled are indicated by gray lines. The black dashed line delineates watershed boundaries of the major rivers in the region.

Table 1. Sampling Dates, Environmental Parameters Measured, and Watershed Land Use of the Study Streams[a]

Sampling Site	Sampling Dates	Water Temperature (°C)	Specific Conductivity (μS)	pH	Dissolved Oxygen (mg/l)	Watershed Land Use Composition	Chlorophyll-a (μg/l)	Nitrate (mg/l)	Ammonium (mg/l)	Watershed Size (km^2)
F1	08-18-2009	23.7	43.9	5.5	6.8	83% forest, 17% cropland	0.03	2.1	b.d.	0.27
F2	05-22-2009	15	92.5	6.2	5.8	100% forest	0.04	0.52	b.d.	0.09
F2	11-09-2009	12.6	158.9	5	3.0	100% forest	0.01	b.d.	b.d.	0.09
F3	11-09-2009	12.6	56.9	5	7.8	100% forest	0.05	b.d.	b.d.	0.28
P1	08-18-2009	24	136.7	6	4.0	70% pasture, 30% forest	6.27	0.69	b.d.	0.29
P1	11-09-2009	18.3	95.9	6	7.9	70% pasture, 30% forest	0.14	2.29	2.49	0.29
P2	08-18-2009	18.5	44.8	5.5	7.3	61% pasture, 39% forest	0.92	0.18	b.d.	0.44
P2	11-09-2009	16.5	73.4	6.5	5.8	61% pasture, 39% forest	0.48	b.d.	b.d.	0.44
C1	05-22-2009	17.8	45.7	4.7	7.0	72% cropland, 28% forest	0.41	17.74	b.d.	0.30
U1	08-18-2009	22.5	713	6.5	5.5	81% urban, 19% forest	0.43	0.73	b.d.	0.67

[a]b.d. = below detection;

specific conductivity, pH, and dissolved oxygen concentration (Table 1).

2.3. Experimental Incubations

[10] Pre-baked GF/F glass fiber filters (nominal pore size of 0.7 μm, 47 mm diameter) were used to remove living and non-living particulate materials and bacterial predators [*Schultz*, 1999; *Raymond and Bauer*, 2000; *McCallister et al*., 2004]. A portion of the 0.7 μm filtrate was subsequently filtered through a 0.2 μm capsule filter (Whatman polycap; pre-cleaned with 10% HCl and distilled water) to remove bacteria and serve as an abiologic control. Three incubation treatments were performed to assess the potential reactivity of stream water DOC and changes in DOM characteristics: (1) 0.7 μm filtrate under light for combined light + bacteria incubations, (2) 0.7 μm filtrate for dark, bacteria-only incubations, and (3) 0.2 μm filtrate (i.e., bacteria-free) for light-only incubations. The incubation experiments were started immediately following filtration, and were conducted in May, August, and November 2009. Two replicate incubation vessels were used for each incubation treatment. The incubation temperature for all experiments was controlled at 22 ± 2 °C in order to eliminate temperature as a confounding variable affecting DOM reactivity.

[11] Light incubations were performed in 500 ml quartz flasks on a rotating light table. The light source consisted of 12 UV 340 bulbs (Q-Panel, Westlake, OH), which have spectral light similar to that of natural sunlight from the UV wavelengths between 295 and 365 nm [*Dalzell et al*., 2009; *Spencer et al*., 2009]. The irradiance of the light source was measured by a photometric meter (Model: IL 1700, International Light, MA, USA) and was approximately one third of seasonally averaged daily solar irradiance in shallow water at 40°N [*Leifer*, 1988]. The samples were exposed to light for 24 h per day during the incubation experiments. Thus, the samples in the 10 day and 15 day incubation experiments received UV exposure equivalent to ~6.6 days and 10 days, respectively, of 12 h daylight at the sampling sites. The dark incubation bottles (1000 ml borosilicate brown glass bottles) were placed in cardboard boxes covered by dark bags to prevent light penetration. The incubation duration was 35–36 days for the dark, bacteria-only treatments and 10–15 days for the light-only and the combined light + bacteria treatments. These incubation times were chosen based on measured changes in DOC concentrations during incubations, with the following considerations: (1) the DOC concentrations at the end of incubation were adequately high for Δ^{14}C-DOC measurements, and (2) the decreases in DOC concentrations were sufficient for the changes in isotopic signatures, if any, to be determined by isotopic mass balance. Sub-samples collected from the incubation vessels were not re-filtered prior to chemical analyses to avoid artifacts associated with additional handling and filtration. Although particle sizes larger than 0.7 or 0.2 μm may potentially interfere with the measurement of DOC concentration and DOM properties described below, this interference should be minor as both filter sizes have been well accepted for all these measurements.

2.4. DOC Measurements and Reactivity Estimates

[12] Throughout the stream water incubation experiments, subsamples (20 ml) at the start (t_0) and end (t_{end}) time points as well as at four to six intermediate time points were collected for DOC concentration to evaluate degradation kinetics. The subsampling frequency varied from 1 to 7 days and was based on the DOC loss pattern during the experiments, which was determined by DOC analysis immediately following each subsampling. Sample water was collected from the incubation vessels and analyzed on a Shimadzu TOC-V_{CSH} total organic carbon analyzer. Glucose was used to construct standard curves, and a consensus seawater reference standard (Hansell laboratory, http://yyy.rsmas.miami.edu/groups/biogeochem/CRM.html) was used to confirm analytical accuracy. Two to three samples were randomly selected for replicate analysis in each run, and the relative standard deviation (RSD) was within 0.7%. The RSD for replicate incubation bottles was ≤ 6%. The percent reactive DOC was calculated as

$$\%\,\text{reactive DOC} = [(\text{DOC}_{t0} - \text{DOC}_{t\text{end}})/\text{DOC}_{t0}]^{*}100 \quad (1)$$

where DOC_{t0} and $\text{DOC}_{t\text{end}}$ refer to the DOC concentrations at t_0 and t_{end}.

[13] The first-order apparent degradation rate constant (k' in day^{-1}) for DOC remineralization during the incubations was calculated as

$$\text{DOC}_t = \text{DOC}_{t0} e^{-k't} \quad (2)$$

where DOC_t is the DOC concentration measured at the various sub-sampling time points (t, in day). The k' values were determined from the slopes of regression lines for ln DOC versus t. Three types of k' values were generated, corresponding to the three incubation treatments: photoreactive k' (k'_P) for the light-only incubations, bioreactive k' (k'_B) for the dark, bacteria-only incubations, and k'_{P+B} for the combined light + bacteria incubations.

2.5. Isotopic Analyses and Mass Balance Calculations

[14] The procedure for extracting water DOC for isotopic analyses is described in detail by *Raymond and Bauer* [2001a] and *Bauer and Bianchi* [2011]. Briefly, ~125 ml of sample was placed in quartz reaction vessels, acidified to pH = 2 with 85% H_3PO_4, and sparged with ultrahigh purity (UHP) He to remove inorganic carbon. The samples were then saturated with UHP oxygen and irradiated with a 2400 W medium pressure mercury arc ultraviolet (UV) lamp for 4 h. The quartz reaction vessels were then connected to a vacuum extraction line to purify and collect CO_2 generated from DOC oxidation. The CO_2 was collected in 6 mm OD Pyrex tubes that were submitted to the University of Arizona Accelerator Mass Spectrometry (AMS) Laboratory for δ^{13}C and Δ^{14}C analyses. δ^{13}C values were reported relative to PDB in standard notation as $\delta^{13}C = [(R_{sample}/R_{standard}) - 1] * 10^3$, where R is $^{13}C/^{12}C$. Δ^{14}C values, defined as the per mil deviation of a sample compared to the ^{14}C activity of nineteenth century wood, were corrected by δ^{13}C for fractionation. Total measurement uncertainty for Δ^{14}C ranged between 4 and 11‰. The SD for duplicate samples and standards (oxalic acid II) was within 0.1‰ for δ^{13}C and 3.1‰ for Δ^{14}C and that for replicate incubation bottles was ≤0.9‰ for δ^{13}C and ≤3.6‰ for Δ^{14}C.

[15] Selected water samples from the combined light + bacteria incubations and the bacteria-only incubations were analyzed for DOC isotopes at t_0 and t_{end}. Samples from the light-only incubations, however, were not measured due to

cost constraints. The $\delta^{13}C$ and $\Delta^{14}C$ values of the reactive DOC pool ($\delta^{13}C_{Reactive}$, $\Delta^{14}C_{Reactive}$) were calculated as

$$\delta^{13}C_{Reactive} = \left(\delta^{13}C_{t0} * DOC_{t0} - \delta^{13}C_{tend} * DOC_{tend}\right)/(DOC_{t0} - DOC_{tend}) \quad (3)$$

$$\Delta^{14}C_{Reactive} = \left(\Delta^{14}C_{t0} * DOC_{t0} - \Delta^{14}C_{tend} * DOC_{tend}\right)/(DOC_{t0} - DOC_{tend}) \quad (4)$$

where $\delta^{13}C_{t0}$ and $\Delta^{14}C_{t0}$ were the isotopic values of DOC at t_0 and $\delta^{13}C_{tend}$ and $\Delta^{14}C_{tend}$ refer to those values at t_{end}.

2.6. Excitation Emission Matrix-Parallel Factor Analysis

[16] Differentiating between allochthonous/terrestrial and autochthonous/aquatic sources of DOM has historically been analytically challenging and in recent years has been facilitated through the application of optical measurements of DOM [e.g., *Jaffé et al*., 2008; *Fellman et al*., 2010]. One technique presently in use to characterize composition is based on the fluorescence characteristics of DOM, and while only a small fraction of DOM is actually fluorescent, these techniques have been shown to be sensitive and appropriate for DOM characterizations and to correlate with DOC concentration in freshwater systems [*McKnight et al*., 2001; *Stedmon et al*., 2003; *Cory and McKnight*, 2005]. In particular, EEM-PARAFAC has the capacity to identify and quantify individual fluorescence components from the overall EEM spectra, which can be assigned to either allochthonous or autochthonous sources [*Stedmon et al*., 2003; *Williams et al*., 2010; *Yamashita et al*., 2011a].

[17] Fluorescence measurements of stream water DOM during experimental incubations were conducted on samples at t_0, t_{end} and one intermediate sampling time (either day 4 or 5). The procedure has been described in detail in *Yamashita et al*. [2011b]. Several post-acquisition steps were involved in the correction of the fluorescence spectra. First, the UV-visible absorption spectra measured by a dual-beam spectrophotometer were used for inner filter corrections according to *McKnight et al*. [2001]. Following this procedure, the EEM of Milli-Q water was subtracted from sample EEMs. Second, the excitation correction factors obtained monthly using rhodamine b, and the emission correction factors supplied by the manufacturers, were applied for correction of our instrument-specific responses, e.g., performance of the gratings and the detector with wavelengths [*Cory et al*., 2010]. Finally, fluorescence intensity was corrected to the area under the water Raman peak (excitation = 350 nm) analyzed daily and then converted to quinine sulfate units (QSU). The PARAFAC model was constructed following a statistical approach described in *Stedmon et al*. [2003], using wavelength ranges of 250 to 450 nm for excitation and 290 to 520 nm for emission. The analysis was carried out in MATLAB using the DOMFluor toolbox according to *Stedmon and Bro* [2008]. A five component EEM-PARAFAC model (C1–-C5) was validated by split-half analysis and random initialization (Table 2). The relative abundance of each of these five fluorescent components (Ci, $i = 1$ to 5) was calculated as

$$\%Ci = F_{ci}/TF * 100 = F_{ci}/\left(\sum_{i=1}^{5} F_{ci}\right) * 100 \quad (5)$$

where F_{Ci} represented fluorescence intensity of each specific fluorescent component and TF was total fluorescence intensity.

2.7. Ancillary Measurements

[18] Chlorophyll-a measurements followed *Parsons et al*. [1984] using a Turner Design TD-700 fluorometer. Dissolved nutrients (phosphate, nitrate, nitrite, and ammonium) were measured by a Dionex ion chromatograph, using an anion and cation mixture (Alltech anion Mix5, Dionex six cation-1 standard) for constructing standard curves and Ion-96.3 river water from the Grand River, Ontario (Environment Canada), for confirming accuracy (measured values $\pm 2\sigma$ of certified values). The RSD for duplicate measurements was within 14.4% for nitrate concentrations and 6.6% for ammonium. Phosphate and nitrite concentrations of all the samples were below the instrument's limits of detection for these solutes (phosphate: 75 μg/l; nitrite: 50 μg/l) (Table 1).

2.8. Statistical Analyses

[19] The streams draining the three types of human land use (i.e., pasture, cropland and urban) were grouped together for certain of the datasets (i.e., k'_P, k'_B, %Ci) for statistical analyses because of the relatively small sample size and the lack of apparent differences in these datasets across the three types of human land use. This grouping ignores the differences among the three types of human-modified land use but identifies differences between forested and human-modified watersheds. Non-parametric Kruskal-Wallis tests were conducted to compare data between treatments or land uses.

[20] A stepwise linear regression model was used to determine parameter(s) that best predict DOC reactivity. k'_P and k'_B were set as dependent variables. All parameters at t_0 were evaluated as predictors, including DOC concentration, relative abundance of the five fluorescent components (%C1 to %C5), $\delta^{13}C$-DOC, $\Delta^{14}C$-DOC, nutrient concentration (nitrate and ammonium), chlorophyll-a, and all in situ environmental variables (i.e., water temperature, conductivity, pH, and dissolved oxygen concentration) (Table 1). Error

Table 2. Characteristics of the Five Fluorescence Components Identified by PARAFAC and Their Attributed Sources

Component	Excitation Maximum Wavelength	Emission Maximum Wavelength	Similar Fluorescence Components Identified in Previous Studies			Major Compound Group Assignment
			Coble et al. [1998]	*Cory and McKnight* [2005]	FCE Model [*Yamashita et al*., 2010]	
C1	<250 (330)	442	A/C	C10	C1 or C6 (Terrestrial)	Fulvic acid-type
C2	260 (380)	504	-	SQ1	C5 (Terrestrial)	Humic acid-type
C3	<250 (305)	388	M	Q3 or C3	C4 (Microbial)	Microbial humic-like
C4	<250	324	B/T	Tyr- or Trp-like	C7 (Protein)	Protein-like
C5	<250	430	A	Q1	C2 (Terrestrial)	Humic-like

assumptions, including constant variance, linearity, and normality, were examined using residuals versusu fitted plots and Q-Q plots. The model selection was primarily based on R-square (RSQ) but also considered that the ratio between the numbers of samples and predictors should be ≥ 5. Samples for which the studentized residue was larger than the Bonferroni correction value were identified as outliers and thus not included in the model. The significance level, α, was set at 0.05.

3. Results

3.1. Ambient Stream DOM Characteristics

[21] Ambient (i.e., t_0) DOM parameters were compared between forested and human-modified streams. Although no statistical differences were found in DOC concentration at t_0 (DOC_{t0}) (Kruskal-Wallis test: $P = 0.8$), DOC_{t0} values in forested streams were generally higher than in the human-modified streams (Figure 2a). The relative distributions of three of the five DOM fluorescent components (i.e., %C1, %C2, and %C4) differed between the two stream types (Kruskal-Wallis test: $P < 0.05$ for %C1, %C2, and %C4) (Figure 2b).

[22] The $\delta^{13}C$ and $\Delta^{14}C$ of DOC at t_0 did not show systematic differences between forested and human-modified streams and thus were presented according to their individual watershed land use types (Figures 3a and 3b). $\delta^{13}C$-DOC values at t_0 ranged from $-31.2‰$ to $-26.0‰$ (Figure 3a). Streams draining forest, pasture, and cropland had enriched $\Delta^{14}C$-DOC values ranging between 65‰ and 114‰. In contrast, the urban stream sample (U1) had significantly depleted ambient $\Delta^{14}C$-DOC ($-202 \pm 4‰$) (Figure 3b).

3.2. Photoreactive and Bioreactive DOC

[23] DOC reactivity varied as a function of the three incubation treatments: light-only (i.e., bacteria-free), dark, bacteria-only, and combined light + bacteria incubations. The percent of photoreactive DOC ranged from 4.8 to 56.9% and was higher than the percent of bioreactive DOC, which varied from 0.3 to 23.9% (Table 3). The percent reactive DOC in the light + bacteria treatment was highest among the three incubation treatments and ranged over nearly an order of magnitude, from 9.8 to 91.5% (Table 3). The mean k' value for each incubation treatment

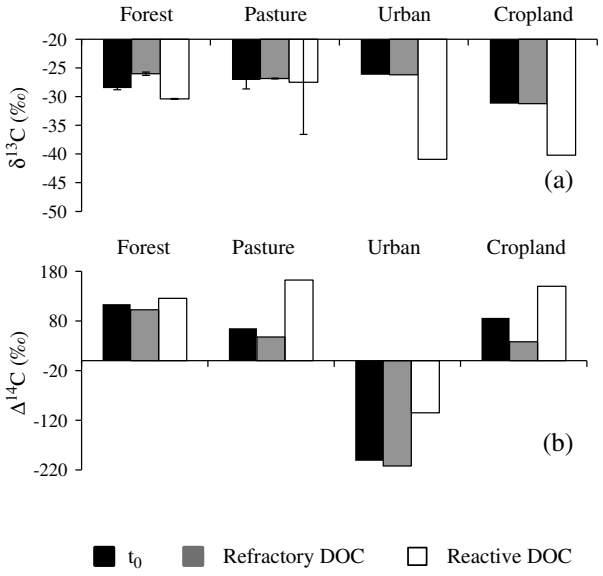

Figure 3. (a) $\delta^{13}C$ and (b) $\Delta^{14}C$ values of DOC at t_0 and of refractory and reactive DOC during the combined light + bacteria incubations. Error bars are $\pm$ SD derived from averaging values of different streams within the same land use type.

reflected the same general pattern, i.e., $k'_{P+B} > k'_P > k'_B$ (Figure 4a), and the mean k' values were significantly different across the three treatments (Kruskal-Wallis test: $P = 0.01$) (Figure 4a). A significant positive correlation was found between k'_P and k'_{P+B} (Pearson $r = 0.9$, $P = 0.001$) but not between k'_B and k'_{P+B} (Pearson $r = 0.4$, $P = 0.3$). Taken together, these data suggest that photochemistry was more effective in removing DOC than bacteria alone. Photochemical processes also played the dominant role when DOC was remineralized in combined photochemical and microbial incubations.

[24] DOC reactivity also varied as a function of land use type. The mean %reactive DOC was higher in forested streams than in human-modified streams during the photoreactive and combined incubations (Table 3). A similar pattern was observed for k' values, where k'_P and k'_{P+B} for forested streams were significantly higher than that for human-modified

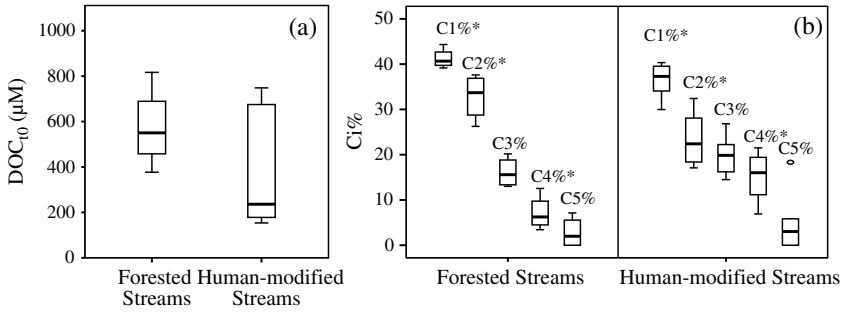

Figure 2. Box plot comparisons of DOM properties between forested and human-modified streams: (a) DOC concentrations at t_0 (DOC_{t0}) and (b) relative distributions of the five fluorescent components (%Ci). Asterisk (*) indicates significant differences between DOC from forest dominated streams and human modified streams by Kruskal-Wallis tests. Open circle (○) represents mild outlier for C5, i.e., data beyond either the upper/lower quartile $\pm$ 1.5 inter-quartile range.

Table 3. DOC Concentrations and Percentages of Photoreactive and Bioreactive DOC in the Study Streams[a]

Site	Incubation Date	DOC at t_0 (μM)	DOC at t_{end} of Photoreactive Incubation[b] (μM)	Photoreactive DOC[b] (%) ± SD[e]	DOC at t_{end} of Bioreactive Incubation[c] (μM)	Bioreactive DOC[c] (%) ± SD[e]	DOC at t_{end} of Photoreactive+ Bioreactive Incubation[d] (μM)	Photoreactive+ Bioreactive[d] DOC (%) ± SD[e]
F1	08-18-2009	377	170	55.0[f]	373	1.1 ± 3.0	144	61.7 ± 0.4
F2	05-22-2009	817	n.d.	n.d.	740	9.4 ± 1.2	411	49.7 ± 0.41
F2	11-09-2009	539	267	50.5 ± 1.4	468	13.2 ± 1.6	46	91.5 ± 0.9
F3	11-09-2009	562	242	56.9 ± 6.0	478	15.0 ± 3.9	83	85.3 ± 0.9
P1	08-18-2009	748	591	21.3 ± 0.5	720	3.8 ± 1.6	602	19.5 ± 0.1
P1	11-09-2009	675	587	12.6 ± 0.01	662	1.9 ± 1.2	568	15.9 ± 2.8
P2	08-18-2009	154	138	10.6 ± 0.3	126	17.9 ± 0.6	130	15.4 ± 0.8
P2	11-09-2009	206	139	32.3 ± 6.1	157	23.9 ± 0.04	103	50.0 ±2.1
C1	05-22-2009	178	n.d.	n.d.	n.d.	14.0 ± 1.2	101	43.1 ± 3.8
U1	08-18-2009	267	254	4.8 ± 5.8	254	0.3 ± 0.4	241	9.8 ± 0.7
Forested Streams (mean ± SD)		574 ± 182	318 ± 187	54.1 ± 3.3	515 ± 157	9.7 ± 6.2	171 ± 165	72.1 ± 9.7
Human-modified Streams (mean ± SD)		371 ± 267	342 ± 231	10.3 ± 9.7	384 ± 285	16.3 ± 10.7	291 ± 234	25.6 ± 16.6

[a] n.d. = not determined.
[b] 0.2 μm filter, 15 days, light.
[c] 0.7 μm filter, 35-36 days, dark.
[d] 0.7 μm filter, 10-15 days, light.
[e] SD (standard deviation) was calculated from the replicate bottles.
[f] SD was not provided because of sample loss.

Figure 4. Box plot comparing first-order DOC remineralization rate constants, k'(day^{-1}) of (a) the three incubation treatments and (b) the three types of k' for forested and modified streams. P = light-only; B = dark, bacteria-only; P + B = combined light + bacteria incubation; Asterisk (*) indicates significant difference between forested and human-modified streams by Kruskal-Wallis tests. Open circle (○) indicates mild outlier; closed circle (●) indicates extreme outlier, i.e., data beyond either the upper/lower quartiles ± 3inter-quartile range.

streams (Kruskal-Wallis test: k'_P: $P = 0.03$; k'_{P+B}: $P = 0.03$) (Figure 4b). In contrast, forested and human-modified streams had comparable values of %bioreactive DOC (Table 3) and showed no significant difference in k'_B values (Kruskal-Wallis test: $P = 0.95$; Figure 4b). Thus, both %reactive DOC and k' values indicate that DOC photoreactivity was higher in forested streams than in human-modified streams, while DOC bioreactivity was not affected by land use type.

[25] The linear regression model selected %C2 as the best predictor of k'_P and in situ stream temperature as the best predictor of k'_B.

3.3. $\delta^{13}C$ and $\Delta^{14}C$ of Reactive and Refractory DOC

[26] During the bacteria-only incubations, changes in both $\delta^{13}C$-DOC and $\Delta^{14}C$-DOC values were within the measurement uncertainties (i.e., ≤0.2‰ for $\delta^{13}C$ and ≤4–11‰ for $\Delta^{14}C$) (Table A1). During the six combined light + bacteria incubations, however, five incubations showed a preferential removal of ^{13}C-depleted DOC (Figure 3a and Table A1) and a concomitant enrichment in ^{13}C in the remaining unoxidized DOC. In addition, four of the combined light + bacteria

incubations demonstrated a selective loss of ^{14}C-enriched DOC (Figure 3b and Table A1).

3.4. Changes in Fluorescence Properties During DOM Degradation

[27] Because changes in DOM fluorescence properties (i.e., %Ci and TF) during light-only and combined light + bacteria incubations were similar (Kruskal-Wallis test: $P = 1$), these two treatments are presented and discussed together (Figures 5 and 6). This similarity further suggests that photochemistry played the dominant role in altering fluorescence properties under the combined effects of light and bacteria.

[28] For stream water of all land use types, TF decreased rapidly over the course of light incubations. TF_t (TF at the sub-sampling time point t) was 13.6–40.6% of the initial TF (TF_{t0}) at day 4 or 5 and decreased to 3.3–24.6% by the end of incubation (Figure 5). In contrast, alterations in TF in the dark, bacteria-only incubations were much smaller, with TF_t within ±15% of TF_{t0} at day 4 or 5 as well as at the end of the incubations (Figure 5).

[29] Based on the EEM spectral characteristics, C1, C2, C3, C4, and C5 were categorized as terrestrial fulvic acid-type, terrestrial humic acid-type, microbial humic-like, protein-like, and humic-like components, respectively (Table 2). In both light treatments (i.e., light-only and combined light + bacteria incubations), significant changes occurred in the relative abundance of most fluorescent components (Kruskal-Wallis test for comparing %Ci at t_0 and t_{end}: $P \leq 0.002$ for percentages of C1, C3, C4, and C5 and $P = 0.1$ for %C2) (Figures 6a and 6b). Samples from forested streams and human-modified streams showed similar patterns: decreases in %C1 and increases in %C4 and %C5 at the end of the light incubations (Figures 6a and 6b). In contrast, no significant changes were observed in the relative abundances of any of the fluorescent components during the dark, bacteria-only incubations (Kruskal-Wallis test: $P \geq 0.2$ for % C1–%C5) (Figures 6a and 6b).

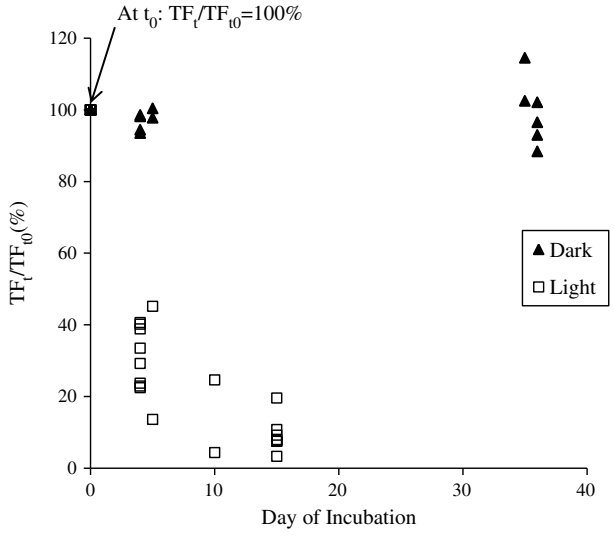

Figure 5. Changes in total fluorescence intensity (TF) over the course of dark (bacteria-only) incubations and light (light-only and combined light + bacteria) incubations. $TF_{t0} = TF$ at the initial time point and $TF_t = TF$ at each subsampling time point.

4. Discussion

4.1. Ambient Properties of DOM From Different Watersheds

[30] Ambient DOM properties at t_0 of incubations established its baseline characteristics during photochemical and microbial degradation. Both similarities and differences in ambient stream water DOM characteristics were found between forested streams and human-modified streams. The similarities were reflected by (1) the dominance of terrestrial humic-like fluorescent components (i.e., C1 and C2) in both stream types (Figure 2b and Table 2) [*Coble et al.*, 1998; *Cory and Mcknight*, 2005], suggesting that terrestrial DOM dominated in all streams, and (2) the ranges of $\delta^{13}C$ values exhibited by forested streams (−28.7 to −28.3‰) and human-modified streams (−31.2 to −26‰) (Figure 3a and Table A1), which both fell within the ranges for C_3 plants, soil organic matter, freshwater algae, and petroleum-derived chemicals [*Faure and Mensing*, 2005; *Ogrinc et al.*, 2008]. Differences in the DOM from the two watershed types were revealed in two ways. First, the larger range in $\delta^{13}C$-DOC in streams from human-modified streams than in forested streams may suggest the former has more variable sources than the latter. Second, %C1 and %C2 were higher in forested stream water DOM, and %C4 was higher in human-modified stream DOM (Figure 2b). C4 has been related to microbial consumption and production [*Balcarczyk et al.*, 2009; *Fellman et al.*, 2009a, 2009b] (Table 2). Thus, forested streams contained higher contributions of terrestrial DOM but lower percentages of microbial DOM than human-modified streams on the basis of %Ci. Similar enrichment of microbial fluorescence components in waters from streams impacted by human activities including agricultural activities or forest management have been reported recently [*Williams et al.*, 2010; *Yamashita et al.*, 2011a].

[31] Besides these general differences in DOM characteristics between forested and human-modified streams, the stream draining the urban watershed (U1) was the only system containing highly aged DOC (mean ^{14}C age = ~1,811 ± 42 years B.P.) (Figure 3b and Table A1). DOC from all of the other study streams was post-bomb in nature, i.e., contained atmospheric CO_2 that was fixed photosynthetically since the period of thermonuclear weapons testing in the 1950s and 1960s. This indicates that the DOC in all streams except U1 was dominated by carbon fixed and exported from watersheds on timescales of years to decades. On the other hand, U1 was potentially influenced by two aged carbon sources in the urban watershed: (1) autotrophic fixation of aged dissolved inorganic carbon derived from the dissolution of sedimentary shell carbonate, which is mostly of Tertiary age [*Roberts*, 1932; *Mixon et al.*, 1989; Geological Map of Virginia, Virginia Department of Mines Minerals and Energy] and (2) fossil fuel-derived organic substances (e.g., petroleum hydrocarbons) released by human activity (Y. H. Lu et al., Effects of land use on sources and ages of inorganic and organic carbon in temperate headwater streams, submitted to *Biogeochemistry*, 2013).

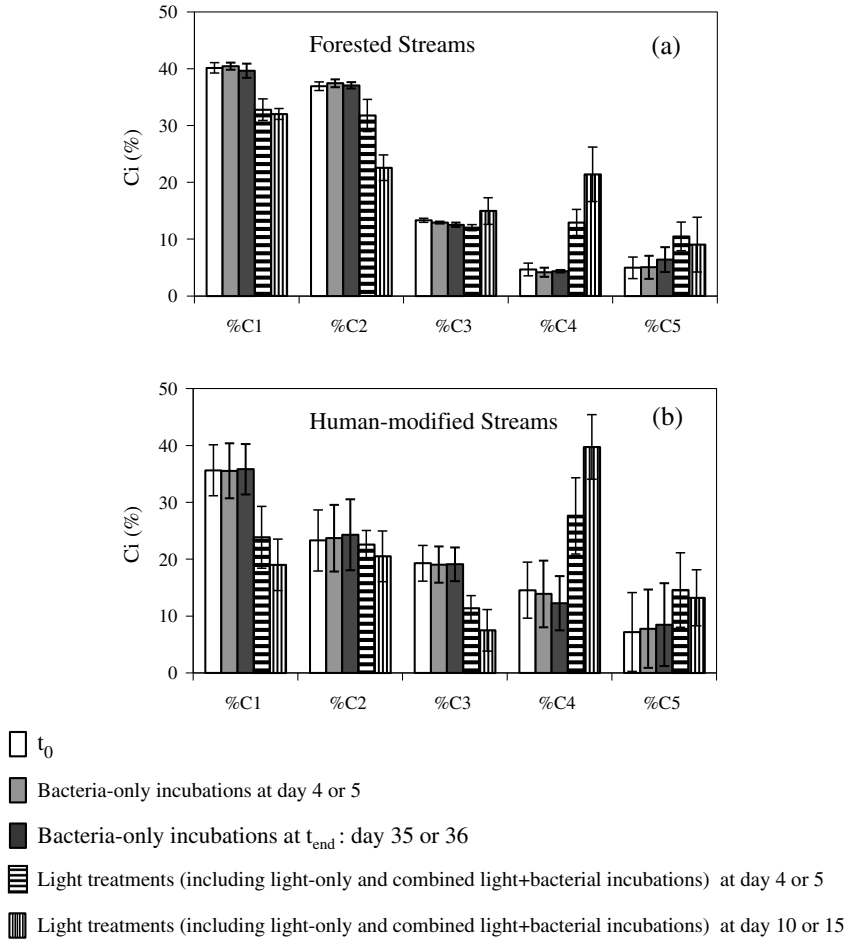

Figure 6. Changes in relative abundances of the five fluorescent components during light and dark incubations of DOC from (a) forest-dominated and (b) human-modified streams. Error bars are ± SD derived from averaging values of different streams of the same land use and replicate bottles for each treatment.

4.2. Factors Impacting Headwater Stream DOC Photoreactivity

[32] The %photoreactive DOC and k'_P varied between 5 and 57%, and 0.045 and 0.055 day^{-1}, respectively (Table 3 and Figure 4a). These values are comparable to ranges previously observed for stream water DOC, which are from below detection to ~50% for % photoreactive DOC and from below detection to 0.062 day^{-1} for k'_P (summarized in Table 4). The % reactive DOC and k' values during the light-only and light + bacteria incubations were overall higher than those during the dark, microbial incubations (Table 3 and Figure 4a), indicating that photochemical processes are more effective in remineralizing DOC than bacteria alone in the study streams. These findings are consistent with the general notion that stream DOC has relatively high photoreactivity and low bioreactivity due to the predominance of terrestrial DOC sources [*McKnight et al*., 2003; *Dittmar et al*., 2006; *Sulzberger and Durisch-Kaiser*, 2009].

[33] Forested streams displayed higher %photoreactive DOC and %photoreactive + bioreactive DOC than human-modified streams (Figure 4b). Consequently, the mean DOC concentration at t_{end} was higher in human-modified streams than in forested streams, although the mean DOC concentration was higher in forested streams at t_0 (Table 3). There are two possible reasons for the higher photoreactivity of DOC in forested streams than in human-modifed streams. First, terrestrial DOM is generally more photoreactive relative to microbial and planktonic materials, due to a higher abundance of aromatic components [*Chin et al*., 1994; *Dittmar et al*., 2006; *Sulzberger and Durisch-Kaiser*, 2009]. The greater proportion of terrestrial materials in forested streams than in human-modified streams (Figure 2b) thus may have led to higher DOC photoreactivity in forested streams. In fact, we found that the variability in DOC photoreactivity for all streams was best predicted by %C2 ($k'_P = -0.042 + 0.003$* (%C2), RSQ = 0.7, $P = 0.006$, $n = 8$) and was reasonably predicated by %C1 (RSQ = 0.6, $P = 0.02$). These quantitative relationships between DOC photoreactivity and the abundance of terrestrial humic-like components indicate the importance of DOM sources to DOC photoreactivity—that is, streams containing a larger percentage of terrestrial-derived DOM tend to have greater DOC photoreactivity. While the importance of the relative contribution of terrestrial DOM to DOC photoreactivity has been long recognized for lake and ocean waters [*Thomas and Lara*, 1995; *Moran and Zepp*, 1997;

Table 4. Comparison of Photoreactive Stream Water DOC and Changes in CDOM From Various Studies[a]

Study Areas	Watershed Land Use	Light Sources	Incubation Duration	Percent Photoreactive DOC	k'_P (day^{-1})[b]	Changes in CDOM	Sources
Virginia	Forest	UVB/UVA/PAR	15 days	51–57%	0.045–0.055	Decrease of TF by ~85% on average and changes of relative abundance of fluorophores	This study
Southern Canada	Forest	UVB/UVA/PAR	6–11 days	11–50%	0.017–0.062	True color[c] decreased by 0–77%	*Molot and Dillon* [1997]
Northern Great Lakes Region	Forest	UVB/UVA/PAR	56 h	6%	0.026	Absorbance at 320 nm reduced by ~10%	*Larson et al.* [2007]
Colorado	Forest and meadow	UVB	24 h	10–20%	0.11–0.22	Specific UV absorbance at 254 nm (SUVa) reduced by ~22 %	*Clements et al.* [2008]
New Jersey	Forest or pasture	UVB/UVA/PAR	21.5–38 h	Below detection	Below detection	n.d.	*Wiegner and Seitzinger* [2001]
Virginia	Agriculture and urban watersheds	UVB/UVA/PAR	15 days	5–32%	0.005–0.022	Decrease of TF by ~91% on average and changes of relative abundance of fluorophores	This study

[a]n.d. = not determined
[b]Generally, only either percent photoreactive DOC or k'_P was provided in references. One of these two values presented here was calculated with the assumption that DOC was remineralized at the first-order rates.
[c]True color: a method designed to emulate Hazen units, which defined water color as 1.35*(broadband absorbance at 405–450 nm)–1.68*(broadband absorbance at 660–740 nm) [*Mierle and Ingram*, 1991].

Obernosterer and Benner, 2004], this is to our knowledge the first study to demonstrate this relationship quantitatively in streams. We further show that the EEM-PARAFAC method may provide a potential tool for predicting stream DOC photoreactivity.

[34] Another factor leading to different photoreactivity between forested stream DOC and human-modified stream DOC is light exposure history, which has been highlighted in several previous studies of freshwater DOC photoreactivity [*Molot and Dillon*, 1997; *Biddanda and Cotner*, 2003; *Larson et al*., 2007]. In the present study, we did not measure the amount of solar radiation to which DOC has been exposed before sample collection and incubation and thus cannot directly evaluate the importance of light exporsure history in determining DOC photoreactivity. However, %C5 may be indicative of photoexposure history of the DOM because the C5 component has been previously found to be photo-stable and considered a photodegradation product of terrestrial humic-like DOM [*Stedmon et al*., 2007; *Chen et al*., 2010; *Cawley et al*., 2012]. The %C5 in stream water DOM at t_0, while not statistically different between forested and human-modified watersheds (Kruskal-Wallis test: $P = 0.8$), was higher in human-modified streams (Figure 2b), agreeing with our observation that light penetration was generally higher in human-modified watersheds than in forested ones that shade streams. Thus, DOM in the human-modified streams may have been photodegraded to a greater extent than in the forested streams, retained lower amounts of photoreactive components, and thus showed lower DOC photoreactivity. However, we did not find a significant correlation between %C5 and DOC photoreactivity (Pearson $r = 0.4$, $P = 0.3$), which may suggest that light exposure history plays a secondary role less important than DOM sources in determining DOC photoreactivity.

[35] Compared to prior work that has focused primarily on streams draining forest-dominated landscapes (Table 4), the present study provides one of the few comparisons of DOC photoreactivity in a regional group of streams draining forested and human-modified watersheds and illustrates the importance of land use on the photoreactivity of DOM exported from upstream watersheds. We attribute the observed difference in photoreactivity of DOC between forested and human-modified watersheds to a combination of higher %terrestrial DOM and lower amounts of previous light exposure for the forested streams. These two characteristics may represent common differences between DOM originating from forested watersheds and DOM form human-modified watersheds. For example, previous studies have also shown that streams in undisturbed forested environments have an enriched terrestrial humic-like fluorescence signature in DOM than those disturbed by human activities [*Williams et al*., 2010; *Yamashita et al*., 2011a]. Further, a fluorescence component possibly representing extensive light exposure has been found to be highly enriched in DOM draining agricultural watersheds [*Yamashita et al*., 2010]. It is thus reasonable to generalize the observations in the present study to other temperate systems that human land use may decrease the photoreactivity of DOC from streams.

4.3. Factors Impacting Headwater Stream DOC Bioreactivity

[36] The % bioreactive DOC and k'_B values ranged between 0.3 and 24%, and $6.2*10^{-4}$ and $1.7*10^{-2}$, respectively, and

were within the range observed in previous studies (Table 5). In the present study, % bioreactive DOC and k'_B in forested and human-modified streams overlapped and did not differ significantly (Table 3 and Figure 4b), suggesting that land use does not play a major role in stream DOC bioreactivity.

[37] We found that in situ stream temperature was the strongest predictor of DOC bioreactivity ($k'_B = -0.018 - 0.001$*(stream temperature), RSQ = 0.8, $P = 0.002$, $n = 9$). The negative slope suggests that greater DOC bioreactivity coincided with lower stream water temperatures. This relationship may be due to DOC being less altered by bacteria in situ under low temperatures. Thus, this less-altered, "fresher" DOC pool may have retained a larger fraction of bioavailable compounds and shown a higher bioreactivity in the laboratory incubations. All experiments were conducted at 22 ± 2 °C, and therefore incubation temperature was not a factor contributing to the observed variations of DOC bioreactivity across samples. Instead, DOC diagenetic status controlled by in situ temperatures may have determined the observed DOC bioreactivity during the incubations. This finding suggests that seasonal variations may play a more important role than land use in determining stream DOC metabolism; i.e., microbial remineralization is a more important process in removing DOC in warmer seasons than in cooler seasons. It also suggests a possible scenario in which DOC exported from terrestrial landscapes during winter may retain bioreactive components until temperatures are high enough for significant biodegradation to occur. This scenario, however, relies on the residence time of stream DOC; that is, whether bioreactive DOC will remain in streams long enough to be degraded in warmer seasons. The residence times of the study streams were not determined, but previous studies have shown that the mean residence time of stream waters can vary from hours to years [*McGuire et al*., 2005]. Thus, the significance of this seasonable variability of DOC bioreactivity in affecting stream DOC metabolism may vary greatly across systems.

[38] Several recent studies of stream water DOC have demonstrated a positive correlation between % bioreactive DOC and the relative abundance of protein-like fluorophores, which indicates that proteinaceous components may be a key factor determining overall DOC bioreactivity [*Balcarczyk et al*., 2009; *Fellman et al*., 2009a, 2009b; *Petrone et al*., 2011] (Table 5). In the present study, no correlation was found between %C4 and DOC bioreactivity (Pearson $r = 0.2$, $P = 0.6$). This may in part be due to our sample sizes being too small to demonstrate this relationship, warranting further work in streams spanning a greater range of geographic regions for establishing robust relations between DOM characteristics and DOC bioreactivity. In addition, the presence of non-colored DOM, such as carbohydrates that may account for a portion of total DOC bioreactivity but were not included in C4, will affect the ability of protein-fluorescence to predict DOC bioreactivity.

4.3. Photochemical and Microbial Alterations of DOC Isotopes

[39] During dark, bacteria-only incubations, both δ^{13}C-DOC and Δ^{14}C-DOC values showed negligible changes, as represented by similarities in the isotopic composition of biorefractory and bioreactive fractions (Figures 3a and 3b

Table 5. Comparison of Bioreactive Stream Water DOC and Potential Driving factors From Various Studies[a]

Study Areas	Watershed Land Use	Incubation Duration	Percent Bioreactive DOC	k'_B (day^{-1})[b]	Potential Factors for DOC Bioreactivity	Sources
Virginia	Forest	15–36 days	1–15%	6.2×10^{-4}–1.0×10^{-3}	Temperature-regulated diagenetic status of OM	This study
Alaska	Upland, Wetland, or Forest	30 days	7–38%	5.9×10^{-3}	Protein-rich DOM	*Fellman et al.* [2009a]
Alaska	Wetland	30 days	6–45%	2×10^{-3}–1.6×10^{-2}	Protein-rich DOM	*Fellman et al.* [2009b]
Alaska	Forest underlain by permafrost	40 days	<20%	$<5.6 \times 10^{-3}$	Protein-rich DOM	*Balcarczyk et al.* [2009]
Virginia	Agriculture and urban land	15–36 days	0.3–24%	7.2×10^{-4}–1.7×10^{-2}	Temperature-regulated diagenetic status of OM	This study
Indiana	Agriculture	6 days	Below detection	Below detection	Not specified	*Warrner et al.* [2009]
Western Australia	Agriculture and urban land	1 month	2–57%	6.7×10^{-4}–2.8×10^{-2}	Protein-rich DOM	*Petrone et al.* [2011]
New Jersey	Urban land	12 days	40–50%	4.3×10^{-2}–5.8×10^{-2}	Not specified	*Seitzinger et al.* [2005]

[a] n.d. = not determined.
[b] Generally, only either percent bioreactive DOC or k'_B was provided in references. One of these two values presented here was calculated with the assumption that DOC was remineralized at the first-order rates.

and Table A1). The absence of any isotopic shift may be due to the generally low amounts of bioreactive DOC (Table 3), which may have been inadequate to produce detectable isotopic changes, rather than documenting an absence of bacterial utilization of isotopically distinct compounds. A few previous studies (Table 6) found significant changes in δ^{13}C-DOC and Δ^{14}C-DOC only when a large fraction of the DOC was microbially remineralized. *Kalbitz et al.* [2003] found negligible changes in δ^{13}C-DOC of soil solutions for samples with low DOC biodegradability (5–9% of DOC) but significant changes for those with higher biodegradability (17–93% of DOC). *Raymond and Bauer* [2001b] also observed that bacteria preferentially degraded younger, ^{14}C-enriched DOC in estuarine waters where 63% of the initial DOC pool was remineralized (Table 6).

[40] In contrast to dark incubations, during the combined light + bacteria incubations where a larger percent of DOC (9.8–61.7%) was degraded, the reactive DOC pool was overall more depleted in ^{13}C than the residual refractory pool (Figure 3a and Table A1). Similar patterns have been observed in various systems, including rivers, bogs, and lakes, and have been attributed to preferential photodegradation of lignin-derived moieties or other aromatic compounds, which are generally more depleted in ^{13}C than bulk DOC [*Opsahl and Zepp*, 2001; *Osburn et al.* 2001; *Spencer et al.*, 2009] (Table 6). This proposed mechanism is consistent with the observed decrease in the relative abundance of terrestrial fluorescence components during photochemical degradation (Figures 6a and 6b).

[41] Preferential utilization of ^{14}C-erniched DOC was observed during the combined light + bacteria incubations (Figure 3b and Table A1). The age of DOC from U1 increased from 1811 ± 42 B.P. at t_0 ($\Delta^{14}C = -202 \pm 4$‰) to 1917 ± 38 yrs B.P. at t_{end} ($\Delta^{14}C = -212 \pm 4$‰), indicating that younger DOC ($\Delta^{14}C = -105 \pm 55$‰; ^{14}C age = 434 to 1667 yrs B.P.; the uncertainty of Δ^{14}C of younger DOC was obtained by propagating measurement uncertainty of Δ^{14}C, i.e., 4‰, in equation (4)) was preferentially utilized. The other light + bacteria incubations from streams draining forest, pasture, or cropland-dominated watersheds remained modern at t_{end}, suggesting that both reactive and refractory DOC were mostly composed of contemporary carbon (Figure 3b and Table A1).

[42] A limited number of studies have investigated changes in DOC isotopes resulting from photochemical and microbial alterations (Table 6). The present study, representing the first such work examining DOC changes in headwater streams, produced several findings similar to those in soil solutions [*Kalbitz et al.*, 2003], rivers [*Opsahl and Zepp*, 2001; *Spencer et al.*, 2009], estuaries [*Raymond and Bauer*, 2001b], bogs, and lakes [*Osburn et al.*, 2001]. These findings include (1) negligible changes in carbon isotopes if only a small percentage of DOC was remineralized; (2) at higher DOC losses, a selective removal of ^{13}C-depleted DOC possibly due to preferential photodegradation of lignin-derived and aromatic-rich compounds; and (3) at higher DOC losses, preferential remineralization of younger, ^{14}C-enriched DOC. These findings suggest that reactive and refractory DOC may share similarities in source-age characteristics across system types and study sites.

4.4. Photochemical and Microbial Alteration of CDOM

[43] Microbial degradation alone did not result in significant changes in either total fluorescence intensity or in the relative distribution of the five fluorescent components (Figures 5 and 6), indicating that these fluorophores were overall resistant to bacterial alteration. This is not surprising given that four out of five fluorescent components (C1, C2, C3, and C5) were either fulvic or humic in nature (Table 2), which are generally considered to be biorefractory due to the presence of condensed aromatic moieties and higher C : N ratios than aquatic, protein-rich materials [*McKnight et al.*, 2003]. In contrast, during light treatments (light-only and combined light + bacteria incubations), the majority of TF (i.e., 75–97%) was removed by the end of all incubations (Figure 5). Such a dramatic decrease, when compared to the 4.8–91.5% of DOC removal at t_{end} (Table 3), suggests that fluorescence components are among the most photoreactive in the DOM pool.

[44] Compared to most prior studies that used absorbance measurements to examine photochemical alteration of stream CDOM [*Molot and Dillon*, 1997; *Larson et al.*, 2007; *Clements et al.*, 2008] (Table 4), the EEM-PARAFAC method provides additional information about the photoreactivity of the fluorescent components. Forested streams and human-modified streams showed similar changes in C1, C2, and C4

Table 6. Changes in Carbon Isotopes During Photochemical or Microbial Incubations of Water Samples From Various Systems

Study Area	System Type	Incubation Condition	DOC Loss (%)	Change in Carbon Isotopes	Sources
Virginia	Stream	Dark, bacteria-only, 35–36 days	1–9	Negligible changes in δ^{13}C and Δ^{14}C of DOC	This study
Virginia	Estuary	Dark, bacteria-only, 2–12 months	63	Decrease of Δ^{14}C-DOC	*Raymond and Bauer* [2001b]
Germany	Soil solutions	Microbial incubation, 90 days	5–93	DOC loss at 5–9%: negligible changes DOC loss at 17–32%: increases of δ^{13}C-DOC DOC loss at 61–93%: decreases of δ^{13}C-DOC	*Kalbitz et al.* [2003]
Virginia	Stream	UVA/UVB/PAR, 15–16 days	10–64	Increase of δ^{13}C-DOC and decrease of Δ^{14}C-DOC	This study
Southeastern United States	River	UVA/UVB/PAR, 17–21 days	21–26	Increases of δ^{13}C-DOC	*Opsahl and Zepp* [2001]
Congo	River	UVA/UVB/PAR, 57 days	45	Increases of δ^{13}C-DOC	*Spencer et al.* [2009]
Pennsylvania	Lake and Bog	UVA/UVB/PAR, 7 days	16	Increases of δ^{13}C-DOC	*Osburn et al.* [2001]

during the light incubations; i.e., the percentages of C1 and C2 decreased and C4 increased (Figures 6a and 6b), indicating terrestrial humic-like fluorophores were relatively more photoreactive than microbially derived fluorophores. This may in part be explained by the presence of a variety of fluorescence structures (i.e., aromatic and unsaturated aliphatic moieties) in fulvic and humic macromolecules derived from terrestrial plants. This observation is also consistent with the increase in δ^{13}C-DOC during the combined light + bacteria degradation, which also suggests a selective removal of terrestrial DOC (Figure 3a). On the other hand, the relatively higher photo-resistance of protein-like fluorophores has also been observed in DOM from streams and lakes draining Arctic tundra [*Cory et al*., 2007]. Furthermore, all of our incubations showed increases in %C5, substantiating the previous explanation of fluorescence components similar to C5 as a product of photodegradation of terrestrial humic-like DOM [*Stedmon et al*., 2007; *Chen et al*., 2010; *Cawley et al*., 2012].

5. Effects of Watershed Land Use on DOM Reactivity and Implications for DOM Metabolism

[45] The present study provides a unique comparison of photochemical and microbial transformations of DOM from streams draining a geographically related set of forested and human-modified watersheds. A number of major differences in the amounts and characteristics of DOM were observed between watershed types, with consequent implications for the metabolism of DOM within stream waters and subsequent downstream fluxes.

[46] First, we found that DOC in streams draining forested systems had significantly higher photoreactivity than in streams draining human-modified watersheds, which led to higher mean DOC concentrations in human-modified streams than in forested streams following photochemical only or photochemical + bacterial incubations. This finding provides another possible mechanism for the decadal increase in surface water DOC concentrations in Europe and North America [*Hejzlar et al*., 2003; *Evans et al*., 2005; *Skjelkvåle et al*., 2005]. The finding has further implications for water quality in downstream environments, where water movement, chemistry, and microbes can be different for the photoresistant upstream DOC to be remineralized and contribute to oxygen consumption [*Sobczak et al*., 2002]. As such, photo-resistant DOC from upstream sources may be an unrecognized pool of OM contributing to the downstream formation of hypoxia that has plagued many coastal areas for decades [*Bianchi et al*., 2010]. Future studies should assess the relative importance of quality *vs*. quantity of upstream DOC in hypoxia formation, which may contribute to the development of more effective watershed management practices.

[47] Second, DOC bioreactivity did not differ significantly among land use types but instead varied as a function of in situ stream temperatures, which may control DOC bioreactivity by regulating its diagenetic status. This finding suggests that temperature is more important than land use in controlling the amount of DOC being remineralized in streams of temperate regions. During colder times of the year, bioreactive DOC components are more likely to persist and be transported to downstream waters than in warmer seasons. From the perspective of alleviating coastal hypoxia, this finding suggests that it is more important to control the amount of DOC exported from upstream watersheds in colder seasons than in warmer seasons. Since management practices do not presently consider the effects of bioreactive DOC from terrestrial sources on hypoxia, we recommend that this organic matter source be incorporated into water quality models.

[48] The third main finding is based on our isotopic and CDOM data, which showed that reactive and refractory DOM pools remineralized during photochemical and microbial alterations shared similar characteristics across watershed land use types. Photochemical alteration, the dominant process contributing to DOC remineralization, alters the isotopic and CDOM properties of DOM, thereby reducing or removing the original source signatures and leaving behind resistant DOM that has similar characteristics across land use types. Consequently, using isotopic and fluorescence signatures to assess the proportion of allochthonous versus aquatic DOM in large, homogeneous downstream systems may underestimate the contributions and importance of upstream, allochthonous DOM to downstream metabolism. Identification and application of novel tracers that are resistant to photodegradation is therefore important for a reliable assessment of transit and metabolism of DOM exported from upstream watersheds.

[49] Last, we emphasize the variability of DOC reactivity in streams as shown in the present as well as previous research, suggesting future work on streams from different environmental settings (i.e., temperature/climatic zones, hydrogeology, and lithology) should strive to understand factors driving this variability. This is a necessary step to better constrain the influence of human land use on stream DOC reactivity and information from such studies should be incorporated into regional or global models of carbon dynamics and management policies. Related to this, it is important to develop rapid, convenient approaches for consistent, long-term monitoring of stream DOC reactivity. We recommend exploring the potential of measuring %terrestrial fluorophores using EEM-PARAFAC as a rapid and inexpensive approach for monitoring stream DOC photoreactivity and stream water temperature for stream water DOC bioreactivity.

[50] **Acknowledgments.** We thank Edward Keesee, Erin Ferer, Christina Pondell, Sarah Schillawski, and Rachel Sipler for helping with field sampling and laboratory work. Jim Kaste provided the access to ion chromatography and helped with the analyses of cations and anions. We also thank Timothy Russell and Stuart Hamilton for their help with ArcGIS. This paper is contribution 3270 of the Virginia Institute of Marine Science, College of William and Mary, and 607 of the Southeast Environmental Research Center. This study was funded by a Mellon Foundation Postdoctoral Fellow Grant from The College of William and Mary and by the National Science Foundation through DEB Ecosystems grant DEB 0234533, Chemical Oceanography grant OCE 0327423, and Integrated Carbon Cycle Research Program grant EAR 0403949 to J.E.B, and in collaboration with the Florida Coastal Everglades Long-Term Ecological Research program under National Science Foundation Grant DBI-0620409 to R.J. E.A.C. acknowledges support from the National Science Foundation during preparation of this manuscript (OCE 0962277, EAR 1003529, and DEB Ecosystems 0542645). Two anonymous reviewers provided constructive comments that have helped to improve the quality of this paper significantly.

References

Aufdenkampe, A. K., E. Mayorga, P. A. Raymond, J. M. Melack, S. Doney, S. R. Aline, R. E. Aaltor, and K. Yoo (2011), Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere, *Front. Ecol. Environ.*, *9*(1), 53–60, doi:10.1890/100014.

Balcarczyk, K. L., J. B. Jones Jr., R. Jaffé, and N. Maie (2009), Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost, *Biogeochemistry*, *94*, 255–270, doi:10.1007/s10533-009-9324-X.

Bauer, J. E., and T. S. Bianchi (2011), Dissolved organic carbon cycling and transformation, in *Treatise on Estuarine and Coastal Science*, *Vol. 5*, *Biogeochemistry*, edited by E. Wolanski and D. S. McLusky, pp. 7–67, Academic Press, Waltham.

Benner, R. (2003), Molecular indicators of the bioavailability of dissolved organic matter, in Aquatic Ecosystems: Interactivity of Dissolved Organic Matter, edited by S. E. G. Findlay, and R. L. Sinsabaugh, (2003), pp 121–138, Academic Press, San Diego.

Bianchi, T. S., S. F. DiMarco, J. H. Cowan Jr., R. D. Hetland, P. Chapman, J. W. Day, and M. A. Allison (2010), The science of hypoxia in the Northern Gulf of Mexico: A review, *Sci. Total Environ.*, *408*, 1471–1484.

Biddanda, B. A., J. B. Cotner (2003), Enhancement of dissolved organic matter bioavailability by sunlight and its role in the carbon cycle of Lakes Superior and Michigan, *J. Great Lakes Res.*, *29*, 228–241.

Cawley K., P. Wolski, N. Mladenov, and R. Jaffe (2012), Dissolved organic matter biogeochemistry along a transect of the Okavango Delta, Botswana, *Wetlands*, doi:10.1007/s13157-012-0281-0.

Chen M., R. M. Price, Y. Yamashita, and R. Jaffé (2010), Comparative study of dissolved organic matter from groundwater and surface water in the Florida coastal Everglades using multi-dimensional spectrofluorometry combined with multivariate statistics, *Appl. Geochem.*, doi:10.1016/j.apgeochem.2010.03.005.

Chin, Y., G. Alken, and E. O'Loughlin (1994), Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances, *Environ. Sci. Technol.*, *28*, 1853–1858.

Clements, W. H., M. L. Brooks, D. R. Kashian, and R. E. Zuellig (2008), Changes in dissolved organic material determine exposure of stream benthic communities to UV-B radiation and heavy metals: Implications for climate change, *Glob. Chang. Biol.*, *14*, 2201–2214, doi:10.1111/j.1365-2486.2008.01632.X.

Coble, P. G., C. E. Del Castillo, and B. Avril (1998), Distribution and optical properties of CDOM in the Arabian Sea during the 1995 Southwest Monsoon, *Deep Sea Res. Part II*, *45*, 2195–2223.

Cole, J. J., and N. F. Caraco (2001), Carbon in catchments: Connecting terrestrial carbon losses with aquatic metabolism, *Mar. Freshw. Res.*, *52*(1), 101–110.

Cory, R. M., and D. M. McKnight (2005), Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter, *Environ. Sci. Technol.*, *39*(21), 8142–8149.

Cory, R. M., D. M. McKnight, Y. P. Chin, P. Miller, and C. L. Jaros (2007), Chemical characteristics of fulvic acids from Arctic surface waters: Microbial contributions and photochemical transformations, *J. Geophys. Res. Biogeosci.*, *112*, G04S51, doi:10.1029/2006JG000343.

Cory, R. M., M. P. Miller, D. M. McKnight, J. J. Guerard, and P. L. Miller (2010), Effect of instrument-specific response on the analysis of fulvic acid fluorescence spectra, *Limnol. Oceanogr. Methods*, *8*, 67–78.

del Giorgio, P. A., and J. Davis (2003), Patterns in dissolved organic matter lability and consumption across aquatic ecosystems, in Aquatic Ecosystems: Interactivity of Dissolved Organic Matter, edited by S. E. G. Findlay, and R. L. Sinsabaugh, (2003), pp 399–424, Academic Press, San Diego.

Dalzell, B. J., E. C. Minor, and K. M. Mopper (2009), Photodegradation of estuarine dissolved organic matter: A multi-method assessment of DOM transformation, *Org. Geochem.*, *40*(2), 243–257.

Dittmar, T., N. Hertkorn, G. Kattner, and R. J. Lara (2006), Mangroves, a major source of dissolved organic carbon to the oceans, *Global Biogeochem. Cycles*, *20*, GB1012, doi:10.1029/2005GB002570.

Driscoll, C. T., K. M. Driscoll, K. M. Roy, and M. J. Mitchell (2003), Chemical response of lakes in the Adirondack Region of New York to declines in acidic deposition, *Environ. Sci. Technol.*, *37*(10), 2036–2042.

Edmonds, J. W., N. B. Grimm (2011), Abiotic and biotic controls of organic matter cycling in a managed stream, *J. Geophys. Res. Biogeosci.*, *116*, G02015, doi:10.1029/2010jg001429.

Evans, C. D., D. T. Monteith, D. M. Cooper (2005), Long-term increases in surface water dissolved organic carbon: Observations, possible causes and environmental impacts, *Environ. Pollut.*, *137*, 55–71.

Faure, G., and T. M. Mensing (2005), Principles of Isotope Geology, third edition, pp 753–802, John Wiley &Sons, Inc., Hoboken, New Jersey.

Fellman, J. B., E. Hood, and R.G.M. Spencer (2010), Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review, *Limnol. Oceanogr.*, *55*, 2452–2462.

Fellman, J. B., E. Hood, D. V. D'Amore, R. T. Edwards, and D. White (2009a), Seasonal changes in the chemical quality and biodegradability of dissolved organic matter exported from soils to streams in coastal temperate rainforest watersheds, *Biogeochemistry*, *95*, 277–293, doi:10.1007/s10533-009-9336-6.

Fellman, J. B., E. Hood, R. T. Edwards, and D. V. D'Amore (2009b), Changes in the concentration, biodegradability, and fluorescent properties of dissolved organic matter during stormflows in coastal temperate watersheds, *J. Geophys. Res. Biogeosci.*, *114*, G01021, doi:10.1029/2008JG000790.

Findlay, S., J. M. Quinn, C. W. Hickey, G. Burrell, and M. Downes (2001), Effects of land use and riparian flowpath on delivery of dissolved organic carbon to streams, *Limnol. Oceanogr.*, *46*, 345–355.

Freeman, C., C. D. Evans, D. T. Monteith, B. Reynolds, and N. Fenner (2001), Export of organic carbon from peat soils, *Nature*, *412*(6849), 785–785.

Gennings, G. C., L. A. Molot, and P. J. Dillon (2001), Enhanced photochemical loss of organic carbon in acidic waters, *Biogeochemistry*, *52*, 339–354.

Hejzlar, J., M. Dubrovsky, J. Buchtele, and M. Ruzicka (2003), The apparent and potential effects of climate change on the inferred concentration of dissolved organic matter in a temperate stream (the Malse River, South Bohemia), *Sci. Total Environ.*, *310*(1–3), 143–152.

Jaffé, R., D. McKnight, N. Maie, R. Cory, W. H. McDowell, J. L. Campbell (2008), Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties, *J. Geophys. Res. Biogeosci.*, doi:10.1029/2008JG000683.

Kalbitz, K., D. Schwesig, J. Schmerwitz, K. Kaiser, L. Haumaier, B. Glaser, R. Ellerbrock, and P. Leinweber (2003), Changes in properties of soil-derived dissolved organic matter induced by biodegradation, *Soil Biol. Biochem.*, *35*(8), 1129–1142.

Kirchman, D. L. (2003), The contribution of monomers and other low-molecular weight compounds to the flux of dissolved organic material in aquatic ecosystems, in Aquatic Ecosystems: Interactivity of Dissolved Organic Matter, edited by S. E. G. Findlay, and R. L. Sinsabaugh, (2003), pp 218–243, Academic Press, San Diego.

Krug, E. C., and C. R. Frink (1983), Acid rain on acid soil—A new perspective, *Science*, *221*(4610), 520–525.

Larson, J. H., P. C. Frost, Z. Zheng, C. A. Johnston, S. D. Bridgham, D. M. Lodge, and G. A. Lamberti (2007), Effects of upstream lakes on dissolved organic matter in streams, *Limnol. Oceanogr.*, *52*, 60–69.

Leifer, A. (1988) The Kinetics of Environmental Aquatic Photochemistry: Theory and Practice, pp 257–264, American Chemical Society, Washington.

McCallister, S. L., J. E. Bauer, J. E. Cherrier, and H. W. Ducklow (2004), Assessing sources and ages of organic matter supporting river and estuarine bacterial production: A multiple-isotope ($\delta^{14}C$, $\delta^{13}C$, and $\delta^{15}N$) approach, *Limnol. Oceanogr.*, *49*, 1687–1702.

McCallister, S. L., and P. A. del Giorgio (2008), Direct measurement of the $\delta^{13}C$ signature of carbon respired by bacteria in lakes: Linkages to potential carbon sources, ecosystem baseline metabolism, and CO_2 fluxes, *Limnol. Oceanogr.*, *53*(4), 1204–1216.

McGuire, K. J., J. J. McDonnell, M. Weiler, C. Kendall, B. L. McGlynn, J. M. Welker, and J. Seibert (2005), The role of topography on catchment-scale water residence time, *Water Resour. Res.*, *41*(5), doi:10.1029/2004WR003657.

McKnight D. M., E. W. Boyer, P. K. Westerhoff, P. T. Doran, T. Kulbe, and D. T. Andersen (2001), Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity, *Limnol. Oceanogr.*, *46*, 38–48.

McKnight, D. M., E. Hood, and L. Klapper (2003), Trace organic moieties of dissolved organic materials in natural waters, in Aquatic Ecosystems: Interactivity of Dissolved Organic Matter, edited by S. E. G. Findlay, and R. L. Sinsabaugh, (2003), pp 71–96, Academic Press, San Diego.

Mierle, G., and R. Ingram (1991), The role of humic substances in the mobilization of mercury from watersheds, *Water Air Soil Pollut.*, *56*, 349–357.

Mixon, R. B., C. R. Berquist Jr., W. L. Newell, G. H. Johnson, D. S. Powars, J. S. Schindler, and E. K. Rader (1989), Geological Map and Generalized Cross Sections of the Coastal Plain and Adjacent Parts of the Piedmont, Geological Survey, Virginia, U.S.

Molot, L. A., and P. J. Dillon (1997), Photolytic regulation of dissolved organic carbon in northern lakes, *Global Biogeochem. Cycles*, *11*, 357–365.

Moran, M. A., and J. S. Covert (2003), Photochemically mediated linkages between DOM and bacterioplankton, in Aquatic Ecosystems: Interactivity of Dissolved Organic Matter, edited by S. E. G. Findlay, and R L. Sinsabaugh, (2003), pp. 244–262, Academic Press, San Diego.

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

Obernosterer, I., and R. Benner (2004), Competition between biological and photochemical processes in the mineralization of dissolved organic carbon, *Limnol. Oceanogr.*, *49*, 117–124.

Ogrinc, N., R. Markovics, T. Kanduc, L. M. Walter, and S. K. Hamilton (2008), Sources and transport of carbon and nitrogen in the River Sava watershed, a major tributary of the River Danube, *Appl. Geochem.*, *23*, 3685–3698.

Opsahl, S. P., and R. G. Zepp (2001), Photochemically-induced alteration of stable carbon isotope ratios ($\delta^{13}C$) in terrigenous dissolved organic carbon, *Geophys. Res. Lett.*, *28*(12), 2417–2420.

Osburn, C. L., D. P. Morris, K. A. Thorn, and R. E. Moeller (2001), Chemical and optical changes in freshwater dissolved organic matter exposed to solar radiation, *Biogeochemistry*, *54*, 251–278.

Parsons, T. R., Y. Maita, and C. M. Lalli (1984), A Manual of Chemical and Biological Methods for Seawater Analysis, pp. 107–109, Pergamon Press, Oxford.

Petrone, K. C., J. B. Fellman, E. Hood, M. J. Donn, and P. F. Grierson (2011), The origin and function of dissolved organic matter in agro-urban coastal streams, *J. Geophys. Res. Biogeosci.*, *116*, doi:10.1029/2010JG001537.

Raymond, P. A., and J. E. Bauer (2000), Bacterial consumption of DOC during transport through a temperate estuary, *Aquat. Microb. Ecol.*, *22*(1), 1–12.

Raymond, P. A., and J. E. Bauer (2001a), DOC cycling in a temperate estuary: A mass balance approach using natural ^{14}C and ^{13}C isotopes, *Limnol. Oceanogr.*, *46*, 655–667.

Raymond, P. A., and J. E. Bauer (2001b), Riverine export of aged terrestrial organic matter to the North Atlantic Ocean, *Nature*, *409*(6819), 497–500.

Roberts, J. K. (1932), The lower York-James Peninsula. Virginia Geological Survey, Bulletin 37, Educational Series No.2.

Schultz, G. E. (1999), Bacterial dynamics and community structure in the York River Estuary, Ph.D. Dissertation, School of Marine Sciences, The College of William and Mary.

Seitzinger, S. P., H. Hartnett, R. Lauck, M. Mazurek, T. Minegishi, G. Spyres, and R. Styles (2005), Molecular-level chemical characterization and bioavailability of dissolved organic matter in stream water using electrospray-ionization mass spectrometry, *Limnol. Oceanogr.*, *50*, 1–12.

Sickman, J. O., C. L. DiGiorgio, M. L. Davisson, D. M. Lucero, and B. Bergamaschi (2010), Identifying sources of dissolved organic carbon in agriculturally dominated rivers using radiocarbon age dating: Sacramento–San Joaquin River Basin, California, *Biogeochemistry*, *99*, 79–96, doi:10.1007/s10533-009-9391-z.

Skjelkvåle, B. L., et al. (2005), Regional scale evidence for improvements in surface water chemistry 1990–2001, *Environ. Pollut.*, *137*, 165–176.

Sobczak, W. V., J. E. Cloern, A. D. Jassby, and A. B. Muller-Solger (2002), Bioavailability of organic matter in a highly disturbed estuary: The role of detrital and algal resources, *Proc. Natl. Acad. Sci. U.S.A.*, *99*, 8101–8105.

Spencer, R. G. M., et al. (2009), Photochemical degradation of dissolved organic matter and dissolved lignin phenols from the Congo River, *J. Geophys. Res.*, *114*, G03010, doi:10.1029/2009JG000968.

Stedmon, C. A., S. Markager, and R. Bro (2003), Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy, *Mar. Chem.*, *82*, 239–254.

Stedmon, C. A., S. Markager, L. Tranvik, L. Kronberg, T. Slatis, and W. Martinsen (2007), Photochemical production of ammonium and transformation of dissolved organic matter in the Baltic Sea, *Mar. Chem.*, *104*, 227–240.

Stedmon C. A., and R. Bro (2008), Characterizing dissolved organic matter fluorescence with parallel factor analysis: A tutorial, *Limnol. Oceanogr. Methods*, *6*, 572–579.

Stern, J., Y. Wang, B. Gu, and J. Newman (2007), Distribution and turnover of carbon in natural and constructed wetlands in the Florida Everglades, *Appl. Geochem.*, *22*, 1936–1948.

Sulzberger, B., and E. Durisch-Kaiser (2009), Chemical characterization of dissolved organic matter (DOM): A prerequisite for understanding UV-induced changes of DOM absorption properties and bioavailability, *Aquat. Sci.*, *71*, 104–126.

Thomas, D. N., and R. J. Lara (1995), Photodegradation of algal derived dissolved organic-carbon, *Mar. Ecol. Prog. Ser.*, *116*, 309–310.

Warrner, T. J., T. V. Royer, J. L. Tank, N. A. Griffiths, E. J. Rosi-Marshall, and M. R. Whiles (2009), Dissolved organic carbon in streams from artificially drained and intensively farmed watersheds in Indiana, USA, *Biogeochemistry*, *95*, 295–307.

Wiegner, T. N., and S. P. Seitzinger (2001), Photochemical and microbial degradation of external dissolved organic matter inputs to rivers, *Aquat. Microb. Ecol.*, *24*, 27–40.

Williams, C. J., Y. Yamashita, H. F. Wilson, R. Jaffé, and M. A. Xenopoulos (2010), Unraveling the role of land use and microbial activity in shaping dissolved organic matter characteristics in stream ecosystems, *Limnol. Oceanogr.*, *55*, 1159–1171.

Wilson, H. F., and M. A. Xenopoulos (2009), Effects of agricultural land use on the composition of fluvial dissolved organic matter, *Nat. Geosci.*, *2*(1), 37–41.

Yamashita, Y., L. J. Scinto, N. Maie, and R. Jaffé (2010), Dissolved organic matter characteristics across a subtropical wetland's landscape: Application of optical properties in the assessment of environmental dynamics, *Ecosystems*, *13*, 1006–1019.

Yamashita Y., B. D. Kloeppel, J. Knoepp, G. Zausen, and R. Jaffé (2011a), Long term effects of watershed disturbance and forest management on dissolved organic matter characteristics in headwater streams, *Ecosystem*, *14*, 1110–1122, doi:10.1007/s10021-011-9469-z.

Yamashita, Y., A. Panton, C. Mahaffey, and R. Jaffé (2011b), Assessing the spatial and temporal variability of dissolved organic matter in Liverpool Bay using excitation emission matrix fluorescence and parallel factor analysis, *Ocean Dynamics*, *61*, 569–579.