Photochemical and Microbial Alteration of Dissolved Organic Matter in Temperate Headwater Streams Associated with Different Land Use

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

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[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.


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].

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reaction, and to assess the underlying mechanisms. 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.

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 [Fremen 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.

The primary objective of the present study was to characterize how DOM exported from varying land uses 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 ($^{13}$C and $^{14}$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

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.serc.com).

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

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 201 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

<table>
<thead>
<tr>
<th>Sampling Site</th>
<th>Sampling Dates</th>
<th>Water Temperature (°C)</th>
<th>Specific Conductivity (μS)</th>
<th>pH</th>
<th>Dissolved Oxygen (mg/l)</th>
<th>Watershed Land Use Composition</th>
<th>Chlorophyll-a (μg/l)</th>
<th>Nitrate (mg/l)</th>
<th>Ammonium (mg/l)</th>
<th>Watershed Size (km²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>08-18-2009</td>
<td>23.7</td>
<td>43.9</td>
<td>5.5</td>
<td>6.8</td>
<td>83% forest, 17% cropland</td>
<td>0.03</td>
<td>2.1</td>
<td>b.d</td>
<td>0.27</td>
</tr>
<tr>
<td>F2</td>
<td>05-22-2009</td>
<td>15</td>
<td>92.5</td>
<td>6.2</td>
<td>5.8</td>
<td>100% forest</td>
<td>0.04</td>
<td>0.52</td>
<td>b.d</td>
<td>0.09</td>
</tr>
<tr>
<td>F3</td>
<td>11-09-2009</td>
<td>12.6</td>
<td>158.9</td>
<td>5</td>
<td>3.0</td>
<td>100% forest</td>
<td>0.01</td>
<td>b.d</td>
<td>b.d</td>
<td>0.09</td>
</tr>
<tr>
<td>P1</td>
<td>11-09-2009</td>
<td>12.6</td>
<td>56.9</td>
<td>5</td>
<td>7.8</td>
<td>100% forest</td>
<td>0.05</td>
<td>b.d</td>
<td>b.d</td>
<td>0.28</td>
</tr>
<tr>
<td>P2</td>
<td>08-18-2009</td>
<td>18.5</td>
<td>44.8</td>
<td>5.5</td>
<td>7.3</td>
<td>61% pasture, 39% forest</td>
<td>0.92</td>
<td>0.18</td>
<td>b.d</td>
<td>0.44</td>
</tr>
<tr>
<td>C1</td>
<td>05-22-2009</td>
<td>17.8</td>
<td>45.7</td>
<td>4.7</td>
<td>7.0</td>
<td>72% cropland, 28% forest</td>
<td>0.41</td>
<td>17.74</td>
<td>b.d</td>
<td>0.30</td>
</tr>
<tr>
<td>U1</td>
<td>08-18-2009</td>
<td>22.5</td>
<td>713</td>
<td>6.5</td>
<td>5.5</td>
<td>81% urban, 19% forest</td>
<td>0.43</td>
<td>0.73</td>
<td>b.d</td>
<td>0.67</td>
</tr>
</tbody>
</table>

*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 Δ14C-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 (t0) and end (tend) 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-VCSH 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

\[ \% \text{reactive DOC} = \left( \frac{\text{DOC}_{t0} - \text{DOC}_{tend}}{\text{DOC}_{t0}} \right) \times 100 \] (1)

where DOCt0 and DOCtend refer to the DOC concentrations at t0 and tend.

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

\[ \text{DOC}_{t} = \text{DOC}_{t0}e^{-kt} \] (2)

where DOCt 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% H3PO4, 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 CO2 generated from DOC oxidation. The CO2 was collected in 6 mm OD Pyrex tubes that were submitted to the University of Arizona Accelerator Mass Spectrometry (AMS) Laboratory for δ13C and δ14C analyses. δ13C values were reported relative to PDB in standard notation as δ13C = [(Rsample/Rstandard) - 1] * 10^3, where R is 13C/12C. δ14C values, defined as the per mil deviation of a sample compared to the 14C activity of nineteenth century wood, were corrected by δ13C for fractionation. Total measurement uncertainty for Δ14C ranged between 4 and 11%. The SD for duplicate samples and standards (oxalic acid II) was within 0.1% for δ13C and 3.1% for δ14C and that for replicate incubation bottles was ≤0.9%o for δ13C and ≤3.6%o for Δ14C.

[15] Selected water samples from the combined light + bacteria incubations and the bacteria-only incubations were analyzed for DOC isotopes at t0 and tend. 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 ($\delta^{13}C_{\text{Reactive}}, \Delta^{14}C_{\text{Reactive}}$) were calculated as

$$
\delta^{13}C_{\text{Reactive}} = (\delta^{13}C_0 + \text{DOC}_{0t} - \delta^{13}C_{\text{tend}} + \text{DOC}_{\text{tend}})/(\text{DOC}_{0t} - \text{DOC}_{\text{tend}})
$$

(3)

$$
\Delta^{14}C_{\text{Reactive}} = (\Delta^{14}C_0 + \text{DOC}_{0t} - \Delta^{14}C_{\text{tend}} + \text{DOC}_{\text{tend}})/(\text{DOC}_{0t} - \text{DOC}_{\text{tend}})
$$

(4)

where $\delta^{13}C_0$ and $\Delta^{14}C_0$ were the isotopic values of DOC at $t_0$ and $\delta^{13}C_{\text{tend}}$ and $\Delta^{14}C_{\text{tend}}$ refer to those values at $t_{\text{tend}}$.

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 $t_0$, $t_{\text{tend}}$ and one intermediate sampling time (either day 4 or 5). The procedure has been described in detail in Yamashita et al. [201b]. 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 ($C_i$, $i=1$ to 5) was calculated as

$$
\%C_i = F_{c_i}/TF \ast 100 = F_{c_i}/(\sum_{i=1}^{5} F_{c_i}) \ast 100
$$

(5)

where $F_{c_i}$ 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 ± 2σ 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., $k_P$, $k_B$, %C) 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>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>&lt;250 (330)</td>
<td>442</td>
<td>A/C</td>
<td>C10</td>
<td>C1 or C6 (Terrestrial)</td>
<td>Fulvic acid-type</td>
</tr>
<tr>
<td>C2</td>
<td>260 (380)</td>
<td>504</td>
<td>-</td>
<td>SQ1</td>
<td>C5 (Terrestrial)</td>
<td>Humic acid-type</td>
</tr>
<tr>
<td>C3</td>
<td>&lt;250 (305)</td>
<td>388</td>
<td>M</td>
<td>Q3 or C3</td>
<td>C4 (Microbial)</td>
<td>Microbial humic-like</td>
</tr>
<tr>
<td>C4</td>
<td>&lt;250</td>
<td>324</td>
<td>B/T</td>
<td>Ty- or Trp-like</td>
<td>C7 (Protein)</td>
<td>Protein-like</td>
</tr>
<tr>
<td>C5</td>
<td>&lt;250</td>
<td>430</td>
<td>A</td>
<td>Q1</td>
<td>C2 (Terrestrial)</td>
<td>Humic-like</td>
</tr>
</tbody>
</table>
assumptions, including constant variance, linearity, and normality, were examined using residuals versus 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 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 streams.
Table 3. DOC Concentrations and Percentages of Photoreactive and Bioreactive DOC in the Study Streams \( ^a \)

<table>
<thead>
<tr>
<th>Site</th>
<th>Incubation Date</th>
<th>DOC at ( t_0 ) (( \mu M ))</th>
<th>DOC at end of Photoreactive Incubation ( b ) (( \mu M ))</th>
<th>Photoreactive DOC ( ^d ) (%) ± SD ( f )</th>
<th>DOC at end of Bioreactive Incubation ( b ) (( \mu M ))</th>
<th>Bioreactive DOC ( ^d ) (%) ± SD ( f )</th>
<th>DOC at end of Photoreactive + Bioreactive Incubation ( b ) (( \mu M ))</th>
<th>Photoreactive + Bioreactive DOC ( ^d ) (%) ± SD ( f )</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>08-18-2009</td>
<td>377</td>
<td>170</td>
<td>55.0 ( ^i )</td>
<td>373</td>
<td>1.1 ± 3.0</td>
<td>144</td>
<td>61.7 ± 0.4</td>
</tr>
<tr>
<td>F2</td>
<td>09-05-2009</td>
<td>214</td>
<td>51.8</td>
<td>740</td>
<td>9.4 ± 1.2</td>
<td>411</td>
<td>159.6 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>11-09-2009</td>
<td>562</td>
<td>242</td>
<td>748</td>
<td>15.0 ± 3.9</td>
<td>83</td>
<td>19.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>08-18-2009</td>
<td>591</td>
<td>22.1</td>
<td>626</td>
<td>3.8 ± 1.1</td>
<td>140</td>
<td>20.4 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>11-09-2009</td>
<td>212</td>
<td>10.6 ± 0.3</td>
<td>126</td>
<td>17.9 ± 0.6</td>
<td>130</td>
<td>15.4 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>11-09-2009</td>
<td>206</td>
<td>3.2 ± 1.1</td>
<td>157</td>
<td>23.0 ± 0.04</td>
<td>103</td>
<td>50.0 ± 2.1</td>
<td></td>
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<tr>
<td>C1</td>
<td>05-22-2009</td>
<td>139</td>
<td>4.8 ± 5.8</td>
<td>254</td>
<td>0.3 ± 0.4</td>
<td>241</td>
<td>9.1 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>U1</td>
<td>08-18-2009</td>
<td>167</td>
<td>254</td>
<td>9.7 ± 6.2</td>
<td>171 ± 165</td>
<td>72.1 ± 9.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Forested Streams (mean ± SD)

\[ 371 ± 267 \quad 10.3 ± 9.7 \quad 384 ± 285 \quad 16.3 ± 10.7 \quad 291 ± 234 \quad 25.6 ± 16.6 \]

Human-modified Streams (mean ± SD)

\[ 342 ± 231 \quad 13.5 ± 10.7 \quad 305 ± 232 \quad 18.5 ± 11.5 \quad 273 ± 224 \quad 21.2 ± 16.6 \]

---

\( ^a \) n.d. = not determined.

\( ^b \) 0.2 \( \mu M \) filter, 15 days, light.

\( ^c \) 0.7 \( \mu M \) filter, 35-36 days, dark.

\( ^d \) SD (standard deviation) was calculated from the replicate bottles.

\( ^e \) SD was not provided because of sample loss.

Figure 4. Box-plot comparing first-order DOC removal rate constants (\( k' \) (day\(^{-1} \))) of (a) the three types of incubations: (i) dark + bacteria \( (B) \), (ii) light + bacteria \( (P+B) \), and (iii) light + bacteria at constant light intensity \( (P+B*) \) for forested and human-modified streams. Open circle \( (O) \) indicates significant difference between forested and human-modified streams by Kruskal-Wallis tests. Open circle \( (O) \) indicates significant difference between forested and human-modified streams by Mann-Whitney tests. Closed circle \( (\bullet) \) indicates extreme outlier. Incubations are (a) dark \( (D) \) and (b) light \( (P) \).
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$_0$) 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$_0$ 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 $t_{final}$: $P\leq0.002$ 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: $P\geq0.2$ for %C1–%C5) (Figures 6a and 6b).

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 watersheds 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 $^{13}$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].
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 $k_p$ 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].

[31] 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-modified 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 predicted 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 Studiesa

<table>
<thead>
<tr>
<th>Sources</th>
<th>Photoactive DOC</th>
<th>Incubation Duration</th>
<th>Light Sources</th>
<th>Water Quality Measure</th>
<th>Changes in CDOM</th>
<th>CDOM % Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>This study</td>
<td>0.045-0.055</td>
<td>15 days</td>
<td>PAR</td>
<td>True color (broadband absorbance at 405-450 nm)</td>
<td>Decrease of TF by ~85% on average and changes of relative abundance of fluorophores</td>
<td>51-57%</td>
</tr>
<tr>
<td>Molot and Dillon [1997]</td>
<td>0.017-0.062</td>
<td>6-11 days</td>
<td>PAR</td>
<td>True color (broadband absorbance at 405-450 nm)</td>
<td>True color decreased by ~22%</td>
<td>10-20%</td>
</tr>
<tr>
<td>Clements et al. [2008]</td>
<td>0.026</td>
<td>24 h</td>
<td>UVB</td>
<td>Absorbance at 320 nm</td>
<td>Decrease of TF by ~91% on average and changes of relative abundance of fluorophores</td>
<td>Below detection</td>
</tr>
<tr>
<td>Larson et al. [2007]</td>
<td>0.11-0.22</td>
<td>Below detection</td>
<td>PAR</td>
<td>True color (broadband absorbance at 405-450 nm)</td>
<td>True color decreased by ~10%</td>
<td>Below detection</td>
</tr>
<tr>
<td>Wiegner and Seitzinger [2001]</td>
<td>0.005-0.022</td>
<td>15 days</td>
<td>PAR</td>
<td>True color (broadband absorbance at 405-450 nm)</td>
<td>True color decreased by ~10%</td>
<td>Below detection</td>
</tr>
</tbody>
</table>

aGenerally, only either percent photoreactive DOC or %C5 was determined. bTrue color: a method designed to emulate Hazen units, which defined water color as 1.35*(broadband absorbance at 405–450 nm) + 1.58*(broadband absorbance at 660–740 nm) [Mierle and Ingram, 1991]. cStudent’s t-test (P < 0.05) was used to assess differences across groups. dFluorescence component possibly representing extensive light exposure (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. eAccording to previous studies, the Eastern US states, including New Jersey, had a higher %C5 than the Western US states. fWestern US states, including Colorado, had a lower %C5 than the Eastern US states. gAnother 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 exposure 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; Yamashita et al., 2011a]. The %C5 in stream water DOM at t0, 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.

4.3 Factors Impacting Headwater Stream DOC Bioreactivity

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.

We found that in situ stream temperature was the strongest predictor of DOC bioreactivity ($k_B = -0.018 \times \text{stream temperature}$, $RSQ = 0.8$, $P = 0.002$, $n = 9$). 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 $22 \pm 2 ^\circ C$, 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 seasonal variability of DOC bioreactivity in affecting stream DOC metabolism may vary greatly across systems.

Several recent studies of stream water DOC have demonstrated a positive correlation between % bioreactive DOM and $\delta^{13}C$-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 biodegradability. In addition, the presence of non-colored DOM, such as carbohydrates that may account for a portion of total DOC biodegradability but were not included in C4, will affect the ability of protein-fluorescence to predict DOC biodegradability.

### 4.3. Photochemical and Microbial Alterations of DOC Isotopes

During dark, bacteria-only incubations, both $\delta^{13}C$-DOC and $\Delta^{13}C$-DOC values showed negligible changes, as represented by similarities in the isotopic composition of biorefractory and bioreactive fractions (Figures 3a and 3b).
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, 1^{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 ^13C 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 ^13C 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 1^{14}C-enriched 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 t₀ (Δ^{14}C = −202 ± 4‰) to 1917 ± 38 yrs B.P. at t_end (Δ^{14}C = −212 ± 4‰), indicating that younger DOC (Δ^{14}C = −105 ± 55‰, ^14C 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).

4.4. Photochemical and Microbial Alteration of CDOM

[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 ^13C-depleted DOC possibly due to preferential photodegradation of lignin-derived and aromatic-rich compounds; and (3) at higher DOC losses, preferential remineralization of younger, ^13C-enriched DOC. These findings suggest that reactive and refractory DOC may share similarities in source-age characteristics across system types and study sites.

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

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

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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 δ13C-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; Cavley 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 microbrial 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.

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