Fluorescence characteristics and sources of dissolved organic matter for stream water during storm events in a forested mid-Atlantic watershed

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[1] The concentrations and quality of dissolved organic matter (DOM) and their sources were studied for multiple storm events collected over a three-year period (2008–10) in a forested headwater (12 ha) catchment in the mid-Atlantic Piedmont region of the USA. DOM constituents were characterized using a suite of indices derived from ultraviolet absorbance and PARAFAC modeling of fluorescence excitation emission matrices. Runoff sources and hydrologic flow paths were identified using an end-member mixing model, stable isotope data, and groundwater elevations from valley-bottom saturated areas. DOM constituents and their sources differed dramatically between base flow and storm-event conditions. The aromatic and humic DOM constituents in stream water increased significantly during storm events and were attributed to the contributions from surficial sources such as throughfall, litter leachate and soil water. Groundwater sources contributed a large fraction of the DOM constituents during base flow and were responsible for the high % protein-like fluorescence observed in base flow. Hydrologic flow paths and runoff sources were critical for explaining the differences in DOM among the storm events. This study underscored the value of studying multiple storm events across a range of hydrologic and seasonal conditions. Summer events produced the highest concentrations for humic and aromatic DOM while the corresponding response for winter events was muted. A large event following summer drought produced a complex DOM response which was not observed for the other events. These extreme events provided important insights into how DOM quality may change for future changes in climate and water quality implications for sensitive coastal ecosystems.


1. Introduction

[2] The amount and quality of dissolved organic matter (DOM) in surface waters has important consequences for water quality and ecological processes [Driscoll et al., 1988; Nokes et al., 1999; Seitzinger et al., 2002; Williamson and Zagarese, 1994]. Exports of DOM from watersheds are typically at their highest during storm events; thus, understanding the storm-event dynamics of DOM is especially critical [Hinton et al., 1997; Inamdar et al., 2006; Raymond and Saiers, 2010]. Most previous work on storm-event DOM has focused primarily on the bulk concentration of DOM and very little attention has been paid to the quality or constituents of DOM. DOM constituents could include aromatic and humic components or non-humic fractions such as amino-acids and carbohydrates [Qualls and Haines, 1991]. Characterizing these constituents and determining their concentration has been difficult since the analytical methods are laborious, time-consuming, expensive, and require large sample amounts. However, recent availability of innovative spectrofluorometric methods [Baker, 2001; Coble et al., 1990; McKnight et al., 2001] allow for rapid characterization of DOM quality using small sample amounts (~4 mL). Knowledge of DOM quality can provide important insights into the mobility, degradability and bio-
availability of DOM [Fellman et al., 2009, 2010; Jaffé et al.,
2008; Kaushal and Lewis, 2005] and DOM constituents can also
serve as tracers for identifying the sources and flow paths for DOM in
watersheds [Hood et al., 2006].

[5] To date, only a handful of studies have explored the
changes in DOM quality for storm events using optical
methods [Austnes et al., 2010; Buffam et al., 2001; Fellman
et al., 2009; Hernes et al., 2008; Hood et al., 2006; Nguyen
et al., 2010; Pellerin et al., 2011]. Some of the optical
indices that have been implemented to characterize DOM
quality include: specific ultraviolet absorbance (SUVA)
[Weishaar et al., 2003], absorption coefficient [Hernes et al.,
2008; Saraceno et al., 2009], spectral slope ratio (Sₘ) [Helms
et al., 2008; Spencer et al., 2010], humification index (HIX)
[Ohno, 2002], fluorescence index (Fl) [Cory and McKnight,
2005], and % protein-like fluorescence [Fellman et al.,
2009] derived from fluorescence-based excitation emission
matrices (EEMs) [Cory and McKnight, 2005; McKnight et
al., 2001]. Studies by Hood et al. [2006], Fellman et al.
[2009] and Nguyen et al. [2010] reported that SUVA va-
ues increased with storm event discharge, suggesting an
increase in the aromatic fraction of DOM during storm
events. The elevated contributions of aromatic DOM were
attributed to the flushing of humic-rich, near-surface soil
waters [Fellman et al., 2009; Hood et al., 2006]. On the
contrary, Austnes et al. [2010] reported a decrease in SUVA
for storm events in a peatland catchment in North Wales,
UK. Storm events may also enhance the exports of high-
molecular weight DOM from watersheds [Li et al., 2005;
Nguyen et al. 2010]. In contrast to the humic and/or aromatic
constituents, the percentage of protein-like fluorescence
(e.g., tryptophan and tyrosine) [Cory and McKnight, 2005]
has been reported to decline during storm events [Fellman
et al., 2009; Nguyen et al., 2010] and was attributed to the
mobilization of allochthonous (versus autochthonous)
DOM during events. Others, however, have not observed a
pronounced change in fluorescence during storm events
[Austnes et al., 2010].

[4] While the studies described above have provided
important insights into DOM quality during events, they
have been limited to only a few storm events. DOM con-
stituents are expected to vary considerably for storm events
occurring under contrasting hydrologic and seasonal con-
ditions. Understanding these differences is critical and re-
quires monitoring DOM responses for multiple storms
across many seasons. Moreover, it is especially important to
identify the hydrologic flowpaths and watershed sources
responsible for the exports of DOM constituents during and
between storm events. New hydrologic flowpaths and sources
of DOM constituents can be “activated” during storm
events which may be considerably different from those dur-
ing base flow conditions. Such information can help us
develop better conceptual or mechanistic models of DOM.

[5] We evaluated the quality of DOM for stream water for
multiple storm events and base flow conditions over a three-
year period (2008–10) in a forested, headwater (12 ha)
catchment in the mid-Atlantic Piedmont region of the USA.
Base flow sampling was performed every 3–4 weeks and 27
storm events were collected over the three-year period. The
quality of DOM was characterized using a suite of spec-
trofluorometric indices that included: absorption coefficient
at 254 nm (a₂₅₄); SUVA at 254 nm, HIX, Fl, Sₘ, and %
protein-like fluorescence derived from an existing EEMs-
PARAFAC model [Cory and McKnight, 2005]. The goal of
this study was to investigate the temporal patterns of DOM
constituents during storm events and determine the runoff
sources and hydrologic flowpaths responsible for their
export in stream runoff. Eight storm events representing
different seasonal and hydrologic conditions were selected
to study the DOM responses. Hydrologic flowpaths and
sources of runoff for these events were identified using a
combination of end-member mixing analyses (EMMA)
[Hooper, 2003; Inamdar, 2011], oxygen-18 stable isotope
data for water (δ¹⁸O-H₂O), and groundwater elevations.
DOM concentrations and quality for the various watershed
sources such as precipitation, throughfall, litter leachate,
soilwater, and groundwater were known from a previous
study [Inamdar et al., 2011]. Knowledge of DOM for these
watershed sources combined with EMMA-derived runoff
sources and contributions were used to explain the storm-
event patterns of DOM observed for stream runoff. Specific
questions that were addressed include: (1) What is the
character of DOM during base flow and how does it differ
from that during storm events? What are the sources and
flowpaths for DOM during base flow? (2) What are the
temporal patterns of various DOM constituents during storm
events? What are the runoff sources and hydrologic flow
paths responsible for DOM during storms? and (3) How
do the patterns and quantity of DOM constituents differ
among storm events? What are the mechanisms behind these
differences?

[6] The innovative aspect of this study is not only the
detailed spectrofluorometric characterization of DOM for a
large set of storm events, but more importantly, the identi-
fication of the sources and flowpaths for DOM constituents
using a rigorous EMMA model supported by supplementary
hydrometric data.

2. Site Description and Methods
2.1. Site Description

[7] The study catchment (12 ha) is located within the
Fair Hill Natural Resources Management Area (NRMA)
(39°42′N, 75°50′W) in Cecil County, MD (Figure 1) and
is part of an intensive, ongoing study, on DOM [Inamdar et
al., 2011]. The catchment is drained via two first-order
tributaries (ST1 and ST2) which merge to form ST3 at the
catchment outlet. Stream ST1 is perennial and originates
from a groundwater seep while portions of stream ST2
occasionally dry up and become hydrologically disconnected
during the driest parts of the year (August–October). The 12
ha catchment drains into the Big Elk creek which lies within
the Piedmont physiographic region and eventually drains
into the Chesapeake Bay. Cecil County has a humid, conti-
nental climate with well-defined seasons. The maximum
daily mean temperature (1971–2000) was 24.6°C (July) and
the daily minimum was −0.6°C (January), with a mean
annual temperature of 12.2°C. For this same period, mean
annual precipitation in this region was 1231 mm with
∼350 mm occurring as snowfall in winter (Maryland State
Climatologist Office Data Page, http://metosrv2.umd.edu/
∼climate/cono/norm.html, accessed January 3, 2009). Late
summer (August–September) tends to be the driest period
of the year while late spring (April–May) is the wettest.
The study area is underlain by the Mt. Cuba Wissahickon formation and includes pelitic gneiss and pelitic schist with subordinate amphibolite and pegmatite [Blackmer, 2005]. The soils in the study area belong to the Glenelg series, which consists of deep, well-drained, nearly level to moderately steep soils. On the hillslopes, soils are coarse loamy, mixed, mesic Lithic Dystrudepts while in the valley bottoms seasonal water saturation leads to the formation of Oxyaquic Dystrudepts. Elevation ranges from 252 to 430 m above mean sea level. Vegetation in the study catchment consists of deciduous forest with pasture along the catchment periphery. Dominant tree species are Fagus grandifolia (American beech), Liriodendron tulipifera (yellow poplar), and Acer rubrum (red maple) [Levia et al., 2010].

2.2. Hydrologic Monitoring

Streamflow discharge at the outlet of the 12 ha catchment at ST3 was monitored using a Parshall flume.
with 15-cm throat width. The water level in the flume was recorded every 15 min using a Global Water (Inc.) logger and pressure transducer. The flow depth was then converted to discharge using an equation provided by the flume manufacturer. Streamflow discharge computed using the equation was checked through manual flow measurement at least twice per year. Depth to groundwater (from the soil surface) was recorded at five locations (Figure 1) in the catchment at 30-min intervals using Global Water loggers (Inc.). Groundwater logging wells consisted of PVC pipes (5 cm diameter) ~2 m below the ground surface that were continuously slotted from a depth of 0.3 m below the soil surface. Precipitation and air temperature data was available at 5-min frequency from a weather station located in the Fairhill NRMA, about 1000 m from the outlet of the 12 ha catchment.

2.3. Water Chemistry Sampling and Analyses

[10] Stream water samples at the outlet of the catchment (Figure 1) were collected during storm events as well as base flow periods. Storm event sampling was performed using an automated ISCO sampler which was triggered to sample when the rainfall amount exceeded 2.54 mm in a one hour period. The samples were collected in the “non-uniform” program model which was adjusted based on the type of rain that was expected (i.e., long-duration versus short-duration high intensity event). The sampling frequency ranged from as low as 15 min on the hydrograph rising limb to 3 h on the recession limb. In addition to stream water, rainfall (one location), throughfall (two locations), and litter (forest floor; two locations) leachate samples were also collected 24 h following storm events [DEOS, 2008]. Rainfall and throughfall samplers consisted of 1L amber glass bottles that collected water through a plastic funnel. Litter samplers consisted of 1L amber glass bottles connected to (via plastic tubing) plastic trays (~1 m²) that contained the O horizon layer.

[11] Non-storm sampling for stream water at the catchment outlet occurred every three to four weeks and involved manual grab sample collection (sampling was performed every 2–3 weeks for 2008–09 and every four weeks in 2010). In addition to the collection of stream water, grab samples from various watershed sources were also collected at this time. The intent was to characterize the DOM concentrations and quality in various watershed sources [Inamdar et al., 2011]. A complete description of the sampling of watershed sources is provided by Inamdar et al. [2011] and only a brief description is provided here. Samples collected included: zero-tension wetland soilwater (WSW); tension soilwater (U); wetland shallow and deep groundwater (SGW and DGW, respectively), riparian groundwater (RGW), groundwater seeps (Seep) and hyporheic water (HY) (Figure 1). Wetland soilwater was sampled at four sites (Figure 1) in the valley-bottom wetlands. The soil water was sampled using zero-tension lysimeters which consisted of screened 5 cm diameter PVC pipes that were inserted at a 45 degree angle to a depth of 30 cm. Tension soilwater samples were collected using two nests of two suction cup tension lysimeters (Soilmoisture Equipment Corp.) each at 10 and 30 cm depths. Shallow groundwater samples were collected from four wells located in the valley-bottom wetlands (Figure 1) and were constructed of 5 cm PVC tubing, augured to 2 m and screened for the full length from 30 cm below the soil surface. Two deep groundwater wells were collocated with shallow groundwater wells in the wetlands and were screened only for the lowermost 50 cm so as to collect only the deeper portion of groundwater (1.5 to 2 m below the soil surface). The riparian groundwater well was identical to shallow groundwater except that it was located in a riparian location which was not a wetland (Figure 1). Hyporheic samples were collected at two stream locations (Figure 1) and consisted of slotted PVC pipes (5 cm diameter) inserted at a 45° angle to a depth of 30 cm in the streambed. All samples from soil and groundwater locations were recovered using a hand-operated suction pump. Seep samples were collected manually at two seep locations from one of the catchment tributaries (ST1) originated (Figure 1).

2.4. Sample Processing and Chemical Analysis

[12] All samples were filtered through a 0.45 µm filter paper (Millipore, Inc.) within 24 h of collection and stored at 4°C. A subsample for nutrients, cations, and anion analyses was stored in HDPE acid-rinsed bottles prior to analysis. Samples for DOM characterization were stored in 40 ml amber glass vials prior to UV and fluorescence measurements. The Biogeochemistry Laboratory at SUNY-ESF, NY, which is a participant in the USGS QA/QC program, performed the following analyses: pH using a pH meter; major cations (Ca²⁺, Mg²⁺, Na⁺, K⁺, Si, Mn, S) using a Perkin-Elmer ICP-AEC Div 3300 instrument; anions (Cl⁻, NO₃⁻, SO₄²⁻) using a Dionex IC; NH₄ with an autoanalyizer using the Berthelot Reaction followed by colorimetric analysis; total dissolved nitrogen (TDN) using the persulfate oxidation procedure [Ameel et al., 1993] followed by colorimetric analysis on an autoanalyzer; and DOC using the Tekmar-Dohmann Phoenix 8000 TOC analyzer. DON concentrations were computed as the difference between TDN and inorganic N (NO₃⁻, NH₄⁺) [Inamdar and Mitchell, 2007]. Analyses for oxygen-18 isotopes (δ¹⁸O) for water were performed using standard procedures by the Environmental Isotope Laboratory at the University of Waterloo, Canada (Protocols for sample analyses, http://www.uwellelab.ca/index.html, last accessed, April 7, 2011).

2.5. UV and Fluorescence Analyses and Metrics

[13] All UV and fluorescence analyses were performed at Inamdar Watershed Laboratory at the University of Delaware. The UV absorption of DOC at 254 nm was measured using a 1 cm quartz window cuvette with a single beam Shimadzu UV-mini 1240 spectrophotometer (Shimadzu Inc.). SUVA (specific UV absorbance) provides a measure of aromaticity of DOM [Weishaar et al., 2003] and was computed by dividing the decadic UV absorbance at 254 nm (m² g⁻¹) by the concentration of DOC (mg C L⁻¹) [Weishaar et al., 2003]. DOM aromaticity increases with increasing values of SUVA. Since iron (Fe) absorbs light at 254 nm, elevated concentrations of Fe (>0.5 mg L⁻¹) can lead to incorrect SUVA values [Weishaar et al., 2003]. We verified this by checking our SUVA values against the sample Fe concentrations (corresponding DOC values were also checked). Most of our data (more than 99%) had Fe concentrations much lower than 0.5 mg L⁻¹.
[14] In addition, the absorption coefficient at 254 nm ($a_{254}$ in m$^{-1}$) was also calculated by using the naperian UV absorption coefficient following the procedures of Green and Blough [1994]. The $a_{254}$ provides a measure of aromaticity but without normalization to C [Helms et al., 2008]. Another UV index, the spectral slope ratio, $S_R$, was also calculated as the ratio of the slope of the shorter UV wavelength region (275–295 nm) to that of the longer UV wavelength region (350–400 nm) [Helms et al., 2008] and was obtained using linear regression on the log-transformed spectral ranges [Yamashita et al., 2010]. The spectral slope ratio, $S_R$, is inversely related to the molecular weight of DOM [Helms et al., 2008].

[15] Three-dimensional fluorescence scans were collected on a Fluoromax-P spectrofluorometer (Horiba Jobin-Yvon Inc.) and corrected for the instrument bias with manufacturer provided correction files. A daily lamp scan, cuvette check and Raman water scan were run to ensure instrument stability. Samples were kept at room temperature (20°C) for two hours prior to any optical analysis. Scans were collected in S/R mode (ratio) for excitation wavelengths between 240 and 450 nm at 10 nm intervals and emission wavelengths between 300 and 550 nm at 2 nm intervals. The integration time for sample collection was set to 0.25 s along with band pass of 5 nm each for excitation and emission ranges. Subsequently, a Raman water blank was subtracted from each scan and resulting EEMs were Raman-normalized using the area under the curve of water Raman peak at excitation 350 nm [Cory and McKnight, 2005]. To avoid inner filter effects, samples were diluted according to Green and Blough [1994]. UV Absorbance, A, at wavelength 254 nm ($A_{254} \geq 0.2$) was chosen as the cutoff threshold. If the samples had $A_{254} \geq 0.2$, then the samples were diluted and the dilution factors (DF) were recorded. The dilution factors were later multiplied to the corrected sample EEM to obtain the intensity of undiluted original sample. In addition, the inner filter correction was also applied [McKnight et al., 2001; Ohno, 2002]. We also investigated the any effects of the plastic ISCO bottles on sample fluorescence by comparing EEMs for deionized water and DOM solutions stored in ISCO and amber glass bottles over a period of 24 h at room temperature (20°C). These comparisons did not yield any noticeable differences in the EEMs.

[16] The fluorescence index (FI) was calculated using the ratio of fluorescence emission intensities at 470 and 520 nm at an excitation wavelength of 370 nm [Cory and McKnight, 2005]. McKnight et al. [2001] have used the FI to differentiate between DOM derived from vascular plants (FI: 1.3–1.4) versus microbial or planktonic sources (FI: 1.7–2.0). The humification index (HIX) was calculated using the normalized HIX equation of Ohno [2002], which reduces the variation introduced by changes in DOM concentration [Ohno, 2002]. The HIX values for this modified equation range from 0 to 1 with higher values indicating a greater degree of humification of DOM [Ohno, 2002]. The EEMs scans were also fitted to the 13-component PARAFAC model developed by Cory and McKnight [2005]. The advantage of using this existing model was that a large sample size was not required and a greater amount of variation in the DOM source and quality was likely to be identified [Miller and McKnight, 2010]. The ability of the Cory and McKnight [2005] PARAFAC model to fit the EEMs was assessed by evaluating the residuals between measured and modeled EEMs. The residuals were found to be less than 10% of the fluorescence intensity values with little coherent structures and no residual peaks were found which confirms that the model fits were good. The percentage of protein-like fluorescence was calculated as the sum of % values for tryptophan-like (component 8) and tyrosine-like fluorescence (component 13) from the Cory and McKnight [2005] model. The sum of these two protein-like components has been found to be a strong predictor of % bioavailable DOM [Fellman et al., 2008, 2009].

### 2.6. Selection of Storm Events and Characterization of Hydrologic Conditions for the Events

[17] A total of 27 storm events were analyzed for all DOM indices. These events were sampled across all possible seasons over the three-year study period including - six in 2008, 14 in 2009, and 7 in 2010. Of these 27 events, eight events representing a range of hydrologic and seasonal conditions were selected; these include (Figure 2): May 20, July 27 and October 25 (all 2008); July 31, October 28, and December 9 (all 2009); and February 22–24 and September 30–October 1, 2010. These eight events were selected: (a) because six of the eight events were representative of storms conditions occurring in a particular season (e.g., short-duration, high intensity events during summer versus long-duration, low-intensity events during spring and fall) and also produced DOM patterns similar to other events within the same season; and (b) two of the events – February 22–24 and September 30–October 1, 2010 represented extreme hydrologic conditions. The event of February 22–24, 2010 was an unusually large snowmelt and rain-on-snow event which was part of an uncommon snow season for the mid-Atlantic region of the USA. The 2009–2010 seasonal snowfall total for Wilmington, Delaware (25 km from our watershed location) was 167 cm and was the highest-ever snow total on record. The long-term seasonal snow total for Wilmington averages around 50 cm. The event of September 30–October 1, 2010 was associated with the tropical depression Nicole [National Oceanic and Atmospheric Administration, 2011], which yielded 151 mm of rainfall in a 28-h period following a very dry summer season.

[18] Hydrologic conditions during and before the storm events were characterized using a number of metrics (Table 1). Precipitation metrics included: total amount for event (mm), maximum 5-min rainfall intensity (mm), sum of 24 h (API24, mm) and 7-day (API7, mm) antecedent precipitation. Streamflow discharge was characterized using total specific discharge for the event (mm per unit catchment area), peak specific discharge (mm/hr), average of streamflow discharge (mm/hr) 24 h prior to event (AR24), and the ratio of total specific discharge (mm) to total precipitation (mm) for the event (runoff ratio). Antecedent moisture conditions in the catchment were also characterized by a 7-day running average of groundwater (GW) elevations (meters below soil surface) for well LW4 (Figure 1). Our visual observations over the three-year study suggested that groundwater elevations for the wetland well LW4 were generally reflective of the overall wetness conditions across the whole catchment.

[19] In addition to storm events, base flow data for the stream at ST3 from the period 2008–2009 was included. This allowed us to evaluate the change in DOM constituents
between storm and base flow conditions and the EMMA-derived sources of runoff for base flow. Flow- or discharge-weighted DOM concentrations and UV and fluorescence indices were also calculated for the eight storm events. The flow-weighted (\(C_w\)) DOM values were computed by:

\[
C_w = \frac{\sum_{i=1}^{n} C_i \cdot Q_i}{\sum_{i=1}^{n} Q_i}
\]

where \(C_i\) and \(Q_i\) were the measured DOM concentrations/indices and discharge values, respectively at time \(i\) during the event.

2.7. DOM Concentrations and Quality for Watershed Sources

A detailed description of the DOM concentrations and fluorescence characteristics for watershed sources has already been provided by Inamdar et al. [2011] and only a brief overview is included here. DOM concentrations and quality indices were determined for nine watershed sources.

Figure 2. Precipitation (mm, 5-min totals), specific streamflow discharge (mm/hr), groundwater elevations for LW4 (meters below soil surface), and DOC concentrations (mg L\(^{-1}\)) for the eight selected events over the three-year (2008–10) study period.
DOC and DON concentrations were highest for throughfall (TF) and litter leachate (LT) and declined dramatically with soil depth. The decrease in DOC was much sharper than that for DON (Figure 3). Lowest DOM concentrations were recorded in groundwater seeps (P) and deep groundwater (DGW) compartments. Similarly, the aromatic content (indicated by SUVA and $a_{254}$) and humic (HIX) components of DOM were highest for surficial DOM sources – throughfall (TF), litter leachate (LT) and soil waters (U and WSW), and declined for DOM sources originating deeper in the soil profile (Figure 4) or along a hydrologic flow path that had greater contact with soil. Inamdar et al. [2011] attributed this decline to the sorption of aromatic and humic DOM constituents along hydrologic flow paths as water percolated through the soil profile. It should be noted though that $a_{254}$ values declined much quickly for deeper sources in the soil compared to the HIX values. In contrast, the values for $S_R$ increased with depth suggesting a decrease in the molecular weight of DOM ($S_R$ is inversely related to DOM molecular weight). This suggests that the aromatic and humic constituents of DOM in surficial watershed compartments were associated with DOM of higher molecular weight. In contrast to the aromatic fractions, the % of protein-like fluorescence and FI

**Table 1.** Data on Precipitation, Specific Stream Discharge, Antecedent Groundwater Conditions, and Antecedent Air Temperature for the Eight Selected Storm Events

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
<th>Total Precipitation (mm)</th>
<th>Max 5-min Intensity (mm)</th>
<th>API24 h (mm)</th>
<th>API7 Days (mm)</th>
<th>Total Stream Discharge (mm)</th>
<th>Peak U (mm/hr)</th>
<th>AR24 Hours (mm)</th>
<th>Runoff Ratio</th>
<th>Groundwater AGI LW4 (m)</th>
<th>Air Temp AT7 Days °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5/20/08</td>
<td>12.9</td>
<td>1.3</td>
<td>2.4</td>
<td>33.6</td>
<td>1.99</td>
<td>0.27</td>
<td>0.04</td>
<td>0.15</td>
<td>0.12</td>
<td>13.5</td>
</tr>
<tr>
<td>2</td>
<td>7/27/08</td>
<td>20.9</td>
<td>6.9</td>
<td>0</td>
<td>68.5</td>
<td>0.96</td>
<td>0.45</td>
<td>0.01</td>
<td>0.05</td>
<td>0.43</td>
<td>22.6</td>
</tr>
<tr>
<td>3</td>
<td>10/25/08</td>
<td>13.0</td>
<td>3.8</td>
<td>13.6</td>
<td>13.7</td>
<td>0.60</td>
<td>0.37</td>
<td>0.06</td>
<td>0.05</td>
<td>1.10</td>
<td>6.6</td>
</tr>
<tr>
<td>4</td>
<td>7/31/09</td>
<td>30.9</td>
<td>11.0</td>
<td>0</td>
<td>37.4</td>
<td>1.85</td>
<td>0.88</td>
<td>0.02</td>
<td>0.06</td>
<td>0.36</td>
<td>21.6</td>
</tr>
<tr>
<td>5</td>
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<td>1.3</td>
<td>17.2</td>
<td>58</td>
<td>6.5</td>
<td>1.16</td>
<td>0.15</td>
<td>0.26</td>
<td>0.42</td>
<td>11.0</td>
</tr>
<tr>
<td>6</td>
<td>12/9/09</td>
<td>44.6</td>
<td>1.0</td>
<td>48.2</td>
<td>17.5</td>
<td>17.5</td>
<td>2.34</td>
<td>0.05</td>
<td>0.39</td>
<td>0.12</td>
<td>2.7</td>
</tr>
<tr>
<td>7</td>
<td>2/22/10</td>
<td>22.0</td>
<td>0.4</td>
<td>0</td>
<td>13.1</td>
<td>17.1</td>
<td>0.08</td>
<td>2.13</td>
<td>0.78</td>
<td>–0.22</td>
<td>0.79</td>
</tr>
<tr>
<td>8</td>
<td>9/30/10</td>
<td>151.0 (43, 108)</td>
<td>5.2</td>
<td>0</td>
<td>20</td>
<td>13.1</td>
<td>3.68</td>
<td>0.18</td>
<td>0.08</td>
<td>0.68</td>
<td>20.1</td>
</tr>
</tbody>
</table>

*API24 and API7 is antecedent precipitation for 24 h and 7 days, respectively; AR24 is average antecedent stream discharge for 24 h; AGI LW4 is 7-day running average for groundwater elevations for well LW4; and AT7 is the average 7-day air temperature preceding the event.

**Table 2.** Data on Precipitation, Specific Stream Discharge, Antecedent Groundwater Conditions, and Antecedent Air Temperature for the Eight Selected Storm Events

**Figure 3.** Concentrations of dissolved organic carbon (DOC) and nitrogen (DON) (mg $L^{-1}$) for the nine sampled watershed sources. The line within the box indicates the median, the bounds of the box indicate 25th and 75th percentile, and the error bars indicate the 10th and 90th percentile of the data set.
values increased with soil depth and was highest for seeps and deep groundwater (DGW). We attributed [Inamdar et al., 2011] the increase in the protein-like fluorescence to the relative decrease in humic fractions, while the increase in FI values suggested either a greater expression of “microbial” DOM or some other unknown influences on groundwater sources.

2.8. End-Member Mixing Analyses (EMMA) Model and $\delta^{18}$O-H$_2$O Data

[21] An EMMA model to identify the sources for stream runoff in the 12 ha catchment was developed following the procedures described by Burns et al. [2001], Hooper [2003] and Inamdar [2011]. A thorough description of the procedures, verification, and testing of the model is provided by S. Inamdar et al. (manuscript in preparation, 2011). Tracers that were used for the EMMA were: sodium (Na$^+$), silica, DOC, and $a_{254}$. These tracers were chosen since they displayed conservative, linear mixing at the time-scale of the storm events (following procedures suggested by Hooper [2003]) and because sodium and silica represented groundwater flowpaths while DOC represented surficial runoff sources. The values for $a_{254}$ helped further discriminate between runoff sources that had similar DOC concentrations. Storm-event and base flow concentrations of selected tracers for stream water were normalized/standardized using mean and standard deviations, and a correlation matrix was developed. A principal component analyses (PCA) was then performed on the correlation matrix. The first two principal components were used (implying a three end-member model) to generate a two-dimensional mixing space [Hooper, 2003]. This allowed us to compare the stream chemistry for storm-events and base flow against the watershed sources and determine how the watershed sources influenced stream chemistry. While a three end-member model explained a large portion (>90%) of the variability in stream chemistry, we hypothesized that the stream chemistry was likely a mixture of a continuum of watershed sources including throughfall, litter leachate, soil water and groundwater and thus did not constrain ourselves to only three end-members and did not determine individual contributions from three selected end-members. Rather, we believed that studying the storm-event chemistry in the U-space with all potential sources included provided a more comprehensive assessment of stream chemistry versus constraining the analyses with a few or selected end-members. In addition to EMMA, we also present data on $\delta^{18}$O for water that was available for a few events in 2008 and 2009. The data on $\delta^{18}$O provides the temporal information on “new” or event water [Buttle, 1994] that can help in further validating the time-source component [Buttle, 1994] of surficial runoff sources derived from EMMA. Groundwater elevations (depth of water below soil surface) recorded in the valley-bottom saturated areas were also used to verify potential end-member contributions from soil or shallow groundwaters.

3. Results

3.1. Hydrologic and Seasonal Conditions for Selected Storm Events

[22] Precipitation, specific stream discharge (discharge per unit catchment area), LW4 groundwater elevations and DOC concentrations for the eight selected events are displayed in Figure 2. Specific metrics characterizing the hydrologic conditions for the eight events are reported in Table 1. The total rainfall amounts for 2008, 2009, and 2010
were 1052, 1238, and 972 mm, respectively. Among the three years, 2009 was the wettest while 2008 was the driest year. The dry conditions for 2008 are highlighted by groundwater elevations for LW4 (Figure 2) which fell below the lower end of the screened well between September, 2008 through January, 2009. Rainfall events during the summer in this watershed (June through September) are typically high-intensity, short-duration events associated with convective storms while storms in autumn (October–November), early winter (December) and spring (March–May) are generally long-duration, low intensity events associated with frontal systems. Based on our visual observations over the three-year period, autumn leaf-fall occurs over a 3–4 week period starting around the last week of October and extends into November. Spring leaf-out occurs over a 2–3 week period from late April to the middle of May. Based on measured data, streamflow discharge (Figure 2) is generally highest during spring (April–May) and lowest in late summer (September). Peak streamflow discharges are associated with high-intensity summer events and/or large events in late fall (December).

Of the eight selected events, the events of December 9, 2009 and September 30–October 1, 2010 produced the largest amounts of rainfall (Table 1). The rainfall yield for the event of September 30, 2010 (151 mm) amounted to 15% of the annual total for 2010. In terms of frequency, the rainfall amount for the September 30, 2010 event was equivalent to a 25-year, 24-h storm for this region (i.e., a return period of 25 years) [Ward and Trimble, 2004, Appendix C]. The specific stream discharge for this event increased 525 times from a pre-event value of 0.007 mm hr$^{-1}$ to a peak event value of 3.68 mm hr$^{-1}$. The summer events of July 27, 2008, July 31, 2009 and September 30, 2010 were the most intense (Table 1) while the winter events (December 9, 2009 and February 22–24, 2010) had the lowest intensity. Runoff ratios indicated that winter events yielded the largest amount of discharge per unit of rainfall (Table 1). The event of September 30, 2010 produced the highest peak discharge.

Figure 5. End-member mixing analysis (EMMA) mixing diagram indicating watershed sources and stream base flow values in U space.
Figure 6. EMMA mixing diagrams for each of the eight selected storm events indicating evolution of stream chemistry during the storm event (indicated by the arrow). The legend from Figure 5 applies to this figure.
3.2. Runoff Sources Identified From EMMA and $\delta^{18}O$-H$_2$O Data

All watershed sources and the stream water chemistry during base flow are displayed in the EMMA mixing diagram (or U-space) in Figure 5. The individual source values in Figure 5 were computed based on the average tracer concentrations for the study period. Stream base flow values were from 2008 to 09. Watershed sources were separated out in different quadrants of the EMMA diagram. The event water sources - rainfall, throughfall, and litter leachate displayed a large range in concentrations along a linear plane extending from quadrants I to IV. In contrast, the groundwater runoff sources - seep, riparian groundwater, hyporheic water, and deep groundwater were tightly grouped in a very small region in quadrants II and III. Saturated and unsaturated soil water and shallow groundwater occupied an intermediate region in the U-space. The close clustering of groundwater sources suggested that the water chemistry for these sources was very similar. Most of the stream base flow values were in the immediate vicinity of groundwater sources, suggesting that stream water during base flow was chemically similar to groundwater and was some mixture of seep, hyporheic, riparian and deep groundwaters.

The within-event evolution of stream water chemistry for each of the eight storm events is reported in Figure 6. Here, watershed source concentrations (other than rainfall, throughfall, and litter leachate) were determined by taking the average of source concentrations measured immediately before and after the storm event (i.e., an average of grab sampling data preceding and following the event). This approach was followed to allow for seasonal/temporal variation in the chemistry of watershed sources. Rainfall, throughfall and litter leachate values, however, corresponded with the individual storms. Litter leachate values were not included in the plots in Figure 6; instead, the coordinate values for litter were listed in Figure 6 so as to allow a closer view of the stream water hysterisis patterns during the event. The watershed sources enclosed stream water chemistry from all sides, highlighting that there was a continuum of multiple sources that contributed to stream runoff.

The EMMA patterns for the eight events revealed some interesting similarities as well as differences. The summer events (July 27, 2008; July 31, 2009; and September 30, 2010) had the largest and most open hysteresis loops, while the winter events (especially February 22-24, 2010) displayed tight linear hysteresis patterns. Large, open loops, while the winter events (especially February 22-24, 2010) displayed tight linear hysteresis patterns. Large, open loops would suggest a greater diversity of runoff sources and a larger change in runoff source contributions; whereas tight, linear loops indicate fewer runoff sources. For both the July 27 and 31 events, stream water evolved in a counterclockwise hysteresis loop starting in the vicinity of the seep groundwater end-member and moving sharply toward the throughfall and/or rainfall end-member, followed by a shift toward soil water (for July 27) or litter leachate (for July 31), and then a return toward seep or hyporheic water on the hydrograph recession limb. Interestingly, the initial shift of stream water toward rainfall/throughfall is also replicated by $\delta^{18}O$ data for the two events (Figure 7), where event water contributions reached a maximum earlier in the event.

The winter events also displayed a pronounced directional shift toward rainfall/throughfall end-members. This directional shift likely indicates a strong expression of rainfall/throughfall in runoff waters during the winter events. This may have been facilitated by the wet, saturated...
catchment conditions at this time (Figure 2 and Table 1). While rainfall or throughfall data (throughfall in winter was similar to rainfall chemistry since there was no forest canopy) was not available for the event of February 22–24, 2010, the snow value (snow sample collected prior to event) in the U-space diagram suggests that snowmelt could have been a likely end-member for this event. It is interesting to note that the linear shift of stream water chemistry away from the seep end-member for the snowmelt event of February 22–24 was much smaller compared to the other winter event (December 9). This suggests that seep groundwater contributed a large proportion of runoff for the February 22–24, 2010 event. The spring event of May 20, 2008 also revealed a counter-clockwise hysteresis loop with stream water evolving from the seep/riparian/hyporheic groundwater end-members toward throughfall and then returning back to the groundwater sources. Compared to the summer events, the hysteresis loop for the spring event was much tighter and smaller.

In comparison to the events described above, the hysteresis loops for both the autumn events (October 25, 2008 and October 28, 2009) were furthest away from the groundwater sources at the start of the event and displayed a more clustered pattern. Although the event of October 25 produced a clockwise hysteresis loop, the evolution in stream chemistry during the event was not pronounced, and a strong influence of any particular end-member was not apparent. In comparison, the October 28 event yielded a clearer counterclockwise loop with a shift in stream chemistry toward throughfall, followed by a return toward the seep end-member.

The large late summer event of September 30–October 1, 2010 was composed of two separate sub-events which generated very different hysteresis loops. The first sub-event (E1, Figure 6) produced a counterclockwise loop with stream water originating in the immediate vicinity of the seep and riparian groundwater sources and then shifting in a direction somewhere between the throughfall and the litter leachate end-members. This was followed by a shift toward soilwater and a return to groundwater sources. The second sub-event (E2) displayed a very pronounced shift in stream water chemistry toward throughfall followed by a return to groundwater sources.

Overall, these EMMA plots suggest that: (a) stream water was very similar to seep, riparian, or hyporheic groundwater sources at the start of the event; (b) throughfall/rainfall contributed to runoff on the rising limb of the hydrograph; (c) litter leachate contributions followed those from throughfall; (d) soil or shallow groundwater sources likely made up the recession limb of the stream discharge hydrograph; and (e) the summer events (e.g., July 31, 2009) had the largest runoff contributions from surficial watershed sources (indicated by the strong shifts toward these sources).

Data on groundwater elevations (LW3 and LW4, Figure 8) from the valley-bottom wetlands (note well

![Figure 8. Streamflow discharge (Q, mm/hr) and groundwater elevations below the soil surface (m) for wells LW3 (cross) and LW4 (empty squares) for the events of July 27, 2008, July 31, 2009 and December 9, 2009.](image)

**Table 2.** Mean, Standard Deviation, Maximum and Minimum Values of Dissolved Organic Matter (DOM) Concentrations and Quality Indices for Stream Base Flow for the 2008–09 Study Period

<table>
<thead>
<tr>
<th>DON (mg L⁻¹)</th>
<th>DOC (mg L⁻¹)</th>
<th>a₂₅₄ (m⁻¹)</th>
<th>SUVA (L mg C⁻¹ m⁻¹)</th>
<th>HIX</th>
<th>FI</th>
<th>% Protein-Like Fluorescence</th>
<th>Sᵣ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>38</td>
<td>38</td>
<td>31</td>
<td>29</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Mean</td>
<td>0.12</td>
<td>1.67</td>
<td>11</td>
<td>3.35</td>
<td>0.84</td>
<td>1.41</td>
<td>5.2</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.08</td>
<td>0.70</td>
<td>4.2</td>
<td>1.17</td>
<td>0.04</td>
<td>0.04</td>
<td>1.49</td>
</tr>
<tr>
<td>Max</td>
<td>0.38</td>
<td>3.88</td>
<td>23</td>
<td>8.13</td>
<td>0.93</td>
<td>1.49</td>
<td>8.14</td>
</tr>
<tr>
<td>Min</td>
<td>0.0</td>
<td>0.85</td>
<td>4.6</td>
<td>2.0</td>
<td>0.75</td>
<td>1.35</td>
<td>3.09</td>
</tr>
</tbody>
</table>
Figure 9. DOM concentrations and quality indices (SUVA, $a_{254}$, HIX, FI, % protein-like fluorescence, and $S_{IX}$) for the events of May 20, 2008 and July 27, 2008.
locations in Figure 1) revealed a slow rise and peak in groundwater following the discharge peak followed by a very slow decrease in groundwater levels through hydrograph recession. This delayed response of groundwater elevations and the elevated values through recession would tend to support the delayed contributions of soil and shallow groundwaters to stream runoff as indicated by EMMA.

### 3.3. DOM in Stream Water During Base Flow

The DOM concentrations and optical characteristics of stream water during base flow (2008–09) are presented in Table 2. The DOC and DON concentrations were fairly low and averaged 1.67 (±0.7) (mg L⁻¹) and 0.12 (±0.08) (mg L⁻¹), respectively. These values were lower than those for surficial sources such as throughfall, litter, and soil water (Figure 3). The $a_{254}$, SUVA and HIX values for base flow averaged 11 (±4.2), 3.35 (±1.17), and 0.84 (±0.04), respectively. These values were again lower than the surficial sources, but greater than the corresponding values for seep, riparian and deep groundwaters (Figure 4). The FI and % protein-like fluorescence for base flow averaged 1.41 (±0.04) and 5.2 (±1.49), respectively and were lower than those for groundwater sources. The value for $S_R$ for base flow averaged at 0.93 (±0.18), over the study period. Among all

![Figure 10. DOM concentrations and quality indices (SUVA, $a_{254}$, HIX, FI, % protein-like fluorescence, and $S_R$) for the events of October 25, 2008 and July 31, 2009.](image-url)
watershed sources, the mean DOM values for hyporheic water (Figures 3–4) were generally closest to those observed for stream base flow.

3.4. Temporal Patterns of DOM Constituents in Stream Water During Storm Events

The within-event patterns for DOM constituents for the eight storm events are presented in Figures 9–12 while the flow-weighted averages for the events are reported in Figure 13. These plots revealed some important similarities as well as key differences for DOM constituents among the events. Except for the event of October 28, 2009, DOC concentrations typically peaked at or after the discharge peak and gradually decreased through hydrograph recession. Although the concentrations for DON were more variable, they generally followed the same pattern as DOC. DOC concentrations for the eight events ranged between 1 and 15 mgC L$^{-1}$; while the corresponding values for DON were 0.02–0.69 mgN L$^{-1}$. Maximum flow-weighted DOC and DON concentrations were observed for the summer event of July 31, 2009 while the lowest values were recorded for the winter snowmelt event of February 22–24, 2010. The storm event DOC concentrations were much greater than the 2-year base flow average of 1.67 mgC L$^{-1}$.

The values for $a_{254}$ followed a trend similar to DOC with an increase in values, a maximum at or after the discharge peak and gradually decreased through hydrograph recession. Although the concentrations for DON were more variable, they generally followed the same pattern as DOC. DOC concentrations for the eight events ranged between 1 and 15 mgC L$^{-1}$; while the corresponding values for DON were 0.02–0.69 mgN L$^{-1}$. Maximum flow-weighted DOC and DON concentrations were observed for the summer event of July 31, 2009 while the lowest values were recorded for the winter snowmelt event of February 22–24, 2010. The storm event DOC concentrations were much greater than the 2-year base flow average of 1.67 mgC L$^{-1}$.

Figure 11. DOM concentrations and quality indices (SUVA, $a_{254}$, HIX, FI, % protein-like fluorescence, and $S_F$) for the events of October 28, 2009 and December 9, 2009.
peak, followed by a decline through event recession. The only event where both DOC and \( a_{254} \) peaked on the rising limb of the hydrograph was the event of October 28, 2009. One other interesting response that was observed for DOC, DON, as well as \( a_{254} \) was the slight dilution of these values at peak streamflow discharge for the very large event of September 30, 2010 (Figure 12). The values for \( a_{254} \) varied from less than 10 to a high of 125 m\(^{-1}\). Similar to DOC, \( a_{254} \) values were highest for the summer and fall events and lowest for the winter events. The flow-weighted \( a_{254} \) value for the event of February 22–24 came closest to the base flow average of 12 m\(^{-1}\) (Figure 12). In contrast to the consistent trends for \( a_{254} \), the temporal patterns of SUVA varied considerably across events. For some events, SUVA values increased on the hydrograph rising limb and peaked before the discharge peak, while for others the peak in SUVA occurred after the discharge peak. The range of SUVA values observed for the events was from less than 2 to 6 L mgC\(^{-1}\) m\(^{-1}\).

The values for HIX, a measure of humic DOM, displayed a fairly consistent trend with an increase on the rising limb of the hydrograph, a maximum at or after the discharge peak, and then a very slow decrease through hydrograph recession. The slow decrease in HIX during streamflow recession was especially interesting compared to the relatively quicker drop in \( a_{254} \) values. For most events, values for HIX did not decline back to their pre-event levels.

**Figure 12.** DOM concentrations and quality indices (SUVA, \( a_{254} \), HIX, FI, % protein-like fluorescence, and \( S_R \)) for the events of February 22–24, 2010 and September 30–October 1, 2010.
Interestingly, the temporal pattern of HIX during storm events was very similar to the temporal pattern for groundwater elevations in the valley-bottom saturated areas (Figure 8). HIX values ranged from 0.71 to 0.92 across the eight events and flow-weighted HIX values were highest for the three summer events and lowest for the winter event of February 22–24, 2010. The base flow average HIX value of 0.84 was more than the flow-weighted event value for the snowmelt event and equal to that for the fall event of October 25, 2008.

Not surprisingly, FI values followed a dilution trajectory, but there were differences among the individual events regarding when FI values reached a minimum. For all events except July 31, 2009, FI values reached a minimum at or after the discharge peak. FI values for the events varied between 1.32–1.48. Flow-weighted event values for FI were lower than the base flow average of 1.41. Similar to FI, the storm-event temporal patterns for % of protein-like fluorescence also followed a dilution trajectory. In general, the protein-like fluorescence also declined through the events and reached a minimum on the recession limb; however, there were two exceptions. First, for some events (e.g., July 31 and December 9, 2009) a small, sharp increase in protein-like fluorescence was observed at the outset of the event. Second, for the first sub-event of September 30, 2010, there was a much larger, sustained increase in the

**Figure 13.** Discharge-weighted values of DOM concentrations and quality indices for the eight selected storm events. Base flow averages (for the 2008–2009 period) are indicated by the dashed line.
protein-like fluorescence which peaked close to the peak in streamflow discharge. This response for the first sub-event of September 30 was in sharp contrast to that for subsequent sub-event which yielded a dilution trend with much lower values of the protein-like DOM fluorescence (Figure 12). Flow-weighted averages for the % protein-like fluorescence indicated that except for the events of October 25 and February 22–24, all other event averages were below the base flow average of 5.2%.

[37] The % protein-like DOM fluorescence has been found to strongly correlate with the % bioavailable DOM derived from incubation assays [Fellman et al., 2009]. To investigate if the mass of % protein-like fluorescence followed the same pattern as the % protein values we determined the temporal pattern for protein mass. The mass of proteins (mg) was computed by multiplying the % protein-like fluorescence with the corresponding values of DOC concentration (mg/L) and the discharge volume (L). We recognize that this may not be the most ideal approach since – (a) we do not know whether the protein-like fluorescence is associated with DOC or DON; (b) the fluorescent DOM fraction typically makes up only a small fraction of the bulk DOM pool; and (c) protein-like DOM may constitute an even smaller fraction of this fluorescent DOM pool. However, it does provide an approximate estimate of the mass of protein-like DOM moieties. The mass value for the protein-like DOM for the event of July 27, 2008 is presented in Figure 14. In contrast to the dilution pattern in % protein-like fluorescence, the mass of protein fluorescence displayed an increase during storm events. Similar patterns were also observed for the other events.

[38] Temporal patterns for S_R were not consistent across the eight events. Overall, there was a dilution trend, with a minimum in S_R on the recession limb of the discharge hydrograph. Since S_R is inversely proportional to molecular weight of DOM, this would suggest that the larger and heavier DOM molecules were being released into stream runoff during hydrograph recession. However, S_R values also displayed sharp increases at the outset of some of the events (July 31, October 28, December 13, and first sub-event of September 30). This sharp increase suggests that some of the low molecular weight DOM constituents were being preferentially flushed out at the start of the events.

Interestingly, both the fall events of October 25, 2008 and 28, 2009 yielded the highest S_R values (0.91 and 0.94, respectively) while the summer event of July 31 produced the lowest value of S_R (0.74). Base flow values for the two-year period for S_R averaged 0.93.

4. Discussion

4.1. Sources, Flowpaths, and Quality of DOM During Base Flow and Differences With Stormflow

[39] Compared to storm events, base flow DOM was less aromatic and humic and had greater proportion of protein-like fluorescence and microbial character (as indicated by FI). Furthermore, based on S_R, DOM in base flow was of lower molecular weight compared to that for storm events. Our EMMA results revealed that base flow runoff was primarily composed of some mixture of groundwater sources that included seep, hyporheic water and/or riparian groundwaters. Our previous work [Inamdar et al., 2011, Figures 3–4] has shown that DOM in groundwater sources was low in humic and aromatic content but had elevated proportions of protein-like fluorescence and microbial DOM. Thus, the EMMA contributions for groundwater sources determined from this study taken together with the DOM character for groundwater sources does explain the low humic and more % protein-like DOM character of base flow observed in our catchment.

[40] Our results agree with previous work by Balcarczyk et al. [2009] who reported elevated % of protein-like fluorescence for groundwater seeps and suggested that these sources could make up a large fraction of the protein-like fluorescence observed in stream water during base flow conditions. Similarly, O’Donnell et al. [2010] compared the DOM quality for blackwater, glacial and clear water streams in Alaska and found that glacial and clear water streams that drained deeper groundwater runoff had lower DOC concentrations and aromatic content. The lower content of aromatic/humic fractions of DOM for base flow and/or for deeper hydrologic flow paths has been ascribed to the preferential removal of aromatic, humic or hydrophobic fractions by sorption and/or alteration of DOM by microbial processes [Qualls and Haines, 1991; Kaiser and Zech, 1998; Ussiri and Johnson, 2004].

4.2. Quality, Sources, and Hydrologic Flowpaths for DOM During Storm Events

[41] The concentrations for both DOC and DON observed in this study increased noticeably during storm events. This increase is not new and has already been reported in many previous studies [Hinton et al., 1997; Inamdar and Mitchell, 2007; Raymond and Saiers, 2010]. Temporal patterns for both DOC and DON were also fairly similar which is consistent with previous studies [Buffam et al., 2001; Hagedorn et al., 2000]. Interestingly however, the similarity in the temporal patterns for DOC and DON in this study was unlike our previous observations [Inamdar and Mitchell, 2007], where we found that DON concentrations consistently increased and peaked before DOC. Unlike this unglaciated Piedmont watershed site, our previous work was conducted in a glaciated, forested watershed in western New York. While the two sites are very different in many aspects including climate, topography, geology (base-rich glaciated
NY site versus un-glaciated site), soils, and vegetation type, currently, we do not have a specific explanation for the differences in DOC and DON patterns for these two sites. DOM quality was not characterized at the western New York site.

The within-event data on DOM quality revealed broad, consistent patterns across the events as well as some key differences among the DOM constituents. Overall, results for $a_{254}$, SUVA, and HIX showed that the humic and aromatic contents of DOM increased dramatically during storms with a maximum in the vicinity of the discharge peak. Previous studies have also reported storm-event increases of aromatic and humic DOM constituents but with much more data on SUVA compared to $a_{254}$ or HIX [Fellman et al., 2009; Hernes et al., 2008; Hood et al., 2006; Nguyen et al., 2010; Saraceno et al., 2009; Vidon et al., 2008]. Vidon et al. [2008] reported an increase in SUVA during storm events in an agricultural watershed and attributed it to increased inputs of DOM from near-surface soil. Work by Nguyen et al. [2010] in a mixed-land use (agriculture, forest, developed land) watershed in South Korea revealed that SUVA values peaked after the peak in streamflow discharge and remained elevated through hydrograph recession. For this same study, the peak in HIX values coincided with the peak in streamflow discharge [Nguyen et al., 2010]. Overall, the range of SUVA values (0.75–8.5) observed in our study are similar to those reported for previous storm-event studies [e.g., Hood et al., 2006; Vidon et al., 2008]. While values for $a_{254}$ have not been reported as often for storms, values for surface and groundwater samples in a watershed in Florida ranged from 17 to 58 [Chen et al., 2010]. HIX values in our study (0.80–0.92) were based on the normalized equation proposed by Olino [2002], while values from the non-normalized version were reported by Nguyen et al. [2010].

Our EMMA observations indicated that throughfall, litter leachate and soilwater were important surficial sources for runoff and that the contributions from these sources occurred in a specific order during the events. For most events, throughfall and litter leachate contributions occurred on the hydrograph rising limb, while litter leachate and soilwater composed the early part of the recession limb. The humic and aromatic content of these surficial sources was also high (Figures 3–4). Thus EMMA-derived contributions from these sources, combined with the elevated humic/aromatic content for these sources, can explain the increase in humic/aromatic character of stream water during storms.

It is interesting to note here that while the values for $a_{254}$ decreased rapidly on the hydrograph recession limb, the corresponding decrease in HIX values was much more gradual. This difference suggests that while both $a_{254}$ and HIX may be derived from surficial sources, there are subtle differences in their sources, release mechanisms and/or kinetics. The steady decline in HIX during recession was similar to the decline in groundwater elevations (Figure 8) for the valley bottom saturated areas. Based on this similarity, we hypothesize that groundwater elevations and their rise into surficial soil layers may have a role in influencing the release of humic DOM material (characterized by HIX) to stream runoff.

Contrary to the pattern of humic and aromatic components of DOM, the values for FI and % protein-like fluorescence displayed an overall dilution pattern during storm events. A dilution pattern for FI has been reported for previous studies [Hood et al., 2006; Nguyen et al., 2010]. FI values for the Kyungan River watershed in South Korea [Nguyen et al., 2010] decreased from a high of 2.06 to a minimum of 1.70 at peak discharge, followed by a recovery during hydrograph recession. The FI values reported by Nguyen et al. [2010] were however much greater than ours and more in the range of the DOM characterized as “microbial” or of autochthonous origin (1.6 to 2.0) [McKnight et al., 2001]. FI values measured in our study were considerably lower (1.32–1.48) and fell in the range of FI that has been characterized as “terrestrial” or of allochthonous origin (1.2–1.5) [McKnight et al., 2001; Mladenov et al. 2005]. A decrease in protein-like fluorescence was also observed by Nguyen et al. [2010], with the minimum protein content coinciding with the peak in streamflow discharge. This led Nguyen et al. [2010] to suggest that DOM produced during storm events was low in % protein-like fluorescence.

At our study site, the values for FI and % protein-like fluorescence were lowest in surficial sources and highest in groundwater sources (Figures 3–4). Thus, the increase in runoff contributions from surficial sources and the corresponding decrease in the proportion of groundwater contributions during events (as indicated by EMMA) would also explain the dilution patterns for FI and % protein-like fluorescence observed for stream runoff. Nguyen et al. [2010] also hypothesized that the decline in protein-like fluorescence during storm events was likely influenced by the input of protein-poor soil-derived DOM. On the other hand, Fellman et al. [2009] attributed the storm-event decrease in % protein-like fluorescence to the dilution of autochthonous production of amino acids by allochthonous runoff sources that were low in protein-like fluorescence. While dilution of protein-rich autochthonous DOM could be a possibility, our EMMA results point toward the dilution of protein-rich groundwater as a more likely explanation.

Fellman et al. [2009] also found that the percentage of proteins was strongly correlated with the % of bioavailable DOM (BDOM) characterized by incubation assays. Furthermore, they also reported that while both % protein-like fluorescence and % BDOM declined during storms for an upland watershed, the absolute amount (or total mass) of BDOM increased. While we did not directly measure BDOM through incubations assays, our data on % protein-like fluorescence (Figures 9–12) and mass of protein-like fluorescence (Figure 14) suggests that while the % protein-like fluorescence may decline during events the mass of protein-like DOM may actually increase during storm events. Thus, storm events could contribute substantially to total BDOM exports from catchments. A similar conclusion was reached by Wiegener et al. [2009], who measured both % BDOM and total BDOM (via incubation assays) for four storm events in a forested watershed in Hawaii. The key point here is that if % protein-like fluorescence is used as a proxy for BDOM, both % as well as the mass of protein-like DOM needs to be reported for storm events.

Data on $S$R indicated that storm events enhanced the exports of high-molecular weight DOM in stream runoff, especially on the early portion of hydrograph recession. Following EMMA and the temporal pattern of humic and aromatic constituents, we attribute this increase in DOM...
molecular weight to the contributions of humic and aromatic constituents from surficial sources; especially litter leachate and soilwater. A few of the events, however (e.g., October 28 and July 31, 2009), produced a quick decrease in molecular weight (sharp rise in \( S_R \)) at the very outset of the event. This early decrease in DOM molecular weight (rise in \( S_R \)) was likely associated with an initial flush of DOM from throughfall. This behavior was however not consistently observed across events.

[49] The increase in the molecular weight of DOM during events has been reported previously. Nguyen et al. [2010] directly measured the molecular weight of DOM (not \( S_R \)) and found that the molecular weight of DOM increased from 1100 Da to ~1500 Da at peak discharge and then declined to 1140 Da toward the end of the event. While Spencer et al. [2010] did not sample individual storm events they did use \( S_R \) as a proxy for molecular weight of DOM and found that \( S_R \) values were lowest following a post-dry flushing period in a tropical rain forest watershed in the Congo Basin of Africa. They attributed this response to the leaching of organic-rich layers containing elevated amounts of aromatic and high molecular-weight DOM moieties. The range of \( S_R \) values recorded by Spencer et al. [2010] (0.79–1.06) are similar to those observed in our current study (0.73 to 1.0).

4.3. Differences in the Patterns and Quantity of DOM Among Storm Events and the Responsible Mechanisms

[50] High-intensity summer events (e.g., July 27, 31, and September 30) produced the highest concentrations of humic/aromatic DOM, whereas low-intensity, long-duration events from winter (e.g., February 22–24) were low in humic DOM but high in % protein–like fluorescence. The events for spring were generally within these two extremes. The events that yielded the highest DOM concentrations and humic fractions also displayed the largest shifts toward the surficial end-members in EMMA space. The intensity of these rainfall events was one potential factor that likely resulted in the elevated contributions of runoff from surficial sources. Thus, hydrolcic flowpaths or runoff sources had a significant role in shaping the storm-event DOM response. These findings have important implications for the seasonal expression of DOM in stream runoff, suggesting that hydrologic flowpaths may play a critical role in regulating the seasonal patterns of DOM. Obviously, biotic factors such as the mineralization/production of DOM that vary with seasons could also influence the seasonal expression of DOM. We explore the seasonal patterns of DOM in greater detail in our subsequent manuscript (Inamdar et al., in preparation) where we include the complete event (all 27 events) and base flow data set for stream water as well as DOM for watershed sources.

[51] Another unique difference observed among the events was the response for % protein–like fluorescence. The events of October 25, 2008 and the long snowmelt event of February 22–24, 2010 produced the highest flow-weighted values for the % protein–like fluorescence. Following Fellman et al. [2009], the elevated protein–like fluorescence for these events would suggest a greater % of bioavailable DOM in runoff waters. Interestingly, the event of February 22–24 occurred during the wet, cold winter season while the event of October 25 followed a very dry summer period. We attribute the elevated flow-weighted protein fluorescence for the winter event to the large proportion of protein-rich groundwater that constituted stream runoff for this event. The significance of groundwater sources for this event is evident from the EMMA diagram which indicated a minimal shift away from the seep groundwater end-member.

[52] The same rationale however cannot be extended to explain the elevated protein–like DOM for the event of October 25, 2008 (Figure 13) since the stream chemistry in EMMA space was already at some distance away from the groundwater end-members at the start of the event. This initial separation from groundwater sources is especially surprising considering that the catchment was extremely dry prior to the event, and one would have expected stream water chemistry to be similar to the chemistry of groundwater sources. The event of October 25, 2008 was however the first substantial event to occur in 2008 after autumn leaffall had started. Previous work has clearly shown that DOM from freshly fallen autumn leaves may be considerably more labile than DOM from other watershed sources [McDowell, 1985; Qualls et al., 1991]. Our fluorescence analyses of DOM extracts from freshly fallen autumn leaves for this catchment (unpublished data, 2011) revealed very high % protein–like fluorescence of 27%, 28%, and 31% for maple, beech and poplar leaves, respectively. Thus, it is very likely that autumn leaf input and the leaching of labile DOM from the leaves may have contributed to the elevated protein–like fluorescence observed for the October 25, 2008 autumn event. While the same elevated protein–like response was not observed for the October 28, 2009 event (Figure 13), a preceding event of October 17–18, 2009 (not included here) did reveal high % protein–like fluorescence values. Thus, the timing of the event with respect to autumn leaf fall may also be a factor in influencing the expression of protein–rich DOM in runoff (e.g., first-flush event versus subsequent events).

[53] In a very atypical pattern for protein–like fluorescence, the first sub–event of September 30, 2010 displayed a sustained increase in the protein–like fluorescence through the event. This increase was unlike the dilution patterns observed for the other events and even different from the second subsequent sub–event for September 30, 2010. The EMMA diagrams for the sub–events also differed considerably, with the second sub–event displaying a much stronger shift toward throughfall. We hypothesize that the increase in protein–like fluorescence during the first sub–event could be attributed to a combination of two factors—minimal dilution of stream runoff by protein-poor event water (rainfall and/or throughfall) and accumulation/production of protein-rich labile DOM in catchment soils prior to the event. The event of September 30 occurred following a dry summer period when the tributary at ST2 had completely dried up and the catchment was thus hydrologically fragmented and disconnected. These extreme conditions would have required a substantial “wetting-up” phase before hydrologic connectivity was restored (ST2 started flowing) and event water was expressed in stream runoff. Simultaneously, long dry periods have been known to promote breakdown of organic matter in surficial soils and yield labile organic matter fractions [Borken and Matzner, 2008]. We speculate that such breakdown and production of labile organic matter may have occurred, especially, in the stream
sediments of the dried-up tributary or in the immediate vicinity in the riparian soils. With the arrival of the pulse of rainfall associated with the first sub-event, the labile DOM from these locations was then likely flushed out with runoff waters and resulted in the sustained increase in % protein-like fluorescence in stream water. The hydrologic dis- and re-connection and the sudden and large expression of protein-like fluorescence in runoff waters could be considered as a hydrologic and biogeochemical “hot moment” as defined by McClain et al. [2003].

[54] Dramatic changes in DOM quality following drought and hydrologic disconnection has also been recently reported by Vazquez et al. [2010]. However, unlike the increase in % protein-like fluorescence as in our case, Vazquez et al. [2010] reported an increase in FI values as the fluvial network became more fragmented and attributed this increase in FI to in situ algal or microbial production in isolated pools of stream water. Our data did not reveal an increase in FI, rather, FI values followed dilution trajectories for both September 30 sub-events. This suggests that while autochthonous production could be happening at our site, the large terrestrial/allochthonous inputs during the event likely shaped the FI response for the event.

[55] With hydrologic connectivity restored, the second, larger, pulse of rainfall associated with the second sub-event of September 30, 2010 resulted in substantial contribution of event water (throughfall) to runoff yielding a dilution pattern in the protein-like fluorescence for stream water. As a matter, the event water contribution at peak discharge was so high that the DOC, DON concentrations and the \(a_{254}\) values indicated a slight depression. However, surprisingly, the same response (depression in values) was not observed for the HIX or SUVA values, further underscoring the subtle but important differences in the kinetics or source pools for the DOM constituents.

[56] It should also be noted that the storm event of September 30–October 1, 2010 occurred at the tail end of a dry summer and the impending arrival of autumn. It is well recognized that summer droughts and associated stress can trigger early autumnal leaf fall [Kozlowski et al., 1991]. Our visual observations in the catchment indicated some early loss of leaves (just prior to September 30, 2010) and their accumulation on the forest floor and in the streams. Thus, in addition to the hydrologic factors (dry soils and hydrologic disconnection), input of leaves on the forest floor and leaching of labile DOM may also be a factor in elevated protein-like response for the first subevent of September 30.

[57] The response of DOM for the extreme events of February 22–24 and September 30, 2010 is especially significant considering the predicted future changes in climate. The Intergovernmental Panel on Climate Change [IPCC, 2007] and other climate studies [Bender et al., 2010] predict that future Atlantic hurricanes and associated storms will become more intense with longer intervening periods of dry weather or droughts. Considering the response of protein-like fluorescence for the first sub-event of September 30, one would then expect that such events would yield greater amounts of labile or bioavailable DOM with runoff. Exports of labile DOM with catchment runoff could have significant implications for downstream aquatic ecosystems and/or coastal water bodies such as the Chesapeake Bay which are already vulnerable to nutrient inputs. Increased runoff along with bioavailable DOM could further exacerbate the density stratification and hypoxic conditions of these sensitive coastal ecosystems [Rabalais et al., 2009].

5. Conclusions

[58] Combining UV and fluorescence metrics and hydrologic flowpaths derived from EMMA for a large data set of storm events this study provided new insights into the patterns and sources of DOM during storm events. Key conclusions that can be derived from this work are:

[59] 1. DOM quality differed dramatically between base flow and stormflow conditions. DOM during storm events was more humic and aromatic while that during base flow had a greater % of protein-like fluorescence and DOM of lower molecular weight. This shift in DOM was attributed to greater runoff contributions from surficial flowpaths and sources including throughfall, litter leachate and near-surface soil water. Base flow was regulated by groundwater sources such as seep, hyporheic water and riparian groundwater.

[60] 2. EMMA indicated a specific sequencing in runoff contributions from watershed sources during storm events. This sequencing of runoff sources played a significant role in shaping the temporal expression of DOM constituents during storm runoff.

[61] 3. While the humic and aromatic DOM constituents were both derived from surficial sources, subtle differences in the temporal patterns of \(a_{254}\) and HIX suggested that there may be important differences in the kinetics of these DOM moieties.

[62] 4. Hydrologic flowpaths appeared to have a significant role in influencing the seasonal storm-event expression of DOM. Summer events that displayed the largest shifts toward surficial runoff sources also produced the highest peak values in aromatic and humic DOM while corresponding responses for the winter events were muted.

[63] 5. This study especially highlighted the value of studying multiple storm events across a range of hydrologic and seasonal conditions. Large events that followed summer droughts produced complex DOM responses that were not typically observed for other events during the year.

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