SPECTRO-FLUOROMETRIC CHARACTERIZATION OF DISSOLVED ORGANIC MATTER (DOM) IN A MID-ATLANTIC WATERSHED

by

Shatrughan Singh

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Geology

Summer 2013

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by

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# TABLE OF CONTENTS

LIST OF TABLES ........................................................................................................... vii
LIST OF FIGURES .......................................................................................................... ix
ABSTRACT .................................................................................................................... xiii

Chapter

1 INTRODUCTION ........................................................................................................ 1

2 METHODS .................................................................................................................. 5
   2.1 Site description ..................................................................................................... 5
   2.2 Watershed sampling ............................................................................................ 8
   2.3 Climate and discharge data ................................................................................ 9
   2.4 UV-Vis and fluorescence spectroscopy ............................................................... 9
   2.5 DOM characterization using FH and CM PARAFAC models ......................... 12
   2.6 Comparison of the two PARAFAC models ..................................................... 17
   2.7 PARAFAC Modeling ......................................................................................... 18
   2.8 EMMA Analyses ............................................................................................... 21
   2.9 Statistical Analyses ............................................................................................ 22
   2.10 Data and Principal Component Analyses ....................................................... 23

3 COMPARISON OF TWO MODELS OF DISSOLVED ORGANIC MATTER FLUORESCENCE FOR A MID-ATLANTIC FORESTED WATERSHED IN THE USA .......... 24
   3.1 Introduction ....................................................................................................... 24
   3.2 Results .............................................................................................................. 27
      3.2.1 Components for the Fair Hill (FH) PARAFAC model ....................... 27
      3.2.2 FH PARAFAC components for watershed sources ....................... 29
      3.2.3 DOM Concentrations and metrics for watershed sources ............. 31
      3.2.4 Comparison of the FH and CM models for humic- and protein-like components ............................................................... 32
      3.2.5 Discriminant analyses for watershed sources using FH and CM PARAFAC models ......................................................... 36
      3.2.6 Correlation between PARAFAC model components and DOM parameters ................................................................. 38
5.3 Discussion........................................................................................................... 112
  5.3.1 Seasonal DOM patterns for watershed sources ............................. 112
  5.3.2 Seasonal DOM patterns in stream runoff ................................. 114

5.4 Conclusions ........................................................................................................... 125

6 OVERALL CONCLUSIONS AND RECOMMENDATIONS .................... 127

REFERENCES ............................................................................................................. 129
LIST OF TABLES

Table 2.1: Catchment characteristics and topographic attributes are presented here for drainage locations in this study.................................................................7

Table 2.2: Specific methods used in each chapter in this thesis are described here. ..........................................................................................................................7

Table 2.3: Seasonal distribution of hydro-climatic variables for the four year (2008-2011) study period is shown here. Data is shown as Mean ± Std Err. ..................................................................................................................10

Table 2.4: Descriptions of the six PARAFAC identified components for the EEM dataset of Fair Hill site and their comparison with previously reported studies......................................................................................................15

Table 2.5: Characteristics of the seven distinct PARAFAC components identified in this study and similar components reported in previous studies........20

Table 3.1: Correlations between DOM Composition (%Humic-Like or %Protein-Like; derived from FH and CM model; see text for details) with measured DOM concentrations (DOC and DON) and DOM metrics (derived from UV and fluorescence measurements) across watershed sources. .....................................................................................39

Table 3.2: Mean (standard deviation) values for the changes in humic- and protein-like DOM composition between FH and CM model across watershed sources in this study. .................................................................44

Table 4.1: Univariate statistical analyses for seven parameters (DOC, % Humic-Like, % Fulvic-Like, % Protein-Like, HIX, FI, and a254) during base flow (five drainage locations) and storm flow (three drainage locations) conditions.........................................................................................56

Table 5.1: Seasonal distributions of DOM concentration (i.e., DOC), and optical parameters (DOM metrics from UV and fluorescence measurements) for stream water during base flow and storm flow are shown for a four year (2008-2011) study period in the mid-Atlantic forested catchment. 98
Table 5.2: Seasonal distributions of DOM concentration (i.e., DOC), and optical parameters (DOM metrics from UV and fluorescence measurements) for each watershed sources are shown for a four year (2008-2011) study period in the mid-Atlantic forested catchment. .................................. 103
LIST OF FIGURES

Figure 2.1: Location of the study site in the mid-Atlantic Piedmont region of northeastern Maryland (inset indicated by filled circle) and sampling locations are marked within the 12 ha forested watershed…………………………… 6

Figure 2.2: Location of the study site in the Piedmont region of Maryland, USA (inset) and sampling locations at multiple catchment scales along the stream drainage network in the study watershed…………………………… 10

Figure 2.3: Spectral positions of excitation and emission loadings maxima derived from the six-component PARAFAC model using split-half validation technique is given in Table 2.4. Solid blue and red lines represent excitation and emission loadings for site-specific FH model. Dashed magenta and black lines represent excitation and emission loadings for a random half of the complete dataset used for model validation. Broken blue and red lines represent excitation and emission loadings for CM model. EEM contour plots of six different fluorescent components identified by the site-specific PARAFAC (FH) model are shown in corresponding insets. ……………………………………………………… 14

Figure 2.4: Spectral positions of excitation and emission loadings maxima derived from the seven-component PARAFAC model using split-half validation technique is given in Table 2.5. Solid blue and red lines represent excitation and emission loadings. Dashed black and magenta lines represent excitation and emission loadings for a random half of the complete dataset used for model validation. EEM contour plots of seven different fluorescent components identified by the PARAFAC model are shown in the corresponding insets…………………………………… 19

Figure 3.1: Spatial distributions of each PARAFAC component (a) FH1, (b) FH2, (c) FH3, (d) FH4, (e) FH5, and (f) FH6 over the two year study period (2008-2009) across watershed compartments. Black lines in the box-plot represent median values and red filled circles represent mean values connected with broken red lines……………………………………… 30
Figure 3.2: Spatial distributions of DOM quantity and quality parameters (a) DOC, (b) DON, (c) $a_{254}$, (d) HIX, and (e) FI over the two year study period (2008-2009) across watershed compartments. Black lines in the box-plot represent median values and red filled circles represent mean values connected with broken red lines. (a), (b), and (c) are log-scaled data for y-axis to capture distinctiveness in data. ........................................ 33

Figure 3.3: Distributions of (a) %Humic-like, (c) %Protein-like DOM composition derived using site-specific PARAFAC (FH model) across watershed sources are shown. Sources represented with same letters are not significantly different. Results for the same using CM model are shown in (b) and (d) respectively. Standard deviation from means is shown using error bars................................................................. 35

Figure 3.4: Scatter plot of discriminant function analysis showing separation of watershed sources for humic-like and protein-like DOM components based on (a) FH model, and (b) CM model results. The extension of biplot rays indicates the strength of correlation between the measured variables................................................................. 37

Figure 4.1: (a) EMMA mixing diagram indicating all watershed sources, (b) stream chemistry for multiple catchments during base flow, and (c) storm flow conditions, over a three year period (2008-10). ......................... 51

Figure 4.2: (a) Dissolved organic carbon (DOC), (b) %Humic-like (HL), (c) %Fulvic-like (FL), (d) %Protein-like (PL), (e) Humification Indices (HIX), (f) Fluorescence Indices (FI), and (f) absorption coefficients at 254 nm, $a_{254}$, for sampled watershed sources. Median base flow (blue lines) and storm flow (red lines) values for corresponding parameters at 12 ha stream outlet is shown for comparison purposes. ......................... 53

Figure 4.3: (a) Dissolved organic carbon (DOC), (b) %Humic-like (HL), (c) %Fulvic-like (FL), (d) %Protein-like (PL), (e) Humification Indices (HIX), (f) Fluorescence Indices (FI), and (f) absorption coefficients at 254 nm, $a_{254}$, for stream waters at multiple drainage locations (five locations) during base flow. Drainage locations are plotted with increasing catchment area from left to right. Same letters in box plots represent no significant differences among catchments for corresponding variable. ........................................................................ 55
Figure 4.4: (a) Dissolved organic carbon (DOC), (b) %Humic-like (HL), (c) %Fulvic-like (FL), (d) %Protein-like (PL), (e) Humification Indices (HIX), (f) Fluorescence Indices (FI), and (f) absorption coefficients at 254 nm, a_{254}, for stream waters at multiple drainage locations (three locations) during storm flow. Drainage locations are plotted with increasing catchment area from left to right. Same letters in box plots represent no significant differences among catchments for corresponding variable. ................................................................. 58

Figure 4.5: PCA plots of (a) loadings, and (b) scores for stream waters at five catchments during base flow. PCA plots of (c) loadings, and (d) scores for stream waters at three catchments during storm flow. .................. 60

Figure 4.6: DOM concentrations and quality indices (DOC, %PL, HIX, FI, and a_{254}) for the events of July 31, 2009 and December 9, 2009 for the two catchment locations ST6 (red symbols and lines) and ST3 (blue symbols and lines). Solid lines represent discharge at corresponding catchments. ................................................................. 63

Figure 4.7: DOM concentrations and quality indices (DOC, %PL, HIX, FI, and a_{254}) for the events of September 30 – October 1, 2010 and August 14, 2011 for the two catchment locations ST3 (blue symbols and lines) and ST12 (green symbols). Solid blue line represents discharge at ST3 catchment. ................................................................. 67

Figure 5.1: Distribution of (a) average daily air temperature, and total daily precipitation (black lines and dark grey bars respectively), and (b) mean daily specific discharge (dark grey lines), are shown for the four study period in the study watershed. Dashed lines represent separation between years. Red cross and blue empty circles represent storm flow and base flow DOC sampling at the 12 ha catchment outlet.............. 94

Figure 5.2: Distributions of mean (a) DOC, (b) a_{254}, (c) HIX, (d) %Humic-like, (e) %Fulvic-like, and (f) %Protein-like DOM composition, for base flow and event flow samples across seasons are shown here. Same alphabets indicate no significant differences for each source (base flow or event flow). Note the changes in y-scale. ......................... 97

Figure 5.3: Mean seasonal distributions of PCA scores and loadings for (a) base flow, and (b) storm flow are presented here. Green represent Spring, red represent Summer, brown represent Fall, and blue represent Winter season. ................................................................. 100
Figure 5.4: Mean seasonal distributions of PCA scores and loadings for (a) throughfall (TF), and (b) litter leachate (LT) are presented here. Green represent Spring, red represent Summer, brown represent Fall, and blue represent Winter season.

Figure 5.5: Mean seasonal distributions of PCA scores and loadings for (a) wetland soil water (WSW), and (b) shallow ground water (SGW) are presented here. Green represent Spring, red represent Summer, brown represent Fall, and blue represent Winter season.

Figure 5.6: Mean seasonal distributions of PCA scores and loadings for (a) seep water (P), and (b) deep ground water (DGW) are presented here. Green represent Spring, red represent Summer, brown represent Fall, and blue represent Winter season.

Figure 5.7: Distributions of mean (a) DOC, (b) a254, (c) HIX, (d) %Humic-like, (e) %Fulvic-like, and (f) %Protein-like DOM composition, for litter leachate (LT) and deep ground water (DGW) samples across seasons are shown here. Same alphabets indicate no significant differences for each source (LT or DGW). Note the changes and breaks in y-scale.

Figure 5.8: Seasonal distribution of PCA scores and loadings for (a) Litter leachate, and (b) deep ground water samples are presented here. Legend for season is same as shown in previous PCA plots. Here all data points are used to plot PCA scores. Specific data in litter samples during storms show seasonal hot moment (in Fall) as labeled.

Figure 5.9: Seasonal distribution of PCA scores and loadings for (a) base flow, and (b) storm flow samples are presented here. Legend for season is same as shown in previous PCA plots. Here all data points are used to plot PCA scores. Specific data in storm flow samples during events show seasonal hot moment (in Fall) as labeled.

Figure 5.10: Relationships between specific discharge rate and (a) DOC concentration, (b) humic-like DOM, and (c) protein-like DOM composition for selected storm events are presented here.
ABSTRACT

Spatial and seasonal pattern of dissolved organic matter (DOM) was characterized using a unique combination of hydrologic flow path analyses and spectro-fluorometric tools. The study was conducted over a four year (2008-11) period in a headwater forested watershed located in the Piedmont region of the mid-Atlantic USA. Sampling was conducted for stream water and variety of watershed sources which include throughfall, litter, soil waters, riparian, shallow, and deep ground waters during base flow and storm flow conditions. Ultraviolet and fluorescence measurements were performed to generate excitation-emission matrices (EEMs). A site specific parallel factor analyses (PARAFAC) EEMs model for DOM was developed and compared against a generic model. Discriminant analyses indicated that the site-specific model was more sensitive to subtle differences in DOM and was able to provide a greater level of differentiation in DOM among the watershed sources. This allowed better insights into how DOM evolved as it moved through the watershed. Spatial analyses indicated that DOM derived from catchment with increased wetland coverage was more humic and aromatic. No influence of drainage area was present in base flow DOM response. Attenuated DOM response during storm flow conditions with drainage area was observed. Water flow paths appeared to be key drivers in shaping DOM response in storm conditions. Seasonal patterns of DOM were evaluated for stream water during base flow and storm flow conditions and for multiple watershed sources. Overall, seasonal pattern in stream water DOM was more pronounced for storm flow versus base flow. Similarly among watershed sources,

xiii
seasonality in DOM sources was more apparent in surficial versus groundwater DOM sources. This pattern confirmed our primary hypothesis that sorption of DOM (to mineral soil surfaces) along deeper hydrologic flow paths damped the seasonal DOM signal. Seasonal “hot” moments such as autumn leaf fall appeared to have a strong influence on seasonal DOM patterns that was apparent in storm runoff occurring immediately following autumn leaf inputs. In conclusion, this study clearly demonstrated the value and benefits of using a suite of optical indices to characterize DOM.
Chapter 1

INTRODUCTION

Dissolved organic matter (DOM) has important environmental implications for terrestrial and aquatic ecosystems. DOM plays an active role in altering chemical and physical properties of aquatic systems and in turn influencing the biogeochemistry of the global carbon cycle [Coble, 2007]. For example, it serves as an energy and nutrient source to microbes and bacteria and forms the basis of the microbial food web [Moran et al., 2000]. DOM has also been found as a major transporter for relocation of nutrients (N and P) in forest ecosystems [Qualls and Haines, 1991]. Nitrogen components of DOM may become bioavailable and stimulate algal growth or eutrophication in an estuarine water bodies such as the Chesapeake Bay [Paerl et al., 2006; Seitzinger et al., 2002]. DOM is an important variable for ecological processes and water quality in forest ecosystem as its characteristics reflect the properties of flora and fauna in the watershed [Fellman et al., 2010a; Sanderman et al., 2009].

Dissolved organic matter (DOM) is a heterogeneous mixture of organic compounds ranging from simple carbohydrates to highly complex aromatic chains of organic moieties [Thurman, 1985]. To date, most research focus in watersheds studies has been on simply determining the DOM quantity or concentrations in runoff waters [Holbrook et al., 2006; Williams et al., 2010]. However, few studies have characterized the composition or constituents of DOM such as carbohydrates, amino acids, polysaccharides, humic and fulvic acids [Ågren et al., 2008; Fellman et al., 2008, 2009c; Mariot et al., 2007; Michel et al., 2006]. These constituents are
especially critical for the transport, processing, and bioavailability of DOM. The presence or absence of these constituents can dictate the sorption of DOM onto clays or mineral oxides [Kalbitz and Kaiser, 2008], microbial breakdown or consumption, and photolysis of DOM [McKnight et al., 2003; Sweeney et al., 2004]. Some of the constituents are preferentially removed from solution (e.g., hydrophobic and/or aromatic compounds) while others persist in solution (e.g., hydrophilic or carbohydrates) [Qualls and Haines, 1991]. Similarly, amino-acids and carbohydrates are especially susceptible to microbial breakdown or consumption [Yamashita and Tanoue, 2003]. Thus, understanding and characterizing the composition of DOM is critical for furthering our understanding of DOM in watersheds.

Characterizing DOM 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 ultra-violet (UV) absorbance [Weishaar et al., 2003] and fluorometric methods [Fellman et al., 2010a; Jaffé et al., 2008] allow for rapid characterization of DOM quality using small sample amounts (~ 4 mL) and can be especially useful for characterizing the quality of DOM for the large number of samples generated from watershed studies. Three dimensional excitation-emission matrices (EEMs) of fluorescence measurements provide information about DOM quality and quantity in terms of its source, age, compositional structure and bioavailability [Fellman et al., 2009b, 2010b; Jaffé et al., 2008; Kaushal and Lewis, 2005] and indices derived from these optical methods can also serve as tracers for identifying the sources and flow paths for DOM in watersheds [Hood et al., 2006]. Some of the optical indices that have recently been used include: specific ultra-violet absorbance (SUVA, [Weishaar et al., 2003]), spectral slope ratio
(SR, [Helms et al., 2008; Spencer et al., 2010]), humification index (HIX, [Ohno, 2002; Zsolnay et al., 1999]), fluorescence index (FI, [Cory and McKnight, 2005]), and % protein-like components [Fellman et al., 2009a] derived from fluorescence-based excitation emission matrices (EEMs, [Cory and McKnight, 2005; McKnight et al., 2001]). While the understanding of optical indices for characterizing DOM composition is new and promising, its use is still in its infancy, and much research still needs to be conducted to allow for reliable assessments of DOM. This research builds on this need by using a suite of spectro-fluorometric analyses to characterize the spatial and seasonal patterns of DOM in a forested study site. The study was conducted in 12 and 79 ha forested watersheds located in the Piedmont region of Maryland. Sampling was performed over a four year period (2008-2011) and included stream water during base flow and storm flow and 10 different watershed sources of DOM. In addition to recording DOC concentrations, a new, site-specific, parallel factor analysis (PARAFAC) model was developed using fluorescence-based EEMs.

Key questions that were addressed were:

- **Question 1:** Can a site- or region-specific fluorescence model (PARAFAC) be developed to characterize the quality of DOM in forested watersheds of the mid-Atlantic USA? What advantages does this region-specific model have over the more general models being currently adopted? What additional insights does this model provide on DOM quality?

- **Question 2:** How does DOM concentration and composition vary spatially along the catchment drainage network? What are the key controls and factors that dictate the spatial variability of DOM?

- **Question 3:** How does DOM vary seasonally for stream water and other watershed DOM sources? How do hydrologic flow paths shape the seasonal expression of DOM?
Each of these questions has been addressed individually in separate chapters and which will be submitted as separate manuscripts for publication. Chapter two provides an overview of the study site and the specific methods that were used in watershed monitoring, sample collection, and DOM analyses. These methods were common to all of the three studies that were conducted. Chapter three describes the comparison of a site-specific PARAFAC model versus a generic model for DOM characterization in a mid-Atlantic forested watershed. Chapter four describes the spatial variation of DOM along a drainage network which extends from the headwater locations of the 12 ha catchment to the outlet of the 79 ha watershed. The role of runoff sources and mixing proportions is investigated in shaping the spatial composition and concentrations of DOM. Seasonal patterns in stream water and watershed DOM sources are explored in chapter five. Finally, chapter six provides overall conclusions and recommendations from this research.
Chapter 2

METHODS

2.1 Site description

The study watershed (12 ha) is located within the Fair Hill Natural Resources Management Area (NRMA) (39°42´N, 75°50´W) in Cecil County, MD (Figure 2.1) and is a part of the Big Elk Creek drainage basin which lies within the Piedmont physiographic region. Big Elk Creek eventually drains into the Chesapeake Bay. Cecil County has a humid, continental climate with well-defined seasons and mean annual rainfall of 1231 mm [Maryland State Climatologist Office, 2008]. The summer months are generally hot and humid while the winter months are colder. Daily mean temperature ranges from -0.6°C (winter) to 24°C (summer) and the average annual temperature is 12.2°C. The study watershed is predominantly forest cover with deciduous canopy dominated by Fagus grandifolia (American beech), Liriodendron tulipifera (Yellow poplar), and Acer rubrum (Red maple) species [Van Stan II and Levia Jr., 2010] while the understory is dominated by Greenbrier (Smilax spp.) and multiflower rose (Rosa multiflora). The study watershed is primarily underlain by Wissahickon formation comprised of metamorphosed crystalline sedimentary and igneous rocks including mica-rich schist, amphibolites, and gneiss. The soils are coarse loamy, mixed, mesic Lithic Dystrudepts, Oxyaquic Dystrudepts, with sub-horizons indicating seasonal water saturation. The soils belong to Glenelg series consisting of deep, well-drained, nearly level to moderately steep soils.
Figure 2.1: Location of the study site in the mid-Atlantic Piedmont region of northeastern Maryland (inset indicated by filled circle) and sampling locations are marked within the 12 ha forested watershed.
More details of the study site can be found elsewhere [Inamdar et al., 2011, 2012, 2013; Levia et al., 2010; Van Stan II and Levia Jr., 2010]. Other catchment attributes are given in the Table 2.1.

Table 2.1:  Catchment characteristics and topographic attributes are presented here for drainage locations in this study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Catchment Locations (in increasing areal extent)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>ST5</td>
</tr>
<tr>
<td>Drainage area (ha)</td>
<td>0.62</td>
</tr>
<tr>
<td>Topographic index – range and mean in brackets</td>
<td>3.9-11.7</td>
</tr>
<tr>
<td></td>
<td>(6.60)</td>
</tr>
<tr>
<td>Elevation (meters) – range and mean in brackets</td>
<td>82-103 (93)</td>
</tr>
<tr>
<td>Slope gradient (degrees) – range and mean in brackets</td>
<td>0.37-20.6 (6.8)</td>
</tr>
</tbody>
</table>

The following sections describe the methods used in the thesis chapters according to the table given below (Table 2.2).

Table 2.2:  Specific methods used in each chapter in this thesis are described here.

<table>
<thead>
<tr>
<th>Methods (Sections numbers)</th>
<th>Methods used in chapters</th>
</tr>
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<tbody>
<tr>
<td>2.2</td>
<td>Chapters 3-5</td>
</tr>
<tr>
<td>2.3</td>
<td>Chapter 5</td>
</tr>
<tr>
<td>2.4</td>
<td>Chapters 3-5</td>
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<tr>
<td>2.5</td>
<td>Chapter 3</td>
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<td>Chapter 3</td>
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<tr>
<td>2.7</td>
<td>Chapters 4-5</td>
</tr>
<tr>
<td>2.8</td>
<td>Chapter 4</td>
</tr>
<tr>
<td>2.9</td>
<td>Chapters 3 (except PCA) and 4</td>
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<tr>
<td>2.10</td>
<td>Chapter 5</td>
</tr>
</tbody>
</table>
2.2 Watershed sampling

Watershed sampling was performed during base flow (1-3 times a month) as well as storm events. Manual grab sampling during base flow over a four-year period (2008-11) was conducted for multiple watershed locations which included: stream (ST), seeps (P), hyporheic zone (HY), wetland soil water (WSW), shallow (SGW) and deep (DGW) ground waters, soil pore water (U), riparian ground water (RGW). However, only two-year (2008-09) data was used in the chapter three for comparison of DOM models between a site-specific and a generic model. Storm samples were regularly collected using automated ISCO sampler during the events for stream water (at the 12 ha watershed outlet), forest floor (or litter leachate, LT), throughfall (TF), and rainfall (R) (Figure 2.1). Water sampling for base flow and storm flow has also been carried out at five and three outlet locations, respectively differing in the drainage areas and other catchment properties (e.g., topography and slope gradient) (Figure 2.2 and Table 2.1) for chapter four. For spatial DOM analyses data for three years (2009-2011) was used, whereas for the seasonal patterns of DOM (chapter five) data from all four years was included. A total of 1770 samples were collected for four year (2008-2011) time period that includes 974 base flow and 796 events samples for total of 43 storm events spanned over four years. However for chapter five, 33 storm events are used for further analysis after dropping some small events. Automated ISCO sampling for stream water during storm events was conducted at ST3 (2008-2011), ST6 (2008-2009) and ST12 (2010-2012). Additional details on storm sampling is provided in Inamdar et al. (2011, 2012).

All water samples were filtered through a 0.45 micron filter paper (Millipore Inc.) within 24 hours of collection and stored at 4°C for further analyses. Dissolved organic carbon (DOC) analysis for the water samples was conducted at the
biogeochemistry laboratory of SUNY-ESF, NY using the Tekmar-Dohrmann Phoenix 8000 TOC analyzer. DOC is reported in mg L\(^{-1}\) unless stated otherwise in this study. Nitrate-N was determined using a Dionex IC; NH\(_4^+\) with an autoanalyzer using the Berthelot Reaction followed by colorimetric analysis; and total dissolved nitrogen (TDN) using the persulfate oxidation procedure [Ameel et al., 1993] followed by colorimetric analysis on an autoanalyzer. Dissolved organic nitrogen (DON) concentrations were computed as the difference between TDN and inorganic N (NO\(_3^-\), NH\(_4^+\)).

2.3 Climate and discharge data

Precipitation and air temperature data was available at 5-minute frequency from a nearby automatic weather station located in the Fairhill NRMA, about 1000 m from the outlet of the 12 ha catchment. Streamflow discharge was monitored at the outlet of the 12 ha catchment (Figure 2.1) using a 6-inch Parshall flume and water flow depths were recorded every 20 minutes using a Global Water (Inc.) logger and pressure transducer as described by Inamdar et al. (2013). The monthly data were organized into four climatological seasons: Winter (December, January, and February); Spring (March, April, and May); Summer (June, July, August, and September); and Autumn (October, and November). Seasonal averages of temperature, precipitation, and discharge were calculated for four year study period (Table 2.3).

2.4 UV-Vis and fluorescence spectroscopy

Absorption spectra (190-1100 nm) were obtained for each sample at room temperature at 1-nm intervals using a UVmini-1240 (Shimadzu Inc.) single-beam
Figure 2.2: Location of the study site in the Piedmont region of Maryland, USA (inset) and sampling locations at multiple catchment scales along the stream drainage network in the study watershed.

Table 2.3: Seasonal distribution of hydro-climatic variables for the four year (2008-2011) study period is shown here. Data is shown as Mean ± Std Err.

<table>
<thead>
<tr>
<th>Season</th>
<th>Temperature (°C)</th>
<th>Precipitation (mm)</th>
<th>Discharge (mm hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>10.7 ± 0.4</td>
<td>282.4 ± 13.6</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Summer</td>
<td>20.5 ± 2.4</td>
<td>478.4 ± 23.0</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>Fall</td>
<td>8.5 ± 2.4</td>
<td>176.9 ± 49.8</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>Winter</td>
<td>-0.6 ± 2.4</td>
<td>243.4 ± 42.1</td>
<td>0.06 ± 0.01</td>
</tr>
</tbody>
</table>
spectrophotometer equipped with a 1 cm path-length quartz cuvette (volume of ~4 ml). The instrument was set up and corrected for scattering and baseline fluctuations after running particle free Nanopure Milli-Q (18.2 MΩ) water on daily basis prior running water samples.

Water samples were treated in a manner similar to absorption measurements. To account for the inner filter effects (IFEs), samples reflecting absorbance greater than 0.2 at 254 nm ($A_{254} \geq 0.2$) were diluted with particle free Nanopure Milli-Q water (also used as a blank). Excitation-emission matrices (EEMs) were generated using a Horiba Jobin Yvon Fluoromax-3P spectrofluorometer equipped with a 150 W ozone-free Xenon arc lamp. The spectrofluorometer was set to collect the signal in ratio mode (S/R mode) with dark offsets using a 5 nm bandpass on the excitation as well as emission monochromators. Factory supplied correction factors were applied to the scans to correct for instrument configuration. The EEM spectra were recorded for excitation spectra from 240 to 450 nm at every 10 nm intervals while the emission spectra ranged between 300 - 550 nm, with data saved for every 2 nm over an integration time of 0.25s. Absorption corrections were applied to account for inner filter effects in “Blank” and sample EEMs. Then, corrected Milli-Q water (Blank) EEMs were subtracted from the sample EEMs to eliminate any influence of Raman peaks. Subsequently, EEMs were normalized to daily-determined water Raman integrated area under maximum fluorescence intensity (350 ex/397 em, 5 nm bandpass) as suggested by Lawaetz and Stedmon (2009). Using this approach, EEMs data were normalized to a comparable Raman Units (R.U.) which in turn is quantitatively independent from any instrumental parameters provided spectrally corrected data used. Finally, the EEMs were multiplied with dilution factor (if samples
were diluted) to obtain the fluorescence intensity for the original undiluted sample [Moran et al., 2000]. Consequently, the corrected EEMs were exported in MATLAB® for pre-model run steps.

2.5 DOM characterization using FH and CM PARAFAC models

Following EEMs exported in MATLAB®, a pre-model step involves a mean centering across the samples to reduce any offsets [Bro and Smilde, 2003]. Then, each EEM scan were normalized to 1.0 by dividing the whole EEM by the maximum recorded fluorescence intensity value for the sample to ensure that no samples dominated the PARAFAC analysis [Westerhoff et al., 2001]. Following the procedures of EEM corrections and normalizations, data were fit to a 13-component PARAFAC model developed previously using 379 samples across the wide range of aquatic environments [Cory and McKnight, 2005]. Residual peaks were less than 10% after fitting EEMs to the 13-component model, confirming that EEMs obtained in this study were well fit to the CM model [Mladenov et al., 2008].

The site-specific FH model was developed using the DOMFluor toolbox (ver. 1.7; Feb. 2009) developed for MATLAB® by Colin Stedmon (NIAR, Technical University of Denmark, Denmark). Based on the Stedmon and Bro (2008) study, PARAFAC constraints, such as non-negativity, and model initialization values derived from singular value decomposition (SVD) were used. The PARAFAC model development was initiated using an EEMs dataset of 747 samples with 121 emission and 22 excitations wavelengths without any assumptions on the number of components (or distinct fluorophores), shape of the resulting spectra, or structure of noise [Stedmon and Bro, 2008]. The number of components (i.e., model validation) was achieved by split-half analysis and by the visual analysis of residuals and
corresponding component loadings [Stedmon and Bro, 2008; Stedmon et al., 2003].

Following this methodology, six components were identified for the dataset with some unexplained variability remaining in the residuals (Figure 2.3). A seven (and eight) component PARAFAC model was rejected as they could not be validated using split-half and random initialization techniques. Further, a close similarity in the PARAFAC excitation-emission loadings obtained for a single split-half against the whole dataset suggests that the selection of the six-component model in this study is justified (Figure 2.3). High scattering Raman and Rayleigh bands were set as missing data and subsequently were removed to avoid any influence by these values in the final dataset according to reported studies [Stedmon and Bro, 2008; Stedmon et al., 2003].

PARAFAC component scores as \( F_{\text{max}} \) are reported for each water sample collected in this EEM dataset. We applied leverage and loading techniques [Stedmon and Bro, 2008] to identify the outliers before a final PARAFAC model. After close inspection of outliers in samples and in excitation-emission loadings, 33 samples, one emission wavelength (300 nm), and the first three excitation wavelengths (240, 250, and 260 nm) were discarded. At the end, final PARAFAC model was developed on the EEM dataset containing 714 samples, 62 emissions, and 19 excitation wavelengths to derive six validated individual components (Figure 2.3). While the PARAFAC model was built using 714 samples collected for storm flow and base flow over a 2-year period, here we present results from 367 samples that include the base flow sampling and storm-event samples for litter leachate and throughfall.

 Similar to the CM model, the \% contributions of the individual PARAFAC components for the FH model were determined by dividing each component \( F_{\text{max}} \) score by the sum of the total fluorescence intensity (sum of \( F_{\text{max}} \) scores of all
Figure 2.3: Spectral positions of excitation and emission loadings maxima derived from the six-component PARAFAC model using split-half validation technique is given in Table 2.4. Solid blue and red lines represent excitation and emission loadings for site-specific FH model. Dashed magenta and black lines represent excitation and emission loadings for a random half of the complete dataset used for model validation. Broken blue and red lines represent excitation and emission loadings for CM model. EEM contour plots of six different fluorescent components identified by the site-specific PARAFAC (FH) model are shown in corresponding insets.
Table 2.4: Descriptions of the six PARAFAC identified components for the EEM dataset of Fair Hill site and their comparison with previously reported studies.

<table>
<thead>
<tr>
<th>Components (Ex/Em in nm)</th>
<th>Designated peaks/components in previous studies</th>
<th>Descriptions</th>
<th>Sources/Environments reported in previous studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH1 (&lt; 250 [330] / 460)</td>
<td>C2&lt;sup&gt;1&lt;/sup&gt;, A peak&lt;sup&gt;2&lt;/sup&gt;, C1&lt;sup&gt;4&lt;/sup&gt;, C1&lt;sup&gt;5&lt;/sup&gt;, C1&lt;sup&gt;6&lt;/sup&gt;, C1&lt;sup&gt;7&lt;/sup&gt;, C3&lt;sup&gt;8&lt;/sup&gt;, C1&lt;sup&gt;9&lt;/sup&gt;</td>
<td>Humic-like / Unknown / UVC-humic-like / Fulvic-like</td>
<td>Terrestrial and marine both (Coble 1996); both oxidized and reduced (Cory and McKnight 2005); River, estuarine and near-shore marine ecosystems; Common to wetlands/forest streams (Fellman et al. 2010b); Terrestrial humic origin (Singh et al. 2010); Terrestrially derived OM , highest concentration in forest streams and wetlands (Stedmon et al. 2003); Observed in bay coastal areas in surface waters, low at the bottom (Yamashita and Jaffé 2008)</td>
</tr>
<tr>
<td>FH2 (&lt; 250 [310] / 400)</td>
<td>C6&lt;sup&gt;1&lt;/sup&gt;, Q3&lt;sup&gt;4&lt;/sup&gt;, C4&lt;sup&gt;5&lt;/sup&gt;, C3&lt;sup&gt;6&lt;/sup&gt;, C4&lt;sup&gt;7&lt;/sup&gt;, C4&lt;sup&gt;8&lt;/sup&gt;, C3&lt;sup&gt;9&lt;/sup&gt;</td>
<td>Unknown – non - humic / Quinone-like (lawsone) / Humic-like</td>
<td>Microbial-origin, aliphatic C; oxidized environment (Cory and McKnight 2005); River, estuarine and near-shore marine ecosystems; aliphatic C (Fellman et al. 2010b); Labile matter; biological production in the water column (Singh et al. 2010); Combination of N and T peaks, autochthonous and biologically labile component (Yamashita and Jaffé 2008); Microbial origin (Yamashita et al. 2010)</td>
</tr>
<tr>
<td>FH3 &lt; 250 [410] / 512</td>
<td>C5&lt;sup&gt;1&lt;/sup&gt;, D region&lt;sup&gt;2&lt;/sup&gt;, SQ1&lt;sup&gt;4&lt;/sup&gt;, C3&lt;sup&gt;5&lt;/sup&gt;, C4&lt;sup&gt;6&lt;/sup&gt;, C2&lt;sup&gt;8&lt;/sup&gt;, C2&lt;sup&gt;9&lt;/sup&gt;</td>
<td>Soil-fulvic-like / Semi-quinone-like / Humic-like</td>
<td>Black Sea (Coble et al. 1990); aromatic C; reducing conditions; higher plant matter (Cory and McKnight 2005); River, estuarine and near-shore marine ecosystems; Higher aromatic content (Fellman et al. 2010b); Derived from agricultural catchments and exists in fresh water environments (Singh et al. 2010); Terrestrial origin, biogeochemical processing of terrestrial POM (Yamashita and Jaffé 2008)</td>
</tr>
<tr>
<td>FH4 &lt; 250 [370] / 460</td>
<td>C1(^1), C peak(^3), SQ2(^4), C2(^5), C2(^6), C3(^7), C5(^8), C4(^9)</td>
<td>Humic-like / Semi-quinone-like / Humic-like</td>
<td>Terrestrial and marine both (Coble 1996); microbial-origin; reducing conditions (Cory and McKnight 2005); River, estuarine and near-shore marine ecosystems; High molecular weight/aromatic (Fellman et al. 2010b); Terrestrial humic origin, related to terrestrial particulate organic matter (Singh et al. 2010); High molecular weight fraction of terrestrially derived humic matter, common to wide range of fresh water environments (Stedmon et al. 2003); Terrestrial origin (Yamashita and Jaffé 2008); Microbial humic (Yamashita et al. 2010)</td>
</tr>
<tr>
<td>FH5 (280 / 328)</td>
<td>C7(^1), T peak(^3), C8(^4), C7(^5), C5(^7), C7(^8), C5(^9)</td>
<td>Protein-like (tryptophan) / UVB protein like (tryptophan)</td>
<td>Terrestrial and marine both (Coble 1996); microbial-origin (Cory and McKnight 2005); River, estuarine and near-shore marine ecosystems; Free-Tryp-like (Fellman et al. 2010b); Combination of N &amp; T peaks, labile matter, autochthonous DOM (Stedmon et al. 2003); Tryptophan like, blue shifted from authentic tryptophan, autochthonous, biologically labile (Yamashita and Jaffé 2008); Freshly produced tryptophan like DOM (Yamashita et al. 2010)</td>
</tr>
<tr>
<td>FH6 (270 / 312)</td>
<td>B peak(^3), C13(^4), C8(^5), C8(^8)</td>
<td>Protein-like (tyrosine)</td>
<td>Terrestrial and marine both (Coble 1996); River, estuarine and near-shore marine ecosystems; Free-tyrosine-like (Fellman et al. 2010b); Tyrosine like, degradation processes important for dynamics of this component (Yamashita and Jaffé 2008)</td>
</tr>
</tbody>
</table>

* [Chen et al., 2010]\(^1\), [Coble et al., 1990]\(^2\), [Coble, 1996]\(^3\), [Cory and McKnight, 2005]\(^4\), [Fellman et al., 2010b]\(^5\), [Singh et al., 2010]\(^6\), [Stedmon et al., 2003]\(^7\), [Yamashita and Jaffé, 2008]\(^8\), [Yamashita et al., 2010]\(^9\).
components). To supplement our assessment of the PARAFAC components we also include the concentrations for DOC and DON and the values for selected DOM metrics such as $a_{254}$, HIX and FI. The $a_{254}$ was calculated using the absorbance values obtained from a one cm path-length quartz cuvette with a single beam Shimadzu UV-mini 1240 spectrophotometer (Shimadzu Inc.) and the equation given by Green and Blough (1994). Humification index (HIX) was calculated according to Ohno (2002) and provides a degree of humification in DOM samples (values ranging from 0 to 1). Fluorescence index (FI) was calculated using a ratio of fluorescence emission intensities computed at 470 and 520 nm with excitation intensity at 370 nm [Cory and McKnight, 2005]. This index has been used to differentiate between terrestrial (FI: 1.2 to 1.5) and microbial sources (FI: 1.6-2.0) [McKnight et al., 2001].

2.6 Comparison of the two PARAFAC models

The two PARAFAC models were compared using multiple approaches. In the first approach, we compared the sum of the two major fluorescing groups - % humic-like and % protein-like DOM from the two models for the watersheds sources. An ANOVA analysis was performed to determine the degree of differentiation among the watershed sources based on data from the PARAFAC models. Additionally, a discriminant function analysis was performed to identify whether watershed sources could be differentiated by their PARAFAC components. Sampling locations were selected as dependent variables whereas humic-like and protein-like DOM compositions were selected as independent variables for the discriminant analysis. Wilks’ lambda distribution was used for the forward stepwise selection of independent variables. Finally, we investigated the correlations (Pearson) between the sum of % humic- and % protein-like components against the DOC and DON concentrations and
the DOM metrics of \( a_{254} \), HIX and FI. The intent here was to determine if the strength of the correlations differed among the two models and how they varied for watershed sources. All statistical analysis was performed with MATLAB® 7.12 (MathWorks Inc., Natick, MA) and a JMP® 9.0 statistical software package (SAS Institute Inc., Cary, NC).

### 2.7 PARAFAC Modeling

Total 1343 EEM scans were used to develop a PARAFAC model. While initial modeling tests identified 5 samples, first three excitation and emission wavelengths as outliers. Subsequently, these were dropped from the dataset before final PARAFAC model run. EEMs used to develop this statistical (PARAFAC) model were not only limited to stream samples at considered drainage locations in this study, rather these EEMs spanned over a variety of catchment sources (e.g., litter leachate, throughfall, rainfall, soil pore waters, seep, wetland soil water, riparian, shallow, and deep ground waters during base flow and streams during both base flow and storm flow; for more description please see Inamdar et al., 2012) for three year study period (2008-2010). Following the development of this model, base flow (285) and storm flow samples (148 samples for 7 events) from the year 2011 were fitted to this model to test the model validity. The fractional contribution of the components identified using three year data were tested for 2011 sampling period. The residuals observed for 2011 data were less than 10% when fitted on this model indicating model captured much of the variation in the 2011 samples [Mladenov et al., 2008].

Total seven distinct fluorescence components were identified by the PARAFAC analysis over the entire dataset of 1338 EEMs (2008-2010), all of which previously have been described in literature (Figure 2.4and Table 2.5). Two of these
Figure 2.4: Spectral positions of excitation and emission loadings maxima derived from the seven-component PARAFAC model using split-half validation technique is given in Table 2.5. Solid blue and red lines represent excitation and emission loadings. Dashed black and magenta lines represent excitation and emission loadings for a random half of the complete dataset used for model validation. EEM contour plots of seven different fluorescent components identified by the PARAFAC model are shown in the corresponding insets.
Table 2.5: Characteristics of the seven distinct PARAFAC components identified in this study and similar components reported in previous studies.

<table>
<thead>
<tr>
<th>Components</th>
<th>Excitation (nm)</th>
<th>Emission (nm)</th>
<th>[Stedmon and Markager, 2005]</th>
<th>[Fellman et al., 2008]</th>
<th>[Balcarczyk et al., 2009]</th>
<th>[Yamashita et al., 2010]</th>
<th>[Mueller et al., 2011]</th>
<th>[Petrone et al., 2011]</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>300</td>
<td>400</td>
<td>C6</td>
<td>C7</td>
<td>C3</td>
<td>C1</td>
<td>C2</td>
<td>C7</td>
<td>Fulvic-like</td>
</tr>
<tr>
<td>C2</td>
<td>340</td>
<td>454</td>
<td>C3</td>
<td>C6</td>
<td>C1</td>
<td>C3</td>
<td>-</td>
<td>C5</td>
<td>Humic-like</td>
</tr>
<tr>
<td>C3</td>
<td>270 (370)</td>
<td>484</td>
<td>C1</td>
<td>C1</td>
<td>C2</td>
<td>-</td>
<td>C1</td>
<td>-</td>
<td>Humic-like</td>
</tr>
<tr>
<td>C4</td>
<td>280 (410)</td>
<td>522</td>
<td>C2</td>
<td>-</td>
<td>C4</td>
<td>C2</td>
<td>-</td>
<td>C2</td>
<td>Humic-like</td>
</tr>
<tr>
<td>C5</td>
<td>270 (380)</td>
<td>462</td>
<td>C4</td>
<td>C3</td>
<td>C6</td>
<td>-</td>
<td>C3</td>
<td>C1</td>
<td>Humic-like</td>
</tr>
<tr>
<td>C6</td>
<td>350</td>
<td>426</td>
<td>C5</td>
<td>-</td>
<td>C8</td>
<td>C4</td>
<td>-</td>
<td>-</td>
<td>Fulvic-like</td>
</tr>
<tr>
<td>C7</td>
<td>280</td>
<td>326</td>
<td>C8</td>
<td>C9</td>
<td>C9</td>
<td>C5</td>
<td>C4</td>
<td>C4</td>
<td>Protein-like (Tryptophan-like)</td>
</tr>
</tbody>
</table>

*In brackets, secondary peaks of excitation wavelengths are represented.
components (C1 and C6) have been associated to fulvic-like fluorophores, since their fluorescence resembles that of lower emission wavelengths (ex = 300 nm/em = 400 nm) and (ex = 350 nm/em = 426 nm). Four humic-like fluorophores located at ex = 340 nm/em = 454 nm, ex = 270/370 nm/em = 484 nm, ex = 280/410 nm/em = 522 nm, and ex = 270/380 nm/em = 462 nm for components C2, C3, C4 and C5 respectively have also been identified for this model, along with one protein-like component (C7; ex = 280 nm/em = 326 nm) spectrally resembling tryptophan-like product. These seven components of our PARAFAC model explained greater than 99% of the total variation in fluorescence EEM dataset. Thus, we conclude that this model is adequate to describe the variability in the DOM fluorescence spectra of these EEMs for various streams draining the forested catchment in this study. Further, following the approach of Fellman et al. (2009), these seven components were classified into three major fluorescing DOM fractions – humic-, fulvic-, and protein-like for simplification and are accordingly discussed. Also, these fractions are represented in relative percentage contribution of total DOM pool such as % Humic-Like (HL), % Fulvic-Like (FL), and % Protein-Like (PL) throughout this study.

2.8 EMMA Analyses

An end-member mixing analysis (EMMA) model to identify the water flow paths in the 12 ha catchment has already been developed. [Inamdar et al., 2011, 2013]. Data from this model was used to characterize the runoff mixing proportions for various stream drainage locations in the catchment. This spatial pattern in runoff mixing was used to evaluate the spatial pattern in DOM. Detailed methodology of performing EMMA analysis is explained in [Hooper, 2003]. EMMA allows identification of water sources or flow paths (end-members) to runoff based upon
water chemistry. For successful EMMA, solutes (or tracers) must (1) differ significantly between end-members, and (2) mix conservatively and linearly. Potential end-members are those water sources which reproduce the concentrations of selected solutes in runoff water, when mixed in appropriate proportions under various hydrological conditions [Hooper, 2003]. For this study, sodium (Na), calcium (Ca), total aluminum (Al), silica (Si), and dissolved organic carbon (DOC) were chosen as they displayed conservative, linear mixing at the temporal scale of the events [Inamdar et al., 2013]. All the potential end-members (e.g., precipitation, throughfall, litter, seep, groundwater, etc.) for the same tracers were normalized to stream waters tracer concentration and projected into EMMA space (or U-space). This approach facilitated to compare the stream water chemistry for base flow and storm events against the catchment sources and determine how the catchment sources influenced stream water chemistry. Further, this approach allowed us to do a comprehensive assessment of stream water chemistry with all potential end-members, especially during events [Inamdar et al., 2011]. However, we did not calculate the actual percent contributions to stream discharge attributable to each of the end-members due to the great variability in the characterization of the differing end members.

2.9 Statistical Analyses

One-way Analysis of Variance (ANOVA) was used for comparison of means as we have more than two groups (drainage locations) for both base flow and storm flow samples. To determine the significant differences among drainage locations, we employed post-hoc Tukey’s HSD (Honest Significant Difference) following ANOVA. Results are represented in box plots with median values displayed by solid lines in the box plots and 5% and 95% of the data is represented using whiskers. A principal
component analysis (PCA) was carried out to further explore the variations in DOM concentration and optical metrics and to determine the differences between drainage locations based on these properties during base flow and storm flow conditions to graphically represent the distribution of the samples from the studied drainage locations. All the statistical analyses were performed at significance level of $p$-value $< 0.05$ with a JMP® 9.0 statistical software package (SAS Institute Inc., Cary, NC).

2.10 Data and Principal Component Analyses

All the statistical analyses were performed on seven DOM parameters: DOC concentration, humic-, fulvic-, and protein-like DOM, HIX, FI, and $a_{254}$ obtained from fluorescence and absorption measurements using JMP® from the SAS Institute (Cary, NC). Statistical tests were considered significant at $p$-value $< 0.05$. One-way ANOVA was utilized to test for statistical differences in mean across seasons for each watershed source. Student’s t test was used for post hoc pairwise analysis at $\alpha = 0.05$ significance level. Prior to statistical analyses, DOC, protein-like DOM, and $a_{254}$ were log-transformed to achieve normality assumptions.

DOM concentration, absorption and fluorescence metrics for each watershed source were also evaluated by applying principle components analysis (PCA) to the data. While PCA was carried out on complete database for each watershed source across seasons, a single mean value for the first two principal components were obtained for each season. The first two principal components of the PCA analyses for each watershed sources generally explained the majority of the variance in sources database.
Chapter 3

COMPARISON OF TWO MODELS OF DISSOLVED ORGANIC MATTER FLUORESCENCE FOR A MID-ATLANTIC FORESTED WATERSHED IN THE USA

3.1 Introduction

Dissolved organic matter (DOM; typically < 0.45µm) is a heterogeneous mixture of aromatic and aliphatic organic compounds, ranging from proteins, carbohydrates, polysaccharides, and lipids, to humic and fulvic acids [Thurman, 1985]. In terrestrial and aquatic ecosystems, DOM not only influences the geochemical and photochemical reactions by participating in carbon (C) and nutrient (N, P, and S) cycles, but also controls microbially-mediated reactions by serving as potential substrate. It also plays a key role in transport and transformation of major contaminants and/or pollutants and their reactivity with the environment [Aiken et al., 2011; Yamashita and Jaffé, 2008]. Additionally, DOM exerts a strong control in the formation of disinfection byproducts (DBPs), for e.g., trihalomethanes (THMs) and haloacetic acids (HAAs), during the drinking water supply treatments with disinfectants [Kraus et al., 2008]. The amount and quality of DOM in terrestrial and aquatic environments influences biological processes such as microbial degradation [Moran et al., 2000; Williams et al., 2010]. It is also a key player in altering the depth of the photic zone in aquatic ecosystems by controlling the incident UV radiation [Wetzel, 2003]. Thus, DOM plays an ecologically important role in various biochemical and physical processes linking terrestrial and aquatic ecosystems.
Recently, the availability of optical measurement techniques such as ultraviolet (UV) absorption \cite{Bricaud1981,Weishaar2003} and fluorescence \cite{Coble1990,McKnight2001} have provided important insights into the character and composition of DOM. Some of the optical indices that have been implemented to characterize DOM quality include: specific ultraviolet absorbance (SUVA; \textcite{Weishaar2003}), absorption coefficient \cite{Hernes2008,Saraceno2009}; spectral slope ratio (SR; \textcite{Helms2008,Spencer2010}), humification index (HIX; \textcite{Ohno2002}), fluorescence index (FI; \textcite{Cory2005}), and % protein-like fluorescence \cite{Fellman2009} derived from absorption measurements and fluorescence-based excitation emission matrices (EEMs, \textcite{Cory2005,McKnight2001}). While UV absorption and fluorescence spectroscopy have provided important insights into DOM composition, these analyses also yield large amounts of data with high dimensionality and non-linearity. To process this data and gain meaningful insights a variety of multivariate statistical tools have been employed \cite{Stedmon2003}. \textcite{Boehme2004} examined the DOM fluorescence variability using EEMs coupled with principal component analysis (PCA). Likewise, parallel factor analysis (PARAFAC) in conjunction with fluorescence EEMs has been used to identify biogeochemically meaningful components of DOM \cite{Stedmon2003}. One fluorescent model adapted by the water science community is the Cory and McKnight model \cite{Cory2005}, which was developed using DOM from a wide range of aquatic environments.

Although the benefits of PARAFAC models to extract biogeochemical information from EEMs are indisputable, there is some uncertainty on whether a new, site-specific PARAFAC model should be developed or whether a previously-validated
model [Cory and McKnight, 2005] is adequate to characterize the variability of DOM at a given site. Fellman et al. (2009c) recently addressed this issue for a study site in Alaska where they compared a new, site-specific PARAFAC model against the Cory and McKnight model [Cory and McKnight, 2005] for EEMs derived from soil and stream waters. While they did not find any significant differences between the two PARAFAC models, they did observe that the site-specific model was more sensitive to particular DOM constituents. They found that the Cory and McKnight model [Cory and McKnight, 2005] was unable to characterize a humic-like component that was specific to soil waters, and that the site-specific model did a better job in characterizing the variability of the protein-like region of DOM. Similarly, Larsen et al. (2010) reported a limited resolving power of the Cory and McKnight model [Cory and McKnight, 2005] in characterizing protein-like and phenolic DOM fractions.

We recently characterized the composition of DOM for multiple watershed sources using UV absorption and fluorescence techniques in a 12 ha forested watershed located in the Piedmont region of Maryland [Inamdar et al., 2011, 2012]. Watershed DOM sources that were studied included throughfall, litter leachate, soil pore water, wetland soil water, hyporheic water, stream runoff, groundwater seeps, and riparian, shallow, and deep ground waters. EEMs for DOM samples from these watershed sources were fitted to the previously-validated Cory and McKnight [Cory and McKnight, 2005] PARAFAC model (hereafter referred to as the CM model). In addition, multiple spectro-fluorometric indices were also determined for the DOM samples which included: absorption coefficient at 254 nm (a254), SUVA at 254 nm (SUVA254), HIX, FI, and S_R. These indices revealed distinct patterns in DOM composition across watershed sources [Inamdar et al., 2012]. The surficial watershed
sources were rich in humic and aromatic DOM constituents while DOM in groundwater or along deeper flow paths was low in humic-like and high in protein-like DOM [Inamdar et al., 2012].

Using the same 714 EEMs collected from our field site, we developed a new, site-specific PARAFAC model (hereafter referred to as the FH model). Our overall objective here was to investigate how this new site-specific FH model compared with the CM model in characterizing the DOM composition at our site. Specific questions that we addressed were: (a) How does the new site-specific FH model characterize the DOM composition? (b) Is the site-specific FH model more sensitive than the CM model for characterizing DOM and does it allow for a greater differentiation among watershed sources? (c) If yes, which model components and watershed sources reveal the largest differences between the FH and CM models? The unique aspect of this study is the availability of strong data set on multiple and distinct watershed DOM sources to assess the differences between the two PARAFAC models.

3.2 Results

3.2.1 Components for the Fair Hill (FH) PARAFAC model

A total of six components were identified by the FH PARAFAC model. Component 1 (FH1) was comprised of a bi-modal excitation maximum peaks at 250 and 330 nm with an emission maximum peak at 460 nm (Figure 2.3a and Table 2.4). This component is identified as a humic-like in character and resembles a mixture of A and C peaks described by Coble (1996). The spectral characteristic of this component is close to the “unknown” component 1 identified by the CM model, despite having a red-shift of 10 nm in emission maximum obtained in our study. High
concentrations of this component (similar to FH1 component) have been reported by Stedmon et al. (2003) in forest streams and wetlands. Likewise, the abundance of this component was reported for wetlands and forest streams by Fellman et al. (2010b).

Component 2 (FH2) showed two peaks of excitation maxima at 250 and 310 nm having an emission maximum at 400 nm (Figure 2.3b and Table 2.4). This component is similar in spectral features to component 12 (Q3) of the CM model, which has a fluorescence comparable to oxidized quinone-like DOM moieties. Previous studies have reported the origin of this component due to the presence of biologically labile organic matter primarily rich in aliphatic carbon content (e.g., Table 2.4, Fellman et al., 2010b; Singh et al., 2010; Yamashita and Jaffé, 2008). Cory and McKnight (2005) suggested that the microbial origin of this component existed typically in oxidized environments whereas Yamashita et al. (2010) attributed the microbial origin to autochthonous production of biologically labile organic matter (Table 2.4).

Component 3 (FH3) is characterized by two excitation maximum peaks at 250 and 410 nm, respectively, with a well-defined emission peak at 512 nm (Figure 2.3c and Table 2.4). This is similar to the semi-Quinone (SQ1) component identified in the CM model. Yamashita and Jaffé (2008) attributed the abundance of this component (C2, Table 2.4) to terrestrial organic matter rich in humic content thus, indicating its origin from higher vascular plants. Component 4 (FH4; Figure 2.3d) had an emission maximum at 460 nm with a bi-modality in excitation wavelengths at 250 and 370 nm. This component is similar to ‘C’ peak in spectral characteristics mainly reported for organic matter of terrestrial origins [Coble, 1996]. Cory and McKnight (2005) reported this component as semi-quinone (SQ2) and attributed its origin to microbial activity in
reducing conditions (Table 2.4). This DOM fraction has also been reported as DOM of high molecular weight and high aromaticity derived mainly from terrestrial inputs [Fellman et al., 2010b].

Component 5 (FH5) and component 6 (FH6) possessed single excitation/emission peaks at 280/328 nm and 270/312 nm, respectively (Figure 2.3e and f; Table 2.4). These two components are comparable to C8 and C13 as reported in the CM model and are attributed to microbial origin. Components, C7 and C8 of the Alaska model of Fellman et al. (2010b) are similar to FH5 and FH6, respectively, and have been reported as ‘free’ tryptophan and tyrosine-like DOM moieties. These protein-like components could together be an indicator of DOM lability and bacterial production in the watershed [Hudson et al., 2007].

3.2.2 FH PARAFAC components for watershed sources

The distribution of FH PARAFAC components for DOM from various watershed sources at our site is illustrated in Figure 3.1(a-f). Median value of component FH1 was highest (0.42 R.U.) for litter leachate followed by wetland soil water (0.37 R.U.) and throughfall (0.36 R.U.) (Figure 3.1a). Riparian water (0.19 R.U.), seep (0.21 R.U.), and deep groundwater (0.14 R.U.) samples recorded the lowest median values for component FH1. The largest variability in FH1 values was observed for riparian, seep and deep groundwater sources. Overall, FH1 displayed a decreasing trend from surface to sub-surface watershed compartments (Figure 3.1a). Median values for component FH2 were highest in soil pore waters and seep (both, 0.30 R.U.), followed by hyporheic (0.29 R.U.), and stream (0.28 R.U.) water samples. However, wetland soil water, shallow, and riparian ground waters (all, 0.25 R.U.), recorded intermediate median values for the component FH2 (Figure 3.1b). The lowest
Figure 3.1: Spatial distributions of each PARAFAC component (a) FH1, (b) FH2, (c) FH3, (d) FH4, (e) FH5, and (f) FH6 over the two year study period (2008-2009) across watershed compartments. Black lines in the box-plot represent median values and red filled circles represent mean values connected with broken red lines.
median value for FH2 was noted for deep ground waters (0.22 R.U.). Again, similar to FH1, a large variability in FH2 values was observed for riparian and seep water samples.

For the soil derived humic-like component (FH3), median values of $F_{\text{max}}$ were highest in the wetland soil water (0.23 R.U.) and litter leachate (0.22 R.U.) and lowest in deep groundwater (0.07 R.U.) (Figure 3.1c). Similar to the trend for FH1, the FH3 values for tension soil pore water (0.16 R.U.) were much lower than the zero-tension wetland soil water. Overall, FH3 displayed a trend similar to FH1 indicating a decrease in humic-like DOM from surficial watershed sources to groundwater DOM sources. Median value for visible-humic-like component (FH4) was higher in throughfall (0.14 R.U.) compared to litter leachate (0.11 R.U.; Figure 3.1d). Thereafter, median values for FH4 were highest for wetland soil water and then decreased for groundwater sources.

The two protein-like components (FH5 and FH6) revealed similar trends across the watershed sources (Figure 3.1e and f). Median $F_{\text{max}}$ values for FH5 and FH6 were lowest for litter leachate and highest for the groundwater sources including riparian, seep and deep ground waters. Median values for throughfall for both components were greater than litter indicating that throughfall was enriched in protein-like DOM. Compared to FH6; FH5 displayed a higher variability for groundwater sources that included riparian, seep and deep ground waters. In contrast, FH6 revealed a larger range of variability for stream and shallow groundwater samples.

### 3.2.3 DOM Concentrations and metrics for watershed sources

DOC and DON concentrations for the entire sample set and values for selected DOM metrics ($a_{254}$, HIX, and FI) are presented in Figure 3.2. Both DOC and DON
concentrations were highest in surficial sources, especially litter leachate (Figure 3.2a and b, respectively) and declined rapidly from soil water to groundwater sources. The lowest DOC concentration was recorded for groundwater seeps (Figure 3.2a). The values for \( a_{254} \) and HIX, which provide a measure of DOM aromaticity and humic content, respectively, were also highest for surficial watershed sources and lowest in deep groundwater (Figure 3.2c and d, respectively). In contrast, FI, which has been used to indicate DOM of microbial origins [McKnight et al., 2001], revealed an opposite trend with low values for surficial sources (except throughfall) and high values for groundwater sources (Figure 3.2e).

### 3.2.4 Comparison of the FH and CM models for humic- and protein-like components

The comparison of the sums of the humic- and protein-like DOM components for the FH and CM models are presented in Figure 3.3. Summation of the humic-like components for the FH model included components FH1, FH3 and FH4 while for the CM model they included components C1, C5, and C7. Similarly, the sum of components FH5 and FH6 represented the protein-like DOM for the FH model while comparable components from the CM model included C8 and C13. Overall, compared to the CM model, the FH model generated a greater contrast in humic-like and protein-like components among the watersheds sources (Figure 3.3). The FH model also displayed greater variability in humic- and protein-like DOM for individual watershed sources (as indicated by the error bars in Figure 3.3) as opposed to the CM model. In addition, the % values for the humic-like DOM fractions in surficial sources were much greater for the FH model versus the CM model. Similarly, the % protein-like DOM values for groundwater sources were considerably greater for the FH model.
Figure 3.2: Spatial distributions of DOM quantity and quality parameters (a) DOC, (b) DON, (c) $a_{254}$, (d) HIX, and (e) FI over the two year study period (2008-2009) across watershed compartments. Black lines in the box-plot represent median values and red filled circles represent mean values connected with broken red lines. (a), (b), and (c) are log-scaled data for y-axis to capture distinctiveness in data.
compared to the CM model. For the FH model, the relative contribution of % humic-like DOM fluorescence ranged from 18% in deep groundwater to 67% in litter leachate samples; reflecting an almost four-fold increase of humic-like DOM for surficial sources (Figure 3.3a). In contrast, for the CM model, the highest % humic-like contribution was only 26% in throughfall, approximately 1.5 times greater than that for deep groundwater (16%, Figure 3.3b). ANOVA on the % humic-like DOM derived from the FH model yielded six distinct classes or categories among the watershed sources (A to F, Figure 3.3a) while the same analyses for the CM model yielded five distinct classes (A to E, Figure 3.3b). The FH model was able to differentiate ($p < 0.05$) between throughfall (TF) and litter leachate (LT) while the same distinction was not possible with the CM model. Similarly, the FH model indicated that litter leachate and wetland soil water were significantly different while the same was not true for the CM model. For groundwater sources, CM model values resulted in a significant difference between the % humic-like DOM for riparian and deep groundwater samples, which was however not replicated by the FH model.

Despite differences, both models produced the same broad trend, i.e., a decrease in humic-like DOM from surficial sources to groundwater sources with a simultaneous increase in % protein-like DOM. With respect to the % protein-like components, the FH model values ranged from 11% for litter leachate to 68% for deep groundwater (Figure 3.3c). In contrast, the range for the CM model was narrower with minimum for litter leachate at 3% to a maximum for deep groundwater at only 15%. However, while the absolute % protein-like DOM values differed substantially among the models, ANOVA analyses indicated that both models classified the watershed sources into five distinct classes. The FH model indicated that throughfall samples
Figure 3.3: Distributions of (a) %Humic-like, (c) %Protein-like DOM composition derived using site-specific PARAFAC (FH model) across watershed sources are shown. Sources represented with same letters are not significantly different. Results for the same using CM model are shown in (b) and (d) respectively. Standard deviation from means is shown using error bars.

were not significantly different from stream, hyporheic, and shallow ground waters for % protein-like DOM (Figure 3.3c). In contrast, the CM model suggested that throughfall was significantly different from all other DOM sources (Figure 3.3d). Similarly, CM model values indicated that % protein-like DOM for the litter samples was not significantly different from that of wetland soil water contradicting the results obtained with the FH model.
3.2.5 Discriminant analyses for watershed sources using FH and CM PARAFAC models

Forward stepwise discriminant function analysis using FH and CM models revealed distinct differences among DOM for watershed compartments (Figure 3.4). For each watershed source, the centroid of the data along with the circle representing the 95% confidence region is displayed (Figure 3.4). Riparian groundwater has a large confidence region, probably due to the small sample size ($n = 12$) whereas stream samples have the smallest confidence region, likely, due to larger sample size ($n = 82$). Biplot rays indicate the direction of variables (humic-like and protein-like DOM compositions) used in space. However, the entire separation (100%, $p < 0.001$) in both the cases (FH and CM models) occurred along the first dimension and humic-like and protein-like DOM characteristic appears to provide good separation. For the FH model, the first discriminant function (Dimension 1), accounted for 81% ($p < 0.001$) of the group (watershed sources) variation, while Dimension 2 accounted for the remaining 19% ($p < 0.001$) (Figure 3.4a). In comparison, the same dimensions (1 and 2) explained 77% ($p < 0.001$) and 23% ($p < 0.001$), of the variability for the CM model (Figure 3.4b). The key observations that come out of this analysis are – (a) overall, both models indicate a similar broader trend with seep, riparian, and deep ground waters clustered in the protein-like region while the remaining watershed sources located in the humic-like region of the plots; (b) in comparison to the CM model, the FH model displays greater separation among watershed sources which is apparent from the reduced overlap of the circular source regions (95% confidence region); and (c) there are slight differences in how the sources are spatially positioned in the discriminant space for the two models. In the CM model space, throughfall is further away from the other humic-like sources; however in the FH model it appears
Figure 3.4: Scatter plot of discriminant function analysis showing separation of watershed sources for humic-like and protein-like DOM components based on (a) FH model, and (b) CM model results. The extension of biplot rays indicates the strength of correlation between the measured variables.

much closer to the other humic-like sources with the exception of tension soil water (U).

Discriminant Analysis was used to determine if separation existed between watershed sources based on DOM characteristics (humic- and protein-like DOM composition). This separation was determined using the Wilks’ lambda multivariate test statistic. Wilks’ lambda values for FH and CM model were 0.11 (F = 75.88, p < 0.001) and 0.17 (F = 56.12, p < 0.001), respectively. Hence, the greater between groups variation (separation between watershed sources) as a proportion of the total variation is explained by larger F statistic observed in case of FH model and the larger the Wilks’ lambda statistic, the greater is the within-group variation as a proportion of the total variation as noticed for CM model (also low F statistic suggesting lower between group variability is explained in the case of CM model). Overall, discriminant function analysis revealed a significant association between watershed sources and
DOM characteristics (based on humic- and protein-like DOM composition) for FH model accounting for 59% of between watershed sources variability, thus correctly classifying the groups (watershed sources) from which samples were collected. In comparison only 51% correct classification (samples predicted belong to the same watershed source) was obtained for CM model. Bi-plot rays showed that DOM characteristics were major factor contributing to the discrimination between watershed sources which is further indicated by the length (or magnitude) of the rays (or vectors) in FH model.

3.2.6 Correlation between PARAFAC model components and DOM parameters

The correlations between the sum of humic-like and protein-like components from the FH and CM models against DOC and DON concentrations and metrics $a_{254}$, HIX and FI are presented in Table 3.1. One of the most significant results coming out of this comparison was that, overall, the correlations between the FH model components and the DOM parameters were much stronger than the corresponding values for the CM model. This suggested that the FH model did a much better job in characterizing DOM than the CM model. Among watershed sources, the correlations for both FH and CM models were generally weakest for litter and throughfall underscoring the wide variability in DOM composition for these watershed sources. In contrast, correlations were strongest for wetland soil water and shallow ground waters. Across most watershed sources, the humic-like components were positively correlated and the protein-like components were inversely correlated with both DOC and DON (the correlations with DOC being stronger). This would suggest that both C and N fractions of DOM contained a greater fraction of humic DOM. Not surprisingly, in most cases, humic-like components were positively correlated with both $a_{254}$ and HIX.
Table 3.1: Correlations between DOM Composition (%Humic-Like or %Protein-Like; derived from FH and CM model; see text for details) with measured DOM concentrations (DOC and DON) and DOM metrics (derived from UV and fluorescence measurements) across watershed sources.

<table>
<thead>
<tr>
<th>Watershed Sources</th>
<th>DOM Composition</th>
<th>DOC</th>
<th>DON</th>
<th>a$_{254}$</th>
<th>HIX</th>
<th>FI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF</td>
<td>%Humic-Like (FH)</td>
<td>0.05</td>
<td>0.34</td>
<td>0.30</td>
<td>0.94</td>
<td>-0.23</td>
</tr>
<tr>
<td></td>
<td>%Humic-Like (CM)</td>
<td>0.13</td>
<td>0.49</td>
<td>0.31</td>
<td>0.41</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>%Protein-Like (FH)</td>
<td>0.01</td>
<td>-0.27</td>
<td>-0.22</td>
<td>-0.99</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>%Protein-Like (CM)</td>
<td>-0.05</td>
<td>-0.27</td>
<td>-0.19</td>
<td>-0.90</td>
<td>0.33</td>
</tr>
<tr>
<td>LT</td>
<td>%Humic-Like (FH)</td>
<td>-0.05</td>
<td>-0.04</td>
<td>0.09</td>
<td>0.88</td>
<td>-0.52</td>
</tr>
<tr>
<td></td>
<td>%Humic-Like (CM)</td>
<td>-0.18</td>
<td>-0.15</td>
<td>-0.23</td>
<td>0.12</td>
<td>-0.20</td>
</tr>
<tr>
<td></td>
<td>%Protein-Like (FH)</td>
<td>0.05</td>
<td>-0.15</td>
<td>-0.27</td>
<td>-0.99</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>%Protein-Like (CM)</td>
<td>0.04</td>
<td>0.05</td>
<td>-0.07</td>
<td>-0.92</td>
<td>0.40</td>
</tr>
<tr>
<td>U</td>
<td>%Humic-Like (FH)</td>
<td>0.66</td>
<td>0.42</td>
<td>0.72</td>
<td>0.97</td>
<td>-0.24</td>
</tr>
<tr>
<td></td>
<td>%Humic-Like (CM)</td>
<td>-0.12</td>
<td>-0.15</td>
<td>-0.04</td>
<td>0.06</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>%Protein-Like (FH)</td>
<td>-0.64</td>
<td>-0.45</td>
<td>-0.68</td>
<td>-0.99</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>%Protein-Like (CM)</td>
<td>0.23</td>
<td>0.43</td>
<td>0.12</td>
<td>-0.32</td>
<td>-0.23</td>
</tr>
<tr>
<td>WSW</td>
<td>%Humic-Like (FH)</td>
<td>0.72</td>
<td>0.38</td>
<td>0.78</td>
<td>0.98</td>
<td>-0.29</td>
</tr>
<tr>
<td></td>
<td>%Humic-Like (CM)</td>
<td>0.31</td>
<td>-0.03</td>
<td>0.39</td>
<td>0.39</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>%Protein-Like (FH)</td>
<td>-0.73</td>
<td>-0.45</td>
<td>-0.78</td>
<td>-0.99</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>%Protein-Like (CM)</td>
<td>-0.55</td>
<td>-0.28</td>
<td>-0.61</td>
<td>-0.93</td>
<td>0.26</td>
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<tr>
<td>ST</td>
<td>%Humic-Like (FH)</td>
<td>0.64</td>
<td>0.46</td>
<td>0.65</td>
<td>0.98</td>
<td>-0.23</td>
</tr>
<tr>
<td></td>
<td>%Humic-Like (CM)</td>
<td>0.11</td>
<td>0.09</td>
<td>0.23</td>
<td>0.32</td>
<td>-0.15</td>
</tr>
<tr>
<td></td>
<td>%Protein-Like (FH)</td>
<td>-0.63</td>
<td>-0.43</td>
<td>-0.60</td>
<td>-0.99</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>%Protein-Like (CM)</td>
<td>-0.53</td>
<td>-0.22</td>
<td>-0.52</td>
<td>-0.79</td>
<td>0.15</td>
</tr>
<tr>
<td>HY</td>
<td>%Humic-Like (FH)</td>
<td>0.42</td>
<td>0.48</td>
<td>0.47</td>
<td>0.96</td>
<td>-0.60</td>
</tr>
<tr>
<td></td>
<td>%Humic-Like (CM)</td>
<td>0.05</td>
<td>0.02</td>
<td>0.05</td>
<td>0.41</td>
<td>-0.36</td>
</tr>
<tr>
<td></td>
<td>%Protein-Like (FH)</td>
<td>-0.47</td>
<td>-0.45</td>
<td>-0.52</td>
<td>-0.99</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>%Protein-Like (CM)</td>
<td>-0.25</td>
<td>-0.18</td>
<td>-0.35</td>
<td>-0.73</td>
<td>0.42</td>
</tr>
<tr>
<td>SGW</td>
<td>%Humic-Like (FH)</td>
<td>0.94</td>
<td>0.71</td>
<td>0.85</td>
<td>0.99</td>
<td>-0.87</td>
</tr>
<tr>
<td></td>
<td>%Humic-Like (CM)</td>
<td>0.89</td>
<td>0.66</td>
<td>0.82</td>
<td>0.90</td>
<td>-0.76</td>
</tr>
<tr>
<td></td>
<td>%Protein-Like (FH)</td>
<td>-0.90</td>
<td>-0.67</td>
<td>-0.79</td>
<td>-0.99</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>%Protein-Like (CM)</td>
<td>-0.84</td>
<td>-0.61</td>
<td>-0.72</td>
<td>-0.92</td>
<td>0.77</td>
</tr>
<tr>
<td>RGW</td>
<td>%Humic-Like (FH)</td>
<td>0.46</td>
<td>0.46</td>
<td>0.83</td>
<td>0.99</td>
<td>-0.52</td>
</tr>
</tbody>
</table>
Table 3.1 continued

| %Humic-Like (CM) | 0.20 | 0.28 | 0.72 | 0.88 | -0.56 |
| %Protein-Like (FH) | -0.48 | -0.44 | -0.82 | -0.99 | 0.53 |
| %Protein-Like (CM) | -0.22 | -0.04 | -0.64 | -0.79 | 0.85 |
| P | %Humic-Like (FH) | 0.47 | 0.11 | 0.30 | 0.99 | 0.20 |
| %Protein-Like (FH) | -0.48 | -0.11 | -0.30 | -0.99 | -0.24 |
| %Protein-Like (CM) | -0.25 | -0.09 | 0.08 | -0.79 | -0.38 |
| DGW | %Humic-Like (FH) | 0.41 | 0.17 | -0.11 | 0.99 | -0.21 |
| %Protein-Like (FH) | -0.31 | -0.17 | 0.12 | -0.99 | 0.09 |
| %Protein-Like (CM) | 0.14 | 0.41 | 0.17 | -0.58 | 0.42 |

All correlations significant at $p < 0.05$

while the protein-like components were positively correlated with FI. This supports the aromatic nature of the humic-like components and the microbial origins of the protein-like DOM.

3.3 Discussion

3.3.1 DOM characterization for watershed sources using the site-specific FH model

This study revealed a marked variability in humic-like components (FH1, FH3, and FH4) across the watershed sources. Surficial sources (litter leachate and wetland soil water) were especially high in humic-like DOM, whereas groundwater sources, namely, riparian, seeps, and deep groundwater were not significant contributors of humic-like DOM. These observations are in accordance with previously reported studies [Chen et al., 2010; Thurman, 1985] as well as our own previous
characterization of DOM for this study site based on the CM model [Inamdar et al., 2012]. The lack of humic DOM pool for groundwater samples could be attributed to dilution [Chen et al., 2010] or processes such as sorption and microbial degradation. For instance, Kalbitz and Geyer (2001) reported that sorption phenomena with soil mineral surfaces could potentially reduce the humic content of DOM along the hydrologic flow path or as runoff water percolated through the soil profile. Similarly, groundwater sources are prone to microbial decomposition which can result in a significant drop in humic-like DOM in groundwater [Chen et al., 2010]. The decrease in humic-like components is also supported by DOM concentrations and metrics like $a_{254}$ and HIX (Figure 3.2c and d, respectively). Both $a_{254}$ and HIX reveal a substantial decrease in values moving from surficial to groundwater sources. Using the UV absorbance values and the equation proposed by Weishaar et al. (2003), we observed lowest aromaticity in sub-surface samples (ranged from 14% in deep ground waters to 21% in seep samples), thus corroborating our finding of low aromaticity in sub-surface or groundwater samples. Similarly, DOC concentrations in groundwater sources were lower than those for surficial sources suggesting that humic-rich DOC is preferentially sorbed to soil along the hydrologic flow path and/or is degraded by microbial decomposition into lower molecular weight DOM [Bolan et al., 2011; Kalbitz et al., 2007; Nguyen et al., 2010]. It should be noted that compared to litter leachate, the character of throughfall DOM was much less humic and more protein-rich, indicating a release of less humic and more degradable compounds for the forest canopy.

In contrast to the decrease in humic-like components, relative percent of the protein-like components (FH5 and FH6) increased from surficial to groundwater sources (Figure 3.1). The increasing trend of protein-like fluorescence is supported by
an increase in FI values indicating a greater influence of DOM of microbial origin of DOM (Figure 3.2). Similar observations have previously been reported by Chen et al. (2010) and Williams et al. (2010). There was a sharp shift in the protein-like components (FH5 and FH6) between shallow ground waters (SGW) and riparian, seep and deep groundwater (Figure 3.1e and f, respectively). The shallow groundwater sampled the full soil profile in the wetlands while the other three sources represented water from deeper in the soil profile. Clearly this change had a substantial impact on the protein-like fluorescence of DOM. Furthermore, it should be noted that there were slight differences in the trend for FH5 and FH6 components across watershed sources. FH5 is considered to represent the relatively “fresher” protein-like DOM moieties (tryptophan) whereas FH6 represents more degraded (tyrosine) protein-like fractions [Santín et al., 2009]. The higher Fmax values for FH6 at riparian, seep and deep groundwater (compared to FH5) locations would tend to suggest the existence of more degraded protein-like DOM at these locations. Chen et al. (2010) have also reported more degraded protein-like DOM for ground waters.

3.3.2 Comparison of site-specific versus pre-validated PARAFAC models for characterizing DOM

The only previous comparison of a site-specific versus a pre-validated model was performed by Fellman et al. (2009c) who compared the CM model against a model (referred to as AK) developed from 307 EEMs of soil and stream water samples from southeast Alaska. They compared the two models by: (i) examining the EEM residuals obtained from CM model, and (ii) determining the correlations between PARAFAC model components and measurements such as DOC and DON concentrations, % bioavailable DOC, and DOM components determined from GC/MS
analyses [Fellman et al., 2009c]. Based on the EEM residuals they found that among stream and soil water samples, the CM model did a poor job in fitting the soil water samples from upland and wetland sites. This result is not surprising considering that the CM model was developed primarily using EEMs from a diverse range of aquatic ecosystems. In addition, while comparing the protein-like PARAFAC components against DOM concentrations, Fellman et al. (2009c) found that while the two models were not significantly different in predicting DOM concentrations, the CM model explained slightly less of the variation than the site-specific model.

We took a slightly different approach than Fellman et al. (2009c) in that we directly compared the ability of the two models (CM versus our own FH model) to differentiate DOM from various watershed sources. We did not use the residual EEMs approach as used by Fellman et al. (2009c) because while fitting the CM model we retained only EEMs that had residuals less than 10%. The direct comparison of CM and FH model was possible because of the wide variety of watershed sources that were sampled in our study and the large data set associated with each source. Our results clearly showed that the FH model was much more sensitive to subtle variations in DOM and did a much better job in highlighting the differences in DOM among watershed sources. This was highlighted both by the model differences in humic-like and protein-like components as well as the spatial separation of watershed sources in discriminant analysis (Figure 3.4). Not only did the FH model allow greater differentiation among watershed sources, but the sum of humic- and protein-like model components also yielded stronger relationships with DOC and DON concentrations, thus providing important insights into the ecological nature of the C and N in watershed pools. Among the humic-like and protein-like components, the
differences between FH and CM model were higher for the protein-like components (Table 3.2). This suggests that in our study the CM model tended to under-predict the protein-like moieties for watershed sources. This assessment was also recently alluded to by Larsen et al. (2010) who implemented the CM model to characterize DOM for the ridge and slough landscape in the Florida Everglades. Larsen et al. (2010) found the highest EEM residuals were associated with the protein-like components which meant that the model had limited capability in resolving these compounds. They further speculated that the inability to correctly resolve these reactive protein-like compounds could hamper our understanding of associated processes such as rapid microbial uptake and photo-degradation.

Table 3.2: Mean (standard deviation) values for the changes in humic- and protein-like DOM composition between FH and CM model across watershed sources in this study.

<table>
<thead>
<tr>
<th>Watershed Sources</th>
<th>Sample Size (N)</th>
<th>Change in % Humic-Like</th>
<th>Change in % Protein-Like</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF</td>
<td>37</td>
<td>0.99 (0.22)</td>
<td>2.94 (0.53)</td>
</tr>
<tr>
<td>LT</td>
<td>42</td>
<td>1.65 (0.14)</td>
<td>2.16 (0.51)</td>
</tr>
<tr>
<td>U</td>
<td>24</td>
<td>1.49 (0.13)</td>
<td>3.34 (0.73)</td>
</tr>
<tr>
<td>WSW</td>
<td>49</td>
<td>1.45 (0.22)</td>
<td>3.74 (0.90)</td>
</tr>
<tr>
<td>ST</td>
<td>82</td>
<td>1.07 (0.30)</td>
<td>4.78 (1.24)</td>
</tr>
<tr>
<td>HY</td>
<td>27</td>
<td>1.14 (0.20)</td>
<td>4.31 (0.96)</td>
</tr>
<tr>
<td>SGW</td>
<td>29</td>
<td>1.12 (0.47)</td>
<td>3.98 (1.16)</td>
</tr>
<tr>
<td>RGW</td>
<td>12</td>
<td>0.27 (0.34)</td>
<td>4.30 (1.61)</td>
</tr>
<tr>
<td>P</td>
<td>37</td>
<td>0.29 (0.31)</td>
<td>4.17 (1.38)</td>
</tr>
<tr>
<td>DGW</td>
<td>28</td>
<td>0.06 (0.22)</td>
<td>3.78 (1.16)</td>
</tr>
</tbody>
</table>
In light of the results from this study and the observations previously made by Fellman et al. (2009c) and Larsen et al. (2010) we suggest that CM model would be appropriate to characterize DOM and should be implemented where – (a) EEMs data is not sufficient to develop a PARAFAC model; (b) broad trends in DOM are required; and/or (c) DOM comparisons between disparate watershed sites are performed. However, when sufficient EEMs are available to develop a PARAFAC model, it should be the preferred approach. Given the sensitivity of our results, a site-specific model could do a slightly better job in characterizing differences in DOM composition among watershed sources, seasonal or spatial patterns in DOM composition and consequently a better understanding of the potential processes (e.g., photo-degradation, microbial uptake, sorption) affecting fate and transport of DOM.

3.4 Conclusions

This study compared a site-specific PARAFAC model (FH model) against a pre-validated PARAFAC model [Cory and McKnight, 2005] for characterizing DOM composition in a forested, headwater watershed. The PARAFAC models were developed for fluorescence EEMs determined for various watershed sources including – throughfall, litter leachate, wetland soil water, stream water, hyporheic zone, shallow and deep ground waters, and groundwater seeps. The site-specific model was more sensitive to subtle differences in DOM and was able to provide a greater level of distinction among watershed sources. The humic- and protein-like constituents derived from the site-specific model displayed more pronounced differences among watershed sources compared to the pre-validated model. DOC and DON concentrations and selected DOM metrics were also more strongly correlated with components of the site-specific PARAFAC model versus the pre-validated model. These results suggest that
while a pre-validated PARAFAC model may capture the broad trends in DOM composition and may allow comparisons with other study sites, a site-specific model will do a better job in characterizing within watershed differences in DOM and consequently provide important insights into processes and mechanisms influencing DOM.
Chapter 4

SPATIAL VARIATION IN OPTICAL PROPERTIES OF DISSOLVED ORGANIC MATTER ALONG A STREAM DRAINAGE NETWORK IN A FORESTED PIEDMONT CATCHMENT

4.1 Introduction

Dissolved organic matter plays an ecologically important role in various biogeochemical and physical processes linking terrestrial and aquatic ecosystems [Aiken et al., 2011; Bolan et al., 2011; Fellman et al., 2009]. In terrestrial and aquatic ecosystems, DOM not only influences the geochemical and photochemical reactions by participating in carbon (C) and nutrient (N, P, and S) cycles, but also controls microbially-mediated reactions by serving as potential substrate [Moran et al., 2000; Spencer et al., 2008]. It also plays a key role in transport and transformation of major contaminants and/or pollutants and their reactivity with the environment [Aiken et al., 2011; Yamashita and Jaffé, 2008]. The amount and quality of DOM in terrestrial and aquatic environments influences biological processes such as microbial degradation [Moran et al., 2000; Williams et al., 2010]. It is also a key player in altering the depth of the photic zone in aquatic ecosystems by controlling the incident UV radiation [Wetzel, 2003]. Thus, understanding how DOM concentrations and composition vary spatially across terrestrial and aquatic ecosystems is critical for assessing their influence on a suite of environmental and ecological processes.

DOM concentration and quality varies with catchment properties such as physiography, vegetation, and wetland cover [Fellman et al., 2009b; Hinton et al., 1998; Hood et al., 2006]. Previous studies have reported spatial variations in DOM character and composition in stream networks in a variety of geographic regions ranging
from boreal, tropical to xeric environments [Ågren et al., 2007; Catalán et al., 2012; Hood et al., 2006]. In addition, few studies encompassed varying hydrologic conditions (e.g., base flow and storm flow) for spatial variability of DOM in stream networks [Buffam et al., 2001; Hood et al., 2006]. DOM variability has also been reported for specific forest cover types or landscape features [Brooks et al., 1999; Dillon and Molot, 1997]. Significant changes in DOM along the stream network have been reported [Dawson et al., 2008, 2011; Fellman et al., 2009b; Hood et al., 2006]. For instance, Hood et al. (2006) reported increased DOC concentration in an unharvested, old growth catchment compared to two harvested forested catchments. Ågren et al. (2008) found wetland coverage as an important contributor in annual DOC export in a study in boreal stream network. Catchment area was another important control in their study for annual DOC export with smallest area displaying almost six times more DOC export than the largest catchment in areal extent. While, Fellman et al. (2009) investigated the DOM exports from two catchments with distinct landscape features (forested wetland and upland forest) during summer and fall storm events in Alaska and reported significant differences in DOM properties. They attributed these differences to dominant landscape features existed in each catchment. Further, in contrast to other findings, they found less aromatic and protein-rich DOM exported from wetland catchment during storm events when compared against base flow conditions and concluded that wetlands could be an important source of labile DOM. Aitkenhead et al. (1999) reported the strongest correlation for stream DOC with soil properties in small size catchments. Factors such as rate of DOM production and/or consumption (in organic soils), adsorption/desorption of DOM (on mineral soils), and hydrologic flow paths through soil profile also play an important role in
dictating DOM concentration and quality in forested ecosystems [Ågren et al., 2007; Frost et al., 2006; Marschner and Kalbitz, 2003].

While the studies described above have provided important insights into the spatial variability of DOM and the potential drivers involved, few of these studies have evaluated the variation of DOM under both base flow and storm flow conditions and have explicitly assessed the influence of hydrologic flow paths and runoff sources. Evaluating DOM variability in light of hydrologic flow paths and other catchment controls (e.g., areal extent of wetlands, stream length, drainage area, etc.) can provide important information on the relative influence of these controls. Such data is crucial for developing reliable and robust mechanistic/conceptual models to characterize fate and transport of DOM in watersheds. Furthermore, few studies have implemented spectro-fluorometric techniques to characterize the spatial variability of DOM in watersheds and much such information is critically required.

We studied the spatial variability of DOM along a stream drainage network within a 79 ha catchment in the mid-Atlantic Piedmont region of the USA. This site is part of an ongoing extensive study of DOM [Inamdar et al., 2011, 2012]. Stream water sampling for DOM was conducted for base flow as well as storm events over a three-year period (2009-2011). Base flow DOM comparisons were performed for five locations along the drainage network ranging from a drainage area of 0.62 to 79 ha while storm flow comparisons were limited to three (3.5 – 79 ha) of these five locations. An end-member mixing model developed for this site [Inamdar et al., 2013] was extended to characterize the runoff flow paths for each of the five drainage locations for base flow and storm flow conditions. This flow path information was used in conjunction with DOM to evaluate the spatial variability of DOM. The
composition of DOM was determined using spectro-fluorometric methods and a new excitation-emission (EEMs) PARAFAC model, specific to our site, was developed to characterize DOM.

The specific questions that were addressed in this study include:

1. How does DOM concentration and composition vary at different locations along the stream drainage network? How does this spatial pattern differ for base flow and storm flow conditions?

2. How does the DOM in the drainage network relate to DOM for watershed sources?

3. What are the key controls and factors that dictate DOM at various spatial scales along the stream drainage network?

Our overarching hypothesis is that the spatial variation in DOM is a function of hydrologic flow paths and catchment features such as percent wetland coverage.

4.2 Results

4.2.1 Runoff sources for stream drainage locations derived from EMMA

Runoff sources and the stream water chemistry during base flow and storm flow are displayed in the EMMA mixing diagram (or U-space) in Figure 4.1 for all drainage locations. Runoff sources were distinctly identified in different quadrants of the EMMA diagram (Figure 4.1a) [Inamdar et al., 2013]. In general, for all drainage locations, precipitation sources – rainfall, throughfall, stemflow, snow, and litter leachate displayed a large range in concentrations along a linear plane extending from quadrants II to III. In contrast, the groundwater runoff sources – seep, riparian groundwater, hyporheic water, and deep groundwater were tightly grouped in a very small region in quadrants I and IV. Saturated wetland soil water (LY) and shallow ground water (SGW) were also tightly grouped and were closer to ground water runoff.
Figure 4.1: (a) EMMA mixing diagram indicating all watershed sources, (b) stream chemistry for multiple catchments during base flow, and (c) storm flow conditions, over a three year period (2008-10).
sources; however unsaturated soil water (U) occupied an intermediate region in the EMMA-space.

Overall, base flow for all stream locations was generally in the vicinity of the riparian, hyporheic, seep and deep ground waters (Figure 4.1b). Among these stream locations, base flow for wetland catchment ST6 was shifted towards wetland soil water and/or shallow ground waters (transient groundwater). ST4 catchment appears to be more influenced by seep and hyporheic waters. In comparisons, ST5 catchment displayed both the influences of shallow groundwater and saturated wetland soil water as well as seep and hyporheic samples for stream chemistry during base flow conditions. Likewise, seep, shallow, riparian and deep groundwater in addition to hyporheic and wetland soil waters characterizes stream chemistry for our reference catchment (ST3) displaying the largest variations in runoff sources. Base flow chemistry for catchment ST12 (79 ha) was shifted to the left and bottom of the graph indicating a substantial influence of deeper groundwater sources.

In contrast to base flow, storm flow chemistry revealed a greater influence of precipitation and surficial runoff sources ((Figure 4.1c). The greatest influence of precipitation water sources was observed for catchment (ST6). Catchment ST12 had the lowest influence of surficial sources while storm flow chemical response for catchment ST3 fell between the responses for ST6 and ST12.

4.2.2 DOM for stream water versus watershed sources

To evaluate how stream water DOM values for ST3 during base flow and storm flow compare against watershed DOM sources, a plot of these values is presented in Figure 4.2. Median base flow DOC and $a_{254}$ increased by more than 400%
Figure 4.2:  (a) Dissolved organic carbon (DOC), (b) %Humic-like (HL), (c) %Fulvic-like (FL), (d) %Protein-like (PL), (e) Humification Indices (HIX), (f) Fluorescence Indices (FI), and (f) absorption coefficients at 254 nm, a$_{254}$, for sampled watershed sources. Median base flow (blue lines) and storm flow (red lines) values for corresponding parameters at 12 ha stream outlet is shown for comparison purposes.
during storm flow conditions for this catchment (Figure 4.2a and g). Significant increases in HL and HIX have also been found (Figure 4.2b and e). In contrast, PL and FI have significantly dropped from median base flow values by 40% and 2% respectively during storm flow conditions (Figure 4.2d and f).

4.2.3 DOM for stream locations during base flow

DOM concentration and metrics during base flow for locations ST3-ST6 and ST12 are presented in Figure 4.3 and Table 4.1. Mean base flow DOC concentration and a$_{254}$ was highest (3.19 and 25.61, respectively) for catchment ST6 and significantly different from other locations No significant differences in DOC and a$_{254}$ were observed for other catchments. The lowest mean values for DOC (0.99) and a$_{254}$ (6.52) was recorded for the smallest catchment ST5. Mean values for HL-DOM for ST6 (55%) was significantly different than those for other catchments except ST3 which had a value of 52%. The lowest HL-DOM was observed for ST5 45%. HIX for ST5 was lowest (0.72) and differed significantly from other catchments. Similarly, FL-DOM at ST12 was highest (33%) and differed significantly from other catchment except ST6 (32%). PL-DOM was highest at ST5 (30%) and lowest at ST6 (13%). FI significantly differed between drainage locations with a highest mean value at ST4 (1.46) and lowest at ST6 (1.40).

Median base flow DOC (1.24) and a$_{254}$ (10.82) for stream water at catchment ST3 were similar to values for ground water sources such as seeps (0.75 and 4.38, respectively), hyporheic (1.42 and 12.09, respectively) (Figure 4.2a and g). Likewise, HL and HIX during base flow displayed stream water (ST3) character similar to hyporheic and unsaturated soil waters (Figure 4.2b and e). Similar runoff sources
Figure 4.3: (a) Dissolved organic carbon (DOC), (b) %Humic-like (HL), (c) %Fulvic-like (FL), (d) %Protein-like (PL), (e) Humification Indices (HIX), (f) Fluorescence Indices (FI), and (f) absorption coefficients at 254 nm, $a_{254}$, for stream waters at multiple drainage locations (five locations) during base flow. Drainage locations are plotted with increasing catchment area from left to right. Same letters in box plots represent no significant differences among catchments for corresponding variable.
Table 4.1: Univariate statistical analyses for seven parameters (DOC, % Humic-Like, % Fulvic-Like, % Protein-Like, HIX, FI, and $a_{254}$) during base flow (five drainage locations) and storm flow (three drainage locations) conditions.

<table>
<thead>
<tr>
<th>Drainage Locations (N)</th>
<th>DOC (mg L$^{-1}$)</th>
<th>% Humic-Like</th>
<th>% Fulvic-Like</th>
<th>% Protein-Like</th>
<th>HIX</th>
<th>FI</th>
<th>$a_{254}$ (m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Base flow</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST5 (24)</td>
<td>0.99 ± 0.29</td>
<td>45.10 ± 4.32</td>
<td>24.29 ± 2.47</td>
<td>30.61 ± 5.43</td>
<td>0.72 ± 0.05</td>
<td>1.41 ± 0.03</td>
<td>6.52 ± 2.17</td>
</tr>
<tr>
<td></td>
<td>(0.60 - 1.67)</td>
<td>(35.66 - 53.99)</td>
<td>(18.17 - 32.02)</td>
<td>(22.50 - 44.24)</td>
<td>(0.58 - 0.80)</td>
<td>(1.34 - 1.49)</td>
<td>(2.99 - 11.05)</td>
</tr>
<tr>
<td>ST6 (24)</td>
<td>3.19 ± 2.73</td>
<td>55.38 ± 4.11</td>
<td>31.82 ± 1.53</td>
<td>12.80 ± 3.90</td>
<td>0.88 ± 0.03</td>
<td>1.40 ± 0.03</td>
<td>25.61 ± 16.48</td>
</tr>
<tr>
<td></td>
<td>(1.22 - 12.68)</td>
<td>(49.68 - 62.09)</td>
<td>(28.02 - 35.19)</td>
<td>(5.93 - 18.27)</td>
<td>(0.83 - 0.93)</td>
<td>(1.33 - 1.45)</td>
<td>(6.68 - 67.48)</td>
</tr>
<tr>
<td>ST4 (27)</td>
<td>1.17 ± 0.39</td>
<td>47.87 ± 4.02</td>
<td>30.66 ± 2.40</td>
<td>21.46 ± 5.17</td>
<td>0.80 ± 0.04</td>
<td>1.46 ± 0.04</td>
<td>8.84 ± 2.66</td>
</tr>
<tr>
<td></td>
<td>(0.72 - 2.06)</td>
<td>(41.19 - 58.45)</td>
<td>(23.38 - 34.56)</td>
<td>(8.95 - 29.25)</td>
<td>(0.74 - 0.91)</td>
<td>(1.35 - 1.54)</td>
<td>(4.61 - 14.97)</td>
</tr>
<tr>
<td>ST3 (44)</td>
<td>1.33 ± 0.50</td>
<td>52.31 ± 6.49</td>
<td>28.60 ± 2.89</td>
<td>20.93 ± 11.75</td>
<td>0.81 ± 0.10</td>
<td>1.40 ± 0.04</td>
<td>11.70 ± 4.03</td>
</tr>
<tr>
<td></td>
<td>(0.44 - 2.99)</td>
<td>(33.37 - 63.43)</td>
<td>(19.10 - 35.60)</td>
<td>(5.86 - 60.88)</td>
<td>(0.48 - 0.93)</td>
<td>(1.34 - 1.45)</td>
<td>(5.76 - 22.57)</td>
</tr>
<tr>
<td>ST12 (41)</td>
<td>1.74 ± 1.02</td>
<td>49.61 ± 8.13</td>
<td>32.74 ± 2.20</td>
<td>17.65 ± 8.06</td>
<td>0.84 ± 0.06</td>
<td>1.43 ± 0.03</td>
<td>11.03 ± 6.90</td>
</tr>
<tr>
<td></td>
<td>(0.80 - 4.63)</td>
<td>(10.52 - 60.20)</td>
<td>(28.14 - 37.69)</td>
<td>(4.76 - 54.21)</td>
<td>(0.58 - 0.95)</td>
<td>(1.32 - 1.52)</td>
<td>(4.38 - 47.21)</td>
</tr>
<tr>
<td><strong>Storm flow</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST6 (106)</td>
<td>8.11 ± 3.30</td>
<td>60.90 ± 2.61</td>
<td>29.90 ± 1.88</td>
<td>9.20 ± 2.37</td>
<td>0.91 ± 0.02</td>
<td>1.36 ± 0.02</td>
<td>76.32 ± 30.53</td>
</tr>
<tr>
<td></td>
<td>(2.79 - 16.43)</td>
<td>(53.58 - 65.70)</td>
<td>(24.34 - 34.51)</td>
<td>(4.10 - 14.57)</td>
<td>(0.86 - 0.94)</td>
<td>(1.31 - 1.41)</td>
<td>(24.64 - 142.56)</td>
</tr>
<tr>
<td>ST3 (192)</td>
<td>6.70 ± 3.11</td>
<td>59.31 ± 3.44</td>
<td>29.61 ± 1.88</td>
<td>10.99 ± 3.67</td>
<td>0.89 ± 0.02</td>
<td>1.37 ± 0.02</td>
<td>59.59 ± 26.33</td>
</tr>
<tr>
<td></td>
<td>(1.59 - 18.35)</td>
<td>(47.76 - 65.35)</td>
<td>(23.95 - 34.31)</td>
<td>(5.85 - 23.98)</td>
<td>(0.82 - 0.93)</td>
<td>(1.31 - 1.45)</td>
<td>(9.44 - 152.00)</td>
</tr>
<tr>
<td>ST12 (81)</td>
<td>5.22 ± 2.05</td>
<td>56.74 ± 4.05</td>
<td>31.44 ± 1.38</td>
<td>11.82 ± 4.20</td>
<td>0.88 ± 0.03</td>
<td>1.39 ± 0.03</td>
<td>46.31 ± 19.58</td>
</tr>
<tr>
<td></td>
<td>(1.17 - 10.89)</td>
<td>(43.50 - 63.88)</td>
<td>(28.55 - 34.45)</td>
<td>(5.07 - 23.10)</td>
<td>(0.75 - 0.93)</td>
<td>(1.33 - 1.50)</td>
<td>(6.68 - 88.90)</td>
</tr>
</tbody>
</table>
dominated the base flow DOC and a$_{254}$ for ST5, ST4, and ST12 locations. However, surface wetland water (SWW) and wetland soil water (WSW) demonstrated larger influence on stream water chemistry at wetland-dominated catchment, ST6 during base flow conditions. For example, median DOC and a$_{254}$ at ST6 (2.32 and 19.81, respectively) lie closer to the median values of same parameters for SWW (3.33 and 32.93, respectively), and WSW (4.36 and 47.33, respectively). Similarly, PL-DOM at ST6 demonstrated a larger influence of wetland surface and soil waters while PL-DOM at other drainage locations seems similar to PL of groundwater characters. In general, dominance of groundwater sources was clearly visible in DOM concentration and in derived DOM metrics for stream water characteristics during base flow at all drainage locations but ST6. This is in accordance with our EMMA analysis, where conservative tracer technique also displayed that stream water chemistry of ST6 seems similar to wetland soil waters (Figure 4.1a and b).

The first two components of PCA explained 75% variability in the dataset (Figure 4.5a and b). Together, PCA loading and scores plot provide further insights about the spatial pattern of DOM and associated characteristics. DOM originating from smallest catchment ST5 is more protein-like whereas wetland dominated catchment ST6 contributed more humic-like and aromatic DOM as indicated by HL and a$_{254}$. ST4 and ST12 appeared to be carrying more FL-DOM primarily derived from microbial mediation as indicated by FL and FI in the Figure 4.5a and b. In general, ST6, ST3 and ST12 contributed more humified DOM (high HIX) compared to ST5 and ST4.
Figure 4.4: (a) Dissolved organic carbon (DOC), (b) %Humic-like (HL), (c) %Fulvic-like (FL), (d) %Protein-like (PL), (e) Humification Indices (HIX), (f) Fluorescence Indices (FI), and (f) absorption coefficients at 254 nm, $a_{254}$, for stream waters at multiple drainage locations (three locations) during storm flow. Drainage locations are plotted with increasing catchment area from left to right. Same letters in box plots represent no significant differences among catchments for corresponding variable.
4.2.4 DOM for stream locations during storm flow

For storm flow, DOM concentration and optical metrics showed considerable differences among drainage locations (Figure 4.4 and Table 4.1). Highest mean values for DOC and a$_{254}$ were noticed at wetland dominated catchment, ST6 (8.11 and 76.32, respectively) followed by the same at ST3 (6.70 and 59.59, respectively), whereas lowest mean values were recorded for the largest catchment, ST12 (5.22 and 46.31, respectively) (Figure 4.4a and g). DOC concentration and a$_{254}$ decreased progressively catchment drainage from ST6 to ST12. Similarly, a decreasing pattern for HL and HIX were noted with increasing catchment size (Figure 4.4b and e). HL-DOM contribution decreased from wetland dominated catchment, ST6 (60.90%) to the largest catchment, ST12 (56.74%) with an intermediate value at our reference catchment, ST3 (59.31%) while the HIX was highest at ST6 (0.91) followed by 0.89 at ST3 and a lowest recorded at ST12 (0.88). No significant difference for FL-DOM existed between ST6 and ST3, but a significantly different and largest mean value was noticed at ST12 (31.44%) for the same variable. In contrast to the patterns shown by DOC and above mentioned metrics, an increasing trend for PL-DOM contributions and FI has been found (Figure 4.4d and f). Although no significant difference was observed for PL-DOM between ST3 (10.99%) and ST12 (11.82%), yet a slight higher value at ST12 is noted (Figure 4.4d). The lowest mean value for PL-DOM was apparent at wetland dominated catchment, ST6 (9.2%). Nevertheless, an inverse trend to HL-DOM contributions was observed in this case which linearly increased with catchment drainage sizes. Likewise, FI also showed an increasing pattern with increasing catchment sizes with a maximum mean value at ST12 (1.39) which was followed by the lowest at ST6 (1.36) with an intermediate FI at ST3 (1.37). Also, FI among drainage locations significantly differed (Figure 4.4f).
Figure 4.5: PCA plots of (a) loadings, and (b) scores for stream waters at five catchments during base flow. PCA plots of (c) loadings, and (d) scores for stream waters at three catchments during storm flow.

Median values of storm flow DOC and $a_{254}$ at ST3 were found closer to throughfall, shallow groundwater, and saturated wetland soil waters (Figure 4.2a and g). Similarly, HL and HIX values also moved more towards precipitation sources such as throughfall and litter leachate. In contrast, significant decreases in PL and FI median values (10.11 and 1.37, respectively) were noticed in storm flow samples that lie closer to median values of litter (4.79 and 1.31, respectively) and saturated wetland soil waters (8.75 and 1.35, respectively) (Figure 4.2d and f). Our largest catchment,
ST12 displayed DOC and $a_{254}$ median values (5.11 and 45.37, respectively) closer to rainfall (4.6 and 11, respectively), throughfall (7.6 and 55.27, respectively), and wetland soil water (4.36 and 47.33, respectively) values. In contrast, median values of PL and FI at ST12 (11 and 1.38, respectively) lie closer to hyporheic (18.43 and 1.42, respectively), soil pore waters (8.74 and 1.50, respectively), wetland surface and soil waters (11.53 & 1.37 and 8.75 & 1.35, respectively). Wetland-dominated catchment, ST6, had median values of 7.5 and 73.58 for DOC and $a_{254}$, respectively. In general, these median values were closer to the median values of litter and throughfall; however, median values of PL in wetland surface and soil water lie closer to median value of the same at ST6 during storm flow conditions suggesting the surficial runoff dominated at this drainage location in DOM composition as well. This was again consistent with our EMMA analysis which showed the influence of precipitation sources was largest in our ST6 catchment (Figure 4.1a and c).

The first two components of PCA explain 90% variability in the dataset which was greater than the same during base flow conditions (Figure 4.5c and d). Storm flow samples from ST6 clustered together and extended towards DOM with high concentration which was rich in humic-like and aromatic character (e.g., high HL-DOM and $a_{254}$). In comparison, storm flow samples of ST12 appeared to be grouped together and inclined more towards FL-DOM which is more influenced by microbial activities as indicated by extension of FI. Storm flow samples of ST3 showed a mixed DOM character as they lie in between ST6 and ST12 samples. One major difference between base flow and storm flow PCA was that DOC and $a_{254}$ were not correlated with HL-DOM and HIX during base flow; however, strong correlations between these parameters existed during storm flow conditions (Figure 4.5a and c). Similarly, DOC...
during base flow showed no correlation with PL-DOM, however a shift in DOM character was found during storm flow conditions when PL-DOM was noticed strongly negatively correlated with DOC.

4.2.5 Storm-event patterns of DOM for stream locations

A total of four storm events were selected to compare within-event patterns of DOM among stream locations, with two storm events (July 31, 2009 and December 9, 2009) for ST3 versus ST6 and two storm events (September 30 – October 1, 2010 and August 14, 2011) for ST3 versus ST12 (Figure 4.6 and Figure 4.7, respectively). While comparing between ST3 and ST12, we used stream discharge values only for ST3 as we do not have any discharge measurements at our largest catchment location at ST12.

During the July 31 event, DOC concentration peaked with increasing discharge both at ST6 and ST3 drainage locations; however DOC concentration was higher (16.43) for ST6 compared to the same at ST3 (14.98) (Figure 4.6a-e). Likewise, HIX and a$_{254}$ also peaked with maximum discharge at both these locations (Figure 4.6c and e). Similar to DOC, peaks in a$_{254}$ and HIX were most noticeable at ST6 corresponding to peak discharge in stream flow. However, for the same storm event, roughly 4% increase in HIX before and after the event conditions was found at both these locations. A larger change in a$_{254}$ (about 95%) was found after the event at ST3, but only 25% increase in a$_{254}$ was noticed at ST6 following the event conditions. In contrast to DOC, a$_{254}$, and HIX patterns at these locations, inverse patterns in PL and FI have been found with peak discharge at both drainage locations (Figure 4.6b and d). In general, greater change in PL was noticeable at ST3 (35%) against the same at ST6.
Figure 4.6: DOM concentrations and quality indices (DOC, %PL, HIX, FL, and $a_{254}$) for the events of July 31, 2009 and December 9, 2009 for the two catchment locations ST6 (red symbols and lines) and ST3 (blue symbols and lines). Solid lines represent discharge at corresponding catchments.
(25%) when compared between pre- and post-event conditions. Whereas similar changes in FI (roughly 2%) occurred before and after event conditions at both locations, but a higher drop in FI (from 1.37 to 1.33) was noticed at ST6 with the maximum discharge (Figure 4.6d). It is important to note here that this event occurred during summer dry conditions when the base flow in the perennial stream (ST3) is generally low. This was also a high intensity short duration event which is typical of this region, particularly during this time of year due to increased convective thunderstorm activities [Inamdar et al., 2011]. Stream at ST6 drainage location which usually does not run during summer may receive enough waters during such precipitation events to start flowing. Thus, similar peak discharge flow at both these locations could have occurred driven from event conditions rather than the base flow conditions. Therefore, a different DOM response could be expected during high discharge season when both these streams (draining ST6 and ST3, respectively) are flowing.

Another winter storm event (December 9) was a low intensity but large duration event (Figure 4.6f-j). During this event, peak discharge between ST6 and ST3 differed by more than 100% (ST6 = 1.01 mm hr\(^{-1}\) and ST3 = 2.28 mm hr\(^{-1}\)). Although the DOC, a\(_{254}\), and HIX patterns were similar that is increasing with peak discharge at both drainage locations, yet a different characteristics were noticed in these responses at different drainage locations, in particular, DOC and a\(_{254}\) responses at ST6 were spiked with discharge peak whereas the same parameters showed a smooth response at ST3 (Figure 4.6f and i). Also, the change in DOC from pre- to post-event condition was again larger at ST3 (as was noticed in summer event of July 31) although with smaller magnitude (11%). One interesting difference in this event compared to
summer event (July 31) was noticed in $a_{254}$ response at both drainage locations (Figure 4.6e and j). In contrary to summer event, where relative increase in $a_{254}$ was observed at both locations, decreases in $a_{254}$ values were noticed at both locations during this winter event for pre- and post-event conditions. However, this decrease was most noticeable at ST6 (26%) as compared to the same at ST3 (3%). Similar to summer event HIX response, increases by roughly 5% were noticed after winter event at both locations (Figure 4.6h). Also, similar to summer event, PL and FI showed an inverse patterns with peak discharge; however a larger decrease at ST6 (40%) was found for PL when compared against the same at ST3 (30%) which was opposite during summer event (Figure 4.6g). No change in FI values were displayed at both these locations before and after storm (Figure 4.6i).

Considerable differences in terms of DOM response were observed between events and across drainage locations for the two events (July 31 and December 9) considered here. During summer events, although DOC, $a_{254}$ and HIX followed a similar pattern of peaking with discharge peak, yet larger differences in response of two drainage locations was absent (Figure 4.6a-e). However, the same drainage locations displayed more differences in DOM response during the winter (high discharge season) event (Figure 4.6f-j). In particular, more separation of DOM responses (DOC and HIX) was visible at least during prior event condition with higher values at ST6 (Figure 4.6f and h). Likewise, more separation in PL was observed with ST3 being higher during winter event (Figure 4.6g). Therefore, DOC, $a_{254}$, and PL were more consistent parameters in the expression of DOM responses, especially when two drainage locations were compared during events. HIX presented a unique
pattern at both locations such as it remained high even after the low flow conditions prevailed following the both summer and winter events.

To determine the differences in DOM patterns between ST3 and ST12 drainage locations, two storm events (September 30 – October 1, 2010 and August 14, 2011) were selected (Figure 4.7). Both these events possessed bi-modal responses in peak discharge corresponding to two precipitation events. DOM responses not only varied with-in events, but also displayed variations among the drainage locations during both these events. Although these events covered only summer season, yet they occurred during different hydrologic conditions in the catchment. For example, September 30 – October 1 event occurred after a long dry spell in 2010 whereas August 14 event occurred following another event on August 9 of the same year. Thus, hydrologic conditions and antecedent soil moisture conditions were different in the catchments during these two events. Interestingly, peak discharge amounts were almost similar for these events (September 30 – October 1 = 3.68 mm hr\(^{-1}\) and August 14 = 3.55 mm hr\(^{-1}\)), but DOM response varied with-in events as well as among catchment locations.

For the September event, the values for DOC and \(a_{254}\) increased sharply during the first event, but then displayed a slight dilution at peak flow during the second sub event (Figure 4.7a-e). However, influence of dilution in DOC and \(a_{254}\) was larger at ST3 when compared against ST12 (Figure 4.7a and e). In particular, the values for DOC dropped by 13% and peaked by 18% during second discharge peak at ST3. Similarly, \(a_{254}\) varied from 14% to 11% during the event peak period at the same location. In comparison, DOC drop and peak were 9% and 4% respectively during second discharge peak at ST12. However, relatively smaller variations in the pattern
Figure 4.7: DOM concentrations and quality indices (DOC, %PL, HIX, FI, and $a_{254}$) for the events of September 30 – October 1, 2010 and August 14, 2011 for the two catchment locations ST3 (blue symbols and lines) and ST12 (green symbols). Solid blue line represents discharge at ST3 catchment.
of a$_{254}$ at ST12 were displayed during the same time period such as a drop of 2% and increase of 6% respectively were noticed. This indicated a quick response shown at ST3 with frequent changes in a$_{254}$ values corresponding to changes in discharge during storm flow. Also, DOC and a$_{254}$ values were higher at ST3 than the same at ST12 during both the peaks of this event.

HIX values displayed a fairly consistent trend, similar to DOC, with an increase on the rising limb of the hydrograph with a maximum at or after the first discharge peak, and then a decrease through event recession for both ST3 and ST12 although the increase at ST12 was larger than at ST3 (Figure 4.7c). During the second discharge peak, HIX values at ST3 were stable but subsequently decreased through the hydrograph recession whereas for the same time period, at ST12, HIX values followed a dilution trajectory during the discharge peak but end up at a relatively higher value when the low flow conditions prevailed following the event.

In contrast, PL and FI followed generally inverse patterns to DOC, a$_{254}$, and HIX at both drainage locations for September event (Figure 4.7b and d). However, some contrasting patterns in PL response were displayed for drainage locations. For example, PL at ST3 peaked with first discharge peak while the same declined at ST12. Prior to second discharge peak, PL increased at ST12 (16.19%) and reduced to roughly half of its preceding value (8.34%) during the maximum peak discharge, followed by a slight increase after the peak (10.45%). PL followed a dilution trajectory throughout the second discharge peak at ST3 and increased substantially by 50% following the event recession (Figure 4.7b). Nevertheless, during the complete storm period from pre-event to post-event conditions, ST3 and ST12, both observed similar decline of PL (roughly 30%), with a four-fold variation at ST3 (6.31 to 23.98%)
compared to a two-fold variation at ST12 (8.02 to 16.19%). Similarly, from pre-event to during event conditions, ST12 experienced a decline of 9% in FI values, almost double of the same at ST3 (5%). For the complete storm duration, roughly 5% drop in FI values at ST12 was noted against 1% at ST3 (Figure 4.7d). Also, an interesting observation for FI values with drainage locations was that ST12 possessed higher FI values than ST3 before both the discharge peaks, and reduced below than the same at ST3 during event recession during both discharge peaks. In fact, lowest FI values for the second discharge peak at ST3 was observed during maximum, whereas the same for ST12 was observed on hydrograph recession limb. In general, both locations displayed a subsequent decrease in FI values following the end of storm period, with a relatively larger decrease at ST12. For instance, from pre- to post-event conditions during storm duration, FI dropped from 1.47 to 1.40 at ST12 when compared against the same at ST3 (dropped from 1.42 to 1.41).

Event of August, 2011 displayed different DOM responses at ST3 and ST12 drainage locations due to varying antecedent moisture conditions for this event (Figure 4.7f-j). DOC and $a_{254}$ values during first discharge peak followed an increase with discharge in general for ST3 and ST12 drainage locations (Figure 4.7f and j). However, a slight dilution for both these parameters was also noticed with larger intensity at ST12. Difference between ST3 and ST12 DOC values was also larger in magnitude for the first discharge peak in comparison to the similar trend shown during September event (Figure 4.7a and f). For instance, DOC increased from its pre-event value of 4.98 to its maximum value of 18.35 during the first peak discharge at ST3; however change in DOC value was noted from 3.70 (pre-event) to 10.89 (just before second peak discharge) at ST12 almost after 5 hours. An interesting pattern in DOC
and $a_{254}$ values was observed with drainage locations such as a steady rise in these parameters at ST12 on the falling limb of the hydrograph recession during first discharge event. In contrast, the same parameters declined at ST3. With the arrival of second discharge peak, DOC and $a_{254}$ again increased on the rising limb of hydrograph at ST3 and achieved their lowest values at the maximum discharge value, then increased on the falling limb of the hydrograph recession before displaying an expected pattern of further drop in DOC and $a_{254}$ values on the falling limb. In contrast, ST12 displayed lowest DOC and $a_{254}$ values just prior to the maximum peak, increased on rising limb, decreased on falling limb, and then increased again before showing a general trend of declining with deceasing discharge. Over the complete duration of storm, DOC at both drainage locations varied similarly such that roughly 44% increase was observed at ST3 (from pre- to post-event) while the same at ST12 was about 39%. However, for the same duration, $a_{254}$ ranged from 21 to 152 at ST3 (167% increase) and 31 to 88 at ST12 (26% increase), indicating a greater export of aromatic DOM from ST3 (Figure 4.7j). It is worth to note here that the lack of discharge data from ST12 may confound this observed pattern of DOC and $a_{254}$, especially at ST12. Nevertheless, highly variable response of DOC and $a_{254}$ with second discharge peak (which was also maximum discharge in magnitude) displayed that ST3 (reference catchment) responded quickly with stream discharges, whereas the same responses at ST12 (largest catchment) were reduced.

HIX values displayed similar variations among the drainage locations (4% change from pre- to post-event conditions at both locations) for the complete duration of the storm (Figure 4.7h). However, an interesting pattern in HIX values was noted at ST12 location, a sharp decrease on the rising limb, just prior to the maximum peak.
discharge for both peaks, whereas, HIX increased at ST3 with the rising limb of the hydrograph during both peak events. Similar to the patterns observed for the September event, HIX values were remained slightly higher than pre-event conditions at both drainage locations. Changes in FI values from pre-event to post-event conditions across drainage locations did not differ; although a minimum FI value was observed at the rising limb of second discharge peak at ST3 in contrast to the same observed at the falling limb for ST12 location (Figure 4.7i). Also, with the second discharge peak, FI increased slightly at ST3 whereas it follows a dilution trajectory at ST12. Following the end of the event, FI values increased at both the drainage locations but were still similar to the pre-event FI values.

Differences in PL during peak discharges among drainage locations were similar and were noted as a decline by approximately 50% from its pre-event values (Figure 4.7g). Also, there were small differences between pre- to post event PL values at both these locations. For example, a decrease by 28% in PL is observed at ST3 whereas the same at ST12 was 26%. However, PL values increased from its lowest values during event to post-event conditions at both drainage locations. But this increase was almost 1.5 times larger at ST3 (63%) than at ST12 (42%). Also, during the peak event conditions, PL values at ST3 were fairly constant while for the same duration, PL follows strong dilution trajectory at ST12 indicating a sharp decline in PL values at the peak discharge conditions. Following the end of first peak discharge, PL at ST3 were slightly higher than the same at ST12; however, this pattern reversed following end of second discharge peak and ST12 displayed higher PL values than at ST3. Nonetheless, PL values although followed a pattern of decrease with increasing
discharge peaks for both the locations, yet they never retained their pre-event values for either drainage locations.

Therefore, again DOC, a$_{254}$, and PL were found as consistent parameters in the expression of DOM responses when two drainage locations (ST3 and ST12) were compared during events similar to what have been noticed while comparing ST6 and ST3. HIX and FI although did not present much differences across drainage locations, yet provided some unique patterns at both locations such as HIX remained high even after the low flow conditions prevailed following events whereas FI values retained its pre-event conditions. However, a$_{254}$ emerged as the strongest parameter for comparison among drainage locations, especially during event conditions. Overall, these results indicated subtle differences in DOM responses from different drainage locations and under varying hydrologic conditions.

4.3 Discussion

4.3.1 Spatial pattern of DOM in the stream network during base flow

DOM concentration and quality observed in this study varied noticeably during base flow conditions among the catchments. Previous studies have shown that the variations in DOM properties can be expressed as a function of catchment attributes [Ågren et al., 2008; Aitkenhead-Peterson et al., 2005; Buffam et al., 2007; Catalán et al., 2012; Graeber et al., 2012; Inamdar and Mitchell, 2006; Worrall et al., 2002]. For example, the proportion of wetland coverage was found to be significantly positively correlated with stream DOC concentration in the Krycklan catchments [Ågren et al., 2008]. In contrast, DOC concentration decreased with increasing channel length and drainage area [Ågren et al., 2007; Buffam et al., 2007; Dawson et al., 2001]. Based on
the results of our study in accordance with these studies, significant variations in the DOM patterns is expected among catchments of varying morphological characteristics during base flow conditions.

Our results revealed marked differences in DOM concentration and quality among catchments of varying hydro-morphological characteristics (Figure 4.3). DOC concentration and $a_{254}$ were higher in a groundwater fed seepage wetland catchment (ST6) than those measured for other catchments ($p < 0.05$). In particular, both DOC concentration and $a_{254}$ at ST6 were twice that of other drainage locations (Figure 4.3a and g). These patterns suggest the stream draining at ST6 is not only rich in high DOC fraction but also showed more aromatic DOM character. Our finding is consistent with a recently reported study showing that the highest DOC concentrations are found in stream waters draining wetlands [Ågren et al., 2008], and that DOC from wetland draining streams is more aromatic compared to streams draining forest [Ågren et al., 2008; Kalbitz et al., 2003] with high DOC and aromaticity reflecting terrestrially derived DOM. In our study, similarly high DOC and aromaticity is noticed for soil, shallow groundwater, and wetland soil water sources during base flow sampling. Wetlands generally represent rich carbon stores due to low rates of decomposition of DOM [Moore et al., 2007]. The carbon stored near the surface of these wetlands can be transformed to dissolved forms via both aerobic and anaerobic decomposition [Kalbitz et al., 2000]. DOM produced from microbial activity during dry periods at ST6 under aerobic conditions when decomposition occurs could have subsequently flushed with the rising water table or water soluble DOM products may have mobilized while water flows through the systems during anaerobic decomposition of DOM in the presence of water table at or close to the surface [Creed et al., 2008;
Moreover, considerable degradation of lignin and phenol (which releases aromatic moieties from lignin macromolecule) containing sources have been reported with highly aromatic DOC content [Kalbitz et al., 2006]. Therefore, we may attribute the largest proportion of wetland coverage and presence of surficial DOM sources nearby catchment outlet at ST6 responsible for highly aromatic DOC character as observed in this study during base flow conditions.

Contrary to the patterns of DOC and \( a_{254} \) observed at ST6, other drainage locations (ST5, ST4, ST3, and ST12) displayed lower values of these parameters. In the forest dominated streams with decreasing proportion of wetland coverage, substantial decrease in DOC concentration and \( a_{254} \) have been reported in other studies [Ågren et al., 2007; Wu et al., 2007]. Our results are consistent with these studies, since other drainage outlets consisted major proportion of forested areas, hence lower DOC and \( a_{254} \) are not unexpected. Our EMMA analysis also suggests that forest draining streams were chemically closer to hyporheic, seeps, riparian and groundwater signals during base flow conditions. Since, the streams primarily originated from seeps, fed by groundwater inputs, the chemical signal of these streams should display similar chemical characteristics. Thus, the influence of these sources in stream waters is obvious, especially during base flow conditions.

The concentrations and composition of DOC at any point in the drainage network can be influenced by the hydrologic flow path and a suite of processes that may alter DOM. Maurice et al. (2002) reported a several fold decrease in DOC concentration and aromaticity in a forest stream during low flow conditions in the Pine Barrens region of the Atlantic coastal plain and attributed these decreases to the dominance of the ground water discharge to the stream. In addition, the low DOC
ground water may dilute the DOC concentration in surface stream waters [Dawson et al., 2001; Laudon et al., 2007]. Other mechanisms for declining DOC in stream waters could be consumption of DOC through soil heterotrophic activity [Bengtson and Bengtsson, 2007; Schiff et al., 1990], in-stream processes [Dawson et al., 2001], and/or adsorption to mineral soils [Kalbitz et al., 2000; Neff and Asner, 2001], thus, lower water DOC concentration at stream drainage locations could be a product of both dilution and abiotic/biotic removal processes. Nevertheless, with the understanding of our drainage locations, we consider the dominance of physical sorption processes to be responsible for lower DOC at ST4 and ST5 as these streams are draining at smaller catchment area and also for smaller channel length. These morphological stream characteristics suggest that due to low residence time of water in these streams, biotic processes may give a muted response over abiotic removal processes. However, dilution by groundwater inputs, in-stream processes in addition to sorption phenomena may dictate the low aromatic DOC patterns at larger catchments, ST3 and ST12. In addition to stream channel length the contribution of groundwater inputs also increase along the stream drainage network for larger catchment area, thus it can be presumed that in-stream processes may become more important for low aromatic DOC at larger catchment scale.

Spatial patterns of changes in humic-like (HL) fluorescence and humification (HIX) characteristics of DOM have been found to be different for drainage locations along the length of the stream during base flow conditions (Figure 4.3b and e). Lowest HL fluorescence and humic character of DOM is noticed for the stream draining smallest catchment area, ST5, whereas ST6 is high in humic character of DOM in addition to its contribution for more humified DOM. Our PCA analysis during base
flow conditions shows strong positive correlation between HL-DOM and HIX. Hence, high humic character of DOM at ST6 is positively associated with more humified DOM at this location. The variations in these parameters at drainage locations also suggest the dominance of humic pools of DOM at ST6 compared to other catchment locations. However, high dominance of HL-DOM at ST6 suggests more allochthonous transport since humic DOM is generally derived from terrestrial sources. Similar results have been reported in Precambrian shield lakes and stream environments of Canada by [Williams et al., 2010; Wu et al., 2007]. Our ST6 catchment drains highest proportion of wetlands; thus, high humic composition of DOM along with high DOC is expected. Williams et al. (2010) have reported high humic composition of DOM with increasing wetland influence. This is also consistent with our understanding that HL DOM composition is generally older and diagenetically altered DOM products possessing more humified and aromatic fractions of DOM [McKnight et al., 2001].

Typically highly humified organic matter is common to wetland draining streams and we have noticed the similar result in our previous findings [Inamdar et al., 2011], therefore, highly humified reduced DOM at ST6 catchment is probable. With respect to our EMMA analysis, wetland soils and shallow groundwater influences at ST6 catchment are dominant. Hence, high humic DOM, especially from allochthonous transport of such sources at this scale should be considered influential for observed DOM character.

In contrast to the high HL and HIX values for ST6 catchment, the smallest ST5 catchment which drains a forest stream shows lower values for these parameters. Relative abundance of less humified DOM at ST5 could be attributed to both its seep and hyporheic dominated source (from our EMMA analysis) and shorter flow path
(small channel length). Ground water seeps are generally low in humic character of DOM [Inamdar et al., 2012] and since the smaller channel length would inhibit humification processes due to less residence time, lower value of HIX at ST5 catchment may hold true in this study. Further, HIX is the representation of carbon to hydrogen (C/H) ratio [Zsolnay, 2003] and since our ST5 catchment is primarily ground water driven seep generated stream, we may hypothesize that the increasing contribution of seep water may lower the carbon to hydrogen ratio resulting in lower HIX value because seep waters are generally poor in carbon sources. Additionally, decline in humic DOM composition and HIX value have been noticed in a lab study while investigating influences of increased solar irradiation and adsorption by mineral surfaces of leaf litter extracts in Han River basin, Korea [Hur et al., 2011]. Both these mechanisms were found responsible for lower HIX values at ST5. Such DOM products are considered more susceptible to microbial degradation as compared to their terrestrial counter parts [Hur, 2011]. Based on these studies and our results, we may hypothesize that lower humic content and HIX value at ST5 catchment could be a product of seep waters DOM quality and multiple processes, such as adsorption of high molecular weight DOM by mineral surfaces and microbial degradation of plant derived DOM, acting in conjunction at ST5 catchment.

Contrary to the patterns of DOC, $a_{254}$, HL DOM, and HIX; protein-like (PL) fluorescence of DOM is highest in ST5 catchment followed by lower values at other forest stream locations (Figure 4.3d). However, the lowest PL DOM is noticed for our wetland dominated ST6 catchment. In general, PL fluorescence of DOM is often associated with biological activities such as phytoplankton blooms and microbial activities in aquatic environments [D’Amore et al., 2010; Wu et al., 2007]. The
increase in PL DOM may indicate specific heterotrophic activities in the stream channel while degrading plant- and algal-derived DOM in addition to ground water inputs rich in PL fluorescence in forest streams [Graeber et al., 2012; Hur, 2011; Inamdar et al., 2012]. Although not significant but with an exception of ST6 catchment, we noticed a gradual decrease in PL DOM along the drainage network with increasing catchment sizes. This suggests the preferential removal mechanism of PL DOM in stream network along the way [Balcarczyk et al., 2009; Fellman et al., 2010a]. Our PCA analysis displays the increased contribution of PL DOM at ST4 and ST5 drainage location (Figure 4.5a and b), while our EMMA analysis suggests that seep, hyporheic, and deep groundwater sourced DOM at these drainage locations (Figure 4.1a and b). Combining these two results, we ascertain that these water sources are rich in PL DOM, in particular at ST4 and ST5 drainage locations. In our previous study for the 12 ha catchment, we did find the high PL DOM contribution with increasing groundwater inputs [Inamdar et al., 2012]. Additionally, relatively higher PL at this catchment could have resulted from the abiotic sorption of HL DOM. Other studies have reported the relationship between PL pool and bioavailability of DOM such as with increase in PL pool, more bioavailable DOM is obtained [Balcarczyk et al., 2009; Fellman et al., 2009a, 2009b]. Although we did not perform any bioavailability experiments via incubation procedures, yet based on the results of these studies we could conclude that DOM at ST5 is more bioavailable and hence would support higher degree of microbial activity in comparison to other drainage locations.

Low protein-like fluorescence of DOM at ST6 catchment may be misleading as the greater contributions of aromatic and humic-like DOM at our wetland...
dominated catchment might have over-shadowed the PL DOM values. Besides, PL DOM is generally considered as most bioavailable fraction of DOM and is readily utilized by microbes in metabolic processes [Balcarczyk et al., 2009; Fellman et al., 2009b]. Therefore, the lower PL at our wetland dominated ST6 catchment could have resulted from microbial decomposition of organic matter. Also, this catchment is seasonally influenced by changing water levels, thus, less ground water influences are common compared to other drainage locations. Because of this ST6 catchment has been visually observed to be drier in many parts of its reaches during low flow conditions, consistent with our previous finding as well as other reported studies [Fellman et al., 2009b; Hood et al., 2006; Inamdar et al., 2012]. The lower contributions of ground water inputs in addition to consumption of bioavailable fraction for microbial utilization are possible explanation for lowest PL DOM at ST6 catchment. However, contrary to our finding, Fellman et al. (2009a, b) reported the production of more labile protein-like DOM in wetland soils than upland forest locations. It is important to note here that this contradiction may have evolved due to very different climatology, geology and ecological conditions at their study site in the glaciated, coastal rainforest of Alaska besides the fact that their wetland location represents more bog like landscape structure. In contrast, our mid-Atlantic site is located in un-glaciated region of NE USA and also our wetland does not represent a true wetland, rather it is a groundwater fed seepage area which gets saturated / unsaturated with the seasonal fluctuations of water table.

4.3.2 DOM patterns and surficial sources influence during storm flow

Our study also revealed marked differences of spatial changes in DOC concentration and DOM quality parameters among catchments during storm flow
conditions. Many studies have reported such changes in DOM variations among spatially varying catchment characteristics during event conditions [Fellman et al., 2009b; Hinton et al., 1997; Hood et al., 2006; Inamdar and Mitchell, 2007; Raymond and Saiers, 2010]. DOC concentration and $a_{254}$ shows a linearly decreasing pattern from ST6 to ST12 catchment with increasing drainage area of these catchments (Figure 4.4a and f). This suggests the removal of aromatic DOC along the stream drainage network. Similar trends have been observed by other studies [Buffam et al., 2007; Dawson et al., 2001, 2011; Laudon et al., 2007]. These studies reported this decrease in DOC along the drainage network due to complexation of DOC with other chemical species (e.g., metals) or biological degradation along the drainage networks and that contribution of longer, deeper flow path of increased ground water inputs at larger catchment size. This was also evident from our EMMA observations which indicated the decreasing influence of surficial sources such as precipitation, stem flow, litter leachate, throughfall for runoff from ST6 catchment to larger ST12 catchment. The other reason for this can be that with increasing DOC poor groundwater inputs for increasing catchment area, dilution of aromatic DOC in surface stream waters could have resulted [Dawson et al., 2011; Inamdar and Mitchell, 2006; Inamdar et al., 2011].

Even though a clear pattern in declining aromatic DOC has been observed here, it is important to note that many fold increases in aromatic DOC is also present during storm flow conditions as compared to those from the base flow conditions. A five times increase in DOC concentration is noticed during storm conditions at ST3 while the same for ST12 was almost four times more from those of base flow levels. The lowest increase of almost double of the base flow DOC value is noted for ST6.
catchment. This suggests that although there was an increase in DOC at ST6 during storm flow condition, yet it was lower than what was observed at other stream locations. We may attribute this lower increase of DOC at ST6 to the contributing runoff sources. Since ST6 showed higher values of aromatic DOC during base flow contributions from wetland soil and shallow ground water sources, it may be possible that with the arrival storm conditions, much of these sources have been flushed into the stream and DOC pool may have gotten depleted of its dominant sources during event conditions. Such flushing mechanism during event condition effectively dilute the solute (e.g., DOC) concentration of surficial sources in wetland dominated catchments [Ågren et al., 2008; Dawson et al., 2011].

The larger increases in storm flow DOC at ST3 and ST12 catchment could be due to increased contribution from near stream riparian zones. It has previously been noticed that during the events infiltrating precipitation waters may lower the water table depth. As a result of infiltrating water aquifers may swell followed by increased contribution of groundwater in the saturated transient riparian zones which then directly contribute to storm flow [Katsuyama and Ohte, 2002]. However, the movement of water from riparian zones to the streams, intersection of this moving water with organic rich riparian soils is expected and hence transport of DOM to the streams [Inamdar and Mitchell, 2006; Inamdar et al., 2011]. Numerous studies have reported the mobilization of aromatic DOM during events from terrestrially derived sources. In particular, Saraceno et al. (2009) using the combination of in situ fluorometric measurements with laboratory optical properties indicated a rapid storm driven flux of soil derived aromatic DOM exiting the Willow Slough Watershed, an agriculturally dominated catchment in the western Sacramento Valley of California.
Similar results have been reported by Austnes et al. (2010) while studying event mobilized DOM and its influence on in-stream DOM processing in a Welsh peatland catchment, UK. Both these studies reported the increased DOC concentration and increased absorbance at high event flow which can be explained by an increased contribution of soil water, due to a rising water table within the upper soil horizons and subsurface flow paths. However, the timing, amount, and intensity of precipitation event is equally important in DOM expression in streams [Hood et al., 2006]. This has been noticed in our storm event of September 30 – October 1, which occurred after a long dry spell during summer of 2010. Contrasting pattern of aromatic DOC is noted with the two peaks of this event. Firstly, DOC followed an increasing concentration with discharge but later a dilution trajectory has been noticed with the arrival of much stronger discharge peak for our ST3 and ST12 catchments (Figure 4.7a). However, higher variations were noticed for ST3 catchment and relatively muted DOM response at our larger catchment. More details for this particular event can be found in our previous study [Inamdar et al., 2011]. Nevertheless, the results here present the evidence of increasing contributions of surficial sources (high aromatic DOC) in all catchments during storm flow conditions with highest influence of these sources at our 12 ha catchment. The drop in DOC from ST6 to ST3 was 17% which increased further downstream from ST3 to ST12 by 22% suggesting removal of aromatic DOC due to sorption on stream-bed and in-stream biological activity along the drainage network. Subsequently, declining aromatic DOC could have resulted from dilution by ground waters and in-stream biotic processes in our largest catchment (ST12). Dilution and depletion of DOM sources at ST6 could have caused relatively less increase of
aromatic DOM during events compared to base flow at our wetland dominated catchment.

Similar to the patterns displayed by DOC concentration and aromatic character of DOM (a$_{254}$) among the catchments during events, humic-like composition and humified DOM character declined linearly from ST6 to ST12 with increasing catchment size (Figure 4.4b and e). However, the decline in humic DOM character should not be attributed to the drainage area of catchments; rather source contribution at different spatial scales may be more plausible explanation for this variability in DOM character. Our wetland dominated catchment (ST6) DOM shows its humic character due to contribution from precipitation and surficial sources such as litter leachate and throughfall. Further downstream, relatively less humic DOM composition at ST3 and ST12 is noticed. This may have resulted from the relatively less influence of terrestrial sources at larger catchments and more from dilutions of humic DOM with contributing ground waters. Also, the decline in humic DOM composition at all drainage locations was in the range of 10-15% from its base flow levels. This suggests that either not much humic DOM is available for transport during events or humic DOM is the dominant fraction during base flow as well, thus there is not much change in humic DOM fraction with changing hydrologic conditions. Another explanation for decreasing humic DOM composition at larger catchments could be due to the oxidation of reduced humic composition which is consistent with other studies [Miller et al., 2006; Williams et al., 2010]. Fellman et al. (2009a) suggested that DOM leached from wetland soils can be an important source of carbon as energy source to downstream microbial community. Based on these studies and our results, we can conclude that even though humic composition is the dominant DOM fraction (nearly
50% of total DOM pool) at our site independent of hydrologic conditions, microbial metabolism and oxidation of reduced humic DOM composition may be occurring at larger catchments due to increased contribution of oxygenated ground waters; therefore, relatively less change in this composition is observed with events. This is also consistent with the hypothesis put forward by Austnes et al. (2010) that at least some part of the aromatic, humic DOM released during events could have rapidly processed close to where it was released.

HIX values also followed the similar decreasing pattern as that of humic DOM suggesting the at larger stream drainage area, reduction in humified organic matter sources occurred. This is not improbable since much humification of organic matter usually occurs in soils in forest than in streams [Kalbitz et al., 2000; Zsolnay, 2003] and at larger catchment drainage area influence of soils is expected to be decreasing compared to small catchment. Hence, lower humified organic matter is obvious in streams draining larger area compared to those draining smaller catchment area. However, post event conditions, a slight rise in HIX values have also been noted compared to base flow levels which is interesting. We have attributed this difference in HIX values to DOM sources, release mechanisms and associated kinetics. In particular, for our September 30 – October 1 event, we noticed a different pattern of HIX among ST3 and ST12 catchment. HIX remained constant with second peak discharge at ST3 while it followed a dilution trajectory for ST12 catchment (Figure 4.7c). Likewise, a different pattern for the same parameter is observed with first peak for August 14 event for these catchments (Figure 4.7h). It is interesting to note here that before August 14 event, already a small event occurred on August 9 which kept the abundant moisture conditions at ST12. Based on our results, we may hypothesize
that if a catchment is already wet, humified organic matter may follow a dilution trajectory with increasing discharge, especially with a larger catchment area; at least this is true for our study site. This is in contradiction to our understanding that with increasing discharge, increasing humified organic matter has been delivered to the streams. The above conclusions need further examination of how the HIX values are being computed using fluorescence EEM data or the dynamics of humified organic matter must be explored by other means (e.g., lab analysis) to better understand their character, especially during events with consideration to catchment properties (e.g., wetness).

We also observed marked differences for protein-like DOM among catchments during events (Figure 4.4d). Additionally larger differences for PL DOM have been noticed during events compared to those during base flow conditions (Figure 4.3d and Figure 4.4d). No significant difference for PL composition of DOM is noticed between ST3 and ST12 catchment ($p > 0.05$). However, wetland dominated catchment displays a significantly different and lowest PL DOM values in our study ($p < 0.05$). An increase of 16% is noticed for PL DOM from ST6 to ST3 along the drainage network while the same from ST3 to ST12 was only 7%. Previously using EMMA analysis at this same site for 12 ha outlet, Inamdar et al. (2011) reported the lowest PL values in surficial sources compared to significantly higher values in ground water sources during event conditions. During storm conditions, PL composition generally followed a dilution trajectory with increasing discharge (Figure 4.6 and Figure 4.7). Thus, with increasing ground water sources at ST3 and ST12, relatively higher PL composition observed is obvious whereas relatively low PL at ST6 is attributed to runoff generated from surficial sources at this location. Relative decline in PL
composition at ST6 during events could also be ascribed to relative increase in humic DOM composition [Fellman et al., 2009b; Inamdar et al., 2011]. However, with increasing groundwater inputs at larger catchments, increase in PL is consisted with other reported studies [Balcarczyk et al., 2009; D’Amore et al., 2010]. A decrease in protein-like fluorescence was also observed by Nguyen et al., (2010), with the lowest protein-like DOM corresponded with the peak discharge in stream in a Korean agricultural-forested watershed and was suggested as DOM produced during storm events was low in protein content. They attributed this low protein content derived from protein-poor sediment sources. Contrary to this, Fellman et al. (2009b) attributed the decrease in total protein-like fluorescence for the upland watershed during storm events resulting from the dilution of high autochthonous production of amino acids by terrestrial DOM inputs.

A clear decline in protein-like DOM during events compared to base flow condition is observed at all drainage locations (Figure 4.3d and Figure 4.4d). ST6 and ST12 displayed roughly 30% decrease in their PL content from those of base flow levels; however, the same reduced to half of its base flow values at ST3. This is an interesting observation because ST12 is our largest catchment and we expected more decreases at ST12. This suggested that the PL DOM sources released into ST3 during events were quickly utilized or that riparian soils near ST3 draining stream could have stored larger PL DOM pools which got flushed during storms. Fellman et al. (2009b) also noticed decrease in protein-like composition during events from base flow levels in forested wetlands in Alaska. The same study indicated a potentially available pool of labile DOM in soil solution could have flushed to streams during initial storm stages. However, in the same study, poor hydrologic connectivity of streams with
nearby riparian soils was attributed for lower protein content in streams which is in contradiction to our study. However, substantial decreases in PL from its base flow levels at all drainage locations may also suggest corresponding relative increases in aromatic, humic DOM during storm conditions. This appears as more plausible explanation as we observed high contribution of aromatic DOM during event conditions at each of these drainage locations. The highest increase was noticed at ST3 and correspondingly highest loss of protein-like DOM at the same drainage location. Therefore, the relative changes in various DOM fractions should be considered in addition to catchment characteristics, especially for stream drainage network at multiple spatial scales.

4.4 Conclusions

This study presented the spatial variations of DOM concentration and quality along a stream drainage network at multiple catchment scales in a Piedmont forested catchment. The variations in DOM concentration and optical properties during base flow and storm flow conditions provided insights about the catchment specific characteristics and related processes. Combination approaches of optically measured DOM quality parameters and EMMA analysis have further been helpful in identifying the dominant sources and flow paths of DOM during different hydrologic conditions pertaining to specific catchments. The key conclusions that can be drawn from this study are:

1. ST6 catchment showed higher DOM concentration and more aromatic, humic character of DOM compared to other drainage locations during base flow condition. Highest protein-like DOM composition is observed at the smallest catchment, ST5 which subsequently decreased downstream.
2. High DOC and highly aromatic, humic character at ST6 during base flow followed a dilution pattern during storm events while increased DOC concentration and more aromatic, humic character of DOM at other stream locations suggests surficial flow paths contributions from near stream riparian zones.

3. Largest increase in DOC concentration and aromatic, humic DOM character is noted at 12 ha (ST3) outlet during storm events while a lowest change for DOM responses is present at ST6.

4. Base flow DOM responses suggest catchment characteristics as key drivers such as a higher proportion of wetland coverage at ST6 exhibiting higher DOM concentration. Relative influence of catchment drainage area is absent during base flow. Attenuated DOM response is observed with respect to drainage area during storm flow conditions suggesting the biotic/abiotic (e.g., sorption and in-stream processes) removal of DOM along the drainage network.
Chapter 5

SEASONAL PATTERNS OF DISSOLVED ORGANIC MATTER (DOM) IN STREAM WATER AND WATERSHED RUNOFF SOURCES

5.1 Introduction

Dissolved organic matter (DOM) exported from watershed sources to streams in forests have manifold effects on the ecology of aquatic ecosystems. It plays important role in the transport and mobilization of nutrients, metals, and organic pollutants [Aiken et al., 2011; Fellman et al., 2010a; McKnight and Bencala, 1990]. DOM also plays a major role in the regulation of photic zones due to its capability of absorbing biologically harmful ultra-violet (UV) light in the aquatic ecosystems [Cory et al., 2004; Fellman et al., 2009a; Jaffé et al., 2008]. More importantly it serves as an energy source to heterotrophic microbial community [Amon and Benner, 1996; Cole et al., 2007; Tranvik, 1992]. While utilizing carbon as energy source, heterotrophic bacteria mineralizes the allochthonous DOM and increases the carbon efflux from streams, thus turning streams into net sources of carbon dioxide to the atmosphere [Butman and Raymond, 2011]. However, the biogeochemical process of production, consumption, and mineralization of DOM largely depends on the quality of organic matter at a micro-site or under transport rather than extensively on its amount or concentration. Moreover, seasonality in catchment flow path dynamics in addition to in-stream biological processes also renders changes in DOM quantity and quality. Therefore, it is critical to understand and identify the chemical composition of DOM and their seasonal controls in terrestrial and aquatic ecosystems.

Numerous studies have shown the variable amounts of DOM from forest ecosystems and related it to seasonal changes [Boyer et al., 1997; Kaiser et al., 2001;
Further these studies have shown the influence of hydrological variations in conjunction with microbial activity throughout the course of year causing variable DOM exports. For instance, Kalbitz et al. (2000) determined the interactions of a range of biotic and abiotic mechanisms governing the production and transport of DOM in soil solutions and runoff waters. They recognized that solubilization of macromolecular forms of DOM (e.g., fulvic and humic acids) and simpler molecules from microbes and vegetation could have resulted from litter decomposition and humification of soil organic matter. Holmes et al. (2008) found biodegradable dissolved organic carbon (BDOC) from three rivers draining into the Arctic Ocean was greatest during the spring freshet and lowest during the summer due to variable biotic demand. Likewise, Fellman et al. (2009) have also found the increased transport of labile DOM during spring and wet fall as compared to dry summer period. They attributed this variation in DOM export to the annual variation in water table, residence times, and biotic demands for the season. Another study using a modeling approach show strong patterns of seasonality in soluble DOM compounds related to temperature, rainfall patterns and soil moisture cycles [Lumsdon et al., 2005]. Worrall and Burt (2010) attributed the influence of flow paths and residence times on the changes in DOM quality released from soils while transport to the stream. Likewise, temporal changes in the sorptive and physicochemical properties of DOM from a small watershed in the northeastern USA have been explored where temporally varying DOM reactivity was determined [Meier et al., 2004]. Based on these studies, it is clear that both biological processes that affect DOM supply (by production and consumption) and hydrological drivers that may influence DOM transport may shape the seasonal expression of DOM. In addition to changes in concentrations these
seasonal changes may extend to changes in DOM quality (or its chemical composition). Thus, it is important to determine the seasonal variations in the DOM that may reflect the seasonal changes in its biochemical properties and in turn its quality to identify the controls over the course of year.

The major challenge in the characterization of DOM existed due to its naturally heterogeneous complex structures; however recent advancement in instrumentation and measuring techniques has substantially overcome this issue [Cory et al., 2011; Fellman et al., 2010a]. Rapidly increasing use of spectroscopic properties of DOM over traditional tedious bio-chemical approaches have aided researchers in quick determination of DOM biogeochemical properties and hence its reactivity within an ecosystem [Chen et al., 2013; Fellman et al., 2009a; Maie et al., 2012]. Many studies have utilized the optical properties of DOM for its characterization and have found strong correlations between traditional characterization methods and optical determinations [Fellman et al., 2009a; McKnight et al., 2001]. Subsequently, previous studies in variety of ecosystems have found seasonal differences in DOM quantity and quality. for example, Catalán et al. (2012) found increased aromatic character in DOM exports during autumn under hydro-morphological influences compared to smaller and freshly produced DOM during winter-spring period indicative of land uses effects in Mediterranean ephemeral washes. Conversely, in European headwater streams, agriculture practices have been found to affect the changes in DOM amount and composition, but inconsistent seasonal patterns were observed here for DOM composition [Graeber et al., 2012]. Further, highly aromatic DOM export in peat-based coastal wetland site during dry period (May-Oct) was reported against wet season (Nov-Apr); whereas DOM in marl-based freshwater
environment was significantly low during dry period indicating different controls that were driving the DOM concentration and composition in two morphologically varying study sites [Chen et al., 2013]. Thus, these and many other studies suggests that seasonal dynamics in DOM composition have been successfully explored using optical techniques [Fellman et al., 2009a; Maie et al., 2012; Miller and McKnight, 2010].

While previous studies have evaluated seasonal patterns of DOM in stream waters, few have explicitly evaluated the difference in seasonal patterns for base flow and storm flow. The hydrologic flow paths for base flow and storm flow differ so one would expect differences in seasonal patterns of DOM. Even fewer studies have explored seasonality of DOM for various watershed runoff sources like throughfall, litter leachate, soil water, riparian, and deep groundwater. For this study, seasons were defined as: Winter (December, January, and February); Spring (March, April, and May); Summer (June, July, August, and September); and Autumn (October, and November). This work builds on our previous studies where we have already characterized the variability in DOM across watershed sources [Inamdar et al., 2012] and identified the flow paths for exports of DOM during storm events [Inamdar et al., 2011, 2013]. Watershed sources that were sampled included – throughfall, litter leachate, soil water, riparian, shallow and deep ground waters, and groundwater seeps. Data was available for a four-year period spanning 2008-2011. DOM composition was characterized using a suite of spectro-fluorometric techniques and a excitation-emission parallel factor analysis (EEMs-PARAFAC) model specific to the site was developed. Specific questions that were addressed:

- How do the seasonal signals vary between stream water base flow and storm flow conditions?
• How does the seasonal DOM pattern vary across various watershed sources?

• Are there particular seasonal events (or hot moments) that display strong signals?

Our overarching hypothesis in this study was that surficial flow paths that bypass the mineral soil DOM sorption filter would produce the strongest seasonal DOM signal. In contrast, hydrologic flow paths that traversed deeper through the soil profile would lose a significant portion of DOM via sorption to the mineral soil and thus result in dampening of the seasonal signal. Thus, we expected that stream water during storm flow would have a pronounced seasonal DOM signal than base flow. Similarly surficial watershed sources such as throughfall, litter leachate, and soil water would display more seasonality than groundwater DOM sources.

5.2 Results

5.2.1 Temperature, precipitation, and runoff across the seasons

Mean seasonal temperatures ranged from -0.6 to 20.5 °C with a highest value in summer and a lowest value in winter (Figure 5.1). Spring and fall seasons showed similar mean air temperature (10.7 and 8.5 °C, respectively). However, both the average precipitation (mm) and specific discharge rate (mm hr\(^{-1}\)) showed considerable variations across seasons. Summer showed highest average precipitation of 478.4 mm and the lowest average specific discharge of 0.03 mm hr\(^{-1}\). The lowest average precipitation of 176.9 mm occurred during fall season with similar average specific discharge as of summer. Winter and spring showed similar average specific discharge rates of 0.07 mm hr\(^{-1}\). Winter and spring of all study years showed high discharge rates than other seasons indicating high water levels during these periods when the
Figure 5.1: Distribution of (a) average daily air temperature, and total daily precipitation (black lines and dark grey bars respectively), and (b) mean daily specific discharge (dark grey lines), are shown for the four study period in the study watershed. Dashed lines represent separation between years. Red cross and blue empty circles represent storm flow and baseflow DOC sampling at the 12 ha catchment outlet.
catchment is wet and water table is generally high. However, average precipitation during winter and spring period was similar with a slightly higher value in spring (Table 2.3).

In general, mean temperatures were high during summer, relatively similar during spring and fall, and lowest during winter. Although average precipitation showed year around distributional pattern, there was considerably higher precipitation received during summer period compared against other seasons. Mean discharge values are generally high during spring and winter and were mostly at its lowest during summer dry conditions which is consistent with our understanding of general hydrologic cycle (Figure 5.1 and Table 2.3).

5.2.2 Seasonal pattern in DOM for stream water during base flow and storm flow

DOM concentration (i.e., DOC) and quality parameters (i.e., UV and fluorescence metrics) were considerably different across seasons in stream samples both during base flow and storm flow conditions (Figure 5.2 and Table 5.1). While not statistically significant, base flow DOC concentration during summer (2.3 mg l$^{-1}$) was higher than the other seasons. The lowest DOC was noted during spring (1.4 mg l$^{-1}$) (Figure 5.2a). The fluorescence analysis of DOM suggested the highest humic-like component was in abundance during spring and summer (roughly around 54%) whereas the lowest was in winter (around 50%) (Figure 5.2d). Fulvic-like DOM component was similar across seasons and showed its contribution of roughly 29% of the total DOM pool (Figure 5.2e). In contrast, protein-like DOM showed slight variations across seasons with a highest value in winter (21%) to a lowest in summer (16%) (Figure 5.2f). No seasonal differences were observed for HIX values (Figure
High variability for \(a_{254}\) in spring and winter was found when compared against summer and fall. While the highest \(a_{254}\) (15.6 m\(^{-1}\)) was noticeable in spring, the lowest corresponds to the fall (11.5 m\(^{-1}\)) (Figure 5.2b). FI was the only parameter that showed significant differences across seasons for the stream water during base flow conditions. The highest FI was observed during fall (1.42) and the lowest was noted in spring (1.39) (Table 5.1).

Seasonal changes in DOM concentration and metrics were more pronounced for stream water during storm flow conditions in stream. DOC concentration ranged from 5.8 mg l\(^{-1}\) during winter to 9.3 mg l\(^{-1}\) in fall (Figure 5.2a and Table 5.1). Also, DOC concentration during summer (9.2 mg l\(^{-1}\)) was similar to fall. Similarly spring DOC concentration (5.9 mg l\(^{-1}\)) was alike to winter. One-way ANOVA revealed significant differences between summer-fall DOC and winter-spring DOC. Humic-like DOM showed significant difference between summer (62%) and fall (58%) while spring and winter (roughly around 60%) were similar to both fall and summer seasons for this parameter (Figure 5.2d). Fulvic-like DOM also increased in summer (30%) as compared to the same in fall (28%) (Figure 5.2e). The largest change was observed for protein-like DOM in fall (14%) with an almost double of its summer value (8%). Spring and winter protein-like DOM were similar (~ 11%) and were intermediate between summer and fall values (Figure 5.2f). HIX also showed a seasonal pattern with a highest value (0.91) in summer and lowest in fall (0.87) (Figure 5.2c). FI ranged between 1.36 (summer and winter) to 1.38 (fall and spring) indicating changes in DOM dynamics, especially during transition periods (spring and fall) of the year (Table 5.1). Similar patterns for \(a_{254}\) was found as of DOC and was significantly higher during fall and summer (78.8 to 79.9 m\(^{-1}\), respectively) while the same was
Figure 5.2: Distributions of mean (a) DOC, (b) a_{254}, (c) HIX, (d) %Humic-like, (e) %Fulvic-like, and (f) %Protein-like DOM composition, for base flow and event flow samples across seasons are shown here. Same alphabets indicate no significant differences for each source (base flow or event flow). Note the changes in y-scale.
Table 5.1: Seasonal distributions of DOM concentration (i.e., DOC), and optical parameters (DOM metrics from UV and fluorescence measurements) for stream water during base flow and storm flow are shown for a four year (2008-2011) study period in the mid-Atlantic forested catchment.

<table>
<thead>
<tr>
<th>Season</th>
<th>DOC (mg l(^{-1}))</th>
<th>% Humic-like</th>
<th>% Fulvic-like</th>
<th>% Protein-like</th>
<th>HIX</th>
<th>FI</th>
<th>a254 (m(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Base Flow</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>1.43 ± 0.32 a</td>
<td>54.39 ± 6.09 a</td>
<td>28.66 ± 1.59 a</td>
<td>16.95 ± 6.52 a</td>
<td>0.85 ± 0.05 a</td>
<td>1.39 ± 0.03 b</td>
<td>15.64 ± 11.71 a</td>
</tr>
<tr>
<td>Summer</td>
<td>2.33 ± 1.93 a</td>
<td>54.88 ± 9.03 a</td>
<td>28.83 ± 1.62 a</td>
<td>16.29 ± 10.35 a</td>
<td>0.85 ± 0.09 a</td>
<td>1.40 ± 0.03 ab</td>
<td>14.10 ± 4.07 a</td>
</tr>
<tr>
<td>Fall</td>
<td>1.50 ± 0.77 a</td>
<td>52.37 ± 2.79 a</td>
<td>28.67 ± 1.41 a</td>
<td>18.96 ± 3.58 a</td>
<td>0.83 ± 0.02 a</td>
<td>1.42 ± 0.03 a</td>
<td>11.51 ± 6.83 a</td>
</tr>
<tr>
<td>Winter</td>
<td>1.91 ± 1.75 a</td>
<td>50.31 ± 5.16 a</td>
<td>28.86 ± 2.66 a</td>
<td>20.84 ± 7.07 a</td>
<td>0.81 ± 0.06 a</td>
<td>1.40 ± 0.02 ab</td>
<td>12.64 ± 11.30 a</td>
</tr>
<tr>
<td><strong>Storm Flow</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>5.87 ± 1.51 b</td>
<td>60.27 ± 3.15 ab</td>
<td>28.55 ± 1.07 b</td>
<td>11.18 ± 2.91 a</td>
<td>0.89 ± 0.02 b</td>
<td>1.38 ± 0.03 a</td>
<td>50.30 ± 14.10 b</td>
</tr>
<tr>
<td>Summer</td>
<td>9.17 ± 1.77 a</td>
<td>61.74 ± 1.35 a</td>
<td>30.33 ± 1.47 a</td>
<td>7.94 ± 1.17 b</td>
<td>0.91 ± 0.01 a</td>
<td>1.36 ± 0.02 b</td>
<td>79.94 ± 12.62 a</td>
</tr>
<tr>
<td>Fall</td>
<td>9.31 ± 1.32 a</td>
<td>57.85 ± 3.83 b</td>
<td>27.89 ± 0.40 b</td>
<td>14.26 ± 4.17 a</td>
<td>0.87 ± 0.03 b</td>
<td>1.38 ± 0.02 ab</td>
<td>78.83 ± 16.94 a</td>
</tr>
<tr>
<td>Winter</td>
<td>5.80 ± 2.78 b</td>
<td>59.20 ± 3.88 ab</td>
<td>29.22 ± 1.20 ab</td>
<td>11.58 ± 3.33 a</td>
<td>0.88 ± 0.03 b</td>
<td>1.36 ± 0.02 ab</td>
<td>54.03 ± 22.21 b</td>
</tr>
</tbody>
</table>

*Data is represented as Mean ± Std. Dev. Same letters are NOT significantly different.
lowest in spring (50.3 m\(^{-1}\)) but was not dissimilar to its winter value (54.0 m\(^{-1}\)) (Figure 5.2b). Nevertheless, stream DOM concentration and quality metrics successfully showed seasonal differences using one-way ANOVA for storm flow conditions which was absent during base flow.

Furthermore, principal component analysis (PCA) also revealed slight differences across seasons. The mean score and loadings for each season during base flow is shown (Figure 5.3a). First two axes of the PCA explained 77.7% of the total variance. The first PC (PC 1) axis explained 55% of the total variance and was positively related with humic-like and HIX and was negatively related with protein-like DOM component. The second PC (PC 2) explained 22.7% of the total variance. PC 2 was positively correlated with FI and fulvic-like DOM components while it was negatively correlated to \(a_{254}\). Close inspection of score and loading plots together revealed that fall and winter seasons are predominantly influenced by protein-like DOM signatures whereas spring and summer season showed rich humic-like DOM character.

Compared to base flow, seasonal mean scores and loading for storm flow displayed much larger separation between seasons (Figure 5.3b). First two PC axes showed 75.7% of the total variance. The first PC axis explained 57.1 % variation while the second PC axis explained 18.6% of the total variance. Loadings showed the positive dominance of humic-like and HIX variables on PC 1. DOC concentration weighed more on the second PC compared to base flow conditions whereas FI showed insignificant loading on the PC2 (opposite to the same during base flow) (Figure 5.3b). An interesting observation was noticed for fulvic-like DOM and \(a_{254}\) during storm flow PCA plots when compared against base flow (Figure 5.3a and b). While during
Figure 5.3: Mean seasonal distributions of PCA scores and loadings for (a) base flow, and (b) storm flow are presented here. Green represent Spring, red represent Summer, brown represent Fall, and blue represent Winter season.
base flow fulvic-like DOM was positively correlated with PC 2, during storm flow a negative correlation was observed. Likewise, $a_{254}$ showed a negative correlation during base flow but changed its character during event and became positively correlated with PC 2 during storm flow conditions (Figure 5.3a and b). These changes in relationship of fulvic-like DOM and $a_{254}$ with PC 2 indicate changes in DOM dynamics with the changes in hydrological conditions (i.e., base flow versus storm flow conditions).

5.2.3 Seasonal distribution of DOM concentration and composition in selected watershed sources

One-way ANOVA demonstrated seasonal differences in DOM parameters for TF and LT samples (Table 5.2). For TF, winter DOC (6.25 mg l$^{-1}$) was significantly different and lower than the same in other seasons. Highest DOC was observed in spring (21.1 mg l$^{-1}$) which was almost double of its values in summer and fall (10.6 and 10.9 mg l$^{-1}$, respectively) but statistically indifferent. Similarly, $a_{254}$ was also lower and different for winter (24.0 m$^{-1}$) than for the same in other seasons. The highest $a_{254}$ corresponded to summer (94.7 m$^{-1}$) that was roughly four times higher than the same in winter. Also, winter $a_{254}$ stand at roughly one-third of the $a_{254}$ values during spring and fall. Another significant seasonal variation in TF was observed for humic-like DOM which followed the similar pattern of DOC. The highest humic-like DOM composition was observed in spring (63%) whereas the lowest was in winter (55%). During summer and fall, humic-like DOM values were between 58-60%. None of the other measured DOM metrics showed any seasonal differences for TF samples (Table 5.2). However, DOC concentration for LT was highest in summer (40.7 mg l$^{-1}$) and lowest in the winter (23.3 mg l$^{-1}$). For spring and fall, DOC concentrations in LT
were 29.9 and 36.7 mg l$^{-1}$, respectively. Significant difference for summer DOC was found with spring and winter while fall DOC was similar. Also, spring DOC was similar to the DOC observed in fall and winter season. Similarly, humic-like DOM in spring (69%) was significantly different than the same in summer (65%) but was relatively high as opposite to the trend for DOC. Fall and winter humic-like DOM ranged from 66-69%, respectively with fall humic-like DOM similar to summer and winter but different to spring. In general, LT samples showed more seasonal variations in DOM properties than TF samples (Table 5.2). For example, spring a$_{254}$ (294.4 m$^{-1}$) was similar to winter a$_{254}$ (257.3 m$^{-1}$) but dissimilar to summer and fall values for LT whereas for the same parameter, spring, summer and fall were similar for TF. Also, the highest a$_{254}$ was observed in fall (410.1 m$^{-1}$) for LT whereas the highest a$_{254}$ was found in summer (94.7 m$^{-1}$) for TF. This also showed four times more aromaticity in LT over TF samples, especially during summer and fall; however the largest was in winter roughly ten times (from 24.0 m$^{-1}$ for TF to 257.3 m$^{-1}$ for LT). Other DOM metrics also showed significant differences across seasons in LT. For instance, protein-like DOM was highest in fall (7%) and lowest in summer (5%) whereas for the same parameter no seasonal difference was noticed in TF. Nevertheless, protein-like DOM in LT was roughly one-third of the same in TF across all seasons.

Near-surface sources (i.e., WSW and SGW) also showed seasonal variations in DOM properties although more pronounced in WSW than SGW (Table 5.2). One-way ANOVA revealed DOC in summer (8.5 mg l$^{-1}$) was significantly higher and the same was lower in winter (2.7 mg l$^{-1}$) for WSW samples. Fall and spring DOC were found similar (6.5 and 4.2 mg l$^{-1}$, respectively) in concentrations but the spring DOC was
Table 5.2: Seasonal distributions of DOM concentration (i.e., DOC), and optical parameters (DOM metrics from UV and fluorescence measurements) for each watershed sources are shown for a four year (2008-2011) study period in the mid-Atlantic forested catchment.

<table>
<thead>
<tr>
<th>Sources</th>
<th>Season</th>
<th>DOC (mg l$^{-1}$)</th>
<th>% Humic-like</th>
<th>% Fulvic-like</th>
<th>% Protein-like</th>
<th>HIX</th>
<th>FI</th>
<th>a$_{254}$ (m$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>THF</td>
<td>Spring</td>
<td>21.13 ± 16.72 a</td>
<td>62.79 ± 7.18 a</td>
<td>19.43 ± 3.77 a</td>
<td>17.78 ± 5.26 a</td>
<td>0.83 ± 0.05 a</td>
<td>1.44 ± 0.11 a</td>
<td>87.41 ± 41.86 a</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>10.63 ± 5.15 ab</td>
<td>60.10 ± 5.16 ab</td>
<td>22.74 ± 5.70 a</td>
<td>17.16 ± 4.91 a</td>
<td>0.85 ± 0.04 a</td>
<td>1.44 ± 0.05 a</td>
<td>94.71 ± 67.30 a</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>10.91 ± 7.77 ab</td>
<td>57.59 ± 3.56 ab</td>
<td>23.10 ± 1.69 a</td>
<td>19.31 ± 4.49 a</td>
<td>0.84 ± 0.03 a</td>
<td>1.46 ± 0.05 a</td>
<td>83.02 ± 41.45 a</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>6.25 ± 0.45 b</td>
<td>54.70 ± 2.89 b</td>
<td>23.38 ± 3.25 a</td>
<td>21.92 ± 3.50 a</td>
<td>0.82 ± 0.03 a</td>
<td>1.40 ± 0.03 a</td>
<td>24.04 ± 10.56 b</td>
</tr>
<tr>
<td>LIT</td>
<td>Spring</td>
<td>29.98 ± 12.01 bc</td>
<td>69.07 ± 2.19 a</td>
<td>25.98 ± 2.19 b</td>
<td>4.95 ± 1.17 bc</td>
<td>0.94 ± 0.01 ab</td>
<td>1.30 ± 0.04 b</td>
<td>294.38 ± 62.10 b</td>
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<tr>
<td></td>
<td>Summer</td>
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<td>65.60 ± 4.16 c</td>
<td>29.55 ± 3.27 a</td>
<td>4.85 ± 1.35 c</td>
<td>0.94 ± 0.01 a</td>
<td>1.34 ± 0.04 a</td>
<td>391.34 ± 99.35 a</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>36.72 ± 14.33 ab</td>
<td>66.10 ± 2.46 bc</td>
<td>27.09 ± 2.09 b</td>
<td>6.81 ± 2.44 a</td>
<td>0.93 ± 0.02 b</td>
<td>1.32 ± 0.03 ab</td>
<td>410.06 ± 91.53 a</td>
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<tr>
<td></td>
<td>Winter</td>
<td>23.32 ± 9.35 c</td>
<td>68.72 ± 1.56 ab</td>
<td>24.74 ± 1.63 b</td>
<td>6.54 ± 2.15 ab</td>
<td>0.93 ± 0.01 ab</td>
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<td>27.08 ± 1.57 b</td>
<td>10.89 ± 4.10 b</td>
<td>0.89 ± 0.04 a</td>
<td>1.34 ± 0.02 b</td>
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<tr>
<td></td>
<td>Summer</td>
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<td>62.54 ± 3.78 a</td>
<td>28.80 ± 2.14 a</td>
<td>8.67 ± 3.04 b</td>
<td>0.91 ± 0.02 a</td>
<td>1.36 ± 0.03 ab</td>
<td>83.25 ± 33.25 a</td>
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<td>Fall</td>
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<td>63.10 ± 6.29 a</td>
<td>27.55 ± 2.33 ab</td>
<td>9.34 ± 4.38 b</td>
<td>0.90 ± 0.04 a</td>
<td>1.35 ± 0.05 ab</td>
<td>65.60 ± 25.47 a</td>
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<td></td>
<td>SGW</td>
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<td>27.93 ± 1.61 ab</td>
<td>17.72 ± 5.84 a</td>
<td>0.84 ± 0.05 b</td>
<td>1.38 ± 0.04 a</td>
<td>23.96 ± 14.65 c</td>
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<td>8.89 ± 1.35 a</td>
<td>0.91 ± 0.01 a</td>
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<td>27.37 ± 3.94 ab</td>
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<td>52.22 ± 39.67 ab</td>
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<td>1.50 ± 0.04 a</td>
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<td>49.54 ± 2.60 a</td>
<td>40.92 ± 2.65 a</td>
<td>9.54 ± 0.13 a</td>
<td>0.90 ± 0.00 a</td>
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<td>23.06 ± 18.60 b</td>
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<td>21.95 ± 3.42 b</td>
<td>57.57 ± 8.15 a</td>
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<td>1.49 ± 0.09 ab</td>
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<td>0.71 ± 0.13 a</td>
<td>1.43 ± 0.05 b</td>
<td>16.15 ± 18.69 a</td>
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<td>37.50 ± 5.34 bc</td>
<td>0.66 ± 0.06 a</td>
<td>1.44 ± 0.02 ab</td>
<td>4.52 ± 0.94 b</td>
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<td>23.86 ± 5.96 ab</td>
<td>53.44 ± 14.77 ab</td>
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<td>45.93 ± 11.48 a</td>
<td>0.58 ± 0.10 b</td>
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<td>Mean ± Std. Dev.</td>
<td>Mean ± Std. Dev.</td>
<td>Mean ± Std. Dev.</td>
<td>Mean ± Std. Dev.</td>
<td>Mean ± Std. Dev.</td>
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<tr>
<td>Fall</td>
<td>0.71 ± 0.37 a</td>
<td>29.93 ± 9.47 ab</td>
<td>29.19 ± 4.11 a</td>
<td>40.88 ± 12.55 ab</td>
<td>0.63 ± 0.10 ab</td>
<td>1.55 ± 0.08 a</td>
<td>5.24 ± 2.24 a</td>
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<tr>
<td>Winter</td>
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<td>34.31 ± 9.15 a</td>
<td>30.45 ± 2.35 a</td>
<td>35.24 ± 9.95 b</td>
<td>0.68 ± 0.08 a</td>
<td>1.50 ± 0.03 a</td>
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<td>DGW</td>
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<td>11.62 ± 5.14 b</td>
<td>25.48 ± 5.47 b</td>
<td>62.90 ± 9.14 a</td>
<td>0.42 ± 0.09 b</td>
<td>1.61 ± 0.10 a</td>
<td>2.47 ± 1.59 a</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>1.64 ± 1.51 a</td>
<td>18.49 ± 7.21 a</td>
<td>27.96 ± 5.14 ab</td>
<td>53.55 ± 10.74 b</td>
<td>0.52 ± 0.09 a</td>
<td>1.59 ± 0.11 a</td>
<td>3.43 ± 1.56 a</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>0.68 ± 0.20 b</td>
<td>19.21 ± 5.20 a</td>
<td>25.38 ± 2.97 b</td>
<td>55.41 ± 5.86 ab</td>
<td>0.50 ± 0.06 ab</td>
<td>1.54 ± 0.07 a</td>
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</tr>
<tr>
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<td>0.64 ± 0.09 b</td>
<td>18.15 ± 4.10 a</td>
<td>31.54 ± 6.78 a</td>
<td>50.31 ± 9.53 b</td>
<td>0.53 ± 0.07 a</td>
<td>1.61 ± 0.08 a</td>
<td>3.03 ± 0.92 a</td>
</tr>
</tbody>
</table>

*Data is represented as Mean ± Std. Dev. Same letters are NOT significantly different.*
significantly different compared to summer and was almost half of its summer values (Table 5.2). Humic-like DOM and HIX were lowest during winter (54% and 0.84, respectively) and the same parameters were highest during summer (63% and 0.91, respectively). In contrast, protein-like DOM was highest in winter (18%) and was half of its value at lowest in summer (9%). Similar to the DOC pattern, a$_{254}$ was found lowest in winter (23.9 m$^{-1}$) and increased to more than three times in summer (83.3 m$^{-1}$) whereas spring a$_{254}$ (42.4 m$^{-1}$) was half of its value in summer. No significant difference between summer and fall a$_{254}$ values was noticed for WSW (Table 5.2). For shallow ground waters (SGW), seasonal variations were less pronounced compared against WSW. For example, DOC was noted lowest in spring (2.7 mg l$^{-1}$) and highest in fall (6.2 mg l$^{-1}$) (Table 5.2). However, no noticeable differences were found for humic-like DOM, HIX, and a$_{254}$ values across seasons for SGW. Protein-like DOM did in fact showed some seasonal differences. For instance, highest protein-like DOM (35%) was found during spring whereas the lowest was during winter (23%) (Table 5.2). Significant drop of protein-like DOM occurred from spring and summer (35-30%) to fall (24%) values. Similarly FI also dropped from 1.5 during spring-summer to 1.4 during fall-winter (Table 5.2).

Seasonal differences from one-way ANOVA were not as strong for deeper sources (i.e., SEEP and DGW) as pronounced the same were found for surficial sources (Table 5.2). Only summer period was distinctly different from other seasons for DGW. DOC concentration in seep waters during winter was highest (1.0 mg l$^{-1}$) whereas the same was lowest in fall (0.7 mg l$^{-1}$) although was similar to summer and spring (both 0.9 mg l$^{-1}$) values. Humic-like DOM and HIX values during spring (27% and 0.60, respectively), fall (30% and 0.63, respectively), and winter (34% and 0.68,
respectively) were similar (Table 5.2). The same parameters (i.e., humic-like and HIX) were significantly different during summer (25% and 0.58, respectively) than winter; however, these two parameters did not differ among winter-spring and winter-fall period. Protein-like DOM showed inverse pattern to humic-like DOM in seep samples: that is, a highest value in summer (46%) and a lowest value in winter (35%) (Table 5.2). Spring and fall protein-like DOM ranged between 41-44% for seep waters. Similar to DOC pattern, a254 varied across seasons for seep waters, that is, the highest a254 was noticed in winter (6.8 m-1) and the lowest was noticed in summer (4.4 m-1). No significant differences were noticed for this parameter in seep samples across seasons (Table 5.2). Analogous statistical analysis (i.e., one-way ANOVA) for deep ground waters (DGW) yielded similar results with slight variations for summer season (Table 5.2). Summer DOC (1.6 mg l-1) was significantly highest compared to the lowest noted during winter (0.6 mg l-1). DOC during spring and fall were found similar to its winter concentration. No significant differences were found in a254 values across seasons; however, slightly higher value corresponded to summer (3.4 m-1) and the lowest during spring (2.5 m-1) (Table 5.2). Humic-like and protein-like DOM showed inverse character in DGW samples across seasons, that is, humic-like DOM values were high in summer, fall, and winter (ranged between 18-19%) and was lowest in spring (12%). In contrast, protein-like DOM was highest in spring (63%) and ranged between 50-55% for other seasons. Also, fulvic-like DOM ranged from 25-32% with a high value during winter and a low value during fall. Evidently, DOM metric responses were generally muted across seasons in deeper sources as compared to the same for surficial sources but to summer season as indicated by humic- and protein-like DOM composition (Table 5.2).
PCA analyses of seasonal mean values for TF and LT showed subtle
differences between these sources across seasons (Figure 5.4). First two PCs explained
66.6% and 79.5% of the total variance for TF and LT samples, respectively. For TF,
PC 1 and PC 2 explained 44.1 and 22.5%, respectively (Figure 5.4a) while for LT, the
same components explained 46.8 and 32.7%, respectively (Figure 5.4b). However,
more clear separation between seasons was observed in LT than the same in TF as can
be seen from seasonal mean scores for both these sources (Figure 5.4). Summer and
fall values fell on positive PC 1 whereas spring and winter values were negative on PC
1 axis for LT (Figure 5.4b). In the case of TF, spring and summer were positive on PC
1 and winter was negative (Figure 5.4a). While DOM parameter loadings on PC axes
also differed for these sources considerably. For example, protein-like DOM weighed
more negatively on the PC 2 and HIX on the positive with the same PC 2 in LT; these
two metrics showed similar relationship for TF but with PC 1 axis. Other DOM
metrics also showed varying relationships with PCs for both these sources. DOC
concentration for both sources (i.e., TF and LT) suggested positive correlation with PC
1. Nonetheless, variations in DOM metrics relation with PCs for these sources
suggests the compositional changes for DOM for TF and LT across seasons which is
not reflected much by DOC concentration alone. PCA results are consistent with one-
way ANOVA results indicating more seasonal variations for LT than TF (Figure 5.4
and Table 5.2).

PCA analyses revealed seasonal differences for WSW and SGW although
milder in the case of SGW (Figure 5.5). First two PCs explained 84.9% of the total
variance for WSW samples whereas the same components together explained 86.5%
for SGW samples. For WSW, PC 1 explained 63.1% and PC 2 explained 21.8%
Figure 5.4: Mean seasonal distributions of PCA scores and loadings for (a) throughfall (TF), and (b) litter leachate (LT) are presented here. Green represent Spring, red represent Summer, brown represent Fall, and blue represent Winter season.
(Figure 5.5a) while for SGW, PC 1 explained 69.3% and PC 2 explained 17.2% (Figure 5.5b) of the total variance for the data. Although for both the sources, PC 1 showed positive loadings for DOC, humic-like DOM, HIX, and a254 while protein-like DOM and FI were negatively associated with PC 1. Also, fulvic-like DOM was positive on PC 2 for these sources. Despite similarities in loadings on PCs, seasonal differences on mean scores differed for WSW and SGW (Figure 5.5). Winter was significantly different and dictated by protein-like DOM for WSW samples (Figure 5.5a) whereas the same season showed more dominance of humic-like DOM for SGW (Figure 5.5b). Similarly, spring and summer were driven by fulvic- and protein-like DOM for WSW (as shown by PC 2 spread) but no difference between spring and summer was found for SGW. Another difference between winter and fall was clearly visible for WSW samples where mean scores lied in opposite quadrants (Figure 5.5a). In contrast, both these seasons clustered together for SGW samples (Figure 5.5b). Thus, from PCA analyses, winter is separated from other seasons for WSW; however winter-fall and spring-summer were separated among themselves for SGW samples.

Seep (P) samples showed spring, summer, and fall weighing negative on PC 1 whereas winter lied in positive quadrant of PC 1 (Figure 5.6a). No separation between seasons occurred along PC 2. While investigation of loadings on PCA axes explained the dominance of DOC, humic-, fulvic-like DOM, HIX, and a254, protein-like DOM was dominant in explaining separation for spring-summer samples weighing more on negative PC 1 (Figure 5.6a). Moreover, together PC 1 (59.6%) and PC 2 (18.3%) explained 77.9% of the total variance in seep samples. Similar analysis for DGW yielded 70.9 % explanation using first two PCs (Figure 5.6b). In particular, PC 1 explained 51.8% while PC 2 explained 19.1% of the total variance for DGW samples.
Figure 5.5: Mean seasonal distributions of PCA scores and loadings for (a) wetland soil water (WSW), and (b) shallow ground water (SGW) are presented here. Green represent Spring, red represent Summer, brown represent Fall, and blue represent Winter season.
Although spring was the only season stood out for DGW primarily due to the dominance of protein-like DOM loading on negative PC 1, other seasons were driven by positive loadings of DOC, humic-like DOM, HIX, and $a_{254}$ on PC 1. DGW samples for winter were similar to summer and fall as opposed to the winter for seep where it was different than summer and spring (Figure 5.6b).

5.3 Discussion

5.3.1 Seasonal DOM patterns for watershed sources

The seasonal DOM signals were found more apparent in surficial sources in comparison to deeper sources. For instance, LT samples showed high DOC concentration and aromaticity in summer samples compared to its winter values. Similarly, TF and WSW were found highly aromatic with high DOM concentrations during summer. Although stream DOM responses did not show larger seasonal differences for base flow condition, seasonal patterns were pronounced during storm conditions (discussed later). In contrast to surficial DOM responses, deeper sources (e.g., DGW) also showed some seasonal DOM dynamics however it was less pronounced. This can be verified from this study as no significant changes in aromaticity were observed for DGW across seasons.

The variable seasonal pattern for DOM responses in surficial and deeper sources thus indicate the presence/absence of different controls responsible for changes in DOM properties with seasons. Both biogeochemical processes and hydrologic changes could cause the changes in observed behavior of DOM. In this study, the seasonal differences in DOM response in surface and deeper sources is attributed to the sorption processes. Many other studies reported the drop in DOC
Figure 5.6: Mean seasonal distributions of PCA scores and loadings for (a) seep water (P), and (b) deep ground water (DGW) are presented here. Green represent Spring, red represent Summer, brown represent Fall, and blue represent Winter season.
concentration from surface layers to deeper sources due to long hydrologic flow paths or reactive sorption mechanisms in temperate forests [Fellman et al., 2009a; Inamdar et al., 2012; Kaiser et al., 2001].

We hypothesized in this study that as DOM moves from surface layers to deeper layers, it may get adsorbed on mineral soil particles or more degradation of DOM would cause a decrease in DOC concentration. Decrease in DOM concentration are generally felicitated by increasing residence time of the water that would provide microbes more time to break high molecular DOM into more soluble byproducts [Kaiser et al., 2001; Kalbitz et al., 2000]. Present study also supports this hypothesis as DOM concentration and its metrics responses were found less pronounced in deeper sources compared against surficial sources (Figure 5.7). Also, surficial sources not only possessed high DOM concentration, they were highly aromatic and humified than deeper sources. In contrast, deeper sources showed abundance of protein-like DOM and were relatively high in FI (compared to surface sources) indicating increased microbial activities. This finding is also consistent with previous reported study by Chen et al.(2010) in Florida Everglades where the authors have reported increasing microbial activity in deep ground waters. However, in this study, the relative drop in humic-like DOM due to sorption mechanism from surficial sources to deeper sources is mainly attributed for a corresponding increase in protein-like DOM rather than an absolute increase for this parameter.

5.3.2 Seasonal DOM patterns in stream runoff

DOM patterns in stream waters during base flow did not show seasonal expression. This is expected as during base flow stream water chemistry is mostly pronounced by the contributions from deep ground water sources. Also, the average
Figure 5.7: Distributions of mean (a) DOC, (b) \( a_{254} \), (c) HIX, (d) %Humic-like, (e) %Fulvic-like, and (f) %Protein-like DOM composition, for litter leachate (LT) and deep ground water (DGW) samples across seasons are shown here. Same alphabets indicate no significant differences for each source (LT or DGW). Note the changes and breaks in y-scale.
base flow DOC concentrations across seasons found in this study remained at low levels (~2.0 mg l\(^{-1}\)) (Figure 5.2 and Table 5.1). This is consistent with DOC concentrations measured in many small forested catchments during base flow or pre-storm conditions [Hood et al., 2006; Mulholland and Hill, 1997; Qualls and Haines, 1991]. For example, pre-storm concentrations of DOC ranged from 1.5 to 2.2 mg l\(^{-1}\) in three small watersheds in Oregon [Hood et al., 2006]. The lower DOC concentration in stream waters is mainly associated with high inputs from DOC-poor ground water contributions. Also, the flow paths of ground water discharges via mineral soils into stream may reduce the DOM concentration.

While not statistically significant, DOC concentration was found highest during summer in stream base flow whereas it was less aromatic in character as compared to spring. Moreover, spring DOC was lowest for stream during base flow. Previous studies have also noted low DOC with low-humic and aromatic character in winter spring DOM in forest stream waters [Ågren et al., 2007; Inamdar et al., 2012]. In contrast few other studies have noticed highly aromatic DOM in spring that was also positive with high DOC concentration and high hydrophobicity [Striegl et al., 2007]. Also protein-like DOM character was lowest in summer base flow conditions in this study suggesting reduced ground water contributions during dry period when water table goes down. Significantly high FI value was associated with low DOC during fall in base flow stream samples suggest the increasing microbial activity, probably via in-stream metabolism (Table 5.1). Similar results have been reported in many past studies [Laudon et al., 2011; McDowell and Fisher, 1976]. Mulholland and Hill (1997) also suggested that in-stream processes may strongly influence during base flow conditions compared against within catchment processes. This seems an
appropriate explanation for an elevated DOM during summer in our catchment resulting from by-products of microbial metabolism in stream channel.

DOM concentration is generally found to increase during storm conditions. We observed three to four fold increases in DOM concentration from base flow to storm flow conditions across seasons. Similar to results of this study, Hood et al. (2006) found a three-fold increase in DOM concentration from pre-storm to storm conditions in small watersheds of Oregon, USA. More seasonal differences were observed for stream during storms in this study. Fall and summer season was clearly distinguishable based on DOM properties for stream during storm events (Figure 5.2 and Figure 5.3). The difference in seasonal DOM pattern in streams during base flow and storm flow may have arisen from the increasing/decreasing contributions from nearby surface and/or deeper sources. For stream during base flow although not significant but high DOC was observed in summer. This could be due to relative drop in water levels in the catchment during summer dry conditions. However, in other seasons as the hydrologic connectivity establishes, increasing contributions from deeper sources (low in DOC) could felicitate the dilution of DOC. During storm condition, relative high humic-like and low protein-like DOM compositions were observed across all seasons (Figure 5.2 and Table 5.1). Because during storms mostly surface sources contribute to stream water chemistry, DOM responses with high terrestrial influence in these conditions are expected. In contrast, base flow stream chemistry would be expected to be more pronounced by the contributions of deeper DOM sources.

Other studies have also noticed the seasonal differences in stream DOM chemistry during base flow and/or event conditions. For instance, Butturini and
Sabater (2000) observed the rate of DOC change with discharge during event condition in Mediterranean stream varied with season. These authors found minimum rate of DOC changes during spring and summer while the maxima corresponded to fall and winter periods suggesting storm inputs of DOM vary between seasons. Approximately doubled DOM concentrations during storms as found in this study have been reported by Buffam et al. (2001) in an Appalachian stream. These authors also did not find any seasonality in DOM responses during base flow conditions thus corroborating the findings of this study. Furthermore, they noticed more bioavailable DOM exports during storm conditions although these laboratory estimates were not significantly different for base flow condition. Even though no laboratory study was performed for the evaluation of bioavailability of DOM in this study, it is speculated from the pattern of DOM in early fall (Figure 5.8a) in litter samples, that storms may export bioavailable DOM at least during early fall events from surficial flow paths. Our results for early fall storm events showed more protein-like DOM characteristics (Figure 5.9b). Using results from Fellman et al. (2009), where authors found strong correlations between bioavailable DOM and protein-like DOM character, we may conclude that early fall events do supply more bioavailable DOM. Also, it has been found that humic-like DOM may supply bioavailable DOM in temperate forests during events [Boyer et al., 1997]. However, this needs to be confirmed via more rigorous chemical analysis.

Nevertheless, storm timing and duration are critical to observe bioavailable DOM in stream water as the production and consumption of such soluble DOM is very rapid ranging from hours to days [Inamdar et al., 2011]. Therefore the signals for soluble DOM could only be seen if the event occurred immediately after the
Figure 5.8: Seasonal distribution of PCA scores and loadings for (a) Litter leachate, and (b) deep ground water samples are presented here. Legend for season is same as shown in previous PCA plots. Here all data points are used to plot PCA scores. Specific data in litter samples during storms show seasonal hot moment (in Fall) as labeled.
Figure 5.9: Seasonal distribution of PCA scores and loadings for (a) base flow, and (b) storm flow samples are presented here. Legend for season is same as shown in previous PCA plots. Here all data points are used to plot PCA scores. Specific data in storm flow samples during events show seasonal hot moment (in Fall) as labeled.
availability of such DOM pool. The differences in event characteristics from seasons (e.g., high intensity short duration in summer; low intensity but long duration in winter) also play major role in influencing DOM characteristics and hence changes in stream water chemistry.

More separation in seasonal DOM patterns were observed for storm flow conditions in stream. For example, DOC concentration was highest in fall but it was less humified indicating increased DOM inputs of freshly fallen leaves which still have not gone under degradation. Also, during the same period, highest protein-like DOM was observed validating the presence of fresh DOM pool lying on the forest floor that was subsequently transported to stream during storm runoff (Figure 5.8a and Figure 5.9b). The humic-like DOM was highest in summer although the DOC concentration was fractionally low than in fall. In fact, the protein-like DOM showed a largest variability for summer where reduction of its value was roughly 50% from fall values (Figure 5.2f and Table 5.1). Therefore, subtle changes in DOM quality were observed in terms of its humic- and/or protein-like DOM character from summer to fall in stream during storm conditions although DOC concentrations remained similar (Table 5.1).

Results from this study support the importance of seasonal events such as autumn leaf fall. During fall and early winter, increasing protein-like DOM in LT samples suggest the availability of more labile DOM from freshly fallen leaves thus providing the necessary energy source for microbial metabolism to heterotrophic community (Figure 5.8a). Also lowest a254 in stream samples during base flow for fall further supports the idea of increased microbial metabolism in the presence of labile
DOM source (Table 5.1). This is further supported by relatively high FI values in stream samples during the same season.

Numerous other studies have highlighted the role of autumn leaf input to stream and soil pore water DOM [Kaiser et al., 2001; McDowell and Fisher, 1976; Mulholland and Hill, 1997]. In this study, drop in DOC concentration in base flow stream water during fall (1.5 mg l\(^{-1}\)) from its highest value in summer (2.3 mg l\(^{-1}\)) also suggest that there may be dilution effects in addition to sorption and microbial degradation. Further, reduced aromaticity as indicated by a\(_{254}\) during this period also suggest enhanced microbial activity in the presence of labile DOM substrates and dilution effect by increasing influence of low-DOC rich ground waters. However, the autumn leaf fall event was more pronounced for stream during storm flow conditions (Figure 5.9b). During early fall event, more protein-like labile DOM substrate was available at the forest floor which was subsequently mobilized during event conditions (Figure 5.9a). Once, this DOM pool was exhausted by early events, the same protein-like DOM signal was missing in subsequent events. This finding underscores the importance of timing of leaf fall and event occurrences in supply of labile DOM substrates to microbial community (Figure 5.8a and Figure 5.9b). An important finding was noted by Inamdar et al. (2011) by for an extreme event (TS Nicole) occurred in late summer-early fall in 2010 where authors have found the increasing protein-like DOM composition on the rising limb of the hydrograph with first sub-event. However, with the arrival of second sub-event of this large event showed an inverse protein-like DOM pattern, that is, a dilution trajectory was observed.

For selected storm events, a relationship between discharge and DOM concentration and character showed important insights about seasonal DOM patterns
in stream waters. Generally DOC concentration and humic-like DOM character increased with increasing discharge for most of the storms irrespective of seasons (Figure 5.10). Other studies have also reported increasing DOM concentration with increasing discharge. This increase of DOC concentration during storm flow at higher discharge rates was attributed to the changes in the hydrologic flow path to the streams from mineral soil horizons during base flow to shallow soil and litter horizons during storm flow [Fellman et al., 2009a; Hood et al., 2006; Wiegner et al., 2009]. However, these storms did respond in different manner in a sense as some early fall storm events showed rapid rise in DOM concentration even at a smaller increase in discharge. Interestingly, they scored more DOM concentration than few of the extreme events noted in this study. An important insight was observed for early fall events that although DOM concentration increased with increasing discharge, humic-like character followed a decreasing trend for these events. Interestingly protein-like DOM was found increasing for these events suggesting the supply of DOM from this pool during early fall events (Figure 5.10). The protein-like DOM may have originated from freshly fallen leaves and could have transported immediately under storm runoff. This underscores the importance of timing and magnitude of the storm events on seasonal events. More insights on DOC dynamics for extreme events can be found in Inamdar et al. (2013) for this catchment.

We also observed a general pattern of decrease in DOC concentration and humic-like DOM per unit specific discharge from summer (low flow) to winter-spring period (high flow) period (Figure 5.10). However, this pattern was not as pronounced for humic-like DOM as for DOC. This could be due to the fact that %humic-like
Figure 5.10: Relationships between specific discharge rate and (a) DOC concentration, (b) humic-like DOM, and (c) protein-like DOM composition for selected storm events are presented here.
DOM is mostly consistent throughout the year ranging from 50-60%. In a recent study, Wilson et al. (2013) suggested that seasonal relationships between DOM properties and discharge may change on event basis. Also, these authors reported the greater export per unit discharge in summer as compared to winter in a forested headwater watershed. Moreover, they noted a progressive response for this pattern across season that is a consistent decline from summer to winter season and subsequently an increase from spring to fall season. Results from this study also concur with these findings. We also noted an inverse pattern for protein-like DOM with some exceptions. Protein-like DOM per unit discharge generally decreased from winter to summer events. However, the exceptions were noted for early fall events when protein-like DOM increased with per unit discharge. This protein-like DOM may have sourced from freshly fallen leaves just after which storm must have arrived. This is evident from the results of this study (Figure 5.8a, Figure 5.9b, and Figure 5.10). Thus we can say that if availability of protein-like DOM pool and timing of events may coincide we may see these interesting DOM patterns which may have further implications in stream water chemistry downstream.

5.4 Conclusions

A four-year record of DOM concentration and composition for a forested catchment in piedmont region of mid-Atlantic USA revealed significant differences across seasons in stream water chemistry. DOM concentration during summer base flow periods were high while for events fall periods showed highest DOM concentration. DOM quality metrics provide further insights into DOM dynamics across seasons. Differences in DOM exports from surficial and deeper watershed sources were also seasonally different. While surface sources showed more influence
in dry summer seasons deeper sources showed muted DOM response across seasons. Storm flow conditions in stream water successfully showed more seasonal patterns than base flow. High DOM concentration and highly humic and aromatic DOM character was seen in event samples while being more abundant in summer and fall season. Conversely, protein-like DOM was dominant for base flow samples with high values corresponding to winter and spring seasons. Autumn leaf fall events were more pronounced but during event conditions and reflected unique DOM patterns related with timing and duration of events. This study further implies the need of long-term data monitoring to evaluate changes in stream chemistry in forest ecosystems in the light of suggested climate change patterns. Since DOM concentration and quality not only influences the heterotrophic community but also plays major role in altering nutrient cycle which is important for downstream community, it is important to monitor and evaluate DOM composition in headwater streams.
Chapter 6

OVERALL CONCLUSIONS AND RECOMMENDATIONS

This project was carried out in a forested piedmont catchment of north-east USA. UV-visible and fluorescence spectroscopy in conjunction with statistical tools were used to study the character of dissolved organic matter in the study area. In this study, spatial and temporal dynamics of dissolved organic matter were explored to understand stream water chemistry during base flow and event flow conditions. In addition, variety of runoff sources was also considered to determine their influence on stream water chemistry both during base flow and event flow conditions. Furthermore, catchment intrinsic properties such as percentage wetland coverage and drainage area were evaluated to determine their influence of variable DOM properties. Generally, catchment with greater wetland coverage contributed larger DOM exports while the catchments with larger drainage area showed a muted DOM response. However, catchment properties were more pronounced during base flow conditions whereas drainage areas have weighted influence in attenuating DOM response during events. Seasonal variations in DOM properties suggested variable DOM dynamics across seasons. Summer showed a high DOM concentration while DOM during dormant (winter and spring) season was many fold lower as reflected in stream chemistry. Although DOM response during events was many fold larger than base flow, DOM quality did change across hydrologic conditions and seasons reflecting variable DOM dynamics at different times of year. While comparing two DOM models, it was found that a site-specific model would be a better alternative than a generic DOM model as it may provide more insights about the DOM origin, processes and transformation mechanisms compared against a generic model.
In summary, this study emphasizes the need to use novel spectro-fluorometric and statistical tools to study DOM character as these are rapid and cheap techniques. Also, determination of DOM properties and exports from a given catchment using multi-dimensional approaches (e.g., EEM-PARAFAC and EMMA) are important. Long-term monitoring of a study site is also important as it will provide important information on changes in DOM properties especially in the light of climate change and predicted extreme weather patterns.
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