DISSOLVED ORGANIC MATTER (DOM) CONCENTRATIONS AND QUALITY FOR WATERSHED COMPARTMENTS IN A FORESTED MID-ATLANTIC WATERSHED, USA

by

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I’m deeply grateful to my younger sister.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

While the importance of dissolved organic matter (DOM) constituents is well recognized we know very little about how these constituents vary across watershed compartments, influence the transport of dissolved organic carbon (DOC) and nitrogen (DON), and determine the mobility and bioavailability of DOM. We explore the concentrations and quality of DOM for ten watershed sources in a 12 ha forested catchment over a two-year period. DOM was evaluated for throughfall, litter leachate, soil water, shallow and deep groundwater, groundwater discharged from seeps, stream water and water in the hyporheic zone. Soil water samples included both free flowing soil water (using zero tension lysimeters) as well as soil pore water (using tension lysimeters). DOM quality was characterized using a suite of indices derived from UV-visible absorbance and parallel factor analysis (PARAFAC) modeling of fluorescence excitation-emission matrices (EEMs). DOM quality displayed a pronounced trend in watershed compartments especially as a function of soil depth. The humic, aromatic, and high molecular weight constituents of DOM decreased with soil depth while there was a concomitant percent increase in the protein-like DOM moieties. Principal component analyses (PCA) revealed that the differences in surficial watershed compartments were dictated by humic components while differences in groundwater sources were dictated by % total proteins. The increase in % total proteins with increasing soil depth indicated that in groundwater a greater fraction of DOM may be bioavailable compared to DOM in litter leachate and soil water. We did not find any conclusive evidence for C or N enrichment in any particular DOM quality pools. In addition, DOM quality displayed pronounced spatial differences. DOM in wetland groundwater was more aromatic and humic than that at the riparian location. This study also suggested that some spectrofluorometric indices (e.g. the humification index, HIX) may be preferable over others (e.g. specific UV absorbance, SUVA) for characterizing DOM quality.

Chapter 1

INTRODUCTION

Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) play key roles in the biogeochemistry of ecosystems. DOC plays an important role in the acid-base chemistry of acid sensitive freshwater systems (Herczeg et al., 1985). It affects the complexation, solubility and mobility of metals such as aluminum and mercury (Driscoll et al., 1998), it is linked to the formation of potentially carcinogenic trihalomethanes when surface water is chlorinated for drinking (Nokes et al., 1999; Siddiqui et al., 1997), it attenuates UV radiation and thus provides protection to aquatic biota (Williamson and Zagarese, 1994) and it connects the C and N cycles of terrestrial and marine ecosystems (Schlesinger, 1997). Similarly, DON constitutes a significant portion of the total N flux for some ecosystems (Campbell et al., 2000; Hedin et al., 1995; Neff et al., 2003), it can contribute to carcinogenic disinfection by-products (Lee et al., 2007), and it can also become bioavailable for estuarine plankton (Seitzinger et al., 2002). Thus, understanding the dynamics and exports of these functional groups of dissolved organic matter (DOM) from watersheds is of considerable environmental significance.

Previous research indicates that concentrations of DOC and DON may differ considerably among watershed sources (Goller et al., 2006; Hagedorn et al., 2001; Inamdar and Mitchell, 2007; Michalzik et al., 2001) which may influence the exports of DOM from watersheds (Campbell et al., 2000; Inamdar and Mitchel, 2006; 2007). In addition, despite being stoichiometrically related, recent studies suggest that carbon- and nitrogen-rich components of DOM may differ with respect to mobility, degradability, and bioavailability (Fellman et al., 2008; Hagedorn et al., 2001; Kaushal and Lewis, 2003; McDowell, 2003; Petrone et al., 2008; Qualls and Haines, 1991, 1992; Yano et al., 2005). These components could include hydrophobic and hydrophilic and/or aromatic and non-aromatic fractions. Hydrophobic fractions include humic and aromatic DOM moieties, tannins and polyphenols, and complexed amino acids while hydrophilic fractions could be composed of carbohydrates, small carboxylic acids and free proteins and peptides (Qualls and Haines, 1991; Yu et al., 2002).

Most studies show that DOM concentrations are highest in surficial watershed sources like throughfall and the forest floor and then decrease dramatically as DOM percolates through the mineral soil profile (Aitkenhead-Peterson et al., 2003; Kalbitz et al., 2000; Michalzik et al., 2001). However, DOC:DON ratios have also been observed to decrease with soil depths suggesting selective removal of carbon rich components (Kalbitz et al., 2000). This has been attributed to the preferential sorption of hydrophobic compounds in soils (Jardine et al., 1989; Kaiser and Zech, 1998; 2000) whereas the hydrophilic fractions have been found to be more mobile and remain in solution (Kaiser and Zech 1998; Ussiri and Johnson 2004). The mobile hydrophilic DOM constituents have also been reported to be N-rich while the hydrophobic constituents were found to contain more C (Kaiser and Zech 1998; Qualls and Haines, 1991; Ussiri and Johnson 2004). Conversely, others have reported that N-containing

DOM may also be hydrophobic (McKnight et al., 1992; Yu et al., 2002). Yu et al. (2002) attributed the hydrophobic behavior to complexation of N-rich amino compounds or proteins/peptides with polyphenols and humic substances. In comparison to these competing observations, Kothawala and Moore (2009) did not observe any preference for either DOC or DON in their sorption experiments.

In addition to the abiotic sorption phenomena, biotic processes such as immobilization and mineralization may also influence DOM dynamics (Aitkenhead-Peterson et al., 2003; Marschner and Kalbitz, 2003). Biotic uptake and release of DOM may occur continuously along the flowpaths as runoff traverses various watershed compartments including the canopy, forest floor, soil profile (e.g. Qualls and Haines, 1992), hyporheic zone, and the stream (Brookshire et al., 2005). Here too, important differences have been noted between the relative bioavailability of C-rich and N-rich DOM (Kaushal and Lewis 2003, 2005; Petrone et al., 2008; Wiegener and Seitzinger, 2001). Petrone et al. (2008) attributed this difference to the greater lability of the N-rich hydrophilic fraction compared to the C-rich hydrophobic portion of DOM. In contrast, Qualls and Haines (1992) did not see a faster decomposition rate for N-rich DOM.

Finally, at the catchment scale, hydrologic flow paths and the movement of DOM along these flowpaths may eventually determine the concentrations and quality of DOM at the watershed outlet. Surficial flow paths in high permafrost watersheds (larger areal extent) yielded higher DOC and DON concentrations and more aromatic DOM compared to low or medium permafrost watersheds which allowed deeper flowpaths and greater opportunity for contact and sorption of DOM on mineral soils (Balcarczyk et al. 2009).

These observations clearly suggest that DOM constituents exert a significant control on DOC and DON concentrations and that these controls could vary significantly across watershed compartments. The recent availability of innovative new spectrofluorometric tools such as ultra violet (UV-visible) absorbance (Weishaar et al., 2003) and fluorescence (McKnight et al., 2001) have especially allowed for a rapid characterization of DOM and has yielded important insights into the quality and constituents of DOM (Fellman et al., 2008, 2009; Hood et al., 2005; 2006; Jaffe et al., 2008; Spencer et al., 2008, 2009). Optical indices that have been used include: specific ultra-violet absorbance (SUVA, Weishaar et al., 2003) and spectral slope ratio (S_R, Helms et al., 2008) from UV absorbance; and humification index (HIX) (Ohno 2002), fluorescence index (FI, Cory and McKnight, 2005), % total proteins (Fellman et al., 2008; 2009) derived from fluorescence-based excitation emission matrices (EEMs, Cory and McKnight, 2005; McKnight et al., 2001).

Our objective in this study was to use a suite of these optical indices to characterize the DOM quality for various watershed sources. Understanding quantity and quality of DOM in watershed sources is critical to assessing the exports of DOM in stream runoff during baseflow and stormflow conditions. Specific attention was paid to exploring how the quality of DOM influenced the relative concentrations of DOC and DON in watershed sources. Sampling was performed over a two-year period in a 12 ha forested catchment located in the Piedmont province of Maryland, USA. Watershed sources that were sampled included: throughfall, litter leachate (O-horizon), wetland soil water, shallow and deep groundwater from wetland and riparian landscape positions, surface seep water, hyporheic water and streamwater. DOM quality was characterized using multiple spectrofluorometric indices. Specific questions that were addressed include:

a) How do DOM concentrations and quality vary across watershed compartments?

b) What role does DOM quality play in influencing DOM concentrations?

c) What do DOM quality indices indicate about the mobility and bioavailability of DOM in various watershed compartments?

The novel aspect of this work is the catchment scale assessment of DOM for multiple watershed compartments through the use of a suite of optical indices.

Chapter 2

SITE DESCRIPTION AND METHODS

Site Description

The study catchment (12 ha) is located within the Fair Hill Natural Resources Management Area (NRMA) (39°42’N, 75°50’W) in Cecil County, MD (Figure 1) and is part of the Big Elk Creek drainage basin. The basin lies within the Piedmont physiographic region and eventually drains into the Chesapeake Bay. Elevation in the study area ranges from 252 to 430 m above mean sea level. Cecil County has a humid, continental climate with well-defined seasons. The maximum daily mean temperature (1971 – 2000) was 24.6°C (July) and the daily minimum was -0.6°C (January), with a mean annual temperature of 12.2°C. For this same period, mean annual precipitation in this region was 1231 mm with ~ 350 mm occurring as snowfall in winter (MD State Climatologist Office 2008). Late summer (August – September) tends to be the driest period of the year while late spring (May – June) is the wettest.

The study area is underlain by the Mt. Cuba Wissahickon formation and includes pelitic gneiss and pelitic schist with subordinate amphibolite and pegmatite (Blackmer, 2005). The compact bedrock is overlain by a zone of weathering which is between 4 and 14.5 m thick in the Fairhill area, and a soil layer of varying thickness (Water Resources of Cecil County, 1958).

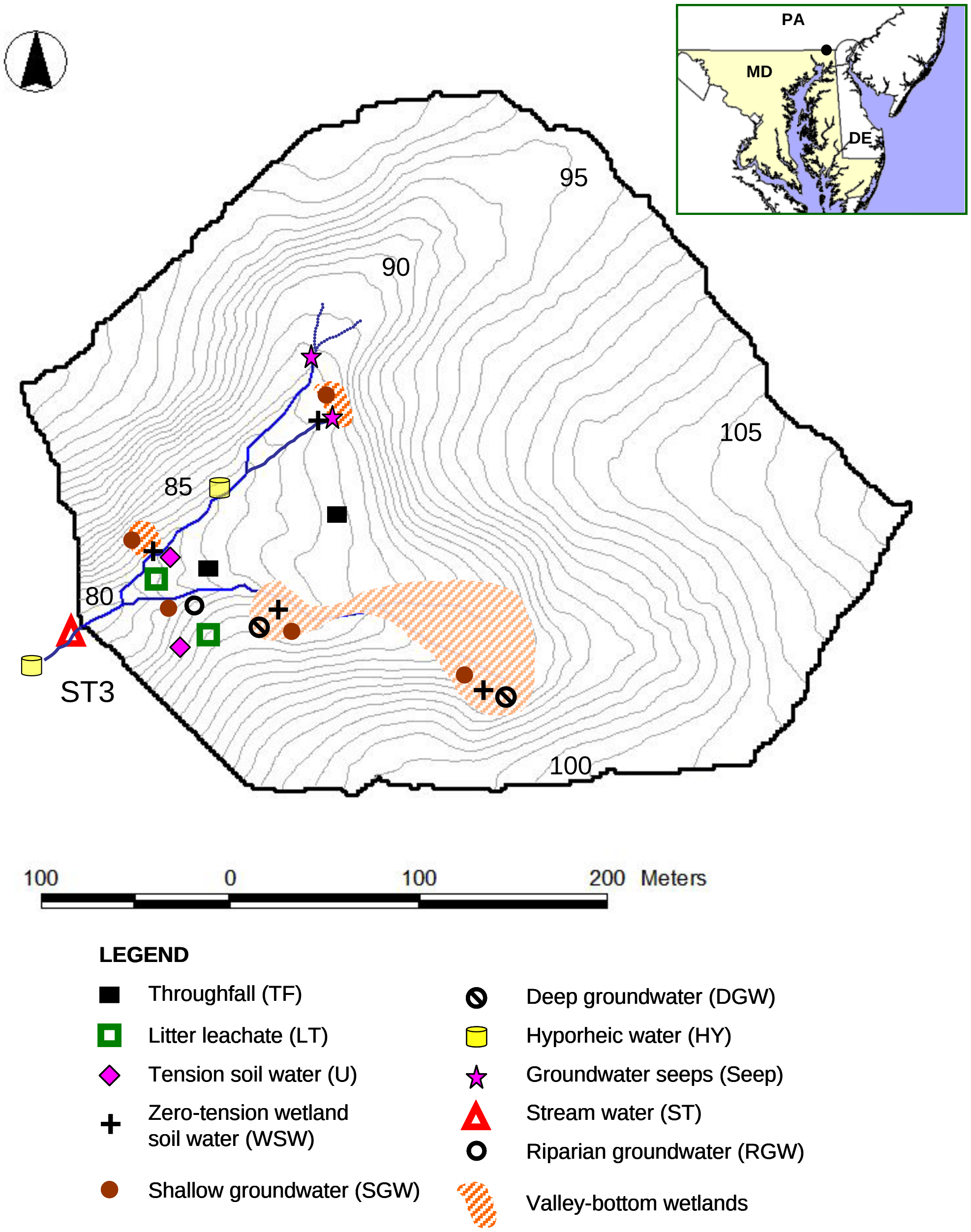

Figure 1: **Location of watershed site in the Piedmont region of northeastern Maryland (inset, indicated by filled circle) and the sampling locations within the 12 ha study watershed.**

The soils in the study area belong the the Glenelg series, which consists of deep, well-drained, nearly level to moderately steep soils. On the hillslopes soils are coarse loamy, mixed, mesic Lithic Dystrudepts while in the valley bottoms seasonal water saturation leads to the formation of Oxyaquic Dystrudepths. The hillslope soil profile has an 8 cm thick organic A horizon. The boundary to the first B horizon (mineral horizon) is diffusive; the boundary to the C horizon at 68 cm depth is gradual. The C horizon is rich in muscovite and biotite and with increasing depth the orange color gradually disappears. The wetland soil profile shows a shallow water table at about 30 cm below surface. The dark A horizon is rich in highly decomposed organic matter. Beneath the A horizon is a grey E horizon (eluvial horizon) which displays specks of orange color, indicative of variable groundwater table depths. It gradually merges into a Bw horizon (water influenced horizon) at about 20 cm depth. The high abundance of muscovite in the Bw horizon may indicate that this is already the parental sediment.

Vegetation in the study catchment consists of deciduous forest with pasture along the catchment periphery. Dominant tree species are *Fagus grandifolia* (American beech), *Liriodendron tulipifera* (yellow poplar), and *Acer rubrum* (red maple). Based on a survey of canopy trees (> 10 cm diameter at breast height), there is a stand density of 225 trees ha^{-1}, a stand basal area of 36.8 m^{2} ha^{-1}, a mean diameter at breast height (dbh) of 40.8 cm, and a mean tree height of 27.8 m (Levia et al., 2010).

Watershed Instrumentation and Sampling

Streamflow discharge measurement was initiated in November 2007 at the 12 ha catchment outlet (Figure 1). A Parshall flume with 15-cm throat-width along with a Global Water (Inc.) logger and pressure transducer was used to measure streamflow stage at 15 minute intervals. Depth to groundwater was recorded at five locations in the valley-bottom at 30-minute intervals using Global Water loggers (Inc.). Groundwater logging wells consisted of PVC pipes (5 cm diameter) ~ 2 m below the ground surface that were continuously slotted from a depth of 0.3 m below the soil surface.

Manual grab sampling was performed every three weeks during non-storm periods from July 2007 to July 2009. While most sampling devices were installed in the spring/summer of 2007, devices for deep groundwater and hyporheic water were installed in October 2007 and April 2008, respectively. Samples collected included: streamwater (ST); zero-tension wetland soilwater (WSW); tension soilwater (U), wetland shallow and deep groundwater (SGW and DGW, respectively), riparian groundwater (RGW), groundwater seeps (Seep) and hyporheic water (HY) (Figure 1). Streamwater samples were collected at the outlet of the 12 ha catchment. Wetland soil water was sampled at four sites (Figure 1) in the valley-bottom wetlands using zero-tension lysimeters which consisted of screened 5 cm diameter PVC pipes that were inserted at a 45 degree angle to a depth of 30 cm thus collecting free pore soil water from the top 30 cm of the soil profile. Valley-bottom wetlands were wettest in late spring (May – June) and driest in late summer (August – September). Tension soilwater samples were collected using two nests of suction cup tension lysimeters (Soilmoisture Equipment Corp.) each. One nest was located at the edge of a valley-bottom saturated area while the other one was located at a drier location at the bottom of a hillslope (Figure 1). At each nest, one tension lysimeter was inserted at a 45 degree angle to a depth of 10 cm while the other lysimeter was inserted vertically to a depth of 30 cm. The tension lysimeters were evacuated to 70 centibars prior to the day of sampling.

Shallow groundwater samples were collected from four wells located in the valley-bottom wetlands (Figure 1). Shallow groundwater wells were constructed of 5 cm PVC tubing, were augured to a depth of 2 m and were screened for the full length from 30 cm below the soil surface. Two deep groundwater wells were collocated with shallow groundwater in the wetlands and were similar except that they were screened for only the lowermost 50 cm so as to collect only the deeper portion of groundwater (1.5 to 2 m below the soil surface). The riparian groundwater well was identical to shallow groundwater except that it was located in a riparian location which was not a wetland. (Figure 1). The riparian soil was well drained and had a thinner O horizon. Hyporheic samples were collected at two stream locations (Figure 1) and consisted of slotted PVC pipes (5 cm diameter) inserted at 45° angle to a depth of 30 cm in the stream bed. All samples from soil and groundwater locations were recovered using a hand-operated suction pump. Seep samples were collected manually at two seep locations from which one of the catchment tributaries originated (Figure 1).

Throughfall and litter (or forest floor/O horizon) leachate sampling was performed following storm events. Throughfall sampling was performed at two locations (Figure 1) and consisted of 1 L amber glass bottles that collected throughfall via a plastic funnel. A wire mesh was placed on the funnel to prevent entry of debris. Litter leachate collectors were placed at two locations (Figure 1) and consisted of 1 L amber glass bottles connected to (via plastic tubing) plastic trays (~ 1 m^2) that contained the O horizon layer. These samples were collected within 24 hours of the storm event.

Sample Processing and Chemical Analysis

All samples were filtered through a 0.45 μm filter paper (Millipore, Inc.) within 24 hours of collection and stored at 4°C. A subsample for nutrients, cations, and anion analyses was stored in HDPE acid-rinsed bottles prior to analysis. Samples for DOM characterization were stored in amber glass bottles prior to UV and fluorescence measurements.

The Biogeochemistry Laboratory at SUNY-ESF, NY, which is a participant in the USGS QA/QC program, performed the following analyses: pH using a pH meter; major cations (Ca^{2+}, Mg^{2+}, Na^+, K^+, Fe_{tot}, Al_{tot}, Si, Mn, S) using a Perkin-Elmer ICP-AEC Div 330 instrument; anions (Cl^-, NO_3^-, SO_4^{2-}) using a Dionex IC; NH_4^+ with an autoanalyzer using the Berthelot Reaction followed by colorimetric analysis; total dissolved nitrogen (TDN) using the persulfate oxidation[1] procedure

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[1] Persulfate digestion method: Nitrate is reduced to nitrite by passage of the sample through a column containing copper coated cadmium. The nitrite (which includes the reduced nitrate plus the original nitrite) is then determined by diazotizing with

(Ameel et al., 1993) followed by colorimetric analysis on an autoanalyzer; and DOC using the Tekmar-Dohrmann Phoenix 8000 TOC analyzer[2]. DON concentrations were computed as the difference between TDN and inorganic N (NO_3^-, NH_4^+) (Inamdar and Mitchell, 2007).

Characterization of DOM Quality Using Spectrofluorometric Indices

While DOC and DON concentrations and UV absorbance was determined for all samples, fluorometric scans and EEMs analyses were performed only on a select subset of the samples. Optical indices used to characterize DOM quality are given in Table 1. The molar UV absorptivity of DOC at 254 nm was measured within 24 hours after sample collection using a 1 cm quartz window cuvette with a double beam Shimadzu UV-mini spectrophotometer (Shimadzu Inc.). SUVA (specific UV absorbance) provides a measure of aromaticity of DOM (Weishaar et al., 2003) and was computed by dividing the UV absorbance at 254 nm (m^{-1}) by the concentration of DOC (mg C L^{-1}) (Hood et al., 2006). Aromaticity increases with increasing values of SUVA. In addition the absorption coefficient at 254 nm (a_{254} in m^{-1}) was also calculated following the procedures of Green and Blough (1994).

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sulfanilamide dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm.

[2] TOC measurement using the Tekmar-Dohrmann Phoenix 8000: The sample is exposed to UV light from a mercury vapor lamp. With sufficient time of exposure, all the dissolved organics may be oxidized to yield CO_2. Detection of the CO_2 is usually by the change in conductivity of the sample. The CO_2 is purged out with a carrier gas and detected by NDIR (non-dispersive infrared sensor) (Furlong et al. 2010)

The a_{254} also provides a measure of aromaticity but without normalization to C (Helms et al., 2008). Another UV index , the spectral slope ratio, S_R was calculated as the ratio of the shorter UV wavelength region (275 – 295 nm) to that of the longer UV wavelength region (350 – 400 nm) (Helms et al., 2008) and was obtained using linear regression on the log-transformed spectral ranges (Yamashita et al., 2010). The spectral slope ratio, S_R is inversely related to the molecular weight of DOM (Helms et al., 2008).

Table 1: Definition and significance of the suite of DOM quality indices used in this study.

DOM Quality Index	**Reference**	**Definition and significance**
UV		
Specific UV Absorbance ($SUVA_{254}$) [mg C L^{-1}]	Weishaar et al. 2003	UV absorbance at 254nm divided by DOC concentration in mg C L^{-1}; provides a measure of aromaticity of DOM. High values of SUVA indicate more aromatic material.
Absorption coefficient a_{254} [m^{-1}]	Green and Blough 1994	(UV absorbance at 254 nm) x 2.303 x 100 Measure of aromaticity of DOM
Slope ratio S_R	Helms et al. 2008	Ratio of the slope of the shorter UV wavelength region (275 – 295 nm) to that of the longer UV wavelength region (350 – 400nm) Can be used as a proxy for molecular weight (MW)

Table 1 continued

DOM Quality Index	Reference	Definition and significance
Fluorescence		
Humification Index HIX	Ohno 2002	HIX $=\sum I_{435\text{-}480}/(\sum I_{300\text{-}345}+\sum I_{435\text{-}480})$ Used to characterize humification status of DOM Ranges from 0 – 1 and increases with increasing degree of humification
Fluorescence Index FI	McKnight et al. 2001	Ratio of fluorescence intensities at 470 and 520 nm at excitation of 370 nm Used to distinguish between terrestrial and microbial sources of DOM Terrestrial or allochthonous DOM: 1.2 – 1.5; Microbial or autochthonous DOM: 1.7 – 2.0
% C3	Cory and McKnight, 2005 Stedmon and Markager 2005	Component 3 in Cory and McKnight (2005) PARAFAC model Indicates DOM of microbial or planktonic origin
% C5	Cory and McKnight, 2005	Component 5 in Cory and McKnight (2005) model Indicates higher plant-derived (versus microbial) organic matter and is also a proxy for aromatic organic matter
% total proteins	Cory and McKnight, 2005	Sum of % tyrosine (C8) and % tryptophan (C13) components from the Cory and McKnight model. Indicates protein-like DOM moieties and has been found to be a strong indicator of bioavailable DOM (Fellman et al., 2008; 2009)
Tryptophan:tyrosine	Cory and McKnight 2005; Fellman et al., 2008	Ratio of the C13 and C8 components from Cory and McKnight model High values of the ratio would indicate less degraded “fresher” proteins while low values would suggest degraded “older” proteins

Three-dimensional fluorescence scans were collected on a Fluoromax-P spectrofluorometer (Horiba Jobin-Yvon Inc.) and corrected for the instrument bias with manufacturer provided correction files. A daily lamp scan, cuvette check and Raman water scan were run to ensure instrument stability. Scans were collected in S/R mode (ratio) for excitation wavelengths between 240 and 450 nm at 10 nm intervals and emission wavelengths between 300 and 550 nm at 2 nm intervals. The integration time for sample collection was set to 0.25 sec along with bandpass of 5 nm each for excitation and emission ranges. Subsequently a Raman water blank was subtracted from each scan and resulting EEMs were Raman-normalized using the area under the curve of water Raman peak at excitation 350 nm (Cory and McKnight, 2005). To avoid inner filter effects, samples were diluted according to Green and Blough (1994). In addition, the inner filter correction proposed by McKnight et al. (2001) was also applied. The EEMs scans were fitted to the 13-component PARAFAC model developed by Cory and McKnight (2005). The advantage of using this existing model as opposed to developing our own PARAFAC model was that a large sample size was not required, and a greater amount of variation in the DOM source and quality was likely to be identified (Miller and McKnight, 2010).

To elucidate differences in the quality of DOM, four of the 13 Cory and McKnight components were selected for further evaluation. Component 3 (% C3) is considered to represent DOM of microbial or planktonic origin (Stedmon and Markager 2005; Cory and McKnight 2005) while component 5 (% C5) is assumed to indicate DOM derived from higher plants (terrestrial origin) and has also been used as a proxy for aromatic material (Cory and McKnight 2005). Total % proteins were calculated as the sum of % values for tryptophan-like fluorescence (component 8) and tyrosine-like fluorescence (component 13) from the Cory and McKnight (2005) model. The sum of these two protein components has been found to be a strong predictor of bioavailable DOM (Fellman et al., 2008; 2009). In addition, we also computed the ratios of the % tryptophan and % tyrosine components. Tryptophan is considered to be less degraded peptide material and contains intact proteins while tyrosine represents more degraded peptide material (Fellman et al., 2009; Mayer et al., 1999; Yamashita and Tanoue 2004). Thus, high values of the tryptophan and tyrosine ratio would indicate less degraded "fresher" proteins while low values would suggest degraded "older" proteins. The fluorescence index (FI) was calculated using the ratio of fluorescence emission intensities at 470 and 520 nm at an excitation wavelength of 370 nm (Cory and McKnight, 2005). McKnight et al. (2001) have used the FI to differentiate between DOM derived from higher terrestrial plants (FI: 1.2 – 1.5) versus microbial or planktonic sources (FI: 1.7 – 2.0). Lastly, the humification index (HIX) was calculated using the normalized HIX equation of Ohno (2002) which reduces the variation introduced by changes in DOM concentration (Ohno, 2002). The HIX values for this modified equation range from 0 to 1 with higher values indicating a greater degree of humification/degradation of DOM (Ohno 2002).

Statistical Analysis

General descriptive statistics including maxima, minima, average and median values and standard deviation was performed on DOC and DON concentrations and DOM quality parameters (DOC:DON, SUVA, a_{254}, HIX, S_R, % C3, % C5, % total proteins, FI and tryptophan:tyrosine ratio) and compiled in the form of box plots. Significant ($p < 0.05$) differences between the watershed compartments for DOM concentrations and quality parameters were determined using the Tukey's parametric test. To further evaluate the differences among watershed compartments and to identify the key parameters influencing these differences a principal component analysis (PCA) was performed using DOM concentrations and quality indices. The compartments were plotted in the PCA space defined by the first two components along with the loadings for the two components. PCA was performed using JMP version 8.0 statistical software.

Correlations between DOM concentrations and quality indices were determined using the Pearson correlation coefficient. Results are reported for significance levels of $p < 0.10$ and all statistical analyses were performed using the statistical software JMP version 8.0.

Chapter 3

RESULTS

DOM Concentrations and Quality Across Watershed Compartments

DOM concentrations revealed pronounced patterns across the watershed compartments (Figures 2 and 3). Significant ($p<0.05$) differences among compartments for DOM are indicated in Table 2. Excitation-emission matrices for six of the ten watershed compartments are presented in Figure 4 to illustrate the change in fluorescence across the watershed compartments. Both DOC and DON concentrations decreased from surficial sources (throughfall and litter) to deep groundwater sources but the decrease in DOC was sharper than the decrease in DON. Median DOC concentrations (Figure 2a) were highest in litter (37 mg L^{-1}) followed by throughfall (14 mg L^{-1}). This was followed by a large decrease and much lower concentrations in tension soilwater (5.9 mg L^{-1}), wetland soilwater (4.1 mg L^{-1}) and shallow groundwater (3.5 mg L^{-1}). Median concentrations were lowest in seeps (0.75 mg L^{-1}) whereas concentrations in deep and riparian groundwater were only marginally higher (0.84 and 1.1 mg L^{-1}, respectively). Median DOC concentrations for stream and hyporheic water were nearly identical (1.5 mg L^{-1}). The greatest variability in DOC was observed in shallow groundwater. Similar to DOC, median DON concentrations were also highest in litter (1.0 mg L^{-1}) followed by throughfall (0.37 mg L^{-1}) (Figure 2 b). Following these two sources, median concentrations of DON were comparable for shallow groundwater (0.33 mg L^{-1}), tension soilwater (0.32 mg L^{-1}) and wetland soilwater (0.30 mg L^{-1}). Lowest DON concentrations were recorded in seep and streamwater (both 0.12 mg L^{-1}) and concentrations were only marginally higher in riparian and deep groundwater (both 0.12 mg L-1). Median DOC:DON ratios (Figure 2c) were highest for throughfall (40) followed by litter (33). Median DOC:DON ratios then decreased systematically from litter to deep groundwater (6). The largest variability in median DOC:DON ratios was observed for stream and hyporheic water. To provide some perspective on how DON compared to dissolved inorganic nitrogen at our watershed site, a plot of DON as a % of total dissolved N was included (Figure 2d). % DON values were highest in tension soilwater (~ 70%) and decreased slightly with soil depth, resulting in a minimum for the seep.

While there was a large range in SUVA values (~ 1 to more then 10, Figure 3a) highest median values for SUVA were recorded for wetland soilwater (4.3) followed by litter (4.2), hyporheic (3.9) and shallow groundwater (3.7). Median SUVA values were slightly lower in stream water (3.3) and much lower in tension soilwater (2.7). The lowest median SUVA values were observed in the groundwater sources – seep (1.7), riparian groundwater (1.7) and deep groundwater (1.7). Median SUVA values for throughfall were lower than that for litter but greater than that of the groundwater compartments. Following the linear model developed by Weishaar et al. (2003), litter leachate with a median SUVA value of 4.2 represented an aromatic C content of 31%. There was considerable variability in SUVA for the litter, hyporheic and shallow groundwater components.

Compared to SUVA, the other aromatic indices – a_{254} and % C5 – displayed a more pronounced and systematic trend across the watershed sources (Figures 3b and c). Median values for both a_{254} and % C5 were highest in litter (391 and 7.8, respectively) and decreased with increasing soil depth in the order of: wetland soilwater (40 and 7.1, respectively), shallow groundwater (22 and 4.6, respectively) and deep groundwater (3.2 and 1.8, respectively). Median values of a_{254} and % C5 for throughfall were less than litter. Intermediate values were observed for the other watershed compartments. The values for SUVA, a_{254} and % C5 show that DOM from riparian groundwater was less aromatic compared to shallow groundwater suggesting that the wetland locations had more aromatic DOM.

While the overall trend for HIX was similar to a_{254} and % C5 there was a sharper drop in HIX for the seep and deep groundwater compartments (Figure 3d). HIX provides a measure of the humification status of DOM and median values for HIX were highest in litter leachate (0.92) followed by wetland soilwater (0.88), shallow groundwater (0.78) and deep groundwater (0.52). Median HIX values for stream and riparian groundwater were 0.84 and 0.55 respectively. Median HIX values for throughfall were lower than those for litter and the soil water components suggesting that DOM in throughfall was not as degraded.

Indices that have been used as a proxy for “microbial” sources of DOM (% C3 and FI) follow a trend exactly opposite to that observed for the aromatic indices (a_{254} and % C5) (Figures 3e and f). In contrast to a_{254} and % C5, both % C3 and FI increased with soil depth from litter to deep groundwater. As reported in Table 1, FI values in the range of 1.2 – 1.5 have been used to represent microbial or planktonic

DOM (or autochthonous DOM; McKnight et al., 2001). Both % C3 and FI were lowest in litter (3.4 and 1.31, respectively) and increased in the order as: wetland soilwater (3.49 and 1.37), shallow groundwater (4.52 and 1.49) and deep groundwater (7.66 and 1.55). Compared to deep groundwater, median values for these two indices were slightly lower in riparian groundwater (6.45 and 1.47) and seep water (6.78 and 1.48). Values for streamwater (% C3 and FI: 4.39 and 1.41, respectively) and hyporheic water (% C3 and FI: 5.21 and 1.45, respectively) were intermediate between wetland soilwater and deep groundwater. Median values for both % C3 and FI in throughfall were greater than the corresponding values for litter.

The trend for % total proteins which includes both the % for tryptophan- and tyrosine-like proteins was similar to the microbial indices (Figure 3g). Similar to % C3 and FI, % total protein values increased with soil depth, starting with litter leachate. Percent total proteins were lowest in litter (3.52) and increased in the order as: wetland soilwater (3.97), shallow groundwater (4.59) and deep groundwater (16.5). A sudden increase in % total proteins can be noted between shallow and riparian groundwater. Compared to the surficial (throughfall and litter) and soilwater DOM, the variability in % total proteins was larger for the deeper groundwater sources. The highest value for the tryptophan:tyrosine ratio was observed for throughfall, the lowest for wetland soilwater and litter (Figure 3h). The soil and groundwater compartments were fairly similar in values for this ratio and there was no systematic trend in this ratio with soil depth.

Finally, the UV-based index, S_R, which is inversely related to the molecular weight of DOM produced a systematic increasing trend with soil depth

(Figure 3i). The values of S_R were lowest for litter leachate (1.87) and increased in the order: wetland soilwater (2.63) < shallow groundwater (3.35) < deep groundwater (4.95). Highest S_R values were found in seeps (6.51) and riparian groundwater (5.69). S_R values for stream (3.68) and hyporheic water (3.51) were intermediate between shallow and deep groundwater. Overall, these values suggest that molecular weight of DOM was highest near the soil surface and decreased with depth into the soil profile. The values of S_R for throughfall were slightly greater that for litter indicating that molecular weight of DOM was lower in throughfall. The median value of S_R for riparian groundwater was considerably greater than that for shallow groundwater indicating a lower molecular weight of DOM at the riparian location.

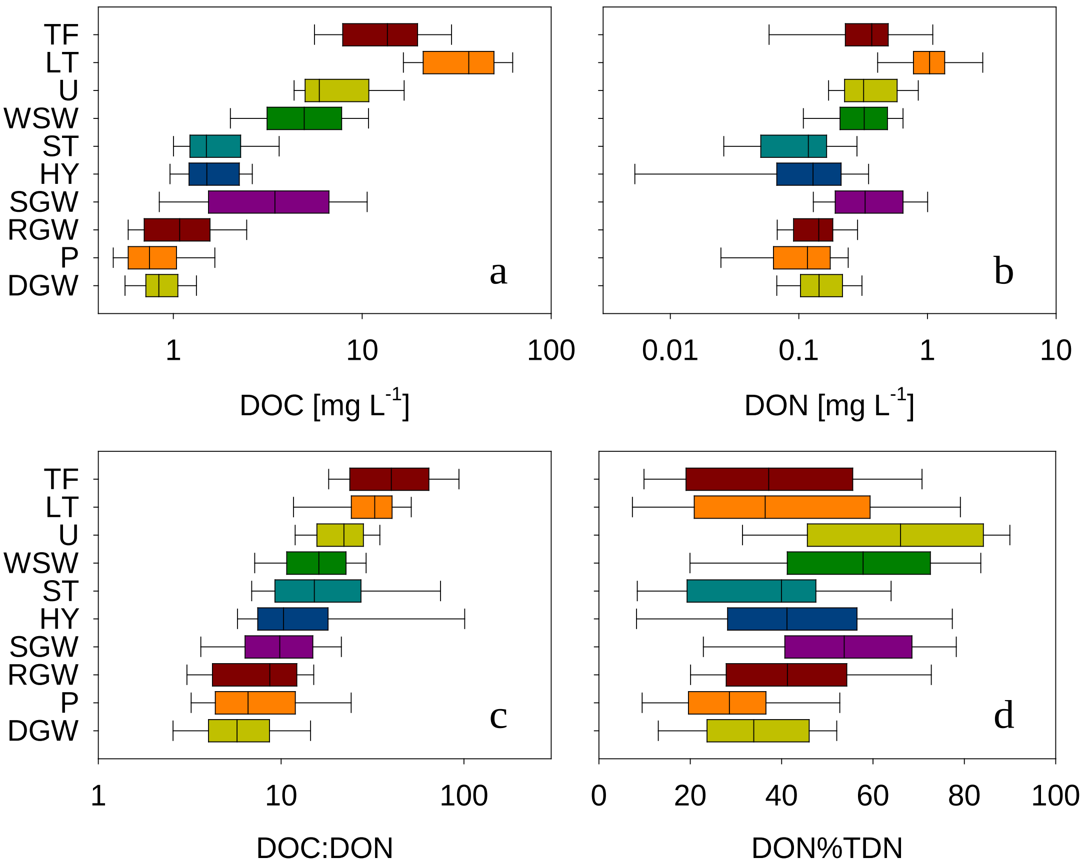


Figure 2: Concentrations of DOC (mg L^{-1}); DON (mg L^{-1}); DOC:DON ratios and DON as % of total dissolved N (DON%TDN) for the various watershed compartments (line = median, box upper and lower bound = 25% and 75%, whiskers = 10% and 90%). Sample numbers are indicated within brackets in the legend – the first value indicates the number of samples used for DOM concentrations and UV while the second value is the number associated with EEMs (for legend see page 25).

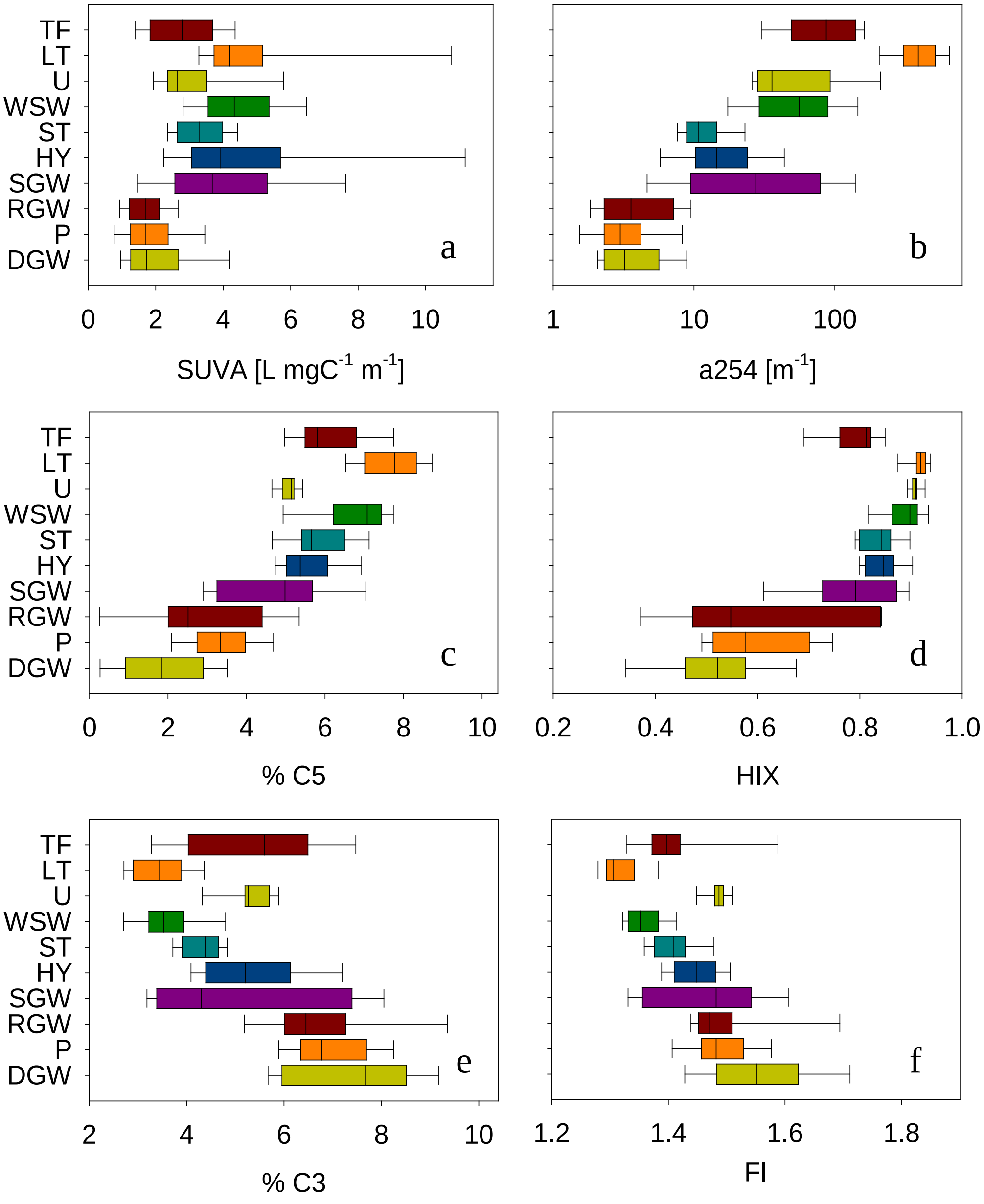


Figure 3: **DOM quality indices for various watershed compartments (line = median, box upper and lower bound = 25% and 75%, whiskers = 10% and 90%, for legend see page 25).**

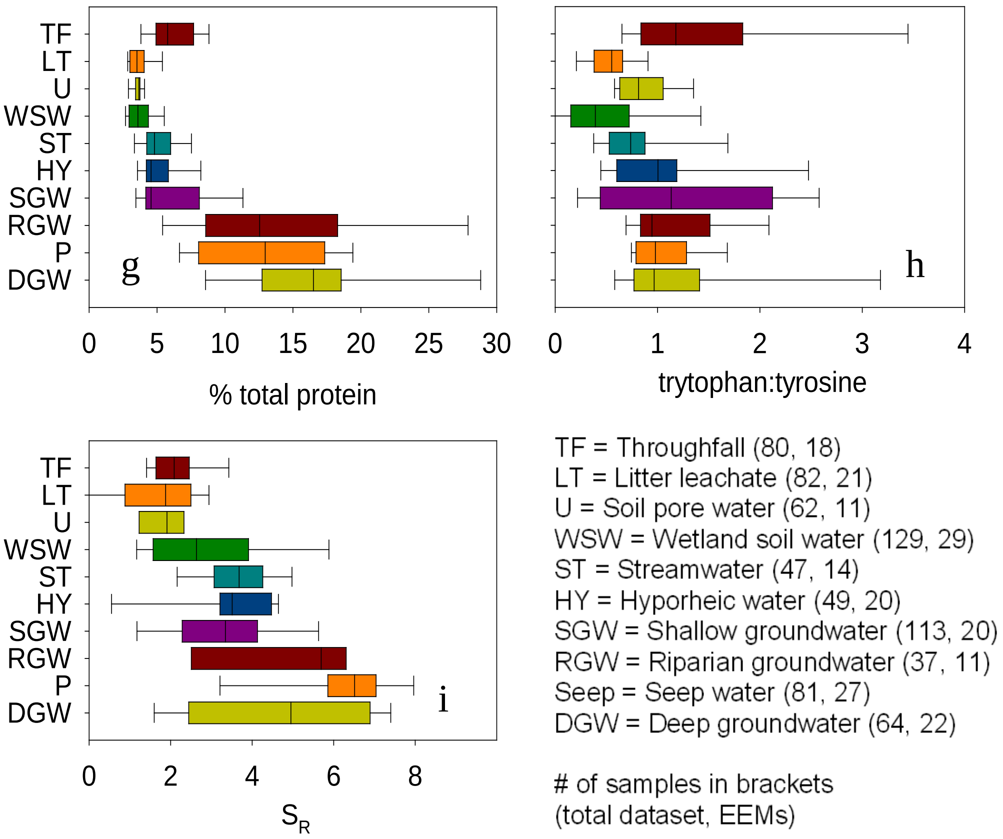


Figure 3 continued

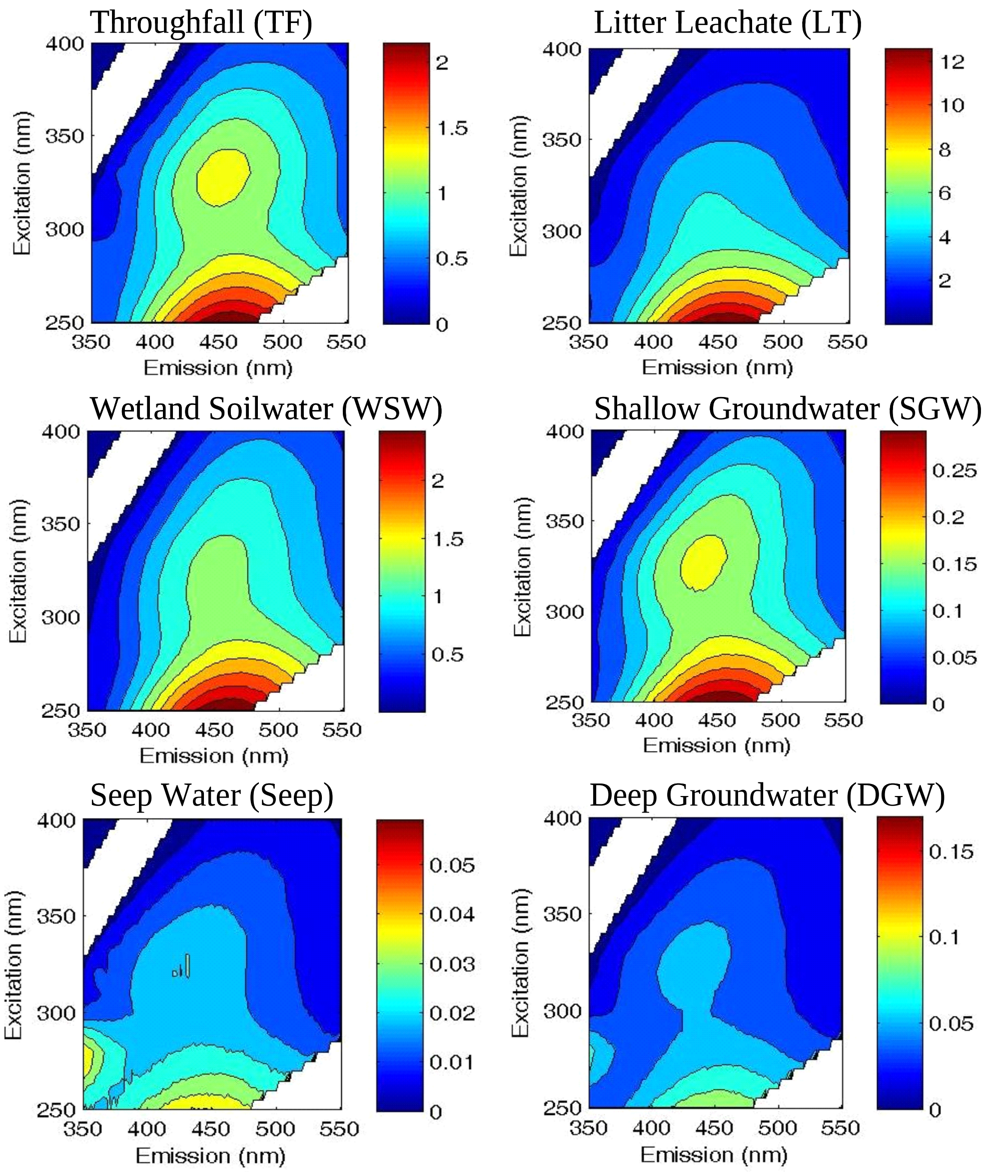
Table 2: Results from Tukey test indicating the significant ($p < 0.05$) differences among the watershed compartments. For each DOM parameter considered, compartment with the same letters are not significantly different. Watershed compartments include – throughfall (TF); litter (LT); tension soil water (U); zero tension wetland soil water (WSW); streamwater (ST); hyporheic water (HY); shallow groundwater (SGW); riparian groundwater (RGW); groundwater seeps (Seep); and deep groundwater (DGW).

	DOC					DON		SUVA				a_{254}					HIX			
TF		B					B				D		B					B		
LT	A					A		A				A					A			
U			C				B			C	D		B	C			A			
WSW			C	D			B	A	B				B	C			A			
ST				D	E		B		B	C	D				D	E	A	B		
HY				D	E		B	A	B						D	E	A	B		
SGW				D	E		B	A	B	C				C	D			B		
RGW				D	E		B				D				D	E			C	
Seep					E		B				D					E			C	
DGW					E		B				D					E				D

Table 2 continued

	% C5	% Protein	% C3	F
TF	B C	C	B C	
LT	A	C	D	
U	C D	C	B C D	A
WSW	A B	C		
ST	B C	C	C D	
HY	C D	C	C	
SGW	D	C	C	
RGW	E	A B	A B	A
Seep	E F	B	A	A
DGW	F	A	A	A

*ratio of % tryptophan to % tyrosine



Throughfall (TF)

Litter Leachate (LT)

Wetland Soilwater (WSW)

Shallow Groundwater (SGW)

Seep Water (Seep)

Deep Groundwater (DGW)

Figure 4: **Excitation-emission matrices (EEMs) for selected watershed compartments highlighting the shift in fluorescence. Intensities are reported in Raman units (RU). Throughfall and litter samples correspond to event of October 25, 2008, while other samples were collected during grab sampling on October 10, 2008.**

Principal Component Analysis (PCA) on DOM Concentrations and Quality Metrics

The PCA analysis for DOM with the property-property plots for first and second factor loadings are presented in Figure 5a while the component scores for the two components are presented in Figure 5b. DOM quality indices rather than DOM concentrations explained most of the variance among the watershed compartments. HIX, % C5 and % C3 explained 48% of the variance in the first principal component, with HIX and % C5 indicating positive and % C3 representing negative first factor loadings (Figure 5a). DOC concentrations explained 14.4% of component 2 loadings followed by DOC:DON ratios explaining 10.2% of component 3 and SUVA explaining 9.6% of component 4 (only component 1 and 2 are plotted in Figure 5).

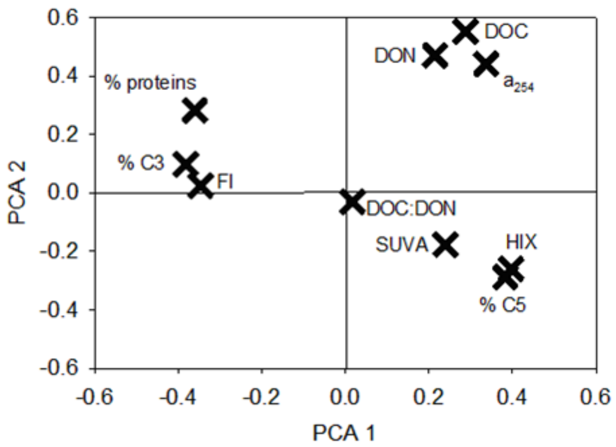


Figure 5a: Principal Component Analysis (PCA) for DOM with property-property plots for first (PCA1) and second (PCA2) factor loadings.

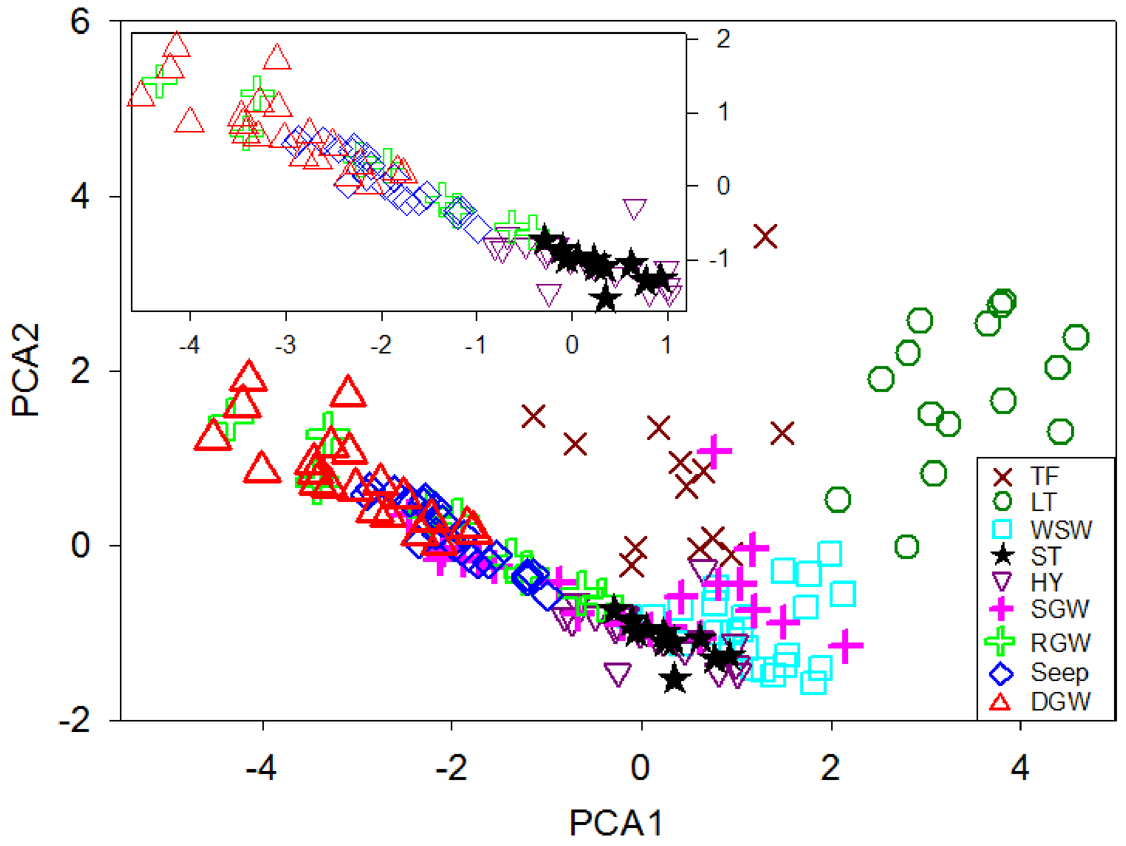


Figure 5b: **Principal Component Analysis (PCA) for DOM with component scores for first (PCA1) and second (PCA2) component. The inset shows a slightly enlarged section of the graph and only contains groundwater sources (riparian groundwater, deep groundwater, seep water), streamwater and hyporheic water.**

Watershed compartments displayed important differences in how they were distributed in the PCA space (Figure 5b). Data from litter leachate, throughfall, wetland soilwater and shallow groundwater were distributed as clusters with relatively similar variances along both component axes. Of these four compartments, litter showed the highest positive values for components 1 and 2. Values were slightly lower for throughfall for both components 1 and 2, followed by wetland soilwater and shallow groundwater with lower values for component 2 but very similar values for component 1. In contrast to the clustered nature of these four compartments, deep and riparian groundwater and groundwater seeps displayed a strong linear distribution with a much greater variance along the PC1 axis than PC2 axis. Stream and hyporheic water samples were intermediate between these two patterns, with a linear trend along PC1 but more clustered distribution along PC2. The linear trend and the orientation of the groundwater sources on the PCA plot (see inset in Figure 5b) suggest a strong influence of microbial and protein indices and a weak influence of the aromatic indices. In contrast, the spatial distribution of the clustered compartments revealed a greater influence of SUVA, HIX and a_{254} (aromatic and humic indices) on these watershed compartments.

Correlations Between DOM Concentrations and Quality Metrics

The intent of these analyses were: (a) to determine if DOC and DON concentrations were preferentially associated with any particular DOM quality pools (e.g., aromatic DOM or % proteins); (b) to investigate if the DOM quality indices corroborated each other; (c) to explore if any particular metrics yielded consistently strong or weak correlations and thus were more or less preferable than others; and (d) to investigate if correlations were weaker or stronger for any specific watershed compartment. While we only report correlations significant at $p < 0.10$ in Table 3, there were a few metric for which the relationships were obvious but not significant at $p < 0.10$.

DOC and DON concentrations were positively correlated across all watershed compartments except riparian and deep groundwater, for which there was no significant correlation. The highest correlation of DOC and DON concentrations was observed for tension soilwater while the lowest values were observed for stream and seep samples. The correlation of DOC concentration and SUVA varied, with negative correlations for throughfall, litter and deep groundwater and positive correlations for tension and wetland soilwater samples. In contrast to SUVA, correlation coefficients for a_{254} and DOC were stronger with positive relationships across all compartments except deep groundwater. Unlike a_{254}, the other humic/aromatic indices – HIX and % C5 did not show a strong relationship with DOC concentration. Correlation coefficients between DOC and HIX were positive for six watershed compartments but negative in throughfall. Correlation coefficients between

% C5 and DOC were positive only for two watershed sources. The “microbial” and protein indices (% protein, % C3 and FI) all reported negative relationships with DOC concentration. Among these three indices, correlations between % protein and DOC were especially strong and negative for six of the ten watershed compartments. The relationship between DOC concentration and S_R was also strong and negative for five watershed compartments indicating that DOC was associated with the high molecular weight DOM.

Similar to DOC, correlations between DON and a_{254} were also positive for nine of the ten watershed compartments, but compared to the relationships to DOC concentrations, correlations between DON and a_{254} were generally weaker (lower correlation coefficients). For the other humic/aromatic indices – SUVA, HIX and % C5, the correlations with DON were positive for four, two and one watershed compartment, respectively. DON was positively correlated with % protein in litter but negatively correlated in tension soilwater, hyporheic and shallow groundwater compartments. With FI, DON was negatively correlated for only two compartments – hyporheic and shallow groundwater. A negative correlation between DON and S_R was observed for throughfall, litter and tension soilwater.

SUVA revealed significant correlations for only a few watershed compartments. Surprisingly, SUVA which is a measure of aromatic DOM did not reveal a strong correlation with the other humic and aromatic metrics (HIX and % C5). The correlations of SUVA with the % protein, % C3 and FI metrics were also mixed. Similarly, significant correlations between S_R and other DOM quality metrics were observed for only a few watershed compartments. S_R was positively correlated with % protein in shallow and riparian groundwater compartments indicating that proteins for these compartments were associated with low molecular weight DOM.

HIX produced the strongest correlations among all DOM metrics. HIX was positively correlated with % C5 across all compartments, suggesting that humified DOM material was also aromatic. HIX also displayed a very strong inverse relationship with % proteins across all compartments, indicating that DOM with protein-like fluorescence was not highly humified or degraded. The relationship between HIX and both % C3 and FI were negative for most compartments.

Finally, as expected, % proteins revealed a strong negative relationship with % C5 across all compartments and a predominantly positive relationship (with the exception of deep groundwater) with the microbial metrics % C3 and FI.

Table 3: Pearson correlation coefficients ($p < 0.10$) for for various watershed compartments. Waters (TF); litter (LT); tension soil water (U); zero t streamwater (ST); hyporheic water (HY); shal groundwater (RGW); groundwater seeps (See

	TF (80,18)*	LT (82,21)	U (62,11)	WSW (129, 29)	ST (47,14)
DOC – DON	0.57	0.37	0.77	0.41	0.30
DOC – SUVA	-0.27	-0.51	0.53	0.22	
DOC – a_{254}	0.59	0.62	0.94	0.84	0.99
DOC – HIX	-0.53			0.38	0.68
DOC – % C5					
DOC – % protein				-0.47	-0.73
DOC – % C3			-0.83	-0.41	
DOC – FI					
DOC – S_R	-0.39	-0.74	-0.93	-0.51	
DON – SUVA			0.66	0.23	0.31
DON – a_{254}	0.31	0.27	0.74	0.43	0.53
DON – HIX					

* Number of samples used for DOM concentrations and UV, followed by number of

Table 3 continued

	TF (80,18)*	LT (82,21)	U (62,11)	WSW (129, 29)	ST (47,14)
DON – % C5					
DON – % protein		0.62	-0.37		
DON – % C3			-0.56		-0.57
DON – FI					
DON – S_R	-0.26	-0.63	-0.82		
a_{254} – HIX				0.43	0.80
a_{254} – % C5					
a_{254} – % protein	-0.36			-0.51	-0.71
a_{254} – % C3			-0.85	-0.51	-0.76
a_{254} – FI					
a_{254} – S_R	-0.44	-0.78	-0.86	-0.58	
SUVA – HIX	0.61				
SUVA – % C5					
SUVA – % protein	-0.49			-0.37	

* Number of samples used for DOM concentrations and UV, followed by number of

Table 3 continued

	TF (80,18)*	LT (82,21)	U (62,11)	WSW (129, 29)	ST (47,14)
SUVA – % C3	-0.40			-0.65	
SUVA – FI					
SUVA – S_R			-0.81	-0.60	
S_R – HIX					
S_R – % C5					
S_R – % protein					
S_R – % C3				0.63	
S_R – FI					
HIX – % C5	0.70	0.47	0.74	0.46	0.63
HIX – % protein	-0.63	-0.95	-0.84	-0.84	-0.90
HIX – % C3	-0.77				-0.50
HIX – FI		-0.38			
% proteins – % C5	-0.42	-0.60	-0.59	-0.44	-0.75
% proteins – % C3				0.44	
% proteins – FI		0.50			

* Number of samples used for DOM concentrations and UV, followed by number of

Chapter 4

DISCUSSION

Changes in DOM Across Watershed Compartments and With Soil Depth

Results from this study revealed marked changes in DOM concentrations and characteristics as throughfall water percolated through the soil profile and entered various hydrologic compartments within the watershed. There were two distinct shifts in DOM – first, from throughfall to litter, and then from the litter layer to sources deeper in the soil profile. Similar to previous studies (Michalzik et al., 2001), DOM concentrations in throughfall were lower than those measured for the litter layer. Furthermore, compared to litter, throughfall DOM was less aromatic and humic (as indicated by SUVA, a_{254} and % C5). Simultaneously, the % protein content of throughfall was also higher than litter and the S_R values suggested that the molecular weight of DOM in throughfall was lower than that in litter. While not many studies have performed fluorescence analysis for throughfall DOM, previous results from sorptive fractionation and other analysis of DOM (Qualls and Haines 1991) corroborate our observations. These studies report that compared to the litter layer, throughfall DOM tends to be more hydrophilic, less aromatic, and may contain a greater proportion of DOM constituents with lower molecular weight (Kalbitz et al., 2000; Qualls and Haines, 1991).

As DOM percolated from the litter layer further into the soil profile a systematic change in DOM occurred: (a) both DOC and DON concentrations decreased with soil depth, but the drop in DOC concentrations was greater; (b) aromatic or humic constituents of DOM decreased as revealed by a_{254}, SUVA, HIX, and % C5; (c) simultaneously, the relative % C3, FI and % protein contents of DOM increased with depth; and (d) the molecular weight of DOM decreased as indicated by an increase in S_R.

High concentrations of DOC and DON in surficial sources such as forest floor or litter layer have been well documented (Kalbitz et al, 2000; Michalzik et al., 2001). Many of these studies have also shown that DOM in forest floors or surficial soil horizons is substantially humic and aromatic (Aitkenhead-Peterson et al., 2003; Qualls and Haines, 1991). Concentrations of DOM have been found to decrease dramatically as DOM percolates through the mineral soil profile and the decreases has been attributed to the preferential sorption of hydrophobic, humic, and/or aromatic C compounds (Jardine et al., 1989; Kaiser and Zech, 1998; Ussiri and Johnson, 2004). Studies have also reported that the proportion of C in DOM decreases much more rapidly as it percolates through the soil profile resulting in decreasing DOC:DON ratios with soil depth (Qualls and Haines, 1991). Some investigators have suggested that this is because the hydrophobic DOM (which is preferentially sorbed) contains more C while the hydrophilic compounds remaining in solution tend to be N rich (Kaiser and Zech, 2000; Qualls and Haines, 1991; Ussiri and Johnson, 2004). This has led to the suggestion that DON may be more mobile than DOC. In contrast, others have reported that N-rich DOM may also be hydrophobic and could be preferentially sorbed (McKnight et al., 1992; Yu et al., 2002). Yu et al. (2002) attributed the hydrophobic behavior of N-rich DOM moieties to complexation of N-rich amino compounds or proteins/peptides with polyphenols and/or humic substances. Contrary to these two competing views, laboratory-scale sorption experiments by Kothawala and Moore (2009) failed to reveal any sorption preference for either DOC or DON.

The decrease in humic and aromatic fractions of DOM with soil depth observed in our study clearly corroborates the results from these previous studies. The decrease in DOC:DON ratios with soil depth is also in line with the previous studies and suggests a greater loss of C-rich DOM with soil depth. Taken together, these two observations imply that a greater amount of DOC was associated with the more humic or hydrophobic fractions of DOM. However, this implication was not necessarily supported by our correlation analyses (Table 3) which did not reveal that DOC had stronger correlations with humic material compared to DON. While the correlation coefficients between a_{254} and DOC were slightly higher than those for DON, the four indices (a_{254}, SUVA, HIX and % C5) taken together did not provide conclusive evidence of C or N enrichment in humic or aromatic DOM pools.

The increase in S_R values with soil depth for our study suggests that molecular weight of DOM decreased with depth into the soil profile. This observation is consistent with previous studies that have found that DOM with high molecular weight (e.g. > 100 Da) and high HIX values is preferentially sorbed while DOM with lower molecular weight (< 1000 Da) is hydrophilic and remains in solution (Banaitis et al., 2006; Guo and Chorover, 2003; Kaiser and Zech, 2000). Recent work of Nguyen et al. (2010) found that the molecular weight of DOM was positively correlated with HIX and the hydrophobic fraction of DOM while it was negatively correlated with the protein-like fluorescence.

With the decrease in molecular weight of DOM, our data also revealed a simultaneous increase in amounts of % C3, FI and protein-like DOM. % C3 and FI have been used as proxies for “microbial” DOM with FI being typically used to differentiate between DOM from microbial or planktonic origins (or autochthonous DOM, FI: 1.7 – 2.0) versus that from higher terrestrial plants (or allochthonous DOM, FI: 1.2 – 1.5) (McKnight et al., 2001). Thus, while our % C3 values indicate a slight increase in the “microbial” fraction of DOM with soil depth, the FI values, even with the increase with depth, remained mostly within the range reported for higher terrestrial plants or DOM of allochthonous origin. We hypothesize that this increase in the % C3 and % total proteins was primarily due to the % decrease of the humic components and not necessarily an absolute increase in microbial or proteinaceous content of DOM. This shift in the DOM character and the importance of protein-like DOM for deeper DOM sources was especially highlighted in our PCA plot (Figure 5b). The clustering and location of the surficial watershed compartments in the PCA diagram clearly suggests that the humic components played a dominant role. However, once the humic components were removed by sorption or biodegradation the variability of DOM for deeper watershed sources was primarily regulated by the “microbial” and the protein contents of DOM.

Yu et al. (2002) determined free and combined amino/protein compounds using high performance liquid chromatography for O horizon leachates in Northern California. They found that proteins and amino compounds could form large complexes with phenols or humic substances resulting in high molecular weight DOM which display a hydrophobic behavior. They further hypothesized that the complexed proteins and amino acids were more likely to be sorbed and the proteins remaining in solution were non-complexed or free amino acids and proteins. Qualls and Haines (1991) also indicated that the proteins remaining in solution (hydrophilic) typically exist as free amino acids, peptides and proteins. Following the rationale of Yu et al. (2002) and our observations of increasing % protein-like moieties and decreasing molecular weight of DOM with soil depth, we hypothesize that the % total proteins observed in groundwater are likely free forms of the proteins. Vazquez et al. (2007, 2010) also concluded that proteinaceous DOM associated with groundwater was of low molecular weight.

The protein-like moieties determined from EEMs – tryptophan and tyrosine, have also been used to characterize the degradation status of proteins and amino compounds (Fellman et al., 2008). Tyrosine is assumed to represent more degraded peptide material, while tryptophan is used as a proxy for less degraded peptide material and intact proteins. Banaitis et al. (2006) evaluated these protein compounds in O-horizon and tree-leaf tissue extracts and found that the sorption potential for tryptophan was greater than for tyrosine (52 and 29 % sorption, respectively). Our observations on the ratio of % tryptophan to % tyrosine (Figure 3h) did not reveal any consistent trend with soil depth. Following the results of Banaitis et al. (2006), we expected that the tryptophan to tyrosine ratio would decrease with soil depth. However, other than the sharp decrease in the ratio from throughfall to litter there was no decrease, rather, the ratio increased slightly with soil depth. Thus the use of this ratio was inconclusive at our study site.

Influence of Wetland and Riparian Conditions on DOM

In addition to the vertical trend in DOM with soil depth, this study also revealed some distinct differences between the well-drained riparian and the wetland groundwater compartments (Figures 2 and 3). Despite the fact that both riparian and shallow groundwater wells sampled DOM from the same soil depth, there were distinct differences in DOM concentrations and quality. Both DOC and DON concentrations were lower in the riparian groundwater versus the wetland groundwater. The DOC:DON ratio was also lower for the riparian versus the wetland position. In addition, DOM for wetland groundwater was much more aromatic and humic (note a_{254}, SUVA, % C5 and HIX values), of higher molecular weight (S_R) and with lower values of % C3 and % total proteins compared to the riparian location (Figures 2 and 3). Following the Weishaar et al. (2003) model, the median SUVA value for the riparian groundwater of 1.8 translated into an aromatic content of 15 % while the corresponding aromatic content of the shallow wetland groundwater came to 27% (median SUVA value of 3.7).

High concentrations of DOM in wetlands have been well recognized (Fellman et al., 2008; Hagedorn et al., 2001; Kalbitz et al., 2000). Many of these studies have also shown that compared to uplands, wetland DOM is more humic, aromatic (high SUVA values) and refractory (high HIX) (Fellman et al., 2008; Geller 1986; Kalbitz and Geyer, 2002). The high concentrations and humic form of DOM in anoxic wetland soils has been attributed to low and inefficient decomposition of organic matter (Mulholland et al., 1990) and the release of DOM due to reductive dissolution of Fe and Al oxides (Hagedorn et al., 2001). We hypothesize that the well drained nature of the riparian location in our watershed likely promoted greater sorption of DOM on mineral surfaces and thus resulted in the lower aromatic and humic DOM observed for the riparian versus the wetland groundwater.

Relationships Between DOM Concentrations and Indices

Correlations between DOM concentrations and quality have been investigated in a number of recent DOM studies, a few of which are summarized in Table 4. Generally, the interest in exploring these relationships has been to: (a) determine if DOC or DON concentrations are associated with humic or non-humic DOM fractions; (b) if optical indices corroborate each other (i.e., SUVA and HIX following the same trend); and/or (c) to determine if the optical indices are related to ecological phenomena (e.g., bioavailable DOM from incubation assays, Fellman et al., 2008) so that these optical indices could then serve as valuable proxies for the phenomena of interest (Jaffe et al., 2008). Yamashita et al. (2010) found strong positive correlation between DOC and a_{350} and a negative relationship between DOC and S_R and DOC and FI. While Sanderman et al. (2009) also reported a significant positive correlation between SUVA and DOC, Jaffe et al. (2008) did not find any such relationship. Others have reported that DOC:DON ratios are positively correlated to SUVA and inversely associated with FI, i.e., DOM rich in C is humic/aromatic while DOM rich in N is less humic and displays a stronger microbial behavior. Bioavailable

DOM has been found to be strongly correlated to the EEM-derived % total proteins and inversely related to SUVA (Fellman et al., 2008). However, in most of these studies the correlations were developed based on data for only one or two watershed DOM sources (e.g. stream or soilwater) and were not investigated across multiple watershed compartments.

Our results across multiple watershed compartments clearly corroborate some of these relationships but also suggest that the strength, and in a few cases the direction (positive or negative correlation) of the relationships, may change with watershed DOM sources. While we found that DOC was associated with humic or aromatic fractions of DOM we also found that DON was associated with the humic or aromatic moieties (though to a lesser extent). Interestingly, both DOC and DON were associated with higher molecular weight DOM fractions (correlation with S_R) for the surficial watershed compartments but the same relationship did not extend to deeper watershed sources. Removal of large molecular weight DOM compounds in surface soil (through sorption) could have been a contributing factor.

DOC was strongly and negatively correlated with % total proteins (note the absence of this correlation for throughfall and litter compartments where humic dominate), but surprisingly, DON also revealed a strong negative correlation with % total proteins. The correlation between % proteins and DON was however weaker than that between % proteins and DOC. Taken together, these results suggest that at our site – (a) both DOC and DON were associated with humic fraction, and (b) that DON was likely composed of many other constituents in addition to proteins. Another interpretation that could explain the negative correlation between DON and %

proteins (as has been alluded to in the previous section) is that the EEM-derived % protein represents only the free forms of proteins. It is very likely that DON at our site is also composed of a large number of combined proteins, but which are not being accounted for by the protein-like fluorescence derived from EEMs. This interpretation would be more in line with the observations of Maie et al. (2006) and Yu et al. (2002) who found that a large proportion of DON was constituted by combined protein and amino compounds.

Our correlation results also reveal that some optical indices may yield stronger relationships than others. This is noticed in the strength and the number of significant correlation coefficients observed for a_{254} and HIX versus those for SUVA. Weak relationships for SUVA have also been reported in a number of previous studies (Fellman et al., 2009; Jaffe et al., 2008; Yamashita et al., 2010). Similarly, while SUVA did not yield a systematic gradient across compartments (Figure 3) the other aromatic metrics (a_{254} and % C5) produced a more pronounced pattern. This would suggest that a_{254} and % C5 may be preferable to SUVA for characterizing the aromatic content of DOM. Finally, our results did not suggest that there were particular watershed compartments that yielded stronger or a greater number of significant DOM correlations compared to others. We had expected that watershed compartments or locations that had a greater potential for hydrologic mixing (intersection of multiple DOM sources and flowpaths) and therefore more complexity would yield weaker DOM relationships versus locations where DOM was less mobile and more intimately in contact with the substrate (e.g., tension soilwater). Clearly, our results suggest that DOM relationships for watershed compartments are influenced by multiple factors.

It is important to note here that in developing these correlations we lumped DOM data from all sampling dates across the year. While most of the data followed the trend indicated by the correlations, there were occasional data points with very high or low DOM. Since the intent of this study was to identify the primary correlations (annual time scale), these few “outliers” were excluded from the correlation analyses. At closer scrutiny, we found that these few outliers corresponded with events of high biogeochemical activity such as leaf-fall in autumn, leaf-out during the spring season, and/or storm events following dry conditions in summer. A few examples of correlations with such outliers are included in Figure 6. These outliers represent events that can be considered as “hot moments” of biogeochemical activity (McLain et al., 2003) and should be given closer consideration especially if the intent is to investigate DOM associated with intense biogeochemical or seasonal activity.

Implications for Bioavailability of DOM

Previous work has shown that DOM with low humic and aromatic content (e.g., consisting of free peptides, proteins, amino acids, etc.) is more bioavailable (Kaushal and Lewis, 2005; Marschner and Kalbitz, 2003; Qualls and Haines, 1992). In contrast, DOM with high molecular weight and containing complexes of proteins and humic material has been found to have very low biodegradation rates (Keil and Kirchman, 1994; Maie et al., 2006). Recent comparisons of DOM incubation assays and optical indices tend to corroborate these observations. Fellman et al. (2008; 2009) found that bioavailable DOC was inversely related to SUVA and DOC:DON ratio, but directly proportional to EEM-derived protein-like fluorescence (or % total proteins) for wetland soils in Alaska (Table 4). Balcarczyk et al. (2009) also noted the strong relationship between % total proteins and bioavailable DOM (BDOM) for discontinuous permafrost soils in Alaska. For both studies, a protein content of 10% corresponded with about 20 – 30% of bioavailable DOC. The range of % protein values at our site (from less than 5 to 30%) was considerably larger than that observed by Fellman et al., (2009) and Balcarczyk et al. (2009) (0 to 15 % in both studies). Whether this translates into higher % BDOM at our site, can however, only be determined after performing DOM incubation assays (BDOM assays are currently being performed for various watershed sources and will be compared with spectrofluorometric indices, Singh et al., in preparation). Nevertheless, the % protein values presented here can still be used to assess the relative bioavailability of DOM among the various watershed sources.

The increasing % of proteins (Figure 3g) with soil depth (LT < WSW < HY < ST < SGW < RGW < Seep < DGW) suggests that the % of BDOM increased with soil depth. This overall assessment is also supported by the trend in other indices like FI, % C3, a_{254} and S_R. However, while the % of BDOM likely increased with soil depth, the total DOM (Figure 2) declined sharply. This indicates that despite the % increase in BDOM, the total amount of BDOM may remain the same or even decline with soil depth. A similar conclusion was reached by Balcarczyk et al. (2009) who found that the proportion of BDOM was highest in groundwater springs, but simultaneously cautioned that the total amount of BDOM may not be high because of the decrease in absolute amounts of DOM with soil depth. Incubation experiments by

Qualls and Haines (1992) showed that while DOM biodegradability decreased from throughfall to the A-horizon it increased with depth below the A-horizon.

A comparison of our % protein values for wetland and riparian groundwater (SGW and RGW, respectively in Figure 3g) at our site suggests that the proportion of BDOM in wetland groundwater was considerably less than that for riparian locations. Again, whether this trend extends to absolute BDOM, would be dictated by the difference in total DOM between the two locations. DOM concentrations in wetland groundwater at our site were much higher than the value for the riparian location (Figure 2a and b). Geller (1986) reported that DOM from wetlands is recalcitrant and is largely unavailable for bacterial degradation. In contrast, Fellman et al. (2009) found that wetland soils produced more labile DOM than upland locations. Observations in the later study were however made in the glaciated, coastal rainforest of Alaska which represents a very different climate, geology, and ecological conditions compared to our unglaciated site in the mid-Atlantic US.

Finally, it should be noted that while sorption may be a dominant phenomenon influencing DOM in watershed compartments (Qualls and Haines, 1992), biodegradation may also simultaneously alter DOM (Qualls, 2000; Marschner and Kalbitz, 2003). Our observations suggest that the relative opportunities for biodegradation may be enhanced in groundwater as humic material is removed during passage through surficial watershed compartments.

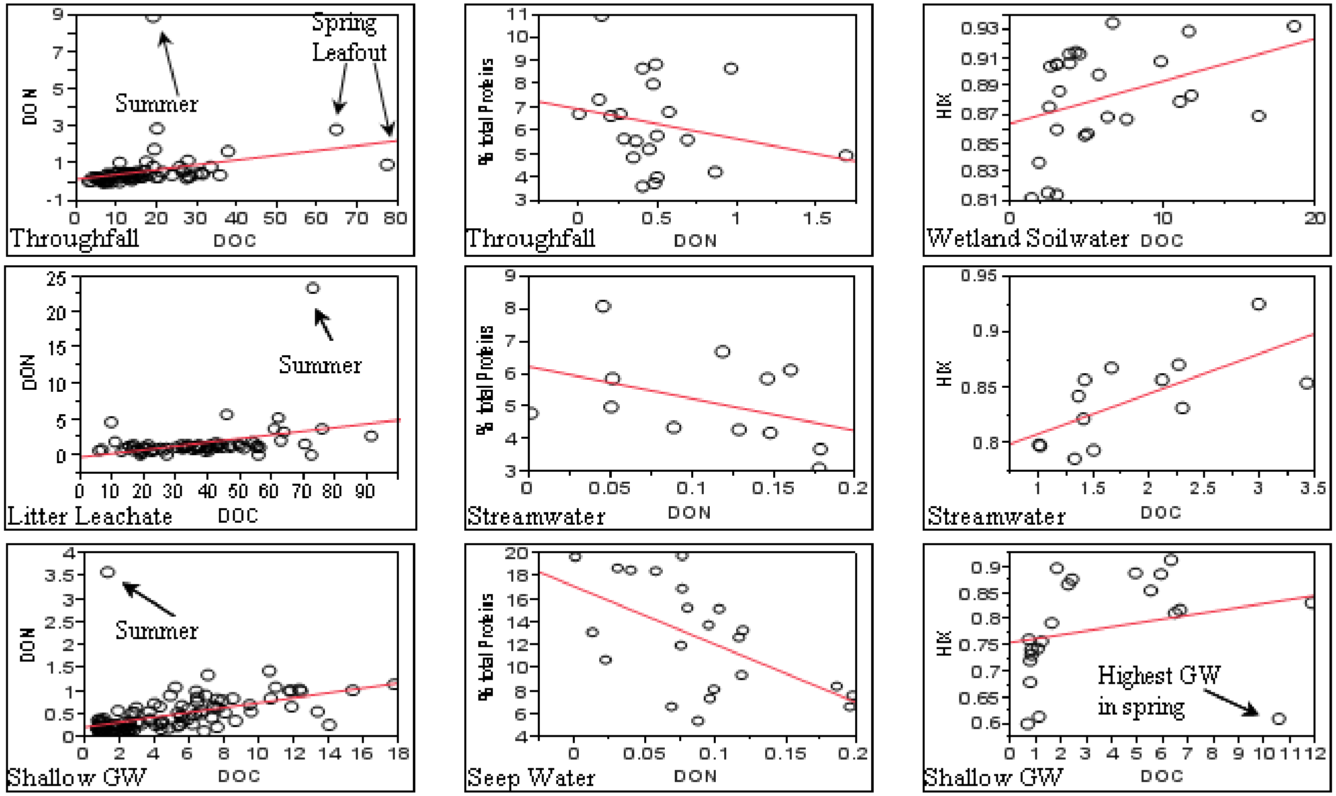


Figure 6: A sampling of correlations observed for various watershed compartments for DOC (mg C L^{-1}), DON (mg N L^{-1}), HIX, and % total proteins. A few outliers associated with summer and spring leaf out can be observed in some of the plots. These outliers were excluded from correlations only if their inclusion dramatically altered the coefficient values.

Table 4: Correlations between various DOM parameters re

Reference	Site and sample type	Parameters correlated
Balcarczyk et al. 2009	Fairbanks, Alaska, USA; discontinuous permafrost soils, streamwater	FI vs. SUVA FI vs. DOC:DON BDOM vs. % proteins
Fellman et al. 2009	Juneau, Alaska, USA; soil water	BDOC vs. % proteins Wetland: BDOC vs. SUVA Upland forest: BDOC vs. SUV SUVA vs. humic-like fluoresce
Jaffé et al. 2008	Multiple sites across US; streamwater, lakes, estuaries	DOC vs. SUVA and FI SUVA vs. FI
Nguyen et al. 2010	Han River basin, Korea; streamwater	HIX vs. molecular weight (MW Protein-like fluorescence vs. m Hydrophobic fraction vs. mole
Sanderman et al. 2009	Marin County California, USA; streamwater	DOC vs. SUVA DOC:DON vs. SUVA
Wu et al. 2007	Ontario, Canada; mixed deciduous forest with large wetland	DOC vs. humic-like componen DOC vs. molecular weight (M DOC vs. FI FI vs. molecular weight (MW)
Yamashita et al. 2010	Tropical streams, Venezuela; Forest and grassy savanna	DOC vs. a_{350} DOC vs. FI DOC vs. S_R DOC vs. $SUVA_{350}$ FI vs. S_R

DOM in Watershed Sources and Implications for Streamwater DOM

Knowledge of DOM concentrations and quality for various watershed sources can be helpful for estimating stream DOM exports if the runoff sources to the stream are known. Alternately, if stream DOM concentrations are known, knowledge of DOM in watershed sources can assist in identifying (or verifying) the runoff sources through the use of end-member mixing models (e.g. Inamdar and Mitchell, 2006; Verseveld et al., 2009). Our observations show that surficial DOM sources (throughfall, litter, wetland soilwater) were clearly more humic and aromatic with a lower content of proteins; and vice-versa for the groundwater DOM sources. Thus streamwater during baseflow (which typically derives runoff from groundwater sources, e.g. Inamdar and Mitchell, 2006) would be expected to be lower in humic and aromatic DOM. Not surprisingly then, DOM chemistry for the streamwater presented in this study (sampled during non-storm periods) was considerably different from that observed for throughfall and litter. Based on Figures 2 and 3, we hypothesize that streamwater DOM during baseflow in our watershed is some combination of wetland soilwater and groundwater sources (SGW, RGW and Seep). We expect that in-stream biotic processes (Brookshire et al., 2005) may further alter the DOM chemistry as runoff traverses the length of the stream to the watershed outlet.

Chapter 5

CONCLUSIONS

To my knowledge, this is the first study that has evaluated DOM concentrations and quality: (a) for multiple watershed compartments; (b) with data across multiple seasons over a two-year period; and (c) implemented a suite of optical indices simultaneously to characterize DOM quality. Key conclusions that can be derived from this research are:

- DOM quality or constituents clearly had a greater influence on DOM dynamics than the concentrations of DOC or DON. Quality of DOM also revealed systematic trends across the watershed compartments, especially with depth. Furthermore, the constituents of DOM differed in their relative influence of DOM dynamics among watershed sources. Humic and aromatic DOM compounds had a greater role in discriminating DOM among surficial sources, while “microbial” DOM and/or protein-like fluorescence became more important for groundwater compartments.

- There was no conclusive evidence for C or N enrichment in DOM quality pools. This highlights the complexity of studying DOM, especially, at the watershed scale. There were distinct differences in DOM quality for wetland and riparian groundwater likely associated with saturated and anoxic conditions at these landscape positions.

- This study demonstrated the value of using multiple optical indices to characterize the mobility, degradability and/or bioavailability of DOM. The results suggest that some spectrofluorometric indices may be more preferable over others for characterizing Dom quality. Indices like a_{254}, HIX, % C5, % proteins and S_R provided distinct trends across watershed sources and were valuable in understanding DOM quality while observations from SUVA were not that helpful.

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