# AN ABSTRACT OF THE DISSERTATION OF

Baek Soo P. Lee for the degree of Doctor of Philosophy in Water Resources Engineering presented on October 29, 2015.

Title: Hydrologic and Land Cover Effects on Sources and Fate of In-Stream Fluorescent Dissolved Organic Matter

Abstract approved: ______________________________________________________

Michael E. Campana

Dissolved organic matter (DOM) is a critical component of the carbon cycle linking terrestrial and aquatic ecosystems. Although many factors influence DOM fluxes and quality in rivers, controls on DOM compositions in catchments of the western U.S. are poorly understood. UV and fluorescent spectroscopy is a simpler, faster, and less expensive DOM fingerprinting technique compared to techniques, such as nuclear magnetic resonance (NMR) spectroscopy or wet chemical fractionation, and could be useful for characterizing complex DOM chemistry. However, only 1 % of DOM is estimated to be fluorescent, and the utility of UV and fluorescent spectroscopy for DOM characterization needs to be further investigated. This dissertation applied UV and fluorescent spectroscopy to examine hydrologic and land cover controls on DOM chemistry in streams of 45 catchments in the forested headwaters and a mixed landscape of the Willamette River Basin, Oregon, based on two years of monitoring. This dissertation contributes three major findings. First, freezing of water alters DOM chemistry by preferentially precipitating aromatic DOM. Second, UV and fluorescent spectroscopy was able to discriminate DOM delivered from highly processed, protein-rich deep subsurface sources during dry seasons especially in forested headwater streams of the H. J. Andrews Experimental Forest (HJA), where nitrogen inputs are very low. In addition, fluorescent DOM chemistry differed among watersheds with varying forest management history. Third, although fluorescent DOM in a headwater forested system differed among land use history, fluorescent DOM composition did not vary among streams draining a well-mixed landscape of urban, pasture/hay, forest, and agricultural land cover types in the central Willamette River Basin, where nitrogen inputs to streams are relatively high. Dissolved organic carbon (DOC) concentration decreased and the fluorescent index indicated an increase in terrestrial sources of DOM from small (1st and 2nd order) to large (4th to 6th order) streams. A protein-like DOM component that was detected in the headwater forested study site (HJA) was not detected in stream samples from the middle basin study site. These findings indicate that in-stream respiration, fueled by nutrient additions from agriculture runoff, consumes bioavailable, labile DOM (proteins) preferentially relative to more recalcitrant, terrestrial sources of DOM along water flow paths from headwater streams to major rivers. This study shows the clear applicability of the fluorescent characterization of DOM in identifying hydrologic and landscape controls as well as varying DOM chemistry and functions throughout watershed ecosystems.

# Hydrologic and Land Cover Effects on Sources and Fate of In-Stream Fluorescent Dissolved Organic Matter

by
Baek Soo P. Lee

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APPROVED:

Major Professor representing Water Resources Engineering

Director of the Water Resources Graduate Program

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Baek Soo P. Lee, Author

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The contributions made by each authors for Chapter 2 are as follows:

Dr. Kate Lajtha assisted with the design of the study,

Dr. Angelicque E. White assisted with the analyses of samples in the Dr. White's laboratory and assisted with data analysis, and

Dr. Lajtha and Dr. White contributed to the writing of the manuscript.

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# TABLE OF CONTENTS

Page

1 Introduction ........................................................................................................1

2 Preservation method effects on nutrients and optical properties of dissolved organic matter in aquatic samples ........................................................................................4

- 2.1 Abstract.................................................................................................5
- 2.2 Introduction ..........................................................................................6
- 2.3 Materials and Procedures .....................................................................9
    - 2.3.1.1 Sample Sites ...............................................................................9
    - 2.3.1.2 Field and laboratory Procedures ..............................................10
    - 2.3.1.3 EEM Analyses ...........................................................................13
- 2.4 Assessment and Results.......................................................................13
    - 2.4.1.1 Nutrients ...................................................................................14
    - 2.4.1.2 DOC...........................................................................................15
    - 2.4.1.3 DOM optical properties.............................................................15
- 2.5 Discussion.............................................................................................16
- 2.6 Conclusions ..........................................................................................18
- 2.7 Acknowledgements ..............................................................................20
- 2.8 References ............................................................................................21
- 2.9 Table.....................................................................................................30
- 2.10 Figures ...............................................................................................31

3 Hydrologic and Forest Management Controls on Dissolved Organic Matter Characteristics in Headwater Streams of Old-Growth Forests in the Oregon Cascades...38

- 3.1 Abstract.................................................................................................39
- 3.2 Introduction ..........................................................................................40
- 3.3 Materials and Methods .........................................................................44
    - 3.3.1 Sample Sites ..................................................................................44
    - 3.3.2 Field methods ................................................................................45
    - 3.3.3 Laboratory methods.......................................................................45
    - 3.3.4 EEM Analysis................................................................................48
    - 3.3.5 Hydrological Analysis ...................................................................48
- 3.4 Results ..................................................................................................49
    - 3.4.1 DOM Components.........................................................................49
    - 3.4.2 Correlations of DOM Components with Other Parameters ..........49
    - 3.4.3 Temporal Variations.......................................................................50
        - 3.4.3.1 DOM optical properties.........................................................50
        - 3.4.3.2 Hydrologic Events.................................................................52
- 3.5 Discussion.............................................................................................53
    - 3.5.1 Overall DOM Sources ...................................................................53
    - 3.5.2 Protein-like DOM and Hydrologic Path.........................................53
    - 3.5.3 Forest Management and Landscape ..............................................56
- 3.6 Acknowledgements ..............................................................................60

# TABLE OF CONTENTS (Continued)

Page

3.7 References ....................................................61
3.8 Tables ....................................................69
3.9 Figures ....................................................73
3.10 Appendix ....................................................83

4 Seasonal and Land Use Effects on UV and Fluorescent Dissolved Organic Matter Characteristics in Streams of a Mixed Landscape ....................................................85
4.1 Abstract....................................................86
4.2 Introduction ....................................................87
4.3 Materials and Methods ....................................................90
4.3.1 Sample Sites ....................................................90
4.3.2 Field and laboratory methods ....................................................91
4.3.3 EEM Analysis....................................................94
4.3.4 Spatial Analysis ....................................................95
4.3.5 Statistical Analysis ....................................................96
4.4 Results ....................................................96
4.4.1 Nutrients ....................................................96
4.4.2 DOC....................................................96
4.4.3 DOM optical properties ....................................................97
4.5 Discussion....................................................98
4.5.1 Spatial and Temporal patterns of DOC and Nutrients....................................................98
4.5.2 DOM optical characteristics in the study site....................................................99
4.5.3 Spatial and temporal patterns of DOM....................................................100
4.6 Acknowledgements ....................................................105
4.7 References ....................................................106
4.8 Tables ....................................................114
4.9 Figures ....................................................116
4.10 Appendices ....................................................131

5 Conclusions ....................................................133

6 Bibliography ....................................................139

# LIST OF FIGURES

Figure Page

Figure 1: Comparison of $NO_3^-$ - N concentrations (mg $L^{-1}$) stored in HDPE bottles in a refrigerator (initial concentrations) against stored in 1) Amber bottles in a refrigerator (y = 1.01x; $R^2$ = 1.00), 2) HDPE bottles in a freezer for a few weeks (FZWK) (y = 0.99x; $R^2$ = 1.00), and 3) HDPE bottles in a freezer for months (FZMO) (y = 0.71x; $R^2$ = 1.00)....31

Figure 2: Comparison of TDN concentrations (mg $L^{-1}$) stored in HDPE bottles in a refrigerator (initial concentrations) against stored in 1) Amber bottles in a refrigerator (y = 0.99x; $R^2$ = 0.99), 2) HDPE bottles in a freezer for a few weeks (FZWK) (y = 0.97x; $R^2$ = 0.99), and 3) HDPE bottles in a freezer for months (FZMO) (y = 1.08x; $R^2$ = 0.98)....32

Figure 3: Comparison of DOC concentrations (mg $L^{-1}$) stored in HDPE bottles in a refrigerator (initial concentrations) against stored in 1) Amber bottles in a refrigerator (y = 1.01x; $R^2$ = 1.00), 2) HDPE bottles in a freezer for a few weeks (FZWK) (y = 0.96x; $R^2$ = 1.00), and 3) HDPE bottles in a freezer for months (FZMO) (y = 0.93x; $R^2$ = 1.00)....33

Figure 4: Comparison of $SUVA_{254}$ (L mg-$C^{-1}$ $m^{-1}$) stored in HDPE bottles in a refrigerator (initial values) against stored in 1) Amber bottles in a refrigerator (y = 0.98x; $R^2$ = 1.00), 2) HDPE bottles in a freezer for a few weeks (FZWK) (y = 0.71x; $R^2$ = -1.47), and 3) HDPE bottles in a freezer for months (FZMO) (y = 0.67x; $R^2$ = -1.13)......34

Figure 5: Comparison of FI stored in HDPE bottles in a refrigerator (initial value) against stored in 1) Amber bottles in a refrigerator (y = 1.00x; $R^2$ = 0.95), 2) HDPE bottles in a freezer for a few weeks (FZWK) (y = 1.02x; $R^2$ = 0.88), and 3) HDPE bottles in a freezer for months (FZMO) (y = 1.01x; $R^2$ = 0.52).......................................................................35

Figure 6: Comparison of HQ (%) stored in HDPE bottles in a refrigerator (initial %) against stored in 1) Amber bottles in a refrigerator (y = 0.99x; $R^2$ = 1.00), 2) HDPE bottles in a freezer for a few weeks (FZWK) (y = 0.81x; $R^2$ = -0.40), and 3) HDPE bottles in a freezer for months (FZMO) (y = 0.82x; $R^2$ = -0.70). ..................................................36

Figure 7: Comparison of Protein (%) stored in HDPE bottles in a refrigerator (initial value) against stored in 1) Amber bottles in a refrigerator (y = 1.00x; $R^2$ = 0.99), 2) HDPE bottles in a freezer for a few weeks (FZWK) (y = 0.87x; $R^2$ = 0.93), and 3) HDPE bottles in a freezer for months (FZMO) (y = 0.89x; $R^2$ = 0.45). ......................................37

Figure 8: Map of the H.J. Andrews Experimental Forest..................................................73

Figure 9: Flow rate and stream temperature at the Lookout Creek Watershed (LOOK) between May 2013 and June 2015 ........................................................................................74

Figure 10: EEMs of four components identified by the PARAFAC model (Figure 10a – d) and their excitation emission loadings (Figure 10e) .....................................................76

# LIST OF FIGURES (CONTINUED)

Figure Page

Figure 11: Time-series of various parameters ........ 80

Figure 12: Comparison of components between harvested and control watersheds in R.U. ........ 81

Figure 13: Comparison of components between harvested and control watersheds in % 81

Figure 14: Map of the monthly sampling locations in the middle Willamette River Basin ........ 116

Figure 15: Land use compositions of a 300-m buffer at each site ........ 117

Figure 16: Average daily flow rate (cfs), total precipitation (mm), and daily temperature ($^{o}$C) for each water year (WY) ........ 118

Figure 17: Time series of inorganic nutrients and TDN based on percent pasture/hay land (a – d) and stream order (e – h) ........ 122

Figure 18: Time series of DOC concentrations based on percent urban land (Figure 18a) and stream order (Figure 18b) ........ 123

Figure 19: Time series of FI based on percent pasture/hay land (Figure 19a), percent urban land (Figure 19b), and stream order (Figure 19c) ........ 125

Figure 20: Time series of $SUVA_{254}$ by percent urban land ........ 125

Figure 21: EEMs of three components identified by the PARAFAC model (Figure 21a – c) and their excitation emission loadings (Figure 21d) ........ 127

Figure 22: Time series of fluorescent components with various land use ........ 129

Figure 23: An example of a protein-like EEM residual ........ 130

# LIST OF TABLES

Table Page

Table 1: The summary of initial sample concentrations stored in HDPE bottles in a refrigerator ........ 30

Table 2: Experimental watersheds properties and history (H.J. Andrews Experimental Forest 2015) ........ 69

Table 3: Biomass (Mg/ha) of total, live tree, and logs in each watershed (Fasth et al., Unpublished Data) ........ 69

Table 4: Four components identified by this study and their previously identified characteristics ........ 70

Table 5: Statistics of measured parameters ........ 70

Table 6: Correlations among key parameters ........ 71

Table 7: Linear correlations of C4 (%) and $SUVA_{254}$ with flow parameters at each watershed ........ 72

Table 8: Logarithmic correlations (coefficient of determination, $R^2$) of C4 (%) with flow parameters at each watershed. ........ 72

Table 9: The summary of sampling period, sample numbers, and detection limit ........ 114

Table 10: Bivariate correlation ........ 114

Table 11. Three unique components (C) identified in the central Willamette River Basin ........ 115

# LIST OF APPENDICES

Appendix Page

Appendix 1: Comparison of components identified by this study and characterized by a previous study (Stedmon and Markager 2005) ................................................................84

Appendix 2: The land use compositions and stream order for each sampling location. . 131

Appendix 3: The comparison of EEM regions of components identified by this study (left) and by *Stedmon and Markager* [2005] (right)........................................................132

# 1 Introduction

Dissolved organic matter (DOM) is a critical component of the carbon (C) cycle in watershed ecosystems and enters water bodies from various sources. Surrounding lands and soils deliver various types of DOM such as leaf litter to streams (allochthonous DOM), and primary producers generates DOM through photosynthesis within streams (autochthonous DOM) (Vannote et al. 1980; Allan and Castillo 2009). Watershed characteristics such as topography, climate, hydrology, and land use create unique mixture of DOM chemistry in streams (Hudson and Reynolds 2007). Nonetheless, the effect of hydrology and disturbances to lands and soil on DOM chemistry and fluxes in streams is poorly understood.

Chemically diverse DOM components play varying roles in watersheds (Barnes et al. 2012). Labile DOM is bioavailable and fuels ecosystem processes (Cory et al. 2011); fulvic acids contribute to metal transport by complexing with copper (McKnight et al. 2001) and redox reactions (Miller et al. 2006); reactive DOM is a byproduct of drinking water disinfection (Weishaar et al. 2003). Therefore, identifying critical chemical properties of DOM may help elucidate factors controlling aquatic ecosystem function.

Unique chemical structures of DOM compounds create distinctive absorbance and fluorescent properties in freshwater samples (Coble 1996; McKnight et al. 2001; Weishaar et al. 2003; Cory and McKnight 2005; Stedmon and Bro 2008); for example, allochthonous DOM has more abundant aromatic compounds than autochtonous DOM because terrestrial vegetation is high in tannins and lignins (McKnight et al. 2001; Allan and Castillo 2009). Such difference in chemical structures of DOM compounds create distinctive UV absorbance and fluorescent properties in freshwater samples (Coble 1996;

McKnight et al. 2001; Weishaar et al. 2003; Cory and McKnight 2005; Stedmon and Bro 2008). UV and fluorescent spectroscopy techniques are simpler, less expensive, and faster than the sophisticated DOM characterization using nuclear magnetic resonance (NMR) spectroscopy (Dai et al. 1996; Kalbitz et al. 2003) or wet chemical fractionation techniques (Yano et al. 2004). Fluorescent intensities of each sample can be observed in 3-D excitation and emission matrices (EEMs) to characterize fluorescent properties of DOM controlled by unique DOM chemical structures. EEMs capture the fluorescent signature at all combinations of excitation and emission wavelengths. Fluorescent DOM components and the fraction of each component are identified from EEMs with statistical analysis, often with a multivariate statistical modeling Parallel Factor Analysis (PARAFAC) (Stedmon and Bro 2008).

Fluorescent characterization of in-stream DOM has been widely studied and accepted in recent years to identify and quantify DOM sources in many freshwater ecosystems of estuaries, wetlands, lakes in the Antarctica, the high altitude of Colorado, and in northern Alaska, headwater forest streams in the eastern U.S., and headwater urban streams in the eastern U.S. (Coble 1996; Cory and McKnight 2005; Miller et al. 2006; Yamashita et al. 2010; Yamashita et al. 2011; Mann et al. 2012; Cawley et al. 2012b; Hosen et al. 2014). However, only 1 % of DOM is estimated to be fluorescent (Cory et al. 2011). The potential for utilizing fluorescent DOM to characterize complex DOM chemistry is of great interest, and further investigation may help determine the applicability of this technique on DOM chemistry characterization in freshwater systems.

The overall goal of the dissertation was to determine how the information gained through UV and fluorescent characterization of in-stream DOM helps understand hydrologic paths and land use disturbance in the Willamette River Basin, Oregon. The specific objectives of each manuscript of the dissertation were as follows:

The purpose of Chapter 2 was to examine the effect of preserving surface water samples frozen at -20 ºC for variable time periods on biogeochemical and optical properties.

The objectives of Chapter 3 were to 1) examine the role of seasonality and hydrology on DOM chemistry and 2) observe the effects of forest management history on DOM chemistry in pristine, forested headwater streams.

The objectives of Chapter 4 were: 1) to characterize DOM properties, 2) to observe spatial variations of DOM properties, and 3) to examine temporal variations of DOM properties driven by seasonal precipitation patterns in the streams of the middle of the Willamette River Basin characterized with uniquely mixed land use composition and stream orders.

# 2 Preservation method effects on nutrients and optical properties of dissolved organic matter in aquatic samples

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## 2.1 Abstract

The use of spectroscopic and fluorescent properties to characterize dissolved organic matter (DOM) has increased in recent years as a means to better understand the transport and processing of carbon in freshwater ecosystems. Because large numbers of samples or samples from remote locations must often be preserved before analysis, various preservation methods have been used, including freezing. This study examined the effects of freezing on biogeochemical properties of freshwater samples for time periods from two weeks to over six months. Samples were collected from pristine waters of the middle Willamette River Basin in Oregon and analyzed for inorganic nitrogen ($NH_4^+$, $NO_3^-$), total dissolved N (TDN), orthophosphate, dissolved organic carbon (DOC), specific ultraviolet absorbance at 254 nm ($SUVA_{254}$), fluorescence index (FI), and 13 fluorescent DOM components identified by the PARAFAC model of Cory and McKnight (2005). TDN and DOC concentrations, originally less than 0.6 mg $L^{-1}$ and 8 mg $L^{-1}$, respectively did not change as a result of freezing at -20ºC, suggesting that freezing is an adequate preservation method in our study site for these analyses. However, $NO_3^-$ declined significantly with freezing. $NH_4^+$ and orthophosphate in our pristine streams were generally under the detection limit, however, orthophosphate often declined after freezing possibly attributable to co-precipitation with DOM. Spectroscopic and fluorescent properties of DOM changed after freezing, likely due to precipitation of aromatic carbon. Thus, storing in a refrigerator at 4 $^{o}$C is recommended for the analysis of inorganic N, orthophosphate, and optical properties of DOM of aquatic samples.

## 2.2 Introduction

Dissolved organic matter (DOM) in freshwater originates from various sources including local exudation by riverine primary producers and via microbial decomposition of plant biomass and soil organic matter that is subsequently transported to nearby streams through hydrologic connections (Allan and Castillo, 2009; Cory et al., 2011). The degree and contribution of source, processes, and transport of DOM depend on the environment and are controlled by the combination of hydrology, climate, and biogeochemistry (Hudson and Reynolds 2007). Thus, understanding DOM characteristics is of great interest to watershed scientists. However, characterization of chemical properties of DOM using conventional methods, such as nuclear magnetic resonance and infrared spectroscopy, are time-consuming and require expensive, specialized equipment, and often very large sample volumes.

Alternative methods to these methods include the characterization of absorbance and fluorescent properties of DOM samples, which are faster, less complicated, and less expensive. Specific UV absorbance at 254 nm ($SUVA_{254}$), or absorbance at 254 nm divided by DOC concentrations, has been shown to approximate the percent aromaticity of DOM (Weishaar et al. 2003). Excitation and emission matrices (EEMs) of DOM samples are used to characterize fluorescent properties of DOM in a 3D format. Light is absorbed by samples over a specific range of excitation wavelengths; this light is then re-radiating over a specific range of emission wavelengths (Stedmon and Bro 2008) . The carbon-bonds of individual DOM components have unique fluorescent properties that lead to distinctive fluorescent intensities and wavelength characteristics (excitation-emission); EEMs allow identification of these DOM components (Stedmon and Bro 2008).

Coble (1996) identified several components of DOM from freshwater and marine environment including humic-like, tyrosine-like, and tryptophan-like components from EEMs. Multiple indices such as fluorescence index (FI) and redox index (RI) were developed to better characterize DOM (McKnight et al. 2001; Cory and McKnight 2005). FI can be used an indicator of the source of the aquatic samples and was derived by characterizing two end members: (1) microbially derived end members refer to the DOM derived by autochtonous processes in lakes and ponds of Antarctica and (2) terrestrially derived end members refer to the DOM from the environment rich in terrestrial plants and soil in creeks and rivers of the U.S. (McKnight et al. 2001). The original FI value (McKnight et al. 2001) has been modified for EEMs with instrument specific corrections to about 1.55 for microbially derived source and 1.21 for terrestrially derived source (Cory et al. 2010a). RI is used to indicate if the aquatic samples were high in reduced quinone-like component (close to 1) or oxidized quinone-like component (close to 0) (Cory and McKnight 2005). A Parallel Factor Analysis (PARAFAC), which is a multivariate statistical modeling technique, has been utilized to easily separate the mixture of DOM in EEMs and characterize each component of DOM (Stedmon et al. 2003; Cory and McKnight 2005). Cory and McKnight (2005) have identified 13 unique DOM components such as oxidized quinone-like, reduced quinone-like, and protein-like components with PARAFAC.

For freshwater samples that cannot be processed quickly after sampling, freezing at -20ºC is a common form of preservation (Motter, 2006; Fellman et al., 2008). Freezing is also an accepted form of preservation for ocean water where immediate analyses are unavailable (Dore et al. 1996). Thus, it is important to understand freezing effects on the optical properties of DOM, nutrients, and dissolved organic carbon (DOC). As freezing under natural conditions appears to change the properties of DOM (Mann et al. 2012), we might expect properties to change under laboratory conditions as well.

Fellman et al. (2008) cautioned against freezing of aquatic samples for later analysis of DOC for samples with DOC concentrations greater than 5 mg C $L^{-1}$ and $SUVA_{254}$ greater than 4 L mg-$C^{-1}$ $m^{-1}$, arguing that constituents of DOC can precipitate following abiotic particle formation in the freezing process. This study, conducted in Alaska, also found a decrease in total dissolved organic phosphorus after freezing of wetland water samples because the combination of high DOM and iron concentrations resulted in the complexation of DOC with P (Fellman et al. 2008). However, it is not clear if these results would be similar under low DOC, Fe, and P conditions of Oregon.

The effects of freezing have been examined for select DOM fluorescent components, identified in peat-rich rural headwater catchments in the UK (Spencer et al. 2007) and in urban and rural freshwaters from central England (Hudson et al. 2009). Spencer et al. (2007) found shifts in the fluorescence intensity and the peak location of DOM components after freezing; this effect was particularly pronounced for tryptophan-like protein components which are less stable than fulvic and humic-like components commonly found in DOM. Hudson et al. (2009) similarly found decreased peak fluorescence intensity in DOM components after freezing, especially for tryptophan-like protein component (-34 ± 24 %). The magnitude of $SUVA_{254}$ has also been found to decrease after freezing, again suggesting that freezing preferentially precipitates the aromatic component of DOM (Spencer et al. 2007; Fellman et al. 2008). Beyond these results, no study has examined freezing effects on the entire suite of 13 fluorescent components identified by the PARAFAC analyses of Cory and McKnight (2005) as well as the spectrofluorecense indices, FI and RI, especially in pristine environments such as rivers in the Pacific Northwest.

The purpose of this study was to examine the effect of freezing surface water samples at -20ºC for variable time periods on the following biogeochemical and optical properties: the concentration of DOC, total dissolved nitrogen (TDN), ammonium as nitrogen ($NH_4^+$-N), nitrite-nitrate as nitrogen ($NO_3^-$ -N), soluble reactive phosphorus ($PO_4^{3-}$-P ) as well as $SUVA_{254}$ , 13 fluorescent DOM components identified by PARAFAC (Cory and McKnight 2005), and FI in the area where relatively low DOC concentrations were found. We hypothesized that DOC concentrations as well as spectrophotometric and spectrofluorometric components would not change significantly as a function of storage methods because of low DOC concentrations in Oregon compared to those from previous studies. However, we hypothesized that those samples with high aromaticity would decrease in aromaticity because of preferential precipitation. We also hypothesized there would be minimal changes in nutrient concentrations between preservation methods because inorganic nutrients would not precipitate during freezing.

## 2.3 Materials and Procedures

### *2.3.1.1 Sample Sites*

Stream water samples were collected from seven sites in June 2013, five sites in July 2013, five sites in August 2013, and five sites in February 2014 (for a total of 22 samples, 13 unique locations) near Corvallis, Oregon in the Willamette River Basin. This sampling scheme was chosen to cover a range of seasonal precipitation patterns, capture various DOC concentrations, and represent a wide variety of land use types. The common land use types were forest, natural grass, urban, pasture, and agricultural grass such as hayfield and grass seed rotation (ISE, 2005, 1999). Spatial precipitation data provided by the PRISM Climate Group (2004) shows the annual average precipitation ranged from 1,102 to 1,304 mm yr-1 in the area for the period 1971 to 2000. During the period that the samples were collected for this study, precipitation ranged from 0 to 46 mm $d^{-1}$ at the nearest rain gage station (NWS ID: 351813) (Western Regional Climate Center, 2014). The climate of the region is characterized as having a wet winter and a dry, warm summer. Stream flow is mainly driven by winter precipitation and snowmelt runoff between November and March.

#### *2.3.1.2 Field and laboratory Procedures*

Samples were collected from the surface of the thalweg, the deepest channel or the most natural course of water, with an acid-rinsed sampler consisting of brown one-liter HDPE NalgeneTM bottles. The sampler was washed with stream water three times before each sample collection. Samples were poured into acid-rinsed and pre-combusted (4 hrs at 490 $^{o}$C) amber glass bottles after pre-rinsing three times with samples. They were stored in a dark icebox packed with ice until samples were taken to the laboratory.

Within about six hours of sampling, each sample was filtered through a pre-combusted 0.7 μm glass-fiber filters (WhatmanTM GF/F) using an acid-rinsed glass filtering system in a darkened room. One liter of filtered sample was divided into 1) an amber glass to be stored in a refrigerator at about 4 $^{o}$C, 2) a high density polyethylene

(HDPE) bottle to be stored in the same refrigerator, 3) a HDPE bottle to be stored in a freezer (about - 20 ºC) for a few weeks ranging between 7 and 18 days, and 4) a HDPE bottle to be stored in the same freezer for several months ranging between 192 and 372 days. Duplicates were prepared every five samples for quality assurance. All the samples were brought to room temperature on the laboratory bench prior to analyses.

All nutrients were measured with a Technicon auto-analyzer. DOC was measured using a Shimadzu TOC-VCSH analyzer. Only samples collected between June to August, 2013 were analyzed for nutrients to examine freezing effects.

A Cary 300 UV-Visible spectrophotometer was used to measure absorbance. Milli-QTM water was used for calibration (Weishaar et al. 2003). $SUVA_{254}$ was calculated as the ultra-violet absorbance at 254 nm normalized for DOC concentrations and reported in L mg $C^{-1}$ $m^{-1}$ (Weishaar et al. 2003). EEMs were measured with a Fluorolog® spectrofluorometer (HORIBA Jobin Yvon, Inc.) and collected over an excitation range of 250 - 400 nm with 10 nm increments and an emission range of 350 - 550 nm with 2 nm increments (Cory and McKnight 2005). EEMs were measured with a band slit of 3 nm. EEM of ultrapure water of Milli-QTM water (Millipore Corporation) was measured each field-work day and subtracted from EEMs of samples for calibration and to remove Raman scattering effects (Cory and McKnight 2005; Stedmon and Bro 2008). The MilliQ EEM was also used to calculate the Raman curve to normalize sample EEMs and enable direct comparison of samples from different dates (Lawaetz and Stedmon 2009). EEMs were corrected for instrument specific errors using the correction file provided by HORIBA Jovin Yvon, Inc. for the instrument. If the absorbance of samples at 254 nm was higher than 0.2 $cm^{-1}$, samples were diluted prior to fluorescence measurements to avoid inner-filter effects (Miller et al. 2010). All optical properties were measured in dim light.

Maximum holding times in a refrigerator for each sample were as follows: 48 hours for inorganic nutrients, 14 days for DOC, and 28 days for TDN following the EPA protocol (Morrison Erway et al., 2004). Absorbance at 254 nm and EEMs were measured on the same day within no more than 4 days after collection. Samples from August 2013 and February 2014 that were frozen for months were thawed and processed for absorbance within 19 days and for EEMs within 40 days of thawing. The effect of longer holding times should be minimal as Ebert (2013) demonstrated stable FI values over a period of five months of refrigerator storage. The average limit of detection was as follows: DOC - C = 0.05 mg $L^{-1}$ (CCAL, 2014), $NO_3^-$ -N = 0.02 mg $L^{-1}$, $NH_4^+$-N = 0.07 mg $L^{-1}$ , $PO_4^{3-}$-P = 0.04 mg $L^{-1}$ , TDN = 0.05 mg $L^{-1}$ . Samples under these detection limits were excluded from analyses.

To determine if ferric iron (Fe (III)) concentrations, found by Weishaar et al. (2003) to significantly influence estimates of $SUVA_{254}$ by increasing absorbance at 254 nm, samples were collected on June 24th, 2015 at all the sites except for two sites that were dry, and filtered through combusted 0.7 μm filters. Samples were preserved with nitric acid ($HNO_3$) and kept in a refrigerator (4 $^{o}$C) and processed by the Cooperative Chemical Analytical Laboratory on July 16th, 2015. The total iron concentration ranged from 0.02 – 1.02 mg $L^{-1}$; all but two locations had concentrations less than 0.5 mg $L^{-1}$ . There was no correlation between the total iron concentrations and absorbance at 254 nm. Additionally, dissolved iron concentrations are low in oxygenated water of neutral pH and much more soluble at low pH in the form of Fe (III) (Drever 1997). In a mildly alkaline environment such as this study site (Raymond et al. 2002), Fe (III) is sparingly soluble (Drever 1997). Hence, we assumed the Fe (III) effect on $SUVA_{254}$ was negligible. Additionally, the maximum of $NO_3^-$ - N concentrations in our samples was 0.59 mg $L^{-1}$, less than the $NO_3^-$ - N effect on absorbance at 254 nm observed by Weishaar et al. (2003). Hence, the effect of $NO_3^-$ - N on absorbance at 254 nm was determined to be negligible for this study.

#### *2.3.1.3 EEM Analyses*

To characterize and quantify changes in DOM from EEMs measured by a spectrofluorometer, the PARAFAC model of Cory and McKnight (2005) was used. Thirteen DOM components identified and examined using the PARAFAC model of Cory and McKnight (2005) include reduced quinone-like components (SQ1, SQ2, SQ3, HQ), oxidized quinone-like components (Q1, Q2, Q3), tryptophan-like component, tyrosine-like component, and 4 additional unknown components (Cory and McKnight 2005).

## 2.4 Assessment and Results

When the samples were stored in a refrigerator less than the maximum holding times (inorganic nutrients: 48 hours, DOC: 14 days, TDN: 28 days, and absorbance and EEM: 4 days), the level of all the parameters was not statistically different between amber and HDPE bottles (rank-sum test at the 5 % significant level conducted with Matlab®(R2013b)). Therefore, from hereon we only compare analyses of samples stored in HDPE bottles. Unless noted, when frozen samples were compared to initial values and/or when percent difference was presented for frozen preservation methods, they were compared to those samples stored in a HDPE bottles in a refrigerator and measured within the maximum holding times.

#### *2.4.1.1 Nutrients*

$NH_4^+$- N levels stored with various methods for our study site were generally below the detection limit of standard autoanalysis (69/78 samples < 0.07 mg $L^{-1}$). While we cannot statistically evaluate the results for the two samples with detectable $NH_4^+$- N levels, we note that freezing for a period of a few weeks to months resulted in a ± 4 % and – 17 % change, respectively, from initial concentrations stored in a refrigerator.

$NO_3^-$ -N concentrations generally declined with increased freezer storage time (Figure 1). The percent change was greater on average for samples having initial concentrations less than 0.1 mg $L^{-1}$. Over a period of up to a year, $NO_3^-$ -N concentrations declined on average by 33.9 % from initial values. When February 2014 samples were compared between freezing for weeks versus months, the average percent change decline was 29.6 %.

Frozen TDN was highly correlated with the original values as indicated by a simple linear regression (Figure 2). For week-long freezer preservation, the percent change ranged between 0.4 and 21.8 % while the average was 6.3 %. For 6 out of 17 samples, a month of freezing caused concentrations to drop below the detection limit. The percent change ranged between 0.1 and 36.7 % while the average was 9.3 %. When February 2014 samples were compared between freezing for weeks and months, the percent change ranged between 5.0 and 46.2%.

Initial $PO_4^{3-}$-P concentrations were generally below detection limit (11/17 samples < 0.04 mg $L^{-1}$). Initial concentrations of only six samples were above the detection limit, and all were below 0.1 mg $L^{-1}$. The percent loss of those samples ranged between 14.0 and 39.1 % for a week-long freezing and 1.7 and 45.0 % for a month-long freezing.

#### *2.4.1.2 DOC*

For most samples, changes in DOC concentrations were small and concentrations mostly decreased when stored in a freezer for a few weeks compared to samples stored in a refrigerator and processed within the recommended holding times (percent absolute difference varied between 1.0 and 14.6 %, Figure 3). The average absolute percent difference was 5.5 %. When frozen for many months, the absolute percent difference was also small ranging between 1.4 and 9.5 % (and mostly decreased) while the average absolute percent difference was 5.7 %.

#### *2.4.1.3 DOM optical properties*

$SUVA_{254}$ of most samples (16/22 samples, 73 %) decreased by 3 to 53 % when frozen for a week (Figure 4). Similarly, $SUVA_{254}$ of most of the samples (17/22 samples, 77 %) decreased by 5 to 57 % when frozen for months, from initial values of 2.2 to 13.6 L mg $C^{-1}$ $m^{-1}$ to 3.3 to 6.4 L mg $C^{-1}$ $m^{-1}$ when frozen for months.

FI did not change for a week-long freezing (Figure 5); the absolute percent difference was less than 7.1 % while the average was 1.9 %. The majority of samples experienced small increases in FI for a month-long freezing; the absolute percent difference was less than 2.6 % while the average was 1.1 %.

The percent oxidized quinone-like components (Q1, Q2, Q3, the average of 12 % combined) did not change with preservation. However, the most dominant component, HQ (a reduced quinone-like component), decreased significantly when frozen (Figure 6). A similar trend of decreased values after freezing was observed for RI. The percent of other reduced quinone-like components (SQ1, SQ2, SQ3) increased after freezing; however, each of these components makes up less than 3 % of the total fluorescing

DOM. This increase after freezing was common among other components that represent a small proportion of fluorescing DOM. For percent protein (the sum of tyrosine and tryptophan components), a decrease was observed after freezing (Figure 7).

## 2.5 Discussion

Although TDN concentrations remained stable after freezing, $NO_3^-$ - N concentrations declined with increasing length of frozen preservation. Some of the highest percent changes were attributed to the low level of original concentrations. Although minimal nutrient losses were found after long-term freezing of freshwater (Avanzino and Kennedy, 1993) and seawater (Dore et al., 1996), it is possible that freezing is more significant for samples with low initial concentrations. Although protein is known to precipitate with tannins to form relatively insoluble complexes, it seems unlikely that the decrease in nitrate is due to co-precipitation with organic constituents, but might be related to salt co-precipitation (Huber et al. 2012). In contrast to a previous study (Fellman et al. 2008), we found minimal changes in DOC concentrations following freezing for up to one year; the average percent difference from initial values was about 6 % when stored in a freezer. This low value is not because our samples contained low aromaticity of $SUVA_{254}$ ($< 4$ L mg-$C^{-1}$ $m^{-1}$) and preventing aromatic DOC from being preferentially removed during freezing as suggested by Fellman et al. (2008). In fact, both our initial DOC and $SUVA_{254}$ levels varied significantly (Table 1), yet DOC concentrations remained relatively constant for this study site with freezing. If our findings can be extrapolated to similar freshwater environments, we would recommend that the recommended maximum duration of freezer preservation for DOC be relaxed to at least one year.

Because FI and percent aromaticity are inversely related (McKnight et al. 2001), an increase in FI in frozen samples indicates that samples became less aromatic. Coupled with a decrease in $SUVA_{254}$ in frozen samples, these observations indicate that aromatic DOM precipitated preferentially during the freezing process and did not re-dissolve when returned to room temperature. Although DOC concentrations did not vary among preservation methods, the optical properties of DOM changed depending on preservation methods. For study sites with highly aromatic DOM ($SUVA_{254}$ higher than 5 L mg-$C^{-1}$ $m^{-1}$), we caution against freezing samples. $SUVA_{254}$ can be used as a criteria to decide whether or not freezing is a proper preservation technique at individual study sites.

Oxidized quinone-like components did not vary significantly because initial oxidized quinone proportions were low and have a lower aromaticity compared to reduced quinone-like components (Cory and McKnight 2005). The reduced quinone-like component, HQ, which was the most dominant component for this study site (an average of 30 % of fluorescent DOM), showed a significant decrease after freezing, likely because of its high aromaticity (Cory and McKnight 2005). The decrease in percent protein, the ratio of SQ1 to SQ1 and SQ2, and RI can also be explained by aromatic components' preferential precipitation during freezing. Large, high molecular weight, hydrophobic DOM components such as lignin and tannins are most subject to precipitation with freezing (Scheel et al. 2007) and these components are also likely aromatic.

Additionally, DOM with high molecular weight such as aromatic compounds with carboxyl groups is easy to precipitate because Al adsorbs to carboxyl groups and the combined weight of carboxyl groups and aromatic compounds promote precipitations

(Scheel et al. 2007; Scheel et al. 2008). Such combination of carboxyl and aromatic groups is commonly generated during the decomposition of lignin (Scheel et al. 2008). The size of precipitated DOM for the study conducted by Scheel et al. (2008) was larger ($3^{-1}$ 10 μm) than the filter pore size used for this study. The aromatic DOM precipitation found in filtered water samples during freezing could have been less if the Willamette River Basin was high in Al, DOM, and $SUVA_{254}$, which result in large size of DOM precipitates. Thus, measuring Al and pH of samples along with aromaticity and DOC concentrations for this type of analysis for future studies would help constrain the potential causes of variable DOM preservation.

## 2.6 Conclusions

Although researchers should remain cautious, this study shows that freezing does not affect TDN concentrations significantly at least for a year. However long-term storage at freezing temperatures did result in the decrease in $NO_3^-$ - N concentrations and should be avoided as a preservation method. When frozen less than 18 days, this decrease in $NO_3^-$ - N concentrations was minimal. While we have shown that DOC concentrations remain stable after months of freezing, we confirm previous findings regarding optical properties of DOM and freezing alters the compositional nature of DOM. This is because aromatic DOM preferentially precipitates while freezing

Thus, to decide whether or not freezing is an appropriate preservation method depends on one's study site context. Although it is best to avoid freezing samples for nutrients, DOC, and fluorescent DOM analyses, if extended storage is necessary, testing the preservation method is highly recommended. It is worth noting that FI did not change significantly after five months in a refrigerator in one study (Ebert, 2013); thus, it is highly recommended to store samples in a refrigerator for the analyses of DOM optical properties. Additional factors such as temperature, quenching by metals (Weishaar et al. 2003; Poulin et al. 2014), pH (Spencer et al. 2007; Poulin et al. 2014), and changes in bond structures contribute to the change in the optical properties of DOM. These environmental changes make interpretation of DOM optical properties challenging and the preservation methods add another layer of complexity.

These changes in DOM optical properties suggest that we need to consider the effects of natural freezing in ecosystems on DOM chemical characteristics. $SUVA_{254}$, percent protein, and reduced quinone-like components will most likely be reduced once water is frozen if there is high aromaticity, although the percent decrease may be highly dependent on DOM source. For example, an aquatic environment rich in Al may have precipitated large DOM particles along the course of water flow in soil or during filtration and may not have much left in solution to precipitate. Accordingly, it may not be appropriate to directly compare DOM optical properties of ice-cores to nearby seawater that has never been frozen. Additionally, samples may appear to decrease in aromaticity during a freezing winter storm, but this may not necessarily mean the aromatic components of DOM decreased in the environment. There may be a high proportion of aromatics after a long freezing event followed by a warm strong flushing event because precipitated DOM during the freezing season is mobilized.

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## 2.9 Table

Table 1: The summary of initial sample concentrations stored in HDPE bottles in a refrigerator
Nutrients and DOC: mg $L^{-1}$, $SUVA_{254}$: L mg-$C^{-1}$ $m^{-1}$

| | $NH_4^+$-N | $NO_3^-$ -N | TDN | $PO_4^{3-}$-P | DOC | $SUVA_{254}$ |
|---|---|---|---|---|---|---|
| Minimum | 0.04 | 0.02 | 0.05 | 0.02 | 1.0 | 2.2 |
| Maximum | 0.12 | 0.59 | 0.59 | 0.08 | 7.9 | 13.6 |
| Average | 0.09 | 0.13 | 0.15 | 0.04 | 2.6 | 5.4 |
| Standard Deviation | 0.04 | 0.17 | 0.14 | 0.03 | 1.9 | 2.7 |
| Median | 0.11 | 0.06 | 0.09 | 0.04 | 1.8 | 4.5 |

## 2.10 Figures

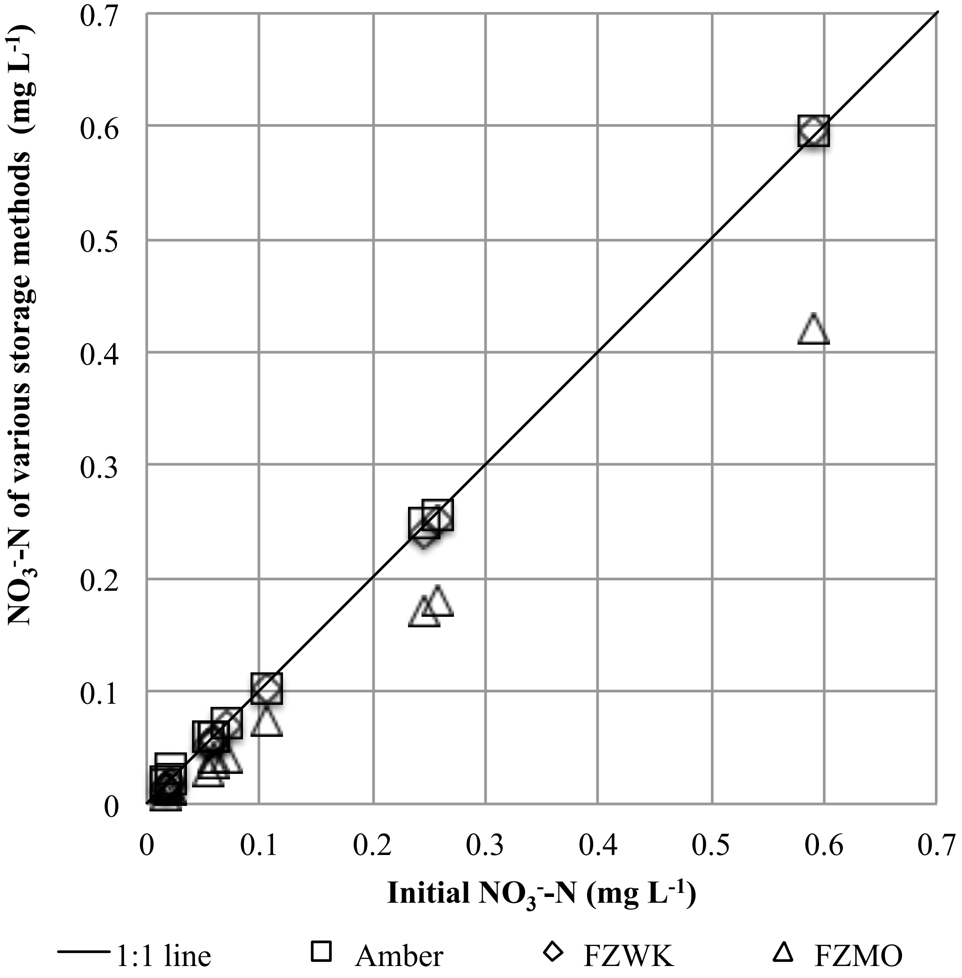

Figure 1: Comparison of $NO_3^-$ - N concentrations (mg $L^{-1}$) stored in HDPE bottles in a refrigerator (initial concentrations) against stored in 1) Amber bottles in a refrigerator (y = 1.01x; $R^2$ = 1.00), 2) HDPE bottles in a freezer for a few weeks (FZWK) (y = 0.99x; $R^2$ = 1.00), and 3) HDPE bottles in a freezer for months (FZMO) (y = 0.71x; $R^2$ = 1.00).

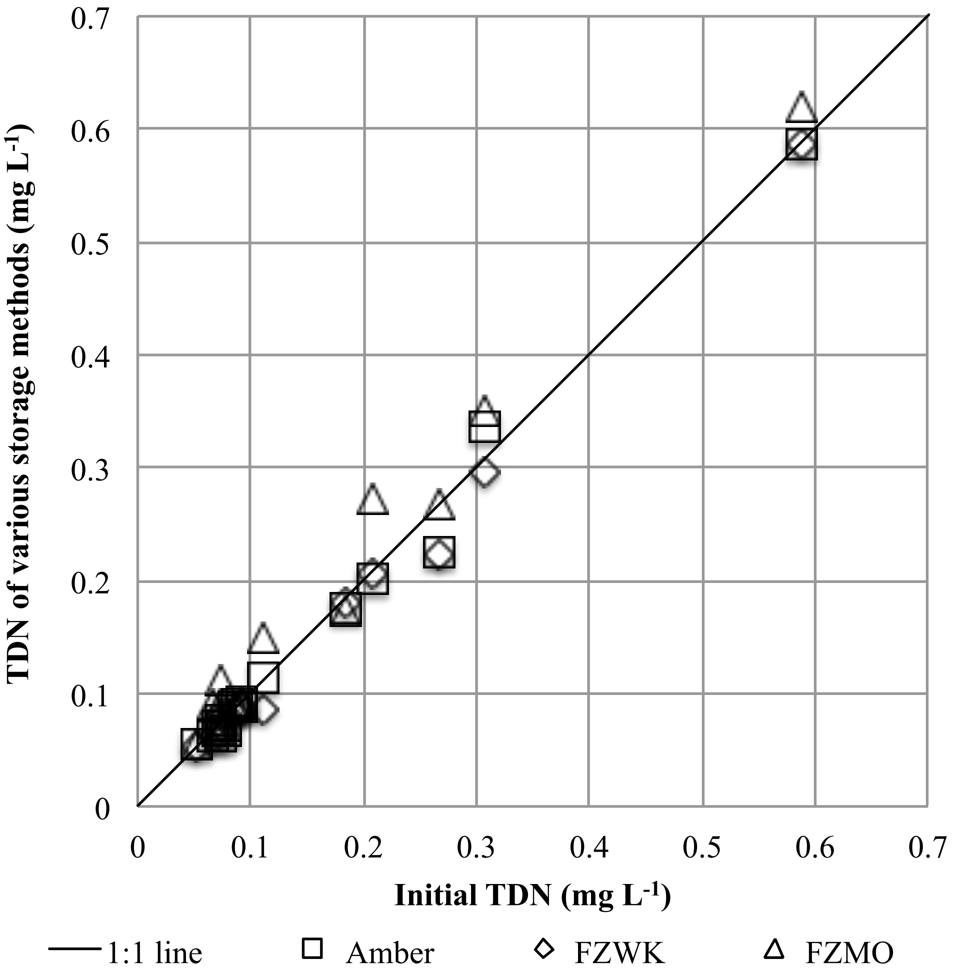

Figure 2: Comparison of TDN concentrations (mg $L^{-1}$) stored in HDPE bottles in a refrigerator (initial concentrations) against stored in 1) Amber bottles in a refrigerator (y = 0.99x; $R^2$ = 0.99), 2) HDPE bottles in a freezer for a few weeks (FZWK) (y = 0.97x; $R^2$ = 0.99), and 3) HDPE bottles in a freezer for months (FZMO) (y = 1.08x; $R^2$ = 0.98).

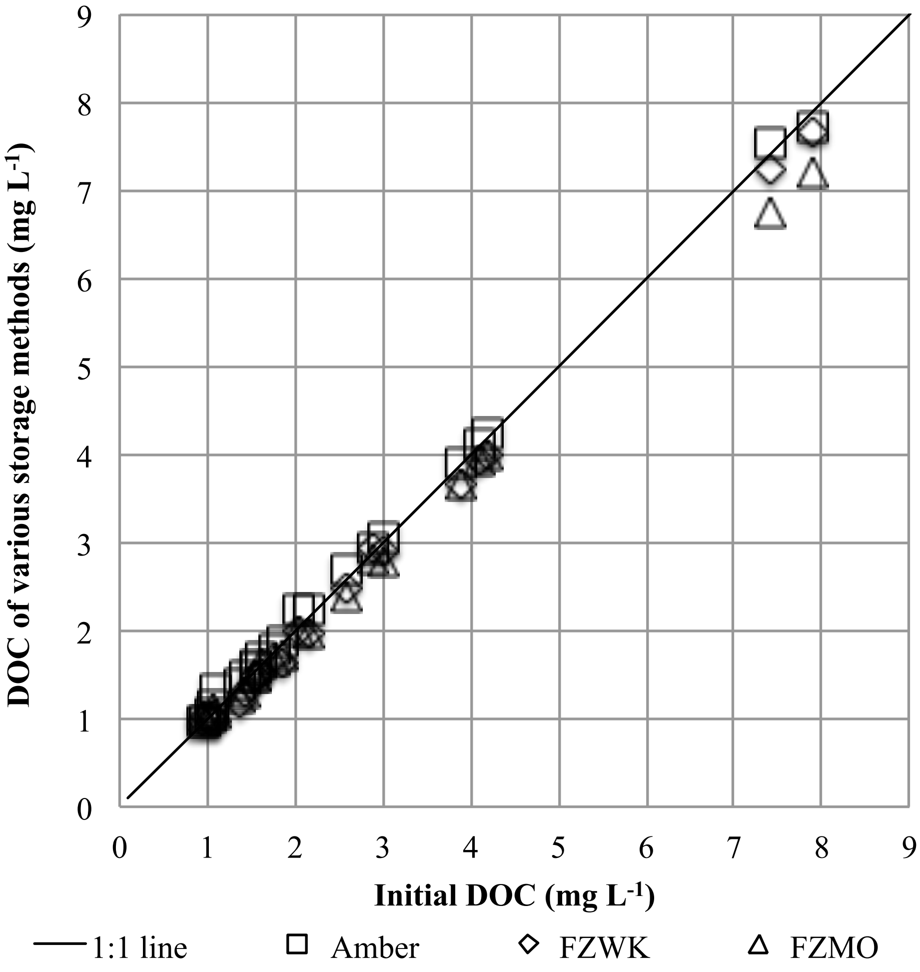

Figure 3: Comparison of DOC concentrations (mg L$^{-1}$) stored in HDPE bottles in a refrigerator (initial concentrations) against stored in 1) Amber bottles in a refrigerator ($y = 1.01x$; $R^2 = 1.00$), 2) HDPE bottles in a freezer for a few weeks (FZWK) ($y = 0.96x$; $R^2 = 1.00$), and 3) HDPE bottles in a freezer for months (FZMO) ($y = 0.93x$; $R^2 = 1.00$).

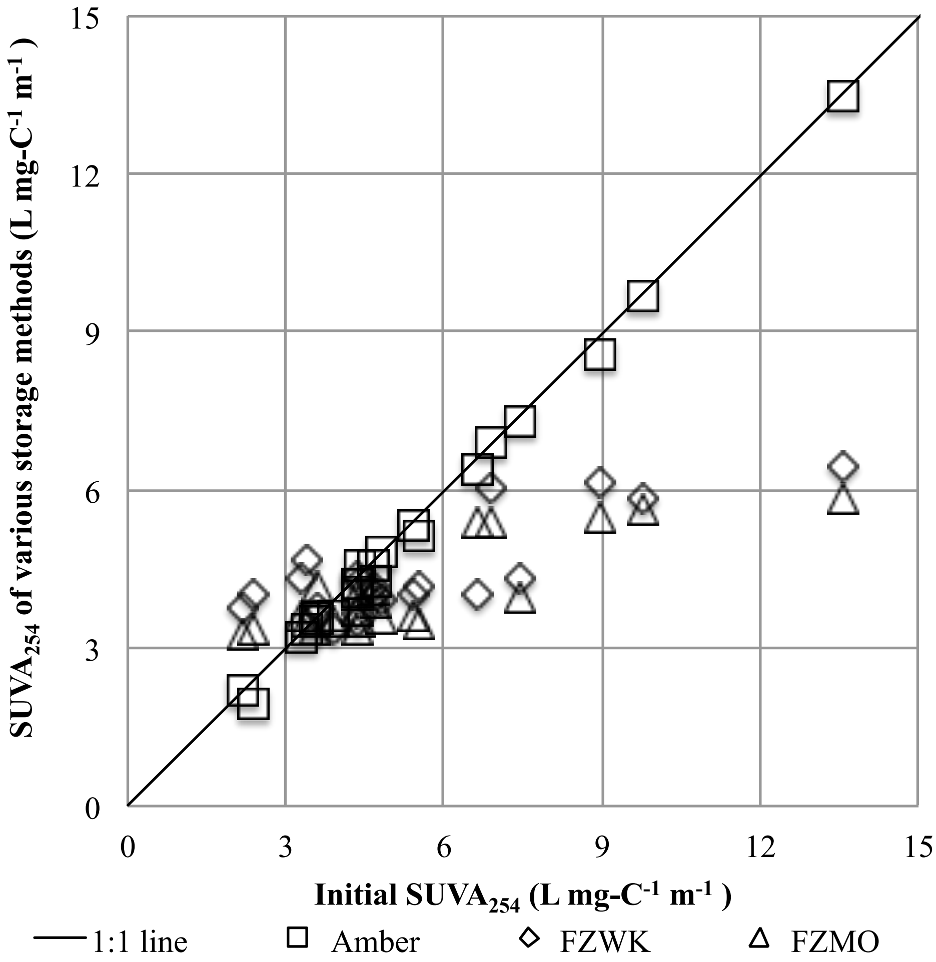

Figure 4: Comparison of $SUVA_{254}$ (L mg-$C^{-1}$ $m^{-1}$) stored in HDPE bottles in a refrigerator (initial values) against stored in 1) Amber bottles in a refrigerator (y = 0.98x; $R^2$ = 1.00), 2) HDPE bottles in a freezer for a few weeks (FZWK) (y = 0.71x; $R^2$ = -1.47), and 3) HDPE bottles in a freezer for months (FZMO) (y = 0.67x; $R^2$ = -1.13).

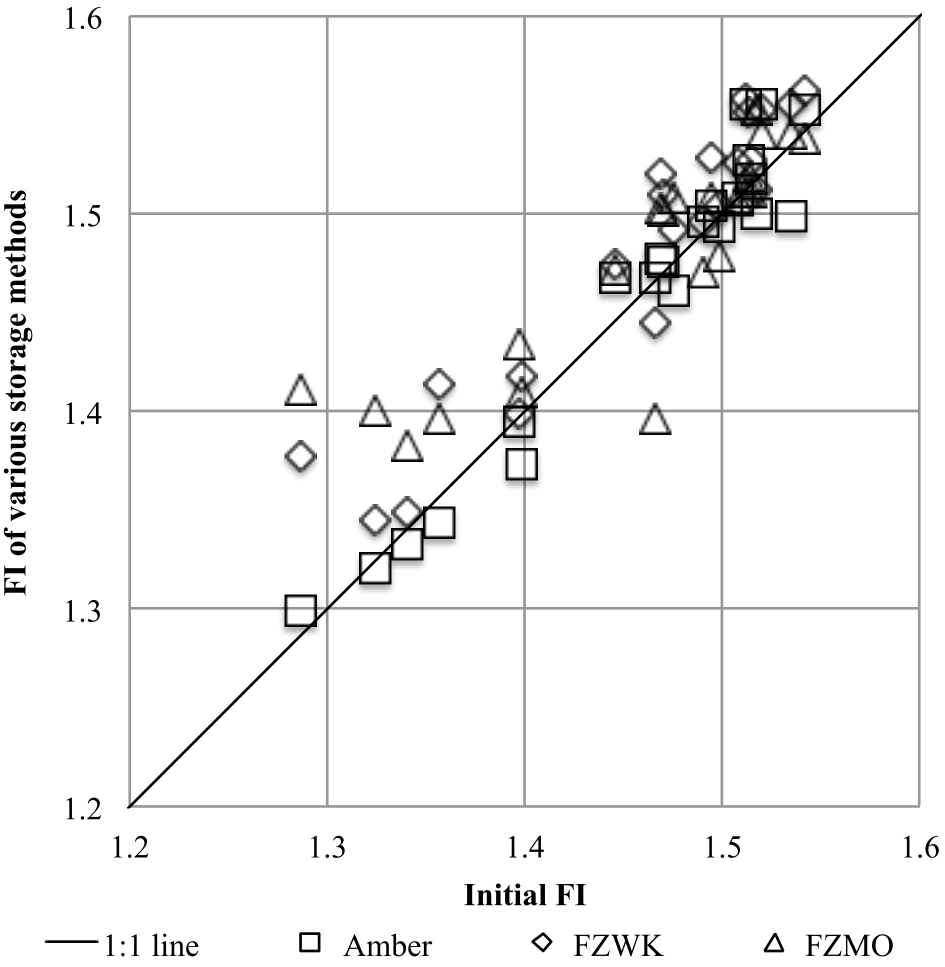

Figure 5: Comparison of FI stored in HDPE bottles in a refrigerator (initial value) against stored in 1) Amber bottles in a refrigerator ($y = 1.00x$; $R^2 = 0.95$), 2) HDPE bottles in a freezer for a few weeks (FZWK) ($y = 1.02x$; $R^2 = 0.88$), and 3) HDPE bottles in a freezer for months (FZMO) ($y = 1.01x$; $R^2 = 0.52$).

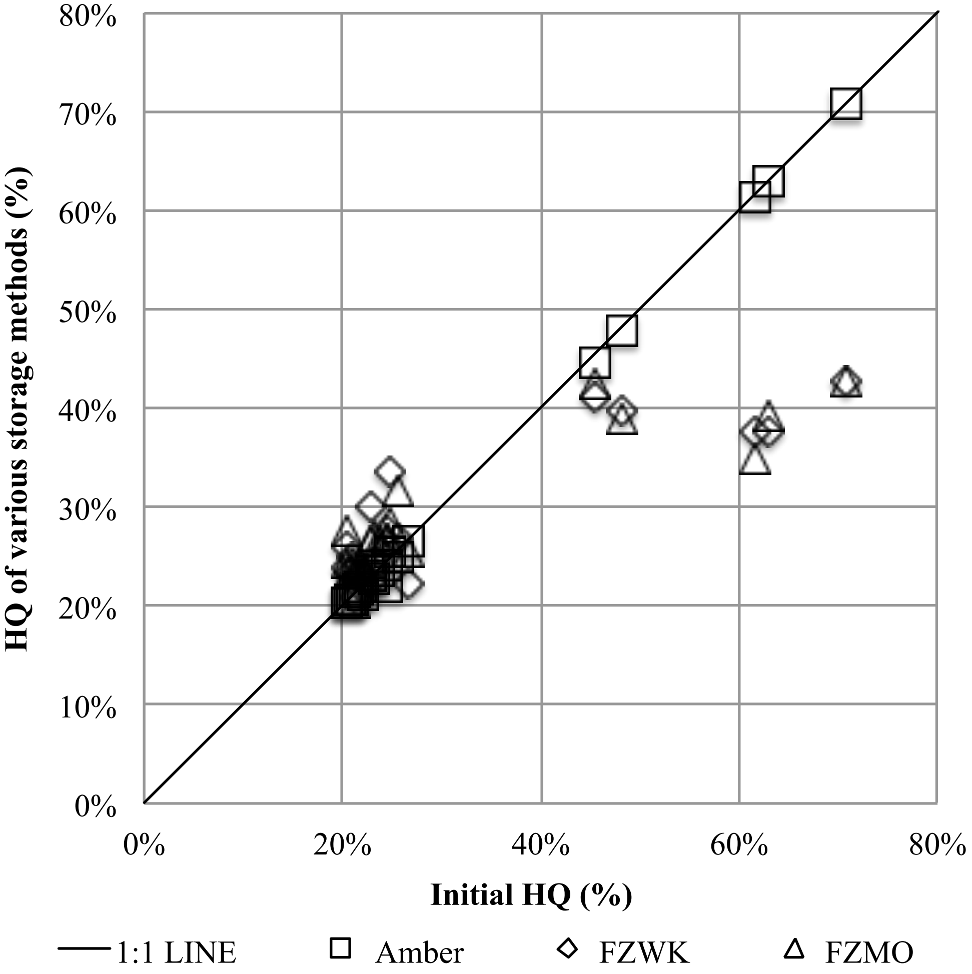

Figure 6: Comparison of HQ (%) stored in HDPE bottles in a refrigerator (initial %) against stored in 1) Amber bottles in a refrigerator (y = 0.99x; $R^2$ = 1.00), 2) HDPE bottles in a freezer for a few weeks (FZWK) (y = 0.81x; $R^2$ = -0.40), and 3) HDPE bottles in a freezer for months (FZMO) (y = 0.82x; $R^2$ = -0.70).

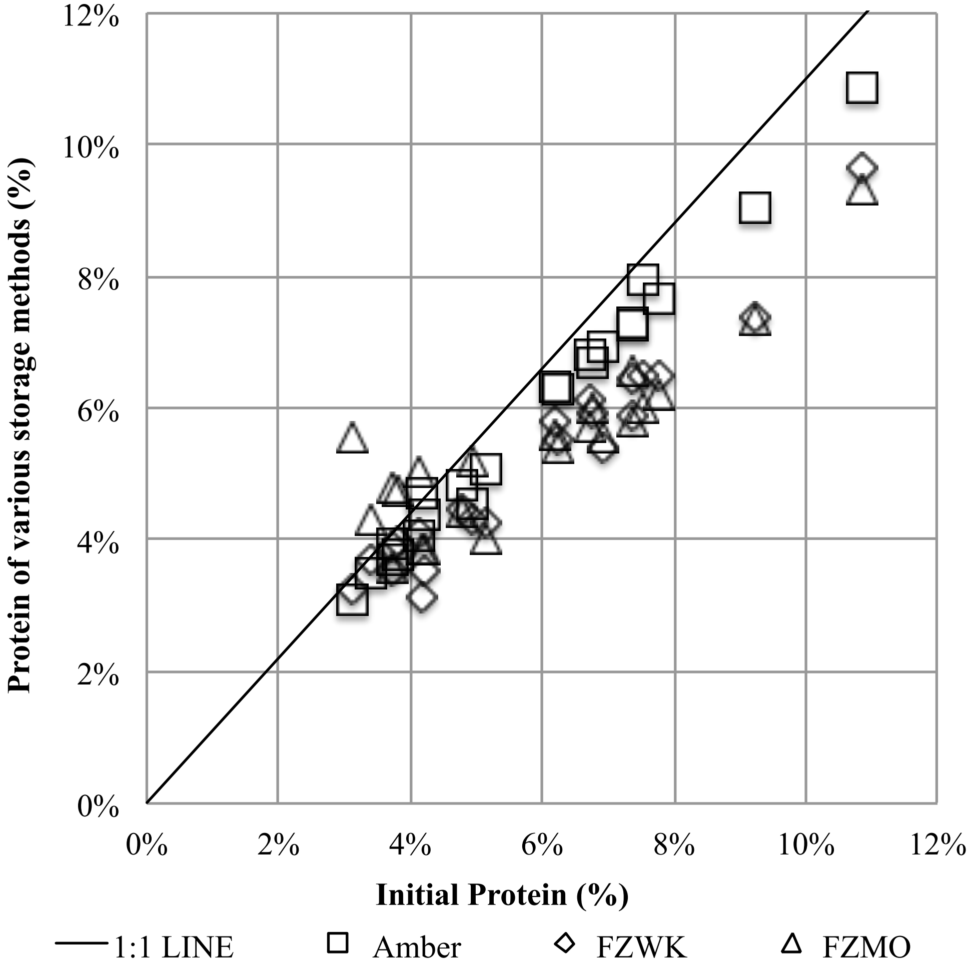

Figure 7: Comparison of Protein (%) stored in HDPE bottles in a refrigerator (initial value) against stored in 1) Amber bottles in a refrigerator (y = 1.00x; $R^2 = 0.99$), 2) HDPE bottles in a freezer for a few weeks (FZWK) (y = 0.87x; $R^2 = 0.93$), and 3) HDPE bottles in a freezer for months (FZMO) (y = 0.89x; $R^2 = 0.45$).

# 3 Hydrologic and Forest Management Controls on Dissolved Organic Matter Characteristics in Headwater Streams of Old-Growth Forests in the Oregon Cascades

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For Submission to
Ecosystems

## 3.1 Abstract

Dissolved organic matter (DOM) is a critical component of the carbon cycle linking terrestrial and aquatic ecosystems. Although many factors have been identified as influencing DOM fluxes and biochemical quality in rivers with different land cover types, controls on DOM composition in forested headwater catchments of the western U.S. are poorly understood. This study examined the effect of hydrologic patterns and forest management history on in-stream DOM chemistry at watersheds located in the H.J. Andrews Experimental Forest of the Oregon Cascades. Specific UV absorbance at 254 nm ($SUVA_{254}$), generally indicative of aromaticity, increased in streams during storms with increasing terrestrial DOM inputs. The relative proportion of a protein-like DOM fluorescent component, identified with Parallel Factor Analysis (PARAFAC), exhibited temporal and spatial variations. Correlation analysis between the protein-like DOM and hydrologic patterns, $SUVA_{254}$, and DOC suggest that stream water during dry seasons come from increased in-stream microbial processing and protein-rich subsurface sources, indicative of more microbially-processed sources for DOM compared to more plant-like surface soil sources observed during high flow. The proportion of the protein-like DOM was also influenced by the abundance of coarse woody debris (CWD), not live tree biomass, and was low among reference watersheds rich in humic-like DOM. The base flow index (the proportion of base flow to total flow) also explained the relative proportion of protein-like DOM. This study shows UV and fluorescent spectroscopy is a viable finger printing method to elucidate DOM sources in pristine headwater streams at the western Cascades of Oregon.

## 3.2 Introduction

Dissolved organic matter (DOM) is a critical component of the global carbon (C) cycle linking the terrestrial and aquatic ecosystems through in-stream microbial metabolism of terrestrial DOM emitting carbon dioxide from freshwater systems (Battin others 2008). Although headwater streams make up the longest river length (53 %) in the U.S. excluding Alaska (Nadeau and Rains 2007), little is known about the contributions of small headwater streams to the global C cycle (Cole et al. 2007; Raymond et al. 2013). To better characterize this role, it is important to understand hydrologic pathways transporting DOM from terrestrial to aquatic ecosystems in headwater watersheds. It is largely known that DOM transport from small watersheds increase with storm and snowmelt (Meyer and Tate 1983; Frank et al. 2000; Raymond and Saiers 2010; Wilson et al. 2013). Similarly, DOM compositions may change under different hydrologic scenarios. For example, Wilson and others (2013) found that bioavailable, less recalcitrant DOM increased with storm events. However, during storm events, van Verseveld and others (2008) saw increases in specific UV absorbance at 254 nm ($SUVA_{254}$), widely used as an indicator of aromaticity of DOM samples (Weishaar et al. 2003) and potentially indicative of more recalcitrant DOM. Further examining changes in DOM chemistry with hydrologic events may help to better characterize DOM transport in small headwater streams.

Fingerprinting fluorescent spectroscopy techniques can be used to identify DOM chemistry in freshwater samples using optical signals created by unique chemical structures of DOM (McKnight et al. 2001; Weishaar et al. 2003). Chemical structures and quantities of DOM create unique 3-D fluorescent spectra at distinctive wavelengths defined as excitation and emission matrices (EEMs) (Stedmon and Bro 2008). The matrices are a complex combination of DOM fluorescent signals. Identification of the chemical structure of the DOM components from the EEMs require statistical analysis, e.g. a multivariate statistical parallel factor analysis (PARAFAC) modeling and principal component analysis (PCA) (Stedmon and Bro 2008). Output from a PARAFAC model results in percentages of DOM signals or identified components, which makes PARAFAC more advantageous for looking at DOM characteristics in natural waters than PCA (Stedmon and Bro 2008). Fluorescent spectroscopy with PARAFAC has been widely used recently to identify and quantify DOM sources in many freshwater ecosystems (Stedmon et al. 2003; Cory and McKnight 2005; Cawley et al. 2012a; Hosen et al. 2014). Fluorescent DOM components have been associated with varying land uses, landscape features, or effluent sources (Stedmon et al. 2003; Cawley et al. 2012a; Hosen et al. 2014). Redox states of quinone-like components have been identified in disparate ecosystems of the Arctic, the Antarctic, Botswana, and Colorado (Cory and McKnight 2005). Technically simple and relatively rapid fluorescent spectroscopy can be useful compared to more complicated and expensive infrared (IR) or nuclear magnetic resonance (NMR) spectroscopy to determine DOM chemistry (Cory et al. 2011).

Studies from eastern forests indicate that DOM optical properties vary seasonally and respond to forest management. In the Coweeta Experimental Forest located in western North Carolina, increased amounts of a protein-like component in forested headwater streams in the early summer and fall were attributed to higher biological activity in the forest floor and/or riparian zone (Yamashita and others 2011). In the Hubbard Brook Experimental Forest in New Hampshire, streams in previously harvested forests had lower dissolved organic carbon (DOC) concentrations and a higher protein-like fluorescence component compared to reference streams (Cawley et al. 2014). These studies were conducted on the East Coast of the U.S. where forests and streams have received relatively high inputs of acidity and pollutants (Evans et al. 2005; Monteith et al. 2007) that can affect both fluxes and composition of DOM. Controls on DOM chemistry in forested headwater ecosystems of the Pacific Northwest with different environment, climate, and forest types compared to the East Coast are less well studied, and it is not clear if they will respond in similar ways to land management and climate variability.

This study was conducted in nine experimental watersheds containing old-growth forest (450-yr-old) and regenerating (50 to 60-yr-old) forest in the H.J. Andrews Experimental Forest (HJAEF) in Oregon. At the HJAEF, DOM chemical characterization has been limited to the fluorescent index (FI) (McKnight et al. 2001) and/or $SUVA_{254}$ during storm events in the limited number of watersheds (Hood et al. 2006; van Verseveld et al. 2008; van Verseveld et al. 2009). Hood and others (2006) and van Verseveld and others (2008) found in-stream DOC was more aromatic during storm events, compared to base flow season. Hood and others (2006) did not see the variation in FI during a week-long storm event. van Verseveld and others (2009) found declining $SUVA_{254}$ in soil water from an organic layer, shallow subsurface flow, deep subsurface flow and stream water, to deep groundwater, respectively. Shallow, mineral soils preferentially adsorb aromatic and carboxyl DOM (Kaiser et al. 1997). This results in deep soil layers with highly hydrophilic, more microbially processed, labile DOM (i.e. less aromatic DOM) (Lajtha et al. 2005), and thus deep soils may be sources of bioavailable and protein-like, DOM to streams. Hence, DOM aromaticity and recalcitrant DOM in streams may be high (high $SUVA_{254}$) during high flow events, when dominant flow paths at the HJAEF are characterized by shallow subsurface flows, whereas bioavailable and protein-like DOM in streams may be high (low $SUVA_{254}$) during low flow periods, when flow is dominantly through deep mineral soil horizons Moreover, optical properties of DOM may reflect seasonal shifts in dominant hydrologic flow paths, as well as shifts in the character of forest floor DOM associated with disturbance.

The objectives of this study were to 1) examine the role of seasonality and hydrology on DOM chemistry and 2) investigate the potential role of forest management history on DOM chemistry using UV and fluorescent spectroscopy. We hypothesized that the proportion of aromatic and terrestrial DOM in streams would be higher during high flow compared to base flow conditions and in watersheds with a low base flow index (BFI) (Santhi et al. 2008), as a result of shifts in dominant flowpaths from deep subsurface flow (in summer, between storm events, and in low-gradient watersheds) to shallow subsurface flow (in winter, during storm events, and in steep watersheds). We also predicted that DOM in streams draining watersheds whose coarse woody debris (CWD) pools have been depleted by forest harvest in the last 50 to 60 years, would have lower $SUVA_{254}$ and a lower proportion of humic-like DOM, but greater relative percentages of protein-like, bioavailable and more labile DOM components, compared to old-growth watersheds.

## 3.3 Materials and Methods

### 3.3.1 Sample Sites

This study examined stream water samples collected between May 2013 and June 2015 (36 events) at the H. J. Andrews Experimental Forest (HJAEF) located in the western Cascades of Oregon (Figure 8). The forest is a 6,400-ha LTER site and encompasses the forested Lookout Creek watershed filled with old-growth Douglas fir and western hemlock (400 – 500 years) (Swanson and Jones 2001; Vanderbilt et al. 2003). The stream water data were collected as a part of the long-term water quality monitoring effort started in 1968 (Swanson and Jones 2001).

The climate of the HJAEF is marine temperate and characterized by dry, warm summers and wet, cool winters (Figure 9) (Swanson and Jones 2001). Elevation ranges between 434 and 1627 m (H.J. Andrews Experimental Forest 2015), which contributes to different precipitation patterns within the forest (Vanderbilt et al. 2003). The average annual precipitation is about 2,500 mm, falling mainly as rain, and a seasonal snow pack accumulates above 1000 m (Swanson and Jones 2001).

The experimental watersheds observed for this study were seven headwater watersheds (WS) 1, 2, 6, 7, 8, 9, and 10 as well as the Mack Creek (MACK, 3rd order) and Lookout Creek (LOOK, 5th order) watersheds (Figure 8, Table 2). All the experimental watersheds except 1, 9, and 10 are nested within LOOK. Each watershed underwent various forest management practices. Northwest-facing WS 1 and 2 are adjacent to each other; WS 1 was clear-cut between 1962 and 1966 and slash was burned in 1966, and WS 2 is a reference watershed with old-growth forest. South-facing WS 6 and 7 are adjacent to each other; WS 6 was clear-cut and slash was burned in 1975, WS 7 received two canopy removal treatments (60 % selective-cut in 1974 and the removal of the remainder of the canopy in 1984), and WS 8 is the reference watershed for WS 6 and 7. West-facing WS 9 is the reference watershed for west-facing WS 10 that was clear-cut in 1975 with no burning. The northwest-facing MACK has not been altered significantly aside from moderate cutting (13 % est.) between 1957 and 1982 and fire that occurred 120 years ago (H.J. Andrews Experimental Forest, 2015). Approximately 25 % of the west-facing LOOK has been patch-cut between 1948 and present. Coarse woody debris (CWD) stocks within watersheds were measured by Fasth and others (Unpublished Data) (Table 3).

### 3.3.2 Field methods

Composite samples of stream water are collected at three-week intervals at gaging stations located at the outlet of experimental watersheds as part of the HJAEF long-term water quality monitoring site. The sampling method is detailed at (http://andrewsforest.oregonstate.edu/lter/; *Johnson* 1984). Samples collected by automated samplers at each experimental watershed are collected weekly and stored in five gallon polyethylene carboys in a refrigerator (about 4 $^{o}$C) or stayed at gaging stations for up to three weeks during cold months (November through March) before being transported to the Cooperative Chemical Analytical Laboratory (CCAL) at Oregon State University every three weeks.

### 3.3.3 Laboratory methods

Analyses for nutrient and DOC were conducted by CCAL; the laboratory methods are described at (http://andrewsforest.oregonstate.edu/lter/). Samples were filtered through GF/F filters (0.7 μm), poured into acid-rinsed and pre-combusted (4 hrs at 490

$^{o}$C) glass vials, and stored in a dark refrigerator (0 – 4 $^{o}$C) until analysis. Inorganic nitrogen (N) species, nitrate-N ($NO_3^-$ - N) and ammonia-N ($NH_3$-N), and total dissolved nitrogen (TDN) were measured with a Technicon auto-analyzer II and DOC were measured with a Shimadzu TOC-VCSH combustion analyzer. The concentrations of DON are obtained by subtracting dissolved inorganic nitrogen N ($NO_3^-$-N and $NH_3^+$-N) from TDN. The ratio between DOC and DON (DOC:DON) was used as an indicator of microbial influence. The method detection limits were as follows: DOC = 0.05 mg $L^{-1}$, $NO^{3-}$-N = 0.001 mg $L^{-1}$, $NH_3^+$-N = 0.010 mg $L^{-1}$, and TDN = 0.01 mg $L^{-1}$ (CCAL 2014). Samples under the detection limit were reported as the measured value.

The optical properties of the samples, absorbance at 254 nm ($Abs_{254}$) and excitation and emission matrices (EEMs), showing fluorescent spectra at the distinctive combinations of excitation and emission wavelengths, were both measured on the same day within 14 weeks of sample collection dates at the HJAEF. The effect of varying holding times is assumed to be minimal for this study, as no significant spectral change for DOM was observed in samples stored for two months in a refrigerator storage (Jaffé et al. 2008). The fluorescent index (FI), developed as an indicator of *terrestrial* versus *microbial* sources (McKnight et al. 2001), stayed stable throughout a five-month refrigerator storage experiment (Ebert 2013), and absorbance, EEMs, and FI remained the same for 70 days for samples collected downstream of this study site (B. Lee, K. Lajtha, J. Jones, A. White, Unpublished Manuscript).

Absorbance over a range of 240 – 560 nm was measured with a Cary 300 UV-Visible spectrophotometer to obtain $SUVA_{254}$ and to correct EEMs for the inner-filter effect (Lakowicz 2006; Miller et al. 2010). Values of $SUVA_{254}$ (L mg $C^{-1}$ $m^{-1}$) were calculated as UV absorbance at 254 nm ($Abs_{254}$) normalized for DOC concentrations (Weishaar et al. 2003). Samples with $Abs_{254}$ greater than 0.2 $cm^{-1}$ were diluted to avoid inner-filter effects before EEMs measurements (Miller et al. 2010). The inner-filter effect was not corrected for samples between May 2013 and October 1st, 2013 because $Abs_{254}$ values of all the samples collected during the period were less than 0.2 $cm^{-1}$ so the range of absorbance necessary for inner-filter correction was not measured. However, samples collected between October 23rd, 2013 and April 2014 were compared for the inner-filter effect. There was no difference for FI and protein identified by the Cory and McKnight (2005) PARAFAC model ($R^2 = 1.0$ and 0.99, respectively) between inner-filter corrected samples and non-inner-filter corrected samples because $Abs_{254}$ of samples was less than 0.2 $cm^{-1}$.

Excitation and emission matrices (EEMs) were measured over an excitation range of 250 - 400 nm with 10 nm intervals and an emission range of 350 - 550 nm with 2 nm inervals with a band slit of 3 nm by a Fluorolog® spectrofluorometer (HORIBA Jobin Yvon, Inc.) (Cory and McKnight 2005). An EEM of Milli-Q™ water was subtracted from EEMs of samples for removing Raman scattering effects (Cory and McKnight 2005; Stedmon and Bro 2008). The MilliQ EEM was also used to calculate the Raman curve to normalize sample EEMs and report EEMs in Raman units (R.U., $nm^{-1}$) and enable the direct comparison of samples from different dates (Stedmon et al. 2003; Lawaetz and Stedmon 2009). The EEMs were corrected for instrument-specific errors using the correction file provided by HORIBA Jovin Yvon, Inc. All optical properties were measured while the lights above the instruments were turned off. The Milli-Q™ water (Millipore Corporation) was used to calibrate samples for both $Abs_{254}$ and EEMs.

### 3.3.4 EEM Analysis

The original FI scale (McKnight et al. 2001) has been modified for EEMs with instrument-specific corrections by Cory and others (2010), and the updated FI (EM 470 nm / EM 520 nm at EX 370 nm) was used for our analyses. The updated FI values of 1.46 or greater are reported to indicate *microbially* derived sources, and 1.21 or lower to indicate *terrestrially* derived sources (McKnight et al. 2001; Cory et al. 2010b). A FI value difference of 0.1 can be used to indicate a different DOM source (McKnight et al. 2001).

Characterization and quantification of fluorescent DOM from a total of 322 EEMs was conducted with the PARAFAC model using the DOMFluor Toolbox (ver. 1.7) (Stedmon and Bro 2008) and the N-way Toolbox (ver. 3.31) (Andersson and Bro 2000) with MATLAB® (ver. R2013b). The model was validated with split half analysis (Stedmon and Bro 2008). Four components identified by the PARAFAC were visually compared to the components determined by previous studies (Figure 10, Table 4). Correlation among DOM parameters was calculated using Pearson's r with SPSS (v. 22) for samples collected from May 2013 to June 2015 (322 samples).

### 3.3.5 Hydrological Analysis

In order to investigate the effect of soil moisture and flow rate on DOM characteristics, the average daily flow rate was summed for the sampling collection date as well as three days (Qsum3), 14 days (Qsum14), 21 days (Qsum21), and 30 days (Qsum30) prior to the sampling collection date. Stream flow is measured at MACK, WS 1, 2, 6, 7, 8, 9, and 10 using trapezoidal flumes and automated stage height recorders; data were obtained from the HJAEF (http://andrewsforest.oregonstate.edu/lter/). Stream flow at Lookout Creek is measured using an automated stage height recorder at a controlled section; data were obtained from the U.S. Geological Survey (USGS; http://waterdata.usgs.gov/nwis/uv?). Missing values were estimated as the average stream flow of the previous and following day. Regression analysis was conducted on DOM optical parameters against Qsum3, Qsum14, Qsum 21, and Qsum30 using SPSS (v. 22). The base flow index (BFI) was calculated for the study period at each watershed using the lfstat package in R (v 3.1.1.) (Gustard and Demuth 2009) to examine the base flow influence on DOM chemistry.

## 3.4 Results

### 3.4.1 DOM Components

Four fluorescent DOM components were identified with the PARAFAC model created for the study site (Table 4, Figure 10). Components 1 to 3 (C1 – C3) were visually associated with previously identified humic-like components; C1 and C2 were humic-like components commonly found in forested streams (Stedmon et al. 2003; Stedmon and Markager 2005). The EEM region of C3 has been previously associated with fulvic acid, commonly found in all types of environments (Stedmon and Markager 2005). Component 4 (C4) was visually associated with an EEM region identified as a protein-like or, more specifically, a tryptophan-like EEM signal (Coble 1996; Cory and McKnight 2005; Yamashita et al. 2011). This EEM region also has been associated with protein-rich, autochtonous DOM sources (Stedmon and Markager 2005).

### 3.4.2 Correlations of DOM Components with Other Parameters

DOC concentrations ranged between 0.3 and 4.0 mg $L^{-1}$ with an average of 1.2 mg $L^{-1}$ and showed a positive and strong correlation ($r > 0.5$, $\alpha = 0.01$) with the percentage of DOM components 1 and 2 (C1 (%) and C2 (%)) and a negative and strong correlation with C4 (%) ($r = - 0.6$, $\alpha = 0.01$) (Table 5 Table 6). The concentrations of DON ranged between 0.00 and 0.12 mg $L^{-1}$ with an average of 0.03 mg $L^{-1}$ and was moderately and positively correlated with C1 (%) and C2 (%) ($r = 0.3 – 0.5$, $\alpha = 0.01$) and moderately and negatively correlated with C4 (%) ($r = -0.47$, $\alpha = 0.01$). The ratio of DOC to DON ranged between 9.3 and 288.3, with an average of 41.9 and was not correlated with the percentages of any DOM components identified from the PARAFAC model (Table 5 Table 6). Values of $SUVA_{254}$ was strongly and positively correlated with C1 (%) and C2 (%) ($r > 0.5$, $\alpha = 0.01$) and strongly and negatively correlated with C4 (%) ($r = -0.53$, $\alpha = 0.01$). C4 (%) and the biomass of coarse woody debris (CWD) were negatively and strongly correlated ($r = -0.72$, $\alpha = 0.07$) (Figure 11).

### 3.4.3 Temporal Variations

#### *3.4.3.1 DOM optical properties*

The FI did not vary significantly among reference watersheds over the sampling season; however, FI at harvested watersheds (WS 1, 6, 7, and 10) increased during low flow seasons and warm months compared to high flow seasons and cold months (Figure 9 &Figure 12a, Table 5 &Table 6). FI increased to 2.1 in October 2014 at MACK (not shown on the figure).

$SUVA_{254}$ ranged between 0.2 and 8.1 L $mg^{-1}$ $m^{-1}$ with an average of 3.5 L $mg^{-1}$ $m^{-1}$ (Figure 12b). Overall, WS 6 and 10 showed the lowest and highest $SUVA_{254}$ throughout the study period, respectively (average = 2.1 and 4.4 L $mg^{-1}$ $m^{-1}$, respectively).

The temporal variations of C1, C2, and C3 in absolute Raman units (R.U.) were similar to the temporal variations in DOC concentrations especially during the first storm event of the high flow season. When a harvested watershed and its corresponding reference watershed were compared, the absolute C1, C2, and C3 in R.U. were lower in harvested, WS 6, 7, and 10, than their reference watersheds (WS 8, 8, and 9, respectively) (Figure 13). The absolute C1, C2, and C3 in R.U. were similar in WS 1 (harvested) and 2 (reference). Values for C4 in absolute R.U. were similar between all combinations of harvested and reference watersheds.

The relative contribution of C1 (%) decreased over summers at some sites (WS 6 – 8) (Figure 12c). The relative contributions of C2 (%) and C3 (%) had low temporal variability and remained near average values of 19 % and 27 %, respectively (Table 5, Figure 12d - e). The relative contribution of a tryptophan-like component, C4 (%), showed the most temporal variation among the four fluorescent components (Figure 12f), ranging between 14 and 46 % with an average of 26 %; mean C4 (%) was the lowest in reference watersheds (17 – 24 %). Amongst harvested watersheds, samples from WS6 and 7 showed higher C4 (%) than those from WS1 and 10 overall. However, C4 (%) in samples from these watersheds decreased and became about the same during the first storm event of each year. When a harvested watershed and its corresponding reference watershed were compared, C4 (%) in samples from WS 6, 7, and 10 were higher than those from their reference watersheds (WS 8, 8, and 9, respectively) and C4 (%) in samples from WS 1 and 2 (control) were highly correlated (Figure 14).

#### *3.4.3.2 Hydrologic Events*

Correlation analysis between all flow parameters and C4 (%) and $SUVA_{254}$ was conducted at each watershed (

Table 7). Values of $SUVA_{254}$ were positively correlated with Q, Qsum3, Qsum14, Qsum 21, and Qsum 30 at all sites. There was a strong linear correlation for $SUVA_{254}$ against all flow parameters at LOOK, WS 1, and WS 10, ($r > 0.5$). A moderate and positive linear correlation for $SUVA_{254}$ against all flow parameters was observed at WS 2, 6, 8, and 9 ($r = 0.3$ to $0.49$). There was a moderate and positive correlation for

$SUVA_{254}$ against Q and Qsum3 at WS 7 (r = 0.3 to 0.49). There was a moderate and positive correlation for $SUVA_{254}$ against Qsum14 and Qsum30 at WS 9 (r = 0.36, 0.38, respectively).

The relative contribution of C4 (%) was negatively correlated with Q, Qsum3, Qsum14, Qsum 21 and Qsum30 (

Table 7) at all sites. There was a strong negative linear correlation for C4 (%) against all flow parameters at LOOK and MACK (absolute r > 0.5). Between C4 (%) against Q and Qsum3, a strong negative correlation was observed at WS 1, 7, 9, and 10 (r

< -0.5); a moderate negative correlation was observed at WS 2, 6, and 8 (r = -0.37 – -0.42). A moderate correlation was observed for C4 (%) against Qsum14, Qsum21, and Qsum30 at WS1, 7, and 9 (r = -0.38 to -0.46). A regression analysis revealed more logarithmic than linear trends with a negative correlation for C4 (%) against all flow parameters at LOOK, MACK, WS1, WS9, and WS10 (Table 8). The relative contribution of C4 (%) was positively and strongly correlated with the base flow index (BFI) (r = 0.69, α = 0.08) ().

## 3.5 Discussion

### 3.5.1 Overall DOM Sources

Although an average FI value of 1.4, as found in this study, has been interpreted as indicative of microbial or autochthonous sources (McKnight et al. 2001; Cory et al. 2010b), other studies of forested headwater streams have reported FI values ranging from 1.2 - 1.4 (Yamashita et al. 2011) at sites which do not freeze to 1.4 - 1.6 at sites where soils are frozen in winter (Cawley et al. 2014). FI has been observed to increase after freezing water samples (B. Lee, A. White, and K. Lajtha, Unpublished Manuscript). The original FI scale was based on end-members that represent a "terrestrial" source (Suwanee River water) and an algal source (McKnight et al. 2001). However, most DOM produced in upland watersheds will be more microbially processed than the low-pH, poorly oxygenated Suwanee River water, and thus we suggest that the FI indices in most stream samples will be more reflective of microbial processing than of autochthonous sources. DOM sources in forested headwater streams have generally been reported to be allochthonous (Vannote et al. 1980), given that they are characterized by significant inputs of forest litter and significant shading of the headwater streams. It is also possible that whole water samples of neutral pH with high humic acids may not necessarily result in a FI index on the same scale as a FI index developed with separated fulvic acids (McKnight et al. 2001) that are soluble at any pH. Hence we restrict our interpretations to relative changes in FI, rather than absolute values.

### 3.5.2 Protein-like DOM and Hydrologic Path

An increase in C4 (%) identified as a protein-like DOM was observed over summers compared to during high flow conditions. One possible explanation for the increase could be an increase within in-stream biological activity with increased temperature, resulting in either greater autochthonous inputs or else greater microbial processing of terrestrial organic matter. FI values similarly increased in harvested headwater watersheds over summers. In alpine and subalpine lakes with low plant inputs, algal and microbial production during low-flow seasons in summer has been shown to increasingly influence DOM chemistry as indicated by elevated FI values (Hood et al. 2003). As our streams are shaded, small, first-order streams draining relatively well-developed forests (50 – 450 years), we hypothesize that autochthonous inputs are extremely low compared to allochthonous inputs during all seasons (Vannote et al. 1980), and are likely much more labile. As microbial activity also increases with temperature in soils (Kaiser et al. 2001), an increased FI value alone cannot differentiate in-stream production from soil microbial processing during summers.

The protein-like component was inversely related to DOC and DON concentrations, $SUVA_{254}$, and flow parameters of Q and Qsum 3 through Qsum 30. This suggests that protein-like DOM increases during low-flow conditions, and decreases during seasons of shallow subsurface flow. As previous studies in this study area report, antecedent soil moisture strongly influences DOC and DON exports, and the exports increase with flow rate during the first precipitation event of the season (Vanderbilt et al. 2003; van Verseveld et al. 2009). High SUVA$_{254}$ values of highly vegetated U.S. river basins coincide with high flow conditions (Butman et al. 2012), indicating a relatively less microbially-processed DOM source. Also, high SUVA$_{254}$ during high flow seasons may indicate that highly aromatic DOM preferentially adsorbed to shallow soil surface (Kaiser et al. 1997) is mobilized by shallow subsurface flow or travels through preferential flow. These findings imply that increased flow during the fall flushes DOC, DON, and highly aromatic or refractory DOM accumulated in soils and valley floors over the summer along with leachate from accumulated leaf litter in and near the stream, producing increased DOC and DON concentrations as well as less microbially-processed, aromatic DOM in stream water. The negative correlation of C4 (%) with these parameters, thus, suggests that the protein-like DOM component does not originate from shallow subsurface flow.

The positive correlation between C4 (%) and the base flow index (BFI) suggests that C4 originates from deeper subsurface flow, characteristic of low flow seasons. Soil organic matter at depth is highly enriched in microbial compounds and is significantly more microbially-processed than surface soil organic matter (Sollins et al. 2009; Rumpel and Kögel-Knabner 2011). Deep soils in the HJAEF contain higher free amino acids and protein than surface soils (Yano and others 2004). Protein or protein-like fluorescent DOM is higher in deep soil and/or groundwater than in surface water (Yano et al. 2004; Sollins et al. 2009; Inamdar et al. 2012; Huang et al. 2015). Higher fluorescent protein % in groundwater and water-extractable soil organic matter (WESOM; 0 – 40 cm deep) compared to more surficial water has been reported from first-order forested catchments (Johnson et al. 2011; Inamdar et al. 2012; Huang et al. 2015). Additionally, several studies have found lower $SUVA_{254}$ in groundwater than surface water (van Verseveld et al. 2009; Inamdar et al. 2012); Gabor and others (2014) reported lower $SUVA_{254}$ at depth than in surface soils. These studies are consistent with the study of Fellman and others (2014) that found that streams with high groundwater sources had a greater contribution of old (i.e. depleted $^{14}C$) and bioavailable DOM from microbial sources in western Australia. Thus, the increased proportion of a protein-like component (C4 (%)) during dry seasons in our study likely indicates an increased proportion of inputs from deep subsurface flow, groundwater, or deep soil sources.

Although DOC:DON commonly decreases with depth in soil at sites including the HJAEF (Yano et al. 2004; Rumpel and Kögel-Knabner 2011), DOC:DON of stream water did not decrease in this study during base flow. The ratio of DOC to DON is often used as an indicator of microbial activity, but did not correlate with C4 (%), our protein-like and microbial component. This could be explained by the low DON in atmospheric deposition and in streams in the HJAEF compared to other sites in the U.S. (Vanderbilt et al. 2003), resulting in DON concentrations at detection limits and with significant noise. Thus, we suggest that a protein-like or specifically a tryptophan-like EEM signal can be used as an indicator of DOC that originates from microbial processing of terrestrial C either in the soil profile or in streams in similar low-N environments.

### 3.5.3 Forest Management and Landscape

We predicted that watersheds with young (50 to 60-yr) forests would have less abundant humic-like components (C1 to C3) and a greater contribution from a protein-like and microbial-like component (C4) in stream water compared to old-growth watersheds (500-yr-old forests). This is because inventories of coarse woody debris (CWD), a source of humic DOM in watersheds with young (50 to 60-yr) forests have been reduced by harvest, slash removal, slash burning, site preparation, and low wood recruitment to the forest floor.

The prediction was supported at harvested watersheds of WS 6, 7, and 10 where higher relative contributions of a protein-like component (C4 (%)) were observed compared to their reference watersheds (i.e. WS 8, 8, and 9, respectively). However, C4 (%) in a harvested watershed, WS 1, was similar to its reference watershed (i.e. WS 2) and lower than other harvested watersheds (i.e. WS 6, 7, and 10). This is probably due to a result of the logistical difficulty of harvesting large, old-growth trees without roads in the early 1960s in WS 1 that resulted in relatively high amounts of CWD compared to other harvested watersheds. Hence, the abundance of CWD and decomposed logs, not live tree biomass, in a watershed also appears to control stream DOM characteristics by increasing highly aromatic, humic-like components in a stream and diluting the protein-like signature from older, deeper and more microbial sources. Although we also hypothesized that the presence of all relatively young trees, found in harvested stands, might result in lower DOC supply due to reduced fine root turnover and root exudation, the lack of difference in DOC between WS1 and WS2 suggests that this is not a significant factor explaining differences in DOM optical properties across watersheds.

Another possibility for the decreased C4 (%) in WS 1 is the steep slope of WS 1. The steep slope may have resulted in higher shallow subsurface flow contribution to streams compared to other harvested watersheds which then diluted the deep flow signal of C4 in WS 1. However, WS 10 is equally as steep as WS 1, and BFI of WS 1 was not the lowest (i.e. not the highest shallow flow contribution), and BFI of the HJAEF remained relatively constant between harvested and reference watersheds; hence, the shallow flow contribution cannot be the sole reason for the low C4 (%) in WS 1.

Our correlation analysis showed that CWD and BFI individually are significant predictors of the protein-like DOM contribution. However, adding both parameters in a multiple linear regression did not improve the prediction. Of more concern is the observation that both significant correlations appear to be driven by WS 6 and 7, and both of these watersheds have high BFI and low CWD.. However, CWD and BFI were not autocorrelated, and WS 8 also has a gentle slope, high BFI, but is a reference watershed with significant stocks of CWD. It is possible that WS 6 and 7 may be showing the perfect scenario to have a high protein-like DOM in streams: high base flow index (i.e. high deep subsurface flow influence) and low CWD (i.e. low humic-like DOM), and that both of these factors need to be present in order to detect a significant difference in DOM chemistry.

In summary, this study shows that a tryptophan-like (protein-like) DOM identified with the fluorescent spectroscopy helps identify sources of DOM and shows important implications of the linkage between terrestrial and aquatic ecosystems. We attribute the increase in a protein-like DOM contribution during summer months to an increased contribution from deeper soil horizons that have an increased microbial (and thus protein) signature. Similarly, the decrease in protein-like signals during the winter can be attributed to increased shallow subsurface flow and/or increased preferential flow that is known to have a more terrestrial, humic and aromatic DOM signal. Increased in-stream microbial activity degrading organic matter inputs to the streams during the warmer summer months at our site also could increase the microbial signature of DOM; our current data cannot distinguish among these hypotheses. Although the combination of CWD and BFI partially explained the fluorescent signature of DOM across these watersheds, our results suggest that the relative contributions of microbially processed, bioavailable DOM (protein) derived from deeper subsurface/groundwater compared to DOM derived from the soil surface horizon, whether due to increased terrestrial inputs such as CWD or else due to increased water flux through surface horizons, can be detected. Our results also show that the imprint of forest harvest, and the reduction in CWD inputs to the forest floor, can be detected for many decades, with implications for the metabolism of DOM in downstream receiving ecosystems.

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## 3.8 Tables

Table 2: Experimental watersheds properties and history (H.J. Andrews Experimental Forest 2015)

| WS no. | Area (ha) | Elevation Min (m) | Elevation Max (m) | Slope (%) | Management History |
|---|---|---|---|---|---|
| Look | 6242 | 434 | 1627 | 40 | 25 % patch-cut, 1948 - Present |
| Mack | 581 | 765 | 1626 | 48 | Control<br>8 units (about 13 %) partially or fully cut, 1957 - 1982 |
| 1 | 96 | 450 | 1027 | 60 | 100 % clear cut, 1962 - 1966<br>Logging debris burn, 1966<br>No roads |
| 2 | 60 | 572 | 1079 | 53 | Undisturbed control for WS1 |
| 6 | 13 | 893 | 1029 | 25 | 100 % clear cut, 1974<br>Planted with Douglas-fir seedlings, 1976<br>A road |
| 7 | 15 | 931 | 1102 | 34 | 60 % selective canopy removal, 1974<br>Logging residue burn at lower half, 1975<br>Planting, 1976<br>Remaining canopy removal, 1984<br>Thinning, 2001 |
| 8 | 21 | 968 | 1182 | 26 | Undisturbed control for WS6 and WS7 |
| 9 | 8.5 | 438 | 731 | 58 | Undisturbed control for WS10 |
| 10 | 10 | 471 | 679 | 58 | 100 % clear cut, 1975 |

Table 3: Biomass (Mg/ha) of total, live tree, and logs in each watershed (Fasth et al., Unpublished Data)

| | Harvested Watersheds | | | | Control Watersheds | | |
|---|---|---|---|---|---|---|---|
| Biomass (Mg/ha) | WS01 | WS06 | WS07 | WS10 | WS02 | WS08 | WS09 |
| Total | 267 | 191 | 130 | 232 | 894 | 906 | 791 |
| Live tree | 142 | 110 | 55 | 134 | 654 | 652 | 585 |
| Logs | 72 | 33 | 29 | 29 | 103 | 115 | 108 |

Table 4: Four components identified by this study and their previously identified characteristics

| C | Ex max (nm) | Em max (nm) | Characteristics |
|---|---|---|---|
| 1 | 260 | 486 | Similar to humic-like DOM commonly found in forested streams (C1, Stedmon and Markager 2005). |
| 2 | 250<br>(310) | 410 | Humic-like DOM common in forested streams (C3, Stedmon and Markager 2005). |
| 3 | 250<br>(330) | 454 | Fulvic acid and found in various environments (C4, Stedmon and Markager 2005). |
| 4 | 250<br>(280) | 350 | Tryptophan-like (Cory and McKnight 2005; C7, Stedmon and Markager 2005; C4, Hosen et al. 2014) and protein-like component (T, Coble 1996; Stedmon et al. 2003). Originated from aromatic amino acids, an indicator of total hydrolysable amino acids, and labile or semi-labile aquatic DOM (C5, Yamashita et al. 2011). Autochthonous source and correlated to terrestrial DOM from forested catchments (Stedmon and Markager 2005). |

Values in the parenthesis indicate the second peak.

Table 5: Statistics of measured parameters

| Parameter | Min | Max | Mean | Median | Stdev |
|---|---|---|---|---|---|
| DON (mg $L^{-1}$) | 0.00 | 0.12 | 0.03 | 0.03 | 0.02 |
| DOC (mg $L^{-1}$) | 0.3 | 4.0 | 1.2 | 1.0 | 0.6 |
| DOC:DON | 9.3 | 288.3 | 41.9 | 34.4 | 28.7 |
| FI | 1.3 | 2.1 | 1.4 | 1.4 | 0.1 |
| $SUVA_{254}$ (L $mg^{-1}$ $m^{-1}$) | 0.2 | 8.1 | 3.5 | 3.7 | 1.0 |
| Abs254 ($m^{-1}$) | 0.2 | 15.6 | 4.2 | 3.6 | 2.6 |
| C1 (%) | 13.1 | 37.7 | 28.8 | 29.4 | 5.3 |
| C2 (%) | 9.2 | 27.9 | 18.7 | 19.2 | 3.1 |
| C3 (%) | 16.1 | 47.1 | 26.7 | 26.6 | 2.9 |
| C4 (%) | 13.8 | 46.0 | 25.7 | 24.4 | 7.2 |

Table 6: Correlations among key parameters
(N = 322, Two-tailed significance test, **: α = 0.01, *: α = 0.05)

| | DOC | DON | DOC/DON | FI | $SUVA_{254}$ | C1 (%) | C2 (%) | C3 (%) | C4 (%) |
|---|---|---|---|---|---|---|---|---|---|
| DOC | 1 | 0.60** | 0.23** | -0.21** | 0.19** | 0.60** | 0.55** | -0.19** | -0.60** |
| DON | | 1 | -0.44** | -0.06 | 0.19** | 0.45** | 0.35** | -0.02 | -0.47** |
| DOC/DON | | | 1 | 0.05 | -0.11* | -0.04 | 0.06 | -0.08 | 0.04 |
| FI | | | | 1 | -0.37** | -0.47** | -0.27** | 0.20** | 0.39** |
| $SUVA_{254}$ | | | | | 1 | 0.55** | 0.51** | -0.23** | -0.53** |
| C1 (%) | | | | | | 1 | 0.74** | -0.31** | -0.94** |
| C2 (%) | | | | | | | 1 | -0.54** | -0.76** |
| C3 (%) | | | | | | | | 1 | 0.06 |
| C4 (%) | | | | | | | | | 1 |

Table 7: Linear correlations of C4 (%) and $SUVA_{254}$ with flow parameters at each watershed

(Two-tailed significance test, **: α = 0.01, *: α = 0.05)

| Site | | C4 (%) | $SUVA_{254}$ | Q | Qsum3 | Qsum14 | Qsum21 | Qsum30 |
|---|---|---|---|---|---|---|---|---|
| LOOK | C4 (%) | 1 | -0.59** | -0.57** | -0.62** | -0.59** | -0.65** | -0.66** |
| | $SUVA_{254}$ | | 1 | 0.51** | 0.55** | 0.60** | 0.64** | 0.62** |
| MACK | C4 (%) | 1 | -0.16 | -0.67** | -0.64** | -0.62** | -0.65** | -0.65** |
| | $SUVA_{254}$ | | 1 | 0.13 | 0.13 | 0.04 | 0.08 | 0.07 |
| WS01 | C4 (%) | 1 | -0.62** | -0.62** | -0.59** | -0.39** | -0.43** | -0.44** |
| | $SUVA_{254}$ | | 1 | 0.54** | 0.49** | 0.59** | 0.61** | 0.60** |
| WS02 | C4 (%) | 1 | -0.39* | -0.42* | -0.42* | -0.11 | -0.10 | -0.04 |
| | $SUVA_{254}$ | | 1 | 0.48** | 0.45** | 0.38* | 0.40* | 0.40* |
| WS06 | C4 (%) | 1 | -0.61** | -0.37* | -0.39* | -0.21 | -0.26 | -0.19 |
| | $SUVA_{254}$ | | 1 | 0.42* | 0.45** | 0.40* | 0.45** | 0.39* |
| WS07 | C4 (%) | 1 | -0.47** | -0.56** | -0.57** | -0.39* | -0.42* | -0.38* |
| | $SUVA_{254}$ | | 1 | 0.36* | 0.36* | 0.24 | 0.27 | 0.25 |
| WS08 | C4 (%) | 1 | -0.14 | -0.42* | -0.42* | -0.31 | -0.33 | -0.27 |
| | $SUVA_{254}$ | | 1 | 0.41* | 0.41* | 0.45** | 0.49** | 0.46** |
| WS09 | C4 (%) | 1 | -0.23 | -0.52** | -0.57** | -0.41* | -0.43** | -0.46** |
| | $SUVA_{254}$ | | 1 | 0.37* | 0.31 | 0.33* | 0.36* | 0.37* |
| WS10 | C4 (%) | 1 | -0.26 | -0.52** | -0.50** | -0.20 | -0.22 | -0.21 |
| | $SUVA_{254}$ | | 1 | 0.55** | 0.53** | 0.68** | 0.79** | 0.75** |

Table 8: Logarithmic correlations (coefficient of determination, $R^2$) of C4 (%) with flow parameters at each watershed.

(a significance test, **: α = 0.01, *: α = 0.05)

| Site | Qsum3 | Qsum14 | Qsum30 |
|---|---|---|---|
| LOOK | 0.69 | 0.77 | 0.68 |
| MACK | 0.72 | 0.70 | 0.55 |
| WS01 | 0.49 | 0.39 | 0.29 |
| WS02 | 0.14 | 0.06 | 0.01 |
| WS06 | 0.20 | 0.14 | 0.07 |
| WS07 | 0.33 | 0.28 | 0.15 |
| WS08 | 0.10 | 0.08 | 0.02 |
| WS09 | 0.48 | 0.41 | 0.35 |
| WS10 | 0.32 | 0.20 | 0.16 |

## 3.9 Figures

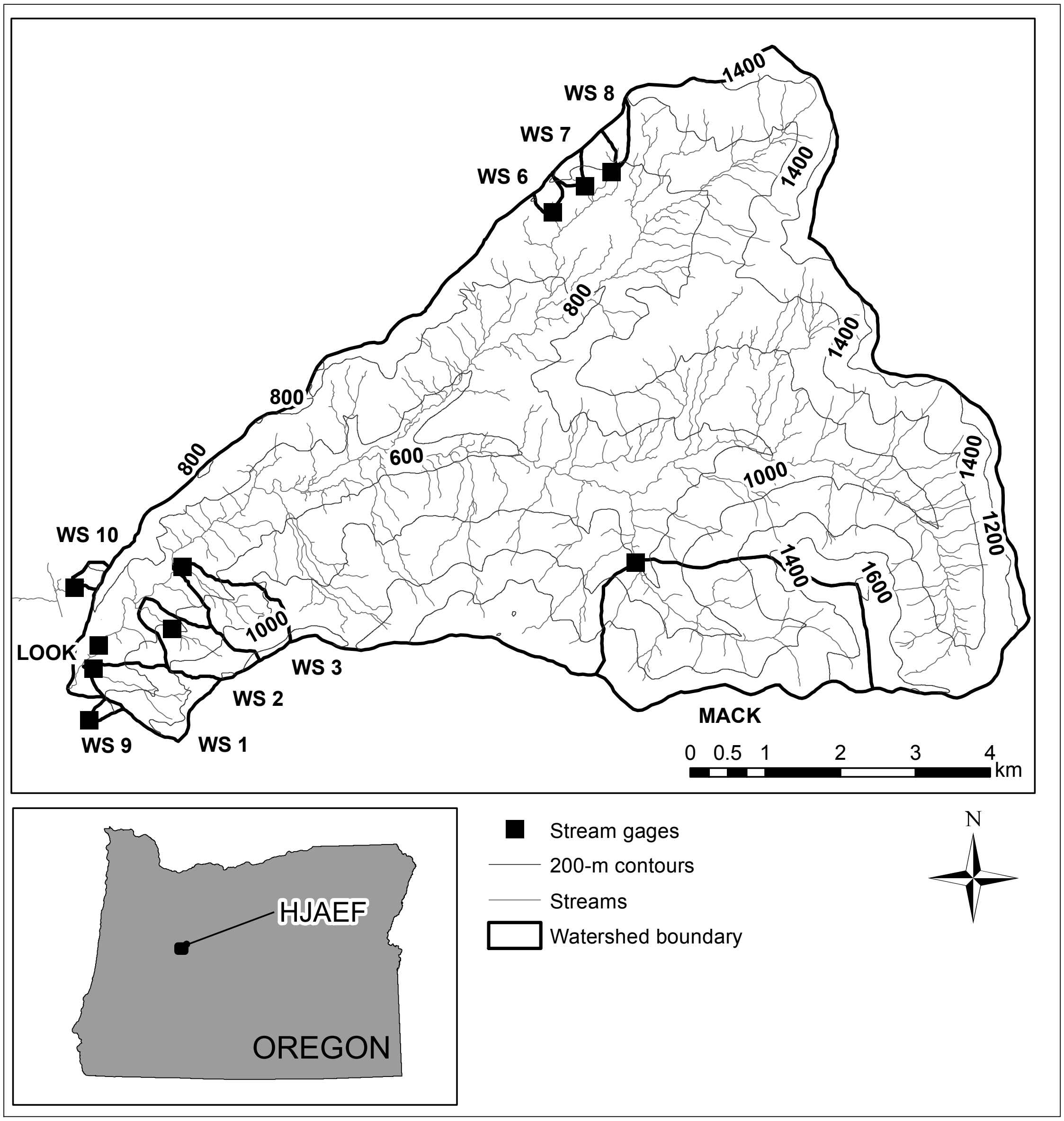


Figure 8: Map of the H.J. Andrews Experimental Forest

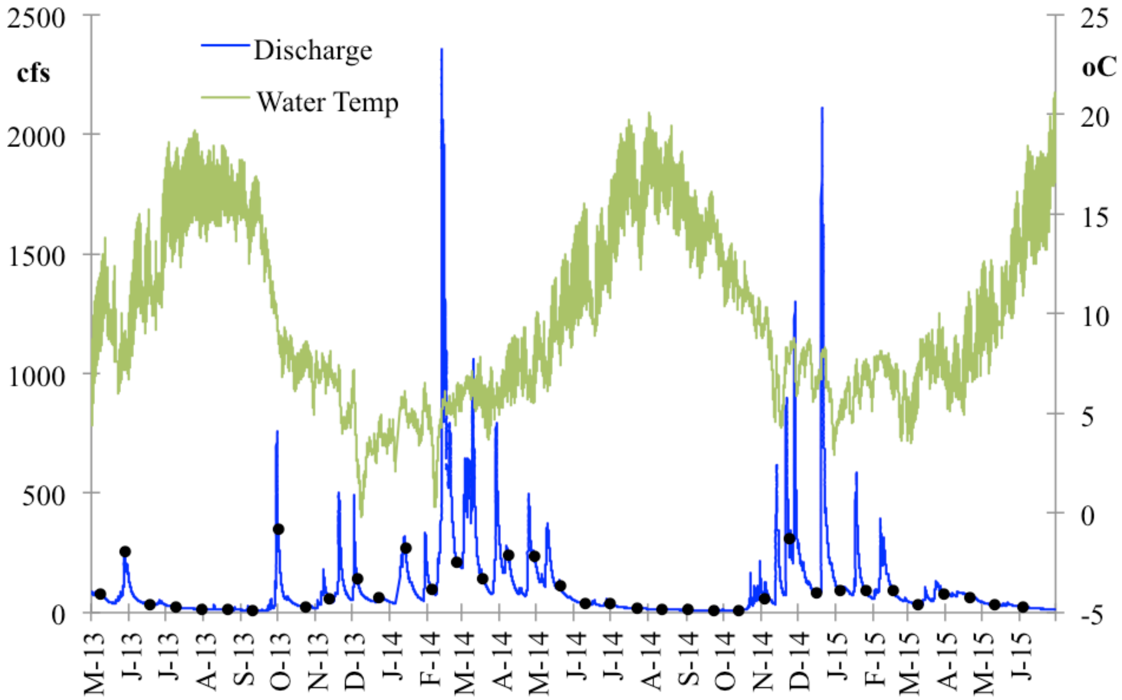
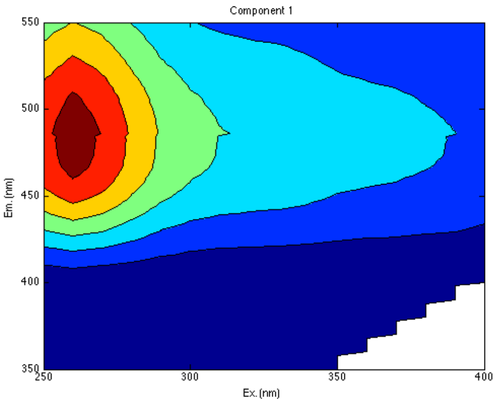
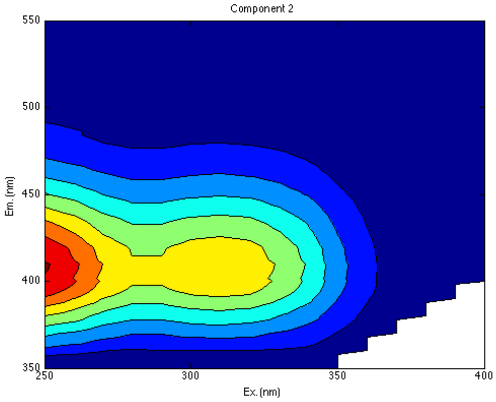


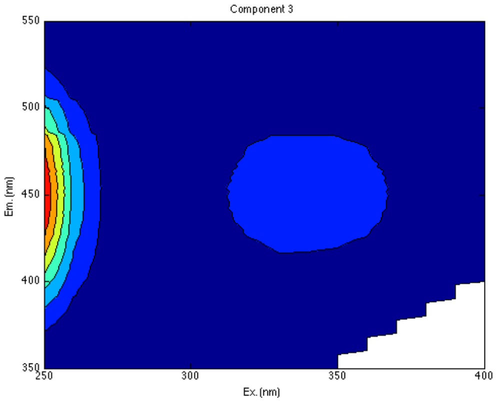
Figure 9: Flow rate and stream temperature at the Lookout Creek Watershed (LOOK) between May 2013 and June 2015
Each dot indicates sampling date. Data measured by USGS (USGS National Water Information System at Lookout Creek Near Blue River, OR (14161500)).



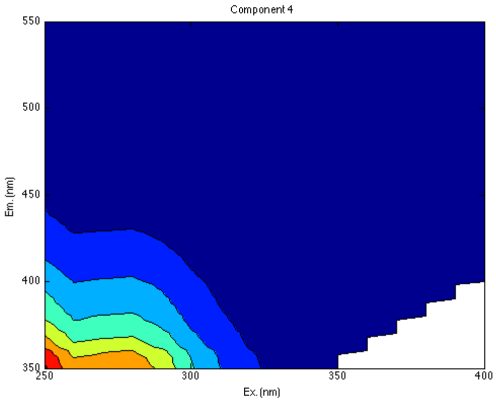
a


Component 2
Em. (nm)
550
500
450
400
350
250
300
350
400
Ex. (nm)

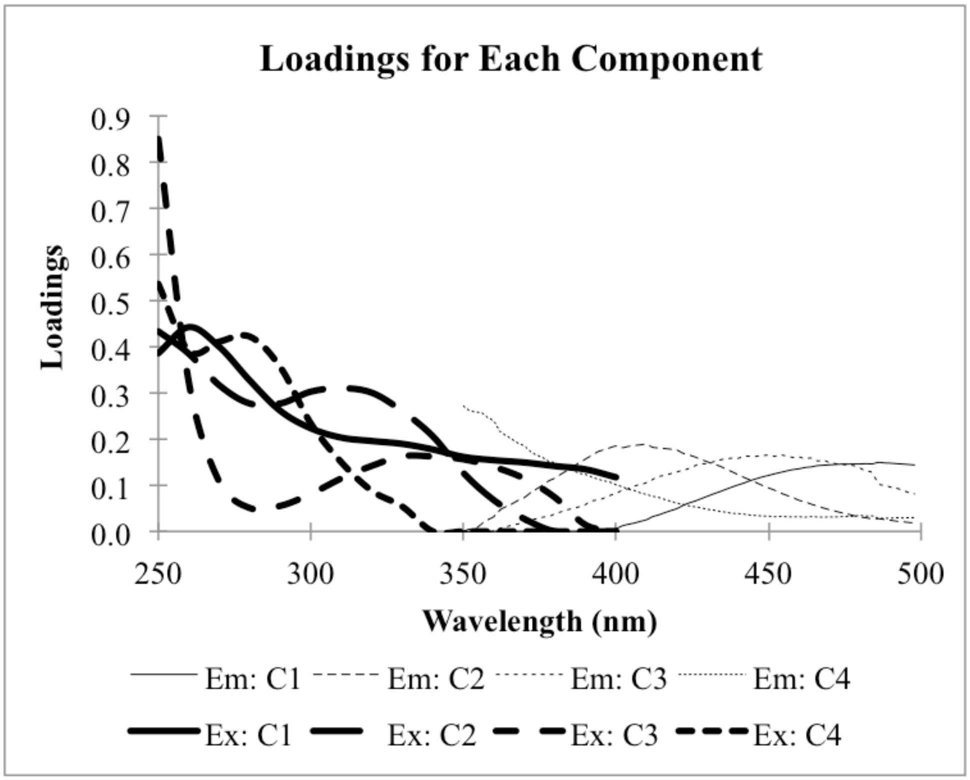
b


Component 3
Em. (nm)
550
500
450
400
350
250
300
350
400
Ex. (nm)

c


Component 4
Em. (nm)
550
500
450
400
350
250
300
350
400
Ex. (nm)

d



e

Figure 10: EEMs of four components identified by the PARAFAC model (Figure 10a – d) and their excitation emission loadings (Figure 10e)

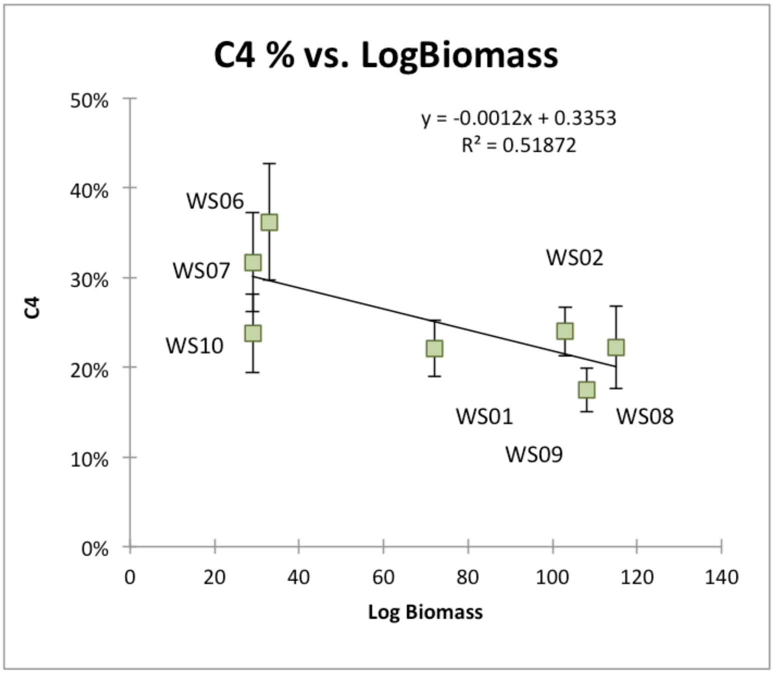
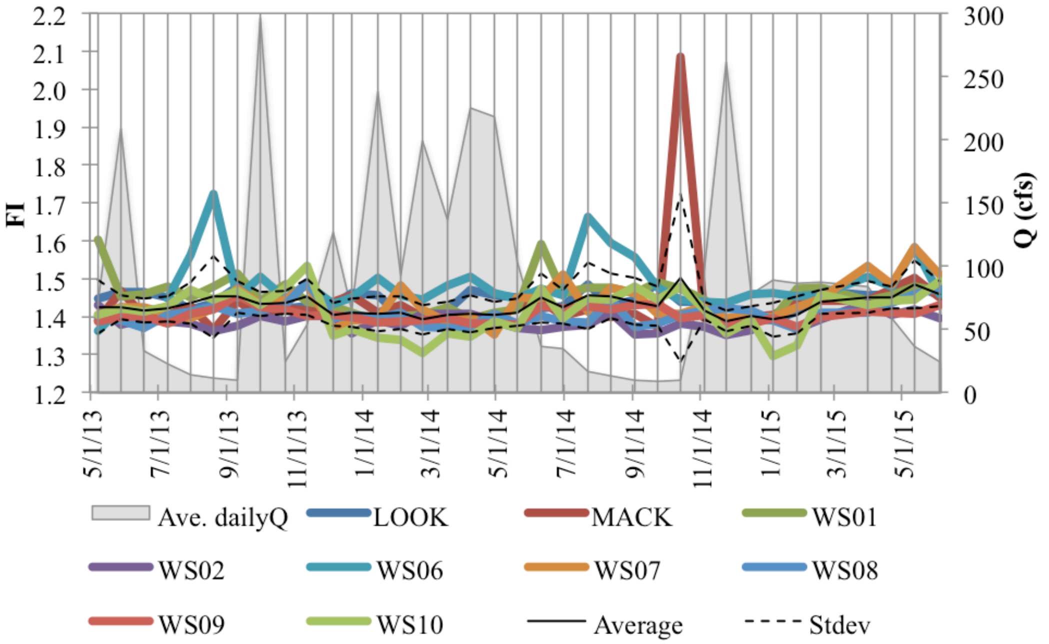
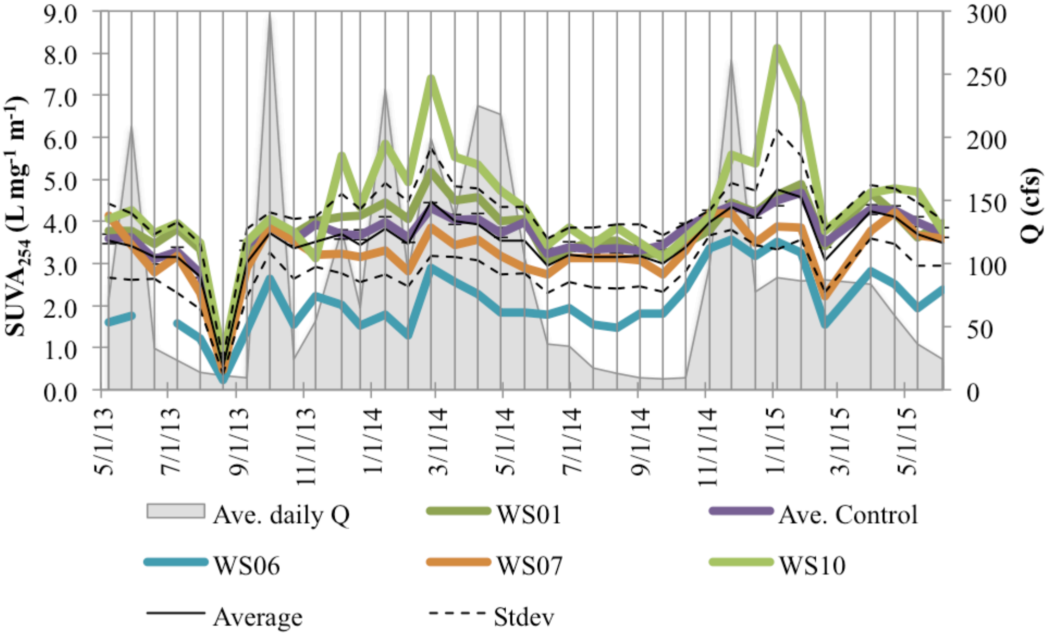


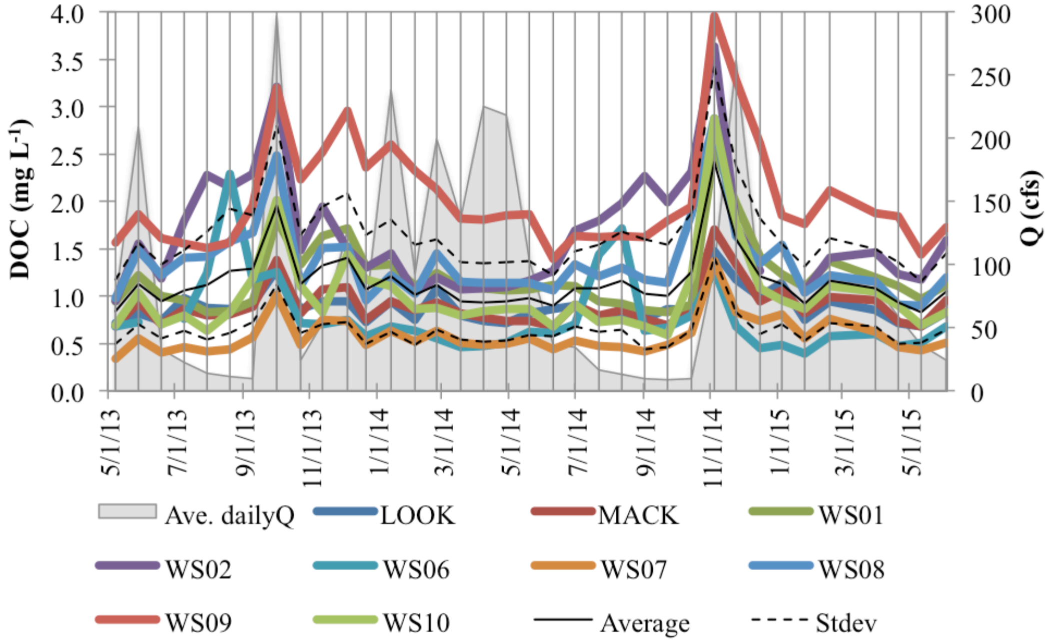
Figure 11: Correlation of C4 (%) and CWD biomass (N = 7)

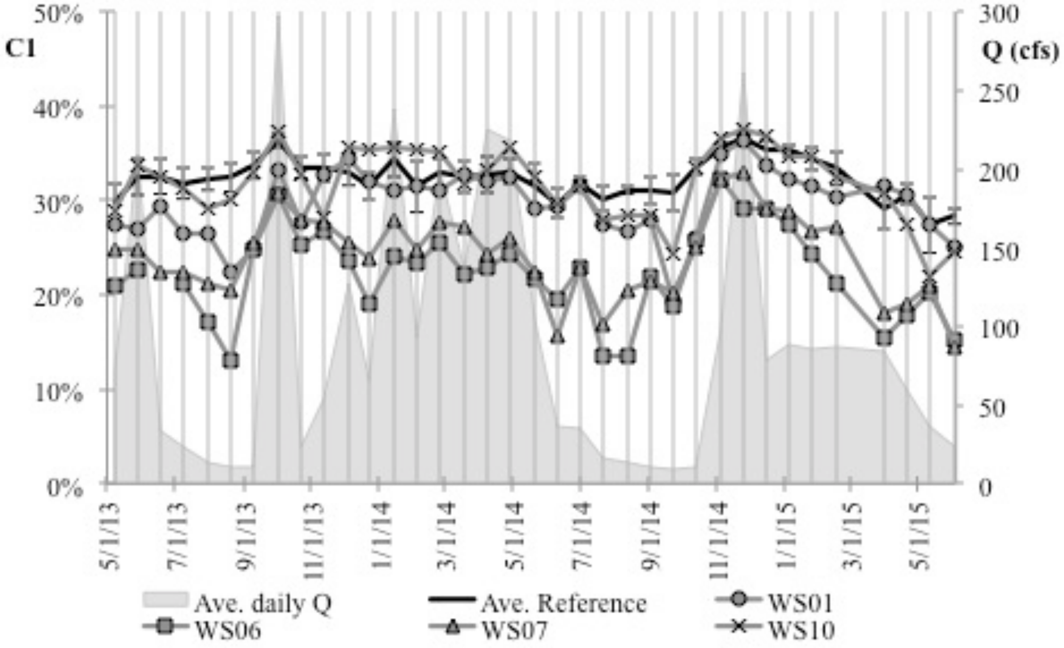

2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
FI
300
250
200
150
100
50
0
Q (cfs)
5/1/13
7/1/13
9/1/13
11/1/13
1/1/14
3/1/14
5/1/14
7/1/14
9/1/14
11/1/14
1/1/15
3/1/15
5/1/15
Ave. dailyQ
LOOK
MACK
WS01
WS02
WS06
WS07
WS08
WS09
WS10
Average
Stdev

a

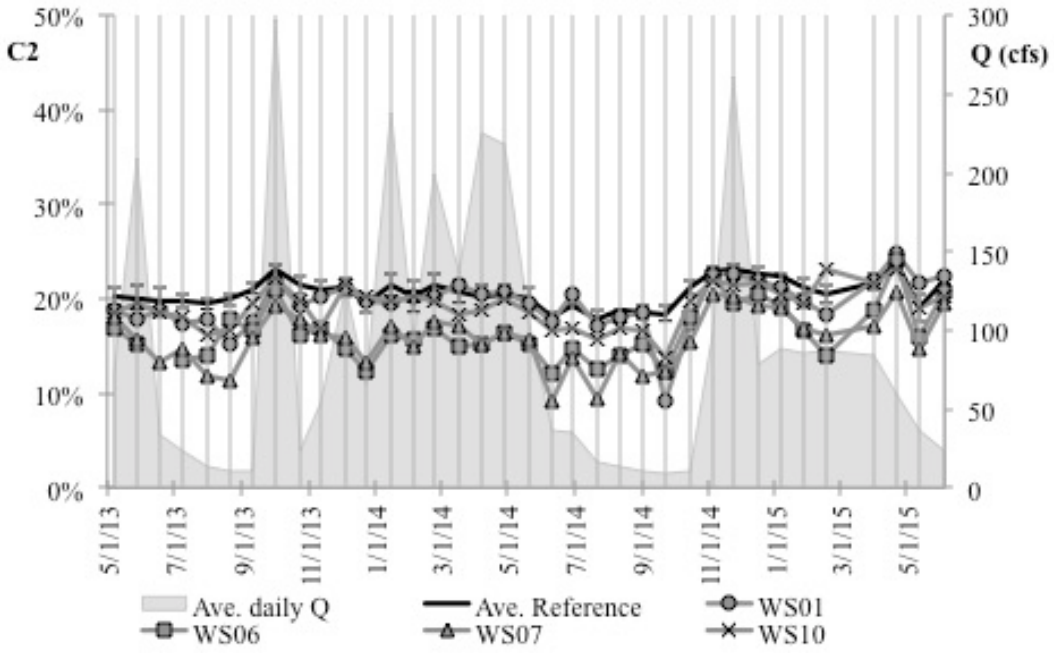

9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
$SUVA_{254}$ (L mg$^{-1}$ m$^{-1}$)
300
250
200
150
100
50
0
Q (cfs)
5/1/13
7/1/13
9/1/13
11/1/13
1/1/14
3/1/14
5/1/14
7/1/14
9/1/14
11/1/14
1/1/15
3/1/15
5/1/15
Ave. daily Q
WS01
Ave. Control
WS06
WS07
WS10
Average
Stdev

b

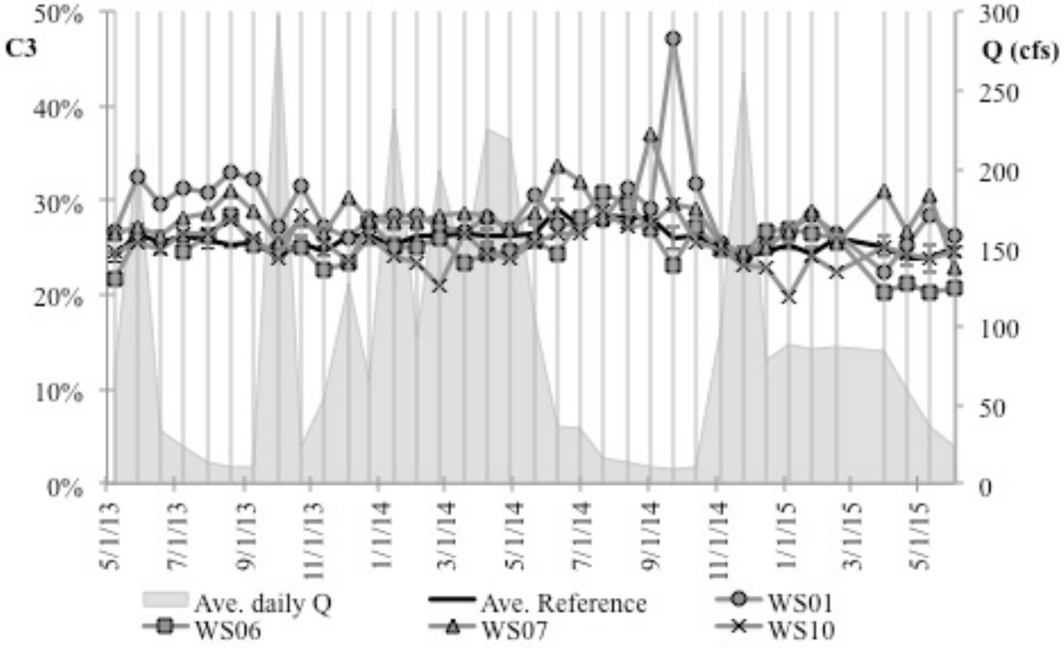

DOC (mg L⁻¹)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Q (cfs)
300
250
200
150
100
50
0
5/1/13
7/1/13
9/1/13
11/1/13
1/1/14
3/1/14
5/1/14
7/1/14
9/1/14
11/1/14
1/1/15
3/1/15
5/1/15
Ave. dailyQ
LOOK
MACK
WS01
WS02
WS06
WS07
WS08
WS09
WS10
Average
Stdev


50%
40%
30%
20%
10%
0%
C1
300
250
200
150
100
50
0
Q (cfs)
5/1/13
7/1/13
9/1/13
11/1/13
1/1/14
3/1/14
5/1/14
7/1/14
9/1/14
11/1/14
1/1/15
3/1/15
5/1/15
Ave. daily Q
Ave. Reference
WS01
WS06
WS07
WS10

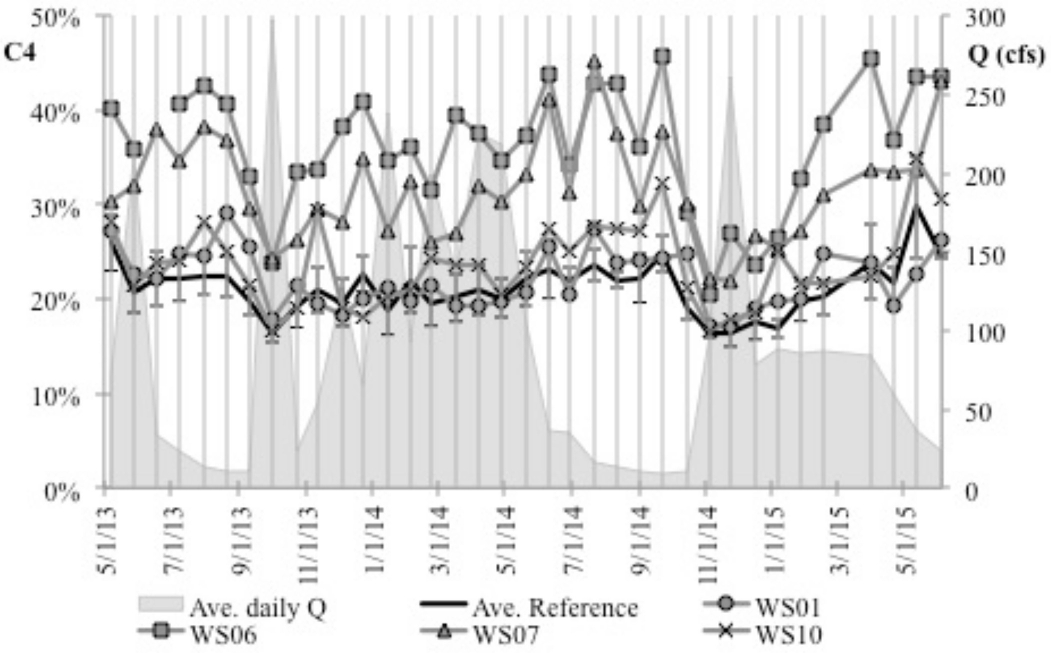
c


C2
Q (cfs)
50%
40%
30%
20%
10%
0%
300
250
200
150
100
50
0
5/1/13
7/1/13
9/1/13
11/1/13
1/1/14
3/1/14
5/1/14
7/1/14
9/1/14
11/1/14
1/1/15
3/1/15
5/1/15
Ave. daily Q
Ave. Reference
WS01
WS06
WS07
WS10

d


C3
Q (cfs)
50%
40%
30%
20%
10%
0%
300
250
200
150
100
50
0
5/1/13
7/1/13
9/1/13
11/1/13
1/1/14
3/1/14
5/1/14
7/1/14
9/1/14
11/1/14
1/1/15
3/1/15
5/1/15
Ave. daily Q
Ave. Reference
WS01
WS06
WS07
WS10

e



f

Figure 12: Time-series of various parameters
Error bars show standard error. Vertical lines indicate sampling dates. Average daily flow rate of sampling date was measured by USGS (USGS National Water Information System at Lookout Creek Near Blue River, OR (14161500)).

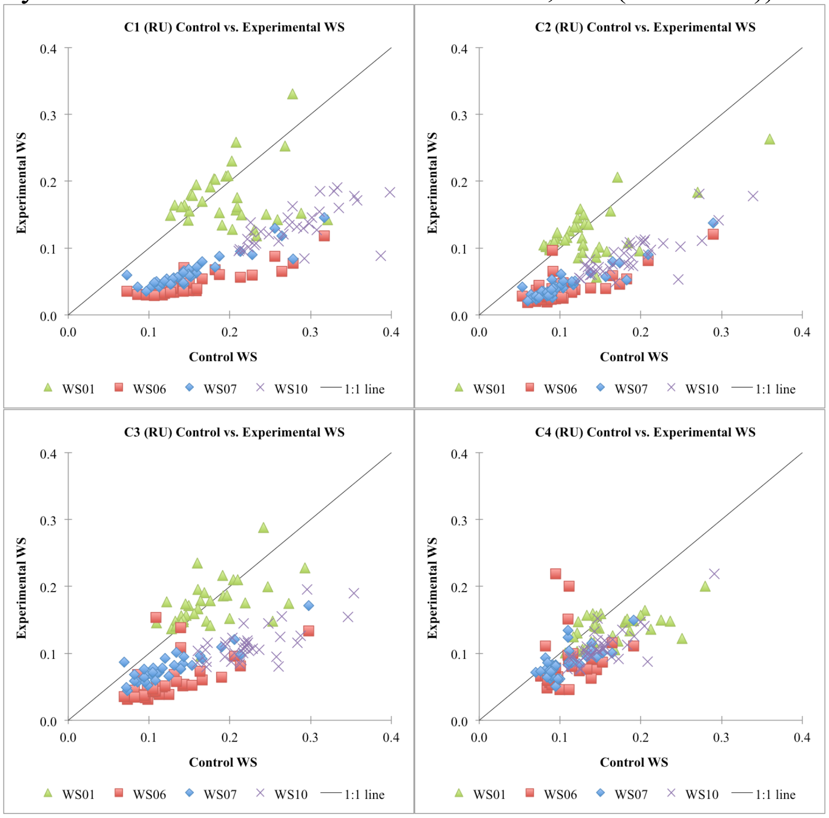


Figure 13: Comparison of components between harvested and control watersheds in R.U.

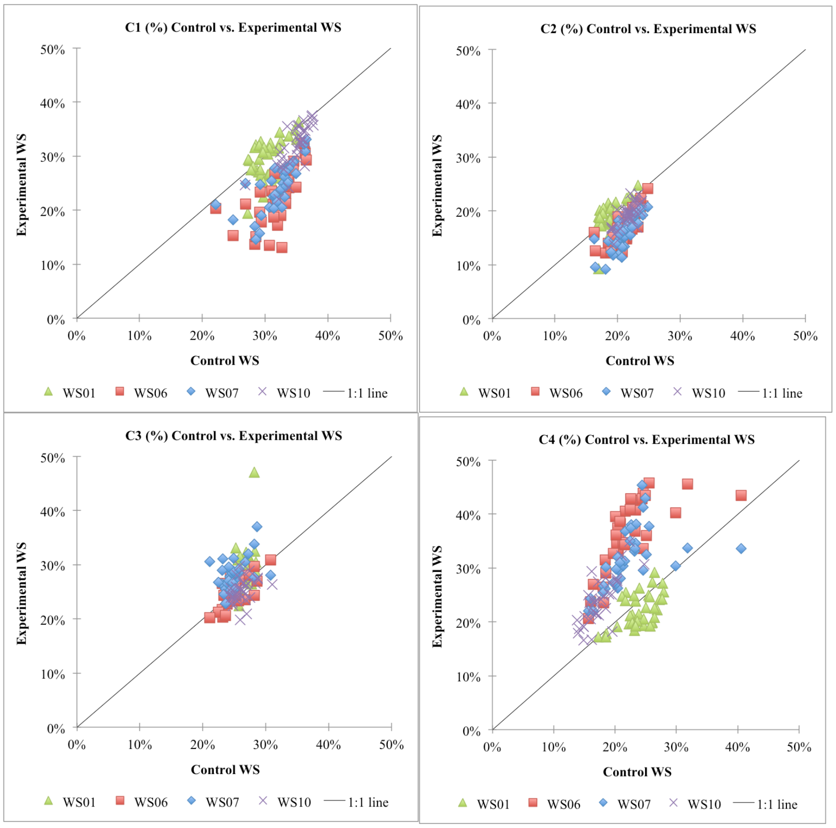


Figure 14: Comparison of components between harvested and control watersheds in %

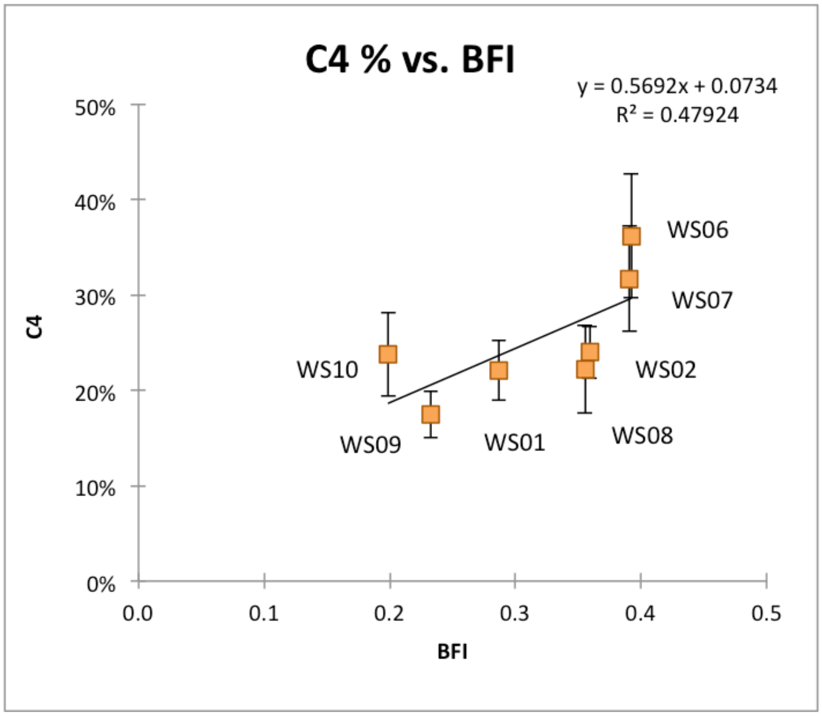
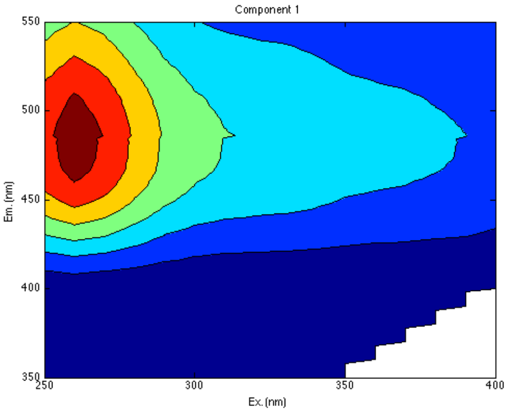
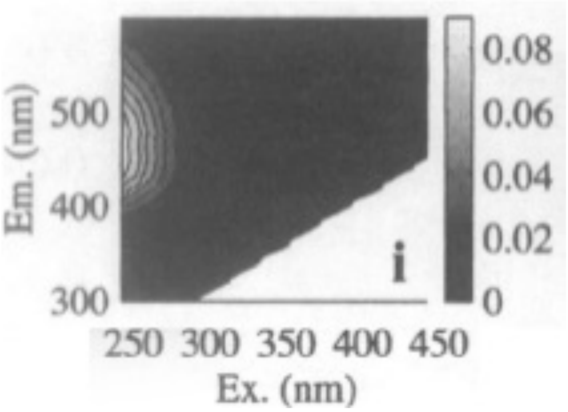
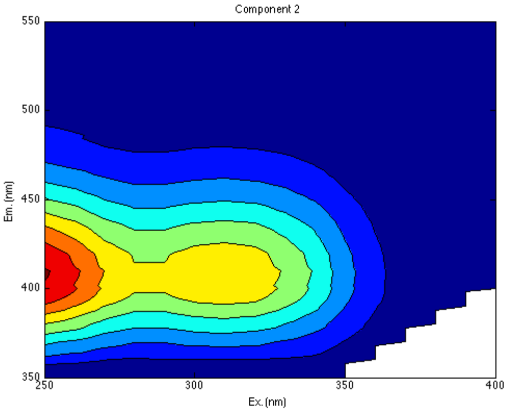


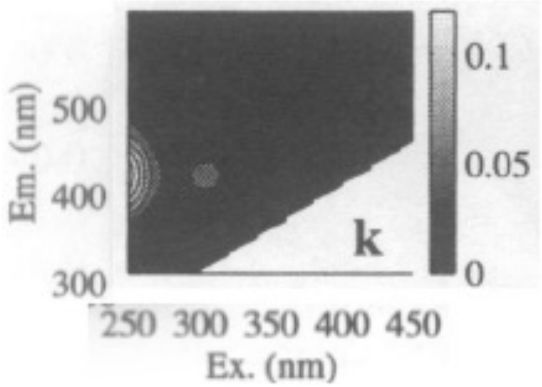
Figure 15: Correlation of C4 (%) and BFI (N = 7)

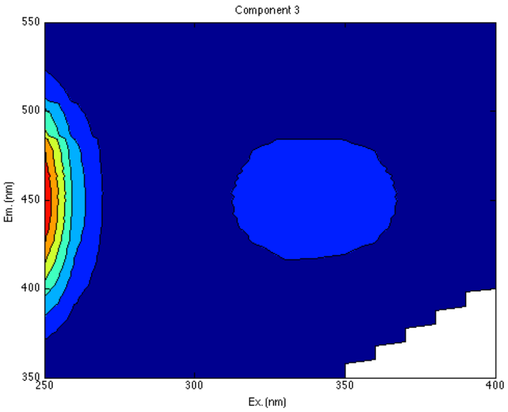
## 3.10 Appendix

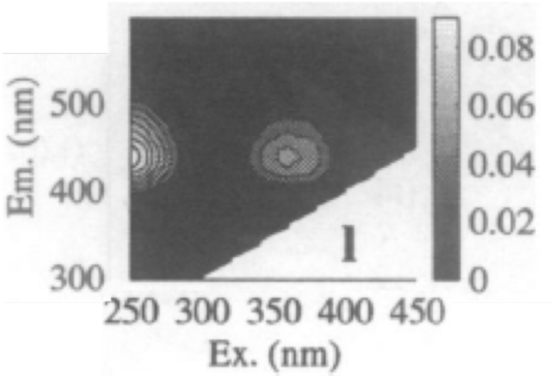


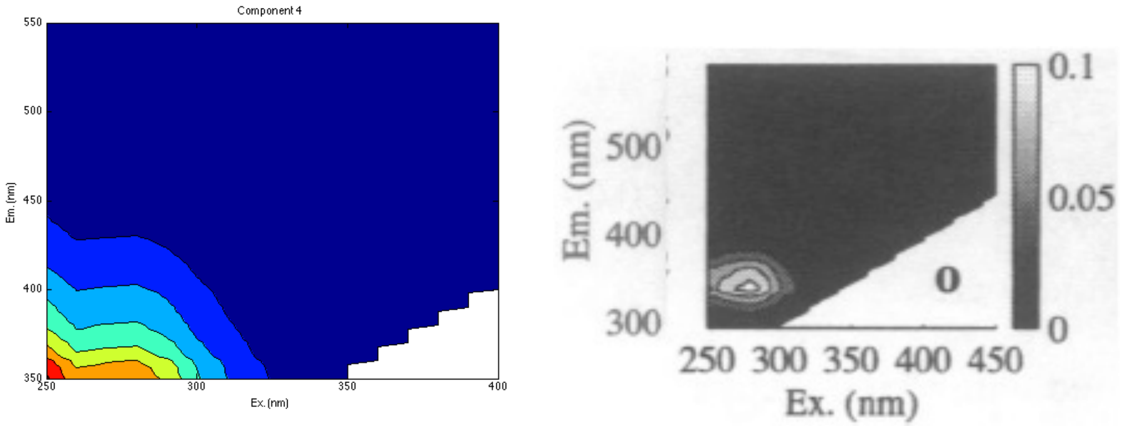












Appendix 1: Comparison of components identified by this study and characterized by a previous study (Stedmon and Markager 2005)

# 4 Seasonal and Land Use Effects on UV and Fluorescent Dissolved Organic Matter Characteristics in Streams of a Mixed Landscape

B. Lee, K. Lajtha, J. A. Jones, and A. E. White

For Submission to
Biogeochemistry

## 4.1 Abstract

Dissolved organic matter (DOM), delivered from terrestrial sources and synthesized within streams, affects carbon composition and metabolism of receiving water bodies. However, little is known about how land use and seasonal flow patterns affect in-stream DOM fluxes and chemistry. This study examined UV and fluorescent DOM characteristics and biochemical properties of streams in contrasting land uses and stream orders to identify the sources and fate of DOM in the Willamette River Basin, Oregon. Nutrient concentrations were the highest among high percentage of pasture/hay land and in headwater streams. Aromaticity measured by $\mathrm{SUVA}_{254}$ and a fluorescent index (FI) indicated streams received higher terrestrial DOM sources with an increase in flow rate and overland hydrologic connectivity. Dissolved organic carbon (DOC) concentrations decreased and FI indicated terrestrial DOM sources with an increase in stream orders while a protein-like DOM signal commonly found by other studies was not identified. These findings indicate that in-stream respiration, fueled by nutrient additions from pasture/hay lands, consumes bioavailable, labile DOM (proteins) preferentially relative to more recalcitrant, terrestrial sources of DOM along water flow paths from headwater streams to major rivers. Contrary to our hypotheses, a hydrologic control was a main driver of fluorescent DOM characteristics compared to land use. The lack of differences in fluorescent DOM characteristics over catchments with varying land use types can be attributed either to a lack of differences in DOM characteristics in soils derived from a relatively small region or to a microbial homogenization of distinct DOM components unique to dominant land use types in streams.

## 4.2 Introduction

Dissolved organic matter (DOM) is a critical component of the carbon (C) cycle in watershed ecosystems and enters water bodies from various sources. Surrounding lands and soils deliver various types of DOM such as leaf litter to streams (allochthonous DOM), and primary producers generate DOM through photosynthesis within streams (autochthonous DOM) (Vannote et al. 1980; Allan and Castillo 2009). Watershed characteristics such as topography, climate, hydrology, and land use create unique DOM chemistry in streams (Hudson and Reynolds 2007). Nonetheless, the effect of disturbances to terrestrial ecosystems on DOM chemistry in streams is poorly understood.

Compared to autochtonous DOM, allochthonous DOM has abundant aromatic compounds because terrestrial vegetation is high in tannins and lignins (McKnight et al. 2001; Allan and Castillo 2009). Such differences in chemical structures of DOM compounds create distinctive UV absorbance and fluorescent properties in freshwater samples (Coble 1996; McKnight et al. 2001; Weishaar et al. 2003; Cory and McKnight 2005; Stedmon and Bro 2008). UV and fluorescent spectroscopy is a simpler and less expensive technique than nuclear magnetic resonance (NMR) spectroscopy (Dai et al. 1996; Kalbitz et al. 2003) or wet chemical fractionation techniques (Yano et al. 2004; Hood et al. 2005) although its use in fingerprinting various DOM characteristics is less well-established.

Fluorescent properties of DOM controlled by unique DOM chemical structures can be characterized using the information from 3-D excitation and emission matrices (EEMs). EEMs capture the fluorescent signature at all combinations of excitation and emission wavelengths. EEMs are then analyzed with statistical analysis (often with a multivariate statistical modeling Parallel Factor Analysis (PARAFAC)) to identify fluorescent DOM components and the fraction of each component present in each sample (Stedmon and Bro 2008).

In recent years, fluorescent characterization of in-stream DOM has been widely used to identify and quantify DOM sources in many freshwater ecosystems (Coble 1996; Cory and McKnight 2005; Miller et al. 2006; Yamashita et al. 2011; Mann et al. 2012; Cawley et al. 2012b; Hosen et al. 2014). However, only 1 % of DOM is estimated to be fluorescent (Cory et al. 2011). The potential for utilizing fluorescent DOM to characterize complex DOM chemistry is of great interest, and further investigation may help determine the applicability of this technique on DOM chemistry characterization in freshwater systems.

Although much is known about land use effects on nitrogen (N) and phosphorus (P) in surface water (Smith et al. 1997; Alexander et al. 2000; Groffman et al. 2004; Alexander et al. 2008; Preston et al. 2011), the relationship of DOM chemistry to land use is poorly understood. And much of the studies, which examined the land use effects on fluorescent DOM chemistry, focused on comparing completely different land use types or variations within a land use type in headwater streams. Fluorescent spectroscopy has been used to describe DOM chemistry of water affected by discharge from a sewage treatment plant in England (Baker 2001) and in a tributary of the Willamette River, Oregon (Goldman et al. 2012). The abundance of fluorescent DOM components has been correlated to impervious cover in headwater watersheds of Maryland (Hosen et al. 2014). EEMS with PARAFAC has been applied to identify fluorescent components of DOM from streams in mainly agricultural lands in and near an estuary in Denmark

(Stedmon et al. 2003; Stedmon and Markager 2005). An index derived from EEMs has been used to relate in-stream DOM characteristics to agricultural land use in south-central Ontario, Canada (Wilson and Xenopoulos 2009). Multiple studies examined the effect of forest management on fluorescent DOM components in headwater streams (Yamashita et al. 2011; Cawley et al. 2014); a protein-like fluorescent DOM was indicative of low log biomass on the forest floor (Lee and Lajtha, in review).

Differences in DOM chemistry identified by UV and fluorescent spectroscopy has also been related to hydrologic regime. Increased aromatic DOM inputs during the spring-melt in Alaska was attributed to increased vascular plant litter inputs during high flows (Spencer et al. 2008). In the H.J. Andrews Experimental Forest in Oregon (HJAEF), in-situ fluorescence and Specific UV absorbance at 254 nm ($SUVA_{254}$), an indicator of aromaticity of water samples (Weishaar et al. 2003), increase during storm events (van Verseveld et al. 2008). $SUVA_{254}$ and a protein-like DOM identified with fluorescent spectroscopy were used to detect DOM delivered from protein-rich groundwater sources during dry seasons at headwater streams of HJAEF where nitrogen inputs are low (Lee and Lajtha, in review). Changes in fluorescent DOM chemistry with hydrologic patterns may be a key factor controlling in-stream DOM chemistry, and long-term monitoring will allow the observation of the hydrologic control on DOM chemistry.

We hypothesized that DOM optical properties would differ among land use types because streams influenced by urban environments receive highly aromatic compounds, e.g. water treatment disinfection byproducts and petroleum products, and streams influenced by nutrient-rich agricultural and pasture promote in-stream DOM processes resulting in less aromatic, more microbial compounds. Additionally, we hypothesized that DOM signatures linked to specific land use types would be more distinct during high flow seasons due to increased hydrologic connectivity. Our objectives were: 1) to characterize DOM properties in streams of the middle Willamette River Basin, Oregon with UV absorbance and fluorescence spectroscopy, 2) to observe spatial variations in DOM properties characterized by unique land use composition, and 3) to examine temporal variations of DOM properties driven by seasonal precipitation patterns.

## 4.3 Materials and Methods

### 4.3.1 Sample Sites

Stream water samples were collected monthly over the period of October 2012 to July 2015 from 21 locations near Corvallis, Oregon in the central Willamette River Basin (Figure 16). Sixteen sites are located within the 803-km$^2$ Marys River Watershed (Raymond et al. 2002); four sites are located in a tributary of the Willamette River located north of the Marys River Watershed; one site is located on the Willamette River. Sample locations were stratified by stream order and land use classes to capture spatial and temporal patterns of stream water chemistry (Appendix 2; Figure 17). The dominant land use types were pasture/hay, urban, forest, cultivated crops, and wetlands according to the 2011 National Land Cover Database (NLCD 2011) (Jin et al. 2013). The regional climate patterns are characterized by dry, warm summers and wet, cool winters, which drive stream flow as winter precipitation and snowmelt runoff between November and March (Figure 18). Some sites completely dried up during summers and froze during the winter of 2014 (Appendix 2).

### 4.3.2 Field and laboratory methods

Samples were collected from the surface of stream above the deepest part of the channel (thalweg) with an acid-rinsed brown one-liter HDPE Nalgene$^{TM}$ bottle. The sampler was washed with stream water three times before each collection. Immediately after collection, the sample was poured into acid-rinsed and pre-combusted (4 hrs at 490 $^{o}$C) amber glass bottles, which have been pre-rinsed three times with sampled water. Duplicate samples were collected from at least two locations during each sampling event for quality control and were averaged for data analysis. Samples were packed with ice and stored in the dark and transported to the laboratory within six hours.

At the laboratory, each sample was filtered through a pre-combusted 0.7 µm glass-fiber filters (Whatman$^{TM}$ GF/F) using an acid-rinsed glass filtering system in a darkened room. Samples were stored in a refrigerator until analysis. All samples were brought to room temperature on the laboratory bench prior to analysis. Samples were analyzed for inorganic nutrients (soluble reactive phosphorus ($PO_4^{3-}$ - P), ammonium as nitrogen ($NH_4^{+}$ - N), and nitrite – nitrate as nitrogen ($NO_3^{-}$ - N)), EEMs, and absorbance.

Analysis periods and storage methods varied (Table 9). TDN and DOC samples collected from October 2012 to April 2013 were frozen for up to eight months. TDN samples from March 2014 were frozen for 23 days before analysis. Samples obtained from May, June, and July 2014 were frozen for 48, 24, and 14 days before analysis for inorganic nutrients. No significant change in TDN and DOC concentrations were found in samples frozen for over a year in this study site for samples with initial concentrations less than 0.6 mg $L^{-1}$ and 8 mg $L^{-1}$ , respectively (Lee et al. in review). Thus, TDN concentrations greater than 0.6 mg $L^{-1}$ and DOC concentrations greater than 8 mg $L^{-1}$ for those frozen samples were excluded from the analysis.

All nutrients were measured with a Technicon auto-analyzer. DOC was measured using a Shimadzu TOC-VCSH analyzer. Absorbance between 240 and 560 nm was measured with a Cary 300 UV-Visible spectrophotometer, and Milli-$Q^{TM}$ water was used for calibration (Weishaar et al. 2003). $SUVA_{254}$ was calculated as the UV absorbance at 254 nm ($Abs_{254}$) normalized for DOC concentrations and reported in L mg $C^{-1}$ $m^{-1}$ (Weishaar et al. 2003). For determination of optical properties ($Abs_{254}$ and EEMs), each sample was placed in a quartz cuvette. Lights above the instruments were turned off during all optical property measurements. EEMs were measured with a Fluorolog® spectrofluorometer (HORIBA Jobin Yvon, Inc.) and collected over an excitation range of 250 - 400 nm with 10 nm increments and an emission range of 350 - 550 nm with 2 nm increments (Cory and McKnight 2005). EEMs were measured with a band slit of 3 nm. An EEM of ultrapure water of Milli-$Q^{TM}$ water (Millipore Corporation) was measured for each analysis day and subtracted from EEMs of samples for calibration and to remove Raman scattering effects (Cory and McKnight 2005; Stedmon and Bro 2008). The MilliQ EEM was also used to calculate the Raman curve to normalize sample EEMs and enable the direct comparison of samples from different dates (Lawaetz and Stedmon 2009). EEMs were corrected for instrument specific errors using the correction file provided by HORIBA Jovin Yvon, Inc. for the instrument. If the $Abs_{254}$ of samples was higher than 0.2 $cm^{-1}$, samples were diluted to avoid inner-filter effects before the fluorescence measurements (Miller et al. 2010).

The effect of $NO_3^-$ and ferric iron (Fe (III)) concentrations on $Abs_{254}$ and $SUVA_{254}$ (Weishaar et al. 2003) was considered negligible for this study as explained by (Lee et al. in review). Out of 19 locations where the water sample was available on July 16th, 2015, the total iron concentration ranged from 0.02 – 2.7 mg $L^{-1}$; all but three locations had concentrations less than 0.5 mg $L^{-1}$. The highest Fe (III) concentration was found at site K.

Maximum holding times in a refrigerator between the date of sampling and the date of analysis were less than 48 hours for inorganic nutrients, 14 days for DOC, and 28 days for TDN, following EPA protocol (Morrison Erway et al. 2004). Optical properties (absorbance and EEMs) were measured within five days of sample collection, except for samples collected from June 2014, October 2014, and August 2015, which were stored in a refrigerator and measured within two months. $Abs_{254}$ and EEMs were measured on the same day except that EEMs for June 2015 samples were measured the day after $Abs_{254}$ measurement. Holding times were expected to have minimal effects on optical properties: *Ebert* (2013) demonstrated stable FI values through five months of refrigerator storage. This was confirmed when a sample from December 2014 was stored in a refrigerator and analyzed for absorbance and corrected EEMs every 7 to 15 days over 69 days. $Abs_{254}$ varied by less than 1 %, FI varied by less than 4 %, and corrected EEMs were mostly within 10% except for higher percent difference (20 %) around the 2nd Rayleigh scattering signals and a lower emission region (about less than 362 nm) compared to the original values.

The average limit of detection was as follows (Table 9): DOC= 0.05 mg $L^{-1}$ (CCAL 2014), $PO_4^{3-}$ - P = 0.03 mg $L^{-1}$, $NH_4^+$ - N = 0.08 mg $L^{-1}$, $NO_3^-$ - N = 0.02 mg $L^{-1}$, and TDN = 0.04 mg $L^{-1}$. Detection limit values were divided by two and used for samples with concentrations under the detection limit before analysis.

### 4.3.3 EEM Analysis

The original fluorescent index (FI) value (McKnight et al. 2001) has been modified for EEMs with instrument specific corrections by Cory et al. (2010), and the updated FI (EM 470 nm / EM 520 nm at EX 370 nm) was used. The updated FI values of 1.46 are reported to indicate *microbially* derived autochtonous sources, and 1.21 to indicate *terrestrially* derived allochthonous sources (McKnight et al. 2001; Cory et al. 2010b). We expand this interpretation to include terrestrially-derived DOM that has been substantially altered by microbial processing and soil sorption as part of the "microbial" pool. A FI value difference of 0.1 can be used to indicate a different DOM source (McKnight et al. 2001).

To characterize and quantify changes in DOM from EEMs measured by a spectrofluorometer, PARAFAC analysis was conducted using the DOMFluor Toolbox (ver. 1.7) (Stedmon and Bro 2008) and the N-way Toolbox (ver. 3.31) (Andersson and Bro 2000) with MATLAB® (ver. R2013b). The model was developed from a total of 546 EEMs: 496 EEMs collected monthly for this study and an additional 51 EEMs (Sept. 2013, Jan. 2014, and Apr. 2014) collected upstream of the study site within the Willamette River Basin to widely represent DOM variability in this basin. Three individual components were identified using this model validated with split half analysis (Stedmon and Bro 2008), and the loadings and the EEMs of these components were visually compared to the loading and the EEM regions of the components identified by previous studies (Appendix 3). Additional inferences about the presence of tryptophan-like protein DOM were made from analysis of the residuals of the PARAFAC analysis over the region of excitation of 280 nm and emission of 345 nm identified as a tryptophan-like DOM by a previous study (Stedmon and Markager 2005).

### 4.3.4 Spatial Analysis

A 300-m buffer was created around each sampling point and the area of each land use type in the upstream portion of this buffer was calculated using NLCD 2011 (Jin et al. 2013). Within these 21 upstream 300-m radius buffer areas, the average land cover and ranges were as follows (Figure 17): urban land 36 % (1 and 100 %); pasture/hay 31 % (0 and 81 %); wetland 14 % (0 and 62 %); forest 9 % (0 and 87 %); and cultivated crops 7 % (0 and 41 %). The 21 sampling sites were grouped into categories of the two dominant land cover types: urban and pasture/hay. Six categories of urban land use were defined: less than 10 % (4 sites), 11 – 15 % (4 sites), 16 – 20 % (3 sites), 21 – 30 % (3 sites), 49 – 70 % (3 sites), and 90 – 100 % (4 sites). Three categories of pasture/hay land use were defined: less than 5 % (7 sites), 10 – 40 % (6 sites), greater than 45 % (8 sites).

Streams and sub-watersheds were delineated using ArcHydro (v. 2.0) in ArcGIS (v. 10.1) based on flow direction and flow accumulation layers from the National Hydrography Dataset Plus (NHDP, v. 2.10), which was developed by the U.S. Environmental Protection Agency and Geological Survey using National Elevation Dataset (NED) topography (30 m) (McKay et al. 2012). Streams were delineated with the stream definition tool based on a flow accumulation layer with a channel initiation threshold of 120 pixels. Sub-watersheds were delineated with the batch sub-watershed delineation tool. The Strahler's stream order for each sampling site was identified from the NHDP, and sampling sites were grouped into three categories of stream order: 1 (8 sites), 2 (7 sites), and 4 – 6 (6 sites). Although upstream water quality and DOM chemistry could potentially affect these parameters downstream, autocorrelation was assumed to be negligible as sampling sites were separated by at least 1.5 km distance on the river network and the 300-m buffers did not overlap. Site I (on a small tributary stream) is about 180 m from site J (the Marys River mainstream) but they are 500 m apart on the river network.

### 4.3.5 Statistical Analysis

To examine the bivariate relationship among all the parameters, Pearson's r was calculated with SPSS (v. 22) for samples collected from June 2013 to July 2015.

## 4.4 Results

### 4.4.1 Nutrients

The concentrations of N species and P varied spatially and temporally over the study period. The highest nutrient concentrations occurred at sites with high percentage of pasture/hay land use and at headwater streams during high flow (fall and winter) (Figure 19). The lowest values of N species and P occurred during low flows (summer) and from sites where pasture/hay land use was not dominant.

### 4.4.2 DOC

Similarly to inorganic nutrients and TDN, DOC concentration showed temporal variations, however, there was no spatial trend with either urban land or pasture/hay land (Figure 20a). DOC increased sharply during the February 2014 sampling at all locations. DOC concentrations were overall higher at small streams with low stream orders of one and two than at large rivers with high stream orders of four to six (Figure 20b).

### 4.4.3 DOM optical properties

FI and SUVA$_{254}$ did not vary among land use types (Figure 21 - 21), however, they were characterized by temporal flow variations. FI stayed near an average of 1.5 during low flow seasons but decreased to 1.4 during high flow seasons (Figure 21). FI was overall higher at the first order streams than at large rivers with high stream orders (4 – 6) (Figure 21c). SUVA$_{254}$ varied over time but stayed within 3 and 6 L mg$^{-1}$ m$^{-1}$ on average, however, there was a significant increase in SUVA$_{254}$ during fall and winter of the 2015 water year and a decrease during the driest time of each water year (Figure 22). Overall, FI showed a negative and moderately linear relationship ($r = - 0.36$, $\alpha = 0.01$) with SUVA$_{254}$ (

Table 10).

Three distinct components were identified by the PARAFAC model (Figure 23). Overall, DOM component 1 (C1) was visually comparable to an EEM region previously characterized as terrestrial, humic-like DOM from forest, wetland, and agricultural lands (Table 11) (Coble 1996; Stedmon et al. 2003; Stedmon and Markager 2005), representing about 45 % of the total fluorescent DOM. Component 2 (C2) was visually comparable to an EEM region previously associated with terrestrial, humic-like DOM dominant in forested streams and wetlands (Coble 1996; Stedmon et al. 2003; Stedmon and Markager 2005), represented about 40 % of the total fluorescent DOM. DOM component 3 (C3) was visually associated with an EEM region previously identified as terrestrial humic-like component influenced by agricultural lands and was about 14 % of the total fluorescent DOM (Stedmon and Markager 2005).

The percentage of DOM component 1 (C1 %) was similarly among various land use composition and stayed near an average of 46 % throughout the sampling seasons (Figure 24a - b). However, C1 % increased slightly (~ 5 %) during high flow seasons. During the summer of 2014, C1% decreased in sites with a high percentage of urban land use (and thus a low pasture/hay land use). C2 % did not vary among different land use composition throughout the study period and stayed near an average of 41 % (Figure 24c - d). However, C2 % increased during the summer of 2014 at the sites with high urban land percentage (i.e. low pasture/hay land). C3 % showed the least variation among DOM components identified by the PARAFAC model and remained closely to an average of 14 % throughout the sampling period regardless of land use composition (Figure 24e).

Among three DOM components identified by the PARAFAC model, C1 % and C3 % were positively and moderately correlated ($r = 0.45$, $\alpha = 0.01$) to each other and C1 % and C2 % were negatively and strongly correlated ($r < -0.95$, $\alpha = 0.01$) to each other (

Table 10). C2 % and C3 % were also negatively and strongly correlated ($r = -0.70$, $\alpha = 0.01$). C1 % was positively and moderately correlated with $SUVA_{254}$ ($r = 0.35$, $\alpha = 0.01$) and negatively and moderately correlated with FI ($r = -0.49$, $\alpha = 0.01$). C2 % was positively and moderately correlated with FI ($r = 0.37$, $\alpha = 0.01$). C3 % was not correlated with $SUVA_{254}$ or FI.

## 4.5 Discussion

### 4.5.1 Spatial and Temporal patterns of DOC and Nutrients

The concentrations of all nutrient species varied with land use and seasonal flow patterns as expected based on previous studies (Smith et al. 1997; Alexander et al. 2000; Groffman et al. 2004; Alexander et al. 2008; Preston et al. 2011). High concentrations of all nutrient species were associated with higher fertilizer and/or animal waste inputs from pasture/hay lands and headwater streams, especially when hydrologically connected during high flow seasons. DOC concentrations were driven by seasonal flow patterns as expected based on other studies (Vanderbilt et al. 2003; van Verseveld et al. 2009;

Raymond and Saiers 2010) rather than land use. We observed increased DOC concentrations during dry months at headwater streams (stream orders 1 – 2) and higher concentrations of DOC in headwater streams (stream orders 1 – 2) than in higher order streams (stream orders 4 – 6) throughout the study period. These observations are consistent with the river continuum concept (Vannote et al. 1980) in that headwater streams (stream orders 1 – 2) receive continuously high DOC inputs relative to size compared to large downstream rivers (stream orders 4 – 6) and DOC declines in higher order streams (stream orders 4 – 6) due to *in situ* microbial respiration.

### 4.5.2 DOM optical characteristics in the study site

In published studies (Coble 1996; Stedmon et al. 2003; Stedmon and Markager 2005), the EEM region of the first component identified by this study (C1) has been characterized as terrestrial, humic-like DOM and found in forested streams and absent in wastewater. Because C1 % was moderately and positively correlated with $SUVA_{254}$ and negatively correlated with FI, our results are consistent with these previous studies, which suggested that C1 is associated with highly aromatic and terrestrial DOM.

The second component (C2) was similar to the EEM region previously characterized as terrestrial, humic-like DOM found in forested streams (Stedmon and Markager 2005). In addition to the previous characterization, this study found the increase in C2 % at sites with high percentage of urban land use during dry seasons possibly due to the continuous surface hydrologic connectivity through urban irrigation over summers compared to low connectivity from other land use composition. Our study did not find any association between C3 % and any of land use types.

The PARAFAC model developed for this study identified three unique fluorescent components; two of the components, C1 and C2, were dominant. Our PARAFAC model did not identify a protein-like DOM although some residuals appear to represent an EEM signal previously identified as protein-like DOM (Figure 25). This missing protein component was unexpected, as a study conducted in a coniferous watershed located in the upper Willamette River Basin found a protein-like DOM component that was indicative of hydrologic flow paths through deep soil, or from groundwater (Lee and Lajtha, in review). Because the EEM signal previously characterized as protein (Stedmon and Markager 2005) was observed among some residuals, the missing protein-like component could be due to a fundamental limitation of the statistical model that cannot detect minor DOM components in the system. However, FI declined with an increase in stream order indicating more terrestrial DOM sources. Because DOC concentrations also declined with an increase in stream order and the concentrations of all nutrient species were the highest at headwater streams, the lack of a protein-like PARAFAC component suggests that labile DOM (i.e. a microbially processed, protein-like DOM) is preferentially processed and lost through microbial respiration, promoted by nutrient inputs from pasture/hay lands, in the study site.

### 4.5.3 Spatial and temporal patterns of DOM

FI remained close to 1.5 during the study period indicating autochtonous DOM sources and high primary producer activities in the streams according to *McKnight et al.* (2001) and *Cory et al.* (2010). This observation was not attributed to nutrient inputs by high pasture/hay lands that could promote in-stream microbial processes. Whole water samples of neutral pH in this study area (Raymond et al. 2002) with high humic acids may not necessarily result in a FI index on the same scale as a FI index developed with separated fulvic acids (McKnight et al. 2001) that are soluble at any pH. Hence caution is necessary before drawing any conclusions about absolute values of FI from whole water samples.

Nevertheless, FI showed temporal variation driven by seasonal flow as observed by previous studies (Jaffé et al. 2008; Catalán et al. 2012). During the high flow seasons, FI decreased by 0.1 indicating changes in DOM sources (McKnight et al. 2001) with more allochthonous DOM sources than in dry seasons. Thus, FI showed shifts in hydrologic pathways from mainly microbially processed groundwater sources (Lee and Lajtha, in review) to mainly overland flow sources during falls and winters and/or shifts in productivity from higher microbial activity during warm months to lower microbial activity during cold months.

The increase in $SUVA_{254}$ during the fall and winter of the 2015 water year also suggests a shift in hydrologic pathway from groundwater to greater overland flow, as surface water is higher in $SUVA_{254}$ than groundwater due to more influence by terrestrial, aromatic DOM (van Verseveld et al. 2009; Inamdar et al. 2012). The lack of increase in $SUVA_{254}$ during the high flow season of the 2014 water year might be attributable to the freezing effect. The high flow timing was delayed during the 2014 water year because the freezing temperatures froze some streams and accumulated snow; aromatic DOM preferentially precipitates with freezing (Spencer et al. 2007; Fellman et al. 2008; Lee et al. in review).

Land use composition did not influence C1 %, although C1 % increased during high flow seasons. The lowest C1 % was observed during summers among the sites with high urban land use. This could be due to the increase in C2 %, which was the other dominant component identified by this study.

The lack of spatial variation in C1% during high flow seasons was surprising as a DOM signal unique to a land use type should increase during high flow seasons (fall and winter). This observation may be attributable to mixing of DOM sources from mixed land use cover in all sampling sites and increased connectivity among sites during high flow periods. In that, a DOM signal unique to a dominant land use type, that was easily observed at headwater streams (Hosen et al. 2014; Lee et al. in review), may be obscured by the increased hydrologic link to multiple land use types during high flow seasons near sampling sites.

C2 % stayed near the average value, but it increased at sites with high percentage of urban land use (i.e. low pasture/hay land) during summer 2014. The driest seasons seem to be limited by hydrologic connectivity, however, urban irrigation could have resulted in continuous hydrologic connectivity over the period with the minimal mixing effects from other land use types. It is not clear why this effect was not prominent in the summers of 2013 and 2015, however, this could be attributed to the delayed onset of high flow and freezing temperatures during the 2014 water year, which may have altered microbial processes and DOM chemistry.

Although a previous study (Stedmon and Markager 2005) found humic-like C3 was associated with agricultural lands, spatial and temporal variation in C3 % were not observed at the study site. This could be the result of equally dominant components, C1 and C2, obscuring the fluorescent signal, associated with the presence of C3.

In summary, this study, conducted in a mixed landscape of the middle Willamette River Basin, found that seasonal hydrologic patterns controlled UV and fluorescent DOM chemistry more than land use. This is contrary to our hypothesis that differences in land use patterns would produce DOM chemistries that were distinguishable by UV absorbance and fluorescent DOM chemistry. One explanation for our unexpected results is that these land use types, developed on similar geologic substrates, that had at one point all been forested, truly produced similar DOM chemistry. Previous studies, which found land use effects on fluorescent DOM components, have compared contrasting environments such as agricultural lands influence observed in an estuary and tributary catchments in Denmark (Stedmon and Markager 2005), the Florida Everglades wetland (Yamashita et al. 2010), and the Florida Bay (Maie et al. 2012). Additionally, fluorescent DOM components have been proven to be useful to differentiate forest and wetlands (Kothawala et al. 2015). Thus, our study conducted in a relatively small area that used to be forest might not have varying DOM characteristics.

Another possible explanation is that DOM chemistry from unique land use disturbances become obscured as the fluorescent DOM signals are truly blended in areas of varying land use types. Unlike other studies examining endmember representing a unique land use type in catchments (Yamashita et al. 2011; Cawley et al. 2014; Hosen et al. 2014; Lee and Lajtha, in review), the observations of this study were conducted in truly mixed landscapes.

Third possible explanation for our results is that while different land uses might produce DOM chemistry that is initially distinguishable with these techniques, microbial activity in soils and streams serves to homogenize any chemical fingerprints of the source material through respiration of the most labile components. This hypothesis that we term the "microbial blender hypothesis" is consistent with results from several previous studies. For example, Fellman et al. (2009) found that a protein-like DOM signal became weaker with an increase in stream order as the stream went downstream further away from the original DOM source (soil). Wickland et al. (2007) observed that while DOM leached from plants differed among varying vegetation types, DOM sampled from soils was more microbially processed and labile. Similarly, Huang et al. (2015) found that soil DOC varied with vegetation type but differences were lost in receiving streams. Finally, Yano et al. (2004) and Strid et al. (in review) found that while different detrital manipulation treatments produced DOC with distinct chemical fingerprints at the soil surface layers, these distinct signals were lost as DOM passed through the soil profile. Thus, as DOM moves from vegetation, to surface soil, to deep soil, and to streams, fluorescent DOM becomes more microbially processed. Hence, DOM in-stream from various land use types become homogenized by microbial processes with increased stream orders and cannot be differentiated with fluorescent spectroscopy. Further research in mixed landscape coupled with detail analyses of soil, soil pore from various the soil profile, and stream water would help to evaluate these hypotheses.

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## 4.8 Tables

Table 9: The summary of sampling period, sample numbers, and detection limit

| Parameters | Sampling period | Sample numbers | Detection limit (average) (mg $L^{-1}$) | Frozen samples |
|---|---|---|---|---|
| TDN | Oct. 2012 – Mar. 2015 | 523 | 0.04 | Oct. 2012 – Apr. 2013 |
| DOC | Oct. 2012 – Jul. 2015 | 573 | 0.05 | Oct. 2012 – Apr. 2013 |
| $PO_4^{3-}$ - P | Jun. 2013 – Mar. 2015 | 399 | 0.03 | May. 2014 – Jul. 2014 |
| $NH_4^+$ - N | Jun. 2013 – Mar. 2015 | 399 | 0.08 | May. 2014 – Jul. 2014 |
| $NO_3^-$ - N | Jun. 2013 – Mar. 2015 | 399 | 0.02 | May. 2014 – Jul. 2014 |
| DOM (EEMs and $Abs_{254}$) | Jun. 2013 – Jul. 2015 | 450 | N/A | None |

Table 10: Bivariate correlation
N = 450 for DOM and N = 399 for nutrients.
Two-tailed significance test, **: α = 0.01, *: α = 0.05

| | $PO_4^{3-}$ | $NH_4^+$ | NN | TDN | DOC | $SUVA_{254}$ | FI | C1% | C2% | C3% |
|---|---|---|---|---|---|---|---|---|---|---|
| $PO_4^{3-}$ | 1 | 0.32** | 0.34** | 0.40** | 0.39** | 0.19** | 0.19** | -0.17** | 0.27** | -0.38** |
| $NH_4^+$ | | 1 | 0.32** | 0.47** | 0.25** | 0.01 | -0.04 | 0.02 | 0.00 | -0.06 |
| NN | | | 1 | 0.96** | 0.09 | -0.05 | 0.27** | -0.26** | 0.20** | 0.03 |
| TDN | | | | 1 | 0.19** | -0.03 | 0.28** | -0.28** | 0.24** | -0.02 |
| DOC | | | | | 1 | -0.03 | 0.03 | -0.23** | 0.41** | -0.66** |
| $SUVA_{254}$ | | | | | | 1 | -0.36** | 0.35** | -0.25** | -0.10* |
| FI | | | | | | | 1 | -0.49** | 0.37** | 0.04 |
| C1% | | | | | | | | 1 | -0.95** | 0.45** |
| C2% | | | | | | | | | 1 | -0.70** |
| C3% | | | | | | | | | | 1 |

Table 11. Three unique components (C) identified in the central Willamette River Basin The excitation (Ex) and emission (Em) maxima in nm of each component are shown along with their characteristics identified by previous studies.

| C | Ex max (nm) | Em max (nm) | Characteristics |
|---|---|---|---|
| 1 | 250 | 486 | Humic-like component (A) (Coble 1996) of terrestrial origins from forested stream and wetland in Denmark (C1) (Stedmon et al. 2003). Additionally, associated with agricultural influence and found absent in wastewater in Denmark (C1) (Stedmon and Markager 2005) (Refer to Figures 2 and 3; Appendix 3). |
| 2 | 250 | 400 | Humic-like component (A) (Coble 1996) with blue-shifted emission (C2) (Stedmon et al. 2003). Additionally identified as terrestrial humic-like component from forested streams and wetlands in Denmark (C2) (Stedmon et al. 2003) and found absent in wastewater (C3) (Stedmon and Markager 2005) (Refer to Figures 2 and 3; Appendix 3) |
| 3 | 330 | 432 | Terrestrial humic-like component with agricultural influence in Denmark (C5) (Stedmon and Markager 2005) (Refer to Figures 2 and 3;). |

## 4.9 Figures

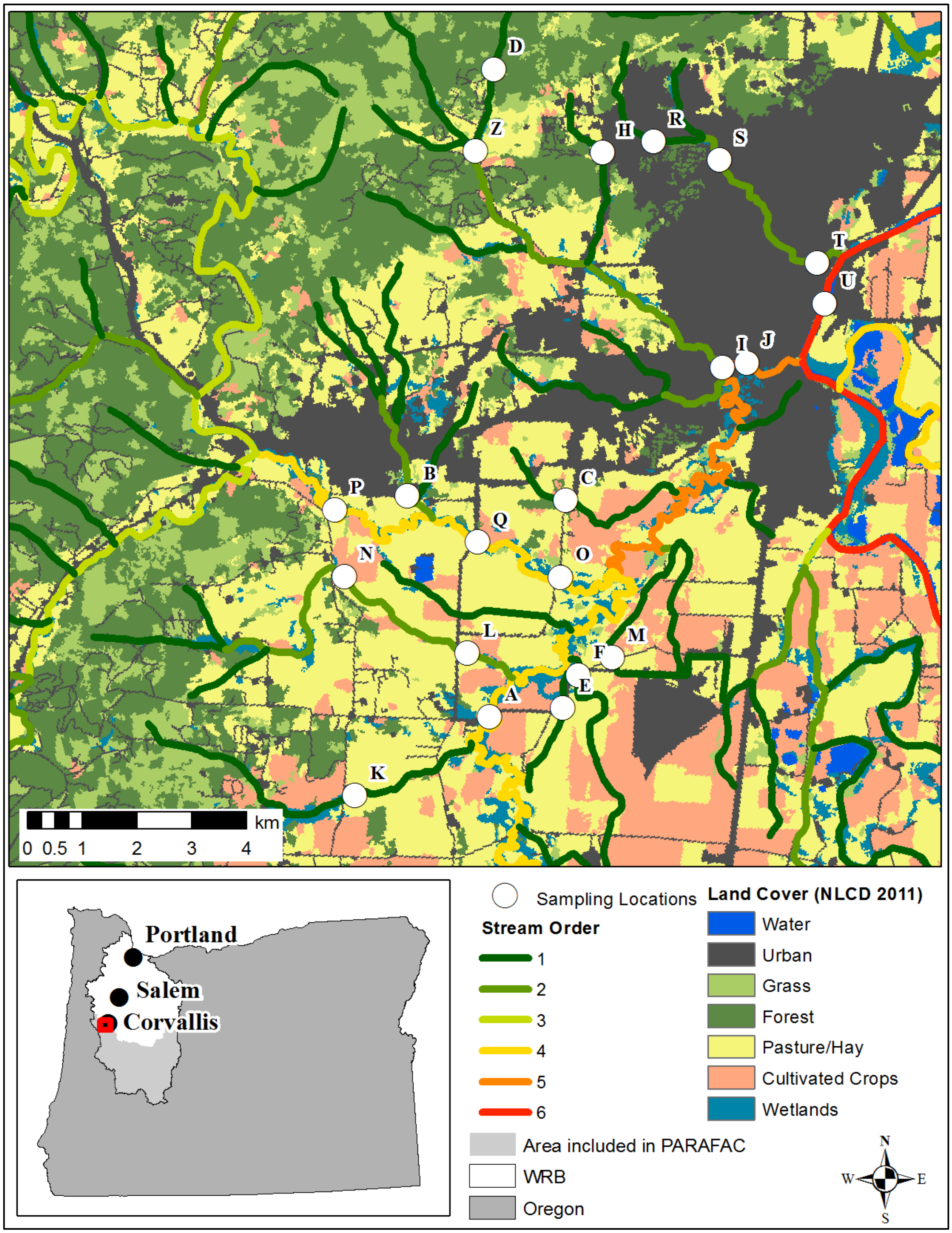


Figure 16: Map of the monthly sampling locations in the middle Willamette River Basin

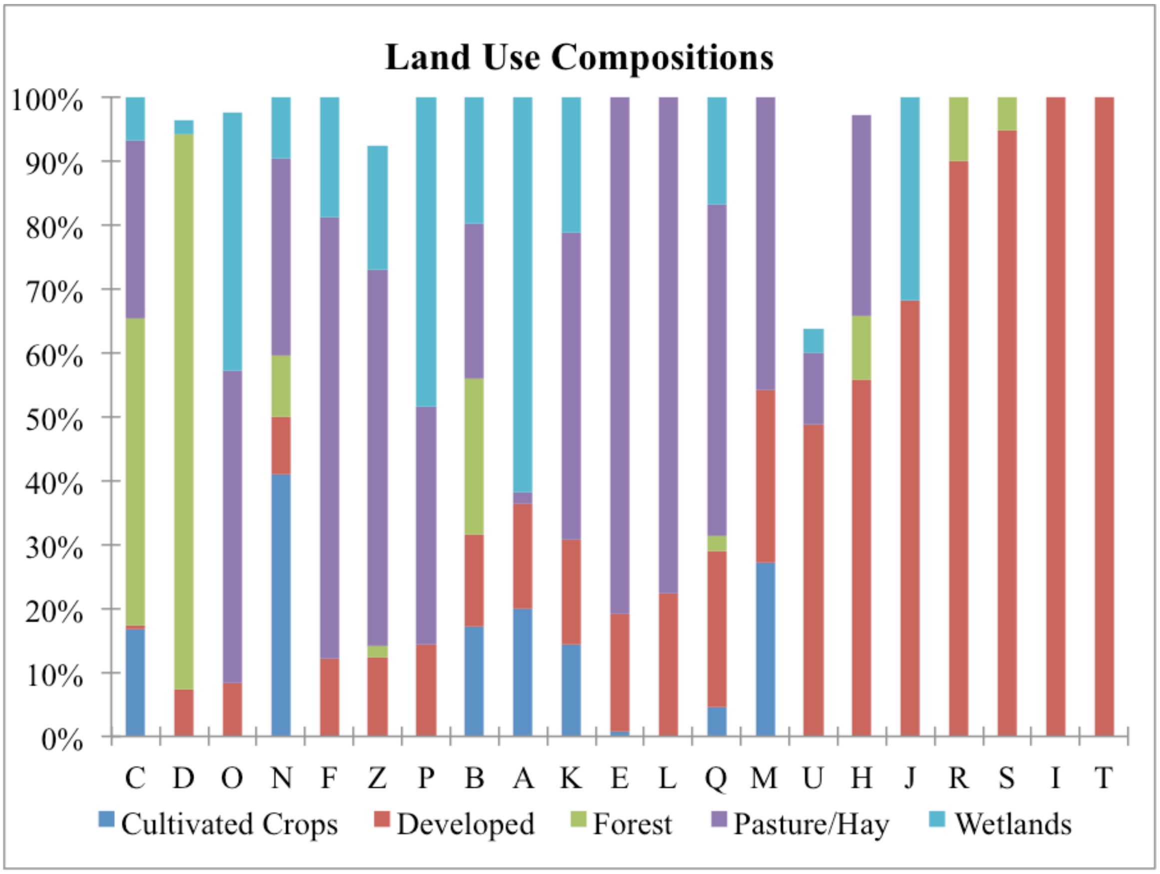


Figure 17: Land use compositions of a 300-m buffer at each site
Sites D, O, Z, and H do not add up to 100 % because of the presence of herbaceous and shrub land use. Site U does not add up to 100 % because of water body.

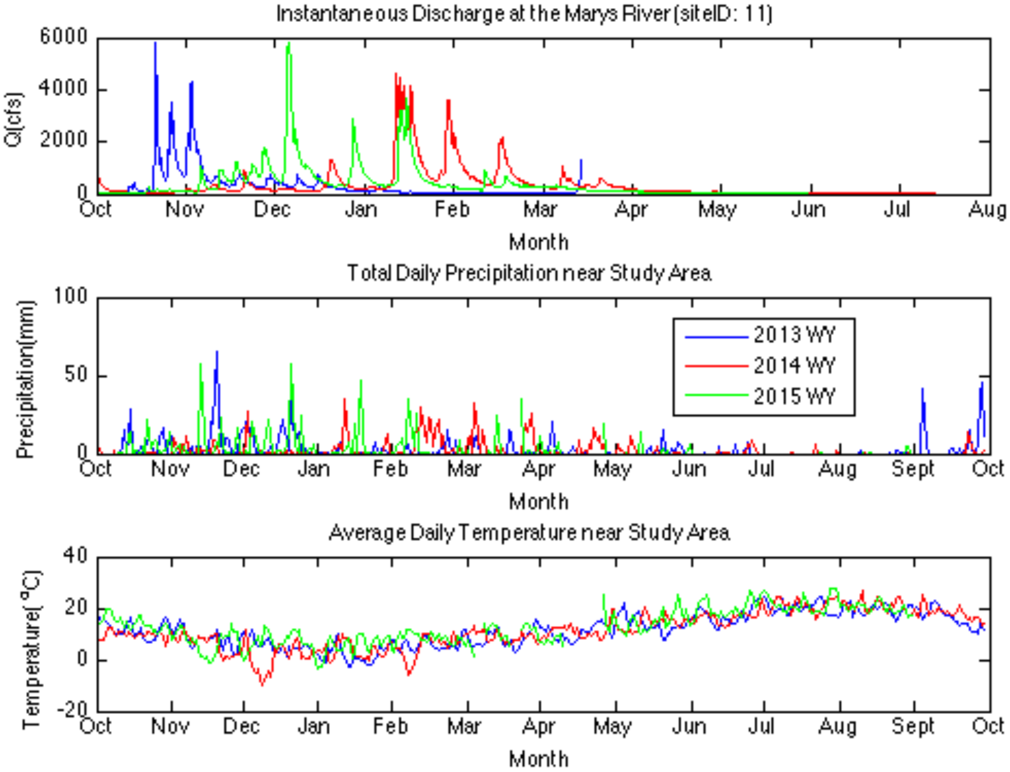
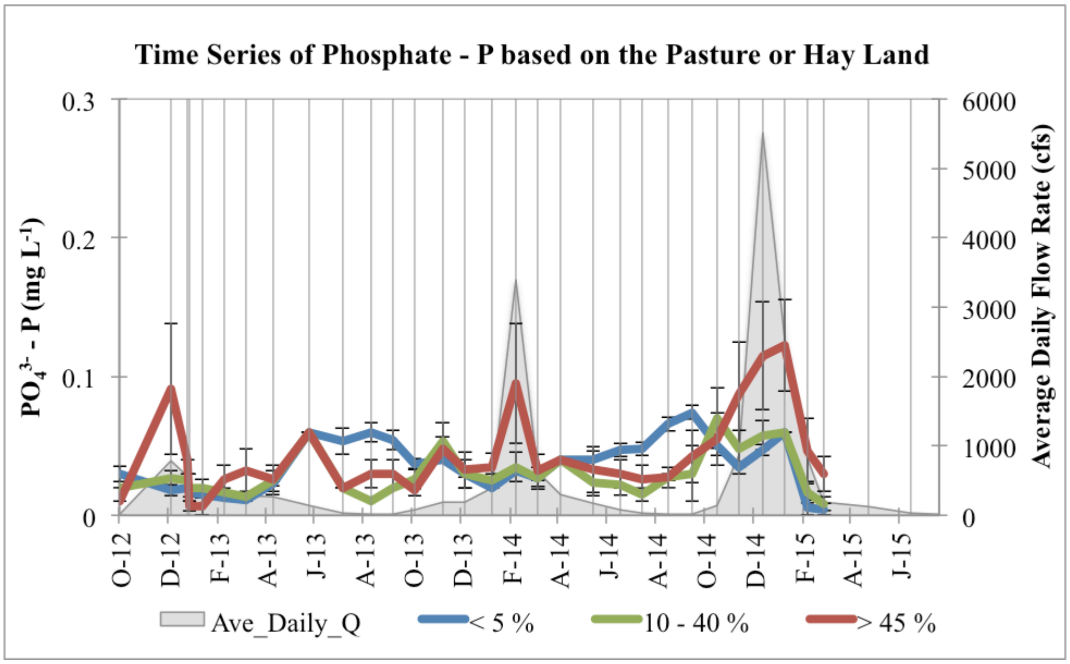
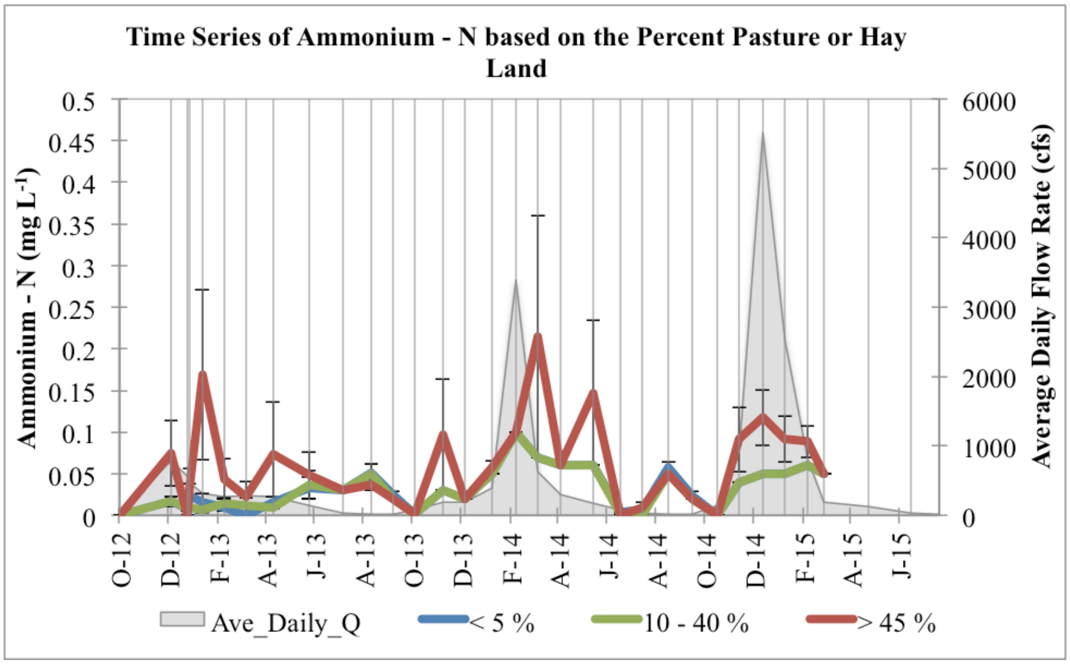


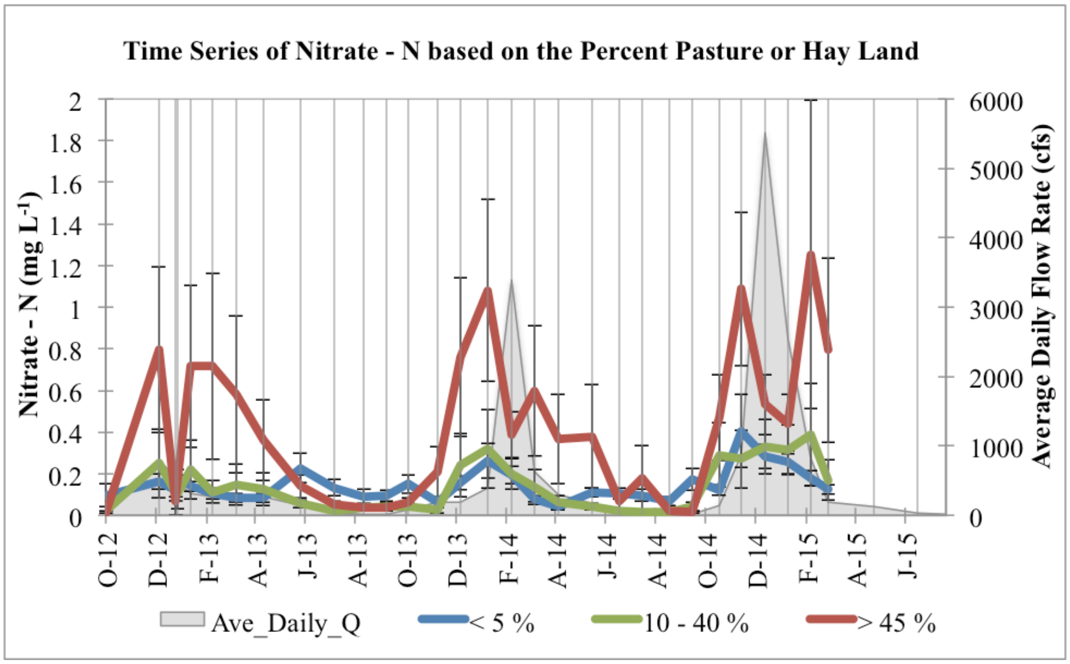
Figure 18: Average daily flow rate (cfs), total precipitation (mm), and daily temperature ($^{o}$C) for each water year (WY)
Average daily flow rate was measured at site Q in the Marys River (USGS Surface-water daily data for the nation, 2015 (ID: 14171000)). Total precipitation (mm) and average daily temperature ($^{o}$C) were measured at the Finley Wildlife Refuge located south of the study site


Time Series of Phosphate - P based on the Pasture or Hay Land
$PO_4^{3-}$ - P (mg L$^{-1}$)
0.3
0.2
0.1
0
Average Daily Flow Rate (cfs)
6000
5000
4000
3000
2000
1000
0
O-12
D-12
F-13
A-13
J-13
A-13
O-13
D-13
F-14
A-14
J-14
A-14
O-14
D-14
F-15
A-15
J-15
Ave_Daily_Q
< 5 %
10 - 40 %
> 45 %

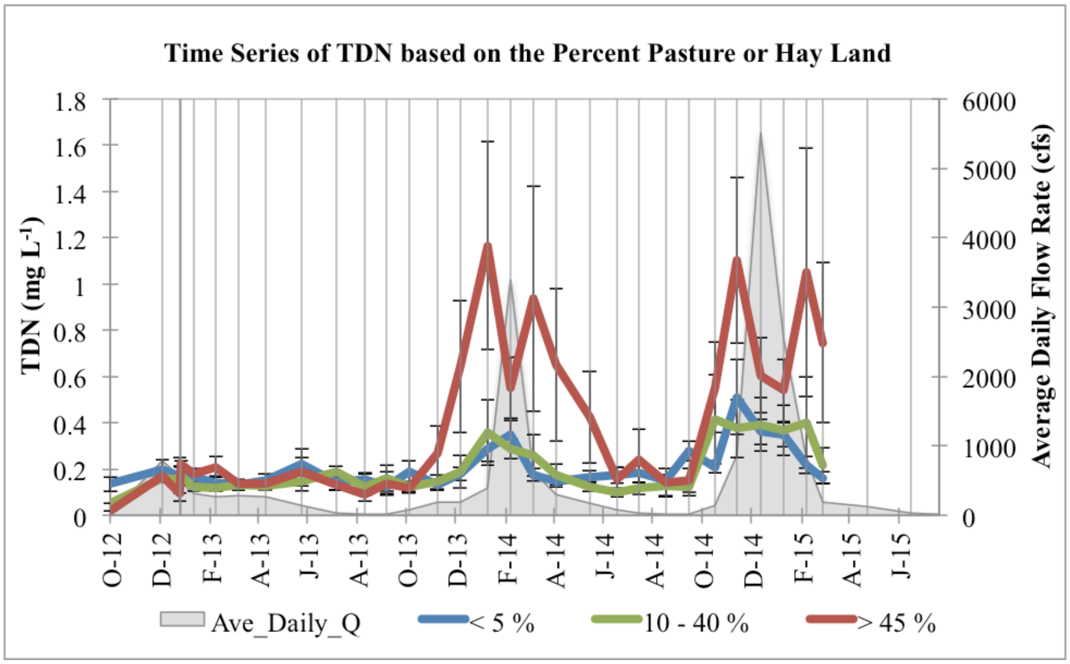
a


Time Series of Ammonium - N based on the Percent Pasture or Hay Land
Ammonium - N (mg L$^{-1}$)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Average Daily Flow Rate (cfs)
6000
5000
4000
3000
2000
1000
0
O-12
D-12
F-13
A-13
J-13
A-13
O-13
D-13
F-14
A-14
J-14
A-14
O-14
D-14
F-15
A-15
J-15
Ave_Daily_Q
< 5 %
10 - 40 %
> 45 %

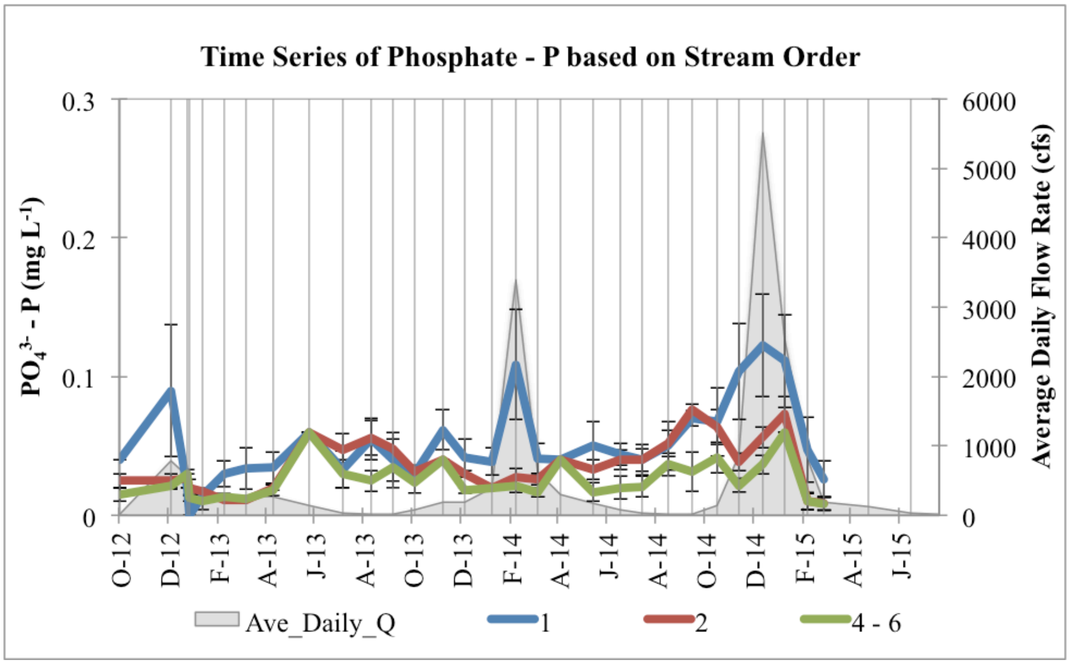
b


Time Series of Nitrate - N based on the Percent Pasture or Hay Land
Nitrate - N (mg L-1)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Average Daily Flow Rate (cfs)
6000
5000
4000
3000
2000
1000
0
O-12
D-12
F-13
A-13
J-13
A-13
O-13
D-13
F-14
A-14
J-14
A-14
O-14
D-14
F-15
A-15
J-15
Ave_Daily_Q
< 5 %
10 - 40 %
> 45 %

c


Time Series of TDN based on the Percent Pasture or Hay Land
TDN (mg L-1)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Average Daily Flow Rate (cfs)
6000
5000
4000
3000
2000
1000
0
O-12
D-12
F-13
A-13
J-13
A-13
O-13
D-13
F-14
A-14
J-14
A-14
O-14
D-14
F-15
A-15
J-15
Ave_Daily_Q
< 5 %
10 - 40 %
> 45 %

d


Time Series of Phosphate - P based on Stream Order
PO$_4^{3-}$ - P (mg L$^{-1}$)
0.3
0.2
0.1
0
Average Daily Flow Rate (cfs)
6000
5000
4000
3000
2000
1000
0
O-12
D-12
F-13
A-13
J-13
A-13
O-13
D-13
F-14
A-14
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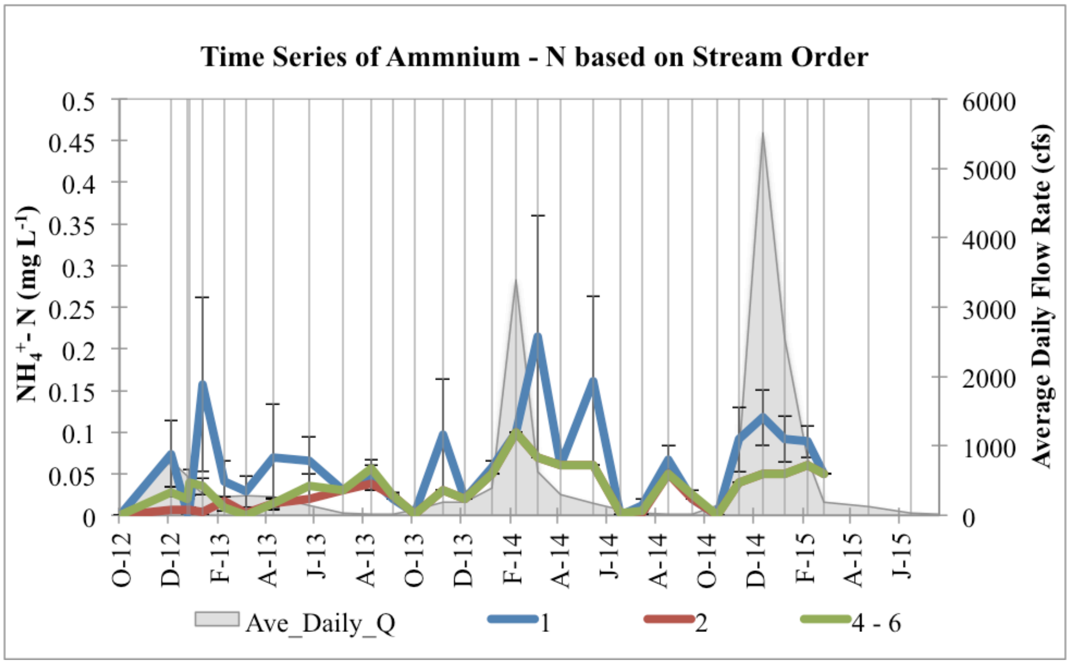
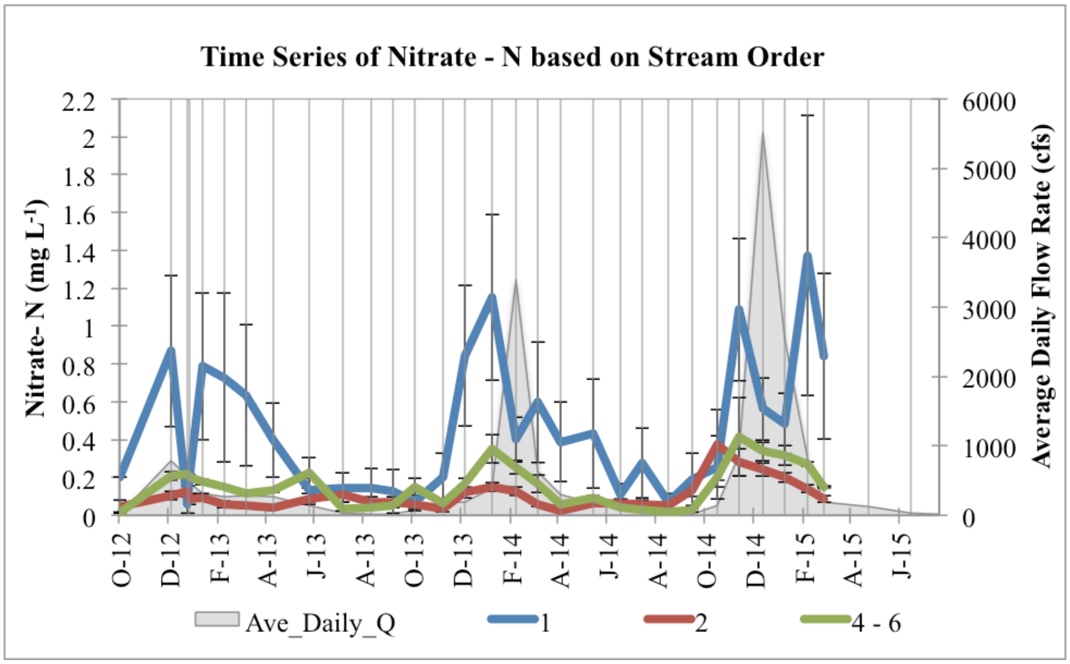
A-15
J-15
Ave_Daily_Q
1
2
4 - 6

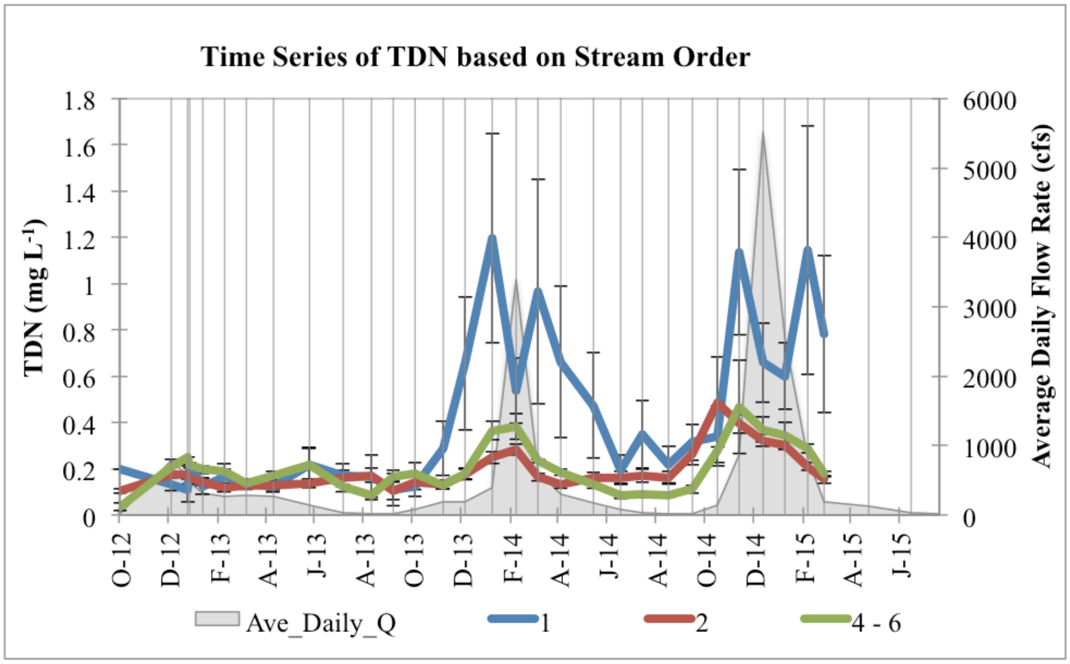
e


Time Series of Ammnium - N based on Stream Order
NH$_4^+$- N (mg L$^{-1}$)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Average Daily Flow Rate (cfs)
6000
5000
4000
3000
2000
1000
0
O-12
D-12
F-13
A-13
J-13
A-13
O-13
D-13
F-14
A-14
J-14
A-14
O-14
D-14
F-15
A-15
J-15
Ave_Daily_Q
1
2
4 - 6

f



g



h

Figure 19: Time series of inorganic nutrients and TDN based on percent pasture/hay land (a – d) and stream order (e – h)

Vertical lines indicate sampling dates. Average daily flow rate was measured at site Q in the Marys River (USGS Surface-water daily data for the nation, 2015 (ID: 14171000)). Error bars show standard error.

Figure 19a: Time series of $PO_4^{3-}$ - P based on percent pasture/hay land

Figure 19b: Time series of $NH_4^+$ - N based on percent pasture/hay land

Figure 19c: Time series of $NO_3^-$ - N based on percent pasture/hay land

Figure 19d: Time series of TDN based on percent pasture/hay land

Figure 19e: Time series of $PO_4^{3-}$ - P based on stream order
Figure 19f: Time series of $NH_4^+$ - N based on stream order
Figure 19g: Time series of $NO_3^-$ - N based on stream order
Figure 19h: Time series of TDN based on stream order

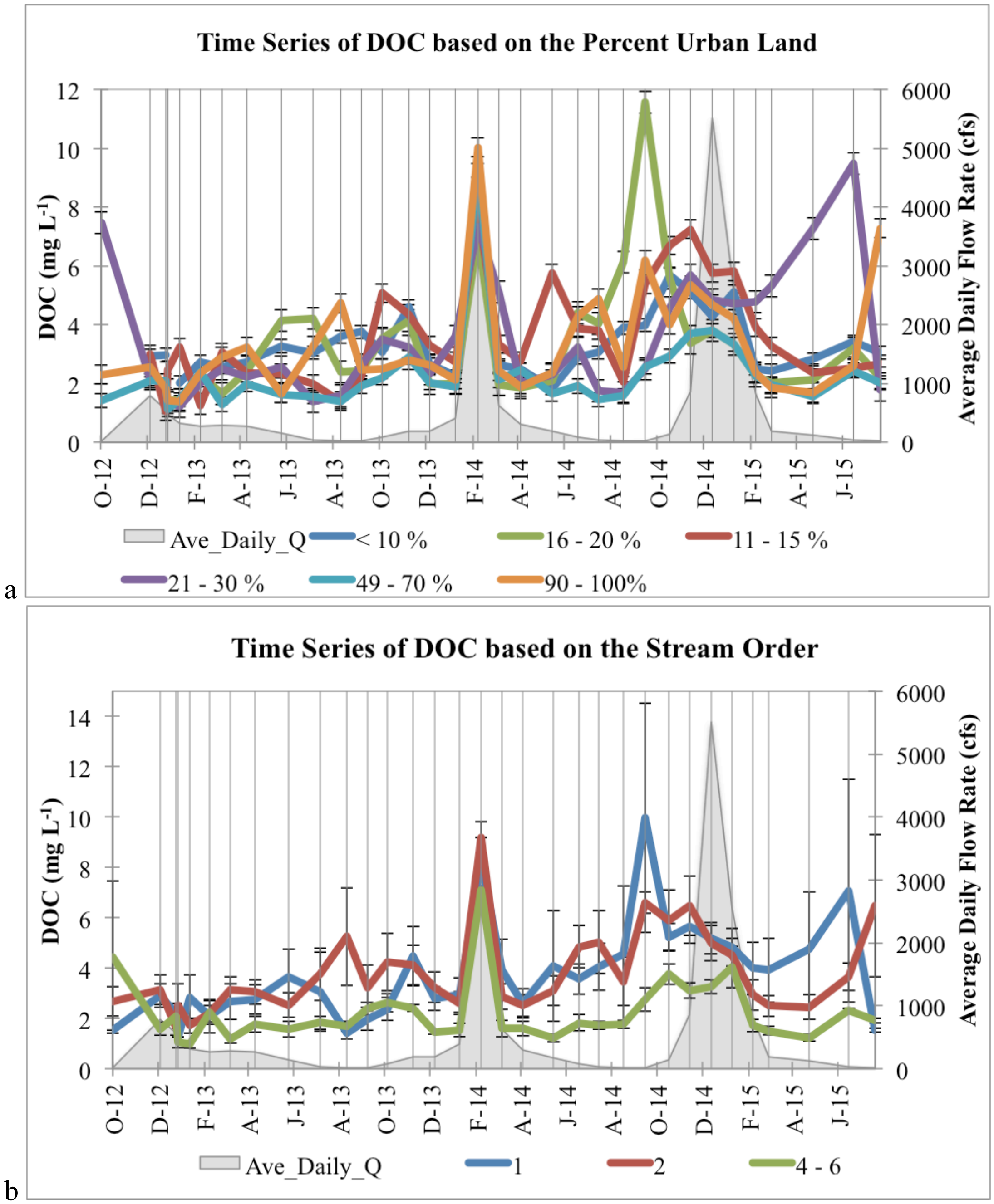
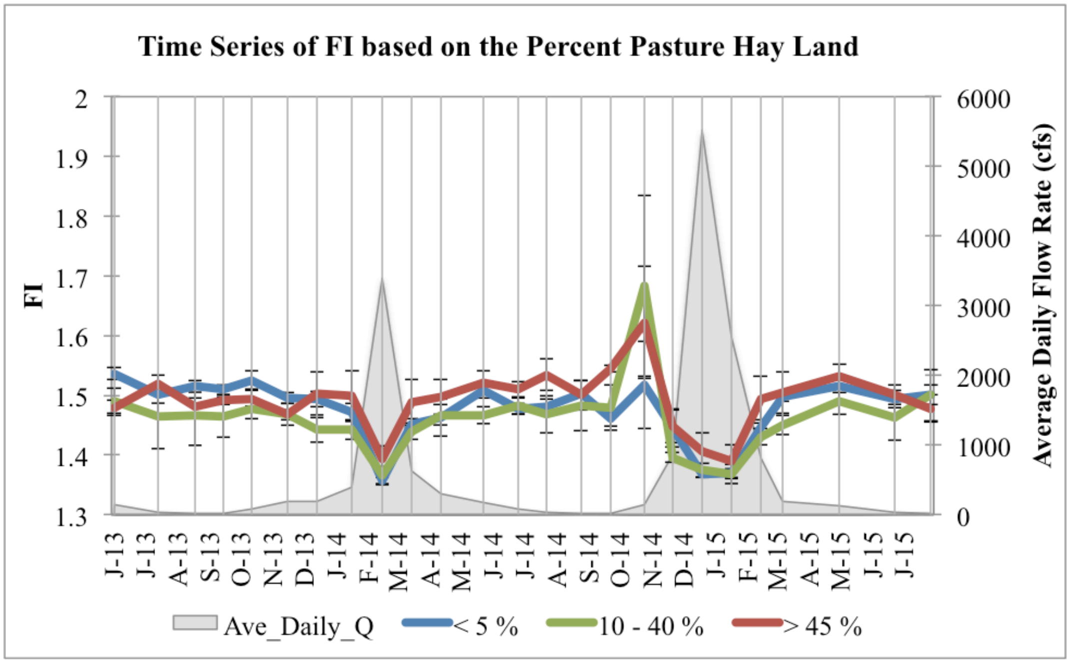
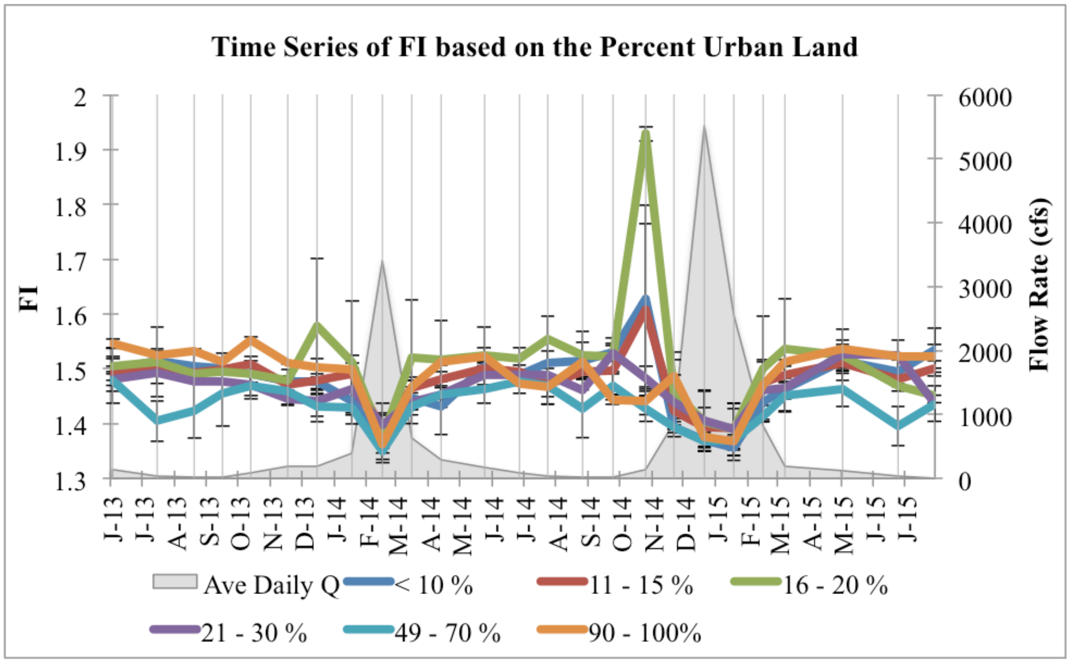


Figure 20: Time series of DOC concentrations based on percent urban land (Figure 20a) and stream order (Figure 20b)

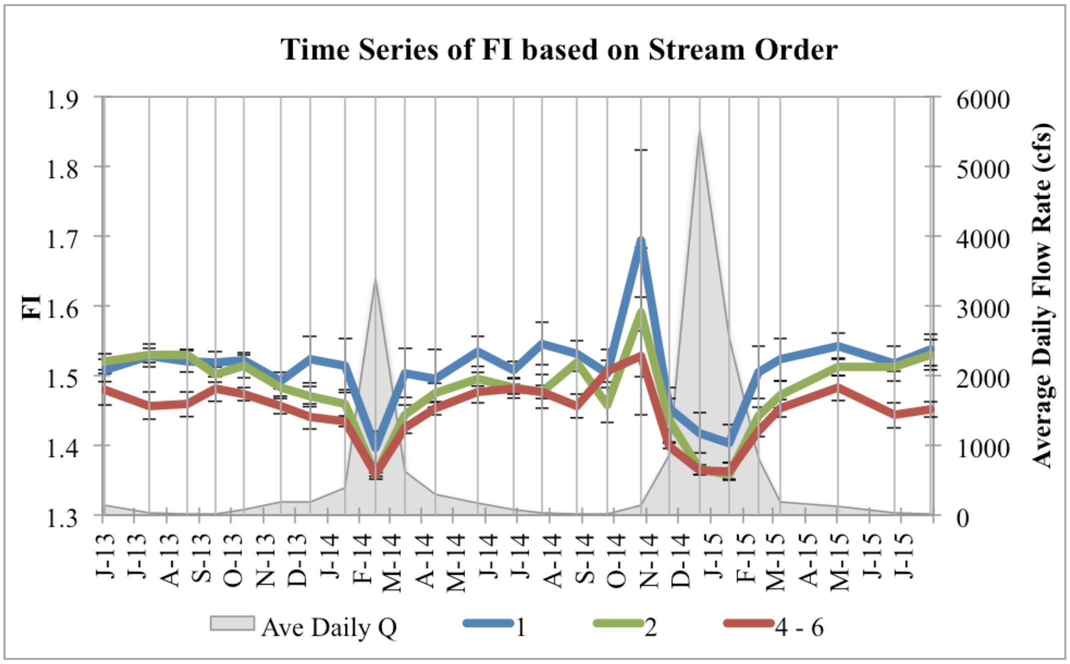
Vertical lines indicate sampling dates. Average daily flow rate was measured at site Q in the Marys River (USGS Surface-water daily data for the nation, 2015 (ID: 14171000)). Error bars show standard error.



a



b



c

Figure 21: Time series of FI based on percent pasture/hay land (Figure 21a), percent urban land (Figure 21b), and stream order (Figure 21c)
Vertical lines indicate sampling dates. Average daily flow rate was measured at site Q in the Marys River (USGS Surface-water daily data for the nation, 2015 (ID: 14171000)). Error bars show standard error.

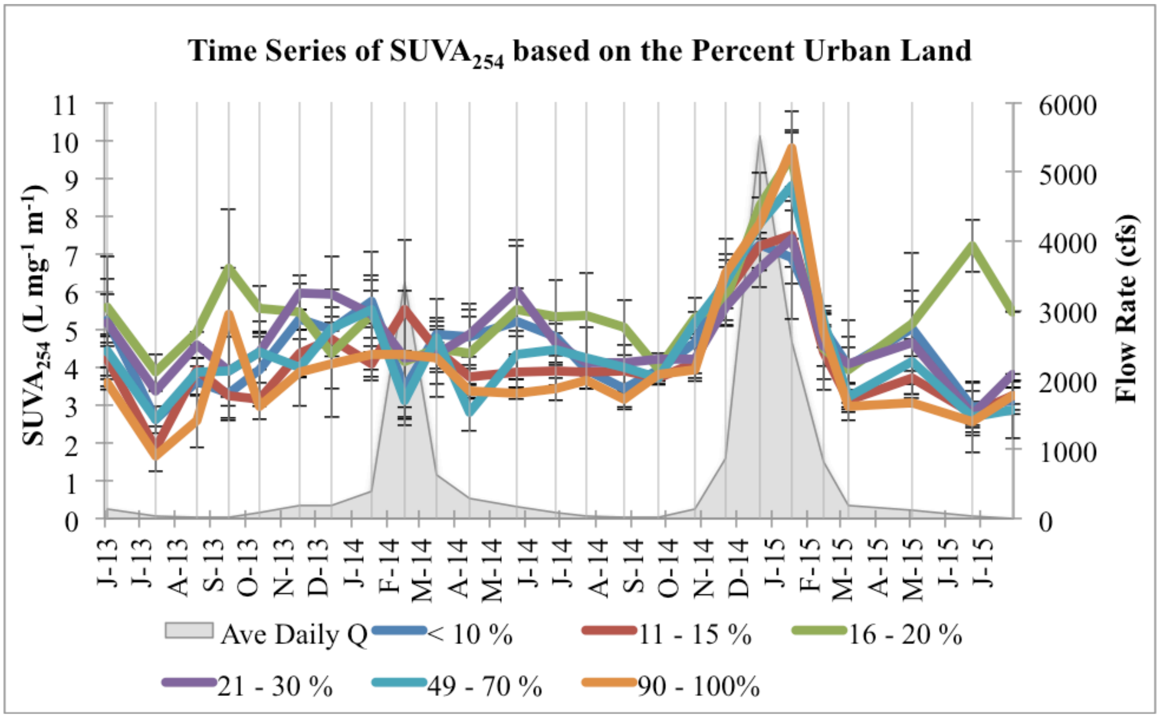
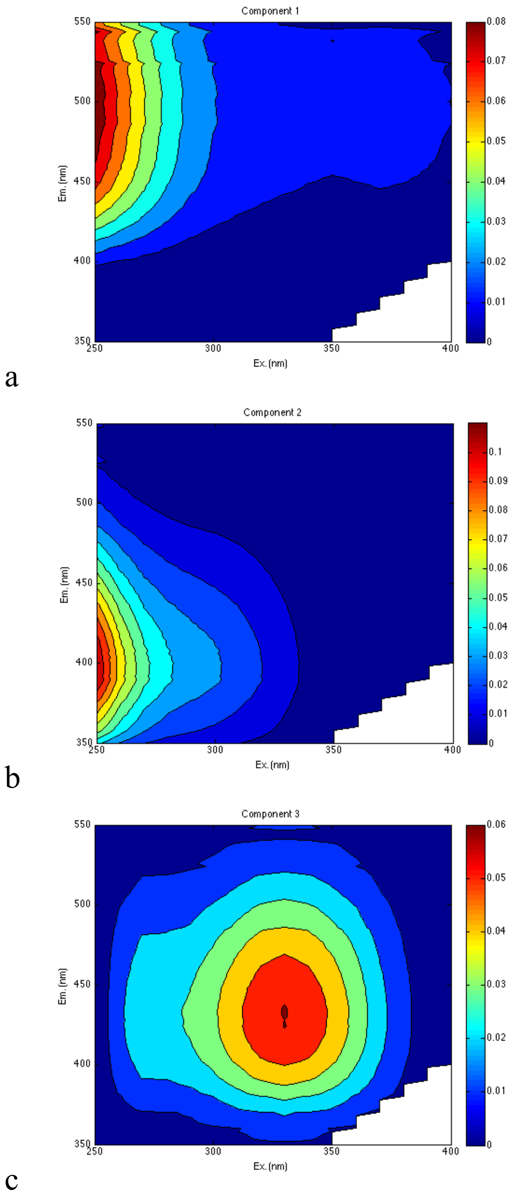
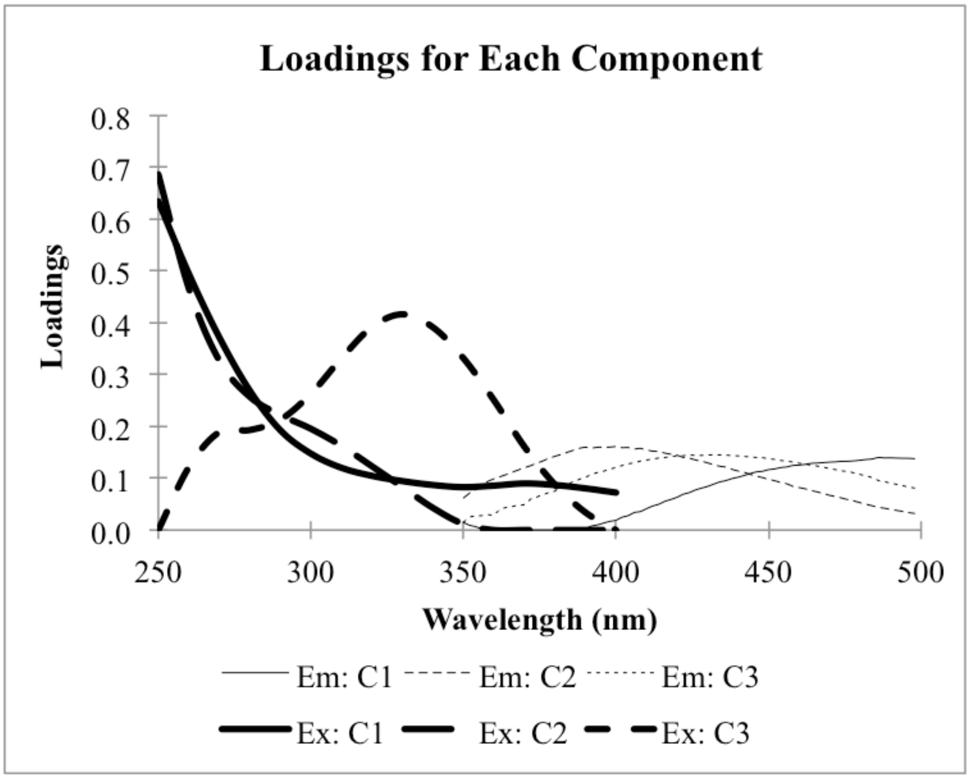


Figure 22: Time series of SUVA$_{254}$ by percent urban land

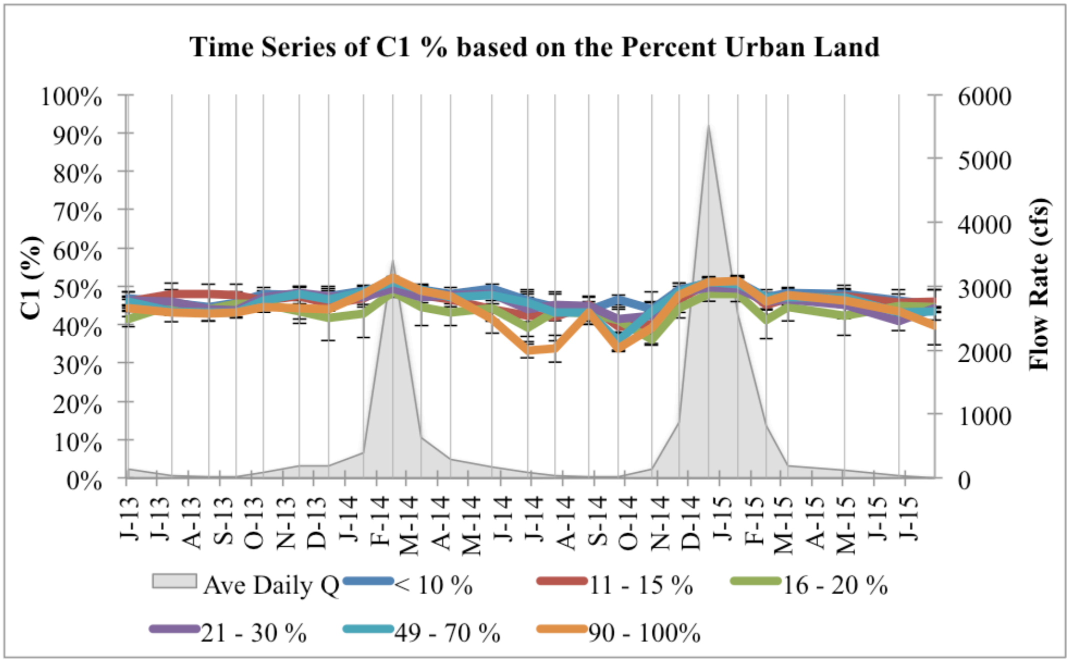
Vertical lines indicate sampling dates. Average daily flow rate was measured at site Q in the Marys River (USGS Surface-water daily data for the nation, 2015 (ID: 14171000)). Error bars show standard error.



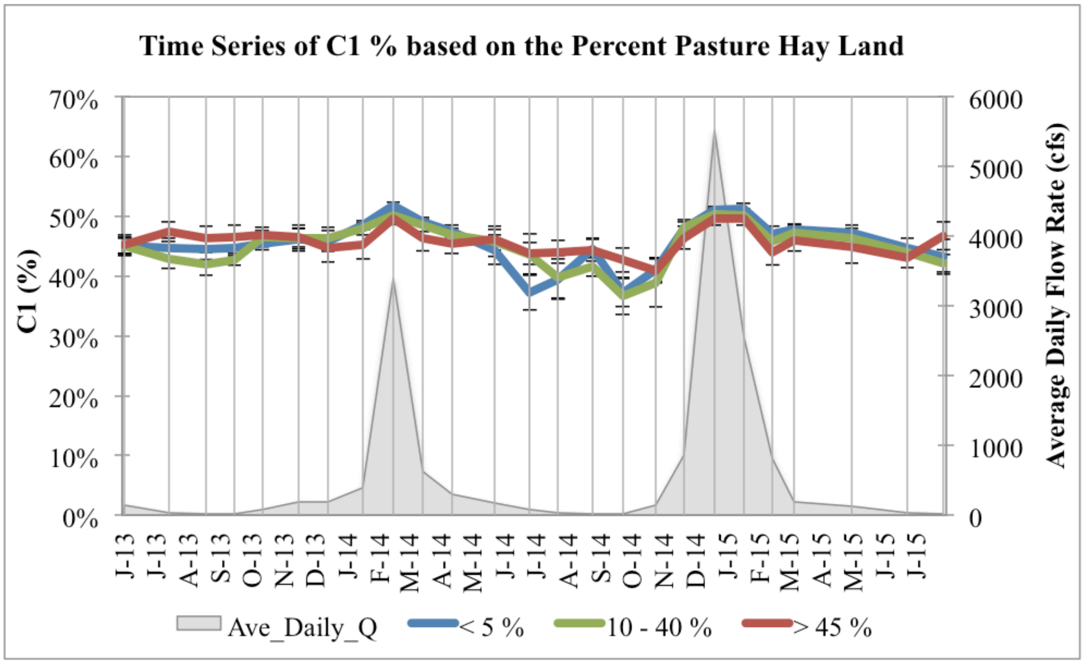


d

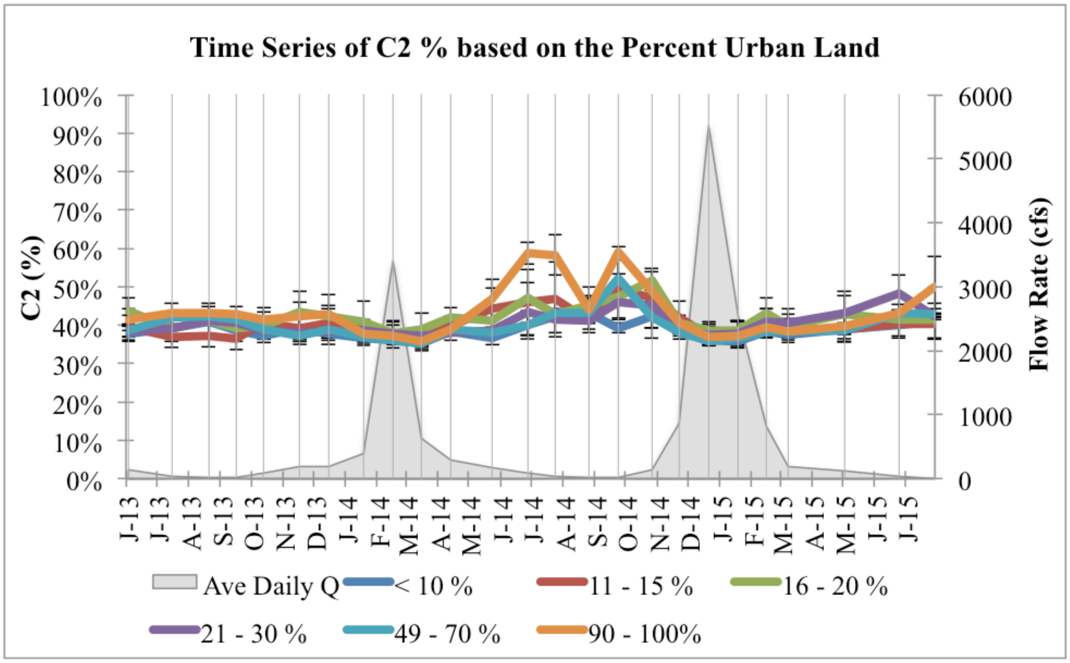
Figure 23: EEMs of three components identified by the PARAFAC model (Figure 23a – c) and their excitation emission loadings (Figure 23d)



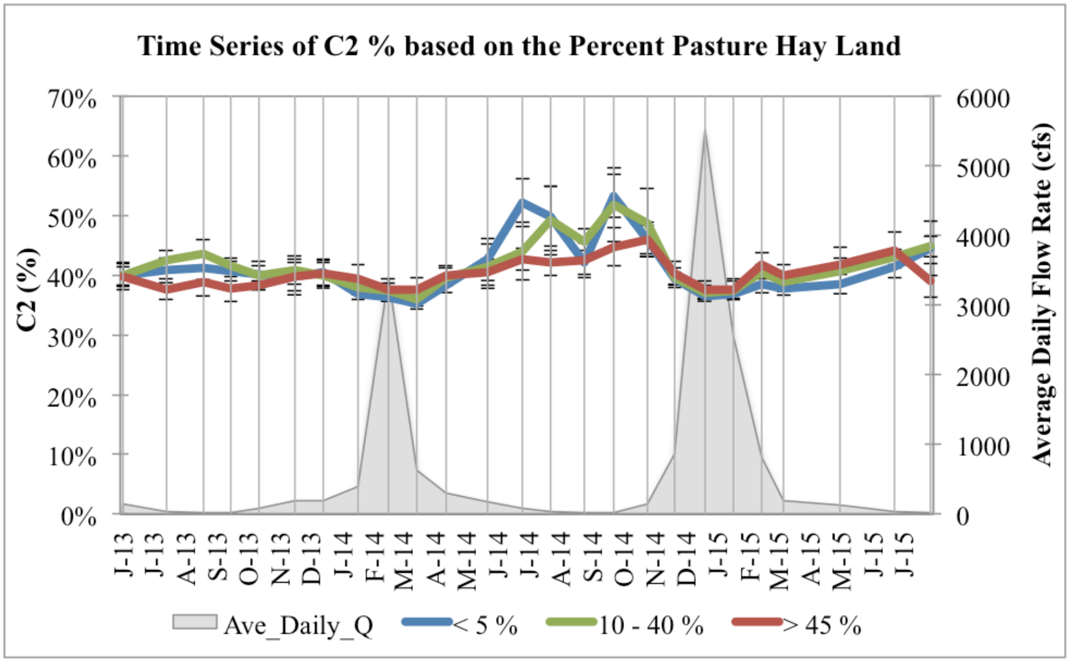
a


Time Series of C1 % based on the Percent Pasture Hay Land
C1 (%)
70%
60%
50%
40%
30%
20%
10%
0%
Average Daily Flow Rate (cfs)
6000
5000
4000
3000
2000
1000
0
J-13
J-13
A-13
S-13
O-13
N-13
D-13
J-14
F-14
M-14
A-14
M-14
J-14
J-14
A-14
S-14
O-14
N-14
D-14
J-15
F-15
M-15
A-15
M-15
J-15
J-15
Ave_Daily_Q
< 5 %
10 - 40 %
> 45 %

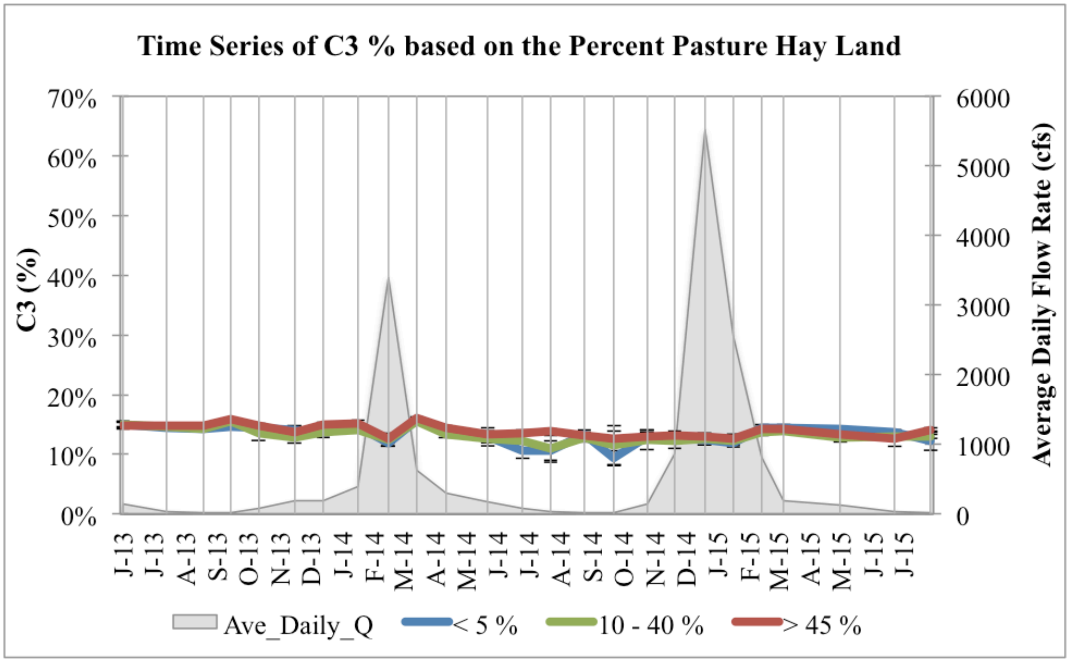
b


Time Series of C2 % based on the Percent Urban Land
C2 (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Flow Rate (cfs)
6000
5000
4000
3000
2000
1000
0
J-13
J-13
A-13
S-13
O-13
N-13
D-13
J-14
F-14
M-14
A-14
M-14
J-14
J-14
A-14
S-14
O-14
N-14
D-14
J-15
F-15
M-15
A-15
M-15
J-15
J-15
Ave Daily Q
< 10 %
11 - 15 %
16 - 20 %
21 - 30 %
49 - 70 %
90 - 100%

c



d



e

Figure 24: Time series of fluorescent components with various land use
Vertical lines indicate sampling dates. Average daily flow rate was measured at site Q in the Marys River (USGS Surface-water daily data for the nation, 2015 (ID: 14171000)). Error bars show standard error.
Figure 24a: Time series of C1% based on percent urban land
Figure 24b: Time series of C1% based on percent pasture/hay land
Figure 24c: Time series of C2% based on percent urban land
Figure 24d: Time series of C2% based on percent pasture/hay land

Figure 24e: Time series of C3% based on percent pasture/hay land

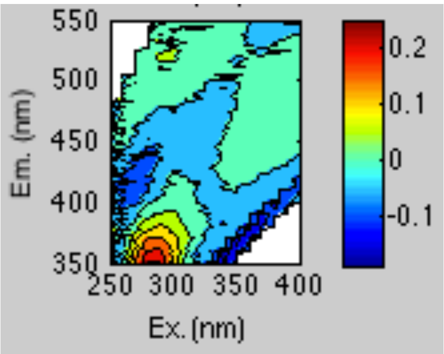


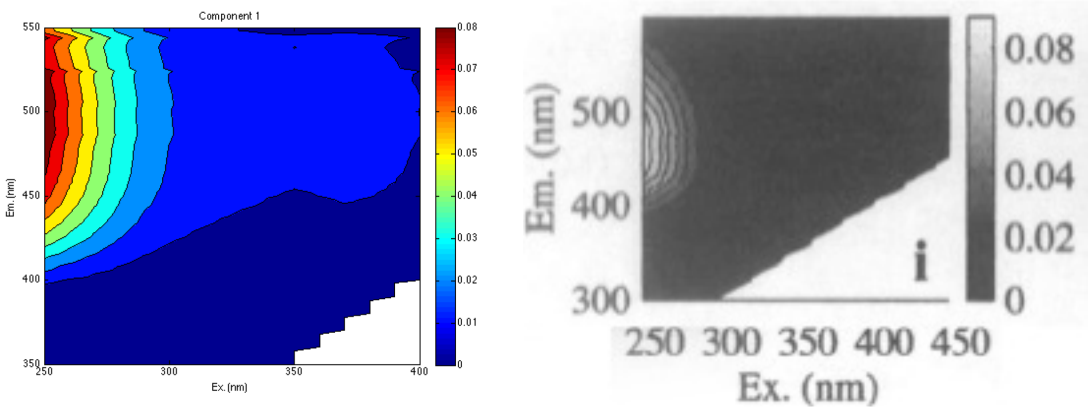
Figure 25: An example of a protein-like EEM residual

## 4.10 Appendices

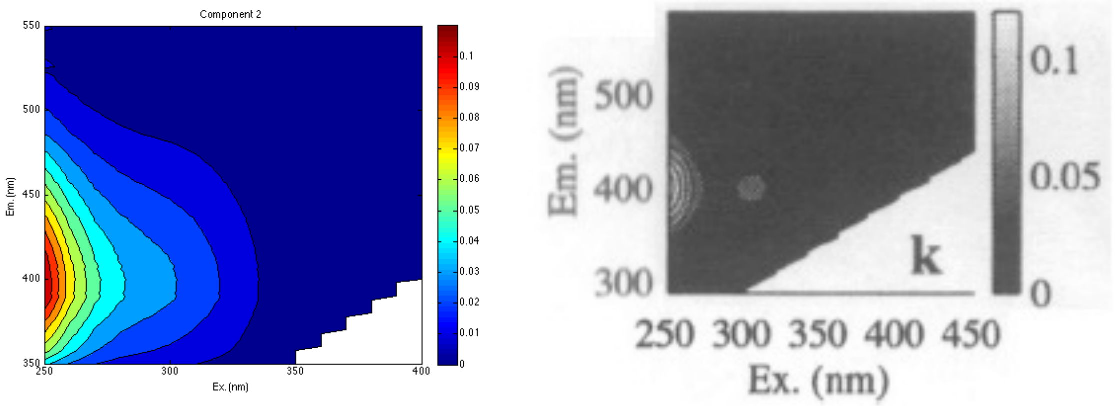
| Sample Location | # of sampling events for DOM | Stream Order | Cultivated Crops | Developed | Forest | Pasture Hay | Wetlands |
|---|---|---|---|---|---|---|---|
| A | 24 | 4 | 20% | 16% | 0% | 2% | 62% |
| B | 22 | 2 | 17% | 14% | 24% | 24% | 20% |
| C | 13 | 1 | 17% | 1% | 48% | 28% | 7% |
| D | 25 | 1 | 0% | 7% | 87% | 0% | 2% |
| E | 17 | 1 | 1% | 18% | 0% | 81% | 0% |
| F | 11 | 1 | 0% | 12% | 0% | 69% | 19% |
| Z | 25 | 2 | 0% | 12% | 2% | 59% | 19% |
| H | 16 | 1 | 0% | 56% | 10% | 31% | 0% |
| I | 24 | 2 | 0% | 100% | 0% | 0% | 0% |
| J | 25 | 5 | 0% | 68% | 0% | 0% | 32% |
| K | 22 | 1 | 14% | 16% | 0% | 48% | 21% |
| L | 18 | 2 | 0% | 22% | 0% | 78% | 0% |
| M | 10 | 1 | 27% | 27% | 0% | 46% | 0% |
| N | 23 | 2 | 41% | 9% | 10% | 31% | 10% |
| O | 25 | 4 | 0% | 8% | 0% | 49% | 40% |
| P | 25 | 4 | 0% | 14% | 0% | 37% | 49% |
| Q | 25 | 4 | 5% | 24% | 2% | 52% | 17% |
| R | 25 | 1 | 0% | 90% | 10% | 0% | 0% |
| S | 25 | 2 | 0% | 95% | 5% | 0% | 0% |
| T | 25 | 2 | 0% | 100% | 0% | 0% | 0% |
| U | 25 | 6 | 0% | 49% | 0% | 11% | 4% |

Appendix 2: The land use compositions and stream order for each sampling location. The highest possible sampling events for DOM per site were 25.

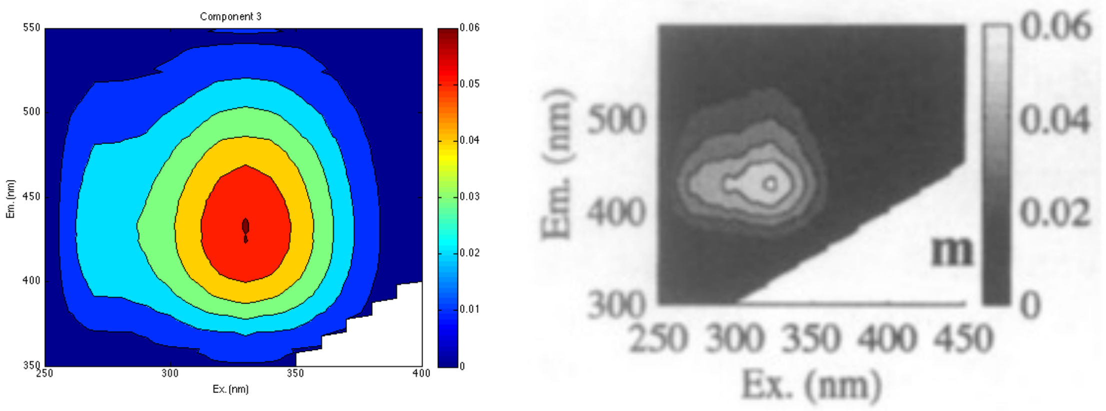
a



b



c



Appendix 3: The comparison of EEM regions of components identified by this study (left) and by *Stedmon and Markager* [2005] (right)
Appendix 3a: Component 1
Appendix 3b: Component 2
Appendix 3c: Component 3

# 5 Conclusions

The main findings of Chapter 2 were that DOC concentrations remain stable but the compositional nature of DOM was altered after months of freezing. This is because aromatic DOM preferentially precipitates while freezing. These changes in DOM optical properties suggest that we need to consider the effects of natural freezing in ecosystems on DOM chemical characteristics. $SUVA_{254}$, percent protein, and reduced quinone-like components will most likely be reduced once water is frozen if there is high aromaticity, although the percent decrease may be highly dependent on DOM source and the percent decrease in protein was less than $SUVA_{254}$. Additionally, samples may appear to decrease in aromaticity during a freezing winter storm, but this may not necessarily mean the aromatic components of DOM decreased in the environment. There may be a high proportion of aromatics after a long freezing event followed by a warm strong flushing event because precipitated DOM during the freezing season is mobilized.

Chapter 3 revealed that fluorescent DOM characterization could indicate shifts in DOM transport and processes with hydrologic events in the pristine forested headwater streams of western Oregon. A high proportion of a protein-like component, identified by fluorescent spectroscopy and characterized as bioavailable and lower in aromaticity compared to other components, is indicative of the increased DOM inputs from groundwater sources. This effect was especially pronounced among harvested watersheds where coarse woody debris, not live tree biomass, was limited compared to reference watersheds as the lack of coarse woody debris resulted in less aromatic and humic-like DOM. The proportion of a bioavailable protein-like component decreased with a shift in hydrologic pathways from groundwater to surface water during fall and winter as highly aromatic and terrestrial DOM inputs from overland flow became dominant in small streams. A protein-like or specifically a tryptophan-like EEM signal can be used as an indicator of shifts in hydrologic pathways in a pristine ecosystem where DOC:DON is not reliable due to low DON concentrations.

Taking the knowledge from Chapters 2 and 3, Chapter 4 was conducted in a mixed landscape and stream orders of the middle of the Willamette River Basin to learn the extent of the information that UV and fluorescent DOM can reveal. This chapter found that seasonal hydrologic patterns controlled UV and fluorescent DOM chemistry more than land use. This is contrary to our hypothesis that differences in land use patterns would produce DOM chemistries that were distinguishable by UV absorbance and fluorescent DOM chemistry. One explanation for our unexpected results is that these land use types, developed on similar geologic substrates, that had at one point all been forested, truly produced similar DOM chemistry. Previous studies, which found land use effects on fluorescent DOM components, have compared contrasting environments such as agricultural lands influence observed in an estuary and tributary catchments in Denmark (Stedmon and Markager 2005), the Florida Everglades wetland (Yamashita et al. 2010), and the Florida Bay (Maie et al. 2012). Additionally, fluorescent DOM components have been proven to be useful to differentiate forest and wetlands (Kothawala et al. 2015). Thus, our study conducted in a relatively small area that used to be forest might not have varying DOM characteristics.

Another possible explanation is that DOM chemistry from unique land use disturbances become obscured as the fluorescent DOM signals are truly blended in areas of varying land use types. Unlike other studies examining endmember representing a unique land use type in catchments (Yamashita et al. 2011; Cawley et al. 2014; Hosen et al. 2014; Lee and Lajtha, in review), the observations of this study were conducted in truly mixed landscapes.

Third possible explanation for our results is that while different land uses might produce DOM chemistry that is initially distinguishable with these techniques, microbial activity in soils and streams serves to homogenize any chemical fingerprints of the source material through respiration of the most labile components. This hypothesis that we term the “microbial blender hypothesis” is consistent with results from several previous studies. For example, Fellman et al. (2009) found that a protein-like DOM signal became weaker with an increase in stream order as the stream went downstream further away from the original DOM source (soil). Wickland et al. (2007) observed that while DOM leached from plants differed among varying vegetation types, DOM sampled from soils was more microbially processed and labile. Similarly, Huang et al. (2015) found that soil DOC varied with vegetation type but differences were lost in receiving streams. Finally, Yano et al. (2004) and Strid et al. (in review) found that while different detrital manipulation treatments produced DOC with distinct chemical fingerprints at the soil surface layers, these distinct signals were lost as DOM passed through the soil profile. Thus, as DOM moves from vegetation, to surface soil, to deep soil, and to streams, fluorescent DOM becomes more microbially processed. Hence, DOM in-stream from various land use types become homogenized by microbial processes with increased stream orders and cannot be differentiated with fluorescent spectroscopy. Further research in mixed landscape coupled with detail analyses of soil, soil pore from various the soil profile, and stream water would help to evaluate these hypotheses.

There are additional opportunities for future research based on the findings of this dissertation. Chapter 3, conducted in the H.J. Andrews Experimental Forest, showed that the contribution of protein-like and bioavailable DOM increased during baseflow season and decreased sharply during the first high flow season in forested headwater streams. Future studies can focus the use of available resources during these two time periods to characterize DOM chemistry in detail using more time-consuming and expensive techniques such as chemical fractionation and measuring bioavailable DOC. The bioavailability of DOM can be measured with the laboratory estimate (Wilson et al. 2013). The DOM chemistry obtained by these techniques will augment the understanding of DOM cycles within forested headwater catchments.

Chapters 3 and previous studies (Fellman et al. 2014; Huang et al. 2015) observed that the contribution of bioavailable/labile DOM is higher in groundwater and soil sources than surface sources. The combination of Chapters 3, 4, and a previous study (Fellman et al. 2014) further found that the contribution of bioavailable/labile DOM was more prominent in headwater streams than downstream rivers. Additionally, Chapter 4 found DOC concentrations decreased and fluorescent index suggested more terrestrial DOM sources with an increase in stream orders. In addition, the concentrations of all nutrient species were the highest at headwater streams. These findings could be due to quick consumption of labile DOC through respiration. The decline in DOC concentrations is contrary to the findings by the Fellman et al. (2014) srudy conducted in rivers of western Australia. These contradicting findings need to be confirmed in a region with different climates as these findings could be key to understanding the poorly understood contribution of headwater streams (Cole et al. 2007) to the global C cycle.

The age and bioavailability of DOM can be measured with $\delta^{18}O$ (Fellman et al. 2014) or the laboratory estimate (Wilson et al. 2013). Additionally, sampling on multiple fluvial networks covering a groundwater source through a first-order stream to a downstream river is necessary to examine the changes in the contribution and utilization of bioavailable DOM along a river stretch.

Previous studies (Yamashita et al. 2011; Cawley et al. 2014; Hosen et al. 2014), Chapter 3, and Chapter 4, conducted among mixed landscapes of the middle of the Willamette River Basin, revealed that fluorescent spectroscopy is a viable technique to find land use disturbance effects on DOM chemistry in headwater streams. This suggests an opportunity for end-member analysis. The equal number of headwater catchments, each representing 100 % of a land use type (e.g. forest, agriculture, pasture/hay, urban), should be selected within the same watershed using the most recent spatial land use dataset, NLCD 2011. In the selection of catchments, groundtruthing would be beneficial to confirm the accuracy of spatial data. Streams must be perennial for statistical analyses. This study setting will allow the direct comparison of varying compositions of EEM regions and components unique to a land use type.

Another interesting and important topic to explore is the effect of temperature on in-stream DOM chemistry and fluxes. The temperature control on DOM in streams, with or without fluorescent characterization of DOM, needs to be examined as climate change increases land temperatures as well as changes precipitation patterns, timing of hydrologic events, ice mass, and snowpack. Dissolved organic carbon concentration increased with stream orders while a protein-like DOM signal commonly found by other studies was not identified with the multivariate PARAFAC statistical model. This observation could be an indication of in-stream respiration quickly consuming labile DOC. This dissertation revealed that both hydrology and temperature affect DOM chemistry and fluxes.  An increase in temperature will affect microbial metabolism within streams.  Examining temperature effects on DOM chemistry will allow a more accurate understanding of river contributions to the global C cycle with changing climate.

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