

ANTHROPOGENIC AND ENVIRONMENTAL DRIVERS OF THE INPUT AND UPTAKE
OF DISSOLVED ORGANIC MATTER IN TEMPERATE STREAMS

by

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ABSTRACT

Dissolved organic matter (DOM) is a complex mixture of organic compounds and plays an essential role in regulating substrate and energy flows in aquatic ecosystems. However, environmental factors and biogeochemical mechanisms mediating the supply and uptake of DOM in streams are not well understood. The overarching goal of this dissertation is to assess the effects of the anthropogenic and natural drivers on the amount, source, composition, and fate of DOM in streams. The objective of Chapter II is to understand the effects of agricultural activities on DOM in a regional group of streams in Southeastern Alabama. The main finding is that agricultural land use increases DOC concentration and the proportions of terrestrial and microbial humic DOM compounds in streams, which suggests that agricultural activities accelerate the mobilization of organic matter from topsoils via enhancing oxidation, erosional transport, and shifting soil-to-stream flow paths. The objective of Chapter III is to identify the environmental drivers controlling the supply of terrestrial DOM in a Coastal Plain stream draining a forest-dominated watershed. The main finding is that discharge can be used to predict DOM supply across timescales, but other environmental drivers could be important at a given timescale. Specifically, the event-scale DOM supply is influenced by antecedent hydrological conditions and the duration of storms. At the diurnal scale, DOM variation is driven by physical dilution and concentration due to evapotranspiration. At the seasonal scale, DOM variation is mediated by organic matter availability from litterfall and discharge. The objective of Chapter IV is to determine the rates of natural DOM removal and identify the associated biogeochemical mechanisms in a second-order stream draining a forest-dominated watershed. The results provide

the first record simultaneously measuring the uptake characters of humic-like and protein-like DOM, which demonstrates that humic-like DOM has a shorter uptake length and higher uptake velocity than protein-like DOM due to the preferential adsorption of humic-like compounds to benthic sediments. This dissertation improves our understandings of the supply and demand of DOM in subtropical streams in response to human land use and hydrological events, contributing to a greater understanding of the factors mediating the aquatic ecosystem response.

DEDICATION

This dissertation is dedicated to my family.

LIST OF ABBREVIATIONS AND SYMBOLS

DOM	Dissolved Organic Matter
DOC	Dissolved Organic Matter
BDOC	Biodegradable Dissolved Organic Matter
MW	Molecular weight
FI	Fluorescence index
SR	Spectral slope ratio
SUVA ₂₅₄	Specific UV absorbance
HIX	Humification index
BIX	Biological index
EEM	Excitation-Emission Matrix
C-Q	concentration-discharge
fDOM	fluorescent DOM
Sw	uptake length
Vf	uptake velocity
TASCC	Tracer Additions for Spiraling Curve Characterization
OTIS	Dimensional Transport with Inflow and Storage
km	kilometer
m	meter
cm	centimeter
nm	nanometer

s	second
m/s	meter per second
mm/min	millimeter per minute
mg	milligram
g	gram
kg	kilogram
$\mu\text{S/cm}$	microsiemens per centimeter
m^3/s	cubic meter per second
mg/L	milligrams per liter
kg/day	kilogram per day
$\text{L mg}^{-1} \text{m}^{-1}$	litter per milligram per meter
δD	hydrogen isotope
$\delta^{18}\text{O}$	oxygen isotope
%	percentage
°	degrees
°C	degree Celsius
>	greater than
<	less than
\geq	greater than or equal to
\leq	less than or equal to
\pm	plus or minus

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CHAPTER 1: INTRODUCTION

Dissolved organic matter (DOM) is the major form of organic matter in aquatic ecosystems, and DOM represents the most abundant and bioavailable form of organic carbon (Findlay and Sinsabaugh, 2003; Battin et al. 2008). Operationally defined as the portion of organic material that can pass 0.2 to 0.7 μm filter pore size, DOM comprises varied organic forms of carbon, nitrogen, sulfur, and phosphorus (Findlay and Sinsabaugh, 2003; Zsolnay, 2003). The molecular weights of DOM typically range from 100 to 100,000 Da, with fulvic acids as the dominant compounds usually accounting for about 45–65% of total DOM (Aikenhead-Peterson et al. 2003). Depending on the source, the chemical composition of DOM varies. For example, the proportion of fluvic acids can range from 80–90% in wetlands dominated by allochthonous DOM to 10–30% in groundwater dominated by autochthonous DOM (Thurman, 1985). DOM plays fundamental and multifaced roles in aquatic ecosystems. DOM is an energy and substrate source to microbial food webs (Azam et al. 1983; Benner 2003; Hood et al. 2009). Heterotrophic bacteria are the primary consumers of DOM, and the organic carbon assimilated from DOM is further exploited by protozoa and metazoan, by which energy and carbon are propagated to higher heterotrophic levels. Owing to the presence of chromophoric compounds, DOM modifies the optical properties of water by adding a yellow color that selectively attenuates UV and visible light in natural water (Williamson and Zagarese 1994; Gergel et al. 1999; Findlay and Sinsabaugh, 2003). The selective attenuation of light (i.e., harmful UV-B radiation) not only alters light regimes and the rates of photosynthesis but also protects

organisms from genetic damages and subsequently maintains the high bioavailability of organic substrates in aquatic ecosystems (Williamson and Zagarese, 1994; Wetzel, 2003). DOM can also protect the aquatic organisms by influencing the solubility and mobility of harmful metals through binding with the aliphatic and aromatic carboxyl and hydroxyl functional groups and forming dissolved complexes (Martell et al. 1988; Maranger and Pullin, 2003).

DOM in aquatic ecosystems can be classified by origins including autochthonous DOM from aquatic microorganisms and allochthonous DOM from terrestrial soils. Autochthonous DOM accounts for the dominant DOM fractions in lakes and oceans, and it is produced through the photoinduced assimilation and respiration of aquatic biomass. Specifically, autochthonous DOM is mainly produced from the release of algae or phytoplankton biomass through processes including extracellular releases by living cells, cell death and lysis, and herbivore grazing (Mostofa et al. 2013). Autochthonous DOM often contains abundant compounds with low molecular weight such as monomeric sugars, carboxylic acids, amino acids, and alditols, and it is usually a high-quality substrate for bacteria (Cole et al. 1982; Bertilsson and Jones, 2003). In comparison, DOM from allochthonous source is more enriched in humic substances (about 50% contribution; such as fulvic and humic acids) when compared to autochthonous DOM, and it is often the dominant fractions in lotic ecosystems with low instream productivity, such as low-order streams (Findlay and Sinsabaugh, 2003; Lu et al. 2013, 2014). Allochthonous DOM in the aquatic ecosystem is typically the product of leaf and root litters, animal remains, and the metabolites of microorganisms from terrestrial organic horizons (Schnitzer and Neyroud, 1975; Kaiser et al. 2001; Aikenhead-Peterson et al. 2003). The export of allochthonous DOM is regulated by multiple environmental factors such as temperature, nutrient and microorganism availability, redox conditions, soil-to-stream flow path, and precipitation conditions (Mostofa et

al. 2013). For example, the soil-to-stream flow path variation in the watershed during storms was found to result in up to five-fold differences in DOC concentration in receiving streams (McDowell and Wood, 1984, Aitkenhead-Peterson et al. 2003). The availability of organic substances, the storage location (e.g., near-channel vs. upland; shallow vs. deep soil), as well as the size of storms also influence the magnitude and timing of DOM export (Pellerin et al. 2012; Vaughan et al. 2017). Owing to the complexity and uncertainty in the generation and transportation of terrestrial DOM, the character of allochthonous DOM in the aquatic ecosystem is rather unpredictable. Low-resolution sampling strategies were commonly used in previous studies and did not capture the high-frequency DOM dynamic during storms, leading to inaccurate carbon flux estimates. In addition to natural DOM sources, anthropogenic activities have significantly modified the DOM composition in natural waters (Wilson and Xenopoulos, 2009; Lu et al. 2013, 2014). At the global scale, fossil fuel burning accelerates global warming, which subsequently enriches DOM in the natural waters either directly through stimulating instream DOM production or indirectly through more frequent mobilizations of allochthonous DOM into streams. At the catchment scale, human wastes such as sewage and industrial effluents can significantly contribute to the increase in DOC concentration in streams (Pal et al. 2010; Yoon et al. 2010), and anthropogenic components such as detergents, fluorescent whitening agents, protein-like DOM components, sterols, and unknown organics were detected in natural waters (Mudge and Duce 2005; Mostofa et al. 2013). Agricultural activities increase the nutrient availability and artificial organic substances that can contribute to the DOM in natural waters such as pesticides, herbicides, dichlorodiphenyltrichloroethane (DDT) (Mostofa et al. 2013). Moreover, agricultural activities alter the landscape of watersheds that can influence the

hydrological retention time, flow path, as well as the source of DOM from soil horizons (e.g., Wilson and Xenopoulos, 2010; Lu et al. 2013, 2014; Hu et al. 2016).

DOM in streams and rivers can be removed by both biotic (e.g., microbial mineralization) and abiotic processes (e.g., adsorption and photo-oxidation) (Findlay and Sinsabaugh, 2003; Lu et al. 2013). The continuous exchange of stream water between the stream channel and hyporheic zone results in the removal of DOM. Humic substances are efficiently removed through adsorption to the surface of iron and aluminum oxides (McKnight et al. 1992; Gu et al. 1995), and the adsorption decreases the biological and photochemical degradability of DOM and facilitates the retention and preservation. DOM can be adsorbed onto biofilms and transported to biofilm bacteria for further microbial degradation. Photodegradation of DOM reduce the average DOM molecular weight and increase the proportions of biologically labile molecules to be directly assimilated by organisms (Zepp et al. 1998; Moran and Covert, 2003). These processes collaboratively remove a significant fraction of DOM during the longitudinal transport from headwater streams to the coastal oceans. Deuser (1988) estimated that the delivery of DOM by the rivers is sufficient to account for much of the ocean DOM as well as the heterotrophy within the oceans, yet terrestrial DOM detected in the oceans only makes up less than 2.5% of the oceanic DOM (Opsahl and Benner, 1997). The uptake of DOM in lotic ecosystems remain understudied due to the complexity of DOM compounds. A few studies have quantified the uptake of DOM with laboratory experiments, and it remains questionable whether these results can realistically represent the natural conditions (Freeman and Lock, 1995; Wickland et al. 2007). Only a limited number of studies have evaluated natural DOM uptake in the field and yielded results applicable to the reach scale, but inconsistent findings were reported (McKnight et al. 2002; Fellman et al. 2009).

The overarching goal of this dissertation is to determine environmental variables and biogeochemical mechanisms controlling the generation, mobilization, and removal of DOM in low-order, subtropical streams. The dissertation contains five chapters including the introduction chapter (Chapter 1), three chapters written in standard scientific manuscript format (Chapter 2, Chapter 3, and Chapter 4), and the conclusion chapter (Chapter 5).

Chapter 2 has been published in *Science of the Total Environment*, and can be cited as “Shang, P., Lu, Y., Du, Y., Jaffé, R., Findlay, R. H., & Wynn, A. (2018). Climatic and watershed controls of dissolved organic matter variation in streams across a gradient of agricultural land use. *Science of the Total Environment*, 612, 1442-1453”. This chapter focuses on understanding the impacts of agricultural activities on the properties of DOM in stream water. The study was carried out in 2014 at the Bear Creek Watershed in northwestern Alabama, Southeast U.S. We collected samples over one year from six first- to fourth-order streams draining watersheds across a gradient percentage of agricultural land use. We assessed DOM properties including the concentration, chemical composition, and biodegradability of DOM. By combining geospatial and multivariate statistical analysis, we established a mechanistic understanding of the effects of agricultural land use on stream water DOM sources and compositions.

Chapter 3, entitled “*Terrestrial Input of Dissolved Organic Matter to a Subtropical Forested Stream across Time Scales from Minute to Year*”, is co-authored with YueHan Lu, Marco Bonizzoni and will be submitted to *Biogeochemistry*. This chapter focuses on identifying the environmental and biogeochemical factors controlling the input of allochthonous DOM into streams. We aim to answer the question of whether terrestrial DOM inputs can be predicted by straightforward environmental and weather variables that are regularly and widely monitored such as temperature and discharge. The study was carried out between 2015 and 2016 in a

second-order stream at the Talladega National Forest of western central Alabama, southeastern USA. We collected a high-resolution time series of fluorescence DOM, conductivity, and water level using *in situ* sensors, in combination with laboratory analysis of DOM source-compositional characters on selected samples. We analyzed the variations in DOM in relation to environmental variables under different temporal scales, including seasonal, monthly, diurnal, and storm event scales, and we identified the environmental variables that can reasonably predict terrestrial input of DOM at different scales. We further suggested biogeochemical mechanisms that were responsible for the linkages between environmental variables and terrestrial DOM. The use of high-resolution sensors also led to a robust estimate of the watershed export of dissolved organic carbon fluxes.

Chapter 4, entitled “*Dissolved Organic Matter Uptake in a Forested Headwater Stream: An Evaluation Combining Reach-Scale Tracer Release Experiments and Numerical Simulation*”, is co-authored with YueHan Lu, Song Wei, Xiaorui He, and Yong Zhang. This chapter is motivated by the limited knowledge on the uptake characters of natural DOM at the reach scale within drainage networks. The most direct example is that reach-scale and network-scale uptake parameters presently being used is mostly derived from laboratory incubations that may not realistically represent dynamic and complex field conditions. The study was carried out between 2017 and 2018 on an 80-meter reach in a second-order stream at the Talladega National Forest of western central Alabama, southeastern USA. We conducted five tracer release experiments using the mixture of natural DOM leached from plant litter. We employed a TASCC (Tracer Additions for Spiraling Curve Characterization) approach that allowed for the collection of uptake parameters on a range of dissolved organic carbon concentrations during one experiment. Our breakthrough curves showed that humic-like DOM and protein-like DOM were transported

differently, and to our knowledge, this discrepant pattern has never been demonstrated in previous studies. DOM uptake velocity at the reach scale was significantly higher than that in laboratory incubation. The OTIS (Dimensional Transport with Inflow and Storage) simulation also suggested a larger uptake rate in the hyporheic zone than that in the water column.

Finally, in Chapter 5, the conclusions from each chapter are summarized, and a specific focus is given to explain how my dissertation research expands our knowledge in the input and removal of terrestrial DOM in aquatic ecosystems.

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CHAPTER 2:

CLIMATIC AND WATERSHED CONTROLS OF DISSOLVED ORGANIC MATTER VARIATION IN STREAMS ACROSS A GRADIENT OF AGRICULTURAL LAND USE

2.1 Abstract

Human land use has led to significant changes in the character of dissolved organic matter (DOM) in lotic ecosystems. These changes are expected to have important environmental and ecological consequences. However, high spatiotemporal variability has been reported in previous studies, and the underlying mechanisms remain inadequately understood. This study assessed variation in the properties of stream water DOM within watersheds across a gradient of agricultural land use with grazing pasture lands as the dominant agricultural type in the southeastern United States. We collected water samples under baseflow conditions five times over eight months from a regional group of first- to fourth-order streams. Samples were analyzed for dissolved organic carbon (DOC) concentration, DOM quality based on absorbance and fluorescence properties, as well as DOM biodegradability. We found that air temperature and antecedent hydrological conditions (indicated by antecedent precipitation index and stream water sodium concentrations) positively influenced stream water DOC concentration, DOM fluorescence index, and the proportion of soil-derived, microbial humic fluorescence. This observation suggests that elevated production and release of microbial DOM in soils facilitated by high temperature, in conjunction with strong soil-stream hydrological connectivity, were important drivers for changes in the concentration and composition of stream water DOM. By comparison, watersheds with a high percentage of agricultural land use showed higher DOC

concentration, larger proportion of soil-derived, humic-like DOM compounds, and higher DOC biodegradability. These observations reflect preferential mobilization of humic DOM compounds from shallow organic matter-rich soils in agricultural watersheds, likely due to enhanced soil erosion, organic matter oxidation and relatively shallow soil-to-stream flow paths.

2.2 Introduction

Dissolved organic matter (DOM) within aquatic environments is an assemblage of heterogeneous compounds derived from terrestrial and aquatic organisms. It plays an important role in a variety of critical environmental and ecological processes, such as providing basal substrate and energy for heterotrophic food webs, protecting aquatic biota through buffering pH and absorbing ultraviolet-B radiation, and influencing complexation, solubility, and mobility of metals (Martell et al. 1988; Williamson et al. 1994; Aitkenhead-Peterson et al. 2003; García-Gil et al. 2004). Allochthonous, terrestrially-derived compounds often account for an important fraction of DOM pool in streams and rivers (e.g., Williams et al. 2010), and thus a number of watershed attributes exert important controls on DOM character (i.e., quantity and quality) within lotic ecosystems (e.g., Mulholland 2003; Mosher et al. 2010; Yamashita et al. 2011; Cawley et al. 2014; Lu et al. 2013, 2014). For example, higher watershed slope gradients generally correspond to lower stream water DOC concentrations because of increased flow rates and shorter soil leaching time (Mulholland 2003; Xenopoulos et al. 2003). Larger watersheds are typically associated with a greater terrestrial carbon pool that can be mobilized and thus higher DOC concentrations in receiving waterways (Mulholland 2003; Frost et al. 2006).

More recently, human land use has been increasingly recognized as an important watershed driver mediating the amount, composition, age, and fate of DOM in streams and rivers (e.g., Jaffé et al. 2008; Wilson and Xenopoulos 2009; Williams et al. 2010; Lu et al. 2013, 2014,

2015a; Butman et al. 2014). The influences of watershed land use, however, are usually confounded with those associated with other environmental and hydrological drivers (e.g., temperature and precipitation). Hence, it remains unclear whether watershed development plays a significant or even primary role in regulating variability in DOM in lotic ecosystems and whether the effects of land use are consistent across a range of spatiotemporal scales (e.g., Jaffé et al. 2008; Hu et al. 2016). Recent studies have reported contradictory impacts of agricultural land use on DOM in streams and rivers. For example, agricultural land use has been shown to increase (Graeber et al. 2012) or decrease (Williams et al. 2010; Stanley et al. 2012) the concentration of DOC in streams, with possible causes including reduced soil OM retention (Graeber et al. 2012), increased production of autochthonous DOM due to enhanced nutrient inputs (Lu et al. 2014), and reduced terrestrial OM pool (Stanley et al. 2012). Contradictory changes have been observed for DOM source and compositional properties as well. Some studies found increases in the proportions of humic-like DOM in agricultural streams (Graeber et al. 2012; Singh et al. 2017) whereas others observed increases in the proportions of protein-like DOM (Lu et al. 2014; Fuß et al. 2017). A recent review by Stanley et al. (2012) focusing mostly on DOC concentrations (i.e., limited information about DOM quality) highlighted this high spatiotemporal variability of DOM in human-influenced streams and rivers, which has led them to conclude that insufficient data existed to provide science-based guidelines for DOC management.

In the present study, our objective was to assess the effects of agricultural land use on DOM character in streams within an agricultural watershed in the southeastern USA. Our study sites included a regional group of first-order to fourth-order streams draining watersheds across a range of percent agricultural land (i.e., 5–64%). We tested the hypothesis that agricultural land

use was the primary driver of stream water DOM character variability in the study region, given that these streams were influenced by similar climatic conditions and their watersheds had similar lithology and soils. We collected stream water DOM five times over the course of eight months under baseflow conditions to minimize the confounding influences of hydrological events. We assessed DOM character by measuring DOC concentration and DOM optical properties (ultraviolet-visible absorbance and 3D fluorescence excitation-emission matrix coupled with parallel factor analysis) and determining DOC biodegradability via laboratory incubation experiments. Additionally, we measured the concentrations of inorganic nutrients and major cations, which provided information on geochemical and hydrological variations to assist with the interpretation of DOM character variability. Our findings provided new insights on the relative importance of agricultural land use versus other environmental drivers on the amount, source, and quality of DOM in regional streams.

2.3 Methods

2.3.1 Sampling sites

Our sampling sites were situated at the Bear Creek Watershed in the northwestern Alabama, Southeast U.S. (Fig. 2.1). Six first- to fourth-order streams (Strahler order based on the National Hydrography Data Version 2) draining watersheds with differing degrees of agricultural land use were sampled five times from March to October, 2014, including two streams draining watersheds with >50% agricultural land, two streams with ca. 40% agricultural land, and two streams with <25% agricultural land. Two additional streams with <25% agricultural land were sampled in March and April (Table 2.1). The watershed boundary for each sampled stream was delineated using StreamStats (<http://water.usgs.gov/osw/streamstats/>), yielding polygons that were then overlain on the National Land Cover Database 2011 map (NLCD 2011;

<http://www.mrlc.gov/nlcd2011.php>) to calculate the percentage contributions of each land use type. NLCD 2011 uses 16-class land cover classification with a spatial resolution of 30×30 square meters (size of one pixel). The percentage contribution of each land use type was calculated as the ratio of the corresponding pixel count over the total count for the entire watershed, with the result rounded to the nearest hundredth. Pasture and cropland were classified as agricultural land, and perennial pasture was the dominant (75% to 100%) agricultural land use type in the study sites. According to the Natural Resources Conservation Service, United States Department of Agriculture (USDA; Russellville, Alabama) and our personal observations, pastures in this area were dominated by Bermuda and Fescue grasses and used for cattle grazing. No tillage was performed on pastures, and no to little tillage was performed on croplands. Irrigation was not applied to most pastures or croplands, and subsurface drainage was rare in this area. Poultry litter was the main fertilizer, and they were generally applied twice a year on pastures (in March/April and August/September) and once a year on croplands (between late February to early April).

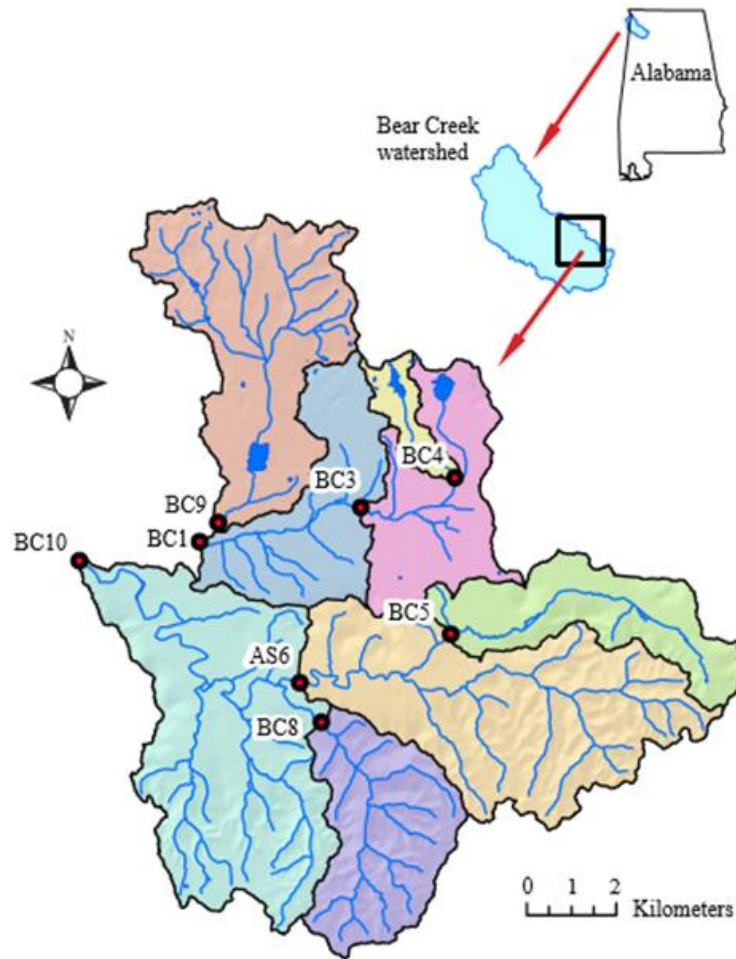


Figure 2.1. Locations of study sites in Bear Creek Watershed, Alabama, USA. Streams are indicated by blue lines, watershed boundaries are indicated by black lines, and sampling sites are denoted by black dots.

Table 2.1. Watershed land use and hydrological parameters for the sampling streams in the present study (mean \pm standard deviation).

Sampling site	Stream order	GPS Coordinates (°)	Watershed area(km ²)	Agricultural land (%) (Pasture+cropland)	Forest (%)	Developed (%)	Lithology ¹	DO (mg/L)	Conductivity (μ S/cm)	pH	Sampling month
BC1	3	N 34.46902	25.68	53%	28%	8%	Limestone,	7.78 \pm 0.79	477.1 \pm 84.3	8.34 \pm 0.58	Mar/Apr/May/ July/Oct
		W 87.72404		(49%+4%)			Sandstone				
BC3	3	N 34.47615	13.23	64%	21%	7%	Limestone,	6.60 \pm 1.18	443.5 \pm 94.3	8.15 \pm 0.86	Mar/Apr/May/ July/Oct
		W 87.68459		(57%+7%)			Sandstone				
BC4	1	N 34.48236	2.07	40%	32%	15%	Sandstone,	6.64 \pm 0.67	305.1 \pm 57.9	8.23 \pm 0.72	Apr/May/July/ Oct
		W 87.66171		(30%+10%)			Limestone				
BC5	2	N 34.45065	11.04	38%	38%	3%	Limestone	11.79 \pm 3.72	408.3 \pm 163.7	8.43 \pm 0.69	Mar/Apr/May/ July/Oct
		W 87.66251		(36%+2%)							
BC10	4	N 34.46495	79.02	18%	59%	3%	Limestone,	7.63 \pm 0.33	330.8 \pm 46.3	8.98 \pm 0.77	Apr/May/July/ Oct
		W 87.75333		(17%+1%)			Shale				
AS6	3	N 34.44123	37.71	19%	61%	2%	Limestone,	7.13 \pm 1.08	405.6 \pm 127.4	8.91 \pm 1.05	May/July/Oct
		W 87.70191		(18%+1%)			Shale				
BC8	3	N 34.432795	13.03	5%	60%	4%	Limestone,	8.36	143.6	NA ²	Mar
		W 87.694251		(5%+0%)			Shale				
BC9	4	N 34.47293	20.79	24%	37%	29%	Sandstone,	8.50	377.8 \pm 62.1	8.25 \pm 0.83	Mar/Apr
		W 87.71948		(22%+2%)			Limestone				

1: Lithologies are reported in a decreasing order of proportions (>20% are reported).

2: NA = data not available

2.3.2 Climate conditions

The sampling streams were less than 9 km apart and hence affected by similar climatic conditions. The Bear Creek Watershed is influenced by a humid, subtropical climate, with annual temperature averaging 18 °C and annual precipitation averaging 1,420 mm (Kottek et al. 2006). The study streams are underlain by Mississippian Limestone and alluvial deposits composed mainly of gravel and sand. The soils are formed by upland sediment deposition and Mississippian Limestone weathering, and they are generally shallow and sandy or clayey (Osborne et al. 1989; McGregor and Cook 2004). Daily precipitation and air temperature of the watershed were recorded by a nearby (1.8–5.5 km from our sampling sites) National Oceanic and Atmospheric Administration (NOAA) monitoring station (RUXA1; <http://raws.wrh.noaa.gov>). The mean air temperature over 15 days before sampling was calculated. Antecedent precipitation intensity over 15 days before sampling (API_{15d}) was calculated as:

$$API_{15d} = \sum_{i=1}^{15} \left(\frac{P_i}{i} \right) \quad (2.1)$$

where P_i (mm) is daily total precipitation on the i th day before sampling, and i denotes integer from 1 to 15 (McDonnell et al. 1991).

2.3.3 Sample collection and filtration

To minimize the influences of storm events, surface stream water samples were collected at least two days after the most recent precipitation. Storm flows were estimated to end within two days in the study watersheds, following an empirical equation that has been commonly used for separating baseflow vs. stormflow: $D = 0.827A^{0.2}$ (D = time between storm crest and end of overland runoff in days, A = drainage basin area in square kilometers; Fetter 2001). The stream discharge and precipitation data from the closest USGS and NOAA gauging stations (40–48 km from the study streams) further confirmed no or little influence of storm flows on the water

samples (Appendix I Figure 1). Samples were collected in duplicate or triplicate with polycarbonate bottles (HDPE; pre-cleaned through >4 h soaking with 3M hydrochloric acid and subsequent rinsing with carbon free ultrapure water). A suite of *in situ* environmental parameters (i.e., dissolved oxygen concentration, conductivity, and pH) were measured during each sampling event (Table 2.1). Stream water depth was less than 1.5 m for all sites throughout our sampling campaigns. Canopy coverage at the sampling site was estimated using two methods — quantifying the percentage of tree coverage within a 30-meter buffer using the NLCD 2011 USFS Tree Canopy map and direct measurement using a spherical crown densiometer. All samples were stored in a cooler with ice and transported back to the laboratory to be filtered on the same day of collection. Whatman 0.7 μm pore size GF/F glass fiber filters (pre-combusted at 500°C for 5 hours) were used to remove particles from stream samples, and the filtrates were stored in pre-cleaned HDPE bottles. The filtrates for DOC and nutrient concentration analyses were stored at -20°C in the dark, and samples for DOM optical measurements were preserved at 4°C in the dark to avoid any potential interference from freeze-thaw processes (Otero et al. 2007; Spencer et al. 2007a) and analyzed within two weeks. The samples for cation concentration analysis were acidified with ultrapure concentrated HNO_3 to 2% by volume and stored in a refrigerator.

2.3.4 DOC concentration

DOC concentration was analyzed using a Shimadzu TOC-V total organic carbon analyzer, following the method described in detail in Lu et al. (2015b). The calibration curve was constructed using potassium hydrogen phthalate solutions with six known concentrations. Carbon free ultrapure water was measured regularly to assess instrumental baseline, and a seawater reference standard (Hansell laboratory,

<http://yyy.rsmas.miami.edu/groups/biogeochem/CRM.html>) was used to confirm accuracy. Two to three samples were randomly selected for replicate measurements in each run, yielding the relative standard deviation ranging between 1.51% and 2.66%.

2.3.5 DOM quality

Absorbance measurement followed the method described in Lu et al. (2015b). Samples were analyzed using a 10-mm path length quartz cuvette and analyzed on a single beam UV-visible spectrophotometer (UV-1800 Shimadzu) under the scanning wavelength from 190 to 670 nm at an interval of 1 nm. Specific UV absorbance (aka. $SUVA_{254}$; $L\ mg^{-1}\ m^{-1}$) was calculated by normalizing the UV decadal absorption coefficient at 254 nm (A_{λ}) in inverse meter (m^{-1}) to DOC concentration (Weishaar et al. 2003). Spectral slope ratio (S_R) of 275–295 nm over 350–400 nm ($S_R = S_{275-295nm}/S_{350-400nm}$) was calculated following Helms et al. (2008), and S (spectral slope gradient) relative to the reference wavelength (λ_{ref}) was defined below:

$$a_{\lambda} = \frac{2.303 \times A_{\lambda}}{l} \quad (2.2)$$

$$a_{\lambda} = a_{\lambda_{ref}} \times e^{-S \times (\lambda - \lambda_{ref})} \quad (2.3)$$

where a_{λ} = absorption coefficient (m^{-1}) at the wavelength of λ , A_{λ} = decadal absorption coefficient at the wavelength of λ , and l = path length (m) (Green and Blought 1994; Twardowski et al. 2004; Spencer et al. 2007b).

DOM fluorescence property was evaluated using the excitation-emission matrix (EEM) coupled with parallel factor analysis (PARAFAC). EEM-PARAFAC allows low-cost, rapid assessments and thus can capture the high spatiotemporal variability associated with natural DOM (Jaffé et al. 2014). Previous studies noted that it provides interpretations on DOM origin and composition consistent with those from molecular-level techniques (e.g., Hernes et al. 2009; Lu et al. 2015a). The fluorescence data were measured on a Horiba Aqualog fluorescence

spectrometer operated at the signal ratio mode (S/R) and corrected using manufacturer's correction factors. Samples were scanned at excitation wavelengths from 240 nm to 621 nm at 3 nm intervals, and emission wavelengths were collected from 213 nm to 621 nm at ca. 1.5 nm intervals. The inner-filter effect (McKnight et al. 2001) was corrected using the UV-absorbance data measured on the Horiba Aqualog fluorescence spectrometer, and the data were subsequently normalized to the area under Raman curve of carbon free ultrapure water at the excitation wavelength of 350 nm (Stedmon et al. 2003; Cory et al. 2010). The drEEM toolbox was used to perform the parallel factor analysis in MATLAB (Murphy et al. 2013). The EEM model was validated via split-half analysis by randomly assigning the samples into four quarter splits and performing three validation tests on the six combined dataset halves (S₄C₆T₃; Murphy et al. 2013). Fluorescence index (FI) was calculated as the ratio of emission intensity at 470 nm to that at 520 nm under the excitation wavelength of 370 nm (Cory et al. 2010). Humification index (HIX) was calculated as the ratio of integrated emission intensity from 435 nm to 480 nm to that from 300 nm to 345 nm, corresponding to an excitation wavelength of 254 nm (Zsolnay et al. 1999).

2.3.6 Nutrients and cations

Samples were analyzed for nitrate, nitrite, ammonium, and orthophosphate with a San⁺⁺ Automated Wet Chemistry Analyzer with standard colorimetric modules designed for each analyte, following the EPA methods 353.2, 350.1, and 365.3, respectively. Relative standard deviation of duplicate measurements varied between 0.48% and 1.77%. The concentrations of major cations, including Na⁺, Ca²⁺, Mg²⁺ and K⁺, were analyzed using a Perkin Elmer Optima 3000 DV ICP-OES, with calibration curves generated from multi-element standards at six

concentrations between 0.25 to 20.0 mg/L. Samples analyzed in duplicate yielded relative standard deviation between 0.05% and 3.95%.

2.3.7 Biodegradable DOC assessment

Microbial degradation experiments were conducted in triplicates on the samples collected from six sites in May 2014 (Table 2.1). Stream water samples that had passed through 0.7 μm pore size GF/F filters were further filtered through 0.2 μm pore size filters (Whatman polycap; pre-cleaned with 10% HCl and carbon free ultrapure water) to remove bacteria and then inoculated with microbes by adding 1% (by volume) *in situ* raw stream water (Servais et al. 1989). Each sample was distributed in three pre-combusted, one-liter amber glass bottles and incubated at 20°C in the dark for 22 days. Subsamples were collected from each bottle at day 0, 5, 12, and 22 and analyzed for DOC concentration and DOM optical property. The percentages of biodegradable DOC (%BDOC) over the 22 incubation days were calculated as:

$$\%BDOC = \left(\frac{DOC_{t0} - DOC_{t22}}{DOC_{t0}} \right) \times 100\% \quad (2.4),$$

where DOC_{t0} and DOC_{t22} were DOC concentrations at day 0 and day 22, respectively. The degradation rate of DOM was assessed under the assumption of the first-order degradation kinetics which has been repeatedly reported in previous studies (e.g., Middelburg 1989; Ogawa et al. 2001). The changes in DOC concentrations during the incubations fit well with the first-order degradation pattern ($R^2=0.89-0.93$). The degradation rates (k') of DOC concentration and DOM component fluorescent intensity (F_{Max}) were calculated as:

$$DOC_t = DOC_{t0} \times e^{-k' DOC \times t} \quad (2.5),$$

$$F_{Max_t} = F_{Max_{t0}} \times e^{-k' F_{Max} \times t} \quad (2.6),$$

where DOC_{t0} ($F_{\text{Max}t0}$) and DOC_t ($F_{\text{Max}t}$) denote DOC concentration (DOM component fluorescence intensity) at the initial (i.e., day 0) and sub-sampling time points (i.e., day 5, 12, 22), respectively.

2.3.8 Statistical analysis

For all statistical analyses, the level of significance, α , was set at 0.05 (two-tailed). Non-parametric Wilcoxon and Kruskal-Wallis tests were used to compare variables between different groups (SPSS). Prior to linear regression and redundancy analyses, Pearson's bivariate correlations were calculated to identify variables that were highly correlated (i.e., Pearson correlation coefficient $|r| \geq 0.7$, $P \leq 0.05$; see details Appendix I Table 1); these correlations were confirmed using principle component analysis (PCA; SPSS). For each group of highly correlated variables, a single representative variable was chosen (typically the variable that was plotted furthest from the origin in the factor map of PCA analysis) for inclusion in subsequent multiple linear regression analyses as potential predictors of DOM character. This was to avoid collinearity in the statistical analysis, as well as, to minimize the possibility of over-interpreting the same process or source as a predictor, given that variables with strong correlations could represent a similar mechanism or common source. For example, percent forest within watersheds was negatively correlated with percent agricultural land ($r = -0.935$, $P < 0.001$), and percent forest was not included. Stream order and watershed area were correlated ($r = 0.728$, $P < 0.001$), and watershed area was not included. The concentrations of inorganic nutrient species including dissolved inorganic nitrogen (DIN) and orthophosphate were significantly correlated ($r = 0.901$, $P < 0.001$), and DIN concentration was retained to represent the influence of inorganic nutrients. Among the major cations, the concentrations of Na^+ and K^+ were strongly correlated ($r = 0.798$, $P < 0.001$), and K^+ was not included. Based on this criterion, eight predictors were included in

subsequent multivariate analyses (Appendix I Table 1). Despite the removal of certain highly correlated predictors prior to statistical analyses, their potential influences, as represented by those selected predictors, were taken into account during data interpretation.

Multiple linear regression analyses were conducted to identify the best sets of environmental and watershed predictors for stream water DOM character variation, including DOC concentration and a suite of DOM quality indices. For BDOC, only DOM quality indices were assessed as potential predictors to identify the labile component responsible for microbial lability. The stepwise method with the criteria of stepping as entry at $P \leq 0.05$ and removal at $P \geq 0.10$ was used, and the model with the lowest Akaike information criterion value (Akaike 1998) was selected as the final model for each dependent variable. Error assumptions, including constant variance, linearity, and normality, were examined using residuals versus fitted plots and Q-Q plots. The redundancy analysis (RDA) was performed using the selected environmental predictors in regression models as explanatory variables to further assess their combined influences on stream water DOM character (Canoco). RDA is a constrained ordination that determines the importance of one set of variables (in our case the environmental variables) in explaining the variation in another set of variables (DOM character) and graphically illustrates the correlations with an ordination diagram. Partial redundancy analysis (pRDA) was used to remove the effects of (a) land use and (b) climate to selectively evaluate the influence of specific environmental predictors.

2.4 Results

2.4.1 DOC concentration and DOM absorbance indices

DOC concentrations averaged 2.10 ± 0.16 mg/L (mean \pm standard error), which is at the lower end of DOC concentration range reported for streams and rivers (i.e., 0.5 to 50 mg/L;

Mulholland 2003). S_R averaged 0.93 ± 0.04 , and $SUVA_{254}$ values averaged $3.23 \pm 0.18 \text{ L mg}^{-1} \text{ m}^{-1}$ (the range of these variables can be seen in Appendix I Table 2). S_R and $SUVA_{254}$ provide approximate estimations of DOM quality, whereby DOM with higher molecular weight (MW) and aromaticity has gentler spectral slopes (lower S_R) and higher light absorption (higher $SUVA_{254}$) under the UV range (Weishaar et al. 2003; Helms et al. 2008). Helms et al. (2008) reported S_R values for contrasting water types, from 0.69 for high MW DOM in wetlands to 3.09 for low MW DOM in coastal oceans. $SUVA_{254}$ values for natural water DOM showed a large range of variability, from 0.6 for highly aliphatic DOM in the Pacific Ocean, to 5.3 for highly aromatic DOM from the Ogeechee River, and to values greater than 10 for highly aromatic DOM in streams in New Mexico and Minnesota (Weishaar et al. 2003; Jaffé et al. 2008; Macdonald and Minor 2013). The range of S_R and $SUVA_{254}$ values for our samples suggests DOM in the study streams is dominated by aromatic, high MW compounds. It is also noted that high iron ($>0.5\text{mg/L}$) and nitrate ($>40 \text{ mg/L}$) concentrations can bias $SUVA_{254}$ values through absorbing light at the wavelength of 254nm (Weishaar et al. 2003). In our samples, dissolved iron and nitrate concentrations were below 0.3mg/L and 12.5mg/L , respectively, suggesting little influence on $SUVA_{254}$ reading.

2.4.2 Fluorescence indices and the source assignment of fluorescence components

Fluorescence index (FI) is an indicator commonly used to aid in identifying DOM sources, and FI values typically range near 1.2 for terrestrially dominated DOM and 1.8 for microbially dominated DOM (Jaffé et al. 2008). HIX measures the humification degree of DOM, for which a value <4 indicates the dominance of autochthonous DOM and a value >16 suggests the dominance of terrestrial DOM (Huguet et al. 2009). Our samples had FI values

averaging 1.66 ± 0.01 , and HIX averaged 5.18 ± 0.30 indicating that DOM was derived from a mixture of microbial and terrestrial organisms (Appendix I Table 2).

EEM analysis yielded four fluorescence components, C₁ to C₄ (Fig. 2.2; Table 2.2). C₁ and C₃ accounted for $50.4 \pm 0.8\%$ and $12.0 \pm 0.3\%$ of total fluorescence, respectively, and they both represented terrestrially-derived, humic-like compounds that have been observed widely within aquatic environments across geographic regions (Chen et al. 2010; Lu et al. 2015b). C₂ comprised $19.9 \pm 0.4\%$ of total fluorescence and was assigned as microbially-derived, humic-like compounds. C₄ ($17.7 \pm 1.1\%$) showed spectra similar to protein-like (tyrosine- or tryptophan-like) compounds that are thought to represent microbially-derived autochthonous DOM (Coble et al. 1998; Cory and McKnight 2005; Stedmon and Markager 2005; Chen et al. 2010; Appendix I Table 2). The source assignment of fluorescence components is further supported by the significant correlations between their relative abundance and absorbance-based source indices. The percentage contribution of C₁ and C₃ were positively correlated with SUVA₂₅₄ (C₁: $r=0.521$, $P=0.004$; C₃: $r=0.493$, $P=0.008$), confirming that they represented terrestrial humic compounds, whereas percent C₄ was negatively correlated with SUVA₂₅₄ ($r=-0.469$, $P=0.012$), confirming the autochthonous origin of C₄. Percent C₂ did not show correlations with SUVA₂₅₄ but had a negative correlation with S_R ($r=-0.446$, $P=0.017$), indicating it represented compounds with relatively high MW.

Table 2.2. Characteristics of the four fluorescence components identified by PARAFAC and the attributed sources.

Component	Excitation maximum wavelength (nm)	Emission maximum wavelength (nm)	Similar fluorescence components identified in previous studies				Present study	
			Coble et al. 1998	Stedmon and Markager 2005	Cory and McKnight 2005	Yamashita et al. 2010		
C ₁	<258 (324)	445	C	4	SQ2 or SQ3	C1	Terrestrial DOM	humic-like
C ₂	<258 (309)	386	M	6	Q3	C4	Microbial DOM from soils	humic-like
C ₃	387 (267)	488	C	2	SQ1	C5	Terrestrial DOM	humic-like
C ₄	273	329	B or T	8	C13	C7	Protein-like DOM	

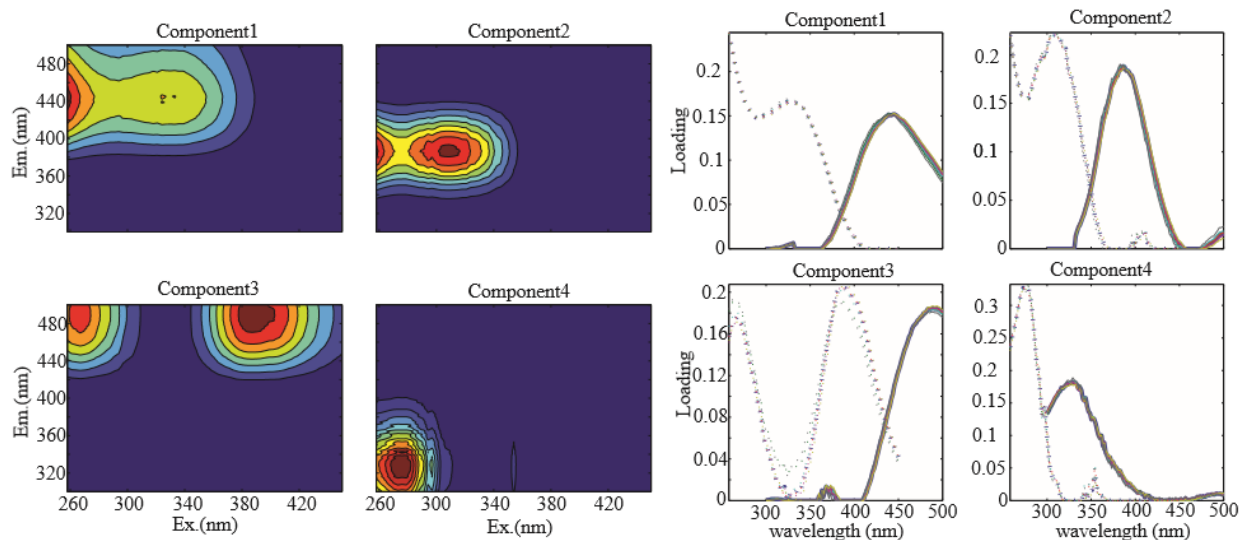


Figure 2.2. Excitation-emission spectra and loading of the four fluorescence components (C₁–C₄) identified by the parallel factor analysis of DOM samples from the Bear Creek Watershed.

2.4.3 Biodegradable DOC and FDOM

In this study, percent BDOC from stream water was greater in watersheds with higher percentages of agricultural land use (Fig. 2.3). Specifically, percent BDOC averaged $11.7 \pm 1.3\%$ ($k'_{\text{DOC}}=0.0062 \pm 0.0010 \text{ day}^{-1}$) for the two streams with <25% agricultural land, $16.3 \pm 2.6\%$ ($k'_{\text{DOC}}=0.0090 \pm 0.0015 \text{ day}^{-1}$) for the two streams with 38–40% agricultural land, and $19.8 \pm 0.4\%$ ($k'_{\text{DOC}}=0.0111 \pm 0.0003 \text{ day}^{-1}$) for the two streams with >50% agricultural land (Fig. 4a). The Wilcoxon test showed significant differences in %BDOC ($P=0.028$) between watersheds with <25% agricultural land and those with >50% agricultural land (Fig. 2.3). In comparison to the degradation pattern of DOC, where the majority of DOC was degraded between day 5 to 12 (Fig. 2.4a), fluorophore degradation was more rapid. All biodegradable fluorophores were removed within the first five days during the incubations (Fig. 2.4b). Among the four DOM components, C₄ was the most labile, exhibiting the largest percentage decrease in fluorescence intensity ($54.4 \pm 1.1\%$ for C₄ vs. $47.3 \pm 0.7 - 53.5 \pm 0.8\%$ for C₁, C₂ and C₃), as well as the

highest rate of biodegradation ($k'F_{Max}$: $0.0291 \pm 0.0003 \text{ day}^{-1}$ for C_4 vs. $0.0236 \pm 0.0002 - 0.0275 \pm 0.0007 \text{ day}^{-1}$ for C_1 , C_2 and C_3 ; Fig. 2.4b).

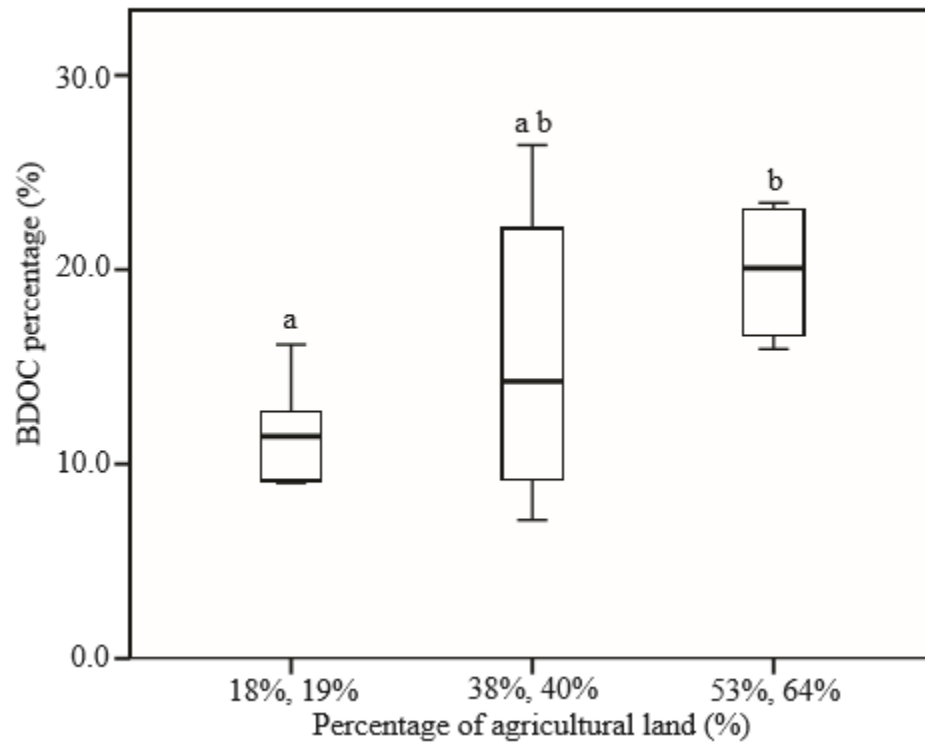


Figure 2.3. Box plot comparing percent BDOC from streams draining watersheds with low (<25%), medium (38–40%) and high (>50%) percentages of agricultural land within watershed. Different letters above the boxes signify significant differences in %BDOC between land use types detected by the Wilcoxon test.

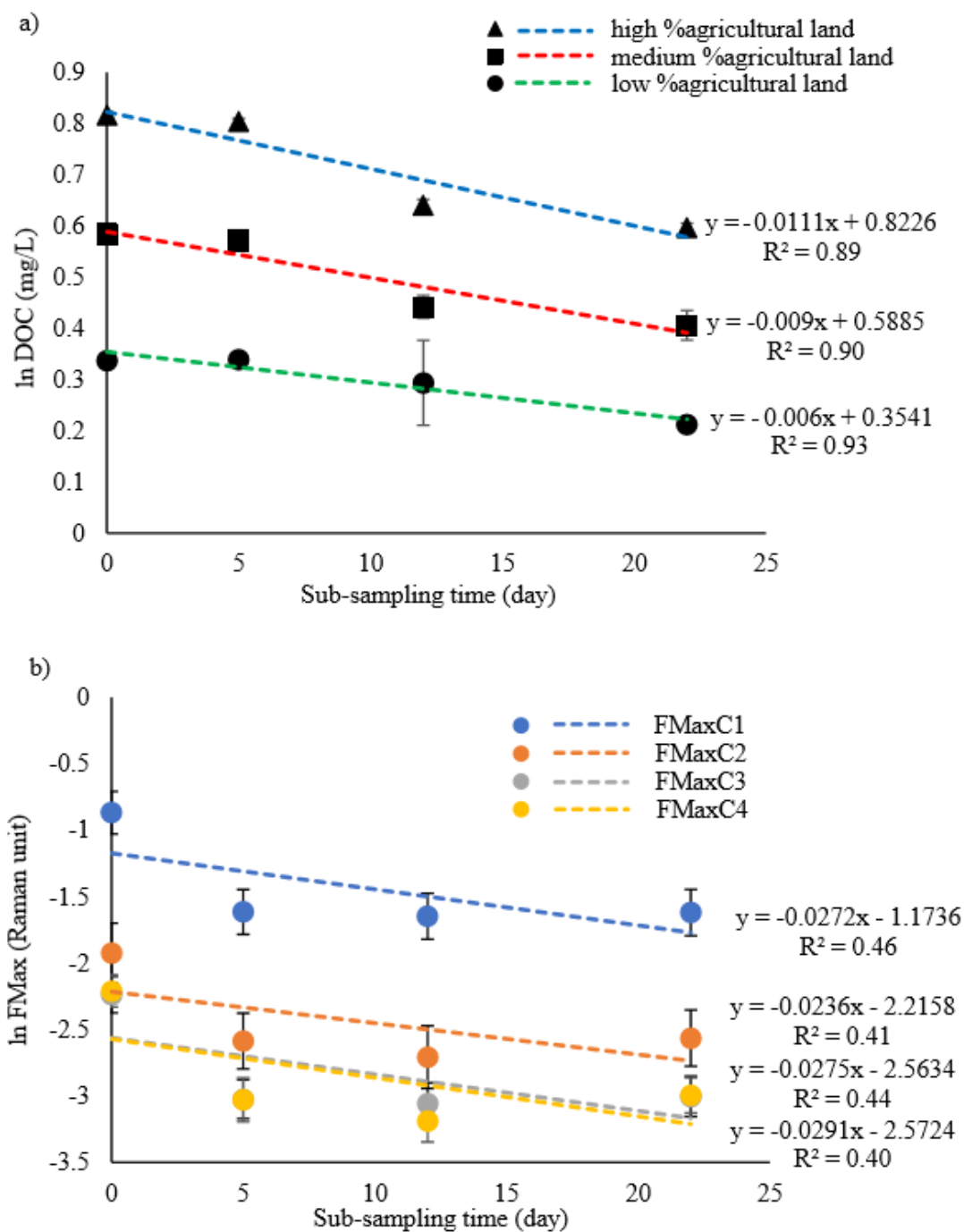


Figure 2.4. Changes in (a) natural log of DOC concentration and (b) natural log of the fluorescence intensities of DOM components (F_{Max} of C_1 to C_4) over the course of the 22-day incubations of DOM samples collected from the Bear Creek Watershed in May 2014. Error bars represent standard error.

2.4.4 Predictors of DOM amount and composition

Based on stepwise linear regression models, DOC concentration was best predicted by Na^+ concentration, temperature, and $\text{API}_{15\text{d}}$, which collectively explained 83.4% of the total variance (Table 2.3). Linear regression models show that the variance in DOM quality indices can also be reasonably explained by a suite of climate and watershed variables (Table 2.3). FI (22.3% of total variance) and HIX (25.9% of total variance) were best predicted by $\text{API}_{15\text{d}}$ and stream order, respectively. Percent C_1 (24.6% of total variance) and percent C_4 (28.1% of total variance) were negatively correlated ($r=-0.962$, $P<0.001$), and they were both best explained by a combination of stream order and $\text{API}_{15\text{d}}$. Percent C_2 (41.3% of total variance) was best explained by sodium concentration and percent agricultural land, whereas percent C_3 (28.6% of total variance) was best predicted by sodium concentration. For percent BDOC, % C_3 was selected as the strongest predictor by the stepwise model, which explained 68.6% of total variance.

Results from the RDA analysis were largely consistent with those from the linear regression models. Each of the six predictors contributed to the explanation of the variance of the response variables describing DOM character (Fig. 2.5a). The two axes, combined, accounted for 38.3% of total DOM character variance. Percent agricultural land was positively correlated with DOC concentration as well as the contribution of microbially- and terrestrially-derived, humic-like DOM (indicated by % C_2 , HIX and % C_1) but negatively with the contribution of low MW, protein-like compounds (indicated by S_R and % C_4). An RDA analysis with percent agricultural land substituted by percent pasture land (the dominant type of agricultural land) showed a similar pattern. Stream order exhibited an opposite influence, that is, decreasing the relative abundance of humic-like DOM but increasing percent protein-like, autochthonous DOM. $\text{API}_{15\text{d}}$, Na^+ , DIN

and temperature all positively influenced DOC concentration and the proportions of microbially-derived DOM (FI and %C₂) and negatively influenced the contributions of terrestrially-derived, humic-like DOM with high aromaticity (SUVA₂₅₄, %C₃ and %C₁). The relative influences of watershed *vs.* climatic drivers were further assessed via pRDA analysis, which reduced the explained variance but led to a pattern similar to the RDA results (Fig. 2.5b, 2.5c).

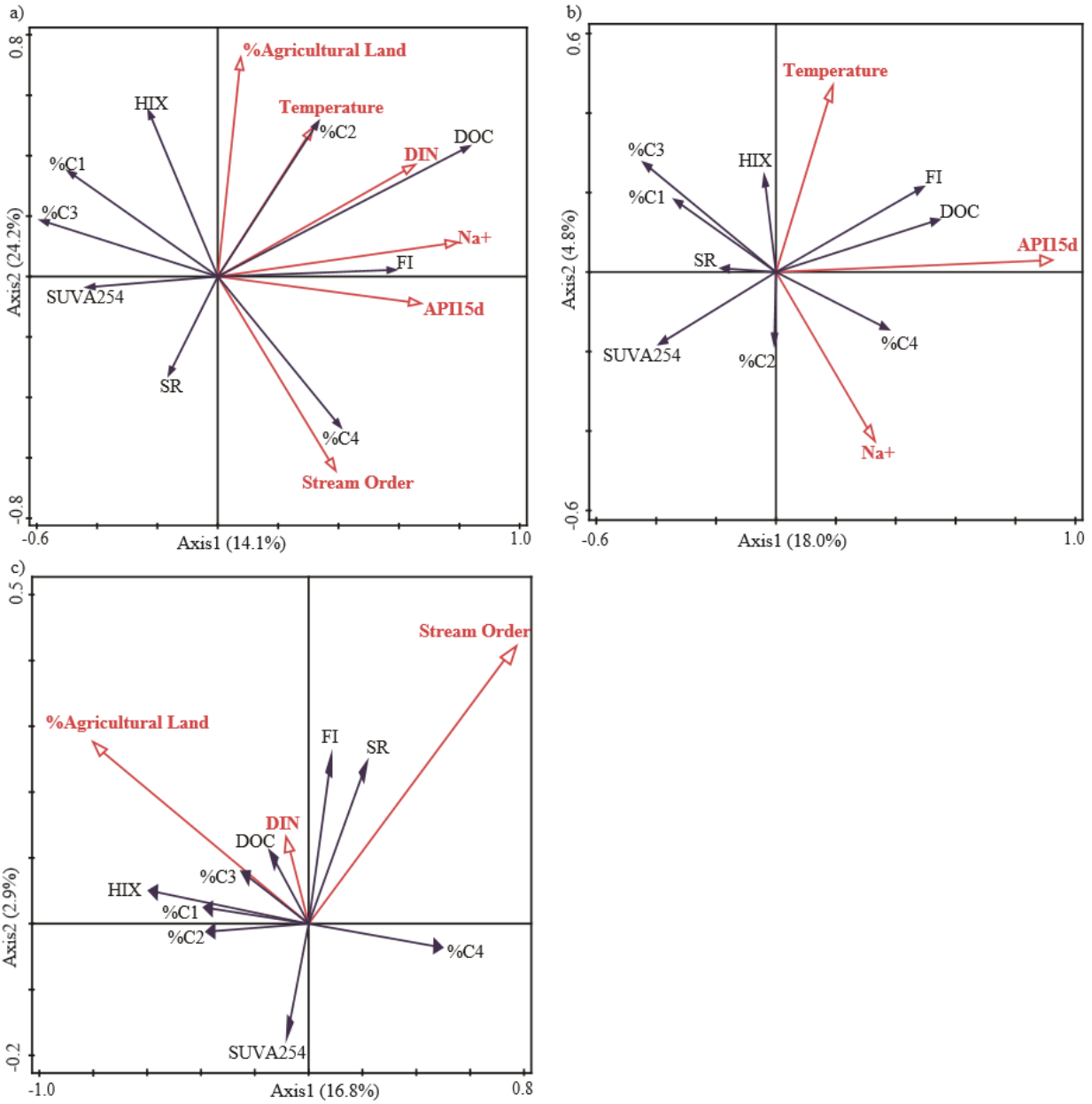


Figure 2.5 (a) Redundancy analysis of climatic and watershed variables as predictors for stream water DOM properties in the Bear Creek watershed; (b) partial redundancy analysis of climate variables, including Temperature, API_{15d} and Na⁺ concentration, as predictors for stream water DOM properties in the Bear Creek watershed; (c) partial redundancy analysis of watershed variables, including %Agricultural Land, Stream Order and DIN concentration, as predictors for stream water DOM properties in the Bear Creek watershed.

Table 2.3. Stepwise regression models predicting stream DOC concentration (n=29), DOM quality indices (HIX, n=29; FI, n=29, DOM composition (%C₁, %C₂, %C₃ and %C₄, n=28), and DOM bioreactivity (n=6). The selection of final stepwise model was based on acquiring the lowest Akaike information criterion value of each dependent variable.

Variable	Adjusted R Square	P value	Model	%Explained by each explanatory variable	AIC
DOC concentration	0.834	<0.001	$=-0.950+0.054(\text{Na}^+)+0.115(\text{temperature})+0.084(\text{API}_{15\text{d}})$	(44.2%), (29.3), (9.9%)	-58.309
FI	0.223	=0.006	$=1.606+0.009(\text{API}_{15\text{d}})$	(22.3%)	-166.898
HIX	0.259	=0.003	$=7.543-0.905(\text{stream order})$	(25.9%)	20.919
%C ₁	0.246	=0.011	$=58.301-1.745(\text{stream order})-0.465(\text{API}_{15\text{d}})$	(12.3%), (12.3%)	73.009
%C ₂	0.413	<0.001	$=17.027+0.103(\text{Na}^+)+0.044(\% \text{ agricultural land})$	(31.0%), (10.3%)	27.372
%C ₃	0.286	=0.002	$=13.217-0.104(\text{Na}^+)$	(28.6%)	16.685
%C ₄	0.281	=0.006	$=6.409+2.448(\text{stream order})+0.682(\text{API}_{15\text{d}})$	(13.3%), (14.8%)	88.911
%BDOC	0.686	=0.026	$=63.449-3.529(\% \text{ C}_3)$	(68.6%)	14.178

2.5 Discussion

2.5.1 DOC concentration vs. DOM quality

In our samples, DOC concentration showed changes coincident with the contributions of microbial DOM — DOC concentrations were positively correlated with FI values ($r=0.475$, $P=0.009$) as well as with the relative abundance of C_2 ($r=0.438$, $P=0.020$), and negatively correlated with $SUVA_{254}$ ($r=-0.371$, $P=0.047$; Fig. 2.5a). None of the other DOM quality proxies showed significant correlations with DOC concentrations. Fluorescence component C_2 has been widely observed within aquatic environments (Stedmon et al. 2003; Cory and Mcknight, 2005; Lu et al. 2013, 2014; Singh et al. 2017), with the general consensus that C_2 represents a microbial degradation product. However, it remains equivocal whether C_2 was derived from soil microbes (i.e., allochthonous) or produced within streams (i.e., autochthonous). In our samples, the proportion of C_2 was best predicted by the concentration of sodium in streams and percent agricultural land, with 31.0% variance explained by Na^+ (Table 2.3). Sodium in streams is derived mainly from soil weathering, and its abundance has been used for indicating inputs of soil solutes to streams (Cerling et al. 1989; Viers et al. 2000). Therefore, the strong correlation between sodium vs. % C_2 indicates that C_2 was derived primarily from microbes in soils. In addition, the strong correlation between % C_2 vs. DOC concentration suggests that microbial DOM produced and released from soils regulated the variability of DOC concentrations in streams. In other words, although the two terrestrial fluorescence components (C_1 and C_3) dominated the fluorescent DOM pool (combined percent contribution > 54.2%), they served more as a baseline of the DOC pool, whereas the less abundant microbially-sourced, humic compounds were responsible for spatial and temporal variability of DOC concentrations in the study streams.

2.5.2 Climatic drivers: temperature and soil-stream hydrological connectivity

Temperature had positive impacts on DOC concentration and the contributions of microbial, humic-like DOM (%C₂) in the study streams (Fig. 2.5a; Table 2.3). This pattern can be explained by that high temperature facilitated the production and release of DOC in soils through enhanced microbial extracellular enzyme activity (K'OH et al. 1996; Götter et al. 1996; Andersson et al. 2000; Park and Matzner 2003). Although temperature rises also enhance the biodegradation rate of DOC in soils, the amount removed is often lower than the amount produced due to accelerated production (Qualls and Haines 1992; Götter et al. 1996). Indeed, nearly all laboratory studies observed positive correlations between temperature and DOC concentration of soil solutions (Kalbitz et al. 2000). In field studies with large spatiotemporal variability, other factors such as organic litter availability and precipitation may sometimes obscure the impacts of temperature (Kalbitz et al. 2000). Overall, temperature is expected to have positive impacts on the concentration of DOC in stream water and soil leachates across ecosystem types and temporal scales (e.g., Götter et al. 1996; Andersson et al. 2000; Raymond and Saiers 2010; Ritson et al. 2014).

In addition to temperature, DOC concentration and microbial DOM contribution were also positively influenced by API_{15d} and Na⁺ (Fig. 2.5a; Table 2.3). These two variables can all be considered as flow-related proxies that are indicative of soil-to-stream hydrological connectivity. API is a proxy used to measure antecedent hydrological conditions and soil moisture (e.g., Saxton and Lenz 1967; Blanchard et al. 1981), and similar to Na⁺ concentration, greater API values usually correspond to larger water fluxes from watersheds into streams (James and Roulet 2007). Our results, therefore, demonstrate the importance of soil-stream hydrological connectivity on stream water DOM character. Soil DOM, especially the newly

produced pool, generally exists in an adsorbed phase but can frequently be mobilized through water leaching and flushing (Park and Matzner 2003). Our data show that stronger hydrological connectivity associated with more abundant antecedent precipitation led to a greater amount of microbially-derived, humic organic compounds being transported from soils to streams.

Although a large number of studies have reported enhanced watershed export of DOC and humic compounds during precipitation events (Vidon et al. 2008; Lambert et al. 2013; Hu et al. 2016), the strong influence of flow-related proxies we observed demonstrate that this effect can persist after the passage of storm flows through streams. Indeed, antecedent hydrological conditions have been found as a significant driver for DOM character variability in various streams (Inamdar et al. 2004; Vidon et al. 2008; Lambert et al. 2013; Mehring et al. 2013; Guarch-Ribot and Butturini 2016). Hydrological events shift flow paths from deep, groundwater flow to shallow soils enriched with organic carbon and humic compounds (Vidon et al. 2008; Lambert et al. 2013; Hu et al. 2016), and this effect can be sustained by a rise of water table that enables continuous mobilization of shallow soil organic compounds. Groundwater table was not determined in this study. Although very few studies have simultaneously evaluated groundwater table fluctuations and stream water DOM character, their findings corroborate our interpretation. For example, Lambert et al. (2013) observed positive shifts in stream water $\delta^{13}\text{C}$ -DOC values when stream discharge returned to baseflows while groundwater table remained high. They explained this pattern as a result of continuous DOC release from soils microbes, and the process was initiated by soil rewetting through rainfalls but sustained by a shallow groundwater table afterward.

2.5.3 Watershed drivers

The effects of watershed attributes on stream water DOM were also identified. Stream order had a negative influence on the contribution of humic DOM compounds (HIX and %C₁) and a positive influence on the proportion of protein-like DOM (%C₄) (Fig. 2.5a; Table 2.3). This observation demonstrates an increasing contribution of protein-like, autochthonous DOM, accompanied by a decline in percent contribution of allochthonous DOM, from low-order to high-order systems. Such a pattern is consistent with a general conceptual trend describing DOM transformations along fluvial continuum. That is, in-stream biogeochemical processing becomes increasingly important from headwaters to large downstream rivers, due to an increased open-canopied area that stimulates photosynthetic microorganisms as well as a longer residence time allowing more thorough biological processing of DOM (Thorp and Delong 1994; Creed et al. 2015). The canopy coverage estimates of the study sites did not vary significantly across stream order (Kruskal-Wallis test: P=0.751 for the values based on 2011 NLCD tree coverage data and P=0.300 for the values determined using a spherical crown densiometer). Stream water residence time was unknown for our study watersheds, and topographic slope gradient was used to provide an approximate measurement, yielding values that did not vary as a function of stream order (Kruskal-Wallis test: P=0.988). However, it needs to be noted that these approximations were only rough estimates of light penetration and water residence time, and hence the regulation mechanism of stream order on DOM in the study sites cannot be determined with our data.

With increases in percent contributions of agricultural land use, stream water showed increases in DOC concentration and humic DOM compounds (%C₁, %C₂, and HIX) and decreases in %C₄ and S_R (Fig. 2.5a; Table 2.3). The changes associated with agricultural land use remained after the removal of the effect of stream order because similar correlations were found

when evaluating only the third-order streams (e.g., %C₂ vs. percent agricultural land, $r=0.692$, $P=0.009$; %C₄ vs. percent agricultural land, $r=-0.635$, $P=0.020$). These effects of agricultural land use can be explained by preferential mobilization of topsoil DOM from agricultural watersheds. Compared to deep soils, shallow soils are usually more enriched in DOC and humic-like DOM compounds, owing to preferential adsorption of high MW, humic-like DOM compounds by mineral particles when cycling downward (Aitkenhead-Peterson et al. 2003; Inamdar et al. 2012; Kaiser and Kalbitz 2012; Hu et al. 2016). It has been widely observed that agricultural soils are more susceptible to erosion than forest soils (e.g., Celik 2005), which accelerates open-air oxidation of topsoil OM and subsequent erosional export (Graeber et al. 2012). This interpretation is further supported by increasing percent contributions of C₁ in agricultural streams (Fig. 2.5a) because C₁ has been identified as a more reduced semiquinone fluorophore produced from the oxidation of other organic compounds such as lignin (Cory and McKnight 2005). Likewise, Williams et al. (2010) noted an enrichment of reduced DOM in agricultural streams and attributed this change to enhanced bacterial production and OM oxidation in agricultural soils relative to forest soils. Besides, agricultural soils are generally more compact than forest soils, due to livestock stomping, heavy instrument use, and reduced rooting networks (Lee and Lauenroth 1994; Reiners et al. 1994; Celik 2005; Harden 2006). These processes collaboratively reduce retention time and infiltration rate of soils (Lee and Lauenroth 1994; Reiners et al. 1994; Celik 2005; Harden 2006; Zimmermann et al. 2006) and in consequence, lead to shorter and shallower soil-to-stream flow paths in agricultural watersheds that further facilitate the export of organic compounds from a shallower soil depth.

The biodegradability of DOC was found higher in streams with higher percentages of agricultural land within watersheds (Fig. 2.3). Similar findings have been widely reported by a

number of studies, although a variety of mechanisms have been proposed to explain these observations, including higher stream water inorganic nutrient concentrations (Wilson and Xenopoulos 2009; Williams et al. 2010), greater proportions of labile, protein-like compounds (Fellman et al. 2008), or a more reduced state of DOM (Williams et al. 2010). In contrast, Lu et al. (2013) found stream water DOC biodegradability did not vary as a function of agricultural land use but was influenced more by temperature variation and the associated diagenetic degrees of organic compounds. For our samples, neither dissolved nutrients (DIN and orthophosphate) nor percent protein-like DOM (%C₄) was a good predictor for %BDOC. Instead, %BDOC was negatively predicted by the contributions of terrestrial fluorophores (%C₃) (Table 2.3). The correlations between %C₁ vs. %BDOC and %C₂ vs. %BDOC were not significant (P=0.154 and 0.137, respectively) but yielded absolute r values greater than 0.6 (r=-0.660 and 0.681, respectively). These data together suggest, on one hand, the refractory nature of terrestrial, humic-like compounds to biodegradation, and on the other hand, ‘fresh’ microbial compounds exported from topsoils through shallower and shorter flow paths were responsible for the greater microbial lability of DOM in agricultural streams. In agreement with this interpretation, previous studies reported large reduction in DOM bioreactivity during the percolation of stream water through soil profiles (Kalbitz et al. 2005; Kaiser et al. 2007, Sanderman and Amundson 2009), and the diagenetic status, or ‘freshness’, was responsible for the variability of DOM biodegradability in streams (Lu et al. 2013).

2.5.4 Climatic vs. watershed drivers on stream water DOM in lotic ecosystems — Is human land use a significant player?

The goal of this study was to evaluate the effects of agricultural land use on DOM character in lotic ecosystems. By comparing the statistical contributions of climatic vs. watershed

drivers in the explanation of DOM character variability, we found 22.8% of total variance of stream water DOM proxies can be explained by the climatic drivers (Fig. 2.4b), in comparison to 19.7% of total variance explained by watershed drivers (Fig. 2.4c). However, these values cannot be taken literally as evidence that climatic drivers and agricultural land use played comparable roles in controlling DOM at our study sites, given the following considerations: (1) the sampling sites were from two nested watersheds, which was reflected in our statistical analyses, and (2) the assignment of each variable as a climatic or land use driver is, at times, ambiguous. For example, DIN was used to represent an agricultural land use driver in the pRDA analysis (Fig. 2.5c), but the mobilization of DIN may also rely on soil-stream hydrological connectivity driven by precipitation. Similarly, sodium mobilization could vary with both land use and hydrology. In addition, interactions between climatic and land use drivers can be expected, such as a higher frequency of irrigation (agricultural land use driver) may correlate to a lower API (climate driver). Taken in total, our data showed that both climate and land use exerted important influences on stream water DOM, with agricultural land use increasing DOC concentration, the contribution of humic-like DOM and DOM biodegradability (Fig. 2.5a; Table 2.3).

Over the past few years, there has been rapidly growing literature on the effects of agricultural land use on DOM within freshwater environments, yielding varied results across broad spatiotemporal scales (see Table 2.4 for examples). These inconsistent or even contradictory findings show the complexity of environmental and watershed drivers in regulating terrestrial-aquatic connectivity and DOM properties in streams and rivers. A few studies reported findings similar to the results from the present study. For example, Graeber et al. (2012) observed agricultural land increased DOC concentrations and the proportions of humic DOM in headwater streams, by comparing them to nearby streams draining forested watersheds. These

findings were thought to be a result of remobilization and oxidation of OM from either former forest soils or a surface agricultural soil layer enriched with humic-like DOM. In comparison, a number of previous studies did not observe systematic impacts on DOC concentrations but reported higher proportions of autochthonous, protein-like DOM in streams more influenced by agricultural land use (e.g., Williams et al. 2010; Lu et al. 2014; Fuß et al. 2017). These observations were attributed to factors such as nitrogen nutrient enrichment from agricultural runoff, increased light penetration, and/or animal grazing (Wilson and Xenopoulos 2009; Fuß et al. 2017; Singh et al. 2017). Fewer investigations have been conducted in large rivers, where heterogeneous watershed sources make it more challenging in relating land use to riverine DOM in a quantitative manner. Hu et al. (2016) sampled DOM over a 160 km river reach in a large agricultural basin in the northwestern China, where they found that agricultural land use (assessed by percent agricultural land within a 1 km buffer) had a positive correlation with percent protein-like DOM at low flows but with percent humic like-DOM at high flows. These researchers concluded that agricultural activities enhanced riverine microbial activities at baseflow and accelerated soil erosion during storm events. Hanley et al. (2013) studied 17 rivers draining large watersheds (>1,000 km²) in North America and reported no correlation between agricultural land use and DOM character in rivers. Instead, they found significant positive impacts of wetland cover on DOC concentration and DOM aromaticity, and these impacts became less significant in rivers with long surface water residence time, highlighting the importance of autochthonous modification of DOM in large rivers.

Given these diverse literature findings, along with the results of this study, we conclude that land use impacts are likely significant and widespread but sensitive to temporal drivers and local watershed heterogeneity. Varied agricultural types and practices (Table 2.4) should be

responsible, at least partially, for these varied findings. In our study area, agricultural practices, overall, were relatively simple and uniform, with little to no irrigation, subsurface drainage, or tillage. An exception, however, existed in the watersheds of BC3 and BC5, where surface water-fed, central-pivot irrigated pastures accounted for 10% of agricultural land in BC3 and 3% in BC5. Since irrigation artificially increases soil-stream hydrological connectivity, these irrigated pastures could have had disproportionately large influences on stream water DOM. Samples from BC5 did not show exceptionally high amount of DOC or %C₂; BC3 showed higher DOC concentrations and %C₂, but it also represented a site with a larger proportion of agricultural land. As such, our data cannot distinguish the influence of irrigated vs. non-irrigated pasture lands. Furthermore, it must be acknowledged that even combining climatic and land use drivers, more than 50% of total variance of DOM proxies remain unexplained (Fig. 2.5a). Other land types, although accounting for a smaller fraction than agricultural land and forest, may also alter stream water DOM character variability. For example, urban land may increase the contributions of protein-like compounds in receiving streams (e.g., Hosen et al. 2014, Parr et al. 2015), and the presence of reservoirs may increase the residence time and thus the processing of terrestrial DOM as well as the production of autochthonous DOM (e.g., Larson et al. 2007). These heterogeneities of agricultural practices and land use should be expected as the ‘usual’ status of most watersheds, and therefore incorporating the human land use – DOM linkage into management decisions and practices will be challenging, perhaps requiring studies that are watershed-specific or at least limited to a small geographic region. Future research efforts on scaling up results from various case studies are essential for understanding the associated environmental and ecological ramifications (e.g., carbon cycles, ecosystem metabolism) at a regional to global scale, but the success will require more empirical studies assessing land use vs.

aquatic DOM across a range of environmental settings (e.g., climatic zones, hydrology, lithology) and temporal scales (month to year to decade).

Table 2.4. Representative examples of studies examining the effects of agricultural land use on freshwater DOM properties.

System	Agricultural land use characterization	DOM responses to agricultural land use*	References
River	%cropland in whole watershed	TON content↑; TOC concentration↑; TOC:TON ratios↓	Mattsson et al. 2005
Stream	%arable cropland in whole watershed	spring DOC export↓	Aitkenhead-Peterson et al. 2007
River	%cropland in whole watershed	DOC concentrations↑; aromatic carbon content→	Chow et al. 2007
Stream	%pasture land in whole watershed and riparian zone (90-m stream buffer), with non-C:P↑; N:P↑; C:N↓ point-source organic waste inputs from poultry, beef and hay production	inDOC concentrations↑; DON concentrations↑; DOP concentrations↑;	Molinero and Burke 2009
Stream	%cropland in riparian zone (100-m stream buffer)	DOC concentrations→; %microbial DOM↑; DOM structural complexity↓	Wilson and Xenopoulos 2009
Stream	% monoculture cropland, mixed cropland, and forage crop and idle land in riparian zone (100-m stream buffer)	DOC concentration↓; %microbial, reduced DOM↑; DOM reactivity for microbes↑	Williams et al. 2010
Stream	%pasture (annually rotated between warm-season and cold-season grasses) and cropland (alternated between corn and soybeans) in whole watershed	DOC photodegradability↓; DOC biodegradability→	Lu et al. 2013
Stream	%pasture and cropland in whole watershed	%terrestrial DOM↓; %autochthonous DOM↑; DOC ages→	Lu et al. 2014
Stream	%cropland in whole watershed (>76% covered by cropland with tile drained and conventional tillage; pure crop or crop in rotation with green manure, oil-bearing seeds or protein-rich plants)	DOC concentration↑; %humic DOM↑; structural complexity↑; reduced DOM↑	Graeber et al. 2012
Stream	%pasture and cropland in whole watershed	DOC concentrations↑; humic DOM↑; structural complexity↑; reduced DOM↑	The present study
River	%cropland (mostly corn lands) within 1 km river buffer	DOC concentrations→; Humic-like DOM↑ in high flow; protein like DOM↑ in base flow	Hu et al. 2016
Stream	%agricultural land in whole watershed (arable land, permanent crops and pastures)	DOC concentrations↑; autochthonous DOM↑	Fuß et al. 2017
Lake	% cropland in whole watershed	Microbially derived humic DOM↑	Singh et al. 2017

*↑=increase; ↓=decrease; →=no apparent change

2.6 Conclusions

Understanding natural and anthropogenic drivers of DOM quantity and quality in streams and rivers is an important topic in regulating and predicting the responses of lotic ecosystems to changing climate and accelerated watershed development. Human land use has been shown as an important driver, but the relative importance and underlying mechanism remain unclear. The present study evaluated factors mediating stream water DOM character variability in temperate watersheds across a gradient of agricultural land use in the Southeast region of the United States. We found that temperature and antecedent precipitation condition positively influenced DOC concentration and the contribution of soil-derived, microbial humic DOM in streams. This finding indicates the importance of soil OM production, release, and mobilization in regulating DOM in the streams. The effects of watershed agricultural land use were also evident, including increases in DOC concentrations and the proportions of terrestrial plant-derived as well as microbially-derived humic compounds in streams. This observation reflects preferential mobilization of OM from top soils in agricultural watersheds due to accelerated oxidation, erosional transport and shallow flow paths. These results, along with a range of literature findings on this topic, demonstrate highly variable influences of watershed development on terrestrial-aquatic connectivity and biogeochemical dynamics in lotic ecosystems and thus the needs of more empirical studies for incorporating DOM into a science-based management framework.

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Appendix I

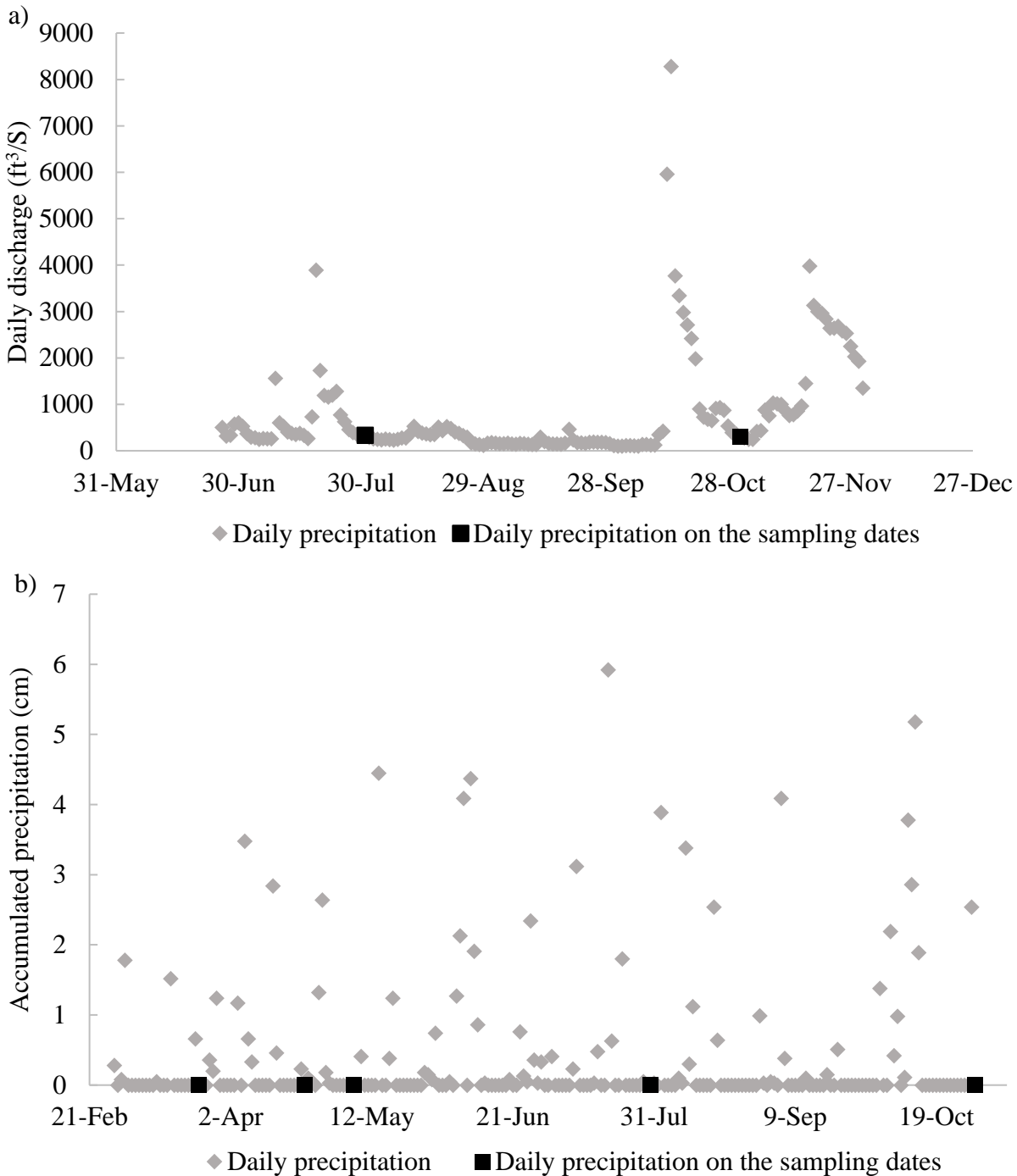


Figure 1. The daily discharge and precipitation data of the sampling area. Sampling dates are 25-Mar, 24-Apr, 8-May, 31-Jul and 31-Oct, 2014. Panel (a) is from USGS gauging station adjacent to the study area (USGS 03592500; <http://waterdata.usgs.gov/nwis>; data prior to 06/26/2014 are not available). Panel (b) is from a NOAA precipitation station adjacent to the study watersheds (RUXA1; <http://raws.wrh.noaa.gov>).

Table 1. Bivariate correlation across explanatory environmental variables. Variables that were included as potential DOM predictors in stepwise multiple linear regression and redundancy analyses were in bold.

		Temperature	API	Watershed area	Stream order	%Agricultural	%Forest	DIN	PO₄³⁻	Na⁺	Ca²⁺	Mg²⁺	K⁺
Temperature	Pearson Correlation	1	-.008	.134	-.105	.025	.073	.326	.360	.087	-.051	-.021	-.084
	Sig. (2-tailed)		.968	.487	.587	.896	.708	.084	.055	.655	.793	.913	.663
	N	29	29	29	29	29	29	29	29	29	29	29	29
API	Pearson Correlation	-.008	1	.063	-.060	.004	.060	.095	.046	.296	.548	.553	.135
	Sig. (2-tailed)	.968		.744	.757	.985	.755	.623	.815	.118	.002	.002	.484
	N	29	29	29	29	29	29	29	29	29	29	29	29
Watershed area	Pearson Correlation	.134	.063	1	.728	-.521	.683	.078	-.088	.013	-.161	-.201	.046
	Sig. (2-tailed)	.487	.744		.000	.004	.000	.686	.651	.945	.405	.297	.814
	N	29	29	29	29	29	29	29	29	29	29	29	29
Stream order	Pearson Correlation	-.105	-.060	.728	1	-.255	.346	.189	.109	.411	-.184	-.170	.465
	Sig. (2-tailed)	.587	.757	.000		.182	.066	.327	.574	.027	.338	.378	.011
	N	29	29	29	29	29	29	29	29	29	29	29	29
%Agricultural	Pearson Correlation	.025	.004	-.521	-.255	1	-.935	.217	.303	.434	.212	.245	.353
	Sig. (2-tailed)	.896	.985	.004	.182		.000	.259	.110	.019	.270	.201	.060
	N	29	29	29	29	29	29	29	29	29	29	29	29
%Forest	Pearson Correlation	.073	.060	.683	.346	-.935	1	-.056	-.178	-.305	-.185	-.211	-.329
	Sig. (2-tailed)	.708	.755	.000	.066	.000		.774	.356	.107	.337	.272	.082
	N	29	29	29	29	29	29	29	29	29	29	29	29
DIN	Pearson Correlation	.326	.095	.078	.189	.217	-.056	1	.901	.674	.070	.180	.349
	Sig. (2-tailed)	.084	.623	.686	.327	.259	.774		.000	.000	.716	.351	.063
	N	29	29	29	29	29	29	29	29	29	29	29	29
PO₄³⁻	Pearson Correlation	.360	.046	-.088	.109	.303	-.178	.901	1	.608	.039	.150	.286
	Sig. (2-tailed)	.055	.815	.651	.574	.110	.356	.000		.000	.841	.437	.132
	N	29	29	29	29	29	29	29	29	29	29	29	29
Na⁺	Pearson Correlation	.087	.296	.013	.411	.434	-.305	.674	.608	1	.322	.428	.798
	Sig. (2-tailed)	.655	.118	.945	.027	.019	.107	.000	.000		.089	.020	.000
	N	29	29	29	29	29	29	29	29	29	29	29	29
Ca²⁺	Pearson Correlation	-.051	.548	-.161	-.184	.212	-.185	.070	.039	.322	1	.986	.212
	Sig. (2-tailed)	.793	.002	.405	.338	.270	.337	.716	.841	.089		.000	.270
	N	29	29	29	29	29	29	29	29	29	29	29	29
Mg²⁺	Pearson Correlation	-.021	.553	-.201	-.170	.245	-.211	.180	.150	.428	.986	1	.267
	Sig. (2-tailed)	.913	.002	.297	.378	.201	.272	.351	.437	.020	.000		.162
	N	29	29	29	29	29	29	29	29	29	29	29	29
K⁺	Pearson Correlation	-.084	.135	.046	.465	.353	-.329	.349	.286	.798	.212	.267	1
	Sig. (2-tailed)	.663	.484	.814	.011	.060	.082	.063	.132	.000	.270	.162	
	N	29	29	29	29	29	29	29	29	29	29	29	29

Table 2. Mean values, standard errors and ranges of DOM character indices assessed in this study.

	Mean \pm standard error	Range
DOC concentration	2.10 \pm 0.16 (mg/L)	0.53 – 3.76 (mg/L)
S _R	0.93 \pm 0.04	0.55 – 1.57
SUVA ₂₅₄	3.23 \pm 0.18 (L mg ⁻¹ m ⁻¹)	2.30 – 5.51 (L mg ⁻¹ m ⁻¹)
FI	1.66 \pm 0.01	1.58 – 1.74
HIX	5.18 \pm 0.30	2.36 – 8.34
%C ₁	50.4 \pm 0.8%	44.4 – 57.8%
%C ₂	19.9 \pm 0.4%	16.4 – 24.2%
%C ₃	12.0 \pm 0.3%	9.8 – 15.3%
%C ₄	17.7 \pm 1.1%	8.2 – 27.0%

CHAPTER 3:

TERRESTRIAL INPUT OF DISSOLVED ORGANIC MATTER TO A SUBTROPICAL FORESTED STREAM ACROSS TIME SCALES FROM MINUTE TO YEAR

3.1 Abstract

The export of terrestrial dissolved organic matter (DOM) to streams plays a central role in mediating inland water carbon cycles, yet the patterns and underlying mechanisms across timescales remain poorly understood. Here, we examined high-resolution time series (every 15 minutes) of fluorescent DOM (fDOM), conductivity, and water depth over an entire year in a second-order forested stream in Alabama, USA. We also collected bi-weekly samples for the analysis of dissolved organic carbon concentration, DOM compositions via characterizing absorbance and three-dimensional fluorescence properties, and a suite of water source and water chemistry parameters including dissolved cations, dissolved nutrients, and stable oxygen and hydrogen isotopes of water. Over the entire year, the export of DOC can be reasonably explained by discharge (Adjusted R^2 : 0.956, $P < 0.001$). Our sensors recorded 25 storm events during the study period, and storm events that occurred only on 8% of days were responsible for 38% of the total export of DOC. The concentration-discharge (C-Q) hysteresis of DOM showed that storm event-scale DOM export was mediated by the durations of antecedent dry periods and storms. Specifically, hysteresis index decreased with the length of the antecedent dry period ($r = -0.434$, $P = 0.030$; $n = 25$) but increased with the discharge prior to the storm ($r = 0.484$, $P = 0.014$; $n = 25$) and the flushing index was positively correlated to the duration of the rising limb of a storm ($r = 0.404$, $P = 0.045$; $n = 25$). At the diurnal scale, DOC was negatively correlated with discharge

($r=-0.627$, $P=0.001$, $n=24$), but DOM compositions, indicated by percent contributions of fluorescence components, were not consistently correlated with discharge, suggesting that physical dilution and concentration due to evapotranspiration were the main drivers. At the seasonal scale, higher discharge in spring and winter and higher DOC concentration in winter resulted in greater DOC fluxes. Storms in winter and spring also showed significantly higher flushing index than in summer and autumn, suggesting a sufficient carbon supply from the watershed. Together, our results show that our study site is a biogeochemically simple system where the supply of watershed DOM is largely unlimited, and discharge dictates the export of DOM across time scales, with little influence from instream microbial and photochemical processing. These results give insights into the mechanisms underlying DOM exports from small forested streams within gentle watershed slope.

3.2 Introduction

Constraining the carbon budgets is important for better understanding and predicting climate change (Cole et al. 2007; Battin et al. 2009, Bradford et al. 2016). Soil carbon is more than two times of the carbon in the atmosphere (Lal, 2004, Bradford et al. 2016). Even the loss of a small proportion of soil carbon may increase atmospheric CO_2 concentration and accelerate global warming (Bradford et al. 2016). The carbon flux from terrestrial carbon pools into inland waters has received increasing attention in recent decades. Battin et al. (2009) estimated that inland waters transport, mineralize and bury a larger amount of carbon than the annual carbon accumulation on land (i.e., 2.7 Pg C yr^{-1} versus 2.2 Pg C yr^{-1} , respectively). After being mobilized from catchments into streams, carbon can become more biochemically reactive, and it is estimated about half of terrestrial carbon input was released as CO_2 and CH_4 into the atmosphere from streams and rivers (Cole et al. 2007; Hagedorn et al. 2008; Mehring et al.

2013). Thus, the loss of organic carbon from terrestrial ecosystems to inland waters redistributes organic carbon sinks and needs to be taken into account in carbon budget description and climate change mitigation strategies. At a regional scale, terrestrial organic carbon fuels the secondary production of heterotrophic biota and has significant and multifaced impacts on lotic ecosystems (e.g., Findlay and Sinsabaugh 2003; Cole et al. 2007). Therefore, it is essential to understand the timing, magnitude and mechanism of organic matter transportation from soils to streams.

DOM is the dominant form of dynamic carbon in terrestrial and aquatic ecosystems (e.g., Findlay and Sinsabaugh 2003; Cole et al. 2007). The generation and mobilization of DOM can be influenced by multiple factors such as physical and chemical characteristics of the soil, organic substance availability and quality, as well as temperature and precipitation. Previous studies found low dissolved organic carbon (DOC) concentrations in soil solutions and suggested that soil particles with larger surface areas and more abundant Fe and Al oxide/hydroxide minerals facilitate the adsorption of DOM (Moore et al. 1992; Gu et al. 1994). Multiple studies related seasonal changes in the concentration and flux of DOC from soil leachates to the availability of litterfall inputs across seasons (e.g., Casals et al. 1995; Currie et al. 1996). In a laboratory leaching experiment, Kuiters (1993) found that more DOM was released from deciduous leaves than from coniferous needles and suggested that litterfall types influenced the DOM characters in leachates. Higher temperature can increase DOC concentration in soils as multiple field studies found that DOC concentrations in soil leachates were higher in summer than winter (e.g., Guggenberger et al.; 1998; McDowell et al. 1998). Higher precipitation rates can decrease DOC concentrations in soil leachates, because of dilution and shorter contact time with soil during the DOM leaching process under fast water flux (McDowell and Wood, 1984). These factors act together, and it is often difficult to resolve their confounding effects. For

instance, the seasonal variation in organic substance availability also coincides with the variation in temperature. Moreover, the mobilization of DOM during precipitation is associated with shifts in hydrological flow paths through soils with different characters.

Discrete sampling strategies have been used in the majority of studies characterizing watershed export of DOM, and contradictory observations were reported among and within various sampling resolutions. Most studies reported positive correlations between discharge and stream water DOC concentration and suggested that precipitation increased the lateral carbon fluxes to streams. For instance, Hinton et al. (1997) and Buffam et al. (2001) collected samples at weekly to monthly intervals and reported positive impacts of stream discharge on DOC concentration. Raymond and Saiers (2010) studied storm events with sub-daily to daily sampling frequencies and found increase in DOC concentration during hydrological events. Others reported negative correlations between stream discharge and DOC concentration, which suggests that mobilization due to precipitation played a less critical role than DOM production and availability. For example, Hornberger et al. (1994) observed an exponential decline in the stream water DOC concentration across the snowmelt hydrograph owing to the dilution effect. Similarly, Mehring et al. (2013) and Humbert et al. (2015) reported negative correlations between DOC concentration and stream discharge with a sub-daily to daily sampling frequency. Along with watershed heterogeneity, sampling on different positions of hydrographs may be responsible for these contradictory observations, because DOM concentration-discharge (C-Q) correlations can behave differently on the rising versus falling limbs (Andrea et al. 2006). Thus, higher-resolution data are needed to better constrain terrestrial carbon fluxes to inland waters.

Indeed, high-resolution data collected using *in situ* water quality sensors have been increasingly employed in recent studies. Cyclic C-Q hysteresis patterns during storms provide

insights into the solute dynamics and catchment functions. However, the C-Q hysteresis of DOM can be highly heterogeneous between storms even within the same watershed. For instance, Butturini et al. (2008) investigated DOM C-Q relationships in a forested watershed for about four years and reported high variability and low predictability in DOM dynamics. Concentrations of DOC were found to increase, decrease or be decoupled from the river discharge during storm events in previous studies (e.g., Koehler et al. 2009; Saraceno et al. 2009; Grayson and Holden, 2012; Pellerin et al. 2012; Wilson et al. 2013). It was suggested that multiple hydrological factors, such as antecedent hydrological conditions and storm magnitude, may regulate DOM export pattern. For example, Guarch-Ribot and Butturini (2016) found that antecedent hydrological conditions including dry period, pre-event baseflow conditions, and the magnitude of the preceding event regulated the timing and magnitude of DOM export during storms. Fovet et al. (2018) compared the timing differences in the C-Q hysteresis of turbidity, nitrate (NO_3^-), and DOM during storms, and they suggested that storm magnitude influenced the surface and groundwater mixing ratios in stream discharge and contributed to the variations in the C-Q hystereses of DOM. The mixing ratio of solutes from watersheds is tightly coupled with the flow path and thus watershed characters such as land use and watershed slope gradient. Vaughan et al. (2017) performed a long-term study on agricultural, urban and forested watersheds and observed the impacts of land use on nitrate export but not on DOM export into receiving streams. In watersheds with gentle watershed slopes, counterclockwise DOM C-Q hystereses were commonly reported, indicating that DOM export was limited by transportation (e.g., Pellerin et al. 2011; Vaughan et al. 2017). By comparison, in a mountainous stream (high average watershed slope of 21°) in northern South Korean, Jeong et al. (2012) found clockwise DOM C-Q hysteresis and suggested that DOM export was limited by DOM supplies from the watershed.

In addition to hydrological and watershed variables, the response of DOM to river discharge shows seasonal variations (e.g., Pellerin et al. 2011; Grayson and Holden, 2012; Wilson et al. 2013). At a diurnal scale, autochthonous production in streams modifies the quantity and quality of DOM, which may conceal the influence of terrestrial DOM input processes (Spencer et al. 2007). Given the complexity of processes regulating the watershed export of DOM, high-resolution time series data are needed.

In this study, we recorded the DOM C-Q dynamics sub-hourly with *in situ* loggers for one year in a small subtropical forested watershed within the Gulf Coastal Plain in Alabama, USA. The high-resolution data were coupled with discrete samples collected about every two weeks, which were assessed for DOM quantity and quality (i.e., composition and bioreactivity) as well as other ancillary water chemistry parameters. Our objective was to evaluate the patterns and mechanisms controlling the export of terrestrial DOM across time scales at in a low order stream.

3.3 Materials and methods

3.3.1 Study area

The study site, Mayfield Creek, is a second-order (Strahler scale) coastal plain stream located within the Talladega National Forest in west-central Alabama, Southeast United States (Fig. 3.1). The creek flows into the Black Warrior River, which is part of an important inland waterway connecting the northern and southern parts of the state of Alabama for the transportation of coal, petroleum, and other goods. Mayfield Creek drains a 17.5 km² watershed with a gentle watershed slope (0.2° on average; Fig. 3.2a). The watershed is dominated by deciduous forest (> 98% of land use) comprised primarily of pine (e.g., longleaf, shortleaf, yellow, and loblolly) and hardwood (e.g., oak, hickory, sweetgum, dogwood). The entire

watershed is underlain by Cretaceous unconsolidated sands, the and benthic sediments in the stream channel are composed of fine sand and gravelly sand (Fig. 3.2b). Based on the Natural Resources Conservation Service Soils Database, the average depth of the soil layer in the study catchment is greater than 2 m. The surface soil is mainly dark yellowish brown sandy and flaggy loam (Fig. 3.2c), and the subsoil is yellowish red sandy and clay loam. The study area has a humid, subtropical climate with a mean annual temperature of 15°C and mean annual precipitation of 13 cm from 2014 to 2016 (data from the most adjacent weather station, Weather Underground KALTUSCA20, which is 21.8 km from the sampling site).

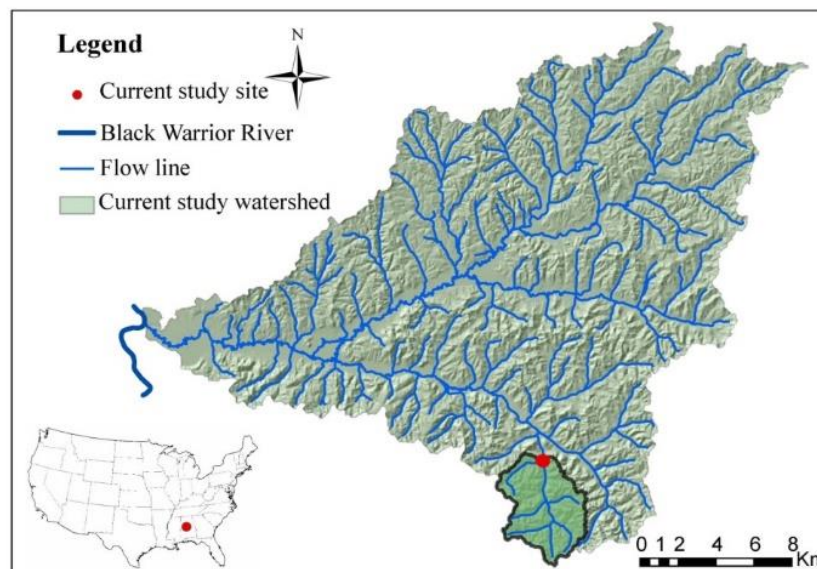


Figure 3.1. Location of the study site in the Talladega National Forest, Alabama, USA. Streams are indicated by blue lines, the watershed boundary is indicated by black lines, and the sampling site is denoted by the red dot.

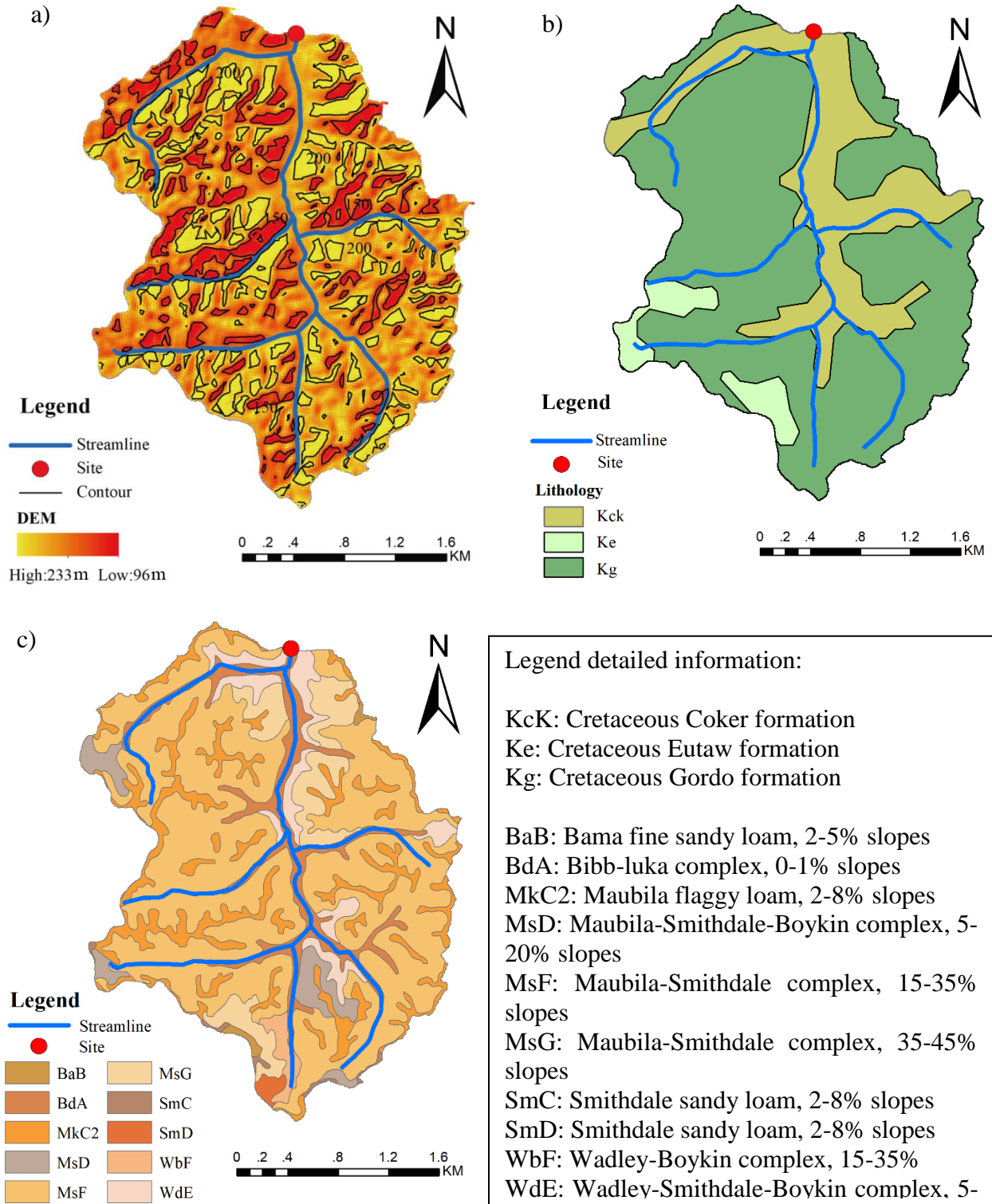


Figure 3.2. Topographic map (a; USGS Digital Elevation Model), lithology map (b; USGS state geologic map), and soil map (c; USDA soil survey) of the study site in the Talladega National Forest, Alabama, USA.

3.3.2 Grab samples

3.3.2.1 Sample collection

Grab samples were collected during baseflow conditions every two weeks, and a total of 35 grab samples were collected over the duration of this study. Additionally, in order to evaluate diurnal DOM variation, a Teledyne ISCO automatic storm sampler was deployed on three of the days (October 02, October 12, and December 5, 2016) to collect grab samples every hour for 24 hours. All water samples were filtered through 0.2 μm polyethersulfone filters and then stored in pre-cleaned polycarbonate bottles on the same day of sample collection. The filtrates for the analysis of DOC and nutrient concentration were stored at -20°C in the dark, and samples for DOM optical measurements were preserved at 4°C in the dark and analyzed within two weeks to avoid any potential interference from freezing-thawing processes (Otero et al. 2007; Spencer et al. 2007). The samples for cation concentration analysis were acidified with ultrapure concentrated HNO_3 (2% by volume) and stored in a refrigerator.

3.3.2.2 DOC concentration and DOM optical property

Dissolved organic carbon concentration was analyzed on a Shimadzu TOC-V total organic carbon analyzer, following the method described in detail by Shang et al. (2018). More than 50% of the samples were collected and analyzed in duplicates, yielding a relative standard deviation between 0% and 3%. Ultraviolet-visible absorbance was collected using a Shimadzu UV-1800 spectrophotometer from the wavelengths of 190 to 670 nm at a 1 nm interval. DOM fluorescence was measured using a Horiba FluoroMax3 fluorospectrometer with the excitation wavelengths from 240 nm to 500 nm every 5 nm and emission wavelengths from 280 nm to 538 nm every 3 nm. The spectra were corrected for blanks, the inner filter effect, and the manufacturer's correction factors and then normalized relative to the area under the water Raman

peak at the excitation wavelength of 350 nm (Cory and McKnight, 2005). DrEEM toolbox was used to acquire and validate fluorescence components (Murphy et al. 2013). A suite of optical indices was calculated including specific UV absorbance ($SUVA_{254}$), spectral slope ratio (S_R) (Weishaar et al. 2003 and Helms et al. 2008), fluorescence index (FI), humification index (HIX), and the index of recent autochthonous contribution (BIX) (e.g., Huguet et al. 2009; Cory et al. 2010).

3.3.2.3 DOM bioreactivity estimates

Four sets of grab samples were collected during baseflow conditions for DOM bioreactivity estimates. The samples were collected on 10/01/2015, 04/26/2016, 11/29/2016, and 03/08/2017, and the bioreactivity incubations followed the method described in Shang et al. (2018). Stream water samples were filtered first through pre-combusted 0.7 μm pore size GF/F filters and then through 0.2 μm pore size filters (Whatman polycap) to remove bacteria. The filtrates were then inoculated with microbes by adding 1% (by volume) *in situ* raw stream water (Servais et al. 1989). Each sample was distributed in three pre-combusted, one-liter amber glass bottles and incubated at 20°C in the dark for 28 days. Subsamples were collected on day 0, 2, 5, 10 and 28 and analyzed for DOC concentration and DOM optical properties. The degradation rate of DOC and DOM was assessed under the assumption of the first-order degradation kinetics as observed in previous studies (e.g., Middelburg 1989; Ogawa et al. 2001).

3.3.2.4 Ancillary parameters: dissolved cations and nutrients and stable oxygen and hydrogen isotopes of water

The concentrations of cations were analyzed using a Perkin Elmer Optima 3000 DV ICP-OES, yielding the relative standard deviation for sample duplicate between 0 % and 2%. Oxygen and hydrogen isotopic compositions of water were measured on a Picarro analyzer (WS-CRDS

at ENRI Stable Isotope Laboratory in the University of Alaska), and the relative standard deviation for sample duplicate ranged between 0% and 3%. The concentrations of inorganic N and P nutrients (nitrate, ammonium, and phosphate) were measured using a QuikChem 8500 Flow Injection Ion Analyzer equipped with standard colorimetric modules designed for each analyte, following the QuikChem methods 10-107-04-1-B, 10-107-06-1-F, and 10-115-01-1-B (https://www.uvm.edu/bwrl/lab_docs/protocols/). The relative standard deviation for sample duplicate varied between 0% and 11%

3.3.3 *In situ* sensor logging of fDOM, conductivity, water level: maintenance and calibration

A Fluorescence DOM (fDOM) logger (Cyclops-7F, TURNER Designs) was deployed in a PVC housing from July 27, 2015, to July 27, 2016, recording data every 10 to 15 minutes (Fig. 3.3). The fDOM loggerhead was wrapped with copper tape to reduce the interference of biofouling. Sensor maintenance was performed every two weeks or more often in the field, including removing debris and gently cleaning the sensor with methanol. Data logged during the maintenance period (approximately 30 to 60 minutes in each field trip) were removed. Occasionally, the sensor was covered by leaves or debris and recorded abnormally low fluorescence intensity (i.e., lower than the values measured in the air), and those values were also removed. The fDOM sensor measured a single excitation/emission pair (325nm/470nm; with 120/60 nm full width at half maximum excitation/emission bandpass filters). The fDOM signal was calibrated against DOC concentrations of grab samples analyzed in the laboratory using a Shimadzu TOC-V total organic carbon analyzer. Stream water level data were recorded by a pressure transducer (HOBO, ONSET) every 10 to 15 minutes, and these data were converted to stream discharge data via a rating curve (Appendix II Table 1). The rating curve was established by directly measuring stream water discharge following the mechanical current-meter method

(Buchanan et al. 1969) twelve times over the study period. A conductivity logger (HOBO, ONSET) was deployed from February 2016 to July 2017, and the readings were directly downloaded from the sensor. The accuracy of the conductivity sensor was verified using laboratory standards before the deployment.

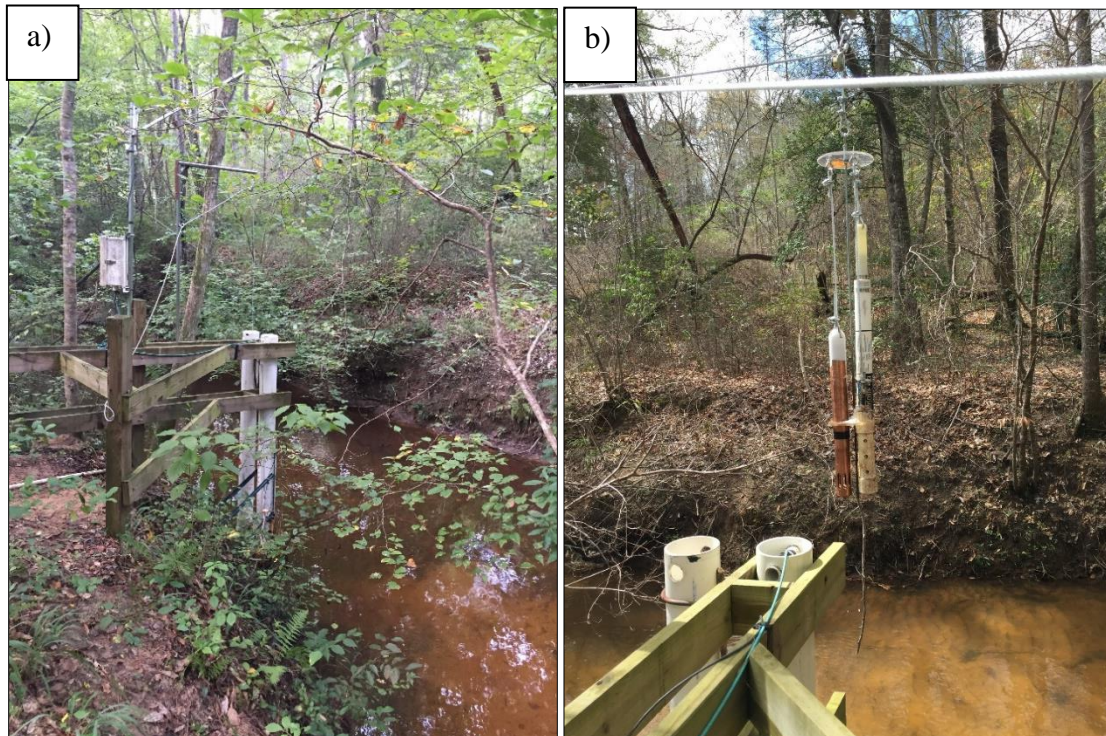


Figure 3.3. *In situ* observation station (a) and deployed sensors (b) deployed from July 27, 2015, to July 27, 2016, recording data every 10 to 15 minutes in the Talladega National Forest, Alabama, USA.

3.3.4 Data analysis

3.3.4.1 Storm event delineation

Varied criteria have been applied to determining the start point of a storm in previous studies. Lloyd et al. (2016) set the beginning of a storm when an increase in stream water discharge was 20% or more above the baseflow; Vaughan et al. (2017) used a minimum rise criterion to determine the start of each storm, and Aguilera et al. (2018) defined precipitation

exceeding 1cm per hour as the start. In this study, we identified the start of a storm event when two conditions were met: 1) an abrupt rise in discharge at the rate of at least $0.05 \text{ m}^3/\text{s}$ per hour, which was about five times greater than the largest diurnal discharge variation observed under baseflow conditions, and 2) discharge maintained an increasing trend over the next two hours. Measurements collected over one hour prior to the start of a storm were considered as the ambient condition for the corresponding storm. The end of a storm has been commonly determined visually by either the returning of discharge to near baseflows or the start of another storm. We identified the end of a storm event when 1) the stream discharge decreased back to the pre-storm level, or 2) another storm (identified based on the same criteria defining a storm) occurred, or 3) the water level stayed relatively constant after the duration of storm flow estimated according to $D = 0.827A^{0.2}$ (D = time between storm crest and end of overland runoff in days, A = drainage basin area in square kilometers; Fetter 2001), which is 1.47 days for the study watershed. Two storms were identified based on condition 3) out of the total of twenty-five storm events identified over the study period. All the storm events identified were compared with the precipitation record at the nearest weather station (KALTUSCA20), and the sharp rises in water discharge corresponded to precipitation that ranged between 0.20 to 5.92 mm/day and averaged 2.36 mm/day.

3.3.4.2 Calculation of storm hysteresis indices

Two hysteresis indices, flushing index, and hysteresis index were determined for each storm event. A negative flushing index (-1 to 0) indicates that storm flows dilute the solutes, and a positive flushing index (0 to 1) indicates that storm flows flush additional solutes into the stream, and the magnitude of flushing index indicates the degree of dilution or flushing (Lloyd et al. 2016). A positive hysteresis index corresponds to a clockwise concentration-discharge loop

that indicates a transport-limited mechanism while a negative hysteresis index corresponds to a counterclockwise loop that indicates source-limited transportation mechanism for DOM export (Lloyd et al. 2016). The magnitude of hysteresis index indicates the solute concentration differences between the rising and falling limbs of a storm hydrograph. The two indices were calculated based on unity-based normalized solute concentration and discharge as follows (Lloyd et al. 2016):

$$\text{Normalized } C_i = \frac{C_i - C_{min}}{C_{max} - C_{min}} \quad (3.1)$$

$$\text{Normalized } Q_i = \frac{Q_i - Q_{min}}{Q_{max} - Q_{min}} \quad (3.2)$$

where C_{min} and Q_{min} were the minimum solute concentration and discharge values, respectively, during a storm, and C_{max} and Q_{max} were the maximum solute concentration and discharge values, respectively. C_i and Q_i were the solute concentration and discharge at timestep i , and each timestep was 10 or 15 minutes, equal to the interval of sensor reading. Normalized C_i and Q_i (C'_i and Q'_i , respectively) were further used to calculate flushing index following Vaughan et al. (2017):

$$\text{Flushing Index} = C'_{Q_{peak}} - C'_{Q_{initial}} \quad (3.3)$$

where the $C'_{Q_{peak}}$ was the normalized solute concentration at the highest discharge and $C'_{Q_{initial}}$ was the normalized concentration at the beginning of a storm. The values for at least every 10% of the discharge range need to be measured to produce robust results of hysteresis index (Lloyd et al. 2016), which can be achieved by interpolating the C'_i to specific percent intervals of discharge when data resolution is not adequate (Vaughan et al. 2017). Our sensor provided a continuous C-Q loop from high-resolution sensor readings, and we thus used discharged-weighted solute concentration on both the rising and falling limbs to achieve the most robust results:

$$C'_{rising} = \frac{\sum_{i=Q_{initial}}^{i=Q_{peak}} \frac{(C'_{i+1} + C'_i) \times |Q'_{i+1} - Q'_i|}{2}}{\sum_{i=Q_{initial}}^{i=Q_{peak}} |Q'_{i+1} - Q'_i|} \quad (3.4)$$

$$C'_{falling} = \frac{\sum_{i=Q_{peak}}^{i=Q_{end}} \frac{(C'_i + C'_{i-1}) \times |Q'_i - Q'_{i-1}|}{2}}{\sum_{i=Q_{peak}}^{i=Q_{end}} |Q'_i - Q'_{i-1}|} \quad (3.5)$$

$$\text{Hysteresis Index} = C'_{rising} - C'_{falling} \quad (3.6)$$

where the C'_i and Q'_i denote the normalized solute concentration and discharge at timestep i , respectively. The hysteresis index of a given storm event was calculated with the hydrograph where the data existed for both rising and falling limbs. In the cases when the stream discharge did not decrease back to the pre-storm level, a fraction of the rising limb during the early stage of a storm with low discharge was not included in the hysteresis index calculation.

3.3.5 Statistical analysis

We conducted all the statistics in SPSS and set the level of significance, α , at 0.05 (two-tailed). Data normality was examined using the Shapiro-Wilk test prior to any parametric statistical analyses. Pearson's bivariate correlation was used to assess the covariance between two variables. Multiple linear regression analysis was performed to identify the best sets of hydro-climate and watershed explanatory variables for the variation in stream water DOM character and DOM C-Q hysteresis indices. The error assumptions of linear regression analysis, including constant variance, linearity, and normality, were examined using residuals versus fitted plots and Q-Q plots. The stepwise method was used, with the criteria of stepping as an entry at $P \leq 0.05$ and removal at $P \geq 0.10$, and the model with the lowest Akaike information criterion (AIC) (Akaike 1998) was selected. To compare if two groups of measurements were significantly different, t-tests were utilized assessing parametric variables, and the Mann-Whitney test was utilized assessing non-parametric variables.

3.3.6 DOC flux and uncertainty estimate

We calculated DOM flux as the product of DOC concentration and stream discharge. The *in situ* fDOM sensor readings were converted to DOC concentrations through an fDOM versus DOC calibration curve ($R^2=0.60$, $n=44$; Fig. 3.4, Appendix II Table 1), and the water level reading was calibrated to discharge using a rating curve ($R^2= 0.92$, $n= 12$; Fig. 3.5). The uncertainties of calculated DOC concentration and stream discharge from the calibration curves were estimated following the method described by Skoog et al. (2007). These uncertainties were propagated into the calculation of stream water DOC flux following Lindberg (2000):

$$\delta F = F \times \sqrt{\left(\frac{\delta Q}{Q}\right)^2 + \left(\frac{\delta C}{C}\right)^2} \quad (3.7)$$

where F, Q, and C denote DOC flux, stream discharge, and DOC concentration, respectively, and δ denotes uncertainties.

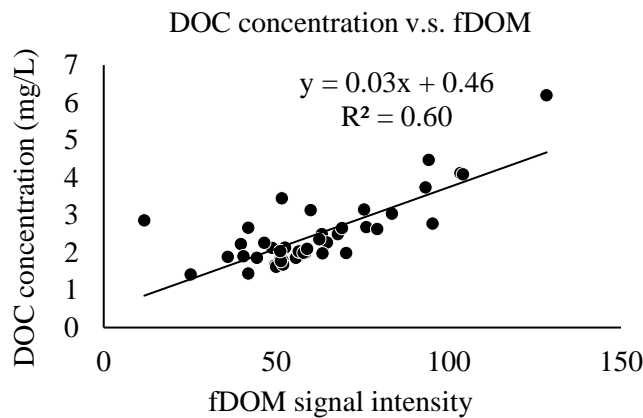


Figure 3.4. Standard curve converting fDOM to DOC concentration based on 44 grab samples collected during the study period from July 2015 to July 2016 in the study site in the Talladega National Forest, Alabama, USA.

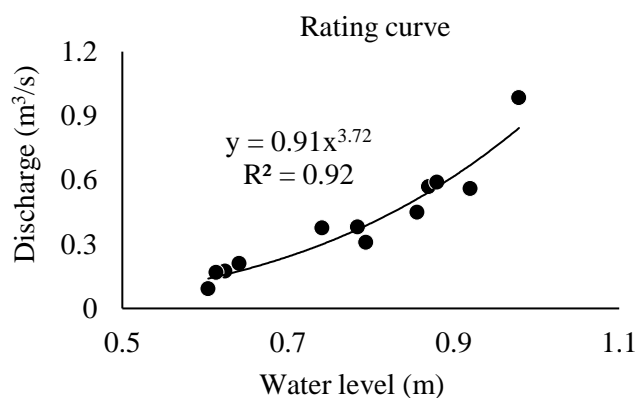


Figure 3.5. Standard curve converting water level to stream discharge based on 12 discharge measurements during the study period from July 2015 to July 2016 in the study site in the Talladega National Forest, Alabama, USA.

3.4 Results

3.4.1 Continuous discharge and DOC recorded from sensors

After removing unreliable data, a total of 47,059 pairs water level and fDOM at 10-minute intervals were recorded from July 2015 to July 2016. Stream discharge ranged between 0.05 and 25.65 m³/s, with uncertainties arising from rating curve extrapolation varying between 20% and 60%. Stream water DOC concentration ranged between 0.36 and 5.63 mg/L, and uncertainties due to DOC versus fDOM conversion ranged between 16% and 252%. These uncertainties were propagated into DOC flux calculation, yielding uncertainties for DOC flux varying between 25% and 259%.

3.4.2 Sources, composition and bioreactivity of DOM

Four DOM components were identified and validated based on 270 samples including grab samples collected at baseflow conditions and bioreactivity incubation samples (Table 3.1, Fig 3.6; Appendix II Table 2), including terrestrially humic-like DOM (C₁ and C₂), protein-like DOM (C₃), and microbial, humic-like DOM (C₄) (Table 3.1). Terrestrial humic-like DOM, i.e.,

C₁ and C₂, were the dominant components and accounted for $31.3 \pm 1.3\%$ and $38.0 \pm 1.1\%$ of the total fluorescence, respectively. Protein-like (tyrosine- or tryptophan-like) DOM (C₃) was thought to be sourced from microbial or autochthonous organisms, and it represented $16.0 \pm 0.9\%$ of total fluorescence. Microbial, humic-like DOM (C₄) comprised $14.7 \pm 2.0\%$ of total fluorescence. All optical indices exhibited little variation over the study period. For the absorbance-based indices, SUVA₂₅₄ and S_R averaged 0.80 ± 0.02 and 2.77 ± 0.09 , respectively (Appendix II Table 3), suggesting the dominance of aromatic, high-molecular-weight (MW) compounds in DOM (Weishaar et al. 2003; Helms et al. 2008). For the fluorescence-based indices, HIX averaged 4.91 ± 0.50 , BIX averaged 0.90 ± 0.03 , and FI averaged 1.69 ± 0.01 (Appendix II Table 3), indicating a mixture of microbial and terrestrial DOM (Jaffé et al. 2008; Huguet et al. 2009). SUVA₂₅₄ and S_R supported the source assignment of fluorescence components. Specifically, percent C₁ was positively correlated with SUVA₂₅₄ ($r=0.351$, $P=0.039$; $n=35$) confirming the high aromaticity of terrestrial humic-like DOM. Percent C₃ had negative correlations with SUVA₂₅₄ ($r=-0.347$, $P=0.041$; $n=35$) and positive correlations with S_R ($r=0.526$, $P=0.001$; $n=35$) supporting the low aromaticity and molecular weight of protein-like DOM.

We evaluated the percent biodegradable DOC (BDOC) on four grab samples from baseflow conditions. BDOC ranged between 0.09 and 2.59 mg/L and percent BDOC ranged between 3% and 23%. Larger concentrations and percent BDOC were observed for the sample collected in November, which had much higher initial DOC concentration (15.91 mg/L) relative to the other three samples (< 3.33 mg/L). Both litterfall and higher stream discharge (Fig. 3.7a) may have contributed to the higher concentrations of initial DOC and BDOC. Changes in fluorescence components were assessed on three sets of incubations. Overall, protein like DOM

(C₃) and microbial humic DOM (C₄) showed larger changes than terrestrial humic-like DOM (C₁ and C₂) (Fig. 3.7b). For the November sample, C₃ was the major biodegradable DOM component (68% removed) relative to the other three components that were degraded less than 4% (Fig. 3.7b).

Table 3.1. Characteristics of the four fluorescence components identified by DrEEM and the attributed sources.

Component	Excitation maximum wavelength (nm)	Emission maximum wavelength (nm)	Similar fluorescence components identified in previous studies				Present study
			Coble et al. 1998	Stedmon and Markager 2005	Cory and McKnight 2005	Yamashita et al. 2010	
C ₁	250 (350)	466	C	4	C1	C1	Terrestrial humic-like DOM
C ₂	250 (310)	388	A	3	SQ3	C3	Terrestrial humic-like DOM
C ₃	280	328	B or T	8	C8 or C13	C7	Protein-like DOM
C ₄	<240 (290)	352	M	6	Q3 or C3	C4	Microbial humic-like DOM

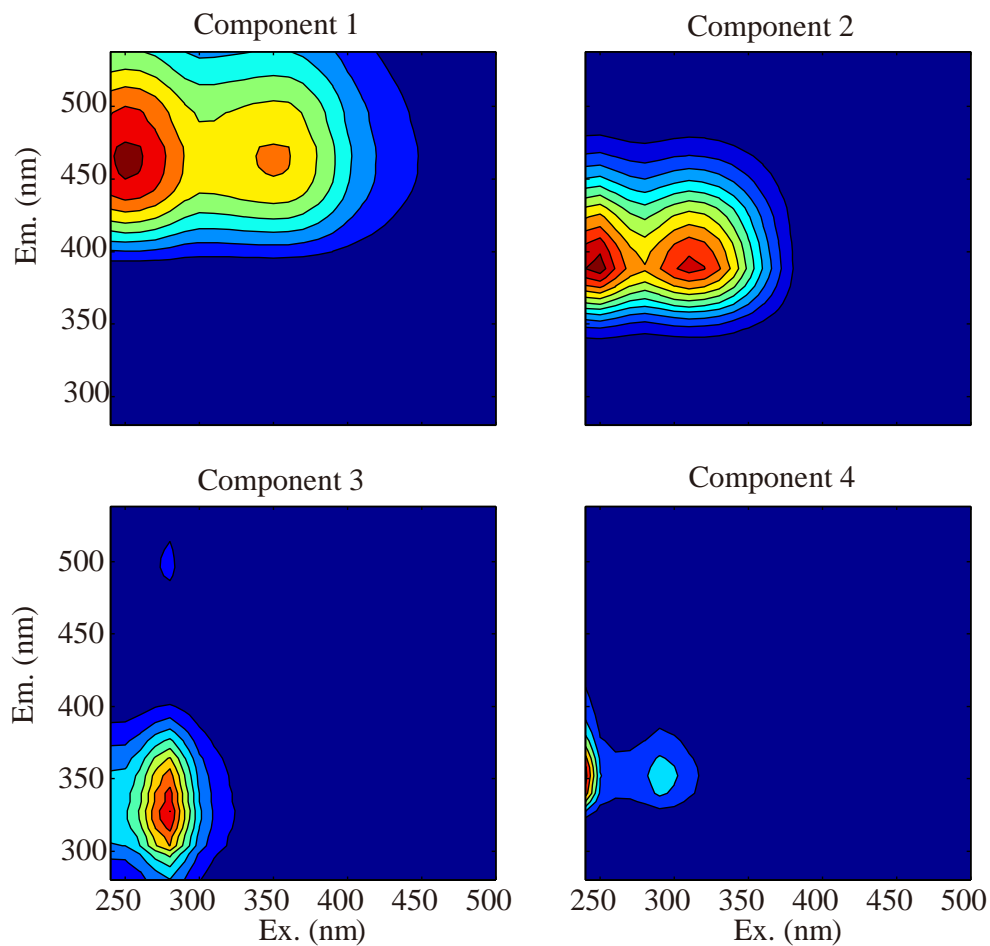


Figure 3.6. Excitation-emission contour plots of the four components (C₁-C₄) identified with DrEEM toolbox based on 270 grab samples collected during the study period from July 2015 to July 2016 in the study site in the Talladega National Forest, Alabama, USA.

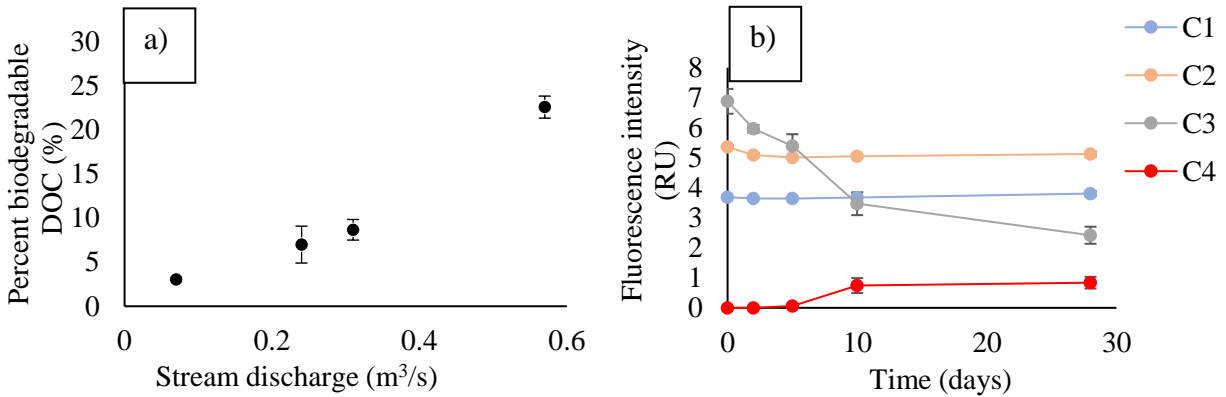


Figure 3.7. Variations of percent biodegradable DOC under different stream discharge conditions (a) and the fluorescence intensity of each component (b; based on the sample collected under high Q) during laboratory incubation based on 4 sets of grab samples collected during the study period from July 2015 to July 2016 in the study site in the Talladega National Forest, Alabama, USA.

3.4.3 Concentration versus discharge hysteresis of DOM during storms

Twenty-five storm events were recorded over the study period. During the majority of storm events (23 out of 25), DOC peaks lagged behind discharge peaks seven hours on average. In contrast, all conductivity peaks appeared before the peak discharge (Fig. 3.8). The C-Q of DOM hysteresis for 23 out of 25 storm events showed counterclockwise rotation and negative hysteresis index ranging from -0.72 to -0.17 (Appendix II Table 4). The flushing index displayed considerable variability from storm to storm, and sixteen (64% of the total) storms had positive values indicating that flushing was the governing process over diluting during the storms (Appendix II Table 4). The hysteresis pattern was a function of the duration of storms and antecedent drought periods (Fig. 3.9). Specifically, flushing index was positively correlated to the duration of the rising limb of a storm ($r=0.404$, $P=0.045$; $n=25$; Fig. 3.9c) indicating more flushing than diluting in DOM input in longer storms, hysteresis index decreased with the length of the antecedent dry period ($r=-0.434$, $P=0.030$; $n=25$; Fig. 3.9a), but increased with the initial discharge prior to the storm ($r=0.484$, $P=0.014$; $n=25$; Fig. 3.9b). The hysteresis pattern also

varied due to storm frequency. During the five pairs of successive storm events we observed (i.e., when a storm started before stream discharge decreased back to the pre-storm level of a preceding storm), lower flushing index and higher hysteresis index values were found in the second storms than the preceding ones (Fig. 3.10). The flushing index also showed seasonal variations, being significantly higher in spring (March 1st to May 31st) and winter (December 1st to February 28th) than in summer (June 1st to August 31st) and autumn (September 1st to November 30th) (t-test, $P \leq 0.033$; $n=25$; Fig. 3.11b), whereas hysteresis index did not differ across seasons (t-test, $P \geq 0.443$; $n=25$).

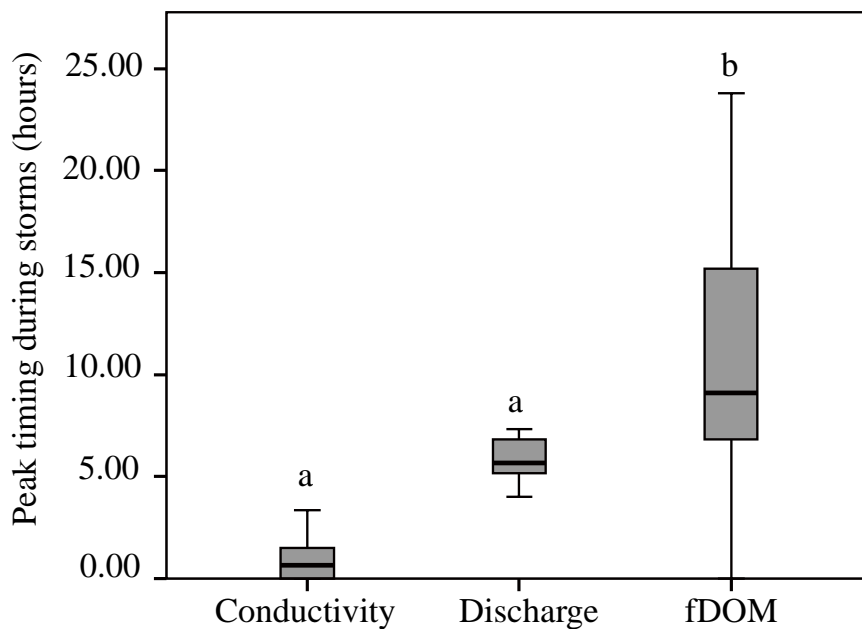


Figure 3.8. Temporal differences in the peak of conductivity, stream discharge and fDOM time series on the hydrographs of storms recorded by *in situ* sensors in the study site in the Talladega National Forest, Alabama, USA.

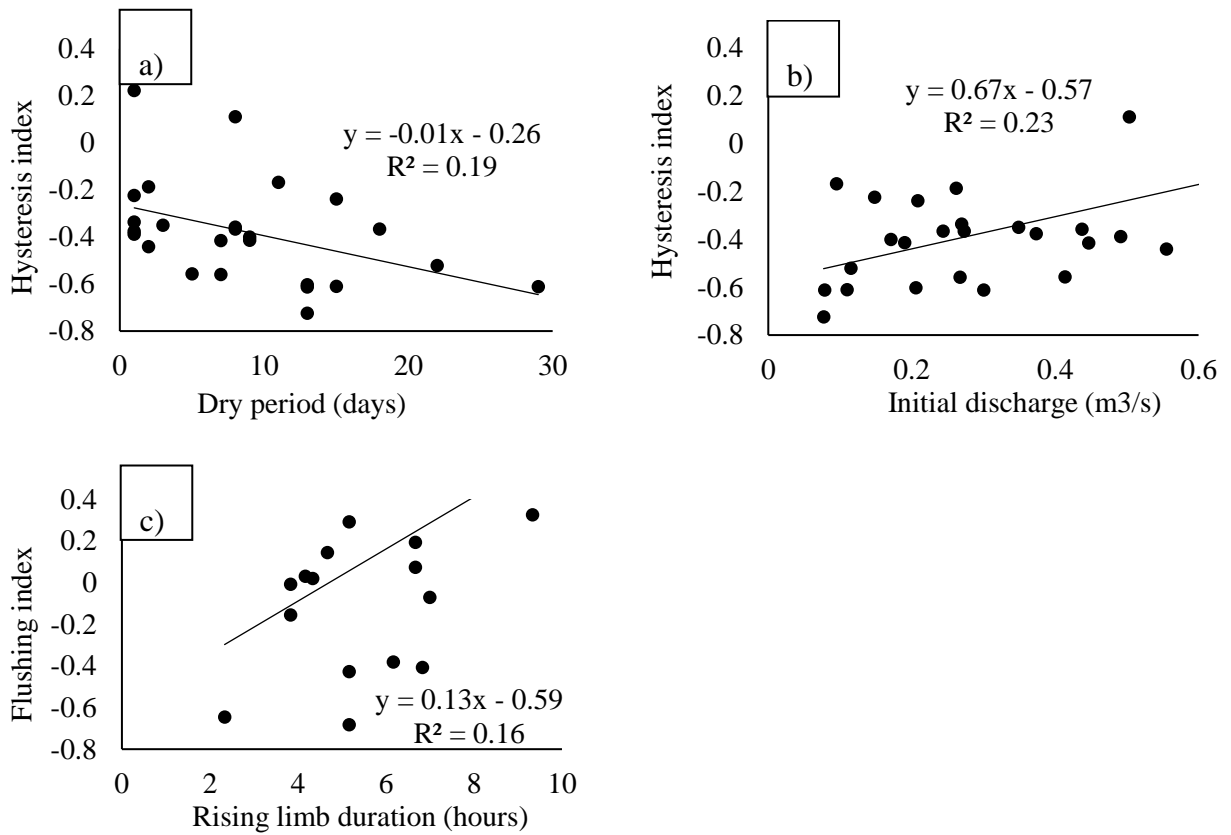


Figure 3.9. Cross-correlations of hysteresis index and dry period prior to a storm (a), hysteresis index and initial discharge prior to a storm (b), and flushing index and the rising limb duration during a storm (c) based on 25 storm events during the study period from July 2015 to July 2016 in the study site in the Talladega National Forest, Alabama, USA.

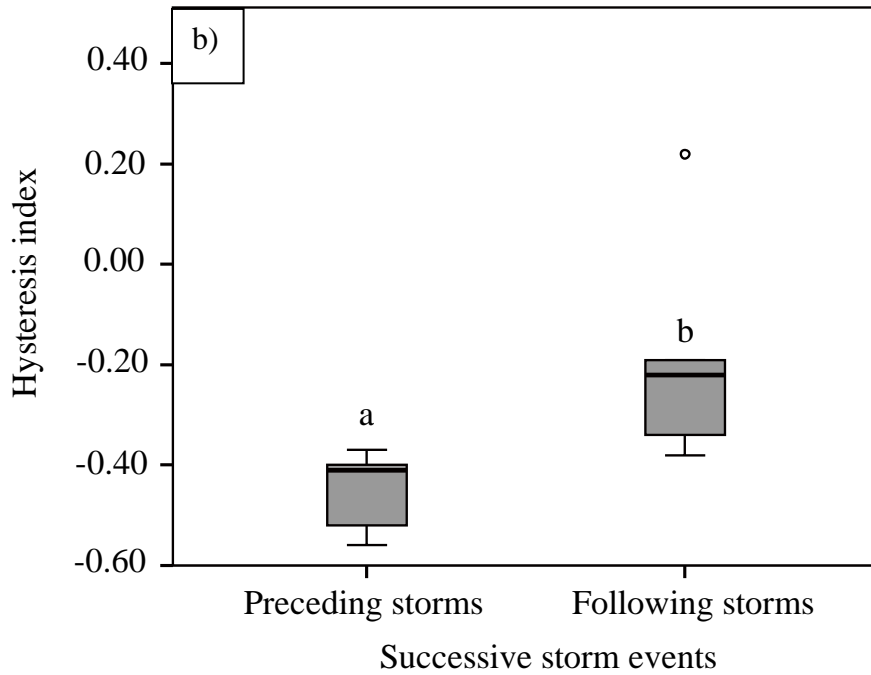
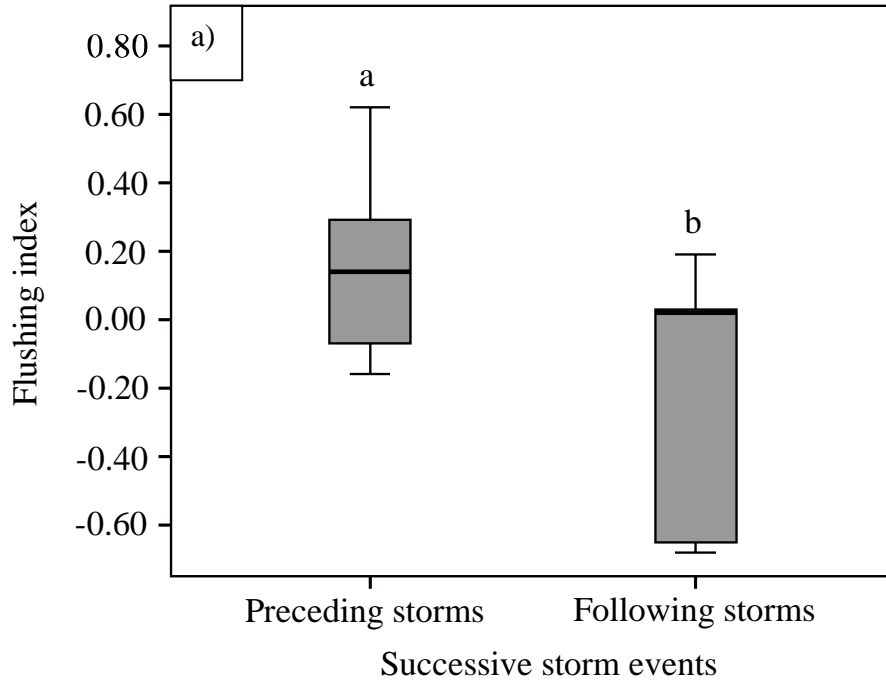


Figure 3.10. Decreasing flushing index (a) and increasing hysteresis index (b) of DOM C-Q hysteresis in five successive storm pairs (preceding versus following) observed during the study period from July 2015 to July 2016 in the study site in the Talladega National Forest, Alabama, USA.

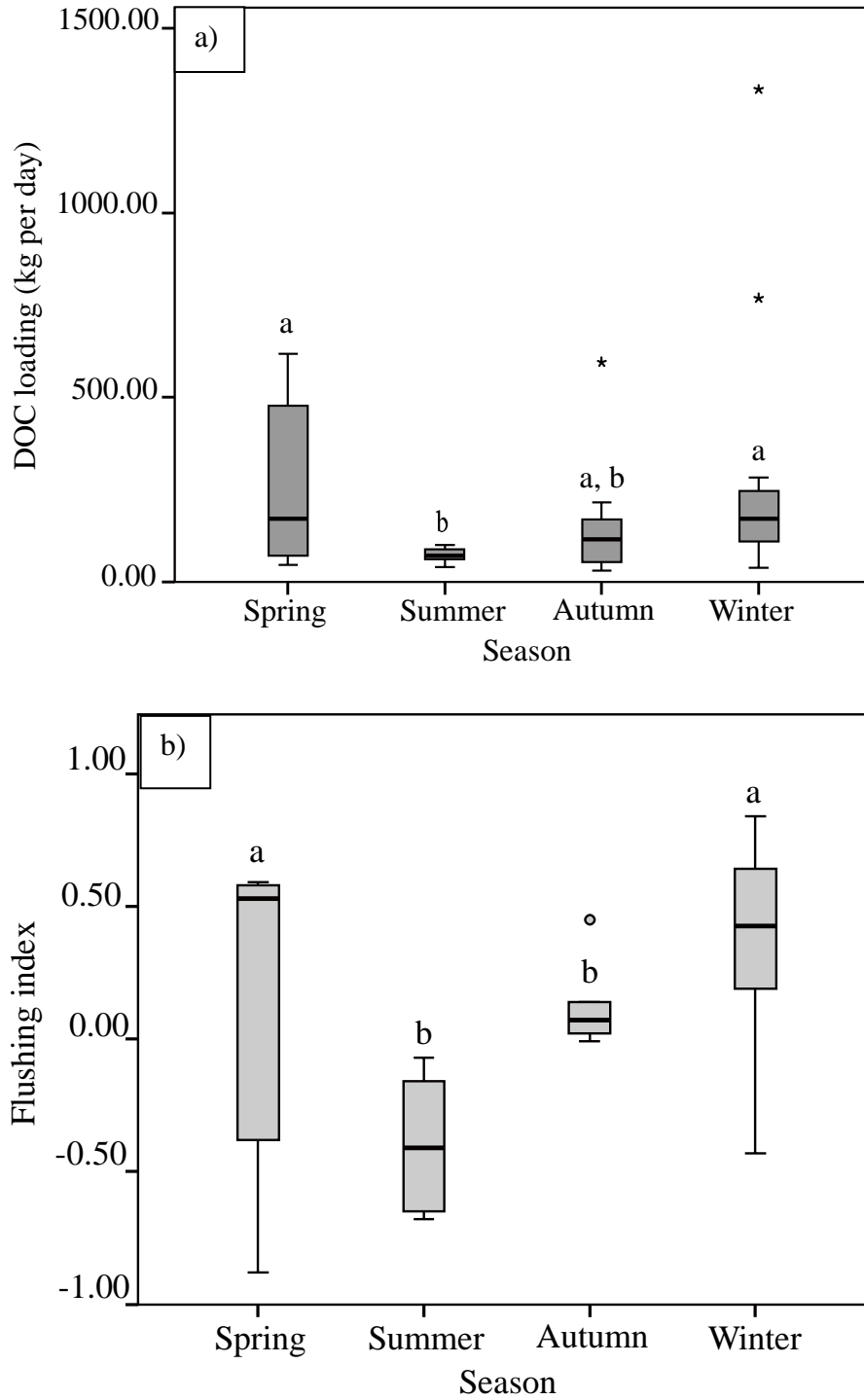


Figure 3.11. DOC loading (a) and flushing index (b) across seasons under storm conditions during the study period from July 2015 to July 2016 in the study site in the Talladega National Forest, Alabama, USA.

3.4.4 Baseflow DOM variation across timescales

3.4.4.1 Diurnal scale

We evaluated diurnal scale variation on days when storm flows did not occur. We calculated hourly DOC concentration, conductivity, discharge, and temperature using the sensor data of a given hour (e.g., 1:00–1:59 am) over the entire study period excluding storm days. Mean hourly DOC concentrations over the study period showed little variation, ranging between 2.19 and 2.24 mg/L. They were negatively correlated with the mean hourly discharge (Fig. 3.12; $r=-0.627$, $P=0.001$, $n=24$) and positively correlated with the mean hourly temperature ($r=0.880$, $P<0.001$, $n=24$). Stream conductivity shows a similar diurnal variation as DOC concentration (Fig. 3.12). We also analyzed DOM sources and compositions on hourly grab samples collected using an automatic stormwater sampler on three different days. Higher proportions of microbially derived humic DOM (C_4) appeared at around 7 am and 5 pm, which corresponded to the time of rising in stream discharge (Fig. 3.12, 3.13). However, the proportions of fluorescence components on each day did not show consistently significant correlations with stream discharge. On one of the three days of autosampler deployment, percent C_1 and C_2 were negatively correlated with discharge (C_2 : $r=-0.80$, $P<0.001$; C_1 : $r=-0.80$, $P<0.001$), and percent C_4 was positively correlated with discharge ($r=0.74$, $P<0.001$). On the other two days, percent C_3 showed significant but opposite correlations with discharge (October 12-13: $r=-0.57$, $P=0.004$; December 5-6: $r=0.66$, $P<0.001$).

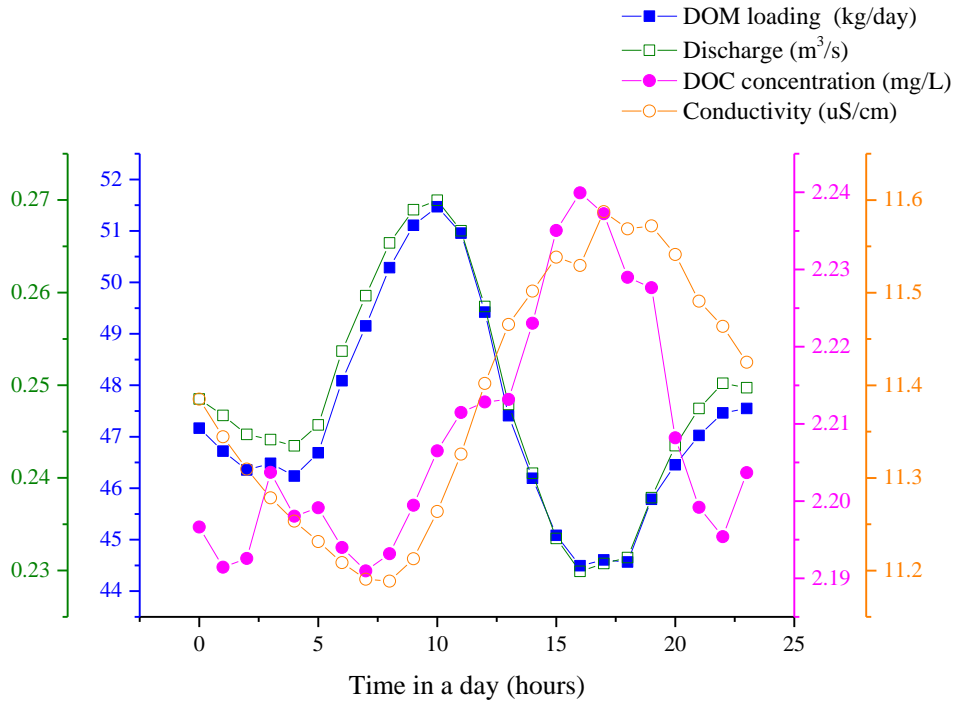


Figure 3.12. Diurnal variations of DOM loading, stream discharge, DOC concentration and conductivity based on the average of one-year *in situ* data collected in the study site in the Talladega National Forest, Alabama, USA (error bar smaller than data symbol).

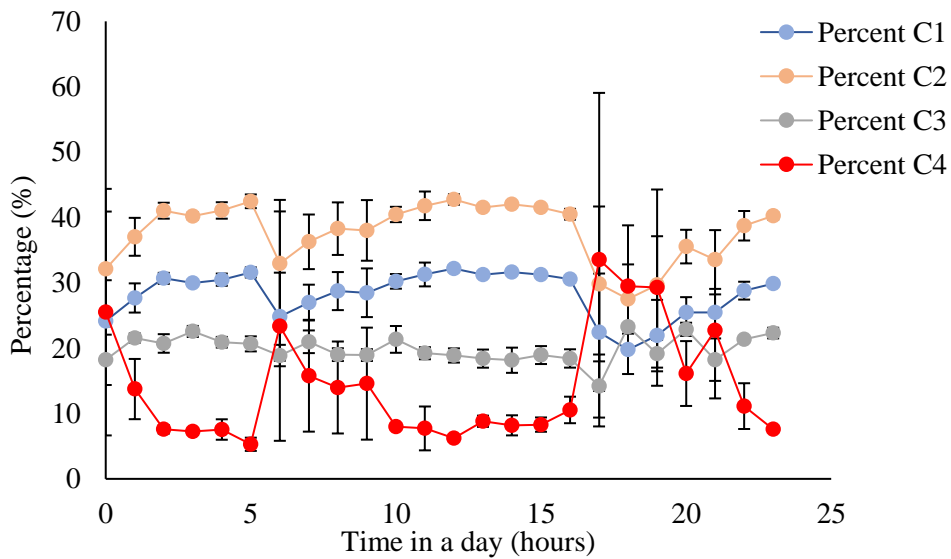


Figure 3.13. Diurnal variations in the percent contribution of four DOM components. Error bars represent the standard error from three observations

3.4.4.2 Monthly to seasonal variation in DOC and DOM

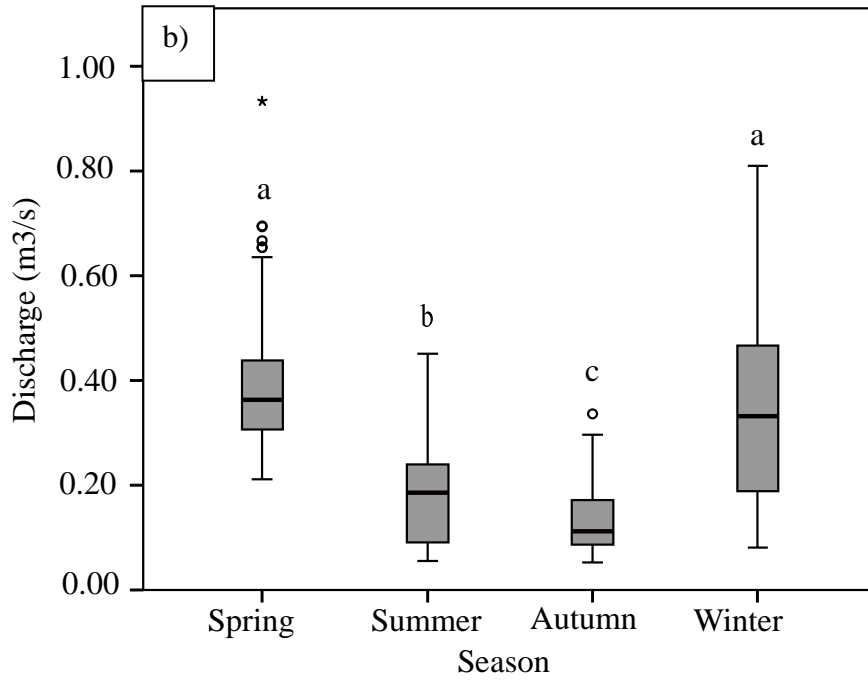
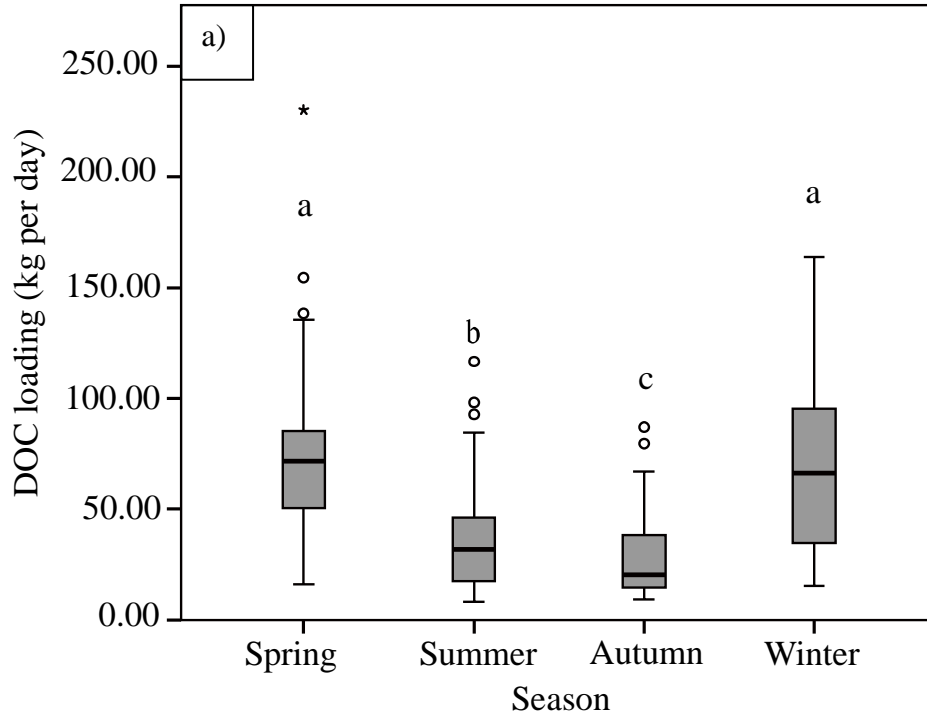
We evaluated monthly to seasonal variation in DOC based on the sensor data. We found that DOC concentration varied due to month, with higher values being observed in November and December (Kruskal-Wallis chi-squared = 9424.6, $df = 11$, $P < 0.001$). DOC concentration for a given month may be significantly correlated with discharge, temperature, or both, but these correlations were not consistent across months. For example, DOC versus temperature correlation was positive in November ($r = 0.56$, $P < 0.001$) and December ($r = 0.48$, $P < 0.001$) but negative in June ($r = -0.09$, $P < 0.001$) and July ($r = -0.28$, $P < 0.001$). For a given season, DOC versus temperature correlation was consistently significant with a positive correlation coefficient in spring ($r = 0.25$, $P < 0.001$) and winter ($r = 0.55$, $P < 0.001$) but a negative coefficient in summer ($r = -0.07$, $P < 0.001$) and fall ($r = -0.09$, $P < 0.001$), while DOC versus discharge correlation was positively correlated for all seasons (spring: $r = 0.19$, $P < 0.001$; summer: $r = 0.17$, $P < 0.001$; fall: $r = 0.35$, $P < 0.001$; winter: $r = 0.08$, $P < 0.001$). DOC concentration varied due to season (Kruskal-Wallis chi-squared = 914.34, $df = 3$, $P < 0.001$), with winter DOC concentration being significantly higher than DOC concentration in summer (Fig. 3.14c).

We evaluated the variations in DOM source and composition using grab samples collected approximately every 7 to 10 days throughout the study period. Among the suite of source-composition proxies evaluated, all but three proxies (fluorescence index, percent C_1 , and percent C_3) varied due to the season. Although the seasonal changes differed across proxies, the general pattern is that DOM in winter had lower contributions of terrestrial-decayed DOM (SUVA₂₅₄, HIX, percent C_2) and lower molecular weight (S_R) and was more labile and larger contribution from recent-produced DOM (BIX, percent C_4) than DOM in summer. Based on the stepwise linear regression models (Table 3.2), DOM characteristics varied as a function of

several environmental factors, including water temperature, stream discharge, and water sources. $SUVA_{254}$ (50.4% of the total variance), S_R (14.2% of the total variance), $\%C_1$ (14.8% of the total variance), and $\%C_3$ (11.3% of the total variance) were best explained by water temperature, whereas BIX (26.7% of the total variance) and FI (14.0% of the total variance) were best explained by stream discharge (Q). HIX (24.5% of the total variance) and $\%C_2$ (12.3% of the total variance) were best explained by the concentration of sodium ions.

Table 3.2. Stepwise regression models predicting stream DOM characters (n=35). The selection of final stepwise model was based on acquiring the lowest Akaike information criterion value of each dependent variable. Potential explanatory variables include temperature, Q, $\delta^{18}\text{O}$, δD , Si, and Na. DOC concentration and %C₄ cannot be predicted by any variables.

Variable	Adjusted R Square	P value	Model	%Explained by each explanatory variable	AIC
SUVA ₂₅₄	0.504	<0.001	=0.014+0.001(Temperature)	(50.4%)	-26.44
SR	0.300	=0.001	=0.669-0.010(Temperature)+0.075(Si)	(14.2%), (15.8%)	-73.45
HIX	0.343	<0.001	=11.824-10.608(Na)+0.194(Temperature)	(24.5%), (9.8%)	31.16
BIX	0.460	<0.001	=-1.355+0.638(Q)+0.617(Na)-0.363($\delta^{18}\text{O}$)	(26.7%), (9.4%), (9.8%)	-57.26
FI	0.147	=0.013	=1.648+0.159(Q)	(14.7%)	-83.46
%C ₁	0.148	=0.013	=20.304+0.622(Temperature)	(14.8%)	62.71
%C ₂	0.123	=0.022	=20.120+18.337(Na)	(12.3%)	59.21
%C ₃	0.218	=0.007	=9.480-0.394(Temperature)+13.815(Na)	(11.3%), (10.5%)	52.99



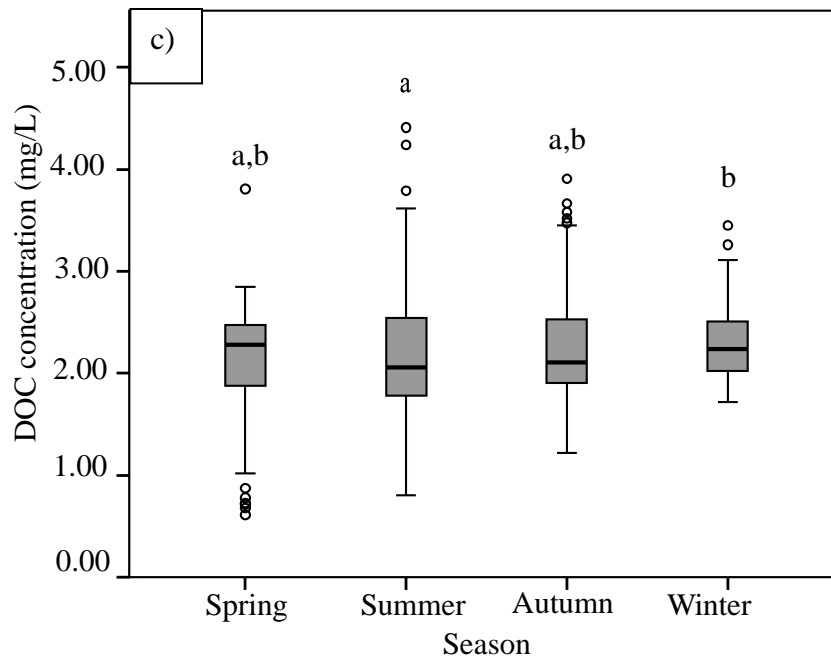


Figure 3.14. DOC loading (a), stream discharge (b), and DOC concentration (c) across seasons under baseflow conditions.

3.4.5 DOC export yield

DOC flux throughout the study year can be well predicted by discharge (Adjusted $R^2=0.956$, $P<0.001$), but not by DOC concentration (Adjusted $R^2=0.037$, $P<0.001$) or temperature (Adjusted $R^2=0.007$, $P<0.001$). DOC flux averaged 47.37 ± 33.81 kg/day and 259.77 ± 531.13 kg/day (mean \pm SD) during baseflows and stormflows, respectively. Storm events accounted for 8% of the study duration but were responsible for around 38% of DOM input. Given that the suspended particles under high turbidity can absorb and scatter light and will reduce both the excitation signal from the fluorescence emitter as well as the resulting emission signal to fluorescence receiver (Downing et al. 2012; Lee et al. 2015), there might be an underestimation of the DOM amount being mobilized during storms. DOM loadings were statistically different among seasons ($P<0.001$, Kruskal-Wallis test; Fig. 3.14a) with the highest loading in winter due to high discharge and the lowest loading in summer due to low discharge.

We acknowledge that the fDOM sensor could be affected by temperature because the high temperature can cause the return of electrons to their original state and result in the underestimation of the fluorescence signal.

3.5 Discussion

3.5.1 Processes regulating storm event export of DOM

The C-Q curves of DOC during a storm event provide information about the sources and processes regulating the watershed export of DOC. The peak time of a given solute indicates the distance between watershed DOM sources and the receiving stream (Creed et al. 2015; Fovet et al. 2018). The lag of DOC peaks relative to the conductivity and discharge peaks indicates a longer traveling time and a more distal source for DOC than ions in Mayfield. That is, DOC export in Mayfield is more limited by transport (i.e., the connectivity between watershed sources and the receiving stream) than by the availability of DOC in the watershed (e.g., McGlynn and McDonnell 2003; Inamdar and Mitchell, 2006; Saraceno et al. 2009; Pellerin et al. 2012; Vaughan et al. 2017; Fovet et al. 2018). Similarly, Pellerin et al. (2012) and Vaughan et al. (2017) compared the C-Q behaviors of NO_3^- and DOM in urban and forested watersheds and found that NO_3^- concentration peaked prior to the discharge (clockwise hysteresis, positive HI), while DOC concentration peaked after the discharge (counterclockwise hysteresis, negative HI). They also suggested a more distal and sufficient supply for DOM than for NO_3^- in the watersheds. Fovet et al. (2018) proposed a similar controlling mechanism that the majority of DOM mobilization occurred when shallow, organic matter-rich soils were connected to the stream via lateral subsurface flow, which took place after the soil profile became saturated, mostly during the falling limb of discharge. In some streams, peak DOC appears before peak discharge during a storm. For example, Jeong et al. (2012) studied a mountainous watershed in

northern South Korean and found that DOC peaked prior to discharge, suggesting that DOC was derived mostly from sources proximate to the streams. This observation may be attributed to greater volumes of surface and shallow subsurface runoff in steep watersheds (Peñuela et al. 2015; Khan et al. 2007) that enables the mobilization of DOM accumulated in topsoils (Kalbitz et al. 2000; Findlay and Sinsabaugh 2003) during the early proportions of the event hydrograph. McGlynn and McDonnell (2003) examined storm DOC dynamics in headwater streams draining forested watersheds with steep slopes and observed the influences of watershed sources in a sequentially spatial order, with riparian and initial hillslope runoff with high DOC dominating the early proportions of a storm hydrograph and hillslope runoff with low DOC dominating the falling limb.

The pattern of C-Q hysteresis gives insights into the timing and magnitude of DOM export. Hysteresis index describes differences in DOC concentrations between the rising and falling limbs of a storm hydrograph, and a lower hysteresis index indicates a sufficient supply of DOM even at the falling limb of stream discharge, implying an unlimited supply of DOM from the watershed during the given storm (Lloyd et al. 2016). In Mayfield creek, hysteresis index varied as a function of antecedent hydrological conditions (Fig. 3.9a, b), with a lower hysteresis index corresponding to a longer duration of dry days before the storm and lower initial discharge of the storm. This observation illustrates that dry periods were important for accumulating and restoring mobilizable DOM in soils after storm events. Successive storms represented extreme cases where there were no dry periods between two storms. Higher hysteresis index was observed in the second storm than the first storm in each pair of successive storms (Fig. 3.10b), suggesting that the first storm lowered the availability of watershed DOM for the second storm. Meanwhile, the second storm showed a lower flushing index than the first storm (Fig. 3.10a),

which indicates a shift from flushing to dilution in DOM mobilization through successive events. Watershed DOM supply was depleted during the first storm but did not have a dry period to restore, and as a result, DOM export during the second storm became limited by the amount of DOM stored in the watershed. Previous studies also found the influence of antecedent dry periods on DOC in streams. Inamdar et al. (2008) found stream water DOC concentrations in stormflow were higher during dry seasons than rainy seasons. Mehring et al. (2013) observed that a dry period with low discharge led to higher stream water DOC concentration in the subsequent hydroperiod. Humbert et al. (2015) noted that low groundwater level during the dry period corresponded to increased mean DOC concentration in streams.

The pattern of C-Q hysteresis also suggests that the duration of precipitation influenced the DOM export in Mayfield. Long-lasting storms (i.e., longer duration on the rising limb of discharge) tended to correlate more with DOM flushing (positive flushing index) than with DOM diluting (Fig. 3.9c), which suggests that a longer-duration rainfall could allow for sufficient time to leach and transport DOM from distal and diffuse sources (e.g., upland or shallow soil). This observation implies that for a given amount of total precipitation, long-lasting storms will lead to a greater amount of DOM export than short but strong storms. Previous studies have not directly assessed the effects of storm duration on watersheds as a potential explanation for inconsistent observations of the export of DOM (e.g., Raymond and Saiers 2010; Mehring et al. 2013; Humbert et al. 2015). Most climate model projections for the southeastern US predict increasing frequency and magnitude of storms. Our data show that the associated watershed export of solutes could be nonlinear and needs further research attention.

3.5.2 DOM dynamics at the diurnal scale

At the diurnal scale, the variation in baseflow DOC closely followed the variation in stream discharge and conductivity, indicating that dilution and concentration due to evapotranspiration were the primary control. Evapotranspiration exerts an important control on water level and discharge in streams, with the highest and lowest discharge generally appearing in early mornings and late afternoons, respectively (e.g., Gribovszki et al. 2010, 2013; Graham et al. 2012). The diurnal variation in DOM composition (percentages of fluorescence components) did not correlate significantly with discharge or temperature, further suggesting physical processes were the primary control of DOM changes within a day (Fig. 3.12, 3.13). Similar diurnal variations in solute concentration were reported in previous studies. For example, Pellerin et al. (2011) observed an inverse correlation between stream flow and NO_3^- concentration and suggested the hydrological control on the diurnal variability of NO_3^- . The dominance of evapotranspiration over photochemical and microbial processes is not surprising in Mayfield, a second-order, heavily shaded stream. However, the influence of photochemical processes and instream production may become increasingly important in modifying DOM when it moves downstream. For instance, Spencer et al. (2007) studied large rivers with discharge approximately two magnitudes higher than Mayfield and reported photochemically and biologically-mediated DOM quality variance at the diurnal scale.

3.5.3 DOM dynamics at the seasonal scale

Significantly higher DOC loadings ($P < 0.001$) were observed in the stream in spring and winter driven by stream discharge (Fig. 3.14). Given that spring and winter were recognized as wet seasons with significant higher discharge ($P < 0.001$) than summer and autumn (Fig. 3.13b) in this study, storms with high frequency and large magnitude were expected to flush a large

amount of terrestrial DOM from terrestrial carbon pool into receiving streams. Multiple previous studies recognized wet seasons as important periods when soil DOM is transported to waterways (e.g., Waterloo et al. 2006; Wilson et al. 2013). Instead of being diluted, DOC concentration in spring and winter had a subtle increase (about 8% of median values, Fig. 3.13c), indicating larger DOM availability in the watershed in spring and winter. However, the higher DOC concentrations in spring and winter were not statistically significant across seasons, except for a higher DOC concentration in winter than summer ($P=0.022$). The majority of litter fell in November (late fall) in the study watershed and resulted in greater organic substance in the following months (i.e., winter and spring). In addition, the decomposition and leaching of DOM from litter have been found to occur predominantly in wet seasons (Wieder and Wright, 1995; Turner et al. 2015), which are spring and winter in this region. We suggest that sufficient carbon resulting from litterfall in late autumn in the watershed (e.g., Zeilhofer et al. 2012; Turner et al. 2015) together with heavier precipitation in spring and winter resulted in larger DOM loading in spring and winter.

The higher percent contribution from microbially derived DOM in spring and winter (18% versus <15% in summer and autumn) may be attributed to the contribution of freshly produced DOM mobilized from terrestrial carbon pool under more reactive hydrological conditions. Lambert et al. (2013) and Jeanneau et al. (2014) characterized DOM quality using molecular biomarkers and optical properties and suggested more microbially derived DOM with lower aromaticity from upland soils. We postulate that DOM with a larger percent contribution of microbially derived compounds from shallower soil was mobilized into streams under conditions with high water table in spring and winters. The contribution of different water sources from the watershed to the stream was supported by the significant correlations between

discharge and cation concentrations as well as between discharge and stable water isotopes (Table 3.3). In previous studies, cation concentrations in streams have been used as indicators for water fluxes from different sources, and Si and Na has been shown to increase with deep and shallow subsurface water fluxes, respectively (D'Arcy and Carignan, 1997). Moreover, high Na concentrations at high flows indicate that the correlation between cation concentration and discharge was not simply a dilution process. Stable water isotopes (i.e., δD and $\delta^{18}\text{O}$) were used in differentiating the contributions from water sources (i.e., old and new) in a watershed (Lambert and Aharon, 2009). In addition to the correlation between discharge and water-source indices, multiple DOM quality proxies also show significant correlations with discharge and water source indices (Table 3.2, 3.3). These correlations support that compared to the DOM from deep soils, DOM from shallow soil is less humified (lower HIX) and has more protein-like DOM (higher %C₃), and a shallower water table leads to the transport of fresh-produced and labile DOM into streams. In addition, temperature was found to affect root exudation and leaf litter degradation in previous studies (Christ and David, 1996; Raymond and Saiers, 2010). This study provided further evidence that at low temperatures (e.g., in spring and winter), DOM was less degraded in soils and, thus, contained higher percentages of biologically labile microbially derived DOM.

No significant difference in hysteresis index was observed across seasons. Similarly, several previous studies suggested C-Q responses cannot be well explained by seasonal or hydrological variables and appeared to be random due to the complex effect of hydrological variables (Butturini et al. 2006, 2008). Wet seasons, i.e., spring and winter in this study, were associated with shorter antecedent dry periods and higher initial stream discharge, which would collaboratively increase hysteresis index values (Fig. 3.9a, b, 3.10b). Our observation suggests

that the impacts of antecedent hydrological conditions on DOM C-Q hysteresis index were buffered by other seasonal variations. We proposed larger DOM availability from litterfall in the watershed during spring and winter might be a possible explanation. The sufficient DOM storage is also in agreement with the more frequent scenarios where flushing, rather than diluting, regulated DOM export in spring and winter than in summer and autumn (Fig. 3.11b). Meanwhile, significant higher DOC loadings in spring and winter further support higher DOC storage in these seasons (Fig. 3.11a).

Table 3.3. Pearson correlations among river discharge, temperature, water isotopes, cation concentrations, and DOM characters of grab samples (n=35) collected under baseflow conditions.

		Temperature	$\delta^{18}\text{O}$	δD	Si	Na	DOC	SUVA254	SR	HIX	BIX	FI	%C ₁	%C ₂	%C ₃	%C ₄
Q	Pearson Correlation	-.363*	.598**	.696**	-.818**	.355*	.161	-.269	-.017	-.469**	.537**	.407*	-.291	.381*	.301	-.171
	Sig. (2-tailed)	.032	.000	.000	.000	.037	.355	.118	.924	.005	.001	.015	.089	.024	.079	.326
Temperature	Pearson Correlation		.077	-.114	.316	-.021	.039	.720**	-.404*	.348*	-.437**	-.387*	.416*	.097	-.372*	-.153
	Sig. (2-tailed)		.661	.515	.065	.905	.823	.000	.016	.041	.009	.021	.013	.579	.028	.381
$\delta^{18}\text{O}$	Pearson Correlation			.897**	-.653**	.531**	-.032	.005	-.316	-.378*	.196	.045	-.134	.301	.204	-.182
	Sig. (2-tailed)			.000	.000	.001	.857	.977	.065	.025	.260	.800	.442	.079	.240	.295
δD	Pearson Correlation				-.769**	.582**	.129	-.093	-.286	-.371*	.286	.107	-.150	.293	.188	-.159
	Sig. (2-tailed)				.000	.000	.462	.597	.096	.028	.095	.539	.389	.088	.281	.361
Si	Pearson Correlation					-.376*	-.256	.325	.271	.415*	-.389*	-.196	.220	-.357*	-.223	.167
	Sig. (2-tailed)					.026	.138	.057	.115	.013	.021	.260	.205	.036	.197	.337
Na	Pearson Correlation						.117	.006	-.139	-.517**	.501**	.335*	-.312	.385*	.361*	-.189
	Sig. (2-tailed)						.502	.974	.424	.001	.002	.049	.068	.022	.033	.278
DOC	Pearson Correlation							.031	-.092	-.244	.277	.187	-.051	.308	.160	-.220
	Sig. (2-tailed)							.861	.598	.158	.108	.281	.770	.071	.358	.204
SUVA254	Pearson Correlation								-.255	.332	-.374*	-.430**	.351*	-.030	-.346*	-.050
	Sig. (2-tailed)								.139	.051	.027	.010	.039	.866	.042	.777
SR	Pearson Correlation									-.221	.280	.377*	-.235	-.134	.529**	-.019
	Sig. (2-tailed)									.202	.103	.026	.175	.444	.001	.913
HIX	Pearson Correlation										-.830**	-.671**	.759**	-.373*	-.759**	.076
	Sig. (2-tailed)										.000	.000	.000	.028	.000	.665
BIX	Pearson Correlation											.869**	-.731**	.489**	.738**	-.151
	Sig. (2-tailed)											.000	.000	.003	.000	.385
FI	Pearson Correlation												-.485**	.563**	.669**	-.324
	Sig. (2-tailed)												.003	.000	.000	.058
%C ₁	Pearson Correlation													.137	-.538**	-.481**
	Sig. (2-tailed)													.434	.001	.003
%C ₂	Pearson Correlation														.440**	-.876**
	Sig. (2-tailed)														.008	.000
%C ₃	Pearson Correlation															-.374*
	Sig. (2-tailed)															.027

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

3.5.4 Ecosystem implications

Understanding the timing and magnitude of terrestrial DOM export to waterways under different temporal scales is important for ecosystem management and confining watershed carbon budgets. Our results are consistent with existing ecological models and frameworks. Data in this study suggest that the subtle discharge variation driven by evapotranspiration powered the lateral exchange of water as well as DOM at the diurnal scale. Freshly produced DOM enriched in microbially derived components was transported to streams at the low water level. Therefore, we suggest that low order streams are a source (instead of the sink) of allochthonous DOM with a diurnal lateral exchange between stream and catchment. Bencala and Walters (1983) suggested the concept of transient storage that described the hydrologic linkages between the channel and riparian zone as the temporary physical retention or delay of solutes. This retention accounts for a significant proportion of intensified biogeochemical activities in the lotic ecosystem (Findlay et al. 1993; Harvey and Fuller, 1996; Zarnetske et al. 2007). Several studies have highlighted the role of the hyporheic zone in controlling transient storage (e.g., Harvey and Wagner, 2000; Packman and Bencala, 2000), and the discharge perturbation had impacts not only on the hyporheic zone but, more importantly, on the overall transient storage of a stream as well (D'Angelo et al. 1993; Hart et al. 1999; Zarnetske et al. 2007). At a whole reach scale, more massive transient storage was expected to enhance the interaction between dissolved nutrients and microbial communities, which increases nutrient uptake and whole-ecosystem metabolism (Fellows et al. 2006). Even though this study was conducted in a second-order stream, the diurnal variation of discharge and DOC observed in this study has theoretical implications to larger scales and needs to be considered in further studies.

During this study we observed that storm events disturbed stream biogeochemical equilibrium by transporting a significant amount of freshly produced, bioreactive, allochthonous DOM (Fig. 3.7) from shallow soils into streams within short temporal durations. High stream discharge during storms can

bypass the majority of DOM from the removal in small streams, as suggested by the pulse-shunt concept (see Raymond et al. 2016). We propose that the chromatography separation effect in the mobilization of different solutes (e.g., ions versus DOM in this study) also facilitates the transport of DOM to large rivers during hydrological events. Even though a significant quantity of nutrients are transported into the streams during storms, biochemical removal of DOM can be restrained due to the temporal and spatial separation of these nutrients in low-order streams (Redfield 1934). Large rivers receive inputs from multiple tributaries, and a longer residence time allows for the formation of well-mixed nutrients as metabolic substrates. Moreover, Graeber et al. (2018) reported that increases in bacteria production had a clear and quick response to the addition of DOC from leaf leachate, and the response ratio of planktonic bacteria was higher than that of benthic bacteria. They further noted that unlike benthic bacteria, planktonic bacteria were transported downstream with a DOC pulse resulting in a longer contact time. Large rivers are less shaded by riparian vegetation, with DOM susceptible to bio- and photo-degradation. Therefore, these factors collaboratively suggest the preferential removal of DOM in large rivers during storms. Storms in the U.S. are expected to increase in frequency and intensity in the future (Walsh et al. 2014), which will influence DOM dynamics and water quality conditions in large rivers.

3.6 Conclusions

High-resolution data in this study provide a more accurate view of the timing and magnitude of DOM export from a small forested watershed across temporal scales, yielding four main findings (Fig. 3.15). First, hydrological events played the dominant role in delivering terrestrial DOM into streams, and the pattern of DOM mobilization was regulated by hydrological conditions including antecedent hydrological conditions, and the duration of storms. Discharge variation can explain DOC export across timescales. Compared to the prompt delivery of ions, the peak of DOM lagged that of stream discharge,

suggesting distal and abundant storage of DOM in the watershed. Second, we speculate that the timing difference in the mobilization of solutes might result in the sequential input of ions, inorganic nutrients, and DOM from catchment into streams that impacts the response of aquatic ecosystems. Third, DOM dynamics at the diurnal scale were driven by the variation in evapotranspiration. Changes in stream discharge shifted the source of DOM from the catchment, resulting in higher contribution of microbial DOM under lower discharge. Lastly, spring and winter were wet seasons and had more abundant carbon availability from litterfall, which together resulted in higher DOC loadings in the stream. Mayfield is a biogeochemically simple system where the supply of watershed DOM is mostly unlimited, and discharge dictates the export of DOM. Similar conditions are expected for numerous small, forested streams within Gulf and Atlantic coastal plains. High-resolution, multiple-parameter data, however, are important in revealing processes regulating short-term watershed exports. The expansion of observation stations acquiring high-resolution water chemistry data such as the National Ecological Observatory Network (NEON) network will yield insights into DOM export dynamics and mechanisms of various spatiotemporal scales.

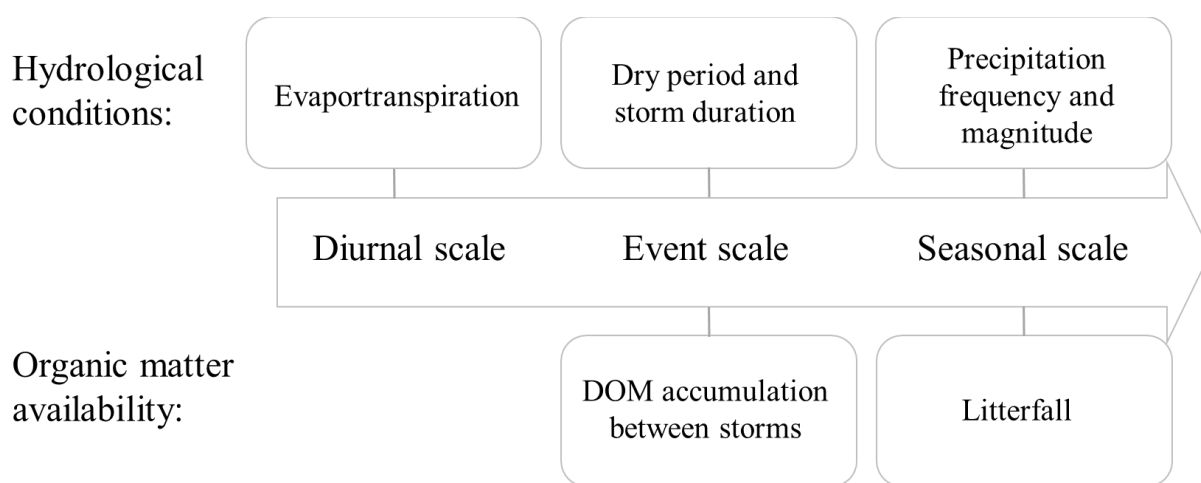


Figure 3.15. Conceptual model illustrating environmental drivers on DOM input in Mayfield creek across temporal scales.

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Appendix II

Table 1. DOC concentration versus fDOM signal

DOC (mg/L)	fDOM (signal strength)
2.49	63.27
1.89	36.03
6.20	128.45
2.12	48.93
3.13	60.03
2.66	41.97
2.26	46.60
1.65	49.76
1.66	50.69
1.86	55.81
1.99	58.48
1.62	50.05
1.84	51.36
1.79	51.91
1.42	25.24
4.12	103.50
3.74	93.38
2.04	56.58
4.09	104.21
2.27	64.79
2.03	56.65
1.99	58.04
1.68	52.07
3.04	83.65
2.77	95.43
2.10	59.05
1.78	51.39
4.47	94.29
1.45	41.97
2.13	52.62
1.86	44.48
2.49	67.90
2.35	62.52
2.66	69.13
2.86	11.78
3.15	75.59
1.99	70.35
1.98	63.51

3.45	51.66
2.68	76.17
2.23	39.84
2.04	51.27
2.63	79.32
1.90	40.61

Table 2. Loadings of the component identified using DrEEM toolbox (n=270)

Mode	nm	Component1	Component2	Component3	Component4
Ex	240	0.305264	0.335166	0.24161	0.785539
Ex	250	0.317322	0.351245	0.246302	0.248463
Ex	260	0.311172	0.303659	0.327684	0.171954
Ex	270	0.291351	0.264376	0.481406	0.177862
Ex	280	0.260756	0.247459	0.574925	0.225451
Ex	290	0.229545	0.272312	0.38285	0.31486
Ex	300	0.214384	0.301877	0.204896	0.26183
Ex	310	0.215274	0.317481	0.119165	0.1667
Ex	320	0.220364	0.306852	0.073807	0.097893
Ex	330	0.226615	0.27775	0.048746	0.062254
Ex	340	0.23237	0.231716	0.02638	0.013643
Ex	350	0.235923	0.174295	0	0
Ex	360	0.232889	0.117617	0	0
Ex	370	0.217637	0.068906	0	0
Ex	380	0.190369	0.030273	0	0.006233
Ex	390	0.159601	0	0	0
Ex	400	0.122739	0	0	0
Ex	410	0.095767	0	0.001172	0
Ex	420	0.075413	0	0.00203	0
Ex	430	0.05777	0	0.002351	0
Ex	440	0.045198	0	0.001626	0
Ex	450	0.036307	0	0.000674	0
Ex	460	0.028098	0	8.74E-05	0
Ex	470	0.023308	0	0	0
Ex	480	0.018633	0	0	0
Ex	490	0.013053	0	0	0
Ex	500	0.007834	0	0.000292	0
Em	280	0	0	0.101901	8.21E-05
Em	286	0	0	0.121323	0.002853
Em	292	0.001306	0	0.152283	0.001911
Em	298	0.006144	0	0.182957	0.004106
Em	304	0.010132	0	0.244442	0
Em	310	0.013309	0	0.258695	0.001066
Em	316	0.013345	0	0.289903	0.007361
Em	322	0.009265	0	0.310152	0.047742
Em	328	0.006698	0.001455	0.31339	0.131915
Em	334	0.001945	0.011311	0.292961	0.248322
Em	340	0	0.028826	0.293354	0.349397
Em	346	0	0.050599	0.273266	0.380784

Em	352	0	0.084132	0.262946	0.412557
Em	358	0	0.125542	0.238617	0.387756
Em	364	0	0.175273	0.213608	0.339298
Em	370	0	0.21943	0.179376	0.275843
Em	376	0	0.264069	0.148398	0.223257
Em	382	0	0.298943	0.116711	0.175191
Em	388	0.002292	0.32835	0.086613	0.139444
Em	394	0.035174	0.319012	0.061418	0.11229
Em	400	0.062181	0.31068	0.039882	0.093147
Em	406	0.092422	0.295531	0.020127	0.077273
Em	412	0.12064	0.275983	0.006016	0.066106
Em	418	0.150732	0.253983	0	0.054861
Em	424	0.175986	0.235896	0	0.043632
Em	430	0.199415	0.211644	0	0.0342
Em	436	0.222192	0.183699	0	0.027878
Em	442	0.237959	0.155999	0	0.021775
Em	448	0.249984	0.129528	0	0.01684
Em	454	0.256322	0.106265	0.003793	0.01286
Em	460	0.258864	0.084147	0.010324	0.009087
Em	466	0.259518	0.063848	0.016567	0.008107
Em	472	0.25794	0.045402	0.02063	0.007848
Em	478	0.248646	0.033098	0.027527	0.007107
Em	484	0.240734	0.021818	0.032746	0.006123
Em	490	0.232533	0.012887	0.037383	0.004583
Em	496	0.227916	0.002259	0.038959	0.003976
Em	502	0.217253	0	0.038712	0.000972
Em	508	0.203269	0	0.037109	6.85E-05
Em	514	0.189038	0	0.034744	0
Em	520	0.173514	0	0.032252	0
Em	526	0.157617	0	0.031271	0
Em	532	0.143629	0	0.031855	0
Em	538	0.130377	0	0.032176	0

Table 3. Values of stream discharge and temperature, water isotope, cation concentration and DOM character indices

Date	Q	Temp	$\delta^{18}O$	δD	Si	Na	DOC	SUVA ₂₅₄	SR	FI ₂₅₄ (HIX)	FI ₃₁₀ (BIX)	FI ₃₇₀ (FI)	F _{max1}	F _{max2}	F _{max3}	F _{max4}	C ₁ %	C ₂ %	C ₃ %	C ₄ %	
	(m ³ /s)	(°C)			(mg/L)	(mg/L)	(mg/L)														
9/13/15	0.10	19.28	-4.05	-18.59	4.42	0.91	2.26	2.99	0.78	6.36	0.56	1.62	1.81	1.35	0.47	0.77	41.18	30.62	10.61	17.59	
9/17/15	0.09	20.81	-4.17	-18.64	4.60	0.78	1.65	2.99	0.83	5.43	0.65	1.56	1.41	0.86	0.25	3.76	22.50	13.65	4.00	59.86	
9/20/15	0.08	20.42	-4.19	-18.01	4.54	0.90	1.66	2.99	0.88	9.63	0.52	1.62	1.41	1.01	0.31	0.00	51.74	36.96	11.30	0.00	
9/24/15	0.09	21.28	-4.18	-18.60	4.56	0.71	1.86	2.98	0.84	8.44	0.59	1.58	1.53	1.17	0.59	0.47	40.68	31.20	15.57	12.56	
10/1/15	0.06	20.33	-4.37	-19.07	4.84	0.75	1.99	3.39	0.75	16.05	0.52	1.58	1.44	0.93	0.08	0.00	58.87	37.96	3.17	0.00	
10/4/15	0.07	16.43	-4.32	-19.34	4.53	0.82	1.62	2.87	0.76	9.24	0.78	1.60	1.33	1.34	0.89	3.24	19.58	19.74	13.08	47.61	
10/8/15	0.10	21.86	-4.42	-20.43	4.61	0.80	1.84	2.89	0.75	12.76	0.59	1.63	1.41	0.96	0.06	1.09	40.17	27.26	1.74	30.83	
10/15/15	0.11	17.09	-4.28	-19.79	4.66	0.82	1.79	2.74	0.80	3.78	0.93	1.71	0.90	1.25	0.56	0.37	29.20	40.62	18.22	11.96	
10/22/15	0.14	17.95	-4.28	-19.88	4.51	0.76	1.42	2.65	0.86	3.59	0.93	1.75	0.68	0.96	0.48	0.12	30.36	42.75	21.41	5.47	
10/29/15	0.10	18.52	-4.13	-18.46	4.63	0.87	4.12	2.69	0.83	3.46	0.91	1.70	1.75	2.38	1.32	0.49	29.47	40.08	22.16	8.29	
11/5/15	0.16	20.04	-4.50	-19.74	4.68	0.95	3.74	2.96	0.88	3.97	0.95	1.70	1.88	2.56	1.07	0.72	30.16	41.12	17.10	11.62	
11/15/15	0.22	11.04	-4.28	-19.85	4.46	0.85	2.04	2.48	1.02	2.55	1.08	1.80	0.74	1.19	0.76	0.32	24.62	39.46	25.37	10.56	
11/19/15	0.29	16.52	-4.51	-20.44	4.08	0.91	4.14	2.44	0.80	3.69	1.13	1.79	1.53	2.27	0.80	0.59	29.52	43.69	15.35	11.44	
12/6/15	0.36	9.57	-4.15	-17.59	4.31	1.10	2.03	2.00	1.20	1.70	1.23	1.82	0.54	0.93	0.81	0.35	20.47	35.51	30.88	13.14	
12/13/15	0.11	15.66	-4.12	-17.26	4.35	1.19	1.99	2.55	0.77	3.30	1.01	1.73	0.92	1.30	0.57	0.66	26.70	37.64	16.54	19.12	
12/20/15	0.38	6.67	-4.07	-16.67	4.48	1.15	1.68	2.14	0.80	2.72	1.03	1.75	0.67	0.97	0.47	0.73	23.53	34.10	16.65	25.71	
12/27/15	0.54	18.52	-3.80	-14.79	3.42	1.03	3.04	3.18	0.79	3.59	0.98	1.70	1.28	1.69	0.74	0.50	30.39	40.23	17.52	11.87	
12/29/15	0.54	15.38	-3.73	-14.22	3.45	1.04	2.77	3.40	0.80	3.35	0.99	1.71	1.23	1.63	0.69	0.97	27.29	36.00	15.17	21.55	
1/17/16	0.20	8.98	-4.16	-17.58	3.84	1.04	1.78	2.08	0.81	2.84	1.00	1.68	0.63	0.89	0.45	0.66	24.13	33.83	17.08	24.95	
1/22/16	0.35	9.87	-4.25	-16.41	3.10	0.94	4.47	2.64	0.75	3.70	0.98	1.70	1.37	1.76	0.72	0.75	29.88	38.22	15.69	16.22	
2/11/16	0.41	7.98	-3.94	-16.82	3.38	0.96	1.45	1.97	0.77	3.35	0.99	1.71	0.47	0.67	0.29	0.24	28.04	40.11	17.25	14.59	
2/25/16	0.71	12.59	-3.96	-16.35	3.16	0.95	2.13	2.21	0.83	4.40	0.99	1.72	0.72	0.98	0.31	0.26	31.65	43.16	13.74	11.45	
3/6/16	0.60	14.04	-3.88	-15.41	3.15	0.95	1.86	1.98	0.77	3.46	0.97	1.74	0.62	0.89	0.43	0.21	28.96	41.33	19.99	9.72	
3/17/16	0.30	18.24	-3.89	-15.46	3.33	1.01	2.49	2.23	0.72	4.87	0.89	1.68	0.98	1.32	0.43	0.26	32.83	43.99	14.35	8.83	
4/10/16	0.41	16.90	-3.74	-15.68	3.34	1.00	2.35	2.06	0.71	4.11	0.91	1.68	0.88	1.19	0.48	0.30	30.89	41.72	16.85	10.53	
4/26/16	0.25	19.28	-3.91	-16.02	3.59	1.38	2.72	2.75	0.75	3.50	0.91	1.67	1.19	1.56	0.83	0.40	29.87	39.21	20.90	10.02	
5/8/16	0.24	19.38	-3.81	-15.95	3.57	1.00	2.86	2.38	0.77	3.62	0.91	1.68	1.14	1.51	0.74	0.27	31.22	41.21	20.08	7.48	

5/15/16	0.40	18.24	-3.91	-17.10	3.71	1.05	3.15	2.28	0.76	3.73	0.91	1.68	1.30	1.71	0.71	0.42	31.41	41.22	17.17	10.20
5/22/16	0.32	20.04	-4.00	-17.38	3.82	1.07	1.99	3.44	0.76	4.43	0.91	1.66	1.29	1.68	0.69	0.35	32.05	41.98	17.14	8.83
5/29/16	0.34	22.24	-4.01	-17.96	3.87	1.09	1.98	3.98	0.78	4.06	0.90	1.66	1.35	1.78	0.75	0.39	31.58	41.75	17.57	9.10
6/19/16	0.38	26.78	-3.89	-16.36	3.90	1.00	2.76	3.43	0.74	4.13	0.95	1.69	1.64	2.14	0.87	0.73	30.47	39.85	16.12	13.56
6/26/16	0.20	24.74	-3.99	-17.33	4.26	1.12	2.23	3.43	0.75	5.34	0.90	1.67	1.45	1.90	0.60	0.40	33.35	43.67	13.83	9.15
7/6/16	0.19	25.51	-4.02	-17.31	4.32	1.07	2.04	2.75	0.71	4.36	1.01	1.81	1.03	1.53	0.45	0.33	30.75	45.73	13.51	10.01
7/16/16	0.24	23.97	-3.79	-16.05	4.29	1.10	2.63	3.74	0.73	4.73	0.93	1.69	1.55	2.07	0.69	0.41	32.86	43.88	14.61	8.65
7/27/16	0.18	25.03	-4.13	-18.18	4.54	1.09	1.90	3.38	0.75	3.77	0.91	1.68	1.24	1.67	0.70	0.44	30.73	41.15	17.31	10.81

Table 4. DOM C-Q hysteresis indices and proxies of hydrological conditions

Date	Season	dry period (days)	delta C	FI	HI	Q start (m3/s)	Q highest (m3/s)	Rising limb duration (hours)	Successive events
08/20/15	Summer	22	-3.33	-0.16	-0.52	0.12	0.34	3.83	1st
08/21/15	Summer	1	-27.93	-0.65	-0.22	0.15	0.39	2.33	2nd
09/05/15	Fall	15	-0.33	-0.01	-0.61	0.11	0.25	3.83	NA
10/31/15	Fall	29	4.03	0.07	-0.61	0.08	2.24	6.67	NA
11/07/15	Fall	7	3.18	0.14	-0.56	0.27	0.41	4.67	1st
11/07/15	Fall	1	0.27	0.02	-0.38	0.37	0.85	4.33	2nd
11/18/15	Fall	11	20.34	0.45	-0.17	0.10	3.49	7.00	NA
12/01/15	Winter	13	28.03	0.64	-0.60	0.21	0.99	6.00	NA
12/14/15	Winter	13	10.65	0.33	-0.72	0.08	0.25	9.33	NA
12/23/15	Winter	9	16.29	0.62	-0.41	0.19	0.48	3.67	1st
12/24/15	Winter	1	5.06	0.19	-0.34	0.27	1.86	6.67	2nd
12/25/15	Winter	1	-18.40	-0.43	-0.39	0.49	25.61	5.17	NA
12/28/15	Winter	3	20.62	0.84	-0.35	0.35	1.22	6.33	NA
12/30/15	Winter	2	11.28	0.52	-0.44	0.56	1.19	7.00	NA
02/15/16	Winter	13	19.52	0.65	-0.61	0.30	0.85	5.17	NA
02/23/16	Winter	8	4.80	0.29	-0.37	0.27	0.74	5.17	1st
02/24/16	Winter	1	0.43	0.03	0.22	0.63	0.89	4.17	2nd
03/03/16	Spring	8	15.63	0.59	-0.36	0.44	3.74	6.33	NA
03/10/16	Spring	7	20.57	0.58	-0.42	0.45	4.32	7.33	NA
03/18/16	Spring	8	-53.98	-0.88	0.11	0.50	0.82	4.00	NA
04/11/16	Spring	5	23.99	0.53	-0.56	0.41	6.00	6.33	NA
04/29/16	Spring	18	-17.31	-0.38	-0.37	0.24	0.63	6.17	NA
06/04/16	Summer	15	-24.94	-0.41	-0.24	0.21	0.58	6.83	NA
06/13/16	Summer	9	-4.39	-0.07	-0.40	0.17	0.47	7.00	1st
06/15/16	Summer	2	-18.23	-0.68	-0.19	0.26	0.80	5.17	2nd

CHAPTER 4:

DISSOLVED ORGANIC MATTER UPTAKE IN A FORESTED HEADWATER STREAM: AN EVALUATION COMBINING REACH-SCALE TRACER RELEASE EXPERIMENTS AND NUMERICAL SIMULATION

4.1 Abstract

Fluvial networks can transport and remove a substantial amount of dissolved organic matter (DOM) in freshwater ecosystems, yet instream processing of DOM and the controlling mechanisms remain poorly understood. Here, we evaluated the uptake of DOM in a second-order forested Coastal Plain stream located in the southeastern United States. We followed the method of Tracer Additions for Spiraling Curve Characterization (TASCC) using slug additions of leaf leachates. We performed five measurements under baseflow conditions at a stream reach of 80 m in length. We further analyzed variations in DOC concentration and DOM composition during the reach-scale uptake and compared with the DOM biodegradation in laboratory incubations to evaluate DOM uptake at different scales. The mean uptake length of DOC, humic-like DOM, and protein-like DOM ranged between 74–257 m, 172–399 m, and 251–589 m respectively, and the mean uptake velocities were 14–27 mm/min, 11–22 mm/min, and 7–21 mm/min, respectively. We found that uptake length and velocity of DOC across the five experiments and found that values from the experiment under the lowest discharge were significantly different from the other four experiments. Discharge increased the uptake length and decreased the uptake velocity of both humic- and protein-like DOM compounds. Humic-like DOM had a significantly shorter uptake length and higher uptake velocity than protein-like DOM. Water column

biodegradation calculated from laboratory incubations were four orders of magnitude lower than the total uptake in the study reach. Results from the One-Dimensional Transport with Inflow and Storage (OTIS) model simulation showed that the uptake rate in the storage zone was more than two orders of magnitude higher than that in the water column. These findings demonstrate that baseflow DOM uptake was regulated by stream discharge and DOM composition, and more than 99% of reach-scale DOM uptake occurred at the benthic sediments with humic-like DOM preferentially removed, suggesting low-order streams with a higher benthic area to water volume ratio are efficient in removing terrestrial DOM.

4.2 Introduction

Freshwater ecosystems, although covering only about 1% of the Earth's surface, play a significant role in transporting carbon from land to sea while transforming and storing terrestrial carbon en route (Battin et al. 2009). Dissolved organic matter (DOM), which is a mixture of soluble organic compounds, accounts for the largest (about 60%) dynamic carbon pool (e.g., Findlay and Sinsabaugh 2003; Cole et al. 2007). Previous studies noted that a substantial amount of terrestrially derived DOM is transported and processed by streams and rivers before it enters the oceans. For instance, Schlesinger and Melack (1981) estimated that approximately 0.4×10^{15} g of terrestrial organic matter was exported to the marine environment every year. Hedges et al. (1997) suggested that about 0.25×10^{15} g of dissolved organic carbon (DOC) and 0.15×10^{15} g of particulate organic carbon (POC) were transported from continents to the ocean every year. The amount of riverine DOC is sufficient to support the turnover of DOC throughout the ocean (Williams and Druffel, 1987), and the amount of riverine POC is more than all organic carbon buried in marine sediments (Berner, 1989; Hedges and Keil, 1995).

Streams and rivers actively transform DOM during the process of transporting DOM to coastal oceans. Cole et al. (2007) and Battin et al. (2009) noted that carbon transported by inland water was removed during the transportation via sedimentary burial (about 10 to 20%) and CO₂ outgassing (about 40%). Along the fluvial network, the removal of DOM is a continuum process with longitudinal variations. Vannote et al. (1980) developed the River Continuum Concept (RCC) framework, which conceptualized the physical controls of biogeochemical processes from headwaters to large rivers and suggested that most organic matter was loaded in low order streams. Creed et al. (2015) suggested a decrease in the compositional variability of DOM as it moves downstream owing to increasing biogeochemical processing in large rivers. The uptake of inorganic nutrients along the fluvial continuum has been suggested to be regulated by both hydrological opportunity and biogeochemical reactivity (Marce et al. 2018). Hydrological opportunity referred to multiple, hydrology-related variables such as discharge, channel morphology, and water transient-storage (Marce et al. 2018). Among these variables, the importance of discharge in regulating DOM removal in streams has been recognized in a large number of empirical and theoretical studies (e.g., Peterson et al. 2001; Raymond et al. 2016; Wollheim et al. 2018). Biogeochemical reactivity is regulated by multiple processes including biological degradation, photooxidation, abiotic adsorption and flocculation (Opsahl and Benner, 1997, Cole et al. 2007, Halbedel et al. 2013). In addition to multiple uptake pathways, the complexity of DOM composition results in a wide range of uptake characters (Mineau et al. 2016). Marce et al. (2018) analyzed data from 82 published studies and found that over 66% of the variability in the retention of inorganic nutrients was explained by biogeochemical reactivity, which suggested that biogeochemical reactivity had a more relevant role on nutrient retention variability than previously thought. Nonetheless, the uptake processes of DOM in relation to

biogeochemical reactivity are understudied, and it remains unclear how the chemical composition of DOM influences the uptake characters.

Laboratory incubations and reach-scale uptake experiments are the commonly used methods for evaluating DOM uptake. In laboratory incubations, the effects of biological degradation, photochemical degradation, and sediment removal are generally evaluated separately (Wickland et al. 2007; Lu et al. 2013). Several studies found that DOM with a larger relative abundance of protein-like fluorescence components was more susceptible to biological degradation, suggesting the composition of DOM was the dominant factor regulating the rate of microbial degradation. For instance, Wickland et al. (2007) incubated soil pore water and reported that biodegradation selectively removed protein-like DOM fluorophores. Fellman et al. (2008) also found a strong positive correlation between DOM bioavailability and the percent contribution of protein-like compounds in DOM from soil solutions, and they further suggested that proteinaceous compound abundance could be used as an indicator of DOM lability to microbial degradation in aquatic ecosystems. The susceptibility of DOM to photodegradation may also be dictated by the composition. Lu et al. (2013) compared photodegradation and biodegradation of DOM in a group of temperate streams draining various land covers and reported that DOM was more readily removed by photodegradation than biodegradation, with the photodegradation rate varying as a function of percent aromatic components in DOM. Cory et al. (2014) studied the fate of DOM released from permafrost soils in shallow arctic streams and lakes and reported that DOM photodegradation rate exceeded biodegradation rate, and photodegradation accounted for 70 to 95% of total DOM removal in the water column. They suggested that the amount and lability of chromophoric DOM had a dominant impact on the susceptibility to photodegradation. Laboratory experiments that evaluated DOM removal due to

physical processes indicated a preferential removal of compounds with high molecular weight and aromaticity. For example, Kaiser and Guggenberger (2000) mixed DOM solution with soil and found the preferential adsorption of aromatic compounds to mineral surfaces. Zhou et al. (2001) characterized the adsorption isotherms of fluvic acid isolated from natural organic matter on goethite and reported the preferential adsorption of high-molecular-weight fractions.

The uptake rate of DOM estimated from laboratory experiments is often extrapolated to estimate DOM uptake at the reach and drainage basin scales. For instance, Moody et al. (2013) used the results from laboratory biodegradation experiments, along with *in situ* DOC concentration and in-stream residence time, to estimate the total loss of DOC from the catchment based on empirical rate laws. Cory et al. (2014) scaled up the laboratory measurements to basin-scale carbon processing via calculating the sum of the areal rates over the study period and multiplying the sum by the surface area of the open water. During the formulation of the Pulse-Shunt concept, DOM decomposition rate was measured from 28-day laboratory incubations was used as a constant to represent the amount of DOC being processed in all orders of streams throughout the drainage networks (Raymond et al. 2016). However, laboratory incubations represented only water column DOM processing but excluded benthic and hyporheic processes (Mineau et al. 2016), and the effects of hydrological dynamics were also not considered in bottle incubations (Acuna et al. 2004). Stream water circulation can facilitate the interaction of DOM with streambed biofilms and change the residence time as well as the equilibrium of DOM adsorption onto benthic minerals (Qualls 2000). Moreover, among biodegradation, photochemical degradation, and sediment removal, the effect of one uptake process can modify DOM composition and, thus, has impacts on the other processes. For example, moderate solar radiation could facilitate the biodegradation of DOM (Tranvik and Bertilsson, 2001; Judd et al.

2007; Cory et al. 2013), whereas overexposure to solar radiation could decrease the biological lability of DOM (Moran et al. 2000). In addition, physical adsorption to soils can significantly reduce the biodegradability of DOM (Kaiser and Guggenberger 2000). Therefore, the removal characters based on laboratory incubations are likely not an accurate representation of DOM removal in streams and rivers.

Compared to laboratory incubations, a smaller number of studies analyzed the uptake characters of DOM at the reach scale (e.g., Bernhardt and McDowell 2008). These studies measured the decline in the quantity of added tracers along a stream reach and incorporated the effects of biogeochemical and hydrodynamic processes in water columns as well as benthic and hyporheic zones (Acuna et al. 2004, Mulholland and Webster 2010). Mineau et al. (2016) summarized the literature values of reach-scale DOM uptake velocity and found they varied as a function of DOM chemical composition. Previous experiments commonly released pure chemicals as DOM tracers, such as potassium acetate (Bernhardt and Likens 2002), urea and glutamic acid (Brookshire et al. 2005), and glucose and arabinose (Newbold et al. 2006), yielding a broad range of DOM uptake rate from below 0.01 to as high as $2.12 \text{ g C m}^{-2} \text{ h}^{-1}$. Only a few researchers studied the reach-scale DOM uptake using natural DOM (e.g., leaf or soil leachates, natural river water), and they reported OM uptake velocities that were overall lower than those from pure chemicals (Bernhardt and McDowell 2008; Mineau et al. 2016). McKnight et al. (2002) investigated DOM uptake along a reach of the Snake River in Colorado by injecting solution highly enriched in recalcitrant, fulvic acid-enriched DOM collected from the Suwannee River. They observed that DOM was removed rapidly by physical processes (i.e., adsorption), where the majority of aromatic compounds were removed within the first 141 m. In contrast, Fellman et al. (2009), using leachates from different types of watershed soils (bog, forested

wetland, and upland forest watersheds), reported that DOM removal was dominated by microbial degradation within the first 25 m of the study reach. Similarly, Bernhardt and McDowell (2008) studied DOM uptake using tracers leached from sugar maple leaves and red spruce needles in streams. They also found a preferential removal of more biologically labile forms of DOM and suggested that both microbial assimilation and abiotic adsorption played a part in DOM uptake. In addition, leaf leachates from various species were removed dissimilarly. For example, the uptake of yellow birch leachate more rapid than that of spruce (Dowell 1985), and the uptake of deciduous leachate more rapid than that of conifer (Lock and Noel Hynes 1975). These studies all demonstrated the significant role that chemical compositions played on the uptake of DOM, it is thus essential to understand the uptake dynamics of different DOM compounds.

Here, we assessed the uptake of natural DOM including DOC and different DOM compositions in a second-order stream under baseflow conditions. We hypothesize that DOM chemical composition dictates the uptake length and rate of DOM when the discharge is relatively constant. The uptake parameters of bulk DOM, humic-like DOM and protein-like DOM were evaluated using the method of Tracer Additions for Spiraling Curve Characterization (TASCC), which was developed for characterizing the uptake of inorganic nutrients (Covino et al. 2010). Compared to the traditional mass-balance method that produces a single uptake length and rate of DOM from one breakthrough curve, TASCC allows for the acquisition of uptake parameters for each sampling point across the full range of tracer concentrations in a breakthrough curve. We further used the One-Dimensional Transport with Inflow and Storage (OTIS) model to numerically simulate the dynamics of DOM in order to elucidate mechanisms regulating DOM uptake.

4.3 Methodology

4.3.1 Study area

The study site, Mayfield Creek, is situated at the Talladega National Forest of western central Alabama, southeastern USA (Fig. 4.1a). The watershed is within the East Gulf Coastal Plain and has a gentle elevation gradient (0.2° watershed slope). The creek is of the second order (Strahler scale) and 5.87 km in length, and it flows into the Black Warrior River. The watershed area is 17.5 km², comprising mostly (> 98%) deciduous forest including pine (e.g., longleaf, shortleaf, yellow, and loblolly) and hardwood (e.g., oak, hickory, sweetgum, dogwood). Surface soil in the study watershed is mainly fine dark yellowish brown sandy and flaggy loam, and subsoil is yellowish red sandy and clay loam based on the United States Department of Agriculture soil survey (https://www.nrcs.usda.gov/Internet/FSE_MANUSCRIPTS/alabama/). Benthic sediment in the study reach is mainly interbedded sands and clays composed of quartz (~50 to 60%), illite (~15 to 18%), montmorillonite (~10%), kaolinite (~10 to 12%) and Iron oxides (~5 to 8%) according to the Geological Survey of Alabama. Woody trunks and organic debris can be observed on the benthic surface in the study stream reach. The study area is influenced by a humid, subtropical climate. The mean annual temperature is 15°C, and the mean annual precipitation is 13 cm, based on the longest available data (from 2014 to 2016) collected by the nearest gauging station (KALTUSCA20; 21.8 km) to our study site. Two observational sites from the National Ecological Observatory Network (NEON) were established in the study area. An aquatic site (Alabama, D08 Ozarks Complex; <https://www.neonscience.org/field-sites/field-sites-map/MAYF>) was in the same tributary and 0.65 km upstream of the study reach. A forest site was 2.22 km from the study reach (<https://www.neonscience.org/field-sites/field->

sites-map/TALL) and was designed to record atmospheric conditions from the top of the vegetation canopy to the ground and soil conditions at multiple depths.

The sediments in the Mayfield Creek are mainly unconsolidated fine sand and gravelly sand. The study reach is 80 m long and about 6 m wide in a straight channel with little variation in width and depth (Fig. 4.1b). According to an *in situ* water level transducer deployed at the study reach from July 2015 to July 2016, the discharge under baseflow conditions varied from 0.05 to 1.00 m³/s and the highest discharge recorded was 25.65 m³/s during storms. We also measured discharge using the velocity-area method. At the study reach, a uniform section of the stream was selected to measure the depth (m) and velocity (m/s) every 25 cm. Stream water discharge was calculated by multiplying the area by the mean velocity of each subsection and then summing across the subsections. Under baseflow conditions, the flow rate in the central point of the stream channel was not higher than 0.5 m/s. Based on grab samples collected every two weeks from the year 2015 to 2016 under baseflow conditions, nitrate and phosphate concentrations in the study stream averaged 13.53 and 2.36 µg/L, respectively, and DOC concentrations ranged between 1.42 and 4.47 mg/L and averaged 2.35 mg/L (Appendix II Table 3). Humic-like compounds were the dominant (mean = 69%) DOM component, and microbially humic DOM and protein-like DOM accounted for 16% and 15%, respectively (Appendix II Table 3).

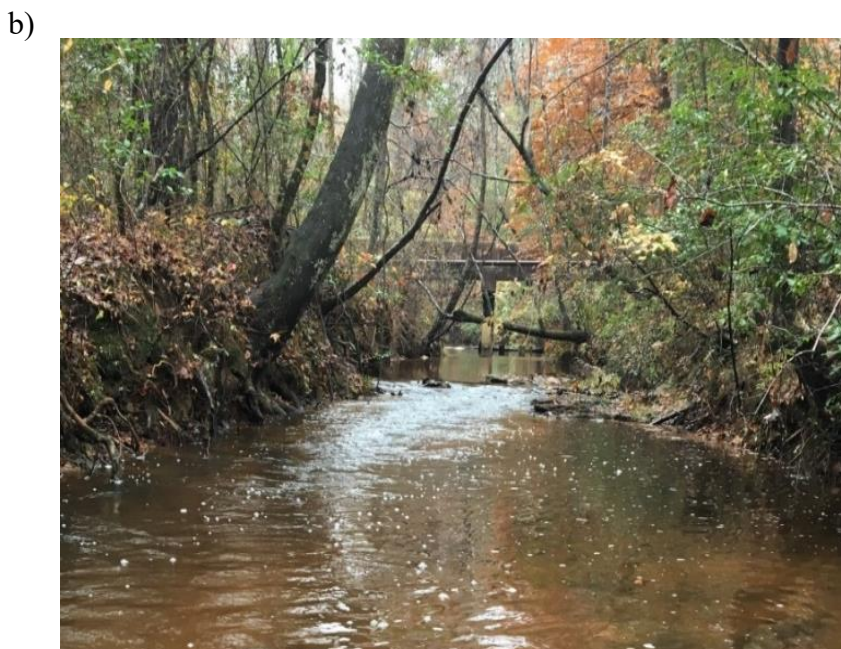
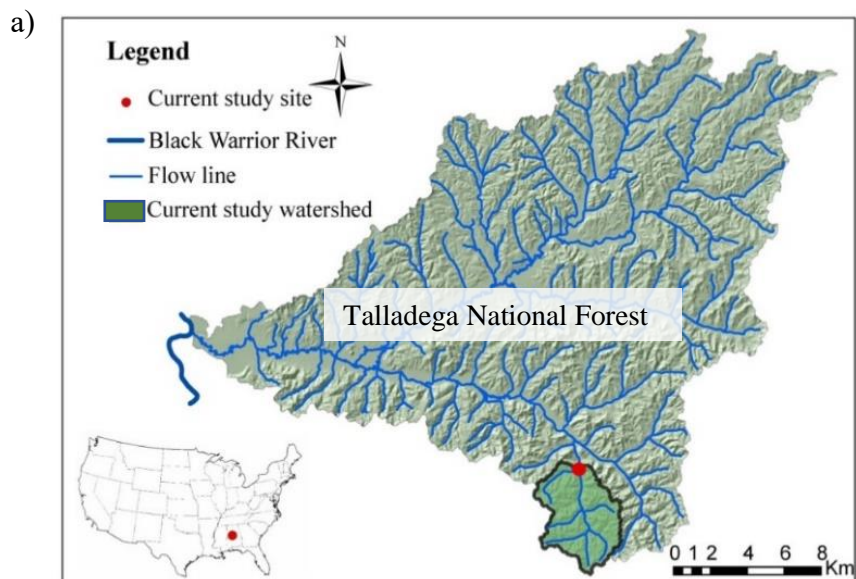


Figure 4.1. a) Location of the study site in the Talladega National Forest, Alabama, USA. Streams are indicated by blue lines, the watershed boundary is indicated by black lines, and the sampling site is denoted by the red dot; b) the study reach of tracer release experiments in a second-order forested stream.

4.3.2 Tracer preparation and release

DOM solutions were made from plant litter and released into the study reach to simulate natural DOM dynamic. Plant litter was collected from the Mayfield watershed and was composed of oak leaves (~80%), sweetgum leaves (~10%) and pine leaves (~10%). The plant litter was placed into two 120-liter buckets and soaked with stream water for seven to ten days at the stream bank under a bridge. The leachates were then filtered through 400 mesh screens (sieve opening 0.037mm) and combined into a 240-liter container. 120g NaCl was added to the leachates as a conservative hydrological tracer. The amount of NaCl was determined based on the estimation based on previous experiments that the released tracer would increase Cl^- by 25% to 50% over the ambient condition at the downstream sampling location. The NaCl enrichment enabled the detection of conservative tracers at the downstream sampling location but would not significantly change the stream salinity (i.e., the increase in stream conductivity by tracer release was not higher than that induced by storm events) and influence the function of stream biota. Our five tracer releases were conducted between December 2017 and March 2018 with small variations in the water temperature (about 5–17 °C), which limited the influences of temperature on DOM uptake. The tracer was released as an instantaneous slug at the same upstream location. Based on the color change of stream water, the tracer was thoroughly mixed with the stream water within ten meters downstream from the releasing point, which ensured a complete mixture between tracer and stream water at the 80 m downstream sampling location.

4.3.3 Breakthrough curve (BTC) construction

The BTCs were recorded every minute 80 m downstream from the releasing point using a conductivity logger (HOBO, ONSET) and a fluorescence DOM (fDOM) logger (Cyclops-7F, TURNER Designs). These loggers were deployed for more than 40 minutes, including at least 10

minutes prior to the tracer release and 10 minutes after the end of BTCs, to ensure the recording of complete BTCs. Meanwhile, grab samples were collected every minute at the downstream location for 25 minutes, and the first grab sample was collected 3 minutes after the tracer release. This sampling strategy, depending on the stream water flow rate, collected at least two samples prior to the start of BTCs and about five samples after the end of BTCs. Grab samples were also collected from ambient conditions (i.e., before the tracer release) and the reservoir of DOM tracer. All grab samples were stored on ice and filtered using 0.2 μm syringe filters (polyethersulfone) on the same day of collection. Stream discharge was measured after each tracer release using the mechanical current-meter method as described by the USGS (<https://water.usgs.gov/edu/streamflow2.html>), where the stream water depth and flow rate were measured every 0.25 m across the stream.

4.3.4 DOM character analysis

Dissolved organic carbon concentration measurements were performed on a Shimadzu TOC-V total organic carbon analyzer, following the method described in detail in Shang et al. (2018). The calibration curve was constructed using potassium hydrogen phthalate solutions with six known concentrations. Carbon-free ultrapure water was measured regularly to assess instrumental baseline, and a seawater reference standard (Hansell laboratory, <http://yyy.rsmas.miami.edu/groups/biogeochem/CRM.html>) was used to confirm accuracy. Two to three samples were randomly selected for replicate measurements in each run, yielding the relative standard deviation lower than 3.69%. DOM ultraviolet-visible absorbance was scanned using a Shimadzu UV-1800 spectrophotometer every 1nm from 190 to 670 nm. Optical indices including specific UV absorbance (SUVA_{254}) and spectral slope ratio (S_R) were calculated (Weishaar et al. 2003 and Helms et al. 2008). DOM fluorescence was measured using a Horiba

fluoroMax3 fluorescence spectrometer under signal ratio mode (S/R) with the excitation wavelength interval of every 5 nm from 240 nm to 500 nm and emission wavelength interval of every 3 nm from 280 nm to 538 nm. Fluorescence index (FI) and humification index (HIX) were calculated based on fluorescence properties (e.g., Huguet et al. 2009; Cory et al. 2010). DrEEM toolbox was used to acquire and validate the compositional information of DOM (n=144, no outlier was removed; Murphy et al. 2013). Chloride concentrations were measured using an Ion Chromatograph (Dionex DX-600), and the relative standard deviation of replicate measurements was lower than 1.96%. The concentrations of DOC and Cl⁻ in grab samples were used to convert the continuous data from *in situ* DOM fluorescence and conductivity sensors into the concentrations of DOC and Cl⁻, respectively.

4.3.5 Biodegradable DOM assessment

The biodegradability of ambient DOM in stream water was assessed using 28-day laboratory incubations. Four sets of grab samples collected between October 2015 and May 2017 were filtered using acid cleaned 0.7 µm (GF/F) and 0.2 µm (Whatman polycap) filters, and then inoculated by adding *in situ* raw stream water (1% by volume) (Servais et al. 1989). Each inoculated sample was distributed into three pre-combusted, one-liter amber glass bottles and incubated at 20°C in the dark for 28 days. Subsamples were collected on day 0, 2, 5, 10, and 28 and were assessed for DOM quantity and quality. The degradation rate of DOM was assessed under the assumption of the first-order degradation kinetics (e.g., Middelburg 1989; Ogawa et al. 2001).

4.3.5.1 DOM uptake characterization

We calculated the uptake length (S_w , m) and uptake velocity (V_f , mm/min) of DOM following the TASCC method (Covino et al. 2010). S_w described the mean distance DOM

traveled in the stream before being removed, and V_f described the uptake efficiency relative to the availability of DOM (Stream Solute Workshop 1990). Data from *in situ* sensors were first converted to the concentrations of DOC and Cl^- based on the calibration curves correlating sensor data with laboratory measurements (Fig. 4.2, 4.3). We then used the central moving average method to smooth out noise in the sensor measurements. Specifically, each smoothed data point was the calculated average of five measures including the current sampling point and two measurements prior to and after the current point (Appendix III Figure 1 for the comparison of data before and after the smooth approach). We followed the approach of TASCSC (Covino et al. 2010) in evaluating the uptake of DOM using the smoothed data, and data from the start (first raising point on the BTC) to the peak of each BTC were used in evaluating DOM uptake. We natural-logarithm transformed the ratios of non-conservative over conservative tracer concentrations (backgrounded corrected) of both tracers and each sample collected downstream. The results were plotted against the stream distance to calculate the slopes of each data pair (injectate and each grab sample) yielding the longitudinal uptake rate of reactive tracer (k_w). The uptake length of reactive tracer (S_w) was calculated as the negative inverse of k_w . The uptake velocity (V_f) of reactive tracer was determined as:

$$V_f = \frac{Q}{S_w \times w} \quad (4.1)$$

where Q is the stream discharge, w is the average width of the experimental reach. The uptake length and velocity were plotted against the geometric mean ($X_{add-dyn}$) of the reactive tracer concentration in the grab sample of interest. $X_{add-dyn}$ can be calculated as:

$$X_{add-dyn} = \sqrt{X_{add-obs} \times X_{cons}} \quad (4.2)$$

where $X_{\text{add-obs}}$ is the background corrected reactive tracer concentration (mg/L) observed in a sample; X_{cons} is the background corrected reactive tracer concentration (mg/L) expected in the sample assuming if the added tracer travels conservatively.

We also calculated percent DOC removal and the uptake length using a simple mass balance approach. The recovered mass (T_{MR}) of DOM and Cl^- were both calculated by integrating the tracer concentration (T_C) across the BTC and then multiplied by discharge (Q):

$$T_{MR} = Q \int_0^t T_C(t) dt \quad (4.3)$$

The uptake length was calculated as dividing the length of the study reach by the natural-logarithm transformed ratios of DOM over Cl^- concentrations of the recovered mass over that in the tracers (Covino et al. 2010). Different from the TASC method that generated multiple uptake lengths corresponding to various DOC concentrations across the BTC, this approach generated only one uptake length from each tracer release experiment.

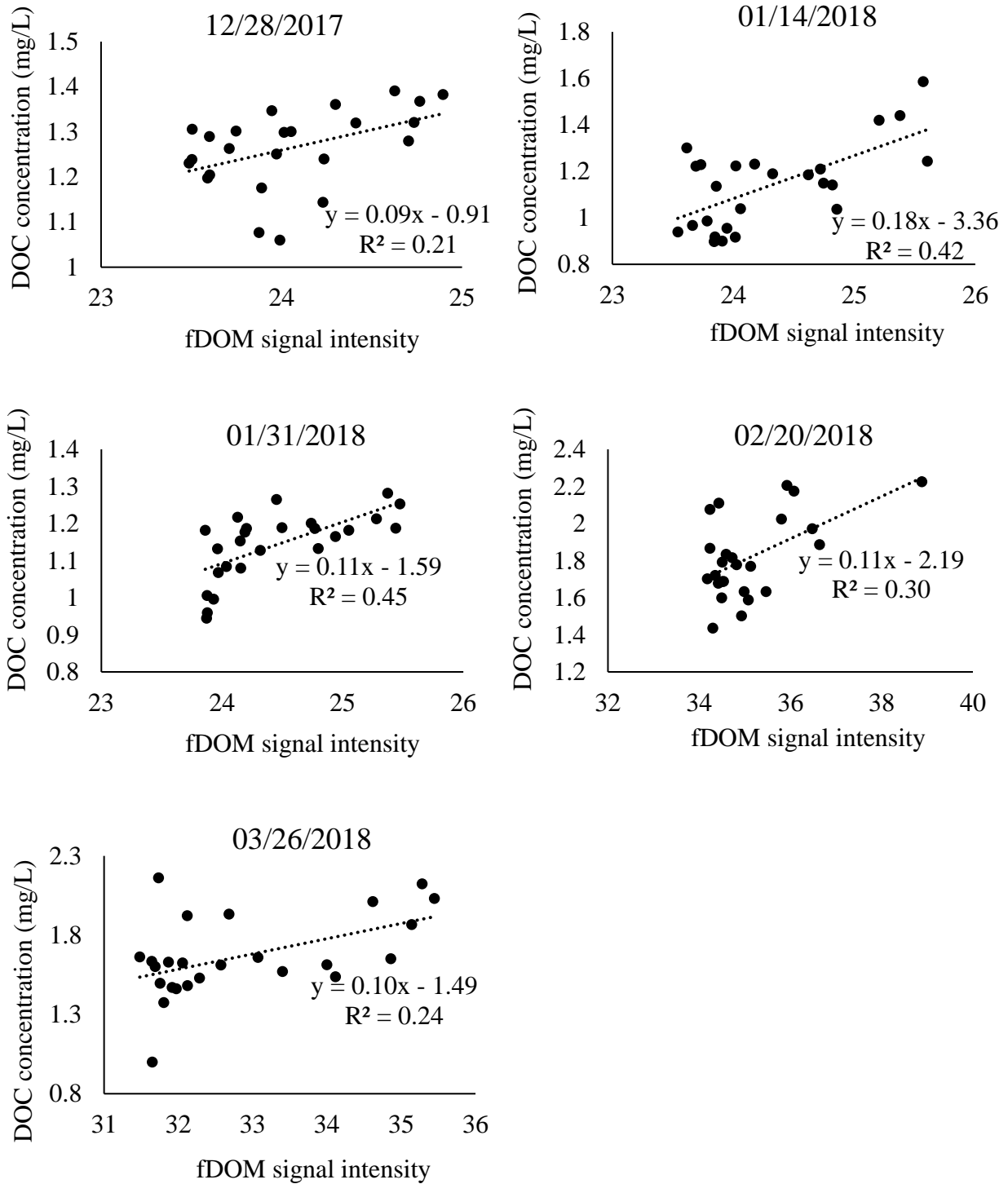


Figure 4.2. Standard curves of DOC concentration in grab samples versus fDOM intensity from *in situ* sensors based on five tracer release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA (n=24 or 25 in each standard curve).

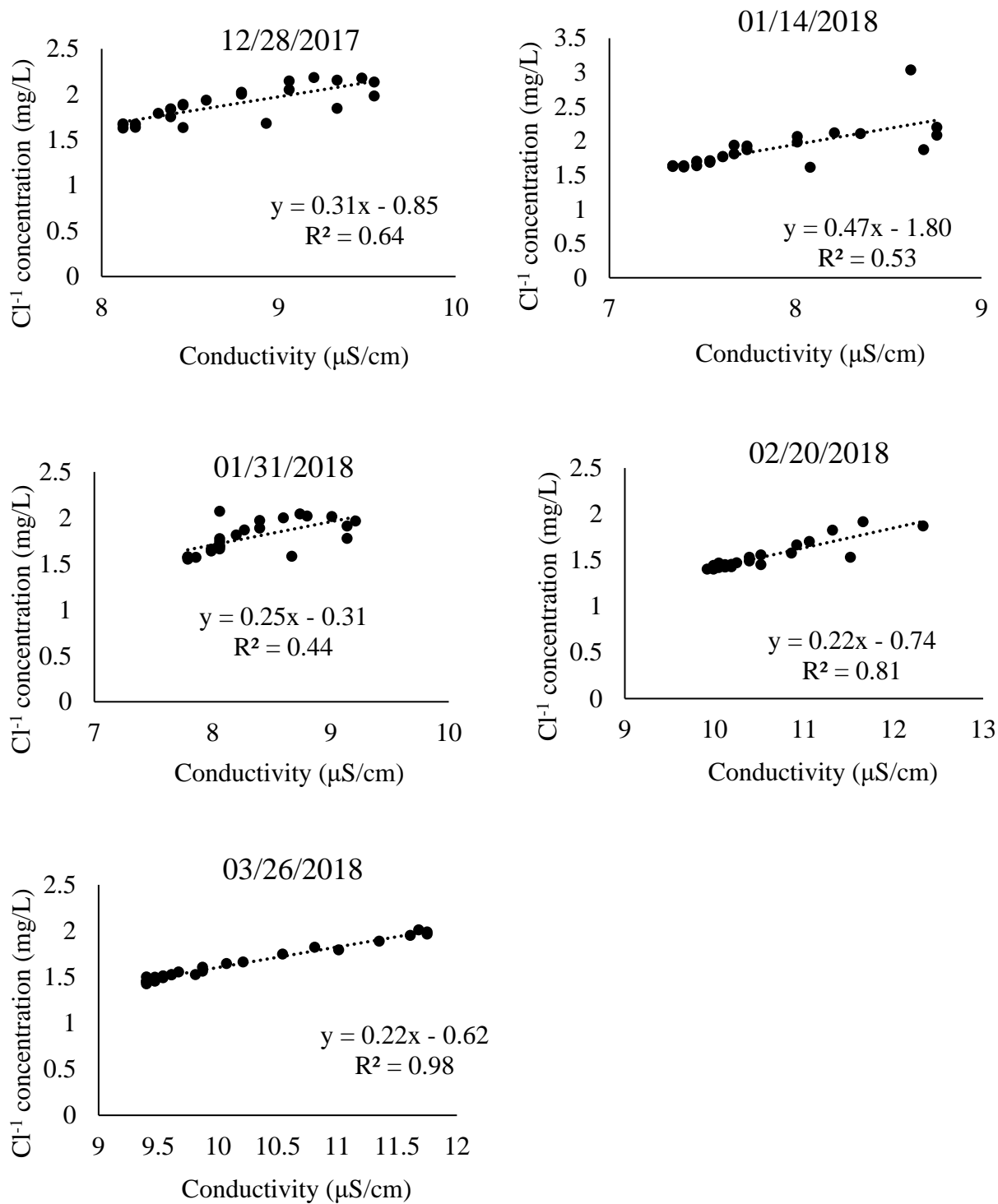


Figure 4.3. Standard curves of Cl^- concentration in grab samples versus conductivity from *in situ* sensors based on five tracer release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA (n=24 or 25 in each standard curve).

4.3.5.2 Numerical simulation of breakthrough curves

In order to quantify the appropriate physical, hydrological and biochemical parameters of the study reach determining the solute transport and to compare the results with those from TASCC, we used the model of One-dimensional Transport with Inflow and Storage (OTIS; Runkel 1998), a solute transport model designed by USGS (<https://water.usgs.gov/software/OTIS/>), in simulating the transportation and fate of non-conservative (i.e., DOM) and conservative (i.e., Cl⁻) tracers. The transportation of chloride ion was assumed to be affected solely by advection and dispersion in the main channel as well as storage effects in the immobile zone, as simulated using the following equations:

$$\frac{\partial C}{\partial t} = -\frac{Q}{A} \frac{\partial C}{\partial x} + \frac{1}{A} \frac{\partial}{\partial x} \left(AD \frac{\partial C}{\partial x} \right) + \frac{q_{LIN}}{A} (C_L - C) + \alpha (C_S - C) \quad (4.4)$$

$$\frac{dC_S}{dt} = \alpha \frac{A}{A_S} (C - C_S) \quad (4.5)$$

where C, C_L, and C_S (mg/L) were solute concentrations in the main channel, lateral inflow, and storage zone, respectively. C_L was assigned as 0 in the present study (no lateral flow), and the initial C and C_S at releasing site were assigned as equal to the concentration of tracer; A and A_S (m²) were cross-sectional areas in the main channel and storage zone. D was the dispersion coefficient (m²/s); Q was the stream discharge (m³/s), which was acquired from direct measurements using the velocity-area method; q_{LIN} was lateral inflow rate (m²/s), which was assigned to 0 in the present work; t was time (s); x was longitudinal distance (m) of the study reach (up to 80m in this study); and α was storage-zone exchange coefficient (s⁻¹). Parameters of A, A_S, D and α were adjusted to acquire the best simulation.

For the simulation of DOM, the stream channel and hydrological condition parameters (e.g., D, A and A_S) were assumed to be the same for conservative and nonconservative tracers,

and the effects of kinetic sorption and first-order decay were added, following the equations below:

$$\frac{\partial C}{\partial t} = (C) + \rho\lambda'(C_{sed} - K_d C) - \lambda C \quad (4.6)$$

$$\frac{dC_S}{dt} = S(C_S) + \lambda'_S(C'_S - C_S) - \lambda_S C_S \quad (4.7)$$

where (C) and S(C_S) represented the physical processes described in equation 4.4 and 4.5. C_{sed} and C'_s (mg/L) are the sorbate concentration on sediment and background storage zone solute concentration, respectively, and were assigned as 0 as initial values; K_d (L/mg) is the distribution coefficient; λ and λ_S (s⁻¹) are the first order decay coefficients in the main channel and storage zone, respectively; λ' and λ'_S (s⁻¹) are the sorption rate coefficients in the main channel and storage zone, respectively; ρ (mg/L) is the mass of accessible sediment by volume water. Parameters of ρ, K_d, λ, λ', λ_S and λ'_S were adjusted to acquire the best simulation.

The variables in the OTIS model in the simulation of conservative tracers were determined using an exhaustive semiautomatic search to acquire the best fitting simulation based on a nonlinear least-square algorithm. The lower and upper limits and the step length in the selection of variables are given in Appendix III Table 1, and the choice of these parameters was aiming to explain the most variation in a BTC (based on R²). Results from the simulation of conservative tracers describing the stream channel were then used in simulating the dynamics of humic-like and protein-like DOM components. The first order decay and sorption rate coefficients in the storage zone (λ_S and λ'_S) and stream water (λ and λ') were fitted to achieve better explanations of the BTCs of DOM components. We assigned that λ= λ' and λ_{S}=λ'_S in order to reduce the variables in the simulation process, and this assumption implied the same rates of first-order decay and sorption. The criterion of the simulation was that at least 80% of the variations in DOM components were explained by the numerical model.}

4.3.6 Statistics and error analysis

We conducted all the statistics using the SPSS software with the level of significance (α) set at 0.05 (two-tailed). Data normality was verified using Shapiro-Wilk tests. All data were normally distributed, and thus parametric tests were used including the Pearson correlation test and the Student's t-test. Errors induced by the standard curve converting *in situ* data to the concentrations of DOC and Cl^- were eliminated during the calculation of uptake parameters. Specifically, all the uptake parameters were calculated based on the ratios of background-corrected concentrations of reactive over those of conservative tracers at each sampling point. The error induced by the uncertainty of the constant in the calibration equation was removed in the process of subtracting ambient condition from the sampling point on the BTCs. The error induced by the uncertainty of the slope in the calibration equation was also removed during the calculation of uptake rate (K_w) of grab samples.

4.4 Results

4.4.1 DOM characters in tracers

The five tracer stocks we prepared had DOC concentrations ranged between 249.23 mg/L and 662.40 mg/L and averaged 401.49 mg/L, which were two orders of magnitude higher than the ambient DOC concentrations that ranged from 1.39 to 3.14 mg/L. Indices based on UV-vis absorbance such as SUVA_{254} and S_R averaged 2.37 and 0.69, respectively, and they were not significantly different ($P > 0.867$, t-test) from those of ambient DOM (mean $\text{SUVA}_{254} = 2.39$; mean $S_R = 0.70$). The FI of tracers averaged 1.72, which was similar to ambient DOM (mean FI = 1.73; $P = 0.690$, t-test). The HIX values of the tracers averaged 1.33, which was significantly lower than ambient DOM (HIX mean = 2.88; $P = 0.023$; t-test) (Fig. 4.4a, Appendix III Table 2). DOM fluorescence coupled with parallel factor analysis identified four components including terrestrial

humic-like DOM (C₁ and C₂), microbial humic-like DOM (C₃) and protein-like DOM (C₄) (Fig. 4.5, Table 4.1, Appendix III Table 3). In agreement with HIX, the compositional data showed that the tracers were more enriched in protein-like DOM compared to DOM in ambient conditions (P<0.001; t-test; 31–72% versus 5–25%; Fig. 4.4b, Appendix III Table 2).

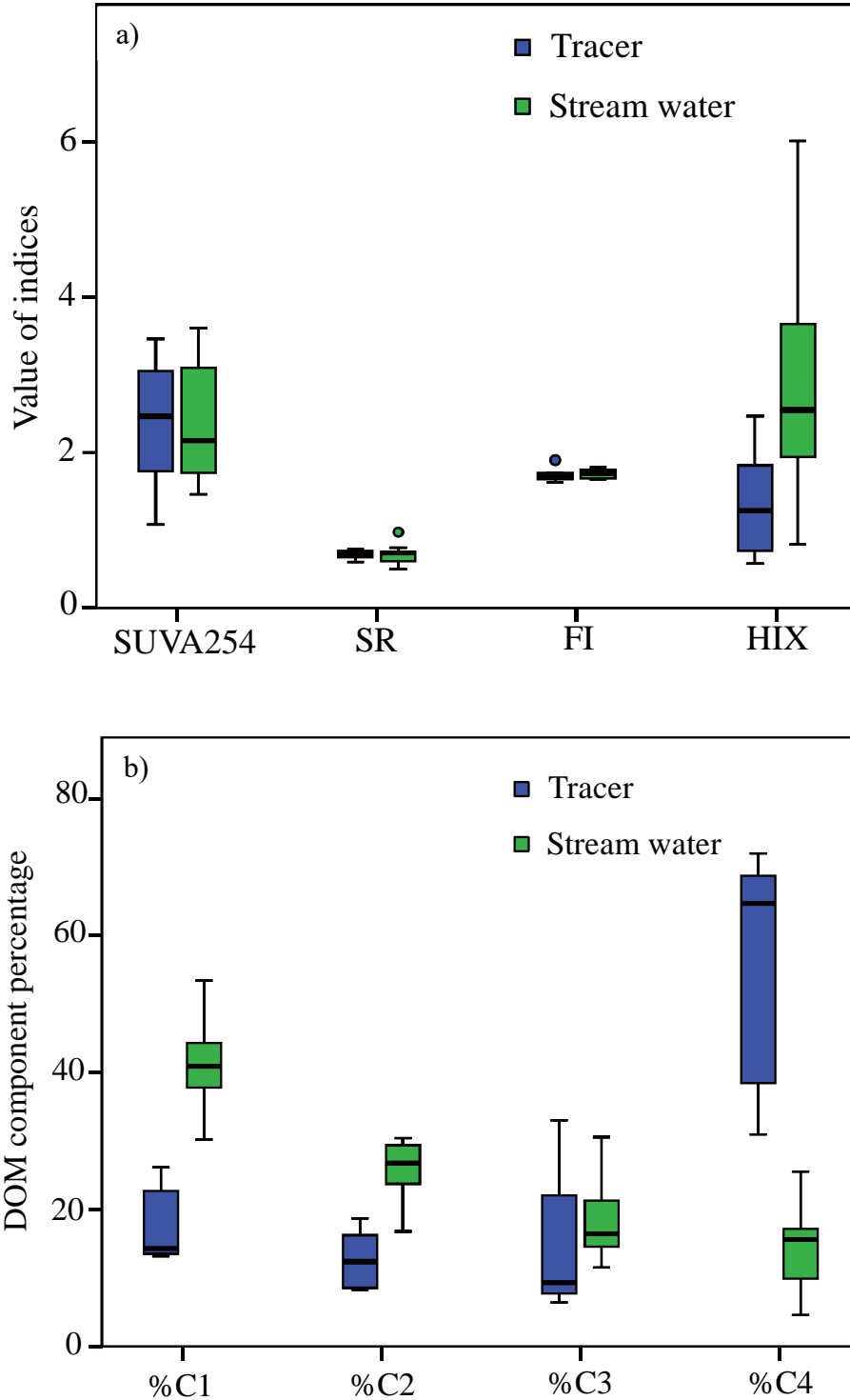


Figure 4.4. Comparison of DOM quality in grab samples from tracer (blue boxes) and stream water (green boxes) in five DOM tracer-release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA

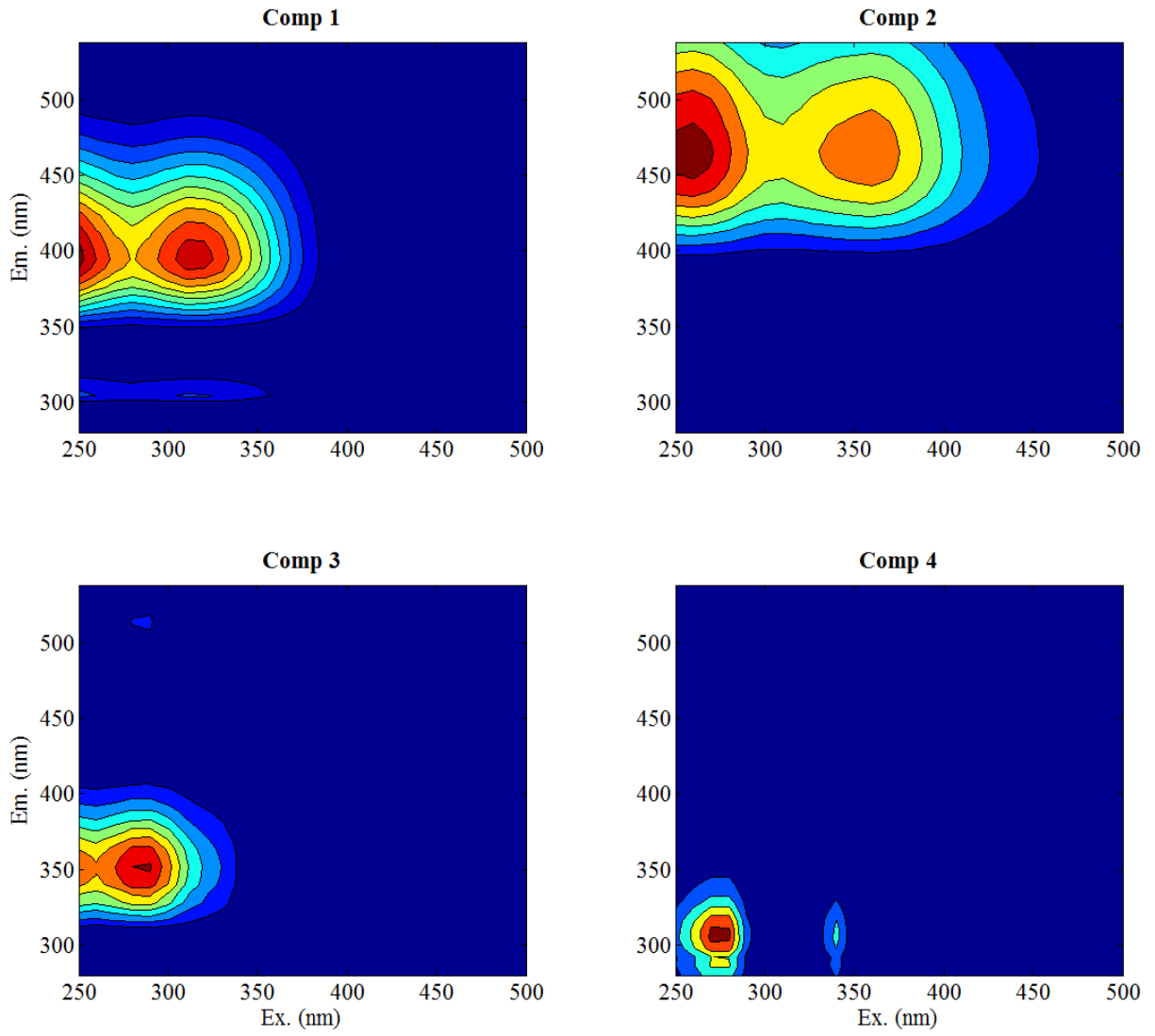


Figure 4.5. Excitation-emission spectra of the four fluorescence components (C_1 – C_4) identified by DrEEM of grab samples from five DOM tracer-release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA.

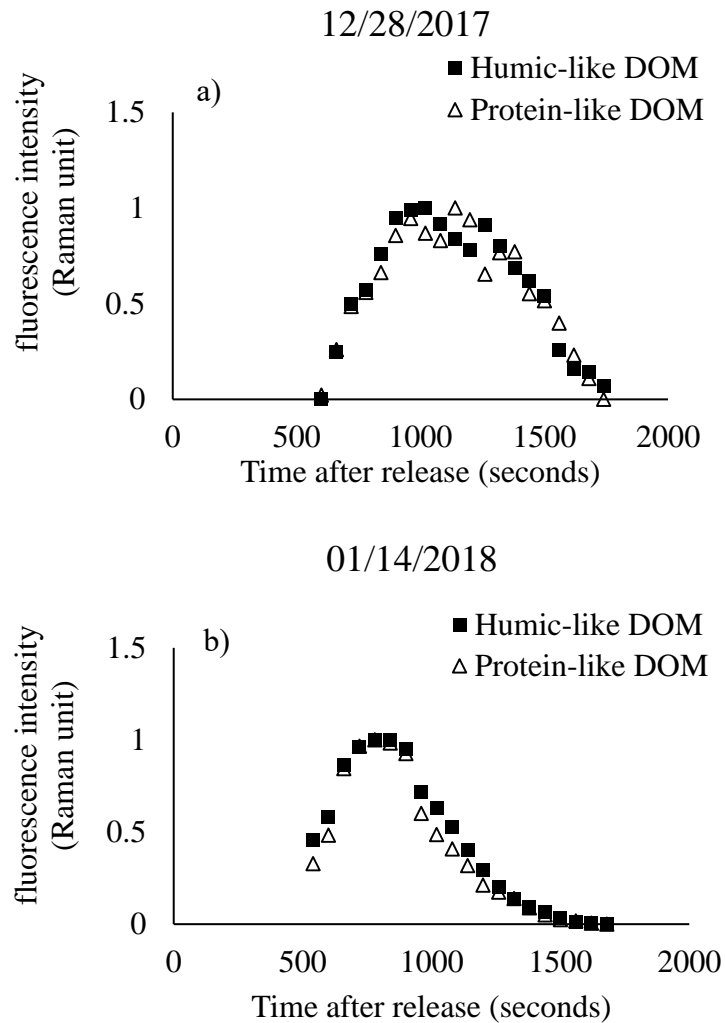
Table 4.1. Characteristics of the four fluorescence components identified by DrEEM and the attributed sources based on five DOM tracer-release experiments conducted in a 2nd order stream the Talladega National Forest, Alabama, USA.

Component	Excitation maximum wavelength (nm)	Emission maximum wavelength (nm)	Similar fluorescence components identified in previous studies				Present study
			Coble et al. 1998	Stedmon and Markager 2005	Cory and McKnight 2005	Yamashita et al. 2010	
C ₁	250 (310)	394	A	3	SQ3	C3	Terrestrial humic-like DOM
C ₂	260 (360)	466	C	4	C1	C1	Terrestrial humic-like DOM
C ₃	290	352	M	6	Q3 or C3	C4	Microbial humic-like DOM from soils
C ₄	270	304	B or T	8	C8 or C13	C7	Protein-like DOM

4.4.2 BTCs in tracer release experiments

The tracer release experiments were conducted under baseflow conditions with stream discharge ranging from 0.19 to 0.31 m³/s. Despite the discharge variation in this study covered a small range of the stream discharge under baseflow conditions (i.e., 0.05 to 1.00 m³/s), based on our one-year *in situ* water level data from the study reach, more than 70% of stream discharge under baseflow conditions fell below 0.31 m³/s. During each experiment, the fDOM recordings from the sensor had a significant positive correlation with the DOC concentrations of grab samples ($r \geq 0.453$; $P \leq 0.026$; Fig. 4.2). Therefore, fDOM data were converted to DOC concentration based on the calibration curves before they were used for BTCs. When DOC concentrations from the grab samples were directly used in BTCs, however, no evident BTCs peaks were observed, probably due to the lower sensitivity of DOC measurements than fDOM sensors. Our tracer release experiments increased the DOC concentration at the sampling location by 0.13 to 0.53 mg/L, which were about 11% to 31% higher than the ambient DOC. Meanwhile, stream water conductivity was also significantly correlated to the Cl⁻ concentrations in grab samples ($r \geq 0.662$; $P \leq 0.001$; Fig. 4.3), and the conductivity in BTCs was converted to Cl⁻ concentration based on the standard curves. More than 96% of the dynamics in Cl⁻ transport could be simulated by the OTIS model solely with parameters related to hydrological transport ($R^2 \geq 0.96$; mean=0.98). The simulated stream channel size (A) ranged between 1.8 and 2.2 m², and the storage zone (As) ranged between 0.6 and 0.8 m² (Table 4.2). In this study, microbial humic-like did not show obvious BTCs during the release experiments, and we used the term, humic-like DOM, as the sum of two terrestrial humic-like DOM components (i.e., C1 and C2). The decay and sorption rate coefficients of both humic-like and protein-like DOM in benthic sediments were more than three orders of magnitude larger than those in stream water (Table 4.3

and 4.4). The BTCs of DOC concentration showed a similar trend as the conservative Cl^- , but different DOM compounds were transported dissimilarly, especially under high discharge conditions. Specifically, under the conditions with stream discharge higher than $0.25 \text{ m}^3/\text{L}$, the intensities of protein-like DOM matched humic-like DOM on the rising limbs of BTCs but had apparent deviations on the falling limbs by maintaining a high percentage of protein-like DOM with a decreasing contribution from humic-like DOM (Fig. 4.6c, 4.6d and 4.6e; data in each BTC were standardized to 0 to 1 scale by subtracting the minimum value and then divided by the difference between the maximum and minimum values).



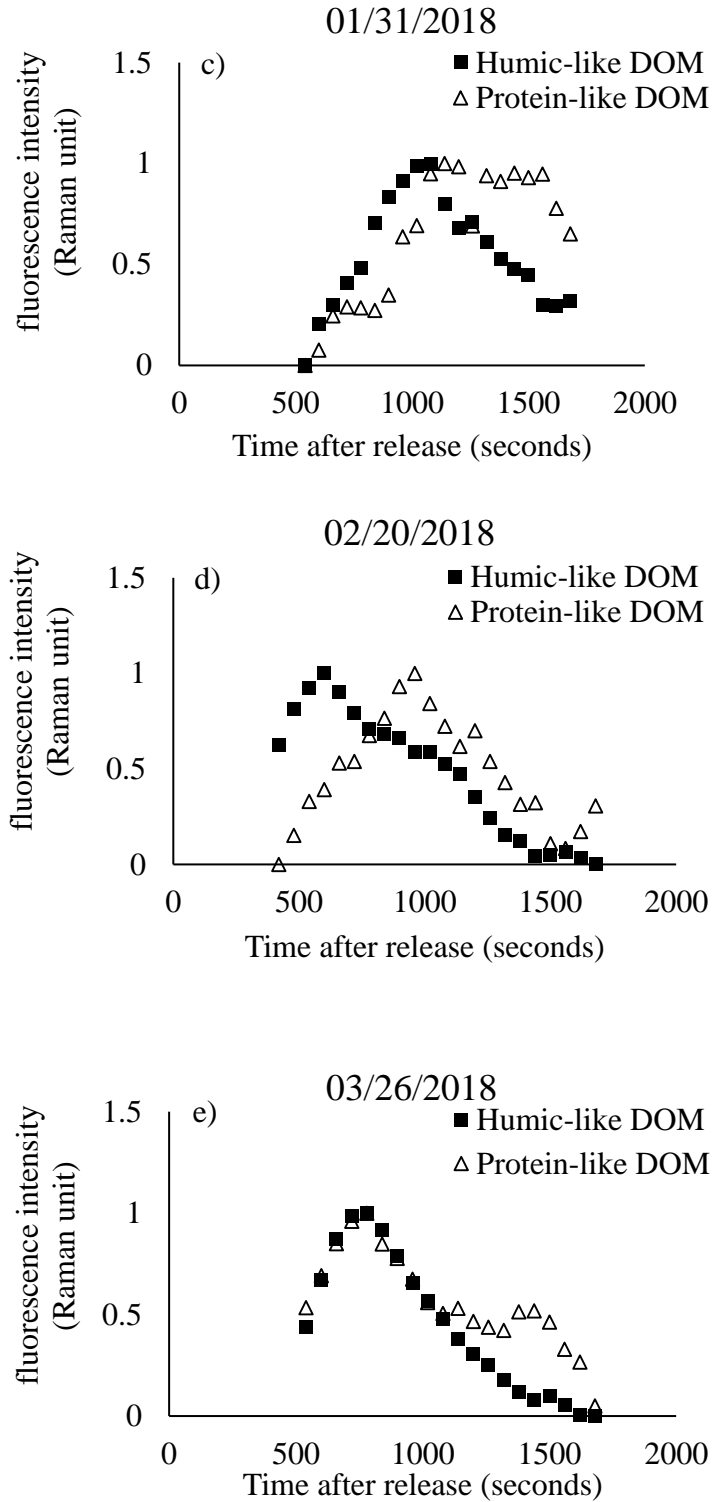


Figure 4.6. Breakthrough curves of humic-like (C1 and C2) and protein-like DOM (C4) (standardized to 0 to 1 scale using min-max normalization) in five DOM tracer-release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA.

Table 4.2. OTIS simulation results based on Cl^- concentration in five DOM tracer-release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA.

date	A (m²)	As (m²)	D (m²/s)	Q (m³/s)	α (s⁻¹)	C and Cs (mg/L)	R²
12/28/17	1.8	0.6	0.04	0.19	0.0016	337.26	0.99
01/14/18	1.8	0.6	0.01	0.23	0.0018	326.44	0.98
01/31/18	2	0.8	0.01	0.26	0.002	329.59	0.97
02/20/18	1.8	0.8	0	0.31	0.00185	326.35	0.96
03/26/18	2.2	0.8	0.08	0.25	0.001	315.60	0.98

Table 4.3. OTIS simulation results based on the fluorescence intensities of humic-like in five DOM tracer-release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA.

date	P	Kd	λ and λ' of humic DOM (s^{-1})	λ_s and λ'_s of humic DOM (s^{-1})	R²
12/28/17	1000	1	10^{-7}	0.002	0.91
01/14/18	1000	1	10^{-7}	0.0005	0.97
01/31/18	1000	1	10^{-6}	0.0026	0.85
02/20/18	1000	1	10^{-7}	0.0009	0.90
03/26/18	1000	1	10^{-6}	0.0004	0.96

Table 4.4. OTIS simulation results based on the fluorescence intensities of protein-like in five DOM tracer-release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA.

date	P	Kd	λ and λ' of protein DOM (s^{-1})	λ_s and λ'_s of protein DOM (s^{-1})	R²
12/28/17	1000	1	10^{-7}	0.0019	0.92
01/14/18	1000	1	10^{-7}	0.0005	0.97
01/31/18	1000	1	10^{-6}	0.025	0.86
02/20/18	1000	1	10^{-6}	0.004	0.82
03/26/18	1000	1	10^{-6}	0.0004	0.87

4.4.3 DOM uptake character vs discharge and DOC concentration

Dissolved organic matter from plant litter leachate was estimated to be removed from the study reach within 74 to 257 m based on the uptake lengths calculated following TASC method. The uptake lengths estimated using traditional mass balance method ranged from 96 to 459 m, and in each experiment, the uptake length derived from mass balance method was within the range of the results from TASC (Fig. 4.7; Dashed lines denote the uptake length estimated based on mass balance method; Appendix III Table 4, 5). Stream discharge had a positive impact on the mean DOC uptake length when the stream discharge increased from 0.19 to higher than 0.23 m³/s ($P \leq 0.039$; Fig. 4.7). Within each experiment, the uptake length increased with the DOC concentration at the corresponding sampling point (Appendix III Figure 2). The mean uptake velocities (V) of DOC concentration ranged from 14.28 to 27.12 mm/min. The highest uptake velocity ($V=27.12$ mm/min) was observed under the condition with the lowest stream discharge ($Q=0.19$ m³/s). Four experiments with slightly higher discharges (0.23 to 0.31 m³/s) had mean uptake velocities from 14.28 and 17.32 mm/min, and three of them showed significantly lower uptake velocity than the one conducted at the lowest discharge ($P \leq 0.033$; Fig. 4.8; Appendix III Table 6). In each experiment, the uptake velocity decreased with the DOC concentration at the corresponding sampling point (Appendix III Figure 3).

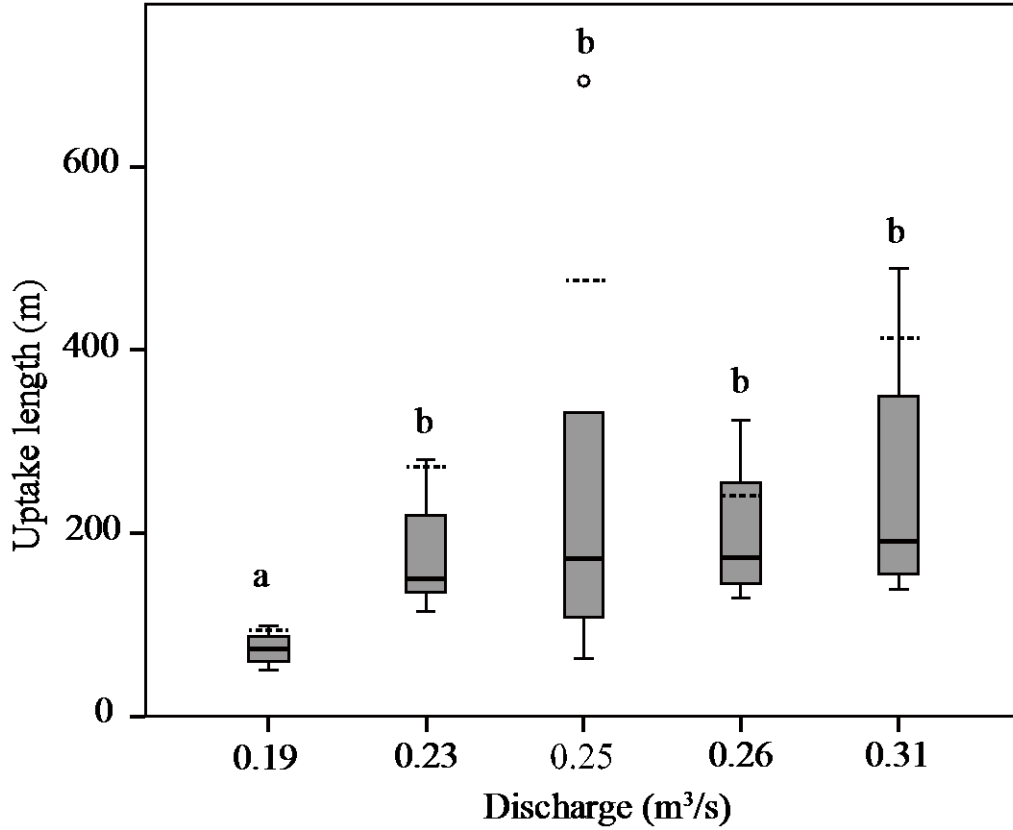


Figure 4.7. DOC uptake length ranges under different stream discharges calculated using TASCSC based on five DOM tracer-release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA. Dashed lines denote the uptake length estimated based on mass balance method. Different letters above the boxes signify significant differences in uptake length between discharge conditions detected by the t-test.

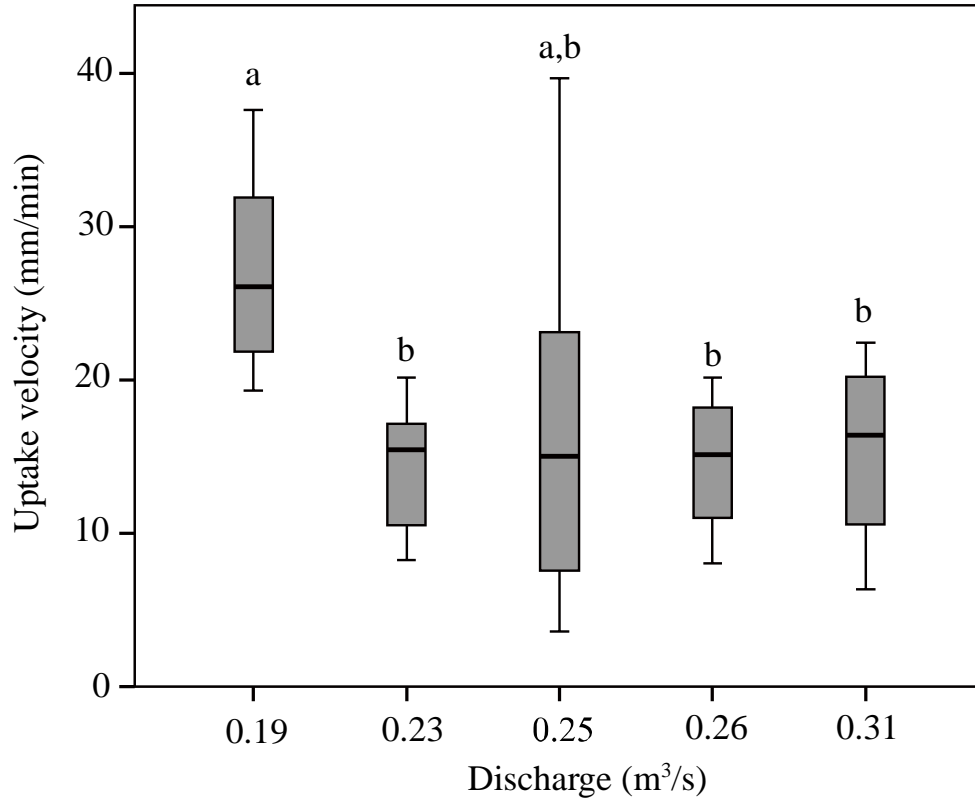


Figure 4.8. DOC uptake velocity ranges under different stream discharges calculated using TASCSC based on five DOM tracer-release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA. Different letters above the boxes signify significant differences in uptake velocity between discharge conditions detected by the t-test.

4.4.4 DOM uptake versus DOM composition

The mean uptake length of humic-like and protein-like DOM ranged between 172 and 399 m and between 251 and 589 m, respectively (Appendix III Table 4). The mean uptake length of protein-like DOM was about 1.6 times higher than that of humic-like DOM and showed an overall significant longer uptake length than humic-like DOM when evaluated lumping all measurements together ($P=0.001$; t-test; Fig. 4.9). Stream discharge increased the mean uptake lengths of both humic-like DOM ($r=0.972$, $P=0.006$) and protein-like DOM ($r=0.911$, $P=0.032$). The uptake length of the humic-like DOM calculated based on mass balance method ranged between 43 and 635 m. However, except for two experiments with positive protein-like DOM

uptake length (83 and 142 m), larger amount of protein-like DOM was recovered at the sampling station than the amount released resulting in negative uptake lengths (Appendix III Table 7). The mean uptake velocity of humic-like and protein-like DOM ranged between 10.91 and 22.25 mm/min and between 7.22 and 21.46 mm/min, respectively (Appendix III Table 6). The uptake velocities of humic-like DOM were significantly higher ($P=0.002$; t-test) than those of protein-like DOM by 1.23 times when evaluated lumping all measurements together (Fig. 4.10). Lower uptake velocities of both humic- and protein-like DOM were observed under conditions with higher stream discharge. Specifically, the mean uptake velocity of humic-like DOM was 15.66 to 22.25 mm/min with the discharge lower than $0.23 \text{ m}^3/\text{s}$ (two measurements) as compared to 10.91 to 11.13 mm/min with the discharge higher than $0.25 \text{ m}^3/\text{s}$ (three measurements). Similarly, the mean uptake velocity of protein-like DOM was 10.24 to 21.46 mm/min with the discharge lower than $0.23 \text{ m}^3/\text{s}$ (two measurements) and 7.22 to 9.64 mm/min with the discharge higher than $0.25 \text{ m}^3/\text{s}$ (three measurements).

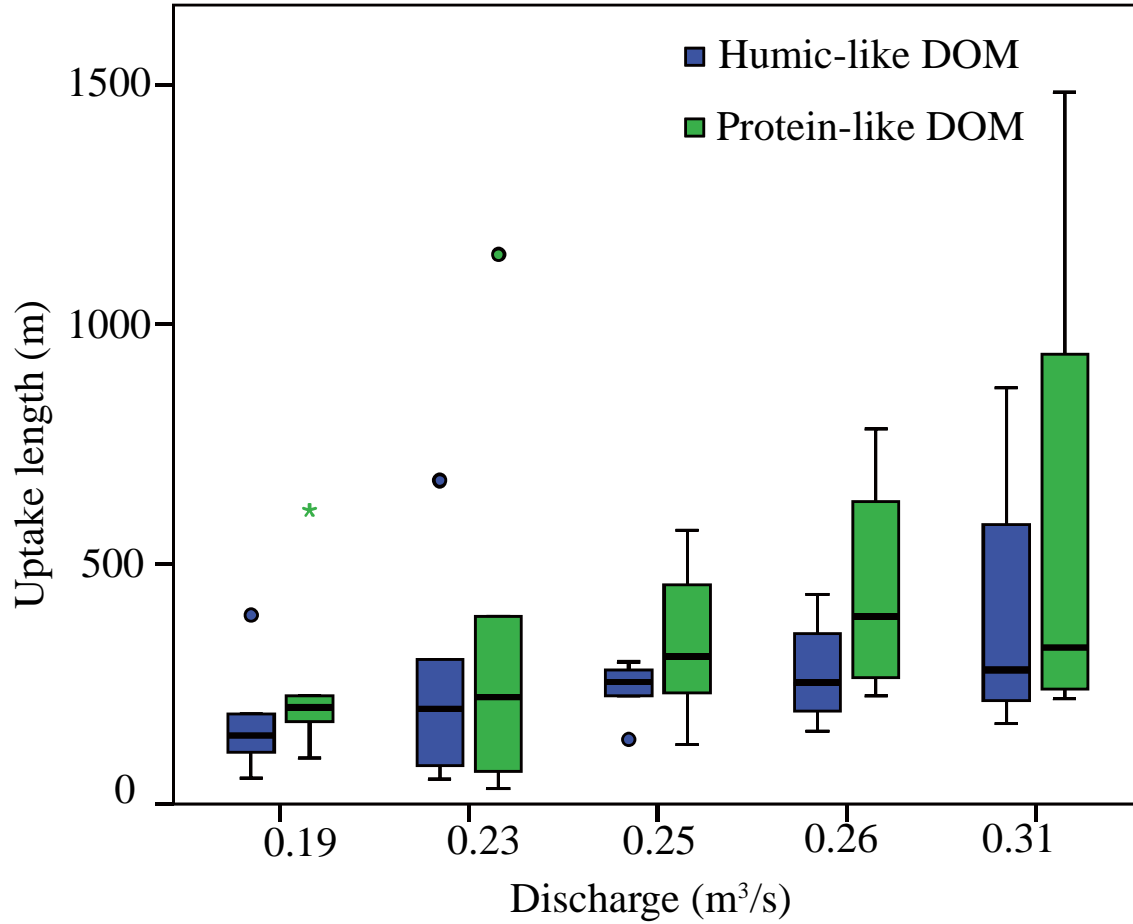


Figure 4.9. Uptake lengths of humic-like (C1 and C2, blue boxes) and protein-like DOM (C4, green boxes) under different stream discharges calculated using TASC based on five DOM tracer-release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA.

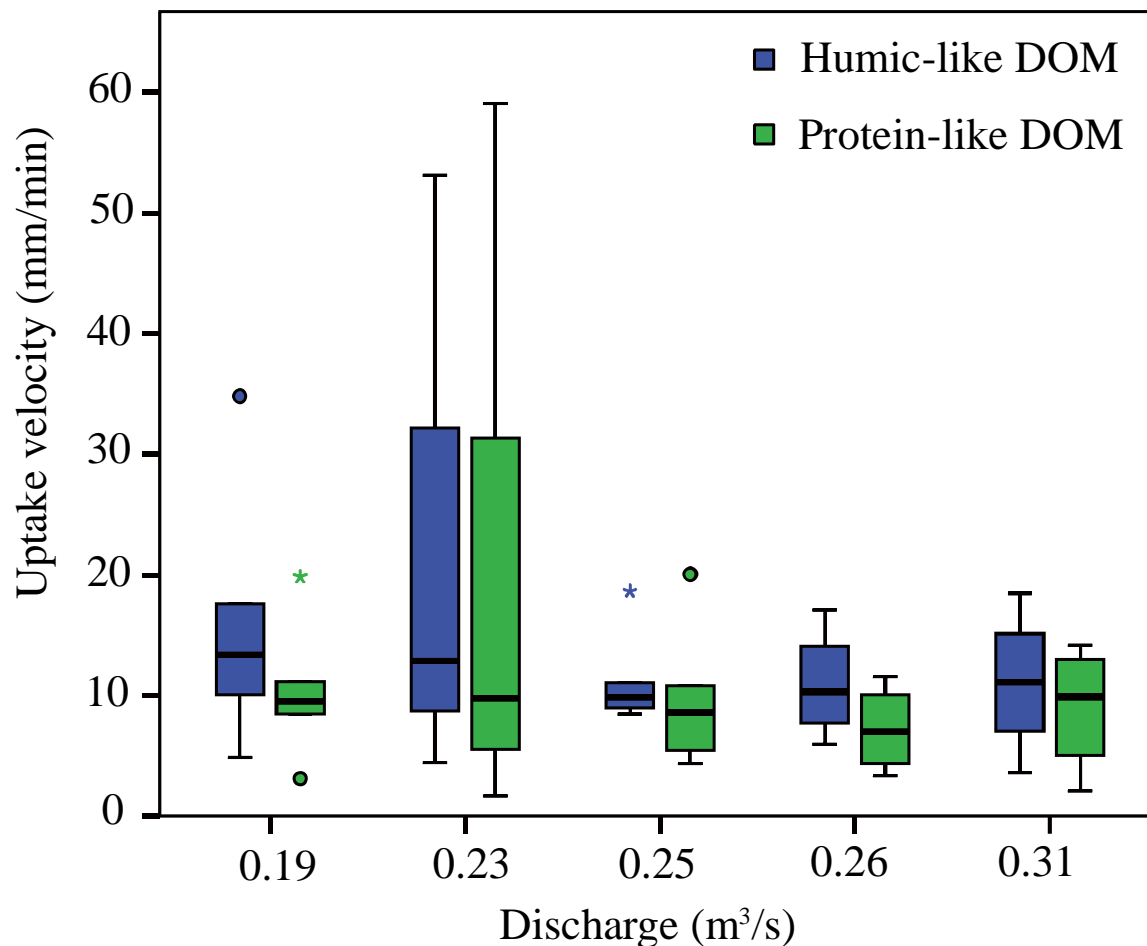


Figure 4.10. Uptake velocities of humic-like (C1 and C2, blue boxes) and protein-like DOM (C4, green boxes) under different stream discharges calculated using TASCSC based on five DOM tracer-release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA.

4.4.5 Biological degradation of DOM

In order to evaluate the relative importance of biodegradation in the water column as compared to the overall uptake of DOM, the microbial degradation rate of DOM removal in laboratory incubation was calculated under the assumption of the first-order degradation (Gregorich et al. 2003; Lu et al. 2013). The uptake rates from 28-day laboratory biodegradation of stream water samples collected under baseflow conditions were between 0.001 and 0.0067 day⁻¹. It is important to note that the stream water samples used for biodegradation estimates

were collected on a different date from the tracer experiments (10/01/2015, 04/26/2016, 11/29/2016, and 03/08/2017). Uptake rates from the incubations were first converted to the uptake velocity using a water depth of 0.27 m (mean stream depth of all tracer release experiments), which yielded an uptake velocity ranging between 0.00019 to 0.00126 mm/min. This uptake velocity was used to calculate percent DOC loss, assuming a flow rate of 0.45 m/s (mean flow rate of all tracer release experiments), yielding %DOC loss due to water column biodegradation ranging between 0.00022 to 0.00064%. In comparison, we observed the percentage loss of DOC was 16 to 57% estimated in our reach-scale experiments (based on the mass balance), suggesting that there was about 9.57 to 49.77 g of DOC removed in benthic or hyporheic sediments during our tracer release experiments. We estimated that about 1.15×10^8 g of sand was stored in the hyporheic zone along the 80-meter study reach based on the assumption that the storage area (A_s) averaged 0.72 m^2 according to the results from OTIS model (Table 4.2) and the density of wet sand as 1992 kg/m^3 (Chukwu and Badejo 2015). Despite the majority of DOM being removed by sediments, DOM stored in the channel-stored sediments was no more than $4.34 \times 10^{-7}\%$ in weight of the benthic sediments.

4.5 Discussion

4.5.1 Factors influencing DOC uptake characters

A substantial amount of DOM (16% to 57%) was retained or removed during downstream transportation in this study, and the uptake was regulated by stream discharge and DOC concentration. The influence of stream discharge was observed for both DOC and DOM compounds and appeared at the lowest discharge captured in this study. The mean uptake length of DOM in this study was within the previously reported range (from about 10 to 5000 m) for organic leachates (i.e., leachates from leaf, soil and fish carcass; Mineau et al. 2016), but the

uptake velocity was overall higher than the reported values of natural organic leachates (0.002 to 7.08 mm/min) (Mineau et al. 2016). Pure organic compounds were found to have higher processing rate than DOM leachates, yet the results in this study fell within the upper range of uptake velocity for pure organic compounds (0.18 to 28.67 mm/min; Mineau et al. 2016). Stream discharge was found as a dominant factor that can increase the uptake length and decrease the uptake velocity of DOM in both previous and our current studies (Mineau et al. 2016; Fig. 4.7, 4.8, 4.9 and 4.10). Given that the specific discharge (discharge/stream width) in our study was below the lower limit of the range reported in previous studies reviewed by Mineau et al. (2016), the high uptake velocity of DOM in this study might result from a more effective DOM retain and removal when water flow was low with prolonged DOM processing time in sediments. The study reach was bounded by a steep bank, and the stream width showed little change as discharge rises (Shang observation). A lower discharge was related to a higher ratio of benthic surface area to water volume, and thus, a greater contribution of benthic sediments to DOM removal. The dominant role benthic sediments played in DOM removal could be supported by the comparison between the reach-scale uptake velocity estimated using TASC method and the biological degradation velocity estimated using laboratory incubation representing the water column processing (Mineau et al. 2016). The reach-scale uptake velocity in this study, with benthic and hyporheic processes included, was four orders of magnitude higher than that in the water column. Hyporheic exchange increased the residence time of the leachate and facilitated the removal of DOM through abiotic adsorption onto mineral surfaces as well as biotic assimilation by contacting with biofilms. In agreement with uptake velocity, the total percentages of DOC loss estimated using the reach-scale mass balance approach were also four orders of magnitude higher than those in the water column estimated from the laboratory incubations. The

OTIS simulation also supported that the uptake rates in benthic sediments (λ_s and λ'_s) were more than three orders of magnitude higher than those in the water column (λ and λ' ; Table 4.3 and 4.4). Therefore, this is consistent with previous studies that have highlighted the importance of hyporheic zone in nutrient removal (e.g., Peterson et al. 2001, Sobczak and Findlay 2002), the benthic sediments appeared to account for more than 99% of the reach-scale DOM removal in this study.

The high uptake velocity may also be related to the low elevation in DOC concentration during the tracer release in this study. The majority of previous studies used the plateau approach, where tracers are applied at a constant rate, and DOM uptake velocities may be underestimated. For instance, in the study of Bernhardt and McDowell (2008), DOM tracer was injected consecutively with the rate that could increase the DOC concentration by about 5mg/L above the ambient condition at the sampling station. In comparison, the pulse release in our study increased the DOC concentration by less than 1 mg/L above the ambient condition at the peak of BTCs. Low DOC concentration might be a factor leading to the high uptake velocity observed in this study, due to the significant negative correlations between DOC concentration and uptake velocity (Appendix III Figure 3). Moreover, continuous injection of DOM leachates might oversaturate the capacity of retaining and removing DOM in the study reach, and thus leading to an underestimation of uptake velocity in previous studies (Mineau et al. 2016).

4.5.2 Preferential removal of humic-like DOM over protein-like DOM

We noted preferential removal of humic-like DOM over protein-like DOM. Despite that fluorescence DOM only accounts for a small fraction of bulk DOM (Findlay and Sinsabaugh 2003), it was shown to correlate with DOC concentration and was commonly used in evaluating DOM compositions (e.g., Stedmon and Markager 2005; Jaffé et al. 2008). It was unclear how

DOM molecular composition influenced uptake in previous studies (Coq et al. 2010), and several reach scale experiments reported contradictory findings (i.e., McKnight et al. 2002; Fellman et al. 2009). For example, some researchers found that labile monomeric carbohydrates and protein-like DOM were rapidly removed, which prompted the suggestion that biological assimilation was the major pathway of DOM removal and DOM with larger proportions of the bioavailable compound would be removed faster in streams and rivers (Bernhardt and McDowell 2008; Fellman et al. 2009). On the other hand, some researchers suggested that DOM was mainly removed by physical processes, and DOM with larger percent contribution from humic-like DOM with high aromaticity was expected to be more promptly removed from streams and rivers (e.g., McKnight et al. 2002). Given that uptake velocity varies as a function of both the concentration and composition of DOM substance, it is possible that the concentration and composition variation in the tracers, along with instream microbial activity and physical watershed geomorphology, has resulted in controversial observations. Our results highlight the importance of studying and comparing the uptake characters of different DOM compounds using a high-resolution sampling strategy in the same release experiment. In consistency with the previous studies that emphasized the importance of benthic sediments on humic substance sorption (Kaiser et al. 1996; Worrall et al. 1997), we attributed the preferential humic-like DOM removal to the high ratio of benthic surface area to water volume in the study reach. Under low discharge conditions, the higher ratios of the benthic surface area to water volume further facilitate humic-like DOM removal and thus result in higher uptake velocities than those under high discharge conditions.

Three of the five tracer release experiments showed that protein-like DOM was becoming more enriched relative to humic-like DOM with time, mostly on the falling limbs of BTCs (Fig.

4.6c, 4.6d and 4.6e). This pattern has not been reported in previous studies, probably due to different tracer release approaches being used. Specifically, with the continuous release of DOM tracers in the plateau approach used in previous experiments, the re-enriched protein-like DOM in the stream water was thoroughly mixed with the newly-added tracer, and thus, protein-like DOM from different sources could not be differentiated at the sampling location. We ruled out the possibility that the re-enrichment of protein-like DOM was directly sourced from the pulse release of tracers because similar enrichment was not observed in the BTCs of the conservative tracer. In addition, there were more protein-like DOM being recovered than released in some of the experiments suggested a new source of protein-like DOM other than the tracer (Appendix III Table 7). Thus, we suggest that the re-enriched protein-like DOM was sourced from the storage zone due to a more active interaction of DOM components with the storage zone. OTIS model results supported this interpretation. The three experiments re-enriched protein-like DOM had larger storage zones than the other two experiments (0.8 versus 0.6; Table 4.2). Our results, however, cannot distinguish the cause of re-enriched protein-like DOM. It could be due to biotic (i.e., a product of biological uptake processes) and abiotic (i.e., desorption from transient storage zones) processes. For example, a number of previous studies reported protein-like DOM as a microbial metabolism product of humic-like DOM uptake (Findlay and Sinsabaugh 2003). The hyporheic zone can efficiently remove or assimilate dissolved nutrients as water flows through because of the biofilms (Mulholland et al. 1997; Harvey and Wagner 2000). Thus, a larger transient storage is considered to correlate with a longer hydraulic residence time in the hyporheic zone and, thus, a greater degree of biogeochemical processing (Findlay 1995). Similarly, several studies suggested that increasing the size of transient storage zones could promote the uptake of nutrients in streams (e.g., Valett et al. 1996; Mulholland et al. 1997).

Therefore, we suggest that the re-enriched protein-like DOM may reflect that the uptake process of humic-like DOM in benthic sediments release protein-like DOM as a product.

4.5.3 Effects of low-order streams on DOM dynamics

The full length of our study stream was about 20 times of the DOM uptake length, and we suggest a thorough removal of DOM prior to being transported into the stream of next order. There have always been inconsistent results regarding the roles of low versus high-order streams in nutrient removal. Seitzinger et al. (2002) suggested that only half of the total nutrient removal took place in the first- to fourth-order streams, which constituted 90% of total channel length. Ye et al. (2017) also suggested that small streams contributed less to nutrient removal than what would be expected based solely on the percent contribution of their lengths to the length of the entire fluvial network. Wollheim et al. (2016) suggested that large rivers contributed more to biological removal of terrestrial nutrients than in small streams. On the contrary, a number of studies reported higher removal rates of nutrients in headwater streams than in large rivers and suggested the relatively high benthic surface area to water volume ratios in small streams as the primary factor increasing the uptake velocity of nutrients (e.g., Alexander et al. 2000; Peterson et al. 2001). Meanwhile, the dominance of headwater streams (70–80% in length) in drainage networks also contributed to the dominant role in removing DOM throughout the networks (Alexander et al. 2000, 2007; Gomi et al. 2002). The mean uptake length of the study reach ranged between 73 and 252 m in our study, which was less than 17% of the estimated average length of first-order streams in the US (1.61km; Leopold et al. 1964) and less than 5% of the total length of the Mayfield Creek (5.87 km from the beginning to the end of this second-order stream). Therefore, our results suggest that under baseflow conditions, low-order streams function as the dominant sink of terrestrial DOM removing the majority of DOM exported from

soils. The influence of discharge was observed on DOC and DOM components, but this study was performed within a restricted range of discharge. Our results, as such, cannot be directly used to test the conceptual models (e.g., Wollheim et al. 2018 Raymond et al. (2016) suggesting that a decreased DOM residence time at higher water flows.

Low-order streams preferentially removed the humic-like components in terrestrial DOM and led to an increasing contribution of protein-like DOM as well as a decreasing diversity in DOM composition along the fluvial network. Similar changes in DOM composition along the fluvial network have been reported in several studies. The River Continuum Concept suggested a continuous decrease in the relative chemical diversity of DOM through the river continuum due to the removal of labile DOM compounds (Vannote et al. 1980). Although the model did not explicitly state which groups of DOM were more labile, it implied that photochemical removal was more important in large rivers. Creed et al. (2015) reported that DOM compositional variability was attenuated by biogeochemical processing during the downstream transport, i.e., a shift from aromatic to aliphatic compounds as indicated by a decrease in SUVA₂₅₄ as stream order increases. In addition to factors including autochthonous DOM production (Hotchkiss et al. 2014) and photodegradation (Lu et al. 2013) that decrease the aromaticity of DOM, our results demonstrate that the preferential removal of humic-like DOM in benthic sediments over protein-like DOM also contributed to the shift in DOM composition from aromatic to aliphatic along the river network. This compositional gradient of DOM along the fluvial continuum supported a longitudinal gradient of the microbial community that was developed to adapt to DOM compositions (Battin et al. 2008).

4.6 Conclusions

In the present study, we evaluated the uptake of DOM in a second-order forested stream under baseflow conditions, using tracer-release experiments assessed with TASC and numerical models. The main findings of this study included: 1) benthic sediment removed DOM with uptake velocity being four orders of magnitude higher than water column biodegradation; and 2) humic-like DOM was removed more rapidly than protein-like DOM, as indicated by an overall shorter uptake length and higher uptake velocity. Our results suggested that small streams that generally have low specific discharge (i.e., high benthic sediment area to water volume ratio) dominate the uptake of terrestrial DOM under baseflow conditions. The composition-selective removal by sediments could also contribute to the widely observed shift from aromatic to aliphatic DOM compounds along the river continuum, yet the common understanding relates this shift only to photochemical and microbial processing. River network models are the most commonly used tools in upscaling the reach-scale estimates to the entire river network, and the models are typically parameterized based on empirical data (Helton et al. 2011; Aguilera et al. 2013; Gomez-Velez et al. 2015). Therefore, our results contribute to the data needed to further refine the carbon budget at the network scale.

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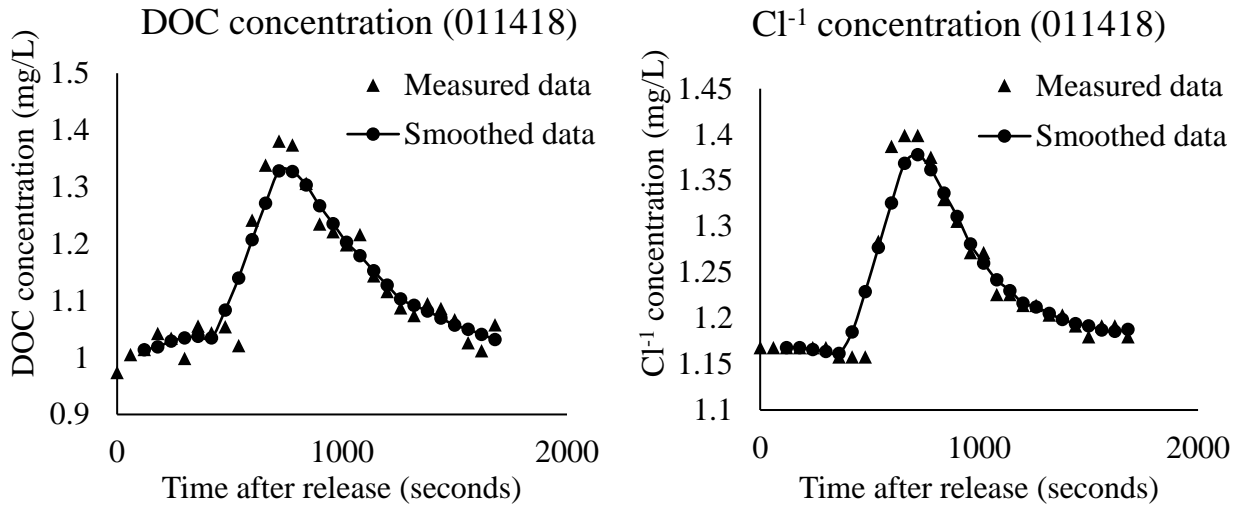


Figure 1. An example of the comparison between measured and smoothed data from one of the five tracer release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA.

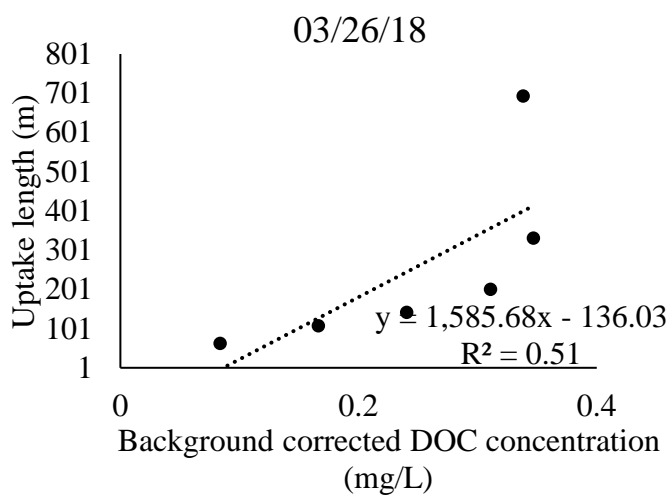
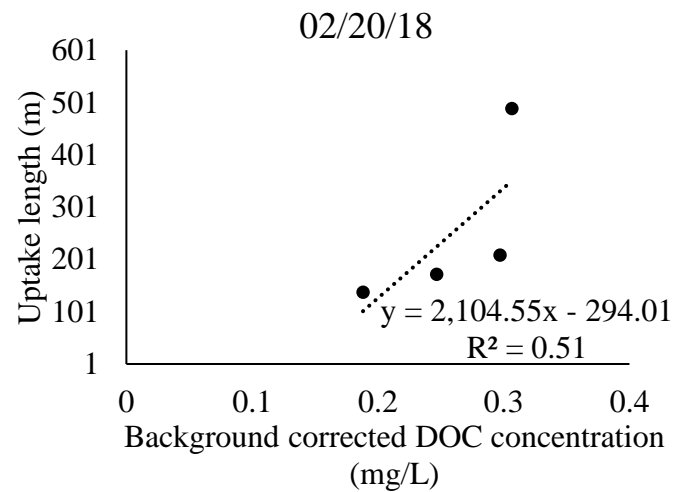
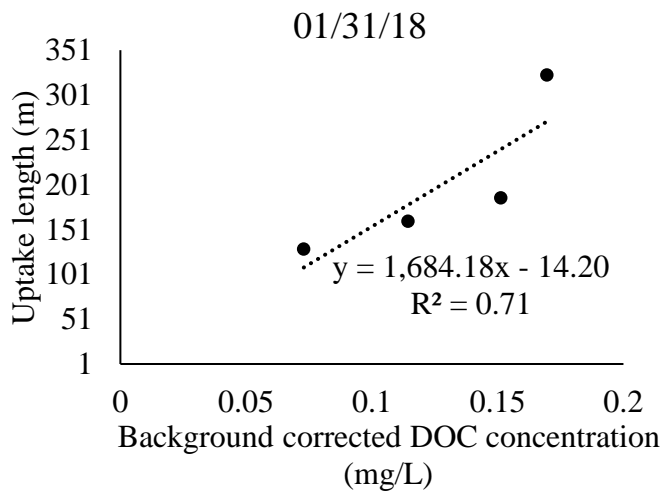
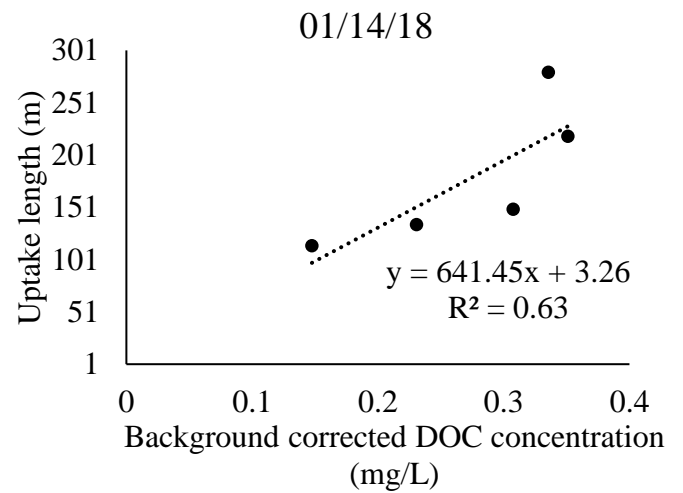
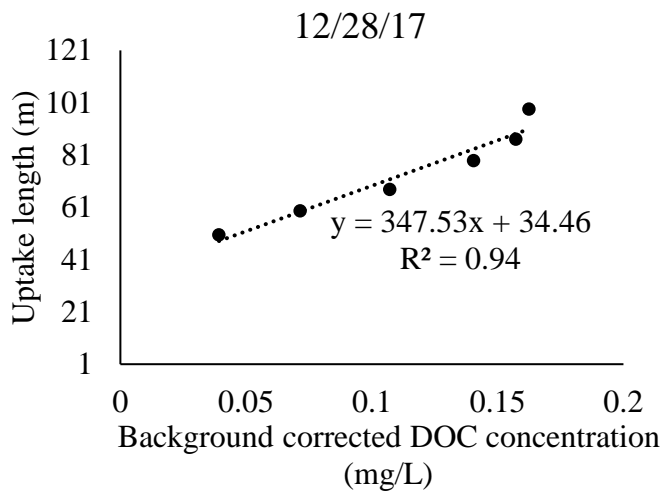


Figure 2. Correlations between uptake lengths and DOC concentrations based on five tracer release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA. Data from the start (first rising point on the BTC) to the peak of each BTC were used in evaluating DOM uptake

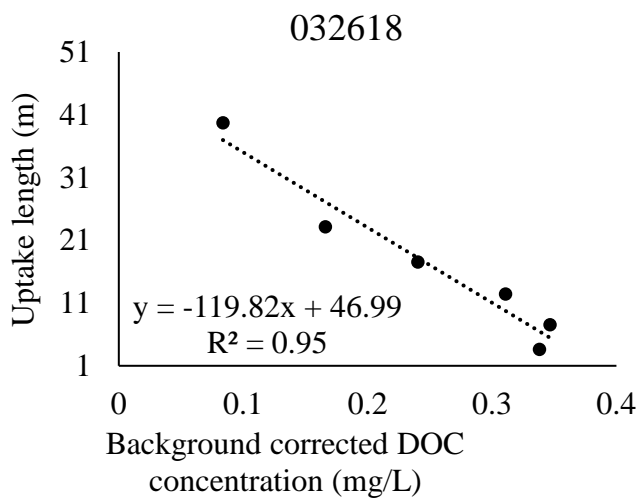
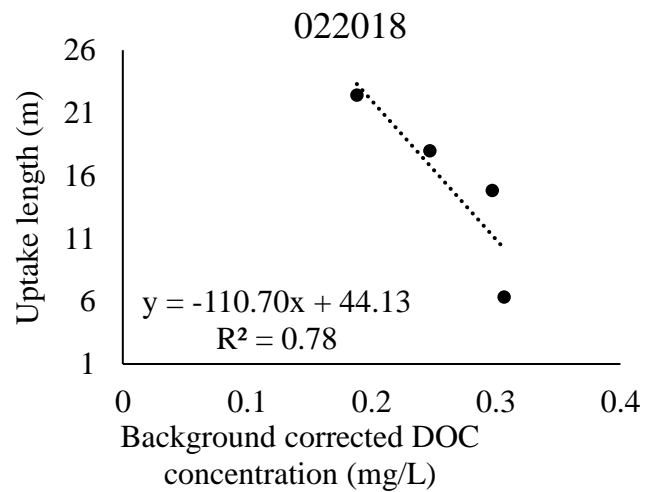
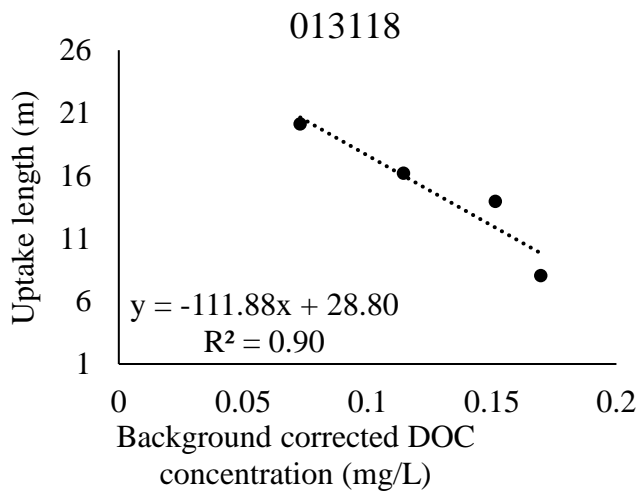
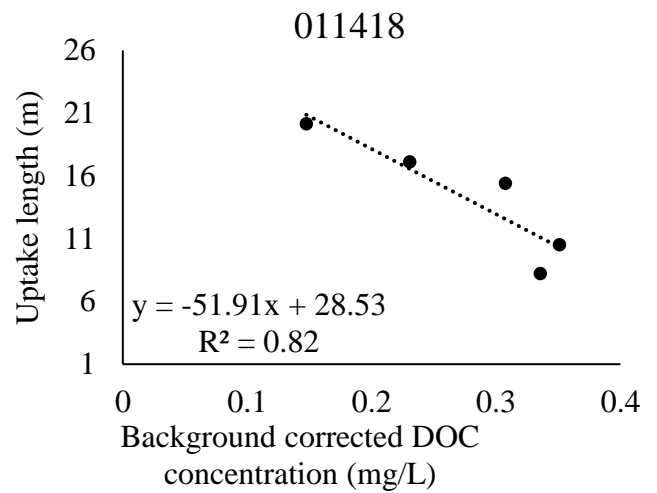
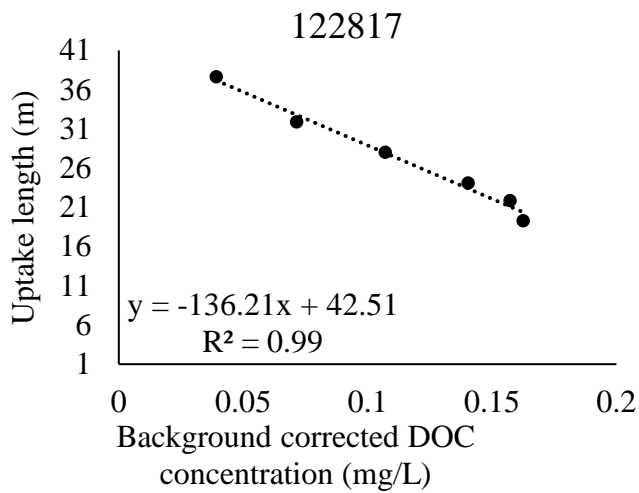


Figure 3. Correlations between uptake Velocities and DOC concentrations based on five tracer release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA. Data from the start (first rising point on the BTC) to the peak of each BTC were used in evaluating DOM uptake.

Table 1. Lower and upper limits as well as the step length in the selection of variables in OTIS model based on the concentration of conservative tracer from five tracer release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA.

	Lower limit	Upper limit	Step length
A (m²)	0	3	0.2
As (m²)	0	3	0.2
C_L (mg/L)	Assigned as 0 (assuming no lateral flow)		
D (m²/s)	0.02	1	0.02
Q (m³/s)	Measured after each experiment		
q_{LIN} (m²/s)	Assigned as 0 (assuming no lateral flow)		
α (s⁻¹)	0.0001	0.005	0.0001

Table 2. DOM quality in tracer and stream water during the five DOM tracer-release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA.

Date	DOC (mg/L)	SUVA254	SR	FI	HIX	Total Fluorescence	%C1	%C2	%C3	%C4	Source
3/26/2018	269.3	2.80	0.72	1.63	1.4	309.77	24.06	16.25	22.43	37.25	tracer
3/26/2018	229.15	3.47	0.75	1.62	1.1	349.00	21.2	14.84	33.03	30.93	tracer
2/20/2018	195	3.41	0.66	1.66	1.84	301.79	22.74	16.79	22.04	38.44	tracer
2/20/2018	224.15	3.06	0.66	1.66	2.47	284.33	26.17	18.73	14.79	40.32	tracer
1/31/2018	107.9	2.46	0.73	1.75	2.02	158.18	14.42	12.75	9.37	63.46	tracer
1/31/2018	98.75	2.48	0.69	1.69	1.83	158.24	14.26	12.07	7.73	65.93	tracer
1/14/2018	112.25	2	0.76	1.73	0.73	147.89	13.38	8.3	6.46	71.86	tracer
1/14/2018	118.95	1.77	0.71	1.69	0.74	149.37	13.21	8.28	6.5	72.01	tracer
12/28/2017	132.85	1.13	0.65	1.9	0.58	116.62	13.54	8.55	9.22	68.69	tracer
12/28/2017	111.6	1.08	0.59	1.91	0.57	93.52	13.65	8.61	8.99	68.75	tracer
3/26/2018	1.53	2.95	0.67	1.67	3.66	2.09	44.35	29.36	16.35	9.93	stream water
3/26/2018	1.25	3.61	0.73	1.69	3.51	2.10	43.57	29.61	16.63	10.19	stream water
2/20/2018	1.62	3.1	0.73	1.66	2.22	2.46	39.31	26.32	14.59	19.79	stream water
2/20/2018	1.57	3.18	0.72	1.67	2.77	2.39	40.81	27.19	15.2	16.79	stream water
1/31/2018	1.49	1.94	0.56	1.72	2.34	1.48	40.98	25.08	18.37	15.56	stream water
1/31/2018	1.39	1.87	0.5	1.75	1.94	1.56	37.8	23.74	21.3	17.15	stream water
1/14/2018	1.10	2.36	0.78	1.81	6.02	1.20	53.48	30.38	11.53	4.62	stream water

1/14/2018	1.84	1.47	0.6	1.81	4.35	1.27	50.75	29.39	12.56	7.31	stream water
12/28/2017	1.86	1.66	0.69	1.79	0.82	2.27	30.18	16.8	27.53	25.49	stream water
12/28/2017	1.72	1.75	0.98	1.79	1.15	1.96	34.35	19.44	30.58	15.63	stream water

Table 3. Loadings of the component identified using DrEEM toolbox (n=144) based on the grab samples from five DOM tracer-release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA.

Mode	nm	Component 1	Component 2	Component 3	Component 4
Ex	250	0.364335488	0.318444163	0.365729976	0.188847031
Ex	260	0.317904542	0.32601101	0.336180417	0.375377728
Ex	270	0.275958364	0.311020328	0.382907678	0.604436927
Ex	280	0.252955702	0.27337179	0.439206736	0.599849171
Ex	290	0.272740298	0.232000358	0.4448944	0.1494382
Ex	300	0.308011441	0.20743304	0.353125661	0
Ex	310	0.335478264	0.203079132	0.231015296	0
Ex	320	0.332710116	0.214397315	0.156210323	0
Ex	330	0.306722571	0.230148639	0.106996109	0.066990721
Ex	340	0.26037151	0.242565581	0.042968817	0.267206027
Ex	350	0.204311118	0.24972193	0	0.006836639
Ex	360	0.14224865	0.255395055	0	0
Ex	370	0.086711395	0.245198538	0	0
Ex	380	0.044638484	0.217785952	0	0
Ex	390	0.013982579	0.185311052	0	0
Ex	400	0	0.150256909	0.002141422	0
Ex	410	0	0.115642055	3.44988E-05	0
Ex	420	0	0.089237337	0.000837047	0
Ex	430	0	0.067686787	0.001764116	0
Ex	440	0	0.052132607	0.001308633	0
Ex	450	0	0.042018517	0.00058513	0
Ex	460	0	0.032192948	0.000175851	0
Ex	470	0	0.026518142	0	0
Ex	480	0	0.021364769	0	0
Ex	490	0	0.015329593	0	0
Ex	500	0	0.009358405	4.39002E-05	0
Em	280	0	0	0	0.202768716
Em	286	0	0	0	0.255467265
Em	292	0	0	0.036082588	0.248466101
Em	298	0.003739926	0	0.018300759	0.373133858
Em	304	0.062591747	0	0	0.431451634
Em	310	0.050044273	0.002929186	0.014240479	0.427371367
Em	316	0.028369824	0.013578524	0.101292483	0.376954963
Em	322	0	0.020767452	0.164825476	0.298779163
Em	328	0	0.002114297	0.24226795	0.209531988
Em	334	0	0	0.279665424	0.151365977
Em	340	0	0.006903237	0.336732723	0.115908503
Em	346	0.008544869	0.015997779	0.355850677	0.074626079
Em	352	0.04524268	0.013897764	0.364575184	0.053340832

Em	358	0.09440966	0	0.348926598	0.037262759
Em	364	0.143662013	0	0.32549821	0.023228818
Em	370	0.189892356	0	0.279145831	0.012749941
Em	376	0.235851495	0	0.234352384	0.009672001
Em	382	0.271716695	0	0.184581163	0.013544067
Em	388	0.300663252	0	0.143225465	0.012470306
Em	394	0.31280674	0.01517606	0.103376558	0.014686273
Em	400	0.309020862	0.043386796	0.073499823	0.013759005
Em	406	0.301157825	0.072929689	0.045662677	0.012244435
Em	412	0.287884164	0.100199019	0.025365951	0.008658227
Em	418	0.271682457	0.132381862	0.008001595	0.009583755
Em	424	0.256153784	0.161695406	0	0.010069499
Em	430	0.231942212	0.190366445	0	0.005158353
Em	436	0.205379818	0.21498117	0	0.008496311
Em	442	0.179297015	0.232484248	0	0.005015324
Em	448	0.15344892	0.246323927	0	0.006400787
Em	454	0.131258931	0.254995962	0	0.003906563
Em	460	0.108962079	0.258923693	0.001540364	0.012503389
Em	466	0.087714113	0.260594645	0.009356499	0.008891995
Em	472	0.068732343	0.257200544	0.017573732	0.006691101
Em	478	0.052840138	0.252949754	0.023931924	0
Em	484	0.038790302	0.246177355	0.031777688	0.004863456
Em	490	0.028169528	0.239325291	0.037365563	0.001239485
Em	496	0.019175543	0.230987299	0.040564169	0
Em	502	0.01195607	0.22286682	0.043257206	0
Em	508	0.006142264	0.211569292	0.044931189	0
Em	514	0.001447402	0.199015857	0.04600461	0
Em	520	0	0.183879236	0.044453312	0
Em	526	0	0.1687085	0.039934413	0
Em	532	0	0.153531001	0.036719904	0
Em	538	0	0.139007537	0.030601451	0

Table 4. Uptake lengths of DOC, humic-like DOM, and protein-like DOM from five tracer release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA.

Date	DOC uptake length (m)	Humic-like DOM uptake length (m)	Protein-like DOM uptake length (m)
12/28/17	51	55	96
12/28/17	60	108	171
12/28/17	68	153	225
12/28/17	79	133	184
12/28/17	87	189	220
12/28/17	99	394	613
01/14/18	114	52	31
01/14/18	134	79	68
01/14/18	149	199	223
01/14/18	219	301	391
01/14/18	280	675	1146
01/31/18	129	152	303
01/31/18	160	273	225
01/31/18	186	234	479
01/31/18	323	436	781
02/20/18	138	168	219
02/20/18	172	263	262
02/20/18	209	296	392
02/20/18	489	869	1484
03/26/18	63	134	125
03/26/18	108	226	231
03/26/18	143	297	376
03/26/18	201	279	458
03/26/18	331	263	571
03/26/18	694	247	239

Table 5. Percent recovery and uptake lengths of DOC evaluated based on mass balance method from five tracer release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA.

Date	DOC percent recovery (%)	DOC uptake length (m)	Stream discharge (m ³ /s)	Temperature (°C)
12/28/17	43%	96	0.19	7.8
01/14/18	75%	274	0.23	5.0
01/31/18	72%	245	0.26	6.7
02/20/18	83%	418	0.31	16.7
03/26/18	84%	459	0.25	15.3

Table 6. Uptake velocities of DOC, humic-like DOM, and protein-like DOM from five tracer release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA.

Date	DOC uptake velocity (mm/min)	Humic-like DOM uptake velocity (mm/min)	Protein-like DOM uptake velocity (mm/min)
12/28/17	37.62	34.81	19.84
12/28/17	31.88	17.56	11.11
12/28/17	28.03	12.46	8.43
12/28/17	24.09	14.25	10.33
12/28/17	21.84	10.08	8.64
12/28/17	19.27	4.82	3.1
01/14/18	20.15	53.14	59.05
01/14/18	17.12	32.17	31.31
01/14/18	15.41	12.86	9.74
01/14/18	10.51	8.69	5.52
01/14/18	8.21	4.4	1.69
01/31/18	20.13	17.06	8.57
01/31/18	16.21	9.51	11.55
01/31/18	13.97	11.1	5.43
01/31/18	8.04	5.96	3.33
02/20/18	22.43	18.47	14.16
02/20/18	17.99	11.77	11.85
02/20/18	14.82	10.48	7.92
02/20/18	6.34	3.57	2.09
03/26/18	39.69	18.64	20.05
03/26/18	23.12	11.08	10.82
03/26/18	17.53	8.43	6.64
03/26/18	12.44	8.96	5.46
03/26/18	7.54	9.52	4.38
03/26/18	3.6	10.13	10.47

Table 7. Percent recovery and uptake lengths of humic-like and protein-like DOM evaluated based on mass balance method from five tracer release experiments conducted in a second-order stream the Talladega National Forest, Alabama, USA. Negative uptake lengths indicated more tracer recovered than released.

Date	Humic-like DOM percent recovery (%)	Humic-like DOM uptake length (m)	Protein-like DOM percent recovery (%)	Protein-like DOM uptake length (m)
12/28/17	87%	561	116%	-529
01/14/18	16%	43	38%	83
01/31/18	88%	635	154%	-184
02/20/18	77%	302	322%	-68
03/26/18	53%	126	57%	142

CHAPTER 5: CONCLUSIONS

In this dissertation, a variety of research tools were employed to investigate the effects of agricultural activity, hydrological variation, and longitudinal transport on the quantity, source, chemical composition, and bioreactivity of natural DOM in streams and rivers. This chapter summarizes the major findings in this dissertation, discusses how natural organic matter responds to anthropogenic and natural drivers, and discusses understanding needed to predict the response of fluvial ecosystems in response to human activities and climate changes

Chapter 2 established a mechanistic understanding of how agricultural land use and climatic variables influence aquatic-terrestrial linkages of DOM. Specifically, I found that temperature and antecedent precipitation condition positively influenced DOC concentration and the contribution of terrestrial, microbially-derived humic DOM in streams. This pattern suggested the importance of soil OM production, release, and mobilization in regulating DOM in the streams. Agricultural land use increased DOC concentration and the proportions of terrestrial and microbial humic DOM compounds in streams, which suggested a preferential mobilization of OM from topsoil in agricultural watersheds owing to accelerated oxidation, erosional transport, and shallow soil-to-stream flow paths. These findings were in general agreement with a few previous studies (e.g., Graeber et al. 2012), but contradictory to the others (e.g., Williams et al. 2010; Lu et al. 2014; Fuß et al. 2017), which is a result of varied agricultural types and practices in combination with climatic variability. Given the highly variable influences of

watershed development on terrestrial-aquatic connectivity and biogeochemical dynamics in lotic ecosystems, our results highlighted the needs for conducting more empirical studies across climate zones to incorporate DOM into a science-based management framework.

In Chapter 3, high-resolution time series were collected to evaluate the impacts of hydrological variation on the quantity and quality of DOM in a small forested stream across time scales. At the event scale, the pattern of DOM mobilization was regulated by antecedent hydrological conditions, as well as the magnitude (duration) of storms. The peaks of DOM lagged those of ions and stream discharge, demonstrating a sequential export of ions and DOM from catchment into streams and suggesting distal and abundant storage of DOM in the watershed. The diurnal scale variation was mainly due to physical dilution and concentration driven by evapotranspiration. The water level change also shifted the source of terrestrial DOM and led to a higher contribution of microbial DOM from lower soil at a lower water level. DOM dynamics at the seasonal scale was driven by soil carbon availability and discharge, with a greater amount of terrestrial DOM export occurring in spring and winter than in summer and fall. Overall, instream processes had little influence on the quantity and composition of DOM in this small forested stream, and the DOM export from this forested watershed can be reasonably predicted by discharge across timescales. These findings are applicable to numerous small, pristine Coastal Plain streams that are hotspots of land carbon being loaded to drainage networks.

In Chapter 4, the removal of natural DOM leachates in a forested headwater stream was assessed for characterizing the uptake features of bulk DOM and DOM compounds. The reach-scale DOM uptake velocity was four orders of magnitude higher than that observed in laboratory incubations, suggesting the dominant role of benthic sediments in DOM removal. In comparison to previous studies providing little information on the effects of DOM molecular composition on

its reach-scale uptake character (McKnight et al. 2002; Fellman et al. 2009; Coq et al. 2010), this study demonstrated that humic-like DOM was more susceptible to reach-scale uptake than protein-like DOM, and humic-like DOM had a significantly shorter uptake length and higher uptake velocity. The importance of low-order streams in nutrient removal remains debatable (e.g., Alexander et al. 2000; Peterson et al. 2001; Seitzinger et al. 2002), and this study demonstrated that low-order streams act as a sink for the natural terrestrial organic matter under baseflow conditions. In addition, low-order streams preferentially removed humic-like components in terrestrial DOM, which can contribute to longitudinal changes in DOM composition along the fluvial continuum. This finding aligned well with the River Continuum Concept conceptual framework proposed in previous studies (Vannote et al. 1980; Creed et al. 2015) but further demonstrated the role of sediments in contributing to the longitudinal shift in DOM composition along the stream-river-coastal ocean continuum.

In carbon budgets of many aquatic ecosystems at regional or global scales, DOM plays an essential role in transporting carbon through the ecosystems. The most commonly used tools in regional to global scale carbon dynamic description are river network models, which upscale reach-scale findings to the entire river network, and the parameters in these models are typically estimated based on conceptual understandings and experimental measurements (Helton et al. 2011; Aguilera et al. 2013; Gomez-Velez et al. 2015). It is critical to evaluate the input and removal rates of natural DOM in aquatic ecosystems and to understand the governing mechanisms. The second chapter in this dissertation demonstrated the impacts of anthropogenic activity, specifically agricultural land use that covers nearly 40% of the earth's ice-free surfaces (Foley et al. 2005), on the input of DOM to streams. This chapter also showed that both agricultural land use and climate influenced stream water DOM and suggested that future studies

were needed to evaluate the impacts of land use on aquatic DOM across a range of environmental settings such as climatic zones and watershed lithologies. The third and fourth chapters in this dissertation evaluated the input and removal of DOM in a relatively biogeochemically simple system, where the supply of watershed DOM was mostly unlimited from soils and regulated by hydrological transport. Chapter 3 demonstrated the significant role of discharge in the input of terrestrially derived DOM in aquatic ecosystems across time scales. The high-resolution, multiple-parameter data further revealed the short-term dynamic pattern of watershed DOM exports within a storm event. The high-resolution uptake data in the fourth chapter provided the first breakthrough curves demonstrating discrepant behaviors of DOM due to the compositional complexity. The conclusions can be used to understand the input and removal of DOM in numerous small, forested streams within the Gulf and Atlantic coastal plains. With more high-resolution water chemistry data becoming available from observation networks, such as NEON (National Ecological Observatory Network), the data and findings from this dissertation can be scaled up to understand the supply and demand of DOM at the continental scale.