Stormwater and Organic Matter in the Urban Stream Continuum

by

Megan L. Fork

Environment
Duke University

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Brian L. McGlynn

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Christopher L. Osburn

Dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Environment
in the Graduate School of Duke University
2017
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Abstract

Dissolved organic matter (DOM) is present in all natural waters and comprises a complex mixture of organic molecules including amino acids, sugars, fulvic acids, and humic material. This mixture of organic solutes modulates aquatic ecosystems by absorbing light and heat, and by acting as a chemical subsidy and stress. DOM is derived from dissolution of organic matter and can be altered by both biotic and abiotic processes that may its structure or mineralize it to CO$_2$.

Urbanization is a widespread agent of landscape change that can alter DOM regimes by changing the amount and types of organic matter in the catchment and by changing the way that water moves through the landscape (transporting DOM from land to stream). This dissertation examines DOM in urban stream networks, exploring its sources, bioavailability, and broad patterns throughout the continental United States.

We determined the role of impervious infrastructure as a proximate source of DOM to stormwater by a) constructing an annual carbon budget for the roof of a house as a small catchment nested within the 60 h catchment of an urban headwater stream and b) comparing the estimated fluxes of solutes and stormwater from impervious infrastructure in the catchment. We found that roofs convert nearly one-third of the leaf litter carbon they receive into dissolved organic carbon (DOC), which
leaves through downspouts. On the event scale, we estimated fluxes of DOC and total dissolved nitrogen from impervious surfaces that generally exceed the fluxes that leave the catchment in stream stormflow.

When we compared the chemical composition of runoff from impervious surfaces to stream stormflow, we found them to be distinct, despite the fact that the we estimated a volume of runoff from impervious surfaces that generally matched the volume of water flowing through the stream during storms. Our findings suggest that a water source other than baseflow and impervious runoff contributes to stream stormflow, and that a considerable proportion of impervious runoff is lost before it reaches the catchment pour point.

An experimental incubation of potential DOM sources in the urban landscape and DOM in stormwater showed that urban DOM is highly bioavailable. The composition of DOM also became more homogeneous over the course of processing.

Finally, we examined continental-scale patterns and long-term trends in riverine DOC. Unlike the widespread 'browning' trends observed in far northern aquatic systems, we did not find evidence for long-term increases in DOC throughout most of the U.S. Instead, we both decreases and increases in long-term DOC concentrations that differed among regions and generally seemed to be driven by changes in wetland cover. We also found evidence for a marginal effect of impervious surfaces that increases DOC concentrations at high canopy cover, consistent with our observations that urban infrastructure can contribute considerable DOM loads in storm runoff.

Together, this research shows that urban stormwater infrastructure functions as the ephemeral headwaters of the urban stream network. In catchments with significant canopy cover, these 'engineered headwaters' collect and transform organic
matter between storms and transport DOM during stormflow.
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List of Abbreviations

AFDM  Ash-free dry mass
ANOVA  Analysis of variance
BIX  Biological index (Index of recent autochthonous production)
CDOM  Colored dissolved organic matter
CPOM  Coarse particulate organic matter
DEM  Digital elevation model
DO  Dissolved oxygen
DOC  Dissolved organic carbon
DOM  Dissolved organic matter
EEM  Excitation-emission matrix
EH  Engineered headwaters
ESI  Electrospray ionization
fDOM  Fluorescent dissolved organic matter
FI  Fluorescence index
HIX  Humification index
HMW  High molecular weight
HUC  Hydrologic unit code
ISC  Impervious surface cover
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<tr>
<td>LTER</td>
<td>Long-term ecological research</td>
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<tr>
<td>MS4</td>
<td>Municipal separated storm sewer system</td>
</tr>
<tr>
<td>NLCD</td>
<td>National Land Cover Database</td>
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<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<tr>
<td>OM</td>
<td>Organic matter</td>
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<td>PARAFAC</td>
<td>Parallel factor analysis</td>
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<td>PCA</td>
<td>Principal component analysis</td>
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<td>Q</td>
<td>Discharge</td>
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<tr>
<td>RCC</td>
<td>River continuum concept</td>
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<td>SUVA</td>
<td>Specific ultraviolet absorbance</td>
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<td>SR</td>
<td>Spectral slope ratio</td>
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<td>TDN</td>
<td>Total dissolved nitrogen</td>
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<td>UV</td>
<td>Ultraviolet</td>
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<td>USGS</td>
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<td>β/α</td>
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Dissolved organic matter (DOM) is a constituent of all natural waters and is often the dominant carbon pool in lakes (Prairie, 2008) and carbon flux in streams and rivers (Mulholland, 1997). DOM includes a diverse array of solutes, from simple sugars and amino acids to complex humic and fulvic acids. Ultimately, DOM in aquatic systems is derived from production of soluble organic matter by organisms either within the system of interest (autochthonous) or in another ecosystem (allochthonous). In addition to differences in source, biotic and abiotic processing of DOM also affects the composition of the DOM pool. The effects that this mixture of molecules has on the water quality and ecological processes of aquatic ecosystems (Prairie, 2008) depend on the amount, composition, and timing of DOM delivery/production in the system.
1.1 Effects of DOM in aquatic environments: carbocentric limnology

Dissolved organic matter affects many aspects of aquatic ecosystem ecology because it can influence the physical, chemical, and biological environment (Prairie, 2008; Solomon et al., 2015; Pace et al., 2004). In addition to its direct effects, DOM can exert a number of indirect effects; Prairie (2008) called dissolved organic carbon (DOC; that portion of the DOM consisting of carbon) the "great modulator" of aquatic ecosystems because of its role in affecting the behavior of other variables in aquatic systems.

First, colored DOM can absorb solar radiation, affecting the light environment and heat balance in aquatic systems (Kirk, 1994; Fee et al., 1996). DOM absorbs strongly in the ultraviolet (UV) range of the spectrum, lending aquatic organisms a degree of protection against damage from UV radiation (Schindler and Curtis, 1997). By absorbing light in the visible and photosynthetically-active areas of the spectrum, DOM can affect the distribution and efficiency of primary producers (Søndergaard et al., 2003) and interactions between visual predators and their prey (Estlander et al., 2010). These direct effects of DOM on light regimes can generate a cascade of indirect effects on ecosystem metabolism (Edwards and Meyer, 1987; Prairie, 2008). Colored DOM also converts incoming solar radiation to heat, altering the overall temperature and heat distribution within aquatic systems (Prairie, 2008; Solomon et al., 2015).

Second, DOM can be an important direct regulator of the metabolism of organisms in aquatic ecosystems. DOM can be an important energy source at the base of the food chain, directly fueling microbial metabolism (Jones, 1992) and can be
transferred to higher trophic levels via the microbial loop (Meyer, 1994). However, some DOM molecules can directly inhibit microbial metabolism (Dodla et al., 2008). Therefore, the effects of DOM on ecosystem function clearly depend not only on concentration, but also on the type of DOM delivered to the ecosystem.

In addition to these physical and chemical effects, DOM can directly impact chemistry of systems by forming complexes with metals and organic contaminants, altering their mobility and bioavailability (Perdue, 1998; Haitzer et al., 1998). Because the DOM pool contains organic acids, DOM can affect the pH of aquatic systems. Through its direct and indirect effects on metabolism, DOM impacts the concentration of dissolved oxygen (DO) and the biogeochemistry of other elements whose cycles are coupled to carbon (Fork and Heffernan, 2014).

In water bodies subject to human use, the amount and composition of DOM may impact the cost and safety of water use. DOM can complicate water treatment by giving undesirable color and taste (Khiari, 2004), providing fuel for coliform bacteria (LeChevallier et al., 1991), or interacting with chlorine to form disinfection byproducts that pose health hazards to humans (EPA, 2012).

1.1.1 Browning of inland waters

Concentrations of DOM in inland surface waters have undergone dramatic and widespread increases over the last two decades, a phenomenon known as browning (Evans et al., 2005; Roulet and Moore, 2006; Monteith et al., 2007). These trends in DOM are best documented in catchments with highly organic soils (Worrall et al., 2004; Hruska et al., 2009) and in high northern latitudes, including Scandinavia, the United Kingdom, and northeastern North America. As an element of global change,
rising DOM concentrations have implications not only for regional carbon balances (including atmospheric CO$_2$ concentrations, as carbon released from soils as DOM may be more likely to be respired), but also for water quality (U.S. EPA, 2003) and for coupled nutrient cycles (Fork and Heffernan, 2014; Sterner and Elser, 2002; Evans et al., 2005). Because of the multiple, interacting driver and effects of increasing DOM concentrations, the ultimate trajectories and impacts of this phenomenon are difficult to predict (Solomon et al., 2015).

1.2 Sources of DOM to aquatic ecosystems

The many effects of DOM on aquatic ecosystems depend on its optical and chemical properties, which in turn are determined by its source and the degree and type of processing it undergoes. Most broadly, DOM may be sourced from within the aquatic system (autochthonous) or from outside the system (allochthonous).

In low order streams, the majority of DOM is ultimately derived from leaching of terrestrial organic matter from soil, vegetation, and litter in the catchment or from particulate matter that has entered the stream channel (Meyer et al., 1998; Hinton et al., 1998; Qualls and Haines, 1992). The dominant autochthonous source of DOM in most stream ecosystems is algae (Kaplan and Bott, 1982; Mulholland and Hill, 1997), with exudation by macrophytes another potentially important source of DOM (Martin et al., 2005).

1.3 Biotic and abiotic processing

The two most important processes that alter DOM in aquatic environments are photochemical degradation and microbial processing, with their relative importance
related both to the composition of DOM and to the properties of the environment. These processes may also interact in causing changes to the concentration and composition of DOM.

Exposure to solar radiation can elicit changes to the chemical and optical properties of the DOM pool, including decreases in color (photobleaching, Moran et al., 2000), breakdown of large molecules to smaller ones (Osburn et al., 2001; Wetzel et al., 1995), and mineralization of OM to CO$_2$ (photooxidation; Osburn et al., 2001; Miller and Zepp, 1995; Amon and Benner, 1996). Photochemical alteration can also cleave and release inorganic nutrients and carbon monoxide from DOM (Valentine and Zepp, 1993; Bushaw et al., 1996; Francko and Heath, 1982). In addition, photochemical processing of the DOM pool has been shown to increase the lability of the pool and stimulate further breakdown by the microbial community (Wetzel et al., 1995; Moran and Zepp, 1997; Kieber et al., 1989). However, photochemical changes may play a smaller role in DOM processing in temperate rivers as compared to the high light environments of estuaries or arctic rivers (Wiegner and Seitzinger, 2001; Cory et al., 2014).

Biological processing also alters the DOM pool. During transport through rivers, microbial processing of DOM can mineralize a large fraction of organic to inorganic carbon (Cory and Kaplan, 2012; Ward et al., 2013) and alter the chemical composition of DOM by removing fractions that are labile and/or rich in organic nutrients (Brookshire et al., 2005; Amon et al., 2001; Kalbitz et al., 2003). Microbial processing of DOM can also generate high molecular weight (HMW) humic and fulvic compounds by through conjugation of extant and new DOM (Repeta et al., 2002).
1.4 Measuring DOM

The amount of bulk DOM in a water sample is operationally defined as the concentration of organic carbon in a filtered water sample (DOC). The concentration of DOC is measured by converting the organic carbon in the sample to CO$_2$ by combustion at high temperature or by chemical oxidation, after purging inorganic carbon from the sample.

Because of the huge diversity of unique compounds in the DOM pool of natural waters, specifically identifying all the molecules present in a sample is impossible. Resins can be used to isolate classes of molecules in the DOM pool; XAD-8 resin is a widely used method of isolating the humic and fulvic fraction DOM (Thurman and Malcolm, 1981). Measurement of the bulk chemical composition of DOM is possible with using 1-D nuclear magnetic resonance (NMR) spectroscopy, while multidimensional NMR can provide information about some of the functional groups and molecular structures present in samples (Sulzberger and Durisch-Kaiser, 2009). Mass spectrometry, combined with electrospray ionization (ESI), has also been used in studies of DOM composition to identify specific molecules across a wide range of molecular weight and complexity (Sulzberger and Durisch-Kaiser, 2009).

1.4.1 Optical measures of DOM composition

Optical properties (absorbance and fluorescence) of a sample can also be used to summarize average characteristics of the composition of the DOM pool. Only a subset of the bulk DOM pool absorbs light (colored DOM or CDOM) and a smaller fraction fluoresces (fluorescent DOM or fDOM), so some care must be taken in interpreting optical measurements of DOM (Coble, 2007). Still, the specific wavelengths of light
absorbed and emitted are determined by the conformation of molecules, and several metrics have been developed that have been shown to have robust relationships with biochemical and physical properties of the DOM. Absorbance-based proxies of DOM composition include the specific UV absorbance of 254 nm light (SUVA_254, an indicator of aromaticity; Weishaar et al., 2003) and the spectral slope ratio (S_R, an indicator of molecular weight; Helms et al., 2008). Fluorescence of water samples is used for measurements such as the humification index (HIX) which indicates average humic content (Zsolnay et al., 1999; Ohno, 2002), and the fluorescence index (FI) which provides information about the source of DOM (McKnight et al., 2001). The freshness index (β/α) and biological index (BIX) are fluorescence measurements that provide information about the mean diagenetic age or "freshness" of DOM in the sample (Parlanti et al., 2000; Huguet et al., 2009).

In addition to these optical indices of OM composition based on single wavelengths or small areas of the spectrum, measurement of excitation-emission matrices (EEMs) and modeling of differences among sample EEMs by parallel factor analysis (PARAFAC; Stedmon and Bro 2008) have gained widespread application in recent years. The EEM-PARAFAC approach can be used to identify the important fluorescence peaks in a data set, and the relative abundance of these peaks can be compared among samples within the data set. Additionally, the identified components can be matched to those observed in other studies to gain wider insight into their ubiquity, biochemical properties, and behaviors (Murphy et al., 2014).
1.5 DOM in human-dominated aquatic systems

At a broad scale, human activity influences DOM regimes through global change, including not only climate change but also changes to biogeochemical cycles and land cover. Browning (Worrall et al., 2004; Evans et al., 2005; Monteith et al., 2007) is one such example of a global change. Another facet of global change is land-use change, such as anthropogenic conversion from forests and wetlands into agricultural fields and cities. These changes affect both the sources of DOM in the catchment as well as the hydrologic processes that transport DOM from land to stream.

On a local scale, urbanization has the potential to alter DOM regimes in streams through changes to the amount, composition and timing of DOM delivery. The trend of increasing urbanization across the globe will subject more and more streams to the effects of urban land uses. The suite of changes to stream chemistry, biology, and geomorphology typically caused by urban development are known together as the Urban Stream Syndrome (Walsh et al., 2005). Among these changes, nutrient and DOM concentrations tend to be higher in urban water bodies as compared to rural reference sites (e.g. in Arizona: Westerhoff and Anning, 2000; Texas: Aitkenhead-Peterson et al., 2009; Georgia: Schoonover et al., 2005; Brazil: Daniel and Montebelo, 2002; Australia: Hatt et al., 2004), and urban DOM tends to be more bioavailable (Harbott and Grace, 2005). Effectively managing urban aquatic ecosystems requires an understanding of how urbanization changes the sources and pathways that deliver nutrients and DOM to urban river networks, and the ecosystem processes that change and remove them.

Urbanization (typically measured as an increase in impervious surface cover [ISC]
within watersheds) alters the potential sources of DOM and nutrients in several ways, most directly by changing the amount and types of soil and vegetation (with differences in phenology, Neil and Wu 2006, and DOM composition) in an urban as opposed to an undeveloped landscape. In addition, novel sources of DOM, like hydrocarbons and pharmaceuticals, and novel forms of nutrients such as pet waste and manufactured fertilizers, are introduced into the urban landscape (Fissore et al., 2011; Martini et al., 2013). Urban development also significantly alters how water moves through the landscape, notably through the construction of impervious surfaces and channels that can rapidly route water to streams during storms ("engineered headwaters" sensu Kaushal and Belt), changing the how and when that DOM and nutrients are transported from uplands to stream.

While the Urban Stream Syndrome has proven useful and applicable to stream reaches in urbanized landscapes throughout the world, an expanded model is needed to understand how urbanization affects freshwater systems at the river network scale. Since increased ISC, soil compaction, and most stormwater infrastructure in urban areas increase the flashiness of urban watersheds, water and solutes (including nutrients and DOM) may exhibit a pulse-shunt behavior (Raymond et al., 2016) in which the majority of solute movement occurs in pulses during high flow events (Doyle et al., 2005). Storm runoff from urban land surfaces is thought to undergo little biochemical transformation in the headwater network because very short residence times characteristic of hydrologically flashy systems. Rather, runoff is ”shunted” to downstream impoundments where solutes in stormwater may then undergo transformations such as biotic sequestration, mineralization, and/or photochemical degradation.
1.6 Outline of the dissertation

In this dissertation, I examine DOM throughout an urban stream continuum, beginning in the uppermost intermittent channels (roof and roadside gutters) down the network through urban streams and into rivers. In Chapter 2, entitled “Engineered headwaters can act as sources of dissolved organic matter and nitrogen to urban stream networks,” my co-authors and I examine organic matter in urban infrastructure through an ecosystem ecology lens; we construct an annual carbon budget for the small headwater catchment comprised by the roof of single-family home in Durham, NC, and we calculate the flux of DOC and total dissolved nitrogen from these and other elements of the headwater infrastructure during storms. Chapter 3, ”Pipes are not pipes: Impervious runoff is not the primary source of stormwater in an urban headwater stream” uses chemical and optical properties of runoff from the engineered headwaters to infer their contribution to stormflow in the stream. Chapter 4, entitled ”Bioavailability of dissolved organic matter in urban engineered headwaters” experimentally measures the biodegradation of leachates from potential sources of urban DOM and from stormflow in the urban headwater continuum. I also characterize changes in the optical properties of DOM over the course of the experiment and describe homogenization of the DOM optical properties as biodegradation progresses. In Chapter 5, ”DOC in U.S. streams and rivers and its decade-scale change,” I characterize differences in the concentration, load, and long-term changes in DOC reported by the USGS, and link these to changes in land use/land cover. Finally, Chapter 6 concludes the dissertation by placing this research in the wider context of urban stream ecology.
Engineered headwaters can act as sources of dissolved organic matter and nitrogen to urban stream networks


2.1 Introduction

As the footprint of urbanization increases worldwide, a growing number streams are subjected to the pressures of draining urban landscapes (Walsh et al., 2005; Grimm et al., 2008). Excess nutrients and labile organic matter (OM) degrade water quality in urban streams and the downstream water bodies into which they flow by promoting eutrophication, harmful algal blooms, and hypoxia (Vitousek et al., 1997; Volkmar and Dahlgren, 2006). High concentrations of dissolved OM (DOM) have a wide range of ecological and biogeochemical consequences (Prairie, 2008; Solomon et al.,
2015), and can also increase the cost of drinking water treatment by interacting with disinfection chemicals to form carcinogenic by-products (Chow et al., 2005). Effectively and efficiently managing water quality in urban streams and downstream water bodies should start with identifying the sources that contribute to high nutrient and OM loads to urban streams.

There are several potential sources of nutrients and DOM to urban streams. Soils and fertilizer used on lawns are generally some of the largest pools of OM and nutrients in urban landscapes (Baker et al., 2001; Aitkenhead-Peterson et al., 2009). Soils underlying irrigated turfgrass in arid regions have been shown to leach high concentrations of dissolved organic carbon (DOC) and nutrients (Steele and Aitkenhead-Peterson, 2012). Nitrogen stored in soils and the application of fertilizer (a dominant N input to urban catchments) have been implicated as sources of the high nitrate concentrations characteristic of many urban streams (Groffman et al., 2004). While they may be large pools of C and N in urban catchments, soils and lawns may not be the most important contributors of these solutes to urban streams if the frequency and degree of their hydrologic connectivity to streams is relatively low as compared to impervious surfaces.

Engineered flowpaths such as stormwater pipes, roof gutters, and roadside gutters are another potential proximate source of nutrients and OM to urban stormwater. Despite storing smaller pools than lawns or soils, these ubiquitous components of urban landscapes may be important sources of nutrient and OM flux since they act as ephemeral headwaters that are highly connected to perennial streams during storms (Kaushal and Belt, 2012). Litter that accumulates in these engineered headwaters (EH) is often protected from wind and has a low surface area exposed to the atmo-
sphere, allowing it to stay damp longer than other parts of the landscape (ML Fork, personal observation). As such, these areas may support greater rates of biogeochemical activity that transforms leaf litter into readily-mobilized particulates and solutes. Previous research in other catchments with extensive tree canopy has shown leaching of litter on roads to be a significant source of nutrients to urban streams (Hobbie et al., 2013; Selbig, 2016; Bratt et al., 2017). During storms, the distinct hydrology of urban catchments promotes the export of DOM derived proximately from infrastructure by short-circuiting infiltration in favor of runoff over and through OM pools that have collected and been processed in EH.

In this paper, we develop an annual carbon budget for the EH catchment comprised by a roof and calculate the contribution of EH to event-scale fluxes of total dissolved nitrogen (TDN) and dissolved organic carbon (DOC) from a small urban watershed in Durham, North Carolina. We characterized inputs, outputs, and transformations of carbon from the roofs of single-family homes. We used DOC and TDN concentrations in flow from roof downspouts, roadside gutters, storm water pipes and catch basins (storm drain inlets with sumps to collect sediment and debris) to calculate the contribution of these engineered headwaters to stormwater solute fluxes from this urban catchment at the event scale. We hypothesized that engineered headwaters may be potentially important sources of dissolved carbon and nutrients derived from the decomposition of leaf litter in gutters and in catch basins.

2.2 Methods

We determined C budgets for the roofs of three single family homes, measured storage of OM and TDN at locations throughout the engineered headwaters and measured
event scale fluxes in a small urban watershed in Durham, NC over the course of a year from November 2015 through November 2016. Our focal area for this study was a medium density neighborhood occupied mainly by single family homes on quarter-acre (~1000 m²) or smaller lots. The 60 ha catchment is in the temperate U.S. Southeast, contains approximately 400 houses, has 39.4% impervious cover (City of Durham Stormwater Services, 2016), and considerable canopy cover (~60%, i-Tree Landscape v2.1.2) with mature willow oaks (Quercus phellos), originally planted in the 1930s, as the dominant canopy tree (Durham City-County Environmental Affairs Board, 2015; Cooper et al., 2016b).

We measured concentrations of DOC and TDN in stormwater for a total of nine storms: We collected stormwater from downspouts of three houses, three roadside gutters, three stormwater pipe outfalls, and the stream at the watershed outlet. A regression of sampled DOC concentration against rainfall prior to collection time showed no significant evidence of dilution over the course of storms (for rainfall up to 22 mm; Figure A.3), despite a very brief first flush of DOC from roofs (Figure A.3). While first flushes are commonly observed in solutes and turbidity in urban stormwater (Sansalone and Buchberger, 1997; Lee et al., 2002), accurately characterizing these requires specialized sampling (Lee et al., 2002). Because the first flush we observed was brief and because we saw no evidence of sustained dilution over storms, we used the mean seasonal concentration for stormwater draining from each infrastructure type (i.e. roof, road, etc.) multiplied by event rainfall volume to determine event-scale DOC and TDN fluxes (see Appendix A).
2.2.1 Annual carbon budgets for the roofs of single family homes

We measured areal rates of litterfall in the yards of five properties (for three of which roof C budgets were determined) and assumed that rates measured in baskets represented areal rates of litter input to roofs and roads (Figure A.1). Litter loading was estimated by multiplying planimetric roof area by measured areal litterfall rate. We estimated DOC inputs to roofs as the sum of contributions from throughfall and rainfall (see Appendix A). Using summer aerial images (Google, 2015), we estimated the proportion of roof covered by tree canopy (Figure A.2) and used these proportions to determine the loads of DOC from throughfall vs. rainfall for each storm, multiplying by measured concentrations and the total rainfall volume for each roof.

To estimate the output of OM as particulate matter and decomposition to CO$_2$, we conducted a litter decomposition experiment. We compared mass loss of senesced willow oak leaves from coarse polyethylene mesh and fine nylon mesh litter bags that were deployed in the roof gutters of five houses and inside five catch basins. We fit decay functions to the proportion of C remaining vs. time and multiplied by the measured C standing stock to infer the total mass loss from each roof or catch basin (Figure A.3). We assumed that mass loss from coarse litter bags was the sum of DOC loss, particulate OC loss, and decomposition to CO$_2$, while the mass loss from fine litter bags was the sum of DOC loss and decomposition to CO$_2$ only, allowing us to estimate particulate loss through downspouts. We independently measured DOC flux from roof gutters and stormwater pipes via grab sampling and were therefore able to determine decomposition to CO$_2$ by difference.

The standing stock of C in roof gutters was assessed by measuring the C content
of accumulated OM in a 50 cm length of gutter and multiplying this mass by the
total gutter length of houses (see Appendix A). Repeated measurements allowed us
to quantify the change in C storage in gutters over the study period.

2.2.2 Estimation of event C and N fluxes from engineered headwaters at the catch-
ment scale

To estimate the total flux of solutes from roofs within the catchment, we began
by converting mean fluxes measured for individual roofs into areal rates. Using
one meter resolution planimetric maps of catchment impervious surface (City of
Durham Stormwater Services, 2016), we identified roofs within the catchment and
then scaled fluxes of DOC and TDN to the total roof area calculated in ArcGIS
10.4. To estimate the total event-scale flux from road gutter sub-catchments and
piped sub-catchments, a one-meter-resolution digital elevation model (DEM) based
on 2016 LiDAR data from the 3D Elevation Program (U.S. Geological Survey, 2016b)
was used to delineate these sub-catchments using Whitebox GAT ‘Montreal’ v. 3.4.0
(Lindsay, 2016). We assumed that only runoff from impervious surfaces contributed
to each of our headwater sampling points (downspouts, roadside grates, and pipe
outfalls) and calculated the impervious area fro each sub-catchment. To estimate
the DOC loading to impervious surfaces from throughfall and direct rainfall we used
the average canopy cover in the catchment (USDA Forest Service, 2016). Measured
concentrations at the sub-catchment pour points during individual events were then
used to determine areal rates of C and N loading to roads. Multiplying by total road
area gave us an estimate of their total contribution to the flux at the pour point.
2.3 Results

2.3.1 Annual carbon budget for a roof

The main annual input of C to roofs was litterfall (10.03 ± 0.53 kg C) with another 2.49 ± 3.71 kg C entering as DOC in throughfall and rainfall (Figure 2.1). Areal litterfall C inputs ranged from a midwinter low of 0.029 ± 0.038 g C m\(^{-2}\) day\(^{-1}\) up to 1.45 ± 0.66 g C m\(^{-2}\) day\(^{-1}\) in late autumn, with a secondary peak of 0.28 ± 0.24 g C m\(^{-2}\) day\(^{-1}\) in mid-spring, coincident with blossom fall and the year’s highest pollen counts.

Export of C via downspouts (as DOC: 5.42 ± 0.05 kg C, as POC: 0.616 ± 0.318 kg C) was the largest measured C loss from roofs. Assuming negligible processing of the DOM that enter roofs in rainfall and throughfall, we can calculate litter-derived DOC export as 291 mg DOC [g litter C]\(^{-1}\), or 29% of litter C inputs.

Litter decomposition in roof gutters was best described by a two-component exponential decay model with slow and fast pools (Figure A.4). The slow proportion comprised 79.2% percent of the OM pool and the decay coefficient did not differ significantly from zero (i.e. no appreciable decomposition of the slow pool), while the fast component was 18.7% percent of the pool with a decay coefficient, \(k\), of 0.044. In catch basins, litter decomposition was best described by simple exponential decay with a \(k\) of 0.00343. Decomposition to CO\(_2\) was a minor annual flux of C from individual roofs (0.202 ± 0.187 kg C) and catch basins (0.875 ± 0.545 kg C). Removal of C from gutters (by wind or gutter cleaning) was determined by difference, and accounts for 6.10 ± 3.67 kg C, a value similar to total DOC export. We measured little change in the storage of organic carbon in roof gutters (0.182 ± 0.146 kg C).
Figure 2.1: Average carbon budget of a roof in Durham, NC. The budget is calculated for a single family home given a mean (planimetric) roof area of 89.6 m\(^2\) for our three study roofs, with mean ± sd pools and fluxes determined from the areal rates we measured at the three houses. *CPOM removal (via wind or cleaning of gutters) was calculated by difference.

over the course of the study.

2.3.2 Longitudinal patterns through the continuum of engineered headwaters

Concentrations of DOC and TDN averaged 88.2 mg DOC L\(^{-1}\) and 5.50 mg TDN L\(^{-1}\) in road runoff and 83.0 mg DOC L\(^{-1}\) and 4.56 mg TDN L\(^{-1}\) in pipe outfalls, with both locations significantly higher than stream concentrations (DOC: p-values <0.001 and
= 0.002, respectively; TDN: p-values <0.001 and = 0.024, respectively, Figure 2.2).

DOC and TDN concentrations ranged from minima of 2.94 mg DOC L\(^{-1}\) and 0.114 mg TDN L\(^{-1}\) in roof outflow to as high as 436.4 mg DOC L\(^{-1}\) and 31.1 mg TDN L\(^{-1}\) in road runoff. DOC concentrations in road runoff were also significantly higher than in roof downspouts (p = 0.039). We observed higher concentrations of DOC and TDN in spring vs. other times of year (Figure 2.2), controlling for differences among location (p <0.001 for both DOC and TDN). These high concentrations of DOC and TDN in mid-spring coincide with blossom fall and the year’s highest pollen counts.

2.3.3 Estimated event-scale fluxes of C and N for the urban catchment

We measured fluxes of water, chloride, DOC, and TDN at the catchment outlet for six storms and estimated the contributions of components of the engineered headwater to these fluxes (Figure 2.3; A.2 & A.3). In general, we found that the rainfall that falls on connected roofs and roads in the catchment can account for an average of 85% of streamflow across a range of storm magnitudes (Figure 2.3; A.1).

Mass flux for an average storm at the catchment outlet was 33.4 ± 30.4 kg Cl\(^{-}\), 51.9 ± 25.3 kg DOC, and 2.42 ± 1.70 kg TDN. On average, areal chloride fluxes from roofs were 26.1 ± 24.9 mg Cl\(^{-}\) m\(^{-2}\), areal DOC fluxes were 580 ± 630 mg C m\(^{-2}\), and areal TDN fluxes were 41.2 ± 46.1 mg N m\(^{-2}\), with high variability resulting from variation in event magnitude. Scaled to total roof area in the catchment, the average event scale yield was 2.2 ± 2.1 kg Cl\(^{-}\), 48.8 ± 52.9 kg C, and 3.5 ± 3.9 kg N from roofs (Appendix A - Tables A.2 & A.3). However, it is estimated that only approximately 10% of roofs have direct hydrologic connections to impervious flowpaths (Miles, 2014), so their actual contribution to solute loads in stream stormwater
is likely much lower; in an average storm, we estimate directly-connected roofs contribute $0.74 \pm 0.65\%$ of the chloride flux, $10.0 \pm 8.6\%$ of the DOC flux, and $14 \pm 15\%$ of the TDN flux through the stream (Figure 2.3).

Solute fluxes through roadside gutters (pour points of the next largest catchments
Figure 2.3: Comparisons of water and solute fluxes for catchment-scale estimates of EH contributions and streamflow measurements for 7 storms. Roof flux estimates assume 10% of roofs are directly connected to impervious flowpaths and therefore contribute quickly during storms, while we assume 100% hydrologic connectivity for roads. Stormwater pipes do not contribute new water sources (because they drain surface infrastructure already considered in our roof and road estimates), but they do store OM in catch basins and can therefore be an additional source of solutes (B, C, and D).

We considered, and in which connected roofs are nested) were 57.1 ± 66.8 mg Cl⁻ m⁻², 938 ± 877 mg C m⁻², and 67.1 ± 83.5 mg N m⁻² on average, respectively. These scale to fluxes of 9.5 ± 11.4 kg Cl⁻, 157 ± 146 kg C and 11.2 ± 14.3 kg N from all roads during the average storm, or 12 ± 13% of chloride, 306 ± 202% of DOC and 430 ± 441% of the average event-scale flux in streamflow (Figure 2.3; Appendix A -
2.4 Discussion

2.4.1 Engineered headwaters can be disproportionate sources of dissolved organic material

We measured the fluxes and transformations of organic matter in an urban catchment and found that EH are often major sources of C and N to the stream during storms. Litter that falls onto impervious surfaces accumulates in catch basins and gutters and is processed into readily-mobile DOM between storms (Hobbie et al., 2014). These areas then become source areas of DOC and nutrients when they are connected to the stream by ephemeral flow (Hobbie et al., 2014; Bratt et al., 2017). We found that about one third of C that enters roof gutters as leaf litter exits as DOC through downspouts, and in some cases these downspouts are routed directly to roads and routed quickly to streams. In addition, we estimated that EH emit more than enough DOC and TDN to account for stormflow fluxes.

We estimate that roofs have the potential to contribute element fluxes disproportionately higher than their areal coverage in the catchment. Although roofs represent only 14.1% of the land cover in this catchment, they would have the potential to contribute up to 100% of the average stormflow DOC load if 1) all roofs allowed litter to collect in gutters and 2) were directly connected to the impervious flowpaths (Table A.2). In fact, parcel-scale BMPs such as cisterns, rain gardens, and simple routing of gutter outflows over vegetated lawns (downspout disconnections) decrease the contribution of roof-derived stormwater to streams (Miles, 2014; Loperfido et al., 2014).
The fluxes of DOC and TDN we measured from roads generally exceed the fluxes measured in the stream (Figure 2.3). While source limitation during very large storms (see Appendix A) may account for some of this discrepancy, retention of DOC and TDN within the stormwater infrastructure and infiltration of stormwater from leaky pipes into the urban groundwater system (Chapter 3) likely play roles in retaining nutrients and DOM in runoff from impervious surfaces. We suggest that this pattern of high DOC and TDN fluxes from EH may hold wherever canopy cover over impervious surface is high, as observed by Janke et al. (2017).

The event-scale mass fluxes we measured in this study indicate a high potential for engineered headwaters to influence catchment-scale organic matter fluxes. If we assume that DOC concentrations in the measured storms (Figure A.5) are representative of DOC dynamics in the 99 storms >1 mm during our study period and that the periodic baseflow samples are representative of inter-storm conditions, the annual areal flux from our 60 ha catchment would be $173 \pm 89$ kg DOC ha$^{-1}$. This is considerably higher than the annual export of DOC from forested watersheds throughout the eastern U.S. (Raymond and Saiers, 2010), but similar to the DOC yield that would be predicted for the highly urban Anacostia watershed near Washington, D.C. given the 1518.9 mm of rainfall during our study period (Smith and Kaushal, 2015). By design, engineered headwaters have high hydrologic connectivity to streams, and lack mineral soils that could contribute to sorption and storage of DOM (Jardine et al., 1989; Kaiser and Guggenberger, 2000). The result is high yields of soluble material from litter that falls on engineered headwaters, and potentially disproportionately contributions to catchment scale fluxes.

Further evidence that EH are important proximate sources of C and N to urban
streams is the fact that stream concentrations of DOC and TDN increase rapidly and remain elevated during storms (Figure A.6), when impervious surfaces become hydrologically connected to the stream. Such behavior has been observed for DOC in other urban catchments (Hook and Yeakley, 2005; Smith and Kaushal, 2015), and suggests that expansion of the stream network during storms connects new sources of DOC and TDN located in EH. These findings do not support a model of impervious surfaces as mere connectors of biogeochemical sources to streams, but rather that these flowpaths are also sources, sinks, and reactors that act as control points for watershed exports (Bernhardt et al., 2017).

The role of gutters and pipes (and indeed, their design) in concentrating and routing precipitation from impervious landscape patches to stream channels is well known. However, prevailing conceptual models have implicitly or explicitly ascribed to pipes the role of simply connecting the sources of OM and nutrients to streams and have not recognized these landscape elements as bioreactors able to process litter into DOM (but see Hobbie et al., 2013; Bratt et al., 2017). While the loads of OM stored in the EH may be smaller than those in lawns and soils, these areas can store readily-mobilizable OM and have the potential to be important proximate sources of dissolved OM and nutrients to streams during storms. This is likely to be true in regions and neighborhoods with high deciduous canopy cover contributing leaf litter to EH, such as older neighborhoods in the Midwest and Piedmont, but may be less important in neighborhoods that are newer (Lowry et al., 2012), of lower socioeconomic status (Schwarz et al., 2015), or in other regions where tree canopy is more sparse.
2.4.2 Implications for urban stream management

Gutter cleaning and street sweeping are potentially significant pathways of OM removal from the headwater stream network. Previous research has shown more dense tree canopy is associated with higher nutrient loads in urban streams (Janke et al., 2017), and that street sweeping can significantly reduce export of N and P during storms (Hobbie et al., 2013; Selbig, 2016), but weather conditions may decrease the effectiveness sweeping as a nutrient removal pathway (Bratt et al., 2017). The effects of litter removal may be especially beneficial in terms of controlling P export to urban waterways (Hobbie et al., 2013; Selbig, 2016), and targeting for P may also yield benefits in terms of C and N. Yard waste collections may also be a significant vector of nutrient and OM removal from urban catchments (Templer et al., 2015), but litter that falls on lawns and gardens may have lower relative connectivity to streams than litter falling on impervious surfaces.

Interventions that remove CPOM from these highly connected headwater flow-paths (e.g. cleaning of catch basins, street sweeping) or that disconnect them from streams (e.g. rain gardens, downspout disconnection) may be effective solutions to mitigate nutrient loads delivered to urban streams in stormwater and provide management options when more typical BMPs such as retention/detention ponds can’t be retrofit into existing developments. These interventions are becoming increasingly common non-structural BMPs implemented by municipalities (Donner et al., 2015; City of South Lake Tahoe, 2013). State, municipal, and community groups encourage interventions managed by individual homeowners such as downspout disconnection and rainwater harvest in this catchment (NC Rules Review Commission 2011; City
of Durham Public Works Department: Stormwater and GIS Services 2015). On the municipal scale, removal of OM from roadside gutters and storm drains are currently being considered as creditable nutrient-reduction strategies by the NC Department of Environment and Natural Resources.

2.4.3 Implications for catchment science

Catchment-scale budgets have been used as a tool to understand how landscapes transport, retain, and transform elements and OM (Bormann and Likens, 1967; Fisher and Likens, 1972; McDowell and Likens, 1988; Schlesinger and Bernhardt, 2013) and can provide insight about the internal processes regulating elements in catchments where loading, soils, and hydrologic connectivity are relatively homogeneous. Such an approach can be especially enlightening when paired catchments can be compared (e.g. Johnson and Swank, 1973; Martin et al., 2000). However, 'black boxing' the catchment as an individual unit may impede mechanistic understanding or inference in systems characterized by high spatial heterogeneity in sources and transport of the element(s) of interest. Disparities in surface hydrologic connectivity can cause significant differences in the potential vs. actual importance of source areas of OM and nutrients in stormwater to streams (Miles and Band, 2015).

Here, we applied a nested catchment mass balance approach that allows us to determine the importance of discrete urban landscape elements as source areas and flowpaths. Although urban soils and lawns have high potential loads of C and N to urban streams, their actual contribution to catchment-scale flux in this system appears to be less important than EH, which have smaller loads but greater hydrologic connectivity to the surface stream. The most valuable applications of this
approach will be in catchments that feature heterogeneity in the source concentrations in patches or in the degree of connectivity to the stream network, as is likely to be common in urban catchments (Hook and Yeakley, 2005; Miles and Band, 2015) and other landscapes (McGlynn and McDonnell, 2003; Laudon et al., 2011; Herndon et al., 2015). In particular, applying this nested catchment mass balance approach to formerly overlooked but relatively bounded subsystems like EH can help provide mechanistic understanding of sources and transport of chemical constituents in urban catchments.
Figure 2.4: Transformation and transport of organic matter along a continuum of engineered headwaters. During periods between storms, gutters, roads and catch basins process OM that has accumulated and produce CO$_2$ and readily-mobile DOC (orange). Roofs have a greater DOC than CO$_2$ yield per gram of litter (mean (sd)), while catch basin show the opposite pattern. When storms generate flow in these ephemeral headwaters, they become connected to the stream (blue) and rapidly transport DOC and nutrients downstream through runoff over impervious surfaces and through engineered channels (gray).
Pipes are not pipes: Impervious runoff is not always the primary source of stormwater in an urban headwater stream

3.1 Introduction

As the number of people living in cities across the globe continues to increase, the catchments of more and more streams will undergo urbanization. Urban development is associated with increased concentrations and loads of nutrients and contaminants in the streams draining these landscapes (as reviewed by Paul and Meyer, 2001; Walsh et al., 2005). Urban stream syndrome describes a commonly observed physical, chemical, and biological degradation in streams with urbanized catchments (Walsh et al., 2005). Urbanization can also significantly alter the hydrology of urban catchments. In general, urban streams have flashier hydrographs with higher peak flows, and are quicker to respond to precipitation events than streams draining non-urban land. This is because a greater proportion of the precipitation that falls
on the catchment is routed to surface runoff as opposed to infiltration (Walsh et al., 2005; Dunne and Leopold, 1978).

3.1.1 The ‘urban karst’

In addition to changing surface hydrology through construction and alteration of channels and reducing infiltration through paving and soil compaction, urbanization also alters subsurface hydrology of catchments. In the earth underlying urban catchments are embedded numerous pipe networks that transmit drinking water, sanitary sewage, and stormwater. Even when these systems are engineered independently of one another, studies have demonstrated significant interactions among these subsurface pipe systems and groundwater, leading Kaushal and Belt (2012) to describe the earth underlying urban catchments as an ‘urban karst’. As in natural karst, the flow-paths of water beneath the urban landscape are complex. Stream burial artificially generates subsurface flowpaths by pushing surface streams underground (Elmore and Kaushal, 2008). Pipes for drinking water and sewage are artificial macropores for water having different sources, but are not entirely separate from the surrounding groundwater, nor from one another.

Subsurface hydrology in urban catchments is relatively poorly characterized. However, research shows that there can be significant interaction among components of the subsurface water infrastructure systems. Drinking water pipes carry water under pressure and have been shown to lose 20-30% of their volume in transit (Garcia-Fresca, 2004). This water infiltrates into the groundwater system and contributes to baseflow in urban streams. Sanitary sewers are also leaky; tracers of sewage can be found in stream flow in Baltimore streams (Kaushal and Belt, 2012),
and sewage can leak from separated sewer pipes through soils and into stormwater pipes (Sercu et al., 2011). Despite the subsurface water infrastructure in cities being termed "impervious", it is clear that the water in these pipes interacts with that in the unconfined flowpaths and with other piped water.

While the current paradigm in stormwater management pushes for structures and strategies that promote slowing and infiltration of stormwater (as opposed to transmission through pipes), many catchments built before these practices came into favor retain infrastructure like subsurface stormwater pipes and concrete-lined channels designed to route stormwater quickly from land to stream (Hale et al., 2014). Like other urban water infrastructure, however, stormwater pipes also interact with surrounding water in the urban karst. Subsurface stormwater pipes (and the gravel-lined trenches that surround them) have relatively high hydrologic conductivity and like agricultural tile drains can act as preferential flow paths that conduct groundwater to the stream channel during baseflow conditions (as reviewed by Bonneau et al., 2017; Kaushal and Belt, 2012).

Leaks through cracks and joints in concrete pipes allow water flowing through to discharge into the surrounding aquifer or allow water in the surrounding aquifer to infiltrate into pipes. In cities throughout the U.S., water infrastructure is aging to the end of its utility (Doyle et al., 2008), and such interactions between piped and non-piped groundwater may be increasingly common.

3.1.2 Sources of solutes and dissolved organic matter in urban streams

In addition to the hard and soft hydrologic changes caused by urbanization are changes in sources of dissolved constituents. Urbanization introduces new sources
of nutrients to urban landscapes, such as lawn fertilizer, pet waste, and detergents (Hobbie et al., 2017). Concentrations of metals such as lead, copper, zinc, and chromium are sloughed from automobile brakes and tires, and other metals including mercury are found in higher concentrations in urban streams than their non-urban counterparts (as reviewed by Paul and Meyer, 2001). Emerging contaminants like pharmaceuticals and personal care products are novel sources of water quality degradation in many urban streams (Rosi-Marshall and Royer, 2012). Winter road salting is a major source of chloride to streams both through runoff and through contamination of groundwater by de-icing salts (Marsalek, 2003; Novotny et al., 2009; Jackson and Jobbágy, 2005).

Urbanization also alters the sources of dissolved organic matter to urban streams. Increased nutrient concentrations and light availability can result in enhanced algal biomass (although more frequent scour may counteract these effects) and shifts in species composition (Walsh et al., 2005; Newall and Walsh, 2005; Sonneman et al., 2001). In addition to changes in autochthonous sources of DOM, urbanization can alter allochthonous sources of DOM to streams. As discussed, leaky sewer infrastructure can contribute sewage to urban streamflow (Sercu et al., 2011; Kaushal and Belt, 2012). Pet waste can be another major source of organic nutrients to urban streams (Baker et al., 2001; Fissore et al., 2011). Urban areas also support different vegetation communities than their non-urban surroundings (Groffman et al., 2014), and litter produced by vegetation can contribute significantly to loads of nutrients and DOM in urban streams (Hobbie et al., 2014; Janke et al., 2017; Bratt et al., 2017; Chapter 2).
3.1.3 Connectivity and contribution of impervious flowpaths

Arguably the most significant change that urbanization makes to the land surface with respect to hydrology is the increase in surfaces impervious to water. Increased impervious surface cover (ISC) decreases the ability of water to infiltrate into the soils, routing it instead as surface runoff (Walsh et al., 2005; Dunne and Leopold, 1978). Large volumes of this runoff from impervious surfaces flow rapidly to urban streams, generating the flashiness is generally considered the main source of hydrologic and geomorphologic degradation to streams in urban landscapes (Walsh et al., 2005). Runoff from impervious surfaces reaches the urban stream through engineered ephemeral channels in the form of gutters and pipes that can be collectively termed the ’engineered headwaters’ (EH) of an urban stream network (Kaushal and Belt, 2012). The EH can be an important proximate sources of solutes in stormwater because they can store and process organic matter and because they have the capacity to rapidly and efficiently transport runoff to urban streams (Chapter 2).

In this chapter, we assess the contribution of EH runoff vs. other potential water sources to stormflow within and among storms in an urban stream in Durham, NC. We also examine changes in the chemistry of stormwater and in the composition of DOM transported in stormflow.

3.2 Methods

3.2.1 Site description

This study was conducted in a medium density residential neighborhood in Durham, NC. The first order stream draining the catchment is a tributary of Ellerbe Creek.
The main land cover in this 60 ha catchment comprises approximately 400 single family homes on quarter acre (~1000 m²) or smaller lots. Overall, the catchment has 39.4% impervious cover, based on 1 m resolution planimetric maps (City of Durham Stormwater Services, 2016), and considerable canopy cover provided primarily by mature Willow Oaks (*Quercus phellos*) planted in the 1930s (Cooper et al. 2016a; Durham City-County Environmental Affairs Board 2015).

Like much of the city, this Durham neighborhood is drained by a Municipal Separate Storm Sewer System (MS4) that includes impervious structures such as roadside gutters and curbs, catch basins (i.e. roadside inlets to subsurface storm drains), and subsurface stormwater pipes (City of Durham Stormwater Services, 2011) that drain to the stream. In addition, the roof gutters of approximately 10% of residences in this area are routed onto roads (Miles and Band, 2015), directly connecting them to the MS4 through engineered impervious flowpaths.

### 3.2.2 Sample collection

We designed sample collection along a continuum of engineered headwaters (EH) beginning with throughfall as water input, to roof gutter outlets, to road runoff, to flow though catch basins, to storm drain outlets, and finally to the catchment pour point, 30 m upstream of the confluence with Ellerbe Creek (Figure 3.1).

We collected throughfall in 1 gal (3.8 L) open-topped plastic buckets (cleaned with Liquinox before deployment) secured to fences at chest level under the tree canopy at each of three focal properties for the duration of three storms. We collected runoff from roofs using Liquinox-clean 5 gal (18.9 L) buckets with lids fitted with black corrugated plastic pipe placed beneath the downspouts of three houses. Road
Figure 3.1: Map of engineered headwater and catchment pour point sampling sites. Approximate boundary of catchment shown in yellow. Image from Google Earth, downloaded on 9 October 2017.
runoff was collected in 1 gal (3.8 L) buckets hung from the corner of catch basin grates (n=3 for each of 8 storms) to collect runoff directly from roadside gutters. We collected stormflow through catch basins either by removing the catch basin grate and sampling flow directly with a 60 mL syringe, or by connecting a weighted flexible PVC tube to the 60 mL syringe and feeding it through the grate to collect water samples. To collect samples from storm drain outlets, we hung 1 gal (3.8 L) buckets from hooks installed in the walls of culverts at the terminus of storm drains.

In all cases, buckets were allowed to overflow until collection, representing a composite sample over the course of the storm up to that point. We collected samples from these by thoroughly mixing the collected volume of water, sampling with a 60 mL syringe, filtering through a Whatman GF/F (nominal pore size: 0.7 µm) into two 60 mL opaque brown plastic bottles which were transported back to the laboratory on ice for chemical and optical analysis.

Stream samples were collected using an automatic sampler (Teledyne Isco 6712) every 5 minutes from the beginning of the rain event for the first 12 samples and every 30 minutes for the remaining 12 samples, and by grab sampling from the same location contemporaneously with EH grab sampling. Autosampler bottles were collected after the conclusion of the sampling program (not more than 16 hours after initialization) and transported back to the laboratory where a subset of samples were chosen for analysis using the time series of water level to choose at least 10 samples spanning the rising and falling limb of the storm. Samples were vacuum-filtered through Whatman GF/F filters into two 60 mL opaque brown plastic bottles for chemical and optical analysis.

Periodic grab samples were collected and analyzed by the urban stream team.
Members of the team collected samples at the stream pour point, near the ISCO intake, every two weeks. Samples were field filtered through Whatman GF/F filter (nominal pore size: 0.7µm) into acid-washed 60 mL plastic sample bottles which were transported back to the laboratory on ice for chemical analysis. In this study, we use only those samples collected between September 2014 and November 2016 at baseflow conditions.

We prepared leachates of potential sources of DOM in urban stormwater as described in Chapter 4. Potential sources for which leachates were prepared included: biofilms, grass, leaves, mulch, pavement, pine needles, plastic (from buckets, pipes, and sample tubing), soil, and roof infrastructure (asphalt shingles and aluminum and vinyl gutter). Other potential sources of urban DOM we considered were throughfall and sanitary sewage. We also included measurements of DOM sources after 60 days of incubation (using the procedure described in Chapter 4) in our analysis.

Sample replicates for chemical analysis were frozen upon return to the lab or upon filtering until analysis, and replicates for optical analysis of DOM were held at 4 C until being transported on ice for analysis at North Carolina State University, within 10 days of collection.

3.2.3 Laboratory measurements

We measured DOC and TDN concentrations on thawed samples using a TOC analyzer with TDN module (Shimadzu TOC-VCPH Analyzer with TNM-1 module). Concentrations of major cations (Ca\(^+\), Mg\(^+\), Na\(^+\), and K\(^+\)) and anions (Cl\(^-\), Br\(^-\), SO\(_4^{2-}\), NO\(_3^-\), and PO\(_4^{3-}\)) were measured by ion chromatography (Dionex ICS 2000).

Optical analyses for DOM composition were performed as described in Chapter
3.2.4 Statistical methods

Processing of EEMs and PARAFAC modeling were conducted in MATLAB (The Mathworks Inc., 2016) using the DOMFluor toolbox (Stedmon and Bro, 2008). All other statistical analyses were conducted in the R environment (R Development Core Team, 2012).

We used analysis of variance (ANOVA) to assess differences in peak concentration, the range of concentration, mean concentration, and load of each solute in stream stormflow among seasons.

Water Balance

To calculate the event discharge in the stream, we used measurements of stage collected using a logging water depth sensor (Onset HOBO U20L) deployed near the ISCO intake to record water depth every 5 minutes. We used a rating curve developed over the same time period (J. Delesantro, pers. comm.) to convert stage measurements to discharge (L per 5 minutes), which were summed to give the event discharge.

We estimated the runoff from EH as the runoff from roads, which we calculated by multiplying event rainfall by road area to give event flux in L. We estimated rainfall in our catchment as the mean measured at the two nearest USGS rain gages (site 3601430785400945, located ~2 km NE and site 355852078572045, located ~4 km SW of the study watershed), which was then summed over each event. Road area was determined using 1 m resolution planimetric maps of catchment impervious
The relationship between stream event discharge and EH runoff was assessed using simple linear regression and validated model assumptions by visual examination of residual and Q-Q plots.

Mixing analysis

We conducted a mixing analysis to assess how the contribution of different potential sources of water to the stream varied among individual storms. The potential sources (end-members) we considered were: 1) runoff from engineered headwaters, 2) baseflow (which we assume to be representative of groundwater in this catchment), and 3) soilwater; samples of soilwater were collected by M. Zimmer between 1-4 October 2015 and between 4-8 March 2016 as part of another study. The Duke Forest site at which these soilwater samples were collected is within 10 km of our study catchment and has similar underlying geology (Carolina Slate Belt) to our urban study catchment.

The solutes chosen as tracers for mixing analysis were potassium, and the sum of magnesium and calcium. Although potassium is not a strictly conservative tracer (Hooper et al., 1990), for the purposes of this analysis we assume K\(^+\) uptake is negligible over the timescale of these events (<6 hours). These tracers discriminated well among the potential end-member sources we considered (Figure 3.8).

In addition to this mixing analyses, we characterized the changes in charge balance time series of stormwater chemistry and for the end-member sources of EH runoff and baseflow for each of five storms over a range of antecedent rainfall condi-
tions.

Ordination

We conducted a principal component analysis (PCA) to assess differences in the DOM composition (as measured by optical properties) among samples. After log-transforming and normalizing data, we conducted PCA using the vegan package in R (Oksanen et al., 2017). We excluded the $\beta/\alpha$ index from the ordination because its loading was nearly identical to that of BIX, given the similarity of these two indices.

3.3 Results

3.3.1 Seasonal patterns in stream stormflow chemistry and DOM composition

In general, we did not observe strong seasonal patterns in solute concentrations measured in stream stormflow (Appendix B). Peak [DOC], range of [DOC] within storms, mean [DOC] within storms, nor DOC load differed among seasons. The same was true for NO$_3^-$, SO$_4^{2-}$, Br$^-$, Cl$^-$, and base cations (although Cl$^-$ showed considerably more variability in winter as opposed to other seasons, likely as a result of road salting; Appendix B). We found lower within-storm range of [TDN] in winter as opposed to other seasons, and larger peak [PO$_4^{3-}$] and PO$_4^{3-}$ loads in fall as compared to other seasons (Appendix B).

Optical properties of DOM in stormwater tended to showed more seasonal variability than solute chemistry. Peak and mean intra-storm SUVA, were lower in winter than in other seasons. The peak fluorescence index and mean slope ratio were significantly higher in fall. We did not observe significant differences among seasons in the HIX or BIX of DOM in stream stormflow (Appendix B). PARAFAC components
also showed seasonal variability. The intra-storm peak and range of component 2 (a red-shifted terrestrial humic-like component) were lower in winter as compared to other seasons. Similarly, the mean contribution of component 4, a protein-like component, was lower in winter than other seasons. The range of component 4 over the course of storms was smaller in summer as compared to other seasons, and its peak was lower in summer and winter than in fall and spring storms (Appendix B).

3.3.2 Water Balance

For the 15 storms included in this study, estimated runoff from EH explains 80% of the variation of the measured discharge in the stream (Figure 3.2). The slope the best-fit line relating streamflow and runoff from EH is 0.83, only slightly shallower than the 1:1 relationship. The plot of this water balance also suggests seasonal variability in the relationship between event-scale EH runoff and stream stormflow; for storms in winter, points tend to fall above the line, suggesting a greater contribution from other flow sources (e.g. baseflow) as compared to EH runoff in winter.

3.3.3 Event-scale patterns in ion chemistry and C-Q relationships of DOC and TDN

Charge balance plots for five storms (Figures 3.3 through 3.7) show that the bulk ion chemistry of streamwater resembled baseflow (left stacked bar in Figures 3.3 through 3.7) at the beginning of each of these storms and becomes more dilute at high flows. However, the event-scale patterns of concentration differ among solutes; the relative and absolute concentration of K⁺ increase over the course of some storms, especially those following dry weeks (Figures 3.4 and 3.5). Nitrate concentrations also increased over the course of each of these five storms, while phosphate increased
Figure 3.2: Event streamflow vs. estimated road runoff for 15 storms between September 2014 and November 2016. Road runoff was estimated assuming 100% water yield for precipitation falling on road surface and on 10% of roof area in the catchment.

over the course of storms with drier antecedent conditions. The net effect of these changes in solute concentrations within events was that the chemistry of streamflow during storms following dry antecedent conditions grew similar to the chemistry of EH runoff (Figures 3.3, 3.4, and 3.5). In contrast, the chemistry of streamflow late in the storms that followed wetter antecedent conditions did not appear to converge of the chemistry of EH runoff (Figures 3.6 and 3.7).
We also examined the concentration-discharge (C-Q) relationships for DOC and TDN (Figures 3.3 through 3.7). The relationship between DOC and discharge for these storms tends to be positive, but the direction of hysteresis differs among storms. For storms following drier antecedent conditions (Figures 3.3, 3.4, and 3.5) the DOC-discharge relationship follows a counter-clockwise hysteresis, while a storm following wet antecedent conditions (Figure 3.7) shows a clockwise hysteresis. Relationships between TDN and discharge also differed among these storms, but distinct hysteresis patterns were not observed for all of the storms. The C-Q relationship for TDN was quite similar to DOC for three storms (Figures 3.4, 3.5, and 3.6), but differed for one storm with dry antecedent conditions (Figure 3.3) and one storm with wet antecedent conditions (Figure 3.7).

### 3.3.4 Mixing analysis

We created mixing plots of the pooled data (Figure 3.8-A) and for individual storms encompassing a range of antecedent rainfall conditions (sum of rainfall in the 7 days preceding the sampled storm; Figure 3.8-B through F). Seven-day antecedent rainfall among these five storms ranged from 0 mm to 32.38 mm. In all of the storms, the chemistry of samples taken early in the rising limb is similar to that of baseflow. For storms with lower antecedent rainfall, the contribution of EH runoff increases over the course of the storm, whereas when antecedent rainfall is high (i.e. the catchment is wetter preceding the sampled storm), the chemistry of streamwater becomes similar to that of median soilwater.

We also generated mixing plots for storms without measurements of EH runoff chemistry using the median of the pooled samples (Figure B.22). Here, the trajec-
Figure 3.3: Charge balance in streamwater over the course of a storm on 15 June 16, and for preceding baseflow and EH runoff samples (top). Concentration-discharge relationships for DOC (bottom left) and TDN (bottom right) for the same storm. Concentration-discharge plots show the hysteresis of the relationship, with symbols ordered from dark to light over the course of the storm.
Figure 3.4: Charge balance in streamwater over the course of a storm on 22 April 16, and for preceding baseflow and EH runoff samples (top). Concentration-discharge relationships for DOC (bottom left) and TDN (bottom right) for the same storm. Concentration-discharge plots show the hysteresis of the relationship, with symbols ordered from dark to light over the course of the storm.
Figure 3.5: Charge balance in streamwater over the course a storm on 14 November 16, and for preceding baseflow and EH runoff samples (top). Concentration-discharge relationships for DOC (bottom left) and TDN (bottom right) for the same storm. Concentration-discharge plots show the hysteresis of the relationship, with symbols ordered from dark to light over the course of the storm.
Figure 3.6: Charge balance in streamwater over the course a storm on 17 December 15, and for preceding baseflow and EH runoff samples (top). Concentration-discharge relationships for DOC (bottom left) and TDN (bottom right) for the same storm. Concentration-discharge plots show the hysteresis of the relationship, with symbols ordered from dark to light over the course of the storm.
Figure 3.7: Charge balance in streamwater over the course of a storm on 31 March-1 April 16, and for preceding baseflow and EH runoff samples (top). Concentration-discharge relationships for DOC (bottom left) and TDN (bottom right) for the same storm. Concentration-discharge plots show the hysteresis of the relationship, with symbols ordered from dark to light over the course of the storm.
ories of stormwater chemistry among storms with varying antecedent rainfall were less clear, likely because the baseflow in many of these storms had much lower concentrations of calcium and magnesium than the median.

3.3.5 Dynamics of DOM composition

We also conducted a principal component analysis on composition metrics of DOM in stream stormflow, EH runoff, baseflow, and potential sources of DOM in this urban stream (N = 336; Figure 3.9). Measures of DOM composition were not available for Duke Forest soilwater. While we do observe distinct DOM compositions between DOM in EH runoff and that in baseflow, stream stormflow spans a greater range of DOM composition than these potential water source end-members. It is worth noting that DOM composition is not a conservative tracer because it changes with biotic and abiotic processing (Chapter 4).

Attributing the DOM in these samples to distinct primary sources based on similarity in composition is difficult because of the wide and overlapping ranges of the PCA scores associated with different potential sources. Even so, it is possible to make some inference about which DOM sources are likely to be important. For example, biologically-processed soil leachate (So.i) has a relatively low score on PC2, as do baseflow and streamflow samples taken during winter storms and at winter and fall baseflow. Washes and leachates of pavement (asphalt and concrete) plot alone in the upper left of this ordination, suggesting that this source of fluorescence is necessary to describe the composition of DOM in many of these samples.

Within individual storm events, DOM composition is considerably less dynamic than cation chemistry (Figure 3.10) and its trajectory does not vary systematically.
Figure 3.8: Mixing of runoff from engineered headwaters (pink triangles), baseflow (gray squares), and soilwater (green diamonds) in stream stormflow (blue circles). Because these potential end-members differ in their concentrations of $K^+$ and the sum of $Ca^{2+}$ and $Mg^{2+}$, these can be used as tracers of mixing in streamflow. In individual storm plots (B-F), the shade of blue circles indicates progression of streamflow chemistry through time, with lighter symbols representing chemistry early in the storm and darker symbols showing the chemistry of later samples. Whiskers show the 10th and 90th percentiles of concentration in microequivalents for the pooled dataset (A) or for each individual storm (B-F). Large end-member symbols (pink triangles, gray squares) show the median of the whole data set, while small symbols show the median for samples within individual events, except for soilwater (green diamonds), which is the median of soilwater samples collected at Duke Forest.
Figure 3.9: Ordination of optical properties of DOM (N = 286) in stormwater. Loadings of different indices and PARAFAC components (A) suggest that samples with lower scores on PC 1 are more humic and conjugated, and higher scores on PC2 indicate more aromatic moieties. The composition of DOM is less distinct among end-members and less dynamic within storms as compared to the cation chemistry (Figure 3.8). Potential primary sources of DOM (B) included: Pv = pavement; Sw = sanitary sewage; Lf = leaf; Ps = plastic; Pn = pine, Gr = grass; TF = throughfall; RI = roof infrastructure; BF = biofilm; Mu = mulch; ‘i’ suffix indicates the composition of a leachate after 60 days of incubation.

3.4 Discussion

Although we found that the volume of water that flows through this small urban stream during storms is often very similar to the estimated volume of runoff from impervious surfaces, the chemistry of these waters are distinct, particularly when the catchment soils are already wet from antecedent rain.

We measured the chemistry of two possible sources of stormflow in this urban stream: runoff from engineered headwaters and baseflow (i.e. the groundwater
source(s) that generate streamflow in the absence of surface runoff). The relative concentration of potassium ion of was greater in EH runoff than in baseflow. Both absolute and relative concentrations of nitrate and phosphate also tend to be higher in EH runoff than stream baseflow. This signature may be the result of application of NPK fertilizer in the catchment that runs off and enters EH flowpaths during storms. We also used measurements of soilwater chemistry from the nearby Duke Forest to represent another potentially important end-member in mixing.

We found that the chemistry of ions was highly dynamics during these storms; relative concentrations of $K^+$ and $NO_3^-$ tended to increase over the course of storms while the bulk chemistry diluted. These changes differed among the storms we measured such that the chemistry of stream stormflow following dry weeks tended to move from baseflow-like to EH runoff-like, while the stream chemistry for storms following wet weeks resembled soilwater much more than EH runoff.

In contrast to cation chemistry, the composition of DOM in impervious runoff and in stream storm- and baseflow are all similar to one another, less dynamic than cation chemistry, and show no distinct patterns among storms with different antecedent rain conditions. This could be because the composition of DOM present in baseflow and EH runoff similar or because the sources contributing the majority of the DOM in stormflow are different than those than contribute water.

3.4.1 Mixing of water from different flowpaths in urban stormwater depends on antecedent wetness

The results of this study suggest that quickflow in this urban stream cannot be explained by two end-member mixing of the efficiently routed runoff from EH with
baseflow. Instead, there seems to be an unmeasured source of water to this urban stream that responds quickly during storms and has distinct solute composition.

Although we did not directly measure soilwater in this urban catchment, we did find that the chemistry of streamwater was more similar to that of soilwater from the nearby Duke Forest when the wetness state of the catchment was high and soils should be able to make greater contributions to streamflow. Zimmer and McGlynn (2017b) observed that quickly-responding shallow flowpaths were important contributors to streamflow generation in a forested headwater catchment in Duke Forest. Our analysis suggests that these quickly-responding shallow flowpaths may also be important contributors to stormflow in this nearby urban stream, particularly when soils are wet from antecedent rainfall. The contribution of these shallow soil flowpaths may even exceed the contribution of runoff from EH in particularly wet conditions.

3.4.2 ‘Missing’ runoff from engineered headwaters - the role of the urban karst

The combination of a) balanced event-scale EH runoff and streamflow and b) significant contribution of additional source of water to quickflow in this stream suggest that a considerable volume of the runoff from EH must be lost before it reaches the stream. Retention of stormwater and infiltration through cracks or other pervious areas along these EH flowpaths could explain this difference in chemistry.

Increased flashiness in urban catchments is generally attributed to the increased proportion of precipitation routed directly to streams as surface runoff from impervious surface. However, this work suggests that impervious stormwater infrastructure does not perfectly convey runoff to the stream in this urban catchment. Instead, loss of surface runoff to groundwater infiltration and replacement of this water vol-
ume by another source in stream stormflow could explain the patterns we observe in stormwater chemistry in this catchment. Particularly when soils in the catchment are wet, the contribution of soilwater seems to be important to stormflow in the stream.

Current paradigms in stormwater management seek to increase infiltration of stormwater through interventions from re-routing of water over pervious surface (Miles and Band, 2015) to engineered green infrastructure such as bioretention cells and rain gardens (Avellaneda et al., 2017). The work presented in this chapter suggests that significant infiltration of stormwater may occur even without these new or retrofitted structures and interventions.
Figure 3.10: Trajectories of OM composition within ordination space for streamflow (blue circles) and the median composition of DOM in baseflow (gray squares) and EH runoff (pink triangles) for four storms. Shade indicates progression through time, with lighter symbols representing chemistry early in the storm and darker symbols showing the chemistry of later samples. Whiskers show the 10th and 90th percentiles of concentration in microequivalents for the pooled dataset (first panel) or for each individual storm (other panels). Large end-member symbols (pink triangles, gray squares) show the median of the whole data set, while small symbols show the median for samples within individual events.
Bioavailability of dissolved organic matter in urban engineered headwaters

4.1 Introduction

Dissolved organic matter (DOM) is present in all natural waters and plays a central role in the ecology of many aquatic environments (Prairie, 2008). Both the quantity and quality of DOM are important in determining how it behaves in aquatic ecosystems. Specifically, the amount and composition of the DOM pool determine its lability to degradation by the action of microbes and by photochemical reactions, while this processing in turn controls the concentration and composition of DOM in the ecosystem. Processing of some kinds of DOM results in conjugation and humification, producing larger and more complex molecules (Repeta et al., 2002). More frequently, microbial and photochemical action degrades DOM by converting large molecules to smaller, simpler ones, or by mineralizing it completely to CO$_2$ (Wetzel
et al., 1995; Moran et al., 2000).

In rivers and streams, processing occurs as DOM is transported through the river continuum, giving rise to a continuum of diagenesis in space and time. Conceptual models have been developed to describe these spatial patterns in DOM composition. Specifically, the River Continuum Concept (RCC) predicts that the composition of the DOM pool becomes less diverse with distance downstream (Vannote et al., 1980), and Creed et al. (2015) observed this pattern in proxies for DOM composition from river continua throughout the continental United States. Vannote et al. suggest that this pattern arises because headwaters, having the greatest interface with terrestrial sources of soluble OM, ought to have the highest diversity in the DOM pool. As photochemical, physical, and biotic processes selectively remove small, labile DOM molecules, the remaining DOM transported downstream becomes more homogenous because it is comprised of the 'left-overs': higher molecular weight and more refractory OM.

Amon and Benner (1996) also consider spatial patterns of DOM molecular size and reactivity along a continuum. Their size-reactivity continuum model describes patterns of biolability of different size fractions of DOM from river to ocean. Along this continuum, the percentage of the DOM pool characterized as high molecular weight (HMW) decreases with distance downstream, and incubation experiments show that this HMW DOM stimulates higher rates of bacterial respiration. Thus, they describe a pattern in which the composition of the DOM pool becomes more refractory and lower in average molecular weight as it is processed and transported downstream.

While the specific predictions regarding patterns in molecular size differ between
these two conceptual models, both the RCC and the size-reactivity continuum rely
on the correlation between distance traveled and transit time that gives rise to pre-
dictable changes in the composition of the DOM pool as processing occurs in concert
with advection. Closer to the terrestrial source, diagenetically young DOM contains
a multitude of different molecules. The most labile of these are quickly mineralized
to CO$_2$ or converted into other compounds, and a less diverse and less labile pool of
DOM continues to move downstream. Thus, the composition DOM at a point in the
continuum is determined by the biotic and abiotic processing it experienced earlier
and upstream.

Statements about relative lability necessarily depend on the boundaries of the
system under consideration. The boundaries of the continuum considered in the RCC
are first order streams at the uppermost and 12$^{th}$ order rivers at the downstream end,
while the size-reactivity continuum model is based on measurements spanning from
river mainstem to open ocean; in other words, the upstream boundary of the system
considered by Amon and Benner is near the downstream terminus of the system
considered by Vannote et al. This difference in reference frame may explain some of
the difference in these authors conclusions regarding the lability of DOM of different
molecular weight.

Because the mechanism that creates patterns in DOM composition in both of
these conceptual models is variation in lability within the DOM pool, it is worth
noting that contextual and compositional changes may alter the lability of OM. The
priming effect occurs when inputs of labile OM facilitate mineralization of otherwise
calcitrant DOM (Guenet et al., 2010). If labile autochthonous DOM is released
into the DOM pool along a river continuum, it may facilitate metabolism of the
'leftover' recalcitrant DOM (Guenet et al., 2010; Rier et al., 2014) and disrupt the predicted patterns. However, the effect of labile OM inputs could also displace the use of other DOM (in effect increasing its recalcitrance, Lutz et al., 2012; Franke et al., 2013). Chemical changes during processing may not necessarily result in mineralization and mass loss, but may make OM more or less labile to further processing. Many studies have demonstrated that photochemical changes to DOM facilitate microbial metabolism of DOM (Lindell et al., 1995; Moran and Zepp, 1997; Bertilsson et al., 1999; Anesio et al., 2000). Similarly, considerable work in stream ecology has demonstrated the importance of microbial conditioning on the decomposition of particulate OM (as reviewed by Tank et al., 2010), as well as the role of the microbial loop in using DOM as a basal resource for aquatic foodwebs (Meyer, 1994). Little work has demonstrated microbial conditioning of DOM (i.e. microbially-mediated changes to DOM chemistry that increase lability), but this may be an important process in determining patterns of DOM composition in a river continuum.

4.1.1 Optical measures of DOM composition

Because of the huge diversity of unique compounds in the DOM pool of natural waters, specifically identifying all the molecules present in a sample is impossible. Instead, approaches have been developed to summarize average characteristics of the DOM pool. Some of the fastest, simplest, and least expensive of these approaches exploit the fact that some DOM absorbs light (colored DOM or CDOM) and a smaller fraction fluoresces (fluorescent DOM or fDOM). The specific wavelengths of light absorbed and emitted are determined by the conformation of molecules, and several metrics that relate these optical properties to average biochemical and
physical properties of the DOM have been developed (Table 4.1).

Table 4.1: Optical indices of OM composition.

<table>
<thead>
<tr>
<th>Index</th>
<th>Definition</th>
<th>Indicator of:</th>
<th>Citation</th>
<th>'Typical' range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific UV absorbance (SUVA&lt;sub&gt;254&lt;/sub&gt;)</td>
<td>Absorbance at 254 nm divided by [DOC] (units: L m&lt;sup&gt;-1&lt;/sup&gt; mg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>Aromaticity</td>
<td>Weishaar et al. (2003)</td>
<td>~0.5 (Pacific Ocean fulvic acid) to ~5.3 (Ogeechee River humic acid)</td>
</tr>
<tr>
<td>Slope ratio (S&lt;sub&gt;R&lt;/sub&gt;)</td>
<td>Best-fit slope of ln(abs) from 275-295 nm divided by best-fit slope of ln(abs) from 350-400 nm (unitless)</td>
<td>Average molecular weight</td>
<td>Helms et al. (2008)</td>
<td>~0.7 (blackwater) to ~10 (open ocean)</td>
</tr>
<tr>
<td>Humification index (HIX)</td>
<td>Area under emission curve between 435-480 nm divided by area under emission curve between 300-345 nm, for excitation at 254 nm</td>
<td>Humification</td>
<td>Zsolnay et al. (1999)</td>
<td>~2 (fumigated mineral soils) to ~16 (fulvic acids)</td>
</tr>
<tr>
<td>Fluorescence index (FI)</td>
<td>Emission at 470 nm divided by emission at 520 nm, for excitation at 370 nm (unitless)</td>
<td>Origin of OM</td>
<td>McKnight et al. (2001)</td>
<td>~1.2 (terrestrial) to ~1.9 (microbial)</td>
</tr>
<tr>
<td>Freshness Index (β/α)</td>
<td>Emission at 380 nm divided by max emission between 420-435 nm, for excitation at 310 nm (unitless)</td>
<td>'Freshness' or degree of processing</td>
<td>Parlanti et al. (2000)</td>
<td>~0.5 to ~1, with higher values indicating 'fresher' OM</td>
</tr>
<tr>
<td>Biological index (BIX)</td>
<td>Emission at 380 nm divided by emission at 430 nm, for excitation at 310 nm (unitless)</td>
<td>Contribution of new autochthonous production</td>
<td>Huguet et al. (2009)</td>
<td>~0.6 (low productivity) to &gt;1 (high autochthonous productivity)</td>
</tr>
</tbody>
</table>

Indices of DOM composition derived from measurements of absorbance include the specific UV absorbance of 254 nm light (SUVA<sub>254</sub>) and the spectral slope ra-
ratio ($S_{R}$). $SUVA_{254}$ is a frequently-used index of the average aromaticity of DOM molecules (Weishaar et al., 2003), and has also been related to the abundance of hydrophobic organic acids (Spencer et al., 2012). The slope of the log-transformed absorbance spectrum is also used as a proxy for properties of the DOM pool; $S_{R}$ is the ratio of the fitted slope of absorbance from 275-295 nm to the slope of absorbance from 350-400 nm and its value is negatively correlated with average molecular weight (Helms et al., 2008).

Fluorescence-based indices of DOM composition include the humification index (HIX), fluorescence index (FI), freshness index ($\beta/\alpha$), and biological index (BIX). The use of HIX as an index relies on the observation that conjugation and humification of organic matter results in red-shifting of the fluorescence emitted by DOM. HIX is a ratio of fluorescence at longer wavelengths to that at shorter wavelengths and its value increases with increasing humic content of DOM (Zsolnay et al., 1999; Ohno, 2002). The fluorescence index was developed as an indicator of the origin of DOM using end member samples of DOM derived entirely from terrestrial vegetation or from aquatic microbial production. The value of FI is also based on a ratio of fluorescence at longer wavelengths to shorter wavelengths and takes on lower values for terrestrial DOM sources and higher values for microbially-derived DOM (McKnight et al., 2001). The freshness index and biological index are calculated using very similar procedure and are used to indicate the freshness or degree of processing and the relative contribution of new autochthonous DOM, respectively. The $\beta/\alpha$ takes on greater values for DOM that is fresher and BIX takes on greater values for samples containing a greater relative proportion of newly synthesized autochthonous DOM (Parlanti et al., 2000; Huguet et al., 2009).
Another approach for characterizing DOM based on its optical properties measures the fluorescence of samples in excitation-emission matrices (EEMs) and applies parallel-factor analysis (PARAFAC) to extract fluorescent components that describe variation among sample EEMs (Stedmon and Bro, 2008). The EEM-PARAFAC approach can be used to identify the important fluorescence peaks in a data set, and the relative abundance of these peaks can be compared among samples within the data set. Additionally, the fluorescent components can be related to those observed by other researchers to gain insight into their ubiquity, biochemical properties, and behaviors in other ecosystems (Murphy et al., 2014).

While optical indices and fluorescent components can be useful summaries of the bulk DOM pool, they must be applied with care, and the conclusions drawn from them not oversimplified. For example, fluorescence peaks around with emission between 300-350 nm and excitation around 275 nm have been widely labeled as protein-like and frequently assumed to be highly labile fractions of DOM (e.g. Balcarczyk et al., 2009; Fellman et al., 2010; Williams et al., 2010). However, recent work has shown that other types of DOM, specifically certain phenols and indoles derived from lignin decomposition, also fluoresce in this region (Hernes et al., 2009; Aiken, 2014), and not all of the fluorescence in this region is associated with highly bioavailable moieties (Cory and Kaplan, 2012). Similarly, aromaticity, as indicated by SUVA measurements, has often been interpreted as a sign of greater relative recalcitrance (Kalbitz et al. 2003; Berggren et al. 2009), but molecules known to be associated with highly bioavailable, such as the amino acids tryptophan and tyrosine contain aromatic groups. Determination and interpretation of spectral slope ratios (Helms et al., 2008) can be complicated by high relative concentrations of absorbing
compounds that create complex structure in absorbance spectra (C. Osburn, pers. comm.). These examples illustrate some of the limitations of assuming bioavailability or ecosystem function of DOM based on individual indices.

4.1.2 DOM in the urban stream continuum

Streams embedded in urban landscapes experience distinct DOM regimes (i.e. timing, amount, and composition of DOM) as compared to non-urban streams, with consequences for their function and that of downstream ecosystems. Urbanization drives differences in the sources and transport of DOM to streams as well as in the processing rates of DOM in the stream network. Better understanding of DOM dynamics in urban streams is particularly important because of their substantial impact on downstream aquatic ecosystems and proximity to sources of drinking water.

There are several reasons to believe that the DOM in urban streams may be distinct in composition as compared to non-urban streams. First, urban landscapes have unique sources of DOM such as petroleum products (including oil and gas from vehicles and tar from roads and shingles), different vegetation (such as exotic and ornamental plants), and different forms of organic matter (e.g. mulch, imported topsoil). Second, impervious surfaces promote accumulation and leaching of OM to the dissolved phase and impede interaction with soils to which DOM might otherwise sorb (Chapter 2; Hobbie et al., 2013; Bratt et al., 2017).

Distinct conditions in urban networks may also lead to differences in the rates of OM processing. Light regimes can be markedly different in urban streams as compared to non-urban streams. On one hand, removal of riparian vegetation and widening of stream channels may increase the light reaching a streams surface, but
this may be countered by stream incision reducing the light reaching the water surface (Walsh et al., 2005). DOM processing rates in urban streams may also be altered by the urban heat island speeding metabolism and enhancing the breakdown of OM (Kaye et al., 2006). Residence time of water in stream networks also differs between urban and non-urban stream networks. Urban streams have highly flashy hydrology, meaning residence times during stormflow are very short (Walsh et al., 2005). However, the infrastructure that makes up the headwaters of the urban stream network may trap and retain water (and the DOM dissolved in it) between storms, where it can have significant residence time before being reconnected by stormflow (2).

The behavior and fate of DOM in urban streams is important because of its influence on the ecological function of urban streams and on downstream aquatic systems. If large loads of urban DOM are metabolized in situ, hypoxic conditions may result. If large pulses of urban DOM are exported to downstream ecosystems in stormwater, hypoxia and eutrophication may shift to these receiving waters. In addition, water bodies receiving runoff from urban systems may be used as drinking water sources, where DOM may interfere with water treatment by reacting with disinfection chemicals to form carcinogenic by-products (Chow et al., 2007).

In this study, we shed light on the potential impacts of urban DOM on streams and receiving waters by determining its lability to microbial degradation. We also characterize changes in the characteristics and composition of the DOM pool over the course of microbial processing, allowing us to infer what patterns of DOM composition may look like over an urban stream continuum. We approach these questions with experimental incubations of DOM in urban stormwater and from leachates of
urban coarse particulate OM (CPOM), pairing measurements of DOC mass loss and changes in optical properties of the DOM pool over the course of processing.

4.2 Methods

4.2.1 Sample collection

Samples were collected in the catchment of a first order tributary of Ellerbe Creek in Durham, NC. Land cover in the 60 h catchment consists primarily of single family homes on quarter-acre or smaller lots, with 39.4% impervious cover (based on 1 m resolution planimetric maps, City of Durham Stormwater Services 2016). The catchment has considerable canopy cover (~ 60%, i-Tree Landscape v2.1.2; i Tree Cooperative 2016) with willow oak (Quercus phellos) as the dominant canopy tree (Durham City-County Environmental Affairs Board, 2015; Cooper et al., 2016b). Sample collection occurred before (for leachate samples) and during (for stormflow samples) two storms on 19 November 2016 and 1 April 2017.

The 75 total samples were categorized into four different groups: 1) primary source leachates, 2) leachates of OM accumulated in engineered headwaters (EH), 3) stormflow in EH, and 4) stormflow in streams. Primary sources that were used to create leachates included: senesced leaves, green leaves, pine needles, mulch, soil, grass clippings, and biofilms in the stream and engineered headwater channels. In engineered headwaters, OM for leachates was collected from accumulation in roof gutters and on top of and inside catch basins. Leachates were prepared by air-drying samples for 24 hours at room temperature and then soaking a known mass of the OM in deionized water for 24 hours in 500 mL opaque brown plastic bottles (Nalgene 2106-0016) with occasional shaking before decanting and filtering the supernatant
liquid.

Stormflow samples were collected at the stream mouth with an automatic sampler (Teledyne Isco 6712) every 5 minutes from the beginning of the rain event and by grab sampling from aboveground stream channels upstream in the network during stormflow. Stormflow from engineered headwaters was collected by grab sampling flow in catch basins, and using Liquinox-washed plastic buckets positioned beneath roof gutter downspouts (covered to exclude throughfall and blow in of OM), hung from catch basin grates (to collect road runoff), and hung in culverts at outfalls of stormwater pipes. All buckets were collected within one hour of the beginning of the rainstorm and returned to the lab for immediate processing.

4.2.2 Bioavailability incubation

After sample collection and leachate preparation, a common inoculant was prepared by filtering 100 mL of raw water or leachate from each sample through a GF/D filter (nominal pore size: 2.7 µm, Whatman 1823-047) to remove large particles. Microbes were then concentrated by filtering this 100 mL through a 0.2 µm PVDF membrane filter (Pall 66477), transferring the filter to a common 500 mL glass media bottle, and re-suspending the filter in 20 mL of filtrate.

We then vacuum filtered the remaining volume of each sample through a GF/D primary filter and then a 0.2 µm PVDF membrane filter to sterilize sample water prior to incubation. For each sample, we prepared three replicates for each of three time points (0 days, 6 days, and 60 days; 9 total vials per sample) by transferring 30 mL of filtered sample into 40 mL acid-washed glass vials with washed septa and lids (I-Chem S146-0040) that were covered with aluminum foil to exclude light. We
then added 1 mL of the inoculant to each replicate and shook vigorously to mix.

Incubation was conducted at room temperature, with samples shaken vigorously every day for the first 6 days and once per week for the remainder of the experiment. At each time point (0 days, 6 days, 60 days) we collected 10 mL from each vial and mixed them in a 60 mL brown plastic bottle (Nalgene 2106-0002) to create a pooled sample. These pooled samples were stored at 4°C until measurement of optical properties, within one week of time point. The remaining 20 mL in each replicate vial was acidified with 0.2 mL of 2M HCl, shaken to mix, and held at room temperature until measurement of DOC concentration at the end of the experiment.

4.2.3 Laboratory measurements

Absorbance and fluorescence were measured using a procedure similar to that described in Osburn et al. (2016). Briefly, absorbance spectra were measured on a Varian Cary 300UV spectrophotometer in 1 cm quartz cells, with dilution as necessary to ensure absorbance at 240 nm was below 0.4, and using ultrapure deionized water as a blank. Fluorescence excitation-emission matrices (EEMs) were measured on a Varian Eclipse fluorometer using the same dilution factor as in absorbance measurements. Excitation was measured from 240 to 450 nm at 5 nm intervals and emission was measured from 300 to 600 nm at 2 nm increments, with a scanning speed of 2400 nm min\(^{-1}\) and an integration time of 0.05 s.

We measured DOC concentrations in replicate samples as non-purgeable organic carbon using a TOC analyzer (Shimadzu TOC-VCPH).
4.2.4 Statistical methods

Processing of absorbance data, EEMs, and PARAFAC modeling were conducted in MATLAB (The Mathworks Inc., 2016) using the DOMFluor toolbox (Stedmon and Bro, 2008). All other statistical analyses were conducted in the R environment (R Development Core Team, 2012).

We calculated six indices of OM composition based on optical properties of samples: SUVA$_{254}$, spectral slope ratio ($S_R$), humification index (HIX), fluorescence index (FI), biological index (BIX), and freshness index ($\beta/\alpha$; see Table 4.1 for definitions and citations).

After normalizing EEMs by total sample fluorescence, we fit a PARAFAC model to the 225 EEMs (three time points for each of 75 samples) as described by Stedmon and Bro (2008). We validated the model by conducting split-half validation and random initialization tests and visually analyzing residuals.

We used one-way t-tests to determine whether changes in DOC concentration and optical indices observed over the course of the experiment were statistically different from zero and a two-way t-test to compare proportional loss of DOC to proportional loss of total fluorescence over the course of the experiment. Analysis of variance (ANOVA) was used to assess differences in responses among sample groups (i.e. primary source leachates, EH leachates, EH flow, and stream flow) with Tukey’s honest significant difference test used to determine pairwise differences post hoc. We used simple linear regression to determine pairwise relationships between changes in optical properties vs. DOC loss during incubation. Assumptions of linear models and t-tests were validated by visual examination of residual and Q-Q plots.
We conducted a principal component analysis (PCA) with the values of indices and [DOC]-related contribution of each of the PARAFAC components using the vegan package in R (Oksanen et al., 2017). We determined the areas of convex hulls around the population of samples for each time point and the magnitude and angle of vectors connecting pairs of time points for each sample (0 to 6 days, 6 to 60 days, 0 to 60 days) in the ordination space. We characterized differences in the vectors connecting sample time points among groups with ANOVA.

4.3 Results

We observed considerable loss of DOC over the course of the incubation (Figure 4.1-A); an average of 11.3% of DOC was lost over the first six days, a proportional loss significantly greater than zero ($p = 0.0036$). In nine of 75 samples, we measured increases in DOC during this first time step. By 60 days, more than half of the DOC had been mineralized on average (51.4%, $p < 0.0001$).

We also measured a loss of total fluorescence during this dark incubation experiment (Figure 4.1-B), with samples losing 14.4% of total fluorescence in the first six days ($p < 0.0001$) and 31.2% by day 60 ($p < 0.0001$). The relative proportions of DOC and total fluorescence loss did not differ over the first six days of the experiment ($p = 0.50$), but a significantly greater proportion of DOC than total fluorescence was lost over the full 60 days of incubation ($p < 0.0001$).

4.3.1 Optical indices

Over the course of the incubation, we observed changes in one of the two absorbance-based indices of OM composition and in all four of the fluorescence-based indices
Figure 4.1: Dissolved organic carbon (A) and total fluorescence (B) in samples over the course of incubation. The proportional loss of bulk DOC is 11.3% between 0 and 6 days (significantly different from 0; p = 0.0036) and 51.5% between 0 and 60 days (p < 0.0001). The proportional loss of total fluorescence is 14.4% in the first six days (p < 0.0001) and 31.2% in 60 days (p < 0.0001). The proportional losses of DOC and total fluorescence do not differ in the first six days (p = 0.50), but after 60 days, a significantly greater proportion of DOC has been lost than total fluorescence in these dark-incubated samples (p < 0.0001).

We measured (Table 4.2, Figure 4.2-A). We also observed significant relationships between initial index values and DOC loss (Figure 4.2-B through D) as well as their magnitude of change and DOC loss. For these calculations, we omitted two outliers in which we measured substantial DOC gains.

**SUVA**

SUVA$_{254}$ is a measure of a sample’s absorbance of light of 254 nm wavelength normalized by DOC concentration, and is used as an indicator of the overall aromaticity.
Table 4.2: Mean initial and final values of optical indices of OM composition (defined in Table 4.1) for population of experimental leachates and water samples.

<table>
<thead>
<tr>
<th>Index</th>
<th>Mean (± sd) in initial samples</th>
<th>Mean (± sd) in samples after 60 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific UV absorbance (SUVA$_{254}$)</td>
<td>5.69 (± 1.67)</td>
<td>8.50 (± 3.20)</td>
</tr>
<tr>
<td>Slope ratio (S$_R$)</td>
<td>0.96 (± 0.23)</td>
<td>1.01 (± 0.24)</td>
</tr>
<tr>
<td>Humification index (HIX)</td>
<td>3.99 (± 2.44)</td>
<td>8.31 (± 2.49)</td>
</tr>
<tr>
<td>Fluorescence index (FI)</td>
<td>1.45 (± 0.24)</td>
<td>1.36 (± 0.13)</td>
</tr>
<tr>
<td>Freshness Index (β/α)</td>
<td>0.48 (± 0.13)</td>
<td>0.55 (± 0.09)</td>
</tr>
<tr>
<td>Biological index (BIX)</td>
<td>0.49 (± 0.13)</td>
<td>0.57 (± 0.09)</td>
</tr>
</tbody>
</table>

of the bulk DOM pool (Weishaar et al., 2003). Among samples, we measured an initial SUVA$_{254}$ of 5.69 ± 1.67 L m$^{-1}$ mg$^{-1}$. SUVA$_{254}$ increased significantly both over the first six days of incubation (by a mean of 1.17 L m$^{-1}$ mg$^{-1}$, p = 0.02) and over the full 60 days (by a mean of 2.84 L m$^{-1}$ mg$^{-1}$, p <0.0001).

The initial value of SUVA$_{254}$ was not a significant predictor of either the DOC mass loss (6 day p = 0.095, 60 day p = 0.46) or proportional DOC loss over the whole experiment (p = 0.12), but was negatively related to proportional DOC loss over the first 6 days (p = 0.042). In contrast, while the change in SUVA$_{254}$ (ΔSUVA$_{254}$) was not significantly correlated with DOC mass loss or proportional loss over the first six days of the experiment (p = 0.13 and 0.49, respectively), we did observe a significant positive correlation between ΔSUVA$_{254}$ and overall DOC mass loss (p = 0.025) but not proportional DOC loss (p = 0.12).
Figure 4.2: Values of optical indices in samples over the course of incubation. Definitions and background information given in 4.1; mean initial and final values given in 4.2. Stars (*) indicate significant changes in the index over incubation (i.e. value of the index measured at 0 days vs. 60 day is significantly different at $p < 0.05$).

**Slope ratio**

A sample’s slope ratio ($S_R$) is a ratio of an exponential best fit slope of its absorbance spectrum between 275 nm and 295 nm to the slope between 350 and 400 nm. $S_R$ is frequently used as an indicator of the average molecular weight of compounds in the bulk DOM pool (Helms et al., 2008). The mean initial $S_R$ in our samples was 0.96
± 0.23. Neither six day nor 60 day incubations produced significant changes in the mean value of $S_R$ ($p = 0.74$ and 0.16, respectively).

The initial value of $S_R$ was positively correlated with DOC mass loss ($p = 0.048$) and proportional DOC loss ($p = 0.047$) over the first six days of incubation. Over the whole experiment, initial $S_R$ was not a significant predictor of the DOC mass loss ($p = 0.91$) but was positively correlated with proportional DOC loss ($p = 0.0092$). In contrast, the $\Delta S_R$ was negatively correlated with the proportion of DOC lost both over the first six days and the entire incubation ($p = 0.005$ and 0.031, respectively), but not with the 6 day or the 60 day DOC mass loss ($p = 0.93$ and 0.77, respectively). Samples in which the $S_R$ decreased over the 60 day incubation lost a higher proportion of their initial DOC than samples in which $S_R$ increased ($p = 0.033$).

**Humification index**

The humification index (HIX) is a fluorescence-based measure calculated by dividing the area under a sample’s emission curve from 435-480 nm by the area under the curve between 300-345 nm, given an excitation wavelength of 254 nm. This index takes on higher values when fluorescence is red-shifted, consistent with greater humification of DOM (Zsolnay et al., 1999). We measured a mean initial HIX of 4.06 ± 2.48 for our samples, and the mean value of this index increased significantly over the first six days of incubation by 0.83 ($p < 0.0001$). Over the whole 60 days of the experiment, the total increase in HIX averaged 4.27 ($p < 0.0001$).

The initial value of HIX was positively correlated with the proportional DOC loss over the first six days of incubation ($p = 0.00084$) but not with DOC mass loss over this time period ($p = 0.85$). In contrast, initial HIX value was negatively
correlated with DOC mass loss over 60 days (p < 0.0001, Figure 4.2-C), but not with proportional DOC loss over this time period (p = 0.089). The change in HIX (ΔHIX) over the first 6 days of incubation was not significantly related to DOC mass loss or proportional DOC loss (p = 0.35 and 0.17, respectively), nor was ΔHIX correlated with the proportional DOC loss over the whole incubation (p = 0.25). Over the whole 60 day experiment, ΔHIX was positively related to the total mass of DOC lost (p = 0.032).

*Fluorescence index*

A sample's fluorescence index (FI) may be used to distinguish broad categories of DOM sources, with terrestrial sources (vegetation and soil) taking lower values and microbial (algal and bacterial exudates) taking higher values (McKnight et al., 2001). FI is calculated using the emission spectra for samples excited at a wavelength of 370 nm, as the ratio of emission at the wavelength of the population of samples’ maximum emission to emission at wavelength where microbially-derived samples’ emission is approximately half of the maximum emission (for consistency with other studies, we used emission wavelengths of 470 nm and 520 nm). The mean value of FI was 1.45 ± 0.24, with a statistically significant decrease of 0.039 over the first six days of incubation (p = 0.004) and 0.090 over the entire 60 day incubation (p = 0.001).

We found a positive correlation between the initial FI and the mass of DOC lost over the whole 60 days (p < 0.0001) as well as a nearly significant negative correlation with and the proportional loss of DOC over the first six days (p = 0.055) and the whole incubation (p = 0.056). The initial value of FI was not correlated with the
initial mass of DOC lost (p = 0.50). The change in FI (∆FI) was not significantly correlated with proportional DOC loss (p = 0.075) or mass loss (p = 0.67) over the first six days of incubation nor with proportional DOC loss over the entire 60 days. DOC mass loss and ∆FI were negatively correlated over the 60 days (p = 0.029).

**Freshness index**

Freshness index (β/α) has been cited as an indicator of recently synthesized DOM, taking lower values for more heavily processed DOM and higher values for more recently synthesized DOM (Parlanti et al., 2000). Its value is the ratio of emission at 380 nm to the maximum emission between 420-435 nm, given an excitation of 310 nm. In our samples, the initial β/α was 0.48 ± 0.13. The freshness index increased significantly by 0.061 in the first six days of incubation (p <0.0001) and slightly more over the entire 60 days (by 0.076, p <0.0001).

The initial value of β/α was not a significant predictor of mass loss of DOC over the first six days of incubation (p = 0.98) nor of the proportional loss of DOC over the whole 60 day incubation (p = 0.09), but initial β/α was positively correlated with proportional DOC loss over the first 6 days (p = 0.00045) and negatively correlated with the overall mass loss of DOC (p <0.0001, Figure 4.2-B). The change in β/α (∆(β/α)) did not show a significant relationship with proportional DOC loss (6 day p = 0.37, 60 day p = 0.074) or with mass loss over the entire 60 days (p = 0.11). However, ∆(β/α) over the first 6 days was positively correlated with mass loss over this time period (p = 0.015).
**Biological index**

The index of recent autochthonous contribution or biological index (BIX) is a slight modification of the freshness index that is determined by taking the ratio of emission at 380 nm to 430 nm for an excitation of 310 nm. Similar to $\beta/\alpha$, it is used to indicate recent production of DOM, particularly from autochthonous sources, taking low values for samples with low autochthonous productivity and higher values for samples from locations of high autochthonous DOM production (Huguet et al., 2009). Given the great similarity between BIX and $\beta/\alpha$, it is unsurprising that the patterns we observe in BIX are consistent with those seen in $\beta/\alpha$: The mean initial BIX was $0.49 \pm 0.13$ in our samples and the value increased significantly by a mean of 0.063 over the first six days of incubation ($p < 0.0001$) and by 0.078 over the whole 60 days ($p < 0.0001$).

Initial BIX value was positively correlated with the proportion of DOC loss over the first six days of incubation ($p = 0.00069$), but not with DOC mass loss over this time period ($p = 0.94$). In contrast, the initial value of BIX was not a significant predictor of proportional DOC loss over 60 days ($p = 0.080$), but initial BIX was significantly negatively correlated with the overall mass loss of DOC ($p < 0.0001$, Figure 4.2-D). The change in BIX ($\Delta$BIX) was not significantly correlated with proportional DOC loss (6 day $p = 0.29$, 60 day $p = 0.062$) or with DOC mass loss over 60 days ($p = 0.071$). Over the first six days of incubation, $\Delta$BIX was positively correlated with DOC mass loss ($p = 0.014$).
4.3.2 EEM-PARAFAC analysis

Given three time points for each of 75 samples, we measured 225 EEMs in total. Visual inspection of these EEMs show, across sample groups, the decrease in a peak centered around Ex: 275 nm/Em: 310 nm (Figure 4.3). We used these EEMs to build and verify a three component PARAFAC model (Figure 4.4). Component 1 (C1; Figure 4.4 - left) resembles a humic-like peak with Ex: <240 nm/Em: 410-430 nm. Component 2 (C2; Figure 4.4 - middle) is humic-like component with a primary peak at Ex: <240 nm/Em: 445-460 nm and secondary peak at Ex: 350 nm/Em: 445-460 nm. Component 3 (C3; Figure 4.4 - right) comprises a protein-like peak, designated peak B by Coble (1996), which we observe decline in our samples over the course of incubation (Figure 4.3-A through D).

To analyze the changes in components’ relative importance during incubation, we divided the maximum fluorescence of each component in a sample by that samples’ DOC concentration; all changes in PARAFAC components refer to changes in [DOC]-relativized fluorescence. During the first six days of incubation, only C1 exhibited a significant change, increasing in relative importance (p = 0.039). Over the entire 60 day experiment, however, the relative contribution of all the components changed significantly: Both C1 and C2 increased (p <0.0001 and p = 0.001, respectively) while C3 decreased (p=0.0001).

Like our analyses of individual optical indices, we assessed whether differences in the initial values of components’ contributions or their changes over incubation were correlated with proportional DOC loss. The initial contribution of C1 was not significantly correlated with proportional loss of DOC over six days (p = 0.57)
Figure 4.3: Examples of changes to EEMs during incubation and the three PARAFAC components fit to the samples in the bioavailability incubation experiment. In leach leachate (row A), leachate of OM accumulated in roof gutters (row B), road runoff (row C), and stream stormflow (row D) the intensity of a protein-like peak decreases over the course of the experiment while humic-like peaks show little change.
Figure 4.4: Fluorescent components of DOM in the bioavailability experiment. We fit and verified a three component PARAFAC model to 225 EEMs (three time points for each of 75 samples).

or 60 days (p = 0.22). In contrast, the initial contribution of C2 showed negative correlations with proportional DOC loss over both incubation time periods (6 day p = 0.0091; 60 day p = 0.028), but explained little variation in the DOC loss data (R² = 0.088 and 0.059, respectively). The initial contribution of C3 was not a significant predictor of DOC loss over the six day incubation (p = 0.11) but was negatively correlated with proportional DOC loss over 60 days, although again, explained little of the variance (p = 0.013, R² = 0.078).

The change in [DOC]-relativized contribution of C1 (∆C1) was positively correlated with the proportional DOC loss over the first six days (p = 0.00066, R² = 0.15) and over the whole 60 days of incubation (p < 0.0001, R² = 0.31). A positive correlation between ∆C2 and proportional DOC loss over the six day incubation was nearly significant (p = 0.063) and these were significantly positively correlated after 60 days of incubation (p = 0.0026, R² = 0.12). Over the first six days, the ∆C3 was positively correlated to DOC loss (p = 0.028, R² = 0.059) but this relationship broke down after 60 days of incubation (p = 0.23).
4.3.3 Differences among sample groups

We compared patterns of DOC loss, optical DOM composition indices, and PARAFAC components among sample groups (Figure 4.5).

Differences in initial values

We began by assessing differences in the initial values of indices and PARAFAC components among sample groups (Figure 4.5-BC, dark bars). While the initial value of SUVA$_{254}$ did not differ among sample groups ($p = 0.17$), the initial value $S_R$ was significantly lower in primary source leachates than in the other sample groups ($p = 0.00085$). Initial values of the fluorescence-based indices (HIX, FI, $\beta/\alpha$, BIX) did not differ among sample groups ($p = 0.080, 1.0, 0.32, \text{ and } 0.39$, respectively). Of the PARAFAC components we identified, only C1 differed among sample groups at the beginning of the experiment ($p < 0.0001$), with values in the leachates (from primary sources and OM accumulated in engineered headwaters) having a lower contribution of C1 than stormwater flow in engineered headwaters or stream channels. The initial values of C2 and C3 did not differ among groups ($p = 0.26 \text{ and } 0.93$, respectively).

Differences in changes during incubation

We then compared how changes in DOC, optical indices of DOM composition, and PARAFAC components contributions over the course of the lability experiment differed among sample groups. In the first six days of the incubation, the proportional change in DOC differed between primary source leachates and stream flow samples (Figure 4.5-A, $p = 0.011$), with primary source leachate sample gaining a mean of 19% of their original DOC concentration while stream stormflow samples lost an
average of 22% of their original DOC. These early differences among groups proportional DOC loss disappeared by the 60th day ($p = 0.19$).
While we did not observe differences in SUVA$_{254}$ on day 0 (above) or day 6 ($p = 0.71$), by day 60, SUVA$_{254}$ was significantly lower in stream flow samples than in leachate or flow from engineered headwaters ($p = 0.0051$ and 0.00088, respectively), both of which showed greater $\Delta$SUVA$_{254}$ than the other groups ($p = 0.011$ and 0.0078, respectively; Figure 4.5-C). $S_R$ showed an opposite pattern, with differences in the initial value of $S_R$ among groups (above) but no differences by day 60 ($p = 0.67$) owing to a larger $\Delta S_R$ in primary leachate samples than other groups ($p = 0.011$; Figure 4.5-C).

The behavior of HIX over the course of the incubation also differed among groups. While the initial value of HIX did not differ among groups (above), after 60 days HIX was significantly lower in primary source leachates than in stream flow ($p = 0.028$) or EH leachates ($p = 0.048$), and $\Delta$HIX was of greater magnitude in EH leachates than in the other sample groups ($p = 0.00087$; Figure 4.5-C). Neither the 60 day values of FI nor $\Delta$FI differed among groups ($p = 0.64$ and 0.95, respectively; Figure 4.5-C). For both $\beta/\alpha$ and BIX, differences among groups developed over the 60 days of the incubation; for both of these indices, values at 60 days differed between EH leachates and stream flow ($\beta/\alpha$ and BIX $p$ both $<0.0001$), between EH leachates and EH flow ($\beta/\alpha$ $p = 0.00077$, BIX $p = 0.00088$), and between source leachates and stream flow ($\beta/\alpha$ $p = 0.031$, BIX $p = 0.015$). Despite these differences, $\Delta(\beta/\alpha)$ did not differ significantly among groups in over the 60 days of incubation ($p = 0.051$), and while $\Delta$BIX did differ significantly among groups ($p = 0.040$), no pairwise comparisons among groups showed significant differences.

The initial differences in the contribution of C1 observed among groups (above) were retained throughout the incubation (Figure 4.5-B), and $\Delta$C1 was significantly
greater in flow samples (EH flow and stream flow) than in leachates (primary source and EH leachates). We observed transient differences in the contribution of C2 among groups although C2 did not differ among groups at 0 days (above) or 60 days (p = 0.42). After 6 days of incubation, the contribution of C2 in primary source leachates was significantly lower than in EH flow (p = 0.0025). The ∆C2 did not differ among groups (p = 0.31). The contribution of C3 exhibited a similar pattern with a higher contribution of C3 in primary source leachates as compared to flow through engineered headwaters on day 6 (p = 0.020), but no differences among groups by day 60 (p = 0.47). We did not observe differences in ∆C3 among groups (p = 0.77).

4.3.4 Principal component analysis

We conducted a principal component analysis (PCA) on the optical indices of DOM composition and [DOC]-relativized PARAFAC components for all sample time points (Figure 4.6-A). Together, the first two principal components (PCs 1 and 2) described 61.01% of the variance in the data. PC 1 explains 42.05% of the variance and has strong positive loading with C3 and strong negative loadings with C1, BIX, and $\beta/\alpha$, suggesting that scores on PC 1 are associated with DOM freshness. PC 2 explains 18.96% of variance in the data, and shows strong positive loadings with $S_R$ and C2, suggesting that scores on PC 2 are associated with conjugation/humification and molecular weight.

Within the ordination space, we connected sample time points with vectors (Figure 4.6-B and C) to analyze how samples’ DOM composition changed over the course of incubation. Vectors connecting samples from day 0 to 6 (Figure 4.6-B) tended to
be relatively small in magnitude (with the exception of two primary source leachates: grass and green leaves) and had a relatively wide distribution of angles with a mean of 272° (Figure 4.7-A). Between day 6 and 60, the magnitude of vectors is nearly significantly greater (p = 0.064) and the distribution of angles is narrower with a mean of 308° (Figure 4.7-B), aligning with the general direction of increasing contribution of C2 (Figure 4.6-A). Finally, the vectors for samples overall changes (i.e. connecting 0 to 60 days) also show a narrow distribution with a mean of 296° (Figure 4.7-C).

We also compared vector characteristics among sample groups and related them to DOC loss over incubation. Over the first six days, vector magnitude for source leachates was greater than for EH leachates (p = 0.017) and EH flow (p = 0.010), while between 6 and 60 days, source leachates had a greater vector magnitude than stream flow (p = 0.035). The magnitudes of vectors connecting samples at day 0 to day 60 did not differ among groups (p = 0.62). The mean of vector angles did not differ among sample groups for any of the pairs of time points (0-6 day p = 0.186, 6-60 day p = 0.99, 0-60 day p = 0.73).

For the whole population of samples, magnitude of the 0 to 60 day vector was positively correlated with proportional DOC loss over the incubation (p = 0.030; R^2 = 0.062), while vector angle was negatively correlated with the proportion of DOC lost and explained a similarly small amount of variation in DOC loss (p = 0.039, R^2 = 0.054). We observed the opposite pattern over the first six days of the experiment, in which proportional DOC loss was negatively correlated with vector magnitude (p = 0.018, R^2 = 0.078) and positively correlated with vector angle (p = 0.0015, R^2 = 0.14). When comparing vector angle and magnitude to proportional DOC loss, we omitted two outliers that gained considerable DOC.
Finally, we compared the areas occupied by convex hulls in the ordination space for each time point (Figure 4.8) to determine whether the population of samples became more homogenous (i.e. convex hull area decreased) over the course of incubation. Between 0 and 6 days, the area of the convex hull increased, but by 60 days the area was smaller than that occupied by the 0 day population of samples.

4.4 Discussion

This experiment demonstrates that the DOM present in urban headwaters is highly bioavailable. In samples from stream stormflow, a mean of 22% of DOC was mineralized in less than a week and more than half of the DOC was gone after two months (Figure 4.5-A). Our value of the initial lability of DOC in streamflow is similar to the measures of bioavailable DOC for riverine samples incubated for similar durations (Søndergaard and Middelboe 1995; Volk and Kaplan 1997).

In general, the changes we observed in indicators of DOM composition over the course of incubation correspond with predictions that arise from the continuum-based conceptual models of riverine DOC bioavailability. Specifically, we observe significant increases in the values of HIX and of SUVA\textsubscript{254}, which is consistent with the removal of aliphatic and less humic compounds in the DOM pool, leaving behind more humic, aromatic DOM. This observation is also consistent with the negative relationship between DOC loss and initial HIX that suggests a more humic DOM pool is less bioavailable to the microbial community. Similar trends in HIX and SUVA\textsubscript{254} were observed by Hansen et al. (2016) in their study of biologically- and photochemically-induced changes in indicators of DOM composition. We also observe increases in BIX and $\beta/\alpha$ over the course of incubation, consistent with the generation of fresh
DOM by aquatic microbes present in the inoculant.

The significant loss of total fluorescence during this dark incubation (Figure 4.1-B) was unexpected, as other work has shown that loss of fDOM tends to be driven mainly by photochemical reactions as opposed to biodegradation (Moran et al., 2000; Chen and Jaffé, 2014). However, the labile DOM present in urban stormwater may condition DOM and facilitate microbial breakdown of fluorescent molecules that may otherwise be recalcitrant to biodegradation (Guenet et al., 2010).

4.4.1 Implications for urban streams and receiving ecosystems

The engineered flowpaths from which we collected stormwater are the headwaters of an urban stream continuum (Kaushal and Belt, 2012) and are capable of storing significant quantities of OM in locations with conditions that facilitate leaching of DOM (Chapter 2). These engineered headwaters therefore act as sources of fresh DOM that has the potential to be processed in place or mobilized to downstream ecosystems during storms. During the course of our study, we rarely observed periods of longer than a week without rain storms of sufficient magnitude to connect engineered headwaters to the stream and mobilize this OM. This suggests that little of the DOM generated in urban headwaters remains in the system long enough for the majority of bioavailable material to be mineralized. Instead, a significant quantity of readily bioreactive DOM is likely transported to downstream ecosystems, as suggested by the pulse-shunt concept (Raymond et al., 2016).

In the case of this system (like many human-dominated catchments), the downstream ecosystem that receives this urban stormwater is a multi-use impoundment. Large pulses of labile DOM and nutrients in urban stormwater can have a variety
of impacts on the ecology and usability of the receiving reservoir, including promoting algal blooms and hypoxia (Carpenter and Caraco, 1998), and interfering with drinking water treatment (Leenheer, 2004; Chow et al., 2007). Changes in storm frequency and intensity in combination with alteration of land cover, particularly impervious surface cover, will likely influence the frequency and severity of these pulsed stormwater disturbances in reservoirs.

4.4.2 Processing conditions and homogenizes the DOM pool

In contrast to the patterns predicted by the RCC and by the size-reactivity continuum model, we did not observe a longitudinal decrease in biolability in our urban stream continuum. In fact, early in the incubation we see the opposite pattern, with greater loss of DOC from streamflow samples (the downstream end of our continuum) as compared to primary source leachates (the upper end of our continuum).

The two continuum models make specific and opposing predictions with regard to differences in average molecular size that should result from DOM processing. Specifically, the RCC predicts that smaller DOM molecules ought to be more labile and therefore removed toward the top of the continuum, leaving larger molecules to be advected downstream, while the size-reactivity continuum model suggests that smaller molecules are those leftover after processing of more bioavailable, larger DOM molecules. In our study, we did not observe any significant trends in average molecular weight (as indicated by $S_R$) over the course of incubations. However, we did observe greater proportional DOC loss in samples where $S_R$ decreased as opposed to increased, suggesting that in these samples a DOC pool with larger average molecular size was left behind.
Like many other studies in which DOM composition is analyzed, we used a multivariate approach to summarize the metrics. We characterized the movement of samples through ordination space during incubation, allowing us to detect general trends in composition change during the experiment. Initially, we observe a wide range of sample trajectories (a wide distribution of vector angles, with significant differences in magnitudes among sample groups; Figure 4.7). We also saw an increase in the overall area of ordination space occupied by leachate samples between 0 and 6 days (Figure 4.8). Combined with the observation that leachate DOM did not exhibit significant mass loss over this initial stage of the incubation, we interpret our results to suggest that DOM leached from particulate OM undergoes a conditioning stage in which significant biochemical rearrangement but little mineralization occurs. Later in the incubation, trajectories of DOM incubation become more similar and the area occupied by samples in multivariate space is smaller, suggesting homogenization of the DOM pool as processing proceeds.

We have shown that DOM in urban headwaters is reasonably labile after leachates of CPOM collected in urban infrastructure undergo a conditioning phase. While we do not consider the role of light in conditioning and oxidizing urban DOM in this experiment, further research could determine the relative importance of bio- vs. photodegradation of DOM in urban streams, particularly at the lotic-lentic transition where urban streams drain to reservoirs, where residence times and light exposure are greater, and eutrophication may cause negative priming.
Figure 4.6: Principal component analysis using optical quality indices (Table 4.1) and [DOC]-relativized PARAFAC components (A). Trajectories through ordination space between 0 and 6 days (B) show a wider range of directions than between 6 and 60 days (C). Ultimately, samples tend to move toward the upper left of the space (C), corresponding with an increase in the value of the humification index (HIX) and a decrease in the contribution of PARAFAC component 3 (C3).
Figure 4.7: Magnitudes and angles of vectors connecting sample time points in two dimensional ordination space. The distribution of vector angles connecting time points 0 days and 6 days (A) is wider than the distribution of vectors connecting time points 6 days and 60 days (B). The vectors connecting the 0 day and 60 day time points (C, i.e. the sum of the two vectors shown in Figure 4.6) have a relatively narrow distribution of angles.
Figure 4.8: Convex hulls in ordination space for the population of samples at each time point. Areas of the convex hulls for the entire population and for each sample group shown at right.
5.1 Introduction

5.1.1 Browning of surface waters

Concentrations of dissolved organic carbon (DOC) in the surface waters have undergone dramatic and widespread increases over the last three decades (Evans et al., 2005; Monteith et al., 2007; Roulet and Moore, 2006). These trends of increasing DOC concentration are best documented in catchments with highly organic soils (Worrall et al., 2004; Hruska et al., 2009) in far northern latitudes, including Scandinavia, the United Kingdom, and northern North America (Evans et al., 2005; Monteith et al., 2007; Roulet and Moore, 2006). This phenomenon has been termed ‘browning’ (or ‘brownification’) because of the brown color that terrestrial DOC can imbue on water with high concentrations (Figure 5.1).
Figure 5.1: Colored DOM absorbs light and gives water a brown color, from which the term “browning” (i.e. long-term increases in DOM concentration) is derived.

DOC is often the largest carbon (C) pool in lakes and the dominant C flux in rivers. Increased C export from terrestrial ecosystems as a part of browning could therefore cause ecosystems such as peatlands and arctic tundra, which currently act as carbon sinks, to become net carbon sources to the atmosphere (Worrall et al., 2004; Hruska et al., 2009; Randerson et al., 2002; Cole et al., 2007). As a part of global change, browning has implications not only for regional and global carbon balances but also for water quality (U.S. EPA, 2003) and for coupled nutrient cycles (Sterner and Elser, 2002; Fork and Heffernan, 2014). Because DOC is comprised of a diverse array of molecules, it influences the physical properties of aquatic ecosystems (visible light, UV penetration, temperature), and modulates many aquatic ecosystem functions (Prairie, 2008; Solomon et al., 2015). Disinfection treatment of DOC-rich water for human drinking water supplies can create by-products that are human health concerns (Chow et al., 2007; Leenheer, 2004; US EPA, 2012) under the Safe Drinking Water Act. Because of the multi-faceted consequences of changes in DOC concentrations, a better understanding of their patterns and causes is critically important.
5.1.2 Causes of browning

Site-specific causes of rising DOC may not always be clear, but many studies implicate drivers that act on large spatial scales, such as changes to climate (Evans et al., 2005; Freeman et al., 2001), atmospheric CO$_2$ concentration (Freeman et al., 2004), and recovery from atmospheric acid deposition (Monteith et al., 2007; Hruska et al., 2009). In very high latitudes, melting of permafrost driven by warming of the global climate also exposes ancient organic C, allowing it to be dissolved and mobilized into surface waters (Abbott et al., 2014).

At more local scales, changes in watershed chemistry (Zech et al., 1994; Pregitzer et al., 2013), including increasing sodicity (the relative contribution of sodium ions to total base cations) resulting from irrigation, (Aitkenhead-Peterson et al., 2009; Steele and Aitkenhead-Peterson, 2012), have been linked to increased soil solution DOC. Land use can also affect the quantity and mobility of DOC in soil solution (EPA, 2012), and therefore concentrations of DOC in streams (Khomutova et al., 2000; Chantigny, 2003; Dawson and Smith, 2007).

While browning has been well-documented in high latitudes, trajectories of DOC concentrations in temperate and more southern aquatic ecosystems (such as those in most of the continental United States) remain largely unreported. Some drivers likely to be important in high latitudes will have little importance throughout much of the U.S. (e.g. melting of permafrost). Instead, changes in land use and land cover, which can alter both the sources and transport of DOC to streams and rivers, may be more important in more populated southern catchments.

In this chapter, we investigate relationships between land cover and DOC in rivers
throughout the contiguous U.S. We characterize differences in mean riverine DOC concentrations among regions, and ask how they are related to urban development, tree canopy cover, and wetland cover of catchments. We also assess the degree to which changes in land cover correlate with changes in DOC concentration and load and characterize regional differences in DOC trends.

5.2 Methods

5.2.1 Data collection

We used the National Water Information System (U.S. Geological Survey, 2016a) of the United States Geologic Survey (USGS) to collect data on streamflow and organic carbon concentration. We downloaded data from all available sites with at least 40 records of TOC or DOC concentration (USGS parameter codes 00680 and 00681, respectively) and concurrent measurements of daily mean streamflow between 1999-12-31 and 2011-01-01. In addition, we compiled similar streamflow and organic matter data made available by the Long Term Ecological Research (LTER) Network. After omitting any sites with a gap in the data of more than 18 continuous months, the dataset included 167 stream sampling sites. Some sites in the data set were situated longitudinally within the same river network. While most of the sites used in this study were located in the eastern half of the U.S. (Figure 5.3), data come from sites located throughout the U.S. in 28 of 48 states as well as the District of Columbia.

We used data from the USGS 3D Elevation Program in order to delineate the catchments for each sampling point in our dataset using ArcGIS 10.4. We then used these catchment shapefiles to determine the land cover in each catchment using spa-
tial data from the National Land Cover Database (NLCD; Homer et al., 2015). We used these NLCD data to determine the %Development, %Wetlands, %Impe-

rvious surface cover (ISC), and %Tree canopy cover for each delineated catchment in 2001 and in 2011, and determined decade-scale change in each land cover type by difference.

5.2.2 Data analysis

For each site, we calculated instantaneous DOC loads by multiplying TOC or DOC concentration by the corresponding day’s mean streamflow. We then tested for trends in DOC concentration and load over the ten year period from 2000-2010 with the Mann-Kendall test using the R package ‘openair’ (Carslaw and Ropkins, 2012). The Mann-Kendall test is a non-parametric test for monotonic trends in data (Helsel and Hirsch, 2002) that has been used in other analyses of long-term changes in DOC concentration (e.g. Monteith et al., 2007). We used the Sen estimate of slope to infer the direction and magnitude of trends in DOC concentration and load.

We used simple linear regression to assess the relationship between DOC concentration and river discharge, and multiple linear regression to relate mean DOC concentration of trends DOC concentration and load between 2000-2010 to hypothe-

sized predictors. Predictors and response variables were log-transformed as necessary to ensure the data met assumptions of linear regression analysis. The initial model for mean DOC concentration included 2-digit HUC, catchment area, and 2001 land cover (%Development, %Wetlands, %ISC, and %Canopy cover), and an interaction term between %ISC and %Canopy cover as predictors. The initial models for trends in concentration and load included 2-digit HUC (categorical), mean [DOC], catch-
ment area, the difference in each land cover predictor between 2001 and 2011, and an interaction term between ∆%ISC and ∆%Canopy cover as predictors. We then serially removed insignificant predictors from the models to obtain a final model.

5.3 Results

5.3.1 DOC concentrations in U.S. Rivers

For the time period between 2000 and 2010, the grand mean DOC concentration in our population of rivers was 5.42 mg L\(^{-1}\) (± 5.54 standard deviation). Mean DOC concentration ranged among rivers from a minimum 0.43 mg L\(^{-1}\) in Icy Brook, a glacial stream in Rocky Mountain National Park, Colorado, to 54.5 mg L\(^{-1}\) in the Suwannee River, a blackwater river in southern Georgia.

The final linear regression model for mean DOC concentration retained HUC2, %ISC, %Canopy cover, and the interaction between %ISC and %Canopy cover as predictors (Table 5.1) and explained nearly 40% of the variation in mean DOC concentration among rivers. The relationship between %ISC and mean DOC concentration was negative, while the relationship between %Canopy cover was positive. The coefficient for the interaction term between these two parameters was positive; at low values of %Canopy cover, DOC concentration and %ISC were negatively correlated, but at high %Canopy cover, %ISC and DOC concentration have a positive relationship (Figure 5.2). In contrast to other studies (Hinton et al., 1998; Gergel et al., 1999; Dick et al., 2015), %Wetlands was not a significant predictor of mean DOC concentration in this population of rivers.
Table 5.1: Final multiple linear regression model for log(mean [DOC]) between 2000-2010 in rivers in the contiguous United States. N = 140; Adj R² = 0.397; p < 0.001. Italic indicates significant parameters at p < 0.05.

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5.3.2 Decade-scale trends in DOC concentration and load

We observed significant trends (Mann-Kendall test with p<0.05) in the DOC concentration for nearly half of the rivers included in this study (80 rivers out of 167; Figure 5.3). DOC increased between 2000 and 2010 in 33 rivers and decreased over this time period in 47 rivers. The relative magnitude of change was greater in rivers
Figure 5.2: Interaction effect of %ISC and %Canopy cover in predicting mean DOC concentration in rivers. Predictions are shown for mean DOC concentration in rivers in HUC 01 at different levels of ISC and tree canopy. At low %Canopy, the relationship between %ISC and DOC concentration is negative, but as %Canopy increases, the relationship between %ISC and DOC concentration becomes positive.

with significant increasing trend (mean increase = 3.35%) as compared to rivers in which DOC concentration decreased (mean decrease = 2.10%; p = 0.002; Figure 5.4). The majority of sites with significant changes in DOC concentration are located in the eastern U.S.

We also assessed trends in DOC load for sites with sufficient discharge data (124 sites) and observed significant trends in 39 sites. In contrast to concentration trends, the majority of rivers with significant trends showed increases in load (N = 29) while only 10 rivers exhibited decreases in instantaneous DOC loads (Figure 5.5).

We observed some correlations among predictors. Unsurprisingly, log(Developed) and log(ISC) were positively correlated. We observed a positive correlation between
log(\%Wetland cover) and log(\%Canopy cover). Log(catchment area) was negatively correlated with both log(\%Developed) and log(\%Wetland cover). These relationships differed between eastern and western catchments. For example, the relationships between \%Developed and \%Impervious and between \%Wetland cover and \%Canopy cover were much stronger in eastern vs. western catchments (Appendix C).

**Figure 5.3**: Map of trends in DOC concentration and load in the contiguous United States between 2000 and 2010

Land cover changed slightly in our study catchments between 2000-2010. Urban development increased by an average of 1.0% (± 1.8), and ranged from no change to an increase of 10.9% cover. Change in ISC was unsurprisingly highly correlated with
Figure 5.4: Histogram of percent change (relative to mean concentrations in 2000) in DOC concentration for sites with significant trends between 2000-2010. The relative magnitude of change is greater for sites that showed increases as opposed to decreases in DOC concentration ($p = 0.002$).

Figure 5.5: Histogram of percent change in instantaneous DOC load (relative to mean in 2000) for sites with significant trends between 2000-2010.

change in development; on average, ISC increased by 0.43% ($\pm 0.70$) and ranged from no change to an increase of 4.0%. Tree canopy cover decreased by an average of 20.5% ($\pm 15.7$), ranging from a decrease of 50.8% to an increase of 36.0%. Wetland cover
changed only slightly in most catchments between 2000 and 2010. The mean change in wetland cover was a slight decrease of 0.085% (± 0.21) and ranged from a decrease of 1.1% to an increase of 0.20%. The change in %Developed and %Impervious were positively correlated, but there were not generally strong correlations among changes in other predictor variables (Appendix C).

We found significant relationships between [DOC] and river discharge in 73 of 124 sites. The mean slope between [DOC] and discharge for sites with a significant relationship was positive (1.04, in log-log space), but there was considerable variability (standard deviation of DOC-Q slope = 1.54; range of slopes = [-1.63, 6.82]).

We used multiple linear regression to relate changes in land cover to the slope of the DOC concentration trend (for sites with significant trends in DOC concentration). The final model retained HUC2 as a categorical variable, as well as positive relationships with both mean DOC concentration and change in %Wetland cover (Table 5.2). The model explained 62% of the variation in DOC concentration trends.

The final model for the change in instantaneous load of DOC retained only HUC2 and catchment area as predictors (Table 5.3), but explained 83% of the variation in the change in instantaneous DOC load among rivers with significant trends. Trend in DOC load was positively related to catchment area.

5.4 Discussion

We observed regional differences in mean DOC concentration (among 2-digit HUCs), with riverine DOC concentrations tending to be lower in the western U.S. as compared to eastern regions. Greater ISC was related to lower mean DOC concentration, while higher levels of tree canopy were positively correlated with DOC concentration.
Table 5.2: Final multiple linear regression model for the change in DOC concentration between 2000-2010 in rivers with significant [DOC] trends. N = 56; Adj $R^2 = 0.617$; p $<0.001$. *Italics* indicate significant parameters at p$<0.05$.

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Table 5.3: Final multiple linear regression model for the change in instantaneous DOC load between 2000-2010 in rivers with significant trends in DOC load. N = 39; Adj $R^2 = 0.83$; p $<0.001$. *Italics* indicate significant parameters at p$<0.05$.

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<tr>
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<td>0.007</td>
</tr>
<tr>
<td>HUC 18</td>
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<td>0.7</td>
</tr>
<tr>
<td>log(Catchment area)</td>
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<td>30.4</td>
<td>$&lt;0.0001$</td>
</tr>
</tbody>
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In addition, a positive interaction effect between canopy and ISC indicates that the relationship between ISC and mean DOC concentration differs at different levels of tree canopy: among catchments with low canopy cover, ISC is negatively related to DOC concentration, but catchments with high canopy cover show the opposite pattern, tending to have greater mean DOC concentrations at greater %ISC. Chapters 2 and 3 and work by Janke et al. (2017), Bratt et al. (2017), and Hobbie et al. (2013) demonstrate that leaf litter on pavement can generate large loads of soluble OM, which may explain the mechanism of the interaction between ISC and tree canopy cover observed among U.S. rivers in this study.

We also observed significant trends in DOC concentration between 2000-2010 in many rivers throughout the contiguous U.S. In contrast to the consistent and widespread browning trend reported in far northern latitudes, we found more frequent decreasing [DOC] trends than increasing in this population of rivers. However, the magnitude of increases in [DOC] was greater than decreases.

A different set of parameters were related to trends in DOC concentration than mean DOC concentration; the change in wetland cover had a large positive slope, indicating that rivers whose catchments lost wetland cover between 2000 and 2010 exhibited considerable decreases in DOC concentration. In addition, mean DOC concentrations were positively related to the decade-scale change in DOC concentration, which is consistent with the fact that browning tends to be observed mainly in organic carbon-rich catchments.

Significant trends in the instantaneous load of DOC were present in a minority of the rivers we studied (39 of 125 rivers). Changes in land cover were not related to the observed changes in DOC load. Instead, we note some significant regional differences
as well as a significant effect of catchment area. The lower prevalence of trends in DOC load as opposed to concentration suggests that changing hydrology may be an important driver of the observed changes in DOC concentration. Rivers with larger catchments had larger increases in their instantaneous DOC load between 2000 and 2010.

It is worth noting that the long-term trends we observe in DOC concentration may not be indicative of differences in underlying processes that generate and transport soluble organic carbon from land to stream. Neither are the trends we observe necessarily indicative of the future behavior of DOC concentration in this population of rivers. As Kirchner and Neal (2013) show, the non-self-averaging behavior of many solutes leads to detection of temporal trends much more often than would be expected by chance; these trends arise from the spectral structure of data and do not necessarily indicate monotonic trends in the underlying sources and transport of solutes. Instead, the processes that govern solute storage, transport, and mixing in catchments operate across a range of timescales that can generate detectable trends, but that don’t necessarily imply changes in external forcing or boundary conditions.
Conclusion: The urban stream continuum

This dissertation adds to our understanding of the ecology of streams, both constructed *de novo* and altered, in the urban landscape. By showing that stormwater infrastructure in the urban landscape performs ecological roles by storing and transforming organic matter (OM), not just transporting stormwater through the catchment, this work expands the notion that "inland waters are not passive transport pipes" (Cole et al., 2007). A full accounting should include engineered water conveyances (including stormwater pipes) as active participants in the biogeochemical work done by stream networks in urban landscapes.

The annual carbon budget for a roof presented in Chapter 2 clearly demonstrates that urban infrastructure stores considerable loads of particulate OM and promotes its transformation into the dissolved phase. Chapter 2 also shows that the OM stored and transformed in infrastructure has the potential produce all of the dissolved OM (DOM) that moves through an urban stream during storms.
Chapter 3 explicitly considers the role of infrastructure as a proximate non-point source of stormwater (and the solutes it carries) during stormflow. This work suggests that the impervious, constructed stormwater conveyances in the urban landscape interact with the "urban karst" (sensu Kaushal and Belt 2012) to a greater extent than previously reported. Stream runoff is not composed mainly of runoff directed through impervious flowpaths, so these pipes must be leaky, allowing stormwater to infiltrate into the urban groundwater system. The source areas that generate stormflow in urban streams are not simply the impervious surfaces that are presumed to have high hydrologic connectivity to the stream.

In Chapter 4, we demonstrate that the DOM that drains from urban infrastructure during storms is highly bioavailable and that its composition becomes more homogeneous as it is processed. In the stream, this biological processing of OM would occur in concert with downstream transport, giving rise to the pattern of decreasing diversity of molecules in the DOM pool with distance downstream described by Vannote et al. (1980) as part of the River Continuum Concept.

Chapter 5 expands the context of the other work by examining land use as a driver of DOM in rivers throughout the United States. We found that in catchments with high canopy cover, the marginal effect of impervious surface is to increase mean DOC concentration. This result is consistent with our observation that impervious infrastructure collects particulate OM and promotes its transformation into soluble and readily mobile compounds.
6.1 Engineered water conveyances as headwaters

Together, the work presented in this dissertation supports the conclusion that the infrastructure designed to convey stormwater in urban landscapes (e.g. roof and roadside gutters, stormwater pipes) is biogeochemically and hydrologically functioning headwaters of urban streams. These engineered headwaters (Kaushal and Belt, 2012) are not acting as passive pipes. Instead, they are active biogeochemical transformers, processing collected particulate matter into soluble, readily mobile compounds and CO$_2$. Much like natural-bed ephemeral headwaters, the engineered headwaters in urban catchments are the interface between the terrestrial and aquatic systems, and failure to appropriately account for their biogeochemical activity may misconstrue element budgets (Benstead and Leigh, 2012).

The work presented here further suggests that the engineered headwaters in urban catchments can have significant hydrologic connections not only longitudinally but also vertically. Like natural-bed ephemeral streams in this region (Zimmer and McGlynn, 2017a), the engineered headwaters in this urban catchment seem to have significant interactions with groundwater, despite being constructed of materials generally deemed impervious. Kaushal and Belt (2012) suggests that aging water infrastructure leads subsurface pipes in urban catchments behave as ”urban karst”, with the water they carry interacting with other pipes and the groundwater pool. Drinking water conveyances can be significant vectors of urban groundwater recharge, losing 20-30% of the water they carry in transit (Garcia-Fresca, 2004). This dissertation suggests that urban groundwater recharge may also be derived from ephemeral flow through subsurface stormwater pipes.
Hydrologically and biogeochemically, engineered channels such as roof gutters, roadsides, and stormwater pipes in the urban landscape behave as ephemeral headwater streams. The same arguments made regarding the need to better understand the and incorporate ephemeral streams into biogeochemical and hydrologic understanding of river networks (Benstead and Leigh, 2012; Zimmer and McGlynn, 2017a) apply to engineered headwaters. Indeed, such understanding of the role of the engineered headwaters in urban stream networks may also be important for effective management of urban water quality.

6.2 Engineered headwaters are part of an urban stream continuum

The engineered headwaters described in this dissertation comprise the upper reaches of an urban stream continuum (Figure 6.1). Like all stream networks (Stanley et al., 1997), the footprints of urban stream ecosystems are dynamic in time, expanding upstream into ephemeral channels during stormflow. This temporary expansion of the stream network not only expands the spatial footprint of the stream network and its biogeochemical action, but also expands the source areas that contribute to longitudinal transport of materials (including dissolved nutrients and DOM) through the landscape (Welter and Fisher, 2016).

The flashy hydrology characteristic of urban streams (Walsh et al., 2005) means these constituents that are connected and transported through urban streams during storms exhibit a pulse-shunt behavior (Raymond et al., 2016). Short residence times within the urban stream network likely means that the nutrients and DOM carried in urban stormwater undergo little processing in the network. Instead, these pulses of stormwater are shunted to a downstream location. In most regions of the world,
it is likely that this downstream receiving ecosystem is an impounded river network (Gregory, 2006). The potential impacts of stormflow as it traverses the lotic-lentic transition into reservoirs is largely unknown, but may potentially have repercussions for the physical, chemical, and biological function of these ecosystems over a variety of timescales. For example, storm runoff may drive important changes in greenhouse gas dynamics in reservoirs by altering the chemical and physical conditions in the reservoir that control greenhouse gas production and evasion.

Conceptualizing the engineered headwaters as part of the urban stream continuum can lead to better understanding of catchment biogeochemical budgets and the water quality in urban groundwater, urban streams, and the downstream systems that receive urban stormwater. Conceptually connecting the ecology of engineered headwaters to other systems through these longitudinal and vertical flowpaths will facilitate management of water quality throughout river networks.
Figure 6.1: The urban stream continuum. Ephemeral headwaters in the urban stream continuum are built channels such as roof and roadside gutters that flow only during storms. Between storms, these engineered headwaters process and transform particulate organic matter that has collected there. During storms, OM is readily dissolved and quickly transported downstream into altered urban streams and into anthropogenic impoundments, where residence time is high and these solutes from the urban landscape can be processed.
Appendix A

Supplemental Material to *Engineered headwaters can act as sources of dissolved organic matter and nitrogen to urban stream networks*


**Figure A.1:** Time series of areal litterfall rate during the study.
Figure A.2: Proportion of roof area of homes beneath tree canopy. Black circles show the proportion of roof area beneath tree canopy for 20 randomly selected homes and red triangles show this proportion for the three focal roofs in this study. Box-plot describes this 23 home sample, with a mean proportion of $0.37 \pm 0.26$ of roof area directly underneath tree canopy, as judged from summer aerial images (Google, 2015).

A.1 Supplemental Methods

A.1.1 Site Description

Our focal area for this study was a low to medium density neighborhood in Durham, NC which drains to a first order tributary of Ellerbe Creek. The neighborhood primarily consists of single family homes on quarter-acre ($\sim 1000 \text{ m}^2$) or smaller lots. The 60 h catchment contains approximately 400 houses and has 39.4% impervious
Figure A.3: Relationships between instantaneous [DOC] and elapsed rainfall in the population of samples used in this study. In roofs (A) there is evidence of a ‘first flush’ effect very early in storms, but there is not strong evidence for ongoing dilution after this initial pulse of DOC. In catch basin outflows (B), we do not observe systematic evidence for dilution of DOC over the course of storms. In both cases, we use seasonal mean concentrations multiplied by event volume to estimate event-scale fluxes from engineered headwaters in the whole catchment.

cover, based on 1 m resolution planimetric maps (City of Durham Stormwater Services, 2016). The catchment has considerable canopy cover (~60%, i-Tree Landscape v2.1.2) with willow oak (*Quercus phellos*) as the dominant canopy tree.
Figure A.4: Proportion of original carbon remaining in leafpacks (air-dried *Quercus phellos* leaves) vs. time deployed in (A) roof gutters and (B) catch basins. Coarse mesh bags consisted of polyethylene mesh with openings of approximately 5 mm and fine mesh bags were made of nylon mesh with 0.5 mm openings. Calculations for decay curves in coarse and fine litter bags deployed in catch basins were done after removing three and two outliers, respectively (open circles).

A.1.2 Field Measurements

Litterfall

To measure areal rates of litterfall, we deployed 10 laundry baskets in front and back yards of 5 homes in the watershed from 20 Nov 2015 through 28 Nov 2016. Litter
was collected from baskets weekly in fall and early winter, fortnightly during late winter to early spring, and monthly during late spring to summer.

We assumed that areal rates measured in baskets represented rates of litter input to roofs and roads. We then determined litterfall input rates to each roof and catch basin by scaling masses of C to basket area (0.5742 m$^2$) and the number of days between collections to yield measurements of litterfall rate in g C m$^{-2}$ d$^{-1}$. Multiplication by catchment area and summation over the year yielded total mass of C inputs from litterfall.

Throughfall and rainfall

Rainfall was calculated as the average of two nearby USGS rainfall gages: site 3601430785400945 is located approximately two km northeast and site 355852078572045 is located approximately four km southwest of the study watershed. We estimated DOC inputs to roofs as the sum of contributions from throughfall and rainfall. To determine the concentration of DOC in throughfall, we deployed 1 gal (3.8 L) open-topped plastic buckets under the tree canopy at each of three focal properties for the duration of three storms and measured the DOC concentration in the aggregate sample. For rainfall DOC, we used the mean DOC concentration of 5.10 ± 7.86 mg L$^{-1}$ reported by Siudek et al. (2015) in Poznań City, Poland. We then used summer aerial images to the proportion of roof area directly beneath tree canopy (Figure A.2) in order to estimate the proportion of precipitation as throughfall and as direct rainfall, assigning the measured throughfall DOC concentration and literature rainfall DOC concentration to each area, respectively. Multiplying by these areas then gave us masses of incoming DOC for each roof.
Road runoff

Stormwater entering catch basins contains DOC derived from precipitation, roof gutters that are directly connected to roads, and soluble OC deposited on roads. To determine the magnitude of this DOC input to catch basins, we hung 1 gal (3.8 L) buckets from the corner of catch basin grates (n=3 for each of 8 storms) to collect runoff directly from roadside gutters. We collected samples from these buckets as soon as possible after runoff generation using a 60 mL syringe and filtered them in the field with GF/F (nominal pore size of 0.7 \( \mu \)m) into 60 mL opaque brown plastic bottles, and froze them until DOC analysis in the lab.

A regression of sampled DOC concentration against total rainfall before collection time showed no significant evidence of dilution of DOC over the course of storms up to 14.6 mm rainfall (Figure A.3), so we used the mean seasonal concentration multiplied by rainfall volume in the specific sub-catchment to determine DOC flux.

Storage and standing stock

We assessed changes in storage over the course of the study by measuring subsamples at points throughout the year. For roofs, we measured the standing stock of C in two 50-cm lengths of gutter on each focal roof at the beginning, mid-point, and end of the year-long study. For catch basins, we surveyed the OM load both inside and on top of the basins with the same sampling pattern as for litterfall. At the beginning of the study, we measured the dimensions of each of the six catch basins studied, and then for each survey measured the depth of OM at 9 points within the basin. To measure the load on top of catch basin grates, we laid a 3 m transect centered on the grid and characterized the width and depth of the accumulated OM load.
Subsamples of basin OM were collected using a 5 cm diameter PVC cylinder at two random points each within and on top of the basin. We used the area of the sampler and depth of OM to determine bulk density of the OM load and transported samples to the lab for analysis of C content.

**DOC outflow (roofs and catch basins)**

We measured the flux of DOC from roofs by deploying clean 5 gal (18.9 L) buckets with lids fitted with black corrugated plastic pipe to collect water from downspouts of houses (n=3 houses for each of 9 storms). Buckets were allowed to overflow until collection, representing a composite sample over the course of the storm up to that point. We collected samples from these by thoroughly mixing the collected volume of water, sampling with a 60 mL syringe, filtering through a Whatman GF/F (nominal pore size of 0.7 µm) into 60 mL opaque brown plastic bottles, and freezing until DOC analysis in the lab. A regression of composite DOC concentration against total rain-fall before collection showed no evidence of dilution of DOC over the course of storms (up to 14.2 mm rainfall; Figure A.3), so we used the mean seasonal concentration multiplied by rainfall volume to determine DOC flux.

To measure DOC outflow from catch basins through stormwater pipes, we hung 1 gal (3.8 L) buckets from hooks installed in the walls of culverts at the terminus of a storm drain. Buckets were allowed to overflow until collection, when they were covered and held until subsampling 60 mL from this composite after thorough mixing. Samples were filtered through Whatman GF/F filters (nominal pore size of 0.7µm) into 60 mL opaque brown plastic bottles, and frozen until analysis.
Particulates and leaf decomposition

To measure the decomposition rates of leaf matter the urban headwaters, we deployed leaf litter packs in roof gutters (5 houses) and catch basins (5 catch basins). In autumn of 2015, we collected senesced leaves from willow oak (*Quercus phellos*) trees on Duke University’s Durham, NC campus. Leaves were air dried at room temperature for one week before 5 g were added to bags made of coarse polyethylene mesh (opening = 5 mm; RMB1000LE-B, Royal via amazon.com) and fine nylon mesh (opening = 0.5 mm; U-CMN-500, Component Supply Company). After assembly, litter bags were weighed and the initial masses recorded. Sets of litter bags were deployed in late November 2015 in each of the 10 locations and 4 bags (2 coarse and 2 fine mesh) collected after 14, 28, 42, 76, 104, and 138 days. After collection, litter bags were gently rinsed with DI water before drying at 40 C until constant weight.

To determine the mass loss due to decomposition, we first fit decay functions to the proportion of C remaining in bags vs time deployed. For each of the coarse and fine sets of litter bags deployed in roof gutters, we fit a two-component exponential decay model of the form:

\[
\text{Proportion remaining} = P_{\text{slow}}e^{k_{\text{slow}} \times \text{days}} + P_{\text{fast}}e^{k_{\text{fast}} \times \text{days}}
\]

Where \(P_{\text{slow}}\) and \(P_{\text{fast}}\) are the proportions of the OM pool that decay slowly and quickly, respectively, and \(k_{\text{slow}}\) and \(k_{\text{fast}}\) are their respective rate constants.

In contrast, the best-fit decay function for coarse and fine litterbags deployed in catch basins was simple exponential decay of the form:

\[
\text{Proportion remaining} = e^{k \times \text{days}}
\]

We then applied the calculated values of proportion remaining to the measured
mass of standing stock of carbon to infer the mass loss from each studied roof or catch basin. We assumed that the mass loss from coarse litter bags was the sum of DOC loss, particulate OC loss, and decomposition to CO$_2$, while the mass loss from fine litter bags was the sum of DOC loss and decomposition to CO$_2$ only. The difference between the proportions of C lost from coarse vs. fine bags was therefore attributed to particulate loss. Because we independently measured DOC flux from roof gutters and stormwater pipes, we were then able to isolate decomposition to CO$_2$ by subtracting the mass flux of DOC.

**OM removal**

From roofs, OM loss due to manual removal or wind of OM was calculated by difference.

### A.1.3 Laboratory methods

For solid samples (standing stock, litterfall, litter bags) material was dried at 40 C, weighed, and combusted at 575 C for 6 hours to determine OM content. We assumed that 50% of the ash-free dry mass (AFDM) consisted of carbon.

Dissolved samples (road runoff, gutter outflow, storm drain outflow) were frozen after filtering on the day of collection and DOC was analyzed within 60 days as non-purgeable organic carbon using a TOC analyzer (Shimadzu TOC-VCBH). Chloride concentrations were by ion chromatography (Dionex ICS 2000).

### A.1.4 Scaling methods

For individual storms, we calculated flux from the watershed outlet by multiplying stream discharge (as determined from 5 minute records of water level and a site spe-
cific rating curve; Delesantro et al. in prep) by concentrations measured in stormflow samples collected using an ISCO autosampler. For each storm, we used a minimum of 10 water samples and used linear interpolation for estimate concentrations for time points between samples.

In order to estimate the total annual flux of DOC and TDN from the catchment, we separated the discharge time series into periods of baseflow and storms, and further divided these among seasons. Because we did not see strong relationships between DOC or TDN concentration and discharge (Figure A.5), we used the mean concentration for each season’s baseflow or storm samples multiplied by discharge to calculate our estimate of annual flux.

In order to convert from measured concentrations to mass fluxes from the nested impervious sub-catchments (i.e. roofs, roads, piped impervious), we started with the assumption that all water falling on impervious service is converted to runoff. Volumes were therefore calculated by multiplying rainfall by the impervious sub-catchment area, and DOC and TDN mass fluxes were determined by multiplying this volume by measured concentrations.

To estimate total fluxes from roofs, we began by converting mean fluxes measured for individual roofs into areal rates. Using 1 m resolution planimetric maps of catchment impervious surface (City of Durham Stormwater Services, 2016) separated by type in ArcGIS 10.4, we were then able to scale fluxes of DOC and TDN to total roof area in the catchment. We used the average canopy cover (USDA Forest Service, 2016) in the catchment to determine the relative contribution of through-fall vs. direct rainfall and therefore determine the magnitude of DOC inputs to the impervious catchment.
We used a one meter resolution digital elevation model (DEM) based on 2016 LiDAR data from the 3D Elevation Program (U.S. Geological Survey, 2016b) to delineate the impervious catchments of catch basins. We filled depressions using a maximum breach length of 80 pixels and used a D8 flow direction algorithm to extract drainage networks from the DEM data in Whitebox GAT 'Montreal' v. 3.4.0 (Lindsay, 2016). Extracted flow paths based on a channelization threshold of 10,000 were then compared to 2010 stormwater pipe maps (City of Durham Stormwater Services, 2010) to ensure alignment. Catchment boundaries were then used to parse planimetric maps for catch basins in which we measured element fluxes to determine areal rates of C and N loading that could be scaled to the whole catchment.

Due to uncertainty in the amount of flow diverted by subsurface pipes within each catchment, to determine the area of solely road surface area draining to individual catch basins, we used Google Earth. Elevation data were used to delimit the catchment boundaries, with the assumption that a catch basin located above the basin of interest diverted all runoff from higher elevations, and that the middle of the road acted as a catchment boundary. We then used the path tool to find the dimensions of the road area draining to each studied catch basin. These catch basin catchment areas were used to determine rainfall volumes flowing into catch basins, which were multiplied by DOC concentrations in the in- and outflows in order to calculated catch basin decomposition rates.
Figure A.5: Concentration-discharge relationships at the watershed outlet among seasons [winter: 28Nov15-28Feb16; Spring: 1Mar16-31May16; Summer: 1Jun16-31Aug16; Autumn: 1Sep16-28Nov16] for DOC (A) and for TDN (C). There were no significant relationships between concentration and discharge except DOC in autumn storms (DOC = 22.833 + 0.0301*Q, p=0.04). However, Q explains only 3% of the variance in DOC concentration ($r^2 = 0.03069$). Violin plots of DOC (B) and of TDN (D) concentrations at the watershed outlet among seasons. The number of storms recorded in each season is given below the season; chemical time series for a total of 14 storms were captured between November 2014 and March 2017.
Figure A.6: Example time series of discharge (blue, left y-axis), DOC concentration (black, right y-axis), and TDN concentration (red, right y-axis) for three storms during the study.
Table A.1: Event-scale fluxes of stormwater for storms used in this study. Total event-scale flux estimates are in units of m$^3$. For roof fluxes, we assume that 10% of roofs have direct hydrologic connection (i.e. some of the stormwater derived from roofs does not contribute to event-scale fluxes because it is not routed to impervious surfaces, but these fluxes are not considered in our estimates). Water fluxes from ”piped” sub-catchment are the sums of connected roof and road runoff calculations. Percents represent the percent of flux measured at the catchment outlet that can be described by flux from each nested infrastructural sub-catchment.

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<tr>
<th>Date</th>
<th>Rainfall (mm)</th>
<th>Roof Total</th>
<th>Road Total</th>
<th>Piped Total</th>
<th>Catchment Total</th>
<th>Roof %</th>
<th>Road %</th>
<th>Piped%</th>
</tr>
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Table A.2: Event-scale fluxes of DOC for storms used in this study. Areal fluxes are in units of mg/m$^2$ and total fluxes are in kg. Total event-scale flux estimates for roofs assume that 10% of roofs have direct hydrologic connection (i.e. some of the material derived from roofs does not contribute to event-scale fluxes because it is not routed to impervious surfaces, but these fluxes are not considered in our estimates). Fluxes from each successive sub-catchment are assumed to include the fluxes of the smaller units. Total roof area = 84,019 m$^2$; Total road area = 166,972 m$^2$; Total catchment area = 674,504 m$^2$. ND = no data.

<table>
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<th>Roof total</th>
<th>Road areal</th>
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<td>165 ± 150</td>
<td>1157 ± 1568</td>
<td>193 ± 408</td>
<td>0.083 ± 0.046</td>
<td>51.9 ± 25.3</td>
</tr>
</tbody>
</table>
Table A.3: Event-scale fluxes of TDN for storms used in this study. Areal fluxes are in units of mg/m² and total fluxes are in kg. Total event-scale flux estimates for roofs assume that 10% of roofs have direct hydrologic connection (i.e. some of the material derived from roofs does not contribute to event-scale fluxes because it is not routed to impervious surfaces, but these fluxes are not considered in our estimates). Fluxes from each successive sub-catchment are assumed to include the fluxes of the smaller units. Total roof area = 84,019 m²; Total road area = 166,972 m²; Total catchment area = 674,504 m². ND = no data.

<table>
<thead>
<tr>
<th>Date</th>
<th>Rainfall (mm)</th>
<th>Roof areal</th>
<th>Roof total</th>
<th>Road areal</th>
<th>Road total</th>
<th>Piped areal</th>
<th>Piped total</th>
<th>Catchment areal</th>
<th>Catchment total</th>
</tr>
</thead>
<tbody>
<tr>
<td>30Nov15</td>
<td>4.19</td>
<td>1.86</td>
<td>0.016</td>
<td>ND</td>
<td>ND</td>
<td>5.76</td>
<td>0.96</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>17Dec15</td>
<td>30.73</td>
<td>85.6</td>
<td>0.72</td>
<td>40.6</td>
<td>6.78</td>
<td>41.9</td>
<td>7.00</td>
<td>0.0052</td>
<td>3.08</td>
</tr>
<tr>
<td>15Jan16</td>
<td>8.13</td>
<td>26.0</td>
<td>0.22</td>
<td>9.11</td>
<td>1.52</td>
<td>16.1</td>
<td>2.68</td>
<td>0.0037</td>
<td>2.22</td>
</tr>
<tr>
<td>1Apr16</td>
<td>22.99</td>
<td>105.8</td>
<td>0.89</td>
<td>159.9</td>
<td>26.7</td>
<td>ND</td>
<td>ND</td>
<td>0.0037</td>
<td>2.22</td>
</tr>
<tr>
<td>22Apr16</td>
<td>21.21</td>
<td>63.3</td>
<td>0.53</td>
<td>199.3</td>
<td>33.3</td>
<td>326.7</td>
<td>54.5</td>
<td>0.0090</td>
<td>5.33</td>
</tr>
<tr>
<td>15Jun16</td>
<td>1.02</td>
<td>3.66</td>
<td>0.031</td>
<td>9.40</td>
<td>1.57</td>
<td>2.53</td>
<td>0.42</td>
<td>0.00060</td>
<td>0.36</td>
</tr>
<tr>
<td>14Nov16</td>
<td>12.19</td>
<td>2.26</td>
<td>0.019</td>
<td>2.19</td>
<td>0.37</td>
<td>35.0</td>
<td>5.84</td>
<td>0.0022</td>
<td>1.31</td>
</tr>
<tr>
<td>Average</td>
<td>14.35 ± 10.91</td>
<td>41.2 ± 0.28</td>
<td>70.1 ± 11.7</td>
<td>66.2 ± 11.9</td>
<td>0.0038 ± 0.0031</td>
<td>2.42 ± 1.70</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table A.4: Proportion of event-scale fluxes of TDN in the stream estimated to come from EH runoff. Fluxes from each successive sub-catchment are assumed to include the fluxes of the smaller units. ND = no data.

<table>
<thead>
<tr>
<th>Date</th>
<th>Rainfall (mm)</th>
<th>Roof %</th>
<th>Road %</th>
<th>Piped %</th>
</tr>
</thead>
<tbody>
<tr>
<td>30Nov15</td>
<td>4.19</td>
<td>19.9%</td>
<td>250%</td>
<td>299%</td>
</tr>
<tr>
<td>15Jan16</td>
<td>8.13</td>
<td>3.92%</td>
<td>32.7%</td>
<td>71.2%</td>
</tr>
<tr>
<td>1Apr16</td>
<td>22.99</td>
<td>22.1%</td>
<td>660%</td>
<td>ND</td>
</tr>
<tr>
<td>22Apr16</td>
<td>21.21</td>
<td>5.84%</td>
<td>320%</td>
<td>784%</td>
</tr>
<tr>
<td>15Jun16</td>
<td>1.02</td>
<td>3.60%</td>
<td>276%</td>
<td>50.8%</td>
</tr>
<tr>
<td>14Nov16</td>
<td>12.19</td>
<td>4.62%</td>
<td>298%</td>
<td>411%</td>
</tr>
</tbody>
</table>

Average ± sd

<14.35 ± 10.91>
<10.0% ± 8.59%>
<306% ± 202%>
<323% ± 299%>

Table A.5: Proportion of event-scale fluxes of TDN in the stream estimated to come from EH runoff. Fluxes from each successive sub-catchment are assumed to include the fluxes of the smaller units. ND = no data.

<table>
<thead>
<tr>
<th>Date</th>
<th>Rainfall (mm)</th>
<th>Roof %</th>
<th>Road %</th>
<th>Piped %</th>
</tr>
</thead>
<tbody>
<tr>
<td>30Nov15</td>
<td>4.19</td>
<td>23.3%</td>
<td>220%</td>
<td>227%</td>
</tr>
<tr>
<td>15Jan16</td>
<td>8.13</td>
<td>9.86%</td>
<td>68.6%</td>
<td>121%</td>
</tr>
<tr>
<td>1Apr16</td>
<td>22.99</td>
<td>40.1%</td>
<td>1204%</td>
<td>ND</td>
</tr>
<tr>
<td>22Apr16</td>
<td>21.21</td>
<td>9.97%</td>
<td>624%</td>
<td>1023%</td>
</tr>
<tr>
<td>15Jun16</td>
<td>1.02</td>
<td>8.58%</td>
<td>438%</td>
<td>118%</td>
</tr>
<tr>
<td>14Nov16</td>
<td>12.19</td>
<td>1.44%</td>
<td>27.8%</td>
<td>445%</td>
</tr>
</tbody>
</table>

Average ± sd

<14.35 ± 10.91>
<15.5% ± 13.9%>
<430% ± 441%>
<387% ± 380%>
Appendix B

Supplementary information for Chapter 3: Seasonal patterns in solute chemistry and DOM optical properties, end-member mixing analysis, and event hydrographs and hysteresis plots
Figure B.1: Peak DOC concentration, range of DOC concentration, mean DOC concentration, and DOC load in storms by season.

Figure B.2: Peak TDN concentration, range of TDN concentration, mean TDN concentration, and TDN load in storms by season.
Figure B.3: Peak nitrate concentration, range of nitrate concentration, mean nitrate concentration, and nitrate load in storms by season.

Figure B.4: Peak phosphate concentration, range of phosphate concentration, mean phosphate concentration, and phosphate load in storms by season.
Figure B.5: Peak bromide concentration, range of bromide concentration, mean bromide concentration, and bromide load in storms by season.

Figure B.6: Peak $K^+$ concentration, range of $K^+$ concentration, mean $K^+$ concentration, and $K^+$ load in storms by season.
**Figure B.7**: Peak sulfate concentration, range of sulfate concentration, mean sulfate concentration, and sulfate load in storms by season.

**Figure B.8**: Peak chloride concentration, range of chloride concentration, mean chloride concentration, and chloride load in storms by season.
Figure B.9: Peak Ca\(^+\) concentration, range of Ca\(^+\) concentration, mean Ca\(^+\) concentration, and Ca\(^+\) load in storms by season.

Figure B.10: Peak Mg\(^+\) concentration, range of Mg\(^+\) concentration, mean Mg\(^+\) concentration, and Mg\(^+\) load in storms by season.
Figure B.11: Peak Na\textsuperscript{+} concentration, range of Na\textsuperscript{+} concentration, mean Na\textsuperscript{+} concentration, and Na\textsuperscript{+} load in storms by season.
Figure B.12: Peak SUVA, range of SUVA, and mean SUVA in stormwater DOM by season.
Figure B.13: Peak slope ratio, range of slope ratio, and mean slope ratio in stormwater DOM by season.
Figure B.14: Peak FI, range of FI, and mean FI in stormwater DOM by season.
Figure B.15: Peak HIX, range of HIX, and mean HIX in stormwater DOM by season.
Figure B.16: Peak BIX, range of BIX, and mean BIX in stormwater DOM by season.
Figure B.17: Peak $\beta/\alpha$, range of $\beta/\alpha$, and mean $\beta/\alpha$ in stormwater DOM by season.
Figure B.18: Peak contribution of PARAFAC Component 1, range of contribution of PARAFAC Component 1, and mean contribution of PARAFAC Component 1 in stormwater DOM by season.
Figure B.19: Peak contribution of PARAFAC Component 2, range of contribution of PARAFAC Component 2, and mean contribution of PARAFAC Component 2 in stormwater DOM by season.
Figure B.20: Peak contribution of PARAFAC Component 3, range of contribution of PARAFAC Component 3, and mean contribution of PARAFAC Component 3 in stormwater DOM by season.
Figure B.21: Peak contribution of PARAFAC Component 4, range of contribution of PARAFAC Component 4, and mean contribution of PARAFAC Component 4 in stormwater DOM by season.
Figure B.22: Mixing plots for storms without measured EH runoff. In individual storm plots, shade indicates progression through time, with lighter symbols representing chemistry early in the storm and darker symbols showing the chemistry of later samples. Whiskers show the 10th and 90th percentiles of concentration in microequivalents for the pooled dataset (first panel) or for each individual storm (other panels). Large end-member symbols (pink triangles, gray squares) show the median of the whole data set, while small symbols show the median for samples within individual events, except for soilwater (green diamonds), which is the median of soilwater samples collected at Duke Forest.
Figure B.23: Hydrograph with DOC concentration in stormwater samples (top) and DOC vs. Q (bottom) for a storm on September 3, 2014. The hysteresis index (bottom) is calculated after Lloyd et al. (2016): Concentration is normalized for each event and the concentration associated with the median Q is linearly interpolated for the rising and falling limb. The index is calculated by subtracting the normalized concentration for the rising limb median Q from that of the falling limb. The hysteresis index takes values between -1 and 1, with negative values implying anti-clockwise rotation and positive values implying clockwise rotation. Larger magnitudes suggest wider loops.
Figure B.24: Boxplots of hysteresis index (calculated as described by Lloyd et al. (2016); see) by solute for all storms in the data set. Arrows indicated significant differences (p < 0.05) in the mean value of the hysteresis index among solutes.
Appendix C

Pairs plots for predictors and response variables in Chapter 5
Figure C.1: Pair plots for log-transformed mean DOC concentration and predictor variables for sites within eastern 2-digit HUCs (01-08). Upper right panels show the absolute value of Pearson r, diagonal panels show histogram for each parameter, and lower left show pair plots.
Figure C.2: Pair plots for log-transformed mean DOC concentration and predictor variables for sites within western 2-digit HUCs (09-18). Upper right panels show the absolute value of Pearson r, diagonal panels show histogram for each parameter, and lower left show pair plots.
Figure C.3: Pair plots for the Sen estimate of slope for rivers with significant trends in DOC concentration and predictor variables for sites within eastern 2-digit HUCs (01-08). Upper right panels show the absolute value of Pearson r, diagonal panels show histogram for each parameter, and lower left show pair plots.
Figure C.4: Pair plots for the Sen estimate of slope for rivers with significant trends in DOC concentration and predictor variables for sites within western 2-digit HUCs (09-18). Upper right panels show the absolute value of Pearson r, diagonal panels show histogram for each parameter, and lower left show pair plots.
Figure C.5: Pair plots for the Sen estimate of slope for rivers with significant trends in instantaneous DOC load and predictor variables for sites within eastern 2-digit HUCs (01-08). Upper right panels show the absolute value of Pearson r, diagonal panels show histogram for each parameter, and lower left show pair plots.
Figure C.6: Pair plots for the Sen estimate of slope for rivers with significant trends in instantaneous DOC load and predictor variables for sites within western 2-digit HUCs (09-18). Upper right panels show the absolute value of Pearson r, diagonal panels show histogram for each parameter, and lower left show pair plots.


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Biography

Megan L. Fork was born on June 16, 1985 in Milwaukee, Wisconsin. She attended the University of Wisconsin - Madison, where she was wooed by ecology under the mentorship of Dr. Emily Stanley and Dr. Monica Turner, in whose lab she worked as a student technician. Fork graduated from the University of Wisconsin - Madison with Bachelor of Science degrees in Zoology, Biological Aspects of Conservation, and Theatre & Drama (with emphasis in Costume Design) in May 2008. After completing her undergraduate degrees, she traveled to New Zealand, where she spent several months as a technician in the University of Canterbury's Freshwater Ecology Research Group, under the supervision of Dr. Angus McIntosh.

Upon returning to the United States in 2009, Fork began a research technician position in the lab of Dr. Jim Heffernan at Florida International University. In 2010, she entered the Master of Biology program at FIU under Dr. Heffernan’s supervision. Her master’s research explored the direct and indirect effects of water column dissolved organic carbon (DOC) on benthic denitrification across a natural gradient of rivers with different DOC concentrations in central Florida. Her thesis showed that benthic denitrification was more likely to be carbon-limited in rivers with greater concentrations of DOC, likely because these excluded aquatic plants that exude labile organic matter into the benthic sediments. Fork completed her
master’s degree in 2012. During her time at FIU, she was awarded Best Poster at the 13th annual Biology Research Symposium (2010) and a Grant-in-Aid of Research from the Sigma Xi Foundation (2011).

Fork began her doctoral studies at Duke University’s Nicholas School of the Environment in 2012 as a James B. Duke fellow, with Dr. Heffernan again serving as her advisor. As a doctoral student, she was awarded a GO! Program Fellowship from Oak Ridge National Laboratory as well as travel and research fellowships and awards from the Duke Graduate School. In 2017, she was awarded the Bass Instructor of Record Fellowship, which allowed her to teach an undergraduate course as Instructor of Record in Fall 2017. Fork will also earn a Certificate of College Teaching as a part of her graduate education at Duke University.

Publications:


