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Urbanization changes the composition and bioavailability of dissolved organic matter in headwater streams

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Abstract

Population growth in cities has resulted in the rapid expansion of urbanized land. Most research and management of stream ecosystems affected by urban expansion has focused on the maintenance and restoration of biotic communities rather than their basal resources. We examined the potential for urbanization to induce bottom-up ecosystem effects by looking at its influence on dissolved organic matter (DOM) composition and bioavailability and microbial enzyme activity. We selected 113 headwater streams across a gradient of urbanization in central and southern Maine and used elemental and optical analyses, including parallel factor analysis of excitation-emission matrices, to characterize DOM composition. Results show that fluorescent and stoichiometric DOM composition changed significantly across the rural to urban gradient. Specifically, the proportion of humic-like allochthonous DOM decreased while that of more bioavailable autochthonous DOM increased in the more urbanized streams. In laboratory incubations, increased autochthonous DOM was associated with a doubling in the decay rate of dissolved organic carbon as well as increased activity of C-acquiring enzymes. These results suggest that urbanization replaces upstream humic material with more local sources of DOM that turnover more rapidly and may drive bottom-up changes in microbial communities and affect the quality and quantity of downstream DOM delivery.

Globally, urban streams and watersheds will become more common as urban lands expand to accommodate the next 2.3 billion humans expected by 2050 (United Nations Population Division 2011). Because small streams comprise the majority of river miles and their structure and function are tightly linked to watershed inputs (Hynes 1975), they should be disproportionately affected by this change in land use. Small streams perform a vital role in the collection, transport, and transformation of organic and inorganic matter (Peterson et al. 2001) produced by natural and human processes, thereby integrating landscape processes and linking them to larger water bodies downstream as a meta-ecosystem (Battin et al. 2008). Ecological function within streams and rivers depends on the quantity and quality of terrestrial organic matter (Wallace et al. 1997). Urbanization, however, supplants the pre-existing, typically more heterogeneous landscape with engineered land uses that alter the flow of organic matter across the landscape (Kaushal and Belt 2012).

Dissolved organic matter (DOM) is typically the largest pool of organic energy in aquatic ecosystems (Webster and Meyer 1997) and plays important roles in shaping ecosystem structure and function. In shaded first–third-order stream food webs where in-stream primary production of organic matter may be limited, allochthonous organic matter provides an important basal energy resource that fuels stream communities (Wallace et al. 1997; Hall and Meyer 1998; Kominoski and Rosemond 2012). Despite the size and seeming abundance of this pool of organic energy, only a small fraction may be bioavailable. Allochthonous DOM is generally considered to be lower quality (higher C : N) and less bioavailable than autochthonous DOM (Kaplan and Bott 1989), but aged fractions of allochthonous DOM may be preferentially consumed (McCallister and del Giorgio 2012). Increases in the abundance of bioavailable autochthonous DOM or some fractions of allochthonous DOM may stimulate bacterial metabolism (McCallister and del Giorgio 2012). Microbial production of enzymes for acquiring this metabolic carbon depends on its composition (Harbott and Grace 2005) and availability of other organic and inorganic forms

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of nitrogen (N) and phosphorus (P) (Brookshire et al. 2005; Harbott and Grace 2005). DOM with multiple aromatic rings (humic-like) can also play an important nonconsumptive role in metabolism by acting as an electron shuttle in redox reactions (Cory and McKnight 2005). Thus, changes that increase or decrease the size of this relatively small pool of bioavailable DOM may alter ecosystem functions, such as respiration and inorganic nutrient cycling, which may have cumulative consequences for organic energy delivery downstream.

Kominoski and Rosemond (2012) and Kaushal and Belt (2012), proposed a number of watershed-scale mechanisms by which urbanization influences stream DOM composition. For example, the removal or replacement of natural land covers with impervious surfaces and turf grass can decrease allochthonous inputs of DOM while increased light and nutrients can stimulate in-stream primary productivity (Bernot et al. 2010). Combined with elevated concentrations of inorganic nutrients in urban areas (Paul and Meyer 2001), this suggests that urbanization may produce more bioavailable DOM leading to net increases in DOM breakdown rates.

Our goal with this study was to use the largely forested or reforested watersheds of Maine, U.S.A. to test whether transitioning from a more “natural” (forested) land cover to an urban land use alters the composition of the DOM pool in streams with consequences for its bioavailability and microbial function. Specifically, we hypothesize that: replacement of native wetland and forest land covers with urban land uses shifts the aquatic DOM pool from less bioavailable humic-like DOM to more bioavailable protein-like DOM; and that changes in resource (C, N, P) abundance due to urbanization result in changes in microbial community allocation of energy and resources for producing C, N, and P acquiring enzymes.

Methods

Overview

We conducted our analysis in Maine where most watersheds are dominated by spodosol (podzol) and inceptisol soils which have been reforested for ~50–70 yr after logging or agriculture. Thus, urbanization typically occurs in a landscape matrix of second growth forest and wetland cover. We tested our hypotheses by sampling 113 stream sites along a gradient of urbanization in the spring, summer, and fall of 2011. At each site, we measured a suite of physicochemical parameters and collected water samples to measure basic water chemistry, DOM composition, and DOM bioavailability. We also collected samples of epilithic biofilms to assay potential microbial extracellular enzyme expression, which reflect microbial resource use and limitation.

Study design and site selection

We defined our urbanization gradient using the percent total watershed impervious surface area (ISA) in the Maine

Land Cover Database impervious cover geographic information systems (GIS) layer (five meter resolution) and selected 113 watershed sites ranging from 0% to 60% ISA. Average watershed slopes ranged from 5% to 13% with high and low slopes equally represented across the urbanization gradient. Sampling sites were limited to first and second-order streams because these should be most tightly connected to surrounding land cover and because headwater streams minimize the effects from natural longitudinal ecological and physicochemical changes within streams. Selecting low order streams also excluded point source discharges from wastewater treatment facilities that can influence DOM composition in larger streams. This, however, does not exclude wastewater inputs from septic systems and leaking wastewater conveyance infrastructure.

Sampling

All sampling was conducted during daylight hours at base flow. Base flow (flow comparable to recent flows at that time of year) was determined from United States Geological Survey stream gages within < 70 km of study watersheds. We collected each set of samples in a 2–3 week period in late spring (May) prior to leaf out, late summer (August) after leaf out, and in the fall (November–December) after leaf abscission to investigate potential interactions between urbanization and season. At each site, we measured basic physicochemical parameters (temperature, dissolved oxygen (DO), conductivity, and pH) with a Hach HQ40d multimeter. Reported pH, conductivity, and DO for spring and summer reflect ~113 sites while fall data reflect 23 sites due to an instrument malfunction. Water samples for optical and chemical analyses were syringe-filtered through precombusted 0.7 μm Whatman GF/F filters into acid-leached and rinsed high density polyethylene bottles.

Chemical analyses

Samples for NH_4^+ , NO_3^- , and soluble reactive phosphorus (SRP) were stored at 4°C and analyzed within seven days of collection. Due to logistics, samples for total dissolved N (TDN), and total dissolved P (TDP) were frozen until analysis. NO_3^- , SRP, TDN, and TDP were analyzed colorimetrically following Standard Methods (APHA 2005) on a Lachat QuikChem 8500 flow injection autoanalyzer. TDN and TDP samples were digested with persulfate. Digested TDN and NO_3^- samples were measured with the cadmium reduction method (detection limit 5 $\mu\text{g N L}^{-1}$). Digested TDP and SRP samples were measured with the molybdate-ascorbic acid method (detection limit 3 $\mu\text{g P L}^{-1}$). Ammonium was analyzed using a fluorimetric method (Taylor et al. 2007) (detection limit 1 $\mu\text{g N L}^{-1}$). Dissolved organic N (DON) and P (DOP) were calculated as the difference between total and inorganic N and P. Dissolved organic carbon was analyzed as the difference between total carbon and inorganic carbon using a Shimadzu 5000A total organic carbon analyzer (detection limit 0.4 mg C L^{-1}).

Optical DOM characterization

We used two optical characterization techniques, ultra violet and visible light absorption (UV–Vis) absorbance and fluorescence, to characterize the composition of DOM. UV–Vis absorbance is an integrated measure of all light absorbing DOM (colored DOM or CDOM). Fluorescence measures the fraction of CDOM that, after absorbing light, reemits it at a longer wavelength (fluorescent DOM or FDOM). Fluorescence scans generate excitation emission matrices (EEMs), which are a collection of emission spectra measured at different excitation wavelengths creating a three-dimensional (excitation, emission, and intensity) fluorescence landscape from which different constituents of the fluorescent DOM pool may be identified (Stedmon and Markager 2005).

Characterization of DOM was conducted on filtered samples stored at 4°C within 1–3 d of collection. The UV–Vis absorbance of DOM was measured in a 0.5 cm quartz cell on a spectrophotometer over the same range of wavelengths scanned for fluorescence. UV–Vis data were analyzed as the specific ultraviolet absorbance at 254 nm (SUVA₂₅₄). Higher SUVA₂₅₄ values (3–5) are considered an indicator of more aromatic ring structures (Weishaar et al. 2003).

Prior to fluorescence analysis, samples with high UV–Vis absorbance were diluted with deionized water (Ohno 2002). Fluorescence EEMs were collected by scanning the emission (em.) spectra (250–535 nm in 3 nm intervals) for a series of excitation (ex.) wavelengths (220–535 nm in 3 nm intervals). All EEMs were corrected for instrumental bias following the manufacturer's method, corrected for inner-filter effects according to Ohno (2002), blank subtracted with deionized water, and Raman normalized (Stedmon and Bro 2008).

The resulting EEM dataset was analyzed by calculating three fluorescence indices and parallel factor analysis (PARAFAC). Fluorescence indices were used to characterize the nature and likely source of DOM. The fluorescence index (FI) describes microbially vs. terrestrially derived organic matter; lower values (<1.4) indicate predominantly terrestrial origins (Cory and McKnight 2005). The humification index (HIX) describes the aromaticity of DOM (Ohno 2002); values range from 0 to 1 and higher values indicate more aromatic DOM. Within a watershed, HIX varies by hydrologic compartment with the lowest values (~0.5) observed in groundwater and higher values observed in-stream, wetland, and soil pore water and litter leachate (0.8–0.95, Inamdar et al. 2012). The $\beta : \alpha$ ratio (Wilson and Xenopoulos 2009) indicates recent autochthonous production; values range from 0 to 1 and relatively higher values indicate DOM with a recent autochthonous source.

PARAFAC analysis was used to gain more detailed insight into DOM composition. PARAFAC resolves EEMs into statistically similar patterns of fluorescence variation called “components.” These components represent chemically distinct structures within the DOM pool (Stedmon and Bro

2008). PARAFAC analysis was performed in Matlab R2012b using the DOMFluor v1.7 toolbox as described by Stedmon and Bro (2008). Due to noise at lower wavelengths, we only modeled the EEM region within ex. 250–535 nm and em. 289–535 nm. The final model was validated with split-half validation. For each component, we reported PARAFAC results as absolute PARAFAC scores (F_{Max} values) as well as proportional scores (individual component score divided by the sum of absolute scores for all identified components).

Finally, because the redox state of DOM may be a useful indicator of microbial ecosystem processes (Cory and McKnight 2005), we computed a redox index (RI) (Miller et al. 2006). The RI is the sum of reduced PARAFAC components (this study: components 4, 6, 7, and 8) over the sum of oxidized (this study: components 1 and 8) and reduced components (Miller et al. 2006). Oxidized vs. reduced state was determined by comparing components identified in our study to previous work (Cory and McKnight 2005; Williams et al. 2010). Lower values of the RI indicate more oxidized DOM and higher values indicate more reduced DOM.

DOM bioavailability

DOM bioavailability was measured as both percent biodegradable dissolved organic carbon (BDOC) and the first order decay rate of dissolved organic carbon (DOC) (k , Eq. 1) during aerobic bottle incubation assays (Servais et al. 1989). The equation for first order decay is:

$$[\text{DOC}](t) = [\text{DOC}]_0 e^{-k \cdot t} \quad (1)$$

where $[\text{DOC}](t)$ is the concentration of DOC measured at time t and $[\text{DOC}]_0$ is the initial concentration of DOC. Assays were conducted in the fall for a representative subset of sites ($n = 55$). Briefly, a 500 mL sample of filter-sterilized (0.2 μm) DOM from each site was individually amended with nutrients (target minimum concentration for NO_3^- [sond]N was 400 $\mu\text{g L}^{-1}$ and SRP was 74 $\mu\text{g L}^{-1}$ P) to alleviate inorganic nutrient limitation. A composite microbial inoculum was created by loosening 20 cm^2 of epilithic biofilm from three rocks with a toothbrush and rinsing the scrubbing into a HDPE bottle with ~20 mL of stream water at two high and two low urbanization sites. The composite inoculum was then homogenized by vortexing and 500 μL (0.1% v/v) was added to each BDOC sample. Bottles were then incubated in the dark at 12°C for 13 d. DO was monitored and was always above 8 mg L^{-1} . Samples were withdrawn at 0 d, 1 d, 3 d, 8 d, and 13 d, filtered, and analyzed for $[\text{DOC}]$. Inorganic nutrients were analyzed on days 0 and 13.

Potential microbial extracellular enzyme activity

As an indicator of DOM composition and bottom-up limitations on stream biofilms, we analyzed biofilms scrubbed from rocks for potential microbial extracellular enzyme activity (EEA) in the same subset of sites used for bioassay. Microbes produce these enzymes to acquire organic forms of

C, N, and P; as such the abundance or activity of these enzymes is responsive to DOM composition and inorganic nutrient availability (Findlay et al. 2001; Harbott and Grace 2005). To characterize the carbon composition bacteria were utilizing, we assessed samples for potential EEA of β -D-glucosidase (BG), α -D-glucosidase (AG), and β -xylosidase (XYL). Nitrogen utilization was assessed as β -N-acetyl glucosaminidase (NAG) and leucine aminopeptidase (LAP) activity. Phosphatase (PHOS) activity measured P demand by both bacteria and algae.

Microbial EEA analysis was conducted on disrupted rather than intact biofilms to better measure potential EEA (Smucker et al. 2009). Samples for enzyme analysis were collected from rock biofilms at the same sites as the BDOC assay during the summer. Three rocks were arbitrarily selected from riffles (when present) and an $\sim 8 \times 8$ cm area of biofilm was scraped with a toothbrush and rinsed into a 50 mL tube. Scrapings were immediately flash frozen in an ethanol-dry ice bath, transported on dry ice, and stored at -70°C until analysis. Although it is known that freezing the samples can introduce some bias in the results, flash freezing samples has been chosen as a compromise since it was not possible to analyze EEA immediately after obtaining the biofilm suspension.

Enzyme activity was quantified using a fluorescence microplate approach (Sinsabaugh et al. 1997). Frozen samples were thawed and homogenized by vortexing for two minutes. Subsamples for assays were removed and buffered using bis-tris adjusted to within 0.5 units of ambient pH. Additional subsamples were dried and combusted for ash free dry mass (AFDM). Black microplates (plates) were prepared with samples, reference standards, and substrate, quench, and sample controls. Reference standards and quench controls used either 7-amino-4-methylcoumarin or 4-methylumbelliferone. For the sample assay, 200 μmolar solutions of enzyme specific substrate were added to sample assay and control wells. Plates were incubated at 20°C during the linear phase of substrate consumption (1–8 h depending on the substrate). Enzyme activity was quench corrected and calculated as $\text{nmol h}^{-1} \text{mg AFDM}^{-1}$ during the linear phase of reaction from a final reading after the pH was raised above 8.5 by adding 10 μL of 0.5 molar NaOH to each well (Sinsabaugh et al. 1997).

GIS watershed analysis

To characterize landscape attributes potentially influencing DOM, we used six GIS datasets describing geography, land cover, geology, watershed geomorphology, and water chemistry. The general description of these datasets is as follows (details in Table 3): “Geography” describes the physical location of sites; “Land cover” comprises the percent (%) coverage of dominant Maine Land Cover Database classes; “Geology” contains surficial geology % coverage from the Maine Geological Survey surficial geology GIS layer;

“Watershed geomorphology” describes watershed size, average slope, and average watershed hydrologic soil group (which describes runoff potential) derived from the United States Department of Agriculture STATSGO2 general soils GIS map; and “Water chemistry” data include physicochemical variables measured at each site.

Statistical analysis

The relations between individual variables and the arcsine square root transformation of ISA (urbanization) were analyzed with linear regressions, with goodness of fit reported as the adjusted R^2 (hereafter R^2). Correlation tables between fluorescence variables and enzymes were constructed using Pearson's r . We used redundancy analysis (RDA) to analyze, evaluate, and separate the influences of multiple explanatory datasets and gradients on DOM composition. RDA was implemented from the *vegan* package in R. We selected the variables explaining the most variation using a bidirectional stepwise selection process. To decrease the chance that multicollinearity would exclude potentially important variables, we transformed (arcsine square root for land cover, surficial geology, soils, and relative PARAFAC fluorescence) or removed collinear variables based on their variance inflation factors (VIF) until the VIFs for all starting variables were < 5 . To improve normality, concentration data (DOC and organic and inorganic N and P) were natural log transformed. For the final model, variable and axis significances were determined using permutational (9999 iterations) ANOVAs.

Explanatory variables used in the final model were selected from 29 potential variables (Table 3) in a bidirectional stepwise process maximizing the R^2 . Adjusted R^2 and p -values are reported for total and partitioned variance of the final model, but adjusted R^2 values less than 0 are not reported. Finally, partial RDAs were conducted to partition out the variance unique to different groups of variables. Significance of the overall RDA, partial RDA, and individual variables was tested using permutation tests.

Results

Water chemistry (inorganic and organic C, N, P)

Basic water chemistry parameters spanned a wide range of values across sites and seasons (Table 1). Average stream temperature was 13°C , 19°C , and 5°C in spring, summer, and fall respectively, but displayed no trends with urbanization. Stream water pH was generally higher in urbanized sites (> 7.0) than reference sites (4.0–6.5) and was positively related to urbanization during all sampling periods (spring $R^2 = 0.35$, summer $R^2 = 0.16$, and fall $R^2 = 0.42$, $p < 0.05$ in all cases). In all seasons, conductivity increased with ISA from $40 \mu\text{S cm}^{-1}$ to $50 \mu\text{S cm}^{-1}$ at reference watersheds to 500 – $1800 \mu\text{S cm}^{-1}$ in urbanized watersheds ($R^2 = 0.60$ – 0.70 in the spring and fall and 0.33 in the summer, $p < 0.05$). $[\text{NH}_4^+]$ and $[\text{NO}_3^-]$ generally increased with increasing urbanization across all seasons (Table 1, $R^2 = 0.09$ – 0.37 , $p < 0.05$).

Table 1. Mean, standard deviation and range of stream water physicochemical variables. R^2 values are from significant ($p < 0.05$) relationships with proportion of total watershed impervious cover (ISA), nonsignificant relationships are indicated with an “ns.” A plus or minus sign in parentheses indicates the direction of that relationship

Parameter	Spring 2011					Summer 2011					Fall 2011				
	N	Mean	Min	Max	R^2	N	Mean	Min	Max	R^2	N	Mean	Min	Max	R^2
pH	113	7.0 (0.7)	4.2	8.3	(+) 0.51	107	6.9 (0.06)	5.6	8.2	(+) 0.20	23	7.4 (0.9)	6.02	9.93	(+) 0.42
Conductivity $\mu\text{S cm}^{-1}$	113	246.6 (290.2)	12.9	1874	(+) 0.69	107	289.6 (269.3)	4.1	1557	(+) 0.43	23	275.8 (213.7)	29.7	922	(+) 0.62
$\text{NO}_3^- \mu\text{g L}^{-1}$	113	216.7 (260.8)	<5	1286	(+) 0.37	107	252.2 (319.0)	<5	2080	(+) 0.22	108	218.9 (267.4)	<5	1790	(+) 0.35
$\text{NH}_4^+ \mu\text{g L}^{-1}$	112	19.7(56.8)	<1	526.3	(+) 0.11	107	14.2 (31.9)	<1	284.2	ns	107	27.1 (75.7)	1.2	565.1	(+) 0.19
TDN $\mu\text{g L}^{-1}$	113	485.8 (295.4)	101	1818	(+) 0.43	103	613.1 (371.1)	134	2378	(+) 0.09	107	541.9 (371.9)	71	2530	(+) 0.22
DON $\mu\text{g L}^{-1}$	113	249.6 (96.9)	77.1	556.3	ns	103	351.1 (219.6)	<5	1673	ns	107	299.2 (156.9)	<5	1063	(+) 0.28
SRP $\mu\text{g L}^{-1}$	113	2.0 (3.4)	<3	29.1	ns	107	6.0 (7.4)	<3	46.8	ns	108	4.8 (8.7)	<3	89.8	ns
TDP $\mu\text{g L}^{-1}$	113	8.2 (5.4)	<3	35.5	ns	104	26.3 (45.1)	<3	261	ns	107	8.0 (11.0)	<3	111	ns
DOP $\mu\text{g L}^{-1}$	113	6.2 (3.7)	<3	21	ns	104	20.4 (43.6)	<3	243.1	ns	107	3.5 (3.5)	<3	21.2	ns
DOC mg L^{-1}	113	5.4 (3.5)	1.1	23.5	ns	107	8.0 (6.5)	1.2	50.7	ns	107	7.8 (5.0)	1.6	33.3	(-) 0.10

[DON] also increased with urbanization, but this trend was only significant in the fall of 2011 ($R^2 = 0.28$, $p < 0.05$). In the spring and summer, [DON] in most watersheds was between $200 \mu\text{g L}^{-1}$ and $400 \mu\text{g L}^{-1}$. In the fall, less urbanized sites (< 10% ISA) remained in this range while more urbanized sites (> 10% ISA) typically contained $> 400 \mu\text{g L}^{-1}$. [DOC] had a weak ($R^2 = 0.10$, $p < 0.05$) negative relationship with urbanization only in the fall of 2011. [SRP] and [TDP] were not related ($p > 0.05$) to ISA.

DOM composition

The molar C : N of DOM (C : N_{DOM}) ranged from ~ 5 to 70 and declined with increasing ISA in all seasons ($R^2 = 0.23$, 0.08, and 0.46, $p < 0.005$, spring, summer, and fall respectively). Sites with > 35% ISA were generally dominated by nonresidential uses and removing these improved the fit in the spring and summer ($R^2 = 0.47$ and 0.15, $p < 0.005$). There was a weak negative relationship ($R^2 \leq 0.10$, $p < 0.005$) between SUVA_{254} and ISA in all seasons (Fig. 1M–O). As the relative impervious cover increased along the gradient, FI increased 15% from an average value indicative of terrestrial source (1.3) to an average value indicative of microbial source (1.5) (Fig. 1A–C). Similarly, $\beta : \alpha$ increased 57% from an average value of 0.35 indicating low autochthonous inputs to an average value of 0.55 indicating relatively higher autochthonous inputs (Fig. 1D–F). In contrast, HIX, an indicator of relative age and aromatic character, decreased 4% from an average value of 0.97 to an average value of 0.92 with increasing urbanization (Fig. 1G–I).

PARAFAC analysis resolved a nine component model (Maine model abbreviated ME1–9, Fig. 2), whose peaks were similar in shape and location to components observed in previous studies (Table 2). Absolute PARAFAC scores of components ME1–4, 6, and 7 increased with increasing [DOC] in

all seasons ($R^2 = 0.5–0.9$, $p < 0.001$). ME5 displayed a similar increase, but this relationship was weaker ($R^2 = 0.11$, 0.35, $p < 0.001$). Absolute scores of protein-like components ME8 and 9 were unrelated to [DOC] in any season.

The C : N_{DOM} generally decreased with increasing abundances of PARAFAC components ME1, 3, 5, 8, and 9 ($R^2 = 0.2–0.27$, $p < 0.005$) and increased in proportion to the abundances of ME2, 4, 6, and 7 ($R^2 = 0.15–0.31$, $p < 0.05$) in all seasons. Overall, the sum of components ME3, 5, and 9 best explained the decrease in C : N_{DOM} ($R^2 = 0.49$, $p < 0.005$). Fluorescence indices were generally good predictors of relative scores of PARAFAC components at least in part because they are derived from the same underlying data. ME2, 4, 6, and 7 were associated with fluorescent indicators suggesting a terrestrial source. Specifically, components ME2, 4, and 7 were negatively related to FI and $\beta : \alpha$ ($p < 0.005$; $R^2 > 0.59$) and positively related ($p < 0.005$; $R^2 > 0.58$) to HIX. ME6 was similarly related to these indices, but to a lesser degree ($p < 0.005$, $R^2 = 0.10–0.39$). ME 1, 3, 5, 8, and 9 were associated with indicators of recent autochthonous production ($\beta : \alpha$, FI) and decreased humic character of DOM (HIX). Of these, ME5, ME8, and ME9 were positively related to FI and $\beta : \alpha$ ($p < 0.005$, $R^2 > 0.69$, despite two outliers in ME9 that decrease the R^2) and negatively related to HIX ($p < 0.005$, $R^2 = 0.29–0.91$). Although ME1 and 3 resemble humic-like components, they exhibited a weak negative correlation with HIX ($p < 0.005$, $R^2 = 0.10–0.22$).

The proportional abundances of individual PARAFAC components were related to impervious cover (Fig. 3), with the proportional abundance of ME8 increasing fivefold to values of 10–15% in sites with the highest impervious cover ($R^2 = 0.51$, $p < 0.005$, Fig. 3H). In contrast, absolute PARAFAC scores displayed little relationship to urban land use with

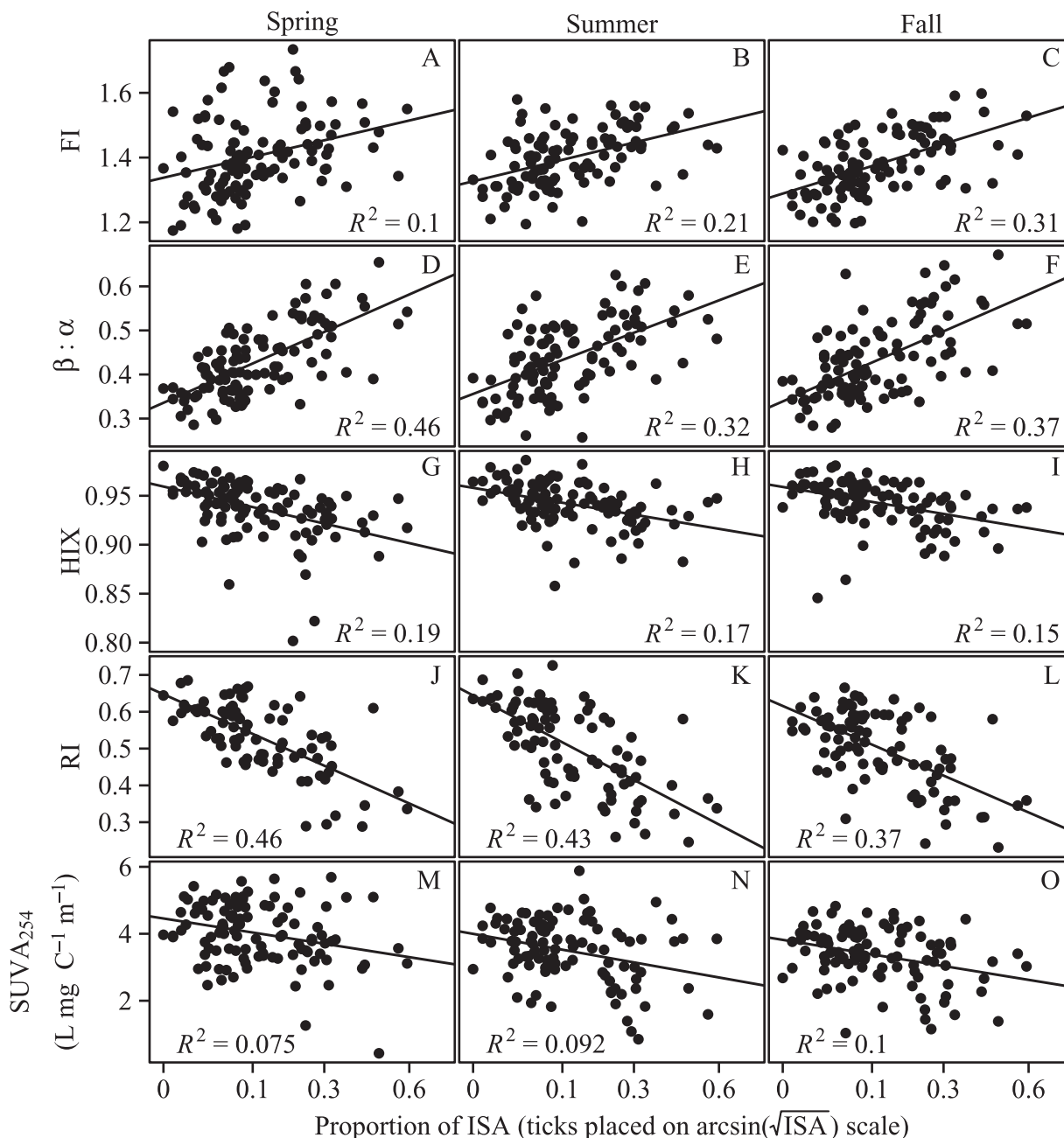


Fig. 1. Relationships of DOM fluorescence and UV-Vis indices with impervious surface area (ISA) across seasons. Larger values of FI (A–C) and $\beta : \alpha$ (D–F) indicate microbial sources while small values indicate more terrestrial sources. Conversely, larger values of HIX (G–I) and $SUVA_{254}$ (M–O) indicate more humic character and terrestrial origins respectively. Higher values of RI (J–L) indicate more chemically reduced DOM. All R^2 values are significant ($p < 0.05$).

the exception of ME8, which increased with increasing ISA ($R^2 = 0.35$, $p < 0.005$). However, the redox-index developed from the absolute scores of oxidized (ME1, 8) and reduced components (ME4, 6, 7) was negatively correlated with increasing ISA, suggesting urbanized streams may have more oxidized DOM ($p < 0.005$, $R^2 = 0.35$ – 0.44 , Fig. 1J–L). This trend was not sensitive to the inclusion or exclusion of oxidized (ME8) or reduced (ME5) components.

These univariate relationships between DOM composition and ISA discussed in the previous paragraphs are further supported by a RDA using a suite of landscape and water chemistry variables (Fig. 4; Table 3) to explain the changes in DOM composition (relative abundance of PARAFAC components). Proportional scores of five PARAFAC components (ME1, 3, 5, 8, 9) were positively associated with metrics of urbanization and four of the components were positively

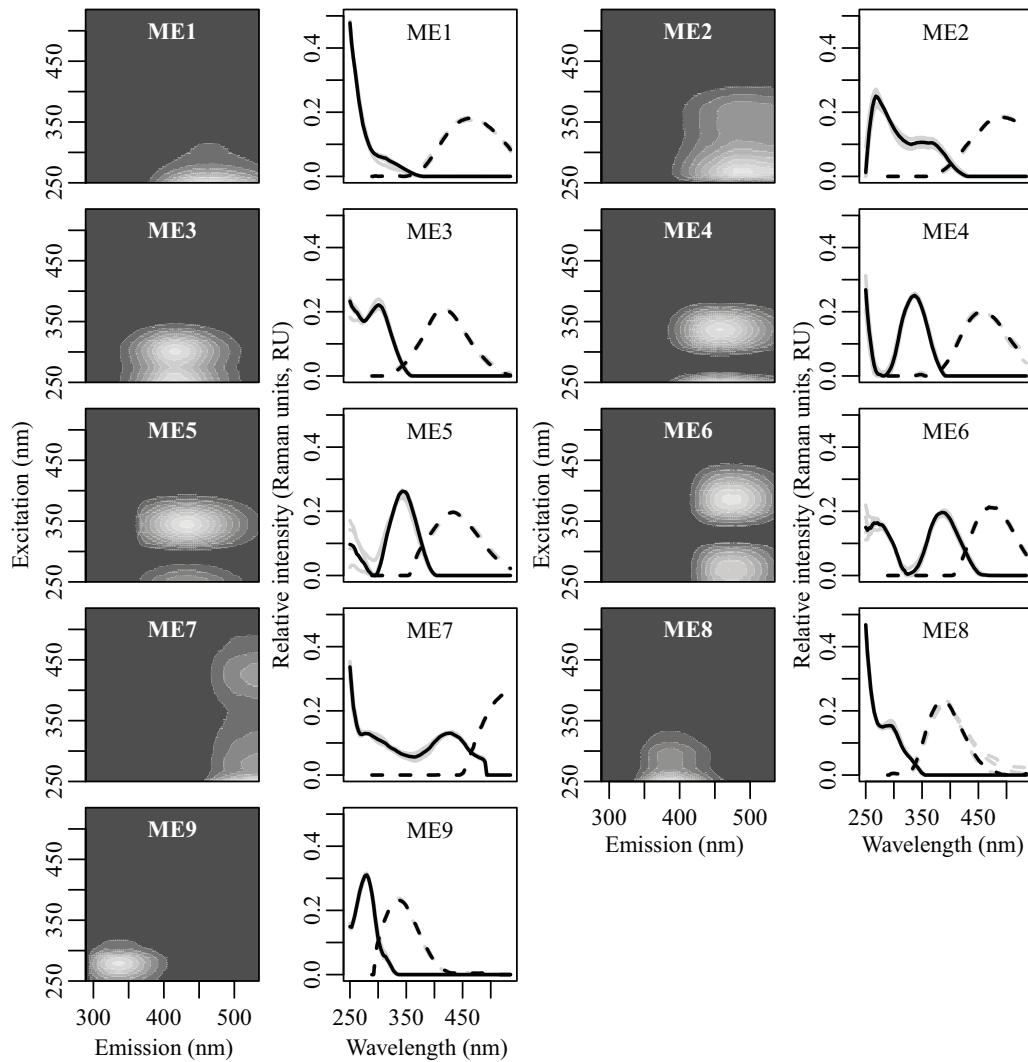


Fig. 2. Fluorescence signatures of nine components identified in the Maine (ME) urbanization gradient dataset. Plots and components are numbered (ME1-9) by decreasing percent of variance explained; overall variance explained was 99.96% of the total variance in the EEM dataset. Both line plots and contour plots are presented. Contour plots are the cross products of the emission and excitation line plots. In contour plots, emission is on the x axis and excitation is on the y axis. In line plots, wavelength is on the x axis and relative intensity is on the y axis. The excitation profile is represented by solid lines and the emission profile by dashed lines. Black lines represent the full model derived from all EEMS and light gray lines are from each of the four validation split halves. See Table 2 for description of components and comparisons with previous studies.

associated with natural land covers (ME2, 4, 6, 7). The variables used in the RDA analysis (Table 3) explained 60% of the overall variation in the fluorescence signatures of DOM. The stepwise variable selection procedure identified six additional variables (%cropland, %forest, %wet forests, and %wetlands, %fine-grained glaciomarine sediment, watershed area) that improved the adjusted R^2 , however permutational ANOVA tests for significance indicated that these variables were not significant ($p > 0.05$). These variables were removed to improve parsimony, but it is worth noting that the natural land covers (forests and wetlands) ordinated positively along the x axis (Fig. 4).

Separating the contributions of individual variables or groups of variables by variance partitioning, we found that

the variance solely attributable to land cover ($R^2 = 0.22$, 37% of explainable variation, Table 3) was approximately 1.5 times the variance attributable to water chemistry variables ($R^2 = 0.15$ and more than four times the variance attributable to season, surficial geology, or geography (each less than < 6%, Fig. 4; Table 3). The first 6 RDA axes were significant at $p < 0.05$ with RDA axes 1–3 accounting for 94% (83%, 7%, and 3%, respectively) of that explainable variation. The strongest axis, RDA 1, describes the urbanization gradient of a landscape transitioning from natural land covers (wetland and forest) to developed land use. As previously discussed, other natural land covers ordinated along the same axis as evergreen forests, but they did not significantly improve the explanatory power of the model ($p > 0.05$).

Table 2. Description and comparison with previous studies of nine PARAFAC components identified in this study. To avoid confusion in comparing studies, we have assigned a unique identifier (this study = ME) to component numbers. (U) indicates that a component was determined to be correlated with urban conditions using redundancy analysis (Fig. 4)

This study	Ex max 1° (2°), Em max (nm)	Description from previous studies	Coble 2007	Similar components
ME1 (U)	<250, 460	Terrestrial humic-like fluorophore observed exported from agricultural catchments as well as wetland and forested streams during warmer months. Positively correlated to % lignin (Fellman et al. 2009).	A	SM1, F1
ME2	268 (~350), 490	Humic-like fluorophore of terrestrial origins ubiquitous in freshwaters, correlated (–) with % phenolic and (+) with % aromatic groups (Fellman et al. 2009).		SQ, SM2, CW1
ME3 (U)	<250 (301), 415	Humic-like component of autochthonous microbial origin (Cory and Kaplan 2012) also attributed to terrestrial origins (SM3, Stedmon and Markager 2005).	A,M	C6, C10, SM3, SM6, CW2, CK1, F3
ME4	<250 (337), 454	Humic-like	A,C	Broadly similar to SQ
ME5 (U)	343 (<250), 433	Humic-like, correlated (+) with % phenolic and (–) with % aromatic groups (Fellman et al. 2009), (+) with anomeric, acetal, and ketal carbon (Cory and McKnight 2005).	C,A	C1, F5
ME6	385 (<250), 469	Terrestrial humic-like; broadly resembles semireduced quinones reported in other studies.		SQ2
ME7	<250 (427), >535	Widespread humic-like; common in wetlands and forest streams.		SQ1 broadly similar to SM2, F2
ME8 (U)	<250 (295), 385	Characteristics of protein-like and microbial humic-like fluorescence; may be produced autochthonously by autotrophs (Coble 2007) or derived from anthropogenic sources (Stedmon and Markager 2005).	A, M	Q3, C8, SM6, CW5
ME9 (U)	280, 337	Protein-like fluorescence resembling tryptophan, associated with recent autochthonous production; often found in anthropogenically affected watersheds (Stedmon and Markager 2005; Williams et al. 2010). Positively correlated to %BDOC (Fellman et al. 2009) and LAP activity (Williams et al. 2010).	T	C8, SM7, F4, CK4

F—Agro-Urban Perth, Australia (Petroni et al. 2011); SM—Denmark (Stedmon and Markager 2005); semireduced quinone (SQ), oxidized quinone (Q), hydroquinone (HQ), component (C)—Alaska and Antarctica (Cory and McKnight 2005); CW—Agricultural, Southern Ontario, CA (Williams et al. 2010); tryptophan-like (T), humic-like peak A (A), marine humic-like peak M (M), humic-like peak C (C)—Marine environment (Coble 2007); CK—White Clay Creek, Pennsylvania (Cory and Kaplan 2012).

Other development classes not selected by the stepwise procedure (high, medium, and low intensity) ordinated with ISA, but did not significantly improve the model ($p > 0.05$). RDA axis 2 may represent a drainage or wetland gradient from north to south (latitude—“utmy”), with more poorly drained soils (high values of “HydroGrp”) in the north and better drained soils (coarse glaciomarine sediments) in the south—however, this axis explains a relatively small proportion of the variance (7%). DOM composition separated most strongly along the primary axis dividing the components into “urban components” ME1, 3, 5, 8, and 9 associated with increasing urban land use and human influence and “nonurban components” ME2, 4, 6, and 7 structuring with natural land cover characteristics (Fig. 4).

DOM bioavailability

In laboratory bioassays, decay rates of DOC (*k*DOC) from highly urbanized watersheds were roughly double the decay rates for DOC from undeveloped watersheds. The *k*DOC in the bottle assays ranged from $5 \times 10^{-4} \text{ d}^{-1}$ to $1.5 \times 10^{-2} \text{ d}^{-1}$, which represents a 0–22% loss of total [DOC], and increased as a function of increasing ISA (Fig. 5B). After removal of three high leverage points (Cook’s distance and site-specific information), *k*DOC increased with both ISA and ME8 (Fig. 5A,B, $R^2 = 0.55, 0.58$ respectively, $p < 0.005$). Other urban components, with the exception of ME3 ($p > 0.05$), displayed similar positive relationships to *k*DOC (ME1, 5, and 9, $R^2 = 0.15, 0.28, 0.08, p < 0.05$). Conversely, nonurban components (ME2, 4, 6, and 7, $R^2 = 0.29, 0.41, 0.28, 0.38$,

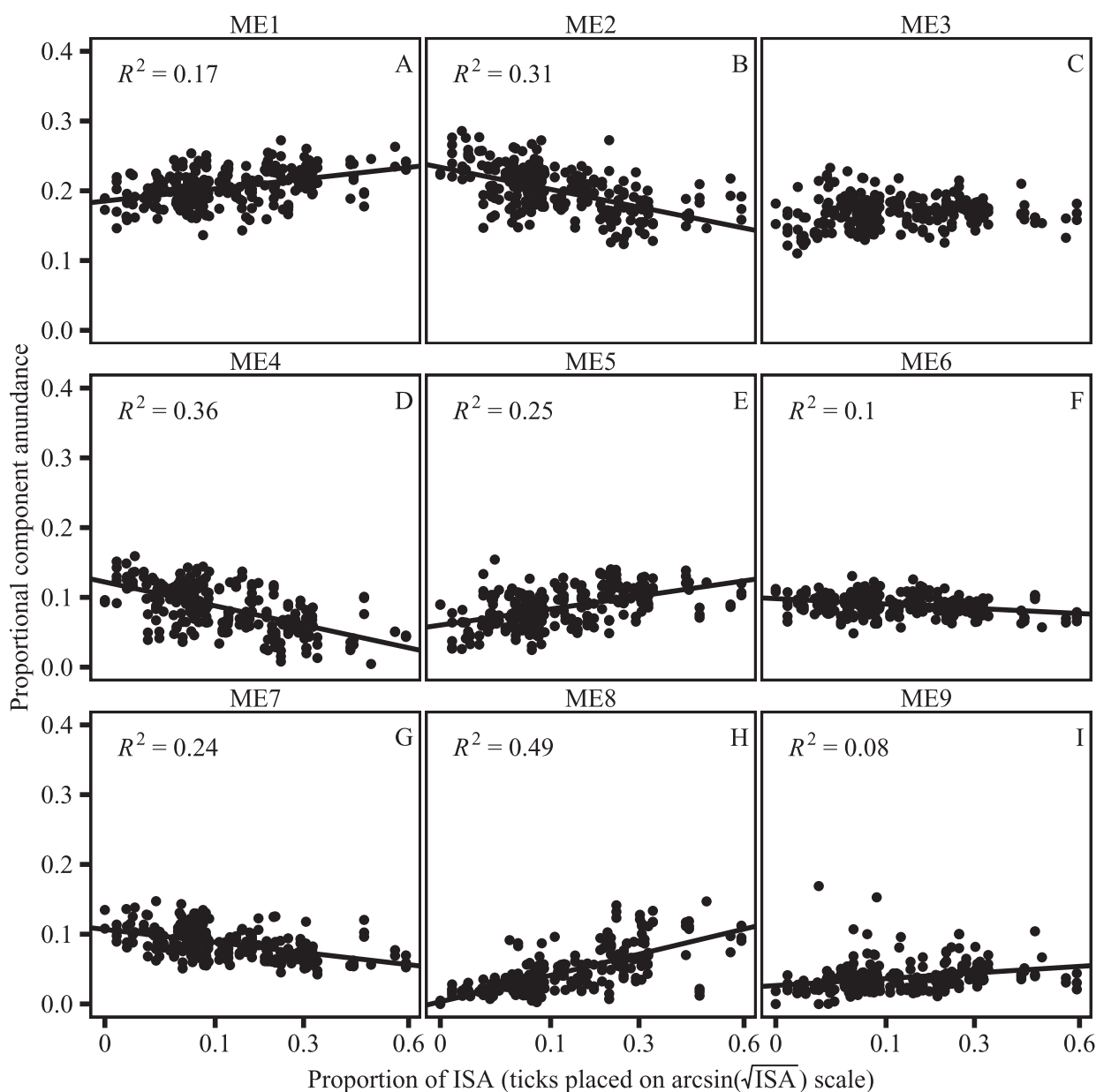


Fig. 3. Relationships between each of the nine components and total watershed imperviousness (ISA) using data from all seasons. Relationships (R^2) significant at the $p < 0.05$ level are shown in each panel. See Table 2 for description of components.

$p < 0.005$) decreased with increasing $k\text{DOC}$. The $k\text{DOC}$ was also higher in samples with DOM characterized by lower aromaticity and lower molecular weight (SUVA_{254} , $R^2 = 0.46$, $p < 0.005$), greater microbial origins (FI, $R^2 = 0.31$, $p < 0.005$), and recent autochthonous production ($\beta : \alpha$, $R^2 = 0.49$, $p < 0.005$). Although it was not significantly correlated to $C : N_{\text{DOM}}$, fastest breakdown rates occurred in samples with an initial $C : N_{\text{DOM}} < 20$.

Potential extracellular enzyme activity

Phosphatase activity was not strongly linearly related to changes in water column [SRP] or [TDP] in the summer

($p > 0.05$) and only a weak negative relationship ($R^2 = 0.12$) between phosphatase and TDP was observed in the fall. However, in both seasons, phosphatase activity declined by $\sim 80\%$ where water column SRP was above $10\text{--}15 \mu\text{g P L}^{-1}$. Phosphatase activity also decreased with increasing urbanization during fall ($R^2 = 0.33$, $p < 0.005$) while other enzymes were not affected by the %ISA in either summer or fall ($p > 0.05$). We did not observe a strong linear relationship between organic nitrogen acquiring enzymes (NAG and LAP) and dissolved inorganic nitrogen or ISA ($p > 0.05$), but LAP declined $\sim 75\%$ with $[\text{NH}_4^+ - \text{N}] > 11 \mu\text{g N L}^{-1}$ in the summer.

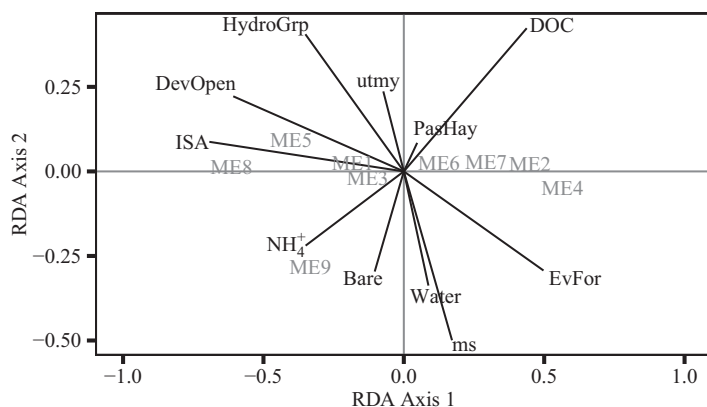


Fig. 4. Results of RDA analysis using a suite of environmental variables to explain variation in DOM composition. The first three axes of the RDA explain 94% of the explainable variation. Clockwise, from the negative x axis, variable codings are as follows: ISA = % of watershed covered by ISA, DevOpen = % of land cover in developed open space, HydroGrp = average soil hydrologic group, utmy = latitude in Universal Transverse Mercator coordinates, PasHay = % of land cover in pasture or hay, DOC = [DOC] in mg L^{-1} , EvFor = % of land cover in evergreen forest, ms = % of surficial geology that is medium-grained glaciomarine deposits, Water = % of surficial geology that is open water, Bare = % of land cover that is bare ground, NH_4^+ = concentration of ammonium in $\mu\text{g L}^{-1}$. Variance partitioning and variable significance is displayed in Table 3.

Enzyme activities and PARAFAC DOM composition displayed the same pattern of correlation in both seasons (Table 4). Generally, phosphatase activity was negatively correlated with urban components, and positively correlated with nonurban components. Two carbon acquiring enzymes (AG and XYL) were negatively correlated with nonurban components and positively correlated with urban components. β -Glucosidase, another C acquiring enzyme, was positively correlated with tryptophan-like ME9, but no other components. Esterase, which indicates general hydrolytic activity, was positively correlated with urban components. Finally, the nitrogen acquiring enzyme LAP was positively correlated to increasing urban component fluorescence and negatively correlated to nonurban component fluorescence. NAG, which can acquire both N and C, was not strongly correlated to any component.

Discussion

Human development has created new ecological systems with distinct biogeochemical characteristics (Kaye et al. 2006). This study provides evidence that urbanization alters the source, composition, and bioavailability of DOM in small streams. Our data demonstrated that urbanization in headwater stream watersheds did not have a strong effect on in-stream DOM concentrations, but significantly increased the proportion of bioavailable DOM derived from autochthonous microbial sources at the expense of less bioavailable

DOM derived from terrestrial sources. These results agree with the current conceptual models (Kaushal and Belt 2012; Kominoski and Rosemond 2012) that predict greater relative abundance of higher quality autochthonously produced DOM in systems subject to anthropogenic land use. In addition, rather than increasing (Kaushal and Belt 2012) or decreasing [DOC] (Kominoski and Rosemond 2012), our results suggest a compensatory mechanism whereby increased autochthonous production balances loss of allochthonous inputs resulting in a relatively uniform [DOC]. Finally, the results of microbial extracellular enzyme analysis (EEA) suggest that urbanization may shift microbial utilization of C and N from allochthonous sources to autochthonous sources. Together, our findings indicate that urbanization potentially has bottom-up consequences for energy transfer through aquatic ecosystems by increasing the abundance of bioavailable microbially derived DOM.

DOM source and composition

Along the urbanization gradient, DOM was derived from a mixture of allochthonous and autochthonous sources (Figs. 1, 3). The shift toward more autochthonous microbial sources with increasing urbanization suggests decreased connectivity with allochthonous source areas and increased contributions from autochthonous microbial sources. While the relationship between ISA and [DOC] or [DON] were variable across seasons, C : N_{DOM} consistently declined from relatively high values (35–60) indicative of terrestrial plant sources to lower values (10–25) indicative of microbial (< 10) and soil sources (~ 14) with increasing ISA. In contrast, researchers studying agro-urban systems have reported increased C : N_{DOM} in surface waters, which they attributed to increased leaching and destabilization of soil organic matter (SOM) due to agriculture and irrigation (Aitkenhead-Peterson et al. 2009; Petrone et al. 2011). However, the increase in $\beta : \alpha$ and FI with ISA (Fig. 1) suggests that the C : N_{DOM} decrease we observed is more likely due to increased autochthonous microbial production rather than increased connectivity with allochthonous inputs from destabilized soil-derived DOM (Williams et al. 2010). This change in source connectivity is further supported by the general decrease in HIX with increasing ISA. Inamdar et al. (2012) showed that HIX values vary by hydrologic compartment within a watershed with lower values generally found in groundwater and higher values observed in wetland surface waters and soil pore water. Thus, the decline in HIX and FI with increasing urbanization we observed suggests that, at low flow, urbanization may interrupt the flow paths connecting streams with allochthonous DOM sources (wetland and upland), thereby increasing the relative dominance of autochthonously produced DOM.

The change in source may also be indicated by the pattern of R^2 values between [DOC] and absolute PARAFAC

Table 3. Results of redundancy analysis indicating variance in DOM components explained by landscape characteristics. Adjusted R^2 values calculated from the full RDAs and portioned out in partial RDAs by group or combination of groups. Interactive effects are the portion of the variance explained by the interaction of the two variable groups. p -values reported are for the significance of the RDA or partial RDA. F values for individual variables are reported in brackets after variable name; all values are significant at $p < 0$.

	Inertia	Adjusted R^2	Proportion explainable variance	p
Total	0.021			
unconstrained	0.008	0.40		
constrained	0.013	0.60	1.00	<0.005
Variance partitioning				
$\text{acrsin}\sqrt{\text{Land Cover (LC)}}$		0.22	0.37	<0.005
% ISA (163.3), % developed open space (8.0), % pasture or hay (7.0), % bare ground (4.0), % evergreen forest (4.1), % open water (13)				
Water Chemistry (WC)		0.15	0.25	<0.005
[DOC] (41.1), $[\text{NH}_4^+]$ (18.2)				
Season (S)		0.06	0.10	<0.005
Spring, summer, fall 2011 (19.0)				
Geography (G)		0.04	0.07	<0.005
Latitude (53.1)				
$\text{acrsin}\sqrt{\text{Surficial Geology (SG)}}$		0.03	0.05	<0.005
% glaciomarine-coarse grain (40.4)				
Watershed Hydrology (WH)		0.01	0.02	<0.005
Average soil hydrologic group (4.1)				
Interactive effects				
LC-WH		0.05	0.08	<0.005
LC-SG		0.00	0.00	ns
LC-G		0.00	0.00	ns
WH-SG		0.02	0.03	<0.005
LC-S		0.02	0.03	<0.005

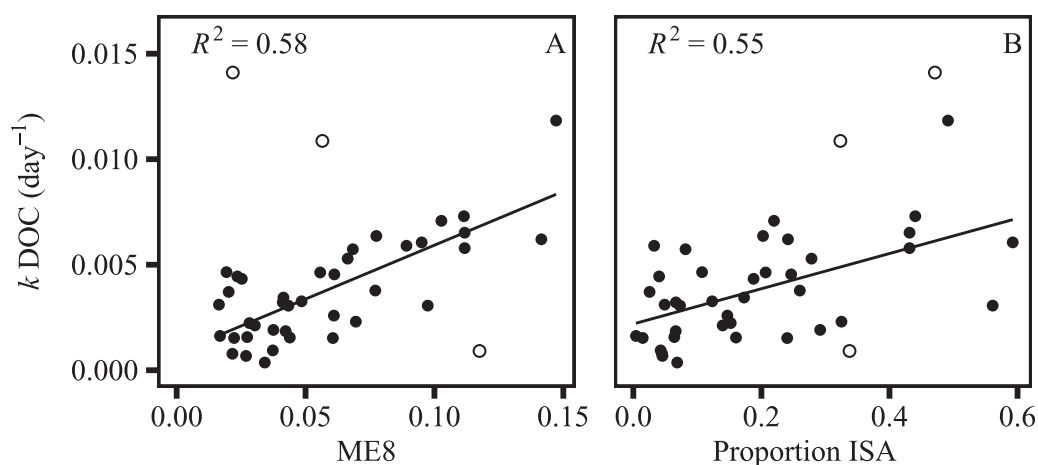


Fig. 5. (A) Breakdown rates of DOC (d^{-1} , k DOC) vs. initial % dominance of fluorophore ME8. Three sites identified as high leverage using quantile-quantile plots are indicated by an open circle in both plots. Removal of these outliers improves fit from $R^2 = 0.16$ to $R^2 = 0.58$, $p < 0.005$ in both cases. (B) Plot of breakdown rate of DOC measured as the first-order decay coefficient of DOC over time (d^{-1} , k DOC). Removal of same three outliers (open circle) as in (A) outliers improves fit from $R^2 = 0.24$ to $R^2 = 0.55$, $p < 0.005$ in both cases.

scores for urban vs. nonurban components in the spring and summer samplings. Of the components displaying a relatively strong correlation to [DOC], the components that

RDA analysis associated with less disturbance and more natural land covers (Fig. 4) displayed a stronger relationship to [DOC] ($R^2 = 0.77\text{--}0.90$) than components associated with

Table 4. Pearson correlation coefficients among enzymes and PARAFAC components. Components have been grouped according to “urban” (upper group in each season) and “nonurban” (lower group) as identified by RDA (Fig. 4). Significant correlations ($p < 0.05$) are indicated with an *

	PHOS	Esterase	BG	AG	NAG	XYL	LAP
Summer 2011							
ME1	-0.13	0.16	-0.17	0.32	0.21	0.02	0.36*
ME3	-0.01	0.08*	0.01	-0.08	-0.12	0.22*	0.11
ME5	-0.31*	0.38*	-0.22	0.24*	0.11	0.21*	0.41*
ME8	-0.23*	0.39*	-0.14	0.32*	0.23	0.19*	0.40*
ME9	0.10	0.36*	0.23*	0.20*	0.06	0.13*	0.15
ME2	0.31*	-0.46*	0.18	-0.31*	-0.21	-0.17*	-0.45*
ME4	0.25*	-0.40*	0.16	-0.32*	-0.18	-0.20*	-0.46*
ME6	-0.07	-0.26*	-0.07	-0.25*	-0.10	-0.19	-0.27
ME7	0.11	-0.32*	0.10	-0.26*	-0.13	-0.24*	-0.37*
Fall 2011							
ME1	-0.09	0.20	-0.22	0.06	-0.19	-0.01	0.09*
ME3	-0.33	0.16*	0.18	0.31	0.07	0.44*	0.15
ME5	-0.41*	0.22*	0.10	0.49*	-0.07	0.44*	0.27*
ME8	-0.27*	0.27*	-0.05	0.38*	-0.13	0.25*	0.23*
ME9	-0.14	0.31*	0.09*	0.33*	-0.01	0.30*	0.21
ME2	0.42*	-0.35*	-0.15	-0.51*	-0.10	-0.48*	-0.37*
ME4	0.32*	-0.31*	-0.03	-0.45*	0.02	-0.37*	-0.25*
ME6	-0.03	-0.15*	0.19	-0.06*	0.31	0.06	0.05
ME7	0.26	-0.14*	0.14	-0.22*	0.38	-0.21*	-0.15*
BDOC	0.19	0.20	-0.09	0.35*	.01	-.11	-.03

urban land cover ($R^2 = 0.03\text{--}0.68$). This may be due to differences in the mechanisms adding allochthonous vs. autochthonous DOM to streams. At base flow, allochthonous DOM reaches streams after interacting with mineral surfaces and microbial enzymes (phenoloxidases and peroxidases) in upland and wetland soils, which likely form “humic” structures (Sinsabaugh 2010) containing highly fluorescent aromatic rings. In comparison, autochthonous DOM microbially produced in situ is not subject to these enzymatic transformations and may have weakly fluorescent aliphatic structures (Aiken et al. 1996). This means that microbial contributions to the DOM pool, if high, may significantly change [DOC] without affecting fluorescence intensity of peaks associated with terrestrial inputs. In urban streams with greater microbial inputs, this would result in the weaker relationship between [DOC] and ME1, 3, 5, 8, and 9 urban associated components that we observed.

The results of the multivariate RDA showed that land use and land cover variables accounted for more variation than any other group of variables (Table 3). In particular, the percentage of ISA appeared to drive shifts in DOM composition (Figs. 3, 4). Similarly, Graeber et al. (2012) found that, in agricultural catchments, land cover was the dominant control on DOM composition whereas in forested catchments land use and soils controlled DOM composition. The dominant control of land use on DOM composition and quantity observed in our study and

Graeber et al.’s (2012) may be due ecological homogenization (Groffman et al. 2014), both modern and historic. In modern times, developers intentionally seek to homogenize the physical characteristics of a site to make it suitable for development by filling wetlands and seasonal drainages, removing organic rich O and A horizons (which are partially redistributed later), and compacting the subsoil (Chen et al. 2013). This process creates spatially homogeneous and poorly characterized “anthrosol” soils and soil processes. At the same time, on the east coast, much of this modern homogenization may be taking place in watersheds where, historically, the land cover had been modified by 17th to early 20th industrial and agricultural activities. The effects of historic land uses have been found to have persistent impacts on modern ecosystems (Foster et al. 2003). These past loci of anthropogenic activity and historical homogenization in the northeastern United States are likely highly correlated with the modern centers of urbanization. Thus, any control they may exert on DOM composition in a disturbed watershed would likely be captured by land cover variables which delineate the modern disturbance (e.g., ISA).

DOM concentration

The land cover variables governing the source and composition of DOM in stream ecosystems may also affect DOM concentration. The individual concentrations of DOM

([DOC], [DON]) displayed no relationship with urbanization in the spring and summer and a negative and positive relationship, respectively, in the fall. Previous work has predicted that [DOC] may increase (Kaushal and Belt 2012) or decrease (Kominoski and Rosemond 2012) due to watershed urbanization. Unlike [DOC], [DON] increased with urbanization in the fall suggesting a seasonal interaction between urbanization and DOM concentration. Wide variation in the direction of the stream water [DOC] vs. anthropogenic land use relationship has been observed in the published literature (Stanley et al. 2012).

One explanation for seasonal variability in the [DON]-urbanization relationship and not in the [DOC]-urbanization relationship may be the “cancellation effect” (Stanley and Maxted 2008; Stanley et al. 2012) and seasonal variability in the processes driving it. The cancellation effect is the offset of losses from one source by gains from another. In our study, the loss of allochthonous DOC inputs from forests and wetlands may be compensated by DOC gains from increased in-stream primary production in urban areas (Bernot et al. 2010). While the contribution of autotrophs to stream [DOC] is variable, it can increase water column [DOC] 24–37% over the daily minimum (Kaplan and Bott 1989). The weak negative relationship in the fall may reflect a seasonal decrease in algal production due to declining water temperatures [mean fall water temperature (3.2°C) was lower than spring (12.5°C) and summer (18.9°C)] and solar flux. Although we did not quantitatively measure bacterial and algal biomass in this study, we did observe thicker epilithic biofilms (pers. obs.), higher $\beta : \alpha$ values, and higher values of ME8, in urban streams suggesting that in-stream production could have contributed to [DOC] in urban streams. A seasonal and disproportionate change in the balance of cancellation effect mechanisms along the urbanization gradient could explain positive relationship between DON and urbanization in the fall. Storm drainage systems can serve as low order streams replacing or extending stream networks and collecting ground water (Kaushal and Belt 2012). Increases in the water table during cool months may increase groundwater inputs to streams via storm drain networks. Groundwater can be nitrogen rich relative to stream water (Inamdar et al. 2012). Additionally these networks may deliver leachates from leaf litter providing a “gutter subsidy” of organic C and N to open channel streams (Kaushal and Belt 2012). Although the C : N_{DOM} of leachates may be high initially (> 30), the greater relative lability of DOC compare to DON in leachates can result in a relative enrichment of DON (C : N_{DOM} < 10) after less than 24 h of processing (Wetzel and Manny 1972). Thus, a gutter subsidy could increase DON while leaving DOC relatively unaffected. Our data clearly show that urbanization changes the source of DOM and seasonally affects its concentration. This seasonal impact on concentration could be important since monitoring efforts by most agencies occur during the summer

months, which potentially overlooks important seasonal fluxes of organic and inorganic nutrients.

Microbial processes and DOM composition

Urbanization has the potential to affect microbial community function in stream biofilms via increased nutrient concentrations and altered DOM composition. While potential N and P acquiring enzyme activity generally decreased with increasing inorganic nutrient availability, nutrients and enzyme patterns displayed varied responses to ISA. This may be due to the timing of resource delivery; previous studies have shown that [P] in storm runoff from developed watersheds in Maine can be 10X higher than adjacent forested watersheds (Dennis 1986). We hypothesize that thicker urban biofilms (relative to forested biofilms in this study) may trap and recycle N and P delivered during storm flow resulting in the weak relationship between low flow enzyme activity and water column nutrient availability. Biofilm P enrichment in response to urbanization (O'Brien and Wehr 2009) and the potential for biofilms to buffer against changes in water column resources (Freeman and Lock 1995) have been previously reported.

Carbon and nitrogen acquiring potential EEAs were positively and consistently correlated to DOM characteristic of microbial sources and urban streams (Table 4). There are two potential mechanisms explaining this. The first is that increased algal primary production common in urban streams (Bernot et al. 2010) broadly stimulated bacterial C and N acquiring enzyme activity (Espeland et al. 2001; Francoeur and Wetzel 2003) due to increased abundance of autochthonous polysaccharidic carbon and trapped allochthonous material in the biofilm matrix (Freeman and Lock 1995; Romani et al. 2004). The second, is a potential release of heterotrophic metabolism from humic acid enzyme activity inhibition with increasing urbanization (Freeman et al. 1990). In this study, carbon acquiring enzyme activities (BG, AG, NAG, and XYL) were negatively correlated with DOM components typical of natural systems. These components typically decreased in relative abundance with increasing urbanization (Table 4). Together, these results suggest a complex feedback between land use and stream microbial biofilms whereby stimulation of primary productivity and decreases in inhibition by humic compounds may increase heterotrophic processing and autotrophic production of DOM resulting in more labile DOM in urban streams.

DOM bioavailability

Our research shows that the pervasive effects of even low levels of land cover change can alter DOM composition and increase its bioavailability. Specifically, we found that as microbial autochthonous DOM increased, so did the bioavailability of DOC (Fig. 5A). The bioavailable fraction of DOC increased from 0% to 10% in forested watersheds to > 20% in the most urban watersheds. This corresponded to a 10–30 fold increase in DOC breakdown rate (d^{-1}). These values are consistent with BDOC values from previous

studies in natural and disturbed aquatic systems which may range from 2% to 60% (Servais et al. 1989; Petrone et al. 2011). The increase in bioavailability we documented was most strongly associated with the increased proportional abundance of microbially derived ME8 (Fig. 5).

Previous studies from agricultural and agro-urban watersheds have hypothesized that anthropogenic watershed disturbance may destabilize stored SOM leading to more reduced redox state of stream DOM (Williams et al. 2010; Graeber et al. 2012). In contrast, our lower RI in urban streams indicated a more oxidized redox state (Fig. 1J–L). Our data support two potentially interactive hypotheses. First, increased respiratory oxidation of DOM would result in a more oxidized residual FDOM pool. Second, thinned or absent riparian canopy cover in urban streams may increase light reaching streams, resulting in more photo-oxidation which decreases the RI (Hood et al. 2003). In support of the microbial respiration hypothesis, DOM in our urban streams was more susceptible to microbial reworking (Fig. 5) and indicators of source suggested microbial origins. With regards to the photo-oxidation hypothesis, while forest cover decreased with urbanization in our study, the % forest in riparian buffers (50 m, 100 m, and 250 m) did not explain the decrease in RI better than total watershed imperviousness (data not presented). We did, however observe a strong correlation between ammonium concentration and ME9, a tryptophan- or amino acid-like component (Fig. 4). Ammonium may be produced from photochemical degradation of amino acid-like components in marine environments (Stedmon et al. 2007). Alternatively, in support of the first hypothesis, increased autotrophic or heterotrophic production and subsequent ammonification of ME9 would produce a similar pattern. While enhanced microbial processing remains the most likely explanation for changes in the RI, we cannot rule out potential photochemical effects on DOM composition and bioavailability.

It is well-known that urbanization manifests itself in ways that degrade the biophysical and ecological condition of streams (Walsh et al. 2005). In this study, we demonstrated that these symptoms may also be accompanied by important changes to processes generating and transforming DOM in urban streams and watersheds. Collectively, the land use driven changes in DOM composition and subsequent alteration of DOM bioavailability and microbial function indicate that urbanization results in bottom-up changes to the stoichiometric and metabolic controls on stream ecosystem function. Increases in labile, microbially produced DOM in the headwaters of a river network may have negative cumulative consequences in downstream reaches and in receiving lakes, estuaries, and oceans, such as exacerbating low oxygen “dead zones” in these larger systems. Future work should strive to couple measurements of microbial production and respiration with higher resolution DOM composition measurements throughout river networks to better understand

the role of composition and source in the fate and transport of DOM. Understanding spatial and temporal linkages among structural and functional characteristics and the composition of DOM in lotic ecosystems will make long-term surface water DOM composition monitoring datasets powerful resources for bottom-up understanding and management of global change phenomena and their effects on aquatic ecosystems.

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