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Dissolved Organic Matter Quality and Bioavailability Changes Across an Urbanization Gradient in Headwater Streams

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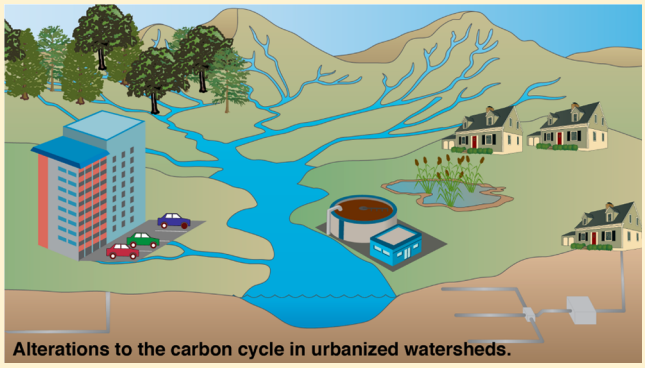
Supporting Information

ABSTRACT: Landscape urbanization broadly alters watersheds and stream ecosystems, yet the impact of nonpoint source urban inputs on the quantity, quality, and ultimate fate of dissolved organic matter (DOM) is poorly understood. We assessed DOM quality and microbial bioavailability in eight first-order Coastal Plain headwater streams along a gradient of urbanization (i.e., percent watershed impervious cover); none of the streams had point source discharges. DOM quality was measured using fluorescence excitation−emission matrices (EEMs) coupled with parallel factor analysis (PARAFAC). Bioavailability was assessed using biodegradable dissolved organic carbon (BDOC) incubations. Results showed that watershed impervious cover was significantly related to stream DOM composition: increasing impervious cover was associated with decreased amounts of natural humic-like DOM and enriched amounts of anthropogenic fulvic acid-like and protein-like DOM. Microbial bioavailability of DOM was greater in urbanized streams during spring and summer, and was related to decreasing proportions of humic-like DOM and increasing proportions of protein-like DOM. Increased bioavailability was associated with elevated extracellular enzyme activity of the initial microbial community supplied to samples during BDOC incubations. These findings indicate that changes in stream DOM quality due to watershed urbanization may impact stream ecosystem metabolism and ultimately the fate of organic carbon transported through fluvial systems.



INTRODUCTION

Freshwater ecosystems bury or return to the atmosphere an estimated 1.0 Pg C y^{-1}[1], with rivers transporting approximately 0.2 Pg C y^{-1} as dissolved organic matter (DOM).[2] Most of the DOM present in freshwaters originates in terrestrial ecosystems.[3] Situated at the origins of river networks, headwater streams represent the transition from terrestrial to aquatic and play an integral role in global carbon cycling as sites of significant organic carbon processing and transport.[4−6] Processing by microbial communities is responsible for a large fraction of total stream metabolism in headwater channels and is driven by the quantity and quality (i.e., molecular structure) of DOM.[7−9] Microbial processing can determine whether headwater stream organic carbon is delivered downstream, enters stream food webs, or is metabolized and released from streams in gaseous form.[6,10] Changes in DOM quality can impact ecosystems through alterations of DOM uptake,[11] food web dynamics,[12,13] and coupled biogeochemical cycles including nitrogen uptake[14] and denitrification.[15]

Stream DOM quantity and quality are related to watershed attributes including soil type,[8] wetland coverage,[16,17] agricultural land use,[10,17,18] and urban point-source inputs such as from wastewater treatment facilities.[12,19−21] Typically this material is highly bioavailable DOM and resembles microbially fixed fulvic acids and protein-like material.[12,19,22] In contrast, nonpoint source inputs of anthropogenically altered carbon from urban landscapes are poorly understood, particularly in terms of the contribution to DOM quality. This knowledge gap is noteworthy given recent findings that urbanization can increase[12,23] and alter[15] labile autochthonous particulate organic matter inputs to streams.

Runoff from asphalt surfaces on roads and parking lots as well as soils and turf grass in urban open areas may contribute substantial amounts of DOM to receiving waters[3,21] and this DOM may be lower in the recalcitrant fractions that characterize forest- and wetland-dominated landscapes. Increased DOM bioavailability in urbanized streams was reported in one study but the source of this altered DOM was uncertain.[23] Organic matter from urban surfaces could have

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been the source since a recent study of runoff from a variety of urbanized landscapes observed DOM that was lower in molecular weight and aromaticity but higher in hydrophobicity compared to forested runoff.[24] Given increasing urbanization,[25] it is important to advance our understanding of the ecological impacts of diffuse, nonpoint source inputs of DOM to streams.

Our goal was to quantify changes in DOM quantity, composition, and bioavailability in response to increasing nonpoint source inputs to streams associated with a gradient of catchment urbanization. We studied eight Coastal Plain streams of Maryland that ranged from completely forested to highly urbanized (>40% impervious cover). Anthropogenic impacts in urbanized watersheds include suburban homes, retail space, parking lots, and stormwater management ponds, but no point-source inputs. We hypothesized that compared to forested or less urbanized streams, DOM in those streams most impacted by urbanization would be (1) more aromatic and autochthonous in nature; and (2) more readily used by microbial communities. We further hypothesized that (3) microbial use of the DOM would not only depend on its composition but on the heterotrophic activity of the microbial community.

MATERIALS AND METHODS

Study Sites. Samples were collected from eight first-order headwater streams located in the Parkers Creek watershed in the Coastal Plain of Maryland (Figure 1 and SI Figure S1). The

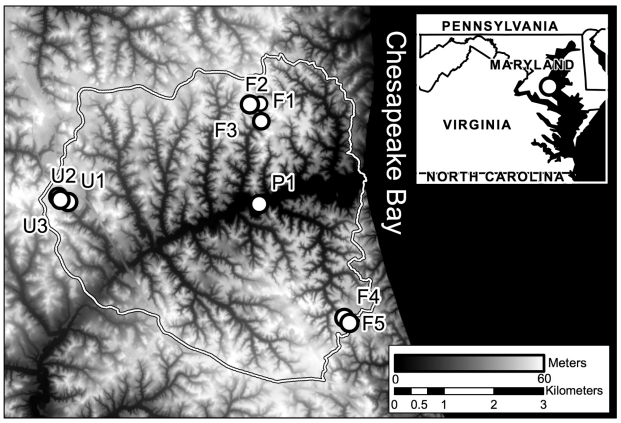

Figure 1. A map based on a digital elevation model (DEM) of the Parkers Creek watershed with study locations indicated. Inset: map of Maryland and surrounding states with the location of the study watershed indicated. Source: LiDAR data provided by Calvert County Government. Inset sources: Esri, TomTom, U.S. Department of Commerce, U.S. Census Bureau.

Parkers Creek watershed is approximately 3107 ha in size and largely composed of alluvial sediments. Five study streams were categorized as forested and have less than 5% impervious cover. Three study streams are located in urbanized watersheds and have at least 10% impervious cover (SI Table S1).

Stream DOM Quantity and Quality. A total of 143 samples were collected on 20 occasions during baseflow conditions from March 2011 through February 2013. For the purposes of this study, baseflow conditions were defined as any period more than 48 h after a rain event. Sites F1, F4, and F5 did not flow during some sampling events that occurred in summer months (July–September), reducing the total number of samples collected. Samples were collected approximately monthly, all within 36 h of each other. There were no precipitation events between the start and finish of any sampling event. Samples were filtered in the field using Whatman GF/F 0.7 μm filters that were precombusted at 450 °C. Water samples for DOM analysis were collected in acid-washed, amber glass bottles that were precombusted at 450 °C; other water samples were collected in acid-washed HDPE bottles. All samples were transported to the laboratory on ice and stored at 4 °C prior to further processing. Dissolved organic carbon (DOC), as nonpurgeable organic carbon, and total dissolved nitrogen (TDN) concentrations were determined using a Shimadzu TOC-vCPH total organic carbon analyzer with attached TNM-1 total nitrogen analyzer.

DOM spectroscopy was conducted following established methods.[19,26,27] Fluorescence excitation–emission matrices were acquired using a Horiba Fluoromax-4 spectrofluorometer. To obtain fluorescence excitation–emission matrices (EEMs), samples were excited from 250 to 450 at 5 nm increments, and emissions were measured at each excitation wavelength from 300 to 550 at 2 nm increments. Blank EEMs were collected routinely using Nanopure water. The mean area under the Raman peak at 350 nm of the Nanopure blanks collected during each fluorescence analysis run was used to normalize EEMs. Fluorescence EEMs for each sample were corrected for machine bias, inner-filter effects, and Raman scatter.[28] UV/vis absorbance spectra for each sample were collected from 200 to 800 at 1 nm increments using a Thermo Scientific Evolution 60 spectrophotometer. Specific ultraviolet absorbance at 254 nm ($SUVA_{254}$), which is positively correlated with DOM aromatic content, was measured by taking the absorbance of a DOM sample at 254 nm and dividing it by the DOC concentration.[29,30] Iron interference of absorbance at 254 nm was identified in some samples. For a subset of samples collected November 2011, April 2012, August 2012, November 2012, and February 2013, total iron was determined using the Hach FerroVer method. Iron concentrations were used to correct absorbance at 254 nm using a previously developed relationship: $A_{254\text{-corrected}} = A_{254\text{-measured}} - 0.0687 \times [Fe^{3+}]$.[36]

To obtain DOM quality metrics from fluorescence EEMs, PARAFAC analysis was conducted using the DOMFluor Toolbox[26] in Matlab version R2013a. An EEM-PARAFAC model based on 417 EEMs from Maryland Coastal Plain streams, including the 143 used in this study, was validated using split-half analysis.[27] Five fractions of DOM were validated using PARAFAC analysis and the form of DOM each component likely represents was identified from previous studies (Table 1; SI Figure S2). Two fluorescence indices, the fluorescence index (FI)[37] and the humification index (HIX),[38] were also used to describe DOM composition. Fluorescence index values were computed as the ratio of emission intensities at 450 and 500 nm at an excitation wavelength of 370 nm.[37] FI is indicative of DOM source: higher values indicate increased auchthonous DOM while lower values are indicative of allochthonous DOM. HIX values were computed based on existing methods[17,38,39] as the ratio of the area of the emission spectrum at 435–480 nm to the emission area from 300 to 445 nm at an excitation wavelength of 255 nm. Increasing HIX values are indicative of more humic, structurally complex DOM.

Microbial Utiltilization of DOM. To determine DOM bioavailability, bioavailable dissolved organic carbon (BDOC) bioassays were conducted following established methods[8] in November 2011, April 2012, August 2012, November 2012, and February 2013, resulting in 36 samples. Bioassays measure

Table 1. Emission and Excitation Maxima and Characteristics of the Modeled EEM-PARAFAC Components[a]

Component	Excitation maxima (nm)	Emission maxima (nm)	Description
C1	<250, (325)	438	humic-like fluorophore of terrestrial origin,[19,22] highest concentrations in forest streams, wetlands, and agriculturally influenced streams,[31] absent from wastewater,[22] photolabile.
C2	<250, (380)	502	ubiquitous fulvic acid,[22] recalcitrant, aromatic, terrestrial humic,[32] humic acid-like UVC/UVA excitation,[19,31] possibly indicative of biogeochemical processing of terrestrial particulate organic matter.[33]
C3	<250, (300)	388	an anthropogenic humic fluorophore dominant in wastewater DOM and linked to DOM originating in agricultural catchments,[22] likely of microbial origin,[33,34] similar to marine humic-like fluorophores.[33,35]
C4	<250, 280	340	tryptophan-like, protein-like fluorescence[19,31,35] indicative of recent production.[31]
C5	<250	310	tyrosine-like, protein-like fluorescence.[8,35]

[a]Previous studies that have described EEM-PARAFAC components with similar properties were identified. Based on this review, a description of the likely characteristics of each DOM fluorophore identified was developed.

the loss of DOC through net bacterial production and respiration over the course of 28-day incubations.[40] Water samples were filtered to 0.22 μm using Millipore polycarbonate membrane filters within 24 h of collection from the field. Microbial inoculum was obtained by collecting sediment and water from site P1 on the main stem of Parkers Creek (SI Table S1) contemporaneously with other sample collection. Approximately 100 g of sediment were combined with 800 mL of streamwater and this slurry was allowed to incubate overnight in the laboratory, after which the mixture was filtered using 0.7 μm precombusted glass fiber filters. A 48 mL aliquot of filtrate and 2 mL of microbial inoculum were added to a precombusted amber glass bottle, which was then thoroughly mixed. For each sample, six replicate incubations were prepared; three replicates for each sample were immediately filtered to 0.22 μm to serve as Day 0 samples. The remaining bottles were incubated for 28 days in the dark at 20 °C with caps vented to allow airflow. Samples were regularly agitated to prevent anoxia. After 28 days, samples were filtered to 0.22 μm, and were analyzed for DOC concentration. Percent BDOC was calculated as the percent DOC lost over the course of the 28-day incubation. To account for potential microbial production of DOM, triplicate control vials that contained Nanopure water in place of streamwater were also incubated with microbial inoculum on each sampling date. Paired t tests that compared the DOC concentrations of 0- and 28-day control samples did not detect any significant changes.

Incubations were conducted without amending samples with nitrogen and phosphorus to prevent nutrient limitation, as has been done elsewhere.[40] This was done to allow microbial lability of DOM to be analyzed in the context of environmental nutrient levels. To account for potential nutrient limitation, nitrate, ammonium, and orthophosphate concentrations were determined using a Lachat QuikChem 8500 Series 2 flow injection analyzer. Molar nitrogen to phosphorus (N:P) ratios were computed as the ratio of dissolved inorganic nitrogen to orthophosphate-phosphorus.

Microbial Heterotrophic Enzyme Production. Extracellular enzyme activities (EEA) measure the forms of DOM utilized by stream microbial communities. These enzymes are produced based on the metabolic demands of microbes and the resources available in the environment.[41] Whole water samples were collected from site P1 contemporaneously with the inoculum used for BDOC assays and were processed for EEA within 24 h of collection. Microplate EEA studies were conducted based on existing methods.[42,43] Activity of extracellular enzymes was measured by assessing the degradation rates of fluorescently labeled model substrates by streamwater. Substrates 4-methylumbelliferone butyrate (MUB)-acetate, 4-MUB-phosphate, and L-Leucine 7-amido-methyl-coumarin were used to measure the activity of esterase, alkaline phosphatase, and leucine aminopeptidase, respectively. The 4-MUB-acetate and 4-MUB-phosphate buffers were prepared in autoclaved 5 mM bicarbonate buffer, L-Leucine 7-amido-methyl-coumarin was prepared in autoclaved Nanopure water. Substrates were refrigerated and stirred continuously for 24 h preceding the experiment to ensure dilution. Equal amounts of streamwater and substrate (150 μL each, 300 μL total volume) were combined in black 96-well plates resulting in a final substrate concentration of 500 μM. Each sample was incubated with each substrate in four replicate wells. Each microplate also included replicate streamwater, buffer, and substrate controls. Microplates were incubated in the dark at 22 °C for 4 h and were measured at regular intervals using a Molecular Devices SpectraMAX Gemini XPS plate-reading spectrofluorometer. Standard curves were developed using MUB and coumarin standards. At the conclusion of the experiment, photoquenching by samples was measured by spiking buffer and streamwater controls with 50 μL of 1 μM standard. All standards and substrates were obtained from Sigma-Aldritch.

Landscape Analysis. A digital elevation model (DEM) of the Parkers Creek watershed was generated from Light Detection and Ranging (LiDAR) data collected in March 2011 and provided by Calvert County, MD Government. Field site watersheds were extracted from the DEM. Impervious, forested, and agricultural land cover were manually delineated using orthophotography collected in March 2011 and provided by Calvert County, MD Government. Septic system geospatial data were obtained from Maryland Department of the Environment reports.[44] All landscape analysis was conducted using ArcGIS 10.1.

Data Analysis. Repeated-measures analysis of variance (ANOVA), analysis of covariance (ANCOVA), and linear regressions were carried out in SAS 9.3 using the *Proc Mixed* package. Normality of ANOVA and ANCOVA variables was evaluated by assessing residuals. Variables that deviated substantially from normality were transformed by taking the

logarithm of the variable plus one. Compound symmetry covariance structures were assumed for all ANCOVA analysis. Multiple mean comparisons for repeated-measures ANOVA were conducted using Tukey's Honestly Significant Difference (HSD) test. For bioavailability analysis, sampling dates were broken into two groups, "Fall/Winter" and "Spring/Summer." A seasonal term and interaction were included for all statistical analyses of bioavailability data. Principal component analysis (PCA) was conducted using the *princomp* function in R version 3.0.2. Normality of all variables used for PCA was assessed with histograms and linearity was assessed with bivariate scatterplots. Prior to analysis, an alpha level of 0.05 was set for all analyses.

RESULTS AND DISCUSSION

DOM Quantity and Quality across Urbanization Gradient. Overall DOM quality at sites, assessed using principal component analysis (PCA) of the five validated PARAFAC components, demonstrated distinct differences in DOM composition between forested versus urbanized streams, as indicated by a plot of the first two PCA components (Figure 2). Forested catchments were characterized by PARAFAC components C1 and C2 and percent fluorescence of both components was positively related to log-transformed percent watershed impervious cover (Figure 3a and b; SI Table S2). Previous studies have linked PARAFAC components similar to C1 and C2 to terrestrial sources of organic matter[19,22,31] with C2 reflecting recalcitrant, terrestrial humic substances[22,32] and C1 more photolabile sources associated with streams in forested[19,22] and agricultural[31] watersheds. This is consistent with an interpretation that the DOM found in Parkers Creek forested headwater streams was represented by compounds originating from forest soils and higher plants.

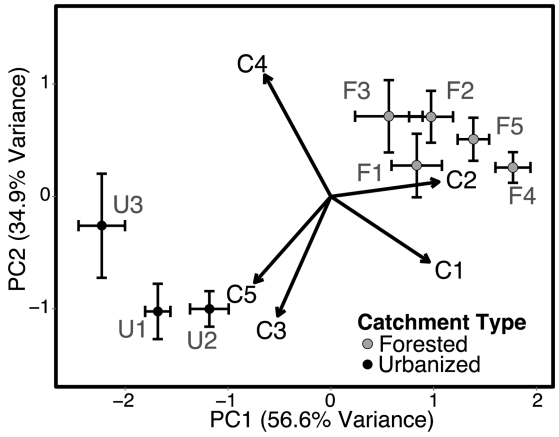

Figure 2. Principal component analysis (PCA) of DOM composition of forested (F1, F2, F3, F4, F5) and urbanized (U1, U2, and U3) stream sites as measured by percent Fmax of DOM PARAFAC components (C1, C2, C3, C4, and C5). Error bars indicate standard error at each site for principal components 1 and 2. The percentages indicated in the axis titles indicate the percent variance explained by each principal component of the PCA.

PARAFAC components C3 and C5 were more prevalent in urbanized sites (Figure 2). Percent fluorescence of component C3 and log-transformed percent fluorescence of component C5 were both positively related to log-transformed watershed impervious cover (Figure 3c and d; SI Table S2). Component C3 most likely represents anthropogenic humic acids that are microbially produced and has been linked to agricultural land use and point sources like wastewater effluent[22] but these impacts are not characteristic of the streams studied. The EEM modeled for component C5 is tyrosine-like[8] and hence likely a product of aquatic carbon fixation and relatively labile.

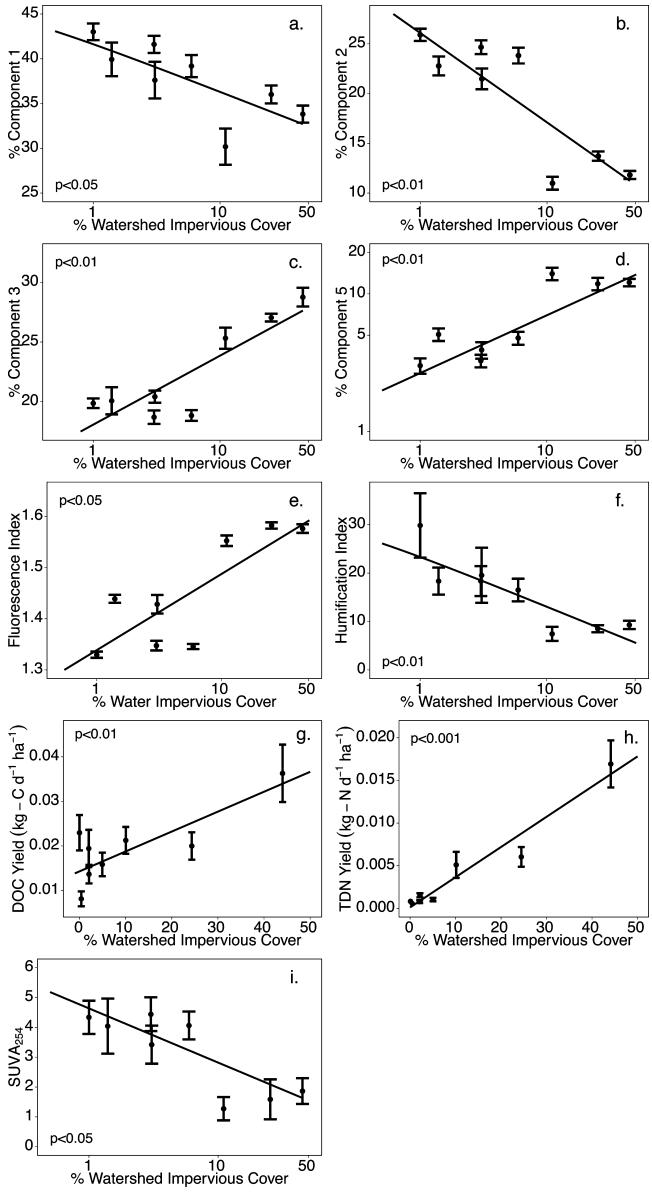

Figure 3. Percent catchment impervious cover as a measure of nonpoint source urban impacts versus mean value by stream sampling site of (a) percent F_{max} component 1, (b) percent F_{max} component 2, (c) percent F_{max} component 3, (d) log-transformed percent F_{max} component 5, (e) fluorescence index, (f) humification index, (g) watershed DOC yield, (h) watershed TDN yield, and (i) $SUVA_{254}$. Impervious cover was related to water chemistry using repeated-measures linear regression. Error bars represent standard error of the mean by site.

The DOM absorbance metrics provided further evidence of a DOM quality shift with increasing impervious cover. Aromaticity of DOM was measured via $SUVA_{254}$, which was negatively correlated with log-transformed impervious cover (Figure 3i). Lower DOM aromaticity in the urban streams provided verification that this material is of recent, microbial origin.

A significant positive relationship between catchment DOC yield and percent watershed impervious cover was found (Figure 3g; SI Table S2). By contrast, neither DOC flux nor concentration was significantly related to catchment impervious cover (SI Table S2), suggesting that increased DOC yield was related to altered hydrology in urbanized catchments.

Possible Urban Sources of DOM. The measured changes in DOM quality (lower molecular weight and aromaticity) reported here support Hypothesis 1, that the urbanized streams have become enriched in the same forms of DOM found in other anthropogenically impacted waters (e.g., agricultural streams and wastewater effluent[10,17,19]). The ecosystem processes driving these changes were less clear. DOM quality across the three urbanized streams was very similar, yet the nature of the anthropogenic impacts was more variable across the urbanized watersheds. The watershed of site U1 is 44.0% impervious and is dominated by commercial space and asphalt parking lots. By contrast, the primary human impacts in watersheds U2 and U3 were residential housing and associated roadways. Site U3 receives water directly from a stormwater management pond; no other watersheds included such a structure.

One possible explanation for the altered DOM composition is increased primary production within and around urbanized streams. Fluorescence index values and log-transformed percent watershed impervious cover were positively correlated (Figure 3e; SI Table S2). Increasing FI values indicate greater prevalence of aquatically fixed DOM in urbanized streams,[37] providing evidence of the autochthonous nature of urbanized DOM. Total dissolved nitrogen (TDN) yields and concentrations were strongly positively related to percent catchment imperviousness (Figure 3h; SI Table S2) which would enhance autochthonous production in streams.[45] Primary production may also have been stimulated by lower canopy cover in urbanized streams.[45]

There are also potential nonpoint sources of anthropogenic DOM in urban landscapes. There are few septic systems within any of the study watersheds (SI Table S1), which precluded examination of this factor. While the urbanized watersheds do not contain farmland or point source effluent inputs, all three urbanized watersheds do contain sanitary sewer infrastructure so it is possible there are sewer pipe leaks into streams. Petroleum-based hydrocarbons from urban landscapes may also be responsible for inputs of small, labile fractions of DOM from urbanized or suburbanized landscapes.[24]

Microbial Use and DOM Quality. Repeated-measures ANOVA comparing bioavailability between urbanized and forested sites incorporating seasons (spring/summer, $n = 2$; fall/winter, $n = 3$) revealed an interaction between season and watershed land cover ($F_{(1,6)} = 15.7$, $p < 0.01$). Bioavailability was significantly higher in urbanized versus forested streams but only during sampling events in spring and summer (Tukey's HSD, $p < 0.05$; SI Table S3).

To determine if seasonal variation in DOM bioavailability was associated with DOM composition, these variables were compared by season using repeated-measures ANCOVA. Protein-like components, C4 and C5, were combined as a single variable, "protein-like DOM," for bioavailability analysis following a previous approach.[8] Significant seasonal interactions were found when bioavailability was compared to percent fluorescence of PARAFAC components C1 and C2, percent protein-like components, HIX, and $SUVA_{254}$ (SITable S3).

Surprisingly, significant relationships between carbon quality and percent BDOC were only found for samples collected in warmer months with no significant relationships identified in colder seasons. Reflecting lower bioavailability of humic substances, PARAFAC components C1 and C2 as well as HIX and $SUVA_{254}$ were negatively correlated with bioavailability for samples collected in spring and summer (Figure 4a, b, f, and g). Percent fluorescence of protein-like DOM was positively correlated to percent BDOC in the spring and summer (Figure 4d), as expected for this typically labile material.

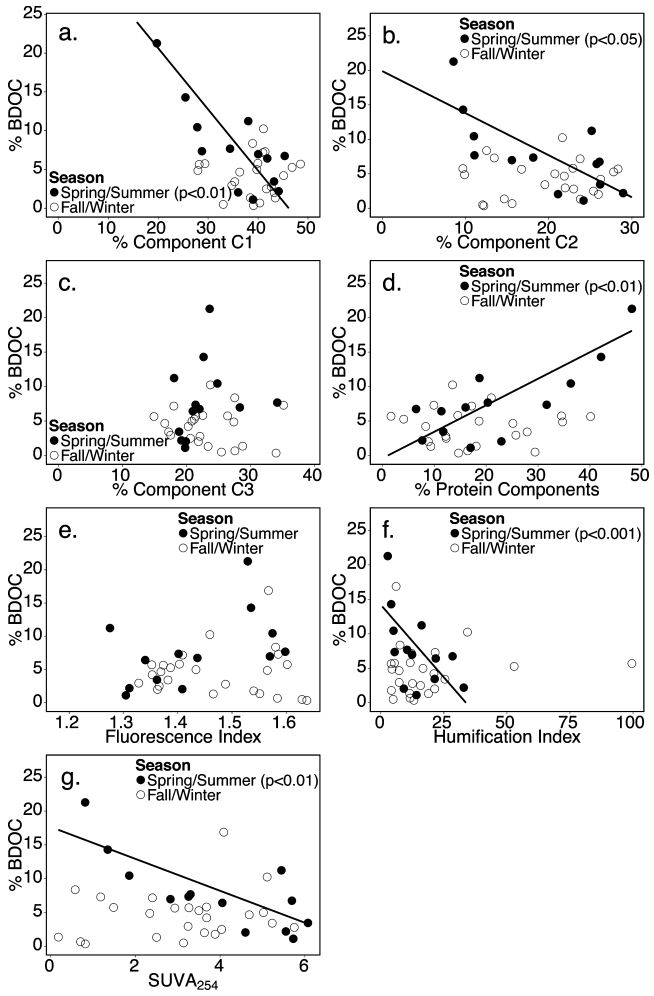

Figure 4. Percent BDOC compared to DOM spectroscopy and water chemistry variables from individual water samples. Statistical comparisons were made between BDOC and (a) percent PARAFAC component C1, (b) percent PARAFAC component C2, (c) percent PARAFAC component C3, (d) percent fluorescence of protein-like PARAFAC components, (e) the fluorescence index, (f) the humification index, and (g) $SUVA_{254}$. Statistics presented are repeated-measures ANCOVA tests. A significant interaction across season was found when percent BDOC was compared to percent PARAFAC component C1, percent PARAFAC component C2, percent protein-like components, humification index values, and $SUVA_{254}$.

These results only partially support the second hypothesis that DOM from urban watersheds would be more readily used by microbes because higher bioavailability was only seen during warmer seasons. During colder months microbial use was

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significantly lower and seemingly decoupled from DOM quality. This suggests that other factors such as nutrient limitation or changes in the heterotrophic activity of the microbial inoculum were responsible for the seasonal differences in DOM bioavailability.

To test for a seasonal nutrient limitation effect, repeated-measures ANCOVA analysis was used to compare BDOC to each fluorescence metric used in this study with dissolved N:P ratios included as a covariate. Sample N:P ratio did not approach significance as a covariate for any PARAFAC component. Stream water N:P ratios were significantly greater in urbanized streams compared to forested streams across all seasons ($p < 0.001$) and the urban N:P ratios did not differ between seasons (SI Figure S4).

Microbial Use of DOM and Heterotrophic Activity. For each bioassay sampling date, a fresh microbial inoculum was collected. Microbial community composition in streams can vary seasonally;[46] thus, if the heterotrophic activity of microbial inocula changed between sampling dates, this may explain observed differences in bioavailability predicted by Hypothesis 3. To test this hypothesis, samples for EEA analysis were taken from site P1 contemporaneously with microbial inoculum collection. Activity of all three enzymes measured—aminopeptidase, phosphatase, and esterase—was greatest in April and August 2012 (Figure 5a.). Increasing EEA coincided with increased percent BDOC in both urbanized and forested streamwater samples (Figure 5b—d.). These results are limited but suggest that seasonal changes in microbial activity can be an important factor determining microbial use of DOM.

Ecosystem Implications. Increasing watershed impervious cover was related to a shift from complex, recalcitrant DOM to smaller, more microbially available compounds. These observed changes are related to increased production of microbially sourced DOM in urbanized streams and watersheds that lack point source inputs. This work supports our first two hypotheses and bolsters suggestions that nonpoint source effects of urbanization are altering organic carbon dynamics both across watersheds and in stream ecosystems.[23,24,47]

Similar relationships between DOM composition, as measured by fluorescence spectroscopy and bioavailability, have been reported elsewhere[8,9,48] (but see ref 49 for contrasting results); however to our knowledge this study is the first to link such changes to urbanization via impervious cover exclusively. The results presented here also demonstrate higher BDOC in streamwater samples during summer months and decreased bioavailability in winter months in streams impacted by urbanization. This seasonal variation has implications for headwater and downstream systems alike. Such increased variability and altered timing of DOM export may interact with other seasonal dynamics in unpredictable ways and represents an important avenue for future research.

Direct evidence of ecosystem impacts in urbanized streams due to altered carbon composition is limited. Experimental additions of labile DOM sources to forested streams demonstrated increases in bacterial production and respiration[14] and subsequent transfer of organic carbon to higher trophic levels.[13] More research is required to determine if urban stream processes respond similarly to increased lability of organic carbon. The change observed in DOM composition and bioavailability in urbanized streams of the Parkers Creek watershed has significant implications for understanding functioning of many stream ecosystems, particularly because these urbanized watersheds lack the point source and septic

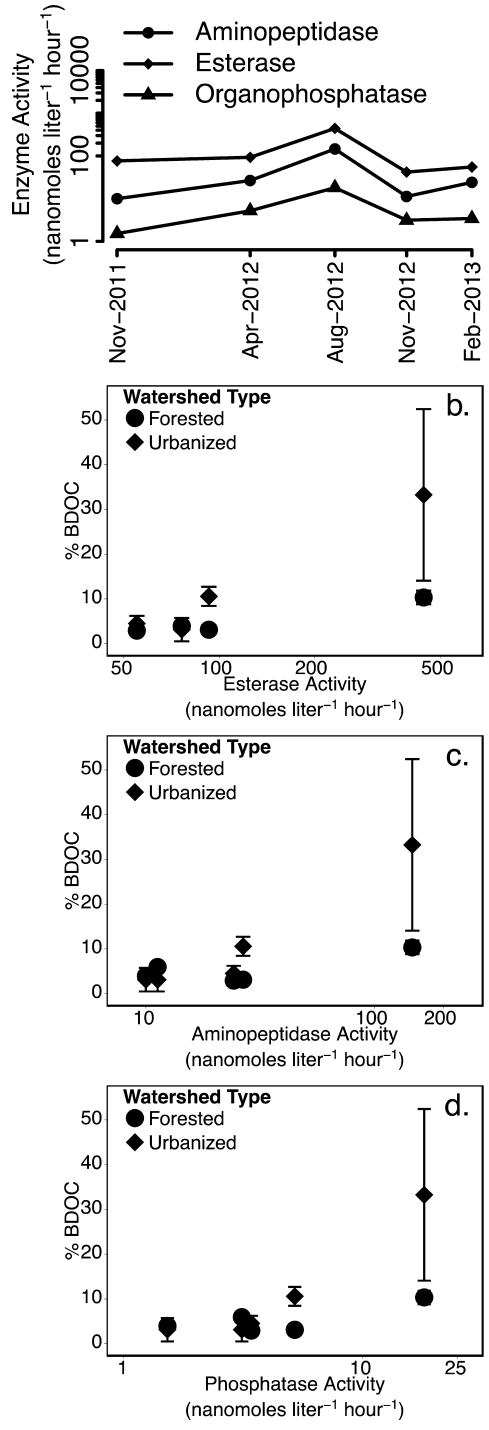

Figure 5. On each bioassay sampling date, an inoculum collected at a common downstream site was amended to the samples at the start of incubations (i.e., a common inoculum was used across sites but varied by date). Aminopeptidase, organophosphatase, and esterase enzyme activities of water collected from site P1 are (a) plotted over time. Activity of (b) phosphatase, (c) esterase, and (d) aminopeptidase extracellular enzymes associated with the microbial inoculum were compared to percent BDOC for all incubations conducted on that date. BDOC of urbanized and forested streams were considered separately. Error bars represent percent BDOC standard error across streams of a given watershed type.

inputs that were assumed to be major sources of anthropogenic DOM in streams. The fact that increased presence of altered DOM found in urbanized streams was related to increased

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heterotrophic use of DOM during spring and summer months also has substantial implications because increases of stream DOM lability have been shown to change stream nutrient dynamics,[14] redirect organic carbon to bacterial growth and respiration,[14] and alter the taxonomic composition of the heterotrophic microbial communities that rely on this energy source.[7,50] Ultimately, the fate of stream organic carbon changes significantly, from being transported downstream to being diverted directly to microbial production and respiration,[6] potentially resulting in greater CO_2 exports from urbanized streams. As urban populations globally continue to expand,[51] increased stream DOM bioavailability may impact not only individual streams but the carbon cycle at large.

■ ASSOCIATED CONTENT

Supporting Information

Content includes Figures S1, S2, S3, and S4 and Tables S1, S2, and S3. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Cole, J. J.; Prairie, Y. T.; Caraco, N. F.; McDowell, W. H.; Tranvik, L. J.; Striegl, R. G.; Duarte, C. M.; Kortelainen, P.; Downing, J. A.; Middelburg, J. J.; et al. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems* **2007**, *10*, 172−185.

(2) Butman, D.; Raymond, P. A.; Butler, K.; Aiken, G. Relationships between Δ14-C and the molecular quality of dissolved organic carbon in rivers draining to the coast from the conterminous United States. *Global Biogeochem. Cycles* **2012**, *26*, GB4014.

(3) Aitkenhead-Peterson, J.; McDowell, W.; Neff, J.; Stuart, E.; Robert, L. *Sources, Production, and Regulation of Allochthonous Dissolved Organic Matter Inputs to Surface Waters*; Academic Press: San Diego, 2003.

(4) Gomi, T.; Sidle, R. C.; Richardson, J. S. Understanding processes and downstream linkages of headwater systems. *BioScience* **2002**, *52*, 905.

(5) Wipfli, M. S.; Richardson, J. S.; Naiman, R. J. Ecological linkages between headwaters and downstream ecosystems: Transport of organic matter, invertebrates, and wood down headwater channels. *J. Am. Water Resour. Assoc.* **2007**, *43*, 72−85.

(6) Battin, T. J.; Kaplan, L. A.; Findlay, S.; Hopkinson, C. S.; Marti, E.; Packman, A. I.; Newbold, J. D.; Sabater, F. Biophysical controls on organic carbon fluxes in fluvial networks. *Nat. Geosci.* **2008**, *1*, 95−100.

(7) Judd, K. E.; Crump, B. C.; Kling, G. W. Variation in dissolved organic matter controls bacterial production and community composition. *Ecology* **2006**, *87*, 2068−2079.

(8) Fellman, J. B.; D'Amore, D. V.; Hood, E.; Boone, R. D. Fluorescence characteristics and biodegradability of dissolved organic matter in forest and wetland soils from coastal temperate watersheds in southeast Alaska. *Biogeochemistry* **2008**, *88*, 169−184.

(9) Cory, R. M.; Kaplan, L. A. Biological lability of streamwater fluorescent dissolved organic matter. *Limnol. Oceanogr.* **2012**, *57*, 1347−1360.

(10) Wilson, H. F.; Xenopoulos, M. A. Effects of agricultural land use on the composition of fluvial dissolved organic matter. *Nat. Geosci.* **2008**, *2*, 37−41.

(11) Sivirichi, G. M.; Kaushal, S. S.; Mayer, P. M.; Welty, C.; Belt, K. T.; Newcomer, T. A.; Newcomb, K. D.; Grese, M. M. Longitudinal variability in streamwater chemistry and carbon and nitrogen fluxes in restored and degraded urban stream networks. *J. Environ. Monit.* **2011**, *13*, 288−303.

(12) Gücker, B.; Brauns, M.; Solimini, A. G.; Voss, M.; Walz, N.; Pusch, M. T. Urban stressors alter the trophic basis of secondary production in an agricultural stream. *Can. J. Fish. Aquat. Sci.* **2011**, *68*, 74−88.

(13) Hall, R. O., Jr; Meyer, J. L. The trophic significance of bacteria in a detritus-based stream food web. *Ecology* **1998**, *79*, 1995−2012.

(14) Bernhardt, E. S.; Likens, G. E. Dissolved organic carbon enrichment alters nitrogen dynamics in a forest stream. *Ecology* **2002**, *83*, 1689−1700.

(15) Newcomer, T. A.; Kaushal, S. S.; Mayer, P. M.; Shields, A. R.; Canuel, E. A.; Groffman, P. M.; Gold, A. J. Influence of natural and novel organic carbon sources on denitrification in forest, degraded urban, and restored streams. *Ecol. Monogr.* **2012**, *82*, 449−466.

(16) Richardson, M. C.; Fortin, M.-J.; Branfireun, B. A. Hydrogeomorphic edge detection and delineation of landscape functional units from lidar digital elevation models. *Water Resour. Res.* **2009**, *45*, W10441.

(17) Williams, C. J.; Yamashita, Y.; Wilson, H. F.; Jaffé, R.; Xenopoulos, M. A. Unraveling the role of land use and microbial activity in shaping dissolved organic matter characteristics in stream ecosystems. *Limnol. Oceanogr.* **2010**, *55*, 1159−1171.

(18) Stanley, E. H.; Powers, S. M.; Lottig, N. R.; Buffam, I.; Crawford, J. T. Contemporary changes in dissolved organic carbon (DOC) in human-dominated rivers: Is there a role for DOC management? *Freshwater Biol.* **2012**, *57*, 26−42.

(19) Stedmon, C. A.; Markager, S.; Bro, R. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. *Mar. Chem.* **2003**, *82*, 239−254.

(20) Baker, A.; Spencer, R. G. M. Characterization of dissolved organic matter from source to sea using fluorescence and absorbance spectroscopy. *Sci. Total Environ.* **2004**, *333*, 217−232.

(21) Sickman, J. O.; Zanoli, M. J.; Mann, H. L. Effects of urbanization on organic carbon loads in the Sacramento River, California: Urbanization and riverine carbon loads. *Water Resour. Res.* **2007**, *43*, W11422.

(22) Stedmon, C. A.; Markager, S. Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. *Limnol. Oceanogr.* **2005**, *50*, 686−697.

(23) Imberger, S. J.; Cook, P. L. M.; Grace, M. R.; Thompson, R. M. Tracing carbon sources in small urbanising streams: Catchment-scale stormwater drainage overwhelms the effects of reach-scale riparian vegetation. *Freshwater Biol.* **2014**, *59*, 168−186.

(24) McElmurry, S. P.; Long, D. T.; Voice, T. C. Stormwater dissolved organic matter: Influence of land cover and environmental factors. *Environ. Sci. Technol.* **2013**, *48*, 49−53.

(25) Baum, R.; Luh, J.; Bartram, J. Sanitation: A global estimate of sewerage connections without treatment and the resulting impact on MDG progress. *Environ. Sci. Technol.* **2013**, *47*, 1994−2000.
(26) Andersson, C. A.; Bro, R. The N-way Toolbox for MATLAB. *Chemom. Intell. Lab. Syst.* **2000**, *52*, 1−4.
(27) Stedmon, C. A.; Bro, R. Characterizing dissolved organic matter fluorescence with parallel factor analysis: A tutorial. *Limnol Ocean. Methods* **2008**, *6*, 572−579.
(28) Cory, R. M.; Miller, M. P.; McKnight, D. M.; Guerard, J. J.; Miller, P. L. Effect of instrument-specific response on the analysis of fulvic acid fluorescence spectra. *Limnol. Oceanogr. Methods* **2010**, *8*, 67−78.
(29) Helms, J. R.; Stubbins, A.; Ritchie, J. D.; Minor, E. C.; Kieber, D. J.; Mopper, K. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnol. Oceanogr.* **2008**, *53*, 955.
(30) Weishaar, J. L.; Aiken, G. R.; Bergamaschi, B. A.; Fram, M. S.; Fujii, R.; Mopper, K. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environ. Sci. Technol.* **2003**, *37*, 4702−4708.
(31) Osburn, C. L.; Handsel, L. T.; Mikan, M. P.; Paerl, H. W.; Montgomery, M. T. Fluorescence tracking of dissolved and particulate organic matter quality in a river-dominated estuary. *Environ. Sci. Technol.* **2012**, *46*, 8628−8636.
(32) Lutz, B. D.; Bernhardt, E. S.; Roberts, B. J.; Cory, R. M.; Mulholland, P. J. Distinguishing dynamics of dissolved organic matter components in a forested stream using kinetic enrichments. *Limnol. Oceanogr.* **2012**, *57*, 76−89.
(33) Yamashita, Y.; Jaffé, R.; Male, N.; Tanoue, E. Assessing the dynamics of dissolved organic matter (DOM) in coastal environments by excitation emission matrix fluorescence and parallel factor analysis (EEM-PARAFAC). *Limnol. Oceanogr.* **2008**, *53*, 1900−1908.
(34) Cory, R. M.; McKnight, D. M. Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. *Environ. Sci. Technol.* **2005**, *39*, 8142−8149.
(35) Coble, P. G. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Mar. Chem.* **1996**, *51*, 325−346.
(36) O'Donnell, J. A.; Aiken, G. R.; Walvoord, M. A.; Butler, K. D. Dissolved organic matter composition of winter flow in the Yukon River basin: Implications of permafrost thaw and increased groundwater discharge: DOM Composition of Winter Flow. *Global Biogeochem. Cycles* **2012**, *26*, GB0E06.
(37) McKnight, D. M.; Boyer, E. W.; Westerhoff, P. K.; Doran, P. T.; Kulbe, T.; Andersen, D. T. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnol. Oceanogr.* **2001**, *46*, 38−48.
(38) Zsolnay, A.; Baigar, E.; Jimenez, M.; Steinweg, B.; Saccomandi, F. Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. *Chemosphere* **1999**, *38*, 45−50.
(39) Plaza, C.; Xing, B.; Fernández, J. M.; Senesi, N.; Polo, A. Binding of polycyclic aromatic hydrocarbons by humic acids formed during composting. *Environ. Pollut.* **2009**, *157*, 257−263.
(40) McDowell, W. H.; Zsolnay, A.; Aitkenhead-Peterson, J. A.; Gregorich, E. G.; Jones, D. L.; Jödemann, D.; Kalbitz, K.; Marschner, B.; Schwesig, D. A comparison of methods to determine the biodegradable dissolved organic carbon from different terrestrial sources. *Soil Biol. Biochem.* **2006**, *38*, 1933−1942.
(41) Hill, B. H.; Elonen, C. M.; Seifert, L. R.; May, A. A.; Tarquinio, E. Microbial enzyme stoichiometry and nutrient limitation in US streams and rivers. *Ecol. Indic.* **2012**, *18*, 540−551.
(42) Sinsabaugh, R. L. Large-scale trends for stream benthic respiration. *J. North Am. Benthol. Soc.* **1997**, *16*, 119−122.
(43) Findlay, S. Dissolved organic matter. In *Methods in Stream Ecology*; Hauer, F. R.; Lamberti, G. A., Eds.; Academic Press, 2007; pp 293−311.
(44) *Chesapeake Bay TMDL Phase 1 Watershed Implementation Plan: Decentralized Wastewater Management Gap Closer Research and Analysis*; Tetra Tech: Research Triangle Park, NC, 2011; p 41.
(45) Bernot, M. J.; Sobota, D. J.; Hall, R. O.; Mulholland, P. J.; Dodds, W. K.; Webster, J. R.; Tank, J. L.; Ashkenas, L. R.; Cooper, L. W.; Dahm, C. N.; et al. Inter-regional comparison of land-use effects on stream metabolism. *Freshwater Biol.* **2010**, *55*, 1874−1890.
(46) Hullar, M. A. J.; Kaplan, L. A.; Stahl, D. A. Recurring seasonal dynamics of microbial communities in stream habitats. *Appl. Environ. Microbiol.* **2006**, *72*, 713−722.
(47) Kaushal, S. S.; Delaney-Newcomb, K.; Findlay, S. E. G.; Newcomer, T. A.; Duan, S.; Pennino, M. J.; Sivirichi, G. M.; Sides-Raley, A. M.; Walbridge, M. R.; Belt, K. T. Longitudinal patterns in carbon and nitrogen fluxes and stream metabolism along an urban watershed continuum. *Biogeochemistry* **2014**, 1−22.
(48) Petrone, K. C.; Fellman, J. B.; Hood, E.; Donn, M. J.; Grierson, P. F. The origin and function of dissolved organic matter in agro-urban coastal streams. *J. Geophys. Res. Biogeosci.* **2011**, *116*, G01028.
(49) Lu, Y.; Bauer, J. E.; Canuel, E. A.; Yamashita, Y.; Chambers, R. M.; Jaffé, R. Photochemical and microbial alteration of dissolved organic matter in temperate headwater streams associated with different land use. *J. Geophys. Res. Biogeosci.* **2013**, *118*, 566−580.
(50) Kirchman, D. L.; Dittel, A. I.; Findlay, S. E. G.; Fischer, D. Changes in bacterial activity and community structure in response to dissolved organic matter in the Hudson River, New York. *Aquat. Microb. Ecol.* **2004**, *35*, 243−257.
(51) United Nations, Dept. of Economic and Social Affairs, Population Division. *World Urbanization Prospects: The 2011 Revision*; UN, 2012.