Biogeochemistry (2023) 164:117–141
https://doi.org/10.1007/s10533-021-00852-1

Anthropogenic landcover impacts fluvial dissolved organic matter composition in the Upper Mississippi River Basin

Derrick R. Vaughn · Anne M. Kellerman · Kimberly P. Wickland · Robert G. Striegl · David C. Podgorski · Jon R. Hawkings · Jaap H. Nienhuis · Mark M. Dornblaser · Edward G. Stets · Robert G. M. Spencer

Received: 19 May 2021 / Accepted: 1 September 2021 / Published online: 7 October 2021

Abstract Landcover changes have altered the natural carbon cycle; however, most landcover studies focus on either forest conversion to agriculture or urban, rarely both. We present differences in dissolved organic carbon (DOC) concentrations and dissolved organic matter (DOM) molecular composition within Upper Mississippi River Basin low order streams and rivers draining one of three dominant landcovers (forest, agriculture, and urban). Streams draining forest and urban landcovers have greater DOC concentrations, likely driven by differences in carbon sourcing, microbial processing, and soil disturbance. Using Fourier transform-ion cyclotron resonance mass spectrometry, 24% of assigned molecular formulae are common across all landcovers. Relative abundances of N-,S- heteroatomic formulae (CHON, CHOS, CHONS) are higher for agricultural and urban streams, with agricultural stream DOM having more N-containing formulae compared to urban stream DOM, which has more S-containing formulae. Higher N-,S- heteroatomic formulae abundance, along with enrichment in aliphatic, N-aliphatic, and highly unsaturated and phenolic (low O/C) compound

Responsible editor: Penny Johnes.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10533-021-00852-1.

Derrick R. Vaughn and Anne M. Kellerman have contributed equally to this work.

D. R. Vaughn (✉) · A. M. Kellerman ·
J. R. Hawkings · R. G. M. Spencer
National High Magnetic Field Laboratory Geochemistry Group, Department of Earth, Ocean, and Atmospheric Science, Florida State University, Tallahassee, Fl, USA
e-mail: dvaughn2@fsu.edu

K. P. Wickland · R. G. Striegl · M. M. Dornblaser
Water Resources Mission Area, U.S. Geological Survey, Boulder, CO, USA

D. C. Podgorski
Pontchartrain Institute for Environmental Sciences, Department of Chemistry, Chemical Analysis and Mass Spectrometry Facility, University of New Orleans, New Orleans, LA, USA

J. R. Hawkings
Department of Earth and Environmental Science, University of Pennsylvania, Philadelphia, Pennsylvania, USA

J. H. Nienhuis
Department of Physical Geography, Utrecht University, Utrecht, Netherlands

E. G. Stets
U.S. Geological Survey, Mounds View, MN, USA

categories within agricultural and urban stream DOM are likely to result from increased anthropogenic inputs, autochthonous production, and microbial processing associated with agricultural and urban impacts. Reduced N-,S- heteroatomic formulae abundances in forested stream DOM, along with enrichments in condensed aromatics, polyphenolics, and highly unsaturated phenolic (high O/C) compound categories, likely reflect greater contributions from surrounding organic-rich forest soil and vegetation. Overall, landcover change from forested to agriculture lowers DOC concentrations and changes from forested to agriculture or urban increases autochthonous, and presumably more biolabile, DOM contributions with ramifications for stream biogeochemical cycling.

Keywords Dissolved organic matter · Dissolved organic carbon · FT-ICR MS · Landcover · Agriculture · Urbanization

Introduction

An increase in global human population from ~ 2.5 billion in 1950 to over 7 billion in 2020 has led to significant landcover alteration, converting historically forested lands into areas dominated by agriculture and urban uses. Currently, around 40% of global land surface is used for crop production and pasture (Alexandratos and Bruinsma 2012). Although urban areas make up a relatively small fraction of Earth's surface, they are, on average, expanding faster than population growth (Seto et al. 2011). With a potential to reach 12.6 billion people globally by 2100 (KC and Lutz 2017), continued expansion of agricultural and urban areas will likely lead to irreversible biophysical changes to the environment.

Dissolved organic matter (DOM) has been the subject of many studies looking at the effects of landcover on freshwater systems because its composition integrates upstream biogeochemical processes. Previous studies indicate streams draining predominantly agricultural (Wilson and Xenopoulos 2009; Tsui and Finley 2011; Stanley et al. 2012; Lu et al. 2014; Graeber et al. 2015; Heinz et al. 2015; Gücker et al. 2016; Spencer et al. 2019) and/or urban landcovers (Sickman et al. 2007; Aitkenhead-Peterson et al. 2009; Silva et al. 2011; Tsui and Finley 2011; Kaushal and Belt 2012; Hosen et al. 2014; Parr et al. 2015; Gücker et al. 2016) display a range of DOM compositions and dissolved organic carbon (DOC) concentrations distinct to streams draining forest landcover, reflecting agricultural and wastewater treatment practices and associated terrestrial and aquatic carbon cycling (Guo and Gifford 2002; Stanley et al. 2012; Gücker et al. 2016). These DOM land use studies have typically addressed differences in DOM centered on one anthropogenic landcover type (e.g., agricultural or urban) versus forested watersheds, and the majority have focused on bulk DOM properties (e.g., DOC concentration and optical measurements such as absorbance and/or fluorescence). Thus, to-date it is still uncertain how agriculture and urbanization within the same watershed will change the composition and biogeochemical fate of exported DOM. Understanding how DOM composition changes with landcover ultimately informs the fate of stream organic matter and in-stream organic carbon (OC) respiration as both are closely linked to composition (Fasching et al. 2014; D'Andrilli et al. 2019).

Here we improve our understanding of anthropogenic landcover impacts on fluvial DOC concentration and DOM composition by comparing streams draining agriculture, urban, and forested/wetland landcovers (referred to hereon as forest or forested streams for simplification) together in the Upper Mississippi River Basin (UMRB). Distinct landcover variability within sub-basins of the UMRB make it an ideal temperate study area in the United States to compare landcover effects on DOC concentration and DOM molecular composition. Landcover changes in the UMRB since the mid-20th century has been dominated by cropland expansion (Ramankutty and Foley 1999; Schnitkey 2013; Wright and Wimberly 2013) and sustained population growth (3.8% between 2000 and 2009; Eathington 2010) has led to increased urbanization, both of which are likely to continue (e.g. DeFries et al. 2004; Tilman et al. 2001; Rajib and Merwade 2017). We hypothesize stream DOC concentrations within UMRB streams will reflect landcover variation based on differences in organic-rich soil and vegetation contributions between landcovers as well as differences with soil disturbance. Using Fourier transform-ion cyclotron resonance mass spectrometry (FT-ICR MS) to fingerprint the molecular composition of stream DOM, we hypothesize the composition of DOM in streams draining agricultural and urban landcovers will reflect higher autochthonous DOM production compared to DOM in streams draining forested landcovers, which are hypothesized to have higher allochthonous DOM inputs.

Methods

Site description and sample collection

The Upper Mississippi River Basin is defined here as the area drained by the Mississippi River at Wabasha, MN (Fig. 1). The UMRB is more than 800 km upstream of the confluence of the Missouri and Ohio Rivers to the Mississippi River and is more sensitive to seasonal variations and source contributions compared to the downstream reaches of the Mississippi River (Voss et al. 2017). Streams and rivers within the UMRB cut though geologies of variable glacial influence (e.g., Kelley et al. 2006; Blumentritt et al. 2009) and drain a wide range of landcover (forested, agriculture, wetland, and urban areas; Table S1). While forested landcovers tend to have less anthropogenic influence than urban and agricultural landcovers, much of the forested area in the UMRB was logged during the mid 1800s to early 1900s and is now covered by secondary forest growth (Stark et al. 2000). Agricultural lands within the UMRB are used to produce row crops, such as corn and soybeans, and many agricultural processes associated with these lands have been linked to alterations in river discharge, including tile drainage, fertilizer use, irrigation, tillage practices, and changes in crop type (Raymond et al. 2008).

Nine streams with varying drainage area in the northern portion of the UMRB were sampled (Fig. 1) and assigned a landcover classification based on

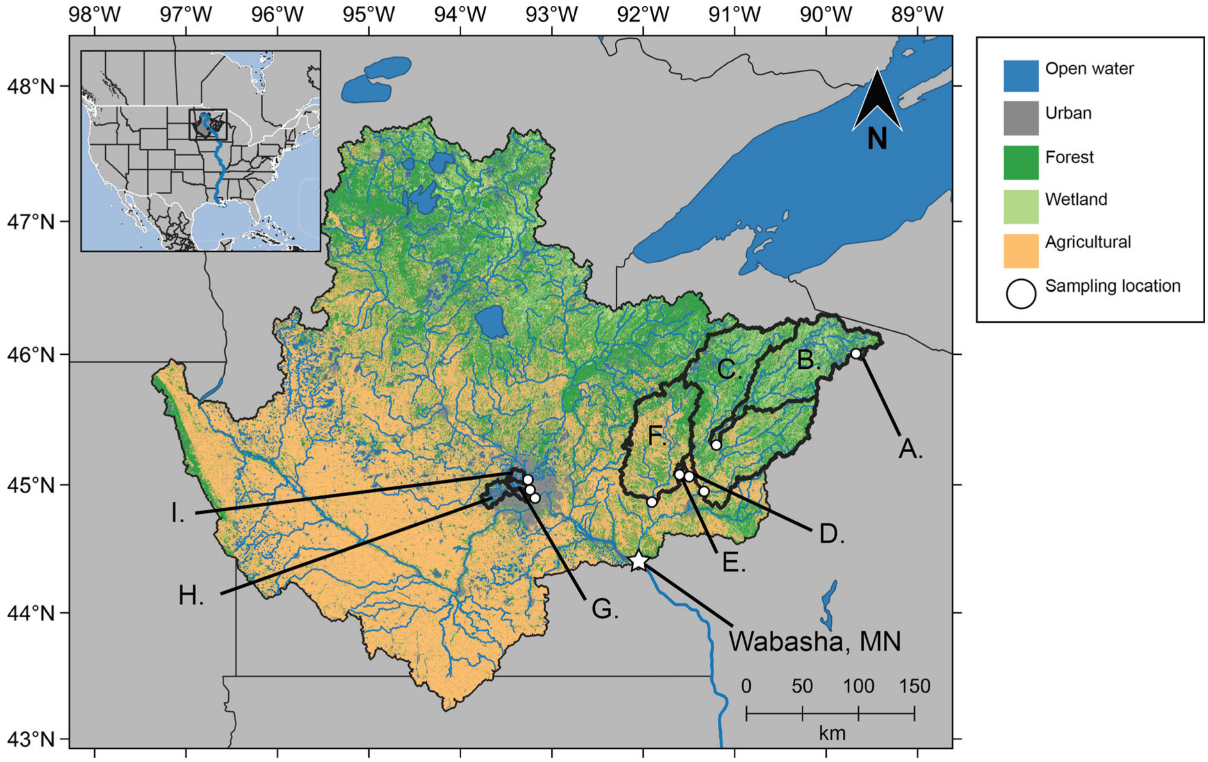

Fig. 1 Study region. Inset map shows region outlined in black and the full length of the main stem Mississippi River outlined in blue. The streams/rivers and their watersheds are labeled as follows: (*A*) Allequash Creek (forest); (*B*) Flambeau River (forest); (*C*) Chippewa River (forest); (*D*) Como Creek (agriculture); (*E*) Trout Creek (agriculture); (*F*) Red Cedar River (agriculture); (*G*) Bassett Creek (urban); (*H*) Minnehaha Creek (urban); (*I*) Shingle Creek (urban). The forest landcover classification for sites is based on the combined forest and wetland areas (light green and dark green colors; see Table S1). (Color figure online)

percent landcover (Table S1). Most sampling sites are within 1 km of a U.S. Geological Survey (USGS) stream gage (Table S2). Only Bassett Creek and Como Creek do not have discharge data from the study period. Landcover was analyzed using QGIS 3.10 software and was based on the 2016 National Land Cover Database (Yang et al. 2018), with watershed shapefiles acquired from the USGS StreamStats program (US Geological Survey 2012). A threshold percentage of 40% was used to indicate whether a stream had a significant portion of land as agriculture or urban landcover to be classified as agriculture or urban streams, respectively. This is consistent with findings from previous studies that examine DOC and DOM changes along gradients of agricultural (e.g. Wilson and Xenopoulos, 2009) and urban (e.g. Aitkenhead-Peterson et al. 2009; Hosen et al. 2014) landcovers. Three streams draining the Minneapolis—Saint Paul metropolitan area are dominated by urban landcover (Bassett Creek, Minnehaha Creek, and Shingle Creek); three streams/rivers in rural Wisconsin are dominated by agricultural landcover (Como Creek, Trout Creek, and Red Cedar River); and another three streams/rivers drain predominantly forested landcover (Allequash Creek, Chippewa River, and Flambeau River; Table S1). The streams draining agricultural and forested landcover are within watersheds previously characterized as outwash plains from recently glaciated areas while streams draining urban landcovers are in watersheds that were characterized as previously glaciated terrain (Schilling et al. 2015). Watershed soils, determined using NHDPlus Version 2.1 (Wieczorek et al. 2018), are predominantly composed of sands ($> 50\%$) for all sites, with silt being the second-most dominant grain size (9–40%) followed by clays (4–17%; Table S1).

Streams were sampled 4–5 times seasonally: in Fall 2015 (October and November), Winter 2016 (February with one early April), Spring 2016 (late April and May), Summer 2016 (August), and Fall 2016 (October and November; Table S2). The winter sample for Allequash Creek was collected in early April, which is still appropriate given lakes are usually still ice-covered at this time. Two sites do not have a Fall 2015 sample, Allequash Creek and Como Creek; however, Allequash Creek has an additional Fall 2016 sample taken during a storm event to capture DOM composition and DOC concentration during high discharge conditions. Samples from the larger rivers (Chippewa River, Flambeau River, Red Cedar River) were collected midchannel from a powerboat, and samples from the smaller streams (all other sites) and all winter samples were collected from the streambank. Water was collected ~ 0.25 m below the surface using a peristaltic pump and was filtered in the field through a pre-rinsed 0.45 μm capsule filter (Geotech Versapor membrane filter) into acid-cleaned polycarbonate or high-density polyethylene sample bottles. Samples were kept cool (4 °C) and in the dark until freezing (within 12 h of collection) and kept frozen until further processing.

Dissolved organic carbon concentration

DOC samples were defrosted and acidified in the lab to a pH of 2 using 12 N analytical-grade HCl. Samples were analyzed using high temperature catalytic combustion on a Shimadzu TOC-L CPH using the non-purgeable OC method, with sample sparging at 75 ml/min for 8 min to remove dissolved inorganic carbon. DOC concentrations are calculated as the mean of at least three injections with a coefficient of variance of $< 2\%$.

Dissolved organic matter composition

DOM samples were defrosted and acidified to pH 2 then prepared for FT-ICR MS analysis by solid-phase extraction on 100 mg Bond Elut PPL (Agilent Technologies) cartridges (Dittmar et al. 2008). Sample volumes used for extraction were adjusted to extract a target C concentration of 50 $\mu g\ mL^{-1}$ in HPLC-grade methanol eluate. Molecular composition of extracted DOM was determined using a 21-tesla FT-ICR MS at the National High Magnetic Field Laboratory (NHMFL; Tallahassee, Fl; Hendrickson et al. 2015; Smith et al. 2018). Negative ions were generated by direct infusion electrospray ionization at a flow rate of 700 $nL\ min^{-1}$. Mass spectra for each sample were generated as the sum of 100 scans.

Molecular formulae were assigned to signals $> 6\sigma$ root mean square baseline noise (Fievre et al. 1997; Stenson et al. 2002; Kujawinski et al. 2002) using custom software (PetroOrg; Corilo 2014). Formulae with elemental combinations of $C_{1-45}H_{1-92}N_{0-4}O_{1-25}S_{0-2}$ with a mass accuracy of < 300 ppb were assigned (e.g. Stenson et al. 2003; Koch et al. 2007; Sleighter et al. 2008) and then classified based on their elemental stoichiometries. The number of formulae detected here, constrained by our techniques, is referred throughout as molecular richness. The modified aromaticity index (AI_{mod}) was calculated for each formula following Koch and Dittmar (2006, 2016), with values of 0.5–0.67 and > 0.67 classified as polyphenolic and condensed aromatic structures, respectively. Other compound classes include: highly unsaturated and phenolic (HUP) (low oxygen) ($AI_{mod} < 0.5$, $H/C < 1.5$, $O/C < 0.5$), HUP (high oxygen) ($AI_{mod} < 0.5$, $H/C < 1.5$, $O/C > 0.5$), aliphatics ($H/C \geq$ 1.5-2.0, $O/C \leq 0.9$, $N = 0$), and N-aliphatics ($H/C \geq$ 1.5-2.0, $O/C \leq 0.9$, $N > 0$) (Stenson et al. 2003; Šantl-Temkiv et al. 2013; Kellerman et al. 2015). Although the formulae assigned to these compound classes can occur in alternate isomeric arrangements, and thus do not necessarily indicate the presence of a structural entity in the DOM sample, we utilize this classification as it provides an overview of the likely basic structural features of the identified molecular formulae. This compound classification technique is supported by other studies that link molecular information with DOM structural properties (e.g., Koch and Dittmar 2006, 2016; Hertkorn et al. 2013).

Relative abundance was calculated by dividing peak signal magnitudes by the sum of all assigned signals. Percent contribution of compound classes was then calculated based on the percent relative abundance of each class to the summed abundance of all formulae. Relative abundance of compounds with only C, H, and O (CHO), compounds with N (CHON), compounds with S (CHOS), and compounds with N and S (CHONS) were also calculated.

Statistical analysis

Statistical analyses were carried out in R (R Core Team 2020), including analysis of variance (ANOVA), t-tests, balanced bootstrap sampling, principal component analysis (PCA), and Spearman rank correlation. DOC and DOM parameters are reported throughout as means ± standard deviations (Tables 1 and 2). One outlier was identified and removed from N-aliphatic compounds using the interquartile range method (see "Dissolved organic matter characterization" and "Agriculture and urban landcover differences" sections for further elaboration). Effects of landcover (forested, agriculture, and urban) and/or seasonality (Fall, Winter, Spring, Summer) on DOC concentration and/or molecular composition of DOM were primarily determined using two-way ANOVA when the groups of data being compared displayed normal distribution for its residuals and equal variances. Levene's test was used to check for the homogeneity of variances and the Shapiro-Wilk test was used to validate that residuals displayed normal distributions. In both tests, a p-value < 0.05 indicates either non-normal residual distribution or non-equal variances and would thus nullify any significance found for the two-way ANOVA testing. ANOVA results were considered significant if they reported a p-value < 0.05. Data that did not satisfy the assumption of equal variances and normal residual distributions (CHOS, condensed aromatics, and aliphatics) were tested for significance using a balanced bootstrap approach with 10,000 iterations, giving 95% confidence intervals (CI) of the bootstrapped means for each landcover and season. Values are significantly different if their 95% CI do not overlap, equivalent to a p-value < 0.05.

We further assessed the combined impacts of landcover and seasonality on overall DOM quality using a PCA on all samples and parameters (DOC concentration and FT-ICR MS compound classes). New relationships may be revealed between samples with different landcovers and seasons since PCA considers correlations among all measured variables at the same time. Sample scores and variable loadings for the first two principal components are plotted against each other for all samples and all DOM parameters measured in this study to investigate potential landcover and seasonality effects. Additionally, sample scores were tested with two-way ANOVA to quantitatively determine if there are significant ($p < 0.05$) differences among samples belonging to different landcovers and/or seasons.

To investigate the potential of landcover and seasonality on individual molecular formulae, we examined the relationship of each molecular formula with DOC concentration, and percent forest, agriculture, and urban coverage (Table S1) using Spearman's rank correlations. Significant correlations ($p < 0.05$) were plotted in a van Krevelen diagram for visualization. t-tests were used to determine if correlations and elemental ratios (H/C and O/C) significantly differed between heteroatomic and CHO formulae.

Table 1 Mean and standard deviations for forest, agriculture, and urban landcover %, dissolved organic carbon (DOC) Concentrations, and percent relative abundance (% RA) of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) parameters

Parameter	Forest (1)	Agriculture (2)	Urban (3)
n	15	14	15
% Forest	83 ± 3	34 ± 14	11 ± 8
% Agriculture	5 ± 6	61 ± 16	6 ± 8
% Urban	3.4 ± 0.3	4.2 ± 1.2	73.4 ± 24.1
DOC (mg/L)$^{1,3>2}$	9.7 ± 3.8	3.7 ± 1.1	7.3 ± 2.90
Formulae (#)$^{2,3>1}$	11,604 ± 1284	16,161 ± 2007	16,087 ± 2836
Mass (Da)$^{1,2>3}$	562 ± 6	564 ± 4	550 ± 9
$AI_{mod}{}^{1>2>3}$	0.34 ± 0.01	0.32 ± 0.01	0.27 ± 0.02
CHO (% RA)$^{1>2>3}$	83 ± 2	68 ± 4	61 ± 6
CHON (% RA)$^{2>3>1}$	11 ± 1	23 ± 4	19 ± 2
CHONS (% RA)$^{3>2>1}$	0.6 ± 0.2	2.0 ± 0.5	2.5 ± 0.6
CHOS (% RA)$^{*3>2>1}$	5 ± 1	7 ± 1	18 ± 6
HUP, High O/C (% RA)$^{1>2,3}$	61 ± 2	56 ± 3	55 ± 6
HUP, Low O/C (% RA)$^{2,3>1}$	21 ± 2	27 ± 3	29 ± 4
Polyphenolics (% RA)$^{1>2>3}$	13 ± 1	10 ± 1	7 ± 2
Cond. Aromatics (% RA)$^{*1,2>3}$	2.6 ± 0.4	2.5 ± 0.4	1.6 ± 0.8
Aliphatics (% RA)$^{*3>2>1}$	2.2 ± 0.4	3.0 ± 0.7	7.1 ± 1.9
N-Aliphatics (% RA)$^{3>2>1}$	0.05 ± 0.03	0.29 ± 0.15	0.52 ± 0.19

AI_{mod} modified aromaticity index, *Da* dalton, *DOC* dissolved organic carbon, *Cond.* condensed, *HUP* highly unsaturated and phenolic

*Indicate significant differences found by bootstrapping, where the assumptions for ANOVA of normality and equal variances were not met

Superscripts next to parameters indicate significant differences using two-way ANOVA (see Table S3), with > indicating the former landcover(s) is(are) significantly greater ($p < 0.05$) than the latter landcover(s). 1 = Forest, 2 = Agriculture, 3 = Urban

Results

Discharge

Discharge varied among sites and landcover, having a wide range in drainage area (Fig. S1; Table S2). Normalizing discharge to drainage area, the lowest normalized mean discharge and low flow occurred in the urban sites (n = 2; Table S2). The normalized low discharge and the normalized mean discharge for forest (n = 3) and agriculture (n = 2) sites were an order of magnitude greater than at the urban sites. Several samples were collected during periods of high discharge, defined here as at least 1.5 times the mean discharge for that creek/river, including the Fall 2016 storm event for Allequash Creek (forested), the Fall 2016 sample for Minnehaha Creek (urban), the Summer 2016 sample for Shingle and Minnehaha Creeks (both urban), and the Spring 2016 samples for Chippewa River (forested), Flambeau River (forested), Red Cedar River (agriculture), and Shingle Creek (urban; Fig. S1).

Dissolved organic carbon concentration variability among landcovers and seasons

DOC concentrations were significantly higher in forested (9.7 ± 3.8 mg C L^{-1}; $p < 0.005$) and urban streams (7.3 ± 2.9 mg C L^{-1}; $p < 0.05$) than in agricultural streams (3.7 ± 1.1 mg C L^{-1}; Table 1; Fig. 2). Greatest mean DOC concentrations were measured in the fall (7.8 ± 4.2 mg C L^{-1}), followed by summer (7.2 ± 3.5 mg C L^{-1}), spring (6.8 ± 3.1 mg C L^{-1}), and winter (5.2 ± 3.6 mg C L^{-1}), which had the lowest concentrations. These

Table 2 Mean and standard seviations for fall, winter, spring, and summer dissolved organic carbon (DOC) Concentrations, and percent relative abundance (% RA) of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) parameters

Parameter	Fall (F)	Winter (W)	Spring (Sp)	Summer (Su)
n	17	9	9	9
DOC (mg/L)	7.8 ± 4.2	5.2 ± 3.6	6.8 ± 3.1	7.2 ± 3.5
Formulae (#)$^{Sp,Su>F;\ Su>W}$	13,503 ± 2462	13,526 ± 3288	15,721 ± 2819	16,536 ± 2894
Mass (Da)	556 ± 8	559 ± 11	562 ± 9	560 ± 8
$AI_{mod}{}^{Sp,Su>W}$	0.31 ± 0.04	0.30 ± 0.04	0.32 ± 0.03	0.32 ± 0.03
CHO (% RA)	71 ± 10	70 ± 12	71 ± 10	69 ± 11
CHON (% RA)	18 ± 6	18 ± 7	17 ± 5	18 ± 6
CHONS (% RA)	1.6 ± 0.9	1.7 ± 1.0	1.7 ± 0.9	1.8 ± 0.9
CHOS (% RA)	10 ± 6	11 ± 7	10 ± 7	11 ± 7
HUP, High O/C (% RA)	58 ± 4	58 ± 7	58 ± 4	55 ± 5
HUP, Low O/C (% RA)	26 ± 4	27 ± 5	25 ± 3	25 ± 4
Polyphenolics (% RA)$^{F,Sp,Su>W}$	11 ± 4	9 ± 3	11 ± 2	12 ± 2
Cond. Aromatics (% RA)$^{*Sp,Su > W}$	2.2 ± 0.8	1.7 ± 0.7	2.4 ± 0.4	2.7 ± 0.4
Aliphatics (% RA)	3.8 ± 2.3	3.9 ± 2.5	4.2 ± 2.9	4.9 ± 2.9
N-aliphatics (% RA)	0.27 ± 0.26	0.26 ± 0.23	0.30 ± 0.27	0.31 ± 0.21

AI

AI_{mod} modified aromaticity index, *Da* dalton, *DOC* dissolved organic carbon, *Cond.* condensed, *HUP* highly unsaturated and phenolic

*Indicate significant differences found by bootstrapping, where the assumptions for ANOVA of normality and equal variances were not met

Superscripts next to parameters indicate significant differences using two-way ANOVA (see Table S3), with > indicating the former season(s) is(are) significantly greater ($p < 0.05$) than the latter season(s). F = Fall, W = Winter, Sp = Spring, Su = Summer

differences in DOC concentration between seasons were not significant (Table 2).

Dissolved organic matter characterization

There were 27,400 molecular formulae assigned for the study sample set, with ~ 24% ($n = 6489$) found in all samples. Within the formulae common to all samples, most (69%) were in the highly unsaturated and phenolic (HUP) compound class. Other dominant compound classes include polyphenolics (13%), aliphatics (8%), condensed aromatics (6%), and N-aliphatics (4%). Most shared formulae were CHON compounds (41%), while 27% were CHO, 24% were CHOS, and 8% were CHONS. Most of the assigned molecular formulae were not ubiquitous (76%, $n = 20{,}911$) across all samples, with the majority (64%) of these being N-,S- heteroatomic compounds (CHON, CHOS, and CHONS).

The molecular richness (number of formulae) of DOM was significantly higher for agricultural (16,161 ± 2007) and urban streams (16,087 ± 2836) compared to forested streams (11,604 ± 1284; $p < 0.005$ for both; Table 1; Fig. 2). Molecular richness was significantly higher in summer (16,536 ± 2894) compared to both fall (13,503 ± 2462) and winter (13,526 ± 3288; $p < 0.05$ for both; Table 2). Spring (15,721 ± 2819) also had significantly higher molecular richness than fall ($p < 0.05$). The modified aromaticity index (AI_{mod}), which is a measurement of the relative aromaticity expected for compounds (Koch and Dittmar 2006, 2016), was highest in forested streams (0.34 ± 0.01), intermediate in agricultural streams (0.32 ± 0.01) and lowest in urban streams (0.27 ± 0.002; $p < 0.005$; Table 1; Fig. 2). The AI_{mod} was significantly higher in spring and summer (0.32 ± 0.03 for both) than winter (0.30 ± 0.04; $p < 0.05$), which had the lowest AI_{mod} of all seasons (Table 2). The weight-abundance average molecular weight was significantly higher in forested (562 Da ± 6 Da) and agricultural streams (564 Da ± 4

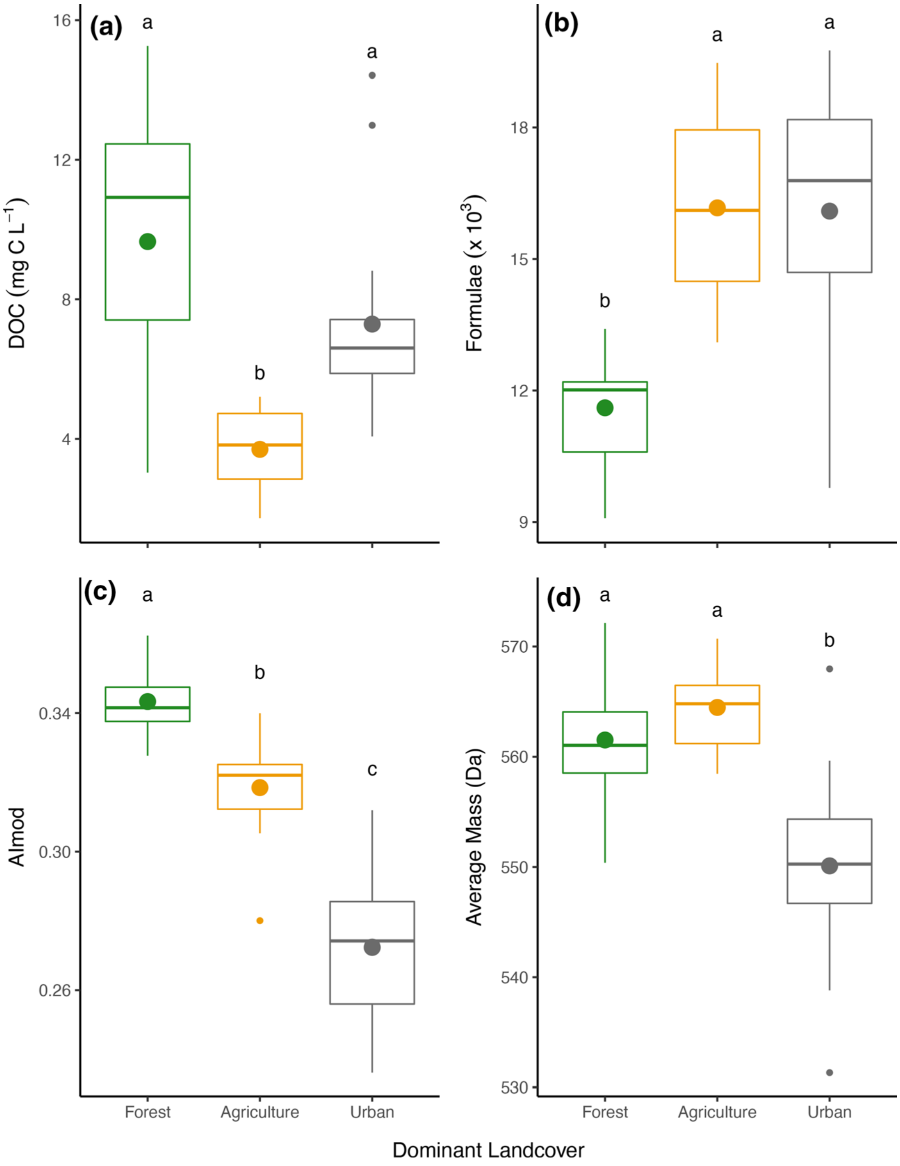

Fig. 2 Box plots for forest (green), agriculture (orange), and urban (gray) landcover. **a** Dissolved organic carbon (DOC) concentrations (mg L^{-1}); **b** Number of assigned formulae (× 10^3); **c** the modified aromaticity index (AI$_{mod}$); **d** Average mass (Da, dalton). Solid circles and the thick horizontal lines represent the mean and median, respectively, for each landcover. Significance letters above each box plot are based on significance testing using two-way analysis of variance (ANOVA), with letters indicating which groups are statistically different from one another. (Color figure online)

Da) compared to urban streams (550 Da ± 9 Da; $p < 0.005$ for both; Table 1; Fig. 2).

CHO formulae were most abundant (by relatively abundance, RA) in forested streams (83% ± 2%), intermediate at agricultural streams (68% ± 4%) and lowest at urban sites (61% ± 6%; $p < 0.005$ for all comparisons; Table 1; Fig. 3). Conversely, heteroatomic formulae (CHON, CHOS, and CHONS) had an overall higher RA in agricultural and urban streams compared to forested streams (Table 1; Fig. 3). CHON RA was the highest in agricultural streams (23% ± 4%), intermediate in urban streams (19% ± 2%) and lowest in forested streams (11% ± 1%; $p < 0.005$ for all comparisons). CHOS relative abundances, using the balanced bootstrap approach to test for significance, were significantly highest at urban

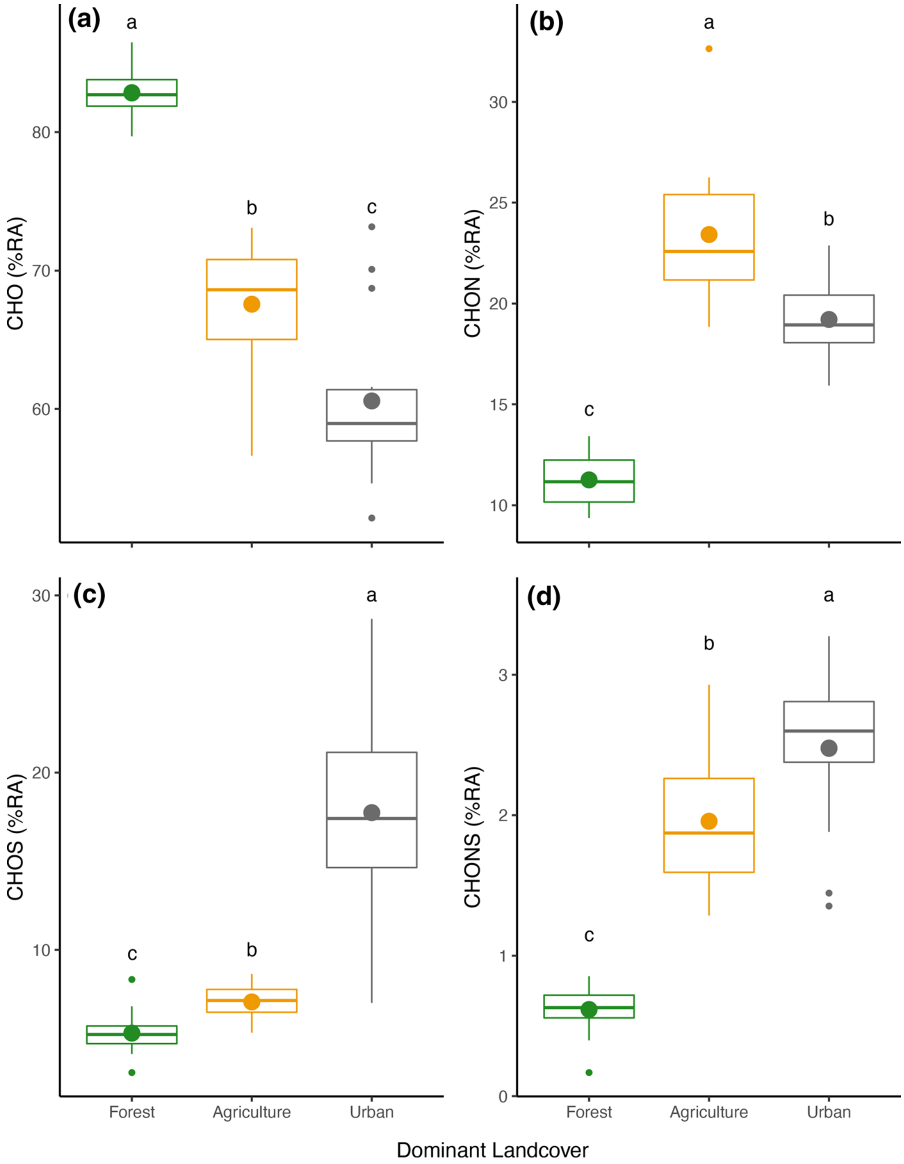

Fig. 3 Box plots for forest (green), agriculture (orange), and urban (gray) landcovers. **a** relative abundance (RA) of C-, H-, and O-containing compounds (CHO; % RA); **b** RA of C-, H-, O-, and N-containing compounds (CHON; % RA); **c** RA of C-, H-, O-, and S-containing compounds (CHOS; % RA); **d** RA of C-, H-, O-, N-, and S-containing compounds (CHONS; % RA). Solid circles and the thick horizontal lines represent the mean and median, respectively, for each landcover. Significance letters above the CHO, CHON, and CHONS box plots is based on significance testing using two-way ANOVA. Significance letters above CHOS box plots is based on significance testing using balanced bootstrapping. (Color figure online)

streams (18% ± 6%), intermediate at agricultural streams (7% ± 1%) and lowest at forested sites (5% ± 1%; $p < 0.05$, for all comparisons; Table 1; Fig. 3). CHONS relative abundances were significantly higher at urban (2.5% ± 0.6%) and agricultural (2.0% ± 0.5%) streams compared to forested streams (0.6% ± 0.2%; $p < 0.005$ for both; Table 1; Fig. 3), and urban streams were significantly higher in CHONS than agricultural streams ($p < 0.05$). CHO, CHON, CHOS and CHONS relative abundances did not differ significantly between seasons (Table 2).

HUP with low O/C were significantly higher in agricultural (27% ± 3% RA) and urban (29% ± 4%

RA) streams compared to forested streams (21% ± 2% RA; $p < 0.005$ for both; Table 1; Fig. 4a). Aliphatics, using a balanced bootstrap approach to test for significance, were highest in urban streams (7.1% ± 1.9%), at intermediate RA in agricultural streams (3.0% ± 0.7%) and lowest RA forested streams (2.2% ± 0.4%; $p < 0.05$ for all comparisons; Table 1; Fig. 4b). N-aliphatic compounds were highest in RA in urban streams (0.52% ± 0.19%), intermediate in agricultural streams (0.29% ± 0.15%), and lowest for forested streams (0.05% ± 0.03%; $p < 0.05$; Table 1; Fig. 4c). One outlier was removed from N-aliphatic

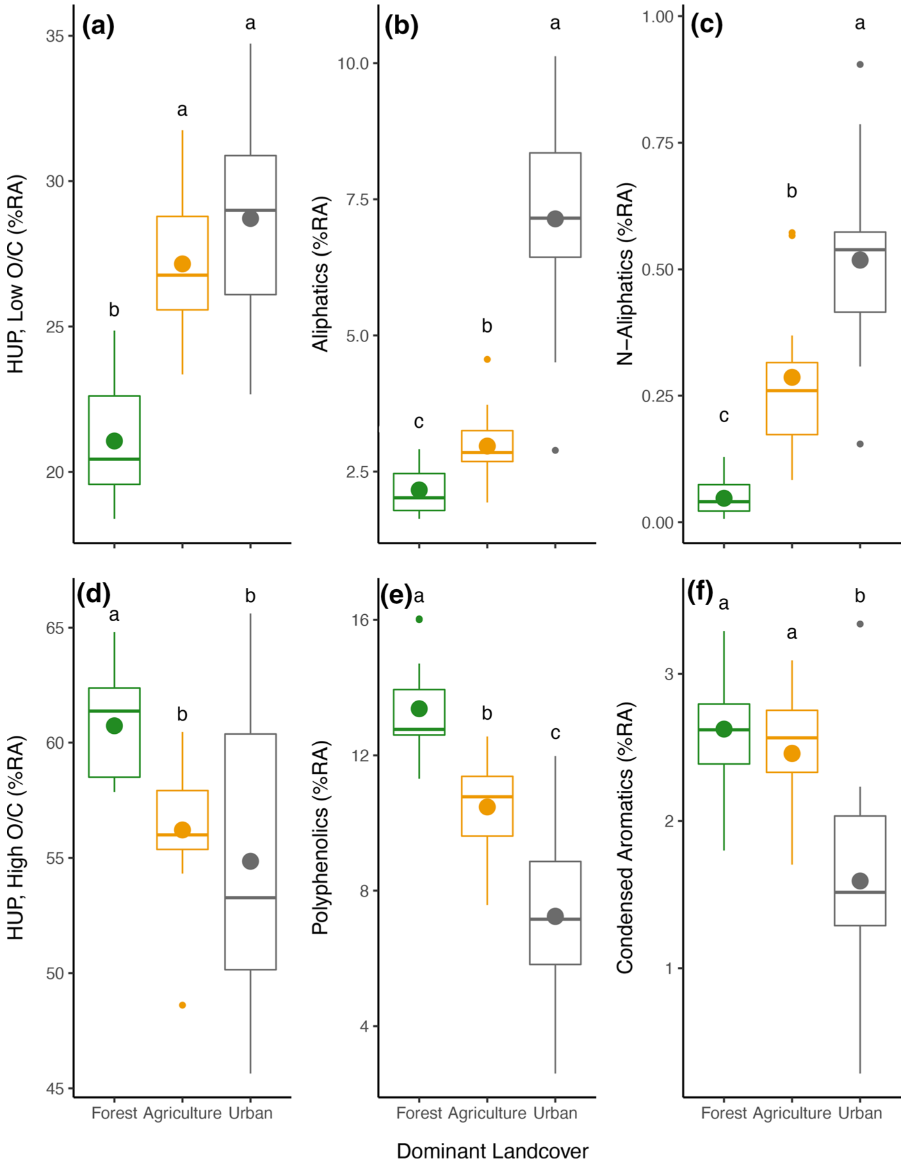

Fig. 4 Box plots for forest (green), agriculture (orange), and urban (gray) landcovers. **a** relative abundance (RA) of highly unsaturated and phenolic (HUP) low O/C (% RA); **b** RA of aliphatics (% RA); **c** RA of N-aliphatic (% RA); **d** RA of HUP high O/C (% RA); **e** RA of polyphenolics (% RA); **f** RA of condensed aromatics (% RA). Solid circles and the thick horizontal lines represent the mean and median, respectively, for each landcover. Significance letters above HUP (low O/C), N-aliphatic, HUP (high O/C), and polyphenolics is based on significance testing using two-way ANOVA. Significance letters above aliphatics and condensed aromatics box plots is based on significance testing using balanced bootstrapping. (Color figure online)

compounds as it was greater than the 75th percentile by a factor of 1.5 times the interquartile range of all N-aliphatics. This sample was found in an agricultural winter sample (Trout Creek). There were no significant differences in HUP with low O/C, aliphatics, or N-aliphatic compounds between seasons (Table 2).

Formulae classified as HUP with high O/C were significantly higher in forested streams (61% ± 2% RA) compared to urban (55% ± 6% RA; $p < 0.005$) and agricultural streams (56% ± 3% RA; $p < 0.05$; Table 1; Fig. 4d). Polyphenolic RA was highest in forested streams (13% ± 1%), intermediate in agricultural streams (10% ± 1%), and lowest in urban streams (7% ± 2%; $p < 0.005$ for all comparisons; Table 1; Fig. 4e). Polyphenolic RA (9% ± 3%) was higher in the fall (11% ± 4%; $p < 0.05$), spring (11% ± 2%; $p < 0.05$), and summer samples (12% ± 2%; $p < 0.005$; Table 2) compared to the winter. Condensed aromatics RA, using a balanced bootstrap approach, was higher in forested (2.6% ± 0.4%) and agricultural streams (2.5% ± 0.4%) compared to urban streams (1.6% ± 0.8%; $p < 0.05$ for both comparisons; Table 1; Fig. 4f). Condensed aromatic RA exhibited some seasonal variation and were significantly higher in the summer (2.7% ± 0.4%) and spring (2.4% ± 0.4%), than in the fall (2.2% ± 0.8%) and winter (1.7% ± 0.7%; $p < 0.05$ for both; Table 2).

Principal component analysis

A PCA was used to further assess impacts of landcover and seasonality on DOM amount and quality. Principal component 1 (PC1) explained 56.0% of the data variance and DOC concentration, average mass, AI_{mod}, CHO, HUP (high O/C), polyphenolics, and condensed aromatics had positive loadings (Table 3; Fig. 5). In contrast, PC1 had negative loadings with molecular richness (number of formulae), heteroatomic formulae (CHON, CHOS, and CHONS), HUP (low O/C), aliphatic, and N-aliphatics. The PC1 scores varied significantly with landcover, from highest in forested streams (PC1: 0.87 ± 0.16), intermediate in agricultural streams (− 0.10 ± 0.36), and lowest in urban streams (− 0.77 ± 0.39; $p < 0.005$ for all; Fig. 5).

Table 3 Principle component analysis structure matrix for dissolved organic carbon (DOC) concentration data and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) parameters

Parameter	PC1	PC2
DOC (mg/L)	0.56	− 0.68
Formulae (#)	− 0.96	0.70
Mass (Da)	0.69	0.84
AI_{mod}	1.14	0.49
CHO (% RA)	1.27	− 0.19
CHON (% RA)	− 0.85	0.72
CHONS (% RA)	− 1.26	0.26
CHOS (% RA)	− 1.10	− 0.35
HUP, High O/C (% RA)	0.97	− 0.41
HUP, Low O/C (% RA)	− 1.16	0.09
Polyphenolics (% RA)	1.07	0.45
Condensed aromatics (% RA)	0.75	0.78
Aliphatics (% RA)	− 1.13	− 0.37
N-aliphatics (% RA)	− 0.57	0.50
% Variance explained	56.0	16.3

AI_{mod} modified aromaticity index, *Da* dalton, *HUP* highly unsaturated and phenolic, *PC1* principal component 1, *PC2* principal component 2

Principal component 2 (PC2) explained 16.3% of the data variance and had positive loadings for molecular richness, average mass, AI_{mod}, CHON, CHONS, polyphenolics, condensed aromatics, and N-aliphatics (Table 3; Fig. 5). PC2 had negative loadings associated with DOC concentration, CHO, CHOS, HUP (high O/C), and aliphatic compounds. Both landcover and seasonality varied significantly with PC2. Scores were significantly higher in agricultural streams (0.84 ± 0.48) than in forested (− 0.24 ± 0.22) and urban streams (− 0.54 ± 0.64; $p < 0.005$ for both; Fig. 5). There is no significant difference between forested and urban PC2 scores. For seasonality, only summer (0.34 ± 0.51) and fall samples (− 0.19 ± 0.73; $p < 0.05$) were significantly different (Fig. 5). Spring and winter samples were not significantly different from the other seasons.

Spearman rank correlations

Spearman rank correlations were used to examine the relationship of the 27,400 formulae to DOC concentration, percent forest coverage, percent agriculture

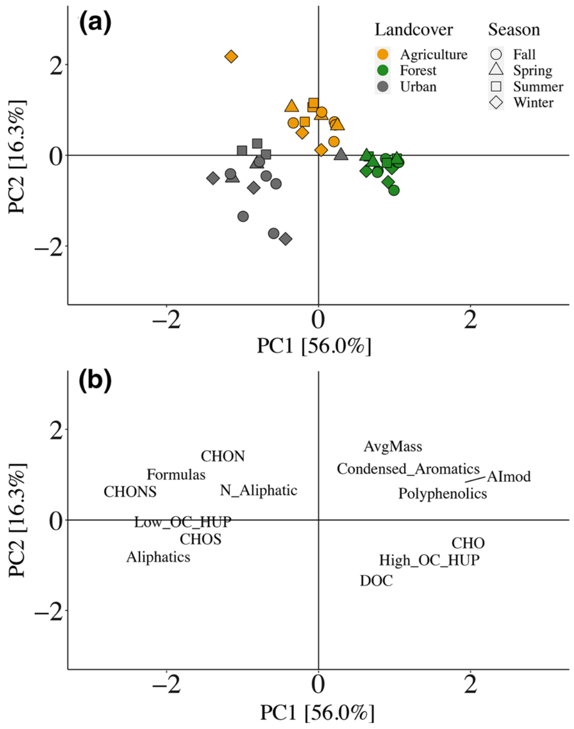

Fig. 5 Dissolved organic carbon (DOC) concentration and dissolved organic matter (DOM) characteristics data principal component analysis. **a** sample scores labeled by landcover (agriculture = orange, forest = green, urban = gray) and season (circle = fall, triangle = spring, solid rectangle = summer, diamond = winter); **b** variable loadings (see Table 3 for variable loading values). *CHO* C-, H-, O-containing formulae, *CHON* C-, H-, O-, N-containing formulae, *CHOS* C-, H-, O-, S-containing formulae, *CHONS* C-, H-, O-, N-, S-containing formulae, *AvgMass* average mass, *AImod* the modified aromaticity index, *Formulas* formulae abundance, *HUP* highly unsaturated and phenolic. (Color figure online)

coverage, and percent urban coverage (Fig. 6a–d). There were significant correlations ($p < 0.05$) for 3374 formulae (12%) with DOC concentration which, when plotted in a van Krevelen space, showed two distinct groupings of Spearman's rank correlation coefficients that separated along the O/C axis (Fig. 6a). Of the 3374 formulae with significant correlations to DOC concentration, only 341 had significant positive correlations, a majority of which were CHO formulae (94%; Fig. S2a). Formulae with significant negative DOC correlations ($n = 3033$) mostly contained N-, S-heteroatoms (89%; Fig. S3a).

Percent forest (16,862 significant correlations, 62%; Fig. 6b) and percent urban landcover (13,408 significant correlations, 49%; Fig. 6d) exhibited an inverse pattern within the van Krevelen. Formulae with significant positive correlations ($n = 3915$) to forest landcover had significantly lower H/C (0.84 ± 0.21) and higher O/C (0.61 ± 0.14) ratios compared to formulae with significant negative correlations ($n = 12{,}947$; H/C = 1.22 ± 0.28; O/C = 0.47 ± 0.14; $p < 0.005$ for both). Most of the formulae that positively correlated with forest landcover were CHO formulae (81%; Fig. S2b). Most of the formulae that negatively correlated to forest cover contained N-, S- heteroatoms (85%; Fig. S3b), with a majority (60%) of these heteroatomic formulae containing S (Fig. S4b).

The opposite trend is seen with urban landcover where formulae with significant positive correlations to urban landcover ($n = 9168$) had significantly higher H/C (1.28 ± 0.27) and lower O/C (0.48 ± 0.15) compared to formulae with significant negative correlations to urban landcover ($n = 4{,}240$; H/C = 0.82 ± 0.20; O/C = 0.58 ± 0.14; $p < 0.005$ for both). Most of the formulae with positive correlations to urban landcover were N-,S- heteroatomic formulae (81%; Fig. S3d), highly enriched in S (Fig. S4d). Formulae negatively associated with urban landcover were mostly CHO-formulae (65%; Fig. S2d), with some formulae containing N (Fig. S5d).

Percent agriculture landcover had 5604 formulae with significant correlations and most were positive ($n = 5402$) with formulae containing a wide range of H/C and O/C values (Fig. 6c). Most (99%) of the formulae with positive correlations to agriculture cover contained N (Fig. S5c), with a few also containing S. Most of the 202 formulae with negative correlations to agriculture landcover were CHO formulae (80%; Fig. S2c). The remaining formulae with negative correlations to agriculture landcover were mostly S-containing formulae (Fig. S4c).

Unique formulae

Another way to explore whether there are clear separations of landcover and/or season with formulae is to examine whether samples from different landcover or season contain unique molecular formulae (e.g. Spencer et al. 2019). None of the seasons had unique formulae; however, fourteen unique formulae were present in all agricultural samples, eight unique formulae present in all urban samples, and one unique formula present in all forested samples (Table 4;

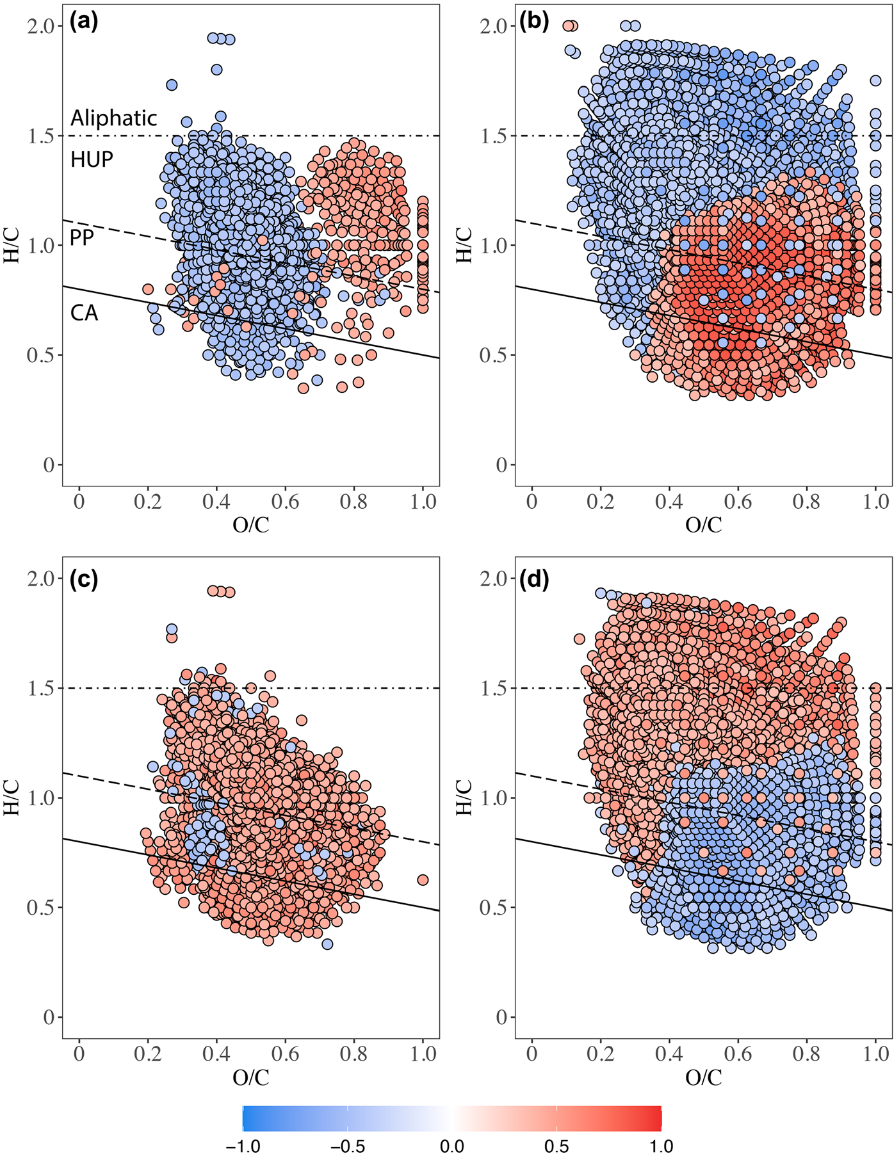

Fig. 6 Spearman-rank correlations between the relative abundance of assigned molecular formulae and **a** dissolved organic carbon (DOC) concentration; **b** % forest in the watershed; **c** % agriculture in the watershed; **d** % urban in the watershed. Axes represent the atomic ratios of H:C (H/C) and O:C (O/C). Colors represent the correlation coefficient (ρ_s) between the relative abundance of each formula and the respective variable with warmer color formulae indicating positive correlations and cooler color formulae exhibiting negative correlations. Scale-bar represents the range of correlation coefficient values. Lines approximately delineate compound groups. *CA* condensed aromatic compounds, *HUP* highly unsaturated and phenolic compounds, *PP* polyphenolic compounds. (Color figure online)

Fig. 7a). Nearly all of these unique formulae had RAs greater than the average formula RA (for all formulae) for their respective landcover (average formula RA for agriculture, urban and forest is 0.31, 0.31, and 0.30, respectively; Table 4), with the only exception being the formula unique to the forest landcover samples. The unique tracers of agriculture were all N-containing formulae classified as either polyphenolics or HUP (both high and low O/C), while urban tracers were a combination of S-containing HUP (both high and low O/C) and S-containing aliphatics. The single unique

Table 4 Unique formulae found in single landcovers (Agriculture, Urban, Forest) and their masses (Da, dalton) and average relative abundances (% RA) for all samples of their respective landcover. The average formula RA for all formulae in agriculture, urban and forest landcovers is 0.31, 0.31, and 0.30, respectively

Unique to	Formula	Mass (Da)	Average Sample Relative Abundance (% RA)
Agriculture	$C_{14}H_{11}O_7N_3S_0$	332	1.04
Agriculture	$C_{15}H_{15}O_7N_3S_0$	348	1.58
Agriculture	$C_{17}H_{16}O_{10}N_4S_0$	435	0.69
Agriculture	$C_{17}H_{17}O_7N_3S_0$	374	1.43
Agriculture	$C_{17}H_{19}O_7N_3S_0$	376	1.65
Agriculture	$C_{18}H_{16}O_9N_4S_0$	431	0.85
Agriculture	$C_{19}H_{16}O_{10}N_4S_0$	459	0.75
Agriculture	$C_{21}H_{16}O_{10}N_4S_0$	483	0.62
Agriculture	$C_{21}H_{20}O_{10}N_4S_0$	487	1.01
Agriculture	$C_{22}H_{15}O_{11}N_3S_0$	496	0.83
Agriculture	$C_{22}H_{18}O_{10}N_4S_0$	497	0.58
Agriculture	$C_{24}H_{22}O_{10}N_4S_0$	525	0.54
Agriculture	$C_{25}H_{24}0_{10}N_4S_0$	539	0.63
Agriculture	$C_{26}H_{21}O_{11}N_3S_0$	550	0.52
Urban	$C_{13}H_{24}O_5N_0S_1$	291	0.74
Urban	$C_{18}H_{26}O_9N_0S_2$	449	0.91
Urban	$C_{18}H_{28}O_9N_0S_2$	451	0.66
Urban	$C_{19}H_{26}O_{10}N_0S_2$	477	0.72
Urban	$C_{19}H_{28}O_{10}N_0S_2$	479	0.69
Urban	$C_{19}H_{28}O_8N_0S_2$	447	0.62
Urban	$C_{20}H_{26}O_{10}N_0S_2$	489	0.65
Urban	$C_{20}H_{26}O_9N_0S_2$	473	0.81
Forest	$C_{32}H_{24}O_{25}N_0S_0$	807	0.30

forest formula was a CHO formula classified as HUP (high O/C).

Discussion

Landcover effects on dissolved organic carbon concentration

The concentration of DOC in all sampled UMRB streams and rivers (Table 1; Fig. 2a) fell within the typical range of DOC for streams and rivers globally (0.5–50 mg C L^{-1}; Mulholland 2003), and for streams in urban and agricultural areas of central and southern Minnesota ($\sim$ 2–13 mg C L^{-1}; Tsui and Finley 2011). Previous studies comparing streams dominated by agriculture (e.g. Stanley et al. 2012; Lu et al. 2014; Graeber et al. 2015; Heinz et al. 2015; Gücker et al. 2016; Spencer et al. 2019) and urban landcovers (e.g. Sickman et al. 2007; Aitkenhead-Peterson et al. 2009; Hosen et al. 2014) to those dominated by forest landcovers had shown variable differences in DOC concentrations between landcovers. In this study, agricultural streams in the UMRB had significantly lower DOC concentrations than forested and urban streams (Table 1; Fig. 2a).

Higher DOC concentrations in the forested streams are likely due to differences in the quantity of DOC inputs. High DOC concentrations found in forested streams (Table 1; Fig. 2a) are associated with the surrounding organic-rich terrestrial ecosystem, including vascular plants (leaching, rhizodeposition, fragmented plant material, and litterfall), and the leaching and erosion of accumulated OM in riparian soils along the stream channel (Thurman 1984; Hope et al. 1994; Meyer et al. 1998; Ledesma et al. 2018). The agricultural streams may have received less terrestrial OC from the watershed, reflective of possible lower vegetation productivity and less litter accumulation (Guo and Gifford 2002) combined with

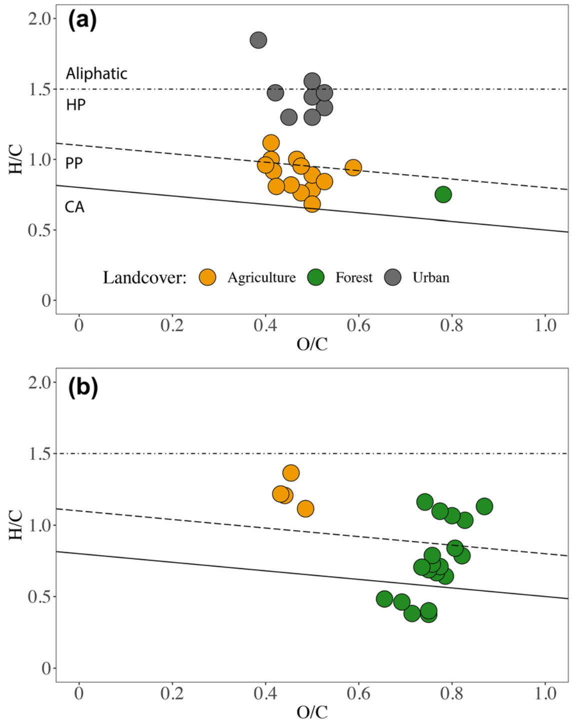

Fig. 7 Unique molecular formulae found solely in forest samples (green), agriculture samples (orange), and urban samples (gray) for **a** the Upper Mississippi River Basin (this study) and **b** forested and agricultural tributaries of the Amazon River (Spencer et al. 2019). Axes represent the atomic ratios of H:C (H/C) and O:C (O/C). Lines approximately delineate compound groups. *CA* condensed aromatic compounds, *HUP* highly unsaturated and phenolic compounds, *PP* polyphenolic compounds. (Color figure online)

smaller surface soil OC stocks (Ogle et al. 2005; McLauchlan 2006; Nagy et al. 2018). Smaller surface soil OC stocks along agricultural streams are associated with lower OC inputs, increased decomposition with intensified soil disturbance (Reicosky et al. 1995; Guo and Gifford 2002), and accelerated soil erosion (Van Oost et al. 2007; Gücker et al. 2016). Additionally, the loss of above-ground vegetation structure and litter in agricultural landcovers compared to forested landcovers can increase soil temperatures (Geiger et al. 1995), which leads to increased in situ OC decomposition (Nagy et al. 2018). While synthetic nitrogen (N) addition to agricultural systems can increase vegetation inputs to the soil, N fertilization can also increase soil OM decomposition rates resulting in either a net loss (e.g. Khan et al. 2007) or no change in soil OC concentrations (e.g. Halvorson et al. 2002).

Significantly greater DOC concentrations in urban streams compared to agricultural streams may relate to higher OC inputs associated with human activity. This human activity includes wastewater from leaky sewer lines or septic drainage fields (Westerhoff and Anning 2000; Verstraeten et al. 2005; Sickman et al. 2007; Pennino et al. 2016), use of petroleum products (oil and gas from vehicles, tar from roads and shingles; Spiker and Rubin 1975), engineered headwaters (e.g. road asphalt, roadside gutters, subsurface pipes; Fork et al. 2018; Niles et al. 2020), imported vegetation and soil, pet waste, and runoff from turf grass in open urban areas (e.g. golf courses and parks; Wright et al. 2005). Increased nutrient inputs associated with these urban sources can also increase DOC concentrations in urban surface waters by stimulating algal and bacterial production (Bernot et al. 2010). Impervious surfaces associated with urban environments promote the export of OM into aquatic systems by impeding DOM interactions with high sorption capacity soils (Hobbie et al. 2014; Bratt et al. 2017; Fork et al. 2018).

Landcover impacts on molecular signatures of stream dissolved organic matter

Landcover impacts on molecular richness

The number of molecular formulae assigned in this study (27,400) is higher compared to other FT-ICR MS studies, such as Spencer et al. (2019), which assigned ~ 15,000 formulae to tropical streams draining forest and agriculture landcovers. This higher number of assigned formulae is likely driven by a diverse sample set (each sample had at least 10,000 formulae assignments and both the agriculture and urban landcovers had high molecular richness; Fig. 2b) as well as the inclusion of an additional landcover (urban) with formulae characterized by different classes as those found in the other two landcovers. A majority of the molecular formulae in this study shared across all samples were highly unsaturated and phenolic (HUP) compounds, which have been found to dominate DOM globally (Riedel et al. 2016; Kellerman et al. 2018; Spencer et al. 2019). Common formulae present in all samples suggests they all share a common DOM pool produced by similar sources that has gone through comparative diagenetic processes during transport into and through streams and rivers (Jaffé et al. 2012; Wagner et al.

2015; Harfmann et al. 2019). This processed pool of DOM may represent a more stable pool of OM as the more labile DOM would be removed through microbial processing (Wickland et al. 2007) and/or photodegradation (Cory et al. 2014; Wagner et al. 2015).

Most of the assigned molecular formulae (76%) were not shared across all samples, driven by contrasting types of N-,S- heteroatomic formulae associated with each landcover. Molecular richness of DOM (Table 1; Fig. 2b), attributed here to increased presence of N-,S- heteroatomic formulae, was significantly greater in agricultural and urban streams compared to forested streams. These N-,S-heteroatomic formulae dominated the formulae with positive Spearman's correlations associated with urban (n = 7409 compared to 1759 for CHO formulae) and agricultural landcovers (n = 5347 compared to 55 for CHO formulae; Fig. S3c/d), demonstrating a potential commonality between agriculture and urban landcover. In contrast, only 23% of formulae with positive Spearman's correlations with forested cover contained N-,S- heteroatoms. Higher abundances of N-containing molecular formulae in agricultural and urban landcovers may be linked to large anthropogenic nutrient inputs (Mattsson et al. 2005; Wilson and Xenopoulos 2009; Wagner et al. 2015; Hertkorn et al. 2016). Nutrients are elevated in agricultural streams in the UMRB due to fertilizer use and animal manure production, and in urban streams due to wastewater inputs (Turner and Rabalais 1991, 2003; Stark et al. 2000; Houser et al. 2015; Voss et al. 2017). Sulfur is less studied in the UMRB but is also associated with the use of fertilizer, the production of sewage, and enhanced oxidative weathering of sulfur-bearing organic materials linked with agricultural practices (Killingsworth and Bao 2015; Wagner et al. 2015; Hertkorn et al. 2016).

Greater allochthonous contributions in streams draining forest landcovers

We found forested streams were more enriched in HUP (high O/C), polyphenolic, and condensed aromatic compounds compared to streams draining agriculture and urban landcovers (Table 1; Fig. 4d, f) and had significantly more positive scores along PC1 compared to agricultural and urban streams (Fig. 5a). Enrichment in the aforementioned compound groups is characteristic of terrestrial carbon sources (e.g. fresh litter layers and organic-rich soil horizons) found in other stream DOM studies (e.g. Stubbins et al. 2010; Williams et al. 2010; Wagner et al. 2015; Kellerman et al. 2018; Spencer et al. 2019). Terrestrial carbon sources typically produce DOM formulae with high O/C and low H/C values (D'Andrilli et al. 2015; Lu et al. 2015; O'Donnell et al. 2016), and these compounds had positive Spearman's rank correlations with percent forest landcover (Fig. 6b). In contrast, autochthonous DOM contribution appears to be lower within forested streams than in agricultural and urban streams as evident by lower HUP (low O/C), aliphatic, and N-aliphatic formulae (Table 1; Fig. 4). Lower autochthonous contributions to forested stream DOM are likely driven by dilution from large allochthonous DOM inputs to the stream, quick mineralization of autochthonous DOM within the streams, and/or low autochthonous DOM production that may be driven by lower nutrient concentrations and heavy in-stream shading by riparian vegetation (Webster and Meyer 1997; Bernot et al. 2006; Voss et al. 2017).

Greater autochthonous contributions in streams draining agriculture and urban landcovers

Agricultural and urban streams are characterized by greater autochthonous DOM contributions to stream DOM compared to forested streams. This is supported by significantly higher relative abundance of aliphatic, N-aliphatic, and HUP low O/C compounds (Table 1; Fig. 4a–c) as well as lower O/C and higher H/C ratios in formulae with significant positive Spearman's correlations to agriculture and urban landcover (compared to formulae associated with forest landcover, Fig. 6b–d). The above associations are indicative of high autochthonous DOM production (e.g. algae and bacteria) and microbial processing (Williams et al. 2010; Graeber et al. 2015; Kellerman et al. 2018) within agricultural and urban streams, as well as possible wastewater inputs to urban streams (e.g. Ye et al. 2019). The association of these compounds with agriculture and urban landcovers is further supported by the more negative scores for these landcovers along PC1 (Fig. 5a) coinciding with the negative loadings on PC1 for these autochthonous-produced compounds (Table 3; Fig. 5b).

High relative contributions of autochthonous-derived compounds in streams draining agriculture and urban landcovers may relate to high nutrient inputs from anthropogenic activity (Delong and Brusven 1992; Corkum 1996; Reche et al. 1998; Bernot et al. 2010) as well as increased light availability and warmer temperatures in smaller, anthropogenically-impacted UMRB streams as a result of reduced shading from the removal of riparian vegetation (Bernot et al. 2006). Higher light exposure also stimulates photodegradation of allochthonous DOM (Spencer et al. 2009; Catalán et al. 2013) and anthropogenically-derived DOM (Meng et al. 2013), leading to increased relative contributions of autochthonous DOM. Autochthonous compounds in streams are typically more bioavailable and more rapidly utilized compared to allochthonous compounds (D'Andrilli et al. 2015; Graeber et al. 2015; Riedel et al. 2016; Textor et al. 2018). Thus, not only does anthropogenic alteration of the landscape through agricultural development likely lead to significant DOC concentration reductions in the UMRB, but agricultural and urban development in the UMRB also significantly alters the DOM composition to make it more biolabile. An increase in biolability has significant ecosystem implications including increased CO_2 production and emissions (Battin et al. 2008; Graeber et al. 2015; Drake et al. 2019), alterations to food web dynamics (Gücker et al. 2011), and alterations to nitrogen uptake and denitrification (Bernhardt and Likens 2002; Newcomer et al. 2012).

Agriculture and urban landcover differences

Although agricultural and urban streams both have larger autochthonous DOM contributions compared to forested streams, there are several distinct differences in DOM composition between them. The separation between agricultural and urban stream scores in the PCA (Fig. 5) is driven by significantly greater contributions from CHON compounds in agricultural streams (Table 1; Fig. 3b). CHON compounds had more positive PC1 and PC2 loadings (Fig. 5b) compared to compounds that were more prevalent in urbanized streams (CHONS, CHOS, and aliphatics; Table 3). This, along with agricultural landcover having significant positive Spearman's correlations with N-containing formulae (Fig. S5c), suggests agricultural streams have greater relative abundances of CHON compounds compared to urban streams. Additionally, the urban (Minnehaha Creek) and forested (Chippewa River) sites with the greatest agricultural cover (Table S1) had significantly ($p < 0.05$) higher relative abundances of CHON compounds compared to the other urban and forested sites, respectively. Similarly, the agricultural site with the lowest percent agriculture (Red Cedar River, Table S1) had significantly lower CHON and CHONS relative abundances ($p < 0.05$) compared to another agricultural site with a higher percentage of agricultural landcover (Trout Creek). The variation of compound classes within sites belonging to a single landcover further supports the influence of agricultural landcover on CHON abundances. In contrast to the agricultural streams, urban streams had significant positive Spearman's rank correlations with S-containing formulae (Fig. S4d) and had greater contributions from CHOS and CHONS compounds (Table 1; Fig. 3c, d), suggesting urban streams have greater S contributions.

The S-containing compounds within urban streams of the UMRB are likely associated with anthropogenic S sources such as fossil fuel emissions (e.g. vehicle combustion sources) and subsequent atmospheric deposition, sewage, groundwater usage and building construction (Killingsworth and Bao 2015). S-containing compounds can also be associated with agricultural practices (Hinckley et al. 2020); however, as seen previously for the Mississippi River basin (Goolsby et al. 1999), agricultural lands do not appear to contribute the highest S fluxes. The highest relative N contributions in agricultural streams are likely due to fertilizer use, animal manure production, soil organic N contribution, and in-stream primary productivity fueled by anthropogenic nutrient inputs (Stark et al. 2000; Panno et al. 2006; Houser and Richardson 2010; Voss et al. 2017). Although urban streams had lower relative abundances of CHON, N concentrations are likely to still be elevated from wastewater inputs, which are commonly associated with elevated concentrations of organic N compounds (Stark et al. 2000; Wasley 2000; Houser and Richardson 2010).

DOM associated with agriculture and urban landcovers can also be differentiated by compound H/C and O/C elemental ratios. Formulae with significant positive Spearman's correlations to urban landcover also had the lowest O/C and highest H/C values of all three landcover correlations (Fig. 6d). The lower O/C and higher H/C ratios for urban formulae in the UMRB may suggest the DOM in urban streams is more characteristic of microbially-derived, bioavailable material (e.g. lipids, proteins, amino sugars; Gonsior et al. 2011; D'Andrilli et al. 2015; Parr et al. 2015; Wagner et al. 2015; Kellerman et al. 2018) compared to agricultural streams.

The low number of formulae negatively associated with streams draining agricultural landcover in the UMRB (Fig. 6c) contrasts with the abundance of high O/C, low H/C formulae with negative associations in tropical agricultural streams (Drake et al. 2019; Spencer et al. 2019). UMRB agricultural streams had a high abundance of formulae with H/C ratios < 1, similar to the formulae associated with DOM in UMRB forested streams. Thus, while agriculture landcover in the UMRB likely increases autochthonous DOM contributions to stream DOM, many formulae within agricultural stream DOM are similar to forest/wetland DOM contributions. This is likely due to the non-trivial contribution of forested area (34 ± 14%) in agricultural watersheds in this study (Table 1), remnants from forest/wetland soils before conversion to agricultural land, and/or from similar DOM produced by agriculture and forest vegetation.

When only formulae found in streams draining agriculture and urban landcovers (removing formulae found in forested streams) are compared, the number of formulae unique to agricultural streams (i.e. not found in urban streams) increased to 27 (+ 13) while the number of formulae unique to urban streams (i.e. not found in agriculture streams) only increased to 9 (+ 1). This supports the occurrence of similar formulae in streams draining agriculture and forested landcovers. Likewise, when only comparing formulae present in forested and agricultural streams (removing urban streams), the number of formulae unique to agricultural streams (not present in forest) increased to 156 but the number of unique forest formulae (not present in agriculture) did not change. DOM in urban streams is thus not compositionally similar to DOM in forested streams, but there is more compositional overlap between agricultural and urban stream DOM.

While DOM composition for the three landcovers is broadly distinct, two samples fell outside the landcover clusters formed in the PCA. First, was an urban sample collected in spring from Bassett Creek that fell between the forest and agriculture landcover groupings in the PCA (Fig. 5a). The Bassett Creek sampling site is just upstream from where the creek discharges to the mainstem Mississippi River and is likely to experience some backflow from the Mississippi River during spring high discharge events. Upstream of Bassett Creek, the Mississippi River drains a mix of forest and agriculture landcovers, potentially giving this Bassett Creek sample a more forest and agriculture signal. Second, a winter agriculture sample from Trout Creek had a more negative loading along PC1 and a more positive loading along PC2 compared to the other agriculture samples (Fig. 5a). This Trout Creek sample was also the outlier previously mentioned for the N-aliphatic formulae, which may have driven the higher molecular richness and higher CHON relative abundances for this sample. High N-aliphatic relative abundance for this winter agricultural sample may relate to livestock manure applications on agricultural fields, which is commonplace in the Midwest United States (Gupta et al. 2004; Loecke et al. 2012) and has been associated with enrichment of N-containing peptides in soils (Mao et al. 2008). Winter application of manure combined with snowmelt could carry manure-derived DOM from these lands to groundwater and surface waters of adjacent waterways (e.g. Lewis and Makarewicz 2009; Zopp et al. 2019). The concurrent low discharge observed during winter months (Fig. S1) means manure-derived DOM may become relatively more concentrated and may give agricultural streams like Trout Creek a DOM signature similar to that of urban streams, which recorded the highest N-aliphatic abundances (Table 1; Fig. 4c).

Global patterns in landcover effects on dissolved organic matter composition

The DOM compositional differences we observed in the temperate UMRB are similar to compositional differences, also derived from FT-ICR MS analysis of DOM, for tropical streams (Drake et al. 2019; Spencer et al. 2019) and other temperate streams (Lu et al. 2015) draining forest and agriculture landcovers. In these study areas, DOM within forested streams also had greater relative allochthonous, terrestrial contributions with greater CHO- and aromatic compound relative abundances, compared to agricultural streams with comparatively higher autochthonous contributions and greater CHON and aliphatic compound relative abundance. These similarities in landcover effects on stream DOM between different climatic regions and localities suggests FT-ICR MS characterization of DOM can help elucidate how global patterns of stream DOM composition relate to upstream catchment landcover.

Global patterns in DOM composition with landcover is also reflected by the types of unique formulae associated with each landcover in two separate climatic regions. The single unique formula associated with all stream samples draining UMRB forested landcover (Table 4; Fig. 7a) was a HUP (high O/C) CHO formula, as were the nineteen formulae that were unique to the tropical forest streams (Fig. 7b; Spencer et al. 2019). The fourteen unique formulae associated with UMRB agricultural streams were HUP and polyphenolic CHON formulae (Table 4; Fig. 7a), which was the same classification as the four unique formulae seen for tropical agricultural catchments (Fig. 7b; Spencer et al. 2019). These HUP CHON formulae likely derive from autochthonous DOM inputs (e.g. Hanley et al. 2013) or degraded proteins (Kujawinski et al. 2004; Schmidt et al. 2009). The streams draining urban landcovers in the UMRB had eight unique formulae all classified as HUP and aliphatic CHOS formulae with high H/C ratios (Table 4; Fig. 7a). There is no comparable data from tropical urban streams, but CHOS formulae have been associated with DOM sourced from wastewater and septic-impacted groundwaters (Gonsior et al. 2011; Arnold et al. 2014). Overall, the similarities between unique formulae found in temperate and tropical climates suggest landcovers in other regions may produce distinct formulae belonging to different formulae classifications. Expanding the characterization of DOM to other climatic zones and landcovers will improve these interpretations.

Seasonal effects on dissolved organic matter composition

Although landcover appears to be the primary driver for DOC concentration and DOM composition, differences in seasonality have a secondary impact on contributions of DOM sources. Spring and summer samples had significantly greater molecular richness, AI_{mod}, and greater relative abundances of polyphenolics and condensed aromatics compared to winter, partially fall, samples. These characteristics all had positive loadings along PC2, which corresponds with the significant positive scores seen for the summer and the non-significant positive scores for the spring on PC2. Overall, this points to greater relative contributions of terrestrial, allochthonous DOM in streams in the spring and summer seasons compared to the winter, and partially compared to the fall. In contrast to the terrestrial, allochthonous contributions, relative autochthonous DOM contributions do not appear to significantly vary by season due to the lack of significant differences in the autochthonous-associated compounds (e.g. HUP low O/C, aliphatics, N-aliphatics) between seasons (Table 2).

There are several possibilities for this difference in terrestrial, allochthonous DOM contributions between seasons. First, the increases in terrestrial DOM contributions in the spring correspond to seasonal increases in discharge, as seen for all forested streams in the spring and for some agricultural and urbanized streams (Fig. S1). Increases in discharge within the UMRB have previously been associated with greater mobilization of terrestrial soil material in streams and diminished autochthonous production (Voss et al. 2017). Additionally, lowered discharge during winter months, with subsequently less mobilization of terrestrial soil DOM, likely increased groundwater DOM contributions to stream DOM during winter months compared to non-winter months (Voss et al. 2017). In contrast to the terrestrial soil DOM, groundwater DOM is leached from deeper soils and is typically dominated by plant-derived degraded compounds and microbial metabolites (Inamdar et al. 2011; Shen et al. 2015; Lambert et al. 2017). It is thus expected that the winter stream DOM reflects this more-degraded groundwater signature with lower relative abundances of condensed aromatics, as well as polyphenolic compounds. Groundwater DOM stream contributions have been characterized by an increase in HUP compounds (e.g. McDonough et al. 2020); however, HUP was insignificantly higher in the winter compared to all other seasons in the UMRB streams. Finally, increased terrestrial, allochthonous contributions in the summer may result, or partially result, from the summer coinciding with the peak growing season for forest landcover vegetation in the northern United States (e.g. Xiao et al. 2004; Sims et al. 2006) and to maximum gross primary production for cropland and grassland ecosystems (Guanter et al. 2014).

Conclusions

This study is one of the first to apply FT-ICR MS to characterize DOM compositional differences within streams of the same region that drain different landcovers (forest, agriculture, and urban) during four different seasons (winter, spring, summer, and fall). Streams and rivers draining agriculture and urban landcovers in the temperate UMRB had lower DOC concentrations than those draining forest landcover. Agriculture and urban stream DOM was also more heteroatomic and contained greater contributions from formulae associated with autochthonous carbon sources, driven by anthropogenic inputs from fertilizers and sewage. Despite this, agricultural stream and forested stream DOM had some common molecular formulae, suggesting the existence of an allochthonous DOM pool in agriculture-dominated watersheds either from patches of riparian vegetation and/or remnant soils of former forest and wetland landcover.

Changes in allochthonous DOM inputs from surface soils and vegetation, as well as increased autochthonous DOM contributions, to streams draining agriculture and urban landcovers have major implications for in-stream biogeochemical cycling. Autochthonous DOM is generally more biolabile and could contribute to an increase in CO_2 production and emissions (Drake et al. 2019). As a result, downstream transport of DOM to higher order streams and rivers may be reduced for streams draining urban and agriculture landcovers. Furthermore, the DOM contributions to waters downstream of urban and agricultural landcover could become increasingly characterized by highly-degraded and highly-stable DOM, warranting further investigation of downstream impacts of landcover alterations. High-resolution molecular-level techniques, such as those we utilized here, have the potential to trace the transfer of headwater stream DOM to larger-order streams and rivers to better understand the consequences of changing landcover in DOM and carbon cycling.

Acknowledgements This work was supported by the USGS Biological Carbon Sequestration Program, the USGS National Research Program, and the American Chemical Society through PRF #59916-DNI8. A portion of this work was performed at the National High Magnetic Field Laboratory ICR User Facility, which is supported by the National Science Foundation Division of Chemistry through DMR-1644779 and the State of Florida. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The authors thank all the helpful researchers at the NHMFL ICR Program who enabled data acquisition and processing. The authors would like to acknowledge Britta Voss, Dane Campbell, Sarah Stackpoole, and Sydney Foks for assistance with field sampling and logistics. The authors would also like to thank Elizabeth Grater and Sarah Ellen Johnston for her help with field prep and laboratory analyses and Amanda L. Cowden for assistance with selection of urban sites. The dataset generated during the current study is available in the EarthChem repository (https://doi.org/10.26022/IEDA/112060).

References

Aitkenhead-Peterson JA, Steele MK, Nahar N, Santhy K (2009) Dissolved organic carbon and nitrogen in urban and rural watersheds of south-central Texas: land use and land management influences. Biogeochemistry 96:119–129. https://doi.org/10.1007/s10533-009-9348-2

Alexandratos N, Bruinsma J (2012) World agriculture towards 2030/2050: the 2012 revision. ESA Working paper No. 12–03. FAO, Rome

Arnold WA, Longnecker K, Kroeger KD, Kujawinski EB (2014) Molecular signature of organic nitrogen in septic-impacted groundwater. Environ Sci Process Impacts 16:2400. https://doi.org/10.1039/c4em00289j

Battin TJ, Kaplan LA, Findlay S, Hopkinson CS, Marti E, Packman AI, Denis Newbold J, Sabater F (2008) Biophysical controls on organic carbon fluxes in fluvial networks. Nat Geosci 1:95–100. https://doi.org/10.1038/ngeo101

Bernhardt ES, Likens GE (2002) Dissolved organic carbon enrichment alters nitrogen dynamics in a forest stream. Ecology 83(6):1689–1700. https://doi.org/10.1890/0012-9658(2002)083[1689:DOCEAN]2.0.CO;2

Bernot MJ, Tank JL, Royer TV, David MB (2006) Nutrient uptake in streams draining agricultural catchments of the midwestern United States. Freshw Biol 51:499–509. https://doi.org/10.1111/j.1365-2427.2006.01508.x

Bernot MJ, Sobota DJ, Hall RO Jr., Mulholland PJ, Dodds WK, Webster JR, Tank JL et al (2010) Inter-regional comparison of land-use effects on stream metabolism. Freshw Biol 55:1874–1890. https://doi.org/10.1111/j.1365-2427.2010.02422.x

Blumentritt DJ, Wright HE Jr., Stefanova V (2009) Formation and early history of Lakes Pepin and St. Croix of the upper Mississippi River. J Paleolimnol 41:545–562. https://doi.org/10.1007/s10933-008-9291-6

Bratt AR, Finlay JC, Hobbie SE, Janke BD, Worm AC, Kemmitt KL (2017) Contribution of leaf litter to nutrient export during winter months in an urban residential watershed. Environ Sci Technol 51:3138–3147. https://doi.org/10.1021/acs.est.6b06299

Catalán N, Obrador B, Felip M, Pretus JL (2013) High reactivity of allochthonous vs. autochthonous DOC sources in a shallow lake. Aquat Sci 75:581–593. https://doi.org/10.1007/s00027-013-0302-y

Corilo YE (2014) PetroOrg software. Florida State University, Tallahassee

Corkum LD (1996) Responses of chlorophyll-a, organic matter, and macroinvertebrates to nutrient additions in rivers flowing through agricultural and forested land. Arch Hydrobiol 136(3):391–411

Cory RM, Ward CP, Crump BC, Kling GW (2014) Sunlight controls water column processing of carbon in arctic fresh waters. Science 345(6199):925–928. https://doi.org/10.1126/science.1253119

D'Andrilli J, Cooper WT, Foreman CM, Marshall AG (2015) An ultrahigh-resolution mass spectrometry index to estimate natural organic matter lability. Rapid Commun Mass Spectrom 29:2385–2401. https://doi.org/10.1002/rcm.7400

D'Andrilli J, Junker JR, Smith HJ, Scholl EA, Foreman CM (2019) DOM composition alters ecosystem function during microbial processing of isolated sources. Biogeochemistry 142:281–298. https://doi.org/10.1007/s10533-018-00534-5

DeFries RS, Foley JA, Asner GP (2004) Land-use choices: balancing human needs and ecosystem function. Front Ecol Environ 2(5):249–257. https://doi.org/10.1890/1540-9295(2004)002[0249:LCBHNA]2.0.CO;2

Delong MD, Brusven MA (1992) Patterns of periphyton chlorophyll a in an agricultural nonpoint source impacted stream. JAWRA 28(4):731–741. https://doi.org/10.1111/j.1752-1688.1992.tb01495.x

Dittmar T, Koch B, Hertkorn N, Kattner G (2008) A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. Limnol Oceanogr-Meth 6:230–235. https://doi.org/10.4319/lom.2008.6.230

Drake TW, Van Oost K, Barthel M, Bauters M, Hoyt AM, Podgorski DC, Six J, Boeckx P, Trumbore SE, Ntaboba LC, Spencer RGM (2019) Mobilization of aged and biolabile soil carbon by tropical deforestation. Nat Geosci 12:541–546. https://doi.org/10.1038/s41561-019-0384-9

Eathington L (2010) 2000–2009 population growth in the Midwest: urban and rural dimensions. Iowa State University, Department of Economics, Ames

Fasching C, Behounek B, Singer GA, Battin TJ (2014) Microbial degradation of terrigenous dissolved organic matter and potential consequences for carbon cycling in brown-water streams. Sci Rep 4:4981. https://doi.org/10.1038/srep04981

Fievre A, Solouki T, Marshal AG, Cooper WT (1997) High-resolution Fourier transform ion cyclotron resonance mass spectrometry of humic and fulvic acids by laser desorption/ionization and electrospray ionization. Energy Fuels 11(3):554–560. https://doi.org/10.1021/ef970005q

Fork ML, Blaszczak JR, Delesantro JM, Heffernan JB (2018) Engineered headwaters can act as sources of dissolved organic matter and nitrogen to urban stream networks. Limnol Oceanogr Lett 3:215–224. https://doi.org/10.1002/lol2.10066

Geiger R, Aron RH, Todhunter P (1995) The climate near the ground. Vieweg, Braunschweig

Gonsior M, Zwartjes M, Cooper WJ, Song W, Ishida KP, Tseng LY, Jeung MK, Rosso D, Hertkorn N, Schmitt-Kopplin P (2011) Molecular characterization of effluent organic matter identified by ultrahigh resolution mass spectrometry. Water Res 45:2943–2953. https://doi.org/10.1016/j.watres.2011.03.016

Goolsby DA, Battaglin WA, Lawrence GB, Artz R, Aulenbach BT, Hooper RP, Keeney DR, Stensland GJ (1999) Flux and sources of nutrients in the Mississippi-Atchafalaya River Basin: topic 3 Report for the Integrated Assessment on Hypoxia in the Gulf of Mexico. NOAA Coastal Ocean Program, Silver Spring

Graeber D, Boëchat LG, Eucina-Montoya F, Esse C, Gelbrecht J, Goyenola G, Gücker B, Heinz M, Kronvang B, Meerhoff M, Nimptsch J, Pusch MT, Silva RCS, von Schiller D, Zwirnmann E (2015) Global effects of agriculture on fluvial dissolved organic matter. Sci Rep 5:16328. https://doi.org/10.1038/srep16328

Guanter L, Zhang Y, Jung M, Joiner J, Voigt M, Berry JA et al (2014) Global and time-resolved monitoring of crop photosynthesis with chlorophyll fluorescence. PNAS. https://doi.org/10.1073/pnas.1320008111

Gücker B, Brauns M, Solimini AG, Voss M, Walz N, Pusch MT (2011) Urban stressors alter the trophic basis of secondary production in an agricultural stream. Can J Fish Aquat 68:74–88. https://doi.org/10.1139/F10-126

Gücker B, Silva RCS, Graeber D, Monteiro JAF, Boëchat IG (2016) Urbanization and agriculture increase exports and differentially alter elemental stoichiometry of dissolved organic matter (DOM) from tropical catchments. Sci Total Environ 550:785–792. https://doi.org/10.1016/j.scitotenv.2016.01.158

Guo LB, Gifford RM (2002) Soil carbon stocks and land use change: a meta analysis. Glob Change Biol 8:345–360. https://doi.org/10.1046/j.1354-1013.2002.00486.x

Gupta S, Munyankusi E, Moncrief J, Zvomuya F, Hanewall M (2004) Tillage and manure application effects on mineral nitrogen leaching from seasonally frozen soils. J Environ Qual 33:1238–1246. https://doi.org/10.2134/jeq2004.1238

Halvorson AD, Wienhold BJ, Black AL (2002) Tillage, nitrogen, and cropping system effects on soil carbon sequestration. Soil Sci Soc Am J 66:906–912. https://doi.org/10.2136/sssaj2002.9060

Hanley KW, Wollheim WM, Salibury J, Huntington T, Aiken G (2013) Controls on dissolved organic carbon quantity and chemical character in temperate rivers of North America. Global Biogeochem Cycles 27:492–504. https://doi.org/10.1002/gbc.20044

Harfmann JL, Guillemette F, Kaiser K, Spencer RGM, Chaung C-Y, Hernes PJ (2019) Convergence of terrestrial dissolved organic matter composition and the role of microbial buffering in aquatic ecosystems. J Geophys Res Biogeosci 124:3125–3142. https://doi.org/10.1029/2018JG004997

Heinz M, Graeber D, Zak D, Zwirnmann E, Gelbrecht J, Pusch MT (2015) Comparison of organic matter composition in agricultural versus forest affected headwaters with special emphasis on organic nitrogen. Environ Sci Technol 49:2081–2090. https://doi.org/10.1021/es505146h

Hendrickson CL, Quinn JP, Kaiser NK, Smith DF, Blakney GT, Chen T, Marshall AG, Weisbrod CR, Beau SC (2015) 21 Tesla fourier transform ion cyclotron resonance mass spectrometer: a national resource for ultrahigh resolution mass analysis. J Am Soc Mass Specrom 26:1626–1632. https://doi.org/10.1007/s13361-015-1182-2

Hertkorn N, Harir M, Koch BP, Michalke B, Schmitt-Kopplin P (2013) High-field NMR spectroscopy and FTICR mass spectrometry: powerful discovery tools for the molecular level characterization of marine dissolved organic matter. Biogeosciences 10:1583–1624. https://doi.org/10.5194/bg-10-1583-2013

Hertkorn N, Harir M, Cawley KM, Schmitt-Kopplin P, Jaffé R (2016) Molecular characterization of dissolved organic matter from subtropical wetlands: a comparative study through the analysis of optical properties, NMR, and FTICR/MS. Biogeosciences 13:2257–2277. https://doi.org/10.5194/bg-13-2257-2016

Hinckley E-LS, Crawford JT, Fakhraei H, Driscoll CT (2020) A shift in sulfur-cycle manipulation from atmospheric emissions to agricultural additions. Nat Geosci 13:597–604. https://doi.org/10.1038/s41561-020-0620-3

Hobbie SE, Baker LA, Buyarski C, Nidzgorski D, Finlay JC (2014) Decomposition of tree leaf litter on pavement: implications for urban water quality. Urban Ecosyst 17:369–385. https://doi.org/10.1007/s11252-013-0329-9

Hope D, Billett MF, Cresser MS (1994) A review of the export of carbon in river water: fluxes and processes. Environ Pollut 84:301–324. https://doi.org/10.1016/0269-7491(94)90142-2

Hosen JD, McDonough OT, Febria CM, Palmer MA (2014) Dissolved organic matter quality and bioavailability changes across an urbanization gradient in headwater streams. Environ Sci Technol 48:7817–7824. https://doi.org/10.1021/es501422z

Houser JN, Richardson WB (2010) Nitrogen and phosphorus in the Upper Mississippi River: transport, processing, and effects on the river ecosystem. Hydrobiologia 640:71–88. https://doi.org/10.1007/s10750-009-0067-4

Houser JN, Bartsch LA, Richardson WB, Rogala JT, Sullivan JF (2015) Ecosystem metabolism and nutrient dynamcis in the main channel and backwaters of the Upper Mississippi River. Freshw Biol 60:1863–1879. https://doi.org/10.1111/fwb.12617

Inamdar S, Singh S, Dutta S, Levia D, Mitchell M, Scott D, Bais H, McHale P (2011) Fluorescence characteristics and sources of dissolved organic matter for stream water during storm events in a forested mid-Atlantic watershed. J Geophys Res 116:G03034. https://doi.org/10.1029/2011JG001735

Jaffé R, Yamashita Y, Maie N, Cooper WT, Dittmar T, Dodds WK, Jones JB, Myoshi T, Ortiz-Zayas JR, Podgorski DC, Watanabe A (2012) Dissolved organic matter in headwater streams: compositional variability across climatic regions of North America. Geochim Cosmochim Acta 94:95–108. https://doi.org/10.1016/j.gca.2012.06.031

Kaushal SS, Belt KT (2012) The urban watershed continuum: evolving spatial and temporal dimensions. Urban Ecosyst 15:409–435. https://doi.org/10.1007/s11252-012-0226-7

KC S, Lutz W (2017) The human core of the share socioeconomic pathways: population scenarios by age, sex, and level of education for all countries to 2100. Glob Environ Change 42:181–192. https://doi.org/10.1016/gloenvcha.2014.06.004

Kellerman AM, Kothawala DN, Dittmar T, Tranvik LJ (2015) Persistence of dissolved organic matter in lakes related to its molecular characteristics. Nat Geosci 8:454–457. https://doi.org/10.1038/NGEO2440

Kellerman AM, Guillemette F, Podgorski DC, Aiken GR, Butler KD, Spencer RGM (2018) Unifying concepts linking dissolved organic matter composition to persistence in aquatic ecosystems. Environ Sci Technol 52:2538–2548. https://doi.org/10.1021/acsest.7b05513

Kelley DW, Brachfeld SA, Nater EA, Wright HE Jr. (2006) Sources of sediment in Lake Pepin on the Upper Mississippi River in response to Holocene climate changes. J Paleolimnol 35:193–206. https://doi.org/10.1007/s10933-005-8686-x

Khan SA, Mulvaney RL, Ellsworth TR, Boast CW (2007) The myth of nitrogen fertilization for soil carbon sequestration. J Environ Qual 36:1821–1832. https://doi.org/10.2134/jeq2007.0099

Killingsworth BA, Bao H (2015) Significant human impact on the flux and δ^{34}S of sulfate from the largest river in North America. Environ Sci Technol 49:4851–4860. https://doi.org/10.1021/esc504498s

Koch BP, Dittmar T (2006) From mass to structure: an aromaticity index for high-resolution mass data of natural organic matter. Rapid Commun Mass Spectrom 20:926–932. https://doi.org/10.1002/rcm.2386

Koch BP, Dittmar T (2016) From mass to structure: an aromaticity index for high-resolution mass data of natural organic matter. Rapid Commun Mass Spectrom 30:250. https://doi.org/10.1002/rcm.7433

Koch BP, Dittmar T, Witt M, Kattner G (2007) Fundamental of molecular formula assignment to ultrahigh resolution mass data of natural organic matter. Anal Chem 79:1758–1763. https://doi.org/10.1021/ac061949s

Kujawinski EB, Hatcher PG, Freitas MA (2002) High-resolution Fourier transform ion cyclotron resonance mass spectrometry of humic and fulvic acids: improvements and comparisons. Anal Chem 74(2):413–419. https://doi.org/10.1021/ac0108313

Kujawinski EB, Del Vecchio R, Blough NV, Klein GC, Marshall AG (2004) Probing molecular-level transformations of dissolved organic matter: insights on photochemical degradation and protozoan modification of DOM from electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Mar Chem 92:23–37. https://doi.org/10.1016/j.marchem.2004.06.038

Lambert T, Bouillon S, Darchambeau F, Morana C, Roland FAE, Descy J-P, Borges AV (2017) Effects of human land use on the terrestrial and aquatic sources of fluvial organic matter in a temperate river basin (The Meuse River, Belgium). Biogeochemistry 136:191–211. https://doi.org/10.1007/s10533-017-0387-9

Ledesma JLJ, Futter MN, Blackburn M, Lidman F, Grabs T, Sponseller RA, Laudon H, Bishop KH, Köhler SJ (2018) Towards an improved conceptualization of riparian zones in boreal forest headwaters. Ecosystems 21:297–315. https://doi.org/10.1007/s10021-017-0149-5

Lewis TW, Makarewicz JC (2009) Winter application of manure on an agricultural watershed and its impact on downstream nutrient fluxes. J Great Lakes Res 35:43–49. https://doi.org/10.1016/j.jglr.2008.08.003

Loecke TD, Cambardella CA, Liebman M (2012) Synchrony of net nitrogen mineralization and maize nitrogen uptake following applications of composted and fresh swine manure in the Midwest U.S. Nutr Cycl Agroecosyst 93:65–74. https://doi.org/10.1007/s10705-012-9500-6

Lu YH, Bauer JE, Canuel EA, Chambers RM, Yamashita Y, Jaffé R, Barrett A (2014) Effects of land use on sources and ages of inorganic and organic carbon in temperate headwater streams. Biogeochemistry 119:275–292. https://doi.org/10.1007/s10533-014-9965-2

Lu Y, Li X, Mesfloul R, Bauer JE, Chambers RM, Canuel EA, Hatcher PG (2015) Use of ESI-FTICR-MS to characterize dissolved organic matter in headwater streams draining forest-dominated and pasture-dominated watersheds. PLoS ONE 10(12):e0145639. https://doi.org/10.1371/journal.pone.0145639

Mao J, Olk DC, Fang X, He Z, Schmidt-Rohr K (2008) Influence of animal manure application on the chemical structures of soil organic matter as investigated by advanced solid-state NMR and FT-IR spectroscopy. Geoderma 146:353–362. https://doi.org/10.1016/j.geoderma.2008.06.003

Mattsson T, Kortelainen P, Räike A (2005) Export of DOM from boreal catchments: impacts of land use cover and climate. Biogeochemistry 76:373–394. https://doi.org/10.1007/s10533-05-6897-x

McDonough LK, Rutlidge H, O’Carroll DM, Andersen MS, Meredith K, Behnke MI, Spencer RGM, McKenna AM, Marjo CE, Oudone P, Baker A (2020) Characterization of shallow groundwater dissolved organic matter in aeolian, alluvial and fractured rock aquifers. Geochim Cosmochim Acta 273:163–176. https://doi.org/10.1016/j.gca.2020.01.022

McLauchlan K (2006) The nature and longevity of agricultural impacts on soil carbon and nutrients: a review. Ecosystems 9:1364–1382. https://doi.org/10.1007/s10021-005-0135-1

Meng F, Huang G, Yang X, Li Z, Li J, Cao J, Wang Z, Sun L (2013) Identifying the sources and fate of anthropogenically impacted dissolved organic matter (DOM) in urbanized rivers. Water Res 47:5027–5039. https://doi.org/10.1016/j.watres.2013.05.043

Meyer JL, Wallace JB, Eggert SL (1998) Leaf litter as a source of dissolved organic carbon in streams. Ecosystems 1:240–249. https://doi.org/10.1007/s100219900019

Mulholland PJ (2003) Large-scale patterns in dissolved organic carbon concentration, flux, and sources. In: Findlay S, Sinsabaug RL (eds) Aquatic ecosystems—interactivity of dissolved organic matter. Academic Press, Cambridge, pp 139–159. https://doi.org/10.1016/B978-012256371-3/50007-X

Nagy RC, Porder S, Brando P, Davidson EA, Silva Figueira AM, Neill C, Riskin S, Trumbore S (2018) Soil carbon dynamics in soybean cropland and forests in Mato Grosso, Brazil. J Geophys Res Biogeosci 123:18–31. https://doi.org/10.1002/2017JG004269

Newcomer TA, Kaushal SS, Mayer PM, Shields AR, Canuel EA, Groffman PM, Gold AJ (2012) Influence of natural and novel organic carbon sources on denitrification in forest, degraded urban, and restored streams. Ecol Monogr 82(4):449–466. https://doi.org/10.1890/12-0458.1

Niles SF, Chacón-Patiño ML, Putnam SP, Rodgers RP, Marshall AG (2020) Characteriation of an asphalt binder and photoproducts by Fourier transform ion cyclotron resonance mass spectrometry reveals abundant water-soluble hydrocarbons. Environ Sci Technol 54:8830–8836. https://doi.org/10.1021/acs.est.0c02263

O’Donnell JA, Aiken GR, Butler KD, Guillemette F, Podgorski DC, Spencer RGM (2016) DOM composition and transformation in boreal forest soils: the effects of temperature and organic-horizon decomposition state. J Geophys Res Biogeosci 121:2727–2744. https://doi.org/10.1002/2016JG003431

Ogle SM, Breidt FJ, Paustian K (2005) Agricultural management impacts on soil organic carbon storage under moist and dry climatic conditions of temperate and tropical regions. Biogeochemistry 72:87–121. https://doi.org/10.1007/s10533-004-0360-2

Panno SV, Hackley KC, Kelley WR, Hwang H-H (2006) Isotopic evidence of nitrate sources and denitrification in the Mississippi River, Illinois. J Environ Qual 35:495–504. https://doi.org/10.2134/jeq2005.0012

Parr TB, Cronan CS, Ohno T, Findlay SEG, Smith SMC, Simon KS (2015) Urbanization changes the composition and bioavailability of dissolved organic matter in headwater streams. Limnol Oceanogr 60:885–900. https://doi.org/10.1002/lno.10060

Pennino MJ, Kaushal SS, Mayer PM, Utz RM, Cooper CA (2016) Stream restoration and sewers impact sources and fluxes of water, carbon, and nutrients in urban watersheds. Hydrol Earth Syst Sci 20:3419–3439. https://doi.org/10.5194/hess-20-3419-2016

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rajib A, Merwade V (2017) Hydrologic response to future land use change in the Upper Mississippi River Basin by the end of 21st century. Hydrol Process 31:3645–3661. https://doi.org/10.1002/hyp.11282

Ramankutty N, Foley JA (1999) Estimating historical changes in global land cover: croplands from 1700 to 1992. Global Biogeochem Cycles 13(4):997–2017. https://doi.org/10.1029/1999GB900046

Raymond PA, Oh N-H, Turner RE, Broussard W (2008) Anthropogenically enhanced fluxes of water and carbon from the Mississippi River. Nature 451:449–452. https://doi.org/10.1038/nature06505

Reche I, Pace ML, Cole JJ (1998) Interactions of photobleaching and inorganic nutrients in determining bacterial growth on colored dissolved organic carbon. Microb Ecol 36(3):270–280

Reicosky DC, Kemper WD, Langdale GW, Douglas CL Jr., Rasmussen PE (1995) Soil organic matter changes resulting from tillage and biomass production. J Soil Water Conserv 50(3):253–261

Riedel T, Zark M, Vähätalo AV, Niggemann J, Spencer RGM, Hernes PJ, Dittmar T (2016) Molecular signatures of biogeochemical transformations in dissolved organic matter from ten world rivers. Front Earth Sci 4:85. https://doi.org/10.3389/feart.2016.00085

Šantl-Temkiv T, Finster K, Dittmar T, Hansen BM, Thyrhaug R, Nielsen NW, Karlson UG (2013) Hailstones: a window into the microbial and chemical inventory of a storm cloud. PLoS ONE 8(1):e53550. https://doi.org/10.1371/journal.pone.0053550

Schilling KE, Wolter CF, McLellan E (2015) Agro-hydrologic landscapes in the Upper Mississippi and Ohio River basins. Environ Manag 55:646–656. https://doi.org/10.1007/s00267-014-0420-x

Schmidt F, Elvert M, Koch BP, Witt M, Hinrichs K-U (2009) Molecular characterization of dissolved organic matter in pore water of continental shelf sediments. Geochim Cosmochim Acta 73:3337–3358. https://doi.org/10.1016/j.gca.2009.03.008

Schnitkey G (2013) Concentration of corn and soybean production in the US. Department of Agricultural and Consumer Economics, University of Illinois, Urbana-Champaign

Seto KC, Fragkias M, Güneralp B, Reilly MK (2011) A meta-analysis of global urban land expansion. PLOS ONE 6(8):e23777. https://doi.org/10.1371/journal.pone.0023777

Shen Y, Chapelle FH, Strom EW, Benner R (2015) Origins and bioavailability of dissolved organic matter in groundwater. Biogeochemistry 122:61–78. https://doi.org/10.1007/s10533-014-0029-4

Sickman JO, Zanoli MJ, Mann HL (2007) Effects of urbanization on organic carbon loads in the Sacramento River, California. Water Resour Res 43:W11422. https://doi.org/10.1029/2007WR005954

Silva JSO, Bustamante MMC, Markewitz D, Krusche AV, Guimarães Ferreira L (2011) Effects of land cover on chemical characteristics of streams in the Cerrado region of Brazil. Biogeochemistry 105:75–88. https://doi.org/10.1007/s10533-010-9557-8

Sims DA, Rahman AF, Cordova VD, El-Masri BZ, Baldocchi DD, Flanagan LB, Goldstein AH, Hollinger DY, Misson L, Monson RK, Oechel WC, Schmid HP, Wofsy SC, Xu L (2006) On the use of MODIS EVI to assess gross primary productivity of North American ecosystems. J Geophys Res 111:G04015. https://doi.org/10.1029/2006JG000162

Sleighter RL, McKee GA, Liu Z, Hatcher PG (2008) Naturally present fatty acids as internal calibrants for Fourier transform mass spectra of dissolved organic matter. Limnol Oceanogr: Methods 6:246–253. https://doi.org/10.4319/lom.2008.6.256

Smith DF, Podgorski DC, Rodgers RP, Blakney GT, Hendrickson CL (2018) 21 Tesla FT-ICR mass spectrometer for ultrahigh-resolution analysis of complex organic mixtures. Anal Chem 90:2041–2047. https://doi.org/10.1021/acs.analchem.7b04159

Spencer RGM, Stubbins A, Hernes PJ, Baker A, Mopper K, Aufdenkampe AK, Dyda RY, Mwamba VL, Mangangu AM, Wabakanghanzi JN, Six J (2009) Photochemical degradation of dissolved organic matter and dissolved lignin phenols from the Congo River. J Geophys Res 114:GB03010. https://doi.org/10.1029/2009JG000968

Spencer RGM, Kellerman AM, Podgorski DC, Macedo MN, Jankowski K, Nunes D, Neill C (2019) Identifying the molecular signatures of agricultural expansion in Amazonian headwater streams. J Geophys Res Biogeosci 124:1637–1650. https://doi.org/10.1029/2018JG004910

Spiker EC, Rubin M (1975) Petroleum pollutants in surface and groundwater as indicated by the carbon-14 activity of dissolved organic carbon. Science 187(4171):61–64. https://doi.org/10.1126/science.187.4171.61

Stanley EH, Powers SM, Lottig NR, Buffam I, Crawford JT (2012) Contemporary changes in dissolved organic carbon (DOC) in human-dominated rivers: is there a role for DOC management? Freshw Biol 57:26–42. https://doi.org/10.1111/j.1365-2427.2011.02613.x

Stark JR, Hanson PE, Goldstein RM, Fallon JD, Fong AL, Lee KE, Kroening SE, Andrews WJ (2000) Water quality in the Upper Mississippi River Basin, Minnesota, and Wisconsin, South Dakota, Iowa, and North Dakota, 1995-98. Summary Circular 1211. US Geological Survey, Reston

Stenson AC, Landing WM, Marshall AG, Cooper WT (2002) Ionization and fragmentation of humic substances in electrospray ionization Fourier transform-ion cyclotron resonance mass spectrometry. Anal Chem 74(17):4397–4409. https://doi.org/10.1021/ac020019f

Stenson AC, Marshall AG, Cooper WT (2003) Exact masses and chemical formulas of individual Suwannee River fulvic acids from ultrahigh resolution electrospray ionization fourier transform ion cyclotron resonance mass spectra. Anal Chem 75:1275–1284. https://doi.org/10.1021/ac026106

Stubbins A, Spencer RGM, Chen H, Hatcher PG, Mopper K, Hernes PJ, Mwamba VL, Mangangu AM, Wabakanghanzi JN, Six J (2010) Illuminated darkness: molecular signatures of Congo River dissolved organic matter and its photochemical alteration as revealed by ultrahigh precision mass spectrometry. Limnol Oceanogr 55(4):1467–1477. https://doi.org/10.4319/lo.2010.55.4.1467

Textor SR, Guillemette F, Zito PA, Spencer RGM (2018) An assessment of dissolved organic carbon biodegradability and priming in blackwater systems. J Geophys Res Biogeo 123:2998–3015. https://doi.org/10.1029/2018JG004470

Thurman EN (1984) Organic geochemistry of natural waters. Nijhoff/Junk, Dordrecht

Tilman D, Fargione J, Wolff B, D'Antonio C, Dobson A, Howarth R, Schindler D, Schlesinger WH, Simberloff D, Swackhamer D (2001) Forecasting agriculturally driven global environmental change. Sciences 292:281–284. https://doi.org/10.1126/sciences.1057544

Tsui MTK, Finlay JC (2011) Influence of dissolved organic matter on methylmercury bioavailability across Minnesota stream ecosystems. Environ Sci Technol 45:5981–5987. https://doi.org/10.1021/es200332f

Turner RE, Rabalais NN (1991) Change in Mississippi River water quality this century. Bioscience 41:140–147. https://doi.org/10.2307/1311453

Turner RE, Rabalais NN (2003) Linking landscape and water quality in the Mississippi River Basin for 200 years. Bioscience 53(6):563–572. https://doi.org/10.1641/0006-3568(2003)053[0563:LLAWQI]2.0.CO;2

US Geological Survey (2012) StreamStats: U.S. Geological Survey website. http://streamstats.usgs.gov/.

Van Oost K, Quine TA, Govers G, De Gryze S, Six J, Harden JW, Ritchie JC, McCarty GW, Heckrath G, Kosmas C, Giraldez JV, Marques da Silva JR, Merckx R (2007) The impact of agricultural soil erosion on the global carbon cycle. Science 318:626–629. https://doi.org/10.1126/science.1145724

Verstraeten IM, Fetterman GS, Meyer MT, Bullen T, Sebree SK (2005) Use of tracers and isotopes to evaluate vulnerability of water in domestic wells to septic waste. Ground Water

Monit Remidiat 25(2):107–117. https://doi.org/10.1111/j.1745-6592.2005.0015.x

Voss BM, Wickland KP, Aiken GR, Striegl RG (2017) Biological and land use controls on the isotopic composition of aquatic carbon in the Upper Mississippi River Basin. Global Biogeochem Cycles 31:1271–1288. https://doi.org/10.1002/2017GB005699

Wagner S, Riedel T, Niggemann J, Vähätalo AV, Dittmar T, Jaffeé R (2015) Linking to molecular signatures of heteroatomic dissolved organic matter to watershed characteristics in world rivers. Environ Sci Techn 49:13798–13806. https://doi.org/10.1021/acs.est.5b00525

Wasley D (2000) Concentration and movement of nitrogen and other materials in selected reaches and tributaries of the Upper Mississippi River System. University of Wisconsin-La Crosse, La Crosse

Webster JR, Meyer JL (1997) Stream organic matter budgets. J N Am Benthol Soc 16(1):3–161. https://doi.org/10.2307/1468223

Westerhoff P, Anning D (2000) Concentration and characteristics of organic carbon in surface water in Arizona: influence of urbanization. J Hydrol 236:202–222. https://doi.org/10.1016/S0022-1694(00)00292-4

Wickland KP, Neff JC, Aiken GR (2007) Dissolved organic carbon in Alaskan boreal forest: sources, chemical characteristics, and biodegradability. Ecosystems 10:1323–1340. https://doi.org/10.1007/s10021-007-9101-4

Wieczorek ME, Jackson SE, Schwarz GE (2018) Select attributes for NHDPlus version 2.1 reach catchments and modified network routed upstream watersheds for the conterminous United States (ver. 3.0, January 2021): U.S. Geological Survey data release. https://doi.org/10.5066/F7765D7V

Williams CJ, Yamashita Y, Wilson HF, Jaffé R, Xenopoulos MA (2010) Unraveling the role of land use and microbial activity in shaping dissolved organic matter characteristics in stream ecosystems. Limnol Oceanogr 55(3):1159–1171. https://doi.org/10.4319/lo.2010.55.3.1159

Wilson HF, Xenopoulos MA (2009) Effects of agricultural land use on the composition of fluvial dissolved organic matter. Nat Geosci 2:37–41. https://doi.org/10.1038/NGEO391

Wright CK, Wimberly MC (2013) Recent land use change in the Western Corn Belt threatens grasslands and wetlands. PNAS 110(10):4134–4139. https://doi.org/10.1073/pnas.1215404110

Wright AL, Provin TL, Hons FM, Zuberer DA, White RH (2005) Dissolved organic carbon in soil from compost-amended Bermudagrass turf. HortScience 40(3):830–835. https://doi.org/10.21273/HORTSCI.40.3.830

Xiao X, Hollinger D, Aber J, Goltz M, Davidson EA, Zhang Q, Moore B (2004) Satellite-based modeling of gross primary production in an evergreen needleleaf forest. Remote Sens Environ 89:519–534. https://doi.org/10.1016/j.rse.2003.11.008

Yang L, Jin S, Danielson P, Homer C, Gass L, Bender SM, Case A, Costello C, Dewitz J, Fry J, Funk M, Granneman B, Liknes GC, Rigge M, Xian G (2018) A new generation of the United States National Land Cover Database: requirements, research priorities, design, and implementation strategies. ISPRS J Photogramm 146:108–123. https://doi.org/10.1016/j.lsprsjprs.2018.09.006

Ye Q, Zhang Z-T, Liu Y-C, Wang Y-H, Zhang S, He C, Shi Q, Zeng H-X, Wang J-J (2019) Spectroscopic and molecular-level characteristics of dissolved organic matter in a highly polluted urban river in south China. ACS Earth Space Chem 3(9):2033–2044. https://doi.org/10.1021/acsearthspacechem.9b00151

Zopp ZP, Ruark MD, Thompson AM, Stuntebeck TD, Cooley E, Radatz A, Radatz T (2019) Effects of manure and tillage on edge-of-field phosphorus loss in seasonally frozen landscapes. J Environ Qual 48:966–977. https://doi.org/10.2134/jeq2019.01.0011