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Bioavailability of dissolved organic matter varies with anthropogenic landcover in the Upper Mississippi River Basin

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ABSTRACT

Anthropogenic conversion of forests and wetlands to agricultural and urban landcovers impacts dissolved organic matter (DOM) within streams draining these catchments. Research on how landcover conversion impacts DOM molecular level composition and bioavailability, however, is lacking. In the Upper Mississippi River Basin (UMRB), water from low-order streams and rivers draining one of three dominant landcovers (forest, agriculture, urban) was incubated for 28 days to determine bioavailable DOC (BDOC) concentrations and changes in DOM composition. The BDOC concentration averaged 0.49 ± 0.30 mg L^{-1} across all samples and was significantly higher in streams draining urban catchments (0.72 ± 0.34 mg L^{-1}) compared to streams draining agricultural (0.28 ± 0.15 mg L^{-1}) and forested (0.47 ± 0.17 mg L^{-1}) catchments. Percent BDOC was significantly greater in urban (10% ± 4.4%) streams compared to forested streams (5.6% ± 3.2%), corresponding with greater relative abundances of aliphatic and N-containing aliphatic compounds in urban streams. Aliphatic compound relative abundance decreased across all landcovers during the bioincubation (average -4.1% ± 10%), whereas polyphenolics and condensed aromatics increased in relative abundance across all landcovers (average of +1.4% ± 5.9% and +1.8% ± 10%, respectively). Overall, the conversion of forested to urban landcover had a larger impact on stream DOM bioavailability in the UMRB compared to conversion to agricultural landcover. Future research examining the impacts of anthropogenic landcover conversion on stream DOM composition and bioavailability needs to be expanded to a range of spatial scales and to different ecotones, especially with continued landcover alterations.

Abbreviations: ΔRA, change in relative abundance; AI_{mod}, modified aromaticity index; BDOC, bioavailable dissolved organic carbon; CHO, compounds with C, H, and O; CHON, compounds with C, H, O, and N; CHONS, compounds with C, H, O, N, and S; CHOS, compounds with C, H, O, and S; DOC, dissolved organic carbon; DOM, dissolved organic matter; HUP, highly unsaturated and phenolic; FT-ICR MS, Fourier transform-ion cyclotron resonance mass spectrometry; NHMFL, national high magnetic field laboratory; OC, organic carbon; PCA, principal component analysis; T0, time point 0 (start of bioincubation); T28, time point 28 (end of bioincubation); UMRB, Upper Mississippi River Basin.

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1. Introduction

Riverine dissolved organic matter (DOM) represents a crucial component of the global carbon cycle, composed of a heterogeneous mixture of compounds derived from terrestrial and aquatic sources (Hopkinson et al., 1998; Raymond and Spencer, 2015). Decomposition of riverine DOM by aquatic microbial communities leads to the preferential biodegradation of bioavailable DOM and the accumulation of less bioavailable DOM during downstream transport (Kim et al., 2006; Kellerman et al., 2018). Rivers also receive bioavailable organic matter from anthropogenic sources derived from agricultural and urban landcovers (e.g. Wilson and Xenopoulos, 2009; Hosen et al., 2014; Drake et al., 2019; Spencer et al., 2019; Vaughn et al., 2021), which, when combined with increasing agricultural and urban lands with a continuously increasing human population, could lead to greater riverine DOM bioavailability globally and potentially increased greenhouses gas emissions (Graeber et al., 2015; Drake et al., 2019).

In the Upper Mississippi River Basin (UMRB), where population growth has led to extensive cropland expansion and increased urbanization (Eathington, 2010; Wright and Wimberly, 2013), we demonstrated streams draining agricultural or urban landcovers had lower dissolved organic carbon (DOC) concentrations and higher relative abundances of compounds characteristic of autochthonous (e.g. algae, phytoplankton, bacteria) and anthropogenic DOM sources compared to streams draining forested and wetland landcovers (referred hereafter as forested; Vaughn et al., 2021). Autochthonous and anthropogenic DOM is generally considered to be more bioavailable than DOM derived from terrestrial plants and organic-rich soils (D'Andrilli et al., 2015; Riedel et al., 2016; Textor et al., 2018); however, it is unknown whether differences in landcover significantly alters overall stream DOM bioavailability with regards to amount and molecular level composition (e.g. changes in relative contributions of aliphatic and condensed aromatic compounds). Additionally, most research on stream DOM composition and bioavailability compares streams draining forested landcovers to streams draining either agricultural or urban landcovers, rarely both.

Here, we investigated DOM bioavailability in the UMRB using bioincubation experiments to measure potential microbial utilization of DOM in streams draining one of three dominant landcovers (forested, agriculture, and urban). DOC loss during the bioincubation represents bioavailable DOC (BDOC), often used to assess DOM bioavailability (Wickland et al., 2007; Guillemette and del Giorgio, 2011). We also utilized Fourier transform-ion cyclotron resonance mass spectrometry (FT-ICR MS), which can differentiate thousands of molecular formulae in a single DOM sample (D'Andrilli et al., 2015; Ware et al., 2022), to assess differences in DOM molecular signatures between pre- and post-bioincubation samples (Textor et al., 2018; Drake et al., 2019). We hypothesized DOM in streams draining agricultural and urban catchments in the UMRB would have greater BDOC than streams draining forested catchments due to greater contributions of bioavailable compounds derived from autochthonous and anthropogenic sources. We also hypothesized the proportion of less bioavailable compounds (e.g. condensed aromatics, polyphenolics) following the bioincubation would increase in all landcovers as bioavailable DOM is degraded, which will have important implications for what types of compounds are transported to downstream ecosystems and water treatment.

2. Methods

2.1. Site description and sample collection

The UMRB is defined here as the area drained by the Mississippi River at Wabasha, MN (Fig. 1). Streams and rivers within the UMRB flow through geologies of variable glacial influence (Blumentritt et al., 2009) and drain a wide range of landcovers (forested, agricultural, wetland, and urban; Table S1). Agricultural lands of the UMRB support row crops (i.e. corn and soybeans) and many agricultural practices used in the UMRB have been linked to alterations in river discharge and water quality (e.g. tile drainage, fertilizer use, and changes in crop type; Raymond et al., 2008).

Nine low-order streams and rivers in the UMRB having distinct landcover were selected for the study (Fig. 1; Table S1). Detailed explanations of the watershed classifications for each stream and river can be found in Vaughn et al. (2021) and in the supplemental material. Three streams drain predominantly urban landcover (Bassett Creek, Minnehaha Creek, and Shingle Creek); three streams/rivers drain predominantly agricultural landcover (Como Creek, Trout Creek, and Red Cedar River); and three streams/rivers drain predominantly forested landcover (Allequash Creek, Chippewa River, and Flambeau River). Streams and rivers were sampled on five occasions over a 1.5-year period (Table S2) and more detailed sampling methods can be found in Vaughn et al. (2021) and the supplemental material. Water was collected ~0.25 m below the surface and filtered through pre-rinsed 0.45 μm capsule filters (Geotech Versapor membrane filter) into pre-combusted 0.5L amber glass bottles (bioincubation) or acid-cleaned polycarbonate/high-density polyethylene bottles (chemical analyses). Unfiltered water for bioincubation inoculation was collected in pre-combusted 60 mL amber glass bottles. Samples for DOC concentration were kept cool (4°C) and in the dark; FT-ICR MS samples were

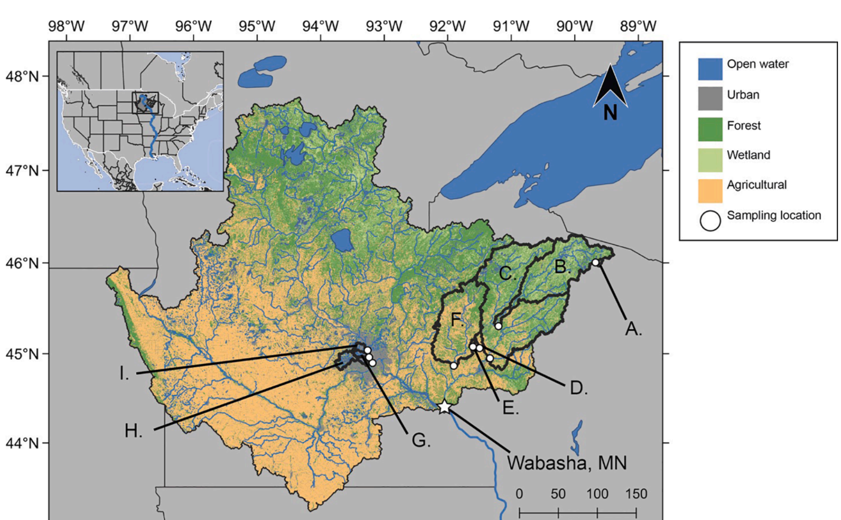

Fig. 1. Study region. The streams/rivers and their watersheds are labeled as follows: (A) Allequash Creek (forest); (B) Flambeau River (forest); (C) Chippewa River (forest); (D) Como Creek (agricultural); (E) Trout Creek (agricultural); (F) Red Cedar River (agricultural); (G) Bassett Creek (urban); (H) Minnehaha Creek (urban); (I) Shingle Creek (urban). Forest landcover classification for Sites A-C is based on combined forest and wetland areas (light green and dark green colors; Table S1). Inset map shows region outlines in black and the full length of the main stem Mississippi River outlined in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

frozen within 12 h of collection until further processing.

2.2. Bioincubation experiments

Bioincubations were set up the same day as sample collection. Filtered water (0.45 µm, 0.5 L) was inoculated with 5 mL of water filtered through pre-rinsed 1.6-µm glass fiber syringe filters, gently mixed for one minute, then divided as 50-mL aliquots into six 100 mL pre-combusted amber glass serum bottles, leaving 50 mL of headspace. Serum bottles were capped with Teflon-lined stoppers and sealed with crimps. Three bottles were immediately injected with 1 mL of 50% H_3PO_4 as a preservative and designated as T=0 days. All bottles were stored in the dark at ambient temperature until returning to the laboratory.

Bioincubation serum bottles acidified at T=0 days (n=3 per site, per date) were refrigerated at 4°C upon returning to the laboratory. Unacidified bottles (n=3 per site, per date) were kept in the dark in an incubator set at 20°C for 28 days from initiation. At T=28 days, the unacidified bottles were acidified with 1 mL of 50% H_3PO_4 and stored in the dark at 4°C to halt biodegradation (Wickland et al., 2007).

2.3. Dissolved organic carbon concentration

DOC samples (T=0 and T=28 days) were analyzed on a Shimadzu TOC-L CPH, detailed in Vaughn et al. (2021) and in the supplemental material. The amount of BDOC (mg C L^{-1}) was calculated as the loss of DOC during the bioincubation, between mean initial (T=0 days) and mean final (T=28 days) DOC concentrations. Percent DOC loss, or the portion of DOC that is bioavailable (% BDOC), was calculated as mean DOC loss divided by the mean initial DOC concentrations and multiplied by one hundred to obtain a percentage (Eq. (1)).

$$\% \ \Delta DOC = \left(\frac{[DOC_{initial} - DOC_{final}]}{DOC_{initial}}\right) * 100 \tag{1}$$

2.4. Dissolved organic matter composition

Initial (T=0 days) and final (T=28 days) bioincubation samples were analyzed via FT-ICR MS to assess which compound classes were utilized by the microbial community during the bioincubation. Details on sample preparation for analysis on the 21 T FT-ICR MS at the National High Magnetic Field Laboratory (NHMFL; Tallahassee, FL) and details on molecular formulae assignments can be found in Vaughn et al. (2021) and in the supplemental material. Briefly, formulae were assigned with elemental combinations of $C_{1\text{-}45}H_{1\text{-}100}N_{0\text{-}4}O_{1\text{-}25}S_{0\text{-}2}$ and then classified based on their elemental stoichiometries. The number of formulae detected here is referred throughout as molecular richness. The modified aromaticity index (AI_{mod}) was calculated for each formula following Koch and Dittmar (2006, 2016) and was used, along with H/C and O/C, to classify the formulae into the following compound classes: polyphenolics, condensed aromatics, highly unsaturated and phenolic (HUP) (Low O/C), HUP (High O/C), aliphatics, and N-aliphatics (Stenson et al. 2003; Kellerman et al. 2015).

Relative abundance was calculated for each compound class and for different elemental combinations (CHO, CHON, CHOS, and CHONS) following methods detailed in Vaughn et al. (2021) and in the supplemental material. Changes in relative abundance (ΔRA) during the bioincubations were calculated following Eq. (2), where the differences between initial (T=0 days) and final (T=28 days) relative abundance are divided by the initial relative abundance and then multiplied by one hundred to obtain a percentage.

$$\% \ \Delta RA = \left(\frac{[RA_{final} - RA_{initial}]}{RA_{initial}}\right) * 100 \tag{2}$$

2.5. Statistical analysis

Statistical analyses were carried out in R (R Version 4.0.1; R Core Team, 2020). Percent change in DOC and DOM parameters during the bioincubation are reported throughout as means ± standard deviations (Tables 1 and 2). t-tests (package "stats"; function "t.test") were used to determine if DOC significantly changed between pre- (T0) and post-bioincubation (T28) for all samples and each landcover. Linear regression (package "ggpubr"; function "stat_cor"; method "pearson") was used to examine relationships between DOC concentration and BDOC (mg C L^{-1} and %), between the relative abundance of aliphatics and % BDOC, and between drainage area and each DOM parameter (e.g. BDOC, % BDOC, % ΔRA of aliphatic and condensed aromatic compounds).

Effects of landcover (forested, agricultural, and urban) on changes in DOC concentration and/or DOM molecular composition were determined using a balanced bootstrap approach with 10,000 iterations, giving 95% confidence intervals (CI) of the bootstrapped means (function can be found in supplemental material). Values are significantly different if their 95% CI do not overlap, equivalent to a p-value < 0.05. The bootstrap mean and CI for each variable and landcover is reported in Table S3.

Finally, to investigate potential relationships of molecular formulae present at the beginning (T0) and end of the incubation (T28) with % BDOC, we examined the relationship of each molecular formula with % BDOC using Spearman's rank correlations (package "stats"; function "corr.test"; adjustment "BH" to control for false discovery). Significant correlations ($p < 0.05$) were plotted in a van Krevelen diagram for visualization. t-tests (package "stats"; function "t.test") were used to determine if correlations and elemental ratios (H/C and O/C) significantly differed between formulae with significant positive and negative correlations to %BDOC.

3. Results

3.1. Dissolved organic carbon (DOC) bioavailability

Initial DOC concentrations (T0) were significantly higher in streams draining forested (9.7 ± 3.8 mg L^{-1}) and urban (7.3 ± 2.9 mg L^{-1}) landcovers compared to streams draining agricultural landcover (3.7 ± 1.1 mg L^{-1}; Table 1; Vaughn et al., 2021). Initial DOC concentrations (T0; Table 1; Vaughn et al., 2021) were also significantly higher than the final DOC concentrations (T28) when grouping samples together by landcover type (p < 0.0001 for each landcover) and when comparing all post-bioincubation samples to their respective pre-bioincubation samples (p < 0.0001). Average DOC loss (T0 – T28), or the concentration of

Table 1
Initial dissolved organic carbon (DOC) concentrations for each landcover at the start (T0) of the bioincubation experiments, the change in DOC from initial to final (ΔDOC), percent bioavailable dissolved organic carbon (BDOC; %), and the initial and change in the number of formulae, average mass (Da), and the modified aromaticity index (AI_{mod}).

Parameter	Forest	Agricultural	Urban	All
n	15	14	15	44
DOC (mg C L^{-1})	9.7 ± 3.8	3.7 ± 1.1	7.3 ± 2.9	7.0 ± 3.7
ΔDOC (mg C L^{-1})	-0.47 ± 0.17	-0.28 ± 0.15	-0.71 ± 0.35	-0.49 ± 0.30
BDOC (%)	5.6 ± 3.2	7.6 ± 3.1	10 ± 4.4	7.8 ± 4.0
Formulae (#)	11,604 ± 1,284	16,161 ± 2,007	16,087 ± 2,836	14,582 ± 2,994
ΔFormulae (%)	-0.11 ± 10.0	-2.1 ± 6.3	1.2 ± 6.3	-0.29 ± 7.6
Mass (Da)	562 ± 6	564 ± 4	550 ± 9	559 ± 9
ΔMass (%)	0.25 ± 0.97	-0.23 ± 1.0	0.25 ± 1.2	0.10 ± 1.1
AI_{mod}	0.34 ± 0.0099	0.32 ± 0.014	0.27 ± 0.022	0.31 ± 0.034
ΔAI_{mod} (%)	0.57 ± 1.5	0.11 ± 0.88	0.84 ± 1.6	0.52 ± 1.4

Table 2

Mean and standard deviations for initial (i) and percent change (Δ) in the percent relative abundances (RA) of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) parameters across all four landcover types (forest, agricultural, urban, and all). C-, H-, and O-containing compounds (CHO); C-, H-, O-, and N-containing compounds (CHON); C-, H-, O-, and S-containing compounds (CHOS); C-, H-, O-, N-, and S-containing compounds (CHONS); highly unsaturated and phenolic (HUP); condensed (Cond.).

	Forest		Agricultural		Urban		All	
Parameter	RA_i (%)	ΔRA (%)	RA_i (%)	ΔRA (%)	RA_i (%)	ΔRA (%)	RA_i (%)	ΔRA (%)
CHO	83 ± 1.9	-0.23 ± 1.6	68 ± 4.4	0.42 ± 1.2	61 ± 5.8	0.75 ± 0.74	70 ± 10	0.31 ± 1.3
CHON	11 ± 1.3	3.5 ± 11	23 ± 3.5	-1.6 ± 3.2	19 ± 2.2	-1.5 ± 3.0	18 ± 5.6	0.15 ± 7.2
CHOS	5.3 ± 1.2	-3.2 ± 6.7	7.1 ± 0.90	2.1 ± 3.7	18 ± 6.0	-0.68 ± 3.2	10 ± 6.6	-0.65 ± 5.2
CHONS	0.62 ± 0.17	8.5 ± 37	2.0 ± 0.51	-0.71 ± 3.8	2.5 ± 0.55	-1.28 ± 3.61	1.7 ± 0.91	2.3 ± 22
HUP, High O/C	61 ± 2.2	0.12 ± 3.1	56 ± 2.8	0.20 ± 1.8	55 ± 6.1	0.39 ± 3.2	57 ± 4.7	0.24 ± 2.7
HUP, Low O/C	21 ± 2.1	-0.91 ± 7.3	27 ± 2.7	-0.050 ± 3.1	29 ± 3.7	1.7 ± 4.9	26 ± 4.4	0.24 ± 5.4
Polyphenolics	13 ± 1.4	2.0 ± 6.3	10 ± 1.4	0.020 ± 1.8	7.2 ± 2.5	2.1 ± 8.0	10 ± 3.1	1.4 ± 5.9
Cond. Arom.	2.6 ± 0.40	3.1 ± 16.4	2.5 ± 0.42	0.070 ± 2.7	1.5 ± 0.77	2.2 ± 8.6	2.2 ± 0.73	1.8 ± 11
Aliphatics	2.2 ± 0.41	-5.9 ± 12	3.0 ± 0.67	-0.76 ± 6.5	7.1 ± 1.9	-5.4 ± 12	4.1 ± 2.5	-4.1 ± 10
N-Aliphatics	0.047 ± 0.034	47 ± 130	0.29 ± 0.15	4.9 ± 40	0.52 ± 0.19	-6.3 ± 28	0.42 ± 0.95	15 ± 84

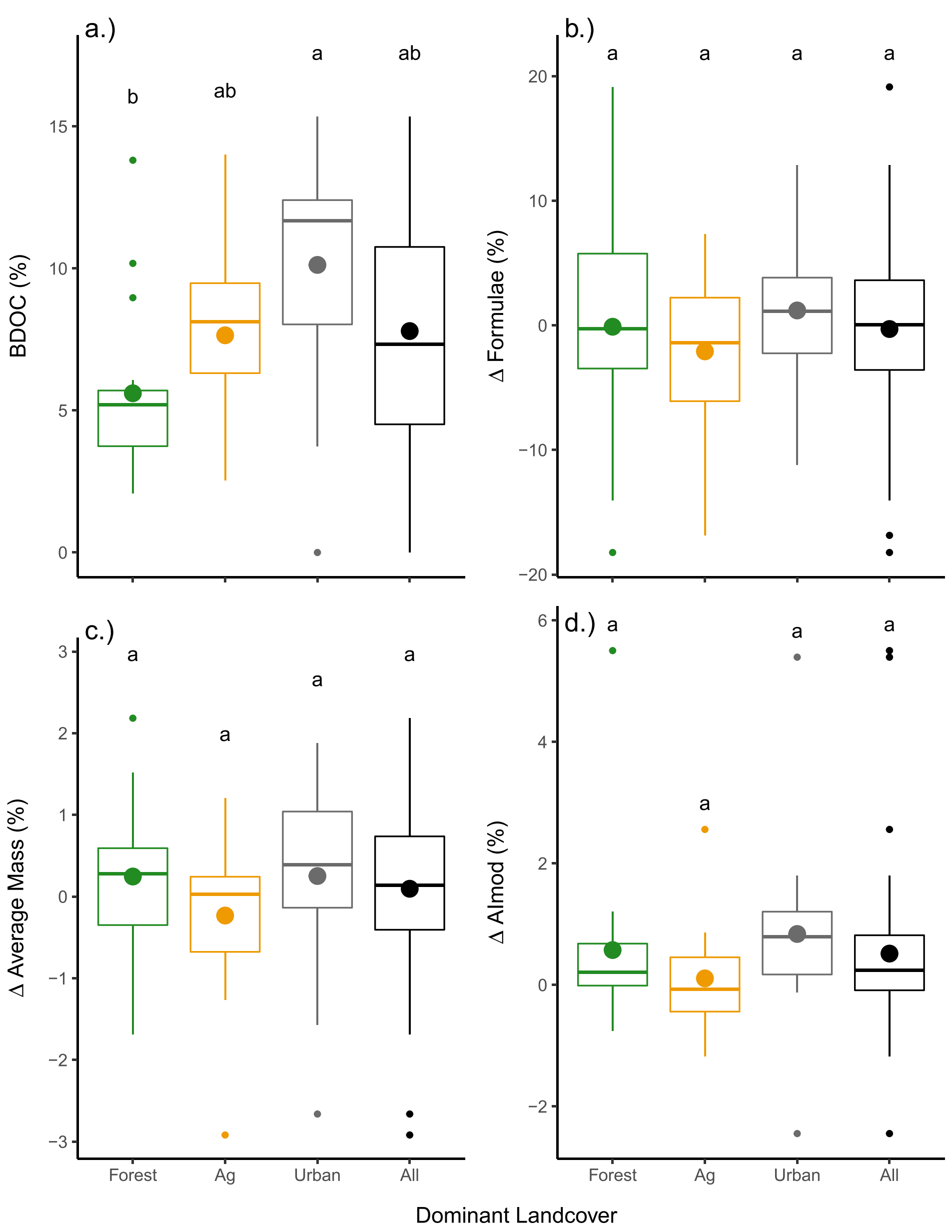

Fig. 2. Box plots for forest (green), agricultural (Ag; orange), urban (gray), and all (black) landcovers. a. Bioavailable dissolved organic carbon (BDOC; %); b. Change in the number of assigned formulae during the bioincubation (%); c. Change in average mass (%) during the bioincubation; d. Change in the modified aromaticity index during the bioincubatioon (AI_{mod}; %). 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 a balanced bootstrap approach, with letters indicating which groups are statistically different from one another ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

BDOC, from all streams during the bioincubations was 0.49 ± 0.30 mg L^{-1}. Urban streams had the largest BDOC (0.71 ± 0.35 mg L^{-1}), significantly greater ($p < 0.05$; Table S3) than BDOC in forested streams (0.47 ± 0.17 mg L^{-1}) and agricultural streams (0.28 ± 0.15 mg L^{-1}; Table 1; Fig. 2a). Forested stream BDOC was also significantly greater than agricultural stream BDOC ($p < 0.05$; Table S3), but there was no significant relationship between BDOC concentration and drainage area (Fig. S1a).

The percentages of BDOC (% BDOC) measured in this study (0.0% to 15%) were comparable to BDOC percentages in blackwater ecosystems from northwest Florida (2.3% to 12%; Textor et al., 2018), agriculturally impacted streams in Australia (1.0% to 27%; Giling et al., 2014) and Alabama (12% to 20%; Shang et al., 2018), and to streams draining urban landscapes within Maine (0.0% to 22%; Parr et al., 2015) and New Hampshire (8.9% to 26%; Coble et al., 2022). The highest % BDOC for the UMRB was in streams draining urban landcovers (10% ± 4.4%), significantly higher than % BDOC found in streams draining forested landcovers (5.6% ± 3.2%; $p < 0.05$; Table S3) but not significantly higher for streams draining agricultural landcovers (7.6% ± 3.1%; Table 1; Fig. 2a). Mean % BDOC of each site decreased with drainage area; however, this decrease was not significant (Fig. S1b). When compared with initial DOC concentrations, % BDOC decreased with increasing initial DOC (Fig. S2a; $R^2 = 0.11$, $p < 0.05$) across all samples while BDOC concentration (mg C L^{-1}) increased with increasing initial DOC (Fig. S2b; $R^2 = 0.19$, $p < 0.005$). When categorizing by landcover, only forested streams demonstrated a significant decrease in % BDOC with increasing initial DOC (Fig. S2a; $R^2 = 0.48$, $p < 0.005$). Similarly, only the agricultural streams had a significant increase in BDOC concentration with increasing initial DOC (Fig. S2b; $R^2 = 0.35$, $p < 0.05$).

3.2. Changes in dissolved organic matter (DOM) composition with biodegradation

A total of 27,400 molecular formulae were assigned in the sample set, with agricultural and urban streams having the highest number of assigned formulae (Table 1; Vaughn et al., 2021). Average percent loss of molecular formulae across all streams was -0.29% ± 7.6% (Table 1; Fig. 2b). Average molecular weight was initially higher for forested and

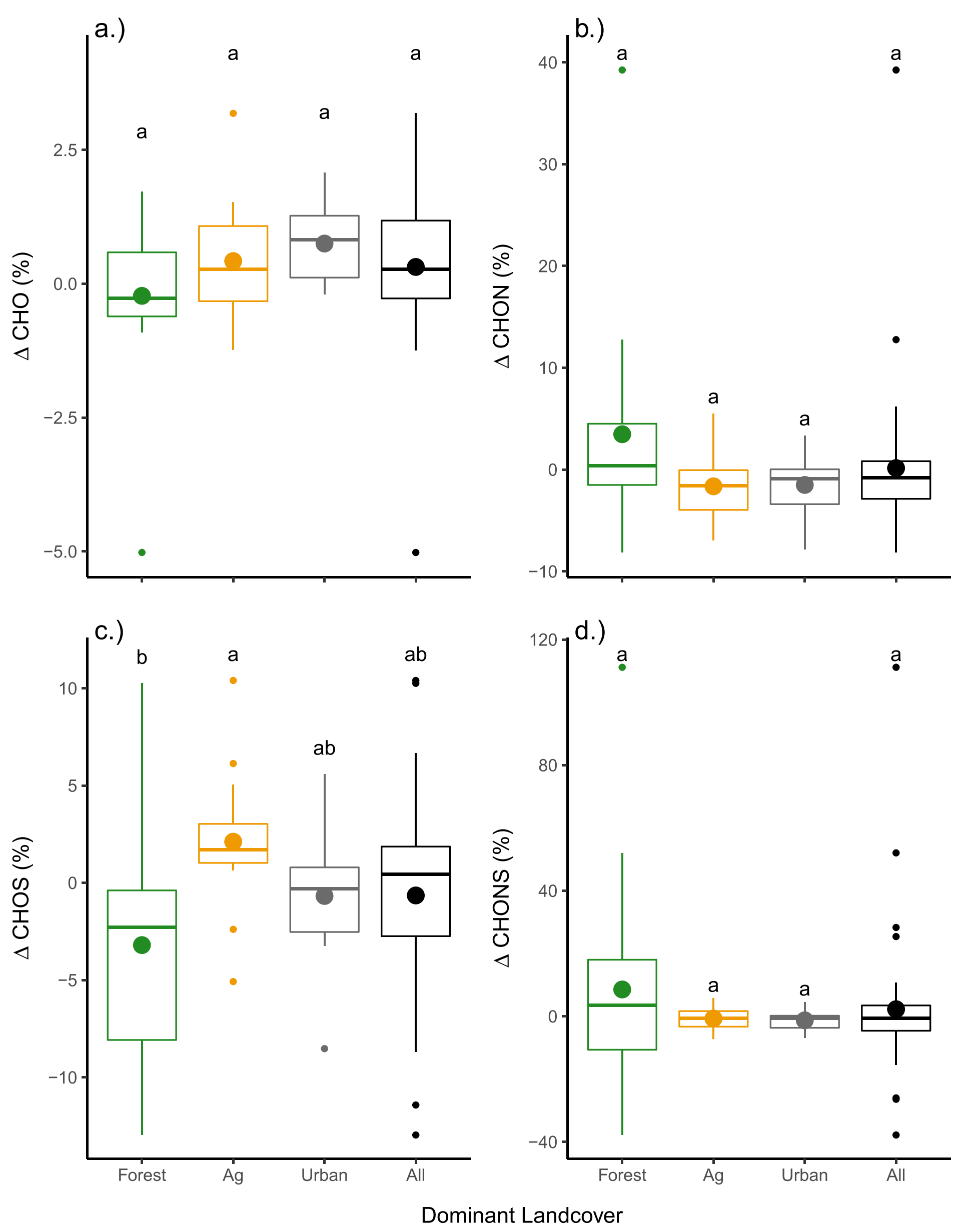

Fig. 3. Box plots for forest (green), agricultural (Ag; orange), urban (gray), and all (black) landcovers. a. Change in the relative abundance (RA) of C-, H-, and O-containing compounds during the bioincubation (CHO; %); b. Change in the relative abundance of C-, H-, O-, and N-containing compounds during the bio-incubation (CHON; %); c. Change in the relative abundance of C-, H-, O-, and S-containing compounds during the bioincubation (CHOS; %); d. Change in the relative abundance of C-, H-, O-, N-, and S-containing compounds during the bioincubation (CHONS; %). Solid circles and the thick horizontal lines represent the mean and median, respectively, for each land-cover. Significance letters above each box plot are based on significance testing using a balanced bootstrap approach, with letters indicating which groups are statistically different from one another ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

agricultural streams compared to urban streams and AI_{mod} was initially highest for forested streams (Table 1; Vaughn et al., 2021). Both the average molecular weight and AI_{mod} increased across all samples during the bioincubation (0.10% ± 1.1% and 0.52% ± 1.4%, respectively; Table 1; Fig. 2c) with the AI_{mod} increase being significant (paired t-test; $p < 0.05$).

3.2.1. Changes in elemental (C, H, O, N, S) composition

Formulae containing only C, H, and O (CHO) dominated the detected formulae (Table 2; Vaughn et al., 2021), consistent with other riverine DOM studies (Wagner et al., 2015; Riedel et al., 2016; Spencer et al., 2019). During the bioincubation, CHO formulae percent relative abundance increased across all samples by 0.31% ± 1.3% (Table 2; Fig. 3a), with the increase in CHO formulae percent relative abundance associated with urban streams being significant ($p < 0.005$; 0.75% ± 0.74%). Formulae containing C, H, O, and N (CHON) were the second most abundant (Table 2) and increased in percent relative abundance during the bioincubation (0.15% ± 7.2%), although both agricultural and urban landcovers decreased in CHON percent relative abundance (-1.6% ± 3.2% and -1.5% ± 3.0%, respectively; Table 2; Fig. 3b).

In contrast to CHO and CHON, formulae containing C, H, O, and S (CHOS) decreased in percent relative abundance across all samples during the bioincubation (-0.65% ± 5.2%; Table 2; Fig. 3c). Agricultural streams, however, increased in CHOS percent relative abundance (2.1% ± 3.7%; Table 2; Fig. 3c), which was significantly higher than the loss of CHOS percent relative abundance in forested streams ($p < 0.05$; Table 2; Fig. 3c; Table S3). Conversely, the number of assigned CHOS formulae for agricultural and forested streams decreased during the bioincubation (from 2,202 ± 356 to 2,181 ± 378 for agricultural and from 1,776 ± 361 to 1,628 ± 367 for forested). While C, H, O, N, and S (CHONS) formulae had the lowest initial percent relative abundance (1.7% ± 0.90%; Table 2; Vaughn et al., 2021), CHONS formulae had the largest, and most scattered, change in percent relative abundance across all samples during the bioincubation, increasing by 2.3% ± 22% (Table 2; Fig. 3d).

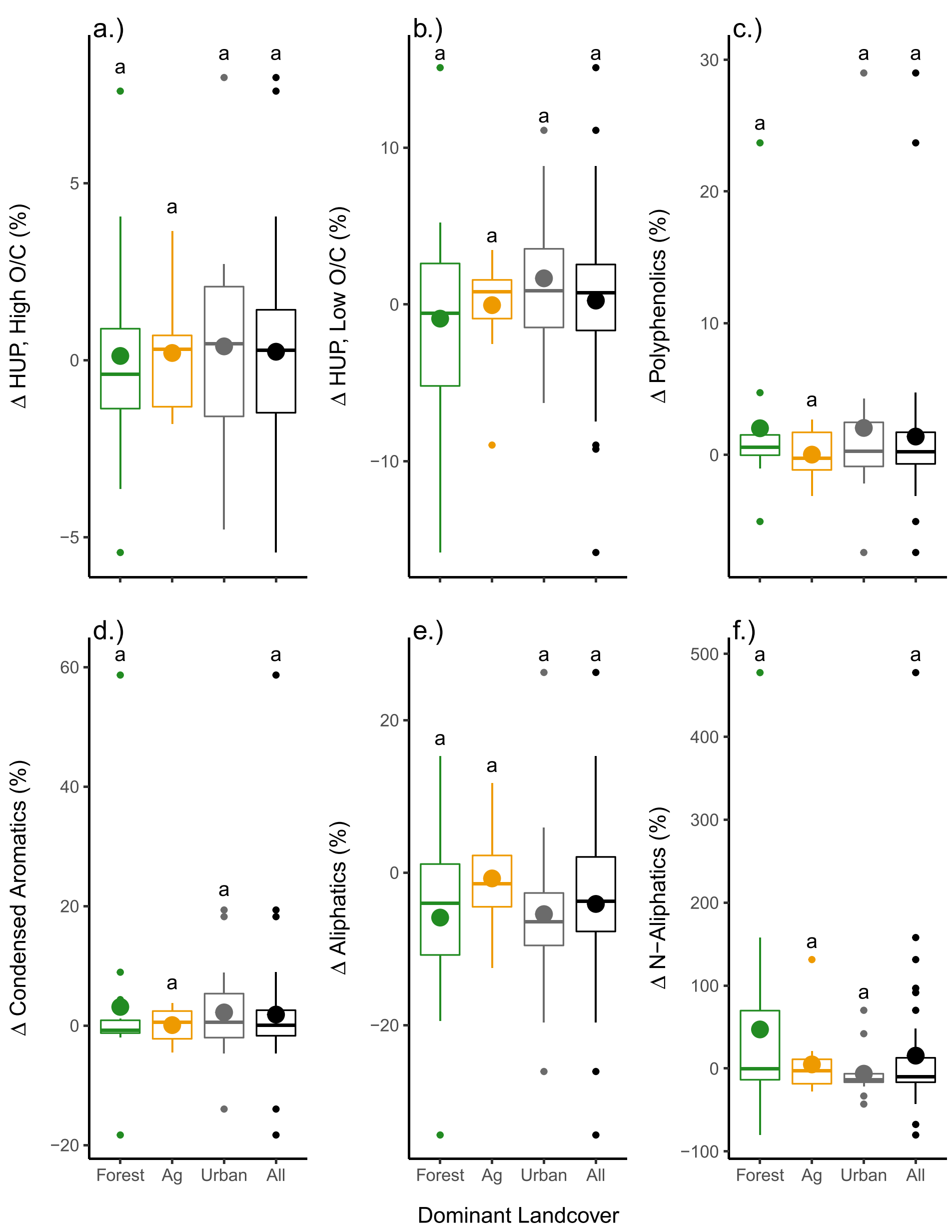

Fig. 4. Box plots for forest (green), agricultural (Ag; orange), urban (gray), and all (black) landcovers. a. Change in the relative abundance (RA) of highly unsaturated and phenolic (HUP) high O/C during the bioincubation (%); b. Change in the relative abundance of HUP low O/C during the bioincubation (%); c. Change in the relative abundance of polyphenolics during the bioincubation (%); d. Change in the relative abundance of condensed aromatics during the bioincubation (%); e. Change in the relative abundance of aliphatics during the bio-incubation (%); f. Change in the relative abundance of N-aliphatics during the bioincubation (%). 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 a balanced bootstrap approach, with letters indicating which groups are statistically different from one another ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2.2. Changes in compound classes

The compound class with the highest initial percent relative abundance prior to the bioincubation was HUP (High O/C) (Table 2; Vaughn et al., 2021), which dominates riverine DOM globally (Riedel et al., 2016; Kellerman et al., 2018; Spencer et al., 2019). Following the bioincubation, percent relative abundance of HUP (High O/C) formulae increased by 0.24% ± 2.7% across all sites (Table 2; Fig. 4a). HUP (Low O/C) had the second highest relative abundance across all samples, with an increase of 0.24% ± 5.4% across all samples during the bioincubation (Table 2; Fig. 4b). Polyphenolics and condensed aromatics also increased in percent relative abundance by 1.4% ± 5.9% and 1.8% ± 11%, respectively, during the bioincubation (Table 2; Figs. 4c and 4d).

In contrast to the aforementioned compound classes, the percent relative abundance of aliphatics decreased across all samples and landcovers following the bioincubation (-4.1% ± 10%; Table 2; Fig. 4e), as well as the number of formulae assigned as aliphatics (T0 = 2,325; T28 = 2,288). Additionally, the decrease in aliphatic percent relative abundance throughout the bioincubation was significant when comparing all post-bioincubation samples to their respective pre-bioincubation samples ($p < 0.005$) and when comparing urban post-bioincubation samples to their respective pre-bioincubation samples ($p < 0.05$). Forested streams had the highest percent relative abundance loss of aliphatics with -5.9% ± 12% and agricultural streams having the smallest loss with -0.76% ± 6.5% (Table 2; Fig. 4e). N-containing aliphatics had the lowest average initial percent relative abundance of all compound classes across all samples (Table 2; Vaughn et al., 2021) and the largest and most varied change in percent relative abundance across all sites during the bioincubation (16% ± 84%; Table 2; Fig. 4f).

In addition to landcover, the percent change in relative abundance of each compound class was compared to drainage area; however, no significant relationships were found between drainage area and each compound class (e.g. aliphatic and condensed aromatic compounds; Figs. S1c and S1d).

3.2.3. Changes in formulae characteristics

Across all landcovers, assigned formulae present only in post-bioincubation samples (T28, n = 385) had significantly lower H/C (0.97 ± 0.32) compared to formulae only present in pre-bioincubation samples (T0, n = 360, H/C = 1.1 ± 0.40) and to formulae common across both T0 and T28 ($p < 0.001$; n = 26,584; H/C = 1.1 ± 0.32; Fig. 5a). Similarly, agricultural and urban streams had significantly lower H/C for the formulae assigned after the bioincubation (T = 28; 1.1 ± 0.35 and 1.0 ± 0.32, respectively) compared to the formulae assigned before the bioincubation (T = 0; H/C = 1.2 ± 0.35 and 1.1 ± 0.38; respectively; $p < 0.001$ for both). Assigned formulae present in only pre- or post-bioincubation samples for forested streams did not significantly differ in H/C (H/C = 1.1 ± 0.32 and 1.1 ± 0.35 for T28 and T0, respectively).

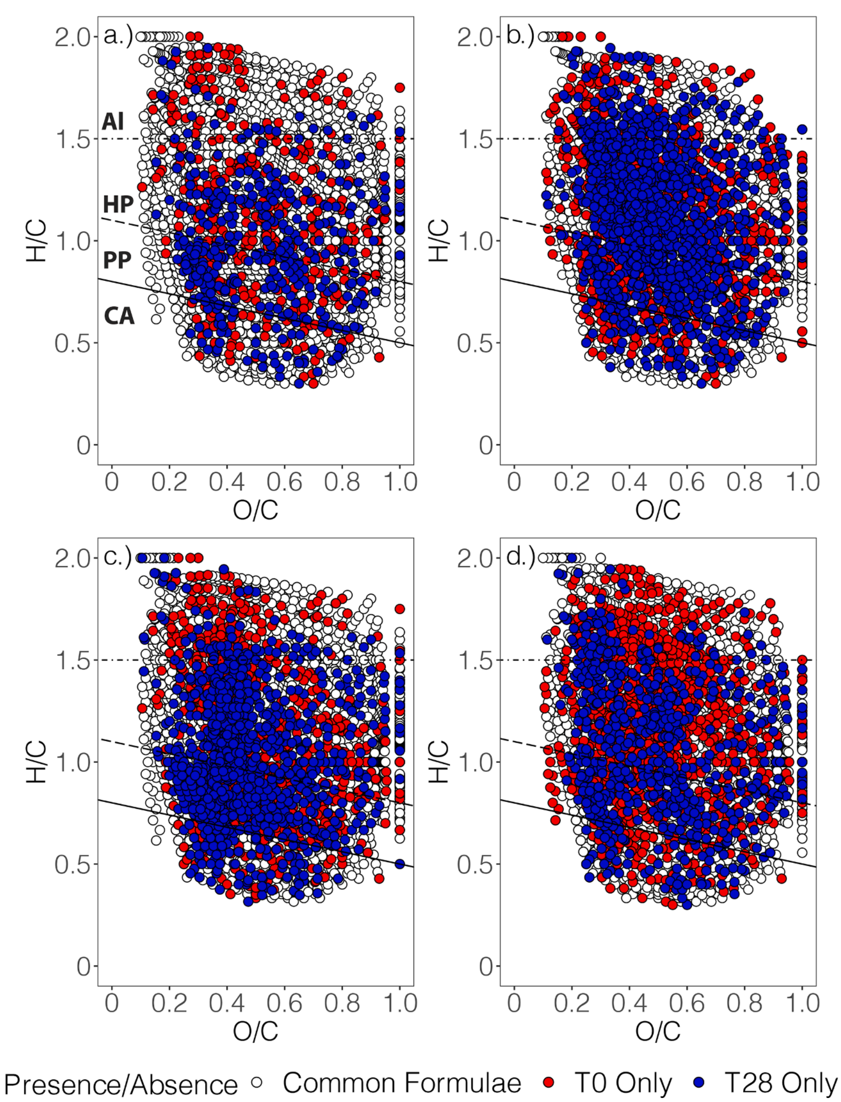

Fig. 5. Presence/absence data for formulae found in all (a), forest (b), agricultural (c), and urban (d) landcovers. Axes represent the atomic ratios of H:C (H/C) and O:C (O/C). Colors represent the presence of formulae in only the pre-incubation samples (T0; red), in only the post-incubation samples (T28; blue), and for formulae present in both pre- and post-incubation samples (white). Lines approximately delineate compound groups: aliphatics (Al), condensed aromatics (CA), highly unsaturated and phenolic (HUP) compounds, and polyphenolic (PP) compounds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Spearman rank correlations were used to examine the relationship of all assigned molecular formulae with % BDOC. There were significant Spearman correlations ($p < 0.05$) for 12,595 formulae present in pre-bioincubation samples (T0; 46% of total assigned formulae) and 11,803 formulae in post-bioincubation samples (T28; 43% of total assigned formulae) with % BDOC which, when plotted in van Krevelen space, showed two distinct assemblages of Spearman's rank correlation coefficients separating along the H/C and O/C axes (Fig. 6). Formulae with negative significant Spearman correlations to % BDOC (23% of all T0 and 23% of all T28 formulae with significant correlations to % BDOC) had significantly lower H/C (0.86 ± 0.19 and 0.87 ± 0.19 for T0 and T28, respectively; $p < 0.001$ for both) and significantly higher O/C (0.62 ± 0.13 and 0.60 ± 0.13 for T0 and T28, respectively; $p < 0.001$ for both) compared to formulae with positive significant Spearman correlations to % BDOC (77% of all T0 and 77% of all T28 formulae with significant correlations to % BDOC; H/C = 1.3 ± 0.30 and 1.2 ± 0.31 for T0 and T28, respectively; O/C = 0.45 ± 0.14 and O/C = 0.45 ± 0.14 for T0 and T28, respectively).

Most formulae with negative significant Spearman correlations to % BDOC were CHO formulae (87% and 93% for T0 and T28, respectively) while most T0 formulae with positive significant Spearman correlations to % BDOC were heteroatomic formulae (CHON, CHOS, and CHONS; 83% and 86% for T0 and T28, respectively). Additionally, all initial (T0) aliphatic formulae, most initial N-aliphatic formulae (99%), and all final (T28) aliphatic and N-aliphatic formulae with significant Spearman correlations to % BDOC had positive correlations to % BDOC.

When % BDOC was compared with percent relative abundance aliphatics from pre-bioincubation (T0) samples, there was a significant increase in % BDOC with increasing % aliphatic relative abundance (Fig. S2c; $R^2 = 0.44$; $p < 0.005$). Breaking this down by landcover, all three landcovers had significant positive increases in % BDOC with increasing % aliphatic relative abundance (forested: R^2=0.50, $p < 0.005$; agricultural: R^2=0.46, $p < 0.01$; urban: R^2=0.55, $p < 0.005$; Fig. S2c). Compound categories from pre- and post-bioincubation samples with significant negative Spearman correlations to % BDOC were HUP (High and Low O/C; 70% and 69% for T0 and T28, respectively), polyphenolics (24% for both T0 and T28), condensed aromatics (6% and 7% for T0 and T28, respectively), and N-aliphatics (<1% for T0).

4. Discussion

To test DOM bioavailability with landcover variability in the UMRB, we quantified changes in DOC concentration and looked at shifts in DOM composition during a 28-day bioincubation. DOC concentrations significantly declined during the bioincubations (-0.49 ± 0.30 mg L^{-1}; Table 1; Fig. 2a), concurrent with significant losses in aliphatic compound relative abundance across all samples (Table 2; Fig. 4e). Energy-rich aliphatic compounds are often characteristic of autochthonous DOM production (e.g. DOM produced from algal, phytoplankton, bacteria growth; Williams et al., 2010; Graeber et al., 2015; Kellerman et al., 2018) and anthropogenic DOM inputs (Wilson and Xenopoulos, 2009; Hosen et al., 2014; Parr et al., 2015) and were likely preferentially utilized by the microbial community during the bioincubation (e.g. Sun et al. 1997, Spencer et al. 2015). This was supported by H/C significantly declining between pre- and post-bioincubation samples ($p < 0.001$; Fig. 5a), suggesting a decline in the abundance of saturated aliphatic compounds (D'Andrilli et al., 2015; Seidel et al., 2015; Kamjunke et al., 2019). Additionally, aliphatic formulae had significant positive Spearman correlations to % BDOC (Fig. 6) and initial aliphatic relative abundance had significant positive correlations to % BDOC across all samples and across each landcover type (Fig. S2c). This indicated streams with greater relative abundance of aliphatic compounds

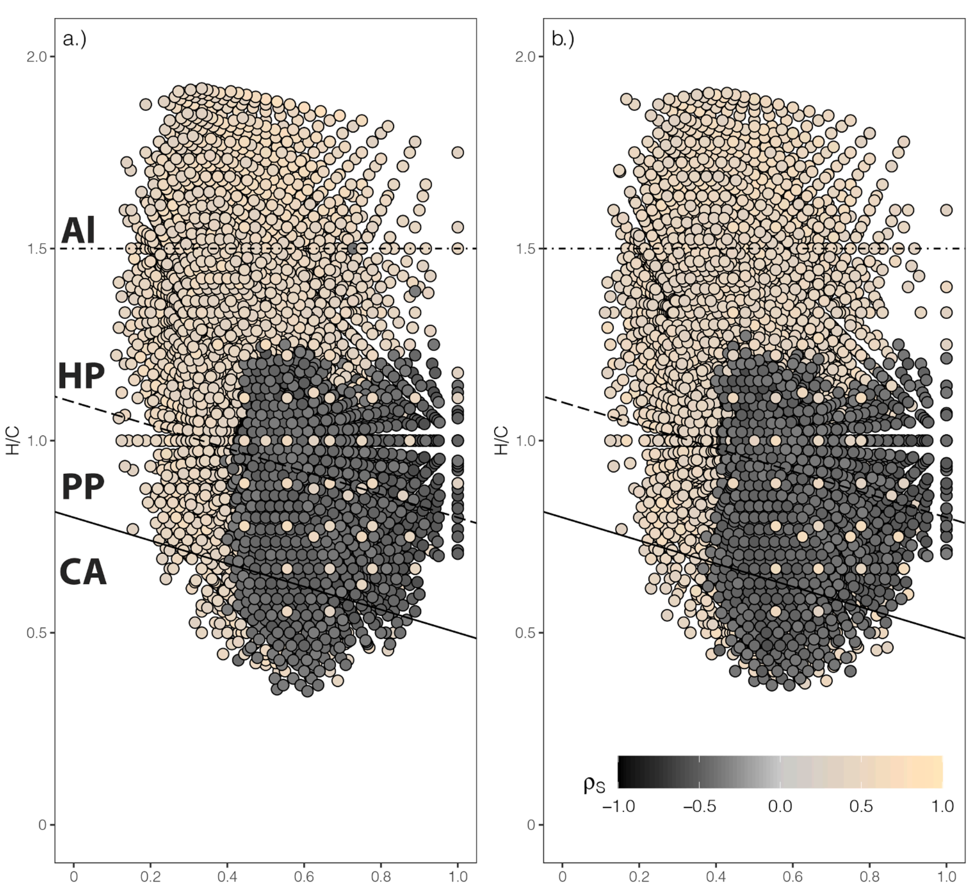

Fig. 6. Spearman-rank correlations between the relative abundance of assigned molecular formulae present in the pre- (a) and post- (b) bioincubation samples and the percent bioavailable dissolved organic matter (BDOC). Axes represent the atomic ratios of H:C (H/C) and O:C (O/C). Colors represent the correlation coefficient (ρ_s) between the relative abundance of each formula and BDOC with gold indicating positive correlations and black indicating negative correlations. Scale-bar represents the range of correlation coefficient values. Lines approximately delineate compound groups: aliphatics (Al), condensed aromatics (CA), highly unsaturated and phenolic (HUP) compounds, and polyphenolic (PP) compounds.

also had greater portions of BDOC within their DOM. Interestingly, N-aliphatic formulae did not significantly change in relative abundance during the bioincubation; however, most N-aliphatic formulae (99% in pre-bioincubation samples and 100% in post-bioincubation samples) had significant positive Spearman correlations to % BDOC. This may suggest N-aliphatic compounds were not as degraded as aliphatic compounds during the bioincubation.

In contrast to aliphatics, HUP (high O/C), condensed aromatics, and polyphenolics, all considered to be less bioavailable compounds and characteristic of terrestrial plant and organic-rich soil sources (Williams et al., 2010; Wagner et al., 2015), did not significantly change in relative abundance during the bioincubation (Table 2; Figs. 4a, 4c and 4d). The lack of change in the relative abundance of these compounds during the bioincubation, along with their negative Spearman correlations to % BDOC, suggest these compounds were not readily degraded during the bioincubation. Most CHO formulae (87% and 93% for pre- and post-bioincubation, respectively) also had significant negative Spearman correlations with % BDOC, suggesting most CHO formulae were not degraded during the bioincubation. Additionally, streams associated with urban catchments had significant increases in the relative abundance for CHO formulae from pre- to post-bioincubation ($p < 0.005$), related to the decrease in the relative abundance of heteroatomic compounds (CHON, CHOS, CHONS) as these compounds are degraded during the bioincubation. N- and S-containing compounds are considered highly bioavailable and have been associated with microbial degradation in urban catchments (Ye et al., 2019; Reid et al., 2020; Gong et al., 2022).

4.1. Greater impact of urbanization on bioavailable DOC (BDOC)

Anthropogenically-impacted streams in the UMRB, including streams draining urban and agricultural lands, had greater % DOC loss and more bioavailable DOM contributions compared to streams draining forested lands. The increase in % BDOC for anthropogenically impacted streams in the UMRB was similar to BDOC increases in other urban (Petrone et al., 2009; Hosen et al., 2014; Knapik et al., 2015; Parr et al., 2015; Shi et al., 2016; Begum et al. 2019; Li et al., 2020) and agricultural watersheds (Royer and David, 2005; Petrone et al., 2009; Shang et al., 2018; Drake et al., 2019). Higher % BDOC in agricultural and urban streams compared to forested streams may be due to greater proportions of bioavailable DOM (i.e. greater relative abundance of aliphatic and N-aliphatic compounds; Vaughn et al., 2021) in these anthropogenically-impacted streams derived from autochthonous (e.g. algal, phytoplankton; Williams et al., 2010; Graeber et al., 2015; Kellerman et al., 2018) and anthropogenic (e.g. fertilizer, animal manure, wastewater) sources (Wilson and Xenopoulos, 2009; Hosen et al., 2014; Kamjunke et al., 2019). This was further supported by H/C ratios significantly declining between pre- and post-incubation in the agricultural and urban landcovers ($p < 0.001$; Figs. 5c and 5d; e.g. Sun et al. 1997, Seidel et al. 2015) but not in forested landcovers.

Despite agricultural and urban streams having greater BDOC contributions to their overall DOC, only urban streams in the UMRB had significantly greater % BDOC compared to forested streams. Additionally, urban streams had significantly higher BDOC concentrations compared to agricultural and forested streams. This all suggests urban landcover likely has a greater impact on stream DOM bioavailability compared to agricultural landcover, which may result from significantly higher initial relative abundance of aliphatic and N-aliphatic compounds in urban streams (Table 2; Vaughn et al., 2021). Urban streams in the UMRB also had significantly greater initial relative abundance of S-containing formulae (CHOS and CHONS; Table 2; Vaughn et al., 2021), which has been susceptible to microbial degradation in other urbanized streams (Ye et al., 2019; Reid et al., 2020; Gong et al., 2022).

Previous studies looking at urban impacts on stream and riverine DOM bioavailability have found similar results of increased bioavailability. At the convergence of the Yangtze River with discharge from a wastewater treatment plant in China, there was greater metabolism of bioavailable amino acids by the local microbial communities compared to upstream locations with less urban influences (Li et al., 2020). In Maryland and Maine, increased algal primary production associated with urbanized streams increased the degradation of DOC and stimulated bacterial enzyme activity (Hosen et al., 2014; Parr et al., 2015). DOM bioavailability has also been linked with increasing percentages of impervious surfaces and development associated with urban areas in New Hampshire (Coble et al., 2022).

Although streams draining agricultural landcover did not significantly differ in % BDOC compared to streams draining forested landcovers in the UMRB, other research looking at the impacts of agriculture on DOM bioavailability globally have found greater stream DOM bioavailability with increasing agriculture activity (e.g. Shang et al. 2018, Drake et al. 2019). The increase in agricultural DOM bioavailability has also been accompanied by increases in aliphatic compounds, N-containing formulae, and formulae with higher H/C ratios (Drake et al., 2019). In contrast, streams within the lower Chesapeake Bay watershed in Virginia (Lu et al., 2013), Australia (Giling et al., 2014); and in southern Ontario, Canada (Kadjeski et al., 2020), showed no impact of agricultural landcover on DOM bioavailability. Overall, DOM within streams draining agricultural landcovers could have significantly greater bioavailability than forested/wetland streams, but the drivers (e.g. different types of agricultural practices, differences in historical landuse), sources (e.g. types of fertilizer and product produced), and other external factors (e.g. nutrient availability, water residence time) for such differences in DOM may vary by location.

For forested streams, low mean % BDOC combined with higher initial contributions from less bioavailable DOM compounds (e.g. polyphenolic and high O/C HUP compounds) and lower initial contributions of more bioavailable DOM compounds (e.g. aliphatic and N-aliphatic compounds; Table 2) could suggest DOM in UMRB forested streams may have already been extensively degraded in soils and upstream aquatic environments (e.g. Textor et al. 2018). The forested streams in this study had some of the largest drainage areas, which may support greater DOM degradation and lower % BDOC (Fig. S2a); however, the regression between % BDOC and drainage area was not significant (Fig. S1b; $p > 0.05$). Forested streams also had higher flow rates compared to urban and agricultural streams (Table S1), which could reduce their residence times and potential DOM degradation.

Despite the decrease in % BDOC with increasing DOC concentrations (Fig. S2a), forested streams with high initial DOC concentrations produced higher BDOC concentrations compared to agricultural streams with low initial DOC concentrations (Fig. S2b). This suggests there is a high concentration of BDOC transported in these forested streams, which can be delivered to microbial communities downstream and potentially degraded. Additionally, while we did not study anthropogenic impacts on forest structure, much of the UMRB forested area was logged in the 1800s and 1900s and is now covered by secondary forest growth (Stark et al., 2000). Secondary forest-growth can produce more bioavailable, proteinaceous DOM compared to old-growth forests with less bioavailable, aromatic DOM associated with deeper O-horizons (Beggs and Summers, 2011; Fegel et al., 2021); thus, future work on the impacts of land management activities on forested stream DOM bioavailability is worth consideration.

4.2. Microbial consumption of compounds independent of landcover

While landcover appears to impact the stream BDOC, as well as the relative abundance of compound classes (Hosen et al., 2014; Drake et al., 2019; Spencer et al., 2019; Vaughn et al., 2021), there is no indication landcover significantly impacts what types of compounds local microbial communities degrade in stream DOM, or how much of each type they degrade. This was indicated by the lack of significant differences between landcovers in how DOM composition changed during the bioincubation (Table 2; Figs. 3 and 4). One exception to this was the percent change in the relative abundance of CHOS formulae being significantly higher in agricultural streams, which had an increase in CHOS relative abundance, compared to forested streams ($p < 0.05$), which had a decrease in CHOS relative abundance. Both landcovers also had decreases in the number of assigned CHOS formulae, suggesting some microbial consumption of CHOS formulae during the bioincubation. The increase in agricultural CHOS relative abundance during the bioincubation may have resulted from the loss of assigned CHON formulae and decrease in CHON relative abundance (Table 2; Fig. 3b), both of which were likely higher than the DOM in forested streams due to anthropogenic nutrient inputs such as fertilizer and animal manure (Stark et al., 2000; Wilson and Xenopoulos, 2009). For the forested streams, the relative abundance and number of assigned CHON formulae increased following the bioincubation, which could reflect the preferential consumption of CHOS formulae (Ye et al., 2019) and the formation of N-containing metabolic intermediates or products during the bioincubations (D'Andrilli et al., 2015). Like CHON formulae, CHONS formulae also decreased in relative abundance and in the number of assigned formulae during the bioincubation for agricultural streams; however, CHONS formulae had smaller relative abundance compared to all other formulae (Table 2; Vaughn et al., 2021) and thus likely had less influence on the relative abundance of CHOS formulae.

5. Conclusions

Very few studies exist comparing the potential impacts of forest, urban, and agricultural landcovers on stream DOM molecular level composition and bioavailability, which hinders our understanding of how anthropogenic landcover modifications may impact in-stream organic matter cycling. Through a 28-day bioincubation, we found significant losses in DOC in stream samples collected across all three landcovers in the UMRB and significantly greater % BDOC in streams draining urban catchments compared to streams draining forested catchments. This pattern in BDOC followed other studies looking at the impact of landcovers on stream DOM bioavailability for other anthropogenically-impacted watersheds in isolation (e.g. Stanley et al. 2012, Hosen et al. 2014, Parr et al. 2015, Kamjunke et al. 2019). However, insignificant difference in % BDOC between agricultural and forested streams suggests the conversion of forested catchments to urban catchments in the UMRB has a bigger impact on stream DOM bioavailability compared to the conversion from forested to agriculture. In the UMRB, greater % BDOC in urban catchments likely reflected high relative abundance of aliphatic and N-aliphatic compounds prior to the bioincubation and the subsequent degradation of these compounds by microbial consumption during the bioincubation. While we found landcover to impact initial contributions of compound classes to stream DOM (Drake et al., 2019; Spencer et al., 2019; Vaughn et al., 2021) and to the proportions of BDOC within streams, landcover did not appear to impact what compounds microbes degrade. Given the paucity of studies relating agricultural and urban impacts to stream DOM molecular level composition and bioavailability, more data are needed from additional stream networks impacted by anthropogenic activity to assess whether our findings are applicable to other locations. Knowing the impacts of agricultural and urban landcovers on stream DOM is a necessity as agricultural and urban areas continue to grow to match the needs of a continuously growing global human population.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Data availability

Repository DOI found in acknowledgments.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.119357.

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