# Influence of Dissolved Organic Carbon on Methylmercury Bioavailability across Minnesota Stream Ecosystems

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S *Supporting Information*

ABSTRACT: Stream ecosystems are widely contaminated by mercury (Hg) via atmospheric transport and deposition in watersheds. Dissolved organic carbon (DOC) is well-known to be the dominant ligand for aqueous methylmercury (MeHg), the bioaccumulative form of Hg in aquatic food webs. However, it is less clear if and how the concentration and character (e.g., aromaticity) of DOC influences the availability of dissolved MeHg to stream food webs. In this work, we analyzed total-Hg and/or MeHg concentrations in water, seston, and macroinvertebrates (filter-feeding hydropsychid caddisflies), and other physiochemical properties in 30 streams along a south—north geographic gradient in eastern Minnesota that corresponds to substantial changes in dominant land cover (i.e., agriculture, urban, wetland, and forest). In general, MeHg concentrations in seston and hydropsychids were higher in watersheds with more forest and wetland coverage, and increased with dissolved MeHg concentration. However, we found that the efficiency of MeHg incorporation into the stream food webs (i.e., bioconcentration factors of MeHg in both seston and hydropsychids, BCF_{MeHg} = solid MeHg ÷ dissolved MeHg) decreased significantly with DOC concentration and aromaticity, suggesting that MeHg bioavailability to the base of food webs was attenuated at higher levels of terrestrial DOC. Therefore, our findings suggest that there is a dual role of DOC on MeHg cycling in streams: terrestrial DOC acts as the primary carrier ligand of dissolved MeHg for transport into surface waters, yet this aromatic DOC also attenuates dissolved MeHg uptake by aquatic food webs. Thus, consideration of MeHg bioavailability and its environmental regulation could help improve predictive models of MeHg bioaccumulation in stream ecosystems.
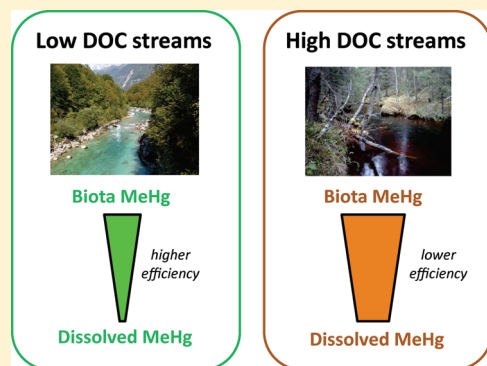
■ INTRODUCTION

Atmospheric deposition represents the dominant source of mercury (Hg) that contaminates the majority of freshwater ecosystems.[1] Watershed processes are known to mediate the bioavailability of atmospherically deposited Hg through transforming inorganic Hg to highly bioavailable methylmercury (MeHg).[2] Since wetlands promote conditions conducive to MeHg production,[3] the proportion of wetlands in the watershed is often found to be a positive predictor of MeHg concentrations in the water and biota of aquatic ecosystems.[2,4−6]

It has been repeatedly observed that positive relationships exist between dissolved organic carbon (DOC) and dissolved total-Hg or MeHg concentrations across numerous aquatic ecosystems.[2,5,7] In fact, the intimate relationship between DOC and Hg is mainly attributed to the extremely strong affinity between DOC and Hg, especially through the reduced thiol group within DOC molecules.[8,9] Nevertheless, streams vary widely in DOC concentration as well as DOC characters (e.g., aromaticity),[10] and these features of DOC may produce variation in not only dissolved MeHg concentration but also in MeHg bioavailability. In fact, variation in MeHg bioavailability as mediated by DOC has been observed in several lake studies such as seston, zooplankton, and fish in Wisconsin lakes,[5] fish in lakes in

Adirondack, New York[4] and freshwater phytoplankton in laboratory experiments.[11,12] Collectively, these studies demonstrated that increasing DOC concentration resulted in lower MeHg bioavailability. Similarly, DOC could attenuate MeHg bioavailability in stream ecosystems but such influence is yet to be demonstrated in the flowing waters.

In this work, we examined the effect of DOC concentration and character (e.g., aromaticity) on MeHg bioaccumulation in streams along a south-north gradient in eastern Minnesota. Land cover varied along this latitudinal gradient with agriculturally dominated watersheds in southern Minnesota and increasingly forested watersheds in the north. There were 30 streams in this study (Figure 1), and these sites span large contrasts in land cover in agricultural land (0−94%), urbanized land (0−96%), wetlands (0−33%), and forest cover (0−92%). All of these streams and their watersheds have no known history of Hg contamination through point-source discharges (e.g., mining and sewage
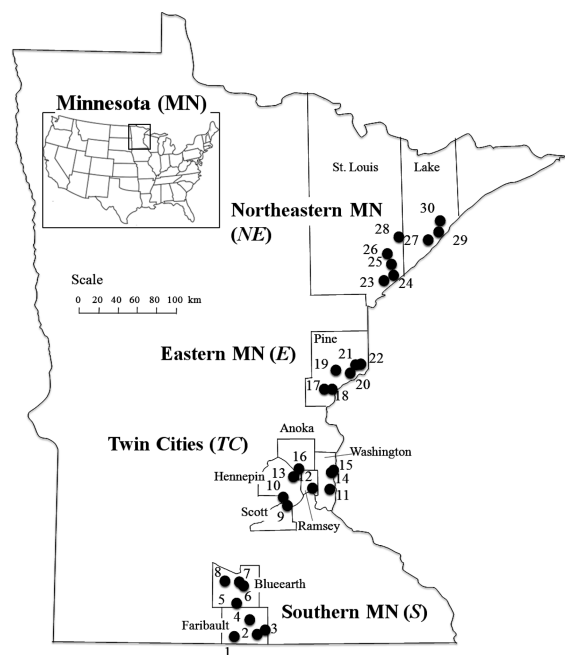
Figure 1. Location of 30 streams in four study zones across a south—north gradient of eastern Minnesota. See site ID in SI, Table S1.

treatment plants) and are assumed to receive Hg predominantly through atmospheric loading to their watersheds.

■ EXPERIMENTAL SECTION

Study Sites and Geospatial Characteristics. We divided the 30 study streams into four specific geographic zones, namely southern MN (*S*), the Twin Cities metropolitan area (*TC*), eastern MN (*E*), and northeastern MN (*NE*) (Figure 1 and Supporting Information (SI) Table S1). For each site, we delineated the watershed boundary, and estimated the drainage area and dominant land cover types using ArcMap GIS software (ESRI, Redlands, CA), along with USGS Seamless Digital Elevation Models and USEPA 2001 National Land Cover Data.

Major land cover types (developed, agriculture, forest, and wetland) in each of the four study zones are presented in SI Figure S1. Developed land was the highest in the *TC* zone but was also highly variable (SI Figure S1A). Agricultural land cover was the highest in *S* zone, followed by sites in both *TC* and *E* zones, and was minimal in *NE* sites (SI Figure S1A). There was increasing forest coverage from *S*, *TC*, *E*, to *NE* (SI Figure S1C). Only sites within *E* zone had substantial wetland coverage in their watersheds (SI Figure S1D).

Sample Collection and Processing. All sample collection was carried out during a relatively low flow period in summer 2008. Since discharge data were not available for most sites, we used streams continuously monitored for discharge by USGS at each of the four study zones to infer the relative flow regime during our sampling period, as shown in SI Figure S2. In the field, personnel wearing nonpowder vinyl gloves collected surface water into two acid-cleaned 1 L Teflon bottles and into two 1 L HDPE bottles. All water samples were transported to the laboratory on ice and processed within 24 h for Hg analyses.[13] Filtration of water sample and the subsequent preservation in the analytical laboratory followed established clean techniques for Hg analysis as described in SI Part I.

At each stream, we filtered water from the Teflon bottles onto acid-cleaned 0.45 μm cellulose nitrate membrane (Millipore) to collect seston for MeHg analysis,[14] or onto muffled Whatman GF/F filter paper in the field to collect seston for determining stable C (δ^{13}C) and N (δ^{15}N) isotopes, as well as C:N elemental ratio. Moreover, we successfully collected late instars of larvae of ubiquitous filter-feeding hydrosychid caddisflies (*Hydropsyche spp.*) from 27 out of 30 study streams. All invertebrates were collected from 1 to 3 riffle(s) in each stream and transported alive in streamwater in acid-cleaned polypropylene vials to the laboratory in a cooler (10–15 °C) within 8 h. In the laboratory, live animals were dissected by clean forceps and their guts were discarded.[15] The dissected tissues were pooled from 4 to 7 individuals per sample and transferred to acid-cleaned HDPE vials, and frozen immediately. Two or three replicate samples were collected at each site.

Water and Animal Analyses. Digested water samples were analyzed for total-Hg by cold vapor atomic fluorescence spectrometry (CVAFS) via double-amalgamation technique.[16] A Hg standard solution (NIST 1641d) was included as a secondary standard check throughout the analysis. Acidified water samples or sestonic particles on a cellulose nitrate membrane [14] were distilled to remove matrix interferences and analyzed for MeHg by CVAFS.[17] The method detection limit for aqueous MeHg was 0.04 ng L^{-1} with 90 mL of sample, and the half of this value (0.02 ng L^{-1}) was used for samples below 0.04 ng L^{-1} in all subsequent calculations and graphical presentation.[18]

Frozen animal tissue samples were freeze-dried, weighed and digested by 4.6 M HNO$_3$ for 12 h at 60 °C.[19] Aliquots of the acid digest were spiked into the sparging flask with neutralization by KOH, and MeHg was analyzed as for the water samples mentioned above. Blanks and NIST 2976 mussel tissue were included along each batch of sample digestion. The digestion blanks had consistently no detectable MeHg while the measured NIST 2976 values were always within 10% of the certified value (i.e., MeHg = 28.09 ng Hg g^{-1} dry wt.). Water samples were determined for common physiochemical parameters as described in SI Part II. Also, both animal and sestonic samples were prepared for determining δ^{13}C and δ^{15}N, and C:N ratio as described in SI Part III.

Statistical Analyses. All regression analyses were performed by SigmaPlot 7.0 (San Jose, CA) while one-way ANOVA with Tukey's post hoc test was performed on SPSS 13.0 (Chicago, IL) to assess significant differences between multiple groups. In all cases, the α value was set at 0.05.

■ RESULTS AND DISCUSSION

Ancillary Streamwater Properties. Stream water properties varied widely among the study zones and less so within individual zones. Interzone variations were mainly driven by the different land cover and surficial geology in the watersheds.[2,10,20] Multiple water parameters differed significantly across zones. Conductivity (a proxy for total ion content) was significantly higher in streams within *S* and *TC* zones than *E* and *NE* zones (Figure 2A), due to the contrasting land cover and surface geology between southern and northern Minnesota.[20] Streams within *TC* zone had significantly higher total suspended solids than streams in other zones (Figure 2B). Sites within *S* zone had highly variable suspended solid levels but during the low flow period when our sampling occurred soil erosion could be minimal, and suspended sediment concentrations were likely much higher during higher
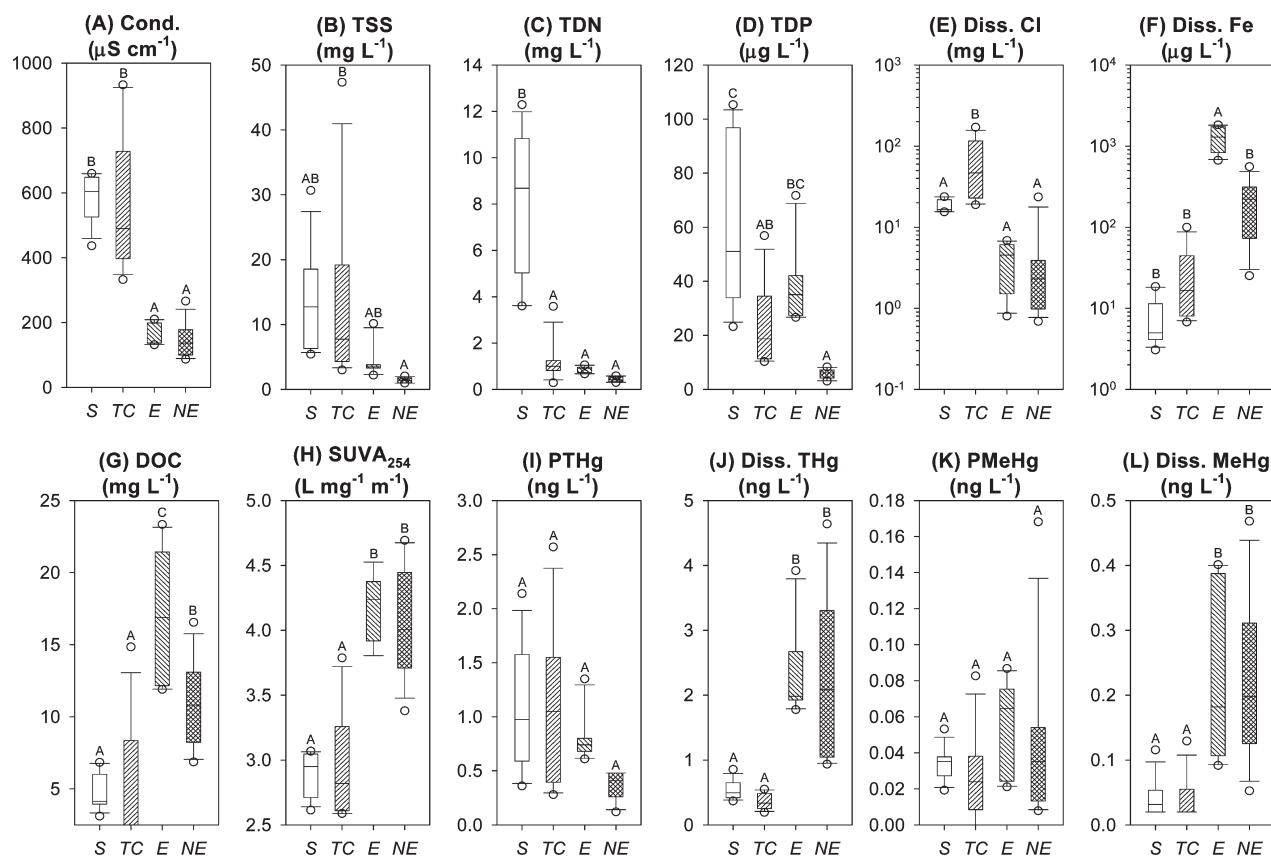
Figure 2. Box plots of selected physiochemical properties and Hg concentrations in stream waters among four study zones (Note: S = southern MN; TC = Twin Cities metro area; E = eastern MN; NE = northeastern MN). (A) conductivity, (B) total suspended solids, (C) total dissolved N, (D) total dissolved P, (E) dissolved Cl, (F) dissolved Fe, (G) DOC, (H) specific UV absorption (SUVA$_{254}$), (I) particulate total-Hg, (J) dissolved total-Hg, (K) particulate MeHg, and (L) dissolved MeHg. Group means bearing different alphabetical letters mean statistically different ($p < 0.05$).

flow period.[21] Due to the high agricultural coverage, streams in S zone were significantly elevated with both N and P (Figure 2C,D), potentially through contamination of the aquifers in these agriculturally dominated landscapes. Also, streams in both TC and E zones were slightly elevated with P compared to streams in NE zone (Figure 2C,D), likely due to agricultural inputs since agricultural coverage were also moderate in their watersheds (SI Figure S1B).

Dissolved Cl concentration was the highest in urbanized streams within TC zone (Figure 2E), due to the contamination of the aquifers during the extensive application of deicing road salts in the winter.[22] The high wetland coverage in E zone likely contributed to high dissolved Fe concentration in stream water,[23] compared to much lower levels in other zones (Figure 2F) with significantly lower wetland coverage (SI Figure S1D). Likewise, the high wetland coverage in E zone led to the highest DOC concentration in the stream waters while streams with high forest coverage in their watersheds in NE zone also had DOC concentrations significantly higher than streams within S and TC zones (Figure 2G). Similarly, stream waters within E and NE zones had higher SUVA$_{254}$ value, implying greater aromaticity of their DOC in the waters than streams within S and TC zones[24] (Figure 2H).

Mercury in Streamwater. Overall, particulate total-Hg and MeHg concentrations (in ng L^{-1}) were not significantly different across zones and were variable within individual zones (Figure 2I, K). In contrast, there were significant differences in dissolved

total-Hg and MeHg concentrations among zones, in which dissolved total-Hg and MeHg concentrations were all significantly higher in both E and NE zones than those in both S and TC zones (Figure 2J,L).

Variation of dissolved Hg concentrations across our 30 study streams is likely related to the availability of dissolved ligands for Hg in streamwater.[2,7,25] Specifically, the large range of DOC concentration across sites (i.e., 1.6 to 23 mg L^{-1}; Figure 2G) was positively and significantly related to both log$_{10}$-transformed dissolved total-Hg and MeHg concentrations (Figure 3A,B). These linear relationships were not surprising given that DOC is the predominant ligand for both inorganic Hg and MeHg in oxygenated surface waters.[8,9] Besides DOC concentration, we found that DOC character such as aromaticity (SUVA$_{254}$) has positive relationships with both dissolved log$_{10}$-total-Hg and MeHg concentrations (Figure 3C,D). One explanation for these relationships is that DOC with higher aromaticity may have higher binding affinity or number of strong binding sites to inorganic Hg and MeHg such as reduced thiol group.[8]

Interestingly, the Hg binding affinity of DOC would be zone-specific and differ significantly among the study zones. Specifically, if we assume all Hg is bound to DOC, DOC from streams in NE zone (0.20 ± 0.03 ng Hg mg OC^{-1}) held significantly more Hg per unit mass of DOC than streams in S (0.11 ± 0.01 ng Hg mg OC^{-1}) and TC (0.14 ± 0.02 ng Hg mg OC^{-1}) zones, then the latter two were not significantly different from streams in E zone (0.16 ± 0.03 ng Hg mg OC^{-1}) (SI Figure S3A).
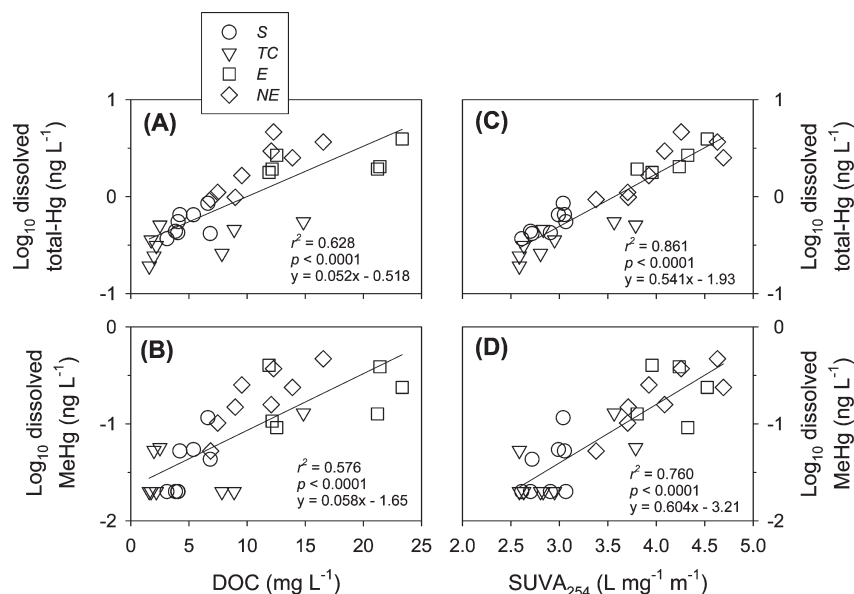
Figure 3. Relationships between DOC concentrations and (A) $\log_{10}$-transformed dissolved total-Hg concentrations, and (B) $\log_{10}$-transformed dissolved MeHg concentrations. Relationships between $SUVA_{254}$ values and (C) $\log_{10}$-transformed dissolved total-Hg concentrations, and (D) $\log_{10}$-transformed dissolved MeHg concentrations. Note: S = southern MN; TC = Twin Cities metro area; E = eastern MN; and NE = northeastern MN.

Moreover, we found that the amount of Hg per unit mass of DOC increased significantly with $SUVA_{254}$ (i.e., aromaticity of DOC) across streams (SI Figure S3B), suggesting that more aromatic DOC held more Hg per unit mass. It should be noted that all of these values fell into the reported ranges summarized previously by Grigal.[7]

Seston and Macroinvertebrates. Stable isotope analysis provides time-integrated information of diet sources (revealed by $\delta^{13}C$) and trophic positions (revealed by $\delta^{15}N$) of stream food webs.[26] The $\delta^{13}C$ of seston and hydropsychids among the study streams varied widely from −36.5 to −22.1 ‰ and from −34.8 to −24.2 ‰, respectively. The $\delta^{13}C$ for both seston and hydropsychids in streams within S zone were significantly more enriched than those in E and NE zones (Figure 4A,B), while not significantly different from those in TC zone. The variation in sestonic $\delta^{13}C$ is likely due to partial contributions of algae to seston because terrestrial $\delta^{13}C$ averages about 28 ‰ in watersheds dominated by C3 plants.[27] Land cover type also had important influences on $\delta^{15}N$ of both seston and hydropsychids, with significantly higher $\delta^{15}N$ in streams within S zone, while similar $\delta^{15}N$ values existed in streams among other zones (with the exception for seston in TC sites) (Figure 4C,D). The elevated $\delta^{15}N$ values in seston and hydropsychids can be attributed to the influence of denitrification in nitrogen-saturated environments.[28]

Across study streams, we found that $\delta^{13}C$ and $\delta^{15}N$ values for both seston and hydropsychids (Figure 5A,B) were positively and significantly correlated, suggesting that seston was the major dietary source for these consumers, which is in agreement with the documented feeding habits of these filter-feeders.[29] For $\delta^{13}C$, the relatively loose relationship between seston and hydropsychid as compared to $\delta^{15}N$ (in terms of r^2 values of the linear regression analysis: 0.522 for $\delta^{13}C$ vs 0.807 for $\delta^{15}N$) may be attributed to selective feeding of the animals on seston (i.e., not all seston is equally consumed by the filter-feeders). Alternatively, the $\delta^{13}C$ relationships between seston and hydropsychids may have been influenced by temporal variation since we only performed one-time sampling of seston, while the animal
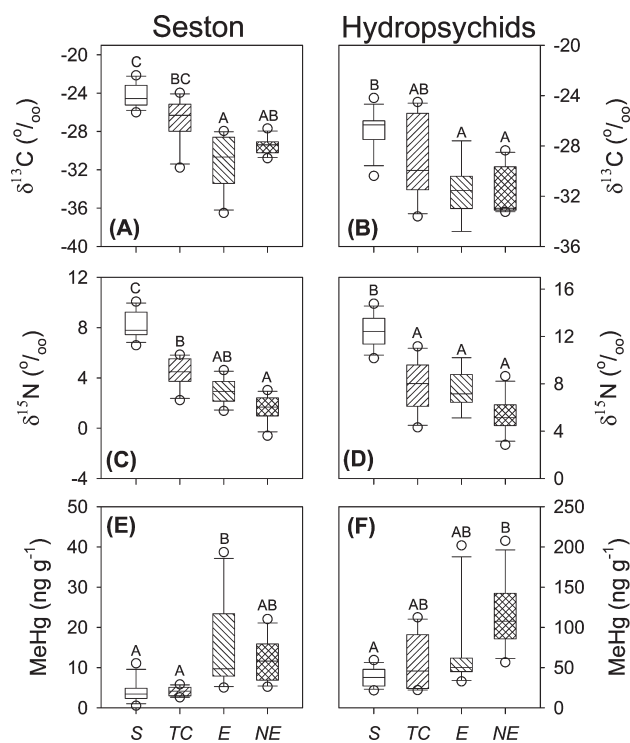
Figure 4. Box plots of (A) sestonic C isotope ratio ($\delta^{13}C$), (B) hydropsychid C isotope ratio ($\delta^{13}C$), (C) sestonic N isotope ratio ($\delta^{15}N$), (D) hydropsychid N isotope ratio ($\delta^{15}N$), (E) sestonic MeHg concentrations, and (F) hydropsychid MeHg concentrations in each of the study zones. Note: S = southern Minnesota; TC = Twin Cities metro area; E = eastern MN; and NE = northeastern MN. Group means bearing different alphabetical letters mean statistically different ($p < 0.05$).

consumers integrated food sources over longer time in the streams.[30] For $\delta^{15}N$, there was consistent enrichment of the $\delta^{15}N$ values in hydropsychids compared to seston across sites
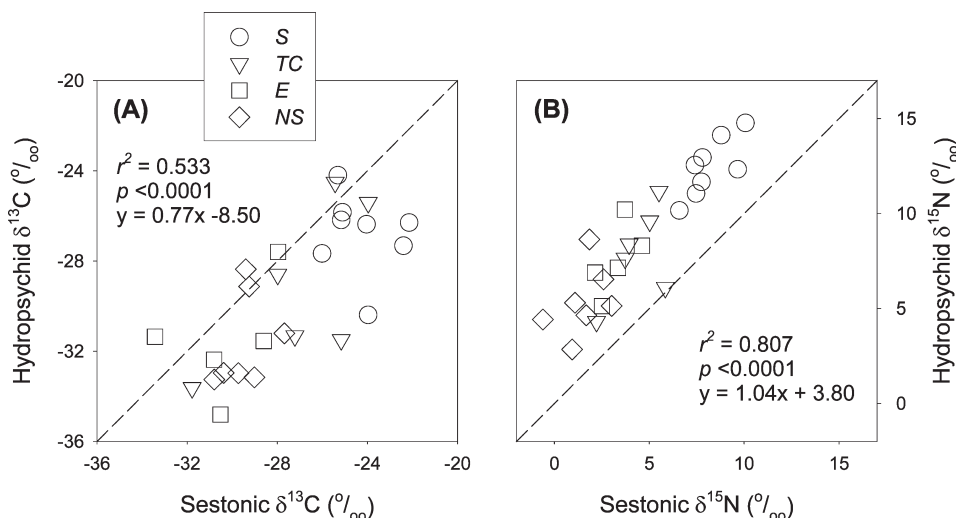
Figure 5. Relationship between sestonic particles and filter-feeding hydropsychid caddisflies across 27 study streams. (A) stable carbon isotopes ($\delta^{13}C$) and (B) stable nitrogen isotopes ($\delta^{15}N$). Dotted lines are 1:1. Note: S = southern Minnesota; TC = Twin Cities metro area; E = eastern MN; and NE = northeastern MN.

(Figure 5B). The mean isotopic enrichment of $\delta^{15}N$ between seston and hydropsychid was 3.77 ‰, close to the mean value observed in the literature of 3.4 ‰.[31] The isotopic enrichment was highly variable, however, ranging from −1.4 to +6.8 ‰ across sites, potentially reflecting the heterogeneity of sestonic particles or the influence of temporal variability in sestonic $\delta^{15}N$.

Sestonic Hg concentrations ranged from 54 to 514 ng g^{-1} for total-Hg (*data not shown*) and from 0.5 to 39 ng g^{-1} for MeHg (Figure 4E). Sestonic MeHg concentrations were significantly higher in streams within E zone, despite of large variations among streams within individual zones. Sestonic C:N ratios, an indicator of dietary quality for macroinvertebrates,[32] varied widely among study zones, with the highest values (i.e., the lowest food quality) in NS zone (seston C:N = 12.7 ± 0.8), intermediate values in E (10.6 ± 0.7) and TC (10.9 ± 1.8) zones and the lowest in S zone (8.4 ± 0.3). However, there was no apparent relationship between C:N and total-Hg/MeHg concentrations for seston across streams (*data not shown*). Tissue concentrations of MeHg in hydropsychids were variable and significantly higher in streams within NE zone (Figure 4F), and the range of MeHg concentrations in these filter-feeding macroinvertebrates is similar to previously published reports for macroinvertebrates in temperate streams receiving primarily atmospheric Hg.[6,15]

In order to better understand the underlying controls on MeHg bioaccumulation, previous studies have successfully used %wetland in watershed, DOC, unfiltered MeHg and dissolved MeHg concentrations to predict Hg concentration in aquatic ecosystems (e.g., refs 5 and 6). We tested the predictability of these variables on MeHg concentrations in hydropsychids across our study streams, and found that the combined percent of wetland and forest covers would be a positive and strong predictor but not %wetland alone (SI Figure S4A,B). However, we found that DOC concentration was a poor predictor of MeHg concentration in hydropsychids across our study streams (r^2 = 0.05; SI Figure S4C), while aqueous MeHg concentration (r^2 = 0.24 for unfiltered MeHg and r^2 = 0.25 for dissolved MeHg) appeared to be a better predictor of MeHg concentration in hydropsychids (SI Figure S4D,E), and somewhat statistically significant. Furthermore, sestonic MeHg concentration appeared

to be even a better predictor of MeHg concentration in hydropsychid caddisflies across sites (r^2 = 0.41; SI Figure S4F), but the relationship can be largely driven by several data points with high MeHg concentrations in seston and hydropsychids. It should be noted that the above relationships between Hg in water/seston and hydropsychids can be strengthened by more frequent sampling of water and seston.[6] The intimate relationships of MeHg between seston and hydropsychids are reasonable because dietary uptake should be the dominant pathway for MeHg accumulation in animal consumers,[33] which is similar to the findings of a previous study on seston and zooplankton in multiple Wisconsin lakes.[5]

Efficiency of MeHg Incorporation into Stream Food Webs. In order to estimate the "efficiency" of dissolved MeHg to be incorporated into the biological compartments, we calculated the bioconcentration factor of MeHg (BCF_{MeHg}) for both seston and hydropsychid caddisflies as below:

$$BCF_{MeHg}(L\ kg^{-1})$$

$$= \frac{MeHg\ concentration\ in\ seston\ or\ consumers\ (ng\ kg^{-1})}{MeHg\ concentration\ in\ dissolved\ phase\ (ng\ L^{-1})}$$

BCF_{MeHg} can be viewed as a positive proxy for the bioavailability of aqueous MeHg in aquatic ecosystems as higher BCF_{MeHg} implies that aqueous MeHg is more efficiently incorporated into the food webs, and vice versa. Interestingly, we found that $\log_{10}$-BCF_{MeHg} for seston decreased (at marginally significant levels) with DOC concentrations (Figure 6A) but decreases in BCF_{MeHg} were much stronger and statistically significant with DOC aromaticity or $SUVA_{254}$ values (Figure 6B). Similar to seston, $\log_{10}$-BCF_{MeHg} for hydropsychids decreased significantly with both DOC concentrations and $SUVA_{254}$ values (Figure 6C,D). These relationships imply that DOC may attenuate the partitioning of dissolved MeHg to the algal/detrital sestonic particles in the study streams. These results were corroborated by the previous studies on the influences of DOC on algal uptake of MeHg [11,12] and the field observation of negative relationships between DOC concentration and MeHg bioconcentration in lake food webs.[4,5] For
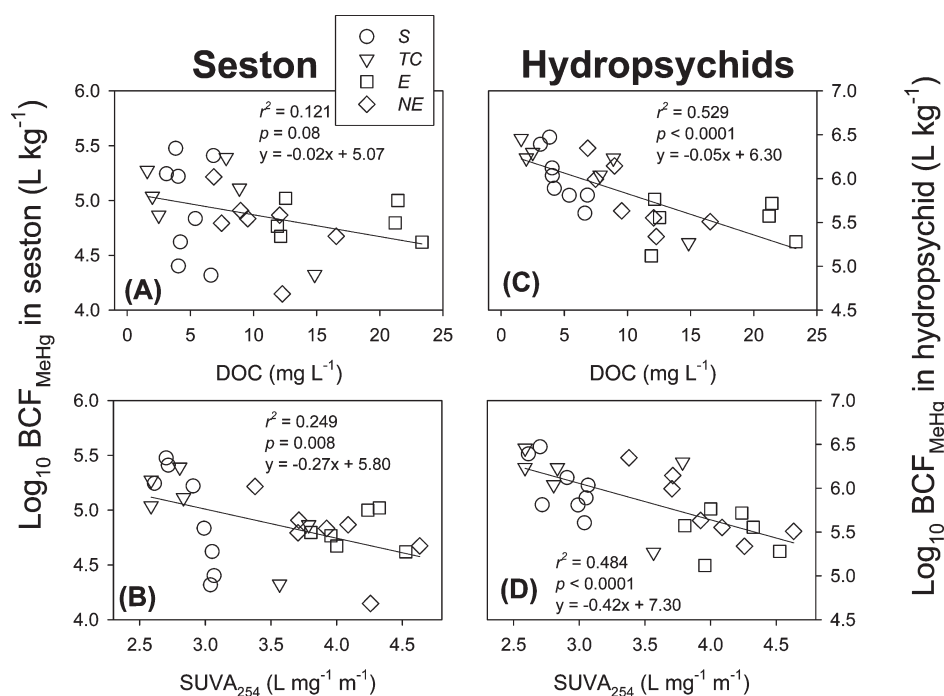
Figure 6. Log_{10}-transformed bioconcentration factor of MeHg (BCF_{MeHg}) in seston as influenced by (A) DOC concentration, and (B) $SUVA_{254}$ values. Log_{10}-transformed bioconcentration factor of MeHg (BCF_{MeHg}) in hydropsychids as influenced by (C) DOC concentration, (D) $SUVA_{254}$ values, Note: S = southern MN; TC = Twin Cities metro area; E = eastern MN; and NE = northeastern MN.

example, Gorski et al. [12] found that BCF_{MeHg} to phytoplankton cells decreased substantially above a threshold DOC concentration of 5 mg L^{-1}. Similarly, in our study the median BCF_{MeHg} for seston from streams of DOC concentration below 5 mg L^{-1} was 2.4×10^5 L kg^{-1} ($n = 8$), as opposed to a median BCF_{MeHg} of 5.8×10^4 L kg^{-1} from streams having DOC concentration above 5 mg L^{-1} ($n = 19$). The DOC-attenuated partitioning of MeHg into the basal resources should propagate to consumers over time,[33] and this effect was in fact reflected by the significant decline of BCF_{MeHg} in hydropsychid animals (Figure 6C,D).

A limitation in this work is that streams with higher wetland and forest cover having higher DOC concentrations also have higher $SUVA_{254}$ values ($r^2 = 0.612$; $p < 0.0001$), reflecting influences of aromatic DOC from the terrestrial landscapes in predominantly forested streams. Therefore, it is difficult to separate the effect of DOC concentration and DOC aromaticity on MeHg bioavailability at the base of food webs. Nevertheless, from the results of this work and the prior knowledge on DOC-Hg interactions, it is likely that higher DOC concentration and/or higher DOC aromaticity would reduce the tendency of dissolved MeHg to partition into the base of food webs (e.g., algae and seston). Future studies should manipulate natural water chemical composition with different combinations of these parameters in influencing MeHg bioavailability (see ref 12) in order to better understand the underlying processes.

Overall, our findings indicate that DOC plays an important role in influencing the incorporation of dissolved MeHg to the base of stream food webs (i.e., seston). Specifically, our data suggest that DOC concentration and aromaticity attenuate the partitioning of dissolved MeHg onto seston which transfers MeHg to the primary animal consumers. Undoubtedly, there is a dominant influence of the aqueous MeHg pool on MeHg

bioaccumulation as suggested in other studies.[6] Our work implies, however, that indirect effect of DOC concentration and character can mediate substantially MeHg bioavailability, and thus influence MeHg bioaccumulation and trophic transfer. Consideration of MeHg bioavailability could thus help improve predictive models of Hg accumulation in aquatic food webs, and should be taken into account for future studies examining Hg cycling in flowing waters.

■ ASSOCIATED CONTENT

Ⓢ **Supporting Information.** SI I: Sample filtration and preservation; SI II: Physiochemical analysis of water samples; SI III: Stable isotope analyses; Table S1: Details of study streams; Figure S1: Land cover types; Figure S2: Relative flow regime; Figure S3: Hg:DOC ratios; and Figure S4: Predicting variables for MeHg in hydropsychids. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

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