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Effects of Stream Water Chemistry and Tree Species on Release and Methylation of Mercury during Litter Decomposition

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Foliage of terrestrial plants provides an important energy and nutrient source to aquatic ecosystems but also represents a potential source of contaminants, such as mercury (Hg). In this study, we examined how different stream water types and terrestrial tree species influenced the release of Hg from senesced litter to the water and its subsequent methylation during hypoxic litter decomposition. After laboratory incubations of maple leaf litter for 66 days, we observed 10-fold differences in dissolved Hg (DHg, $<$ 0.45-μm) concentrations among different stream water types and more than 50-fold differences in dissolved methylmercury (DMeHg) concentrations. Percent MeHg (i.e., DMeHg $\times$ 100 $\div$ DHg on day 66) varied from 23−102% across seven natural stream water types. In general, stream waters with higher dissolved sulfate, suspended solid, and chlorophyll-*a* concentrations (e.g., eutrophic streams draining agricultural land) are associated with higher Hg release and methylation compared to more pristine sites (e.g., clear waters from coldwater trout stream). Across six tree species collected at the same site and incubated with the same source water, litter from slower decomposing species (e.g., cedar and pine) yielded higher DHg concentrations than those with more labile carbon (e.g., maple and birch). Percent MeHg, however, was relatively similar among different leaf species (i.e., 61−86%). Our study is the first to demonstrate that stream water chemistry and terrestrial plant litter characteristics are important factors determining Hg release and methylation during hypoxic litter decomposition. These results suggest that certain watershed and aquatic ecosystem properties can determine the levels of MeHg inputs during litterfall events.

Introduction

Methylmercury (MeHg) is the most bioaccumulative form of mercury (Hg), and its production in the environment is mainly mediated through the activities of sulfate-reducing bacteria (SRB) (*1, 2*). Surface sediments remain the major focus of studies of Hg methylation in freshwater environments (e.g., refs *3*−*5*). Nonsedimentary compartments are potentially important in MeHg formation but are much less studied. For example, organic matter sources such as periphyton (*6*) are associated with high net Hg methylation rates.

A particularly important source of organic materials entering streams and small lakes is terrestrial leaf litter (*7*). Terrestrial inputs often greatly exceed in situ primary production so that total production in small streams and lakes may be closely linked to that of surrounding forests (*7, 8*). There is some evidence that this organic carbon input could also represent an important pathway for Hg entering into aquatic ecosystems. Balogh et al. (*9*) observed an episodic pulse of MeHg (up to 5 ng L^{-1}) following a litterfall event because of in situ MeHg production in an agricultural stream during baseflow. Moreover, Hall and St. Louis (*10*) found that incubation of terrestrial plant litter in a reservoir elevated litter MeHg concentrations over time. Therefore, the production of MeHg can be strongly affected by the inputs of organic materials such as leaf litter, yet these processes remain poorly characterized.

Mercury enters terrestrial plants mainly through stomatal exchange from the atmosphere (*11, 12*) or by direct deposition onto leave surfaces (*13*). In general, foliar Hg concentrations are positively related to atmospheric Hg levels and the duration of growing season (*11*). However, the major Hg species in living leaves and recently senesced leaves is inorganic Hg (*10, 12*), instead of MeHg. Thus, the incorporation of litter Hg into aquatic biota ultimately depends on its release from litter and subsequent transformation to MeHg.

In the present work, we address two fundamental questions relating to the processes that may regulate the conversion of litter Hg to aqueous MeHg under saturated conditions. First, given the markedly different water chemistries present in different stream ecosystems, do stream water characteristics affect the release and methylation of Hg from litter? Second, given that riparian vegetation composition varies within and among watersheds (*14*), do litter species of different lability and potential inherent decomposition rates (i.e., different C:N ratios; ref *15*) influence the release and methylation of Hg? Through examining these questions, our study can provide important information regarding the MeHg production potential of litter inputs to the aquatic environment.

Experimental Section

Leaf Litter and Stream Water. Newly fallen leaf litter from six species [i.e., maple (*Acer*), oak (*Quercus*), birch (*Betula*), loosestrife (*Decodon*), cedar (*Thuja*), and pine (*Pinus*)] were collected in late September and mid-October, 2007 near Cedar Bog Lake in the Cedar Creek Natural History Area, a National Science Foundation Long Term Ecological Research site located 60 km north of Minneapolis, Minnesota. Litter was air-dried for 1−2 days inside a class 100 laminar-flow bench and analyzed for carbon (C), nitrogen (N), and Hg as described in Supporting Information, section I. In addition, water from seven Minnesota streams and rivers was collected and used (see Incubation Experiments) to examine the effect of water chemistry on Hg dynamics in litter. The sites included urban streams in Minneapolis (Shingle Creek) and Fridley (Rice Creek), a cold groundwater fed stream (Valley Creek), a warm-water stream east of St. Paul (Valley Branch), a stream with a forest and wetland dominated watershed near St. Croix State Park (Sand River), and two rivers in agriculturally dominated watersheds in south-central Minnesota (Big Cobb River and Maple River) (Supporting Information, Table S1).

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For analysis of aqueous Hg and other ancillary parameters, surface water was collected into two 1 L acid-cleaned Teflon bottles (i.e., duplicate). Briefly, the preserving solution (1% ultrapure HCl) in the Teflon bottle was discarded, and the bottle was rinsed with stream water, and then filled up with stream water by personnel wearing nonpowdered cleanroom gloves. Caps were then tightly closed, and all samples were transported to the laboratory on ice within 10 h. Water was also collected into two 1 L HDPE bottles for determining total suspended solids (TSS). For litter incubation, surface water was collected directly into new, sterile, and Hg-free Nalgene 500 mL PETG (polyethylene terephthalate glycol) bottles. All PETG bottles were rinsed with stream water one time before actual collection. The processing of water samples for chemical analyses in the laboratory is summarized in Supporting Information, section II.

Incubation Experiments. There were two sets of incubation experiments, with experiment 1 starting prior to experiment 2.

Experiment 1. Effect of water types. The seven stream waters described above and Millipore ultrapure water (0.2 μm-filtered and 18.0 MΩ, as a control) were incubated with maple leaf litter to compare the effect of different water types on Hg release and MeHg production. Maple leaf litter was selected because of its ubiquity in the Midwest area and high rate of decomposition (*16*).

Experiment 2. Effect of leaf species. The six litter species described above were incubated with Valley Branch water, which was chosen because of its intermediate TSS (7.5 mg L^{-1}) and dissolved sulfate concentrations (4.7 mg S L^{-1}) (Supporting Information, Table S1). Because the leaf morphologies of the species used were somewhat variable (e.g., some species have large petioles), we incubated only the intact lamina (i.e., leaf blade).

For all experiments, the initial density of litter was 2 g L^{-1}, which was 20-fold lower than a previous incubation experiment (i.e., 46.7 g L^{-1}; ref *9*). The incubation lasted for a total of 66 days at 25 °C in the dark, and the bottles were tightly closed so that hypoxic decomposition proceeded. Such oxygen-limited conditions may be present in certain stream microenvironments, such as the hyporheic zone (*17*) and an area with massive accumulation of leaf litter (*9*), which may be affected by the actual hydrological conditions. The incubation period (66 days) was chosen to allow adequate litter decomposition and observable Hg transformations across treatments, which is partially based on the findings of a previous laboratory experiment (*9*). On days 1, 6, and 66, the bottles were opened to retrieve samples (7, 100, and 110 mL, respectively) without replacement. All treatments were conducted in triplicate. Control samples (water without litter, one replicate per water type) were also incubated and analyzed to determine whether water-only respiration would greatly change Hg concentration and other water parameters. Over time, we found that dissolved oxygen, dissolved organic carbon (DOC), and DHg concentrations in these controls only decreased slightly and remained largely stable (Supporting Information, Figure S1).

Water Analyses and Statistics. All water samples and leaf extracts were preserved and measured for (total-)Hg or MeHg using cold vapor atomic fluorescence spectrometry (*1*). Both unfiltered and filtered Hg were analyzed for the original waters; however, all leaf extracts were filtered through 0.45 μm to remove particulate matters, which would complicate interpretation because of the varying amounts of particulate matters that can contain Hg across treatments, that is, only dissolved Hg (DHg) and dissolved MeHg (DMeHg) were analyzed. Other water parameters were measured for the original waters, with details provided in Supporting Information, section III. The method detection limits (MDL) were established for Hg (0.08 ng L^{-1}) and MeHg (0.054 ng L^{-1}) (see also Supporting Information, section III), samples with Hg or MeHg below the MDL were assigned a value equal to the half of the MDL for all calculations (*18*). One-way ANOVA with Tukey's post hoc test was performed on SPSS 13.0 (Chicago, IL) to assess significant differences between multiple treatments, where p value was set at 0.05. All regression analyses were performed using SigmaPlot 7.0 after passing normality test (Point Richmond, CA).

Results and Discussion

Stream Water and Litter Characteristics. Overall, aqueous Hg levels were low (0.2−4.7 ng L^{-1}), while three and six sites had unfiltered MeHg (TMeHg) and DMeHg levels, respectively, below the established MDL (Supporting Information, Table S1). Percent MeHg in unfiltered samples ranged only from 1.3−5.5%. The measured aqueous Hg and MeHg concentrations are typical of surface waters in undisturbed watersheds (*11, 19*). There were wide ranges in TSS (2.5−64 mg L^{-1}) and chlorophyll-*a* (chl-*a*) (0.4−28 μg L^{-1}) among the stream waters (Supporting Information, Table S1). We did not quantify the abundance of SRB in stream water, but they are generally ubiquitous in heterogeneous aquatic environments (*20*). All six litter species had similar C contents (i.e., 450−500 mg g^{-1}) but varying N contents (i.e., 3−18 mg g^{-1}). Consequently, C/N ratios were variable ranging from 32−206 (Supporting Information, Table S2). Litter Hg concentrations ranged from 24−65 ng g^{-1} (Supporting Information, Table S2). Interestingly, litter C/N ratios were negatively correlated to litter Hg concentrations ($r^2 = 0.683$, $p = 0.043$), suggesting that differences in plant N metabolism may affect Hg accumulation in leaves.

Dissolved Organic Carbon and Oxygen During Incubations. Following litter additions, DOC levels increased dramatically and dissolved oxygen levels decreased substantially over time (Supporting Information, Figure S2). Among different water types (experiment 1), the DOC increase ranged from 212 to 286 mg L^{-1} over 66 days for maple litter (Supporting Information, Figure S2-A), with the highest values for waters from two pristine streams (Valley Branch and Valley Creek) and lowest values for the ultrapure water treatment and waters from the agricultural rivers (Big Cobb River and Maple River). The final DOC level reflects the net difference between leaching of DOC from the litter and consumption of DOC by different microbial communities. We made no attempt to distinguish between these two processes. Across the six litter species incubated with a single water type (experiment 2), there was a wide range of final DOC increase, ranging from 33 mg L^{-1} for pine to 332 mg L^{-1} for loosestrife litter (Supporting Information, Figure S2-B). The increase in DOC was negatively correlated with litter C/N ratios, with the exception of maple litter, which had a relatively high C/N ratio and a high DOC increase. Because of the microbial respiration and the release of DOC from litter, solution pH at day 66 (4.6−6.5) was much lower than that of the original waters (7.2−8.1) in both experiments. Indeed, the decrease in pH was significantly correlated with the increase in DOC in both experiments 1 ($r^2 = 0.598$; $p < 0.0001$) and 2 ($r^2 = 0.984$; $p < 0.0001$).

In experiment 1, dissolved oxygen at the beginning of the experiment was near saturation for all waters (mean = 7.5 mg L^{-1}) but dropped quickly during incubation with maple litter to only 4−8% of initial level at day 66 (Supporting Information, Figure S2-C). Therefore, all treatments (including the ultrapure water treatment) had considerable microbial respiration during the experiment and conditions became hypoxic by the end of incubation. For different litter species (experiment 2), high C/N litter (e.g., pine) had a slower rate of oxygen consumption compared to species with more labile carbon (e.g., birch) (Supporting Information, Figure S2-D). However, by day 66, dissolved oxygen was nearly depleted

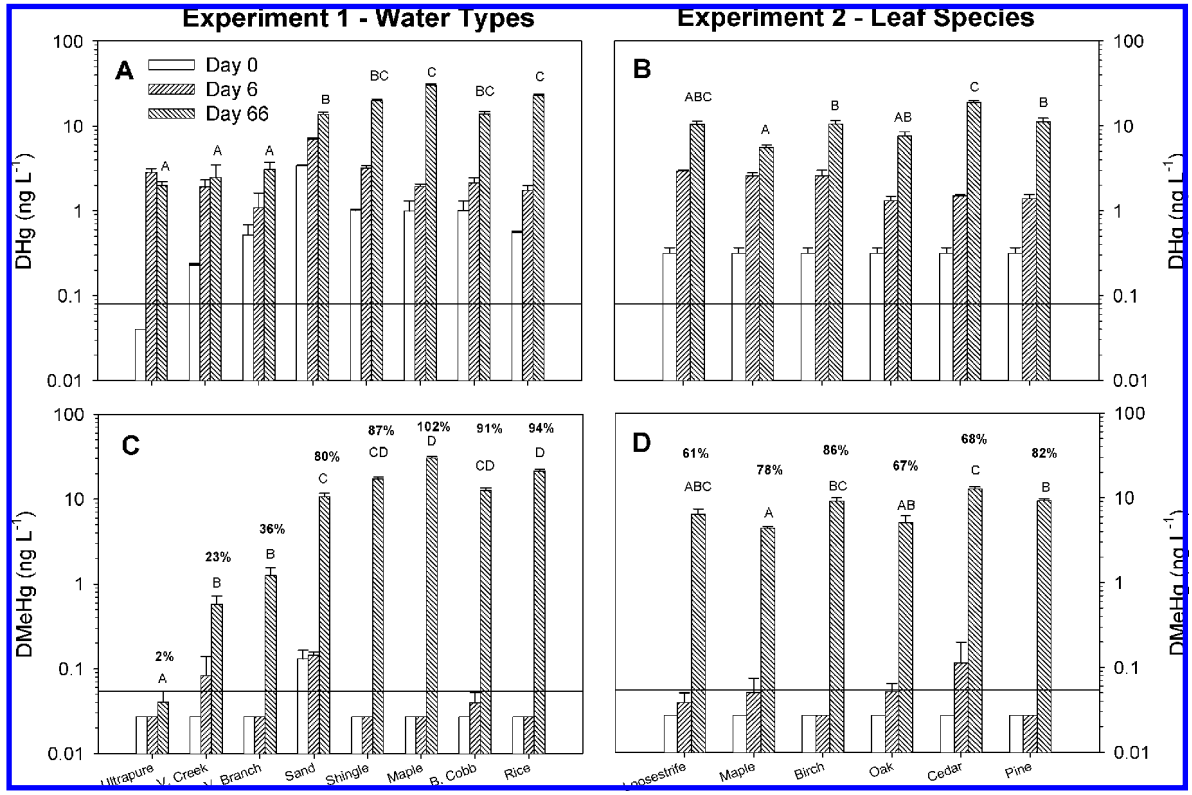

FIGURE 1. DHg concentrations over time among (A) different water types and (B) litter species and DMeHg concentrations over time among (C) different water types and (D) litter species. The numbers in panels C and D indicate the %MeHg for the particular treatment on day 66. The horizontal line represents the method detection limits for Hg (0.08 ng L^{-1}) and MeHg (0.054 ng L^{-1}). Error bars represent standard error of means. Site abbreviation: V. Creek = Valley Creek; V. Branch = Valley Branch; B. Cobb = Big Cobb. In each panel and on day 66 data only, means for a treatment are not significantly different ($p > 0.05$) if they bear the same alphabetical letter.

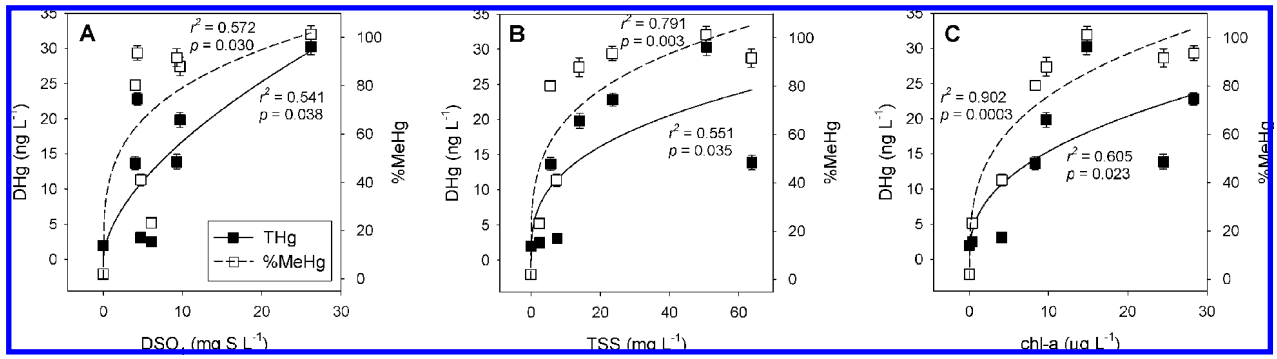

FIGURE 2. Relationship between DHg or %MeHg on day 66 from treatments of different water types (experiment 1) and (A) dissolved sulfate (DSO_4), (B) total suspended solids (TSS), (C) chlorophyll-*a* (chl-*a*). Error bars represent standard error of means.

for all treatments to only 2.6−8.5% of the initial level, suggesting that microbial activities may be very different among litter species during the early stages of incubation but that oxygen was almost entirely depleted in all treatments by the end of incubation (i.e., hypoxic for all treatments) (Supporting Information, Figure S2-D). A strong sulfurous (H_2S) odor also developed by day 66 across all treatments in both experiments, with the exception of ultrapure water treatment in experiment 1, which suggests that there is an active sulfate reduction (*9*).

Mercury. Among different water types incubated with maple leaf litter (experiment 1), there was a substantial increase in DHg concentrations later in the experiment (Figure 1A). By day 66, there was an increase of 1.9 ng L^{-1} in DHg concentrations for ultrapure water treatment. For natural water treatments, there were small increases in DHg level from day 0 to 66 for Valley Creek (2.3 ng L^{-1}) and Valley Branch (2.6 ng L^{-1}) but much larger increases over the same period for forested (Sand River 10.2 ng L^{-1}), urban (Shingle Creek 18.8 ng L^{-1}; Rice Creek 22.3 ng L^{-1}), and agricultural (Maple River 29.3 ng L^{-1}; Big Cobb River 12.9 ng L^{-1}) stream waters. These data show that some aspect of stream water chemistry considerably influences the extent and rate of release of litter Hg. Among different parameters, we found that dissolved sulfate, TSS, and chl-*a* of the original water could explain 54−60% of the variation in releasing litter Hg at the end of the experiment (Figure 2A−C). Overall, it appears that stream water with higher loading of particulate matter would lead to higher release of litter Hg (see Potential Mechanisms). It should be noted that the regression lines follow a power function (Figure 2), which may imply that the efficiency of these materials (e.g., TSS) in releasing litter Hg would decrease as the abundance of these materials increase in the waters.

For all species studied (experiment 2), DHg concentrations increased over time (Figure 1B). At the end of the experiment, the species with slower decomposition rate (i.e., lower final DOC level) had the highest DHg concentration (19 ng L^{-1} for cedar), while the faster decomposing species had the lowest (e.g., 5.6 ng L^{-1} for maple). DHg increased 18−60-fold from the initial concentration (0.32 ng L^{-1}) during the experiment. Nevertheless, there was no significant relationship between DHg concentrations on day 66 and litter Hg concentrations ($r^2 = 0.036$; $p = 0.718$) or litter C/N ratios ($r^2 = 0.232$; $p = 0.334$) across treatments. Since different litter species had different initial Hg concentrations (experiment 2), we also calculated the fraction of litter Hg that was released into the dissolved phase over 66 days, by taking the initial aqueous Hg pools (both dissolved and particulate) into account with the following equation

$$\text{release of litter Hg (\%)} = \frac{[\text{DHg in extract (ng)}]_{\text{day66}} \times 100\ \%}{[\text{THg in water (ng)}]_{\text{day0}} + [\text{litter Hg (ng)}]_{\text{day0}}}$$

where THg refers to unfiltered Hg.

Cedar and pine, the two conifer species examined, had the highest fraction of litter Hg that became DHg after 66 days of incubation (21% and 16%, respectively), while birch (9.4%) and oak (7.9%) were intermediate, and loosestrife (5.4%) and maple (4.4%) were the lowest. Interestingly, the fraction of litter Hg that was released was negatively correlated to the litter organic carbon released by day 66 into the solution, although the relationship is marginally significant ($r^2 = 0.612$; $p = 0.066$; Supporting Information, Figure S3). This may imply that the release of DOC from litter is uncoupled with Hg release among litter species, which is in contrast to the common observation that DHg is positively correlated with DOC in surface waters (*19*). One of the possible explanations is that in natural freshwaters DHg and DOC do not originate from the same source such as litter. However, it should be noted that these litter species also vary in many other traits that potentially results in different binding affinity of Hg inside the litter tissue. For example, the geometry of the litter differs widely in this experiment (e.g., pine vs maple), which may have an effect on the distribution of Hg on the litter surface versus in the litter tissue (*13*).

Methylmercury. In contrast to results for Hg observed in experiment 1, where incubation of litter with ultrapure water resulted in a relatively high DHg concentration (i.e., 1.98 ng L^{-1}), the ultrapure water treatment had only very low DMeHg concentration on day 66 (i.e., mean = 0.040 ng L^{-1}). This indicates a very low net rate of Hg methylation for the ultrapure water treatment, which is supported by the absence of a sulfurous odor, and is similar to a previous observation (*9*). Otherwise, the resulting DMeHg in the ultrapure water treatment could result from direct release of litter MeHg (*9*). The natural water treatments displayed DMeHg trends similar to those observed for DHg (Figure 1A and C). Overall, the increase in MeHg ranged from 0.55 to 30.6 ng L^{-1} for incubation with natural stream waters (Figure 1C). Patterns in DMeHg concentrations (Figure 1D) for different litter species (experiment 2) were similar to those for DHg (Figure 1B) at the end of the experiment. Compared to DMeHg in the original waters (i.e., < 0.054 ng L^{-1}), increases ranged from 162- to 344-fold for the six studied species on day 66. As for DHg, there were no significant relationships between DMeHg concentrations and litter Hg concentrations ($r^2 = 0.120$; $p = 0.500$) or C/N ratios ($r^2 = 0.283$; $p = 0.277$).

For litter incubated with different stream waters (experiment 1), the fraction of DHg as DMeHg (i.e., %MeHg) varied widely from 23 to 102% (Figure 1C). In contrast, in experiments with different litter species incubated with the same source water (experiment 2), %MeHg was relatively similar across treatments (61−86%) (Figure 1D). It should be noted that waters from Valley Branch when incubated with maple litter showed different %MeHg in experiment 1 (36%) and experiment 2 (78%). These two experiments started at different times (late September for experiment 1 and mid-October for experiment 2), and it is unknown if water, litter, or both collected for these two experiments were very different to result in such discrepancy. Otherwise, these may be caused by the variability of methylation efficiency of the microbes in the incubation. Overall, Hg methylation may be more strongly governed by the stream water properties than by litter species. In contrast, the data suggest that both stream water and litter species are important in determining Hg release from the litter. Among different water types (experiment 1), we found that dissolved sulfate, TSS, and chl-*a* of the original waters explained a substantial variation in the methylation (57−90%) at the end of the experiment (Figure 2A−C). Similar to DHg, the power function used in the regression analysis for %MeHg (Figure 2) suggests that the efficiency of these materials (e.g., sulfate) in methylating Hg would decrease as the abundance of these materials increase in the waters.

In this study, litter MeHg concentrations were not directly measured because previous studies reported very low and variable %MeHg (0.2−6.4%) across litter species in uncontaminated areas (*10, 12*). There is a possibility that DMeHg measured in the litter incubation came directly from the litter, but not as a result of methylation of litter Hg. However, we can eliminate this possibility because if we assume that the litter has MeHg concentration of 0.5 ng g^{-1} (at the high end of litter MeHg concentration reported in ref *10*), then only a maximum of 1.0 ng L^{-1} of DMeHg would be expected to be derived from the litter. From our observation, five out of eight treatments in experiment 1 had final DMeHg concentrations at least an order of magnitude higher than this calculated DMeHg concentration (Figure 1C). Therefore, we concluded that DMeHg in the litter extract was derived mainly from the methylation of litter Hg.

Potential Mechanisms. To produce MeHg from litter, inorganic Hg from litter must be first released into the dissolved phase (i.e., DHg) and then taken up by methylating microbes (e.g., SRB) to transform to dissolved MeHg (i.e., DMeHg) (*2*). In this study, we only measured DHg and DMeHg at days 6 and 66 after the litter incubation, and therefore, we could not evaluate release of inorganic Hg or production of MeHg associated with the particulate phases (i.e., litter, suspended particulate matters, and microbes larger than 0.45 μm). Therefore, the following discussion is based on the responses of DHg and DMeHg to experimental treatments.

In experiment 1, we found that three original water parameters (i.e., dissolved sulfate, TSS, and chl-*a*) can partially explain the variation of DHg concentrations among water types (Figure 2) while other parameters were unrelated to measured DHg concentrations (data not shown). It is possible that waters with higher TSS and chl-*a* could also have higher abundance of microbial communities because of the often observed positive associations between algae and bacteria in aquatic ecosystems (*21, 22*). Therefore, higher abundance of heterotrophic organisms in these waters may promote a more extensive decomposition of the litter than the water with lower microbial abundance. Interestingly, our final DOC measurements revealed a narrow range of increase of DOC (213−286 mg L^{-1}) among seven natural water types incubated with maple leaf litter in experiment 1, but the variation in increase of DOC was negatively related to DHg concentrations on day 66 (Supporting Information, Figure S4). It is possible that treatments with lower DOC values on day 66 indicated higher decomposition because the DOC generated may be

consumed by the decomposing microbes. Thus, this data suggests that differences in microbial abundance among treatments may determine the release of litter Hg. Although dissolved sulfate of the original waters also appeared to be related to the variation of DHg among treatments in experiment 1 (Figure 2A), sulfate may be more relevant and important to anaerobes such as SRB for Hg methylation (*2, 5*). Because dissolved sulfate, TSS, and chl-*a* among our water types also covaried with each other (Supporting Information, Table S1), these parameters cannot be isolated to account for the observed DHg pattern.

For Hg methylation, the importance of both sulfate and labile organic carbon on this process has been well established in previous studies in anaerobic microenvironment such as peatland porewater (*5*). In experiment 1, labile carbon was apparently unlimited (as shown by high and excess DOC at the end), while sulfate supply varied widely among water types. In addition to sulfate and labile carbon, the maximum amount of MeHg produced is ultimately constrained by DHg. It appears that three of the five water types showing high %MeHg (80−102%) at the end of incubation had relatively high initial dissolved sulfate concentrations (9.3 mg S L^{-1} for Big Cobb River; 9.7 mg S L^{-1} for Shingle Creek; 26.2 mg S L^{-1} for Maple River). In contrast, both Rice Creek (4.3 mg S L^{-1}) and Sand River (4.0 mg S L^{-1}) resulted in high %MeHg but had relatively low and similar dissolved sulfate as Valley Creek (6.1 mg S L^{-1}) and Valley Branch (4.7 mg S L^{-1}), the latter two stream waters resulted in relatively low %MeHg (22 and 36%, respectively). Therefore, it is possible that dissolved sulfate concentration is important for Hg methylaytion but may not be the only factor in determining methylation during litter incubation. For example, the presence of specific methylating microbes in the water may be another important factor for determining Hg methylation, which may positively correlate with the density of autotrophic organisms in the water as revealed by a strong correlation between chl-*a* and %MeHg (Figure 2C). Overall, the high %MeHg for several incubation treatments suggest that dissolved inorganic Hg from litter is highly bioavailable for methylating microbes. This observation is corroborated by a field study that %MeHg in natural stream water increased up to 77% during high leaf litter input and baseflow condition (*9*).

In experiment 2, where different litter types were incubated with a single source water (i.e., same initial TSS, chl-*a*, and dissolved sulfate), the resulting DHg on day 66 was in a relatively narrow range (i.e., 5.6−11.3 ng L^{-1}) compared to that observed in experiment 1 (Figure 1). As shown above, litter that released greater amount of organic carbon released proportionally less Hg (Supporting Information, Figure S3). Thus, among different species the properties of the litters themselves are important in determining the lability of litter Hg. Alternatively, this could be caused by the varying pH values in the litter incubation because final pH (range: 5.2−6.5) negatively correlates with final DOC levels in these treatments ($r^2 = 0.989$; $p < 0.0001$). Interestingly, %MeHg was similar (i.e., 61 to 86%) across different litters incubated with the same stream water (Figure 1D), and therefore, it is likely that water characteristics such as dissolved sulfate and chl-*a* are more important than leaf litter in determining the degree of methylation.

Future studies investigating the release and methylation of litter Hg should experimentally study potential mechanisms to account for water chemistry effects observed here via laboratory and field manipulation. Moreover, the origin of methylating microbes in these processes should be identified; they can be present on the litter surface or in the waters, and it is important to understand why these oxic substrates can accommodate potentially anaerobes for Hg methylation, such as SRB. Since sulfide was not measured directly in the current study (but sulfurous odor was found in all natural water treatments), and such measurement in the future studies would allow us to understand if sulfide can control the bioavailability of litter Hg for methylating microbes (*2*).

Litter Mercury in the Environment. Litter represents an important pathway for Hg entry into aquatic ecosystems for two reasons. First, Hg concentrations are elevated in litter because the foliar burden of Hg increases during the growing season (*11*). Consequently, litterfall Hg flux is higher on an areal basis than either wet deposition or throughfall in temperate and boreal forests (*11*). Second, Hg transported to aquatic ecosystems via litterfall is accompanied by a large supply of labile organic matter. Therefore, litter entering lakes, wetlands, or streams under low flow conditions may rapidly drive the microenvironment to hypoxic conditions, induce anaerobic microbial activities, and possibly elevate in situ net Hg methylation rates because of high organic carbon and inorganic Hg availability from litter (*9*). However, variation in other ambient factors, such as sulfate concentrations, TSS and chl-*a* may simultaneously control the availability of inorganic Hg and methylation as implied in the present study.

The litter-to-water ratio employed in most of the experiments in this study (i.e., 2 g L^{-1}) was much lower than used in a previous experiment (46.7 g L^{-1}; ref *9*), and it is important to evaluate the relevance of our laboratory experiments to natural environmental conditions. The resulting DOC levels from maple litter in this study ranged from 212 to 289 mg L^{-1}, and for high C/N litter (i.e., cedar and pine), the DOC ranged only from 37 to 50 mg L^{-1} (Supporting Information, Figure S2-A and -B). Although the DOC levels of leaf extracts are much higher than many natural waters (e.g., 1−16 mg L^{-1}, this study), these DOC levels have values similar to aqueous phases such as sediment porewater from peatlands (e.g., 2−140 mg L^{-1}; ref *4*), small agricultural ponds (e.g., 2−70 mg L^{-1}; ref *23*), and saturated organic soils (e.g., 20−120 mg L^{-1}; ref *24*). Therefore, while our laboratory experiments were not meant to simulate aquatic habitat conditions, they are analogous to natural conditions encountered under some saturated conditions, especially during autumn leaf inputs.

Our findings suggest that streams enriched with algal seston, suspended particles, or sulfate are more vulnerable to pulses of Hg flux via litter, an observation supported by a field study on an agricultural stream during baseflow conditions (*9*). However, whether these high algal conditions can lead to higher Hg bioaccumulation in the food webs is perplexing because recent research established that high algal density could reduce Hg concentrations in the food webs through growth biodilution (*25*). Thus, the ultimate transfer of litter Hg to the aquatic food webs also depends on other ambient processes such as food abundance and quality. In addition, Hg release also varied among tree species and appeared to be related to litter quality or plant physiology. Riparian zones can have variable dominant tree species (e.g., deciduous vs coniferous) (*14*), and such differences may influence Hg release from litter and subsequent in situ Hg methylation.

In summary, our study provides the first systematic analyses of environmental controls over Hg release and methylation during hypoxic litter decomposition. Our study shows orders of magnitude of differences in potential in MeHg production among water types. In addition, among different species of a wide range in litter C/N ratios and a small range in litter Hg concentrations, we observed only a factor of 2−3 of differences in Hg release and similar net Hg methylation. Therefore, it appears that chemical and microbial constituents in the water are key factors in the complex biogeochemical processes that regulate Hg bioavailability from litter, while terrestrial plant species plays a smaller role in these processes and mainly through effects on Hg release.

The step of transferring Hg from litter to water and the subsequent methylation is complicated and warrants further studies for detailed examinations and manipulations, both in the laboratory and the field.

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Supporting Information Available

Elemental and Hg analyses of plant litters, processing of natural water samples, water and leaf extract analyses, tables showing physicochemical properties and Hg concentrations of different water types and elemental and Hg concentrations of litter species, and figures showing water parameters for water-only treatments, changes in DOC and oxygen during litter incubation, the relationship between litter Hg and C release (experiment 2), and the relationship between DHg and increase of DOC (experiment 1). This material is available free of charge via the Internet at http://pubs.acs.org.

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