Microbial Pathways of Mercury Methylation During Litter Decomposition

By: Elaine Chow and Martin Tsz-Ki Tsui

Chow, Elaine; Tsui, Martin Tsz Ki. Elucidating Microbial Pathways of Mercury Methylation During Litter Decomposition. *Bulletin of Environmental Contamination and Toxicology*. v103 n4 (Oct 2019) 617-622. https://doi.org/10.1007/s00128-019-02700-3

Abstract:

Tree foliage sequesters gaseous elemental mercury (Hg) through stomatal uptake, when the foliage senesces and falls into the water, Hg from leaf litter can be released into the water and/or microbially methylated into a highly toxic form, methylmercury. The dominant groups of microbial communities that can methylate Hg during litter decomposition are, however, less certain. We conducted a microbial inhibition experiment to identify the primary methylators of leaf litter Hg during 28-day decomposition of two litter species of contrasting quality (pine and maple). We demonstrate that sulfate-reducing bacteria are the dominant microbial groups for Hg methylation during anoxic litter decomposition, and our study also indicates that methanogens may have a minor role in mediating Hg methylation during litter decomposition. Thus, aquatic environment with extensive litter accumulation and decomposition (e.g., wetlands, ponds, and river pools) can be hotspots of Hg methylation through sulfate-reduction and, to a lesser extent, methanogenesis.

Keywords: Litter decomposition | Mercury | Methylation | Microbial communities | Aquatic environment

Article:

Mercury (Hg) is a global pollutant due to its gaseous emission, and long-range atmospheric transport and deposition in the environment (Fitzgerald et al. 1998). Sources of atmospheric Hg include natural origins (e.g., degassing of rocks and volcanic activities) and anthropogenic origins (e.g., coal burning and artisanal gold mining) (UNEP 2013). Since atmospheric Hg (as gaseous elemental Hg) can accumulate in foliage through stomatal uptake (Ericksen et al. 2003), thus forests can enhance sequestration of atmospheric Hg (Jiskra et al. 2018).

Upon litterfall, litter represents an important flux of Hg to the forest floor (i.e., dry deposition), or in nearby aquatic environment (Grigal 2002). For litter decomposition in the soil, external Hg

(e.g., in gas or soil) can be further sequestered by the decomposing litter (Pokharel and Obrist 2011), elevating Hg levels in the highly decomposing organic matter (Obrist et al. 2011). For litter decomposition in the water, some portion of inorganic Hg [Hg(II)] from litter can be quickly released into the water as this pool of Hg is associated with labile dissolved organic matter (DOM) derived from the litter (Allan and Castillo 2007).

Recently, Hg methylating microbes have been identified to possess a two-gene cluster (*hgcA* and *hgcB*) (Parks et al. 2013). Since then, capability of methylating Hg has been inferred to be present in diverse microbial groups besides the well-known sulfate-reducing bacteria (SRB) and iron-reducing bacteria (FeRB), such as methanogens and *Firmicutes* (Gilmour et al. 2013). Also, the environment conducive to microbial methylation of Hg has been implied to be widespread including newly identified environment such as invertebrate guts, coastal "dead zones", etc. (Podar et al. 2015). Once produced, methylmercury (MeHg) can be strongly concentrated into the base of aquatic food webs (e.g., algae), and biomagnified along the trophic food chain (Tsui and Wang 2004), posing a worldwide concern for human and wildlife to expose to this neurotoxin through fish consumption (Scheuhammer et al. 2007).

Leaf litter contains labile organic matter and Hg(II), and thus during litter decomposition in water (e.g., standing water) can quickly provide an ideal condition for Hg methylation such as low dissolved oxygen and abundant energy sources. For example, Balogh et al. (2002) first observed very high levels of aqueous MeHg in a slowly-moving stream in southern Minnesota (USA) with massive amount of litter accumulation in the autumn, and later the authors showed that incubating litter with freshly collected streamwater in a closed container can quickly produce high levels of MeHg over short period of time (e.g., 1 week) along with strong sulfidic smell ("egg-rotten"), suggesting the presence of sulfate reduction along with Hg methylation. Interestingly, Tsui et al. (2008) later demonstrated that litter of different plant species (e.g., different quality, or C:N ratio) can be an important factor in regulating the release and methylation of Hg(II) during anoxic incubation, as well as if the incubation of the same litter species with different streamwater types (e.g., different nutrients and suspended particle levels). These initial studies thus suggest there are complex interactions between litter, water, and microbes during the incubation to determine the extent of Hg release from litter and the subsequent Hg methylation. However, one question that remains unresolved during litter decomposition in natural water is: what is the dominant microbial pathway of Hg methylation during anoxic litter decomposition? Since different pathways such as sulfate reduction (Gilmour et al. 1992), iron reduction (Kerin et al. 2006) and methanogenesis (Hamelin et al. 2011) have been implied to be important Hg methylation pathways, thus resolving this question would have important implications on improving our understanding of the complex biogeochemical Hg cycle.

In the work reported here, we performed a 28-day litter incubation experiment with two foliage species common to the piedmont region of North Carolina (USA), *Pinus taeda* (Loblolly pine) and *Acer saccharum* (Sugar maple). In general, pine needles (C:N ratio ~ 204) have slower decomposition rate and are expected to produce higher dissolved Hg(II) levels as observed in our previous study (Tsui et al. 2008) while maple litter (C:N ratio ~ 124) is more labile and would decompose faster, and is expected to produce lower dissolved Hg levels as observed before (Tsui et al. 2008). Both litter species were incubated with the same source of streamwater, from a local

stream with relatively low levels of aqueous Hg (total-Hg: ~ 1–2 ng/L; Tsui, *unpublished data*) in the city of Greensboro, North Carolina, USA. Therefore, our study can provide a comparison for dominant microbial pathways for Hg methylation when two different common litter species are concerned as source of Hg for the microbial methylation.

Materials and Methods

Freshly fallen, dry leaf litter of two tree species were collected from the Eno State Park in Durham, North Carolina, USA, on separate occasions in late September and October 2014. The state park is distant from any currently operating coal-burning power plants in the region and thus Hg in these leaf litter are considered to be derived mostly from relatively well mixed regional/global sources of Hg. Pine litter and maple litter were both light brown in color when collected (Fig. 1). Samples were collected by personnel wearing powder-free vinyl gloves and placed into clean ziploc bags, and subsequently air-dried for 2 days in a class 100 laminar-flow bench in the laboratory.

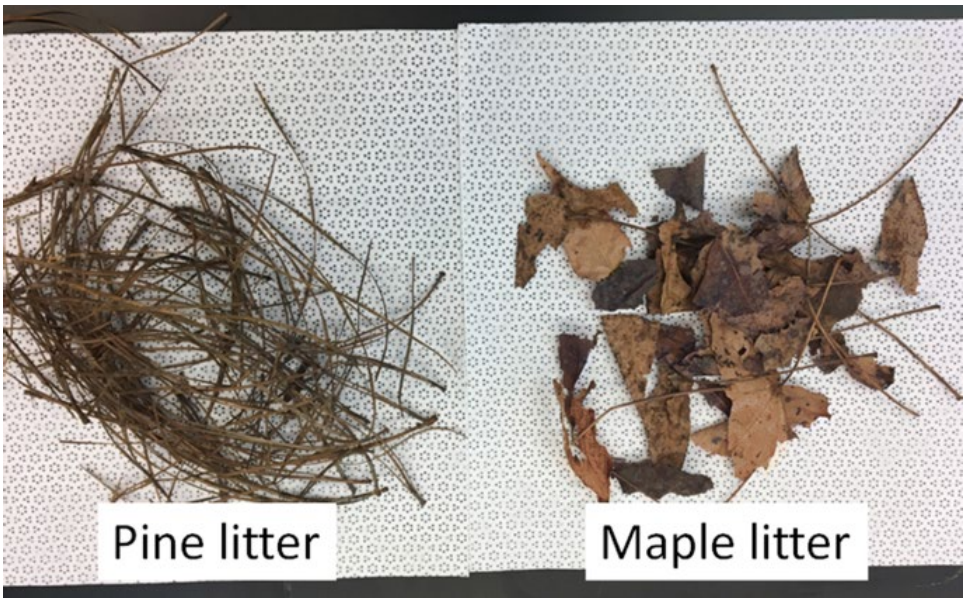

Figure 1. Pictures of both leaf litter used for this incubation study. Photo taken by M. Tsui

Water used for the incubation experiments was collected from a local urban stream, North Buffalo Creek, in Greensboro, North Carolina, USA, one day prior to the beginning of incubation experiments. We used "freshly-collected" streamwater without filtration for litter incubation because in another incubation study we found that aged streamwater (> 3 months in the lab) or filtered freshly-collected streamwater appeared to end up the incubation with very minimal Hg methylation (Ku et al. 2018), suggesting that some anaerobic microbes capable of methylating Hg should be originally present in the streamwater, even though the water was highly oxygenated.

In this work, we used microbe-specific inhibitors similar to another recent study in our laboratory (Blum et al. 2018) as well as other published work (e.g., Hamelin et al. 2011), to determine if MeHg production would be mediated by a specific microbial group such as sulfate-

reducing bacteria, methanogens, and/or other microbial groups (e.g., iron-reducing bacteria). Three specific inhibitors were used and obtained from Alfa Aesar (Ward Hill, Massachusetts, USA): 2-bromoethane-sulfonic acid (BESA, at 5 mM) was used to inhibit the activities of methanogens, sodium molybdate (Na_2MoO_4, at 20 mM) was used to inhibit the activities of sulfate-reducing bacteria, and chloramphenicol (at 2 mM) was used as a general bacterial inhibitor.

Dried, intact pine and maple litter were separately incubated in new Hg-free Nalgene 1 L Polyethylene Terephthalate Glycol (PETG) square media bottles filled with unfiltered streamwater. Specifically, we added 5.0 ± 0.1 g of dried litter in 1 L of streamwater while microbe-specific inhibitors were added and well mixed with the streamwater. All treatments were then placed in the dark at the room temperature (between 21 and 24^{o}C), and mixed daily to homogenize the content inside each bottle (Tsui et al. 2008; Blum et al. 2018; Ku et al. 2018). The incubation became anoxic quickly due to the rapid consumption of dissolved oxygen by aerobic microbes (Tsui et al. 2008). The incubation was replicated three times and conducted for a total of 28 days. Control treatments without any microbial inhibitors were also placed under the same conditions.

On day 14 and 28 of incubation, about 100 mL of water samples were taken from each experimental container and filtered through Hg-free filter membrane (pore size: 0.45-µm; cellulose nitrate membrane) in a disposable filter unit (Nalgene) to remove any particulates for analyzing both dissolved total-Hg and MeHg (Tsui et al. 2008). Filtered samples were split and transferred into two acid-cleaned 40 mL I-CHEM borosilicate glass vials (Thermo Scientific). For total-Hg analysis, samples were oxidized by an acidic permanganate/persulfate mixture at 60^{o}C overnight to completely break down ligands (e.g., DOM) binding to Hg(II) and/or MeHg (Woerndle et al. 2018). For MeHg analysis, samples were preserved by 0.4% trace metal grade HCl (Parker and Bloom 2005), and distilled to remove matrix interference prior to analysis (Horvat et al. 1993). Both litter samples were digested by trace metal grade concentrated HNO_3 and H_2SO_4 (1:1 v/v) in a Teflon digestion vessel (Savillex, Eden Prairie, Minnesota, USA) at 60^{o}C overnight, followed by complete oxidation with the addition of 1% BrCl to analyze for total-Hg content.

For total-Hg measurements, completely oxidized samples (as shown by the persistent purple color in water samples; or yellow color in the acid digest of solid samples) were neutralized by aliquots of 30% NH_2OH, and known volume of aliquots was added into a glass bubbler. Hg(II) was reduced by the addition of 20% $SnCl_2$ and Hg(0) was purged onto gold-coated traps by Hg-free N_2 gas. We used two certified standard reference materials (SRMs): NIST3133 and NIST1641d, for calibration and quality assurance and quality control (QA/QC) procedures. Total-Hg was analyzed by the double amalgamation technique and quantified by cold vapor atomic fluorescence spectrometer (CVAFS, Brooks Rand Model III) (USEPA 2002). For MeHg measurements, known volume of distilled samples were added into a glass bubbler, maintained at pH 4.9 with acetate buffer, ethylated with 1% sodium tetraethylborate, and purged onto Tenax traps by Hg-free N_2 gas. MeHg was quantified by CVAFS after being separated by an isothermal gas chromatography column and pyrolyzed (Bloom 1989; Liang et al. 1994). Our MeHg calibration standard was obtained from CEBAM Analytical Laboratory (Bothell, Washington,

USA), and QA/QC procedures involved the measurements of a digested solution (by 25% KOH in methanol) of SRM NRCC DORM-4 (fish protein).

We performed one-way analysis of variance (using Microsoft Excel) to test the significant difference among inhibitor treatment, litter species, and time of incubation. The significance level for all statistical analyses was set at $\alpha = 0.05$.

Results and Discussion

Streamwater collected from the local stream for incubation experiment had low levels of Hg during baseflow period in the summer (total-Hg: ~ 1–2 ng/L; Tsui *unpublished data*), it is expected that Hg(II) in litter would be the major contributor of total-Hg observed in the filtered phase in the incubation container, since pine and maple litter used in this study had total-Hg of 28.1 and 38.1 ng/g dry wt., respectively.

Consistent to our previous study (Tsui et al. 2008) and the expectation of faster decomposition, we found that DOC concentrations in incubation were significantly higher ($p < 0.05$) in maple than pine incubations but we found no significant difference in DOC concentrations between 2 and 4 weeks of incubation for individual leaf litter type (Fig. 2).

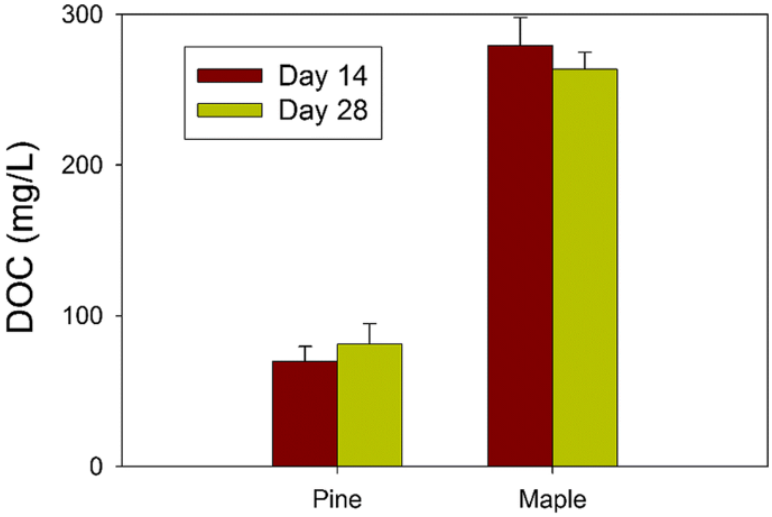

Figure 2. Leaf litter incubation on pine and maple and their dissolved organic carbon (DOC) concentrations. Data are means ± SD ($n = 3$).

Overall, dissolved Hg concentrations differed between incubation experiments for pine litter (Fig. 3) and maple litter (Fig. 4). For pine litter incubation, dissolved total-Hg concentrations in general increased over time, from Day 14 to Day 28 (Fig. 3a). However, total-Hg concentrations were different among treatments, being significantly higher for control and BESA treatments ($p < 0.05$), potentially indicating that treatments with molybdate and chloramphenicol may decrease the microbial decomposition of litter and thus the release of inorganic Hg from the litter. Nevertheless, such differences seem to be non-existent for Day 28 samples ($p > 0.05$). For MeHg, it is very clear that treatments with both molybdate and chloramphenicol resulted in very low MeHg or close to our analytical detection limit (~ 0.04 ng/L) while the control treatment resulted in the highest MeHg followed by the BESA treatment (Fig. 3b).

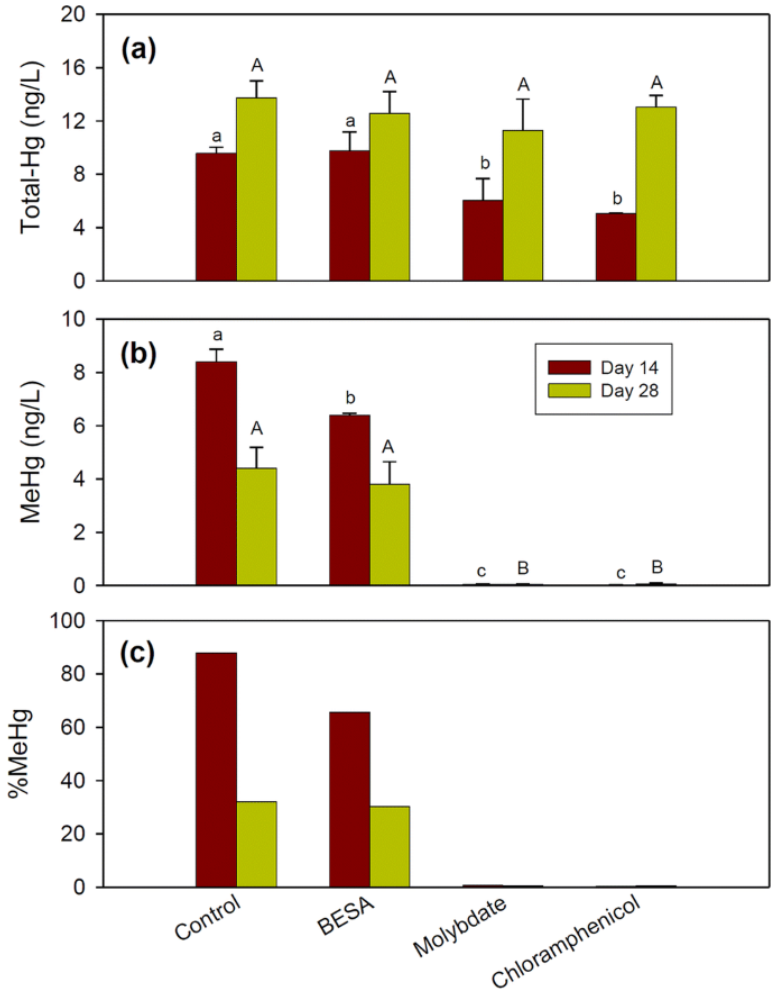

Figure 3. Pine litter incubation results on Day 14 and 28: **a** total-Hg concentration, **b** MeHg concentration, and **c** %MeHg, in filtered incubation solution. Data are means ± SD ($n = 3$). In each figure and on the same day, means for a treatment are not significantly different ($p > 0.05$) if they bear the same alphabetical letter

Interestingly, we observed that MeHg concentrations decreased from Day 14 to Day 28 in both control and BESA treatments, suggesting that there could be either microbial demethylation or re-partitioning of dissolved MeHg to decomposing detrital debris, since we incubated the bottles in the dark and thus there should not be any photochemical demethylation of MeHg (Seller et al. 1996), and we also filtered the incubation solution during the collection and thus any MeHg associated with particles would not be measured. We also calculated the fraction of total-Hg as MeHg (or %MeHg), and found that the majority of Hg in both control and BESA treatments existed as MeHg on Day 14 but their %MeHg became lower on Day 28, contributed by both decrease of MeHg and increase of Hg(II) (or total-Hg) (Fig. 3c).

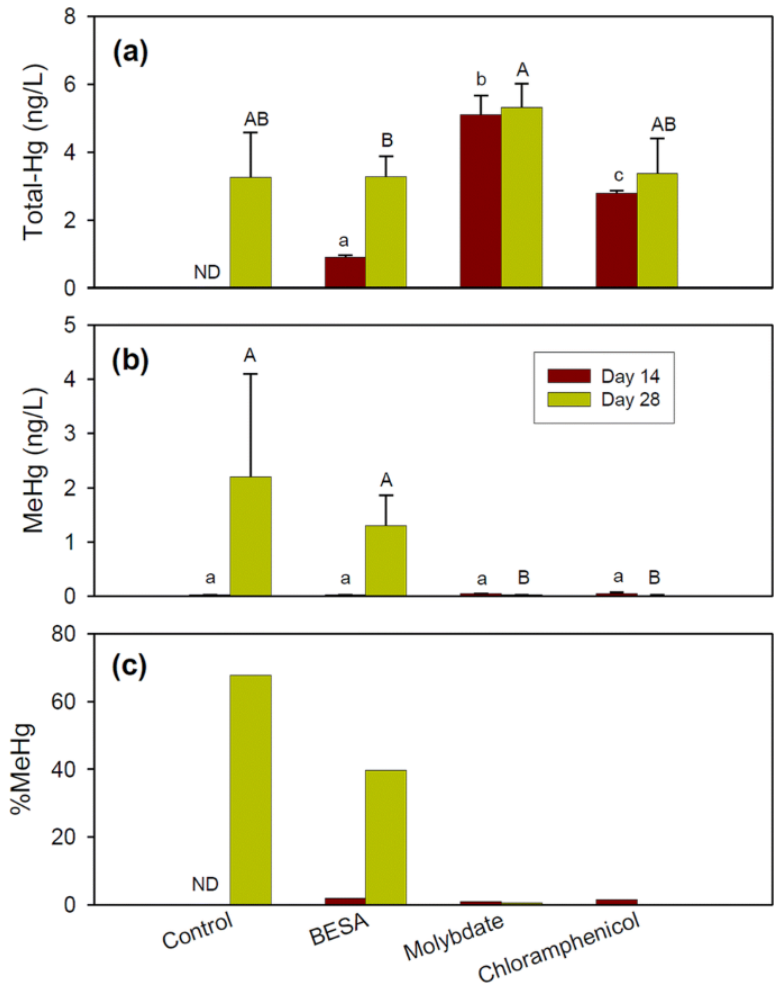

Figure 4. Maple litter incubation results on Day 14 and 28: **a** total-Hg concentration, **b** MeHg concentration, and **c** %MeHg, in filtered incubation solution. Data are means ± SD ($n = 3$). In each figure and on the same day, means for a treatment are not significantly different ($p > 0.05$) if they bear the same alphabetical letter.

For maple litter incubation, the results seem to be different from the incubation with pine litter, in which we found significantly higher levels of total-Hg ($p < 0.05$) in treatments with molybdate and chloramphenicol on Day 14 compared to BESA treatment (note that we did not have data for total-Hg for control treatment on Day 14, denoted as "ND") (Fig. 4a). However, these differences among treatments became smaller for Day 28.

Overall, it appears that pine litter incubation releases more Hg(II) than maple litter incubation, a finding similar to a previous study after 66 days of incubation (but under different litter to water ratio) (Tsui et al. 2008). Interestingly, MeHg level is close to or below detection limits for all treatments on Day 14 in maple litter incubation (Fig. 4b), which is very different from the pine litter incubation (Fig. 3b), suggesting that Hg(II) from pine litter is more readily available for Hg methylation, perhaps this may be attributed to the much higher dissolved total-Hg levels (Figs. 3 and 4). On Day 28 it became clear that only the control and the BESA treatments resulted in high concentrations of MeHg in the incubation solution but not the molybdate and

chloramphenicol treatments (Fig. 4b), both with $p < 0.05$. When expressed as %MeHg, only the control and the BESA treatments had intermediate and high percentages on Day 28, respectively.

In this study, we observed that the microbial inhibitors have some effects on the release of Hg(II) from the litter, but our findings do not indicate a specific trend or pattern (Figs. 3a and 4a). It is very clear, however, that the control treatments would result in high levels of MeHg, findings consistent to previous studies of litter incubation (Balogh et al. 2002; Tsui et al. 2008; Ku et al. 2018) and sediment incubation (Hamelin et al. 2011; Blum et al. 2018). It is also obvious from these studies that methylating microbes are potentially present in streamwater since litter incubated with laboratory reagent water, aged streamwater, and filtered (sterilized) streamwater did not produce significant amount of MeHg during closed incubation of litter (Ku et al. 2018).

From both incubation experiments, we found that molybdate and chloramphenicol treatments almost resulted in no MeHg production after 28 days, but both the control and the BESA treatments produced substantial amount of MeHg at the end of 28-day period (Figs. 3b and 4b). Compared to the control treatment, the BESA treatment produced lower levels of MeHg when we compared their %MeHg (Figs. 3c and 4c), on Day 14 for pine incubation and Day 28 for maple incubation. These data are interesting as it may suggest that methanogens are potentially present and contribute to Hg methylation during litter decomposition, findings similar to a recent sediment incubation study including the stream in this study at Greensboro, North Carolina, USA (Blum et al. 2018). Several studies have also implied that methanogens are capable of methylating Hg in the environment based on field samples (Hamelin et al. 2011), laboratory cultures (Yu et al. 2013), and/or the presence of *hgcA* and *hgcB* gene cluster (Gilmour et al. 2013; Podar et al. 2015).

However, it is very clear from this work that the largest contributor of Hg methylation during litter decomposition would be sulfate-reducing bacteria, a long known microbial group of Hg methylators in aquatic sediments including freshwater reservoir (Gilmour et al. 1992) and wetlands (Jeremiason et al. 2006). These findings suggest that the streamwater used in our study would contain at least two major groups of methylating microbes, sulfate reducing bacteria and methanogens, results corroborating a recent sediment study on Hg methylation (Blum et al. 2018). The leaf litter would provide labile carbon sources for their metabolic activities, and thus stimulating their Hg methylating activities.

It should be noted that in pine incubation experiment we observed decreasing MeHg concentrations and %MeHg from Day 14 to Day 28 (Fig. 3b, c), even though total-Hg concentration increased during the same time span (Fig. 3a). Despite of the possibility of re-partitioning of MeHg to the solid phase (e.g., detrital debris), these data could indicate microbial demethylation of MeHg, potentially driven by anaerobes as observed in freshwater sediments (Oremland et al. 1991) or oxidative demethylation system as demonstrated in previous studies in Hg-contaminated systems (Marvin-DiPasquale et al. 2000), as *mer*-detoxification system would be dominant in more contaminated system. However, if we compare the percent decline of MeHg concentrations between control and BESA treatments for pine litter incubation, we found that the control treatment had ~ 10% higher rate of decline in MeHg (i.e., 64% in control treatment *vs.* 54% in BESA treatment), thus implying a role, despite being small, that methanogens may play in demethylation of MeHg, which warrants further investigation.

In summary, our study demonstrated that sulfate-reducing bacteria are the dominant microbial group for Hg methylation during anoxic litter decomposition, and it is very likely that well-oxygenated streamwater would already have these methylating microbes present as implied by several litter incubation studies (Balogh et al. 2002; Tsui et al. 2008; Ku et al. 2018; *this present study*). Our results also indicate that methanogens (or any microbes inhibited by BESA) have a potential, despite minor, role in mediating Hg methylation during litter decomposition, a finding that is in line with our recent recognition of methanogens for Hg methylation in the environment (Gilmour et al. 2013; Hamelin et al. 2011; Yu et al. 2013; Blum et al. 2018). Decline in MeHg also occurred during litter decomposition in this study, which implies that demethylation is more substantial than methylation during the later phase of litter decomposition, but it is potentially mediated by certain types of microbes, which require future studies to identify. Therefore, the data of our study suggests complicated interactions between methylating microbes and demethylating microbes, and these potentially diverse communities would control the fate of litter Hg during decomposition in saturated environment with limited oxygen in the aquatic environment. Once produced, MeHg can have very high potential to be bioaccumulated and biomagnified in the aquatic environment such as in primary producers (biofilm), macroinvertebrates, and fish (Tsui et al. 2009).

Acknowledgements

This study was supported by National Science Foundation awards (DEB-1354811 and EAR-1711642) and funding from Department of Biology, the University of North Carolina at Greensboro. We appreciated the constructive comments from two anonymous reviewers on the draft of the manuscript.

References

Allan JD, Castillo MM (2007) Stream ecology: structure and function of running waters, Second edn. Springer, Netherlands Google Scholar

Balogh SJ, Huang Y, Offerman HJ, Meyer ML, Johnson DK (2002) Episodes of elevated methylmercury concentrations in prairie streams. Environ Sci Technol 36:1665–1670 Article Google Scholar

Bloom N (1989) Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. Can J Fish Aquat Sci 46:1131–1140 Article Google Scholar

Blum PW, Hershey AE, Tsui MTK, Hammerschmidt CR, Agather AM (2018) Methylmercury and methane production potentials in North Carolina Piedmont stream sediments. Biogeochemistry 137:181–195 Article Google Scholar

Ericksen JA, Gustin MS, Schorran DE, Johnson DW, Lindberg SE, Coleman JS (2003) Accumulation of atmospheric mercury in forest foliage. Atmos Environ 37:1613–1622 Article Google Scholar

Fitzgerald WF, Engstrom DR, Mason RP, Nater EA (1998) The case for atmospheric mercury contamination in remote areas. Environ Sci Technol 32:1–7 Article Google Scholar

Gilmour CC et al (2013) Mercury methylation by novel microorganisms from new environments. Environ Sci Technol 47:11810–11820 Article Google Scholar

Gilmour CC, Henry EA, Mitchell R (1992) Sulfate stimulation of mercury methylation in freshwater sediments. Environ Sci Technol 26:2281–2287 Article Google Scholar

Grigal DF (2002) Inputs and outputs of mercury from terrestrial watersheds: a review. Environ Rev 10:1–39 Article Google Scholar

Hamelin S, Amyot M, Barkay T, Wang Y, Planas D (2011) Methanogens: principal methylators of mercury in lake periphyton. Environ Sci Technol 45:7693–7700 Article Google Scholar

Horvat M, Liang L, Bloom NS (1993) Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low level environmental samples. Part II. Water. Anal Chim Acta 282:153–168 Article Google Scholar

Jeremiason JD et al (2006) Sulfate addition increases methylmercury production in an experimental wetland. Environ Sci Technol 40:3800–3806 Article Google Scholar

Jiskra M et al (2018) A vegetation control on seasonal variations in global atmospheric mercury concentrations. Nat Geosci 11:244–250 Article Google Scholar

Kerin EJ, Gilmour CC, Roden E, Suzuki MT, Coates JD, Mason RP (2006) Mercury methylation by dissimilatory iron-reducing bacteria. Appl Environ Microbiol 72:7919–7921 Article Google Scholar

Ku P, Tsui MTK, Nie X, Chen H, Hoang TC, Blum JD, Dahlgren RA, Chow AT (2018) Origin, reactivity, and bioavailability of mercury in wildfire ash. Environ Sci Technol 52:14149–14157 Article Google Scholar

Liang L, Horvat M, Bloom NS (1994) An improved speciation method for mercury by GC/CVAFS 165 after aqueous phase ethylation and room temperature precollection. Talanta 41:371–379 Article Google Scholar

Marvin-DiPasquale M, Agee J, McGowan C, Oremland RS, Thomas M, Krabbenhoft D, Gilmour CC (2000) Methyl-mercury degradation pathways: a comparison among three mercury-impacted ecosystems. Environ Sci Technol 34:4908–4916 Article Google Scholar

Obrist D et al (2011) Mercury distribution across 14 U.S. Forests. Part I: spatial patterns of concentrations in biomass, litter, and soils. Environ Sci Technol 45:3974–3981 Article Google Scholar

Oremland RS, Culbertson CW, Winfrey MR (1991) Methylmercury decomposition in sediments and bacterial cultures: involvement of methanogens and sulfate reducers in oxidative demethylation. Appl Environ Microbiol 57:130–137 Google Scholar

Parker JL, Bloom NS (2005) Preservation and storage techniques for low-level aqueous mercury speciation. Sci Total Environ 337:253–263 Article Google Scholar

Parks JM et al (2013) The genetic basis for bacterial mercury methylation. Science 339:1332–1335 Article Google Scholar

Podar M et al (2015) Global prevalence and distribution of genes and microorganisms involved in mercury methylation. Sci Adv 1:e1500675 Article Google Scholar

Pokharel AK, Obrist D (2011) Fate of mercury in tree litter during decomposition. Biogeosciences 8:2507–2521 Article Google Scholar

Scheuhammer AM, Meyer MW, Sandheinrich MB, Murray MW (2007) Effects of environmental methylmercury on the health of wild birds, mammals, and fish. Ambio 36:12–18 Article Google Scholar

Seller P, Kelly CA, Rudd JWM, MacHutchon AR (1996) Photodegradation of methylmercury in lakes. Nature 380:694–697 Article Google Scholar

Tsui MTK, Finlay JC, Nater EA (2008) Effects of stream water chemistry and tree species on release and methylation of mercury during litter decomposition. Environ Sci Technol 42:8692–8697 Article Google Scholar

Tsui MTK, Finlay JC, Nater EA (2009) Mercury bioaccumulation in a stream network. Environ Sci Technol 43:7016–7022 Article Google Scholar

Tsui MTK, Wang WX (2004) Uptake and elimination routes of inorganic mercury and methylmercury in *Daphnia magna*. Environ Sci Technol 38:808–816 Article Google Scholar

UNEP (2013) Global Mercury Assessment 2013: sources, emissions, releases and environmental transport. United Nations Environment Programme, Chemicals Branch, Geneva Google Scholar

USEPA (2002) Method 1631, revision E: mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry. office of water. U.S. Environmental Protection Agency, Washington, DC Google Scholar

Woerndle GE, Tsui MTK, Sebestyen SD, Blum JD, Nie X, Kolka RK (2018) New insights on ecosystem mercury cycling revealed by stable isotopes of mercury in water flowing from a headwater peatland catchment. Environ Sci Technol 42:1854–1861 Article Google Scholar

Yu RQ, Reinfelder JR, Hines ME, Barkay T (2013) Mercury methylation by the methanogen *Methanospirillum hungatei*. Appl Environ Microbiol 79:6325–6330 Article Google Scholar