Controls of Methylmercury Bioaccumulation in Forest Floor Food Webs

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Abstract:

Compared to the extensive research on aquatic ecosystems, very little is known about the sources and trophic transfer of methylmercury (MeHg) in terrestrial ecosystems. In this study, we examine energy flow and trophic structure using stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios, respectively, and MeHg levels in basal resources and terrestrial invertebrates from four temperate forest ecosystems. We show that MeHg levels in biota increased significantly ($p < 0.01$) with $\delta^{13}C$ and $\delta^{15}N$ at all sites, implying the importance of both microbially processed diets (with increased $\delta^{13}C$) and trophic level (with increased $\delta^{15}N$) at which organisms feed, on MeHg levels in forest floor biota. The trophic magnification slopes of MeHg (defined as the slope of $\log_{10}$MeHg vs $\delta^{15}N$) for these forest floor food webs (0.20–0.28) were not significantly different ($p > 0.05$) from those observed for diverse temperate freshwater systems (0.24 ± 0.07; $n = 78$), demonstrating for the first time the nearly equivalent efficiencies with which MeHg moves up the food chain in these contrasting ecosystem types. Our results suggest that in situ production of MeHg within the forest floor and efficient biomagnification both elevate MeHg levels in carnivorous invertebrates in temperate forests, which can contribute to significant bioaccumulation of this neurotoxin in terrestrial apex predators.

Keywords: forest food webs | methylmercury | forest ecosystems | bioaccumulation

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Controls of Methylmercury Bioaccumulation in Forest Floor Food Webs

Martin Tsz-Ki Tsui,*[,†,▽] Songnian Liu,[†,▽] Rebecka L. Brasso,[‡,▽] Joel D. Blum,[§] Sae Yun Kwon,[∥] Yener Ulus,[†] Yabing H. Nollet,[⊥] Steven J. Balogh,[⊥] Sue L. Eggert,[#] and Jacques C. Finlay[⊗]

[†]Department of Biology, University of North Carolina at Greensboro, Greensboro, North Carolina 27402, United States

[‡]Department of Zoology, Weber State University, Ogden, Utah 84408, United States

[§]Department of Earth and Environmental Sciences, University of Michigan, Ann Arbor, Michigan 48109, United States

[∥]Division of Environmental Science and Engineering, Pohang University of Science and Technology, Pohang 37673, South Korea

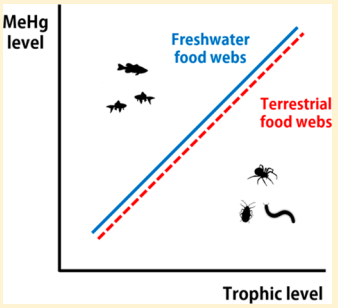
[⊥]Metropolitan Council Environmental Services, St. Paul, Minnesota 55106, United States

[#]U.S.D.A. Forest Service, Northern Research Station, Grand Rapids, Minnesota 55744, United States

[⊗]Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, Minnesota 55108, United States

Supporting Information

ABSTRACT: Compared to the extensive research on aquatic ecosystems, very little is known about the sources and trophic transfer of methylmercury (MeHg) in terrestrial ecosystems. In this study, we examine energy flow and trophic structure using stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios, respectively, and MeHg levels in basal resources and terrestrial invertebrates from four temperate forest ecosystems. We show that MeHg levels in biota increased significantly ($p < 0.01$) with $\delta^{13}C$ and $\delta^{15}N$ at all sites, implying the importance of both microbially processed diets (with increased $\delta^{13}C$) and trophic level (with increased $\delta^{15}N$) at which organisms feed, on MeHg levels in forest floor biota. The trophic magnification slopes of MeHg (defined as the slope of $\log_{10}$MeHg vs $\delta^{15}N$) for these forest floor food webs (0.20−0.28) were not significantly different ($p > 0.05$) from those observed for diverse temperate freshwater systems (0.24 ± 0.07; $n = 78$), demonstrating for the first time the nearly equivalent efficiencies with which MeHg moves up the food chain in these contrasting ecosystem types. Our results suggest that in situ production of MeHg within the forest floor and efficient biomagnification both elevate MeHg levels in carnivorous invertebrates in temperate forests, which can contribute to significant bioaccumulation of this neurotoxin in terrestrial apex predators.



INTRODUCTION

The concentration of mercury (Hg) in environmental media has been greatly increased by human activities, and long-range atmospheric transport and deposition leads to contamination of virtually all ecosystem types.[1,2] Anaerobic microbial methylation of deposited inorganic Hg produces highly toxic methylmercury (MeHg),[3] which can lead to extensive bioaccumulation and biomagnification in natural food webs, often resulting in elevated levels of MeHg in apex predators (e.g., wildlife and humans) and causing worldwide health concerns related to this global pollutant.[4] Aquatic ecosystems, often tied to elevated Hg methylation in reduced sediments[5−7] and high MeHg levels in fish,[8−11] have been studied extensively for MeHg production, degradation, and food web bioaccumulation, and include freshwater ponds, lakes, reservoirs, wetlands, streams,[8−13] and riparian zones with strong aquatic connections.[14−18]

Overall, basal resources play an important role in transferring and incorporating MeHg into the base of animal food webs, such as herbivores, which ultimately leads to trophic transfer and biomagnification of MeHg in animals of higher trophic levels.[19,20] In general, basal resources in various aquatic ecosystems have been found to have much higher MeHg levels (e.g., seston in Great Lakes with a mean of 33 ng/g dry wt.;[9] periphyton in boreal lakes with a mean of 11.0 ng/g dry wt.;[21] filamentous algae in a California river with a mean of 19.2 ng/g dry wt.[15]) than their terrestrial counterparts (e.g., foliage and fresh litter in different North American forests with a range of 0.01−0.45 ng/g dry wt.).[22−24] Such discrepancies in MeHg levels between aquatic and terrestrial basal resources may be attributed to the very efficient bioconcentration of dissolved MeHg from ambient water by aquatic bacteria or algal cells (e.g., to the order of 10^5−10^6),[25] in addition to the elevated production of MeHg in saturated, anoxic surface sediment, in comparison to dry, oxic forest floors.[5−7]

However, Tsui et al.[15] analyzed MeHg in a river and forest food web in a northern California watershed without point

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source pollution, and found that MeHg levels in the forest invertebrates of similar trophic positions were comparable to their local aquatic counterparts (e.g., carnivorous invertebrates had similar ranges of MeHg levels among rivers and forests), and similar observations were made in another comparison of food webs between a forest and a lake in northern Michigan[26] (see Supporting Information (SI) Table S1). A few other studies also documented concentrations of total Hg and MeHg in terrestrial invertebrates similar to the above two studies.[27−30] Thus, the processes governing in situ production and trophic transfer of MeHg in forests, which are largely unknown at present, may play crucial roles in driving bioaccumulation and efficient biomagnification of MeHg among forest invertebrates, given the very low MeHg levels in terrestrial basal resources (e.g., fresh litter) compared to their aquatic counterparts (e.g., seston).

In this study, we compared concentrations and trophic transfers of MeHg in four temperate forest reserves in the United States (SI Table S2), and elucidated their potential dietary and MeHg sources to the base of forest floor food webs that are independent of aquatic diets (i.e., solely terrestrially based food webs).

MATERIALS AND METHODS

Study Sites and Sample Collection. Our study sites were in four temperate forest reserves in the continental United States, including: (i) Angelo Coast Range Reserve (Angelo Reserve) of University of California Natural Reserve System in Branscomb, CA; (ii) University of Michigan Biological Station (UMBS) in Pellston, MI; (iii) Coweeta LTER (Coweeta) in Otto, NC; and (iv) Hubbard Brook Experimental Forest LTER (Hubbard Brook) in North Woodstock, NH. The forests are all in the temperate zone but vary in annual temperature and rainfall (SI Table S2). At Coweeta and UMBS, we collected samples among coniferous and deciduous forests while at Angelo Reserve and Hubbard Brook we sampled multiple sites of mixed tree stands.

At each forest, we established 2−5 sampling locations away from nearby streams (>27 m; with the majority of sites being >100 m). We determined that these locations received little, if any, aquatic inputs to consumer diets as the abundance of emerged aquatic insects decreases exponentially with distance from stream edge.[31] We sampled these sites from 2011 to 2015 (see years of sampling in SI Table S2). Within each location, we collected fresh litter wearing clean nonpowder vinyl gloves, and we spent 1−3 days in the field sampling diverse forest invertebrates mainly using three strategies: (i) pitfall traps (during the day and night), (ii) direct capture using tweezers and/or dip nets (during the day), and (iii) light traps (at night) with dry ice to anesthetize biota. For each sampling, we composited samples of the same taxa into a single sample per location during each field trip. Since the collected invertebrates covered a wide range of expected dietary sources and trophic positions, we classified commonly encountered taxa into nine major groups for presentation of the results: moths (Lepidoptera; n = 23), slugs (Gastropoda; n = 8), millipedes (Polydesmida/Spirobolida; n = 15), grasshoppers/crickets (Orthoptera; n = 13), harvestman (Opiliones; n = 7), spiders (Araneae; n = 17), beetles (Coleoptera; n = 28), centipedes (Lithobiomorpha; n = 12), and scorpions (Scorpiones; Angelo Reserve only; n = 9). Samples collected in Angelo Reserve (CA) in 2011 and 2012 were previously analyzed for total-Hg (THg), MeHg and stable Hg isotope compositions,[15,32] while all other data have not been reported. A summary showing invertebrate collection (total = 141 composited samples; including 9 samples not classified above) and number of samples in different sites and groups is presented in SI Table S3.

Sample Processing and Mercury Analyses. Field samples were transported to the laboratory, and frozen immediately at −20 °C. All samples were later freeze-dried in the laboratory and dried samples were homogenized either by an agate mortar and pestle or a mixer mill (SPEX SamplePrep) cleaned between samples by multiple steps using Barnstead Nanopure water and isopropyl alcohol. All dried and homogenized samples were then stored in acid-cleaned glass vials with PTFE-lined septa (Thermo Scientific) or Hg-free polypropylene centrifuge vials (Falcon or Corning).

Individual samples were extracted for MeHg using 4.6 M nitric acid at 60 °C for 12 h,[33] and the remaining acid digest was then completely oxidized by $KMnO_4$ and $K_2S_2O_8$ for subsequent THg analysis.[34] Sample MeHg or THg was quantified by cold vapor atomic fluorescence spectroscopy (Brooks Rand; for both MeHg and THg) or cold vapor atomic absorption spectroscopy (Nippon Instruments Corporation; for THg analysis on samples collected in 2011 and 2012). All acid digestions for THg and MeHg analyses were accompanied by standard reference materials (SRMs; NIST-1515 Apple Leaves, NRCC TORT-2 lobster hepatopancreas, NRCC DORM-3 and DORM-4 fish protein) and reagent blanks. All THg and MeHg concentrations were reported on a dry weight basis. Detailed analytical procedures and QC/QA data can be found in the SI Methods.

Stable Isotope Analyses and Trophic Level Estimates. All samples were prepared for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analyses by gas isotope-ratio mass spectrometry at Colorado Plateau Stable Isotope Laboratory at Northern Arizona University (Flagstaff, AZ), to estimate energy sources and trophic positions, respectively.[35,36] Trophic levels (TLs) of forest invertebrates in this study were estimated following Post[35] and Jardine et al.:[36]

$$TL_{invertebrate} = (\delta^{15}N_{invertebrate} - \delta^{15}N_{freshlitter})/3.4 + 1$$

where $\delta^{15}N_{fresh\ litter}$ is the mean value from each forest site while 3.4 ‰ is a commonly adopted trophic enrichment factor for stable N isotopes in food web analyses.[36] The repeated analyses of SRM NIST-1547 peach leaves (n = 78) along with our samples produced 2 SE of 0.018 ‰ for $\delta^{13}C$ and 2 SE of 0.017 ‰ for $\delta^{15}N$.

Statistical Analyses. Sample MeHg concentrations were log-transformed, and $\log_{10}$MeHg was plotted against $\delta^{15}N$ values in each forest site to calculate the slope, which is defined as the trophic magnification slope of MeHg (TMS_{MeHg}).[37] All linear and multiple regression analyses were performed using SigmaPlot 12.5 (Systat), in which a normality test (Shapiro-Wilk) was passed. We compared regression slopes and tested for significant differences using ANCOVA on Prism 5.03 (GraphPad). The significance level for all statistical analyses was α = 0.05.

RESULTS AND DISCUSSION

Mercury Concentrations in Litter and Invertebrates. Consistent with published data from other North American forests,[23,24,38,39] THg and MeHg concentrations of all fresh litter samples were low and had relatively narrow ranges, with

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THg ranging between 18 and 61 ng/g dry wt. (47.1 ± 19.4 ng/g; mean ± S.D.; n = 17) and MeHg ranging between 0.12 and 0.24 ng/g dry wt. (0.17 ± 0.03 ng/g) in our study forests.

In contrast to fresh litter, we observed a much wider range of THg (3.00−1509 ng/g), MeHg (0.20−291 ng/g), and percentage of THg as MeHg (i.e., %MeHg; 0.70−100%) among invertebrate consumers (n = 141). Clear group-to-group differences among forest invertebrates were evident for THg, MeHg, and %MeHg (Figure 1). In particular, we found

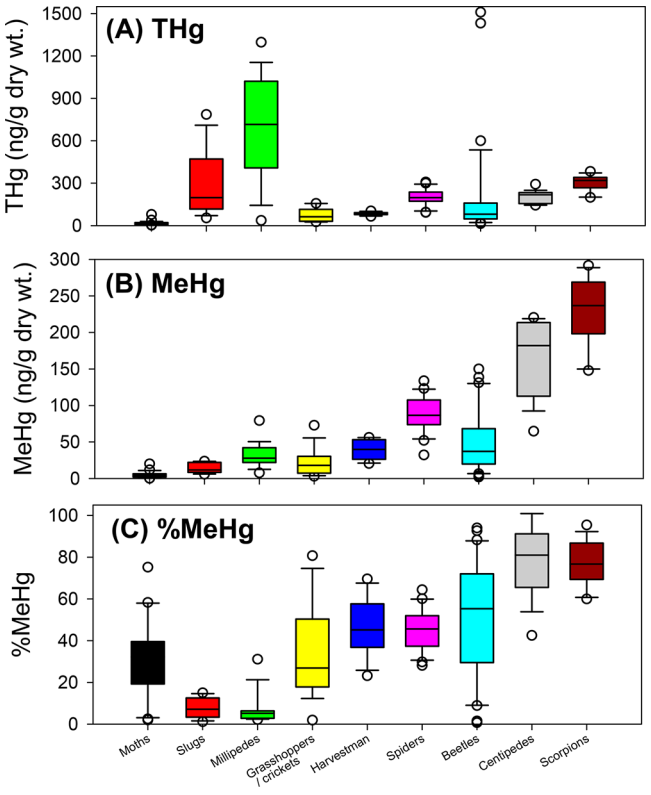

Figure 1. Boxplots of (A) total mercury (THg), (B) methylmercury (MeHg), and (C) percentage of THg as MeHg (%MeHg) among nine major groups of invertebrates (on dry weight basis) collected over the four study forests: Angelo Coast Range Reserve in California, University of Michigan Biological Station in Michigan, Coweeta LTER in North Carolina, and Hubbard Brook Experimental Forest in New Hampshire. Number of samples used in the boxplots: n = 23 for moths, n = 8 for slugs, n = 15 for millipedes, n = 13 for grasshoppers/crickets, n = 7 for harvestman, n = 17 for spiders, n = 28 for beetles, n = 12 for centipedes, and n = 9 for scorpions.

that groups of slugs and millipedes (and three samples of beetles, belonging to the family of Scarabaeidae in two study sites) showed elevated levels of THg in their tissues (Figure 1A), up to >1000 ng/g dry wt., which is considered very high for invertebrates of low TLs in noncontaminated environments. For comparison, THg at such high levels (e.g., >1000 ng/g dry wt.) in aquatic invertebrates are found almost exclusively in highly contaminated aquatic environment such as streams impacted by Hg mining.[40] The reasons underlying the very high inorganic Hg accumulation in these forest invertebrates are unknown at present but we speculate on the presence of insoluble granules[41] in the tissues of these forest invertebrates that may sequester inorganic Hg (and potentially other metals) from their diets; this mechanism has been used to explain the extremely high zinc accumulation in marine barnacles (e.g., with reported values up to 1.6% of body weight).[42]

MeHg levels increased with TL from moths to centipedes and scorpions (Figure 1B). Strikingly, when we examined the overall relationship between THg and MeHg among these invertebrates, except the groups with very high THg and low MeHg (slugs, millipedes, and three samples of beetles belonging to the family of Scarabaeidae), we found a significant and positive relationship over different sites (Figure 2),

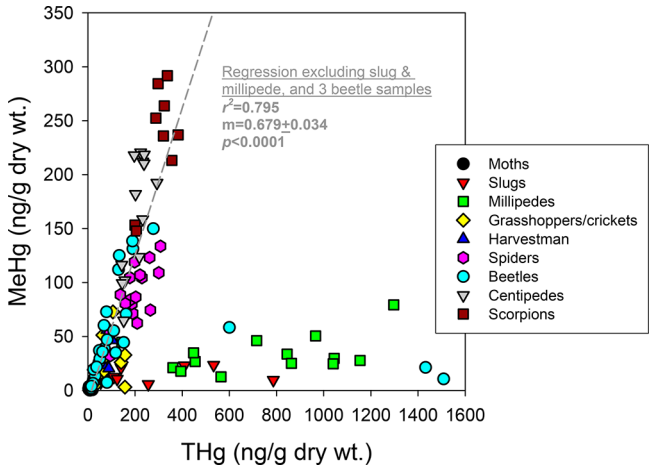

Figure 2. Relationships between total mercury (THg) and methylmercury (MeHg) concentrations (on dry weight basis) in invertebrate samples as separated by groups. Linear regression was performed by excluding the groups of slug and millipede, and three samples of beetle.

indicating that MeHg is driving overall THg bioaccumulation for the majority of invertebrate consumers in forest floors. Similar to studies of aquatic macroinvertebrates,[11,15,43] % MeHg spans a wide range among forest invertebrates but % MeHg in general becomes elevated at higher TLs (Figure 1C).

Characterization of Food Web Structures Using Stable Isotopes. We found that fresh litter (O_i layer) had little variation in $\delta^{13}C$ signatures within and among sites (−29.2 ± 0.83‰; mean ± S.D.; n = 17; SI Tables S4−S7), which was similar to C_3 plants around the world (−25 to −29‰).[44] Compared to fresh litter, invertebrate $\delta^{13}C$ values varied more widely (from −34.5 to −21.4‰) with the distribution of values skewed toward much higher values relative to those of litter (−29.2 ± 0.83‰; n = 17), and with an overall mean of −26.0‰ among all invertebrate samples (n = 141; Figure 3). The exception to this was the group of moths in which we found a mean (±S.D.) of −29.3 ± 2.04‰ (n = 22) (SI Figure S1) that matches well to those of fresh litter, implying that there may not be a direct trophic connection between moths and many of the other forest invertebrates that we investigated.

Because the average trophic enrichment of $\delta^{13}C$ is expected to be ∼0.5‰ per trophic level (TL) in natural food webs,[45] these data suggested that fresh litter itself is not the major, direct diet for many of these forest invertebrates (excluding moths). Similar results were found in a previous study on forest floor food webs in Switzerland.[46] In fact, incorporation of decomposed litter into soil organic matter could elevate the $\delta^{13}C$ value of organic carbon by approximately 1.6−2.3‰,[47] and the assimilation of organic carbon by saprotrophic fungi can even further increase $\delta^{13}C$ values of residual organic

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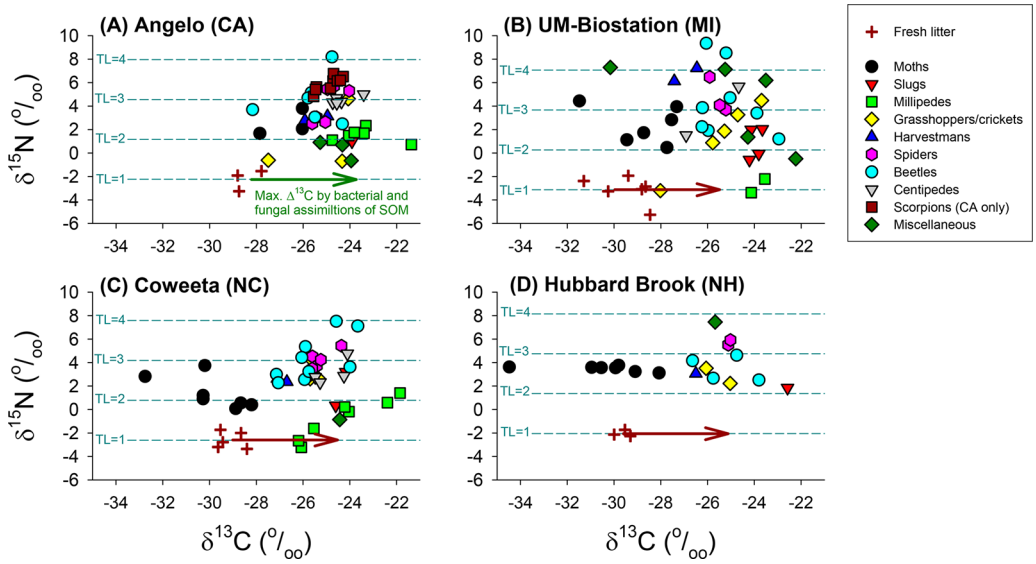

Figure 3. Relationship between stable carbon isotope compositions ($\delta^{13}C$; as a proxy of dietary sources) and nitrogen isotope compositions ($\delta^{15}N$; as a proxy of trophic positions) of biota samples in each study forest: (A) Angelo Coast Range Reserve in California, (B) University of Michigan Biological Station in Michigan, (C) Coweeta LTER in North Carolina, and (D) Hubbard Brook Experimental Forest in New Hampshire. The arrows indicate the maximum enrichment of $\delta^{13}C$ for litter decomposition and incorporation into soil organic matter (SOM)[47] and for the utilization of SOM by saprotrophic fungi[48] from fresh litter. The dashed lines indicate the calculated trophic level (TL) at each forest.

carbon by approximately 3.0‰ (see arrows in Figure 3 to denote the maximum enrichment of $\delta^{13}C$).[48] Some direct evidence connecting $\delta^{13}C$ in microbial diets of forest invertebrates comes from fungal samples at our California forest site (Angelo Reserve). Specifically, $\delta^{13}C$ values of soil hyphae and fruiting bodies were found to be -24.69 ± 1.40‰ and -24.82 ± 0.09‰ ($n = 2$ each), respectively, which matches well to those of invertebrate consumers in this and other study sites (Figure 3). Thus, microbial (bacterial and/or fungal) decomposition of organic matter can lead to increases of $\delta^{13}C$, and consumption of these dietary sources with higher $\delta^{13}C$ values likely account for the large enrichment of invertebrate $\delta^{13}C$ relative to that of C3 litter in these forests.

We used the site-specific mean $\delta^{15}N$ values of basal resources (−3.14 to −2.05‰) to estimate the TL of invertebrate consumers by adopting a mean trophic enrichment of 3.4‰ per TL (Figure 3).[36] Overall, our sampled invertebrate food webs have TL spanning from 0.9 to 4.5 ($n = 143$) among the four study forests (shown by horizontal dashed lines in Figure 3).

Influence of Food Web Structures on Methylmercury Bioaccumulation. Since MeHg is the main Hg species that biomagnifies,[37] here we focus on the trophic transfer of MeHg (rather than THg) in forest food webs. As invertebrates at higher TLs tended to have $\delta^{13}C$ shifted toward higher values among sites (Figure 3), it is not surprising to observe highly significant and positive relationships ($p < 0.01$) between $\delta^{13}C$ and MeHg in all sites (Figure 4A−D). Thus, we suggest that invertebrates with higher $\delta^{13}C$ values than fresh litter might consume diets/prey based on carbon derived from more decomposed organic matter, which can be associated with higher MeHg content[22,23] and higher $\delta^{13}C$ values (and our $\delta^{13}C$ measurements of fungal biomasses at Angelo Reserve).[47,48]

Consistent with biomagnification of MeHg widely observed in aquatic food webs, all forest invertebrate consumers showed highly significant increases of MeHg ($p < 0.0001$) along the food chain in each site with increasing $\delta^{15}N$ (Figure 4E−H). We calculated TMS_{MeHg} (defined as slope of $\log_{10}$MeHg vs $\delta^{15}N$; including fresh litter and invertebrate samples) among the four forest sites and found only a small range from 0.202 to 0.281, with the lowest value at the UMBS (MI) and the highest in mixed forests at Hubbard Brook (NH). We did not detect significant differences in TMS_{MeHg} among the four forest floor food webs ($p > 0.05$). Importantly, the TMS_{MeHg} values estimated from four temperate forests were within the range (or within 1 SD of the mean value) reported by a recent data synthesis of freshwater studies in temperate regions in which the authors found a mean TMS_{MeHg} of 0.24 ± 0.07 ($n = 78$; 1 SD).[37] We also did not find significant differences in TMS_{MeHg} between our forest floor food webs and the diverse aquatic food webs compiled by Lavoie et al.[37] ($p > 0.05$). Thus, these results demonstrate for the first time that MeHg has a similar efficiency for biomagnification along freshwater vs forest floor food webs under similar climate conditions (i.e., temperate).

We also performed multiple regression analyses to assess the relative contributions of $\delta^{13}C$ (after correction for trophic enrichment)[45] and $\delta^{15}N$ to MeHg levels in invertebrates. We showed that both isotope signatures were significant predictors ($p < 0.001$) of MeHg levels in forest biota, but $\delta^{15}N$ was still a stronger predictor (higher t statistic values) than $\delta^{13}C$ (SI Table S8). This result underscores the importance of TL and the internal microbial processing of dietary sources (and the associated in situ MeHg production) on the ultimate MeHg bioaccumulation in forest invertebrate food webs.

Explanations for "Higher-than-Expected" Methylmercury Levels in Forest Invertebrates. As discussed above, the reported MeHg tissue concentrations are similar between terrestrial carnivorous invertebrates (from limited studies) and freshwater carnivorous invertebrates in the same systems unaffected by point sources or wetlands (SI Table S1). The question remains: why and how MeHg is taken up at such levels in terrestrial invertebrates given that the basal resources (i.e., fresh litter) have very low MeHg levels (i.e., < 0.2 ng/g)

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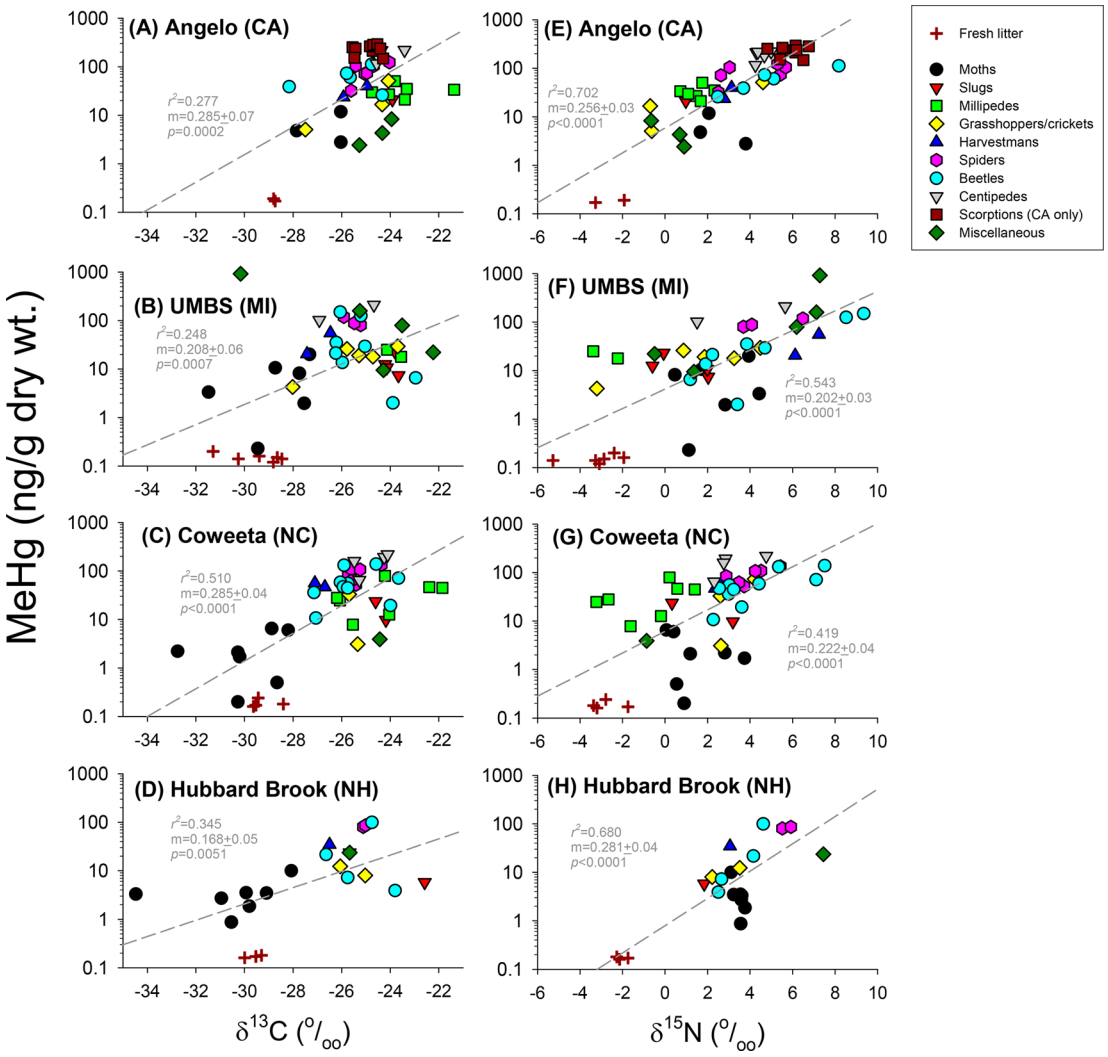

Figure 4. Relationships between stable carbon isotope compositions ($\delta^{13}C$; uncorrected for trophic enrichment; as a proxy of dietary sources) and methylmercury (MeHg) tissue concentrations (on dry weight basis) of biota samples in each study forest: (A) Angelo Coast Range Reserve in California, (B) University of Michigan Biological Station in Michigan, (C) Coweeta LTER in North Carolina, and (D) Hubbard Brook Experimental Forest in New Hampshire. Relationships between stable nitrogen isotope compositions ($\delta^{15}N$; as a proxy of trophic positions) and MeHg tissue concentrations of biota samples in each study forests: (E) Angelo Coast Range Reserve in California, (F) University of Michigan Biological Station in Michigan, (G) Coweeta LTER in North Carolina, and (H) Hubbard Brook Experimental Forest in New Hampshire.

compared to aquatic basal resources? Given the strikingly similar TMS_{MeHg} between temperate forest and freshwater ecosystems, we argue that the actual diets for the base of forest invertebrate food webs must have considerably higher MeHg levels than those found in fresh litter (i.e., ≫0.1−0.2 ng/g) for the invertebrates to achieve MeHg levels comparable to aquatic consumers at the same trophic levels. Here, we provide two different (but mutually inclusive) reasons that alone or in combination may explain the "higher-than-expected" MeHg levels in forest invertebrates.

First, MeHg can be generated during organic matter decomposition processes. While we lack direct data to address this mechanism, some support is found from observational and experimental studies that have found that partially decomposed organic matter contained higher MeHg levels than fresh organic matter in the forest floor. For example, Obrist[23] found that decomposed litter layers (O_e and O_a horizons) had elevated (10 times higher), yet variable, MeHg levels compared to fresh litter (O_i horizon). Hall and St. Louis[22] similarly showed that tree litter and wood block placed in unsaturated forest soils in a decomposition experiment developed higher (4−30 times higher) MeHg levels over time (see values in SI Table S1).

Further support for a role of litter decomposition processes on terrestrial MeHg production comes from our $\delta^{13}C$ data (Figure 3), which strongly suggests influences of microbial degradation of litter (by bacteria and/or fungi) within the forest floor. Reliance on highly decomposed organic matter and its associated fungal and microbial biomass would be expected to increase $\delta^{13}C$ of the organic matter diets of the invertebrates.[47,48] For example, mycorrhizae are known to be very effective at decomposing organic carbon and remineralizing nutrients in forested ecosystems.[49] Mercury methylation has also been demonstrated in some fungal groups under controlled conditions,[50] likely through the association of ubiquitous anaerobic microbes possessing *hgcA/hgcB* gene clusters.[51] Thus, we hypothesize that decomposition of the soil organic matter pool (especially with the participation of tree roots or their exudates)[46] introduces additional MeHg to the diets of invertebrates (i.e., decomposed organic matter) that

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can be routed into terrestrial food chains, as shown in the elevated and highly variable MeHg levels in decomposed plant materials (SI Table S1). Additional research is needed to identify the spatial distribution of these biogeochemical pools of MeHg in forest floors; we suggest that this mechanism could provide higher MeHg dietary inputs to forest food webs.

A second possible explanation for higher than expected MeHg in terrestrial invertebrates is the high TLs of some of the terrestrial invertebrate consumers found in this study (up to TL = 4.0 or higher) (Figure 3), which may be explained by the prevalence of detrital (or "brown") food webs in forest floors.[52] The presence of microbial components would lengthen (or inflate) the trophic food chain as detritus would be initially transformed into bacterial and fungal biomass,[52] which can be consumed by very small predators (e.g., protists) resulting in elevated MeHg levels through biomagnification.[37] Since the two processes discussed above can operate at the same time, we suggest a combination of them can lead to higher MeHg levels in terrestrial invertebrate consumers, but their relative importance needs to be better resolved by additional research.

In summary, the work presented here shows that MeHg biomagnifications (i.e., the increase per TL) are similar among forest floor and freshwater food webs in temperate regions. We do not know at present if these findings can be extrapolated to other terrestrial ecosystems such as grasslands and tropical rainforests. However, our work shows that apex animal consumers such as birds and mammals in temperate forests can still obtain large amounts of MeHg from terrestrial invertebrate prey at high TLs. It should be noted that terrestrial arthropods have a very large global biomass (i.e., 0.2 Gt C),[53] thus they can represent a large, concentrated pool of MeHg (i.e., up to sub-ppm level in tissues) that is readily available to move up the food chain in forest ecosystems.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b06053.

Detailed analytical methods for total mercury and methylmercury analyses, QC/QA data, compilation of methylmercury levels in aquatic and terrestrial carnivorous invertebrates from two previous studies, basic characteristics of the forest study sites, summary of sample collection in this study, raw data for individual samples, results of multiple linear regressions between stable isotopes and methylmercury levels in forest floor food webs, and a comparison of stable carbon isotope ratios among moths vs all invertebrates in this study (PDF).

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 336-256-0087; e-mail: tmtsui@uncg.edu.

ORCID

Martin Tsz-Ki Tsui: 0000-0003-2002-1530

Joel D. Blum: 0000-0001-5389-8633

Steven J. Balogh: 0000-0003-2606-0450

Author Contributions

∇Equal contribution/cofirst authors.

Notes

The authors declare no competing financial interest.

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