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Biomagnification and trophic transfer of total mercury and methylmercury in a sub-tropical montane forest food web, southwest China

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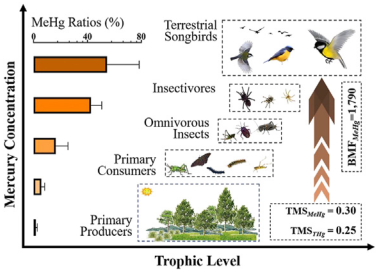
HIGHLIGHTS

- THg and MeHg increased with trophic positions in forest food webs.
- THg exceeded adverse effect thresholds in insectivorous songbirds.
- MeHg significantly biomagnified in subtropical forest food webs.

GRAPHICAL ABSTRACT



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ABSTRACT

Little is known about the bioaccumulation and trophic transfer of total mercury (THg) and methylmercury (MeHg) via food webs in terrestrial ecosystems, especially in subtropical forest ecosystems. In the present study, THg and MeHg were determined as well as the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope composition in samples of soils, plants, invertebrates, and songbird feathers to construct food webs in a remote subtropical montane forest in Mt. Ailao, southwest China and assess the bioaccumulation, biomagnification, and trophic transfer of Hg. Results showed that the trophic levels (TLs) of all consumers ranged from 0.8 to 3.3 and followed the order of songbirds > spiders > omnivorous insects > herbivorous insects > plants, and THg and MeHg exhibited a clear biomagnification up the food chain from plants-herbivorous/omnivorous insects-spiders-songbirds. The lowest MeHg concentration was observed in pine needles ranged from 0.104 to 0.949 ng g^{-1} with only a 1.6% ratio of MeHg to THg (MeHg%), while the highest MeHg concentrations ranged from 425 to 5272 ng g^{-1} in songbirds with MeHg% values of up to 96%. High values of trophic magnification slope (TMS) for THg (0.22) and MeHg (0.38) were observed in plant-invertebrate-songbird food chain, verifying the significant bioaccumulation of Hg, particularly MeHg, in the remote subtropical forest ecosystem. This study confirmed

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the production and efficient biomagnification of MeHg in remote subtropical montane forest and the significant bioaccumulation of MeHg in terrestrial top predators.

1. Introduction

Mercury (Hg) is one of the most hazardous and widespread contaminants in natural ecosystems, arising from its long-range atmospheric transport and deposition (Tsui et al., 2012; Driscoll et al., 2013). Once it enters the environment, inorganic Hg (IHg) can be microbially converted into the highly toxic organic form, methylmercury (MeHg). MeHg readily bioaccumulates and is biomagnified in food webs (Lavoie et al., 2013), leading to high concentrations in apex predators such as fish, seabirds, and mammals (Abeysinghe et al., 2017; Eagles-Smith et al., 2018; Chételat et al., 2020).

Songbirds (*Passeriformes*) are considered as a potential sentinel to monitor Hg pollution owing to their widely distributed habitats, abundant populations, and sensitivity to Hg exposure (Keller et al., 2014; Jackson et al., 2015; Sauer et al., 2020). They can increase the length of the food chain/web by preying on predatory invertebrates like spiders, aggravating the biomagnification of Hg (Cristol et al., 2008). Elevated concentrations of Hg were recently reported in feathers of songbirds; for example, Abeysinghe et al. (2017) found concentrations as high as 123.3 ± 34.2 mg kg^{-1} THg in feathers of the spot-breasted scimitar babbler dwelling in a Hg-contaminated site, which was considerably higher than the highest value of 91.6 mg/kg documented in wandering albatross feathers (Renedo et al., 2017). A further study indicated that most Hg (95.9%) in terrestrial songbird feathers exists as cysteine-bound MeHg (Xu et al., 2019). Thus, a better understanding of Hg burdens in songbirds is of great significance to elucidate biogeochemical processes of Hg, and particularly MeHg, in terrestrial ecosystems, although certain part of songbird Hg may be derived from aquatic preys (Tsui et al., 2018).

Biomagnification factors (BMFs) and the trophic magnification slope (TMS) are the most relevant bioaccumulation metrics to quantify the potential Hg biomagnification in food webs (Conder et al., 2012; Borgå et al., 2012). To date, investigations on the bioaccumulation and trophic transfer of Hg in food webs have usually focused on aquatic ecosystems (Jaeger et al., 2009; Lavoie et al., 2010, 2013; Hall et al., 2020); however, several studies have been conducted on terrestrial ecosystems and have indicated the efficient biomagnification and bioaccumulation of MeHg via food chains (Abeysinghe et al., 2017; Yung et al., 2019; Tsui et al., 2019; Rodenhouse et al., 2019; Luo et al., 2020). Tsui et al. (2019) found that, at low Hg levels in temperate forests, there could be significant bioaccumulation and biomagnification of MeHg in invertebrates. According to the clear food chain of pine needle-caterpillar-tit nestling, Luo et al. (2020) found that the TMS of MeHg (0.36–0.38) in a sub-tropical forest was higher than that observed in temperate forests (0.20–0.28; Tsui et al., 2019) and freshwater ecosystems (0.24; Lavoie et al., 2013).

It is widely accepted that forest ecosystems play a crucial role in the global cycling of Hg (Obrist, 2007; Keenan et al., 2015; Zhou et al., 2016; Lu et al., 2020). Rodenhouse et al. (2019) considered that humid deciduous forests are favorable for Hg methylation and bioaccumulation. To date, several studies have identified elevated Hg levels in evergreen broadleaf forests in China (Shi et al., 2013; Luo et al., 2014), but there have been few studies on the terrestrial biogeochemical processes of Hg bioaccumulation and biomagnification in food chains/webs.

Mount Ailao (Mt. Ailao) is a typical remote subtropical montane forest site in southwest China, which has few industrial inputs and contains pollution-free regions. However, as the terrestrial biogeochemical processes of MeHg bioaccumulation and biomagnification via food chains/webs have little been studied, the investigation on Hg cycling in subtropical forest ecosystems in Mt. Ailao may contribute to a better understanding of Hg bioaccumulation and biomagnification in the terrestrial food webs. In the present study, songbird feathers, invertebrates, forest soils, and plants from the Mt. Ailao ecosystem were collected to study the transfer and biomagnification of Hg in forest food chains. The objectives were to: (1) characterize the montane forest food web structure using stable isotopes of carbon and nitrogen; (2) ascertain variations in THg and MeHg in organisms occupying different trophic positions; and, (3) quantify the TMS for THg and MeHg in the montane forest food webs.

2. Materials and methods

2.1. Study area

Study sites are located in the Mt. Ailao Field Station (24°32′N, 101°01′E), which is subordinate to the Chinese Ecosystem Research Network and principally engaged in montane forest ecosystem research. The Xujiaba Reservoir is a major aquatic system adjacent to the Station, of which the liner distance between the nearest edge and the Station is approximately 0.5 km. Mt. Ailao is central within Yunnan Province in southwest China. It is the dividing line between the Yun-Gui Plateau and Cross-Mountain Range as well as the watershed of the Yunjiang and A'Mo rivers. Its altitude is approximately 2443 m. The climate in the region is controlled by the southwest monsoon and has distinct a rainy season (May to October) and dry season (November to April), with an average temperature of 13.0 ± 5.0 °C, annual precipitation of 1400 ± 300 mm, and relative humidity of 84 ± 5% (Wang et al., 2016). Typical forest is old-growth and montane *Pinus* mixed with broadleaf forest. The dominant tree species include *Pinus armandii Franch*, *Castanopsis orthacantha Franch, Schima wallichii (DC.) Choisy,* and *Lithocarpus truncatus (King) Rehd. et Wils.*

The dominant Hg source of Mt. Ailao is from atmospheric deposition of regional air movements and long-range transport (Zhou et al., 2013; Wang et al., 2019). The annual average atmospheric Hg concentration is 2.09 ± 0.63 ng m^{-3}, which is slightly higher than the background value of 1.5–1.7 ng m^{-3} in the northern hemisphere and of 1.1–1.3 ng m^{-3} in the southern hemisphere (Zhang et al., 2016; Sprovieri et al., 2016).

2.2. Sample collection and preparation

Samples of songbird feathers, invertebrates, surface soil, and plants were collected in September 2018 and October 2019. Base on field observations, the number of invertebrate species occurred and population size of each species are abundant during this period. To minimize lateral inputs of aquatic Hg via insect movements into the forests, all sampling sites are located at least 500 m away from the nearest edge of Xujiaba Reservoir. Avian sampling was permitted by agreement between the Institute of Geochemistry, Chinese Academy of Sciences, and the Forestry and Grassland Administration of Jingdong County, Yunnan Province.

Songbird feathers ($n = 102$). Given advantages of noninvasive and simple sampling procedure, feathers were selected as bio-indicator to reflect Hg burden and exposure risk of songbirds in the present study. Songbirds were captured randomly by mist nets within a 1 km × 1 km plot according to Chu and Zheng (1993). After capture, chest feathers from each individual were collected unharmed. Species of songbirds were identified and classified into three categories—insectivorous, omnivorous, and frugivorous—according to the Handbook of the birds of China and Handbook of the Birds of the World online (https://www.hbw.com). Eleven species of songbird were identified: the stripe-throated yuhina (*Yuhina gularis*), brown-breasted bulbul (*Pycnonotus xanthorrhous*), black-throated parrotbill (*Suthora nipalensis*), dark-backed sibia (*Heterophasia melanoleuca*), grey-headed canary-flycatcher (*Culicicapa ceylonensis*), Manipur fulvetta (*Fulvetta manipurensis*), rusty-capped fulvetta (*Schoeniparus dubia*), common stonechat (*Saxicola torquatus*), Bianchi's warbler (*Phylloscopus valentini*), rufous-bellied niltava (*Niltava sundara*) and snowy-browed flycatcher (*Ficedula hyperythra*).

Detailed information on the songbirds is provided in Table S1. In the laboratory, feather samples were thoroughly washed with tap water and detergent, then cleaned with acetone to remove extraneous organic contaminants, and finally rinsed with deionized water and air dried to a constant weight. All feather samples were cut into fragments of about 0.1–0.3 mm prior to analysis.

Invertebrates ($n = 171$ individuals). *Lepidoptera* spp. (caterpillars, moths and butterflies), *Orthoptera* spp. (small grasshoppers, large grasshoppers, katydids, and gryllulus), *Hemiptera* spp. (stinkbugs), and *Araneae* spp. (small and large spiders) were collected within the same plot as songbirds. Those selected species were according to the diet of songbirds as reported by Cristol et al. (2008) and Rimmer et al. (2010). After collection, the invertebrates were starved in centrifuge tubes covered with gauze for approximately 24 h, then cleaned with tap water followed by ultrapure water, and finally dried at −80 °C in a freeze-dryer (LGJ-10, China). The lyophilized samples were ground to powder using an agate mortar and stored in clean PVC bags prior to analysis.

Plants ($n = 40$). Twenty newly emerged pine needles of *Pinus armandii Franch*, the dominant tree species with the frequency of pine individuals ranging between 728 and 1200 individual per hm^2 in the sampling sites, and 20 leaves of the dominant grass (*Arthraxon prionodes*) were collected. The plant samples were washed four times with ultrapure water, then freeze-dried (LGJ-10, China), ground by a pulverizer (IKA A11; IKA, Germany), and finally sieved through 100 mesh prior to analysis.

Soil ($n = 36$). Surface forest soil (5–20 cm) samples were collected and packed in PVC bags, then air-dried in a ventilated place, ground with a ceramic mortar, and finally sieved through 200 mesh prior to analysis.

2.3. Analytical methods

2.3.1. THg analyses

For each feather, invertebrate, and plant biota sample, approximately 0.05–0.2 g was weighed into a 50 mL centrifuge tube and digested with HNO_3 (5 mL) in a water bath at 95 °C for 3 h. The digestion solution was then oxidized with BrCl (25%, 0.5 mL) for at least 24 h, then neutralized by $NH_2OH\cdot HCl$ (25%, 0.2 mL) and reduced with $SnCl_2$ (20%, 0.2 mL) prior to analysis. A suitable volume of the resulting solution was used for measurement of THg via cold vapor atomic fluorescence spectrometry (CVAFS, Brooks Rand Model III, USA) following Method 1631E (USEPA, 2002).

For each soil sample, approximately 0.2 g of soil was weighed into a 50 mL centrifuge tube and digested with aqua regia (HNO_3:HCl = 1:3 v/v, 5 mL) in a water bath at 95 °C for 3 h. The resulting mixture was oxidized with BrCl (25%, 0.5 mL) for 24 h, neutralized by $NH_2OH\cdot HCl$ (25%, 0.2 mL) and then reduced with $SnCl_2$ (20%, 0.2 mL) prior to analysis. A suitable volume of digestion solution was measured by CVAFS (Brooks Rand Model III), as for the determination of THg in biota samples. The limit of detection was 0.023 ng g^{-1} for THg.

2.3.2. MeHg analyses

For each feather and invertebrate sample, approximately 0.01–0.04 g was weighed into a 50 mL centrifuge tube and digested with KOH/methanol solution (25%) in a water bath at 75 °C for 3 h. Adequate digestion solution was taken for MeHg analysis. Following $NaBEt_4$ ethylation, purging, and Tenax trapping, the resulting solution was analyzed by gas chromatography − cold vapor atomic fluorescence spectrometry (GC-CVAFS, Brooks Rand Model III) following Method 1630 (USEPA, 2001).

For each plant sample, approximately 0.2–0.4 g was weighed into a 50 mL centrifuge tube and digested with KOH/methanol solution (25%) in a water bath at 75 °C for 3 h. The MeHg in the resulting solution was extracted with CH_2Cl_2 (10 mL) then back-extracted from the solvent phase to the aqueous ethyl phase, and finally analyzed by GC-CVAFS as for the determination of MeHg in feather and invertebrate samples.

For each soil sample, approximately 0.2–0.4 g was weighed into a 50 mL centrifuge tube, then leached with $CuSO_4$ (2 M, 1.5 mL) and HNO_3 (25%, 7.5 mL) according to the method of Liang et al. (2004). MeHg in the sample was extracted with CH_2Cl_2 (10 mL), then back-extracted from the solvent phase to the aqueous phase, and finally measured by GC-CVAFS according to Method 1630 (USEPA, 2001). The limit of detection was 0.0025 ng g^{-1} for MeHg.

2.3.3. Stable isotopes analyses

The carbon and nitrogen isotope composition ($\delta^{13}C$ and $\delta^{15}N$) were measured by continuous flow mass spectrometry (MAT 253, Thermo Finnigan Instrument, Germany) equipped with a flash analyzer (EA, 2000; Thermo Scientific, Germany) in the State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences. The precision of the analytical measurements was <0.1‰. KNO_3 (IAEA-NO_3, $\delta^{15}N = 4.7‰$) and cellulose (IAEA-C3, $\delta^{13}C = -24.7‰$) were used to calibrate the $\delta^{15}N$ and $\delta^{13}C$ values, respectively. The isotope values for $\delta^{15}N$ and $\delta^{13}C$ were converted based on standard atmospheric nitrogen and Vienna Pee Dee Belemnite (V-PDB), respectively. The results are expressed in standard delta notation according to Equation (1):

$$\delta X = \left(R_{sample} \middle/ R_{reference} - 1\right) \times 1000 \tag{1}$$

Here, X represents isotopes of ^{15}N and ^{13}C, R_{sample} represents the isotope ratio ($^{15}N/^{14}N$, $^{13}C/^{12}C$) of a sample, and $R_{reference}$ represents the isotope ratio ($^{15}N/^{14}N$, $^{13}C/^{12}C$) of the reference.

2.3.4. QA/QC

Quality assurance/quality control (QA/QC) for both THg and MeHg was implemented using method blanks, triplicates, and certified references. For THg, yellow-red soil (GBW07045), citrus leaves (GBW10020), and human hair (GBW09101b) were selected as certified references for soil, plant, and feather/invertebrate samples, respectively. Recovery of THg was 103 ± 1.4%, 115 ± 7.5%, and 100 ± 4.0% from soil, plants, and feather/invertebrate samples, respectively. Each relative standard deviation (RSD) was lower than 10% for triplicate samples.

For MeHg, lobster hepatopancreas (Tort-2, NRCC, Canada) and estuarine sediment (CC580, ERM, Europe) were selected as certified references for biota and soil samples, respectively. Recovery of MeHg from biota and soil were 103 ± 1.4% and 115.2 ± 7.5%, respectively. The RSD was less than 10% for triplicate samples.

2.4. Calculation of TL, BMF, and TMS

2.4.1. TL

In the present study, the average $\delta^{15}N$ of primary producers (pine needle and grass) was taken as a representative baseline. A value of 3.4‰ is a commonly applied trophic enrichment factor for $\delta^{15}N$ in food web analyses according to Post et al. (2002) and Jardine et al. (2006); however, because of the tissue-diet turnover rate of songbirds, 3.7‰—as recommended by Becker et al. (2007)—was used as the average fractionation value between songbird feathers and diet. The TL value was calculated according to Equation (2):

$$TL = \lambda + \left(\delta^{15}N_{consumer} - \delta^{15}N_{base}\right) \Big/ \Delta \mathrm{n} \tag{2}$$

Here, $\delta^{15}N_{consumer}$ and $\delta^{15}N_{base}$ are the isotope ratio of consumers or primary producers, respectively, λ is the trophic position of the organism used to estimate $\delta^{15}N_{base}$, and Δn is the mean trophic fractionation.

2.4.2. BMF

The BMF is the ratio of the concentration of Hg between predator and prey, as recommended by Gray et al. (2002). BMF >1 indicates the presence of specific elements in the biological biomagnification process. BMF values were calculated using Equation (3):

$$BMF_{(\mathrm{THg\ or\ MeHg})} = \frac{[THg\ \ or\ \ MeHg]_{predator}}{[THg\ \ or\ \ MeHg]_{prey}} \tag{3}$$

Here, $[THg\ or\ MeHg]_{predator}$ is the concentration of THg or MeHg in the predator, and $[THg\ or\ MeHg]_{prey}$ is the concentration of THg or MeHg in prey.

2.4.3. TMS

TMS is expressed as the slope (b) of the linear regression between $\delta^{15}N$ and $\log_{10}$[Hg] or $\log_{10}$[MeHg] and is commonly used to quantify Hg biomagnification in food webs. TMS >0 suggests that significant biomagnification of Hg exists across the food web, with larger TMS values indicating more significant Hg biomagnification. This value was calculated according to Equation (4), as recommended by Lavoie et al. (2013):

$$log_{10}[THg\ \ or\ \ MeHg] = \delta^{15}N(b) + a \tag{4}$$

2.5. Statistical analysis

Data analysis was conducted using Excel (Microsoft, USA) and Origin 9.0 (Origin Lab Corporation, Northampton, USA). The differences between all taxa in terms of THg and MeHg, $\log_{10}$[Hg] and $\delta^{15}N$ were evaluated using SPSS 24 (IBM, New York, USA), with $P < 0.05$ regarded as significantly different. Figures were obtained using Origin 9.0 and R v3.6.1 (R Core Team, 2019).

3. Results and discussion

3.1. THg and MeHg in forest ecosystems

3.1.1. THg

Songbird feathers. THg concentrations in feathers of the various songbirds investigated in the present study are listed in Table 1. There was a wide range of THg concentrations across all feather samples, 354–5272 ng g^{-1}, and large inter-species difference with an average of 1659 ± 1027 ng g^{-1} (n = 102). The highest THg of 4666 ± 857 ng g^{-1} (n = 2) on average was observed in the grey-headed canary-flycatcher, followed by the snowy-browed flycatcher with an average of 2998 ± 665 ng g^{-1} (n = 15). The lowest average value of 453 ± 65 ng g^{-1} (n = 5) was found in the stripe-throated yuhina.

As expected, feathers of primarily insectivorous songbirds had higher THg concentrations than those of primarily omnivorous (2.1 times, $p < 0.0001$) and frugivorous (2.8 times, $p = 0.0002$) songbirds (Fig. S1). Omnivorous songbird feathers exhibited slightly higher THg concentrations of those of frugivorous songbird feathers, although this difference was not significant ($p > 0.05$). THg increased in the order of frugivorous-omnivorous-insectivorous, and which was consistent with previous studies (Table S2; Keller et al., 2014; Abeysinghe et al., 2017; He et al., 2019; Xu et al., 2019; Ackerman et al., 2019; Stenhouse et al., 2020), which verify the fact that levels of THg are dependent on bird dietary or trophic position.

In the present study, THg concentrations in songbird feathers were closest to the concentration of 1799 ± 1296 ng g^{-1} in California, USA reported by Ackerman et al. (2019), but higher than those reported in the Southern Appalachians (average: 558 ± 279 ng g^{-1}; Keller et al., 2014) and Alaska, USA (average: 660 ± 674 ng g^{-1}; Stenhouse et al., 2020), and even higher than those observed in regions impacted by intensive nonferrous smelting activities in South China (average: 1270 ± 2100 ng g^{-1}; He et al., 2020). Compared with THg levels in feathers of raptors, the present results were slightly lower the concentration of 1950 ± 1370 ng g^{-1} reported in the Marin Headlands of California, USA (Bourbour et al., 2019), but significantly higher than recently reported in the Tibetan Plateau, China (average: 56.7 ± 1.09 ng g^{-1}; Liu et al., 2020). Compared with levels of THg in feathers of seabirds, the present results were similar to those recently reported in the Arctic Ocean (average: 1877 ± 908 ng g^{-1}; Renedo et al., 2020), but were lower than those reported in the Southern Ocean (average: 3854 ± 3554 ng g^{-1}; Renedo et al., 2018).

For bird feathers, THg concentrations of 5000 ng g^{-1} have been recommended as the threshold of adverse effects and reduction in reproductive success for seabirds (Burger and Gochfeld, 2000). Jackson et al. (2011) reported the THg concentration of 3000 ng g^{-1} have been recommended as the threshold of adverse effects and reduction in reproductive success for songbirds. Although only the grey-headed canary-flycatcher exceeded the adverse effect threshold value of 5000 ng g^{-1}, two of eleven species in the present study exceeded the threshold of 3000 ng g^{-1}, suggesting that songbirds suffered from elevated Hg exposure risks even living in low basal Hg input montane forest ecosystems.

Invertebrates and plants. As shown in Table 1, THg concentrations in invertebrates ranged from 32.6 to 404 ng g^{-1}. The highest value of 459 ng g^{-1} was observed in large spiders, followed by gryllulus (average: 94.7 ng g^{-1}), while the lowest value of 32.6 ng g^{-1} was observed in moths. Overall, the average THg concentration in spiders was as high as 261.7 ± 197 ng g^{-1}, which was comparable to previously reported data (Table S3; Zhang et al., 2010; Kwon et al., 2015; Abeysinghe et al., 2017; Rodenhouse et al., 2019; Tsui et al., 2019). The highest concentrations of THg

Table 1
Stable carbon isotope ($\delta^{13}C$) and nitrogen isotope ($\delta^{15}N$) composition of biotic samples, and concentrations of THg, MeHg, and MeHg% for all samples from a pine forest in Mt. Ailao, Yunnan Province, southwest China.

Sample	Diet	THg (ng g^{-1})		MeHg (ng g^{-1})		MeHg%		$\delta^{13}C$‰	$\delta^{15}N$‰	TL
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Mean ± SD	Mean ± SD
Common Stonechat	Insectivorous	1761 ± 151	1665–1934	410 ± 312	229–771	23.9 ± 19.03	11.8–45.8	−22.2	8.22	3.32
Green-crowned Warbler	Insectivorous	2105 ± 522	1512–2869	1267 ± 271	797–1573	61.0 ± 9.6	52.7–77.0	−24.8 ± 0.327	5.56 ± 0.0134	2.60 ± 0.000
Grey-headed Canary-flycatcher	Insectivorous	4666 ± 857	4060–5272	3576 ± 1357	2617–4536	76.7 ± 15.3	64.5–86.0	−24.5 ± 0.09	7.37 ± 0.0170	3.09 ± 0.00459
Manipur Fulvetta	Insectivorous	1423 ± 879	425–2936	673 ± 346	172–1542	52.9 ± 18.2	31.3–92.2	−25.1 ± 1.07	3.90 ± 0.123	2.15 ± 0.0333
Rufous-bellied Niltava	Insectivorous	1572 ± 795	576–2926	1223 ± 634	275–2313	76.7 ± 14.5	47.7–90.7	−24.5 ± 0.965	5.6 ± 0.834	2.60 ± 0.225
Rusty-capped Fulvetta	Insectivorous	1765 ± 667	678–2299	1378 ± 760	248–2193	70.8 ± 22.9	36.6–96.4	−26.3 ± 0.281	6.89 ± 0.344	2.96 ± 0.0929
Snowy-browed Flycatcher	Insectivorous	2998 ± 665	2122–3849	2066 ± 688	1181–3355	68.6 ± 14.7	48.1–96.1	−24.5 ± 1.09	6.39 ± 0.608	2.82 ± 0.164
Dark-backed Sibia	Insectivorous	945 ± 164	685–1144	461 ± 211	251–954	49.5.0 ± 22.2	22.7–87.2	−23.5 ± 0.249	6.40 ± 0.522	2.83 ± 0.141
Black-throated Parrotbill	Omnivorous	991 ± 465	574–1547	156 ± 40.9	112–217	17.6 ± 5.6	10.0–24.4	−29.6 ± 0.551	4.07 ± 0.445	2.20 ± 0.120
Brown-breasted Bulbul	Frugivorous	1375 ± 451	1056–1694	539 ± 80.2	482–596	42.5 ± 19.8	28.5–56.5	−22.5 ± 0.115	8.01 ± 0.0741	3.28 ± 0.0510
Stripe-throated Yuhina	Frugivorous	453 ± 65.4	354–515	122 ± 23.2	104–163	28.0 ± 10.2	22.3–46.1	−29.6 ± 0.551	4.07 ± 0.445	2.20 ± 0.120
Small spiders	Insectivorous	48.9 ± 0.344	48.7–49.2	23.2 ± 1.40	22.2–24.2	47.4 ± 3.22	45.6–49.2	−26.2 ± 0.116	5.19 ± 0.326	2.63 ± 0.0958
Large spiders	Insectivorous	404 ± 48.1	373–459	157 ± 26.3	140–188	39.5 ± 9.23	31.5–49.6	−25.6 ± 0.0653	5.60 ± 0.113	2.75 ± 0.0333
Months	Herbivorous	32.6 ± 0.400	32.3–32.9	3.30 ± 1.08	2.54–4.07	10.1 ± 3.20	7.84–12.4	−29.3 ± 0.401	2.41 ± 0.926	1.81 ± 0.272
Butterflies	Herbivorous	47.3 ± 6.82	34.1–50.0	1.80 ± 0.124	1.71–2.21	3.90 ± 0.00900	3.22–6.77	−30.2 ± 0.426	0.473 ± 0.167	1.24 ± 0.0492
Caterpillars	Herbivorous	36.1 ± 23.8	42.7–44.0	1.71 ± 0.873	0.496–3.55	5.52 ± 2.49	1.76–10.0	−28.5 ± 0.475	−0.220 ± 0.707	1.17 ± 0.208
Small Grasshoppers	Herbivorous	41.2 ± 0.299	41.0–41.4	1.84 ± 0.140	1.74–1.94	4.47 ± 0.372	4.21–4.73	−27.8 ± 0.113	−0.800 ± 0.196	1.10 ± 0.0577
Katydids	Omnivorous	61.7 ± 0.928	61.1–62.4	3.74 ± 0.389	3.46–4.01	6.05 ± 0.540	5.67–6.44	−28.6 ± 0.176	5.05 ± 0.170	2.588 ± 0.0501
Gryllulus	Omnivorous	94.7 ± 0.000	94.7–94.7	25.1 ± 0.561	24.7–25.5	26.5 ± 0.59	26.1–26.9	−26.7 ± 0.220	4.63 ± 0.508	2.46 ± 0.149
Stinkbugs	Omnivorous	71.4 ± 0.884	70.8–72.0	11.9 ± 1.23	11.1–12.8	16.70 ± 1.52	15.6–17.8	−27.1 ± 0.256	0.755 ± 0.317	1.33 ± 0.0932
Large Grasshoppers	Herbivorous	43.3 ± 0.134	43.2–43.4	2.67 ± 0.0967	2.61–2.74	6.17 ± 0.242	6.00–6.35	−17.6 ± 0.279	0.407 ± 0.181	1.22 ± 0.0532
Pine needles	–	19.1 ± 6.82	11.1–34.8	0.320 ± 0.273	0.104–0.949	1.59 ± 0.946	0.529–3.91	−27.7 ± 1.09	−0.66.7 ± 0.406	–
Grasses	–	91.0 ± 60.2	13.9–192	1.04 ± 0.869	0.281–2.81	1.71 ± 1.40	0.141–4.36	−29.6 ± 0.995	−0.13.7 ± 0.453	–
Soil	–	124 ± 38.3	72.1–204	1.04 ± 1.46	0.122–6.44	0.883 ± 1.12	0.0868–4.70	–	–	–

in spiders suggested the high accumulation ability of Hg, verifying the important role of food chains in Hg transfer.

THg concentrations in pine needles ranged from 11.1 to 34.8 ng g^{-1} with an average of 19.1 ± 6.82 ng g^{-1}. This was close to the concentration of THg in balsam fir needles (average: 20.0 ± 14.5 ng g^{-1}) reported in Stratton Mountain, northeastern USA (Rimmer et al., 2010), but significantly lower than the concentrations (needles or leaves of five plants, average: 135 ± 52.2 ng g^{-1}) reported in Tieshanping, a subtropical forest in Chongqing, southwest China (Zhou et al., 2016). Grasses exhibited slightly higher THg concentrations with an average of 91.0 ± 60.2 ng g^{-1}, which was approximately five times greater than in pine needles. Plants can accumulate Hg from both soil and atmosphere via root and leaf stoma (Laacouri et al., 2013; Manceau et al., 2018; Yuan et al., 2019). Generally, the translocation of IHg from roots to shoots is low due to the barrier from root iron/manganese plaques (Ericksen et al., 2003; Wang and Greger, 2004). In the present study, pine needles have less stoma area compared to broad leaves of grasses. The significant difference of Hg concentrations observed between pine needles and grasses may suggest that most of Hg in plants are from atmosphere (Fleck et al., 1999; Ericksen et al., 2003; Millhollen et al., 2006; Assad et al., 2016).

Soils. THg concentrations in soil ranged from 72.1 to 204 ng g^{-1}, with an average of 124 ± 38.3 ng g^{-1}; this was close to previously reported values (range: 118–279 ng g^{-1}) in forest soil in Mt. Ailao (Zhou et al., 2013; Lu et al., 2016). Although there is an impact from the long-range and transboundary transport of anthropogenic Hg emitted from China, south and southeast Asia (Zhang et al., 2016), Hg concentrations observed in soils from Mt. Ailao were slightly lower than those reported for temperate forest soils (average: 164 ± 30.0 ng g^{-1}) in central New Hampshire, USA (Rodenhouse et al., 2019). The Hg concentrations observed in soils as well as that observed in primary producers verified that the montane forest food chain in Mt. Ailao is characterized by a low basal Hg input.

3.1.2. MeHg and MeHg%

Songbird feathers. MeHg concentrations in each sampled taxon are listed in Table 1. MeHg in feathers ranged from 104 to 4536 ng g^{-1} with an average of 987 ± 868 ng g^{-1}. Similar to THg, the highest value of MeHg was found in the grey-headed canary-flycatcher (average: 3576 ± 1357 ng g^{-1}), followed by the snowy-browed flycatcher (2066 ± 688 ng g^{-1}), and the lowest value of 122 ± 23.2 ng g^{-1} was in the stripe-throated yuhina with a range of 104–163 ng g^{-1}.

As expected, MeHg concentrations in songbirds showed an obviously increasing trend from frugivorous to omnivorous to insectivorous (Fig. 1). MeHg in insectivorous songbird feathers was 3.6-fold and 5.3-fold that in omnivorous ($p < 0.0001$) and frugivorous ($p < 0.0001$) songbird feathers, respectively. No significant difference existed between MeHg content in omnivorous and frugivorous songbird feathers ($p > 0.05$).

The ratio of MeHg to THg (MeHg%) showed a wide range of 10.0%–96.1% with an average of 55 ± 24%. MeHg% significantly increased with increasing TLs (Fig. 1). Among all species, the rufous-bellied niltava recorded the greatest average MeHg% value of 76.7 ± 14.5%. In contrast, species of the common stonechat, black-throated parrotbill, and stripe-throated yuhina exhibited MeHg ratios of less than 50.0%, ranging from 10.0% to 45.8%. In the present study, MeHg% in songbird feathers were consistent with results recently reported in tit nestling feathers, ranging between 12.9% and 43.9% (Luo et al., 2020). In comparison with reported MeHg% values (74%–96%) from heavily Hg contaminated sites (Abeysinghe et al., 2017; Xu et al., 2019), the ratios of MeHg to THg in feathers from remote regions were significantly low. The significant

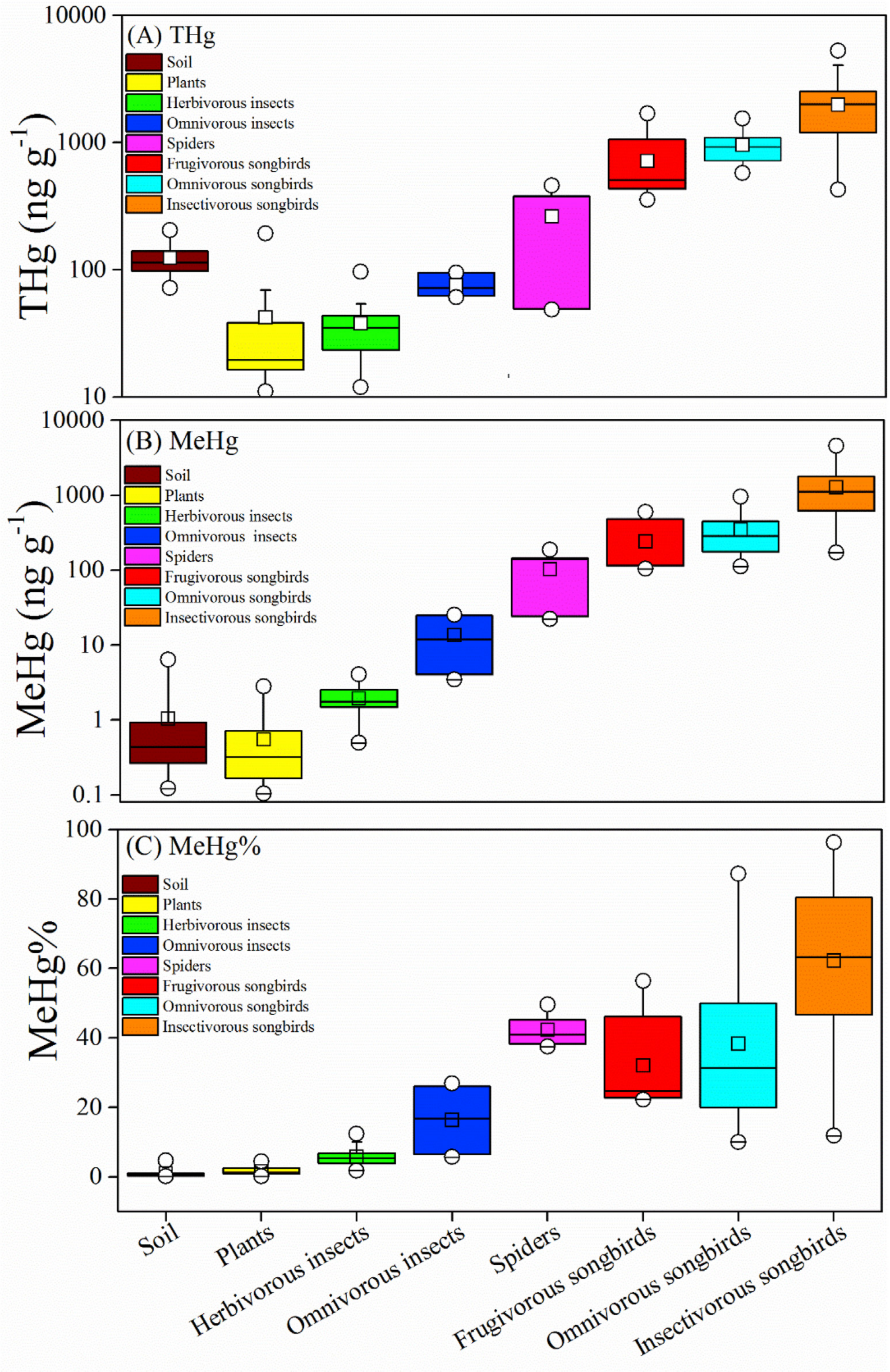

Fig. 1. Concentrations of THg and MeHg, and MeHg% in all samples (Plants-pine needles and grasses; Herbivorous insects-months, butterflies, caterpillars, small grasshoppers, and large grasshoppers; Omnivorous insects-katydids, gryllulus, and stinkbugs; Frugivorous songbirds-Brown-breasted Bulbul and Stripe-throated Yuhina; Omnivorous songbirds-Black-throated Parrotbill and Dark-backed Sibia; Insectivorous songbirds-Common Stonechat, Green-crowned Warbler, Grey-headed Canary-flycatcher, Manipur Fulvetta, Rufous-bellied Niltava, Rusty-capped Fulvetta, and Snowy-browed Flycatcher). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

difference of MeHg% in feathers may associate with their diets. Considering low MeHg% observed in feathers of songbirds from remote regions, the mechanism needs to be further investigated in the future.

Invertebrates and plants. MeHg concentrations in invertebrates and plants ranged from 1.04 to 188 ng g^{-1}. Lower values were observed in pine needles (average: 0.320 ± 0.273 ng g^{-1}) and grasses (average: 1.04 ± 0.869 ng g^{-1}), and the highest values were found in large spiders with an average of 103 ± 75.6 ng g^{-1}. Other invertebrates—such as butterflies, grasshoppers, moths and katydids—exhibited average MeHg concentrations of less than 5.00 ng g^{-1}, which was slightly lower than those of invertebrates form similar taxa in temperate forests (Tsui et al., 2019). In the present study, MeHg levels in invertebrates showed an increasing trend from herbivorous insects to omnivorous insects to spiders. In comparison to IHg, MeHg is readily bioaccumulated and biomagnified via food chains, and organisms at high trophic levels have more energy requirements and tend to eat more (Lavoie et al., 2013); this is conducive to the trophic transfer of MeHg in diets, leading to high levels of MeHg occurred in predators.

Invertebrates had low MeHg% values ranging from 3.9% to 47.4%, with an average of 16.6 ± 15.9%. The highest average MeHg% (47.4 ± 3.22%) was found in spiders and was consistent with previous studies in temperate forest food webs (Tsui et al., 2012, 2019; Kwon et al., 2015; Rodenhouse et al., 2019). However, except for spiders, the MeHg% values for most invertebrates were less than 10.0%, and the lowest MeHg% was observed in small grasshoppers with an average of 4.47 ± 0.372%, indicating low MeHg biomagnification from their diet.

A significantly positive correlation ($R^2 = 0.95$, $p < 0.001$) between THg and MeHg was found in all sampled taxa groups (Fig. 2), which was consistent with recent data reported by Rodenhouse et al. (2019) and Tsui et al. (2019) in temperate forest food webs. The higher THg concentrations the greater MeHg burdens in multiple biota species highlights potential pathways of MeHg bioaccumulation in those forest ecosystems. This phenomenon is also possibly related to the high ability for accumulation, transfer and biomagnification of MeHg in organisms, which may eventually affect biological MeHg loads in food webs.

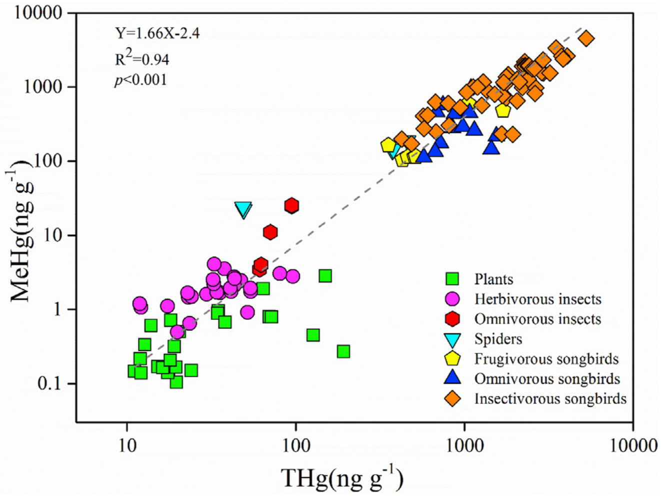

Fig. 2. Correlation between THg and MeHg in all biota samples (Plants-pine needles and grasses; Herbivorous insects-months, butterflies, caterpillars, small grasshoppers, and large grasshoppers; Omnivorous insects-katydids, gryllulus, and stinkbugs; Frugivorous songbirds-Brown-breasted Bulbul and Stripe-throated Yuhina; Omnivorous songbirds-Black-throated Parrotbill and Dark-backed Sibia; Insectivorous songbirds-Common Stonechat, Green-crowned Warbler, Grey-headed Canary-flycatcher, Manipur Fulvetta, Rufous-bellied Niltava, Rusty-capped Fulvetta, and Snowy-browed Flycatcher). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Soils. MeHg concentrations in soil samples ranged from 0.12 to 6.4 ng g^{-1} with an average of 1.0 ± 1.5 ng g^{-1}. It is worth noting that a MeHg ratio as high as 4.7% was observed in soils; this suggested an active methylation process in the soil, which verified that humid deciduous forest soils are favorable for Hg transformation and methylation (Rodenhouse et al., 2019).

3.2. *Characterization of forest food web structure*

3.2.1. *Food sources*

In the present study, the primary producers—pine needles and grasses—exhibited a slight variation in $\delta^{13}C$ values ranging from −30.5‰ to −26.7‰ with an average of −28.2 ± 0.89‰. The $\delta^{13}C$ values in those plants suggested that they belong to C_3 plants, which usually range between −34‰ and −22‰ (O'Leary, 1988). Values of $\delta^{13}C$ in invertebrates (except for large grasshopper) ranged from −30.6 to −22.2‰, which overlapped with the $\delta^{13}C$ range observed in C_3 plants; this indicated that these invertebrates, or their prey, mainly feed on C_3 plants. In contrast, large grasshoppers exhibited high $\delta^{13}C$ values with an average of −17.6 ± 0.28%, suggesting that their diet was primarily dependent on C_4 plants, which have $\delta^{13}C$ values ranging from −20‰ to −10‰ (O'Leary, 1988). Our previous study indicated that grasshoppers contributed little to songbird diets (Luo et al., 2020), thus, the following discussion on Hg transfer via food chains was based on C_3 plants (i.e., pine needles, grass).

Songbird feathers exhibited a wide range of $\delta^{13}C$ values, ranging from −30.4 to −22.2‰ with an average of −25.4 ± 2.4‰. All data for $\delta^{13}C$ fell in the range between large grasshoppers (C_4 plant sources) and other taxa (C_3 plant sources). Additionally, a significant difference in $\delta^{13}C$ values of up to 4.7‰ was observed in songbird feathers; this suggested diverse food sources, which feed on C_3 and/or C_4 plants.

3.2.2. *Trophic levels and food web structure*

To identify the trophic level of an organism, $\delta^{15}N$ is usually employed as an effective indicator. Among all taxa groups, $\delta^{15}N$ differed significantly ($p < 0.005$) and varied widely from −4.4 to 8.2‰ (Fig. 3). The highest value of $\delta^{15}N$ was found in songbird feathers (average: 5.9 ± 1.5‰, range: 3.4–8.2‰), indicating the high trophic position of these taxa in food webs. In addition, high values of $\delta^{15}N$ were observed in spiders, which had an average of 5.4 ± 0.22‰. The lowest value of $\delta^{15}N$ was found in the primary producer pine needles and grasses, which had an average of 0.35 ± 0.49‰.

Based on the $\delta^{15}N$ value of 0.35 ± 0.49‰ for pine needles and grasses, which have a TL of 1.0, TLs of invertebrates and songbirds were estimated as shown in Table 1. Herbivorous insects had the lowest TLs (average: 1.3 ± 0.29), followed by omnivorous insects (average: 2.1 ± 0.70), spiders (average: 2.5 ± 0.08), and songbirds (average: 2.7 ± 0.38). Based on TLs and $\delta^{15}N$ values, the food chain of plant-herbivorous/omnivorous insect-spider-songbird was established in the sub-tropical montane forest ecosystems of Mt. Ailao.

3.3. *Biomagnification and transfer of THg and MeHg*

3.3.1. *BMFs*

BMFs were evaluated for THg and MeHg between prey and predators in food webs (Table S4). The highest BMFs were observed between invertebrates and songbirds, ranging from 6.4 to 60 and 11–878, respectively. The lowest BMFs for THg and MeHg were observed between plants and herbivorous insects, which ranged

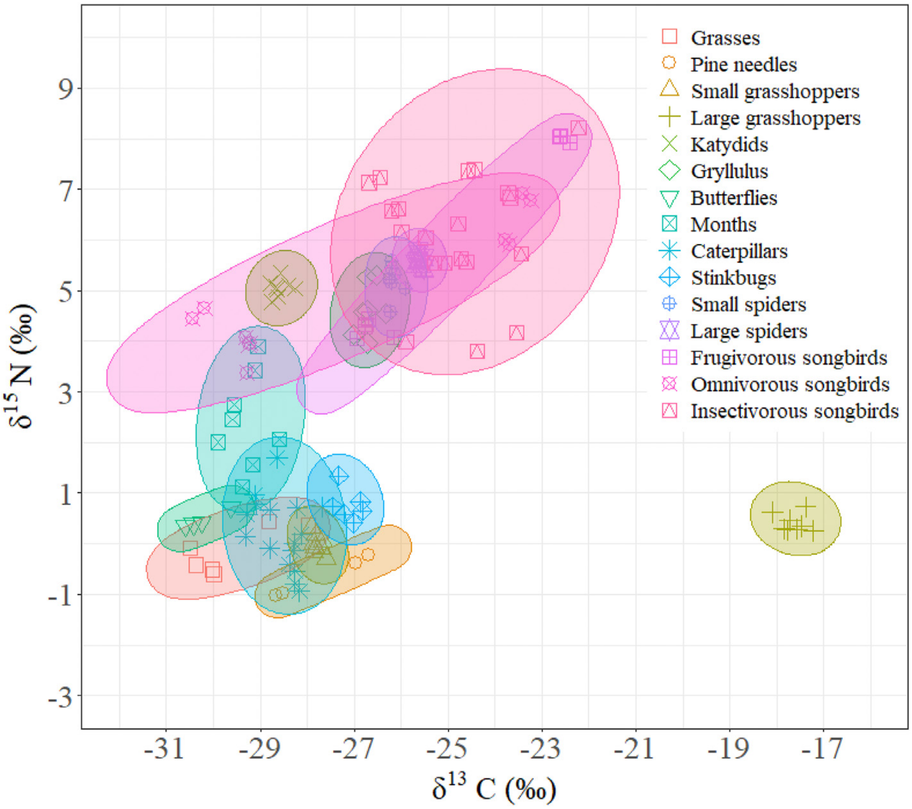

Fig. 3. Distribution of stable carbon isotope (δ^{13}C) and nitrogen isotope (δ^{15}N) in all biota samples. Each ellipse represents a category, different symbols represent different categories, and each symbol represents a specimen (Frugivorous songbirds-Brown-breasted Bulbul and Stripe-throated Yuhina; Omnivorous songbirds-Black-throated Parrotbill and Dark-backed Sibia; Insectivorous songbirds-Common Stonechat, Green-crowned Warbler, Grey-headed Canary-flycatcher, Manipur Fulvetta, Rufous-bellied Niltava, Rusty-capped Fulvetta, and Snowy-browed Flycatcher). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

from 0.6 to 0.8 and 2.5–4.9, respectively. BMF results showed a clear tendency of efficient biomagnification of both THg and MeHg from prey to predator (BMF > 1). Furthermore, the BMFs (THg: 39.5; MeHg: 1795) of plant–songbird in this study were significantly higher than those found in our previous study (THg: 16.3; MeHg: 114) on great tit nestling in a nearby manmade monoculture pine forest (Luo et al., 2020).

It is worth noting that BMFs for MeHg were significantly higher than BMFs for THg, verifying the greater efficiency of MeHg biomagnification in subtropical terrestrial forest ecosystems.

3.3.2. TMS

The trophic magnification slopes (TMSs) of both THg and MeHg in subtropical montane forest food webs in Mt. Ailao are shown in Fig. 4. Significantly positive correlations between log-transformed [Hg] or [MeHg] and δ^{15}N (THg: $R^2 = 0.85$, $p < 0.001$; MeHg: $R^2 = 0.52$, $p < 0.001$) were observed, suggesting a significant biomagnification pattern of THg and MeHg from invertebrates to songbirds. Based on the regressions, the values of TMS_{THg} and TMS_{MeHg} were 0.22 ± 0.03 and 0.38 ± 0.05, respectively. In addition, a significantly positive correlation was also observed between MeHg% and δ^{15}N ($R^2 = 0.70$, $p < 0.001$), which further confirmed the significant bioaccumulation and biomagnification of MeHg in terrestrial food chains.

Recent results for TMS_{THg} and TMS_{MeHg} from terrestrial ecosystems are summarized in Table 2. For temperate forest ecosystems, Tsui et al. (2019) reported comparable TMS_{MeHg} values to the present study, with an average range of 0.20–0.28 for litter-invertebrate food webs. Much lower TMS values (THg: 0.14, MeHg: 0.09) were reported in malaise-detritivore-litter arthropods-spiders-salamanders-birds in forest food chains in central New Hampshire (Rodenhouse et al., 2019). For an Hg-contaminated terrestrial ecosystem, Abeysinghe et al. (2017) reported comparable TMS_{THg} (0.40) and TMS_{MeHg} (0.36) values from rice-herbivorous insects-carnivorous invertebrates food webs in an abandoned Hg mining region, while higher values of TMS_{THg} (0.45) and TMS_{MeHg} (0.80) from the nettle-insect food web in chlor-alkali sediment landfill were reported by Yung et al. (2019). In the present study, the TMS values in the plants-herbivorous/omnivorous insects-spiders-songbirds food chains/webs of subtropical forest ecosystems in Mt. Ailao were high, especially for MeHg, and were even higher than those observed in temperate freshwater ecosystems ($TMS_{MeHg} = 0.24$; Lavoie et al., 2013).

Significant variations in TMS values among different ecosystems might be due to factors such as food chain/web composition, taxa groups, and habitats, etc. (Lavoie et al., 2013). In comparison, TMS values for food chains of plant-invertebrate-songbird, plant-invertebrate, and invertebrate-songbird are shown in Table 2 and Figs. S2 and S3. The TMS (MeHg: 0.25; THg: 0.079) in the food chain of plant-invertebrate was significantly lower than the TMS (MeHg: 0.36; THg: 0.21) in the food chain of invertebrate-songbird. Interestingly, the TMS value for MeHg in the food chain of pine needle & grass-invertebrate from subtropical forest ecosystem in Mt. Ailao exhibited no difference from those observed in food chains of fresh litter-invertebrate from the temperate forest ecosystems (Tsui et al., 2019). Clearly, when songbirds were included in food chains the TMS values for MeHg become to be higher, and even were comparable to previous results reported in aquatic food chains (Clayden et al., 2015).

High TMS values for MeHg estimated in food chains with

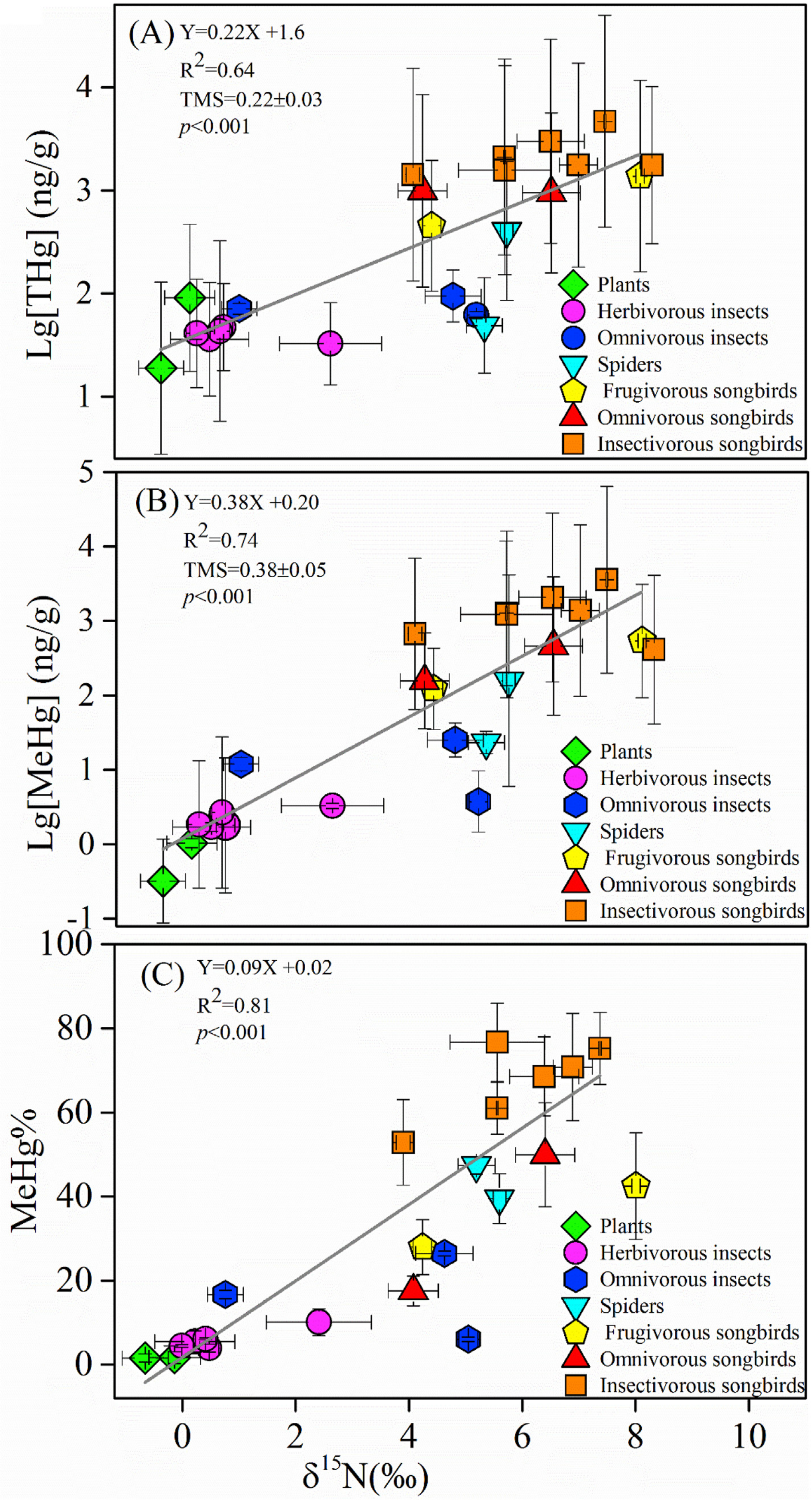

Fig. 4. Relationship between $\delta^{15}N$ values and log-transformed concentrations of (A) THg, (B) MeHg, and (C) MeHg% in all biota samples (Plants-pine needles and grasses; Herbivorous insects-months, butterflies, caterpillars, small grasshoppers, and large grasshoppers; Omnivorous insects-katydids, gryllulus, and stinkbugs; Frugivorous songbirds-Brown-breasted Bulbul and Stripe-throated Yuhina; Omnivorous songbirds-Black-throated Parrotbill and Dark-backed Sibia; Insectivorous songbirds-Common Stonechat, Green-crowned Warbler, Grey-headed Canary-flycatcher, Manipur Fulvetta, Rufous-bellied Niltava, Rusty-capped Fulvetta, and Snowy-browed Flycatcher). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
The reported trophic magnification slope (TMS) values of THg and/or MeHg in food webs.

Food chain/web composition	TMS		Habitat	Location	Reference
	MeHg	THg			
Invertebrates/Fish	0.15	0.036	Arctic polynya	Nasaruvaalik Island, Canada	Clayden et al. (2015)
Fish/Seabirds	0.26	0.095	Arctic polynya	Nasaruvaalik Island, Canada	
Fresh litter/Invertebrates	0.26	–	Temperate forest	California, USA	Tsui et al. (2019)
Fresh litter/Invertebrates	0.20	–	Temperate forest	Michigan, USA	
Fresh litter/Invertebrates	0.22	–	Temperate forest	North Carolina, USA	
Fresh litter/Invertebrates	0.28	–	Temperate forest	New Hampshire, USA	
Insect/Spider/Salamanders & Songbirds	0.09	0.14	Watersheds	New Hampshire, USA	Rodenhouse et al. (2019)
Soil/Nettle/Insects	0.80	0.45	Chlor-alkali sediment landfill	Bourgogne Franche-Comté, France	Yung et al. (2019)
N/A	0.24	0.16	Freshwater	Global	Lavoie et al. (2013)
Rice/Insect/Invertebrates	0.36	0.40	Mercury mining region	Wanshan, China	Abeysinghe et al. (2017)
Pine needle/Caterpillars/Nestlings	0.36	0.18	Subtropical Pine forest	Jingdong, China	Luo et al. (2020)
Pine needle/Invertebrate/Nestlings	0.38	0.18	Subtropical Pine forest	Jingdong, China	
Pine needle & Grass/Invertebrate/Songbirds	0.30	0.25	Subtropical montane forest	Mt. Ailao, China	This study
Pine needle & Grass/Invertebrates	0.25	0.079	Subtropical montane forest	Mt. Ailao, China	
Invertebrate/Songbirds	0.36	0.21	Subtropical montane forest	Mt. Ailao, China	
Invertebrate/Insectivorous songbirds	0.38	0.24	Subtropical montane forest	Mt. Ailao, China	
Invertebrate/Omnivorous songbirds	0.14	0.14	Subtropical montane forest	Mt. Ailao, China	

including songbirds would be likely associated to potential incorporation of aquatic-derived MeHg in the songbird tissues. Given the fact that songbirds usually have a longer-range movement, it is very hard to make sure that all their body MeHg is derived solely from terrestrial prey only; hence, songbirds may also take up certain amount of MeHg via aquatic preys (Tsui et al., 2018). Thus, the derive part of MeHg from aquatic sources may change the MeHg and δ^{15}N signals, then changing the TMS estimation in the food chains that extended by songbirds. Furthermore, different tissue types of songbirds used in the analysis might also change the TMS estimation. Moreover, birds are endotherms and have high energy requirements to maintain their metabolism; the higher the energy requirements, the higher the potential intake of Hg, especially MeHg (Lavoie et al., 2010, 2013).

3.4. Implications for terrestrial wildlife

Numerous studies verified that variations of Hg concentrations exist between inner tissues and feathers, and the order of magnitude higher Hg concentrations in feathers than inner tissues will lead to an overstate Hg burden to birds (e.g., Herring et al., 2009; Low et al., 2020). In comparison with other tissues, however, feathers deposited the most largely proportion (i.e., the highest concentrations) of Hg originated from dietary (Rubio et al., 2016; Zabala et al., 2019). In the present study, we focused on the main Hg transfer through food chains of remote subtropical montane forest with a low basal Hg input, hence, we employed the feather as indicator to elucidate the biomagnification of Hg in songbirds.

In the present study, higher TMS values were observed in the terrestrial food webs compared with those reported in food chains/webs in temperate ecosystems and even those in aquatic food chains. These results suggested that terrestrial songbirds tend to have higher Hg intake and are subject to a particular MeHg exposure risk because of their high trophic position in food chains. The high Hg concentrations observed in feathers of grey-headed canary-flycatchers exceeded threshold levels, which may affect their flight ability and reproduction. Although invertebrates exhibited slightly lower Hg levels compared to songbirds, a long-term exposure may also affect their health and populations owing to the ready bioaccumulation of MeHg. Hence, the bioaccumulation of Hg in invertebrates, particular spiders, is another important issue because of their trophic position and importance in food chains.

Forest is regarded as a large sink for atmospheric Hg and accounts for approximately 31% of the terrestrial surface (Obrist, 2007; Keenan et al., 2015). Therefore, elevated atmospheric Hg deposition may cause a higher risk of Hg accumulation and biomagnification in forests (Blackwell and Driscoll, 2015; Wang et al., 2019). Given the elevated TMS values observed in the sub-tropical montane forest of Mt. Ailao in the present study, the Hg flow among different trophic levels of wild animals plays a significant role in the biogeochemical cycling of Hg in this forest ecosystem. Investigations of this type are necessary to deepen understanding of Hg cycles in terrestrial ecosystems on the global scale.

4. Conclusions

As far as is known, this work is the first study of the transfer and biomagnification of Hg in subtropical montane evergreen broadleaf forest food chains. The present study determined the concentration of THg and MeHg in free-living songbirds, and constructed a clear food web of plants-herbivorous/omnivorous insects-spiders-songbirds in the subtropical montane forest of Mt. Ailao. Results showed that the TLs of consumers ranged from 0.8 (butterflies) to 3.3 (common Stonechat), and followed the order of songbirds > spiders > omnivorous insects > herbivorous insects > plants in the food web. A clear transfer of both THg and MeHg from lower to higher trophic level organisms was also found. The TMS values of both THg and MeHg in the present study were higher than those found in temperate forest and freshwater food webs. Some of the free-living songbirds in this subtropical montane forest—which is characterized as having low basal Hg input—were found to have THg concentrations that were greater than the adverse-effect threshold value of 5000 ng g^{-1}, which deserves more research attention. However, further studies on the mechanism of terrestrial MeHg accumulation and biomagnification are urgently required in the future.

Credit author statement

Chan Li: Investigation, Formal analysis, Methodology, Visualization, Data curation, Writing – original draft. Zhidong Xu: Investigation, Visionary, Methodology, Writing – review & editing. Kang Luo: Investigation, Methodology, Resources, Formal analysis. Zhuo Chen: Supervision, Validation, Funding acquisition. Xiaohang Xu: Investigation, Methodology, Writing -review & editing. Chengxiang Xu: Validation, Writing – review & editing, Funding acquisition. Guangle Qiu: Supervision, Conceptualization, Project administration, Writing-review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the manuscript–“**Biomagnification and trophic transfer of total mercury and methylmercury in a sub-tropical montane forest food web, southwest China**”.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.130371.

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