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# The fate of mercury and its relationship with carbon, nitrogen and bacterial communities during litter decomposing in two subtropical forests

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ABSTRACT

Forests play a significant role in the biogeochemical cycling of mercury (Hg). Litterfall represent a dominant pathway for Hg to reach the ground surface under a forest canopy. In order to understand Hg accumulation in the litterfall, the dynamics of Hg, carbon (C), nitrogen (N), microbial C and N, as well as bacterial community in the decaying litterfall of two typical subtropical forest stands in southwest China were investigated for one year. THg levels in the litterfall after one-year increased to 124.64% and 135.90%, and MeHg levels in the litterfall increased up to 295.65% and 209.38% of the initial values in the mixed broadleaf-conifer forest and evergreen broadleaf forest respectively. Differently, the concentrations of THg and MeHg in the organic layer of the underlying soil were quite stable. The concentrations of THg in the decaying litterfall corresponded negatively with C/N ratios. Bacterial community analysis found that the bacteria previously being confirmed as Hg methylators did not occur in the genera of the decomposing litterfall in the two forest stands, which might imply that the increase of MeHg during decomposing did not mainly due to the contribution of confirmed Hg methylators, and other sources of MeHg might exert certain roles.



## 1. Introduction

Mercury (Hg) is regulated as a hazardous pollutant, which is released from both natural and anthropogenic sources. Large amounts of Hg have been emitted into the environment, which makes it a global health concern due to its toxicity (Abelson, 1970; Boening, 2000; Driscoll et al., 2013). Forest ecosystem is the largest terrestrial ecosystem, the area of which is approximately one third of the Earth, and the biomass of which accounts for about 85% of the terrestrial ecosystems (Abella, 2015). Forest ecosystem is generally considered to be an active library of Hg, and environmental behavior of Hg in the forest ecosystem is believed to be an important part of global Hg cycle (Ericksen et al., 2003; Kim et al., 1995). Forest canopies can uptake atmospheric Hg more rapidly than other landscapes due to their large leaf areas and rough surfaces (Risch et al., 2012). Mercury input to the forest floor includes dry deposition on the leaf surface, litterfall, throughfall, dry deposition directly on the forest floor and wet deposition during leaf-off periods (Choi, 2007; Demers et al., 2007). Mercury dry deposition through litterfall means that the leaves become contaminated via atmospherically deposited Hg and accumulated Hg externally and internally as a function of leaf age (Ericksen et al., 2003; Rea et al., 2000; Tyler, 2005; Tyler and Olsson, 2006; Zhang et al., 2005). Previous studies have indicated that Hg deposition through litterfall is significantly greater than wet deposition (Demers et al., 2013; Grigal, 2002; Jiskra et al., 2015) and represents a dominant pathway for Hg to reach the ground surface under a forest canopy (Lindberg et al., 1994; Munthe et al., 1995; Rea et al., 1996, 2001). Namely Hg deposition through litterfall have much greater effect than wet deposition on the size of Hg storage in the forest soil. Therefore, Hg dry deposition from the air through litterfall had attracted more

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and more attention from researchers worldwide (Fu et al., 2015; Risch et al., 2012; Zhang et al., 2012). The annual quantity of global Hg deposition through litterfall is estimated to be 1180 ± 710 mg $yr^{-1}$ through statistical analysis based on published data, approximately 70% of which occurs in the tropical and subtropical regions (Wang et al., 2016). This systematic evaluation on the role of Hg deposition through litterfall pointed out the scarce studies on the fate of litterfall Hg in the tropical/subtropical forests, and significantly highlighted the role of tropical/subtropical forests in global Hg cycling (Wang et al., 2016). The distribution and dynamics of soil Hg of Asian subtropical forest have been documented for seven years (Lu et al., 2016), and Hg in leaf litter in typical suburban and urban broadleaf forests were also reported (Niu et al., 2011). However, there is still limited understanding about the dynamics and fate of accumulated Hg through litterfall in the subtropical forests.

Moreover, researchers found that MeHg concentrations in the litterfall increased in the decaying process, depending on the plant type (Hall and St Louis, 2004). Our unpublished results also found this interesting phenomenon and proposed a hypothesis that MeHg levels in the decomposing litterfall increased in the subtropical forests. If this hypothesis establishes, then it implies that the risk of Hg migration from the forest system to the aquatic system may also increase. Previous research speculated that THg levels corresponded with microorganisms that involved in nitrogen fixation, especially fungi (Demers et al., 2007; Hall and St Louis, 2004), and found that the quantities of bacteria during litter decomposing were much larger than fungi (Wang et al., 2006). Moreover, MeHg is produced in the environment via biotic and abiotic processes (Li and Cai, 2013), among which a series of anaerobic bacteria and archaea is the predominant way (Raposo et al., 2008). That is to say, the concentrations of THg and MeHg change with the degradation of litterfall, in which a series of microorganisms are mainly involved. Therefore, it is important to research on the biotic formation of MeHg and understanding the role of the bacterial community structure in the litterfall decomposing process (Strickman and Mitchell, 2016; Macalady et al., 2000; Bae et al., 2014). In addition, nitrogen fixation seems to play significant roles in Hg emission and accumulation, which means that it is essential to research on the dynamics of Hg and its relationship with the concentrations of nitrogen during litter decomposing. Therefore, we need to explore the relationship of Hg concentrations with microorganisms that maybe involved in this process. However, little is known about whether bacteria play certain roles in the emission and accumulation of Hg during the degradation of litterfall and what kind of bacteria dominate in this process, even the bacterial community structures was unknown so far. To date, less research on the dynamics of bacteria during litterfall decaying had been conducted. Thus, the objectives of this study were: 1) to characterize Hg dynamics and fate in the litterfall and forest soil in two typical subtropical forest in southwest China; 2) to analyze the relationship between the dynamics of litterfall THg and carbon nitrogen ratios during litterfall decaying; 3) to determine the relationship between the dynamics of litterfall MeHg and bacterial community structure during the whole degradation process.

## 2. Methods

### 2.1. Study area description

Chongqing is situated at the transitional area between the Qinghai-Tibet Plateau and the plain on the middle and lower reaches of the Yangtze River in the subtropical climate zone. This study was conducted at the Simian Mountain, a national nature reserve (106° 22′–106° 25′ E, 28° 35′–28° 39′ N) located about 200 km from urban Chongqing. Mt. Simian is a typical subtropical forest of southwest China. It possesses the largest and most well preserved primitive subtropical evergreen broadleaf forest at 28° north latitude in the northern hemisphere, and the forest coverage has reached 95.8%. It is known as the “natural species gene pool” by the specialists of ecological protection of the United Nations (Zhang et al., 2011). The dominant land over of Mt. Simian is natural or secondary vegetation with more than 1422 species of vascular plants, mainly including mixed broadleaf-conifer forest (90%) and evergreen broadleaf forest (8%). The natural conditions are suitable for vegetation growing and landscape resources are abundant in this area. The topography is characterized by a low mountainous landscape. The climate is predominantly subtropical humid monsoon with a relative humidity over 90%, annual average temperature and rainfall of 13.7 °C and 1127 mm, respectively. At present, there are no significant point source discharges of Hg in the forest floor of Mt. Simian. In our research, the mixed broadleaf-conifer forest (M forest for short) and evergreen broadleaf forest (B forest for short) were selected as the two forest stands. The two forest stands are two adjacent sites, about 500 m apart from each other. Within the organic horizon, the *Oi* horizon means soil at the range of 0–10 cm, and *Oe* horizon is the soil at 10–20 cm in thickness in each stand. The background information about the two forests are shown in Table S1.

### 2.2. Decomposition experiment design and sample collection

Experimental research on the litter decomposition at the two forest stands was investigated by litterbag technique. A total of 10 nylon-mesh-lined baskets were acid cleaned and randomly placed throughout each forest stand to collect fresh leaf litter. Leaves were collected during rain-free periods to make sure that Hg was not leached from the fallen litter before the deployment of litterbags (Demers et al., 2007). Leaves from each stand were weighed into 108 acid-cleaned 1-mm mesh bags (10*15 cm, 10.0 g/bag), labeled, and heat-sealed, with 36 bags per plot. Nine litterbags (3 bags per plot) were immediately removed from each forest stand for analysis of initial mass, carbon, nitrogen, THg and MeHg contents on a dry mass basis and DNA extraction. The remained 99 litterbags were returned to the field and split between three 1 × 1 m plots (33 bags per plot) in each forest stand. Each litterbag was pinned to the ground with small plastic stakes at the four corners. Litterbags were not covered with additional fresh litter upon deployment. The litter was collected monthly for one year from March 2014 to February 2015. Namely 9 decomposing litterbags were taken from the three plots of each forest stand every month. For the three bags moved to the lab from one plot each month, one bag was used to measure various environmental factors, the second was used to analyze the concentrations of THg, MeHg, microbial C and N, and the last one was used to extract DNA. For each sampling, the litter samples were shipped to the laboratory and air dried within 2 h, and then weighed to determine dry mass loss. The corresponding soils under the bags were also collected at depth of 0–10 cm ($O_i$) and 10–20 cm ($O_e$) each month together with bags.

According to the exponential regress model, the relative rates of decomposition ($k$) were calculated after Olson model (Olson, 1963):

$$X_t = X_o\, e^{-kt} \quad (1)$$

where: $X_o$ is a weight of initial material, $Xt$ is a weight of decaying material after time $t$, $K$ is a rate of decomposition, $e$ is a base of natural logarithm.

The mass of THg and MeHg in each litterbag was calculated by multiplying the THg or MeHg concentrations by the final freeze-dried weight of litter tissues in the litterbags (Hall and St Louis,

2004). The percent mass of THg or MeHg remaining in each litterbag at the time of collecting was standardized to the initial THg or MeHg mass in each litterbag.

### 2.3. Analytical methods for carbon and nitrogen

Litters were also collected every month from each site for the estimation of carbon and nitrogen concentrations, and microbial biomass C and N with three replicates. Carbon and Nitrogen concentration in plant tissues from each litterbag were analyzed by an Exeter Analytical Model 440 elemental analyzer. Microbial biomass (C and N) were measured by fumigation extraction method (Anderson and Ingram, 1994). Microbial biomass C was calculated by modified Walkley Black method (Vance et al., 1987).

$$\text{Microbial biomass C} = K_c \times 2.64 \quad (2)$$

Microbial biomass N was determined by micro-kjeldahl method (Page, 1982).

$$\text{Microbial biomass N} = K_n \times 1.46 \quad (3)$$

*Kc* and *Kn* are the difference between C and N extracted from fumigated and unfumigated soils.

### 2.4. DNA isolation and bacterial 16S rRNA sequencing analysis

The DNA was extracted from 0.05 g litterfall mixed samples from the three plots each month with the PowerPlant DNA Isolation kit (MO BIO Laboratories, Inc., USA) according to the manufacturer's protocol. The purity and concentration of total DNA extracted were measured by 1% agarose gel electrophoresis (Rio-Rad, USA) and a Nanodrop 2000 Spectrophotometer (Thermo Fisher, USA) respectively. The DNA was then stored at −20 °C before use.

The isolated DNA was sent to Shanghai Majorbio to perform high-throughput sequencing of 16S rRNA amplicons by Illumina MiSeq PE300 platform. Primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) targeting both the V3 and V4 regions of the bacterial 16S rRNA genes were selected for PCR amplification. A total of 24 samples were sequenced, with the number of sequences ranging from 32273 to 112459. Low quality reads were removed by Trimmomatic before chimera detection. OTUs were classified by Usearch (v7.1) using a 97% sequence similarity threshold.

### 2.5. THg and MeHg analysis

For THg analysis, approximately 1.0 g litterfall sample was dissolved at 95–140 °C with a mixture of $HNO_3$ and $H_2SO_4$ (4:1, v/v) (Qiu et al., 2006). Litterfall samples were measured using cold vapor atomic fluorescence spectroscopy (CVAFS) in accordance with Method 1631 (USEPA, 2002). For litterfall MeHg determination, approximately 0.5 g sample was weighed for digestion using 25% KOH-methanol at 75–80 °C for 3 h. Then MeHg in litterfall samples was leached with $CH_2Cl_2$ and back-extracted into water phase for determination based on Method 1630 (USEPA, 2001). Soil MeHg concentrations were determined by $HNO_3$ leaching/$CH_2Cl_2$ extraction, ethylation, trapping on a Tenax trap, isothermal gas chromatographic (GC) separation, and the CVAFS detection method (Liang et al., 2004). Soil THg concentrations were determined by acid (1:3 HCl + $HNO_3$) digestion followed by CVAFS detection (Qiu et al., 2006). All the litter and soil samples have three replicates.

### 2.6. Quality assurance and statistical analysis

Field blanks, system blanks, spike recoveries and triplicate samples were used for quality assurance of the analytical processes. Field blanks and duplicates were regularly collected throughout each sampling campaign. The equipment blanks for THg and MeHg were 0.04 ng $g^{-1}$ and 0.02 ng $g^{-1}$ respectively. Detection limits were based on three times the standard deviations of the blank. The detection limits of THg and MeHg in the litterfall and soil samples were 0.02 ng $g^{-1}$ and 0.01 ng $g^{-1}$, respectively. The method blank was lower than the detection limits in all cases. The precision was determined by relative standard deviations (RSDs) for duplicate samples, which were 6% and 6.4% for the THg and MeHg analyses. Recoveries for matrix spikes ranged from 94% to 111% for THg and from 91% to 114% for MeHg. The unilateral test was used to compare the concentrations of Hg in the growing and dormant seasons, with significant level of 0.05. One-way ANOVA was used to compare the difference of microbial C or N in different seasons. Student's t-test was used to compare of the dominant bacteria genera between the two forest stands. Origin 8.0 was used for drawing.

## 3. Results and discussion

### 3.1. THg and MeHg accumulated during the decomposition of litterfall

The dynamic changes of the mass loss and concentrations of carbon, nitrogen, THg, and MeHg of the litterfall during the whole decomposing process were shown in Fig. 1. The mass of the litterfall decreased gradually as predicted, and the final mass were 72.00% and 68.25% of the original mass for the M and B forests respectively. Carbon content decreased during the whole degrading process, while the nitrogen content increased slightly. Initial THg concentrations in the studied litterfall were 69.13 ng $g^{-1}$ and 78.30 ng $g^{-1}$ for the M and B forest respectively, which were relatively higher comparing with other studies in the forest stands (Almeida et al., 2009; Fu et al., 2010). Both the concentrations of THg and MeHg increased with the degradation of litterfall. THg levels after one-year increased to 124.64% and 135.90% of the initial values for the M and B forests respectively. The concentrations of THg increased from 69.13 ng $g^{-1}$ to 86.3 ng $g^{-1}$ (SE = 7.5, $p < 0.0001$) and 78.3 ng $g^{-1}$ to 106.4 ng $g^{-1}$ (SE = 7.6, $p < 0.0001$) in the M and B forest stand respectively during the whole decomposing process. The increase of THg concentration in this research was less than that in the broadleaf and needle litter of a forested site in northern Ontario, Canada (Hall and St Louis, 2004). They showed that THg concentrations increased from 7.1 ng $g^{-1}$ to 40.9 ng $g^{-1}$ and 14.1 ng $g^{-1}$ to 35.4 ng $g^{-1}$ for the broadleaf and needle litter respectively after decomposing 800 days (Hall and St Louis, 2004). The concentrations of MeHg when decomposing for one-year increased to 295.65% and 209.38% of the original values of the litterfall for the M and B forests respectively, which were much more remarkable comparing with THg levels. The change of MeHg concentrations in the process of litterfall degradation was more obvious than that of THg, C, N and mass. These results were consistent with our observations of the previous year and the statistical data worldwide (Wang et al., 2016). In addition, the half-life of the litter mass from the M and B forests were 3.1 and 3.7 years respectively based on the exponential regress model ($r^2 = 0.75$ and 0.86 for M and B forests), which were comparable to the results of previous studies (Gosz et al., 1973; Melillo et al., 1982; Rustad, 1994).

*Oi* means the undecomposed organic layer of the soil, and *Oe* means the decomposed organic layer of the soil.

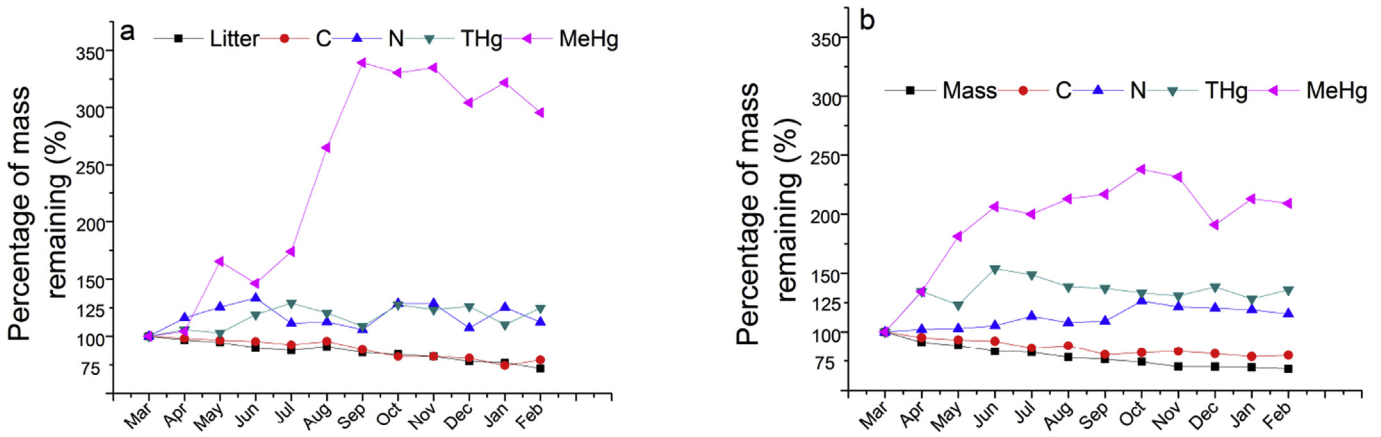


**Fig. 1.** Percentage of mass remaining of carbon (C), nitrogen (N), THg and MeHg in the decomposing litterfall of the M forest (a) and B forest (b) stand during the whole decaying process.

### 3.2. Comparison of THg and MeHg concentrations in the decomposing litterfall and underlying soil layers

After decomposing for one year, THg concentrations in the litterfall in the two forest stands were 86.7 ng $g^{-1}$ (SE = 18.5, n = 3, $p < 0.0001$) and 106.7 ng $g^{-1}$ (SE = 20.9, n = 3, $p < 0.01$) for the M and B forest respectively, which were still much less than that in the *Oi* and *Oe* layers of the organic matter of the soil (Table 1). THg levels in the organic layers (*Oi* and *Oe*) of the soil of the B forest stand were lower than that in the M forest. While the concentrations of MeHg in the decomposing litterfall in the two forest stands were 0.68 ng $g^{-1}$ (SE = 0.12, n = 3, $p < 0.0001$) and 0.97 ng $g^{-1}$ (SE = 0.09, n = 3, $p < 0.01$) for the M and B forest respectively, which were higher than that in the *Oi* and *Oe* layers of the soil (Table 1). The variation of MeHg levels in the organic layers of the soils of the two forest stands did not have significant difference ($p > 0.05$). The variation of THg concentrations in the decomposing litterfall contributed to the paradox of soil Hg pool. The larger input of Hg, faster decomposition rate, and larger net Hg accumulation in the litterfall might result in the accumulation of Hg in the underlying soil of the B forest stand. Generally speaking, THg and MeHg concentrations in the underlying soil were stable, with only slightly changes throughout the year.

During the whole decomposing process, THg and MeHg concentrations accumulated gradually in the growing season, especially MeHg concentrations in the M forest which increased sharply from March to October. Results from the piecewise regression model indicated that the accumulation of THg had seasonal difference (Table 2). In the dormant season, there was a slight, not significant decrease (−1.1%/month, Table 2) in the accumulation of THg. During the growing season, the accumulation of THg appeared to increase significantly (+5.5%/month, Table 2). This indicated that the seasonal accumulation of THg was a continuous phenomenon, and would occur in the growing season of the two forest stands. The reasons perhaps are that the decaying of litterfall is primarily dominated by microorganisms, and the temperature and humidity in the growing season are conducive to the survival of microorganisms. In addition, such a humid and muggy condition in the subtropical forest might favor the decomposition of litters.

**Table 2**
Results of piecewise regression model of THg concentrations in the two forest stands.

| forest type | Growing season (Mar.–Oct.) | | | Dormant season (Nov.–Feb.) | | |
|---|---|---|---|---|---|---|
| | Mean | SE | *p* | Mean | SE | *p* |
| M | 4.8 | 1.1 | 0.304 | 0.5 | 1.9 | 0.046 |
| B | 5.7 | 1.8 | 0.284 | −1.1 | 1.5 | 0.006 |

Note: p value were the result of unilateral test.
M: Mixed broadleaf-conifer forest; B: evergreen broadleaf forest.

### 3.3. THg and MeHg concentrations in relation to C and N

It is speculated that the correlation between nitrogen, carbon and Hg flux is suggestive of a biotic mechanism, such as microbial immobilization, for Hg accumulation in decaying leaf litter (Demers et al., 2007; Melillo et al., 1982; Wang et al., 2006). The binding of Hg in organic matter, including decomposing litterfall is inferred to be associated with nitrogen accumulation via microbial uptake or immobilization (Demers et al., 2007). Therefore, the relationship between THg concentrations and C/N ratios for the two forest stands were analyzed in our research. The C/N ratios decreased with the increase of THg levels, which indicated that the

**Table 1**
THg and MeHg concentrations in litters and different soil layers before and after decomposition.

| Samples | Month | M | | B | |
|---|---|---|---|---|---|
| | | THg (ng·$g^{-1}$) | MeHg (ng·$g^{-1}$) | THg (ng·$g^{-1}$) | MeHg (ng·$g^{-1}$) |
| Litter | Initial | 69.13 | 0.23 | 78.30 | 0.32 |
| | Final | 86.3 | 0.68 | 106.4 | 0.97 |
| $O_i$ | Initial | 208.2 | 0.35 | 186.5 | 0.35 |
| *(0-10 cm)* | Final | 213.3 | 0.51 | 193.2 | 0.37 |
| $O_e$ | Initial | 138.1 | 0.25 | 125.1 | 0.29 |
| *(10-20cm)* | Final | 114.8 | 0.38 | 114.8 | 0.31 |

M: Mixed broadleaf-conifer forest; B: evergreen broadleaf forest.

concentrations of THg corresponded negatively with C/N ratios, especially in the M forest (Fig. 2). The increase of nitrogen in the M forest was more obviously (Fig. 1 and Fig. S1), which implied that stronger nitrogen fixation effect and more active nitrogen fixing microorganisms probably existed in the M forest stand (Demers et al., 2007). Our research found that the C/N ratios in the M forest were higher than that in the B forest. Previous research indicated that the forest with higher C/N ratios always had higher nitrogen fixation rate (Park and Matzner, 2001). Therefore, the M forest probably had higher nitrogen fixation rate than the B forest, which contributed to its fast increase of THg. Presumably the nitrogen fixation bacteria could sequester Hg and other metals (Demers et al., 2007). Moreover, the M forest stand also had higher soil carbon content and larger carbon pool, which was beneficial to the accumulation of Hg. This probably because that a high carbon pool is equivalent to a high dissolved organic matter (DOM) pool to a certain extent. The DOM is very easy to combine with Hg (Jiang et al., 2017). Therefore, the higher carbon pool means that more Hg is accumulated in the forest stand. In addition, litterfall is the main path for the new produced and recycled Hg entering into the soils of the forest stand (Demers et al., 2007), but Hg in the soils may also be historic. This would explain why the soils with high DOM have higher THg levels than the soils that received high-Hg litter. Moreover, the decomposing litterfall could probably capture the net Hg flux through throughfall during leaf-out periods (Choi, 2007).

### 3.4. Microbial C, N and bacterial community structures of the decomposing litterfall

In the M forest stand, the microbial C and N ranged from 83.7 to 1112.6 mg g-1, and 36.5–95.4 mg g-1 respectively (Fig. 3). In the B forest stand, the microbial C and N ranged from 185.4 to 904.2 mg g-1, and 32.4–71.2 mg g-1 respectively. In the M forest stand, the microbial C contributed 1.84–2.63% of the total organic C in the soil, with the maximum appearing from May to October and the minimum during winter season. The percentage contribution of microbial biomass N to total N ranged from 152 to 163% for the M forest. In the B forest stand, the microbial C contributed 1.27–1.42% of the total organic C in the soil, with higher values occurring from May to October and lower values in the winter season, which was the same with the M forest. The percentage contribution of microbial biomass N to total N ranged from 112 to 124% in the B forest. Overall, Microbial C and N increased with the decomposition process, especially in the M forest stand. Previous research found that the microbial C and N were significantly higher in summer, and lower in winter in two stands of mixed-oak forest ecosystem of Manipur, Northeast India (Devi and Yadava, 2006). In our research, the microbial C was significantly higher in summer comparing that in winter in the B forest stand (Fig. 3, $^{**}p < 0.01$). The microbial N was significantly higher in summer comparing with that in winter in the M forest stand (Fig. 3, $^{*}p < 0.05$). The reason probably is that the surface litter in summer could often maintain an optimum temperature, favoring the process of microbial decomposition (Gilmour et al., 2013; Hall and St Louis, 2004). Further, the microorganisms activated in the decomposing litterfalls could immobilize more nutrients as decomposition rates and microbial activities are at peak in summer, and thus contributing to the soil microbial biomass. Therefore, the concentration of THg increased along with this process, especially MeHg, which increased exponentially.

It is known that the biotic methylation of Hg is under anaerobic environments (Parks et al., 2013; Hsu-Kim et al., 2013). Sulfate-reducing bacteria (SRB), iron-reducing bacteria (IRB), and several methanogens are confirmed to methylate Hg under anaerobic conditions, such as *Desulfovibrio desulfuricans* ND 232, *Geobacter sulfurreducens* PCA and *Methanospirillum hungatei* JF1 (Gilmour et al., 2013; Hall and St Louis, 2004; Yu et al., 2013). The litterfall placed in the litterbags in the observation plots may form anaerobic microzones, supposedly to promote MeHg production. Additionally, Hall and St. Louis (2004) also observed that MeHg concentrations in decomposing litter placed on unflooded soils and reservoirs exhibited differently, dependent on litter species; Alder leaves, jack pine needles, wood, and old wood (decomposed wood) placed on unflooded soils experienced increases in MeHg mass. In our research, MeHg concentrations in the decomposing litter of Chinese subtropical M and B forest stand increased remarkably with the decomposing of litterfall, especially the M forest. In addition, the positive correlation between Hg accumulation and nitrogen immobilization possibly caused by additional biotic mechanisms of Hg transformation in decaying litterfall related with reduced sulfur groups (Demers et al., 2007) or other undiscovered mechanisms.

As we know that the methylation of Hg is mainly regulated by anaerobic bacteria, especially the SRB and IRB (Colombo et al., 2013; Gilmour et al., 2013; Hsu-Kim et al., 2013; Parks et al., 2013). Therefore, bacterial community structures of the decomposing litterfall were analyzed. Rarefaction and specaccum curves based on a 97% phylogenetic cluster similarity showed a good sampling depth of our sequencing results (Fig. S2). Dynamics and comparison of the

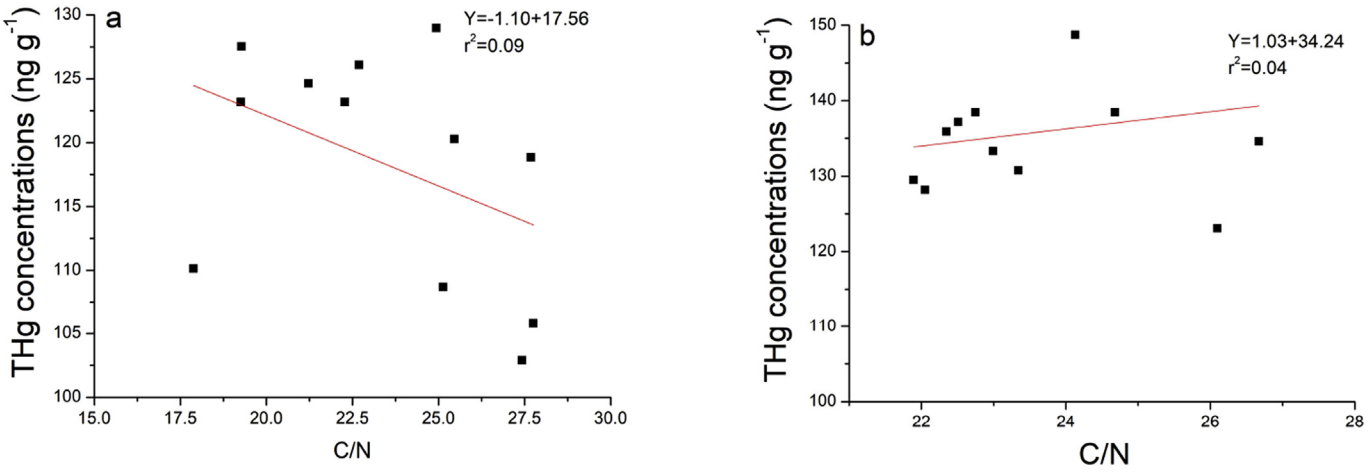


**Fig. 2.** Relationship between carbon/nitrogen ratios and THg concentrations in the litterfall of the M forest (a) and B forest (b) during the whole decomposing process.

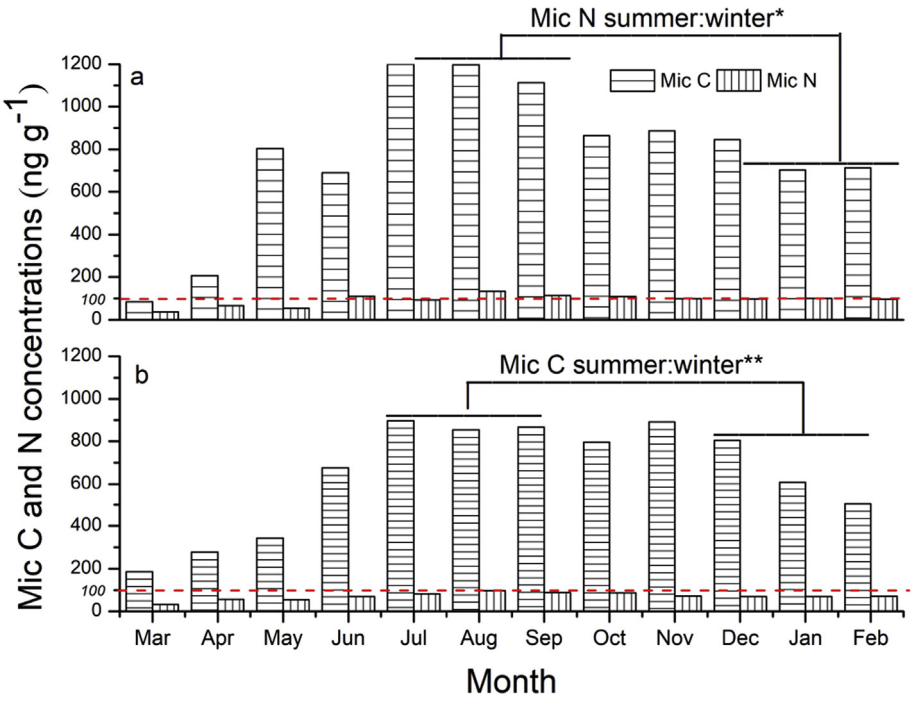


**Fig. 3.** Dynamics of microbial C and N in the litterfall of M forest (a) and B forest (b) during the whole decomposing process. The asterisk indicates the difference of microbial C or N based on one-way ANOVA (*: $p < 0.05$; **: $p < 0.01$).

relative abundance of bacteria in the decomposing litterfall of the two forest stands at the genus level were shown in Fig. 4a and b. The bacterial community structures differed remarkably between M and B forest stands. Within the bacteria domain, *Pseudomonas* (8.06%), *Sphingomonas* (7.72%), *Mucilaginibacter* (7.55%), *Burkholderia* (4.75%) and *Granulicella* (4.09%) were the dominant genera in the litterfall from the M forest. While the top five genera predominant in the B forest were totally changed to *Bradyrhizobium* (4.60%), *Rhizomicrobium* (3.68%), *Actinoplanes* (2.81%), *Sphingomonas* (2.21%) and *Tardiphaga* (2.09%), which had relatively higher abundances during the whole decomposing process. The genus of *Mucilaginibacter* and *Acitinoplanes* had extremely significant difference (Student T-test, $p \leq 0.001$ ***) between the two forest stands (Fig. 4b). *Sphingomonas* was remarkably higher in the M forest than that in the B forest (Student T-test, $0.001 < p \leq 0.01$ **), while *Bradyrhizobium* and *Burkholderia* were remarkably higher in the B forest than that in the M forest (Student T-test, $0.001 < p \leq 0.01$ **). Principle coordinate analysis (PCoA) results found that samples from the two types of litterfall were seasonly clustered, especially the M forest stand (Fig. 5), indicating that different dominant bacteria were involved in the samples in different seasons. The bacteria being confirmed as Hg methylators previously did not occur in the genera of the decomposing litterfall in the two forest stands, which may imply that the increase of MeHg during decomposing did not mainly due to the contribution of confirmed Hg methylators. Since previous work had found that Hg methylation occurred at normal rates even when microbial groups hosting confirmed Hg methylators were very rare (Strickman and Mitchell, 2016), as the elevated MeHg in the decaying litterfall may originate from other possible sources. The sources of MeHg in the decaying litterfall may come from the biotic methylation of new methylators, abiotic methylation of the litterfall, as well as the biotic or abiotic methylation of the underlying soil layers. First, our research showed that the anaerobic bacteria known to methylate Hg were notably absent from the litterfall sequencing results. However, there may be some new Hg methylation bacteria, archaea, or even fungi that have not yet been discovered in the decomposing litterfall. After the indispensable Hg-methylating gene cluster *hgcAB* being found, researchers have tested the methylation capabilities of current known methylators, and found that not all of the strains with the ability had *hgcAB* gene clusters (Gilmour et al., 2013). Their work significantly expands the range of Hg methylators and our knowledge of MeHg producing habitats. In the past few years, some scholars found several new bacteria or archaea had strong Hg methylation capacities (Gilmour et al., 2013; Gionfriddo et al., 2016; Yu et al., 2013). Some scholars even put forward the hypothesis that aerobic bacteria and facultative anaerobic bacteria in *γ-proteobacteria* have strong Hg-methylation abilities (Tao et al., 2016). Therefore, there are probably some unknown and not yet recognized Hg methylation strains in the decomposed litterfall need to be found. Secondly, abiotic methylation of the litterfall may also dominant in the decomposing litterfall of the subtropical forest stand. The humid, warm and sometimes anaerobic litterfall-soil interface in the subtropical forest stand may also become important sites for abiotic methylation. Meanwhile, sufficient illumination may also provide proper conditions for the synthesis of MeHg in the decomposing litterfall. Thirdly, in the litterfall-soil interface, the underlying soil had indivisible relation with the litterfall. Biotic and abitic methylation might exist in the underlying soil or the microenvironment formed by the decaying litterfall and the underlying soil. So it is necessary to research on the metagenomes of the litterfall-soil to find the biotic sources.

The ultimate fate of Hg accumulated in the decaying leaf litter may depend on how Hg enters into the soil and whether Hg is lost by draining or volatilization. The source of Hg in decomposing litterfall mainly includes the dry and wet depositions of gaseous elemental Hg (RGM) and particulate Hg (HgP), and the migration of native Hg from the lower soil layers to the surface under the role of microorganisms. Microbial nitrogen fixation of Hg(0) and the newly

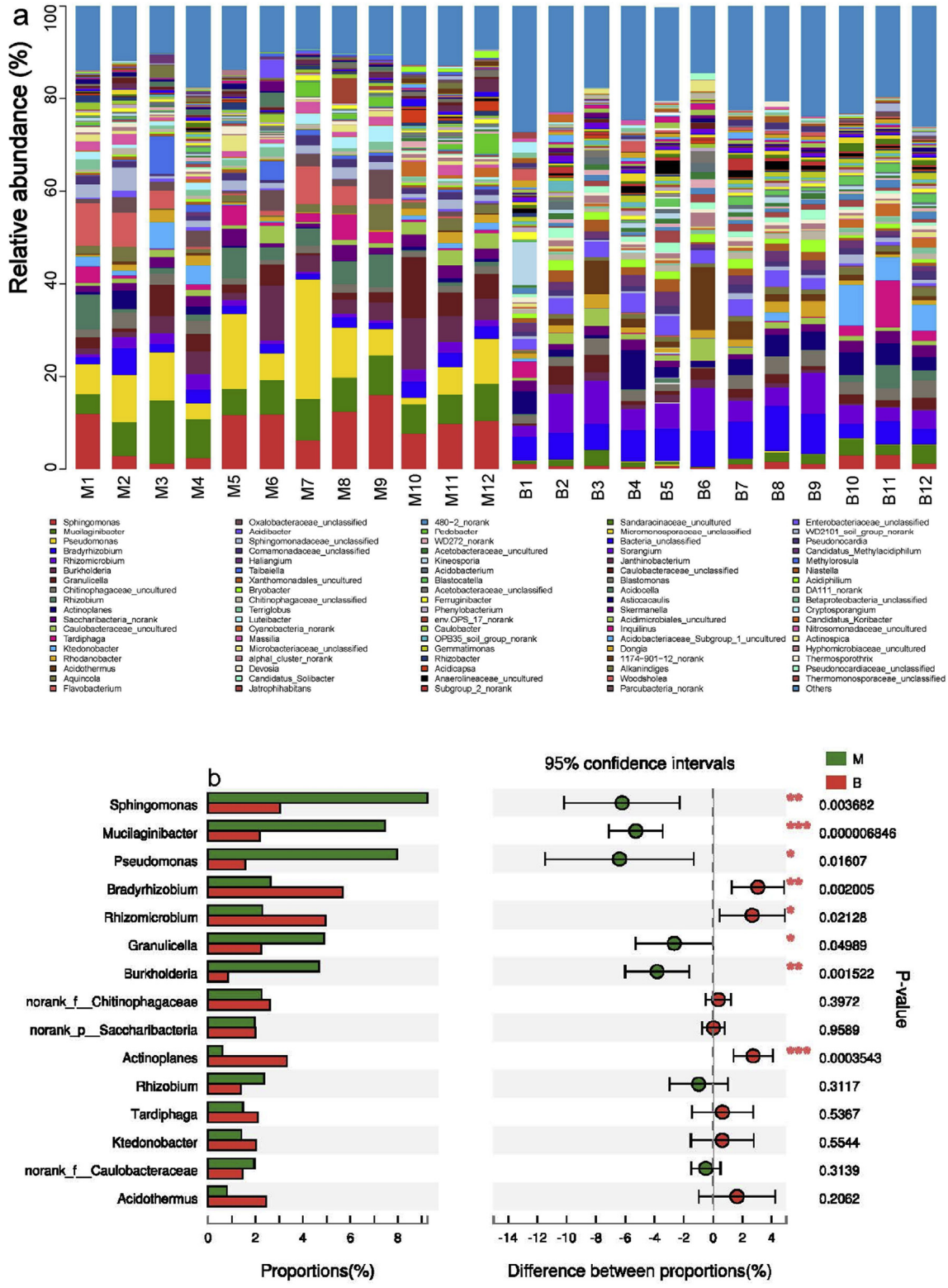


**Fig. 4.** Dynamics (a) and comparison (b) of the dominant bacteria genera in the decomposing litterfall of the two forest stands. M: Mixed broadleaf-conifer forest; B: evergreen broadleaf forest. Fig. 4b were results of Student T-test, $0.01 < p \leq 0.05$ *; $0.001 < p \leq 0.01$ **; $p \leq 0.001$ ***.

deposited Hg in the litterfall surface is presumably to be a new source of Hg in the decomposing litterfall (Demers et al., 2007). RGM and HgP accumulated on the surface of the canopy can be washed away by rains and into the litterfall. Other conditions, such as the thin organic layer, saturated soil, and litterbags with mesh size larger than 1 mm, may also impact the accumulation of Hg in decaying leaf litter (Devi and Yadava, 2006; Jiang et al., 2017). As we know that the complexation of organic matter in the soil can adsorb

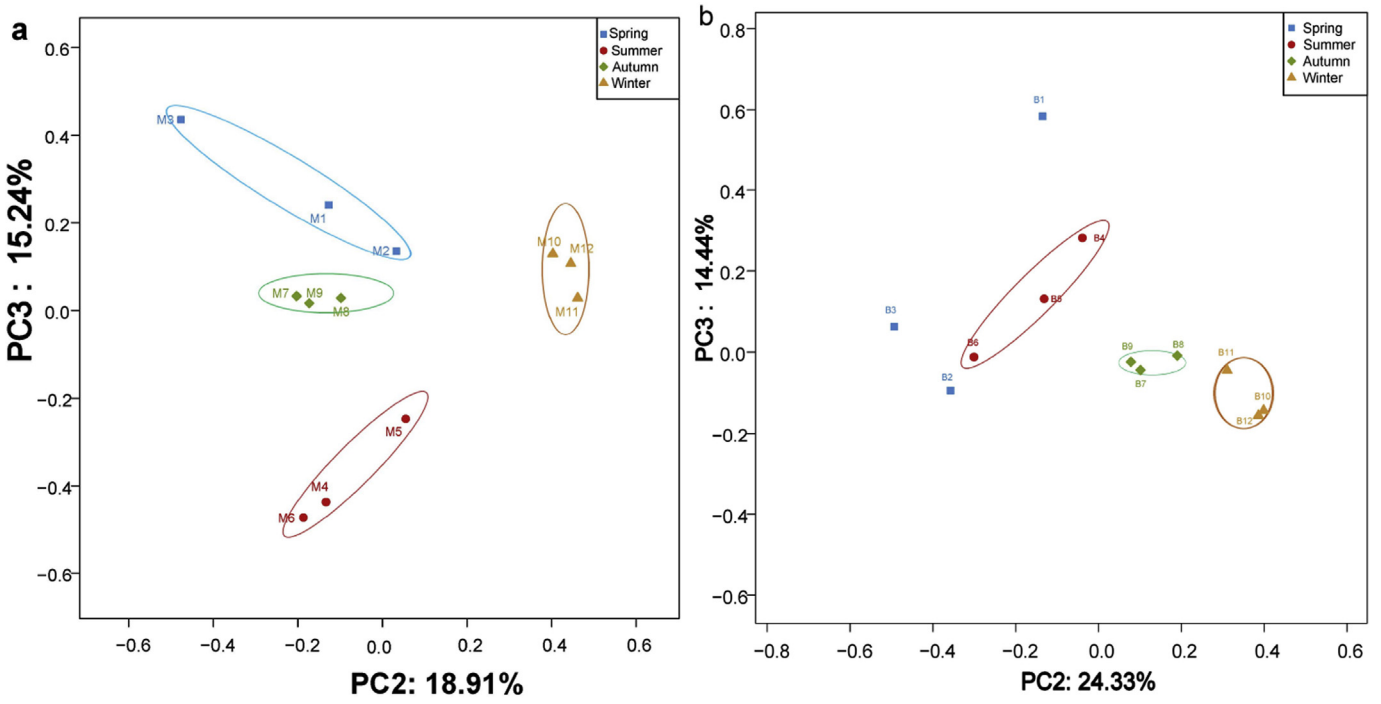


**Fig. 5.** Principle coordinate analysis (PCoA) of the microbial community structures in the degraded litterfall of the M (a) and B forest (b) during the whole decomposing process. M: Mixed broadleaf-conifer forest; B: Evergreen broadleaf forest.

or fix Hg, and the aggregation structure of the soil can also fix Hg (Jiang et al., 2017). When the absorbed or fixed Hg by the soil reaches saturation, the Hg may transfer to the newly deposited litterfall in the forest stands (Jiang et al., 2017). In addition, previous research found that the mesh size of the litterbags greater than or equal to 1 mm would not impede the transport of Hg between the litter and soils (Devi and Yadava, 2006). Research found that the newly deposited dissolved Hg was easily retained on the surface of vegetation and litter (Hintelmann et al., 2002). If the Hg accumulated in the decaying litterfall is only absorbed by the litter tissues or fixed in the litter surface by microorganisms, this part of Hg will eventually enter into the forest as residues, easily covered by the subsequent cohorts of litterfall. However, if Hg from the lower soil horizons and more decomposed litter layers is translocated by microorganisms, this would constitute an internal recycling within the forest floor and finally Hg would be accumulated in the upper layer of the soil, delaying its transportation through the forest soil and increasing its residence time in the forest stands (Demers et al., 2007). Additionally, the litterfall Hg degrading data provided in this research might be helpful for understanding MeHg impacts on forest ecosystems, which was less researched previously.

## 4. Conclusion

The litterfall mass, and concentrations of carbon, nitrogen, THg and MeHg varied in the decomposing litterfall of the M and B forest during the whole decaying process. Both the concentrations of THg and MeHg in the litterfall increased with the degradation of litterfall. THg levels in the litterfall after one-year increased to 124.64% and 135.90% of the initial value for the M and B forests respectively, while the concentrations of MeHg in the litterfall increased up to 4 and 3 times of the initial values in the M and B forest stands. However, the concentrations of THg and MeHg in the organic layer of the soil were stable, with only slight changes. The C/N ratios decreased with the increase of THg levels, which indicated that THg levels corresponded negatively with C/N ratios. The increase of nitrogen in the M forest was more obviously, which implied that stronger nitrogen fixation effect and more active nitrogen fixing microorganisms probably existed in the M forest stand. Analysis of bacteria associated with MeHg accumulation supplied that bacterial community structures differed remarkably between the M and B forest stands. The bacteria that had been confirmed as Hg methylators did not occur in the genera of the decomposing litterfall in the two forest stands, which may imply that the increase of MeHg during decomposing might not mainly due to the contribution of confirmed Hg methylators and other possible MeHg sources might exert certain roles.

In addition, we admitted that our research still have some limitations. For example, it is painful to differentiate the microbial accumulation of Hg and the effective accumulation of Hg simply due to the loss of organic matter through decomposition. Moreover, confirmed Hg-methylators include both bacteria and archaea. However, our research does not mention the analysis of archaea and their relationship with Hg concentrations during litterfall decomposing. More importantly, the source of the elevated MeHg in the decomposed litterfall cannot be easily identified due to the deficiency of the meta-analysis of microorganisms in the underlying soil. It is indispensable to research on the metagenomes of the litterfall-soil interface to identify the source of the elevated MeHg in the decayed litterfall. Therefore, these issues should be solved in the future research. Nevertheless, our research indeed showed the fate of Hg and its relationship with carbon, nitrogen and bacterial communities during litter decomposing in two subtropical forests, southwestern China. Results of our research had significant meaning for understanding the geochemical cycling of Hg in the forest ecosystems.

## Competing financial interests

The authors declare no competing financial interest.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.apgeochem.2017.09.008.

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