Fine Root Mercury Heterogeneity: Metabolism of Lower-Order Roots as an Effective Route for Mercury Removal

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S *Supporting Information*

ABSTRACT: Fine roots are critical components for plant mercury (Hg) uptake and removal, but the patterns of Hg distribution and turnover within the heterogeneous fine root components and their potential limiting factors are poorly understood. Based on root branching structure, we studied the total Hg (THg) and its cellular partitioning in fine roots in 6 Chinese subtropical trees species and the impacts of root morphological and stoichiometric traits on Hg partitioning. The THg concentration generally decreased with increasing root order, and was higher in cortex than in stele. This concentration significantly correlated with root length, diameter, specific root length, specific root area, and nitrogen concentration, whereas its cytosolic fraction (accounting for <10% of THg) correlated with root carbon and sulfur concentrations. The estimated Hg return flux from dead fine roots outweighed that from leaf litter, and ephemeral first-order roots that constituted 7.2−22.3% of total fine root biomass may have contributed most to this flux (39−71%, depending on tree species and environmental substrate). Our results highlight the high capacity of Hg stabilization and Hg return by lower-order roots and demonstrate that turnover of lower-order roots may be an effective strategy of detoxification in perennial tree species.
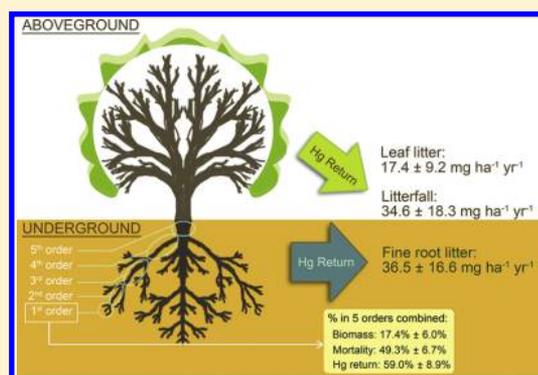
INTRODUCTION

Mercury (Hg) biogeochemistry may affect natural ecosystems resulting from the metal's high phyto- and vertebrate toxicity and severe ecological effects.[1−3] Intensive field work and lab experiments have shown that Hg in leaves and roots originated primarily from atmosphere and soil, respectively,[4−9] and that a very small fraction of Hg in roots (<5%) could be translocated to the shoot in various tree species.[3,10−14] Higher soil Hg concentration commonly led to higher root Hg concentration, and was likely to enhance the percentage of total plant Hg allocated to roots.[8,9,15] Millhollen et al.[9] suggested that root Hg accumulation and release were important processes in underground Hg cycling. However, few efforts have been made to quantify these underground processes, particularly as related to forest tree species.

Hg uptake by organisms in the rhizosphere is an essential process in soil Hg phytoremediation,[16,17] and the base of food webs where Hg bioaccumulation starts.[18] In the rhizosphere, a series of chemical and biological processes (e.g., Hg reduction and Hg methylation) may occur,[19−21] thereby shifting the Hg's chemical species and bioavailability.[18,22,23] Roots are important in these processes, and their coexistence with rhizospheric microbial community may promote the process of Hg methylation[24] and the accumulation of Hg in tissues of wetland plants.[22] However,

there are large gaps in our understanding of the mechanisms of absorption, translocation, and stabilization of Hg within the fine root architecture of various plant species.[14]

Fine roots have been increasingly demonstrated to be heterogeneous in structure and functions. Within the root branch system, the distal orders, relative to proximal orders, usually have smaller diameter, higher nitrogen (N) concentration (and respiration rate), shorter lifespan, and higher frequency of mycorrhizal fungi infection, with each being closely related to root function as water and nutrient uptake.[25−32] Hg uptake by roots of plant is thought to be a physiological process closely related to root structure and function. Cocking et al.[15] reported that the root total Hg (THG) concentration in several herbs significantly corelated with root diameter, however, other root morphological traits and the fine root Hg heterogeneity within 1.5 mm diameter class were not investigated. Close associations between Hg concentration and carbon (C) level in soil has been well documented[4,33,34] but little is known about the patterns within the root system. Moreover, Hg may bind to

sulphydryl groups at mouths of aquaporin and can physically block root channels, significantly reducing the hydraulic conductance,[35−37] and thus its passive uptake with water flow may be limited. In fact, different regions of roots were observed to respond differently to water transportation after application of $HgCl_2$,[35,38] but it remains unclear how the cellular partition of Hg differs within the fine roots. To our knowledge, no study has yet addressed the root Hg heterogeneity by branch order and how the fine root's morphology and chemistry correlate with the root Hg species and distribution.

In terrestrial ecosystems, root senescence is an important route for chemical return (typically C and N) from plants to the soil.[39,40] Yet, Hg return via root death has rarely been considered. Root longevity is a key parameter to estimate dead root return but it may vary from <1 year for the first-order roots to >9 years for higher-order roots.[32,41,42] However, the traditional methods to estimate root turnover usually ignored the fine root heterogeneity (e.g., ref 31), so that the accurate estimation of root return flux (the rate of return flow from phytosphere to pedosphere by root) is difficult. Guo et al.[42] showed that the branch order approach may substantially reduce errors in estimating the longevity of fine root guild. They also found that the first-order roots may account for ~50% of the total biomass mortality and >60% of the N flux for the first three root orders combined.[31] Still, degrees of root Hg return flux from different branch orders of any species and how they compare with the recycling part of above-ground litterfall flux remain unexplored. Thus, we studied the remote forest at the Dinghu Mountain, where anthropogenic Hg deposition is relatively low, to estimate the root Hg return and compare it with the potential litterfall return.

The objectives of this study were to determine how Hg concentration varied with branching order and responded to fine root morphology and chemistry in six selected tree species, and to estimate the fine root and leaf/litterfall return fluxes and relative contribution of each branch order to fine root Hg return flux. Two primary hypotheses were tested: (1) there would be high variability of Hg by branch order and higher-order roots possess high Hg concentration because of the longer duration of exposure; (2) root Hg return flux caused by root senescence is comparable to leaf or litterfall Hg return flux and the relative contribution of root return flux is branch-order specific.

■ MATERIALS AND METHODS

Study Site and Sample Collection. Dinghu Mountain, the first national nature reserve in South China, is located in the subtropical zone (23°09′−23°11′ N, 112°30′−112°33′ E). The mean temperatures in summer and winter are 28 and 12.6 °C, respectively, whereas the annual precipitation of the reserve averages 1929 mm. The zonal soil type is mainly latosolic red soil, followed by yellow soil in some regions.[43] Six dominant plant species (*Ardisia quinquegona* (ARQU), *Cryptocarya concinna* (CRCO), *Canthium dicoccum* (CADI), *Cinnamomum camphora* (CICA), *Cryptocarya chinensis* (CRCH), and *Cleistocalyx operculatus* (CLOP)) from four sites in mixed evergreen broadleaf forest were selected and sampled in mid-August 2010. Three to five fine root lateral branches of each species (including at least 5 branch orders) were collected from intact soil blocks (30 cm long × 20 cm wide × 10 cm deep) (except those of *Cleistocalyx operculatus* from river). Simultaneously, corresponding mature leaves (except the leaf of *Cinnamomum camphora*, which was too high to collect) and rhizospheric soils (pH 3.84−4.27;

SOM 3.55−5.76%; TN 0.26−0.36%; TS 0.05−0.06%) of each species were collected. Water from river flow where the roots of *Cleistocalyx operculatus* grew was also sampled. The plant samples were rinsed and preserved using the method as described by Guo et al.[28] Briefly, the root branch networks were carefully placed in Milli-Q water (1 °C), gently stirred to dislodge the soil from the roots, and rinsed three times. Afterward, they were placed under 10× magnification where residual soil, organic matter particles, and dead root fragments were carefully removed by Teflon-coated forceps, and then preserved at −80 °C.

Root Morphology Determination. Cleaned root networks were isolated strictly by branch orders.[27,28,44−46] That is, the distal roots were categorized as the first order, the root from which two first-order roots branched were categorized as second order, and so on. Besides the branch ratio and total biomass, other parameters including root length, root diameter, and individual biomass (dw) of different orders were measured based on 20 segments for the first three orders and 10 segments for the fourth and fifth orders. Specific root length (SRL), specific root area (SRA), and tissue density were also calculated. For the third-, fourth-, and fifth-order roots of *Ardisia quinquegona* and *Cinnamomum camphora*, we randomly took away parts of the samples and further subsampled them by isolating the cortex (commonly including epidermis, cortex, and phloem) from the stele. Meanwhile, the biomass of cortex and stele from different orders were also recorded.

Macroelements and Total Hg (THg) Determination. All solid samples were lyophilized (48 h), ground, and sieved through 200-mesh screen. The sample C, N, H, and S concentrations were then measured by elementary analytical instrument (Vario EL CUBE), with sulfanilamide as standard material. For THg measurement, the soil and plant samples were microwave-digested (using Ethos 1, Milestone) by aqua regia and H_2O_2 + HNO_3, respectively (Table S1). Digested solutions were filtered and analyzed by CVAFS (Mercur, Analytic Jena), using $SnCl_2$ as reductant. The water sample was filtered, acidified by superpure HCl, and then measured by CVAFS. Certified materials of GBW10020 (plant sample) and GBW07403 (yellow soil) and blank were measured in parallel for quality control. All of the samples were measured as least in triplicate. All quality control met EPA limits (Table S2).

Buffer Soluble and Insoluble Hg Determination. The buffer soluble (cytosolic) and insoluble (bound to cell walls and membranes) mercury (SHg and IHg) were measured by adapting a previously reported protocol.[47,48] Briefly, rinsed and frozen plant sample was first homogenized by mortar and pestle on ice with a freshly prepared buffer (100 mM HEPES pH 8.6, 1 mM PMSF, and 0.2% Tween 20 (v/v)). After the suspending liquid was centrifuged (16 000 rpm, 4 °C for 10 min), the supernatant was carefully collected. The pellet was resuspended in another extraction buffer (10 mM HEPES pH 8.6, 0.04% Tween 20 (v/v)), and washing and centrifuging were repeated six times. All six resulting extracts were incorporated into the first one. Then the SHg and IHg were isolated in the final pellet and supernatant, respectively. The Hg content was determined by the same means with the THg determination. All constituents of the buffers were in high purity to ensure a low Hg background. Blank and replicate tests were also conducted in parallel.

Calculation of Hg Return Flux. Roots of different orders have been well established to have distinct life spans. Guo et al.[42] built up an accurate calculation model for actual root biomass
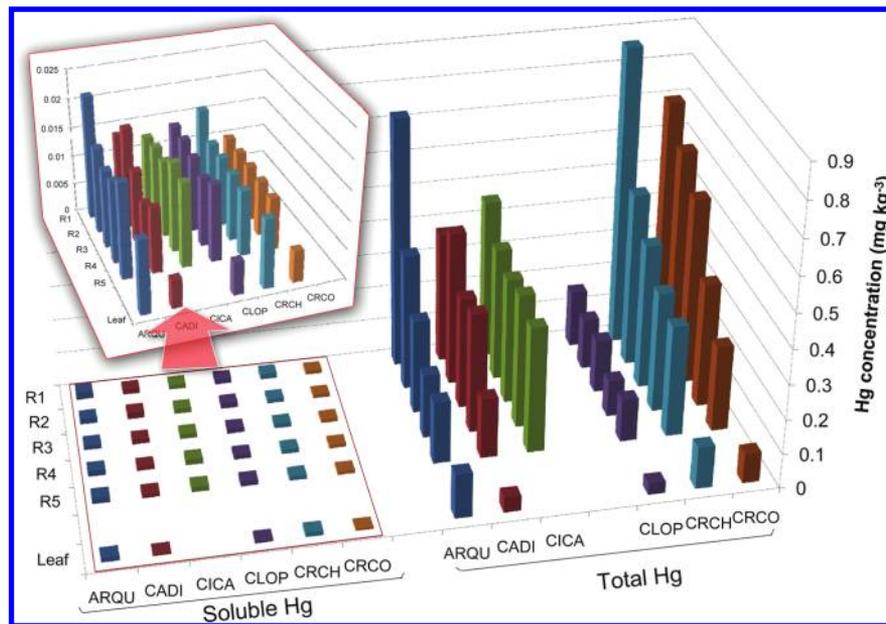
Figure 1. Root soluble and total mercury concentrations by branch order and leaf mercury concentrations in six Chinese subtropical tree species: *Ardisia quinquegona* (ARQU), *Canthium dicoccum* (CADI), *Cinnamomum camphora* (CICA), *Cleistocalyx operculatus* (CLOP), *Cryptocarya chinensis* (CRCH), and *Cryptocarya concinna* (CRCO). R1–R5 refer to root categories from the first order to the fifth order. The SE of all soluble Hg concentration was 4.5% ± 4.6% (range: 0.3–11.3%) whereas that of all total Hg concentration was 10.3% ± 11.8% (range: 2.3–31.7%).

mortality (M, in kg ha^{-1} yr^{-1}) as

$$M = \sum_{k=1}^{D} \sum_{i=1}^{N_k} \left(B_{ki} \cdot \frac{1}{L_{ki}} \right) \quad (1)$$

where k and i are the branch order and individual roots in each order, respectively; D and N_k are the total number of branch order and total number of roots, respectively; B is the root biomass (in kg ha^{-1}) and L is the root longevity (in yr).

Accordingly, we proposed the root-order based models for calculation of actual root Hg return flux (F_{Hg}, in mg ha^{-1} yr^{-1}) and estimated root Hg return flux (F'_{Hg}, in mg ha^{-1} yr^{-1}) as follows:

$$F_{Hg} = \sum_{k=1}^{D} \sum_{i=1}^{N_k} \left(C_{Hg-ki} \cdot B_{ki} \cdot \frac{1}{L_{ki}} \right) \quad (2)$$

$$F'_{Hg-k} = \sum_{k=1}^{D} \left(\overline{C}_{Hg-k} \cdot B_k \cdot \frac{1}{L_k} \right) = \sum_{k=1}^{D} \left(\overline{C}_{Hg-k} \cdot \overline{M}_k \right) \quad (3)$$

where C_{Hg-ki} and $\overline{C}_{Hg-ki}$ (both in mg kg^{-1}) are the root THg concentration of the individual root i of root order k and mean root THg concentration of root order k, respectively. Due to the difficulty to measure all individual root concentrations (i.e., there are a large number of roots of different orders, individual roots of the first order may weigh too little to be detected, and it is difficult to collect all individual roots from a big tree), we used eq 3 for estimation. In this case, the relative contribution of order root k to total root return flux (%F'_{Hg-k}) can be calculated as follows:

$$\%F'_{Hg-k} = \frac{\overline{C}_{Hg-k} \cdot \overline{M}_k}{\sum_{k=1}^{D} \overline{C}_{Hg-k} \cdot \overline{M}_k} \quad (4)$$

Besides, the estimation here was also based on four assumptions: (1) the Hg would not be recycled during both leaf and root senescence; (2) the litter biomass was in proportion to their dominances and each species constituted 5% of the total biomass mortality according to their dominance;[49] (3) the Hg concentration in growing season was representative for the mean annual Hg concentration;[12] (4) the root life span increases by a factor of 2.0 with increasing branch order according with Guo's model.[39,42] For calculation, the annual averages of biomass of leaf litter (4260 kg ha^{-1} yr^{-1}), the total biomass of litterfall (8450 kg ha^{-1} yr^{-1}) and fine root (<2 mm) biomass mortality (1590 kg ha^{-1} yr^{-1}) were cited from the long-term monitoring at the same study forest station.[49,50] All other parameters were measured from the present study.

■ RESULTS

Variations in Root Hg by Branch Order. Across all six species, the THg concentrations generally decreased with ascending root order (Figure 1), with the first-order roots ranging from 0.868 ± 0.044 mg kg^{-1} in *Cryptocarya chinensis* to 0.163 ± 0.014 mg kg^{-1} in *Cleistocalyx operculatus*, and the fifth-order roots ranging from 0.364 ± 0.018 mg kg^{-1} in *Cinnamomum camphora* to 0.121 ± 0.007 mg kg^{-1} in *Cleistocalyx operculatus*. The largest concentration difference between the first and fifth orders amounted to more than 4 fold in *Ardisia quinquegona*. However, *Cleistocalyx operculatus*, the only water-inhabiting species, had both the lowest mean root THg concentrations and standard deviation along five orders (0.142 ± 0.026 mg kg^{-1}). Across all six species, there were significant correlations ($P < 0.05$) between the mother and daughter roots (e.g., second-order and first-order roots) (Table 1). This pattern was similar to the correlations of root diameter,[43] N concentrations, and respiration rates[51] across different root orders in other tree species.

In contrast, decreasing trends of SHg concentration with root order were much less significant in most species. Root SHg concentration was less dependent on either branch order ($P > 0.1$) or tree species ($P = 0.002$) compared to root THg concentration (both $P < 0.001$) based on ANOVA, and these two

Table 1. Correlation Coefficients among Hg Concentrations of the First Five Order Roots and Leaf in Six Plant Species (R1−R5 Refer to Root Categories from the First Order to the Fifth Order)

	total Hg					buffer soluble Hg				
	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5
R2	0.76**a					0.43				
R3	0.68*	0.95**				0.49	0.60			
R4	0.42	0.78**	0.82**			0.26	−0.29	0.77**		
R5	0.51	0.49	0.62*	0.77**		0.60	0.37	0.94**	0.89**	
leaf b	0.93**	0.51	0.42	0.23	0.66	0.90**	−0.10	0.50	0.90**	0.80*

a*Correlations are significant at $P < 0.1$. **Correlations are significant at $P < 0.05$. bThe correlations of leaf Hg concentration against root Hg concentrations were based on 5 plant species because the leaf data of *Cinnamomum camphora* are lacking.

types of Hg concentrations were not significantly correlated ($P > 0.1$). Among all species, the root SHg/THg ratio generally rose with increasing order ranging from 3.5% ± 2.6% in the first order to 6.3% ± 3.1% in the fifth order, all lower than that of the leaf samples (9.1% ± 4.0%). *Cleistocalyx operculatus* had the highest ratios both in roots (9.7% ± 1.3%) and leaf (17.4%). Compared to the results of THg, the correlations of SHg concentration between mother and daughter roots in low orders were less significant (Table 1).

Leaf Hg concentration ranged from 0.033 ± 0.001 mg kg^{-1} in *Cleistocalyx operculatus* to 0.127 ± 0.006 mg kg^{-1} in *Ardisia quinquegona*, with the soluble fraction ranging from 4.8 ± 1.6 μg kg^{-1} in *Canthium dicoccum* to 0.013 ± 0.001 mg kg^{-1} in *Ardisia quinquegona*. It was significantly lower than root THg concentrations of any order in all tree species ($P < 0.01$). For example, the THg concentration of the first-order root amounted to 6.6 ± 1.8 times that of leaf. Although no significant correlation between the leaf and root THg concentration (except the first order) was observed ($P > 0.1$), the leaf SHg were significantly correlated with higher-order roots ($P < 0.1$).

Variations in Root Hg by Cortex and Stele. Although the *Ardisia quinquegona* and *Cinnamomum camphora* do not belong to the same family, they presented similar patterns in Hg species distribution in root components (Figure 2). Specifically,
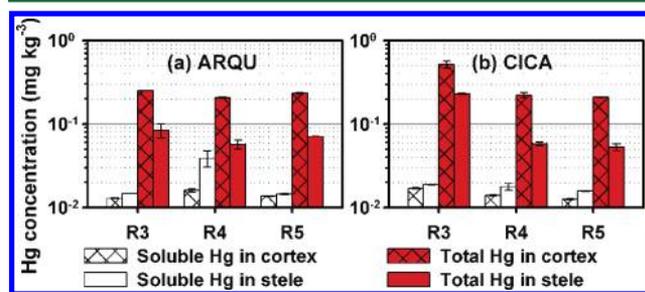

Figure 2. Root mercury concentrations in cortex and stele samples of the third-, fourth-, and fifth-order roots of (a) *Ardisia quinquegona* and (b) *Cinnamomum camphora*. Note that the trends of soluble and total Hg concentrations with root order here are not exactly consistent with those in Figure 1 because these two tests were measured from two separate sets of root samples.

the cortex always accumulated more THg in concentration compared to the stele throughout all 3 orders of both species (3.0−3.6 times differences for ARQU and 2.2−4.0 times for CICA), but contained less SHg in concentration (0.4−0.9 time for ARQU and 0.8−0.9 time for CICA). Therefore, the SHg/THg ratio of stele (ARQU: 35.3% ± 28.3%; CICA: 22.9% ± 12.7%) was consistently higher than that of cortex (ARQU: 6.3% ± 1.3%; CICA: 5.2% ± 1.7%).

Correlations between Hg Concentration and Root Characteristics. Root length, diameter, SRL, SRA, TD, and C, H, N, and S concentrations of all 6 species were measured and found to have similar trends when organized by root branch order as reported in other plant species, i.e., increasing trends for root length and diameter and decreasing trends for SRL, SRA, and root N concentration with increasing root order.[27,28] No significant correlation ($P > 0.1$) between Hg concentration and any measured root parameter except root order was observed in the water-inhibiting *Cleistocalyx operculatus*, highly different from the other 5 soil-inhibiting species. Using linear, power, and exponential models, we built up regressions for THg concentration and SHg concentration against the aforementioned 10 root traits for 5 tree species excluding *Cleistocalyx operculatus* (Tables S3 and S4). The results showed that THg had significant regressions with root order, length, diameter, SRL, SRA, and root N concentration ($P < 0.01$), whereas SHg had significant regression with only root C concentration and S concentration ($P < 0.01$) (Figures 3 and 4). To eliminate the difference caused by soil Hg, bioconcentration factor (BCF, the root THg concentration divided by soil THg concentration) was also calculated and tested for regression, yet quite similar regressions to those of THg were found (Table S3).

DISCUSSION

Mercury Absorption, Translocation, and Storage. There was up to 4-fold difference in THg concentration among different branch orders (Figure 1 and Figure S1), as hypothesized. However, contrary to our expectation, the lower-order roots generally have higher Hg concentrations than higher orders, despite the much shorter life span (i.e., shorter exposure time with the Hg-containing soil solution) and lower C level (which positively associated with Hg concentration in soil[4,33,34]) in the lower order roots. This finding suggests that during the passive Hg uptake with water flow the higher-order roots have less specific root area and thus much weaker absorptive potential,[30] as supported by the significant correlations between root THg concentration and most root morphological traits (Figure 3). These significant correlations also suggested that besides the hotspots of root tips,[18] the outer epidermis and cortex of fine roots also perform certain absorptive functions that diminish rapidly with increasing root order. The lower THg concentration in higher order roots may be due to the "dilution effect". Since more than 90% of the Hg was bound to the cell walls and membranes of fine roots (Figure 1), the Hg translocation was weak, and thus the previously absorbed Hg would be diluted to some extent as the higher-order roots grew coarser and longer.

Compared to morphological traits, root N concentration can explain a significant fraction of the variability of both Hg concentration and BCF (51.8% and 69.3%, respectively).
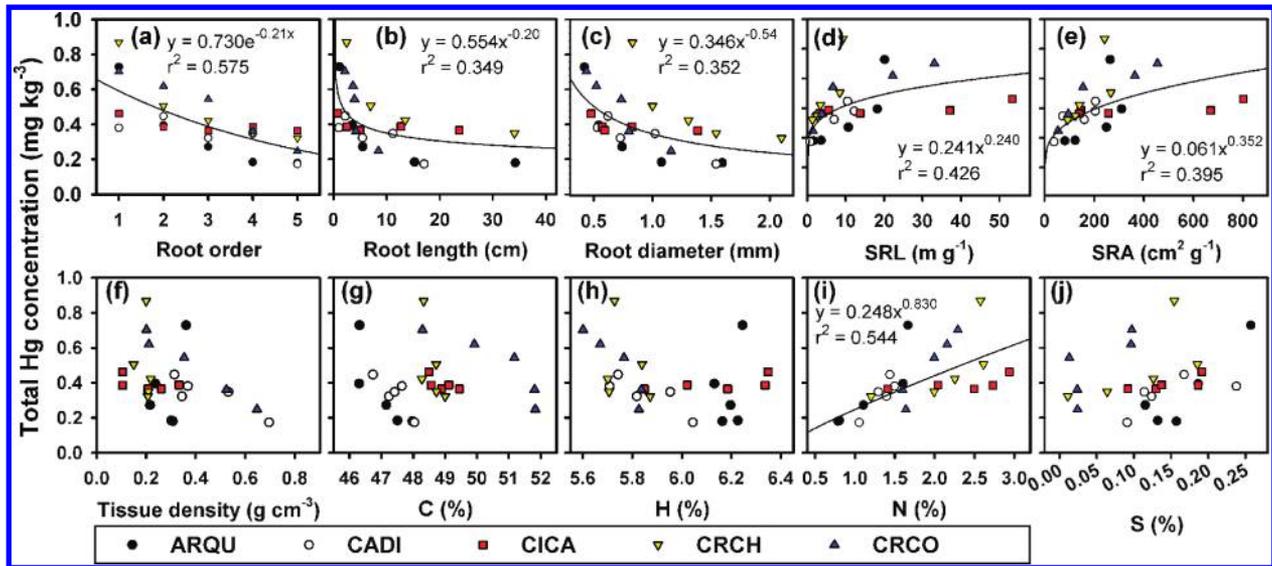
Figure 3. Relationship and best fitting regression models of root total Hg concentration against different root parameters ($n = 25$). The abbreviations used for plant species are the same as those in Figure 1.
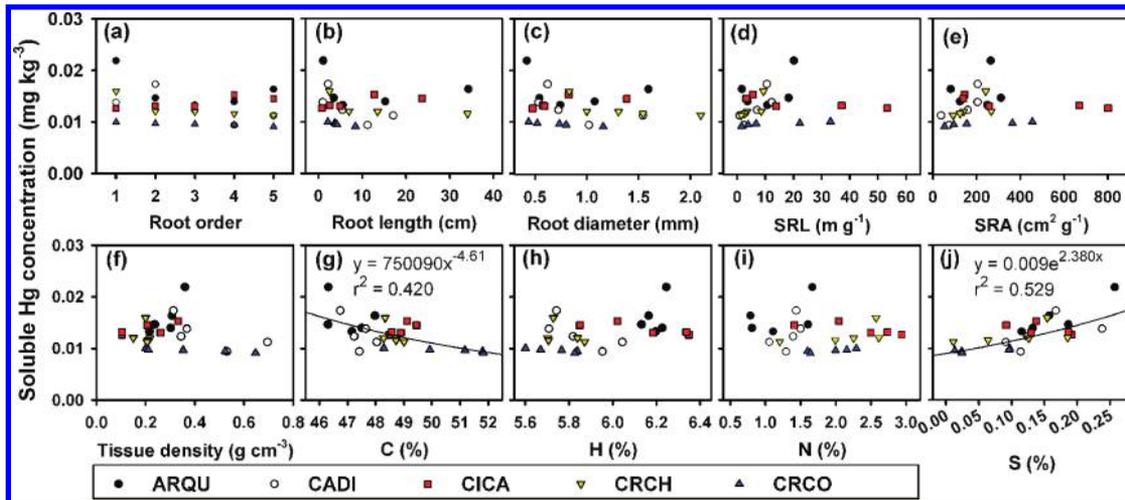

Figure 4. Relationship and best fitting regression models of root soluble Hg concentration against different root parameters ($n = 25$). The abbreviations used for plant species are the same as those in Figure 1.

We also found collinearities between root N concentration and root morphological traits (N concentration vs root diameter: $P = 0.025$; vs SRL: $P = 0.001$; vs SRA: $P < 0.001$; vs tissue density: $P = 0.008$), suggesting that the observed THg concentration may result from coabsorption or costorage of Hg with N rather than the simple effects of root morphology proposed by Cocking et al.[15] In another words, root THg concentration is likely to increase with increasing root N demand and root N uptake, and is not simply determined by passive diffusion which depends on root morphology. Indeed, Hg can be bound to transporter proteins,[37,52] making it possible to accumulate in roots accompanying to the N uptake processes.

It has been well documented that only a small amount of Hg in roots (<5%) can be translocated to the shoot in various tree species.[3,10−14] This is also confirmed by the large Hg concentration differences between roots and leaves (Figure 1) as well as the poor correlations between THg concentration of leaf and those of most roots in the species studied here (Table 1). In addition, we found no evidence for both the long-distance Hg translocation from root to leaf, and the short-distance translocation within fine roots (<2 mm) of different orders as a large proportion of Hg was bound to cell walls and stabilized (Figure 1). The weak correlations between root orders that are not directly connected across the 6 tree species (Table 1) also suggest that the root THg in certain order can not strongly influence roots 2 orders away despite the short physical distance between them.

The buffer soluble (cytosolic) fraction of Hg is quite mobile in the organisms, and thus plays a vital role in Hg translocation.[53] Here, we found that SHg concentration depended on root S and C concentrations rather than other root parameters (Figure 4 and Table S4), thus root morphological traits that may influence Hg storage may not affect Hg translocation to any large extent. The effects of root S concentration on Hg translocation (Figure 4j) may be associated with the complexation reaction of Hg in cytoplasm with the excessive cytosolic −SH groups of phytochelatins or metallothionein (competing with binding sites in cell walls or membranes). If this is true, the weak Hg translocation was at least partially due to the lacking of cytosolic −SH containing chelators. This possibility is partly

supported by the observation that although inorganic Hg was commonly recognized and used as aquaporin inhibitor,[1,54] application of mercaptoethanol or dithiothreitol usually reversed the inhibition.[55−57] The significant but relatively weak regression of SHg and root C concentration, however, may involve a series of direct or indirect biochemical mechanisms dependent on both C quality and quantity, and its principles still await further investigation.

Species-Specific Difference and Environmental Impact. The degree of heterogeneity in root Hg distribution is supposed to depend on the type of environmental media the roots inhabit. Indeed, we found that the Hg concentration and its distribution in water-inhibiting fine roots of *Cleistocalyx operculatus* were markedly different from those of other plant species (Figure 1), and did not have any significant correlation with most of the root morphological and stoichiometric traits, suggesting that the aquatic plants may have a different mechanism or pattern of root Hg uptake compared to terrestrial plants. Many possible factors may have contributed to this phenomenon. First, in our study the river water had quite low Hg concentration (<5 ng L^{-1}) for roots to accumulate. Second, Hg in natural water bodies appears primarily in soluble forms,[58,59] highly different with that in soil.[60] Third, flooding in water may lead to anoxia and regulate water transport by aquaporin gating,[61,62] thereby limiting the Hg transport. Finally, the wet habitat for this species may inhibit mycorrhizal fungi infection which may closely affect Hg uptake.[63]

On the other hand, fine root Hg heterogeneity across the remaining 5 tree species showed that the Hg concentration and distribution depend on the species identity and soil conditions ($P < 0.05$). Belonging to the same family of *Lauraceae*, *Cryptocarya chinensis* and *Cryptocarya concinna* had similar variation treads of both THg and SHg, quite different from those of *Canthium dicoccum* (belonging to *Rubiaceae*) (Figure 1) at the same sampling site. However, another *Lauraceae* species, *Cinnamomum camphora*, sampled at a different site, had an Hg pattern different from its sibling species, suggesting that soil Hg may also have influenced fine root Hg uptake and distribution. Specifically, at the location where soil Hg concentration was higher (site of CRCH and CRCO: 0.394 ± 0.041 mg kg^{-1}), the variation between root orders appeared to be more significant compared to that at a site of lower soil Hg concentration (site of CICA: 0.237 ± 0.017 mg kg^{-1}). This may be attributable to the more bioavailable soil Hg causing inhibition of water channels to the larger extent.[35−37]

The similar impact of soil Hg concentration on variation of Hg concentration between roots of different diameter categories in *Asclepias syriaca* was also observed by Cocking et al.[15]

Roots Respond Differently to Hg Uptake. Whereas the lower-order roots that have large specific surface area and N concentration were proved to be the most critical for Hg absorption, the significant correlations between SHg concentrations of leaf and higher-order roots rather than lower-order roots (Table 1) suggested that higher-order roots mainly provided structural and transport functions, which is consistent with the heterogeneity of water uptake within fine roots.[30,64] Besides, the results of Hg cellular partitioning in cortex and stele suggest that the outer cortex serves as the major storage pool of IHg or THg (the cortex Hg mass accounts for 84−89% of the total root Hg amount in either the third-, fourth-, or fifth-order roots) probably by providing more binding sites (higher root S concentration in cortex than in stele, data not shown), whereas the stele primarily functions as the pathway of upward transportation for SHg by providing the xylem structure. As the current knowledge about the physiological function of root orders is scant,[30] this study further supports the increasingly recognized functional heterogeneity within the branching root system of fine roots.

Errors Caused by Root Hg Heterogeneity. Roots have been intensively studied for environmental monitoring and assessment, ecological remediation (e.g., hyperaccumulator screening), ecophysiology, and even food safety. However, ignoring the root heterogeneity in certain species may lead to substantial error. Scientists often collected parts of the roots as samples to represent the population, especially for large tree species that have extensive root systems. The fine roots, especially the first-order roots are usually very small (some may be <0.5 mm in diameter), rather easy to break off, and thus difficult to collect during sampling. Here, we found if the first-order roots were excluded from the samples, the overall mean fine root Hg concentration would be underestimated by 1.6−19.3% (mean $\pm$ SE: 9.0% $\pm$ 7.5%). Thus, here we advise that the most distal roots should be carefully sampled in future studies to improve the accuracy of the measurements.

Estimated Fine Root Hg Return Flux Vs Litterfall Flux. Mercury concentrations in roots are commonly much higher than in shoots in many forest ecosystems,[10,14,47,53,65] which suggests forest roots constitute a large Hg pool. As our calculation showed (Table 2), the fine root Hg return flux (12.0−53.5 mg ha^{-1} yr^{-1}) caused by root mortality significantly

Table 2. Estimated Hg Return Flux of Leaf, Litterfall, and Fine Roots (Assuming Each Species Constitutes 5% of the Whole Forest) and the Relative Contribution of Different Root Orders to Fine Root Return Flux

	estimated Hg return flux (F'_{Hg-k} in mg ha^{-1} yr^{-1})			relative contribution of root orders (%F'_{Hg-k} in %)				
species	leaf litter	litterfall[a]	fine roots	R1	R2	R3	R4	R5
ARQU	27.1	53.8	35.8	61.9	22.1	3.8	3.5	8.6
CADI	9.2	18.3	31.3	47.2	40.6	8.1	3.4	0.8
CICA			32.3	38.6	22.1	12.6	14.0	12.8
CLOP	7.0	13.9	12.0	54.4	26.8	10.2	4.5	4.1
CRCH	25.7	51.0	53.5	71.2	14.4	8.0	3.4	3.0
CRCO	18.2	36.1	49.9	60.2	24.9	10.6	2.7	1.6
mean[b]	17.4	34.6	36.5	59.0	25.8	8.1	3.5	3.6
SE[b]	9.2	18.3	16.6	8.9	9.5	2.7	0.6	3.1

[a]The litterfall Hg flux was calculated assuming the total litterfall had the same Hg concentration with leaf litter, which may slightly overestimate the true values because leaf litter commonly had higher Hg concentration compared to other litters such as fruit or twig. [b]Values of *Cinnamomum camphora* were not taken into calculation for means or standard errors.

surpassed the leaf litter return flux (7−27.1 mg ha^{-1} yr^{-1}), and was at least comparable to the estimated litterfall Hg flux (13.9−53.8 mg ha^{-1} yr^{-1}), which are inconsistent with our second hypothesis. These large fluxes indicate that there is not a net Hg input from pedosphere to phytosphere in the upland forest but the Hg exchange in the rhizospheric interface is rather dynamic. After being absorbed and stabilized by roots, the majority of Hg in roots is difficult to translocate to more vulnerable shoots,[48] and is returned to soil when roots die, so that the plant will not continuously accumulate Hg in vivo to high and toxic levels. This route for Hg removal should be especially important for perennial tree species to respond to potential Hg stress and maintain their long survival (up to 400 yr in the present study) despite continuously absorbing Hg-containing soil solution. Furthermore, the fine roots of lower order, which have shorter longevity,[31,41,66] have larger contribution to the root Hg return flux (Table 2). Particularly, the first-order roots accounted for only 17.4% ± 6.0% of the root biomass, but contributed 49.3% ± 6.7% of root biomass mortality and 59.0% ± 8.9% of total fine root Hg return (all 5 orders combined). Sacrificing the minimum biomass (lower-order roots) to realize maximum Hg return seems to be an effective metabolism for Hg removal, which may also be an important route for Hg detoxification in trees.

Based on the branch order approach, underground Hg biogeochemical processes were explored and quantified, and the potential limiting factors were identified. Within the root branch architecture, the Hg accumulation and turnover was highly heterogeneous with the lower-order roots accumulating higher Hg concentration and contributing disproportionately larger return flux across all 6 species. Our results also showed that the metabolism of lower-order roots was an important route for tree species to effectively and rapidly remove Hg that was previously taken up. Because this study examined a limited number of plant species in a specific subtropical forest environment, it is unclear whether the mechanism identified here also applies to other natural environments. Future studies on root−Hg interaction concerning more ecosystems and plant species will likely expand our knowledge on underground Hg biogeochemistry at larger scales.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information
Figures S1 and S2, and Tables S1−S4. This information is available free of charge via the Internet at http://pubs.acs.org/.

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