Fine Root Branch Orders Contribute Differentially to Uptake, Allocation, and Return of Potentially Toxic Metals

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Supporting Information

ABSTRACT: Growing evidence has revealed high heterogeneity of fine root networks in both structure and function, with different root orders corporately maintaining trees’ physiological activities. However, little information is available on how fine root heterogeneity of trees responds to environmental stresses. We examined concentrations of seven potentially toxic metals (Cr, Ni, Cu, Zn, As, Cd, and Pb) within fine root networks and their correlations with root morphological and macro-elemental traits in six Chinese subtropical trees. The contributions of different orders of roots to fine-root metal storage and return were also estimated. Results showed no consistent pattern for the correlation among different metal concentration against root traits. Unlike root metal concentration that generally decreased with root order, root metal storage was commonly lowest in middle root orders. Root senescence was at least comparable to leaf senescence contributing to metal removal. Although the first-order roots constituted 7.2–22.3% of total fine root biomass, they disproportionally contributed to most of metal return fluxes via root senescence. The two distinct root functional modules contributed differentially to metal uptake, allocation, and return, with defensive (lower-order) roots effectively stabilizing and removing toxic metals and bulk buffering (higher-order) roots possessing a persistent but diluted metal pool. Our results suggest a strong association of physiological functions of metal detoxification and metal homeostasis with the structural heterogeneity in fine root architecture.

INTRODUCTION

Fine roots in a forest ecosystem are of great interest due to their disproportionately important role in the global carbon (C) and nitrogen (N) cycling, as well as key functions for maintaining normal tree physiology. Although fine roots have been long considered the same as all roots of less than an arbitrary diameter (such as 2 mm), increasing evidence based on the root branching approach has shown that a fine root network is a highly heterogeneous structure in morphology, anatomy, chemistry, and physiology. These studies have shown that the most distal roots (or the lower-order roots) commonly possess the following characteristics: smaller root diameter and length, higher specific root area and specific root length, lower stele proportion, higher mycorrhizal colonization rate, higher N concentration, lower C (also cellulose) concentration, stronger absorption ability, higher respiration rate, and shorter longevity. These root traits across different branch orders endow them with distinct functions for nutrient and water absorption to maintain the tree’s physiological activities; that is, low-order roots play a key role for water uptake, while high-order roots mainly provide transport and structural functions. Despite recent progress, little research in fine root heterogeneity has been conducted on how these roots corporately respond to environmental stresses. Among various potential stresses in the natural environment, metal stresses may escalate rapidly resulting from urbanization and industrialization, especially in rapidly developing countries. As the phytotoxic effects have been well identified, ranging from DNA damage and organ development inhibition to individual mortality, metal deposition has been considered an important contributor to forest decline (e.g., in Europe and North America), leading to considerable losses in plant
productivity. Moreover, heavy metals accumulated in edible plant organs also pose significant health risks through the food chain to human beings, giving rise to some regulations on metal concentrations in edible plants. Highly sensitive to metal stresses, the root is the paramount organ for plant metal detoxification and tolerance by limiting the root-to-shoot translocation through metal stabilization and metal removal. As fine roots possess the most absorptive capacity and highest turnover rate, exploring the metal distribution patterns along the root longevity gradient within the branching architecture would provide us a more quantitative understanding of the metal biogeochemical cycle and an explanation of the role of roots in this process.

Recently, Wang et al. reported that a considerable amount of mercury (Hg) was retained in lower-order roots within the fine root structure, and its uptake may be associated with N demand or root morphological traits. However, the allocation dynamics for different metal species within the plant are highly varied due to their different physical and chemical properties. Moreover, the tree species commonly requires trace amounts of essential metal elements (e.g., Cu, Zn, and Ni) but no nonessential elements (e.g., Cr, Cd, Hg, and Pb). Specifically, Cu is of great importance for reproductive growth and root metabolism, facilitating protein utilization; Zn participates in membrane integrity, enzyme activation, and gene expression; while Ni is required for urea metabolism, iron absorption, and N fixation. To satisfy its physiological demand, the plant may have developed diverse strategies for absorbing different metal species. However, whether the root uptake, allocation and return among various metal species depends upon fine root architecture is poorly understood.

In the present study, we investigated the concentration, content, and return flux of seven metals (Cr, Ni, Cu, Zn, As, Cd, and Pb) by branch order in fine roots of six Chinese subtropical tree species and analyzed the relation and difference across roots of different orders. Three hypotheses were examined: (1) As fine root systems are increasingly revealed to be highly heterogeneous branching networks instead of hitherto considered homogeneous, the root metal concentration and content should decrease with increasing root order within fine roots because lower-order roots commonly have higher absorptive ability. (2) Because lower-order roots have much shorter longevity, they are supposed to contribute more to root metal return. (3) Due to diverse properties of different metal species, the root metal heterogeneity across different orders and its controlling factors should not be the same.

### MATERIALS AND METHODS

#### Study Site and Sample Collection.
This study was conducted at Dinghu Mountain (23°09′~23°11′N, 112°30′~112°33′E) in the middle of Guangdong Province, China. The local mean temperature in summer and winter are 28 and 12.6°C, respectively, and the average annual precipitation amounts to 1929 mm. The zonal soil type is latosolic red soil with low soil pH (3.84~4.27), SOM 3.55~5.76% (total N: 0.26~0.36%; total S: 0.05~0.06%), annual mean relative humidity (81.5%), and redox potential (400~800 mV). Six plant species, including Ardisia quinquegona (ARQU), Cryptocarya concinna (CRCO), Canthium diococcus (CADI), Cinnamomum camphora (CICA), Cryptocarya chinensis (CRCCH), and Cleistocalyx operculatus (CLOP), were selected and sampled in mid-August 2010 from four sites in mixed evergreen broadleaf forests.

Three-to-five fine root networks of each species containing at least five root orders were collected from soil blocks (except Cleistocalyx operculatus, collected from a river), gently rinsed with Milli-Q water, and carefully cleaned using Teflon-coated forceps under 10X magnification as previously described. At the same time, rhizospheric soils of each species, mature leaves (except the leaf of Cinnamomum camphora, which was too high to collect), and water from river flow where the roots of Cleistocalyx operculatus grew, were also sampled.

#### Root Morphology Determination.
Cleaned root networks were dissected carefully into different branch orders according to Pregitzer et al.; that is, the distal nonwoody branch order was categorized as the first order. Average root length, root diameter, and individual biomass (lyophilized for 48 h), tissue density (TD), specific root area (SRA), and specific root length (SRL) of different orders were measured or calculated. To calculate the metal partitioned in cortex and stele, we took parts of the third-, fourth-, and fifth-order roots of Ardisia quinquegona and Cinnamomum camphora, carefully subsampling them by separating the cortex and stele and recording their dry weights.

#### Macro-elements and Trace Metal Determination.
All solid samples were lyophilized, ground, and sieved through a 200-mesh screen. The concentrations of C, N, H, and S in dry weight were measured by elementary analytical instrument (Vario EL CUBE). Metal concentrations in each root order were determined by ICP-MS after microwave digestion (Ethos 1; operation conditions illustrated in Table S1), following EPA methods 3052 and 6020A. Samples were measured at least in triplicate. The accuracy and precision of the analytical method was checked with Certified Reference Materials of GBW10020 (plant sample) and GBW07403 (yellow soil), as well as by spike tests. All quality control met EPA limits.

#### Calculation of Metal Return Fluxes.
Root longevity of different orders can be highly different within fine root networks, making it an important parameter to be included in the return flux calculation. Here, we adopted a previously proposed rough model for the estimation of root metal return fluxes (F_M in mg ha⁻¹ yr⁻¹) and relative contribution of order root k to total root return flux (%F_Mk) as follows:

\[
F_M^r = \sum_{k=1}^{D} \left( C_{M-k} B^r_{k} \right)^{-1} \sum_{k=1}^{D} (C_{M-k} M_{k})
\]

\[
%F_{M-k} = \frac{C_{M-k} M_{k}}{\sum_{k=1}^{D} C_{M-k} M_{k}}
\]

where \( C_{M-k} \) is the root metal concentration in mg kg⁻¹; \( k \) is the branch order; \( D \) is 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). For estimation, the life growth rate of each root order was assumed to be 2.0 based on Guo's model and the annual averages of biomass of leaf litterfall (4260 kg ha⁻¹ yr⁻¹), total biomass of litterfall (8450 kg ha⁻¹ yr⁻¹), and fine root (<2 mm) biomass mortality (1590 kg ha⁻¹ yr⁻¹) were obtained from the reported literature. Notably, this model did not consider the possible recycling of trace metals by the plant during root senescence and thus may slightly overestimate the accurate fluxes.

#### Statistical Analyses.
The mean, standard deviation (SD), and coefficient variance (CV) of all roots of five orders for each plant species were calculated. The translocation factor (TF) from the first-order root to the fifth-order root for each species was also calculated as
Differences between metal concentrations among metal species, plant species, or root orders were determined using ANOVA and a post hoc Tukey’s test. When parameters did not conform to normal distribution and homogeneity of variance, Kruskal–Wallis tests and nonparametric Tukey-type multiple comparison tests were used. To explore the relation between root metal concentrations and root morphological and macroelement traits, nonparametric Spearman’s correlation analysis was performed. The levels of significance for all tests were set at 0.05. Hierarchical cluster analysis for each plant species was conducted using Ward’s method, based on squared Euclidean distance.
distance measure for roots of different orders by using different metal species as variables. The metal concentrations were all standardized to eliminate the different weights of various metal species before cluster analysis was conducted. Two groups within the cluster analysis were identified by segregating the data by certain rescaled distance.

## RESULTS

### Variations in Root Metal Concentrations and Contents by Branch Order.

The root metal concentration for different plant species is summarized in Table 1. Some of them had much higher concentrations than the critical toxicity level (CTL) in plants \(^{33,34}\) (e.g., the Cu in CICA (\(>3\) times CTL) and the Zn (\(>4\) times) and the Pb (\(>10\) times) in ARQU). Regardless of different metal species, most root metal concentrations generally decreased with root order across all six plant species with few exceptions (Figure 1 and Table 1), such as basically remaining constant (Cu in CAD1), slightly increasing with root order (Cr in CRCH), or having no obvious trends (Ni, Zn, As in ARQU). The root metal concentration was highly dependent on soil conditions (Supporting Information Figure S1) and tree species (Table 1), with distinctive ranking orders for different metals. This translocation factor can well explain the variation coefficient of the root metal concentration (\(R^2 = 0.884\)), reflecting the heterogeneous degree of fine root metal concentration. From the results of regression analysis of metal concentrations across different orders, it was found that as the distance between root orders increased the difference between metal concentration of higher order and that of lower order consistently decreased. At the same time the significance of regression gradually declined (Supporting Information Figure S3). Within the same order, root metal concentrations in the cortex were much higher than those in the stele in the third-, fourth-, and fifth-order roots of both ARQU and CICA (\(P < 0.05\); Figure 2).

Metal contents of different root orders were calculated from the root metal concentrations multiplied by the root biomass (Figure 3). They did not simply increase or decrease with root order but had lowest values in a middle root order in most cases. Specifically, root orders contributing the lowest metal content for ARQU, CICA, CLOP, CRCH, and CRCO were all located between the second order and the fourth order only, except Cu, Zn, and As in CICA. Although CAD1 had the lowest metal storage in the highest order of the first five orders we studied, it still had the lowest root metal storage in the middle root order within the whole root system. This was because the coarse root metal storage of any higher root order (\(\geq 6\)th order) was examined to be higher (data not shown).

### Correlations between Metal Concentration and Root Traits.

Correlations between fine root metal concentrations and root length, root diameter, specific root area, specific root length, tissue density; and root carbon, nitrogen, and sulfur (S) concentrations are listed in Supporting Information Table S2. Root Cu concentration highly correlated with root N concentration and most root morphological traits. Root Zn, Cd, and Pb concentrations significantly correlated with C and S concentrations (\(P < 0.05\)), and Zn concentration also correlated with root length and root diameter significantly (\(P < 0.05\)). Root Ni and C concentrations were significantly correlated (\(P < 0.05\)), whereas As concentration only significantly correlated with tissue density (\(P < 0.05\)). Root Cr concentration did not show significant correlation with any root traits studied (\(P > 0.05\)).

### Variations in Root Metal Return Flux by Branch Order.

Metal return fluxes of leaf and fine roots were comparable with variations less than 2 orders of magnitude (Table 2). Generally, the Cr, Zn, Cd, and Pb return fluxes contributed by fine roots were higher than those by leaves with only two exceptions: Cr for CRCH and Cd for CRCO. However, the difference between leaf and root return fluxes of Ni, Cu, and As varied by tree species. The relative contribution of different root orders to fine root metal return flux did not depend on sampling location, tree species, or metal type (all \(P > 0.1\)) but decreased dramatically with root order (\(P < 0.001\); Figure 4). The first-order roots contributed the highest return percentages ranging from 40% to 70%, much higher than any other order. More specifically, among six species, the metal return fluxes contributed by first-order roots were as follows: Cr: 53% ± 5.1%; Ni: 57.0% ± 10.7%; Cu: 56.5% ± 9.0%; Zn: 57.2% ± 9.1%; As: 52.4% ± 10.6%; Cd: 57.3% ± 9.1%; and Pb: 60.4% ± 13.8%, respectively.

### Cluster and Regression Analysis by Branch Order.

Based on the cluster analysis of root metal concentrations across different root orders of the six tree species, most could be divided into two sharply different categories at <10% rescaled distance (except CLOP at about 12%) (Supporting Information Figure S2). Generally, the most similar roots were mother and daughter roots, which were mutually connected, such as the last two orders of ARQU, CICA, and CRCH, the first two orders of CADI and the third-and fourth-orders of CLOP. The boundary location highly differed for different plant species, for example, located between the third and fourth orders for ARQU but between the first and second orders for CICA (Supporting Information Figure S2).

## DISCUSSION

### Metal-Specific Controlling Factors on Fine Root Heterogeneity.

To our knowledge, no previous report has linked multiple metal concentrations with fine root traits of different branch orders to explore the mechanism of fine root metal uptake. Fine roots are quite small but the major absorptive fraction of the whole root system.\(^{1,5}\) If metals were proportionally

\[\text{Figure 2. Metal concentrations of cortex and stele samples of the third-, fourth-, and fifth-order roots of (a) Ardisia quinquegona and (b) Cinnamomum camphora. *The Ni concentration of the forth-order root of CICA was lower than the detection limit. The error bars refer to standard deviation of three replicates.}\]
absorbed with soil solution, then they should follow a similar concentration pattern and depend on similar fine root traits. However, we observed metal-specific concentration along the root orders \((P < 0.05;\) Figure 1) and no consistent pattern for the correlation among different metal concentrations against root traits (Supporting Information Table S2), indicating that the controlling factor of fine root metal uptake is highly metal specific even within the <2 mm fine root networks.

The fine root metal uptake is unavoidably influenced by the metal bioavailability which was affected by texture and chemical composition of the soils.\(^{35,36}\) A comparison of the first-order root metal concentrations and soil metal concentrations is shown in Supporting Information Figure S1. Most of the metal species, especially the essential elements Cu and Zn, had much higher concentrations in the first-order roots than in soils, implying high bioabsorption and bioaccumulation of these metals. Among the nonessential elements, Cr, As, and sometimes Pb concentrations showed opposite phenomena, which may have been caused by the low bioavailability in soils or their relatively weak translocation ability. However, Cd had the considerable bioaccumulation probably due to its well-known higher mobility.\(^{37,38}\) CRCH and CRCO, both belonging to Lauraceae and growing at the same site, had the same ranking of relative abundance of metals (Supporting Information Figure S1). However, their patterns were quite different from CICA at another site, even though CICA also belongs to the family Lauraceae. The dependence of root metal concentration on sites \((P < 0.05)\) indicated that soil properties should have contributed to this difference of root metal concentration between sites; for example, the higher soil Pb level in Site 2 caused much higher root Pb levels in Site 2 than those in Site 1 (Supporting Information Figure S1). Compared to CRCH and CRCO, the

Table 2. Estimated Metal Return Flux (mg ha\(^{-1}\) yr\(^{-1}\)) Contributed by Leaf and Fine Roots

<table>
<thead>
<tr>
<th>Plant</th>
<th>Fine Roots</th>
<th>Leaf</th>
<th>Fine Roots</th>
<th>Leaf</th>
<th>Fine Roots</th>
<th>Leaf</th>
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<th>Leaf</th>
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<td>217</td>
<td>423</td>
<td>461</td>
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<td>CADI</td>
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<td>4265</td>
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<td>3907</td>
<td>9770</td>
<td>2292</td>
<td>4255</td>
<td>3384</td>
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<tr>
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<td>34860</td>
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<td>13103</td>
<td>15880</td>
<td>17252</td>
<td>16315</td>
<td>37175</td>
</tr>
<tr>
<td>CLOP</td>
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<td>5210</td>
<td>2481</td>
<td>759</td>
<td>1624</td>
<td>828</td>
<td>1939</td>
<td>7175</td>
</tr>
<tr>
<td>CRIA</td>
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<td>105</td>
<td>190</td>
<td>189</td>
<td>129</td>
<td>2528</td>
<td>33</td>
<td>70</td>
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<tr>
<td>CRCO</td>
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<td>1394</td>
<td>3827</td>
<td>101</td>
<td>915</td>
<td>20650</td>
<td>278</td>
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<td>565</td>
<td>10411</td>
<td>18660</td>
<td>18660</td>
<td>18660</td>
</tr>
</tbody>
</table>

*Values of Cinnamomum camphora were not taken into calculation for means or standard errors.

Figure 3. Percentages of fine root metal contents in different orders of six tree species. The abbreviations used for plant species are the same as those used in Figure 1.
Root metal uptake was probably affected by the metals' chemical properties as well. The essential elements Zn, Cu, and Ni showed commonly higher bioaccumulation in first-order roots from soil compared to the nonessential elements (Supporting Information Figure S1), which highlighted a remarkable discrimination for plants from higher-order root to lower-order root versus decreasing concentrations, together with the decreasing slope with increasing root order with few exceptions (Figure 1). These hypothesized, root metal concentrations generally decreased from their lithophile and siderophile nature, respectively. Cr showed rather different concentration did not correlate with any root traits and Ni correlated to root C and S concentrations, similar to that of observation that root Zn, Cd, and Pb concentrations all showed commonly higher bioaccumulation in first-order roots (Supporting Information Figure S3), suggesting that root metal absorption also depended on plant species besides soil properties.

Metal Concentration along Root Order Gradient. As we hypothesized, root metal concentrations generally decreased with increasing root order with few exceptions (Figure 1). These decreasing concentrations, together with the decreasing slope from higher-order root to lower-order root versus first-order root (Supporting Information Figure S3), indicated that the metal uptake capability commonly dropped with increasing root order. Consequently, the translocation factor from the first- to fifth-order roots was a potential indicator for the variability (heterogeneity) of fine root metal concentrations ($R^2 = 0.884$, $P < 0.001$), besides its original function to represent the translocation ability from lower-order roots to higher-order roots.

Here, the significance of linear regression between root metal concentrations of different orders continuously decreased with increasing order interval (Supporting Information Figure S3), for example, dropping from 0.99 between the first and second orders (no interval) to 0.90 between the first and fifth orders (four-order interval). In this case, the $R^2$ between the mother and daughter roots was always higher than nonconnected roots (Supporting Information Figure S3), which agreed with the correlations of different root orders continuously decreased with increasing order interval (Supporting Information Figure S3), for example, dropping from 0.99 between the first and second orders (no interval) to 0.90 between the first and fifth orders (four-order interval). In this case, the $R^2$ between the mother and daughter roots was always higher than nonconnected roots (Supporting Information Figure S3), which agreed with the correlations of root diameters, N concentrations and respiration rates, and Hg concentrations across different root orders in various tree species. Our results, together with the mentioned previous reports, consistently show that the physical connection within fine roots is the determinant for their chemical connection.

Metal Allocation along Root Order Gradient. Unlike root metal concentration that agreed with our hypothesis, root metal content (i.e., metal storage) did not simply increase or decrease with root order, but commonly had the lowest value in a middle root order (from the second to fifth order depending on different plant species; Figure 3). The difference in root heterogeneity between metal storage and metal concentration was largely influenced by the irregular biomass distribution patterns across root orders. Although biomass distribution across root orders often differed in various plant species, most of them follow a similar pattern, i.e., decreasing or remaining similar in the first several root orders and then increasing rapidly in subsequent root orders to the tap root. Resulting from this biomass distribution pattern and the decreasing trend of metal concentrations with root order, most root metal was allocated to both lower-order roots and higher-order roots but not to some middle-order roots.
In addition, root metal storage is much higher in the cortex than in the stele. For instance, the metal content in ARQU’s cortex accounted for 87.0% ± 7.2% \((n = 21)\) of the total. The cortex metal content was much higher because its abundant cellulose, hemicellulose, pectins, and glucoprotein (containing abundant negative charges) can serve as metal binding sites or cation exchangers, compared with the stele with many vascular cylinders. It has been well documented that the metal immobilization and stabilization of roots were important to plant metal tolerance,\(^{40,45,46}\) and the cortex was found in this study to play an important role in the process.

**Metal Return along Root Order Gradient.** Root metal return is an important process at least comparable to leaf metal return contributing to metal removal as shown in the estimated fluxes (Table 2). The relative contribution of root metal return flux is highly branch-order specific \((P < 0.001)\) and not influenced by sampling location, tree species, or metal category \((all \ P > 0.1)\). Lower-order roots disproportionately contributed to most of the metal return fluxes via root senescence. For example, although the ephemeral first-order roots only constituted 7.2–22.3% of total fine root biomass, they were estimated to contribute 53.0% ± 5.1% of Cr, 57.0% ± 10.7% of Ni, 56.5% ± 9.0% of Cu, 57.2% ± 9.1% of Zn, 52.4% ± 10.6% of As, 57.3% ± 9.1% of Cd, and 60.4% ± 13.8% of Pb return fluxes, respectively, highlighting the important role of first-order roots for tree detoxification as we have proposed.\(^{43}\) In addition, lower-order roots were recently found to be more palatable to herbivores.\(^{44}\) If it is true for most tree species, then the lower-order roots with high metal concentration consumed by herbivores would also contribute to tree detoxification.

**Root Modules-Linking Function to Structure.** Xia et al.\(^{41}\) recently proposed the concept of “ephemeral root modules” based on the highly synchronous changes in anatomical, nutritional, and physiological patterns of *Fraxinus mandshurica*. Due to the continuous change of metal concentration with root order, the similarity-based cluster analysis could provide a boundary dividing the first five root orders into two distinct categories at a certain rescaled distance, indicating certain “modularity” for metal distribution within fine root networks. This threshold of rescaled distance is a potential indicator for fine root modularity, with lower rescaled distance for classification showing the higher significance of fine root modularity. In the present study, although the five root orders of all six tree species could be divided into two different categories, the boundary location varied greatly for different tree species (Supporting Information Figure S2), suggesting the modularity is highly plant species specific. The boundary line seems to be physically close to the lowest-metal-containing root order for most plant species (Figure 3 and Supporting Information Figure S2). For example, ARQU had boundary a line between the third and fourth order; the lowest-metal containing root order was either the third or fourth order. Similar patterns are also shown in CADI, CICA, and CLO, but this observation is less significant in both CRCH and CRCO.

Two modules divided by a boundary line seem to have distinct strategies in response to the potentially toxic metal stress, namely defensive roots (lower-order roots) and buffering roots (higher-order roots). Defensive roots absorbed and stabilized the majority of metals by possessing the highest metal concentrations so that metal translocation upward to higher-order roots and vulnerable shoots could be limited. They also returned the metals quickly back to the soil via rapid root senescence\(^{9,28,29}\) so that metals could be effectively removed and the plant would not accumulate metals to toxic levels. As we have proposed,\(^{23}\) preferentially sacrificing high-metal-concentration defensive (lower-order) roots to largely remove toxicants at the low expense of biomass mortality is likely to be an important strategy for tree detoxification. Buffering roots (higher-order roots) provided a vast amount of biomass to dilute metal concentration once the metals reached them and thus largely relieved the potential stress despite the longer metal residence in these roots. They provided good buffering effects for the lower-order roots and constituted large and diluted phytospheric metal pools and continued to reduce upward metal transportation. Compared with defensive roots, buffering roots have much more persistent metal pools and are of greater importance in maintaining metal homeostasis.

Because this study only examined several plant species in a specific subtropical forested biome, it is not yet known whether the fine root metal heterogeneity and the corresponding controlling factors identified here also apply to other natural environments. Future studies on more ecosystems and plant species at larger spatial and temporal scales and controlled mechanistic studies are desirable to expand our knowledge on the underground metal biogeochemistry and its molecular mechanisms.

### ASSOCIATED CONTENT

Supporting Information
Figures S1, S2, and S3 and Tables S1 and S2 as described in the text. This information is available free of charge via the Internet at http://pubs.acs.org/.

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The authors declare no competing financial interest.

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