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Earthworm impacts on trace metal (Al, Fe, Mo, Cu, Zn, Pb) exchangeability and uptake by young *Acer saccharum* and *Polystichum acrostichoides*

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Abstract Non-native earthworms are a continued source of environmental change in the northeastern United States that may affect trace metals in the plant-soil system, with largely unknown effects. We assessed earthworm impacts on exchangeable and strong acid extractable (total) concentrations and pools of Al, Fe, Cu, Zn, Mo, Pb in non-point source polluted, forest soil horizons (Organic, A, and B) and foliar metals concentrations in young (< 3 years) *Acer saccharum* and *Polystichum acrostichoides* at four proximal forests in the Finger Lakes Region of New York. We observed decreasing total trace metal Organic horizon pools and increasing total trace metal A horizon concentrations as a function of increasing earthworm biomass. Earthworms had limited effects on exchangeable concentrations in A and B horizons and total metal concentrations in the B horizon. Foliar trace metal concentrations in *Acer* were better explained by earthworm biomass than soil concentrations but foliar concentrations for *Polystichum* were poorly predicted by both earthworm biomass and soil metal concentrations. Our results suggest that earthworms can affect trace metal uptake by some plants, but not by increasing soil trace metal exchangeability or from changing soil properties (pH, %SOM, or cation exchange capacity). Instead, non-native earthworms may indirectly alter understory plant uptake of trace metals.

Keywords Micronutrients · Trace elements · Aluminium · Foliar tissue · European earthworms

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Introduction

Trace metals are ubiquitous in terrestrial environments, originating primarily from weathering of minerals and dust deposition (Essington 2015; Adriano 2001; Hue et al. 1986) but their biogeochemical cycles have been influenced by domestic and industrial sources, (Kabata-Pendias 2001; Steinnes and Friedland 2006; Richardson et al. 2015; Van Hook et al. 1977; Adriano 2001). Trace metals that function as micronutrients (Cu, Mo and Zn) are essential for plant growth because of their role as enzyme cofactors and oxidation–reduction reactions (Kabata-Pendias 2001). Loss of micronutrients can reduce growth of economically important tree species such as *Acer saccharum* Marsh. (sugar maple) (St. Clair et al. 2008; Dauer et al. 2007; Berger et al. 2009). However, at elevated concentrations, trace metals may cause toxicity in plants and animals due to induction of biochemical and physiological stress. For example, Heale and Ormrod (1982) found that elevated Cu concentrations in soil caused interveinal chlorosis, severe leaf drop, and stunted leaf size in red maple (*Acer rubrum*) and yellow-stem dogwood (*Cornus stolonifera* 'Flaviramea') seedlings.

Changes to trace metal sequestration in forest soils by decreased accumulation or increased mobilization may impact vegetation (St. Clair et al. 2008; Richardson and Friedland 2016), terrestrial invertebrates (Milleret et al. 2009; Richardson et al. 2015), or downslope aquatic ecosystems (Campbell et al. 2009). Over the past few hundred years, human activities have distributed non-native earthworms from Europe and Asia throughout many forest ecosystems in the northeastern United States, resulting in alterations to soil structure and function (Scheu and Parkinson 1994; Bohlen et al. 2004). In forests soils, earthworms increase decomposition of the Organic horizon and vertically mix the organic and mineral soil horizons (Bohlen et al. 2004; Marhan and Scheu 2006; Frelich et al. 2006; Hale 2008). The effects of earthworms on biogeochemistry of elements in soils is dependent on soil type, earthworm ecophysiology (epigeic, endogeic, anecic), time of invasion, and earthworm community composition (Bohlen et al. 2004; Suárez et al. 2006; Filley et al. 2008; Laossi et al. 2010; Görres and Melnichuk 2012).

We know comparatively little about earthworm effects on trace metal biogeochemistry in forest soils compared to carbon and nitrogen since previous studies of earthworm effects on soils have primarily focused on changes to C, N, and P (e.g. Bityutskii et al. 2002; Bohlen et al. 2004; Suárez et al. 2004; Hale et al. 2005; Wironen and Moore 2006; Costello and Lamberti 2009). Earthworms may affect trace metal accumulation in soil. For example, accelerated decomposition of the Organic horizon could decrease retention of metals by decreasing soil organic matter (SOM) and total cation exchange capacity (CEC) of a soil and disrupt physical protection of organometallic compounds (Berggren and Mulder 1995; Jones and Kochian 1996; Suárez et al. 2006; Sizmur and Hodson 2009; Bityutskii et al. 2012).

Furthermore, earthworms may impact trace metal bioavailability and uptake by plants (e.g. Lukkari et al. 2006). Understory plants play a foundational role in forest trophic interactions (Gilliam 2014). Exposure of understory plants to trace metals could serve as sources of micronutrients and pollutants to herbivores (e.g. Scheifler et al. 2006). Moreover, some plants may have a higher tolerance or actively phytoaccumulate pollutant trace metals (e.g. As accumulating ferns; Zhao et al. 2002). Bityutskii et al. (2012) found *Aporrectodea caliginosa* (Savigny) increased the mobility and uptake of micronutrients (Fe, Mn, and Zn) in cohabitating cucumber plants in a mesocosm study. Thus, earthworms can potentially influence trace metal accumulation and retention in soils, with potential impacts for uptake by plants but with species specific effects. Earthworms also have been shown to affect plant competition by reducing species diversity and promoting certain plant species over others by changing germination and plant fitness (Lukkari et al. 2006; Wurst et al. 2005; Laossi et al. 2011; Hale et al. 2005). Northeastern North American understory plants have been particularly affected, as earthworms consume and mix the characteristic deep Organic horizons upon which these communities have established (Bohlen et al. 2004).

Our study investigates how biogeochemical changes from earthworm invasion affects plant biochemistry and how nutrient and potentially toxic metals are cycled. First, we sought to explore how earthworms impact trace metals concentrations (metal mass per soil mass) and pools (metal mass per volume) in forest soils. Trace metal concentrations provide information on abundance in samples, but pools provide absolute changes at the ecosystem level. This is of importance as concentrations cannot observe volumetric differences in trace metals. We studied both micronutrient (Fe, Cu, Zn, and Mo) and potentially toxic (Al and Pb) trace metals. Secondly, we aimed to quantify if earthworm disturbance affected trace metal uptake by two common understory plant species in a typical hardwood forest that we expected to have differing responses to earthworms: *A. saccharum* (herein referred to as *Acer*) and *Polystichum acrostichoides* (Michx.) Schott (herein referred to as *Polystichum*). *Acer* is among the most ecologically, economically and culturally important tree species in northeastern North America (Godman, et al. 1990). It is potentially sensitive to earthworm invasion due to nutrient and pH requirements, reliance on soil symbionts, and dependence on organic horizons for germination (Cooke et al. 1993; Kolb and McCormick 1993; Côté et al. 1995; Horsley et al. 2002; Juice et al. 2006) *Polystichum* is a common evergreen fern that we expected to benefit from earthworm invasions and be released from competition from other understory species due to its low nutrient requirements and low mycorrhizal specificity (Bard 1946; West et al. 2009). We tested two hypotheses regarding the influence of earthworms on trace metal biogeochemistry in forest soils: (1) earthworm presence decreases exchangeable and total metal concentrations and pools in Organic, A and B horizons; and (2) earthworms decrease uptake of metals by understory plants. We expected these decreases in both plants and soil to be due to decreased SOM and higher pH in earthworm-invaded plots.

Materials and methods

Study area

We studied four proximal forests (Bobolink Hill (BOB), Hammond Hill State Forest (HAM), Ringwood Preserve (RIN) and Yellow Barn State Forest (YB)) in Tompkins and Tioga counties in the Finger Lakes Region of New York State (Fig. 1). All four forest sites fall within the Allegheny section of the Appalachian Plateau at 42.4°N, − 76.5°W. Soils are Fragiaquepts and Dystrochrepts in the Mardin and Volusia series (SoilWeb, USDA-NRCS & UC Davis California 2010). Soil textures ranged between loamy and sandy loam. Land use history varied between forests, however all plots within a forest had similar land-use history. Mixed hardwood canopies were predominantly closed (Leaf Area Index 5–7), and dominated by *A. saccharum*, *Fagus grandifolia* Ehrh. and *Quercus rubra* L. In each of the four forests we selected six plots (15 m in diameter) representing an earthworm gradient from uninvaded areas devoid of earthworms or earthworm impact to earthworm-invaded areas at each site (a total of 24 plots). Each plot contained both *Acer* and *Polystichum* individuals in the understory. Sample plots were upland in geomorphic positions that were low angle (0–3°) shoulder or foot slopes and were approximately down-slope linear and cross-slope convex to linear.

Seedling and earthworm sampling

In July 2016, we collected one to three *Acer* saplings (10–30 cm high) and one reproductive *Polystichum* individual in each of the six plots at each site. At this stage, both species were rooting in Organic and upper 15 cm of the A horizon. We rejected any plants with signs of senescence or damage from insects or disease. Foliar samples from each plant (2–5 leaves) were triple-rinsed and air-dried in a greenhouse for 1 week. We separated leaves (removing petioles and stems) and ground each sample using a coffee grinder (Fresh Grind, E160BY, Proctor Silex, Southern Pines, NC, USA).

We sampled earthworms at 10 m intervals along two diagonal transects encompassing the seedling sampling locations (n = 5 earthworm samples per transect) in autumn 2012, 2013 and 2015 using liquid mustard extraction (3 g powdered mustard 3.79 L^{-1} water) poured into a 0.5 × 0.5 m sampling frame (Lawrence and Bowers 2002). We collected all surfacing earthworms and preserved them in formalin for 48 h before transferring them into ethanol for storage. We then pooled all 10 samples from within each sampling area and dried them at 60 °C for 72 h to determine dry biomass. We used the 3-year mean of this pooled earthworm biomass for analyses to minimize influence of annual variability and microhabitat heterogeneity.

Soil sampling

At each plant sampling plot, we excavated one soil monolith near the approximate center of each plot. To avoid direct effects from mature trees and surface erosion, monoliths were at least 1 m away from any trees and drainage areas. For the excavation, we

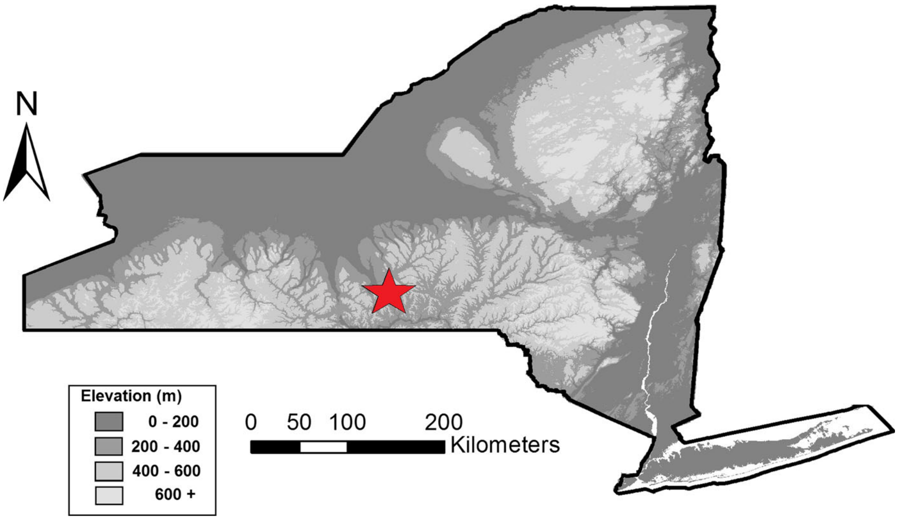

Fig. 1 Digital elevation map of New York State, USA with study area denoted with the red star. (Color figure online)

randomly placed a 15 × 15 cm template on the soil surface and then cut the Organic horizon using a stainless steel handsaw. We removed soil surrounding the template to expose the soil monolith, noting soil horizon depths and morphological features. We then collected the soil monolith by each master horizon. In the laboratory, we air dried each master horizon (Organic horizon, A horizon, and B horizon) and sieved to < 2 mm. We quantified rock mass (> 2 mm) and root mass (> 1 mm) for each soil horizon. We calculated bulk density by determining dried rock, root, and soil mass by field depth measurement and area for each master horizon. We also determined fine-fraction soil density by dividing the air dried, sieved soil mass for each horizon by the horizon depth measured in the field. Only the fine-fraction soil density was used to determine soil trace metal pools, particularly since large rocks have much less surface area for trace metal sorption.

Plant and soil analyses

We measured soil pH using a buffered extraction using 4 g of soil to 10 g of 0.01 M $CaCl_2$ solution for a 2:5 soil–water gravimetric ratio, shaking for 1 h and allowing to equilibrate for 2 h. We centrifuged this solution at 3000 rpm for 30 min and measured pH of the supernatant using a pH meter (8015 VWR). To measure % soil organic matter (SOM), we oven-dried soil subsamples at 60 °C for 48 h, followed by grinding to a homogeneous powder (DynaCrush; Customer Laboratory Inc., Orange City, FL, USA) and sieved to < 1.18 mm. We weighed 4 g of soil subsamples down to ± 0.001 g, combusted samples at 600 °C for 6 h in a muffle furnace, and then re-weighed them to determine mass loss.

To characterize exchangeable trace metal fraction and cation exchange capacity (CEC), we extracted 2 g of soil using 20 mL of 1 M ammonium acetate (Ciesielski et al. 1997). Samples were shaken for 1 h and allowed to equilibrate for 24 h. We centrifuged resulting slurries at 3000 rpm for 30 min and decanted the extraction solution. Total trace metal concentrations in soil and plant samples were determined using a strong acid digestion following EPA method 3051 A. This method is often referred to as a pseudo-total digestion because of the inability to dissolve crystalline silicates and other refractory compounds. Since our study focuses on plant available trace metals not in silicate minerals, we henceforth refer to pseudo-total concentrations as total concentrations. In the digestion method, we air-dried and digested 0.500 g of material in 5 ml of 8 M reverse aqua regia (9:1, HNO_3: HCl) at 90 °C for 45 min on an insulated hot plate in sealed Teflon vials. We further diluted digestate and analyzed via Spectroblue ICP-OES (SPECTRO Analytical Instruments, Kleve, Germany) for total soil samples but analyzed total plant tissues and exchangeable soil concentrations using an Element 2 ICP-MS (Thermo Fisher Scientific, Waltham, MA, USA).

Every 25 samples included a digestion blank, a duplicate and two standard reference materials (SRM). We used matching sample matrices using Montana

Soil 2711 for mineral soil samples and Peach Leaves 1547 for plant tissue samples (National Institute of Standards and Technology, Gaithersburg, MD). The %relative standard deviations (RSDs) among SRMs and duplicate samples were $< 8\%$ for all total concentrations in plants and soils and $< 9\%$ for exchangeable soil concentrations. Preparation blanks for plant and soil analyses had elemental concentrations below detection limit for Al, Fe, Cu, Zn, Mo, Pb. As expected, total concentration recoveries for Montana Soil SRM were between 60–80% for all nutrients particularly due to indigestion of silicate and other residual compounds. Plant leaf tissue recoveries for Peach Leaves SRM via ICP-MS analysis were Al (92%), Fe (87%), Cu (106%), Zn (103%), Mo (86%), and Pb (97%).

Data analyses

We did not explore relationships between organic horizon concentrations and soil physical and chemical properties because many plots were lacking an Organic horizon ($n = 14$). Instead, we considered organic horizon trace metal pools ($n = 24$). To determine Organic horizon pools, Organic horizon trace metal concentrations were multiplied by the average dry mass of the Organic horizon over the 225 cm^2 templates. To determine A horizon and B horizon trace metal pools, trace metal concentration data was multiplied by their fine-fraction bulk density. We also compared summed total soil profile trace metal pools, which were calculated as summation of organic, A, and B horizon pools. Because some soil monoliths were deeper than others, the summed depth of A and B horizons were adjusted to fit a 0–20 cm total depth interval, primarily by only considering a limited portion of the total B horizon depth.

We tested the association of earthworms with (1) exchangeable and total soil concentrations and pools, (2) *Acer* and *Polystichum* foliar concentrations, and (3) trace metal sorption promoting properties using generalized linear mixed models (GLMMs) with forest site as a random effect in all models. We analyzed trace metal concentrations in the A ($n = 24$) and B horizons ($n = 20$) with earthworm biomass and soil properties (CEC, pH, %SOM).

Again using GLMMs, we tested the effects of soil variables on earthworm biomass, with forest site as a random effect. We examined the relationship between foliar trace metal concentrations and soil properties (earthworm biomass, CEC, root biomass, pH, %SOM, exchangeable trace metal concentrations, and total trace metal concentrations) within the A horizon using multiple regressions. Variables found to be significant from GLMM analyses and all soil variables in the A horizon were utilized in two sets of multiple regressions to explain foliar concentrations in *Acer* and *Polystichum*. GLMM analyses were performed in MATLAB (Matlab 2016, Mathworks, Natick, MA).

Results

Earthworm community

Three-year mean dry earthworm biomass ranged from 0.0 to 9.1 g per 2.5 m^{-2}, (cumulative total of ten 0.25 m^{-2} annual samples) in our plots. Each site had all three ecophysiological groups (anecic, epigeic and endogeic) present (Supplemental Fig. 1). Each group was present in similar proportions across sites, with the exception of the most heavily invaded site (Hammond Hill), which had a higher proportion of endogeic earthworms (Supplemental Fig. 1). Earthworm biomass was correlated with higher soil pH ($R = 0.57$, $p < 0.05$) and soil CEC ($R = 0.59$, $p < 0.05$) but the latter was driven by a single data point and when removed was no longer significant. Earthworm biomass was not predicted by total root biomass or %SOM for both A and B horizons ($p > 0.10$). The composition of earthworms was primarily European with some Asiatic species. *Lumbricus terrestris* L. and *Lumbricus rubellus* Hoffmeister were the most numerous earthworms at all sites, while species in the *Aporrectodea calignosa* complex (*Aporrectodea tuberculata* Eisen, *Aporrectodea calignosa* Savigny, *Aporrectodea trapezoids* Dugés) were co-dominant with *Lumbricus* sp. at Hammond Hill and Bobolink Hill (Dobson and Blossey 2015). *Octolasion tyrtaeum* Savigny, *Octolasion cyaneum* Savigny, *Aporrectodea rosea* Savigny and *Dendrobaena octaedra* Savigny and *Dendrodrillus rubidus* Savigny were also observed across the sites. Additionally, we sporadically found *Amynthas agrestis* Kinberg (Yellow Barn and Ringwood) and *Lumbricus castaneus* Savigny at Yellow Barn only. Within sites, low-earthworm biomass plots included *D. octaedra*, *D. rubidus* and *L. rubellus* (for further information about earthworm populations see Dobson and Blossey 2015).

Soil physicochemical properties

Forest soil physical properties were generally similar among the four forests. The Organic horizon ($\sim$ 3.4 cm), A horizon ($\sim$ 7 cm) and B horizon ($\sim$ 11 cm) thicknesses were similar among forests ($p > 0.10$; Table 1). However, Organic horizon areal mass was lower with increasing earthworm biomass ($R^2 = 0.49$, $p < 0.01$) but A horizon thickness was higher with increasing earthworm biomass ($R^2 = 0.32$, $p < 0.01$). Soil pH was lowest in the Organic horizons (pH 3.5) and highest in B horizons (pH 4.9) (Table 1). Similarly, %SOM was highest in the Organic horizons ($\sim$ 53%) and lowest in the B horizons (14%) (Table 1). Conversely, areal soil mass and CEC were lowest in the Organic horizons and greater in the A and B horizons (Table 1). Total root biomass was not significantly different between A and B horizons for each forest site ($p > 0.05$) (Table 1). Soil pH and %SOM were significantly correlated with each other in the A and B horizons ($p < 0.05$; data not shown). Similarly, %SOM and CEC were both significantly correlated with each other in the A and B horizons ($p < 0.05$; data not shown; using separate GLMMs). However, pH, %SOM, and CEC were not significantly correlated in the Organic horizons ($p > 0.10$; data not shown).

Exchangeable and total metal concentrations and pools

Overall, exchangeable trace metal concentrations (metal mass per soil mass) generally did not vary in A and B horizons as a function of earthworm biomass while total trace metal concentrations in A horizons increased with increasing earthworm biomass (Table 2). Only exchangeable Al and Fe

Table 1 Soil properties at four forest sites

	Depth (cm)	Total root biomass ($kg\ m^{-2}$)	Soil pH (pH units)	%SOM ($g\ g^{-1}$)	CEC ($kmol_c$)	Areal soil mass ($kg\ m^{-2}$)
Bobolink Hill						
Organic horizon	2.1 ± 0.9	N/A	3.4 ± 0.4	54 ± 4	0.3 ± 0.1	3.7 ± 1.5
A horizon	8.7 ± 1.8	1.3 ± 0.4	3.8 ± 0.3	12 ± 1	4.2 ± 1.2	91 ± 17
B horizon	12.0 ± 2.0	0.9 ± 0.2	4.8 ± 0.3	17 ± 3	1.2 ± 0.4	69 ± 11
Hammond Hill State Forest						
Organic horizon	4.3 ± 1.8	N/A	3.5 ± 0.3	51 ± 8	0.3 ± 0.0	3.5 ± 1.4
A horizon	9.2 ± 1.7	0.8 ± 0.2	4.2 ± 0.4	11 ± 2	2.3 ± 0.9	63 ± 13
B horizon	9.3 ± 2.1	0.4 ± 0.1	5.1 ± 0.3	14 ± 2	1.5 ± 0.7	102 ± 21
Ringwood preserve						
Organic horizon	3.2 ± 1.7	N/A	3.7 ± 0.3	51 ± 7	0.4 ± 0.1	5.4 ± 2.5
A horizon	6.5 ± 2.8	0.9 ± 0.3	4.3 ± 0.3	8 ± 2	2.7 ± 1.1	55 ± 14
B horizon	9.7 ± 1.8	1.0 ± 0.3	4.8 ± 0.4	11 ± 2	6.1 ± 1.3	101 ± 16
Yellow Barn State Forest						
Organic horizon	4.1 ± 1.6	N/A	3.6 ± 0.3	59 ± 6	0.3 ± 0.0	4.2 ± 1.1
A horizon	4.0 ± 1.4	1.6 ± 0.4	3.9 ± 0.3	8 ± 1	1.2 ± 0.5	38 ± 8
B horizon	11.7 ± 3.0	1.5 ± 0.5	5.0 ± 0.4	12 ± 2	1.5 ± 0.3	109 ± 17

Data are means ± 1 SE with n = 6 per site, except for organic horizons, where n = 3 per site. %SOM (soil organic matter) was measured using LOI (loss-on ignition)

CEC (Cation Exchange Capacity) was determined using 1 M NH_4Ac extraction concentrations decreased as earthworm biomass increased in A horizons (Table 2; Supplemental Fig. 2). Total Pb concentrations in A horizons were lower as earthworm biomass increased but total Al, Fe, Zn, and Mo concentrations in A horizons increased as earthworm biomass increased (Table 2; Supplemental Fig. 2). In B horizons exchangeable and total trace metal concentrations did not vary as a function of earthworm biomass except for exchangeable Fe (Table 2; Supplemental Fig. 3). Organic horizons trace metal concentrations were not compared across plots because they were absent at many earthworm invaded plots (however, we provide organic horizon metal concentration in Supplemental Table 1).

We compared soil trace metal pools (metal mass per unit area) along the earthworm biomass gradients. Exchangeable Organic horizon pools decreased as earthworm biomass increased for Fe, Cu, Zn, and Mo (Fig. 2). Total Organic horizon pools of Al, Fe, Cu, Zn, Mo, and Pb decreased as earthworm biomass increased (Fig. 2). In A horizons, exchangeable trace metal pools did not respond to changes in earthworm biomass while total Al, Fe, Cu, Zn, and Mo pools increased as earthworm biomass increased (Supplemental Fig. 4). For B horizons, exchangeable and total pools were unaffected by changes in earthworm biomass (Supplemental Fig. 5). When considering metal pools for the entire soil profile (summed Organic, A, and B horizons for the upper 20 cm), we observed only summed exchangeable Al pools decreased but summed total Zn and Mo pools

Table 2 R^2 values for generalized linear mixed models for exchangeable and total soil metal concentrations (A and B horizons) as a function of soil and ecosystem properties

	Earthworm biomass	Total root biomass	%SOM	pH	CEC
Exchangeable A horizon concentrations					
Al	0.40(−)	–	–	0.24(−)	–
Fe	0.41(−)	–	–	–	–
Cu	–	–	0.35(−)	–	–
Zn	–	–	–	–	–
Mo	–	–	–	–	–
Pb	–	–	–	–	–
Total A horizon concentrations					
Al	0.45(+)	–	–	–	–
Fe	0.37(+)	0.38(−)	–	–	0.27(+)
Cu	–	0.26(−)	0.33(+)	–	–
Zn	0.40(+)	–	–	–	–
Mo	0.32(+)	–	–	–	–
Pb	0.35(−)	–	–	–	–
Exchangeable B horizon concentrations					
Al	–	–	–	–	–
Fe	0.27(−)	–	–	–	–
Cu	–	–	–	–	–
Zn	–	–	–	–	–
Mo	–	–	–	–	–
Pb	–	–	–	–	–
Total B horizon concentrations					
Al	–	–	0.26(+)	–	–
Fe	–	0.37(+)	–	–	–
Cu	–	–	–	–	–
Zn	–	0.26(−)	–	–	–
Mo	–	–	–	–	–
Pb	–	–	0.34(+)	–	–

Only significant models ($p < 0.05$) are presented and (+) indicates a positive relationship while (−) denotes a negative relationship

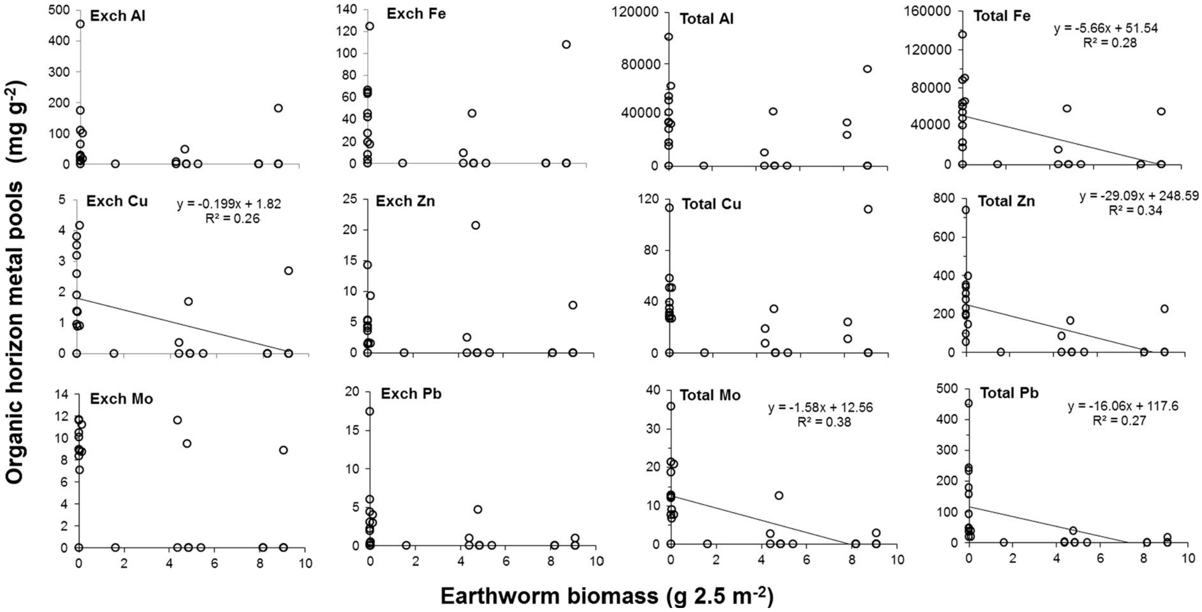

Fig. 2 Organic horizon exchangeable (Exch) and total metal pools (mg m^{-2}) as a function of earthworm biomass (3 years mean of ten, 0.25 m^2 quadrats per site per year) at all 24 sample plots across four forests. Only significant regressions ($p < 0.05$) are shown

increased with increasing earthworm biomass (Supplemental Fig. 6).

Overall, exchangeable and total trace metal concentrations in the A and B horizons generally did not correspond with differences in %SOM, soil pH, total root biomass, or CEC using GLMMs (Table 2). The A horizon exchangeable Al concentrations decreased as soil pH increased and exchangeable Cu concentrations decreased with increasing %SOM (Table 2). Total trace metal concentrations exhibited significant trends among A horizons with increasing earthworm biomass.

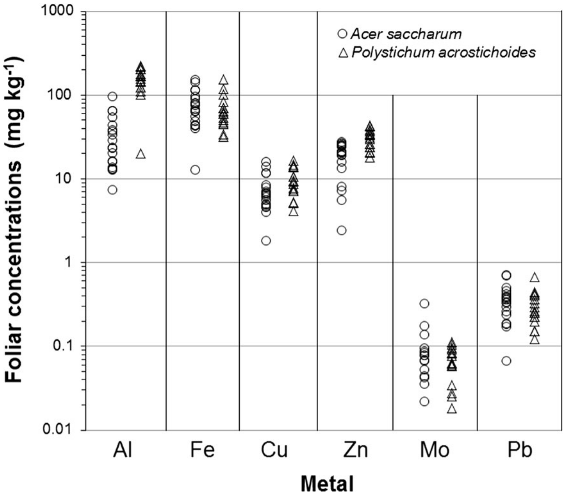

Fig. 3 Foliar metal concentrations (mg kg^{-1} dry weight) for *Acer saccharum* and *Polystichum acrostichoides* sampled at 4 sites

Foliar trace metal concentrations

Foliar concentrations of trace metals varied by over an order of magnitude for *Acer* and *Polystichum* (Fig. 3). Foliar concentrations were comparable for Fe, Cu, Mo, and Pb between *Acer* and *Polystichum* (Fig. 3). However, foliar Al and Zn concentrations were significantly greater for *Acer* than *Polystichum*, (non-parametric Wilcoxon rank-signed test, $p < 0.05$). Foliar trace metal concentrations were not affected by variations in A horizon total root biomass, soil pH, %SOM, and CEC. The only exceptions were the negative relationships between soil pH and foliar Zn concentrations for *Polystichum* and total root biomass and foliar Fe concentrations for *Acer* (Table 4).

Earthworm biomass was able to explain 29–47% of the variation in foliar trace metal concentrations for *Acer* (Table 4), while multiple regressions using all variables found to be significant with GLMMs explained 43–72% of the variation in *Acer* foliar metal concentrations (Table 5). Earthworm biomass was only a significant variable for *Polystichum* foliar Zn concentrations (Table 5). GLMMs of significant variables were not significant for Al, Fe, and Pb. However, using all soil variables (earthworm biomass, exchangeable and total metal concentrations, total root biomass, pH, %SOM, and CEC) increased explanatory power of the multiple regressions for Al, Fe, and Pb up to 0.37, 0.47, and 0.38% (Table 5). Moreover, 98% of the variation in foliar Zn concentrations for *Polystichum* was explained using all variables in the multiple regression model (Table 5).

Discussion

Earthworm effects on soil trace metals

Our results indicate that earthworms have decreased exchangeable and total trace metals pools in Organic horizons and their activities appear to have shifted metal distributions downward, from the organic horizon to the A horizon, leading to increased total trace metal concentrations and especially pools (Fig. 2, Table 2). We did not present trace metal concentrations for the Organic horizon because comparable concentrations in the Organic horizon cannot be considered as evidence for a lack of change, for example in the thickness or volume of the Organic horizon. This finding supports our hypothesis that earthworms are affecting trace metals in soils and agrees with observations from previous studies, especially Resner et al. (2011). In their study, Resner et al. (2011) found enhanced downward transport of atmospheric radionuclides during earthworm invasion along disturbance gradients in a hardwood forest in north central Minnesota. These effects appear to be limited to the upper-most portions of the soil, since we did not detect significant relationships of earthworm biomass and B horizon exchangeable and total concentrations or pools.

On the basis of lower Organic horizon mass, decreased Organic horizon total trace metal pools, and higher A horizon trace metal concentrations and pools all as a function of higher earthworm biomass, we conclude that earthworms are consuming the organic horizon while translocating trace metals into the A horizon. Epigeic and endogeic (*Lumbricus* spp. and *Aporrectodea* spp.) species are known to consume organic matter and mix materials in surface soil horizons, and were widespread in our plots (Bohlen et al. 2004; Suárez et al. 2006; Sizmur et al. 2011; Snyder et al. 2011; Görres and Melnichuk 2012; Ziemba et al. 2015). We consider the possibility that earthworms chose soils with lower Organic horizon pools and greater A horizon concentrations unlikely as plots at each site had similar physical and chemical soil properties (Table 1), were located in close geomorphic proximity to each other at each forest site, and we avoided sampling sites with variations in microtopography. Thus, we conclude that earthworms have decreased trace metals in the Organic horizon and increased their concentration and pools in A horizon. However, the source of the substantially increased Fe, Zn, and Mo pools in the A horizon remains unclear. Translocation of trace metals from the organic horizon to the A horizon is likely an important source. However, it only accounts for a fraction of the increases in the A horizon, as organic horizon trace metal pools were 1–2 orders of magnitude smaller than A horizon pools. Earthworms are likely promoting increased trace metal retention in surface mineral soil horizons through other mechanisms by increasing the thickness and mass of the A horizon (Sizmur and Hodson 2009; Sizmur et al. 2011; Richardson et al. 2017).

We observed limited changes in trace metal exchangeability in mineral soil horizons, which contradicts previous findings that earthworms increase trace metal exchangeability (e.g. Sizmur and Hodson 2009). We primarily attribute this difference to our focus on non-point source polluted, forest soils with limited disturbance. Existing literature on the effect of earthworms on metal biogeochemistry has primarily focused on point-source contaminated soils in mine-tailings (e.g. Morgan and Morgan 1992; Ruiz et al. 2009; Sizmur et al. 2011) or disturbed soils either from collection and mixing or perturbations for agriculture (e.g. Sizmur et al. 2011; Bityutskii et al. 2012). These previous studies were conducted in locations that have 10–1000 times greater soil metal concentrations than soils investigated in our study or found earthworm activity can increases trace metal mobility and availability in soils that received metal amendments or point source contamination from mining and other industrial activities (e.g. Wen et al. 2004; Ruiz et al. 2009; Sizmur et al. 2011; Bityutskii et al. 2012). Our study is one of few to examine non-point source contaminated or metal amended forest soils (Ernst et al. 2008; Richardson et al. 2015). By studying natural soils, our data suggest that the trace metals undergoing natural biogeochemical cycling in forest soils are more resilient to mobilization than those affected by point source soil pollution or transformative human manipulation. Further, it cautions against generalizing earthworm effects obtained studying metal cycling in contaminated soil to less polluted field soils.

Our data showed that earthworms are decreasing Al accumulation in Organic horizons and are increasing total Al concentrations and pools in the A horizon due to translocation and downward mixing from the organic horizon or potentially upward mixing from the B horizon. An alternative explanation is that earthworms avoided areas with higher Al. Earthworms are known to respond to soil pH and calcium availability, which is inversely related to Al concentrations (Cronan and Grigal 1995; Kobe et al. 2002; Reich et al. 2005; Dobson et al. 2017). In addition, exchangeable Al is toxic for earthworms. Exchangeable Al concentrations of < 10 mg kg^{-1} water soluble Al has been observed to cause $> 50\%$ earthworm mortality under laboratory conditions (Zhao and Qui 2010). For Pb, earthworms may also be enhancing leaching out the Organic and A horizons, on the basis of negative correlation between earthworm biomass and total Pb pools in the organic horizon and concentrations in the A horizon. Lead is strongly sorbed to SOM and dissolved transport in soil is very limited as an exchangeable or dissolved phase (Miller and Friedland 1994; Johnson et al. 1995; Kaste et al. 2006). For this reason, earthworms would likely need to physically mix the Organic and A horizons to facilitate greater downward movement.

Similar to Al, an alternative explanation for the negative relationship between earthworms and Pb concentrations and pools may be that earthworms prefer soils with lower Pb. However, the Pb concentrations were likely not high enough to induce toxicity. Earthworms are capable of sustaining populations in soils with 2–2000 times higher Pb concentrations (e.g. Morgan and Morgan 1989; Fraser et al. 2011; Nannoni et al. 2014; Richardson et al. 2015). This is due to decreasing bioaccumulation at higher Pb concentrations because of removal by inorganic ligands within digestive tissues (e.g. Morgan and Morgan 1989) and calciferous glands that can excrete Pb within $\mathrm{CaCO_3}$ nodules (Fraser et al. 2011). We conclude earthworms have decreased the retention of Pb in the Organic and A horizons.

Soil trace metals and physicochemical properties

We expected changes in exchangeable and total soil trace metals to be directly tied to shifts in soil physicochemical properties. Our results demonstrate that earthworms significantly decreased the mass of the Organic horizon and increased thickness of the A horizon. However, earthworm biomass was not related to variations in chemical properties that influence sorption and retention of trace metals in soils (CEC or %SOM) for both A and B horizons ($p > 0.10$). We conclude that we did not observe evidence that earthworm abundance substantially changed forest soil chemical properties generally responsible for trace metal adsorption and retention in soil. Earthworms may not been present for a sufficient duration to substantially to impact the soil properties at our sites to the extent observed in late-stages of earthworm invasion (Resner et al. 2011). It is generally recognized that in late-stages of earthworm invasion, soils have marked decreases in %SOM and increased soil pH (Hale et al. 2005; Suárez et al. 2004; Sizmur and Hodson 2009). Earthworms may require many years to inflict change in forest soils, particularly Organic horizons. Richardson et al. (2016) noted that after 80 days of habitation, *L. rubellus* and *A. agrestis* did not significantly impact the exchangeability of nutrients or toxic metals in the Organic horizon soil materials in a laboratory experiment. Instead of affecting the sorption and retention, earthworms may influence the processes that govern the rate trace metals move into and out of the A horizon. Our set of variables was not exhaustive enough to detect the mechanism earthworms are changing that have affected trace metal biogeochemistry (e.g. we did not measure aggregate strength, hydrologic soil properties, dissolved organic carbon concentrations, etc.). Earthworms can even affect the microbial communities associated with trace metal redox and soil metabolic processes (Wen et al. 2004; Sizmur et al. 2011). Even in controlled column experiments, the exact mechanism of earthworms altering trace metal mobility has been difficult to demonstrate experimentally (Sizmur et al. 2011). Thus, there are many ways earthworms can influence the processes that control trace metal retention in the A horizon without affecting dominant adsorption mechanisms such as CEC or %SOM.

Earthworm influence on plant trace metal uptake

Our results suggest that *Acer* and *Polystichum* were affected by earthworms, with differences in metal concentraitons for Organic and A horizons. The uptake of micronutrients (Fe, Cu, Zn and Mo) by *Acer* were negatively impacted by earthworm abundance (Fig. 4; Tables 3, 4). Conversely, *Polystichum* uptake of trace metal concentrations was not strongly tied to earthworm presence or trace metals in any soil horizon (Table 4). Our results bolster arguments by Wurst et al. (2005); Laossi et al. (2011), and other investigations that conclude certain plant species benefit from earthworms while others remain unaffected or were negatively affected. In our study, trace metal uptake by *Acer* was negatively impacted while *Polystichum* was largely unaffected. These different responses shed light on the potential responses understory plant species may have to earthworm invasion, with *Acer* generally being negatively affected and ferns such as *Polystichum* benefitting or being unaffected by earthworm presence (Lawrence et al. 2003; Corio et al. 2009; Laossi et al. 2010; Burke et al. 2011; Dobson et al. 2017). *Polystichum* may be buffered from the influence of earthworms on understory habitat because it has lower micronutrient requirements and can associate with a broad spectrum of arbuscular mycorrhizal species (Siccama et al. 1970; Lawrence et al. 2003; Milleret et al. 2009; West et al. 2009). In addition, *Polystichum* may have a higher tolerance to excess Al than *Acer*.

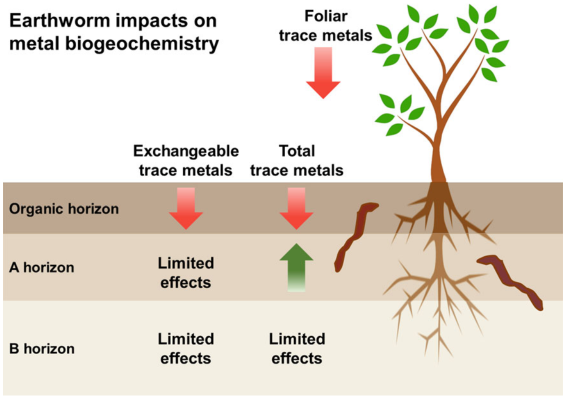

Fig. 4 Conceptualization of conclusions from our results of earthworm impacts on metal biogeochemistry for young *Acer saccharum*

Earthworm biomass was a strong predictor of variations in *Acer* foliar metal concentrations (Table 5). When combined with Organic horizon metal pools, A horizon metal concentrations, %SOM, CEC, soil pH, only explained and additional 1–22% of the variation in foliar trace metal concentrations compared to the explanatory power using earthworms biomass alone (Table 5). Earthworms were a more dominant factor in *Acer* foliar metal concentrations than soil trace metal concentrations and soil properties. Thus, neither changes in metal exchangeability, total availability, nor alterations in measured soil properties were strongly responsible for differences in *Acer* foliar trace metal concentrations. Although our study and others (e.g. Sizmur et al. 2011; Laossi et al. 2010) could not identify a mechanism for differences in foliar trace metal concentrations, we suggest that earthworms are affecting the uptake by plants through alterations of biological relationships not determined in this study rather than changes in exchangeability in soil. Earthworm biomass did not affect A horizon bulk density or fine-fraction soil density (data not shown, $R < 0.10$; $P > 0.05$) but earthworms could be impacting the size of the aggregates and physical protection of trace metals (Bossuyt et al. 2006; Piron et al. 2012; Blouin et al. 2013; Ma et al. 2014; Knowles et al. 2016). Earthworms may also be affecting fine roots involved in nutrient uptake both directly through root herbivory and indirectly by changing soil properties (Fisk et al. 2004; Hale et al. 2005; Laossi et al. 2011; Gilbert et al. 2014; Dobson et al. 2017). Moreover, earthworms could be impacting microbial activity that may increase trace metal bioavailability and the extent of fungal hyphal networks, which plants utilize for nutrient acquisition (Lawrence et al. 2003; Bityutskii et al. 2012; Bati et al. 2015; Paudel et al. 2016). Further biogeochemical studies linking abiotic nutrient uptake and microbial communities are needed to explore this potential negative feedback system.

Many *Acer* seedlings in this study had Fe, Cu, and Zn concentrations below previously published values for lower estimates of healthy foliage (Table 6, Fig. 3). Since *Acer* foliage Fe, Cu, Mo and Zn concentrations were lower in areas with higher earthworm biomass, we assume that earthworms are decreasing uptake of these micronutrients. Thus,

Table 3 R^2 values for generalized linear mixed models for foliar metal concentrations in *Acer saccharum* and *Polystichum acrostichoides* as a function of metal soil concentrations or pools

	Exchangeable organic horizon pools	Total organic horizon pools	Exchangeable A horizon concentration	Total A horizon concentration	Exchangeable B horizon concentration	Total B horizon concentration
Acer saccharum						
Al	–	–	0.26(−)	0.24(+)	–	–
Fe	–	–	–	0.31(+)	–	–
Cu	0.34(+)	–	0.38(−)	0.30(−)	0.30(+)	–
Zn	0.30(+)	0.32(+)	0.36(−)	0.48(−)	–	–
Mo	–	0.48(+)	–	–	–	–
Pb	–	–	–	–	–	–
Polystichum acrostichoides						
Al	–	–			–	–
Fe	0.44(+)	0.48(+)			–	–
Cu	–	–	0.48(+)	–	–	–
Zn	0.74(−)	0.77(−)	0.58(−)	0.44(−)	–	–
Mo	–	0.45(+)	–	–	–	–
Pb	–	–	–	–	–	–

Only significant models ($p < 0.05$) are presented, and (+) indicates a positive correlation while (−) denotes a negative correlation

Table 4 R^2 values for generalized linear mixed models for foliar metal concentrations in *Acer saccharum* and *Polystichum acrostichoides* as a function of soil and ecosystem properties

	Earthworm biomass	Total root biomass	Soil pH	%SOM	CEC
Acer saccharum					
Al	0.36(+)	–	–	–	–
Fe	0.32(−)	0.24(−)	–	–	–
Cu	0.29(−)	–	–	–	–
Zn	0.47(−)	–	–	–	–
Mo	0.29(−)	–	–	–	–
Pb	–	–	–	–	–
Polystichum acrostichoides					
Al		–	–	–	–
Fe		–	–	–	–
Cu	–	–	–	–	–
Zn	0.76(−)	–	0.25(−)	–	–
Mo	–	–	–	–	–
Pb	–	–	–	–	–

Soil chemical properties are only for the A horizon. Only information for significant models ($p < 0.05$) are presented, (+) indicates a positive correlation while (−) denotes a negative effect

earthworms may be inducing abiotic micronutrient stresses on *Acer* seedlings (Fig. 4), a feature that has been noted for other macronutrients (e.g. Ca by St. Clair et al. 2008). In addition, this abiotic stress is likely metal dependent. For example, the molar ratio of Ca:Al ranged from 40 to 295 mol mol^{-1}, which suggests Al toxicity is not a concern (Thornton et al. 1986; Cronan and Grigal 1995). Since published data for foliar concentrations for healthy understory plants such as *Polystichum* are limited, further investigation into the impacts of earthworms on soil fertility for understory plants is warranted.

Table 5 R^2 values for multiple regressions for foliar metal concentrations with significant variables from GLMM linear regressions

	Variables Significant GLMM variables	R^2	Variables All variables	R^2
Acer saccharum				
Al	Earthworm biomass (+) A horizon exch. (−) A horizon total (+)	0.53	Exch. and total O and A horizons, earthworm biomass, total root biomass, pH, %SOM, and CEC	0.54
Fe	Earthworm biomass (−) Root biomass (−) A horizon total (+)	0.61	Exch. and total O and A horizons, earthworm biomass, total root biomass, pH, %SOM, and CEC	0.79
Cu	Exchangeable Cu (−) O horizon Exch. (+) A horizon Total (−)	0.55	Exch. and total O and A horizons, earthworm biomass, total root biomass, pH, %SOM, and CEC	0.64
Zn	Earthworm biomass (−) O horizon Exch. (+) O horizon Total (+) A horizon Exch. (−)	0.72	Exch. and total O and A horizons, earthworm biomass, total root biomass, pH, %SOM, and CEC	0.89
Mo	Earthworm biomass (−) O horizon Total (+)	0.43	Exch. and total O and A horizons, earthworm biomass, total root biomass, pH, %SOM, and CEC	0.65
Pb	–	N.S.	Exch. and total O and A horizons, earthworm biomass, total root biomass, pH, %SOM, and CEC	0.43
Polystichum acrostichoides				
Al	–	N.S.	Exch. and total O and A horizons, earthworm biomass, total root biomass, pH, %SOM, and CEC	0.37
Fe	O horizon Exch. (+) O horizon Total (+)	N.S.	Exch. and total O and A horizons, earthworm biomass, total root biomass, pH, %SOM, and CEC	0.47
Cu	A horizon Exch. Cu (+)	0.48	Exch. and total O and A horizons, earthworm biomass, total root biomass, pH, %SOM, and CEC	0.74
Zn	Earthworm biomass (−) O horizon Exch. (−) O horizon Total (−) A horizon Exch. Zn (−)	0.81	Exch. and total O and A horizons, earthworm biomass, total root biomass, pH, %SOM, and CEC	0.98
Mo	O horizon Total (+)	0.27	Exch. and total O and A horizons, earthworm biomass, total root biomass, pH, %SOM, and CEC	0.32
Pb	–	N.S.	Exch. and total O and A horizons, earthworm biomass, total root biomass, pH, %SOM, and CEC	0.38

(+) indicates a positive while (−) denotes a negative effect

Conclusions

Ecological communities co-occurring with different earthworm communities experience drastically different micronutrient cycling. Of all the variables we measured, earthworms influence the vertical distribution, abundance and exchangeability of metals, though the influence is predominantly to non-exchangeable forms. Earthworms have minimal influence on soil and foliar Pb, and though earthworms were associated with an increase in foliar Al concentrations, it did not reach toxic Ca:Al or absolute concentrations in foliage. Foliar micronutrient concentrations of different plant species vary in their response to earthworm invasion,

Table 6 Comparisons of measured and published values for *Acer saccharum* foliar trace metal concentrations

	Measured in this study		Forest values[a]	Metropolitan and park values[b]	
	Mean (mg kg^{-1})	Range (mg kg^{-1})	Range (mg kg^{-1})	Mean (mg kg^{-1})	Range (mg kg^{-1})
Acer saccharum					
Al	32 ± 5	7–95	23–60	70	22–138
Fe	75 ± 8	13–152	51–119	146	75–311
Cu	7.3 ± 0.8	1.8–15.8	3–11	7	0.9–14
Zn	18.5 ± 1.8	2.4–27.5	28–72	21	7.4–132
Mo	0.09 ± 0.02	0.01–0.32	N/A	0.05	N/A
Pb	0.35 ± 0.04	0.07–0.71	N/A	17	N/A

Mean values are presented ± 1 standard error

[a] Data were obtained from Burton et al. (1993), Bernier and Brazeau (1988), Likens and Bormann (1970), and Mader and Thompson (1969)

[b] Nursery data were obtained from Smiley et al. (1985) and Hanna and Grant (1962)

though these differences cannot be explained by earthworm-induced changes to soil micronutrients alone. Earthworm invasion can create stressful rooting conditions for some plants such as *Acer*, impeding their ability to take up micronutrients. Since plant communities are the major conduit through which nutrients pass to higher trophic levels, we expect earthworms influence on whole-ecosystem micronutrient cycling to be considerable and far-reaching.

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