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RESEARCH ARTICLE

Forest floor decomposition, metal exchangeability, and metal bioaccumulation by exotic earthworms: *Amynthas agrestis* and *Lumbricus rubellus*

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Abstract Earthworms have the potential to reduce the retention of pollutant and plant essential metals in the forest floor (organic horizons) by decomposing organic matter and increasing exchangeability of metals. We conducted a laboratory experiment to investigate the effects of two exotic earthworms, *Amynthas agrestis* and *Lumbricus rubellus*, on forest floor decomposition, metal exchangeability, and metal bioaccumulation. Eighty-one pots containing homogenized forest floor material were incubated for 20, 40, or 80 days under three treatments: no earthworms, *A. agrestis* added, or *L. rubellus* added. For earthworm treatments, *A. agrestis* and *L. rubellus* were stocked at densities observed in previous field studies. Pots containing either *A. agrestis* or *L. rubellus* had lost more forest floor mass than the control plots after 40 and 80 days of incubation. Forest floor pots containing *A. agrestis* had significantly lower % C (16 ± 1.5 %) than control pots (21 ± 1.2 %) after 80 days. However, *L. rubellus* consumed more forest floor and C mass than *A. agrestis*, when evaluated on a per earthworm biomass basis. Exchangeable (0.1 M KCl + 0.01 M AcOH extractable) and stable (15 M HNO_3 + 10 M HCl extractable) concentrations of Al, Ca, Cd, Cu, Mg, Mn, Pb, and Zn in forest floor material were measured. Stable concentrations and % exchangeable metals in forest floor material were similar among treatments. Although exchangeable metal concentrations varied significantly for most metals among treatments (except Mg and Zn), we conclude that earthworms did not increase or decrease the exchangeability of metals. However, earthworms bioaccumulated Cu, Cd, Zn, and Mg and had potentially hazardous tissue concentrations of Al and Pb. This was best illustrated by calculating bioaccumulation factors using exchangeable concentrations rather than total concentrations. Future research is needed to understand the effect of earthworms on metals in other soil types.

Keywords Nonnative earthworms · Forest floor mass · Heavy metals · Exchangeability · Trace metal · Plant nutrients

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Introduction

Metals serve multiple roles in terrestrial ecosystems. For example, some metals (e.g., Ca, Cu, Mg, Mn, Zn) are essential for plants and animals as nutrients and enzyme cofactors, while other metals (e.g., Al, Cd, and Pb) are not required by organisms and acute or chronic ingestion may lead to toxicity (Adriano 2001; Alloway 2013). Many of these metals (e.g., Cd, Cu, Pb, Zn) have been enriched in the forest soils of the northeastern USA due to increased atmospheric deposition of pollution from human sources (Likens and Bormann 1995; Steinnes and Friedland 2006). In particular, concentrations of Cu, Pb, and Zn have been enriched in forests in close proximity to cities and in rural areas (Friedland et al. 1986; Richardson et al. 2015a; Evans et al. 2005). The rate of movement of metal pollution to waterbodies and to vegetation is generally controlled by soil properties. The high soil organic matter (SOM) concentration in surface horizons allows forest

soils to act as large reservoirs for terrestrial cycling of metals. As a prime example, the forest floor (organic horizons) of the soil can be a repository of metals, and their subsequent fate might ultimately be plant and animal uptake, leaching to groundwater, or transfer to surface waters (Pritchett and Fisher 1987; Likens and Bormann 1995; St. Clair et al. 2008; Richardson and Friedland 2016).

Earthworms are an expanding source of environmental change for the forests of northern New England, USA, and recent evidence suggests that they are disturbing major element and metal cycling of the forest floor (Edwards and Bohlen 1996; Fahey et al. 2013; Resner et al. 2014; Richardson et al. 2015b). Multiple European and Asiatic earthworms have invaded the northern forests as a result of human activities, particularly from fishing, logging, waste management, and agriculture (Bohlen et al. 2004; Görres and Melnichuk 2012). Two species of interest are *Amynthas agrestis* and *Lumbricus rubellus*, due to their prevalence across northern New England, USA, and their ecophysiology as epi-endogeic earthworms. *A. agrestis* was first reported in the northern New England, USA, by Görres and Melnichuk (2012). *A. agrestis* is a relatively large, invasive earthworm from Asia that belongs to the Megascolecidae family, has been known to consume the entire forest floor (Edwards and Bohlen 1996; Bohlen et al. 2004; Görres and Melnichuk 2012), and creates a distinctive layer of loose castings (Ziemba et al. 2015). *L. rubellus* is a smaller earthworm from Europe, which has been widely distributed across North America, from the Great Lake States of Minnesota and Michigan to Maine and southern Canada (Edwards and Bohlen 1996; Burtelow et al. 1998; Holdsworth et al. 2007; Addison 2009). *A. agrestis* and *L. rubellus* can alter forest soils by mixing soil horizons and decreasing SOM (Edwards and Bohlen 1996; Bohlen et al. 2004; Frelich et al. 2006). These actions have been shown to alter the cycles of major elements, such as C, N, and P, in the forest floor (Suárez et al. 2003; Bohlen et al. 2004; Frelich et al. 2006; Fahey et al. 2013). Physical changes to the forest floor and chemical changes to major element cycles may reduce the retention of trace elements (Resner et al. 2011), potentially resulting in a loss of plant essential metals required by northern hardwood forests (Frelich et al. 2006; Alban and Berry 1994; St. Clair et al. 2008; Resner et al. 2014) and cause the leaching of potentially hazardous metals that are stored in the forest floor (Steinnes and Friedland 2006; Sizmur and Hodson 2009).

Decomposition, mineralization, and other transformations of the forest floor caused by earthworms may affect the retention of metals. Previous laboratory studies on earthworm effects on metals have not addressed strongly acidic, nonpoint source-contaminated, organic matter-rich forest soils that cover much of the northeastern USA. We conducted a laboratory experiment to investigate *A. agrestis* and *L. rubellus* effects on forest floor decomposition, metal exchangeability, and metal bioaccumulation. The first objective of this study was to determine if adult earthworms are able to inhabit and enhance decomposition of a strongly acidic forest floor that contains elevated metal concentrations. We hypothesized that both species would survive and enhance the decomposition rate of forest floor material, showing that forests of New England, USA, are susceptible to invasion by both species. The second objective was to determine if the earthworms impacted the exchangeability of metals in the forest floor. We hypothesized that the ingestion of forest floor soil and bioturbation would increase the exchangeability of metals, allowing for mobilization of trace metal pollution. The final objective of this study was to determine if earthworms were bioaccumulating the metals. We hypothesized that Ca, Cd, Pb, and Zn would be bioaccumulated in the earthworm tissue based upon previous studies and their physiological role in the organism. This information is vital for environmental scientists, ecotoxicologists, forest managers, and policy makers on the impact of exotic earthworms on forest ecosystem services in New England, USA.

Methods

Forest floor material and earthworm collection

Forest floor was collected from a complex of Becket and Monadnock soil series Spodosols in Lyme, NH. Vegetation at this site was primarily red maple (*Acer rubrum* L.), red spruce (*Picea rubens* Sarg.), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britt.). The forest floor was separated from the underlying E horizon using a hand spade and collected in 0.5 × 0.5 m mats and immediately taken to the laboratory. Recognizable leaf material (Oi horizon), rocks >5 mm, and roots >5 mm in diameter were removed. The forest floor samples were placed into paper bags and allowed to air dry to a constant weight at 25 °C for >3 weeks. The forest floor material, consisting of Oe and Oa horizon, was milled to <2-mm particles using a Wiley Mill (Richardson et al. 2014) and thoroughly mixed to achieve homogeneity.

Adult earthworms were collected from a forest soil in late June 2014, which was a complex of Hartland-Hitchcock soil series Inceptisols in Norwich, Vermont, USA (Soil Survey Staff 2010). The soil had been under agricultural fields during the early 1900s, when it was abandoned. Physical and chemical soil properties of the forest soil are given in Table 1. Currently, the soils support an uneven-aged forest comprised primarily of northern hardwoods (*Acer saccharum* L., *Betula papyrifera* Marshall, *B. alleghaniensis* Britt., and *Fagus grandifolia* Ehrh.) interspersed with coniferous vegetation (*Pinus* spp. and *Tsuga canadensis* (L.) Carrière). Earthworms were collected using the hand-sort method, stored alive in their horizon material. *L. rubellus* was

Table 1 Soil concentrations and physicochemical properties

Soil	Soil series	Horizons	Texture	% SOM	% C	% N	pH	Al (mg kg^{-1})	Ca (mg kg^{-1})	Cd (mg kg^{-1})	Cu (mg kg^{-1})	Mg (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Pb (mg kg^{-1})
Field soil	Hartland-Hitchcock	Ap, A, Bw	Silt Loam	22 ± 3	8 ± 2	0.4 ± 0.1	6.88 ± 0.02	1370 ± 110	12,340 ± 470	0.26 ± 0.04	18 ± 4	4680 ± 140	620 ± 12	53 ± 7	16 ± 3
Forest floor	Becket-Monadnock	Oe, Oa	N/A	49 ± 2	20 ± 1	2 ± 1	4.72 ± 0.14	1740 ± 120	8500 ± 230	0.35 ± 0.01	5.2 ± 0.4	1180 ± 140	210 ± 12	28 ± 3	26 ± 2

The field soil is the material from which the earthworms were collected. The forest floor is the material in the pots

identified live in the laboratory using a dichotomous key (Great Lakes Worm Watch, University of Minnesota, 2011). *A. agrestis* had previously been identified here (Richardson et al. 2015b). *A. agrestis* and *L. rubellus* were chosen for study because they are two epi-endogeic earthworms that live in the forest floor-mineral soil interface in forest soils. Only earthworms with clitella, i.e., adults, were utilized in this study.

Forest floor decomposition experiment

Prior to introduction to the forest floor experiment, the earthworms were washed of surface particles and depurated to remove material ingesta from their bowels (Nahmani et al. 2007). Earthworms were rinsed in deionized water and incubated for 7 days in plastic Petri dishes on moist Whatman No. 1 filter paper disks (3 earthworms per dish) to allow for evacuation of their digestive tracts. The Petri dishes were kept in the dark at 22 °C with daily filter paper changes to prevent coprophagy. Fifteen earthworms from each species served as a control group and were analyzed to determine the tissue concentration and amount of metal included in this pool. Individuals in the control group of earthworms were individually weighed for wet mass, preserved using cryodesiccation, and weighed three times to attain a dry weight (dw) biomass after drying.

Plastic containers used to hold the forest floor material (forest floor pots) measured 29.2 cm in diameter and 26.7 cm in height. The forest floor pots included drainage holes in the bottom of the container, which were covered with a fine synthetic mesh fabric to prevent earthworm escape. In addition, the top of the pots was open to the ambient room conditions (25 °C), and concentric rings of wire were secured to the inside of the containers to prevent earthworm escape by breaking their adhesion to the plastic surface (Melnichuk, personal communication). Each pot contained 500.0 ± 0.1 g of dry, homogenized forest floor material (consisting of Oe and Oa horizon). The mean loss-on-ignition of the forest floor material at 550 ° C for 8 h was 49 ± 2 %. The soil pH using a 2:5 soil/water slurry in 0.01 M $CaCl_2$ was 4.72 ± 0.14 log units. The forest floor pots were wetted to 60 % of their water holding capacity (WHC) and incubated without earthworms for 10 days to allow for establishment of microbial community and settling of materials.

We compared two earthworm treatments (*A. agrestis* and *L. rubellus*) to a set of 27 control forest floor pots with no earthworms added. Twenty-seven forest floor pots were stocked with *A. agrestis* and another 27 forest floor pots were stocked with *L. rubellus*. After the 10-day incubation period, *A. agrestis* was stocked at 2.0 ± 0.1 g dw (~29 g m^{-2}) per forest floor pot at 0.21 ± 0.07 g dw per individual or ten *A. agrestis* were added per pot. This is equivalent to 145 *A. agrestis* individuals m^{-2}, a typical density of *Amynthas* spp. in sugar maple dominated forests from July to

September (Görres et al. 2014). *L. rubellus* were added to at 0.7 ± 0.1 g dw (~10 g m^{-2}) per forest floor pot at about 0.4 ± 0.1 g dw per individual or two earthworms per pot. This matches approximately 25 individuals m^{-2}, at the high end of natural occurrence in northern hardwood forests (Suárez et al. 2006). Forest floor pots were maintained at 40–60 % WHC throughout the experiment with deionized water. Drainage was minimized by adding water at a slow rate from a nebulized water source. However, percolation of water did occur but was not collected due to differences in preferential flow and water storage among treatments.

Previous studies have observed that earthworm tissue metal concentrations reach pseudoequilibrium with their soil medium between 40 and 80 days of incubation (Neuhauser et al. 1995; Nahmani et al. 2007). Thus, forest floor pots were destructively sampled after 20, 40, and 80 days of decomposition to determine earthworm metal tissue concentration with time. Forest floor pot material was hand sorted for earthworms after 40 and 80 days of incubation. Forest floor material was then oven dried at 40 °C to a constant mass. Prior to tissue analysis, earthworms collected from the 40- and 80-day pots were treated using the same depuration techniques to remove ingesta from their bowels as previously described.

The change in forest floor mass was modeled with a first-order, exponential model (Eq. 1) for each treatment. In Eq. 1, a is an asymptote, b is the amplitude of the curve, and k is the decay rate at a given time, t, during the experiment. This model has been commonly applied to decomposition studies (e.g., Andrén and Paustian 1987) and allows for decay rates and pseudoequilibrium levels of remaining forest floor mass to be estimated.

$$\text{Forest floor mass} = a + b \cdot e^{-k \cdot t} \quad (1)$$

Chemical analyses

Soil pH was determined using a 2:5 soil/water slurry in 0.01 M $CaCl_2$. The % C concentration in the forest floor material was measured using a Carlo-Erba elemental analyzer. In brief, 6 ± 1 mg of sample ground to <0.5 mm were analyzed in duplicate for all samples. Every 20 samples included one blank, one atropine SRM, and a triplicate. Total C and N concentrations in atropine SRMs were with 3 % of its certified value and <10 % relative percent difference among duplicates and triplicates.

We utilized a weak acid-salt, strong acid sequential extraction to quantify weakly and strongly adsorbed metals in the soil columns following decomposition. The weak acid-salt extraction used 0.1 M KCl with 0.01 M acetic acid to get cation exchangeable and other weakly adsorbed metals into solution. Two grams of soil was shaken in 10 mL of the weak acid-salt extraction solution for 24 h. The slurry was centrifuged at 6000 rpm for 20 min and the supernatant was decanted. This extraction process was repeated but with 10 mL of deionized water, and the resulting supernatant was combined with the previous supernatant. For the strong acid digestion, 5 mL of strong acid (9:1, HNO_3/HCl, trace metal grade) was added to the slurry from the weak acid-salt extractions. After 12 h of degassing, the samples were microwave digested at 90 °C for 45 min. Earthworm tissue concentrations were measured using strong acid digestion following EPA method 3051A. Entire earthworms were lyophilized to a constant weight (typically ranging between 100 and 400 mg dw) and digested overnight in 5 mL of strong acid (9:1, HNO_3/HCl, trace metal grade).

Soil weak acid-salt extractions, soil strong acid digestions, and earthworm digestions were further diluted with deionized water and analyzed with an Agilent 7700x ICP-MS (Agilent Technologies, Santa Clara, CA) for Al, Ca, Cd, Cu, Mg, Mn, Pb, and Zn. Every 25 samples included a digestion blank, a randomly spiked sample, a duplicate, and one standard reference material (SRM). Digestion blanks were below detection limits. A random sample was spiked with 50 μL of multi-element standard 71A from Inorganic Ventures (Inorganic Ventures, Christiansburg, VA) and recoveries of the added elements ranged between 85 and 102 % with an average of 94 % for all spiked samples. Matching sample matrices from the National Institute of Standards and Technology were used: San Joaquin 2709 for soil digests and Mussel Tissue 2976 for earthworm tissue analysis (National Institute of Standards and Technology, Gaithersburg, MD). Exchangeable + strong acid metal concentrations for SRMs were within 12 % of their total certified concentration values.

Bioaccumulation factor (BAF), or the synonymous bioconcentration factor, is a comparison of the concentration of a metal within the earthworm relative to the soil it inhabits (e.g., Dai et al. 2004; Ernst et al. 2008). The conventional method for calculating the BAF is using Eq. 2. BAF values >1.0 suggest that the organism is bioaccumulating the metal, while a value <1.0 indicates that the earthworm is actively excreting the metal from its tissue (cf. Neuhauser et al. 1995; Morgan and Morgan 1999; Nannoni et al. 2014).

$$\text{BAF} = [\text{Earthworm}] \,/\, ([\text{Exchangeable soil}] + [\text{Stable soil}]) \quad (2)$$

In addition to studying the bioaccumulation with BAF values in the conventional manner, we also investigated bioaccumulation with respect to the exchangeable concentration (BAF-exchangeable) for each metal with Eq. 3.

$$\text{BAF-exchangeable} = [\text{Earthworm}] \,/\, [\text{Exchangeable soil}] \quad (3)$$

The purpose of comparing exchangeable metal concentration rather than strong-acid extractable metal concentrations is

to better assess the bioaccumulation of potentially bioavailable metals by earthworms. Thus, the BAF-exchangeable does not take into account metals that are stable or strongly sorbed to particles in the soil and less likely to be incorporated into earthworm tissue.

Statistical analyses

Descriptive statistics for soil physicochemical properties, soil metal concentrations, and earthworm metal concentrations were calculated in MATLAB R2011b (MathWorks Inc, Natick, MA). For the figures and in text data, mean values are given ±1 standard error. Differences among earthworm treatments were compared using one-way ANOVA with post hoc Student's *t* test, after evaluating the normality of their distribution using the Lilliefors test (Lilliefors 1967). Repeated measures one-way ANOVA was added to determine if there was a significant different among treatments for the duration of the experiment in MATLAB 2015a.

Results

Forest floor mass and % C decreases

Physicochemical properties of the forest floor material used in the study are given in Table 1. Soil pH did not vary among treatments for any of the collection dates. The mass of forest floor material in all forest floor pots decreased with time for all treatments. However, forest floor mass was significantly lower for pots containing *A. agrestis* and *L. rubellus* ($P < 0.05$, Fig. 1) than the control pots. Specifically, after 80 days, forest floor pots containing *A. agrestis* and *L. rubellus* had on average <85 % of the original material remaining, while the control pots had >90 % remaining on average. It appears the most

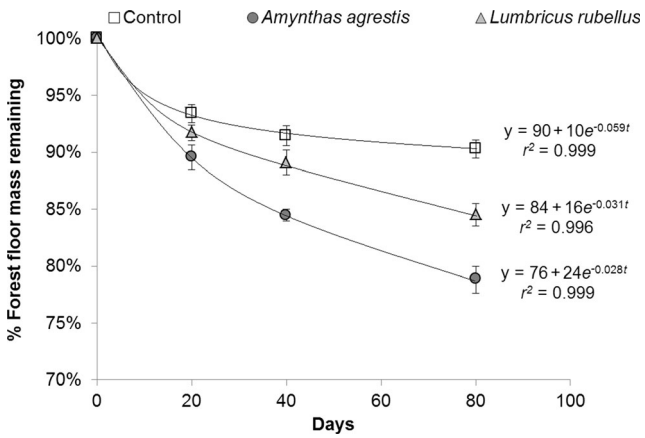

Fig. 1 Change in forest floor mass remaining in pots with *Amynthas agrestis*, *Lumbricus rubellus*, and control pots that contain no earthworms. Exponential regressions were calculated to estimate the rate of loss of forest floor among treatments. *Error bars* are ±1 standard error, $n = 9$

rapid decomposition happened during the first 20 days of the experiment, particularly for control pots (Fig. 1). Using first-order, exponential models, we observed that the control forest floor pots had significantly greater decay rates (0.059 ± 0.002) than pots containing *A. agrestis* (0.028 ± 0.001) and *L. rubellus* (0.031 ± 0.007) ($P < 0.05$). However, the asymptotic boundary (*a* in Eq. 1) of exponential models estimates that control pots will reach a significantly higher pseudoequilibrium proportion of remaining forest floor mass (90 ± 1 %) than pots containing *L. rubellus* (84 ± 1 %) and *A. agrestis* (77 ± 2 %) ($P < 0.01$). The amplitude (*b* in Eq. 1) of the models suggests a similar greater loss for *L. rubellus* (16 ± 2 %) or *A. agrestis* (23 ± 1 %) compared to the control pots (10 ± 1 %) ($P < 0.01$). In sum, the exponential models show that the control pots initially lost forest floor mass more rapidly, but forest floor pots *L. rubellus* and *A. agrestis* treatments will decompose a greater portion of the forest floor after 40 days and this trend continued into the 80-day collection.

After 80 days of decomposition, forest floor pots containing *A. agrestis* had lost significantly more mass (106 ± 5 g) than forest floor pots containing *L. rubellus* (12 ± 5 g) ($P < 0.05$). Both earthworm treatments lost significantly greater mass than the control forest floor pots (49 ± 4 g) over the 80 days. However, earthworms were stocked at different densities. When considered on a per earthworm basis, forest floor pots with *L. rubellus* lost greater mass (191 g forest floor per gram earthworm) than *A. agrestis* forest floor pots (30 g forest floor per gram earthworm) over the 80-day period.

The forest floor % C for the treatments at the three collection times is shown in Fig. 2. There was no significant difference among the treatments for the first 20 days of the experiment. However, after 40 days, forest floor pots containing *A. agrestis* had significantly lower % C than control pots. Forest floor pots containing *L. rubellus* were similar to control and *A. agrestis* treatment (Fig. 2). At the end of the 80-day experiment, *A. agrestis* still had a significantly lower % C than control pots, and *L. rubellus* was still similar to the other two treatments (Fig. 2). Using the forest floor mass, we were able to calculate the mass of C lost during the decomposition experiment. Forest floor pots containing *A. agrestis* had lost significantly more mass (42 ± 4 g) than forest floor pots containing *L. rubellus* (31 ± 5 g) ($P < 0.05$). Both lost significantly greater C than the control forest floor pots (12 ± 5 g) over the 80 days. When considered on a per earthworm basis, forest floor pots with *L. rubellus* lost greater C (66 ± 5 g forest floor C per gram earthworm) than *A. agrestis* forest floor pots (30 ± 4 g forest floor per gram earthworm) over the 80 days (Fig. 3). The loss of forest floor mass and C was modeled with a linear regression as opposed to the first-order, exponential decay model in Eq. 1. The change in forest floor mass and C was best modeled as a linear relationship as the differences were consistent through time.

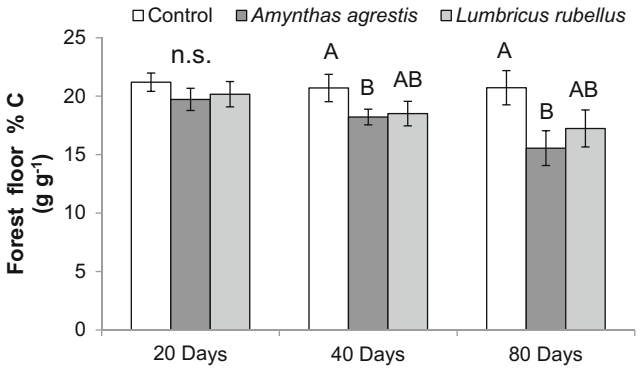

Fig. 2 The change in forest floor C—concentration among the three treatments at three times during the study. *Letters* (*A*, *B*) indicate significant differences among treatments ($P < 0.05$) and *n.s.* indicates no significant difference among treatments. *Error bars* are ±1 standard error, $n = 9$

Stable and exchangeable metal concentrations

Exchangeable concentrations varied widely among metals. Calcium, Mg, and Mn had the highest exchangeable concentrations of the metals in the soil, while all other metals were at trace concentrations <100 μg g^{-1} (Table 2). Stable concentrations of Al, Ca, and Mg concentrations were the highest and all other metals were in trace concentration (Table 3). One-way ANOVA comparing treatments was performed on exchangeable concentrations and stable concentrations for each metal at 20, 40, and 80 days. Generally, forest floor pots with *A. agrestis* or *L. rubellus* had significantly lower exchangeable concentrations of Ca, Mn, and Pb (Table 2). However, exchangeable metal concentrations in forest floor pots containing *L. rubellus* after 80 days of decomposition had higher exchangeable concentrations of Al, Cd, Cu, Mg, and Zn (Table 2). Stable concentrations of all metals in forest floor pots did not vary among treatments for any of the collection dates (Table 3).

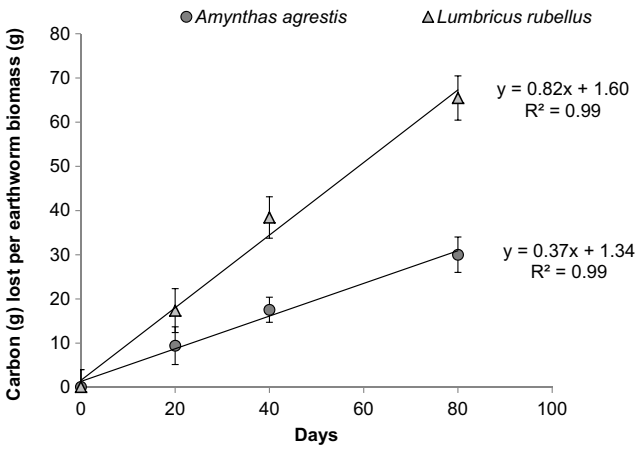

Fig. 3 Forest floor C mass lost normalized per gram of *Amynthas agrestis* and *Lumbricus rubellus* dry weight biomass. Linear regressions were calculated to estimate the rate of C loss among treatments through time. *Error bars* are ±1 standard error, $n = 9$

We utilized a repeated measures one-way ANOVA to determine if there was a significant difference in stable and exchangeable metal concentrations among treatments for the duration of the experiment. On the basis of repeated measure results, we observed no significant difference for any stable metal concentration among treatments during the experiment (Table 2). However, most exchangeable metal concentrations (excluding Mg, Zn) were significantly different than the control treatments. Aluminum and Cd exchangeable concentrations in *L. rubellus* pots were significantly greater than the control pots. Other metals had significantly higher exchangeable metal concentrations in the control pots than earthworm pots at day 20 but became significantly lower for control pots than earthworm pots by day 80 (e.g., Ca, Cu, Mn). Lead exchangeable concentrations were significantly lower for control pots than earthworm pots at day 20, but control pots became significantly higher than earthworm pots for days 40 and 80 (Table 2).

To investigate the relative exchangeability of each of the metals and if it varied among treatments, we calculated the % exchangeable using Eq. 4.

$$\%\ \text{Exchangeable} = \left([\text{Exchangeable}] \Big/ ([\text{Exchangeable}] + [\text{Stable}])\right) \times 100\ \% \quad (4)$$

One-way ANOVA was performed on % exchangeable for each metal at 20-, 40-, and 80-day collection points among treatments (Table 4). The % exchangeable strongly varied among metals, ranging from ~70 % for Ca and Mn to ~3 % for Al and Pb (Table 4). The % exchangeable only varied among treatments for Cu, in which forest floor pots with *L. rubellus* were lower than the control at the 20-day collection and lower than *A. agrestis* pots at the 40-day collections (Table 4). However, the % exchangeable for all other metals did not vary significantly among treatments.

Earthworm metal concentrations and bioaccumulation

Earthworms were able to inhabit the forest floor material; earthworm survival was >50 % for all forest floor pots (Supplemental Table 1). Metal concentrations in earthworm tissue at 0, 40, and 80 days of incubation in the forest floor pots are compared to average forest floor metal concentrations in Fig. 4. Generally, earthworm tissue concentrations of Ca and Mg did not significantly vary between earthworm species (Fig. 4). We observed that *A. agrestis* and *L. rubellus* Mn concentrations decreased significantly through the experiment ($P < 0.05$, Fig. 4). Earthworm tissue concentrations of Al and Pb significantly increased through time ($P < 0.05$, Fig. 4), reaching or exceeding total forest floor concentrations

Table 2 Exchangeable (0.1 M KCl + 0.01 M AcOH extracted) metal concentrations in the forest floor pots among the three treatments

No. of days	Treatment	Number[a]	Al (μg/g)	Ca (μg/g)	Cd (μg/g)	Cu (μg/g)	Mg (μg/g)	Mn (μg/g)	Pb (μg/g)	Zn (μg/g)
20	Control	9	62 ± 4	6750 ± 480*	0.14 ± 0.01	0.41 ± 0.07*	520 ± 40	160 ± 11*	0.57 ± 0.07	16 ± 3
20	*Amynthas agrestis*	9	68 ± 4	4290 ± 530	0.14 ± 0.01	0.38 ± 0.03*	510 ± 30	114 ± 6	0.84 ± 0.11*	13 ± 1
20	*Lumbricus rubellus*	9	87 ± 10*	5590 ± 450	0.16 ± 0.01*	0.17 ± 0.01	530 ± 30	143 ± 6	0.72 ± 0.08	15 ± 2
40	Control	9	55 ± 5	5910 ± 490*	0.15 ± 0.02	0.28 ± 0.02*	530 ± 40*	140 ± 12	1.21 ± 0.28*	14 ± 2
40	*Amynthas agrestis*	9	58 ± 3	5420 ± 300*	0.15 ± 0.01	0.36 ± 0.03*	520 ± 30	149 ± 8*	0.98 ± 0.13	14 ± 2
40	*Lumbricus rubellus*	9	56 ± 4	4000 ± 190	0.14 ± 0.01	0.20 ± 0.02	430 ± 20	106 ± 6	0.69 ± 0.07	13 ± 1
80	Control	9	30 ± 2	4540 ± 280	0.13 ± 0.01	0.15 ± 0.02	440 ± 30	114 ± 8	1.05 ± 0.23*	11 ± 1
80	*Amynthas agrestis*	9	31 ± 2	3800 ± 220	0.13 ± 0.01	0.25 ± 0.03*	390 ± 20	95 ± 6	0.56 ± 0.03	12 ± 1
80	*Lumbricus rubellus*	9	59 ± 5*	5030 ± 170*	0.16 ± 0.01*	0.23 ± 0.02*	590 ± 30*	132 ± 8*	0.79 ± 0.08	15 ± 3*

Using repeated measures one-way ANOVA, exchangeable concentrations of Al, Ca, Cd, Cu, Mn, and Pb were determined to be significant among treatments for the duration of the experiment

*$P < 0.05$ indicates if a treatment is significantly greater than one or both of the other treatments based on one-way ANOVA with post hoc *t* test

[a] The number of replicate forest floor pots

(Fig. 4). *A. agrestis* and *L. rubellus* tissue concentrations responded differently for Cu and Zn. Copper tissue concentrations did not vary for *L. rubellus* but Cu significantly decreased with time for *A. agrestis* ($P < 0.05$, Fig. 4). Zinc tissue concentrations significantly increased for *L. rubellus* but did not vary significantly for *A. agrestis* ($P < 0.05$, Fig. 4).

We found that the BAF values for *A. agrestis* after 80 days of incubation were Cd > Zn > Pb > Cu > Al > 1.0 > Mg > Ca > Mn and BAF values for *L. rubellus* after 80 days were Cd > Zn > Cu > Pb > 1.0 > Al > Ca > Mg > Mn. The BAF-exchangeable values were different from the conventional BAF values and exhibited a different pattern of accumulation. The BAF-exchangeable values for *A. agrestis* after 80 days were Pb > Al > Cu > Cd > Zn > Mg > 1.0 > Ca > Mn and BAF-exchangeable values for *L. rubellus* after 80 days were Pb > Cu > Cd > Zn > Mg > 1.0 > Al > Ca > Mn (Table 5). The BAF-exchangeable values generally agree with the BAF values calculated conventionally.

Discussion

Forest floor mass and % C decreases

Both species of earthworms were able to inhabit the forest floor material despite the low pH and concentration of potentially hazardous metals (e.g., Al and Pb). Soil pH values <5 have been observed to limit the colonization of earthworms (Addison 2009) and soil Al concentrations >50 mg kg^{-1} have been shown to increase mortality of earthworms (Zhang et al. 2009). At high concentration, Al has been observed to impact uptake of Ca and affect cation regulation in invertebrates (Sparling and Lowe 1996). However, the exact mechanism for Al toxicity and other metals in earthworms has not been well characterized. The high Al concentration in the forest floor may not have been as impactful on earthworm health as in Zhang et al. (2009) and Zhao and Qui (2010) due to the high soil C concentration for sorption of Al.

Table 3 Stable (9:1 HNO_3/HCl extractable) metal concentrations in the forest floor pots among the three treatments

No. of days	Treatment	Number[a]	Al (μg/g)	Ca (μg/g)	Cd (μg/g)	Cu (μg/g)	Mg (μg/g)	Mn (μg/g)	Pb (μg/g)	Zn (μg/g)
20	Control	9	1680 ± 120	1760 ± 120	0.19 ± 0.01	4.8 ± 0.3	660 ± 49	48 ± 4	25 ± 2	13 ± 1
20	*Amynthas agrestis*	9	1760 ± 100	1810 ± 110	0.19 ± 0.01	5.0 ± 0.3	698 ± 42	50 ± 4	26 ± 1	14 ± 2
20	*Lumbricus rubellus*	9	1730 ± 120	1810 ± 110	0.18 ± 0.01	5.0 ± 0.3	679 ± 44	50 ± 4	26 ± 1	14 ± 1
40	Control	9	1730 ± 160	1780 ± 180	0.19 ± 0.02	4.9 ± 0.4	680 ± 65	49 ± 4	25 ± 2	13 ± 2
40	*Amynthas agrestis*	9	1860 ± 150	1940 ± 160	0.20 ± 0.01	5.3 ± 0.4	729 ± 46	54 ± 5	28 ± 2	14 ± 2
40	*Lumbricus rubellus*	9	1720 ± 130	1820 ± 130	0.18 ± 0.01	5.0 ± 0.3	672 ± 40	50 ± 3	26 ± 2	14 ± 2
80	Control	9	1610 ± 110	1690 ± 120	0.19 ± 0.01	4.6 ± 0.3	635 ± 45	47 ± 3	24 ± 2	13 ± 2
80	*Amynthas agrestis*	9	1640 ± 110	1720 ± 110	0.18 ± 0.01	4.8 ± 0.3	649 ± 43	48 ± 3	25 ± 2	13 ± 1
80	*Lumbricus rubellus*	9	1890 ± 150	1980 ± 140	0.21 ± 0.02	5.4 ± 0.3	747 ± 42	55 ± 4	28 ± 2	15 ± 2

There were no significant differences in stable metal concentrations among treatments, using repeated one-way ANOVA tests

[a] The number of replicate forest floor pots

Table 4 %Exchangeable (exchangeable/exchangeable + stable) concentrations in the forest floor pots among the three treatments

Treatment	Number[a]	No. of days	Al (%)	Ca (%)	Cd (%)	Cu (%)	Mg (%)	Mn (%)	Pb (%)	Zn (%)
Control	9	20	3.5 ± 1.0	79 ± 10	43 ± 4	7.9 ± 1.0*	44 ± 11	77 ± 9	2.2 ± 1.2	55 ± 9
Amynthas agrestis	9	20	3.7 ± 0.8	70 ± 7	45 ± 7	7.0 ± 0.5	42 ± 8	69 ± 7	3.2 ± 1.8	50 ± 7
Lumbricus rubellus	9	20	4.8 ± 1.7	76 ± 6	41 ± 6	5.3 ± 0.6	44 ± 8	74 ± 7	2.7 ± 0.5	51 ± 6
Control	9	40	3.1 ± 1.6	77 ± 13	45 ± 3	5.4 ± 1.4	44 ± 10	74 ± 12	4.5 ± 0.9	51 ± 12
Amynthas agrestis	9	40	3.0 ± 0.7	74 ± 8	43 ± 6	6.3 ± 0.6*	42 ± 8	74 ± 8	3.4 ± 1.2	49 ± 8
Lumbricus rubellus	9	40	3.2 ± 0.8	69 ± 8	42 ± 7	3.8 ± 0.5	39 ± 7	68 ± 7	2.6 ± 0.8	49 ± 8
Control	9	80	1.9 ± 0.8	73 ± 9	41 ± 5	3.2 ± 0.8	41 ± 10	71 ± 11	4.2 ± 0.9	47 ± 9
Amynthas agrestis	9	80	2.0 ± 0.8	69 ± 8	41 ± 8	5.1 ± 0.7	38 ± 9	67 ± 8	2.2 ± 0.8	49 ± 8
Lumbricus rubellus	9	80	3.0 ± 0.7	72 ± 8	43 ± 8	4.0 ± 0.6	44 ± 8	71 ± 8	2.7 ± 0.7	50 ± 8

Only Ca exhibited a significant difference among treatments for any collection period

*$P < 0.05$ indicates if a treatment is significantly greater than one or both of the other treatments based on one-way ANOVA with post hoc *t* test

[a] The number of forest floor pots

From our forest floor mass and % C results, we can conclude that earthworms have accelerated the decomposition of forest floor material (Figs. 1 and 2). At the earthworm densities observed in field studies, the effect was more pronounced for *A. agrestis* than *L. rubellus* (Figs. 1 and 2). However, on a per earthworm biomass basis, *L. rubellus* consumed more SOM and C than *A. agrestis* (Fig. 3). These results support the first objective of this study of determining if adult earthworms are able to survive in and enhance decomposition of the northern New England forest floor, despite its high acidity and trace metal concentration. *L. rubellus* may be more effective at consuming forest floor material especially with higher C concentration, potentially due to preferential consumption of more humified SOM than *A. agrestis* (for information about preferential consumption of earthworms, see Edwards and Bohlen 1996; Morgan and Morgan 1999). Moreover, the soil microorganisms associated with mucus layer and intestine of *L. rubellus* may be better decomposers than those cohabitating with *A. agrestis* (Bityutskii et al. 2016). The linear change in forest floor C suggests that feeding or decomposition of C compounds was consistent through time.

The impact of earthworms on the forest floor and storage of C in soil has been well documented. For example, Bohlen et al. (2004) showed approximately an 80 % decrease in C storage in the forest floor due to earthworms such as *Lumbricus terrestris*. Similarly, Fahey et al. (2013) found that *L. terrestris* and *L. rubellus* had decreased soil carbon pools by 37 % by eliminating the forest floor. By conducting our experiment in the laboratory, we showed that earthworms increased the decomposition of the moderately and highly degraded Oe and Oa forest floor material, either by consumption or priming the microbial community. Our results suggest that enhanced decomposition of the forest floor does not need to be primed through mixing of fresh litter, which was described by Fahey et al. (2013) as one of the main mechanisms of SOM loss in earthworm-invaded soils. It remains unclear if the mass loss is strictly due to earthworm digesting the forest floor material, or if their activities stimulated microbial communities to mineralize the organic material (Groffman et al. 2004).

The decomposition of the forest floor and the decrease of soil C by *A. agrestis* and *L. rubellus* have ramifications for pollutant metal and nutrient metal accumulation and retention. SOM is important for sorption and retention of metals. Decreased accumulation and increased mobility could result in greater mobility for pollutant metals from the forest floor and decreased concentrations of nutrient metals for plant uptake. This is of concern if the rooting zone is primarily the forest floor, such as with small herbaceous understory plants (Gilliam 2007). Future studies are needed to evaluate how varying earthworm biomass and the composition of earthworm communities affect the decomposition rate of SOM and C in the forest floor.

Forest floor stable and exchangeable metal concentrations

Concentrations of Al, Ca, Mg, and Mn in the forest floor were within the typical range of forest soils in the northeastern USA (Adriano 2001; Herndon et al. 2011; Alloway 2013; Smith et al.2014; Richardson and Friedland 2016). Concentrations of Cu, Zn, and Pb were within the range reported for other forests soils in northern New England, USA (Evans et al. 2005; Richardson et al. 2014, 2015a), but are generally higher than forest floor concentrations in other regions of the USA (Smith et al. 2014). Concentrations of Cu, Zn, and Pb were higher than typical values for US soils due to atmospheric deposition rates which peaked between 30 and 40 mg m^2 $year^{-1}$ in the mid-1970s (Siccama and Smith 1978; Johnson et al. 1982; Richardson et al. 2014).

In our second hypothesis, we expected that earthworms would increase the exchangeable concentration of metals due to enhanced decomposition of SOM. This could have

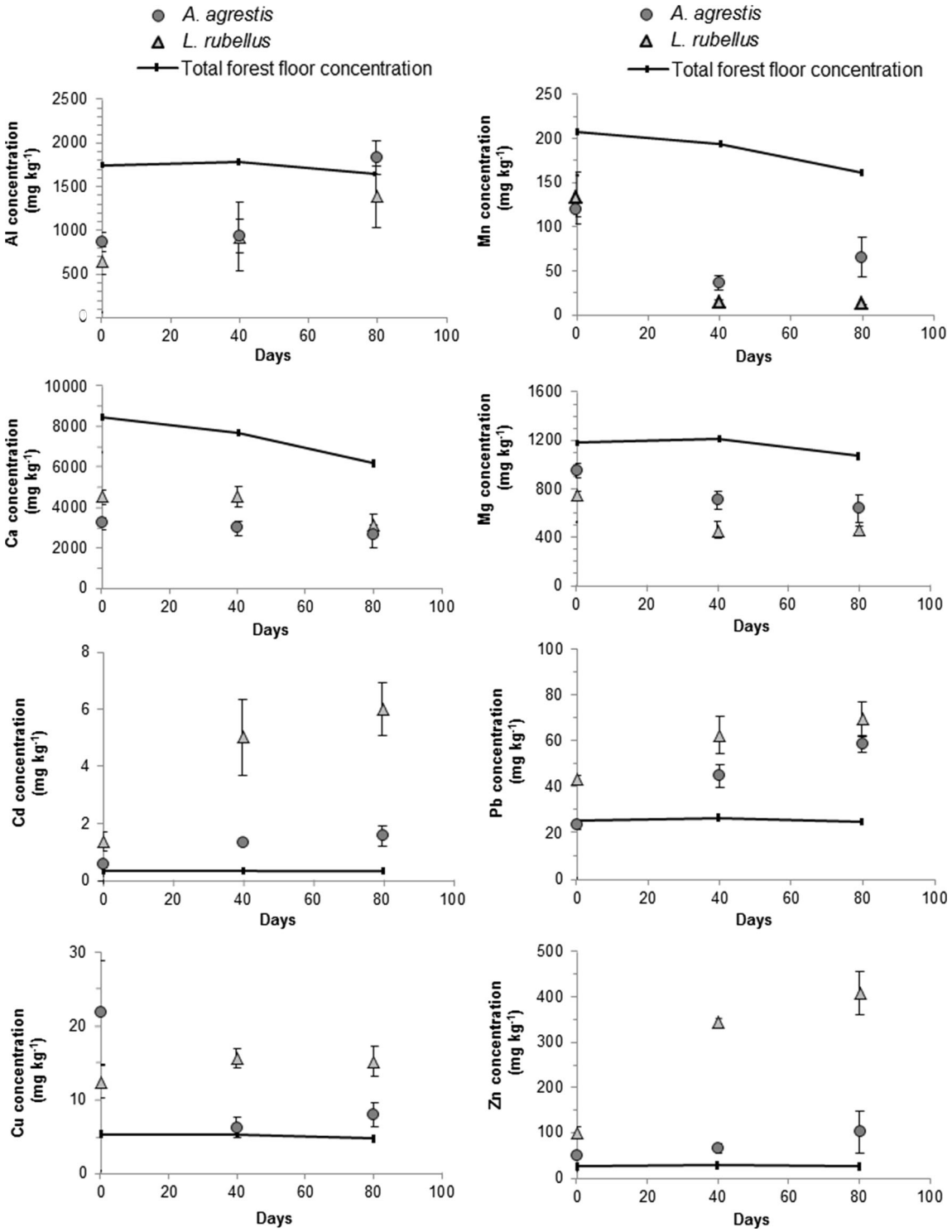

Fig. 4 Average total (exchangeable + stable) forest floor concentrations across all treatments compared with earthworm concentrations through the 80-day pot incubation. Earthworm metal concentrations were measured prior to introduction to the forest floor pots and after 40 and 80 days of incubation in the forest floor pots. *Error bars* are ±1 standard error, $n = 9$

been manifested as a decrease in stable concentrations or an increase in exchangeable concentration, depending on the intensity of the effect. Although our previous results show a significant decrease in forest floor mass and soil C concentrations, stable metal concentrations did not vary among earthworm treatments for any of the metals using one-way ANOVA for each collection date and repeated measures one-way ANOVA for the duration of the experiment

Table 5 Displayed are the BAF (bioaccumulation factor) and BAF-exchangeable for *Amynthas agrestis* and *Lumbricus rubellus* through time

Treatment	Number[a]	No. of days	Al (μg/μg)	Ca (μg/μg)	Cd (μg/μg)	Cu (μg/μg)	Mg (μg/μg)	Mn (μg/μg)	Pb (μg/μg)	Zn (μg/μg)
A. agrestis BAF	9	80	1.09	0.47	5.0	1.59	0.62	0.46	2.32	4.03
L. rubellus BAF	9	80	0.71	0.44	16.4	2.69	0.35	0.07	2.39	13.87
A. agrestis BAF-exchangeable	9	80	53.8	0.69	11.8	31.5	1.64	0.69	104.2	8.17
L. rubellus BAF-exchangeable	9	80	0.78	0.61	38.0	67.5	23.6	0.10	88.3	27.8

BAF values were calculated using the strong-acid extractable metal concentrations in earthworm tissues and soils. BAF-exchangeable values were calculated using the strong-acid extractable metal concentrations in earthworm tissues and exchangeable metal concentrations in soils

[a] The number of replicate forest floor pots

(Table 2). This suggests that earthworms did not significantly decrease the stable pool of metal in the forest floor material to an observable extent (Table 4), and these results agree with other studies on earthworms and trace metals (e.g., Sizmur and Hodson 2008; Natal-da-Luz et al. 2011).

We did observe significant differences in exchangeable metal concentrations in forest floor pots among earthworm treatments. However, these differences were inconsistent. For example, exchangeable Cu concentrations in control pots were significantly greater than in pots containing *L. rubellus* at 40 days ($P < 0.05$), but the opposite effect was observed at 80 days ($P < 0.05$). Irregular patterns through time were observed for exchangeable concentrations of Ca, Mg, Mn, and Pb, for both earthworm treatments (Table 2). However, the repeated measures one-way ANOVA results do indicate that earthworms significantly affected exchangeable metal concentrations. Exchangeable Al and Cd were significantly increased by the presence of *L. rubellus*. The presence of *A. agrestis* and *L. rubellus* significantly decreased exchangeable Ca, Cu, Mn, and Pb concentrations. However, the overall effect when considered as % exchangeable earthworms did not significantly increase or decrease. This is partly attributable to the fact that stable concentrations were generally greater than the exchangeable concentrations (except for Ca and Mg) and were not affected by earthworm treatments.

Although significant differences were observed among treatments, our results suggest that earthworms do not affect forest floor metal exchangeability in a clear, predictable manner (Tables 3 and 4). This agrees with previous studies that also did not find a significant, predictable effect of earthworms on metal availability (e.g., Udovic and Lestan 2007). For example, Lukkari et al. (2006) did not find significantly greater exchangeable metal concentrations in egesta compared to undigested soil. This stands in contrast to other studies that have found that earthworms increased metal availability in soils (cf. Sizmur and Hodson 2009). There are three key unique aspects to this study that may have led to results that differ from other studies. First, our experiments utilized *A. agrestis* and *L. rubellus* earthworm species, while previous studies focused on other species such as *Eisenia fetida*, *Aporrectodea caliginosa*, or *L. terrestris*. Second, our study focused on the strongly acidic forest floor material, as opposed to mineral soil horizons with lower organic matter concentrations and higher pH values. Lastly, other studies have conducted their experiments with soils that are point-source contaminated (in close proximity to a known pollution sources). These factors control the rate of metal uptake and excretion by earthworms (Morgan and Morgan 1999; De Vries et al. 2007) and are likely the source of variation in studies identifying increased exchangeability in soil by earthworms. In our strongly acidic, organic-dominated, moderately contaminated soils, we did not observe an increase in exchangeability.

Variations in exchangeable and stable concentrations could have potentially been caused by earthworm bioaccumulation, leaching, or stabilization. Uptake and bioaccumulation in earthworm tissue may have contributed to lower exchangeable metal concentrations in forest floor pots with earthworms. However, the earthworm masses (0.3–2.0 g) were far smaller than the forest floor mass (380–500 g) and unlikely to significantly increase or decrease exchangeable metal concentrations. Uptake and bioaccumulation of metals is discussed in greater detail in the section "Earthworm bioaccumulation." Increased leaching from the forest floor pots due to bioturbation was a possibility but was unlikely the cause of differences in metal concentrations. Leachate was minimized but did occur as <50 mL of solution in total, as per pot. However, leachate metal concentrations were not measured. Increased stabilization of metals in egesta is another possibility, particularly for metals Ca, Mg, Pb, and Zn and other metals potentially stabilized as carbonates by the calciferous gland of *L. rubellus* (Sizmur and Hodson 2009; Nannoni et al. 2014). However, an increase in stable metal concentrations was not observed and may not have been detectable due to the relative difference in stable and exchangeable concentrations.

Earthworm bioaccumulation

For our last hypothesis, we expected that earthworms would bioaccumulate metals from forest floor material. The BAF of metals were similar to several other studies on metal bioaccumulation (Ireland 1979; Morgan and Morgan 1999; Ernst et al. 2008; Nannoni et al. 2014; Richardson et al. 2015b). The Cu, Zn, and Pb BAF values in this study are similar to previous studies (e.g., Morgan and Morgan 1999; Dai et al. 2004; Ernst et al. 2008; Richardson et al. 2015b). Similarly, our Cu, Zn, and Pb BAF values for *L. rubellus* are also in agreement with our hypothesis and previous studies (e.g., Morgan and Morgan 1999; Ernst et al. 2008; Richardson et al. 2015b). In general, the uptake of metals by earthworms may have been affected by the material they ingested, or physiological differences in digestion (Morgan and Morgan 1999; De Vries et al. 2007). The material ingested may have affected earthworm metal tissue concentrations in multiple ways. First, the change in tissue concentration during the experiment (especially from 0 to 40 days) was primarily driven from a dietary change of the forest soil to the forest floor material, which contained different metal concentrations (Table 1). This change in soil material affected earthworm tissue concentrations of each metal differently. When earthworms were transitioned from their "native" forest soil to the forest floor material used for the pot experiment, which contained much lower Mn and Cu concentrations (Table 1), earthworm tissue concentrations decreased. This was evident for Mn in both earthworms and for Cu with *A. agrestis* (Fig. 4). However, this did not occur for the other metals. For example, instead of earthworm Ca tissue concentrations decreasing, they were similar throughout the experiment. This suggests that the earthworms' Ca was not affected by this transition, likely due to earthworm regulation of their tissue Ca concentrations (Edwards and Bohlen 1996). An additional change may have been preferential consumption of soil with varying metal concentrations during the experiment (Morgan and Morgan 1990).

Physiological differences in digestion between earthworms may have affected their tissue concentrations of Cd, Cu, and Zn. Uptake and bioaccumulation of Cd, Cu, and Zn by many earthworm species has been observed in previous studies (e.g., Neuhauser et al. 1995; Morgan and Morgan 1999; De Vries et al. 2007; Nannoni et al. 2014). The differences in Cd, Cu, and Zn tissue concentrations between *A. agrestis* and *L. rubellus* may have arisen from physiological differences in their digestive tract. Differences in surface area or enzymatic activity could affect the rate of assimilation of metals into tissues. Specialized organs, such as the calciferous glands, may aid in the formation of carbonate precipitants in the earthworm (Edwards and Bohlen 1996; Piearce 1972; Nannoni et al. 2014). Additionally, the tissue concentrations of Cu and Zn in *L. rubellus* may have exceeded *A. agrestis* due to a preference to consume material that had higher Cu and Zn, whether deliberately or unintentionally. *L. rubellus* and other earthworms have been shown to be selective feeders (Morgan and Morgan 1999). The consumption of highly decomposed material containing higher metal concentrations could lead to varying uptake rates, among individuals or among genera.

Other processes that may affect earthworm tissue concentration are decreasing biomass or metal excretion. In decreasing biomass, the concentration of metal in the earthworm tissue is increased due to the loss of biomass at a greater pace than it uptakes the metal. Although earthworm biomasses were decreasing throughout the experiment, it was not significantly different. Additionally, metals may be lost from the earthworm via excretion. In particular, earthworm tissue Mn concentrations decreased through the first 40 days of the experiment. As mentioned earlier, this was the result of being introduced to a soil matrix with lower Mn concentration, and excess Mn was lost via excretion to the forest floor material. However, the excreted Mn was not enough to influence the observed exchangeable or stable forest floor Mn concentrations.

The bioaccumulation of Pb by *L. rubellus* has been observed in previous studies (e.g., Neuhauser et al. 1995; Morgan and Morgan 1999; Ernst et al. 2008; Richardson et al. 2015b). The bioaccumulation and toxicity of Al in earthworms have been previously reported for other species by Zhao and Qui (2010). Toxic metals such as Al and Pb are accumulated in earthworm tissues due to a general lack of physiological processes of excretion (Morgan and Morgan 1999; Nannoni et al. 2014). Interestingly, Al concentrations were within the range shown to be lethal for other species of earthworm such as *Eisenia andrei* (Zhao and Qui 2010) and *Eisenia fetida* (Zhang et al. 2013). However, survival of *A. agrestis* and *L. rubellus* was >50 % for all treatments (Supplemental Table 1). Whether the high concentrations of Al contributed to the death of earthworms is unclear. The high organic matter concentration of the forest floor may have alleviated much of the Al toxicity at the measured soil Al concentration based upon Zhang et al. (2013). However, other mesocosm experiments have also reported similar mortality rates (e.g., Bellitürk et al. 2015).

Metal concentrations of Ca, Cd, Cu, Mg, Mn, and Zn did not exceed concentrations known to be toxic, which is in agreement with previous studies in other regions (e.g., Beyer and Cromartie 1987; Neuhauser et al. 1995). However, earthworms can attain concentrations potentially hazardous to animals (e.g., Beyer and Cromartie 1987; Richardson et al. 2015b). In this study, tissue concentrations of Al and Pb for *A. agrestis* and *L. rubellus* exceeded the maximum tolerable levels in feed for mice and poultry as described by the Committee on Minerals and Toxic Substances in Diets and Water for Animals from the National Research Council (Klasing et al. 2005). In addition, our observed *L. rubellus* tissue concentrations of 6 μg Pb g^{-1} of tissue do approach

the 10-μg-g^{-1} concentration that can lead to organ damage in mammals and fowl from chronic ingestion (Fig. 4). Similarly, *A. agrestis* and *L. rubellus* exceeded the 1000-μg-g^{-1} concentration maximum tolerable levels after 80 days of incubation (Fig. 4). Thus, the earthworms have or may attain potentially hazardous concentrations of Al and Pb for small mammals and birds within 80 days of inhabiting the forest floor pots.

BAF values of ≤1.0 show that soil concentrations exceed earthworm TMM concentrations, suggesting dilution or nonaccumulation. BAF values >1.0 suggest active bioaccumulation by earthworms (Table 5). Using this premise, Al, Ca, Mg, and Mn were actively removed to weakly bioaccumulated in earthworm bodies, while Cu, Zn, and Pb were weakly to strongly bioaccumulated. From Fig. 4, it is clear that earthworm concentrations of Al, Cu, Pb, and Zn are able to exceed or match the total forest floor metal concentration due to bioaccumulation. The differences in BAF values between *A. agrestis* and *L. rubellus* may have arisen from genus-specific factors such as calciferous glands, which may affect the excretion of metals (Edwards and Bohlen 1996; Piearce 1972).

Overall, our exchangeable BAF values were similar to the conventional BAF values for many metals (Table 5). However, differences between species and among metals with varying exchangeability were more pronounced. For example, Mg was not bioaccumulated by either earthworm on the basis of a <1.0 BAF value. However, the BAF-exchangeable value suggests that Mg was relatively weakly bioaccumulated by *A. agrestis* (BAF-exchangeable = 1.64) but strongly bioaccumulated by *L. rubellus* (BAF-exchangeable = 23.6) (Table 5). In addition, Al which was weakly bioaccumulated by *A. agrestis* according to the conventional BAF value (1.09) now shows a very strong BAF-exchangeable value of (53.8). The BAF-exchangeable suggests this is likely a species-unique property because *L. rubellus* Al BAF value (0.71) and BAF-exchangeable value (0.78) were <1 and nearly identical. Hobbelen et al. (2006) suggest that uptake of Cu and Cd by *L. rubellus* is strongly controlled by its exchangeability but can uptake metals from stable phases as well. Thus, uptake and subsequent bioaccumulation is co-dependent on stable concentrations and exchangeable concentrations. This implies that measuring BAF-exchangeable can help interpret the phase of the source of the bioaccumulated metal.

The transplanting of earthworms between soils with dissimilar metal concentrations and pH may have influenced our results, but they may have also highlighted that earthworms had the capacity to respond to pollutants. For three metals, the transfer of earthworms from the forest soil to the forest floor pot material caused greater exposure to Al, Cd, and Pb. For the other metals, there was a decrease in exposure. After 80 days, large BAF-exchangeable values were found for Al, Cd, and Pb for both earthworms, with the exception of Al and *L. rubellus*. However, Cu, Mg, and Zn exposure was reduced but had high BAF-exchangeable values for at least one of the earthworm species. Thus, there was no consistent pattern in BAF-exchangeable response to change in exposure. Further careful studies of the effects along pollutant gradients may increase our knowledge of how earthworms respond to changes in exposure (e.g., a pollution event or chronic accretion of soil metals through atmospheric deposition).

Whether to utilize total extractable metal concentrations or exchangeable metal concentrations when calculating bioavailable metal fraction is still a topic of debate. Arguments can be made for other methods to measure the most bioavailable fraction, such as water extraction. Studies such as Hobbelen et al. (2006) found that strong acid extractable concentrations best correlated with uptake into earthworms, while Vijver et al. (2007) found water extractable concentrations to be a better predictor of metal uptake by earthworms. Other parameters besides the speciation of metals in soil can affect their uptake into earthworms. To further complicate predictions of bioaccumulation, Sizmur and Hodson (2009) have shown that field studies and laboratory studies can provide contradicting results on which extraction method better predicted metal accumulation by earthworms. Moreover, metal uptake by earthworms varies widely among genera and even species due to differences in dermal and intestinal uptake as the primary method for inclusion into earthworm tissue (Sizmur and Hodson 2009; Nannoni et al. 2014). For these reasons, our study provides a case study for those interested in strongly acidic, moderately contaminated forest soils. Future research should consider other common soil types and soil physicochemical parameters for earthworm uptake studies conducted in the laboratory.

Conclusions

This study assessed the effect of *A. agrestis* and *L. rubellus* on forest floor decomposition, metal exchangeability, and bioaccumulation of plant essential and toxic metals. Our results show that both species of earthworms are able to live within the acidic forest floor of northern New England, USA, and increased its decomposition and loss of C. Although we observed significant differences in exchangeable Al, Ca, Cd, Cu, Mn, and Pb concentrations, we did not observe a significant alteration to the stable concentrations or overall exchangeability of metals in the soil. These results show that earthworms can affect the storage of metals by decomposing the forest floor, but not necessarily by increasing its solubility or mobility. From our assessment of bioaccumulation patterns, we observed that earthworms may attain concentrations of Al and Pb that are potentially hazardous to small mammals and ground-foraging birds. Quantifying the exchangeable concentration of a metal provides greater insight into the bioaccumulation of a metal as

opposed to examining only total concentrations in a soil. In order to validate our laboratory-based results, further research in locations where earthworms are being introduced is needed to assess their impact using field studies.

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