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Exotic Earthworms Decrease Cd, Hg, and Pb Pools in Upland Forest Soils of Vermont and New Hampshire USA

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Abstract Exotic earthworms are present in the forests of northeastern USA, yet few studies have documented their effects on pollutant metals in soil. The objective of this study was to identify if Cd, Hg, and Pb strong-acid extractable concentrations and pools (bulk inventories) in forest soils decreased with the presence of exotic earthworms. We compared 'Low Earthworm Abundance' (LEA) sites ($\leq$10 g m^{-2} earthworms, n = 13) and 'High Earthworm Abundance' (HEA) (>10 g m^{-2} earthworms, n = 17) sites at five watersheds across Vermont and New Hampshire. Organic horizon Cd, Hg, and Pb concentrations were lower at HEA than LEA sites. Organic horizon and total soil pools of Cd and Hg were negatively correlated with earthworm biomass. Soil profile Cd and Hg concentrations were lower at HEA than LEA sites. Our results suggest earthworms are decreasing accumulation of Cd, Hg, and Pb in forest soils, potentially via greater mobilization through organic matter disruption or bioaccumulation.

Keywords Trace metals · *Aporrectodea* · *Lumbricus* · *Dendrobaena* · Toxic metal · Bioturbation

In the northeastern USA, concentrations of Cd, Hg, and Pb in forest soils have been elevated due to atmospheric deposition from regional pollution by smelting, industrial activities, automobiles, and coal combustion (Friedland et al. 1986; Johnson et al. 1995; Richardson et al. 2013). Mobilization of Cd, Hg, and Pb from organic and mineral soil horizons can negatively affect terrestrial biota as well as surface and groundwater. Moreover, forest soils play a particularly important role in the regional and global cycling of Hg, an element with a substantial gaseous phase, by sequestering atmospheric Hg.

Exotic and invasive earthworms have been introduced to the northeastern USA as a result of human activities: deforestation, recreational fishing, and agriculture (Bohlen et al. 2004; Görres et al. 2014). Although earthworms are beneficial in agricultural systems (van Groenigen et al. 2014), their effects on forest ecosystems in North America are generally negative, with considerable consequences for plant communities, macrofauna, and soil biogeochemistry (Gundale et al. 2005; Bohlen et al. 2004). Previous studies have shown that earthworms are capable of mobilizing SOM or stimulating microbial decomposition of SOM following disturbance of soil aggregates (Bityutskii et al. 2012). However, the effect of earthworms on pollutant metal accumulation in soils has not been widely characterized in forested ecosystems (Sizmur and Hodson 2009).

Field observations of earthworm effects on pollutant metals in forest soils are limited and are needed to quantify earthworm-induced changes to forest ecosystems and environmental quality. In our previous study Richardson et al. (2015), we examined metal and metalloid concentrations within earthworm tissues. The objective of this study was to determine if Cd, Hg, and Pb concentrations and pools (bulk inventories) in forest soils changed as a result of the presence of exotic earthworms. We hypothesized that soils with greater earthworm population densities would have lower Cd, Hg, and Pb concentrations and pools in organic

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and mineral soil horizons due to bioturbation and decomposition of SOM by earthworms.

Materials and Methods

Sampling sites from five watersheds in Vermont and New Hampshire between the Green Mountains of Vermont and the White Mountains of New Hampshire were studied (Fig. 1). Watersheds in this study correspond with Study Sites 1, 2, 3, 5, and 6 from Richardson et al. (2015). Study sites 4, 7, 8, and 9 from Richardson et al. (2015) were not included in this study due to earthworm dominance and proximal human management. Elevation ranged from 250 to 500 m.a.s.l. The watersheds were chosen from a previous regional sampling by Kamman et al. (2003), for their extensive watershed information. In total, six sampling sites were studied at each of the five watersheds (N = 30). Vermont and New Hampshire, USA are continental with warm summers and cold, dry winters (DfB) according to the Köppen–Geiger classification.

Vegetation in all watersheds was secondary-growth, uneven-aged mixed composition stands consisting of northern hardwoods with interspersed coniferous vegetation. The soil parent material at sampling sites 1–24 was basal glacial till. The soils are well-drained, upland soils generally with an average depth of 46 cm until glacial-till. Soils in sampling sites 25–30 were derived from glacial lake sediments. Soil textures were all sandy loam, with clay fractions <10% for all watersheds. Rock fragments gravel to cobble sized (2 mm–10 cm) were common in sampling sites 1–24 particularly in the BC, Bs, Bw and Bw2 horizons. The textures of soils at sampling sites 25–30 are predominately sandy loams dominated by fine sand and there are very few rock fragments until >46 cm in depth. The soils at sampling sites in 1–24 were Spodosols with Albic (E, AE, EA) horizon and Spodic (Bh, Bhs or Bs) horizons and Inceptisols with Ap and multiple Bw horizons (Soil Survey Staff 2010).

All sampling was conducted in September and October of 2013. Soil pits were excavated at each sampling sites within the watersheds. At each sampling site, one morphological and one quantitative soil pit (quadrat) were excavated [see Richardson and Friedland (2016) for methodology]. Earthworm casts were included in their respective soil horizon, most commonly an A horizon. Sampling sites within a watershed were at least 100 m apart and in a representative location to avoid microtopographic low points. To avoid edge effects, selection of soil pit locations was restricted to areas greater than 50 m from any human development or road and at least 100 m away from any water body or stream and >50 m into the forest away from the edge boundary. The soil profile for each site was described from the morphological pits following the USDA taxonomic guide (Soil Survey Staff 2010). Live earthworms were hand-sorted from the organic horizons and mineral soil in the 0.25 m² quantitative soil pit. Earthworms were stored alive in their horizon material and identified live using a dichotomous key (Great Lakes Worm Watch, University of Minnesota 2011). Information on earthworm species, ecophysiological classification, abundance, biomass and trace element composition is available and discussed in Richardson et al. (2015) within text and supplementary materials.

Soil samples from the morphological soil pit at each site were analyzed for physicochemical properties. Soil pH was determined using a 2:5, soil:water slurry in 0.01 M CaCl$_2$. The % SOM was determined using loss on ignition, in which 4 g of soil was held at 475°C for 8 h. Soil samples from the morphological soil pit were analyzed for strong-acid extractable trace metals following EPA method 3051A. The use of a strong-acid digestion does not reveal any information on bioavailability, but does allow us to quantify concentrations of metals in non-primary mineral phases that may become dissolved or exchanged. In brief, 0.25 g of air-dried material was and microwave digested at 90°C for 45 min in 5 ml of strong acid (9:1, HNO$_3$:HCl), degassed overnight, and analyzed with an Agilent 7700× ICP-MS (Agilent Technologies, Santa Clara, CA) for (Cd, Pb, and Hg). See Richardson et al. (2015) for details on digestion QA and QC methods.

Descriptive statistics were calculated using Matlab (Matlab Inc, Natick, MA). Sampling sites with ≤10 g m^{-2} earthworms in the quantitative soil pits were considered 'Low Earthworm Abundance' (LEA) while 'High Earthworm Abundance' (HEA) sites had >10 g m^{-2} (roughly greater than 20 earthworms m^{-2}; see Supplemental Table 6 in Richardson and Friedland 2016). Metal concentrations in LEA and HEA soils were compared using the non-parametric Kruskal–Wallis test. Soil horizons were grouped by
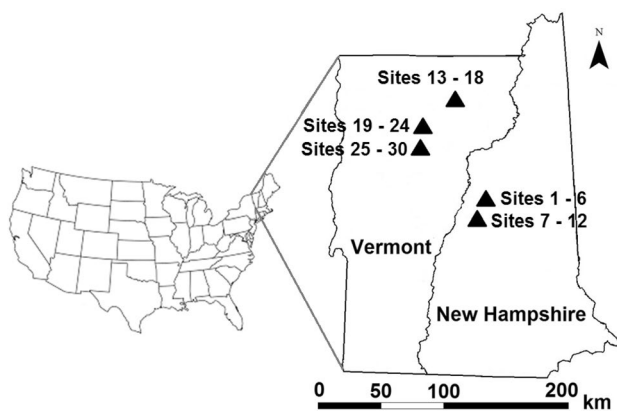

Fig. 1 Location of sampling sites in Vermont and New Hampshire, Sites 1–6 (Lake Armington watershed), Sites 7–12 (Upper Baker Pond watershed), Sites 13–18 (Great Hosmer Pond watershed), Sites 19–24 (Hardwood Pond watershed), and Sites 25–30 (Curtis Pond watershed)

epipedons and subsurface order (i.e. A and E surface horizons were compared). Differences in soil physicochemical properties, soil trace metals concentrations were compared between LEA (n = 13) and HEA sites (n = 17) using the non-parametric Wilcoxon signed-ranks test.

Results and Discussion

Exotic European earthworms were present at 17 of the 30 sampling sites. LEA and HEA sites were generally evenly distributed, LEA sites were (1, 2, 4, 5, 8, 10, 11, 12, 16, 17, 19, 20, 21, 22, 24, 28, 29). Five European species were observed and *Aporrectodea rosea* was most prevalent at 11 sampling sites. *Dendrobaena octaedra* was present at 7 sampling sites. *Lumbricus* genera were expected to be prevalent due to recreational fishing (Addison 2009) but were only observed at four sampling sites. Moreover, *Lumbricus terrestris*, which is one of the most commonly used earthworm for fishing bait (Addison 2009; Resner et al. 2011), was the least encountered earthworm, present at only 1 sampling site. *Dendrobaena octaedra, Aporrectodea rosea*, and *Aporrectodea tuberculata* had the highest population densities, with an average of approximately 50 individuals per m^{-2} (see Richardson et al. 2015). Sites with *Amynthas Agrestis* in Richardson et al. (2015) were not present in the subset of watersheds included in this study. Additional information about earthworm biomass and juveniles can be found in Richardson et al. (2015).

The Oe and Oa horizons at HEA sites were approximately 50% thinner than LEA sites (Table 1). Moreover, the % SOM at HEA sites was 4% higher in the A and E horizons but was 3% lower for BC and Bw2 horizons at HEA sites than LEA

sites ($p < 0.05$)(Table 1). When SOM was considered on an area-mass basis, the organic horizons at HEA sites had lower SOM (4.2 ± 0.5 kg m^{-2}) with earthworms compared to LEA sites (5.6 ± 0.7 kg m^{-2}). Mineral soil SOM mass was not significantly different between LEA and HEA sites. These results suggest that earthworms are enhancing decomposition of SOM and consuming the organic horizons, matching observations described in previous studies (e.g. Lyttle et al. 2011). Earthworms have likely increased the % SOM in the A and E horizons through bioturbation and/or by mixing SOM from the organic horizons into the mineral soil (Langmaid 1964; Bohlen et al. 2004; Lyttle et al. 2011).

Soil pH was 0.2–0.5 pH units higher at HEA sites for nearly all organic and mineral horizons ($p < 0.05$) (Table 1) which agrees with Burtelow et al. (1998). The thinner organic horizons and lower total organic horizons mass at HEA sites may have caused the underlying mineral soil horizons to be less acidic than at LEA sites (Strobel et al. 2005). This may have occurred either from the earthworms decomposing the litter responsible for acidification or due to the secretion of calcium carbonate from their calciferous glands (Langmaid 1964; Bohlen et al. 2004; Resner et al. 2011). However, earthworms may be found in greater abundance in the higher pH soils due to greater survival and fitness (Addison 2009). Physical and chemical soil properties were not significantly different among watersheds ($p > 0.05$), indicating watersheds likely did not confound differences between LEA and HEA sites.

We hypothesized that Cd, Hg, and Pb concentrations and pools would be lower at HEA sites than at LEA sites due to changes in SOM as a result of earthworms. Cadmium concentrations were lower in Oe, Oa, A and E, and Bw and Bs horizons at HEA sites compared to equivalent horizons

Table 1 Description of physical and chemical soil properties by soil horizon at low earthworm abundance (LEA) (n = 13) and high earthworm abundance (HEA) (n = 17) sites

Earthworm abundance	Horizon	Depth (cm)	pH 2:5 soil:water	SOM (%)	Soil mass kg m^{-2}
LEA	Oi	2.1 ± 0.1	4.0 ± 0.1	83 ± 3	0.9 ± 0.2
LEA	Oe	3.1 ± 0.3*	3.4 ± 0.2	75 ± 5	2.0 ± 0.3
LEA	Oa	5.3 ± 1.0*	3.3 ± 0.2	41 ± 4	8.2 ± 3.2
LEA	A and E	6.2 ± 0.9	3.6 ± 0.2	10 ± 1	75 ± 23
LEA	Bs and Bw	16.8 ± 1.8	4.0 ± 0.1	11 ± 2	179 ± 23
LEA	BC and Bw2	19.0 ± 1.9	4.4 ± 0.1	8 ± 1*	142 ± 31
HEA	Oi	1.8 ± 0.3	4.3 ± 0.1*	70 ± 4	0.6 ± 0.1
HEA	Oe	1.4 ± 0.3	3.7 ± 0.2*	65 ± 5	2.2 ± 1.3
HEA	Oa	2.7 ± 0.4	3.8 ± 0.2*	39 ± 5	6.0 ± 1.7
HEA	A and E	7.7 ± 0.9	4.0 ± 0.1*	14 ± 1*	81 ± 21
HEA	Bs and Bw	14.4 ± 1.3	4.3 ± 0.1*	9 ± 1	154 ± 32
HEA	BC and Bw2	17.8 ± 2.3	4.5 ± 0.1	5 ± 1	135 ± 36

Mean values are shown $\pm$ 1 standard error

Asterisk indicates a significant difference between LEA and HEA sites using the non-parametric Wilcoxon signed-ranks test ($p < 0.05$)

at LEA sites (Fig. 2). Mercury concentrations were lower in Oa, Bw and Bs, and Bw2 and BC horizons at HEA sites compared to equivalent horizons at LEA sites (Fig. 2). Lead concentrations were only relatively lower in the Oa horizons at HEA sites than LEA sites (Fig. 2). However in the mineral soil, Hg and Pb concentrations in the A and E horizons were the opposite of the hypothesized effect and were higher at HEA sites than LEA sites (Fig. 2). When considering metal pools, we observed significant negative correlations between Cd, Hg, and Pb organic horizon pools with earthworm biomass at the 30 sampling sites ($p < 0.05$) (Fig. 2). Total soil pools of Cd and Hg were significantly lower with greater earthworm biomass ($p < 0.05$) (Fig. 3). Total soil Pb pools, however, did not exhibit a significant trend with earthworm biomass. Metal concentrations and pools did not vary significantly among watersheds ($p > 0.05$), suggesting differences among watersheds did not confound differences between LEA and HEA sites.

Our findings suggest that earthworms are decreasing the accumulation of Cd, Hg, and Pb in forest soils. Earthworms are most likely increasing the mobilization of pollutant metals from organic and mineral soil horizons by decomposition and disruption of SOM (see Sizmur and Hodson 2009; Resner et al. 2011). We observed significantly lower SOM mass in the organic horizons with greater earthworm biomass. However, there are additional potential pathways for decreased Cd, Hg, and Pb accumulation and retention in forest soils. As shown in Richardson et al. (2015), earthworms bioaccumulate Cd, Hg, and Pb from soils. Thus, earthworms may enhance losses of metals from soils through
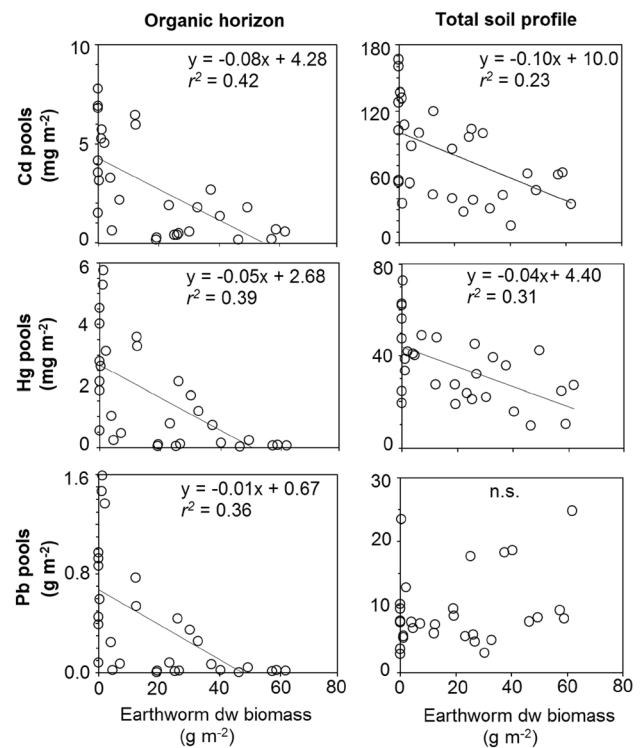
Fig. 3 Organic horizon and total soil profile Cd, Hg, and Pb pools plotted against earthworm biomass mass for each site among the five watersheds. For the organic horizon pools, Oi, Oe, and Oa horizons were summed while all soil horizons were summed for the total soil profile pools. (n.s.) indicates the linear regression was non-significant ($p \geq 0.05$)
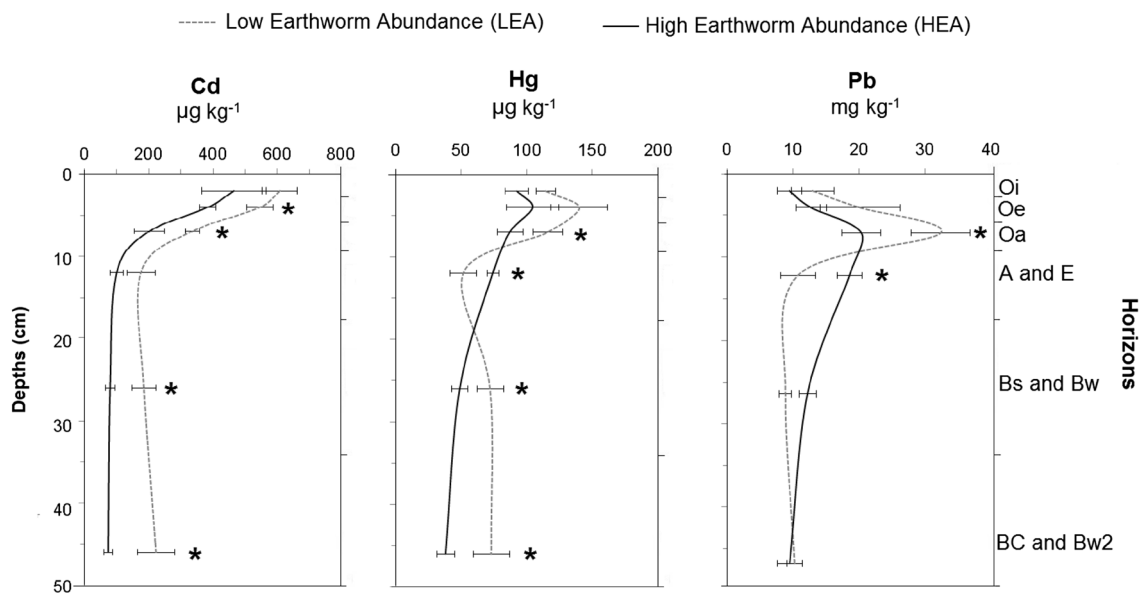
Fig. 2 Concentrations of Cd, Hg, and Pb in Low Earthworm Abundance (LEA) (n = 13) and High Earthworm Abundance (HEA) (n = 17) forest soils were compared. Mean values are shown ± 1 standard error. (*) indicates a significant difference between LEA and HEA sites using the non-parametric Wilcoxon signed-ranks test ($p < 0.05$)

bioaccmulation and incorporation into biomass and subsequent predation. Earthworms may be decreasing the accumulation of Cd and Hg from litterfall in the soil by altering their immobilization pathway in soil. Litterfall is one of the major sources of Cd and Hg to forest soils in Vermont and New Hampshire (e.g. Richardson and Friedland 2016). The decomposition of leaves and SOM containing Cd and Hg by earthworms as opposed to microorganisms may affect the formation of immobile SOM-metal compounds and their retention in the soil profile (Langmaid 1964; Bohlen et al. 2004; Sizmur and Hodson 2009). We hypothesized that Pb from 1960s and 70s combustion of leaded gasoline (see Johnson et al. 1995) has been mobilized from Oa horizons into A and E horizons at HEA sites. Lastly, earthworms may prefer forest soils with properties that promote less Cd and Hg retention. Further studies are needed to determine if less Cd and Hg are being stabilized in earthworm inhabited soils or if Cd and Hg are being lost from the soil profile as mobile compounds or transferred to the predators. In addition, more field experiments are required to determine the spatial and temporal extent by which earthworms are changing trace metal biogeochemistry.

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