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Invasive Earthworms Deplete Key Soil Inorganic Nutrients (Ca, Mg, K, and P) in a Northern Hardwood Forest

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Abstract

Hardwood forests of the Great Lakes Region have evolved without earthworms since the Last Glacial Maximum, but are now being invaded by exotic earthworms introduced through agriculture, fishing, and logging. These exotic earthworms are known to increase soil mixing, affect soil carbon storage, and dramatically alter soil morphology. Here we show, using an active earthworm invasion chronosequence in a hardwood forest in northern Minnesota, that such disturbances by exotic earthworms profoundly affect inorganic nutrient cycles in soils. Soil nutrient elemental concentrations (Ca, Mg, K, and P) were normalized to biogeochemically inert Zr to quantify their losses and gains. This geochemical normalization revealed that elements were highly enriched in the A horizon of pre-invasion soils, suggesting tight biological recycling of the nutrients. In the early stage of invasion, epi-endogeic earthworm species appeared to have been responsible for further enriching the elements in the A horizon possibly by incorporating leaf organic matter (OM). The arrival of geophagous soil mixing endogeic earthworms, however, was associated with near complete losses of these enrichments, which was related to the loss of OM in soils. Our study highlights that elemental concentrations may not be sufficient to quantify biogeochemical effects of earthworms. The geochemical normalization approach, which has been widely used to study soil formation, may help when determining how invasive soil organisms affect soil elemental cycles. More generally, this approach has potential for much wider use in studies of belowground nutrient dynamics. The results support the existing ecological literature demonstrating that invasive earthworms may ultimately reduce productivity in formerly glaciated forests under climate change.

Key words: hardwood forest; inorganic nutrient cycling; biological invasion; earthworms; geochemical mass balance; calcium; phosphorous; potassium; magnesium.

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Author contributions K.R. conducted part of the laboratory and data analyses, and wrote the first draft of the manuscript. K.Y. conceived and designed the study, led and supervised field and laboratory works, data analysis, and paper writing. A.A. and S.D.S. assisted with study design, laboratory analysis, and manuscript writing. C.H. supervised earthworm survey and identification and coordinated the 2009 field trip. A.L. participated in determining earthworm biomass and identification. A.B. conducted and interpreted quantitative XRD.

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Introduction

Soil bioturbators have received considerable attention as ecosystem engineers that modify the flow of materials and energy (for example, Gutierrez and Jones 2006). Although their roles in shaping soil morphology have been widely documented (for example, Hole 1981; Johnson and others 2005), quantifying their effects on nutrient availability and biogeochemical cycling has been challenging because of direct and indirect feedbacks among physical perturbation, feeding activities, and soil types that are altered by bioturbators. This challenge is particularly acute with regards to invasive soil bioturbators because of expectations that they may negatively and nonlinearly affect forest ecosystems where introduced (Jones and others 1997; Eisenhauer and others 2008).

Earthworms are well-known bioturbators. About 3,700 earthworm species have been identified, and 120 species are dispersed globally or regionally largely due to human activity (Hendrix and others 2008). Northern hardwood forests in North America are examples of how exotic earthworms dramatically affect ecosystem structure and function. Since the last glacial retreat in approximately 9,000 years BP, northern forests in the glaciated regions had evolved without native earthworms until the recent introduction of exotic earthworms through agriculture, fishing, and logging (Hendrix and Bohlen 2002; Bohlen and others 2004b). Even though history and rates of invasion have large uncertainties (Stoscheck and others 2012), it has become increasingly difficult to find glaciated forests without the presence of exotic earthworms in the Great Lakes Region (Hale 2008).

In contrast to perceived benefits of earthworms to agriculture, exotic earthworms are considered nuisance species in the formerly glaciated northern forests that have evolved without native earthworms until recently. Consumption of litter by exotic earthworms has been shown to be detrimental to some native plants (Hale and others 2006; Frelich and others 2006; Holdsworth and others 2007) and animals (Maerz and others 2009; Loss and Blair 2011). Exotic earthworms have also been found to affect soil organic matter (OM) contents and distributions and homogenize soil profiles in hardwood forests (for example, Hale and others 2005b; Fahey and others 2013a, b; Lyttle and others 2014).

In the formerly glaciated northern hardwood forests, where earthworm invasion is widespread, a number of studies have shown that inorganic nutrients, particularly calcium (Ca), limit net primary production (for example, McLaughlin and Wimmer 1999). Mineralization of OM in the forest floors is the major mechanism of furnishing nutrient elements (for example, Likens and others 1998), and exotic earthworms, depending on their species compositions and biomasses, may significantly alter mineralization (for example, Hale and others 2005b; Fahey and others 2013a, b; Crumsey and others 2013). Here, we quantify losses and gains of key inorganic nutrient elements such as calcium (Ca), magnesium (Mg), potassium (K), and phosphorus (P) as a function of exotic earthworm communities across an earthworm invasion chronosequence in a sugar maple forest in Northern Minnesota: environmental conditions affecting soil formation, except the different compositions and arrival times of exotic earthworm species, were virtually homogeneous across the transect. We then discuss how the changes in the earthworm-affected fluxes of inorganic nutrients are tied to previously studied effects of exotic earthworms on other critical ecosystem properties such as plant diversity and soil carbon (C) contents.

Unlike previous studies that have focused on N and P availabilities and concentrations (Suarez and others 2003; Bohlen and others 2004a; Hale and others 2005a, b, 2006), we applied a geochemical normalization approach (for example, Brimhall and Dietrich 1987) to quantify losses and gains of inorganic nutrient elements (Ca, Mg, K, and P). We propose that geochemical normalization is critical particularly in accounting for fluxes of inorganic nutrient elements where OM is substantially more abundant. For example, even when lost from a soil, their concentrations may still increase if OM is being simultaneously lost at a faster rate. By normalizing the elemental concentrations relative to those of biogeochemically conservative elements, we can detect losses and gains of these elements. Geochemical normalization has been successfully and widely used to determine element enrichments and depletions during soil formation and ecosystem development over time scales from centuries to millions of years (for example, Chadwick and others 1999). To our knowledge, however, the geochemical normalization approach has not been applied to studying soil biogeochemical responses to earthworm bioturbation or more generally, for understanding belowground nutrient dynamics.

Methods

Study Site and Sampling

The Ottertail study site is located near Leech Lake in north central Minnesota (Figure 1). In 1998, a

transect of 150 m length was established (Hale and others 2005a, b, 2006; Frelich and others 2006; Larson and others 2009). In 2009, we extended this transect to 190 m (Figure 2). In this transect, the location nearest to the presumed source of invasion (that is, a road that may have facilitated introduction of earthworms, (Figures 1, 2) was set as the 0 m point. The location that we presumed to be most recently invaded was at the distance of 190 m east of that road. The composition of overstory trees was homogeneous and was dominated by sugar maples (*Acer sacharuum*) with interspersed birch (*Betula papyrifera*, *Betula alleghaniensis*) and basswood (*Tilia americana*). The area is generally flat, but seasonal vernal pools occur in local topographic depressions.

The soil at the site was mapped as the Warba soil series and classified as a fine-loamy, mixed, superactive, frigid Haplic Glossudalf (Soil Survey Staff 2014). The A horizon was thin (<5 cm) in areas without exotic earthworms (Figure 3). The underlying E horizon was highly homogeneous in thickness (~40 cm), silty texture, morphology, elemental chemistry, and mineralogy despite the presence of localized wetlands and vernal pools due to subtly undulating terrain (<3 m of topographic relief). The E horizon thus exhibited the characteristics of aeolian loess homogeneously blanketing the land-surface. Henceforth, we refer it as loess material (Figure 3). The C horizon was a calcareous glacial till, and the depths to C horizons varied between 80 and 150 cm.

In 2009, six soil pits were dug at distances of 0, 50, 100, 150, 160, and 190 m (Figure 2). Soils were sampled by 2.5-cm depth increments for the A horizon, 5-cm increments for the loess layer and 10-cm increments thereafter. At each soil pit, leaf litter thickness was measured, and litter was collected from a 0.09 × 0.09 m quadrat for determining biomass and elemental compositions. Our sampling of soils and leaf litter was based upon previous studies at the same transect regarding the effects of different exotic earthworm species on N and P nutrient status, soil C contents, soil horizon depths, and bulk densities (Hale and others 2005a, b, 2006; Frelich and others 2006; Larson and others 2009). Although there were relatively few soil locations examined as necessitated by the intensive soil excavation process and laboratory analyses, correspondence of findings between past and current studies suggests that we detected representative trends across the earthworm invasion chronosequence.

We used liquid mustard to extract earthworms every 10 m across the transect (Figure 2) during 2009. One gallon of water was mixed with 1/3 cup of powdered mustard and applied to 0.3 m^2 plots (Hale and others 2005a, b). Earthworms were

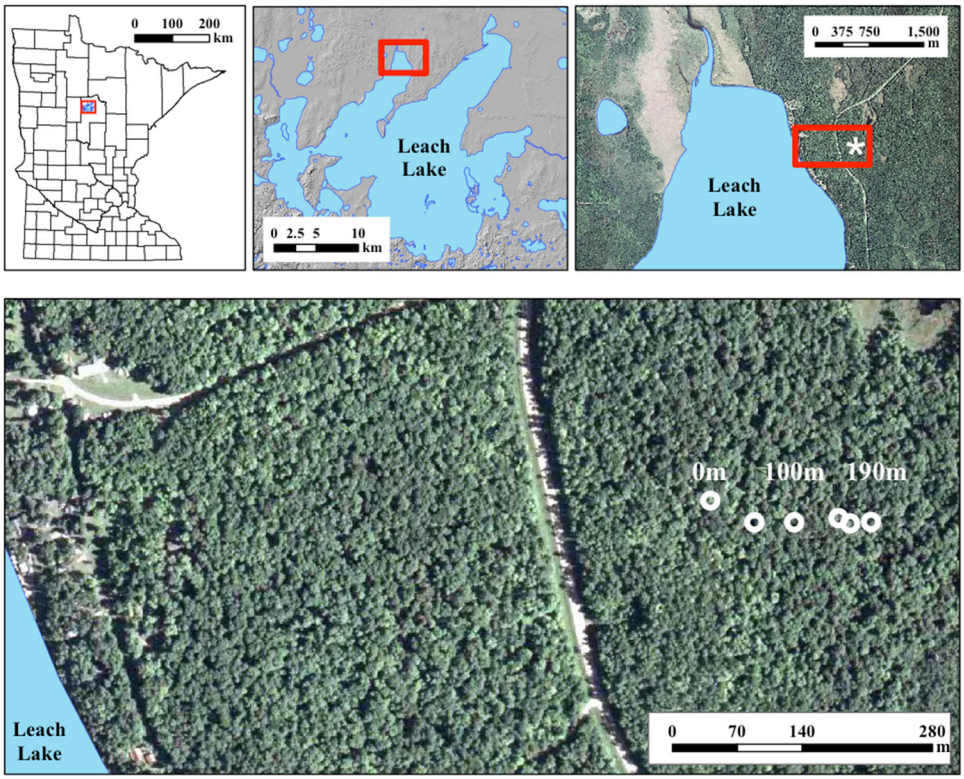

Figure 1. Maps of study area near Leech Lake, Minnesota and soil pits along the Ottertail earthworm invasion chronosequence.

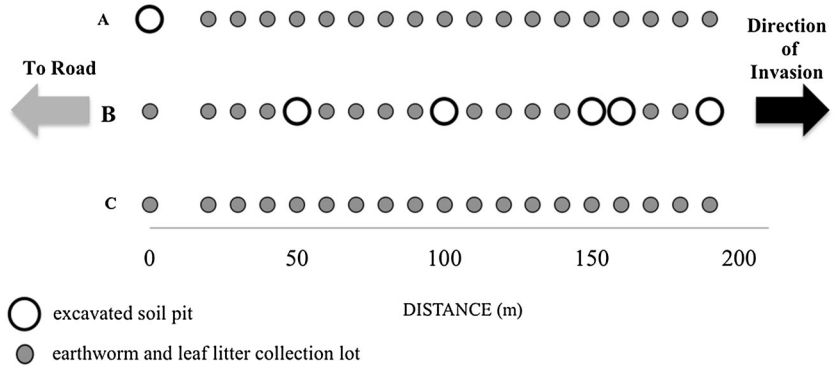

Figure 2. Sampling plots along the earthworm invasion chronosequence. The soil pit with the longest history of earthworm invasion (0 m) is located at the distance of 145 m from the road. The Ottertail sampling transect has 19 plots along three transects (**A**–**C**). *Open circles* represent the soil pits that were excavated in 2009, and the soil pits are divided into front (190, 169, and 150 m) and rear (100, 50, and 0 m) groups. Nineteen locations in *gray circles* along the transect B were used for earthworm sampling in 2009.

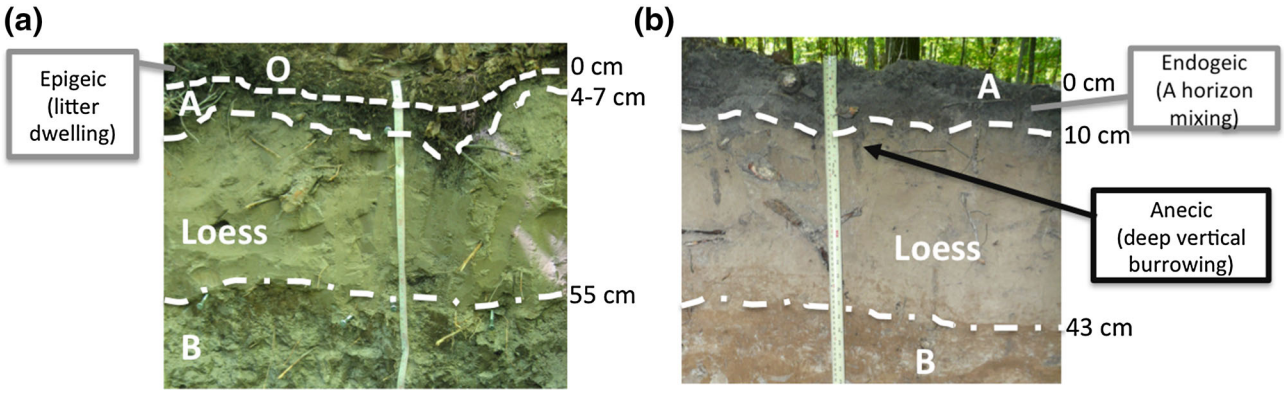

Figure 3. The top about 50 cm of two soil profiles at 190 m (**A**) and 0 m (**B**) along the studied transect with functional earthworm groups present. Removal of litter layer (O horizon) and thickening of A horizon with increasing degree of earthworm invasion are evident. The soil pit at the distance of 190 m shows a leaf litter layer, about 5 cm thick A horizon, and loess layer on top of B horizon (**A**), and the soil pit at 100 m exhibits thickened A horizon on top of the loess layer (**B**). The difference in the color between (**A**) and (**B**) is owing to the different lighting conditions.

collected for approximately 5 min after the application. Ash-free dry (AF) masses of earthworms were determined as described in Hale and others (2004). Earthworms were identified to species level and then grouped by their burrowing and feeding habits following Schwert (1990), Reynolds (1977), and Hale (2007). Anecic species build vertical and permanent burrows and consume leaf litter; endogeic species burrow and ingest OM and mineral material in the A horizon; and epigeic species only dwell in the litter layer feeding primarily on fungi and bacteria (Bohlen and others 2004b). Epi-endogeic earthworms (*Lumbricus rubellus*) show characteristics of both epigeic and endogeic species. *L. rubellus* and *Lumbricus terrestris* were undistinguishable when not sexually mature, so *Lumbricus* juveniles were grouped into the epi-endogeic category.

Geochemical Normalization

Even though an element may be lost from a soil, its elemental concentration may still increase if other elements were lost in greater quantities. Therefore, to correctly assess elemental losses or gains, we normalized each element relative to an index element that is biogeochemically inert, zirconium (Zr). Fractional mass depletion (negative values) or enrichment (positive values) for a given element in the A horizon (subscript, s) relative to its parent material (subscript, p) is

$$\tau_j = \frac{c_{i,\mathrm{p}}}{c_{i,\mathrm{s}}}\frac{c_{j,\mathrm{s}}}{c_{j\mathrm{p}}} - 1, \qquad (1)$$

where c is the mass concentration (kg kg^{-1}) of an element of interest (j) and index element (i). For

instance, τ_{Ca} of −0.5 indicates that 50% of Ca originally present in the parent material has been lost, whereas doubling of the original Ca due to addition from external sources would result in τ_{Ca} of 1.0. We confirmed that Zr was the least mobile by calculating the fractional changes of Zr relative to other potentially insoluble elements such as titanium and niobium at the site (Kurtz and others 2000). We used the loess as parent material for the A horizon because the A horizon was a physical mixture of OM and the loess at the study site. Across the studied transect, we did not observe any indication of physical mixing of soil materials proceeding beyond the depth of the loess materials. Variations in the elemental compositions of the loess materials were propagated to determine the standard errors in the calculated fractional mass changes.

Laboratory Analysis

Well-mixed soil samples (n = 102) were sub-sampled with soil-splitter and treated with a lithium borate fusion and analyzed for elemental chemistry by inductively coupled plasma-mass spectrometry (ICP-MS) with method number ME-ICP06 at ALS Chemex (ALS Chemex 2014). Leaf litter elemental concentrations were determined by inductively coupled argon plasma optical emission spectrometry (ICP-OES) (Fassel and Kniseley 1974) at the University of Minnesota Research Analytical Lab (http://ral.cfans.umn.edu/) for the materials collected from each soil pit in 2009 (n = 12), 0.5 g of dried plant biomass was predigested with 2 mL hydrogen peroxide (H_2O_2) and 0.5 mL nitric acid (HNO_3). The predigested material was then digested in a microwave oven prior to ICP-OES analyses (Munter and Grande 1981). Losses on ignition (LOI) were calculated from about 1.0 g of samples before and after muffling in an oven at 1,000°C for 1 h (method number: OA-GRA05 in ALS Chemex 2014). LOI was used as a proxy for OM contents. The coarse soil fractions (>2 mm) contributed less than 5% to sample masses and were thus ignored. We determined mineralogical compositions of selected soil materials by quantitative X-ray diffractometry (XRD) analysis at the United States Geological Survey in Boulder, Colorado. Exchangeable cations were determined for a subset of soil samples (n = 29). Three grams of fine fractions (<2 mm) of air-dried soils were mixed with 30 mL of 1 M ammonium acetate (NH_4OAc) at pH 7, shaken for 20 min, and centrifuged (Thomas 1982). Cation concentrations of extracts were then measured by ICP-OES at the University of Minnesota Research Analytical Lab. Non-exchangeable cations were calculated as the difference between exchangeable and total elemental contents.

Results

To mirror the chronological order of invasion, below we present results in the reverse order of distance. For instance, the results are introduced in the order of 190–160 m instead of 160–190 m.

Characteristics of Earthworm Invasion and Basic Soil Properties

The 2009 earthworm survey revealed the distribution of earthworm species (Figure 4), which was largely consistent with earlier observations (Hale and others 2005a). Plots at 190–160 m had few to no earthworms present. Plots from 190 to 120 m had litter dwelling epigeic earthworms, but no adult endogeic earthworms. The mid-section of the transect (100–70 m) was populated with endogeic and epi-endogeic earthworms as well as interspersed epigeic species and *Lumbricus* juveniles. Plots from 60 to 0 m were heavily populated with anecic species, in addition to endogeic and epigeic species.

The soil at 100 m marked the forefront of adult endogeic earthworms, and the transition from thick forest litter layer to bare mineral soil occurred at 150 m. Based on the distinct alteration of soil morphology associated with endogeic species, we divided the points along the transect into two groups (Figures 2, 4). Soils at distances 190, 160, and 150 m, all in front of the furthest presence of the endogeic earthworms are hereafter termed the front-group. The front-group had low earthworm biomasses comprised of epigeic, epi-endogeic *Lumbricus* juveniles, or no earthworms. Likewise, soils at distances of 0, 50, and 100 m, which were to the rear of the furthest presence of the endogeic earthworms, were in the rear-group (Figure 2).

Endogeic mixing of leaf litter and mineral soil is consistent with the observed thickening of the A horizon from less than 5 cm in the front-group to about 10 cm in the rear-group. Front-group soils had clear boundaries between the A horizon and the loess layer, in contrast to the rear-group soils that had diffusive boundaries (Figure 3). The loss of O horizon is also an important difference between the soils in the front- and rear-groups. Front-group soils had loam to silty loam textures in the A horizon, but the A horizon showed loam to sandy loam texture in the rear-group soils.

Soil mineralogy was mainly quartz, plagioclases, and K-feldspars (see Supplementary Data). The

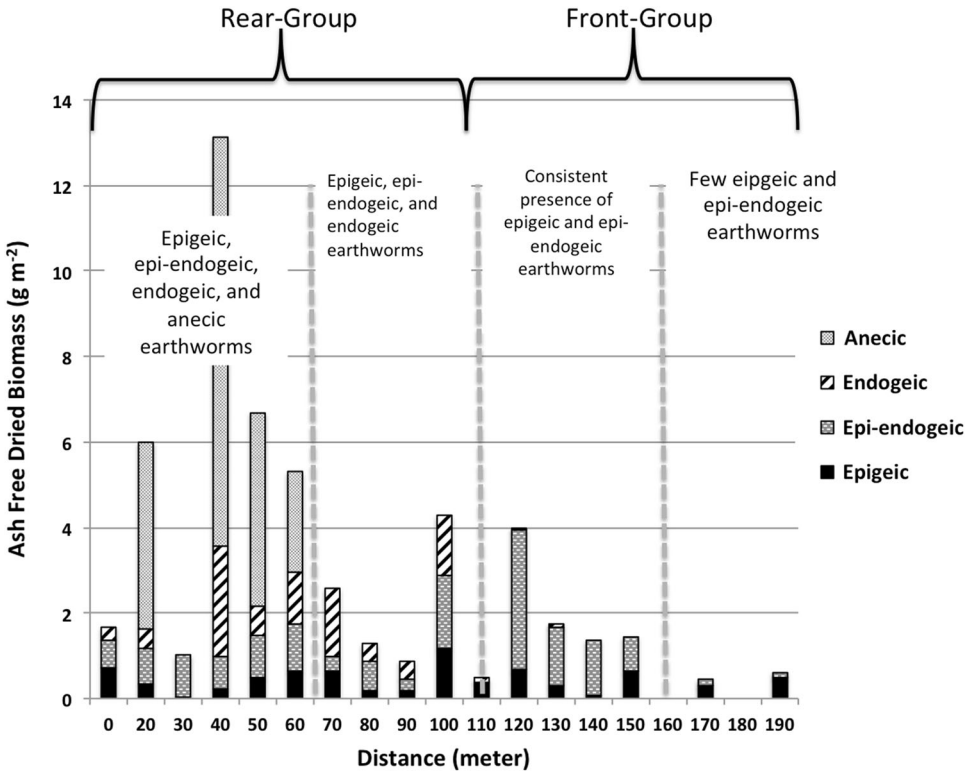

Figure 4. Earthworm biomass by ecological groups along the invasion chronosequence. Earthworm biomass is generally higher next to the road and decreases with increasing distance from the road. Based on the earthworm biomass survey and identification and field observation of soil morphology, the excavated soil pits (at 0, 50, 100, 150, 160, and 190 m) are divided into two groups, the front- and rear-groups. Presence of endogeic earthworm species largely divides the two groups. In each group, the soils are further distinguished by different associations of functional groups inhabiting the soils.

mineralogical depth profiles in the upper 0–10 cm were more uniform in the rear-group soils. Mineralogical profiles in the loess and the underlying B horizon remained unaltered across the transect. The mineralogical data show that consistent changes in elemental compositions in the A horizon (described below) are independent of the loess mineralogy. Calcite and dolomite were found in the glacial till C horizon but were absent in the A horizon and loess parent material across the entire transect (Resner 2013). In agreement with this segregation of calcareous minerals, which were not affected by the presence of earthworms, we observed that earthworm burrows did not extend below the A horizon and loess.

Elemental Compositions and Changes in Soils and Leaf Litter

Depth profiles of elemental concentrations in the A horizon changed substantially along the transect (Figure 5). All elemental concentrations exhibited less variation with depth in the rear-group soils. In the front-group, the concentrations of Ca and P exponentially declined with increasing depth, and their concentrations in the A horizon increased at 160 and 150 m. In the rear-group, in comparison to the front-group, the A horizon had reduced concentrations of Ca and P.

Potassium contrasted with Ca and P in that its concentration increased with depth and that its concentrations in the A horizon were greater in the rear-group than in the front-group. Magnesium concentrations were not clearly patterned along the transect. LOI decreased with increasing soil depth and was nearly absent in the loess layer. LOI in the A horizon were lower in rear- than front-group soils.

Figure 6 shows the results of fractional mass changes. Calculated values of fractional mass changes and associated standard errors propagated from the variations in elemental compositions of loess material are in the Supplementary Material. All elements were enriched in the A horizon relative to the loess parent material in the front-group soils (Figure 6). Within the front-group, these enrichments further increased at the distances of 160 and 150 m. Such enrichments of all elements,

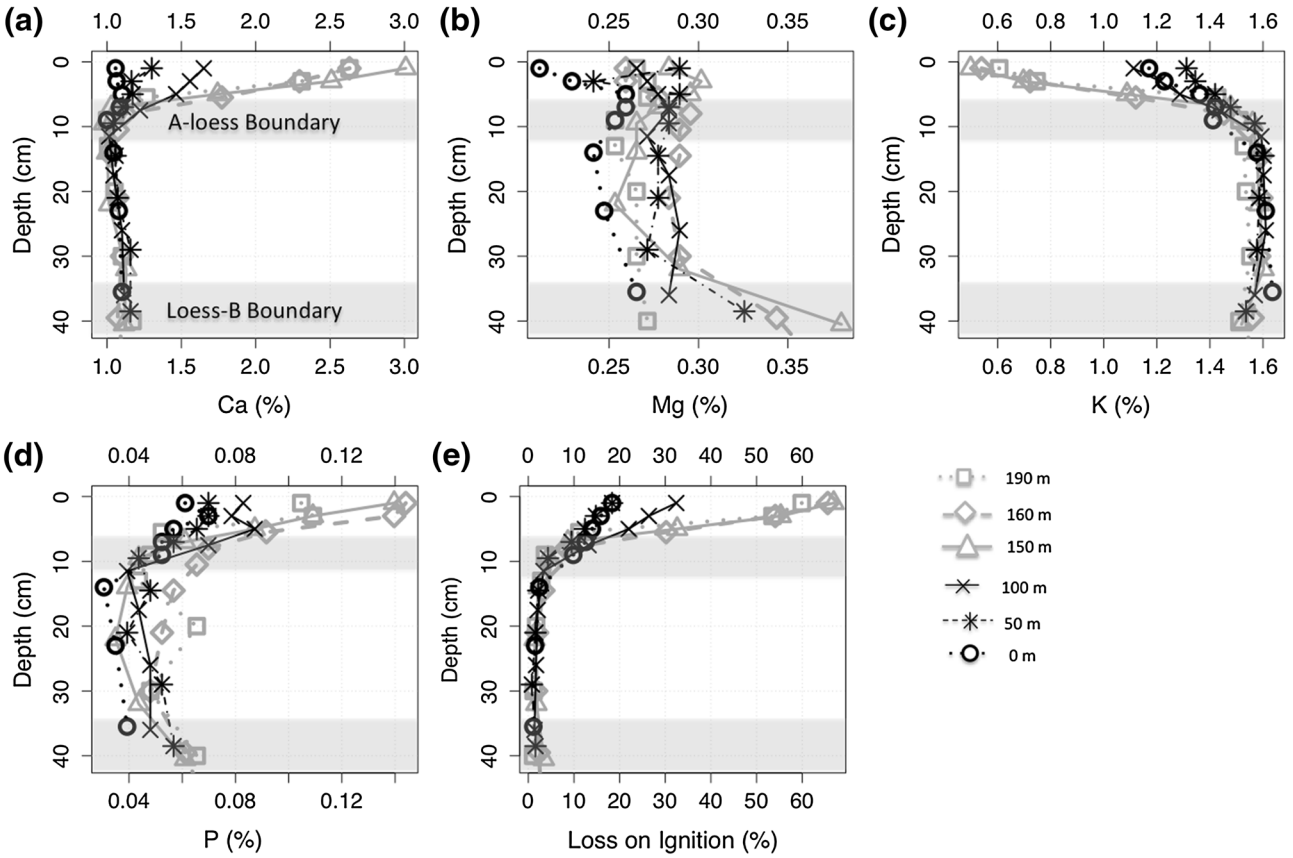

Figure 5. Depth profiles of mass concentrations of Ca (**A**), Mg (**B**), K (**C**), P (**D**), and loss on ignition (**E**) along the earthworm invasion chronosequence. The *gray divisions* indicate the depth ranges in the A horizon to loess boundaries and loess to B horizon boundaries.

however, substantially decreased in the rear-group soils. For instance, the Ca enrichment levels decreased from the range of 7–15 in the front-group soils to the values of less than 2 in the rear-group soils (Figure 6A). These abrupt changes in the enrichment levels were particularly noticeable in light of the relatively modest changes observed in elemental concentrations.

Depth profiles of exchangeable Ca, Mg, and K varied consistently along the transect (Figure 7). The rear-group soils had lower percentages of exchangeable cations and less variation throughout the depth profile compared to the soils in the front-group. All exchangeable cations were positively and tightly correlated with LOI and converged to zero with negligible LOI (Figure 8; see Supplementary Data for Mg and K.).

As a proxy for the leaf litter mixed into the A horizon by epi-endogeic earthworms, we examined leaf litter samples collected from the front-group (n = 6). Leaf litter contained approximately 40% C, 3.23 ± 0.11% Ca, 0.14 ± 0.00% P, 0.19 ± 0.01% K, and 0.21 ± 0.01% Mg.

Discussion

Earthworm Invasion Sequence

Because all of the biogeochemical results are compared to the observed earthworm population, we begin the data analysis by interpreting a probable sequence of earthworm invasion. Based on our earthworm sampling in 2009 and the published earthworm survey from 1998 to 2001 (Hale and others 2005a), we found that the leading edge of earthworm invasion, where an abrupt loss of leaf litter layer occurred, advanced at the rate of 5 m y^{-1} in the direction away from the road. Based on the observed patterns of earthworm biomass, we infer that the forefront of earthworm invasion transect is first populated by epigeic species, followed by epi-endogeic species, endogeic species, and last anecic earthworm species (Figure 4).

The details of this sequence, however, may be incompletely captured in our survey in 2009. Even though epi-endogeic *L. rubellus* were not found at 160 m in 2009, this species was likely present beyond 160 m in 2009. This speculation was based on

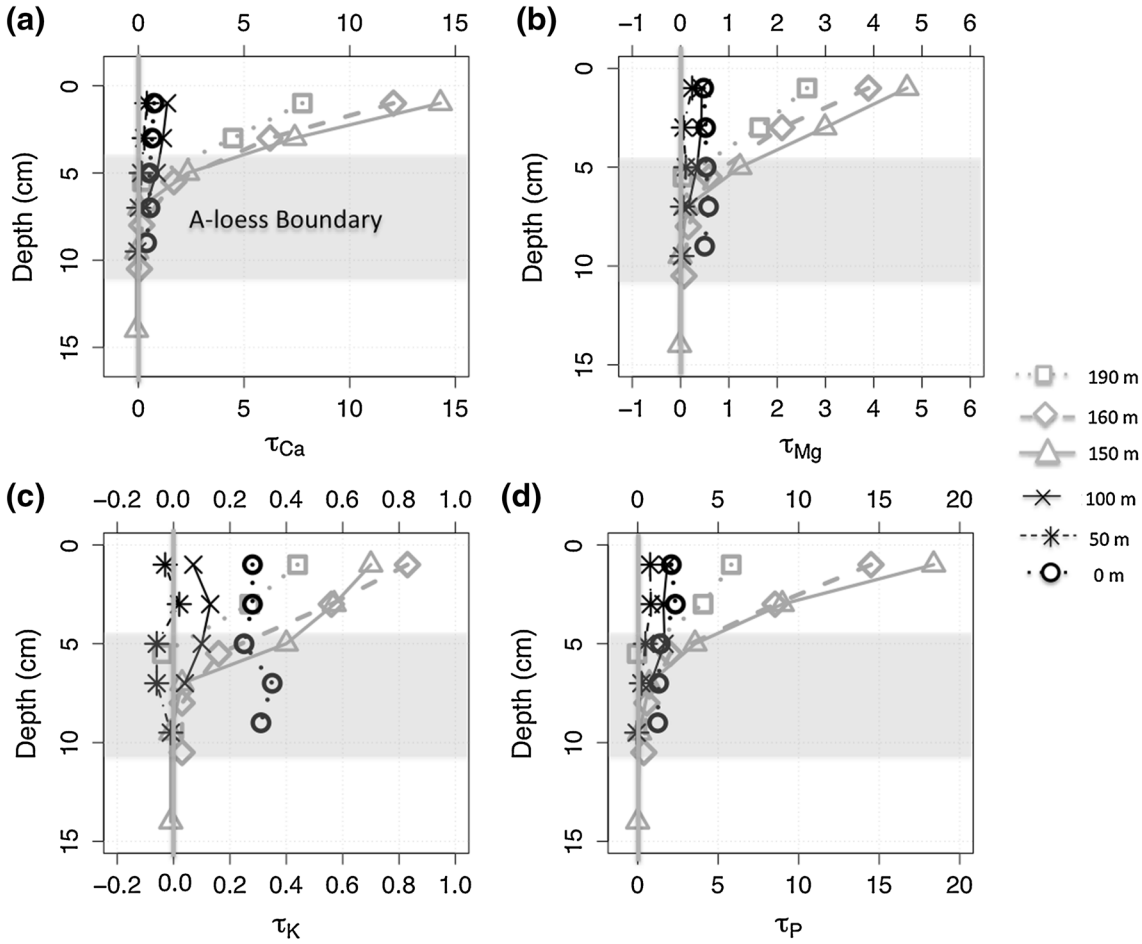

Figure 6. Fractional mass gains (positive values) or losses (negative values) (τ) of Ca (**A**), Mg (**B**), K (**C**), and P (**D**) in the *A horizons*. The *vertical gray lines* indicate no mass gains or losses. The *gray divisions* indicate the depth ranges in the A horizon to loess boundaries and loess to B horizon boundaries.

the observation that they were present up to 150 m in 2000 (Hale and others 2005a). Additionally, liquid mustard may have been less effective in extracting *L. rubellus* during the unusually dry summer of 2009 because the mustard solution may have replenished soil moisture rather than infiltrated through transient endogeic burrows that were not well connected to the ground surface. Liquid mustard extraction has been most effective for anecic species probably because of their vertical burrows connected to the ground surface (Bartlett and others 2006). Furthermore, *L. juveniles* were sampled over the entire transect in 2009, and the ones in the front-group were probably *L. rubellus* rather than *L. terrestris* given that *L. terrestris* were only found at distances less than 70 m. Therefore, we consider that epi-endogeic species were present at 160 m, and this interpretation is consistent with our biogeochemical results. Likewise, in 2009, we observed *L. terrestris* at less than 70 m, but this anecic species was found at up to 110 m in 1999–2000 (Hale and others 2006). The habitat range of *L. terrestris* may have been reduced in the dry 2009, or, as noted above, our sampling may have been less effective. Nonetheless all three soils in the rear-group soils had at some period been inhabited by all of the three earthworm functional groups.

Nutrient Dynamics in the Front-Group Soils

Enrichments of Ca, Mg, K, and P in the A horizon at 190 m likely reflected the combined effects of litter inputs and subsequent recycling of nutrients by trees and understory plants in the soils least affected by earthworms. For example, sugar maple and basswood have high foliar and litter Ca concentrations, which have a feedback on Ca biogeochemical cycling in the forest floor (Holdsworth

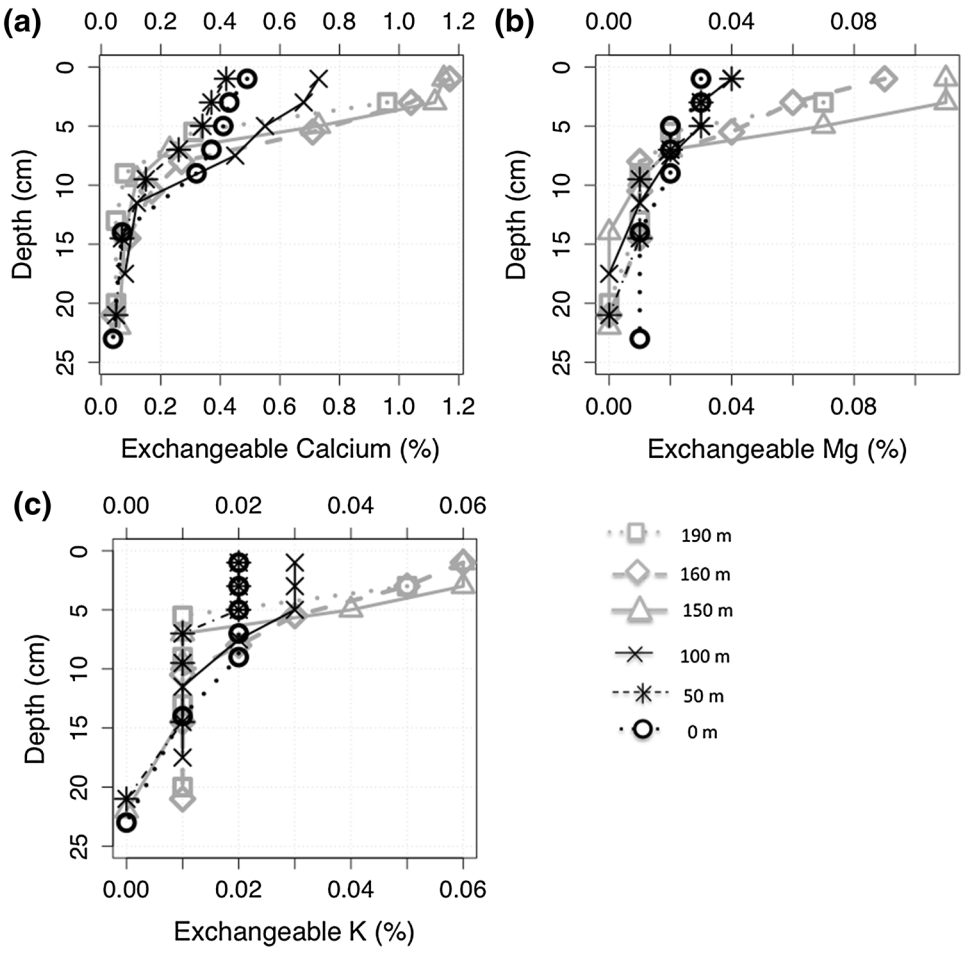

Figure 7. Depth profiles of exchangeable Ca (**A**), Mg (**B**), and K (**C**) concentration (%) along the studied earthworm invasion chronosequence. Exchangeable Ca data are from Resner and others (2011).

and others 2008; Likens and others 1998). Litter deposition and subsequent translocation of the litter materials into the A horizon were likely primary sources of basic cations and P that were enriched in the A horizon at 190 m.

The enrichment levels of Ca, Mg, K, and P in the upper A horizon (0–2.5 cm) were even greater at 160 and 150 m than at 190 m. This increase coincided with the observed increase in LOI from 60% at 190 m to 65% at 160 and 150 m. These simultaneous changes suggest that OM input from leaf litter further enriched the inorganic nutrients, possibly from reworking of the A horizon by epi-endogeic *L. rubellus*.

Although we did not measure the composition of OM that was newly incorporated into the A horizon by epi-endogeic species, exchangeable Ca (Figure 8) offers insight into the OM composition. As LOI contents became negligible, so did the exchangeable Ca concentration (for other cations, see Supplementary Data). Virtually all exchangeable cations in the A horizon were associated with OM (that is, LOI) rather than 2:1 secondary clay minerals. This finding may be interpreted as minerals being a primary source of non-exchangeable cations. However, such supposition is contradicted by the observation that non-exchangeable Ca and LOI positively co-varied where LOI exceeded approximately 30–40% (Figure 8). Therefore, we suggest that fragmented leaf litter and roots—which have Ca as structural components rather than as exchangeable ions—may be important sources of OM in the A horizon of the front-group soils, where LOI values exceeded 30–40%, but were no longer important fractions in heavily invaded soils in the rear-group.

This finding led us to investigate leaf litter to determine how LOI, elemental concentrations, and fractional mass changes varied within the front-group where the first 190 m differed from the 160 to 150 m zone. Figure 5 shows that soil mineral masses contain ~1.1% Ca, ~0.27% Mg, ~1.6% K, and ~0.04% P where OM (that is, LOI) is nearly absent. When these values are compared to the elemental concentrations of leaf litter, physical incorporation of leaf litter into minerals is expected to further concentrate Ca and P in the A horizons, marginally affect Mg concentrations, and to dilute

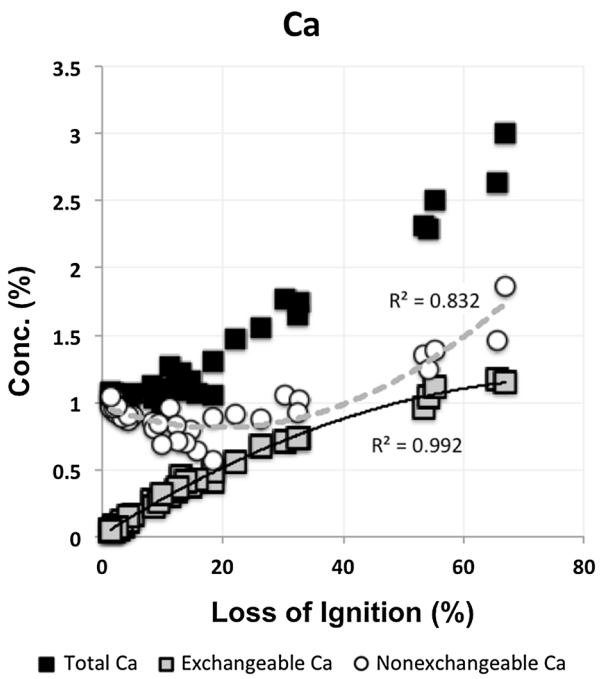

Figure 8. Total Ca concentrations, exchangeable cations, and non-exchangeable cations relative to the loss on ignition. For Mg and K, see Supplemental Data.

K in the A horizons. However, as leaf litter contained all of these elements, earthworms may have mixed those elements from leaf litter into the A horizon, increasing their enrichments (Figure 6).

Although previous studies have shown that understory plant abundance and species richness have dramatically decreased in the presence of epi-endogeic species such as *L. rubellus* (Hale and others 2004; Holdsworth and others 2007), we observed small epi-endogeic biomasses (Figure 4) and largely intact understory plant communities in the front-group soils relative to rear-group soils. Therefore, the initial mixing of OM from leaf litter to the A horizon by epi-endogeic species may have resulted in greater enrichment of nutrient elements in the upper A horizon at 160 and 150 m (Figure 6). Still the enrichment was largely constrained to the upper A horizons due to the absence of endogeic soil mixing.

Dynamics of Nutrient Elements in the Rear-Group Soils

LOI in the upper A horizon (0–2.5 cm) dramatically declined from the front to rear-groups (Figure 5E), and such reduction was almost entirely explained by the loss of light fraction OM (<2 g C cm^{-3}) (Lyttle 2013). The near complete removal of forest floor cover at approximately 150 m corresponded well with abrupt reductions in LOI and elemental enrichment levels between 150 and 100 m. This loss of OM was also concurrent with the significant loss of understory vegetation, which was visually evident in the rear-group soils during this study. This loss is consistent with earlier studies where densities of native understory plant species were reduced in areas where exotic earthworms consumed the litter layer (Gundale 2002; Hale and others 2006, 2008). Decreased fine root and fungal biomasses have also been associated with earthworm disturbance in a sugar maple forest in central New York State US (Fisk and others 2004; Dempsey and others 2011).

Indeed, in the rear-group, where LOI was less than 30–40%, non-exchangeable Ca was largely explained by minerals (Figure 8), indicating that OM in the rear-group soil was primarily microbially processed OM, in which nearly all Ca was exchangeable, rather than fine roots and plant debris. The loss of nutrient elements in the rear-group may have resulted from combinations of many factors including the reduction in OM that was consumed by endogeic and anecic species and less nutrient uptake by plants. At the study site, biomass of understory plants had been shown to decrease with the arrival of endogeic earthworms possibly because of their negative impacts on the forest floor and mycorrhizal fungi (Hale and others 2006). Such leaching losses apparently outweighed the accelerated physical mixing of leaf litter and associated nutrient elements into and within the A horizon by endogeic and anecic earthworms.

Utility of Geochemical Normalization Approach

The comparative benefits of the geochemical mass balance approach over simple assessment of elemental concentrations can be effectively highlighted with K, which showed contrasting responses to earthworm invasion in concentrations versus fractional mass changes (Figures 5C, 6C). In the front-group soils, K concentrations were lower in the A horizon than in the underlying loess (Figure 5C), yet K was enriched in the A horizon relative to the loess layer (Figure 6C). This excess K was derived from the leaf litter that simultaneously added more of other elements such as C and Ca into the A horizon and thus diluted K. The rear-group soils, in contrast, showed greater K concentrations in the A horizon, whereas K enrichments declined or disappeared in the rear-group soils. These patterns illustrate that losses of K from the A horizon were smaller relative to losses of OM.

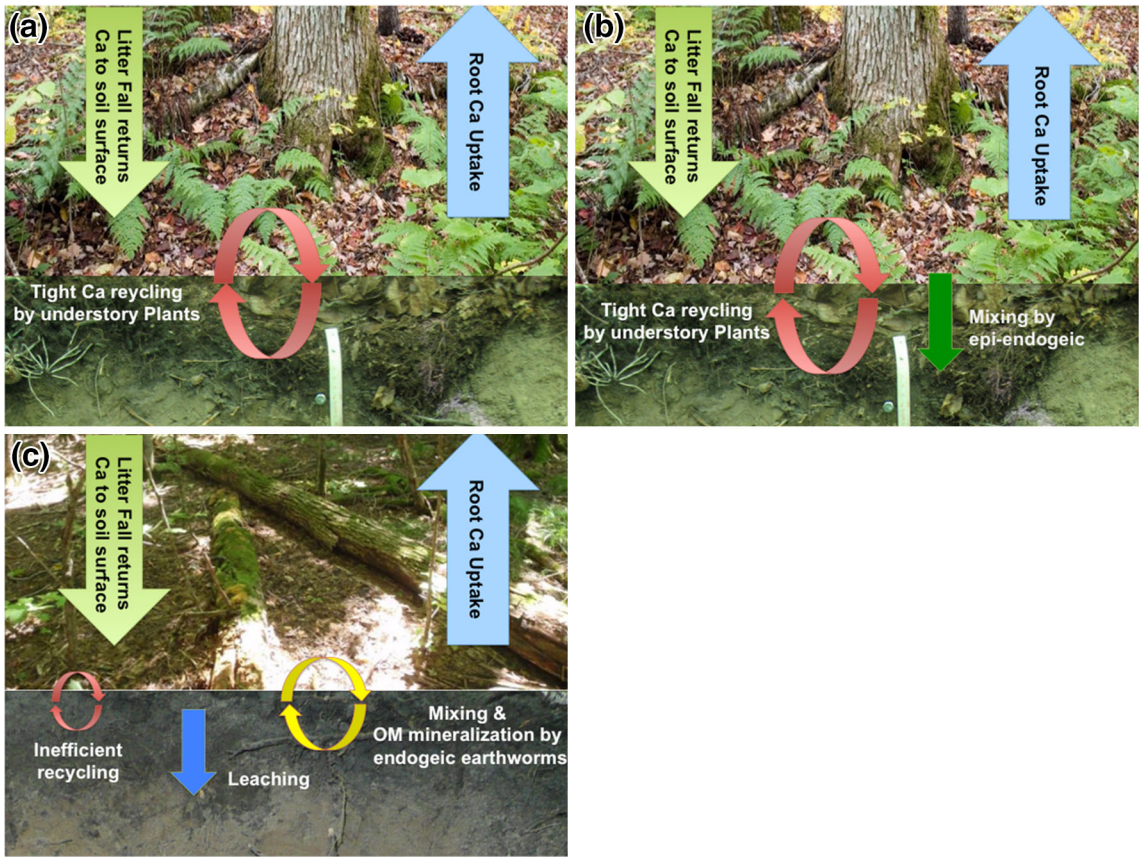

Figure 9. Three stages of the soil Ca cycling in responses to exotic earthworms at the study site: (**A**) pre-invasion and initial invasion with epigeic earthworms, (**B**) arrival of epi-endogeic earthworms, (**C**) presence of all functional groups of earthworms with dominant impacts from endogeic earthworms. The smaller sizes of the trees and fallen trees in **C** are not meant to indicate reduced tree sizes and increased tree mortality or plant carbon inputs—which are not within the scope of this study—in the rear-group area.

The utility of geochemical normalization was also evident for other elements. For example, fractional Mg enrichments varied from a factor of 5 to nearly zero (Figure 6B) whereas there was little change in Mg concentration along the transect (Figure 5B). Exotic earthworm effects on enrichment factors of Ca and P were also greater than the effects on their elemental concentrations. Overall, all inorganic nutrient elements, once normalized against Zr, showed identical response patterns to stages of earthworm invasion.

Implications for Forest Nutrient Dynamics and the C Cycle

The section between 150 and 100 m, where dramatic shifts in factional mass changes and exchangeable cations occurred, represented about 12 years of earthworm invasion. This rate of change is exceptionally fast relative to typical pedogenic processes in these post-glacial landscapes, yet is probably not unique to our site. A study of a northern hardwood forest in Connecticut found high concentrations of exchangeable Mg and Ca in top mineral soils under sugar maple and attributed them to the large quantities and high Ca concentration of sugar maple litterfall (Finzi and others 1998). In these young glaciated deciduous forest soils with limited abundances of secondary clay minerals, OM is often the dominant source of exchangeable nutrients such that rapid OM loss due to exotic earthworms may results in immediate and dramatic losses of exchangeable cations as shown in our study.

Previous work by Hale and others (2005a) at our site showed that phosphate availability was lower in soils with high earthworm biomass than soils with low earthworm biomass. These spatial patterns of lowered P availability correspond to our observation of loss of total P enrichment in heavily invaded soils (Figure 6D). This result differs from past speculation that P released from OM mineralization by

earthworms may be fixed on secondary clay or iron oxides, which would have reduced biological availability of P (Pare and Bernier 1989). If such mineralogical sorption of P was quantitatively important, enrichment levels of the total P should have been homogeneous across the transect, which we did not find. Additionally, *L. terrestris* did not burrow below the loess, which precluded mining of P-bearing primary minerals by that deep burrower (Surarez and others 2003) and mixing from that source into the A horizon.

It should be noted that earthworms only mixed the A horizon and the upper boundary of an underlying loess at our site. Silty loess material or dense clay in B horizon, which may have been a primary control on seasonally high water tables that flooded the nearby seasonal ponds, may have inhibited deeper burrowing. Had endogeic or anecic earthworms burrowed into the clay-rich B and calcareous C horizons, the geochemical results would have differed and should have reflected upward translocation of calcium carbonates and secondary clay minerals with substantial cation exchangeable capacities. Our work therefore highlights the importance of recognizing that initial soil properties and the behavioral responses of the exotic earthworms to the soil properties have strong legacy effects on the degree to which invasive earthworms affect soil biogeochemistry.

From the combination of earthworm demographics and geochemical normalization, we developed a conceptual model of how earthworms affect soil elemental cycles at our study site over time related to the invasion sequence (Figure 9). First, prior to earthworm invasion, tight recycling of nutrients may have contributed to enrichment of inorganic nutrient elements in the thin A horizon (Figure 9A). Second, epi-endogeic species, by incorporating fresh leaf litter into the A horizon, may have further enriched inorganic nutrients in the A horizon but had little effect on exchangeable cations (Figure 9B). Third, epi-endogeic, endogeic, and anecic species accelerated OM mineralization, which resulted in the release of organically bound Ca, Mg, K, and P. Additionally, understory plants and fungi were also negatively affected by the earthworms at our study site (Hale and others 2006) and other glaciated deciduous forests in the US (Dempsey and others 2011), which may have resulted in inefficient recycling and increased leaching of nutrients (Figure 9C).

There may be repercussions for the soil C cycle along with the changes in nutrient elemental cycling. Polyvalent ions like Fe^{3+} and Ca^{2+} bridge soil OM and negatively charged clay minerals and complex OM, which protect OM from microbial degradation (Oades 1998; Schmidt and others 2011). Losses of base cations like Ca^{2+} that we observed may thus make OM more vulnerable to microbial decomposition. Subsequently, there could be a dramatic positive feedback between losses of cations and OM in responses to exotic earthworms. And this positive feedback may contribute to the observations that exotic earthworms have depleted the enriched pools of nutrients in the time span of approximately 12 years.

Conclusions

Even though initial mixing by epi-endogeic earthworms may have transiently led to the highest levels of inorganic nutrient enrichment in the upper A horizon, leaching losses may have ultimately outweighed the bioturbation-driven incorporation of those elements from the litter layer and underlying loess layer. These losses could be understood in light of previously reported declines in the fine roots, fungi, and understory plants in earthworm-infected areas (Gundale 2002; Fisk and others 2004; Dempsey and others 2011). Consistent patterns between soil nutrient status and earthworm populations will be useful in predicting how hardwood forests in the region will respond to earthworm invasions. Ecological understanding of exotic earthworm biomasses and species composition can be more effectively integrated with existing soil and geological surveys of the glaciated forests in the North America.

Steep declines in enrichments of inorganic nutrients with earthworm disturbance may not persist. As the population and species composition of exotic earthworms reach a new equilibrium with a reworked soil and trophic structure at the site, we expect the pools and fluxes of the inorganic nutrients to do the same. The time scale of this re-stabilization is unknown, especially with regard to interactions of exotic earthworm and climate change effects (Frelich and others 2012; Frelich and Reich 2009). Regardless, pre-existing conditions may not be the end points of the re-stabilization. Such re-stabilization may provide unique opportunities to examine interactions between aboveground versus belowground biological communities, and these interactions may propagate to deeper B horizons, though not detected in our study due to site-specific soil properties. In addition to the quasi-irreversible nature that is likely true for the biogeochemical impacts of exotic earthworms, these impacts are also superimposed on the pedogenesis unique to the study location. The future evolution of these soils, therefore, presents a unique opportunity to study soil

genesis in relation to time-dependent biological factors.

This study shows that earthworms have dramatic effects on the fluxes of major inorganic elements within the rhizosphere. That these changes occur in a matter of years to decades, rather than the millennia over which the soils initially developed, is important. Provided that the elemental concentrations of inorganic nutrients are sensitive to the contents of abundant OM in the A horizons, we propose that geochemical mass balance has wide utility in assessing the degree to which ecological disturbances affect the gains and losses of the nutrient elements. Thus, this normalization method may also be useful in reexamining published relationships between C and inorganic nutrient cycling. The broadest implication is that colonization of terrestrial environments by soil bioturbators has been critically important to global scale biogeochemistry and may rapidly reset soil properties relative to the conditions in which the landscape and contemporary forest structure developed.

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