

Invasive earthworms change nutrient availability and uptake by forest understory plants

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Abstract

Background and aims Assess whether invasive earthworms alter nutrient dynamics in habitats they colonize. **Methods** We investigated nutrient dynamics of forest soils and three native plant species (*Acer saccharum*, *Polygonatum pubescens*, *Polystichum acrostichoides*) along four earthworm invasion gradients in central New York.

Results Earthworm biomass (a proxy for earthworm impact) was related to distribution and concentration of soil and plant nutrients. At shallower depths, earthworms were associated with lower total and exchangeable P, but higher Ca, K, Mg and Mn. Earthworm-invaded plots showed higher soil Ca and higher foliar Ca in *A. saccharum* and *P. acrostichoides*, and lower soil P with lower foliar P in *P. pubescens*. Presence of earthworms substantially decreased rooting volume in the A horizon, co-occurring with a build up of exchangeable nutrient concentrations and pools.

Conclusions Overall, earthworm biomass was a better predictor of foliar nutrient concentrations than either exchangeable or total nutrient concentrations and pools. Earthworms may create stressful rooting conditions, limiting rooting of native plants in the A horizon. The resulting plant-accessible nutrient pool that builds up in the A horizon of earthworm-invaded soils could provide a mechanism for the invasive success of non-indigenous plants that have an evolutionary association with earthworms in the native range and that follow earthworm invasions.

Keywords Macronutrients · Calcium · Phosphorus · Foliar tissue · Root tissue · European earthworms

Introduction

Historically, north-eastern North American forests represent nutrient-limited systems with occasional temporal and spatial nutrient flushes (Gilliam 2014). As a result, native plants have evolved unique strategies such as complex mycorrhizal associations, gender shifts and extended dormancy to maintain optimal stoichiometry across a range of soil fertility (Doust and Cavers 1982; Boerner 1986; Kery and Gregg 2004). Following the last glacial maximum approximately 22,000 yrs. ago, north-eastern North American forests have developed in the absence of earthworms (James 1995; Hendrix and Bohlen 2002). These forest soils are often starkly stratified, and organic inputs build up to create a well-

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developed organic horizon (Bohlen et al. 2004c; Frelich et al. 2006; Hale et al. 2008).

As a result, macro (such as N, P, K, S) and mesonutrient (Ca, Mg, Mn) cycles are tightly coupled with forest plants, mycorrhizal symbionts, and microorganisms to maximize nutrient retention in shallow horizons through close coupling of decomposition and uptake processes (Leonard and Field 2004; Suarez et al. 2004; Gilliam 2014). P cycling is particularly tightly regulated due to its commonly limiting concentration in acidic forest soils, and little P is leached from the A horizon (Wood et al. 1984; Yanai 1992; Gilliam 2014). As such, the understory is the source of 80% of P available to trees (Yanai 1992; Suarez et al. 2006b). Calcium, Mg, K and S availability have varying degrees of limitation in many forest ecosystems in the region, and acid rain has caused a substantial decrease in exchangeable Ca in forest soils (Huntington et al. 2000; Kobe et al. 2002; Jenkins et al. 2005). These nutrients serve many physiological functions, and are crucial for biological processes such as photosynthesis, chlorophyll production, chemical signaling, root foraging, or mycorrhizal colonization (Ellsworth and Liu 1994; Long et al. 1997; Horsley et al. 2000; Kobe et al. 2002; Clair and Lynch 2005; Juice et al. 2006). In addition to physiological problems of Ca, Mg and K deficiencies, soils may lose their pH buffering capacity, and Al and Mn toxicity can become problematic (Elliott 2009). Calcium losses limit photosynthetic capacity, contributing to issues such as canopy dieback, and is so severe that growth simulations predict that Ca fertilization of 10 g m^{-2} led to more than a doubling of *A. saccharum* basal area in forests in one tree generation (Ellsworth and Liu 1994; Kobe et al. 2002).

In Appalachian forests, exchangeable Mn is associated with increased understory plant diversity (Newell and Peet 1998). Plants preferentially take up Ca over Mn when it is available, but acid deposition and subsequent Ca deficiency (as well as high Mn availability) in soils can lead to Mn toxicity (Juice et al. 2006). In acidic forests, higher Mn (and lower Mg) foliar concentrations are associated with sugar maple decline (Horsley et al. 2000). Tree species display a breadth of susceptibility to Mn toxicity, suggesting soil Mn could be an important driver of forest species assemblages (St. Clair and Lynch 2005).

A myriad of anthropogenic influences alter nutrient cycling, which threatens this closely coupled system (Côté et al. 1995). To conserve species, ecosystems,

and diversity of forests, it is vitally important that we understand the consequences of novel perturbations to nutrient dynamics. The understory has received relatively less consideration in forest ecology, but acts as a filter of future species pools by determining which seeds and propagules can establish (Dorning and Cipollini 2006; Royo and Carson 2008; Gilliam 2014). It is diverse (up to 90% of plant biodiversity in temperate forests), and is the interface of below and above-ground interactions (Gilliam 2014).

Though low in aboveground biomass (<1%) relative to canopy trees, herbaceous understory plants are important components of nutrient cycling (Gilliam 2014). Litter from understory plants is typically high quality, and can contribute up to 20–25% of litterfall in a season (Gilliam 2014). Understanding nutrient dynamics is important to project future species assemblages. For example, decreased nutrient availability has been strongly linked with sugar maple decline, a socially and economically important species (Kolb and McCormick 1993; Horsley et al. 2002). Additionally, disturbances can promote invasive plants or early successional species that are better able to take advantage of elevated labile macronutrient concentrations (Huenneke et al. 1990; Schaberg et al. 2006; Szlavecz et al. 2011).

European and more recently Asian earthworms have been introduced by human activities (Scheu and Parkinson 1994; Burtelow et al. 1998; Bohlen et al. 2004c). As soil engineers, the influence of earthworm invasion propagates throughout entire ecosystems. In forests, the most noticeable change is rapid decomposition and redistribution of organic matter, often leading to a complete loss of the O horizon (Bohlen et al. 2004c; Hale et al. 2008). The outcome of earthworm invasion on individual soil nutrients is inconsistent, and depends on soil type, earthworm species assemblages, time since invasion and existing soil community (Bohlen et al. 2004a; Suarez et al. 2006a; Filley et al. 2008). Despite this complexity, earthworm-associated changes to C and N cycling have been well described, although other important forest nutrients have received considerably less attention (Scheu 1994; Bityutskii et al. 2002; Bohlen et al. 2004b; Wironen and Moore 2006; Weihua and Xiuqin 2007; Umarov et al. 2008). Resner et al. (2015) observed an initial enrichment of Ca, K, Mg and P in the A-horizon along an earthworm invasion gradient, but an overall depletion of these nutrients in the soils with the longest earthworm invasion history. Availability of nutrients to native understory plants may

decline with increased leaching out of the rooting zone (Bohlen et al. 2004a; Hale et al. 2005; Bal et al. 2017).

The effect of earthworms on P cycling is particularly complex, and is dependent on soil type, nutrient fraction, earthworm species, and time since invasion (Bohlen et al. 2004c; Suarez et al. 2004). Previous studies in tropical forests have observed earthworms increase total and plant available P (De Vleeschauwer and Lal 1981; Zhang et al. 2000; Kuczak et al. 2006; Chapuis-Lardy et al. 2011). In temperate agricultural systems earthworms have been associated with a decrease in inaccessible organic P, but increased water-soluble inorganic P (Coulis et al. 2014). In central New York forests, Suarez et al. (2004) found earthworms influence on different P fractions to be variable in direction and scale. In a mesocosm study, Hale et al. (2008) demonstrated that diverse earthworm communities increased available phosphates, although individual earthworm species had no effect. Taken together, this could suggest initial increases in organic P mineralization in early stages of earthworm invasions may be followed by a decrease in available P due to leaching (Bohlen et al. 2004c).

Earthworm influences on soil nutrient dynamics will determine understory plant assemblages, as some species are sensitive to changes while others may thrive. Including plants in the conceptual model of impacts of earthworm invasion on nutrient cycling is the next step to understanding their fundamental impact to forest taxa. We investigated *A. saccharum*, *Polygonatum pubescens* (Willd.) Pursh and *Polystichum acrostichoides* (Michx.) Schott, three important understory species with contrasting life histories and variable survival and growth responses to earthworm invasion. By including a tree, an herbaceous perennial and a fern, we were able to understand whether the stoichiometry of plants with different growth strategies vary in response to earthworm invasion. While an understanding of precisely what causes a plant to be vulnerable to earthworm invasion is still limited, we chose study plants that vary in traits that may be associated with increased sensitivity to earthworm invasion such as high mycorrhizal dependence and specificity, shallow rooting architecture and roots lacking chemical and physical protection from herbivory (Gundale 2002; Lawrence et al. 2003; Hale et al. 2006a; McLean et al. 2006; Drouin et al. 2016).

In previous experiments, *P. acrostichoides* benefited from earthworms (Bowe et al., unpublished), while survival of the congener *Polygonatum biflorum* was negatively affected by earthworms (Dobson and

Blossey 2015). Negative relationships between invasive earthworms and *A. saccharum*, have been identified through field and mesocosm studies in the Midwest (Holdsworth et al. 2007a; Hale et al. 2008; Corio et al. 2009; Bal et al. 2017). *Polystichum acrostichoides* is an evergreen fern with branched roots growing from a perennial rhizome, variable (and possibly facultative) degrees of mycorrhizal colonization, and broad mycorrhizal compatibility (Berch and Kendrick 1982; West et al. 2009). The root biomass of *P. pubescens* consists of a large storage rhizome with a few small, largely unbranched roots, and arbuscular mycorrhizal colonization ranges from moderate to very high (Boerner 1986; Brundrett and Kendrick 1988; Dobson, unpublished). Roots of *A. saccharum* are highly chemically and physically protected, and have moderate levels of arbuscular mycorrhizal colonization (Brundrett and Kendrick 1988).

Objectives

The objective of this study was to quantify macro and mesonutrients in soils and plants to assess the potential impacts on understory vegetation by earthworm invasions. We tested several related hypotheses: (1) earthworm presence decreases total and exchangeable soil nutrients; (2) earthworms mix soil horizons, causing vertical homogenization of soil nutrient concentrations; and (3) earthworm-associated changes in exchangeable soil nutrients will trigger analogous changes in nutrient concentration of plant foliage.

Materials and methods

Study area

We selected four forests (Bobolink Hill, Hammond Hill State Forest, Ringwood Preserve and Yellow Barn State Forest) in Tompkins and Tioga counties in the Finger Lakes Region of New York State (Supplementary Table 1). All forests fall within the Allegheny section of the Appalachian Plateau at approximately 42°N, 76°W with acidic (pH 3.9 – 5.0) Fragiaquepts and Dystrochrepts soil in the Mardin and Volusia series (SoilWeb, USDA-NRCS & UC Davis California 2010). Mixed hardwood canopies are predominantly closed (Leaf Area Index 5-7), and dominated by *A. saccharum*, *Fraxinus* spp., *Fagus grandifolia* Ehrh. and *Quercus rubra* L.

We selected six plots in each forest along a 0.5–2 km gradient from earthworm-free to earthworm-invaded areas. Land use history varied among forests, however all plots within a forest had similar land-use history (Supplementary Table 1). Earthworm-invasion histories are unknown, however all plots at Ringwood, Yellow Barn and Bobolink Hill retained traces (<5 cm depth) of an organic horizon. Each plot contained all three study species: *A. saccharum*, *P. pubescens*, and *P. acrostichoides* within a 15 m diameter sampling area. Despite their different responses to earthworms, all plant species were present at all plots with and without earthworm invasions, with the exception of *A. saccharum* in the uninvaded plot at Yellow Barn State Forest.

Plant sampling

In July 2016, we collected *A. saccharum* saplings (15–50 cm), non-reproductive *P. pubescens* and reproductive *P. acrostichoides* individuals in each plot, taking care to excavate the entire root mass. We rejected any plants showing signs of senescence, insect or pathogen damage. To ensure sufficient foliar material for nutrient analyses, we collected one to three *A. saccharum* saplings and *P. pubescens* stalks in each plot. We triple-rinsed and removed all visible soil from roots, and air dried samples in a greenhouse for 1 week. We separated leaves (removing petioles and stems) and roots, and ground each sample using a coffee grinder (Fresh Grind, E160BY, Proctor Silex, Southern Pines, NC, USA). For *A. saccharum*, we separated and discarded the primary root from actively growing roots. We used roots, but not rhizomes of *P. acrostichoides*, but due to low biomass of secondary roots, we ground entire *P. pubescens* root masses.

Earthworm sampling

All plots were chosen from within large, long term sampling sites (Dobson and Blossey 2015). Therefore, we were able to use earthworm biomass data from 10 locations along two diagonal 50 m transects around each plant sampling plot in autumn 2012, 2013 and 2015 using liquid mustard extraction (3 g powdered mustard 3.79 L⁻¹ water poured into a 0.5 m⁻¹ sampling frame) (Lawrence and Bowers 2002). We collected all surfacing earthworms and preserved them in formalin for 48 h before transferring them into 70% ethanol for storage. We identified all adult individuals to species and juveniles to genus, and classified them to ecophysiological

group, including epigeic (litter-dwellers), endogeic (soil-dwellers), anecic (deep burrowing surface dwellers), and epi-endogeic (soil/litter dwellers) (Edwards and Bohlen 1996; Deleporte 2001; Hale et al. 2005). We then pooled all 10 samples from within each plot and dried them at 60 °C for 72 h to determine dry biomass. We used the 3-year mean of this pooled plot-level earthworm biomass for analyses to minimize influence of annual variability and microhabitat heterogeneity.

Soil sampling

At each plot, we excavated one soil monolith at a random location near the approximate plot center. To avoid direct effects from mature trees, we excavated monoliths at least 1 m away from any trees. A 15 cm² template was randomly placed on the soil surface. We cut O horizons using a stainless steel handsaw and soil surrounding the template was removed to expose the soil monolith. We noted horizon depths and soil morphological features for each soil monolith. We then separated the soil monolith by master horizons and collected each entire horizon. In the laboratory, we air dried each master horizon (O horizon, A horizon, and B horizon) and sieved to <2 mm. Only two earthworm-invaded pits had sufficient O horizon mass to analyze, thus we could not include it in further evaluations. We quantified rock mass (> 2 mm) and root mass (> 1 mm) for each soil horizon. We calculated bulk density by dividing air dried, sieved soil mass for each horizon by horizon depth measured in the field.

Plant and soil analyses

We included plot-level pH measurements recorded in summer 2014 (Dobson and Blossey 2015). To measure % soil organic matter (SOM), we dried horizons separately at 60 °C for 48 h, followed by grinding (DynaCrush; Customer Laboratory Inc., Orange City, FL, USA) sieving (1.18 mm) and igniting at 360 °C for 2 h in a muffle furnace. This temperature combusts organic material, while maintaining inorganic soil constituents.

To characterize exchangeable/weakly bound metal fractions, we extracted 2 g of soil with 20 mL of 1 M ammonium acetate (Chapman 1965; Ciesielski et al. 1997). Samples were shaken for 1 h and allowed to equilibrate for 24 h. We then centrifuged soil slurries at 3000 rpm for 30 min and the extraction solution was

decanted. A pseudo-total digestion was used to measure total nutrient concentrations in plant tissues and soil samples following EPA method 3051A. This method is often referred to as a pseudo-total digestion due to the inability to dissolve silicates and other refractory compounds. These refractory compounds are dominant in soils but may also be present in plants. Since our study focuses on plant available nutrients, we henceforth refer to the pseudo-total concentrations as total concentrations. In the digestion method, 0.500 g of air-dried material was digested in 5 ml of 8 M reverse aqua regia (9:1, HNO₃:HCl). Materials were digested at 90 °C for 45 min on an insulated hot plate in sealed teflon vials. We further diluted digestate and analyzed via ICP-OES (SPECTRO Analytical Instruments, Kleve, Germany). Every 25 samples included a digestion blank, a duplicate and 2 standard reference materials (SRM). We used matching sample matrices from the National Institute of Standards and Technology: Montana Soil 2711 for mineral soil samples and Peach Leaves 1547 for plant tissue samples (National Institute of Standards and Technology, Gaithersburg, MD). Relative standard deviations (RSDs) among SRMs and duplicate samples were less than <4% for all total concentrations in plants and soils and <8% for exchangeable soil concentrations. Preparation blanks had elemental concentrations below detection limit for Ca, Mg, Mn, P and S. Potassium concentrations within blanks were <4 µg L⁻¹, or roughly a 0.2% of sample concentrations. As expected, total concentration recoveries for Montana Soil SRM were between 60 and 90% for all nutrients particularly due to the indigestion of silicate and other residual compounds. Total concentration recoveries for Peach Leaves SRM were Ca (77%), Mg (87%), K (103%), Mn (76%), P (72%), and S (65%). Similar to soil extractions, recoveries were <100% for many elements due to residual compounds within plant tissues.

Data analyses

We tested the influence of earthworm activity on three response variables: (1) soil horizon nutrient concentration, (2) 0 – 10 and 10 – 20 cm nutrient pools, and (3) plant nutrient concentration using linear mixed models (LMMs) (package lme4). Each set of models included dry earthworm biomass as a fixed effect, with site as a random effect. We analyzed nutrient concentrations as separate response variables for each nutrient and each horizon. Each pit had an A horizon ($n = 24$), however the sample size for B horizons ($n = 18$) is depreciated

because pits from heavily invaded soils contained one deep, homogenous A horizon, consistent with earthworm mixing. Since A horizon and B horizon depths varied significantly among plots and strongly influenced total volume of soil horizons, we calculated nutrient pools using standardized depths of 10 cm and 10–20 cm, henceforth referred to as normalized A horizon and normalized B horizon, respectively. We adjusted normalized A horizon and B horizon pools to 10 cm intervals using their bulk density. Due to orders of magnitude difference in nutrient content among pits, we log-transformed normalized horizons for analysis with LMMs. For plant nutrient models, we analyzed root and leaf tissue for each species separately. In addition to LMMs, we qualitatively compared foliar seedling nutrient concentrations to the minimum published concentration for healthy July *A. saccharum* foliage following Burton et al. (1993), Kolb, T.E.; McCormick (1993) and Ca: Al toxicity from Cronan and Grigal (1995) to infer whether different patterns emerged above and below these thresholds.

Next, we tested whether earthworms altered plant-soil relations, again using LMMs. We evaluated concentrations of each nutrient (response variables) of each plant species with independent models, with site as a random effect. We used the interaction between earthworm presence/absence or earthworm biomass and nutrient pools from the top 10 cm of soil (to reflect rooting depth of our plants) as fixed effects. Using root biomass as a response variable, we coarsely modeled vertical distribution of roots in our soil pits using the interaction between earthworm biomass and horizon (A and B only) as fixed effects, with site as a random effect.

Finally, we tested the relationship between earthworms biomass (fixed effect) and cation exchange capacity (CEC), pH and % soil organic matter (SOM) (independent response variables), with site as a random effect. We tested CEC, and pH of the top 10 cm only, but ran separate models for SOM in each horizon. We performed all analyses using R statistical software (R Core Team 2014).

Results

Earthworm sampling

Lumbricus terrestris L. and *Lumbricus rubellus* Hoffmeister dominated earthworm populations at all sites, while endogeic species in the *Aporrectodea*

caliginosa complex (*Aporrectodea tuberculata* Eisen, *Aporrectodea caliginosa* Savigny, *Aporrectodea trapezoids* Dugés) were co-dominant with *Lumbricus* sp. at Hammond Hill and Bobolink Hill (Supplementary Fig. 1). At all sites except Ringwood, we observed moderate abundances of *Octolasion tyrtaeum* Savigny, *Octolasion cyaneum* Savigny, *Aporrectodea rosea* Savigny and *Dendrobaena octaedra* Savigny and *Dendrodrillus rubidus* Savigny (Supplementary Fig. 1). Within sites, low-earthworm biomass plots ($0.004 - 0.64 \text{ g m}^{-2}$, $0 - 10$ individuals m^2) included *D. octaedra*, *D. rubidus* and *L. rubellus*. Moderately invaded sites ($1.76 - 2.16 \text{ g m}^{-2}$, $29 - 43$ individuals m^2) were dominated by *Lumbricus* sp., and *Octolasion* sp. and *D. octaedra*, and heavily-invaded plots ($3.27 - 3.63 \text{ g m}^{-2}$, $21 - 63$ individuals m^2) maintained a diverse assemblage of species from all ecophysiological groups (Supplementary Figs. 1 & 3).

Earthworms and soil

Consequences of earthworm invasion were variable for nutrient concentrations and pools in the A and B horizons. In the A horizon, higher earthworm biomass was associated with higher concentrations of total Ca, K, and Mg and exchangeable Ca, K, Mg, and Mn but lower total P (Fig. 1; Supplementary Fig. 2; Supplementary Table 2). In contrast, exchangeable K, Mn, and P in the B horizon were depleted with increasing earthworm biomass, although higher total Ca and K was maintained in addition to higher S (Fig. 1; Supplementary Fig. 2; Supplementary Table 2). Earthworm biomass was also associated with lower ratios of exchangeable:total Ca, K, P and S in the B horizon (Fig. 1; Supplementary Fig. 2; Supplementary Table 2). In the A horizon, earthworm biomass was associated with higher exchangeable:total concentrations of Mg and Mn, and small but significantly higher S concentrations (Fig. 1; Supplementary Fig. 2; Supplementary Table 2). We observed a negative effect of earthworm biomass on P at 10-20 cm depth, but also observed a positive effect on total Ca and exchangeable Mg (Fig. 3; Supplementary Table 3). Nutrient pools were consistent with concentration patterns within the A horizon, with greater earthworm biomass predicting higher Ca, K, Mg and exchangeable Mn in the top 10 cm (Fig. 2; Supplementary Table 3).

Earthworm influence on plant nutrition

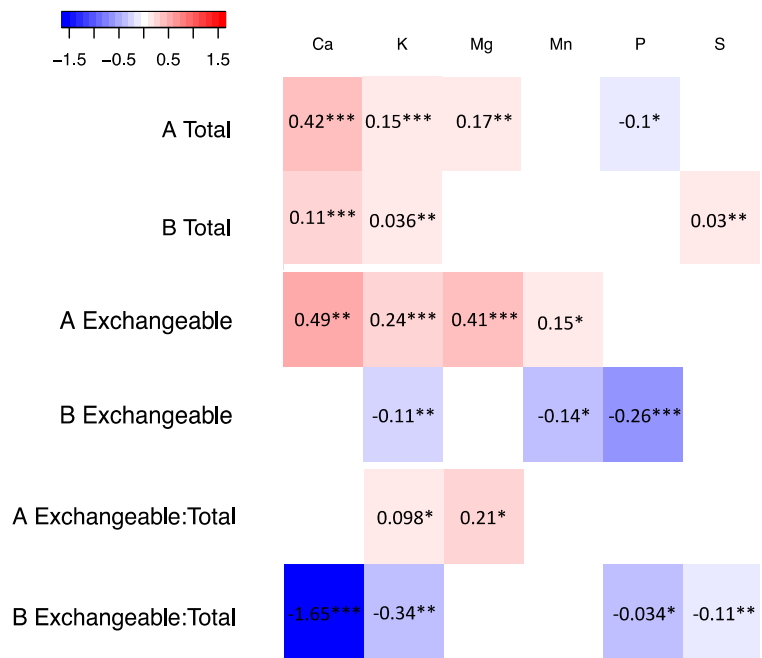
With exception of *P. pubescens*, increased earthworm biomass predicted higher concentrations of foliar (Fig. 3; Supplementary Table 4) and root (Fig. 4; Supplementary Table 4) Ca and lower P concentrations in all roots, and *P. pubescens* leaves. Despite having a positive effect on exchangeable soil Mn in the A horizon (Figs. 1 and 2), both foliar and root Mn concentrations declined with increasing earthworm biomass for all species (Figs. 3 and 4; Supplementary Fig. 4; Supplementary Table 4). Further, higher soil exchangeable Mg, and K in earthworm-invaded plots did not translate into higher tissue Mg or K concentrations (Figs. 3 and 4; Supplementary Fig. 4; Supplementary Table 4).

Soil nutrient concentration and pools were highly variable and were poor predictors of plant tissue concentrations. Further, we did not find evidence for earthworm x soil nutrient interactions for *P. pubescens* or *P. acrostichoides*. We found a positive relationship between soil and *A. saccharum* tissue Ca in non-earthworm-invaded plots (Supplementary Fig. 5; Supplementary Table 5). Surprisingly, for most other species and many other nutrients, earthworm biomass alone was a much better predictor of plant tissue nutritional concentrations (Fig. 3; Supplementary Table 4).

Earthworm influence on other soil conditions

Earthworm biomass had a positive, though nonsignificant relationship with CEC ($R^2_{\text{m}} = 0.14$, $p = 0.057$). However, this was driven by a single datum with a CEC one order of magnitude larger than the rest, and the trend remained nonsignificant when the single datum is removed from analysis ($R^2_{\text{m}} = 0.08$, $p = 0.14$). Earthworm biomass was positively associated with soil pH ($R^2_{\text{m}} = 0.44$, $p < 0.001$), but did not correspond with any trends with SOM for any horizon. Interestingly, we found a significant interaction between earthworm presence and horizon in our pits, with roots preferentially growing in the B horizon in earthworm-invaded conditions and in the A horizon in the absence of earthworms (Fig. 5; Supplementary Table 6). Similarly, continuous earthworm biomass x horizon was a good predictor of root biomass ($R^2_{\text{m}} = 0.19$, $R^2_{\text{c}} = 0.30$, $p = 0.009$).

Fig. 1 Effect of earthworm biomass on total, exchangeable and exchangeable:total soil nutrient concentration (mg kg^{-1}). Colored boxes represent parameter estimates of earthworm biomass from mixed models fitted with maximum likelihood. Earthworm biomass was the sole fixed effect and site is the random effect. To compare across nutrients, raw response variables were standardized by dividing by their mean. Red and blue represent direction of the relationship (positive and negative, respectively), and saturation represents the parameter itself. Raw plots in Supplementary Fig. 2, summary statistics are in Supplementary Table 2. *P*-values are estimated from a normal distribution (* $P < .05$; ** $P < .01$, *** $P < .001$)



Discussion

Our results support a growing body of evidence that earthworm-invaded forests have conspicuously different nutrient dynamics than uninvaded forests. On the basis of previous studies of earthworm impact on N cycling, we hypothesized that exotic earthworms would negatively impact exchangeable and total macro and mesonutrients soil concentrations, but observed that the effect varied among nutrients and between soil horizons (Bohlen et al. 2004b; Wironen and Moore 2006; Szlavecz et al. 2006; Eisenhauer et al. 2007). Earthworm-invaded plots were characterized by higher total Ca, K and Mg and exchangeable Ca, K, Mg and Mn, particularly for A horizons (Figs. 1, 2, and 6). This is likely due to mineralization of the O horizon and incorporation of those nutrients in the A horizon, which is consistent with patterns of early epi-endogeic earthworm invasions observed by previous studies such as Resner et al. (2015) in Midwestern hardwood stands. Although we do not have information about the time since earthworm invasion, plots at three of four sites retained traces of an organic horizon, either because the invasion is relatively new or the habitat is only conducive to low earthworm densities.

Assuming Ca and Mg concentrations at uninvaded plots are estimates of baseline, we approximate that earthworms have increased Ca and Mg concentrations

and pools 75–526% (Figs. 1 and 2). It is possible that elevated Ca and Mg occurred due to earthworms affecting the chemical nature of SOM. For example, Knowles et al. (2016) found *A. tuberculata* altered the physicochemical properties of soil C and restructured SOM. These physicochemical changes to SOM can directly impact SOM sorption capacity and exchangeability for cations like Ca and Mg (Reich et al. 2005). However, we did not observe a significant relationship between earthworm biomass and cation exchange capacity nor SOM (Supplementary Fig. 5). Thus, it is unlikely that differences in exchangeable or total nutrient concentrations were caused by earthworms affecting the sorption capacity of SOM. An additional hypothesis is that earthworm distributions may follow nutrient heterogeneity in the landscape. Earthworm preference for high-Ca substrates can be remarkably finely tuned, with earthworm biomass responding to individual trees with Ca-rich litter (Reich et al. 2005), although this may be exclusive to anecic earthworms such as *L. terrestris* (Schelfhout et al. 2017). Additionally, earthworm biomass is positively associated with pH, possibly due to higher soil Ca to buffer acidity, which Elliott (2009) proposes supports a more diverse understory community with higher quality litter. With evidence that earthworms, Mg/Ca concentrations, and higher pH co-vary, it is possible that they create a positive base-cation-earthworm feedback loop (Supplementary Fig. 5).

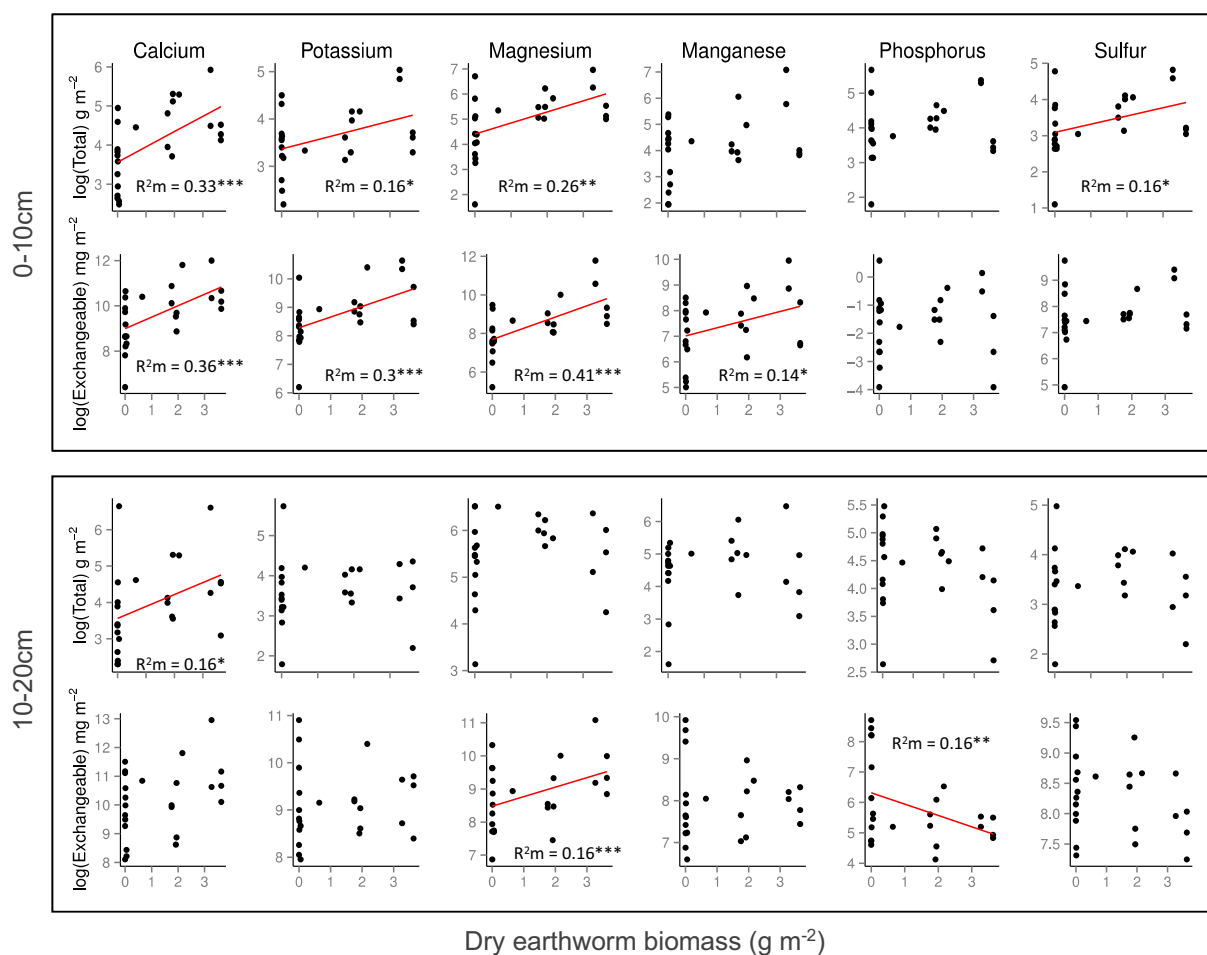


Fig. 2 Elemental total (g m^{-2}) and exchangeable nutrient pools (g m^{-2}) in shallow (0 - 10 cm soil depth; top panel) and deep (10 - 20 cm soil depth; lower panel) as a function of dry earthworm biomass (g m^{-2}). Data are log-transformed standardized ($N = 24$

plots). Panel with lines represent significant linear mixed model predictions. Summary statistics are provided in Supplementary Table 3. P-values are estimated from a normal distribution (* $P < .05$; ** $P < .01$, *** $P < .001$)

Although many of the nutrient metals had higher concentrations in earthworm-invaded soils, total and exchangeable P declined with increasing earthworm biomass in A and B horizons, respectively (Figs. 1, 2, and 6). Assuming P concentrations at uninvaded plots are baseline estimates, we estimated that earthworms decreased P concentrations from 2 to 25%. Even small declines in P are profoundly important in these forests, where many plants are near or below the P deficiency threshold (Fig. 3). Our results suggest earthworms have a negative impact on P, and similar declines in P were found by Paré and Bernier (1989) where forest soils with earthworm-mixed horizons lost exchangeable P under acidic conditions. Depleted soil P is consistent with later stages of earthworm invasion, due to

mineralization and leaching of the O horizon or rapid mobilization into microbial biomass once earthworms have established (Le Bayon and Binet 2006; Resner et al. 2015). The inverse relationship between Ca and P is notable because soil Ca and P often covary (Figs. 1 and 2). For example, Kobe et al. (2002), saw foliar P, Mg and K increase in *A. saccharum* following Ca fertilization, and Boerner (1986), observed a significant positive correlation between natural Ca and phosphate concentrations in soil in oak-maple forests of Ohio. However, this may not be universally true, as Long et al. (1997) found Ca and Mg fertilization decreased exchangeable soil P. They hypothesized that under fertilization treatments, P formed insoluble Ca phosphates or was taken up by stimulated roots. However, our

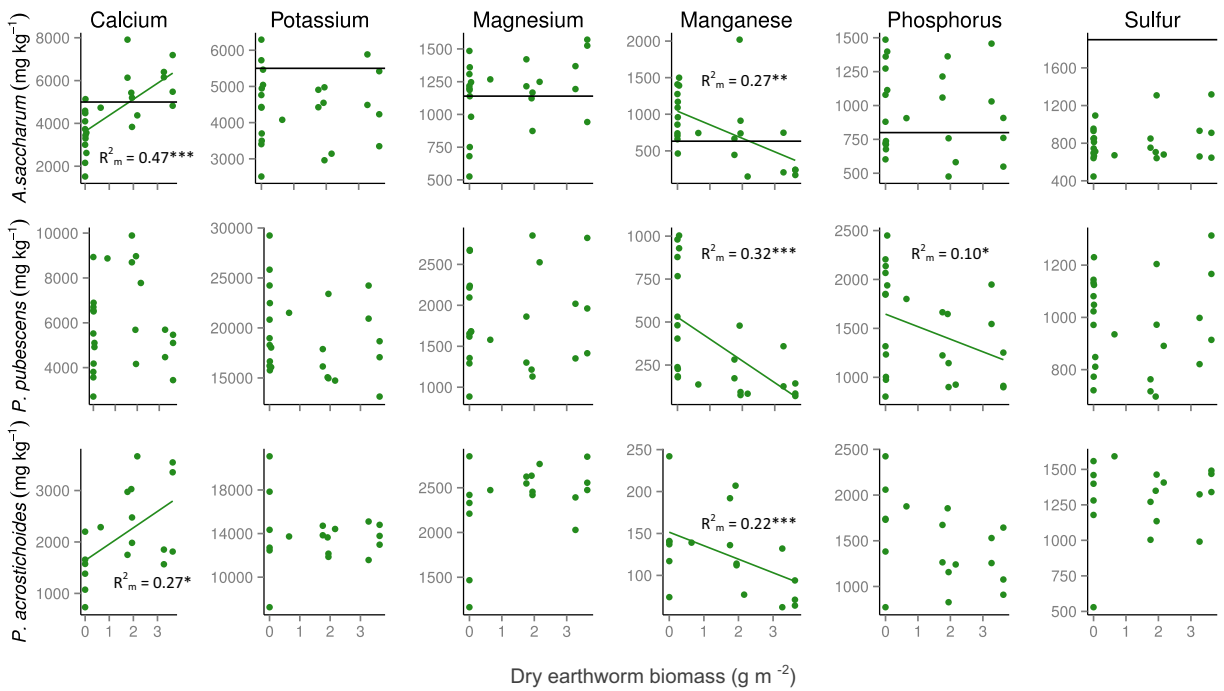


Fig. 3 Relationship between plant leaf tissue (mg kg^{-1}) and earthworm biomass (g m^{-2}). Horizontal line represents minimum published range for healthy *Acer saccharum* trees grown in unfertilized forest conditions in July/August (Burton et al. 1993; Kolb

and McCormick 1993). Summary statistics are provided in Supplementary Table 4. P-values are estimated from a normal distribution (* $P < .05$; ** $P < .01$, *** $P < .001$)

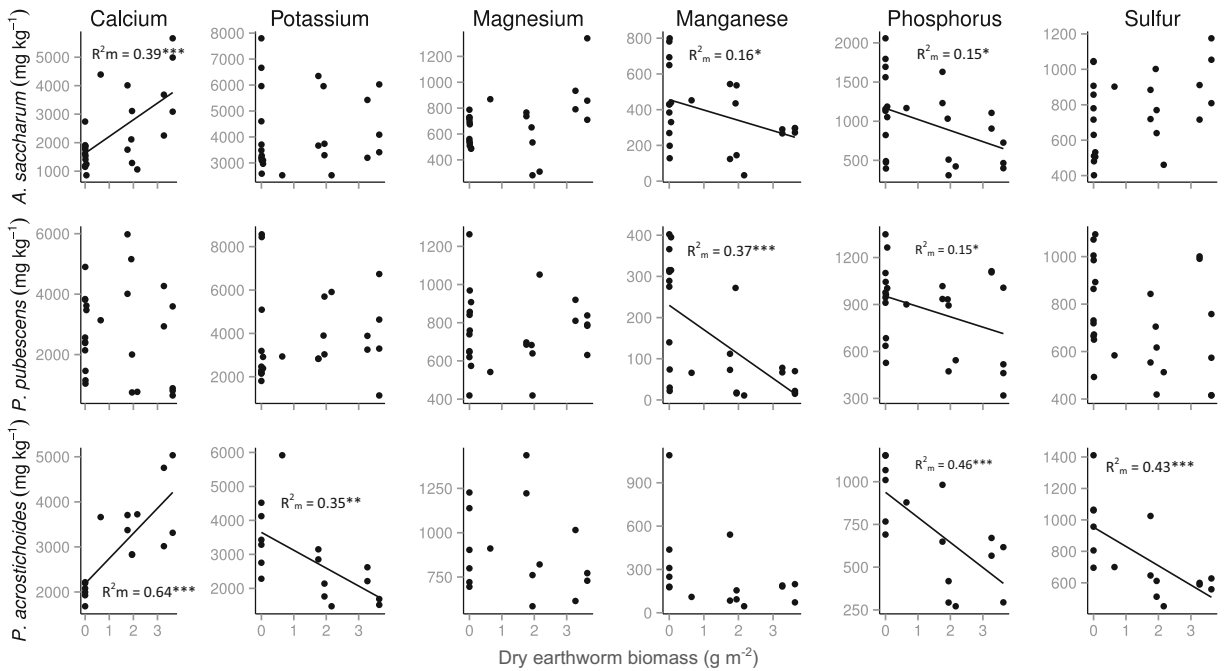


Fig. 4 Relationship between plant root tissue (mg kg^{-1}) and earthworm biomass (g m^{-2}). Summary statistics are provided in Supplementary Table 4. P-values are estimated from a normal distribution (* $P < .05$; ** $P < .01$, *** $P < .001$)

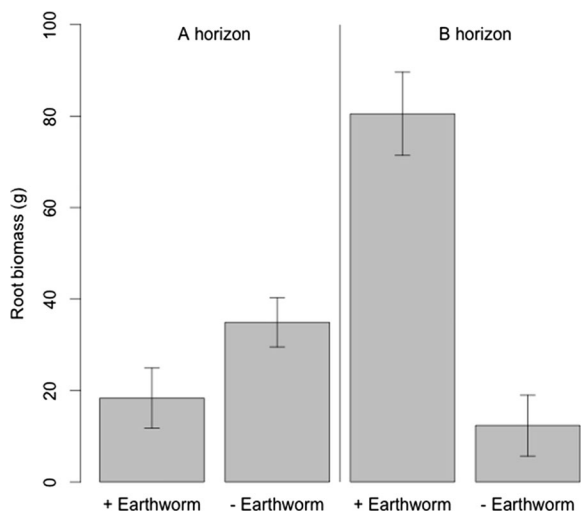


Fig. 5 Biomass of roots recovered from A and B horizons in earthworm-invaded and uninvaded pits ($N = 24$). Linear mixed models include a significant horizon \times earthworm interaction ($p = 0.019$). Summary statistics are provided in Supplementary Table 6

results suggest that while Ca is retained, P has been lost from the organic and mineral soil horizons at earthworm-invaded plots.

Independent of Ca, the magnitude and direction of invasive earthworm influence on soil P has been conspicuously variable in previous studies (Suárez et al. 2004; Le Bayon and Binet 2006; Coulis et al. 2014; Vos et al. 2014). Therefore, we must consider biotic and abiotic differences between these studies to interpret our

data and understand earthworm impact on P cycling. Earthworm ecophysiological groups influence the pattern of soil mixing, and can increase or decrease nutrient availability depending on dominant earthworm species. Suarez et al. (2006b) found sites dominated by anecic *L. terrestris* maintained higher total P as earthworm's added ions from the deep mineral horizon. In contrast, those dominated by epi-endogeic *L. rubellus* had lower total P as mineralized P was leached away from rooting zones. Although both *Lumbricus* species were represented at all of our sites (Supplementary Figs. 1, 4), most individuals are juvenile, and cannot be identified to species. Our sites follow the patterns of epi-endogeic dominance, likely because juvenile *L. terrestris* behave more like epigeic or epi-endogeic feeders (Daniel 1990; Edwards 2004; Asshoff et al. 2010). Taken all together, earthworm-associated increases in soil Ca, Mg, K, and Mn do not support earthworm-associated depletions suggested in hypothesis (1). However, decreases in P suggest that earthworm-invaded plots could be on the threshold of short-term earthworm-derived enrichments giving way to lower fertility as the remaining organic horizon is consumed, as predicted by our first hypothesis. Additional monitoring through time will be required to quantify these long-term changes to nutrient cycling.

We reject our second hypothesis since earthworm presence did not homogenize the vertical nutrient distribution, and earthworm biomass was associated

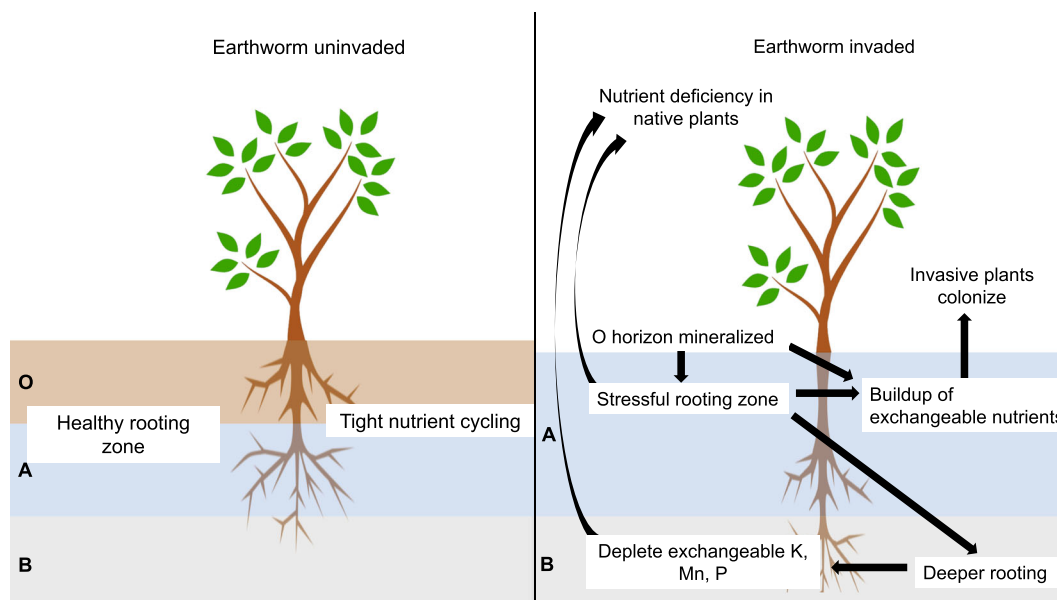


Fig. 6 Proposed mechanism for earthworm disruption of nutrient cycling in forests

with higher exchangeable Ca, K, Mg and Mn concentrations and pools in the A horizon, and lower K, Mn and P concentrations in the B horizon (Fig. 1; Supplementary Fig. 5). Further, earthworms influenced partitioning of nutrients between exchangeable and total pools, with a higher relative concentration of exchangeable nutrients in the A horizon, and lower relative concentration in the B horizon. Surprisingly, this appears to be driven by root absorption, and not mixing of SOM or increased sorption capacity (Fig. 5; Supplementary Fig. 5). This has important implications for understanding the role of invasive earthworms in selecting for certain plant species over others (Fig. 6).

Earthworms create stressful rooting conditions for many shallow-rooted native plants, requiring those not pre-adapted to these conditions to adapt or perish (Gundale 2002; Bohlen et al. 2004c; Hale et al. 2008). In our earthworm-invaded pits, we observed more rooting in the B horizon, corresponding with depleted exchangeable nutrient pools (Fig. 5). Conversely, in the absence of earthworms, roots predominantly appear in the A horizon (Fig. 5). This is somewhat surprising, as one might expect root biomass to correspond to high exchangeable nutrient concentration (Brady and Weil 2008; Giehl and von Wiren 2014). Direct and indirect stress on shallow-rooted species has been widely suggested as an explanation for the destructive effect of invasive earthworms on native forest plants, including disruption of mycorrhizal symbioses, decreased soil moisture, production of phytohormones, altered microbial community and root herbivory (Lawrence et al. 2003; Hale et al. 2006b; Holdsworth et al. 2007b; Laossi et al. 2010a; Laossi et al. 2010b; Gilbert et al. 2014; Whitfield et al. 2014; Dobson and Blossey 2015). As plants root deeper in response to earthworms, they consume available nutrient pools, which likely explains lower exchangeable pools in the B horizon of earthworm-invaded plots. This alternative deep-rooting strategy coupled with depletion in the B horizon could further explain the plant deficiencies of K, Mn, P and S, and lower concentrations of K, Mn and P B horizons of earthworm-invaded soils (Figs. 1, 3, 4, and 6). In other words, vertical root distribution is inversely related to exchangeable soil fertility in earthworm-invaded plots, strongly suggesting that large pools of biologically available nutrients build up in the A horizon of earthworm-invaded soils that are inhospitable to roots of native understory plants. This could provide the missing link to explain how earthworms facilitate

invasive plants at the expense of some native perennials (Heneghan et al. 2007; Nuzzo et al. 2009).

Invasive plants often thrive with increased soil fertility (Huenneke et al. 1990), and those that have co-evolved with earthworms may have germination and rooting strategies conducive to earthworm activity (i.e. physical and chemical defenses to herbivory and desiccation, deeper rooting, low mycorrhizal dependence). Conversely, many native plants have evolved shallow roots that rely on the unique microhabitat of the O horizon (Huenneke et al. 1990; Corio et al. 2009). Indeed, the presence of earthworms prevents native plant communities from taking advantage (measured as biomass and species richness) of increased soil fertility in the A horizon (Whitfield et al. 2014). Considering this facilitation of invasive plants by earthworms in light of previous studies finding earthworms preferentially degrade invasive shrub litter (Heneghan et al. 2007) and removal of invasive shrubs lowers earthworm biomass (Madritch and Lindroth 2009), co-invasion of invasive plants and earthworms may facilitate each other.

In addition to changes in total and exchangeable soil nutrients, we have demonstrated that earthworm invasion has ramifications for plant nutrition, which may ultimately influence forest understory nutrient dynamics. We hypothesized that earthworm impacts on exchangeable and total concentrations would influence foliar and root nutrient concentrations. However, our results did not show a consistent, significant relationship between exchangeable or total nutrient concentrations with root and foliar concentrations. Higher Ca and lower P in both exchangeable and total soil pools of earthworm-invaded soils were also observed in roots and leaves and among all species (with the exception of Ca in *P. pubescens*) (Figs. 3, 4, and 6). Similarly, in a P-deficient Quebec forest, *A. saccharum* growing at earthworm-invaded sites had lower foliar P (Paré and Bernier 1989). However, this pattern may be difficult to identify in nutrient-rich forest ecosystems (Suarez et al. 2006b). Higher exchangeable soil Mg and K concentrations were associated with greater earthworm biomass, but were not associated with plant Mg and K tissue concentration (Figs. 3 and 4). This disconnect between soil and plant tissue stoichiometry in forest soils is surprisingly common (Bard 1949; Boerner 1986; Gilliam and Adams 1995; Schaberg et al. 2006). Just as with our foliar tissue, Schaberg et al. (2006) found that while foliar Ca correlated well with soil Ca

(Supplementary Fig. 3), other nutrients such as K, Mg and Mn did not. Overall, earthworm biomass was a better predictor of foliar nutrients than soil nutrient pools in explaining foliar nutrient concentrations. This could mean that earthworm effects are either more important than soil fertility in plant nutrition, or that earthworm biomass better captures the heterogeneous soil conditions than our soil pits, though these are not mutually exclusive mechanisms.

As ecosystem engineers, it is possible that earthworm invasion overrides site fertility control on plant nutrient dynamics (Fig. 6). In addition to indirectly affecting plants by altering soil parameters controlling nutrient availability, earthworm invasion can strain mycorrhizal associations, enhance moisture and temperature stress and directly feed on fine roots (Lawrence et al. 2003; Fisk et al. 2004; Larson et al. 2010; Gilbert et al. 2014). Historically, understory plants have maintained remarkably consistent foliar stoichiometry across varying environments through unique evolutionary strategies such as seasonal luxury consumption, root foraging and mycorrhizal associations (Johnson 2010; Hawkesford et al. 2011; Giehl and von Wiren 2014; Gilliam 2014). We propose that the disruption of these strategies by earthworms enhances site-to-site variation in foliar nutrition, which could ultimately explain why earthworms are associated with losses in native species richness, cover and seedling recruitment (Hale et al. 2006b; Holdsworth et al. 2007a; Drouin et al. 2014). Our study provides additional support that earthworms drive physicochemical changes to soil and influence plant nutrition (Fig. 6).

Our results show that plant responses to earthworm invasion were variable for different nutrients and species. Many *A. saccharum* seedlings had nutrient concentrations below the lowest value published for healthy foliage, particularly for Ca, Mg, Mn and P (Fig. 3; Burton et al. 1993; Kolb, T.E.; McCormick 1993). Nearly all *A. saccharum* seedlings growing in the absence of earthworms are Ca-deficient, reaching healthy Ca concentrations at an earthworm biomass of $4 \pm 0.2 \text{ g m}^{-2}$. Many (but not all) are within the range of Mn for healthy foliage, becoming deficient beyond an earthworm biomass of $4 \pm 0.2 \text{ g m}^{-2}$ (Fig. 3). Only one individual (in the earthworm-invaded plots) demonstrated possible Mn toxicity, exceeding healthy Mn levels of $1632 \mu\text{g kg}^{-1}$ by $\sim 400 \mu\text{g kg}^{-1}$ (Fig. 3; Kolb, T.E.; McCormick 1993). The molar ratio of Ca:Al ranged from 40 to 295 mol mol^{-1} , which is well above the threshold of toxicity where Ca:Al ratios < 9.9 has 50%

chance of Al toxicity (Thornton et al., 1986; Cronan and Grigal 1995). Therefore, although many *A. saccharum* seedlings were deficient in several nutrients, Al and Mn toxicity is not a concern.

For both *A. saccharum* and *P. acrostichoides*, earthworm biomass was strongly associated with root and foliar Ca (Figs. 3 and 4). Tissue Mn concentrations simultaneously decreased, suggesting that these species may be able to preferentially take up Ca over Mn in earthworm-invaded soils. *Polygonatum pubescens* was the only species that did not have higher tissue concentrations at plots with higher soil Ca and greater earthworm biomass (Figs. 3 and 4). While root P content was lower with increasing earthworm biomass in all species, *P. pubescens* was also the only species with coinciding declines of P in foliage (Fig. 3). Taken together, differences in nutrient availability and uptake may explain this species' susceptibility to earthworm invasion (Dobson and Blossey 2015). Although *P. acrostichoides* thrives with earthworm invasion, earthworm biomass was a negative predictor of foliar Mg, and root K, P and S. It is possible that given their lower nutrient requirements, earthworm impacts on nutrient cycling are less important to fern success compared to angiosperms (Siccama et al. 1970).

Alternatively, Ca could be the most limiting nutrient to *P. acrostichoides*'s growth, such that elevated Ca supersedes the effect of lower concentrations of other nutrients (Bernier et al. 1989; Côté et al. 1995). Foliar nutrients in *A. saccharum* were similar to *P. acrostichoides*, although they are known to be susceptible to earthworms, likely through root herbivory and disruption of mycorrhizae (Lawrence et al. 2003; Fisk et al. 2004; Corio et al. 2009; Gilbert et al. 2014; Bal et al. 2017). However, the negative impact of earthworms on *A. saccharum* is not universal, and young seedlings may not be affected (Drouin et al. 2014). It is possible that decreases in P in earthworm-invaded plots negate any benefits from higher Ca, or that higher Ca was not enough to elicit a response in this calciphilic species. The large proportion of *A. saccharum* individuals that are potentially deficient in multiple nutrients could support either possibility. Overall, our results suggest native plants differ in their ability to incorporate nutrient resources under earthworm invasion. Species most at risk such as *P. pubescens* are unable to benefit from higher soil Ca in earthworm-invaded forests, while simultaneously becoming more deficient in other macronutrients. This supports previous research suggesting

P. pubescens, and species with similar life histories will likely be disproportionately affected by earthworm invasion, and may warrant special conservation concern (Dobson and Blossey 2015).

Conclusions

In this study, we present evidence that nutrient cycles in forests are altered by earthworm invasion, and earthworms create a different set of selection pressures for plants growing in these forests. Earthworm biomass was a better predictor of plant nutrition than soil nutrient content or concentration. This may be because earthworm invasion overrides site fertility, or that the soil is so heterogeneous that earthworm biomass is a better indicator of site fertility than soil pits. Overall, a plant species' ability to persist or thrive with earthworms may depend on its ability to take advantage of rapidly mineralized nutrients in earthworm-invaded soils.

Our results suggest earthworm invasion creates a stressful rooting zone for many native species, and some understory plants may adapt, while others may disappear. Evidence of the latter has been well documented (Gundale 2002; Hale et al. 2008; Dobson and Blossey 2015), but our observation that rooting volume was highest in the B horizon in the presence of earthworms suggests some plants may simply root deeper (or be replaced by deeper-rooted plants). The lack of shallow roots in earthworm-invaded soils corresponds to a build-up of exchangeable nutrients. Therefore, early successional and non-native species that can establish successfully in the absence of an organic horizon may benefit from pools of exchangeable nutrients from mineralization of the organic horizon.

What remains to be seen is consequences for herbivore preference, which can in turn impact plant communities (Hunter et al. 1991; Joern et al. 2012). Impacts on other trophic levels and adjacent ecosystems such as streams and waterways are vitally important to consider. In addition to macronutrients, earthworm invasions impact micronutrient and metal cycling, which could have ramifications for higher trophic levels (Joern et al. 2012; Richardson et al. 2015; Richardson et al. 2016).

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