

Exotic earthworm effects on hardwood forest floor, nutrient availability and native plants: a mesocosm study

Cindy M. Hale · Lee E. Frelich · Peter B. Reich ·
John Pastor

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Abstract A greenhouse mesocosm experiment, representing earthworm-free North American *Acer*-dominated forest floor and soil conditions, was used to examine the individual and combined effects of initial invasion by three European earthworm species (*Dendrobaena octaedra*, *Lumbricus rubellus* and *Lumbricus terrestris*) on the forest floor and upper soil horizons, N and P availability, and the mortality and biomass of four native understory plant species (*Acer saccharum*, *Aquilegia canadensis*, *Aralia racemosa*, and *Carex pensylvanica*). All the three earthworm species combined caused larger impacts on most variables measured than any single earthworm species. These included loss of O horizon mass, decreased thickness of the O horizon and increased thickness of the A horizon, and higher availability of N and P. The latter finding differs from field reports where nutrients were less available after invasion, and probably represents an initial transient increase in nutrient supply as earthworms consume and incorporate the O horizon into the A horizon. Earthworms also increased mortality of plants and decreased total mesocosm plant biomass, but here the impact of all the three

earthworm species was no greater than that of *L. terrestris* and/or *L. rubellus* alone. This study corroborates field studies that European earthworm invasions alter North American forest ecosystem processes by initiating a cascade of impacts on plant community composition and soil properties.

Keywords European earthworms · Invasive species · *Lumbricidae* · Northern hardwood forests · Plant mortality

Introduction

Earthworms affect many aspects of the plant growth environment. They alter the populations of beneficial microorganisms, pests and diseases, plant growth regulating substances, cause physical changes in fine root systems and soil structure, and change the availability of water and nutrients, improving plant growth in the vast majority of cases studied, especially in regions where earthworms are native and in agricultural fields (Blouin et al. 2006; Brown et al. 2004).

In stark contrast, earthworms can cause forest decline and loss of native plant species in cold-temperate forests that were previously earthworm free (Frelich et al. 2006). Across the cold temperate regions of North America, European earthworms have changed ecosystem structure and function by consuming the previously thick forest floors (also referred to as the O horizon; Nielsen and Hole 1963; Alban and Berry 1994), and mixing the organic matter into the A horizon, increasing its thickness and bulk density (Nielsen and Hole 1964; Hale et al. 2005b). The net effect of these changes has been mortality of plants that were rooted primarily in the O horizon due to physical disruption and exposure of their root systems (Gundale 2002; Hale et al. 2006), and decline in nutrient availability and fine root biomass in the upper horizons of the mineral soil (Fisk et al.

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C. M. Hale · L. E. Frelich · P. B. Reich
Department of Forest Resources,
University of Minnesota, 1530 Cleveland Avenue North,
115 Green Hall, St Paul, MN 55108-6112, USA
e-mail: freli001@umn.edu

C. M. Hale (✉) · J. Pastor
The Natural Resources Research Institute,
University of Minnesota Duluth,
5013 Miller Trunk Highway,
Duluth, MN 55811-1442, USA
e-mail: cmhale@d.umn.edu

2004; Suárez et al. 2004; Bohlen et al. 2004b; Hale et al. 2005b). However, it is possible that variability in the soil environment in the field could account for some effects attributed to earthworm invasion, a situation that could be remedied by controlled experiments to examine the effects of exotic earthworms on soil properties and plant species growth and survival without other confounding factors.

In addition to confounding environmental factors, several earthworm species commonly invade concurrently (Alban and Berry 1994; Dymond et al. 1997; Bohlen et al. 2004a), each with specific burrowing and feeding behaviors that can lead to different impacts (Bouché 1977; Shaw and Pawluk 1986; Lavelle 1997), so it is difficult to determine the importance of any particular earthworm species on the changes in soil properties and plant species composition (Bohlen et al. 2004a). Again, controlled experiments with different earthworm species or combinations thereof are needed to understand whether the different burrowing and feeding behaviors have different effects on soils and plant communities.

For this study, greenhouse mesocosms that represent earthworm-free forest floor and soil conditions seen in field sites prior to invasion (Hale et al. 2005a) were used to examine the effects of invasive earthworms. The first objective was to see whether the impacts found in the field also occur under controlled conditions, and the second was to disentangle the impacts of individual earthworm species. The impacts of three invasive earthworm species were studied on: (1) the forest floor (O horizon thickness and mass); (2) properties of the upper mineral soil horizons (i.e., A horizon thickness and the availability of N and P); and (3) the mortality and biomass of selected native plant species.

Three common European earthworm species with different feeding and burrowing behaviors (*Dendrobaena octaedra*, *Lumbricus rubellus* and *Lumbricus terrestris*) were included in the experiment. *D. octaedra* is a small (~2–4 cm) litter-dwelling (epigeic) species that feeds primarily on microorganisms associated with decaying surface litter (Scheu and Parkinson 1994; McLean and Parkinson 2000). In previous field studies, *D. octaedra* has not been associated with changes in forest floor and soil horizon thicknesses or changes in plant communities (Gundale 2002; Hale et al. 2005a, b). *L. rubellus* is a medium sized (~3–7 cm) surface-dwelling (epi-endogeic) species that feeds on surface litter but also burrows and casts in the upper mineral soil layer (Rozen 1988; Hendriksen 1990; Hendrix et al. 1999) and is commonly found intimately associated with plant roots, suggesting that this species actively feeds in the rhizosphere (Pearce 1978; C. M. Hale, personal observations). In field studies, *L. rubellus* has been associated with rapid removal of the O horizon and changes in the abundance and composition of forest understory plant communities (Gundale 2002; Hale et al. 2005b, 2006). *L. terrestris* is a large (~8–15 cm) soil-dwelling (anecic) species that feeds preferen-

tially on freshly fallen leaf litter, rather than the accumulated forest floor layer (Hale et al. 2005a), and forms deep, persistent vertical burrows. Large amounts of cast material are deposited in the burrows and at the surface (Pitkänen and Nuutinen 1997). Although *L. terrestris* can consume large amounts of surface litter (Knollenberg et al. 1985; Cothrel et al. 1997) its burrowing habits and the pattern of forest floor removal in the field during invasion may be less disruptive to the upper soil horizons where most plant roots are concentrated than *L. rubellus* (Hale et al. 2005b).

Based on these previous studies we hypothesized that the magnitude of impacts to O horizon mass, O and A horizon thickness, nutrient availability and plant mortality and biomass would, among species, be least for *D. octaedra*, intermediate for *L. terrestris* and largest for *L. rubellus*. Because the three earthworm species are in different functional groups, with different types of impacts, we hypothesized that the impacts would be greater for the treatments including all three species than for any one species, even when biomass is taken into account.

We studied four native understory plant species that were shown to have differing responses to earthworm invasion in previous studies where plant community composition was compared on either side of earthworm invasion fronts at four hardwood forests sites in Minnesota (Hale et al. 2006). *Carex pensylvanica*, a non-mycorrhizal native sedge species, was the dominant native plant species following earthworm invasion in the field (Hale et al. 2006). *Aralia racemosa* is a large, perennial herbaceous species that was mostly eliminated following earthworm invasion in field sites. The abundance of *Acer saccharum*, the dominant tree seedling in the field sites, also declined following earthworm invasion. *Aralia* and *Acer* are strongly mycorrhizal species with much thicker fibrous root systems than *Carex* (Brundrett and Kendrick 1988; C. M. Hale, personal observations). *Aquilegia canadensis* is a mid-sized perennial herbaceous species that is common in temperate hardwood forests, including some forests that have been invaded by earthworms for several decades (Hale et al. 1999, personal observations) suggesting that this plant species may be more tolerant of earthworm invasion than *Aralia*. Based on these field studies we hypothesized that the effects of earthworms on plant mortality and total plant biomass would be greatest for *Acer* and *Aralia*, intermediate for *Aquilegia*, and least for *Carex*.

Materials and methods

Experimental design and mesocosm assembly

A randomized and fully crossed block design consisting of five plant treatments and five earthworm treatments was

used to test our hypotheses. The plant treatments were the four individual species and a no-plant control. The earthworm treatments were each of the three species alone, all three together, and a no-worm control. *Carex*, *Aquilegia* and *Acer* had 14 replicates in each earthworm treatment while *Aralia* and the no plant control had seven replicates in each earthworm treatment because of space limitations on the number of mesocosms ($n = 280$). For each earthworm and plant treatment, the total number and biomass of earthworms and plants were standardized across all replicates.

Earthworm-free conditions similar to field sites without earthworm invasion (Hale et al. 2006) were created in each mesocosm. The mesocosms were 30-cm-diameter by 38-cm-deep plastic buckets with a 5-cm-diameter PVC ventilation tube in the center. The inside surfaces were sprayed with polyurethane and coated in coarse sand to discourage earthworms from preferentially burrowing along them. In addition, to further discourage earthworm burrowing along the soil–mesocosm interface, each mesocosm was filled with ~22 cm of mineral soil and the outer edge (~1.5 cm) of soil compacted. The mineral soil used was a silty clay loam Eutroboralf (Warba series; USDA 1997). Soil was collected from the B horizon (10–50 cm depth) of earthworm-free field sites (Hale et al. 2005a); the soil was spread on tarps, sun-dried for 48 h and then sifted and mechanically mixed until homogeneous. The surface of the mineral soil in each mesocosms was raked to create a rough surface and a disc of intact forest floor (~5–7 cm thick by 30 cm diameter) was placed on top. The forest floor discs were collected randomly from a 1-ha area within an earthworm-free, sugar maple-dominated hardwood forest in Tettegouche State Park in northeastern Minnesota. Mesocosms were watered to field capacity, weighed to the nearest 0.25 kg and randomly placed in a greenhouse at the Natural Resources Research Institute in Duluth, Minnesota, USA.

Plants used in the study were either collected from field sites or germinated and started in a greenhouse. In the first week of June, *Acer* seedlings were collected from Tettegouche State Park in northeastern Minnesota and *Carex* seedlings were collected from Magney–Snively Natural Area in Duluth Minnesota. *Aralia* and *Aquilegia* seedlings were established in spring 2001 from seed in a greenhouse by a regional native plant nursery (Steve Banovetz, Agrecol, Madison, Wis.). *Aralia* seeds were collected in fall 2000 from sites previously used in earthworm invasion studies (Hale et al. 2005a) and *Aquilegia* seeds from central Wisconsin were purchased from Agrecol. One of five plant treatments was randomly assigned to each mesocosm. Planting occurred between 15 and 21 June 2001. In each mesocosm, six seedlings (~7–15 cm in height) of the assigned species were planted, evenly spaced, by piercing the forest floor layer to the top of the mineral soil with a

hand trowel and planting the roots in each furrow. The forest floor was similarly pierced in the no plant control treatment. Plants were allowed to grow for 8 weeks before earthworms were added. During plant establishment, each mesocosm was weighed and watered to field capacity weekly. Plants that died in the first 3 weeks were replaced.

Earthworm treatments were randomly assigned and implemented during 17–20 August 2001. *L. rubellus* and *L. terrestris* were purchased at local fishing bait shops. Separation of these two species was determined by size using sexually mature individuals. *D. octaedra* were collected from a field site known to contain only that species and identification of 83 individuals collected confirmed that only *D. octaedra* was present. A mixture of juvenile and adult *D. octaedra* individuals was used. The number of individual earthworms included in each treatment reflected the average densities from field sites (Hale et al. 2005a; three *L. terrestris*, 15 *L. rubellus*, 40 *Dendrobaena*). The fresh biomass of earthworms added to each mesocosm was recorded. Mesocosms were incubated for 13–18 weeks, depending on plant species.

Throughout the experiment, greenhouse air temperature was maintained between 15 and 35°C, with supplemental lighting to maintain 16 h of daylight, similar to summer conditions in northern Minnesota. Soil temperatures were maintained between 13 and 21°C (again, similar to summer conditions in northern Minnesota) by circulating cool water in water baths as needed. Mesocosms were monitored daily and watered as needed to maintain near field capacity moisture conditions. Visual indications of earthworm activity, forest floor condition and the general health of each plant were recorded weekly. Any foliage shed during the experiment was collected and labeled for each plant. All mesocosms were rotated in the greenhouse on a weekly basis to randomize the effects of any localized conditions within the greenhouse.

Sample collection and data calculations

Initial fresh biomass of the forest floor layer, individual plants and total earthworms added to each mesocosm were recorded. Initial forest floor dry biomass values for each mesocosm were estimated based on the average fresh to dry weight conversion of ten samples oven dried for 48 h at 60°C.

After 13–18 weeks of exposure to earthworm treatments, the plants, soils, and earthworms in the mesocosms were harvested. Because harvesting of the mesocosms would take over a month, all mesocosms containing each plant species were harvested together, over approximately 7 days, to minimize the time variation within each plant species. Mesocosms were exposed to earthworms for a mean of 13, 16, 17, 17 and 18 weeks for *Acer*, no plant control, *Aralia*, *Aquilegia* and *Carex*, respectively.

In each mesocosm, O and A horizon thickness were measured at three evenly spaced locations. All remaining forest floor material was collected and doused in a mustard solution (Hale et al. 2005a) to extract any earthworms. The forest floor material was then oven-dried at 60°C for 48 h and dry mass was measured. All surviving plants were collected individually and fresh biomass of above-ground plant parts, fine roots (≤ 1.0 mm) and coarse roots was recorded. All plant parts were oven dried for 48 h at 60°C and then final dry biomass recorded. Finally, the soil from each mesocosm was sifted to collect remaining earthworms. All earthworms collected from the forest floor material and mineral soil were killed in 70% isopropyl alcohol and preserved in 10% formalin. Ash-free dry biomass of earthworms collected was estimated from their preserved length (mm) using previously determined allometric regression equations (Hale et al. 2004).

Individual plant above-ground biomass (grams dry weight) was the sum of all foliage collected during the course of the experiment and the final above-ground biomass collected. Mean individual plant above-ground biomass, fine root, coarse root, total root and total plant biomass were calculated for each mesocosm. The total plant biomass in each mesocosm was the sum of the biomass of all surviving plants.

N and P availability were measured using ion exchange resin bags, containing 5 g of Rexyn I-300 (Fischer Scientific, Fairlawn, N.J.) mixed-bed, cation-anion exchange resin (Binkley 1984). One resin bag was buried (~ 15 cm deep) in each mesocosm during assembly 4–7 June 2001 and removed during final harvesting 16 November–20 December 2001. The resin bags were rinsed with de-ionized water and stored in Ziplock bags in a refrigerator until processing.

NH_4 , NO_3 and PO_4 were extracted from 3-g subsamples of resin with 100 ml of 1 mol/l KCl; the resins were shaken for 15 min in 25 ml KCl, decanted, shaken again in another 25-ml aliquot, poured into small Buchner funnels (5.5 cm diameter) equipped with pre-washed no. 1 Whatman filters, and washed several times with additional KCl before the filtrate was brought to 100 ml volume. The extracts were analyzed by standard methods for $\text{NH}_4\text{-H}$, $\text{NO}_3\text{-H}$ and $\text{PO}_4\text{-P}$ on a Lachat autoanalyzer (Lachat Instruments, Milwaukee, Wis.).

Statistical methods

A combination of two-way ANOVA with least squared mean comparisons and one-way paired comparisons were used to test for the effects of earthworm and plant treatments (SAS 2001). To meet assumptions of normality, the square root of the percent mortality of the plants was tested. Earthworm number and biomass, nutrient availability and all plant biomass parameters were log transformed [$\ln(x + 1)$]. No transformations were necessary for forest floor mass loss or A and O horizon thicknesses.

Results

Soil characteristics

The presence as well as the species of earthworms caused significant differences in forest floor mass and O and A horizon thickness (Table 1). The forest floor (gram dry weight) lost more mass when all earthworm species or *L. terrestris* or *L. rubellus* alone were present compared to

Table 1 Summary statistics: two-way ANOVA of plant and soil parameters among earthworm treatments and plant species. The five earthworm treatments and five plant treatments are shown. NS Non-significant

Plant/soil parameters	Full model ^d (R^2)	ANOVA parameters		
		Earthworm treatment	Plant treatment	Plant \times earthworm
Forest floor mass loss	0.27	***	*	*
O horizon thickness	0.49	***	*	NS
A horizon thickness	0.46	***	NS	NS
N availability (NH_4)	0.26	***	***	NS
N availability (NO_3)	0.36	***	***	NS
P availability (PO_4)	0.19	*	***	NS
Plant mortality (%)	0.63	***	***	NS
Mean plant total biomass ^b	0.78	NS	***	NS
Mesocosm total plant biomass ^c	0.78	†	***	NS
Mesocosm above plant biomass ^c	0.77	†	***	NS
Mesocosm total root biomass ^c	0.77	*	***	NS
Mesocosm coarse root biomass ^c	0.78	†	***	NS
Mesocosm fine root biomass ^c	0.50	*	***	NS
Final earthworm numbers	0.54	***	***	*
Final earthworm biomass	0.78	***	***	*

† $P \leq 0.10$, * $P \leq 0.05$, *** $P \leq 0.001$

^a All models $P \leq 0.001$

^b Mean of all surviving plants for each mesocosm

^c Sum of all surviving plants for each mesocosm

D. octaedra alone and the no earthworm control ($P < 0.0001$; Table 1, Fig. 1). All earthworm species reduced O horizon thickness and increased A horizon thickness compared with the no earthworm control ($P < 0.0001$; Table 1, Fig. 2). *D. octaedra* had the least effect on forest floor thickness followed in increasing order of effect by *L. rubellus*, *L. terrestris* and all earthworm species combined, respectively ($P < 0.0001$; Table 1, Fig. 2).

Nutrient availability

NO_3 dominated the available inorganic forms of N. Nitrogen (NH_4 and NO_3) and phosphorous (PO_4) availability were highest when all earthworm species were present compared to mesocosms with only one species or no earthworms. *L. rubellus* was the only earthworm species that elevated nutrient availability above the no earthworm control and only for NO_3 ($P < 0.0001$; Table 1, Fig. 3). Soils planted with *Carex* had the highest N availability (dominated by NO_3) among plant species while soils planted with *Acer* had the highest phosphorous availability (PO_4) ($P < 0.0001$; Table 1, Fig. 3).

Plant mortality and biomass

Overall mortality was highest for *C. pensylvanica* followed by *Aralia racemosa*, *Aquilegia canadensis* and *Acer saccharum*, respectively ($P < 0.0001$; Table 1, Fig. 4). Among earthworm treatments, mortality for all plant species combined was highest when all earthworm species were present compared with *L. rubellus*, *D. octaedra* or the no earthworm control, and intermediate when *L. terrestris* was present ($P = 0.0009$; Table 1, Fig. 4). Mortality was significantly higher for *Aquilegia* when all three earthworms were

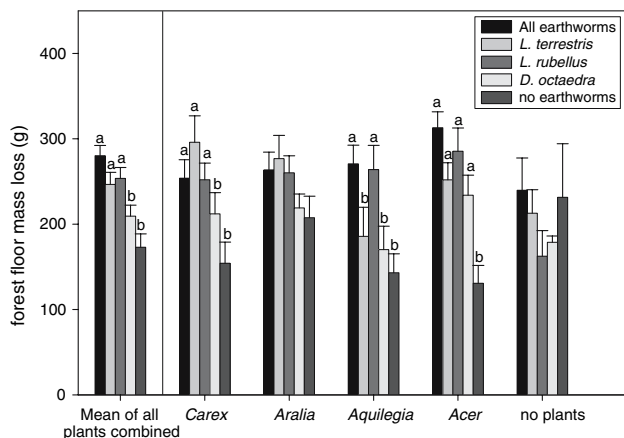


Fig. 1 Mean forest floor mass loss (g dry weight) compared among earthworm treatments for each plant species and the mean of plants combined. In least squared mean paired comparisons among earthworm treatments within each group, significant differences are indicated by different letters ($P \leq 0.05$)

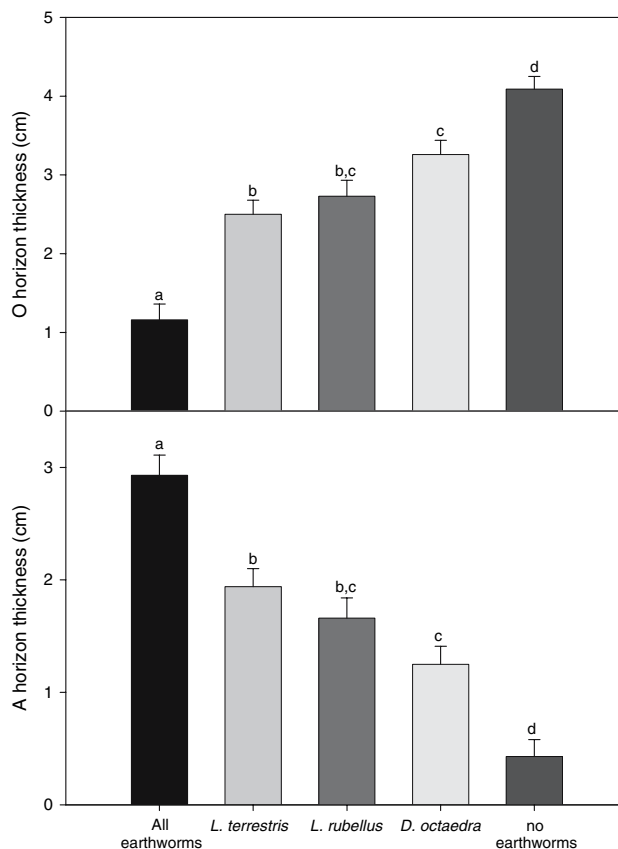


Fig. 2 Mean O and A horizon thickness (cm) compared among earthworm treatments across all plants combined. In least squared mean paired comparisons among earthworm treatments, significant differences are indicated by different letters ($P \leq 0.05$)

present, and for *Acer* when all three earthworms were present or *L. terrestris* was present. There was a non-significant trend toward higher mortality for *Aralia* when all three earthworm species were present, and there were no trends among earthworm treatments for *Carex* (Fig. 4).

The addition of earthworms did not change mean individual plant total biomass compared with the no earthworm control (Table 1). However, total plant biomass (grams dry weight) decreased significantly when all earthworm species were present compared with no earthworms, and single earthworm species caused intermediate biomass values ($P \leq 0.10$; Table 1, Fig. 5). Mesocosm above-ground plant biomass, fine root, coarse root, and total root biomass had the same patterns as total plant biomass (data not shown; Table 1). *Aralia* had the highest mesocosm plant biomass values followed by *Aquilegia*, *Carex* and *Acer*, respectively ($P < 0.0001$; Table 1, Fig. 5).

Discussion

This experiment corroborates evidence from the field that earthworms decrease mass and thickness of the O horizon,

Fig. 3 Mean N (NH_4 and NO_3) and phosphorous (PO_4) availability among earthworm treatments and plant species. Least squared mean paired comparisons are of log-transformed data and significant differences among earthworms or plant species are indicated by *different letters* ($P \leq 0.05$)

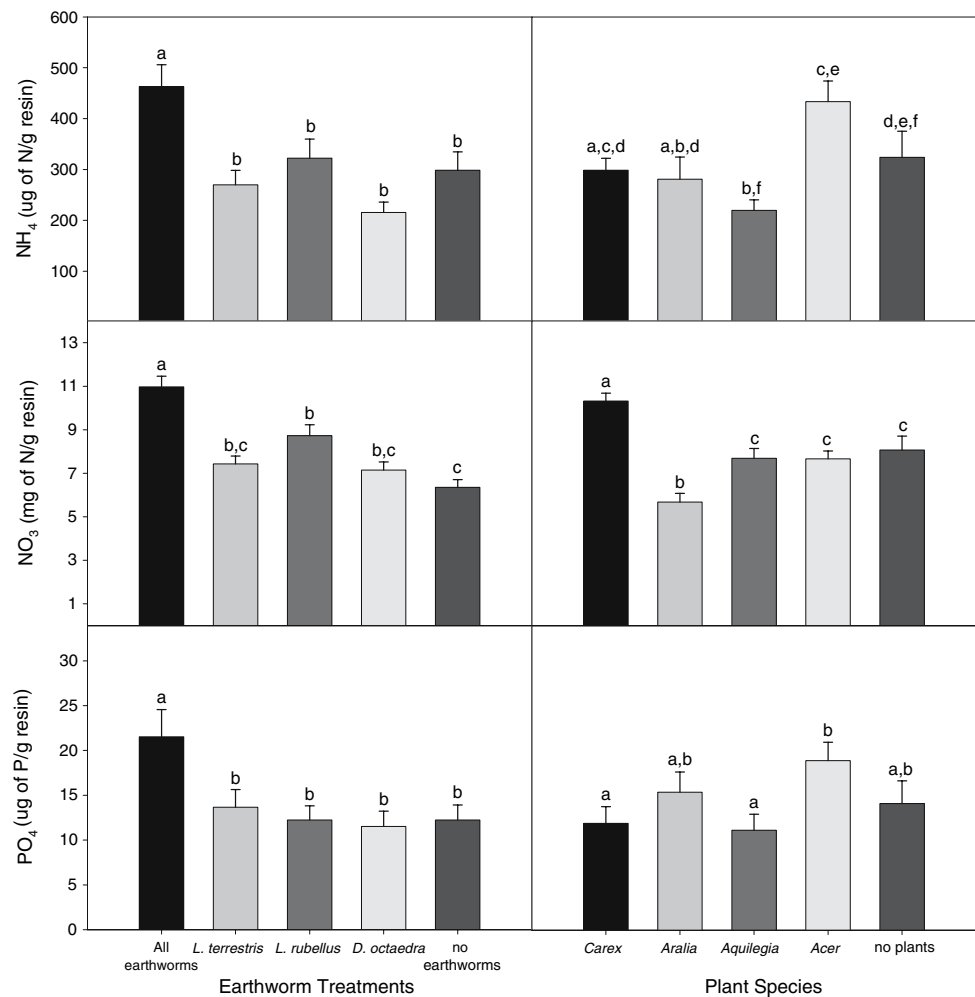
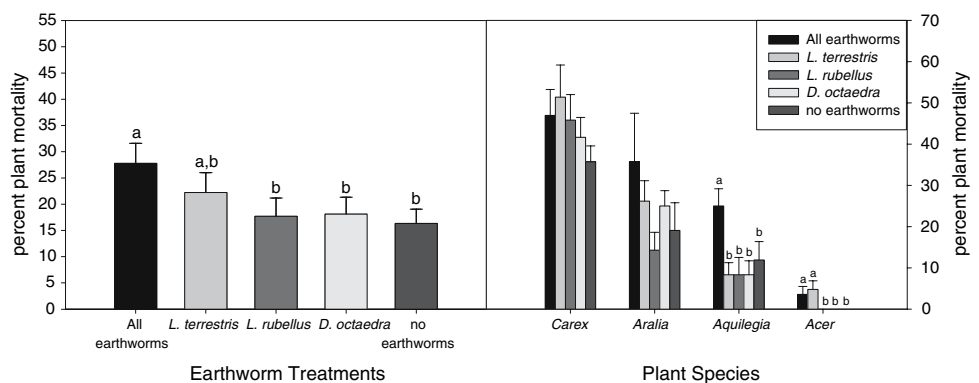


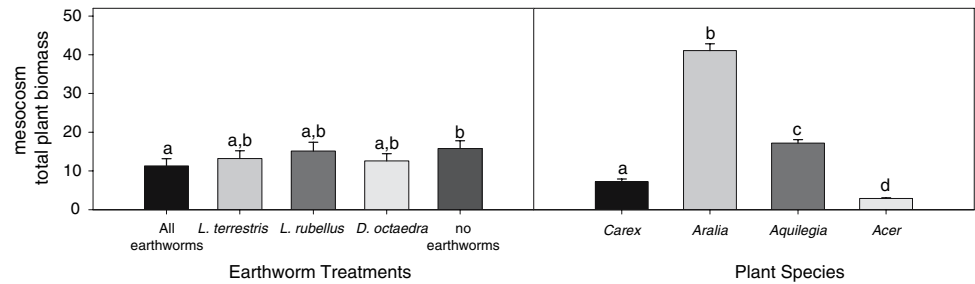
Fig. 4 Mean percent plant mortality among earthworm treatments and plant species. Least squared mean paired comparisons are of square root-transformed data and significant differences among earthworms are indicated by *different letters* ($P \leq 0.05$)



increase A horizon thickness, have complex influences on nutrient dynamics, increase plant mortality and decrease plant biomass. The presence of all three earthworm species generally caused larger impacts than any single earthworm species. Thus, this study reinforced field evidence that impacts of earthworm invasion on plant growth were context specific; although mostly positive earthworm–plant interactions have been demonstrated in agricultural settings, negative impacts on plant growth have occurred in

woodland settings. The results are consistent with previous studies that have found large negative impacts of multi-species earthworm invasion on plant growth and species richness in previously earthworm-free, cold-temperate forests (e.g. Hale et al. 2006; Holdsworth et al. 2007), a situation that contrasts with native earthworms in warm-temperate and tropical woodland settings where positive impacts on herb and tree growth have been found (Pashanasi et al. 1992; Callaham and Hendrix 1998).

Fig. 5 Mean mesocosm total plant biomass (g dry weight) among earthworms and plant species. Least squared mean paired comparisons are of In-transformed data and significant differences among earthworms or plant species are indicated by different letters ($P \leq 0.05$)



How representative of field conditions were the earthworm treatments in this experiment? Although this question may not apply to single-species earthworm treatments, which were meant to tease apart individual species' impacts that could not be seen in the field, the all-earthworm treatment was designed to represent field conditions. Holdsworth (2006) surveyed exotic earthworm ash-free dry mass on 39 *Acer*-dominated forests in northern Wisconsin and Minnesota, USA, and found that values of 10–22 g/m² were typical in forests near human settlements. Earthworm mass at the end of incubation ranged from 11.6 to 22.6 g/m² among replicates of the all-earthworm treatment. Although the species composition in the field data set was not identical to the all-earthworm mesocosm treatment, in both data sets *L. terrestris* and *L. rubellus* contributed a large majority of the total earthworm biomass. Thus it is reasonable to conclude that earthworm biomass in the mesocosm experiment was representative of heavily invaded forests in the north-central US.

Earthworm species effects

As predicted, *D. octaedra* had the smallest effects on soil horizons among the three earthworm species, and did not impact forest floor mass or plant mortality (Fig. 2). This is consistent with field studies suggesting that *D. octaedra* may have minimal short-term effects on forest soil structure and understory plant communities (Gundale 2002; Hale et al. 2006, 2005a, b). However, changes in microbial communities associated with invasion by *D. octaedra* may have important long-term effects that are still poorly understood (Scheu and Parkinson 1994; McLean and Parkinson 2000), particularly in conifer-dominated forests where *D. octaedra* is often the only European earthworm species present (Dymond et al. 1997; Reich et al. 2005; Tiunov et al. 2006).

In contrast to our prediction that *L. rubellus* would have the greatest effects among the three individual earthworm species, *L. rubellus* and *L. terrestris* decreased O horizon thickness and mass and increased A horizon thickness by about the same amount (Figs. 1, 2). The explanation for the slight elevation of NO₃ in the *L. rubellus* treatment is not clear, although there was some mortality of *L. rubellus*

during the experiment (unlike *L. terrestris*; data not shown), and mineralized earthworm biomass could have contributed. The overall lack of significant impacts on nutrient availability, plant mortality and plant biomass for the two *Lumbricus* species (Figs. 3, 4, 5) was surprising because declines in nutrient availability and plant abundance have been documented in the field when these species are present (Bohlen et al. 2004b; Hale et al. 2005b, 2006). However, in these and other field studies, *L. rubellus* and *L. terrestris* have been found with at least one other species and never in isolation (Alban and Berry 1994; Bohlen et al. 2004a; Reich et al. 2005; Holdsworth et al. 2007).

We predicted *L. rubellus* would have larger effects than *L. terrestris* in this experiment because in field studies *L. rubellus* was the species most strongly correlated with changes in soil characteristics and plant species composition, although *D. octaedra* was also found at the field sites (Gundale 2002; Hale et al. 2005b, 2006). Additionally, in this experiment *L. rubellus* removed the same amount of forest floor material in much less time (~6 weeks) than did *L. terrestris* (~13 weeks) (C. M. Hale, personal observation). Therefore, greater impacts to nutrient dynamics and plant species might have been expected.

There was some evidence that earthworm impact did not always depend on biomass. Total earthworm biomass for *L. terrestris* treatment was the same at the end of the experiment as when all three species were present (data not shown), but the effects were greater when all three species were present. In particular, only when all earthworm species were present did nutrient availability and plant mortality increase and mesocosm plant biomass values decrease significantly as compared to some of the other earthworm treatments and/or the no earthworm control (Figs. 3, 4, 5). In addition, *L. rubellus* alone had a similar magnitude of effect on nutrients as *L. terrestris* alone, which had 2–3 times the total biomass (Fig. 3). These results support the conclusion from other studies that more impacts will occur when earthworms from more than one functional group occur together (Lavelle et al. 1998; Hale et al. 2005a, b). In this case, with the presence of *Dendrobaena*, which compacts but does not consume litter, *L. rubellus* which rapidly consumes older litter and burrows in the upper mineral soil, and *L. terrestris*, which consumes fresh litter and burrows

more deeply, it is reasonable to conclude that the three earthworm species in combination will have greater impacts than an equivalent earthworm biomass of one species because of the differences in their foraging and burrowing behaviors.

Plant species responses to earthworms

In field studies that document changes in native plant communities following European earthworm invasion, increased plant mortality is inferred from the lower population densities of native understory plant species behind the invasion front compared to in front of it (Kourtev et al. 1999; Gundale 2002; Hale et al. 2006). This study confirms, under controlled conditions, that mortality of mature native understory plants increases following earthworm invasion.

Because of differences in incubation time among plant species and the lack of significant interaction between plant species and earthworm treatment in the ANOVA (Table 1) we must add a note of caution to interpretation of the magnitudes of mortality of individual plant species. However, there was a suggestion in the data that mortality of *Aquilegia* and *Acer* was higher when all earthworm species were present than for the other earthworm treatments (Fig. 4), and that earthworms had no effect on mortality of *Carex* (Fig. 4), suggesting that *Carex* was less affected by earthworm invasion than the other species. This lack of increased mortality coupled with possible release from competition from species whose mortality increases may be the reasons for the relative success of *Carex* following earthworm invasions in field sites compared to most other native understory plant species (Holdsworth et al. 2007; Hale et al. 2006).

Earthworm presence did not significantly increase mortality of *Aralia* in this experiment even though the root crowns and much of the fine root system of *Aralia* plants were exposed and plants lost their leaves when *Lumbricus* was present (C. M. Hale, personal observation). The protective conditions of the greenhouse allowed re-establishment of the root system and re-growth of new leaves. However, it is unlikely that such re-growth would occur under field conditions where a few days without rainfall and grazing by herbivores would lead to mortality (Augustine 1997; Frelich et al. 2006).

Despite increased nutrient availability in the presence of earthworms, mean individual plant biomass did not change (Fig. 3, Table 1). It is widely assumed that increased nutrient availability resulting from earthworm activity would increase plant productivity (Lee 1985; Edwards et al. 1995). However, most research on response of plant productivity to earthworm introductions has been conducted with agricultural plants and these results may not apply to

native forest plant species (Scheu 2003). The results of other potted plant experiments are contradictory and few have involved plant species from natural communities (James and Seastedt 1986; Blair et al. 1995; Alpei et al. 1996). Furthermore, the observed increase in nutrient availability in potted plant experiments like this one may be short term, immediately following consumption and incorporation of the forest floor into the A horizon (Lavelle et al. 1992). Field studies conducted in areas invaded by earthworms for at least a few years have documented declines in nutrient availability following earthworm invasion and these declines appear to be the result of increased immobilization during the initial phases of decomposition as well as increased leaching rates from the rooting zone (Scheu and Parkinson 1994; Bohlen et al. 2004b; Hale et al. 2005b). Rapid production of highly leachable nitrates during initial earthworm invasion could contribute to decreased nutrient availability in the long run.

Total plant biomass decreased in response to earthworms through increased plant mortality rather than through decreases in individual plant biomass (Table 1, Fig. 5). Increased mortality may be an important cause of change in plant populations associated with earthworm invasion, even if growth of individual plants remains unchanged. In addition, reduced plant population densities make the remaining plants more susceptible to grazing by deer and other herbivores (Augustine et al. 1998), and reduced forest floor thickness caused by earthworm invasion is also likely to change seed bed conditions and moisture levels in the A horizon, causing further change in plant community composition. Thus, the findings of this experiment are consistent with field studies showing that European earthworm invasion in North American hardwood forests affects fundamental ecosystem processes that start a cascade of changes in the ecosystem.

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