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Invasive earthworms as seed predators of temperate forest species

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Abstract: Soil seedbanks play a key role in forest plant communities, contributing to regeneration and acting as a refuge from seed predators. This study provides evidence that seeds entering the soil seedbank are vulnerable to granivory by invasive earthworms in temperate forests. Overall, 73% of seeds of 6 ecologically important forest species were removed from the soil surface over two weeks in a *Lumbricus terrestris* microcosm experiment; 30% vanished entirely, and presumably were destroyed. The invasive garlic mustard, *Alliaria petiolata*, was subject to the highest rates of removal. In contrast, results from a field exclosure experiment using 23 species of seed indicate that while seed predation by worms is still detectable, predation by rodents often may mask impacts of earthworms under natural conditions. Worms and rodents preferred different sizes of seeds: while seed predation by rodents was high in mid- to large-seeded species, earthworms tended to prefer smaller seeds. These findings suggest that although rodents are the main driver of seed predation, invasive earthworms may act as an additional ecological filter, and potentially may further influence the species composition of forest plant communities.

Keywords: Invasive earthworms; seed predation; granivory; seed bank; *Lumbricus terrestris*; exotic species.

INTRODUCTION

Seed predation during the post-dispersal period can be critically important to determining a seed's ultimate fate (Fenner 1985; Crawley 1992; Chambers and MacMahon 1994; Fenner and Thompson 2005). For instance, some of the most dominant temperate trees, such as maples and oaks, suffer post-dispersal predation exceeding 80%, and sometimes nearly 100% (Myster and Pickett 1993; Meiners 2005). Despite the fact that individuals of some species are capable of producing millions of seeds over their lifetime, a recent meta-analysis of seed-addition experiments has shown that many communities are indeed seed-limited (Clark *et al.* 2007). This suggests that such high levels of seed loss can have important consequences for the structure of many plant communities.

One of the most influential groups of seed predators consists of small mammals. In particular, rodents, such as grey squirrels (*Sciurus carolinensis*), eastern chipmunks (*Tamias striatus*), and deer mice (*Peromyscus* spp.), are common and widespread throughout North American forests where they are widely viewed as the seed predators responsible for the greatest volume of seed removal (Hulme 1998; Falls *et al.* 2007; Hsia and Francel 2009). For example, in a three year observational study, between 48% and 55% of white spruce (*Picea glauca*) cones were lost to pre-dispersal predation by cone-caching red squirrels (*Tamiasciurus hudsonicus*) (Peters *et al.* 2003), and the authors expected that nearly all seeds that escaped red squirrel granivory were lost to post-dispersal seed predators such as red-backed voles (*Clethrionomys gapperi*) and deer mice (*Peromyscus maniculatus*) (Peters *et al.* 2003). At the same time, rodents can also act as important seed dispersal agents, particularly for medium to larger sized seeds (Vander Wall 2001). Rodent seed caches regularly go unexploited and can result in seedlings occurring in microsites with favourable conditions for growth (Vander Wall 2001).

The role of invertebrate seed predators in temperate forests is less well understood. Some

carabid beetles are known seed predators (Honek *et al.* 2003), but a limited amount of research has been conducted regarding their importance in temperate forest systems. The ability of these beetles to remove a substantial volume of seed in temperate forests is likely constrained by their limited size and abundance (relative to other granivores) and in some cases generalist foraging behaviour (Honek *et al.* 2003). Harvester ants have been identified as important seed predators in arid systems (e.g., Mull 2003) but are absent from most temperate areas of North America; however, other ant species widely found in North America have been noted as important dispersers of eliasome-bearing species (Lengyel *et al.* 2010).

In contrast, a very different group of invertebrates may play an underappreciated role as seed predators. Although they have been identified as granivores for over 100 years (Darwin 1881), the effects of earthworms on seed populations remain understudied, despite their potential importance (Frelich *et al.* 2006; Forey *et al.* 2011). For instance, Hopfensperger *et al.* (2011) collected soil cores from two forest transects and found that fewer seeds germinated from soil cores collected from areas containing adult *Lumbricus* species than sites that contained no adult earthworms (but see Nuzzo *et al.* 2015). Other experiments have specifically examined earthworms as seed predators in petri dishes and other artificial environments (e.g., McRill & Sagar 1973; Eisenhauer *et al.* 2010). For example, Quackenbush *et al.* (2012) were able to document species selectivity within one *Lumbricus* species, attributing at least some of their preferences to seed size and morphology; in particular, seeds of the invader garlic mustard (*Alliaria petiolata*) were preferred. Earthworm ingestion is not necessarily fatal to a seed, as it may be egested along with the worm's castings (Forey *et al.* 2011; Clause *et al.* 2015). Nonetheless, many seeds are killed, either directly by digestion, or indirectly by burial to depths from which successful germination is impossible (Forey *et al.* 2011; Quackenbush *et al.* 2012; Drouin *et al.* 2014). However the net effects of earthworms on seed survival remain unclear; in a

correlative study, Nuzzo *et al.* (2015) report larger and more diverse seed banks from areas with high earthworm populations. Reasons for this discrepancy are uncertain, but some other studies have also found positive or variable results (e.g., Eisenhauer *et al.* 2009a, Dávalos *et al.* 2014).

Understanding the impacts of earthworms is especially important given their global success as invasive species (Hendrix *et al.* 2008). For instance, native earthworms are believed to have been eliminated from much of northeastern North America by the Wisconsinian glaciation (Reynolds 1977). Since then, this region has been invaded by a variety of Eurasian species (Frelich *et al.* 2006; Addison 2009; Evers *et al.* 2012); Reynolds (1977) considered only two of Ontario's 19 known species of earthworms to be native to North America. Such invasive worms are widespread and often very abundant in temperate North American forests where they act as "ecosystem engineers", altering many key characteristics of forest ecosystems, often with negative consequences for biodiversity (Bohlen *et al.* 2004a,b; Frelich *et al.* 2006; Addison 2009; Dobson and Blossey 2015). In particular, diversity and abundance of many herbaceous species such as spring wildflowers often severely decline in the understory of invaded forests (Bohlen *et al.* 2004b; Frelich *et al.* 2006; Hale *et al.* 2006; Addison 2009; Fisichelli *et al.* 2013; Dobson and Blossey 2015). These impacts extend to tree seedlings as well: abundance and performance can be strongly reduced by earthworms (Frelich *et al.* 2006; Hale *et al.* 2006; Griffith *et al.* 2013; Drouin *et al.* 2014), perhaps reducing canopy tree recruitment and forest regeneration. These changes have been attributed to a variety of mechanisms including soil changes, disruption of mycorrhizal networks, and exposure of seeds to predation by removal of leaf litter (Bohlen *et al.* 2004a,b; Frelich *et al.* 2006); could consumption of seeds also play a role?

In this study, we use microcosm and field exclusion experiments to quantify the potential that invasive earthworms have to act as post-dispersal seed predators in a temperate forest. Specifically, we ask:

- 1) Can earthworms remove substantial numbers of seeds from the forest floor, compared to vertebrate seed predators?
- 2) What is the fate of seeds removed by earthworms?
- 3) Do worms prefer different species of seeds than vertebrates?

This is the first earthworm exclosure field experiment of which we are aware. Our results suggest that although their impacts generally are small compared to those of rodents, earthworms may act as significant predators of some seeds, particularly those too small to be preferred by vertebrates.

METHODS

Site Information

Field work was conducted at the University of Toronto's Koffler Scientific Reserve at Jokers Hill (KSR) (website: www.ksr.utoronto.ca), King Township, Ontario (44°02' N, 79°32' W). This property falls within the Mixedwood Plains Ecozone (Ecoregion 6E), as defined by the Ontario Ministry of Natural Resources and Forests, and is situated on the Oak Ridges Moraine.

Research was conducted within the forested section of the property. Nearly all native forest stands are mature secondary growth, often dominated by *Acer saccharum*, *Fagus grandifolia*, *Tsuga canadensis* and *Quercus rubra*. Soils are sandy loams with a thin but well-developed litter layer overlaying 10-30cm of grey-brown podzolic soil on top of very deep morainal sand. Earthworms are ubiquitous and abundant at this site; species include *Aporrectodea rosea* (Savigny), *Aporrectodea tuberculata* (Eisen), *Aporrectodea turgida* (Eisen), *Dendrobaena octaedra* (Savigny), *Dendrodrilus rubidus* (Savigny), *Lumbricus rubellus* (Hoffmeister), *Lumbricus terrestris* (Linnaeus), and *Octolasion* spp (Choi 2012).

Species Information

A total of 23 locally occurring and ecologically important woody and herbaceous forest species were selected for use in our experiments (see Table 1 for details and full scientific

names); subsets of these species were used in each experiment, depending on the its goals and seed availability. These species represented a variety of seed sizes and both natives and exotics. Species with eliaosomes were not used so as to mitigate any effect of myrmecochory. Wings were removed from samaras of *Acer saccharum* before use.

All seeds used in each experiment were marked using day-glow highlighter pens with inconspicuous UV-fluorescent dyes to facilitate recovery and to help distinguish experimental seeds from any naturally-occurring seeds. Seeds were examined with an ultraviolet flashlight (HQRP 390nm UV LED); the effectiveness of the dye-marking method did not diminish over the duration of the experiment. Trials indicated UV-marking did not affect rates of seed removal, as also reported by Wrobel and Zwolak (2013).

Microcosm Experiment

A microcosm experiment was conducted to estimate the potential of worms to remove seeds from the upper 1cm of the soil. This experiment was established in a 10m x 10m plot of mixed forest (>25% deciduous and <75% coniferous by canopy) in the first week of September 2013. The plot had a nearly level grade and was dominated by mature *A. saccharum*, *Pinus strobus*, and *Tsuga canadensis* while containing <5% herbaceous ground cover.

PVC tubing was used to create 60 (length 30cm, diameter 15cm) earthworm microcosms. Fiberglass 1mm window screening was fixed to the bottom of each tube to permit drainage; each tube was then sunk into the soil to an approximate depth of 15cm and filled with soil sieved from the site using a 1cm sieve to remove any large earthworms and coarse debris.

Common nightcrawlers, *Lumbricus terrestris*, were purchased from a local fishing bait distribution company (nationalbait.com). *L. terrestris* was selected as the most appropriate earthworm species due to its high abundance relative to other exotic species of earthworms at this site (Choi 2012), its use in other related research (e.g., Eisenhauer and Scheu 2008; Eisenhauer *et*

al. 2010; Quackenbush *et al.* 2012), and its large size, suggesting it as a potential predator of seeds over a large range of sizes. After purging for 24 hours on moist filter paper, a single earthworm was added to 54 of the 60 microcosms; the remaining 6 were designated as control units allowing estimation of rates of seed burial in the absence of worms. Tubes then were left undisturbed for 7 days to allow the added earthworms to acclimate to their environments. Each of the 54 tubes containing an earthworm was then randomly assigned to receive fifteen seeds from one of six previously selected species, so each of the six species of seed was replicated across nine tubes. These six species included four native trees, one exotic shrub and, and one herbaceous invader (Table 1). Each of the remaining 6 earthworm-free control tubes received ten seeds from all 6 species. After seed addition, the top of each tube was covered with fiberglass screening secured with a rubber band.

Two weeks after seed addition the microcosms were removed from the field and transported to the lab. The soil-filled tubes were randomized and stored in two 4°C refrigerators for no longer than five days as processing took place; cold storage was used to slow the metabolic rate of the earthworms and reduce the likelihood of further seed movement. Soil was excavated from each tube in predetermined layers (<1cm, 1-3cm, 3-5cm, etc. to a maximum depth of 25cm) using a small trowel and then examined for seeds using a UV flashlight. Once the soil from each microcosm had been exhaustively searched, it was spread evenly (~3cm depth) within a standard greenhouse flat (28cm x 54cm x 6cm) and transported to a growth chamber. Soils were then exposed to 12-hour day/night cycles for 6 weeks and watered daily to promote germination of any unrecovered seeds; as well, soil was disturbed after 4 weeks to expose any buried seeds.

Field exclosure experiment

This experiment was designed to compare actual removal rates of seeds of different

species by earthworms with removal by above-ground seed predators. In the summer of 2013, twelve sites were selected throughout the property's mixed hardwood forest, spaced at least 100m apart. Each site consisted of a 10m x 10m plot containing 0.1m² (diameter 36cm) treatment units. We conducted two trials. For the first (summer) trial, we used four treatment units per site: two earthworm exclosures, and two controls that permitted unrestricted access to both above- and below-ground seed predators. Earthworm exclosures consisted of 3 cm tall open-topped 36cm cylinders constructed of 1.25cm² metal hardware cloth fastened with plastic zip ties and anchored with ground staples; earthworms were excluded by lining the floors and sides of these units with 1mm fiberglass window screen. The upper 3cm of the leaf litter and O horizon were carefully removed from the experimental plot, the exclosure was set into this space, and the removed material was replaced into the exclosure unit, making it flush with the surrounding leaf litter. For controls, leaf litter was manually disturbed in a 36cm circular plot delineated with wire fixed to the litter surface using 2 ground staples. For the fall trial, we added two replicates of a third, rodent-exclusion treatment to each site. These consisted of roofed but bottomless cylinders (height 0.25m, diameter 0.36m) of hardware cloth, with a 10cm strip of metal flashing around the perimeter of each cage buried flush into the soil.

Plots were left undisturbed for two weeks before treatments were applied. For the summer trial, seeds were added to each experimental unit on 29 July, early in the annual cycle of seed dispersal; for the fall trial, seeds were added on 5 September, well into the annual dispersal cycle. For each trial, a slightly different set of seeds was selected representing a range of seed sizes, and both natives and exotics (Table 1). Seeds were marked with an UV fluorescent highlighter, and then added to the ground surface. To establish how granivory changes over time, one replicate of each treatment type was randomly selected for collection 2 weeks after seed addition in each trial (12 August / 19 September respectively). The remaining units were collected 4 weeks after seed

addition (26 August / 3 October); previous work at this site suggested this would be sufficient time for significant seed removal to have occurred.

During both summer and fall trials, motion-sensitive trail cameras (Primos[®] Truth Cam Blackout model) were placed at 5 randomly selected sites prior to seed addition. These cameras took photos both in daylight and at night, using an invisible infrared flash. Photos indicated our exclosures were regularly visited by both diurnal (eastern grey squirrels, *Sciurus carolinensis* Gmelin, red squirrel, *Tamiasciurus hudsonicus* (Erxleben), eastern chipmunk, *Tamias striatus* (Linnaeus) and nocturnal (deer mice, *Peromyscus* sp., southern flying squirrel, *Glaucomys volans* Linnaeus) rodent seed predators.

To sample our plots, any cages were removed and leaf litter and the top 3cm of soil were collected. These samples were then returned to the lab and stored in a -4°C freezer until further processing, when they were emptied onto a tray and searched for seeds with an UV flashlight. Whole seeds were scored as ‘viable’, while any fluorescing seed fragments were pieced together to estimate the number of seeds destroyed.

Statistical Analysis

All analyses were conducted using JMP 11 (SAS Inc. 2012). Large numbers of zeroes and ones made logit transformation problematic; instead, the number of recovered seeds was converted into a proportion which was then arcsine square-root transformed as required to better meet the assumptions of Analysis of Variance. Means are reported as untransformed values $\pm$ SEM.

For the microcosm experiment, ANOVAs followed by Tukey-Kramer HSD *a posteriori* tests were performed comparing recovery of each species at each burial depth (<1cm, 1-5cm, >5cm, and removed). An omnibus repeated-measures test was not used since the effects of depth differed strongly among species. In cases when ANOVA assumptions were not met, results from

non-parametric Kruskal-Wallis ranked-sum tests are also reported; these generally are very similar.

Analyses of the enclosure experiments used randomized block factorial ANOVAs (Kirk 1995) with a restricted maximum likelihood (REML) approach. "Species" and "treatment" (protected from worms, rodents, or open) were treated as crossed fixed effects. "Site" was treated as a random blocking factor; "plot" was not directly included in the model, since plot effects were equivalent to the residual 3-way interactions. Each species also was analyzed separately using a randomized block model ("treatment" as a fixed factor, and "site" as a random block), followed by Tukey-Kramer HSD tests. Given the exploratory nature of these analyses and the relatively small sample size, we did not attempt Bonferroni correction, but instead present exact p values; marginal significance should be treated with caution. In cases where ANOVA assumptions were poorly met, results from nonparametric Wilcoxon Rank-Sum tests with "site" as a blocking factor also are shown; these generally were very similar to results of parametric statistics.

RESULTS

Microcosm Experiment

Effectively all (99.9%) of the 1093 seeds recovered were discovered using visual inspection aided by UV light. Only a single seedling (*B. thunbergii*) germinated in the growth chamber; this seedling has been omitted from all analysis. Seeds rarely moved in the absence of worms; overall recovery of seeds from the soil surface (<1cm) of tubes without added worms was $97.8\pm0.7\%$, and species did not differ in removal rates ($p > 0.05$); consequently, the rest of this discussion focusses on seed removal from the worm addition treatments.

Recovery in the presence of earthworms for all species combined <1cm from the soil surface was $27.2\pm4.1\%$, with another $18.5\pm2.9\%$ from 1-5cm, and $23.8\pm3.7\%$ from depths below 5cm. Finally, a total of $30.5\pm5.0\%$ of seeds were removed outright.

ANOVAs indicated significant differences ($p < 0.0001$) among species in rates of recovery from the soil surface ($<1\text{cm}$: $F_{5,48} = 9.90$), from 1-5cm ($F_{5,48} = 9.42$), from $>5\text{cm}$ ($F_{5,48} = 12.64$), and in seeds removed entirely ($F_{5,48} = 69.59$). Data poorly met ANOVA assumptions, but results of Kruskal-Wallis tests were essentially identical ($X^2_5 > 24$, $p \leq 0.001$ in all cases). Tukey HSD tests found seeds of *P. strobus* were more likely to be recovered at the surface than those of other species (Figure 1). The three largest-seeded species (*Acer saccharum*, *Prunus serotina*, and *Berberis thunbergii*) were the most likely to be recovered at deeper depths, while the smallest seeds (*Alliaria petiolata* and *Betula allegheniensis*) were significantly more likely than the others to not be recovered at all: 89% and 68% respectively were lost, while the four larger species were removed at rates lower than 15% (Figure 1).

Field Exclosure Experiment

Treatment effects

For the summer trial of this experiment, exclusion of belowground seed predators was compared to controls in which seed was available to both aboveground and belowground predators. At the four-week sampling, more seeds were recovered from plots that excluded belowground granivores than from control plots ($F_{1,451} = 38.37$, $p < 0.0001$). Recovery also differed significantly among species ($F_{20,451} = 47.89$, $p < 0.0001$), though there was a significant interaction term ($F_{20,451} = 2.12$, $p = 0.004$), indicating that the effect of treatment differed among species (Figure 2). When analyzed separately, F-tests indicated 9 of 21 species had significantly ($p < 0.005$) greater recovery when belowground seed predators were excluded (Table 2; Figure 2). Data often imperfectly met ANOVA assumptions; however, all of these differences also were identified by Wilcoxon Rank-Sum tests; in addition, results for *Pinus banksiana* were marginally significant ($p = 0.049$) (Table 2). In all cases, recovery was higher in below-ground exclosure treatments than controls (Figure 2).

After four weeks of exposure to granivores, significant differences again were detected in the summer trial between exclusion treatments ($F_{1,451} = 11.54$, $p = 0.0007$) and among species ($F_{20,451} = 45.55$, $p < 0.0001$), though their interaction was not significant ($F_{20,451} = 0.87$, $p = 0.624$). As expected, many species showed recovery rates that were lower than those found after two weeks; however, only one species (*Betula allegheniensis*) differed among treatments according to separate F-tests, likely because higher background rates of removal by vertebrate seed predators obscured any patterns (Table 2, Figure 2). Wilcoxon Rank-Sum tests also identified this difference, and in addition found differences between treatments in recovery of *Larix laricina* ($p = 0.007$), *Maianthemum racemosum* ($p = 0.020$), *Berberis thunbergii* ($p = 0.044$), and *Betula pendula* ($p = 0.048$). In all cases, recovery was higher in below-ground exclosure treatments than controls (Table 2, Figure 2).

For the fall trial, an above-ground exclosure treatment was added to the worm exclosure treatment. At the two-week sampling, recovery differed among treatments ($F_{2,715} = 422.88$, $p < 0.0001$): seeds were recovered at the lowest rate in exposed control plots, and at the highest rate in above-ground exclosures, while recovery from plots protected from worms was often close to that of the controls (Figure 3). Species differed significantly in their responses ($F_{21,715} = 15.88$, $p < 0.0001$), and a significant species x treatment interaction ($F_{42,715} = 19.40$, $p < 0.0001$) indicated that the effect of the exclosures depended upon species identity. When species were considered separately (Table 3), ANOVAs found 14 differed significantly among treatments; Wilcoxon Rank-Sum Tests added *Pinus banksiana* ($p = 0.016$) and *Tsuga canadensis* ($p = 0.024$). In all of these cases, above-ground protection improved recovery over one or more other treatments (Table 3, Figure 3). In contrast, rates of recovery from below-ground exclosures seldom exceeded recovery of controls, though in some cases (*Berberis thunbergii*, *Pinus sylvestris*, *Rhamnus cathartica*) they also were indistinguishable from the above-ground exclosure treatment.

Results at the four-week sampling of the fall trial were similar, though as expected, rates of recovery were lower (Figure 3). Recovery again differed among treatments ($F_{2,695.6} = 289.22$, $p < 0.0001$), with seeds recovered at the lowest rates in exposed controls and the highest rates in above-ground exclosures (Figure 3). Again, species differed significantly in their responses ($F_{21,693.1} = 16.75$, $p < 0.0001$), and the effect of the exclosures varied among species ($F_{42,693.1} = 14.22$, $p < 0.0001$). When species were considered separately (Table 3), ANOVA detected a significant ($p < 0.0001$) difference among treatments for 14 species; Wilcoxon tests added *Tsuga canadensis* ($p = 0.029$) and *Alliaria petiolata* ($p = 0.046$), even though one site needed to be dropped from these analysis due to damage by a fallen tree. In most of these cases, above-ground protection improved recovery over one or more other treatments (Table 3), while for *Lonicera canadensis*, below-ground protection also increased recovery relative to controls (Figure 3).

It is possible that any effects of earthworms may have been statistically concealed by the much stronger effect of the above-ground exclosures. When this treatment was deleted from parametric analyses, differences between controls and below-ground exclosures were detected at 2 weeks for *Betula papyrifera* ($p = 0.040$) and at 4 weeks for *Lonicera canadensis* ($p = 0.002$), *Pinus banksiana* ($p = 0.031$) and *Tsuga canadensis* ($p = 0.0147$). Wilcoxon tests similarly detected significant differences between open and below-ground exclosure treatments for *Betula papyrifera* ($p = 0.003$) and *Pinus sylvestris* (0.048) at 2 weeks, and for *Fraxinus americana* (0.017), *Lonicera canadensis* ($p = 0.0002$), *Pinus banksiana* (0.002), *Alliaria petiolata* ($p = 0.010$) and *Tsuga canadensis* ($p = 0.002$) after 4 weeks. For *Alliaria* and *Betula*, recovery was actually higher in controls, likely reflecting random error; in the other 5 cases it was lower, consistent with mild earthworm impacts.

Seed size

The effects of exclosures depended in part on seed size. In both summer and fall trials,

larger-seeded species tended to have higher rates of seed loss unless protected from above-ground seed predators (Figures 2,3). When treatment effect size (mean recovery in exclosures - mean recovery in controls) was plotted against seed size (Figure 4), the effects of below-ground exclosures were greatest for small seeds and diminished with seed size at all four sampling dates, significantly so at the first sampling ($p = 0.009$) and overall (Fisher's combined probability test: $X^2_8 = 15.529$, $p = 0.050$). In contrast, the protective effects of above-ground exclosures increased strikingly with seed size ($p < 0.0001$, Fig 4).

DISCUSSION

Our results indicate that introduced earthworms potentially may act as important seed predators in temperate forests. In practice, their effects often may be eclipsed by the much stronger effects of rodent seed predators; nonetheless, they can remove significant amounts of seed, while the fact that they prefer a different size spectrum of seeds implies they may still have significant incremental effects. Although other studies have shown that earthworms are indeed capable of ingesting and digesting some species of seed (e.g., Eisenhauer and Scheu 2008; Eisenhauer et al. 2010; Quackenbush *et al.* 2012), evidence of seed removal by earthworms has not previously been demonstrated in a realistic field exclosure experiment.

Microcosm Results: Potential Of Earthworms As Granivores

Earthworms are potentially significant agents of mortality for seeds of forest species. In our microcosm experiment, in the absence of other seed predators, 73% of seeds placed on the soil surface were removed in only two weeks when exposed to earthworms. These rates of seed loss are comparable with losses observed in other earthworm seed predation studies (Eisenhauer and Scheu 2008; Quackenbush *et al.* 2012).

While ingestion and removal from the soil surface are not necessarily fatal (Eisenhauer *et al.* 2009b; Forey *et al.* 2011), seeds of many species are unable to germinate without light

(Baskin and Baskin 1998; Fenner and Thompson 2005), and few seedlings are likely to have the resources to reach the soil surface if buried to depths of more than a few cm (Fenner 1985; Leck et al. 1989; Fenner 2000; Fenner and Thompson 2005). The depth of burial required to prevent emergence is likely to be less for smaller seeds with correspondingly smaller resource stores (Fenner 1985; Leck *et al.* 1989; Fenner 2000); they also may be at more risk of ingestion and destruction in worms' digestive systems (Forey *et al.* 2011). In our study, the two smallest-seeded species (*Betula allegheniensis* and *Alliaria petiolata*) were the species that were most likely to be removed from the soil surface, and the most likely to disappear entirely. Seeds of at least *Betula* are unlikely to emerge if buried to depths of more than a few cm (Burns and Honkala 1990), while the effectiveness of our seed detection methods (<2% of seeds escaped detection in worm-free controls) suggests that most of the vanished seeds were indeed destroyed, as reported in other studies (Quackenbush *et al.* 2012). In contrast, some larger-seeded species may be able to rarely emerge from greater depths: in a greenhouse trial *Pinus strobus* rarely emerged from as deep as 7 cm. As well, even smaller seeds occasionally may be transported to the soil surface once again. Although the possibility some seeds may ultimately emerge clouds the interpretation of our results, similar issues apply to seed-caching rodents such as squirrels.

Earthworms can discriminate amongst seed species, and their dietary choices may be influenced by factors such as seed size, shape, surface structure, and chemistry (Shumway and Koide 1994; Eisenhauer and Scheu 2008; Eisenhauer *et al.* 2009b, 2010; Quackenbush *et al.* 2012). In our experiment (though not always in preliminary trials), *Pinus strobus* appeared to be less prone to removal than other species. Rates of removal were much higher for the remaining species, even the relatively large-seeded *P. serotina*, *B. thunbergii*, and *A. saccharum*; this suggests that earthworms are capable of directly or indirectly removing at least some quantity of the majority of species commonly found in temperate forests. Notably, the problematic invader *A.*

petiolata was removed in significantly greater numbers than all other species in our microcosm experiment (though not our enclosure experiment). This agrees with Quackenbush *et al.* (2012) who used a series of simple choice experiments to show that *L. terrestris* ingested *A. petiolata* at a significantly greater rate than three of six other species tested.

Enclosure Experiments: Relative Role Of Earthworms & Other Granivores

In the summer trial, exclusion of earthworms (including *L. terrestris*) significantly increased the number of seeds recovered, both overall and for particular species. This effect was weaker after four weeks, possibly because it was increasingly masked by predation by rodents and other seed predators. In the fall trial, a rodent exclusion treatment was added, and directly demonstrated that rodent predation was indeed much stronger than earthworm predation - so strong that it masked most evidence of earthworm effects. Additionally, earthworm populations tend to exhibit decreased activity towards the end of the summer months (Edwards and Bohlen 1996), when small mammal populations tend to peak (Falls *et al.* 2007); these phenological trends may be at least partially responsible for an apparent decrease in seed removal by earthworms in the fall trial compared with the summer trial. For instance, after only 2 weeks, rodent enclosure plots (exposed exclusively to belowground seed predators) lost 35% of their seeds - a considerable fraction. However, earthworm exclusion plots lost 75% of their seeds to above-ground predators in the same period, while controls (exposed to both above & belowground predators) exhibited nearly identical levels of seed removal (77%) suggesting either only minor additional effects of worms, or that worm and rodent granivory were compensatory. Patterns at 4 weeks were similar: 47%, 80%, and 84% for rodent exclusion, earthworm exclusion, and control treatments respectively. Nonetheless, if the rodent exclusion treatment was removed from the analysis, earthworm effects could still be detected.

Although seed predation by rodents clearly exceeds losses to earthworms, these results

demonstrate worms have measurable effects on seed removal, even in plots exposed to rodent predation. As well, our results indicate that rodents and earthworms selected different species of seeds. In particular, smaller-seeded species were more preferred by earthworms than larger-seeded species, as has previously been reported (Milcu *et al.* 2006; Eisenhauer *et al.* 2009b, 2010). In contrast, rodents strongly preferred larger seeds, as also has often been reported (Thompson 1987; Hulme 1998): each of the largest 8 species of seed used in this experiment were subject to >90% removal when exposed to aboveground granivores. These patterns also provide evidence that our enclosure treatments were effective in excluding their intended targets: these patterns of size-related loss make sense only if below-ground enclosures indeed excluded predators preferring small seeds, and above-ground enclosures excluded predators preferring large seeds.

CONCLUSIONS

The results of this study provide evidence that invasive earthworms may have direct effects on invaded plant communities through seed consumption, in addition to their indirect impacts via soil modification (Frelich *et al.* 2006; Bohlen *et al.* 2004b; Larsson *et al.* 2010). We also found evidence that earthworms are capable of removing different species at different rates. Not all buried seeds necessarily are ingested, and not all ingestion necessarily is fatal, but earthworms nonetheless can act as significant seed predators (Forey *et al.* 2011). Impacts of earthworms may be masked by strong effects of small rodents (e.g., Schnurr *et al.* 2002; Falls *et al.* 2007), but they are still capable of selectively removing seeds with certain traits, and in particular the small-seeded species (Forey *et al.* 2011) typically less preferred by rodents (Reader 1993; Blaney and Kotanen 2001; Maron *et al.* 2012).

Earthworms are known to have negative effects on many understory plants, though the mechanisms are not entirely clear. Many explanations have been proposed (Frelich *et al.* 2006;

Eisenhauer and Scheu 2008; Griffith *et al.* 2013), notably impacts on mycorrhizae and complex consequences of removal of the litter layer (Lawrence *et al.* 2003; Bohlen *et al.* 2004b; Frelich *et al.* 2006, Hopfensperger *et al.* 2011). Although Darwin was aware of the ability of earthworms to act as seed predators as long ago as 1881, this aspect of invasive earthworm ecology has been largely overlooked in recent ecological studies. Effects of earthworms on seeds are not all negative (Forey *et al.* 2010). For example, burial may protect seeds from rodents, and some studies have found earthworms may actually increase the seed bank (Nuzzo *et al.* 2015); this study differs from ours in that we directly assessed seed removal, rather than sampling the abundance of those species that escaped predation. Nonetheless, the negative impacts of earthworms may be in part due to their direct effects on seed survival.

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Table 1 Native and exotic species used in microcosm and granivore exclusion experiments, as ordered by seed mass. Seed mass represents the mean weight of 200 individual seeds randomly selected from the same seed stock used in each experiment. “*” indicates use of the noted species in each experiment.

Origin	Species	Common name	Seed mass (mg)	Lifeform	Experiment		
					Microcosm	Summer enclosure	Fall enclosure
Native	<i>Betula papyrifera</i> Marshall	Paper Birch	0.3	Tree		*	*
	<i>Betula alleghaniensis</i> Britton	Yellow Birch	1.1	Tree	*	*	*
	<i>Larix laricina</i> (Du Roi) K. Koch	Tamarack	1.7	Tree		*	*
	<i>Tsuga canadensis</i> (L.) Carrière	Eastern Hemlock	2.6	Tree		*	*
	<i>Lonicera canadensis</i> Bartram ex Marshall	American Fly Honeysuckle	3.0	Shrub	*	*	*
	<i>Pinus banksiana</i> Lamb.	Jack Pine	3.8	Tree		*	*
	<i>Pinus strobus</i> L.	White Pine	10.8	Tree	*	*	*
	<i>Acer rubrum</i> L.	Red Maple	12.9	Tree		*	*
	<i>Maianthemum racemosum</i> (L.) Link	False Solomon's-seal	28.5	Herb		*	*
	<i>Fraxinus americana</i> L.	White Ash	56.3	Tree		*	*
	<i>Prunus serotina</i> Ehrh.	Black Cherry	76.6	Tree	*	*	*
	<i>Acer saccharum</i> Marshall	Sugar Maple	92.7	Tree	*	*	*
	<i>Tilia americana</i> L.	American Basswood	117.8	Tree		*	*
	<i>Carya cordiformis</i> (Wangenh.) K. Koch	Bitternut Hickory	2905.0	Tree		*	*
	<i>Carya ovata</i> (Miller) K. Koch	Shagbark Hickory	4331.0	Tree			*
	<i>Quercus rubra</i> L.	Red Oak	4790.0	Tree		*	*
Exotic	<i>Betula pendula</i> Roth	Silver Birch	0.1	Tree		*	
	<i>Alliaria petiolata</i> (M. Bieb.) Cavara & Grande	Garlic Mustard	2.6	Herb	*		*
	<i>Pinus sylvestris</i> L.	Scotch Pine	6.2	Tree		*	*
	<i>Berberis thunbergii</i> DC.	Japanese Barberry	17.0	Shrub		*	*
	<i>Robinia pseudoacacia</i> L.	Black Locust	19.4	Tree		*	*
	<i>Rhamnus cathartica</i> L.	Common Buckthorn	23.1	Shrub		*	*
	<i>Acer platanoides</i> L.	Norway Maple	97.7	Tree		*	*

Table 2 Results of randomized block ANOVAs for recovery of seeds of each species in a summer enclosure experiment. Significant results ($p < 0.05$) are indicated in **bold**. Also shown are results of F-tests comparing controls (C) and below-ground enclosures (treatment "B"); "B>C" means more seeds were recovered from below-ground enclosures than controls, while "-" means no significant result was detected ($p > 0.05$).

Origin	Species	2 Weeks			4 Weeks		
		F _{1,11}	p	Treatments	F _{1,11}	p	Treatments
Native	<i>Acer rubrum</i>	0.13	0.7236	-	0.58	0.4632	-
	<i>Acer saccharum</i>	0.00	1.0000	-	1.00	0.3388	-
	<i>Betula alleghaniensis</i>	10.09	0.0088*	B>C	6.21	0.0300*	B>C
	<i>Betula papyrifera</i>	2.37	0.1520	-	1.55	0.2395	-
	<i>Carya cordiformis</i>	0.00	1.0000	-	0.00	1.0000	-
	<i>Fraxinus americana</i>	1.00	0.3388	-	1.39	0.2632	-
	<i>Larix laricina</i>	23.81	0.0005*	B>C	3.93	0.0728*	-
	<i>Lonicera canadensis</i>	5.39	0.0405*	B>C	2.75	0.1257	-
	<i>Maianthemum racemosum</i>	1.30	0.2785	-	3.23	0.0996*	-
	<i>Pinus banksiana</i>	2.34	0.1544*	-	1.16	0.3045	-
	<i>Pinus strobus</i>	6.68	0.0254*	B>C	0.09	0.7737	-
	<i>Prunus serotina</i>	1.00	0.3388	-	1.00	0.3388	-
	<i>Quercus rubra</i>	0.00	1.0000	-	0.00	1.0000	-
	<i>Tilia americana</i>	0.52	0.4849	-	0.01	0.9107	-
	<i>Tsuga canadensis</i>	9.62	0.0101*	B>C	1.73	0.2146	-
Exotic	<i>Acer platanoides</i>	0.00	1.0000	-	.	.	-
	<i>Berberis thunbergii</i>	7.63	0.0185*	B>C	3.72	0.0800*	-
	<i>Betula pendula</i>	10.52	0.0078*	B>C	1.71	0.2177*	-
	<i>Pinus sylvestris</i>	0.16	0.6951	-	1.24	0.2888	-
	<i>Rhamnus cathartica</i>	7.61	0.0186*	B>C	0.66	0.4336	-
	<i>Robinia pseudoacacia</i>	14.68	0.0028*	B>C	0.0044	0.9483	-

* Significant in Wilcoxon Rank-Sum tests ($p < 0.05$)

Table 3 Results of randomized block ANOVAs for recovery of seeds of each species in a fall enclosure experiment. Significant results ($p < 0.05$) are indicated in **bold**. Also shown are results of Tukey HSD tests comparing controls (C), above-ground enclosures (treatment "A"), and below-ground enclosures (treatment "B"); "B>C" means more seeds were recovered from below-ground enclosures than controls, while "-" means no significant result was detected ($p > 0.05$). Results failed to converge for *Betula allegheniensis* at 4 weeks; however, means were very similar across all three treatments.

Origin	Species	2 Weeks			4 Weeks		
		F _{2,22}	p	Treatments	F _{2,21(approx.)}	p	Treatments
Native	<i>Acer rubrum</i>	18.53	<0.0001*	A>B,C	4.79	0.0190*	A>B,C
	<i>Acer saccharum</i>	77.86	<0.0001*	A>B,C	20.18	<0.0001*	A>B,C
	<i>Betula allegheniensis</i>	0.49	0.6189	-	-	-	-
	<i>Betula papyrifera</i>	3.98	0.0334*	-	0.033	0.9677	-
	<i>Carya cordiformis</i>	4015.44	<0.0001*	A>B,C	115.66	<0.0001*	A>B,C
	<i>Carya ovata</i>	4015.44	<0.0001*	A>B,C	111.67	<0.0001*	A>B,C
	<i>Fraxinus americana</i>	23.51	<0.0001*	A>B,C	17.35	<0.0001*	A>B,C
	<i>Larix laricina</i>	0.49	0.6218	-	2.26	0.1290	-
	<i>Lonicera canadensis</i>	0.88	0.4275	-	7.22	0.0040*	A,B>C
	<i>Maianthemum racemosum</i>	1.313	0.2894	-	5.13	0.0151*	A>C
	<i>Pinus banksiana</i>	2.55	0.1010*	-	3.82	0.0379*	A>C
	<i>Pinus strobus</i>	16.05	<0.0001*	A>B,C	6.77	0.0052*	A>B,C
	<i>Prunus serotina</i>	72.43	<0.0001*	A>B,C	79.05	<0.0001*	A>B,C
	<i>Quercus rubra</i>	4015.4	<0.0001*	A>B,C	108.45	<0.0001*	A>B,C
	<i>Tilia americana</i>	147.87	<0.0001*	A>B,C	46.26	<0.0001*	A>B,C
	<i>Tsuga canadensis</i>	1.7528	0.1966*	-	2.70	0.0899*	-
Exotic	<i>Acer platanoides</i>	112.25	<0.0001*	A>B,C	20.79	<0.0001*	A>B,C
	<i>Alliaria petiolata</i>	0.79	0.4672	-	1.62	0.2209*	-
	<i>Berberis thunbergii</i>	4.81	0.0185*	A>C	1.22	0.3159	-
	<i>Pinus sylvestris</i>	4.73	0.0196*	A>C	5.00	0.0164*	A>C
	<i>Rhamnus cathartica</i>	6.14	0.0076*	A>C	2.03	0.1550	-
	<i>Robinia pseudoacacia</i>	1.39	0.2690	-	2.28	0.1264	-

* Significant in Wilcoxon Rank-Sum tests ($p < 0.05$)

Figure Captions

Figure 1 Mean proportion of seeds recovered + SEM (n=9) at various depth profiles within microcosms exposed to the earthworm *Lumbricus terrestris*. For each depth, species sharing the same letter do not significantly differ. Seed species are ordered by increasing seed size.

Figure 2 Mean proportion of seeds recovered + SEM, after 2 and 4 weeks exposure to various granivore treatments in a summer exclosure experiment (n=12). Treatments: "Below" = below-ground exclosure, "Control" = no protection. For both natives and exotics, species are ordered by increasing seed size.

Figure 3 Mean proportion of seeds recovered + SEM, after 2 and 4 weeks exposure to various granivore treatments in a fall exclosure experiment (n=12). Treatments: "Above" = above-ground exclosure, "Below" = below-ground exclosure, "Control" = no protection. For both natives and exotics, species are ordered by increasing seed size.

Figure 4 Effect of exclosures vs. seed size. "Below-ground - Control" = mean recovery of seeds in plots protected from earthworms - mean recovery in unprotected controls; "Above-ground - Control" = mean recovery of seeds in plots screened from rodents - mean recovery in controls. Each point represents the value for a single species; larger values indicate greater removal by that group of granivores.

Figure 1

Figure 2

Figure 3

Figure 4