Invasive Earthworms Ingest and Digest Garlic Mustard Seeds at Rates Equal to Native Seeds

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Abstract - The European and Asian earthworms introduced to eastern North American forests have great potential to facilitate plant invasions, in part through selective seed predation and dispersal. The invasive plant *Alliaria petiolata* (Garlic Mustard) contains secondary metabolites that may deter earthworms from eating its seeds. In 2 growth-chamber experiments, I determined whether the invasive earthworms *Lumbricus terrestris* (Nightcrawler) and *Eisenia fetida* (Red Wiggler) could aid the spread of Garlic Mustard by ingesting its seeds at lower rates than the similar-sized seeds of the native forest herb *Geranium maculatum* (Wild Geranium). Earthworms had similar rates of seed ingestion regardless of earthworm or plant species and digested the majority of seeds they ate (67–73%). There was no interaction between earthworm and plant species. Given a choice between Garlic Mustard and Wild Geranium, seed selectivity cannot explain positive associations between earthworm abundance and Garlic Mustard invasion.

Introduction

Invasive species often facilitate the establishment, spread, or impacts of other invasive species, either directly, through mutualistic interactions, or indirectly, through environmental modifications (Bourgeois et al. 2005, O'Dowd et al. 2003). This synergy may increase overall impacts beyond the sum of the impacts of individual species and accelerate community change, a process called invasional meltdown (Simberloff 2006, Simberloff and von Holle 1999). The European and Asian earthworms now distributed throughout the forests of northeastern North America and the Great Lakes region have great potential to aid other invasive species, especially plants (Bohlen et al. 2004c, Frelich et al. 2006). Several studies have observed spatial correlations between earthworm abundance and plant invasion (Clause et al. 2015b; Heneghan et al. 2007; Kourtev et al. 1998; Nuzzo et al. 2009, 2015), and the cover of non-native plant species increased with earthworm biomass in a meta-analysis of 645 observations across North America (Craven et al. 2017). In seed-addition experiments, the presence of earthworms often increased the biomass of invasive plants (Eisenhauer and Scheu 2008b, Roth et al. 2015, Whitfeld et al. 2014; but see Belote and Jones 2009). This growing evidence supports a positive association between invasive earthworms and invasive plants in eastern North American forests.

The causes of this pattern remain less well known. Invasive earthworms and plants may track common environmental factors, such as disturbance. Instead, or

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in addition, earthworms may facilitate plant invasion or vice versa. The presence of earthworms clearly can favor certain plant species over others (Aira and Pearce 2009, Eisenhauer and Scheu 2008a, Laossi et al. 2009, Milcu et al. 2006), and earthworm presence could potentially benefit invasive plants in several ways. First, earthworms radically transform forest-floor habitats. They consume leaf litter, soil organic matter, and microorganisms; redistribute organic matter from the surface to deeper levels; and often eliminate the organic horizon entirely (Bohlen et al. 2004a, Hale et al. 2008). Within soils, earthworm invasion can change chemical properties, speed nutrient cycling, and shift the composition of microbial communities, including mycorrhizal fungi (Bohlen et al. 2004b, Groffman et al. 2004). These changes could favor nutrient-demanding or non-mycorrhizal plants, including certain native species (Frelich et al. 2012), and invasive species such as *Alliaria petiolata* (M. Bieb.) Cavara & Grande (Garlic Mustard; Scheiner and Koide 1993). In addition to indirect effects via habitat modification, earthworms interact directly with plant populations by consuming roots, seeds, and seedlings. Seeds likely contribute substantially to earthworm nutrition (Eisenhauer et al. 2010), and earthworm populations may defecate as many as 860,000 seeds per hectare per year (Edwards and Bohlen 2008, Willems and Huijsmans 1994). Earthworms act as selective seed predators and dispersers; thus, they impose an important ecological filter on plant establishment (Forey et al. 2011). If invasive plant seeds benefit from earthworms' selectivity, this mechanism could increase the establishment and growth of invasive plant populations.

Ingestion by earthworms may affect the fate of seeds in several ways. A substantial proportion are fully digested, often more than half (Clause et al. 2011; Eisenhauer et al. 2009, 2010; Quackenbush et al. 2012; Willems and Huijsmans 1994). Seeds that pass through the gut can have increased or decreased germination rates, depending on the species (Clause et al. 2015a, Decaëns et al. 2003, Drouin et al. 2014, Eisenhauer et al. 2009). Burial in the soil could protect seeds from predation and harsh conditions, but could also prevent germination if they are too deep; deposition in casts and middens provides favorable microsites for establishment (Forey et al. 2011, Milcu et al. 2006). Earthworms ingest seeds selectively based on their seed size, shape, and surface structure (Aira and Piearce 2009; Cassin and Kotanen 2016; Clause et al. 2011; Eisenhauer and Scheu 2008b; Eisenhauer et al. 2009, 2010; Milcu et al. 2006; Quackenbush et al. 2012; Regnier et al. 2008; Shumway and Koide 1994). There is also evidence that earthworms use chemical cues such as smell and taste to select seeds (Willems and Huijsmans 1994). The net effects on plant establishment and the composition of soil seedbanks thus differ among seed and earthworm species (Aira and Piearce 2009, Clause et al. 2015b, Eisenhauer et al. 2012, Hopfensperger et al. 2011, Milcu et al. 2006, Nuzzo et al. 2015).

Here, I attempt to determine whether invasive earthworms could facilitate the spread of invasive Garlic Mustard by ingesting its seeds at lower rates than seeds of native species. Garlic Mustard produces a suite of secondary metabolites that deter herbivores, including glycosides, glucosinolates, and cyanide (Barto and Cipollini 2009). I hypothesized that these compounds would cause earthworms to avoid

Garlic Mustard seeds. A standard technique for collecting earthworms involves using a solution of ground *Sinapis alba* L. (Yellow Mustard) seeds to irritate their skin (Hale 2013). I experimentally compared earthworms' ingestion and digestion of Garlic Mustard seeds to seeds of the native forest herb *Geranium maculatum* L. (Wild Geranium), which are very similar in surface texture and in size; both are ~2 mm in diameter (Quackenbush et al. 2012). This experimental design allowed me to control for seed size while evaluating the importance of secondary metabolites to earthworms' feeding preferences. I assessed responses to these seeds by 2 common, invasive earthworm species, the large, anecic (burrowing) *Lumbricus terrestris* L. (Nightcrawler) and the moderate-sized, epigeic (surface-dwelling) *Eisenia fetida* Savigny (Red Wiggler). I predicted that both earthworm species would preferentially eat Wild Geranium over Garlic Mustard seeds, and digest a high percentage of the seeds they ingested, suggesting that their presence would increase the ratio of Garlic Mustard seeds to seeds of native species in forest soil seed-banks.

Methods

Factorial experiment

With students in an introductory biology class, I conducted a factorial experiment to compare the ingestion and digestion of Garlic Mustard and Wild Geranium seeds by Nightcrawlers and Red Wigglers. We obtained Garlic Mustard seeds from Plant World Seeds, Devon, UK; Wild Geranium seeds from Prairie Moon Nursery, Winona, MN; and earthworms from Rodmakers Shop, Strongsville, OH. We set up 41 petri dishes with each combination of earthworm and plant species (n = 164 dishes). In each 15-cm dish, we placed damp filter paper, a thin layer of potting soil (80% peat, 20% sand), and 20 seeds of 1 plant species. We added the soil to simulate earthworm habitat and to provide sand particles that normally help grind organic matter in the earthworm's gizzard (Marhan and Scheu 2005). We added 1 earthworm to each dish and allowed it to eat for 24 h in a growth chamber at 10 °C with no light. We then transferred each earthworm to a fresh petri dish with damp filter paper and allowed it to void its gut for 5 d. We counted the seeds remaining in the first dishes and subtracted from 20 the number of seeds remaining to determine seed ingestion. We counted intact seeds in the earthworm casts in the second dishes and determined seed digestion by subtracting the number of seeds in the casts from the number of seeds ingested. We performed two-way ANOVAs to assess the effects of earthworm species, plant species, and their interaction on seed ingestion and digestion. The data met assumptions of normality and equality of variances.

Choice experiment

In this experiment, we allowed individual earthworms to choose between seeds of the 2 plant species. The methods were exactly as in the factorial experiment except that we placed 10 seeds of Garlic Mustard and 10 seeds of Wild Geranium into each petri dish. We had 36 replicates for each earthworm species (n = 72 dishes). A slight difference in shape allowed us to distinguish Garlic Mustard from Wild Geranium seeds in the dishes; Garlic Mustard seeds are longer and narrower (averaging 1 × 3 mm), and Wild Geranium seeds are rounder (averaging 2 × 2 mm; Quackenbush et al. 2012). For each dish, we subtracted the number of Garlic Mustard seeds ingested from the number of Wild Geranium seeds ingested, and the number of Garlic Mustard seeds digested from the number of Wild Geranium seeds digested. To assess the effect of plant species on seed ingestion and digestion, we tested whether these quantities differed from zero using 1-sample *t*-tests. To assess the interaction between earthworm species and plant species, we tested whether these quantities differed between the earthworm species using 2-sample *t*-tests. The samples met the assumption of normality.

Results

Factorial experiment

Earthworms ingested 36% of seeds regardless of earthworm or plant species (Table 1). They digested 73% of the seeds they ate, regardless of earthworm or plant species; thus, 27% of seeds passed intact. There was no interaction between earthworm and plant species.

Choice experiment

In this experiment, earthworms ingested 35% of seeds and digested 67% of the seeds they ate, passing 33% of ingested seeds intact. The worms ingested and digested the 2 plant species at similar rates (1-sample *t*-tests: ingested, $t = 1.04$, df = 71, $P = 0.30$; digested, $t = 0.15$, df = 71, $P = 0.88$). There was no interaction between earthworm species and plant species (2-sample *t*-tests: ingested, $t = 0.79$, df = 71, $P = 0.44$; digested, $t = 0.34$, df = 71, $P = 0.73$).

Discussion

The results of both experiments clearly refuted my hypothesis; Nightcrawlers and Red Wigglers did not discriminate against Garlic Mustard seeds. The earthworms digested the majority of seeds they ate (67–73%); thus, they would have equally negative effects on populations of the 2 plant species. This digestion rate was slightly higher than previously observed (Quackenbush et al. 2012). In the factorial experiment, the earthworms' lack of preference could be attributed to the absence of other food sources, but the choice experiment showed that the earthworms did not avoid Garlic Mustard seeds even when other food was available. Earthworms' indifference to the secondary metabolites in Garlic

Table 1. Results of two-way ANOVAs testing the effects of earthworm species, plant species, and their interaction on the number of seeds ingested and digested.

	Seeds ingested		Seeds digested	
	F	*P*	*F*	*P*
Worm species	0.224	0.637	0.005	0.942
Plant species	0.448	0.504	0.188	0.665
Worm × plant	0.256	0.614	0.131	0.718

Mustard seeds is surprising given their response to ground Yellow Mustard seeds (Hale 2013). However, our finding is consistent with the studies of Quackenbush et al. (2012), in which Nightcrawlers actually preferred Garlic Mustard seeds to those of Wild Geranium and 3 other plant species with larger seeds. The latter result could be explained by earthworms' demonstrated preference for smaller seeds (Aira and Piearce 2009; Clause et al. 2011; Eisenhauer and Scheu 2008b; Eisenhauer et al. 2009, 2010; Milcu et al. 2006; Shumway and Koide 1994). Cassin and Kotanen (2016) also found that Nightcrawlers ingested Garlic Mustard seeds at higher rates than seeds of 4 plant species with larger seeds. Evidently, seed size is a more important determinant of earthworms' feeding preferences than seed chemistry.

Given a choice between Garlic Mustard and Wild Geranium, seed selectivity cannot explain the observed association between earthworm abundance and Garlic Mustard invasion (Nuzzo et al. 2009, 2015). The distributions of the 2 taxa might be spatially correlated rather than causally related. Alternatively, earthworms could facilitate Garlic Mustard invasions in other ways. Removal of the leaf litter and organic horizon may aid establishment of Garlic Mustard and other invasive plants that germinate well on bare mineral soil. As a non-mycorrhizal species (Scheiner and Koide 1993), Garlic Mustard may tolerate earthworms' transformation of the soil microbiota better than other plants, or earthworms may avoid eating its roots. It is also possible that Garlic Mustard facilitates earthworm population growth, perhaps via its litter quality. Garlic Mustard and other European and Asian plant invaders have a history of coevolution with earthworms, and may have multiple adaptations for survival in earthworm-rich habitats. Future research should examine other possible interactions between invasive earthworms and Garlic Mustard and other invasive plants of eastern North American forests. Understanding the ecological relationships among invasive species will help us protect and restore native forests from their manifold impacts.

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