Earthworm invasion in North America: Food resource competition affects native millipede survival and invasive earthworm reproduction

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ABSTRACT

The invasive non-native earthworm Amynthas agrestis (Goto and Hatai, 1899) has recently been documented invading forests of the Appalachian Mountains in the southeastern United States. This epigeic earthworm decreases the depth of organic soil horizons, and this may play a role in the decrease of millipede richness and abundance associated with A. agrestis invasion. To investigate the mechanisms behind these effects, A. agrestis and the millipede Sigmoria ainsliei (Xystodesmidae) were placed into microcosms with soil and either L horizon, F and H horizon, or a combination L/FH treatment. Microcosms were destructively sampled and reconstructed with the same treatments every four weeks to assess faunal fresh weight change and survival. Soils from earthworm treatments were wet-sieved for cocoons to assess treatment effects on reproduction. On average, millipede mortality occurred 88 days sooner in treatments that did not have FH horizon material, and within all litter treatments millipedes tended to survive longer when A. agrestis was absent. Earthworms maintained higher fresh weight in L/FH than FH or L treatments. With a single exception, no A. agrestis cocoons were recovered from microcosms that also contained S. ainsliei. The results suggest that A. agrestis and S. ainsliei may compete for food resources, particularly the smaller particle material in the FH horizons of the forest floor. Millipedes may exert some biotic resistance to A. agrestis invasion, as diminished earthworm fecundity was observed in experimental units containing both species.

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1. Introduction

Non-native earthworm invasion is a truly global phenomenon in which invasive earthworm species are invading every continent, except Antarctica (Hendrix et al., 2008). These earthworm species also have origins on every continent, except Antarctica. In North America, earthworms of Asian origin (the genera Amynthas, Metaphire, Pheretima, and Pithemera) have recently been documented in the northeastern (Steinberg et al., 1997; Burtelow et al., 1998; Bohlen et al., 2004a,b), central (Snyder, unpublished results), and southeastern (Callaham et al., 2003; Snyder et al., 2011) regions of the United States, although these earthworms have been known in North America since the early 20th century (Garman, 1888; Gates, 1937).

Earthworm invasion can significantly alter forest ecosystems. Physical changes to the forest floor through consumption of organic horizons, mixing of organic and mineral horizons, and burrowing and casting activities can impact biogeochemical cycling (Bohlen et al., 2004a,b,c). Earthworm invasion can also impact soil fauna communities through competition and through the significant alteration of soil profile and structure (Bohlen et al., 2004b,c; Frelich et al., 2006). Although much is known about the interactions of invasive earthworms with soil micro- and mesofauna, less is known about interactions with detritivorous macrofauna, such as millipedes (Migge-Kleian et al., 2006). Bonkowski et al. (1998) found that earthworms (Octolasion lacteum) benefited from consuming millipede (Glomeris marginata) fecal pellets in a European Beech forest. However, in a microcosm experiment, millipedes were negatively affected by earthworms (Amynthas corticis), but earthworms may have similarly consumed millipede fecal material (Snyder et al., 2009). Snyder et al. (2009) found that although the millipede Pseudopolydesmus erasus was epigeic and A. corticis was endogeic, P. erasus acquired less C during the four-

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week course of the experiment in the presence of *A. corticis*, and it is likely that over longer time scales, this C deficit could affect growth, weight maintenance, survival, and/or reproductive output.

Amynthas gracilis invasion in forests of New York, USA was found to reduce O horizon organic matter (Steinberg et al., 1997; Burtelow et al., 1998). Similarly, invasion of *Amynthas agrestis* in the Great Smoky Mountains, USA, reduced the depth of the FH horizon (a combination of the F and H horizons) (Snyder et al., 2011). Millipedes reside in and consume FH horizon, and Snyder et al. (2011) found that millipedes were negatively affected by this *A. agrestis* invasion, both in terms of abundance and species richness. The field observations of Snyder et al. (2011) motivated us to explore the mechanisms behind this interaction, and a microcosm experiment was performed to test whether these two taxa competed for food resources in the L or FH horizons, and whether earthworms and millipedes benefited from the presence of these resources. This microcosm experiment was novel in its approach toward creating a longer-term study (i.e., months instead of weeks). Earthworms, and to a lesser extent millipedes, burrow in the soil and this prevents regular monitoring of faunal survival and fresh weight without causing disturbance. In order to facilitate data collection, all microcosms were destructively sampled every four weeks and fauna were put into newly constructed microcosms of the same treatment.

2. Methods

Millipedes and earthworms were collected by manually searching through leaf litter at the Great Smoky Mountains Institute at Tremont (Blount Co., Tennessee, USA; 35°38′22″N, 83°41′17″W), within the Great Smoky Mountains National Park (GSMNP) in early June 2007. Earthworms and millipedes were kept separate during transport to the laboratory. The two taxa were also stored separately until the beginning of the experiment in containers with soil and litter from the collection site.

Microcosms consisted of 1 l transparent plastic containers with perforated snap-on lids. Each microcosm received 500 ± 5 g of air-dried soil that was then mixed with 70 ± 5 mL tap water. Soil was a commercially acquired ultisol (USDA soil taxonomy) from the top 25 cm of a recently cleared forested site in Clarke Co., GA, USA. Soil was screened through a 4.75 mm sieve to remove large aggregates and rocks. Litter was previously collected from GSMNP and defaunated via Berlese extraction for 72 h, followed by air-drying. Dominant tree species at the litter collection site were *Acer* spp., *Quercus* spp., *Liquidambar styraciflua*, *Liriodendron tulipifera*, and *Pinus strobus* (Snyder et al., 2011).

Litter treatments were defined by particle size: litter was 4.75 mm sieved to separate unfragmented leaves (L horizon) from fragmented and partially decomposed organic matter (FH, combined F and H horizons). Large rocks, twigs, seeds and nuts were discarded. Organic layer treatments were L (15 ± 0.1 g of L horizon), L/FH (7.5 ± 0.1 g each of L and FH horizon), or FH (15 ± 0.1 g of FH horizon). Litter was misted with a standard quantity (~ 7 mL) of tap water when microcosms were constructed. Three fauna treatments were established: two *A. agrestis* individuals (mean fresh weight 0.86 ± 0.036 g each); one adult male *Sigmoria ainsliei* (mean fresh weight 2.26 ± 0.038 g); and two *A. agrestis* and one *S. ainsliei* together. *A. agrestis* were all clitellate or pre-clitellate. All individuals were approximately the same size and due to the annual nature of their life cycle (Reynolds, 1978; Callaham et al., 2003; Snyder et al., 2011), all individuals were similar in age. Individuals were randomly assigned to treatments with 6–7 replicates for a total of 76 microcosms. However, at the end of the experiment four experimental units were found to contain *A. corticis* rather than *A. agrestis*; these were excluded from subsequent analyses.

All microcosms were kept in the dark at 20 °C (± 2 °C). Each microcosm was misted with tap water weekly, except early in the incubation when microcosms were misted every 3 d. Incubation began in June 2007 and continued until all fauna died (except *A. corticis* mentioned above).

Microcosms were destructively sampled every four weeks. After destructive sampling, new microcosms were constructed and the surviving fauna were weighed and placed into the new microcosms. Earthworms were rinsed in tap water to remove soil and gently dried on a paper towel prior to weighing. If any fauna (earthworm or millipede) from the original treatment were alive, then a new microcosm was constructed, if all fauna in a particular microcosm had died, then that microcosm was terminated. In this way, longevity of every individual could be assessed. Soils from treatments that included earthworms were wet-sieved through a 2 mm sieve to assess cocoon production. After the first cocoons collected were found to be only slightly larger than 2 mm in diameter, a 1.4 mm sieve was employed to ensure cocoon capture.

Millipede and earthworm survival and fresh weight data were analyzed using a general linear model (GLM), with the LSMEANS option for post-hoc tests. Data used in the GLM analysis for earthworm survival were the calculated average days of survival for the two worms in each microcosm. Fresh weight changes through 12 weeks (millipedes) and 16 weeks (earthworms) were analyzed using a repeated measures analysis; beyond these points there were insufficient replicates for robust analyses. Cocoon production was assessed with a *t*-test comparing between Months 1–3 and 4–7, and GLM comparing between Months 4, 5, 6, and 7. All statistical analyses were completed in SAS (Version 9.2).

3. Results

3.1. Survival and growth

Millipedes lived a mean time of 136.8 ± 10.6 d ($n = 36$) from the beginning of the experiment (Fig. 1). The overall model testing fauna and litter effects was significant ($P = 0.0002$). Millipede survival was significantly affected by litter ($P < 0.0001$), with survival time significantly decreased in L relative to FH ($P < 0.0001$) and L/FH ($P = 0.0012$). However, differences in millipede survival times between L/FH and FH treatments were not statistically significant ($P = 0.0567$). There was a trend for earthworm presence to decrease millipede survival time, but this was not statistically
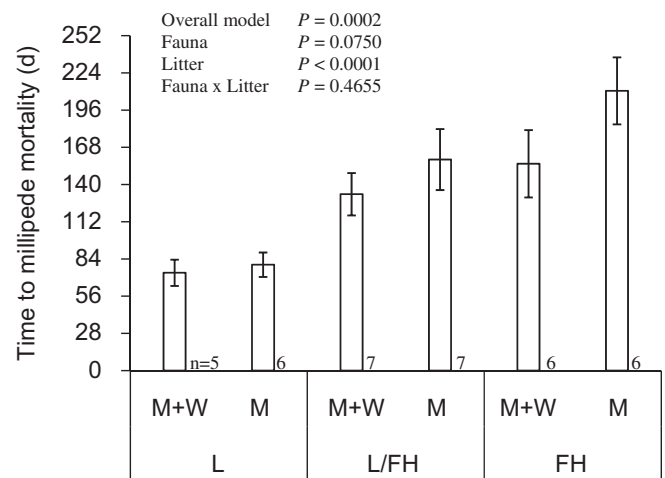

Fig. 1. Mean survival ($\pm$SE) of *Sigmoria ainsliei* from initiation of the incubation with (M + W) and without (M) earthworms. Litter treatments were litter (L), litter and FH material (L/FH), and FH only (FH).

significant ($P = 0.0750$). Specifically, when *A. agrestis* was absent, millipedes survived an average of 26 days longer in L/FH and 54 days longer in FH (Fig. 1). Overall, millipedes survived 47.4% (69 days) longer in the L/FH treatment and 58.1% (106 days) longer in the FH treatment relative to the L treatment. There was no interaction between litter and fauna ($P = 0.4655$).

Mean time to *A. agrestis* mortality was 117.9 ± 4.1 d ($n = 36$ experimental units) from the initiation of the experiment. The first and second *A. agrestis* mortality within each experimental unit were 31.8 ± 5.6 d apart ($n = 36$). There was no evidence for earthworm survival being affected by millipede presence or litter type ($P = 0.2771$, Fig. 2). In microcosms with both earthworms and millipedes, at least one earthworm survived longer than the millipede in every replicate.

Millipede fresh weight (Fig. 3A) did not differ either between treatments at the beginning of the experiment ($P = 0.5294$) and at the last measurement before mortality ($P = 0.9010$). There were significant differences between litter treatments but earthworms did not impact millipede fresh weight (Fig. 3A, Table 1, analyzed through week 12). Millipede fresh weight increased significantly more in FH relative to L treatments ($P = 0.0100$), but neither were significantly different from L/FH treatments (FH vs. L/FH, $P = 0.1351$; L vs. L/FH, $P = 0.1412$). Within-subjects tests for effects over time and interactions with time were all non-significant (data not shown).

Earthworm fresh weight (Fig. 3B) did not differ between treatments at the beginning of the experiment ($P = 0.6190$). A significant impact of litter ($P = 0.0180$), but not of millipede treatments ($P = 0.9531$, Table 1), was observed in fresh weight changes through week 16: *A. agrestis* maintained a higher fresh weight in L/FH than in FH and L treatments. Earthworm fresh weight decreased over time (Table 2, Fig. 3B, $P < 0.0001$), and a time by litter interaction was also significant ($P = 0.0211$).

3.2. Earthworm cocoon production

Cocoons were detected beginning in the fourth month and in every subsequent month of incubation (Fig. 4). In Months 4–7, microcosms in which cocoons were recovered contained 2.06 ± 0.44 cocoons; this was a significant increase over the zero cocoons recovered during Months 1–3 (*t*-test, $n = 17$, $P = 0.0003$). Numbers of cocoons recovered in Months 4–7 were not significantly different from one another (GLM, $P = 0.8952$). Cocoons were recovered from microcosms that began a month with either one or
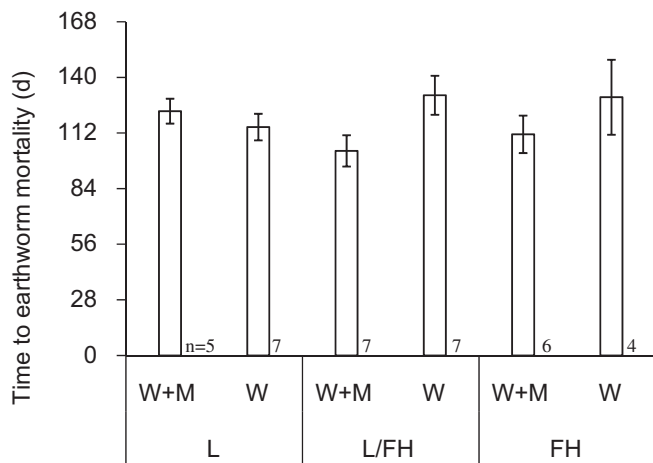

Fig. 2. Mean survival ($\pm$SE) of *Amynthas agrestis* from initiation of the incubation with (W + M) and without (W) millipedes. Litter treatments were litter (L), litter and FH material (L/FH), and FH only (FH).
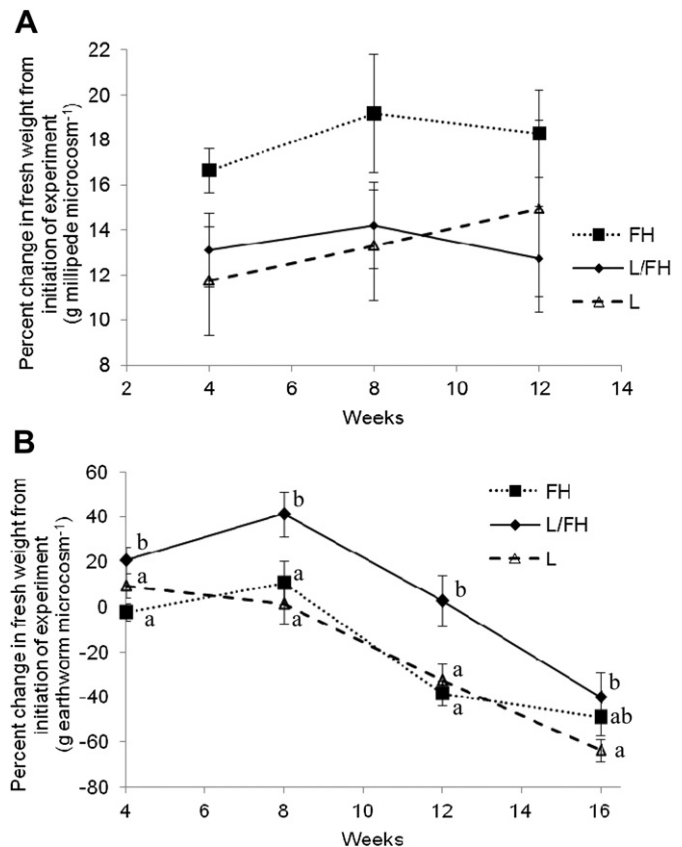

Fig. 3. Mean fresh weight change of surviving fauna ($\pm$SE) from the beginning of the experiment, expressed as percent of initial mass: millipedes (A) and earthworms (B) in different litter treatments through 12 weeks (millipedes) or 16 weeks (earthworms) of the incubation. Different letters indicate significant *P*-values at $\alpha = 0.05$ within one sampling time.

two earthworms, but the number of cocoons per microcosm was not significantly different due to this factor ($P = 0.5381$). There were a total of 28 cocoons recovered during the experiment, and only one of these was recovered from a microcosm that also contained a live millipede. Three cocoons were recovered in millipede treatments after millipede mortality. Litter treatment did not influence the number of cocoons recovered per microcosm ($P = 0.7868$).

4. Discussion

Based on inferences from field (Snyder et al., 2011) and microcosm (Snyder et al., 2009) studies that Asian invasive earthworms

Table 1
Results of repeated measures GLM analyses tests of hypotheses for between subjects effects on fresh weight change from initiation of the incubation. Fresh weight was measured every four weeks. Millipede data were analyzed until week 12 and earthworm data until week 16. Significant *P*-values at $\alpha = 0.05$ indicated by an asterisk.

Source	df	SS	MS	F	P
Millipede fresh weight					
Fauna treatment	1	3.72×10^{-6}	3.72×10^{-6}	0.00	0.9912
Litter treatment	2	0.21553	0.10777	3.59	0.0438*
Fauna*litter	2	0.03781	0.01891	0.63	0.5411
Error	23	0.68954	0.02997		
Earthworm fresh weight					
Fauna treatment	1	0.00274	0.00274	0.00	0.9531
Litter treatment	2	7.73328	3.8666	5.00	0.0180*
Fauna*litter	2	1.19285	0.59642	0.77	0.4761
Error	23	14.6819	0.77273		

Table 2
Results of repeated measures GLM analyses tests of hypotheses for within-subjects effects on earthworm fresh weight change from initiation of the incubation. Fresh weight was measured every four weeks and analyzed until week 16. Significant P-values at $\alpha = 0.05$ indicated by an asterisk.

Source	df	SS	MS	F	P
Time	3	19.2486	6.41619	86.54	<0.0001*
Time*fauna	3	0.18038	0.06013	0.81	0.4930
Time*litter	6	1.21529	0.20255	2.73	0.0211*
Time*fauna*litter	6	0.07883	0.01314	0.18	0.9820
Error (time)	57	4.22590	0.07414		

may compete with native North American millipedes, we designed a microcosm experiment to evaluate longer-term interactions between two species focusing on the potential for food competition. 'Longer-term' in this case is relative to most microcosm experiments and also to the putative life-span of these taxa, i.e., the experiment continued for months rather than weeks. This methodology had the advantage of allowing measurement of fresh weight and survival while limiting the frequency of disturbance. Although the disturbance to the microcosms may seem substantial — the entire microcosm was destroyed and replaced — in reality the stress to the organisms was quite brief and limited as much as possible. In practice, each organism was quickly located, weighed, and placed into a new microcosm in a matter of seconds. The alternative of searching for surviving individuals, weighing, and returning to the same container would have the potential of differentially disturbing experimental units depending on how quickly and easily individual organisms were discovered, collected, and measured.

Presence of FH material was important for *S. ainsliei*: millipede survival time decreased greatly without FH material and biomass increased the most in the FH treatment. However, earthworm fresh weight, but not survival, was highest in the treatment with both particle sizes (L/FH). In FH and L/FH, we observed that *A. agrestis* consumed nearly all FH material within each four-week time

Fig. 4. Cocoon production by *Amynthas agrestis* over the duration of the incubation. Mean (±SE) number of cocoons recovered per microcosm (A) and number of earthworm-containing microcosms from which cocoons were (shaded) and were not (open) recovered (B). Cocoon recovery in Months 4–7 was greater than 0 (Months 1–3, $P = 0.0003$; $n = 17$).

period. This finding is consistent with field observations and data showing that a decrease in FH horizon correlates with *A. agrestis* invasion (Snyder et al., 2011), and supports the hypothesis that *A. agrestis* directly causes this decrease through consumption.

In L/FH and FH treatments, there was also a trend that *S. ainsliei* survived a shorter amount of time when *A. agrestis* was present, but this was not statistically significant. However, we propose that from a biological standpoint, this may indeed be a relationship worthy of further exploration. Interestingly, when both species were present, millipedes almost always died first, and this suggests that when the two are in close proximity, the invasive earthworms may outcompete millipedes and eventually exclude them. In these same litter treatments (FH and L/FH) there was also a very weak trend that *A. agrestis* survived longer in treatments without millipedes. However, in L treatments, *A. agrestis* tended to survive longer in the presence of millipedes, suggesting that earthworms may benefit from millipede presence in L treatments, probably through consumption of litter that had been processed by millipedes, as has been observed in other studies (Bonkowski et al., 1998; Snyder et al., 2009). Earthworms were also observed to burrow into mineral soil during the incubations, and may have been able to exploit organic matter in the mineral soil in addition to resources supplied on the soil surface (Zhang et al., 2010; Callaham et al., unpublished results). This behavior is consistent with the findings of Zhang et al. (2010) who showed that *Amynthas* had substantial dietary flexibility, and this possible additional source of organic matter may help explain why earthworm survival was not affected by litter treatment, as well as why the effect of millipede presence was not statistically significant. When availability of aboveground resources was limited, *A. agrestis* may have burrowed and consumed soil organic matter, while adult *S. ainsliei* were restricted to feeding on surface organic horizons. Millipedes appear to have inhibited reproductive potential, possibly through this same mechanism. In the presence of millipedes, earthworms may have spent more energy burrowing to access lower quality food resources. This combination may have led to less energy being available to devote to cocoon production.

4.1. Conclusions and future perspectives

Overall, the data from the present study are consistent with, and help to elucidate, observations from field studies (Snyder et al., 2011), and other lab experiments with invasive earthworms and millipedes (Snyder et al., 2009). *A. agrestis* invasions being associated with decreased F/H horizon depth and decreased millipede abundance (Snyder et al., 2011) served as a starting point to ask questions about what the potential mechanisms behind these relationships might be. From our microcosms, we now have evidence that two of the organisms involved in the field study will consume the same food sources, and that when they are kept in proximity to one another, these organisms affect one another's longevity and reproductive output. Although microcosms are, of necessity, quite simple relative to the natural systems they are meant to simulate, they can nevertheless offer important insights particularly into mechanistic relationships (Drake and Kramer, 2012; Cadotte et al., 2005). We suggest that our study has uncovered just such a mechanistic relationship between *A. agrestis* and *S. ainsliei*, but we also recommend that much more detailed work should be undertaken to examine the trophic ecology and resource use of these organisms in their native habitats. Such work will be crucial if we are to have fuller understanding of the effects of earthworm invasion, and imperative to the future development of successful management approaches to control earthworm invasions in the Southern Appalachian Mountains.

Our study lends support to the hypothesis that earthworms and millipedes compete for partially decomposed leaf material, but
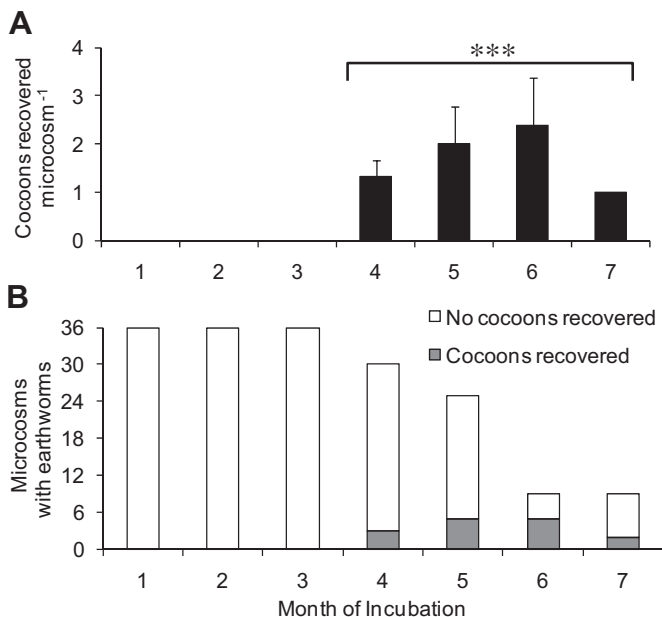
many questions remain. Greater cocoon production in the absence of millipedes supports the competition hypothesis and suggests that millipedes may provide some biotic resistance to invasion. Future studies could offset the natural variability in earthworm and millipede mortality, and improve their statistical power, by including more replicates. Additionally, initiating treatment conditions on younger individuals may produce stronger responses. Maintenance of laboratory cultures (Lowe and Butt, 2005) will be a critical step in our ability to perform more of these experiments.

Acknowledgments

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