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# Responses of non-native earthworms to experimental eradication of garlic mustard and implications for native vegetation

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**Abstract.** Recent studies in invasion biology suggest that positive feedback among two or more introduced organisms facilitate establishment within a new range and drive changes in native plant communities. Here, we experimentally tested for relationships between native plants and two non-native organisms invading forest habitats in North America: garlic mustard (*Alliaria petiolata*, Brassicaceae) and earthworms. In two forested sites, we compared understory vegetation and earthworm biomass in plots where garlic mustard was removed for three years, plots without garlic mustard invasion, and plots invaded by garlic mustard that was not removed. Earthworm biomass was highest in the plots with garlic mustard, and long-term eradication of garlic mustard reduced earthworm biomass to levels similar to those observed in the uninvaded control plots. Invasion treatment, and the interactions between earthworm biomass and treatment, explained most of the variation in plant community composition and diversity—suggesting that earthworms alone do not necessarily drive forest understory floristic patterns. In contrast to broader geographic patterns indicating earthworms as the main driver of vegetation change in the presence of non-native plants, we show that garlic mustard solely, or in conjunction with earthworm biomass, drives changes in native plant composition and diversity at the scale of individual forests. From a local management perspective, our data suggest that garlic mustard eradication can directly assist in the conservation of native plant communities and simultaneously reduce earthworm biomass.

**Key words:** *Alliaria petiolata*; co-invasion; earthworms; garlic mustard; invasive species.

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## INTRODUCTION

It is now widely accepted that the introduction of non-native species into new habitats has profound ecological implications for biodiversity and ecosystem function, but the mechanisms that determine the success or failure of a given species beyond its home range remain elusive (Levine et al. 2003, Facon et al. 2006). An emerging hypothesis in conservation biology is that

positive feedback between one or more non-native species can occur during biological invasions (Eisenhauer et al. 2012, Roth et al. 2015). The invasion meltdown hypothesis (Simberloff and Von Holle 1999) and the notion of ecosystem engineers (Frelich et al. 2006, Eisenhauer et al. 2009) also predict that invasive organisms create conditions under which others can successfully invade in a new range. Identifying the major players in a cascading invasion process and

establishing causal relationships are fundamental goals in invasion ecology and have great potential to inform the management and conservation of native biodiversity.

Here, we report experimental evidence for a direct effect of an invasive plant on native plants and non-native earthworm biomass, but little correlation between earthworm biomass and many native plant community parameters. Our data challenge the emerging idea that non-native earthworms are primary drivers of increased abundance of non-native plants and of declines in native plant diversity in northern North America (Nuzzo et al. 2009, 2015, Craven et al. 2016, Duncan and Whitfeld 2018). Forests in the northeastern and mid-Atlantic region of the United States have historically had no native earthworms since the last glaciation, but they have become increasingly invaded by several earthworm species introduced as early as the time of European settlement and through their modern use as recreational fishing bait (Bohlen and Hendrix 2002). Earthworms are considered ecosystem engineers that accelerate nutrient cycling (Szlavecz et al. 2006, Sackett et al. 2013, Ewing et al. 2015), alter soil physical and chemical properties (Hendrix et al. 2008, Eisenhauer 2010), and drive changes in forest plant community diversity and composition (Hale et al. 2006, Eisenhauer et al. 2007, Fisichelli et al. 2013). A compelling case that earthworms engineer forest soils in a manner that facilitates plant invasions has been made in recent meta-analyses and correlative studies; earthworm biomass is broadly associated with increases in non-native plant species and declines in the presence of native plant species across a range of forest types and invaders (Nuzzo et al. 2009, Craven et al. 2016). However, experiments investigating responses of earthworms to the removal of invasive plants, and their respective associations with native plant communities, are limited to a small number of studies, mainly with invasive shrubs (Madritch and Lindroth 2009, Lobe et al. 2014, Roth et al. 2015).

*Alliaria petiolata* (garlic mustard, Brassicaceae) is an invasive plant that co-occurs with earthworm invasions (Nuzzo et al. 2009). Garlic mustard is a biennial forb that was introduced into North America in the 1800s, and while it has become a prominent species in forest

understories (Nuzzo 1991), it is unclear whether garlic mustard benefits from the activity of earthworms or whether earthworms proliferate in soils invaded by garlic mustard. Land managers and conservation groups throughout North America invest significant time and resources into hand-pulling and other eradication measures to reduce garlic mustard densities in forest habitats that are simultaneously invaded by earthworms. In these habitats, garlic mustard can form dense monocultures that reduce the abundance, growth, and diversity of native species through competitive effects as well as phytochemical suppression of mycorrhizal symbioses (Stinson et al. 2006, Anthony et al. 2017). In recent studies, garlic mustard presence was correlated with earthworm abundance, but the response was site specific (Nuzzo et al. 2009, Craven et al. 2016, Duncan and Whitfeld 2018), and it remains unclear whether earthworms facilitate garlic mustard invasion or whether earthworms co-occur with garlic mustard for other reasons, like changes in soil pH and organic matter contents that may vary across sites (Kalisz and Dotson 1989). Garlic mustard is known to increase soil pH to a neutral range that may favor earthworms (Levine et al. 2003, Rodgers et al. 2008), but shifts in soil pH occur along a site-level gradient (Anthony et al. 2017). Though earthworms ingest and metabolize soil microbes, they also eat the seeds of many plant species and have been shown to use the abundant seeds produced by garlic mustard as a food source (Quackenbush et al. 2012, Nuzzo et al. 2015, Cassin and Kotanen 2016). Although earthworm introductions pre-date that of garlic mustard and create conditions that may benefit garlic mustard establishment (Bohlen et al. 2004, Nuzzo et al. 2009), garlic mustard may also promote earthworms, and it is possible that garlic mustard actually facilitates earthworm invasions.

Here, we tested for relationships between garlic mustard and earthworms and their interactive effects on native plants in the context of an ongoing, long-term garlic mustard eradication study at two forested sites in Western Massachusetts (Anthony et al. 2017; Haines et al., *in press*). We hypothesized that there would be a positive relationship between earthworm biomass and garlic mustard densities across our plots. By eradicating garlic mustard, we would expect earthworm

biomass to be unchanged if garlic mustard does not positively influence earthworms. Lower earthworm biomass with garlic mustard eradication, in contrast, would provide new evidence that garlic mustard promotes earthworm invasions. Likewise, if garlic mustard is the main driver of changes in native vegetation, we would expect eradication of garlic mustard to alter the diversity, abundance, and composition of native vegetation regardless of potential earthworm effects on vegetation.

## MATERIALS AND METHODS

### *Study sites and eradication treatments*

We noticed earthworm presence in research plots that are part of a larger study on plant and fungal community responses to eradication of the widespread invasive plant, *Alliaria petiolata* (garlic mustard), in Western Massachusetts (Anthony et al. 2017). We conducted earthworm surveys at two replicate forests undergoing garlic mustard eradication since May 2013: Harvard Forest in Petersham (Worcester County, 42°31'45.82" N), and the Trustees of Reservations McLennan Reservation (Berkshire County, 42°13'17.44" N) on the border of Tyringham/Otis, Massachusetts, USA. Both sites have a similar understory and are dominated by sugar maple (*Acer saccharum*), white ash (*Fraxinus americana*), white pine (*Pinus strobus*), birch (*Betula allegheniensis* and *Betula papyrifera*), and black cherry (*Prunus serotina*), a canopy structure typical of secondary-growth forests of this region. We chose sites with similar land use history by (1) verifying with the landowners that garlic mustard has been present for the past twenty years; (2) confirming that plots were situated on soils indicative of use as unimproved pasture and/or woodlot (i.e., shallow and disorganized AB horizon; Motzkin et al. 1996); (3) mapping spatial coordinates of the sites to available historical maps to confirm a similar forest age of approximately 140 yr (Foster and Motzkin 2009).

At each site, we established nine 3-m<sup>2</sup> plots, three in each of the following treatments: invaded by garlic mustard with a field density of >20 plants/m<sup>2</sup>; invaded by garlic mustard at the same density but with all garlic mustard plants completely removed via annual hand-pulling in the spring around the same time in late May and

early June; and nearby reference plots with similar canopy structure, slope, and aspect, but with garlic mustard absent and with no known prior history of garlic mustard presence. Plots with garlic mustard were selected by establishing transects through the full length of each invaded area and randomly selecting points along the transect. The uninvaded control plots were selected in a similar manner by establishing transects through uninvaded areas within 20–50 m of same forest understory invaded by garlic mustard. Eradication treatments consisted of hand-pulling every garlic mustard seedling and adult plant by the stem to remove the entire plant (including roots) from the eradicated plots. Initial garlic mustard densities averaged 43 (standard error [SE] 7.1) plants per square meter prior to the eradication treatments across all plots containing garlic mustard. All eradicated garlic mustard biomass was bagged and removed from the site each year. No garlic mustard was present at the control plots, and the initial garlic mustard density was not altered in the invaded plots. Annual censuses of garlic mustard confirmed invasion status at the invaded plots at a mean density of 32.33 (SE 16.24) plants per square meter. The main eradication was conducted in May 2014, and pulling was repeated once per year through 2017 to remove increasingly lower densities of garlic mustard seedlings that continued to disperse from surrounding plots and/or emerge from the seed bank.

### *Environmental data*

Volumetric soil moisture was quantified at one random point per experimental plot with a ThetaProbe soil moisture sensor (Delta-T Devices, Houston, Texas, USA) to generate plot-level estimates prior to the sampling of earthworm biomass in July 2017. Soil pH was measured in distilled water (1:10 wt : vol) using a digital pH meter. Total soil organic carbon and nitrogen (N) contents were analyzed on air-dried, finely ground soils using dry combustion (Perkin Elmer 2,400 Series II CHN elemental analyzer [Waltham, Massachusetts, USA]).

### *Earthworm biomass*

We used earthworm biomass as a measure of overall earthworm activity (Duncan and Whitfeld 2018). A 0.25-m<sup>2</sup> quadrat was established in

the northwest corner of each plot. Earthworms were coaxed to the surface on a single day in July 2017 by saturating the soil in the quadrat with 3.76 L of a solution of Coleman's Mustard Powder and water (sensu Lawrence and Bowers 2002). During a fifteen-minute waiting period, any earthworms that appeared were collected into labeled plastic containers with distilled water for transportation back to the laboratory. The procedure was then repeated at each quadrat directly following the first fifteen-minute waiting period, and earthworms were counted and weighed. This procedure is known to bring epigeic, endogeic, and anecic worms to the soil surface (Hale and Host 2005). While we did not identify individual earthworms to functional group or species, we estimate that we observed species from all three groups: the anecic *Lumbricus terrestris*, the epigeic *Lumbricus rubellus* and *Dendrobaena* species, and the endogeic *Octolasion* species at all of our plots (Hale 2013).

#### Vegetation surveys

We conducted a complete vegetation census in each plot three years after the initial eradication (May 2017). We identified and counted the stems of each plant below 1 m in height. We identified each plant to species, following the species nomenclature in *Flora Novae Angliae* (Haines 2011). We calculated the abundance of woody plants and forbs (graminoids were scarce in our dataset), the total abundance of native plants, and the Shannon-Weaver diversity index ( $H'$ , Shannon and Weaver 1949) of native plants in each plot.

We also counted the number of garlic mustard seedlings and adults present at the invaded and eradicated plots prior to the annual eradication (zero garlic mustard present in the control plots and garlic mustard plants temporarily present in the eradication plots prior to pulling). The only other non-native species present in our study besides garlic mustard was the shrub *Euonymus alatus*, which occurred at low densities across the treatments at the Harvard Forest site.

#### Statistical analyses

All statistical analyses were conducted in R 3.4.2 (R Core Team 2017), and significance was set at  $P \leq 0.05$ . We used general linear models with invasion status (treatment) and site as the main explanatory variables and soil pH, soil

moisture content, percent soil N, soil C, and earthworm biomass as the response variables. To test the relative effects of garlic mustard eradication treatments and earthworms on native vegetation, we constructed models with treatment as the main effect and earthworm biomass as a covariate. We explored the effects of treatment, earthworm biomass, and their interaction on the following response variables: (1) native plant diversity, (2) abundance of woody native plants, (3) abundance of native forbs, and (4) total native plant abundance. To account for site-level variation, we included site as a main effect. Total stem abundance and native woody plants were log-transformed, and native forb abundance was log ( $x + 1$ ) transformed to meet assumptions of normality. To account for potential heterogeneity in plot-level invasion status within and among sites, we constructed a linear model with site and plot as main effects and number of garlic mustard stems as the response variable.

To analyze the effects of earthworm biomass, treatment, and the interaction between earthworm biomass and treatment on plant community composition, we ran a PERMANOVA on the Bray-Curtis distance matrix derived from absolute plant abundance (adonis function, vegan package; Oksanen et al. 2018). We constrained the permutations within sites by using the strata option. We visualized differences in plant communities between plots using non-metric dimensional scaling (NMDS; metaMDS and ordiplot functions).

## RESULTS

There was no effect of garlic mustard removal on soil moisture, soil C, or inorganic N, but soil pH was slightly higher in invaded plots than in eradicated and control plots (Table 1). Volumetric soil moisture ranged from  $0.18 \pm 0.10$  to  $0.62 \pm 0.36$ ;

Table 1. Results of general linear models testing the effects of treatment and site on soil pH, inorganic N concentrations, and moisture.

Effect	df	pH		Nitrogen		Moisture	
		F	P	F	P	F	P
Treatment	2	3.63	<b>0.05</b>	0.08	0.09	0.51	0.61
Site	1	7.00	<b>0.02</b>	3.08	0.10	0.45	0.51

Note: Values in bold indicate that the null hypothesis can be rejected with 95% confidence ( $P < 0.05$ ).

temperature ranged from  $16.27 \pm 0.17$  to  $19.70 \pm 0.10^\circ\text{C}$ ; pH ranged from  $4.72 \pm 0.02$  to  $5.07 \pm 0.17$ ; soil C ranged from  $6.63\% \pm 0.77\%$  to  $10.78\% \pm 1.62\%$ ; and soil N ranged from  $1.25\% \pm 0.68\%$  to  $3.13\% \pm 1.25\%$  (Appendix S1: Table S1). Earthworm biomass was highest in the invaded plots and was similar between the eradicated and the non-invaded plots (Fig. 1; Table 2).

Plot-level plant diversity and mean abundance of plant functional groups were variable but without clear patterns across invasion statuses (Appendix S2: Table S1). Native plant diversity ( $H'$ ) ranged from  $0.76 \pm 0.39$  to  $1.80 \pm 0.05$  across treatments. Ferns only occurred at the Harvard Forest site at a density of  $3.67 \pm 2.73$ ;

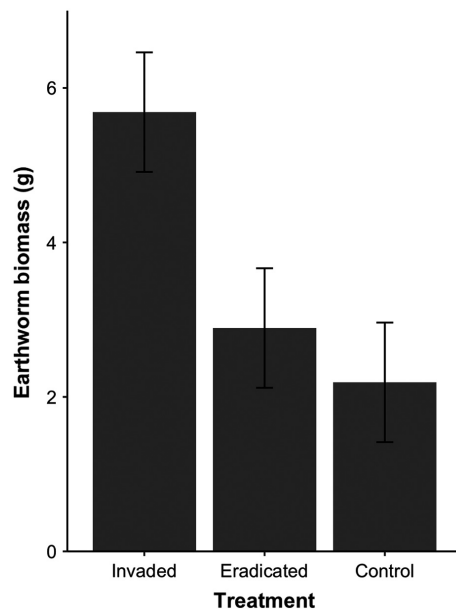


Fig. 1. Mean earthworm biomass in experimental plots by treatment: invaded; garlic mustard eradicated annually since 2013; and control (no history of garlic mustard present) at two forests in Western Massachusetts.

Table 2. Results of general linear model testing the effect of eradication treatment and site on earthworm biomass.

Effect	df	F	P
Treatment	2	5.72	<b>0.02</b>
Site	1	0.29	0.60

Note: Values in bold indicate that the null hypothesis can be rejected with 95% confidence ( $P < 0.05$ ).

non-native plant abundance ranged from 0 to  $58.67 \pm 24.66$  individuals per plot; and woody plants ranged from  $15.00 \pm 1.15$  to  $70.33 \pm 26.31$  plants per plot. Forbs occurred at densities ranging from  $13 \pm 4.73$  individuals to  $318.33 \pm 266.03$  individuals per plot, driven by very high numbers of a single species, *Erythronium americanum* (trout lily; 847 individuals). There were no effects of garlic mustard eradication treatments on native plant diversity, total native plant abundance, or abundance of plant functional groups other than forbs (Table 3). The effect of invasion status on forbs appears to have been driven by the high densities of trout lily in a single plot as described above. There was no significant effect of plot or site on garlic mustard density ( $F_{\text{plot}} = 0.389$ ;  $P_{\text{plot}} = 0.815$ ;  $F_{\text{site}} = 0.5402$ ;  $P_{\text{site}} = 0.4649$ ), indicating that patchiness of garlic mustard densities did not increase heterogeneity across the invaded plots and was not likely to have affected our findings.

There was no general relationship between earthworm biomass and plant diversity because the regression slopes varied by treatment (Fig. 2; Treatment  $\times$  Earthworm interaction  $P = 0.07$ ; Table 3). There was a positive correlation between earthworm biomass and native plant diversity within the eradicated (garlic mustard removal) treatment ( $R^2 = 0.745$ ,  $P < 0.05$ ; Fig. 2). The control and invaded treatments showed no individual correlations between earthworm biomass and plant diversity.

However, there were effects of eradication treatment and significant treatment  $\times$  earthworm biomass interaction on native plant community composition (Table 4). The interaction between treatment and earthworm biomass explained 17% of the variation in native plant community composition ( $P = 0.003$ , Table 4). Site explained 14% of the variation in native plant community composition, and sites showed some separation in community composition in the NMDS plot (Fig. 3).

## DISCUSSION

Understanding ecological linkages between co-occurring invasive species is rapidly becoming a central question in ecology and conservation biology (Kuebbing et al. 2013, Tekiel and Barney 2017). Broad-scale correlative patterns

Table 3. Effects of general linear model testing the effects of site, eradication treatment, earthworm biomass, and the treatment by earthworm biomass interaction term on native plant diversity.

Effect	df	Native plant diversity		Total native plant abundance		Native woody plant abundance		Native forb abundance	
		F	P	F	P	F	P	F	P
Earthworm biomass	1	0.67	0.43	0.54	0.48	0.27	0.61	1.46	0.25
Treatment	2	1.55	0.26	0.60	0.57	0.78	0.48	0.02	0.98
Site	1	0.06	0.80	0.33	0.58	0.34	0.57	0.72	0.41
Treatment × Earthworm biomass	2	3.35	0.07	0.87	0.45	0.75	0.50	2.79	0.10

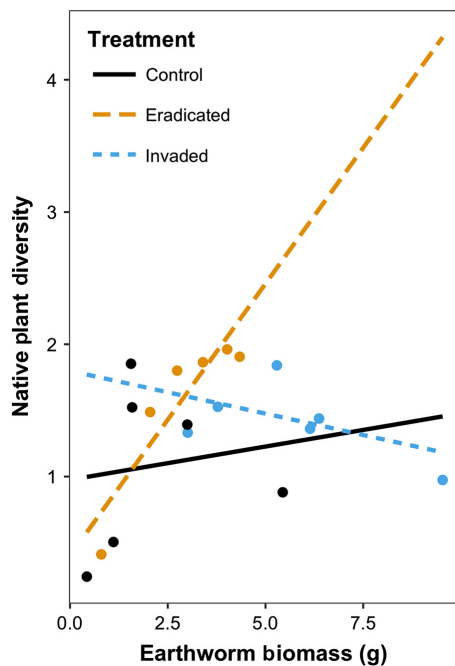


Fig. 2. Native plant diversity (measured with the Shannon diversity index [ $H'$ ]) as a function of earthworm biomass separated by treatment at our experimental garlic mustard eradication plots. The relationship between earthworms and native plant diversity was significant only for the eradicated plots.

suggest that earthworms facilitate the success of non-native plant species in North American forests (Nuzzo et al. 2009, Roth et al. 2015). However, it is also possible that invasive plants create conditions that facilitate earthworm invasion (Madritch and Lindroth 2009). Further, it is unclear whether earthworms or invasive plants are the main drivers of changes in native plant community diversity and composition.

Table 4. PERMANOVA model effects of eradication treatment, earthworm biomass, site, and the treatment × earthworm biomass interaction term on community dissimilarities between plots.

Effect	df	F	R <sup>2</sup>	P
Treatment	2	1.73	0.169	<b>0.003</b>
Earthworm biomass	1	0.81	0.039	0.600
Treatment × Earthworm biomass	2	2.09	0.205	<b>0.001</b>

Note: Values in bold indicate that the null hypothesis can be rejected with 95% confidence ( $P < 0.05$ ).

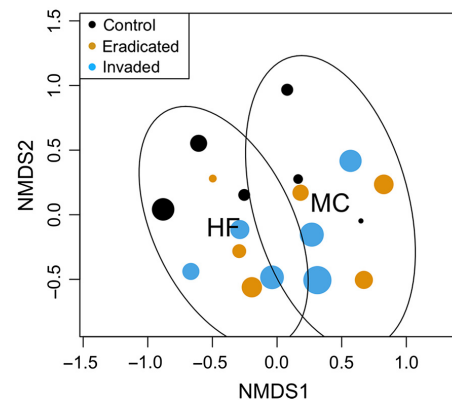


Fig. 3. Non-metric dimensional scaling (NMDS) plot of native plant communities. Each point represents a single plot, the size of the point corresponds to relative earthworm biomass, and the color of the point corresponds to experimental treatment. Differences in plant community composition between sites are shown by standard deviation ellipses. Plot stress = 0.19.

Here, we conducted surveys of earthworm biomass in response to experimental eradication of garlic mustard to better understand their interactions with garlic mustard and native plants.



### *Effects of garlic mustard eradication on earthworm biomass*

Garlic mustard eradication are a time-consuming but common ad hoc management strategy in Massachusetts and throughout the invaded range, but the efficacy of this approach for restoring native vegetation remains unclear. Experimental eradication of garlic mustard has been shown to increase the diversity of native plant communities and abundance of native plants after just one year (McCarthy 1997, Stinson et al. 2007), but long-term studies are generally lacking. Spring perennials, tree seedlings, and other woody species tend to increase, in particular, after garlic mustard eradication (Meekins and McCarthy 1999, Hochstedler et al. 2007, Stinson et al. 2007). A greenhouse experiment conducted by Barto and Cipollini (2009) showed that the eradication of garlic mustard by hand-pulling augmented the growth of native jewelweed (*Impatiens pallida*), but the effectiveness of field eradication on native plant communities is mixed (Stinson et al. 2007, Pardini et al. 2008, Chapman et al. 2012). Earthworms are a management concern for their effects on plant community structure as well (Hale et al. 2006, Eisenhauer et al. 2007, Fisichelli et al. 2013), but eradication of non-native earthworms is difficult and is focused on public education efforts to limit and contain their introduction into natural ecosystems (Hale 2013).

Earthworms were more abundant in invaded plots than non-invaded plots, supporting prior correlative work showing positive associations between invasive plants and earthworms (Eisenhauer et al. 2009, 2012, Nuzzo et al. 2009, Duncan and Whitfeld 2018). However, lower earthworm biomass at plots undergoing garlic mustard eradication compared to plots without garlic mustard suggests further that plant invasion might also be facilitating earthworm invasions (Fig. 1). Similar findings have been reported elsewhere, with earthworm declines following eradication of the invasive shrubs such as buckthorn and honeysuckle (Madritch and Lindroth 2009) and privet (Lobe et al. 2014).

The mechanisms by which garlic mustard invasion might favor earthworm invasions remain unclear. Though soil pH values were slightly higher at invaded plots, there was otherwise little evidence to suggest that either garlic

mustard presence or earthworms affected soil conditions (Table 2), so it is unlikely that the soil properties we measured are involved at the spatial scale of this study, though we did not measure phosphorus or macronutrient contents which may affect earthworms. Elsewhere, garlic mustard has been shown to raise pH and may be altering pH in such a way that earthworms favor invaded sites (Levine et al. 2003, Rodgers et al. 2008). Another possibility is that garlic mustard seeds boost food resources available to earthworms (Quackenbush et al. 2012, Cassin and Kotanen 2016, Flinn 2017), and thus, earthworms depart from the eradication treatments in search of other resources. However, the emergence of garlic mustard seedlings in the eradication plots indicates that seeds may still arrive from surrounding plots and/or were present in a persistent seed bank within the timeframe of our experiment. Some earthworms are negatively influenced by consumption of ectomycorrhizal fungi, but others are positively influenced by consuming pathogenic fungi (Montecchio et al. 2015). Garlic mustard invasions can reduce ectomycorrhizal fungal abundance (Wolfe et al. 2008) and increase pathogenic and pathotrophic fungal abundance (Anthony et al. 2017), which may confer a benefit to earthworm nutrition. Further, the antimycorrhizal effects of garlic mustard on soil biota may also be beneficial to earthworms that harbor fungal pathogen loads (Ghosh 2018), and garlic mustard invasion has been shown to be associated with higher fungal pathogen abundance (Anthony et al. 2017), although this hypothesis requires further investigation. The phytochemical suppression of certain fungal groups can also persist for several years post-eradication (Lankau et al. 2014). It is further possible that hand-pulling of garlic mustard disturbs the soil in such a way that disrupts earthworm activity, although the heaviest disturbance occurred three years prior to our earthworm sampling, with low densities of recurring garlic mustard removed in subsequent years to maintain the eradication treatment. In addition, since earthworms themselves are known to churn soils and are often found in disturbed sites (Kalisz and Dotson 1989, Bohlen and Hendrix 2002, Levine et al. 2003), the minor disturbance of garlic mustard pulling is unlikely to be a factor. Overall, in contrast to correlative data in prior

studies, our findings suggest that earthworms in this study are responding to, rather than creating, conditions of garlic mustard invasion.

#### *Interactions between garlic mustard eradication and earthworms on native plants*

Our study showed mixed evidence that non-native earthworms are a major driver of native plant diversity and community composition. In contrast to prior correlative work at broad geographic scales (Nuzzo et al. 2009, 2015, Craven et al. 2016, Duncan and Whitfeld 2018) and observed associations between earthworms and plant diversity in our study region (Eisenhauer et al. 2009, Duncan and Whitfeld 2018), we found no relationship between earthworms and the diversity or abundance of native plants within invaded or control plots. It is possible that the finer scale of measurement in the present study was not sufficient to capture patterns of plant diversity as a function of earthworms, though observational and experimental work has demonstrated variation in invaded and uninvaded plant communities at this scale (Haines et al., *in press*). That our data demonstrate direct effects of an invasive plant removal on earthworms indicates a clear need for additional experimental work to test the mechanisms and generality of observational correlations between earthworms and forest plant species.

The positive relationship between earthworm mass and plant diversity in the eradicated plots does suggest, however, that earthworms interact with native vegetation and related invasion management practices. The increase in plant diversity with increasing earthworm biomass is opposite that of broader-scale patterns in which earthworms are associated with lower native plant diversity (Nuzzo et al. 2009). One possibility is that soil-churning by earthworms that do remain in eradicated plots opens niche space for disturbance-tolerant species, though our data did not suggest specific functional groups were favored by the presence of earthworms. In terms of overall plant community composition, the treatment and treatment  $\times$  earthworm biomass interaction in the PERMANOVA further supports the idea that native vegetation responds to both plant and earthworm invasions. Despite the evidence that earthworms play a role in both plant diversity and community composition, the clustering

of invaded plots in Fig. 3 suggests that native plant community composition is better explained by garlic mustard presence rather than earthworm biomass, though their interaction also explained some of the variation.

Finally, it is important to note that there were site effects on plant composition that were visually detectable in the NMDS (Fig. 3), indicating some floristic differences in understory vegetation between the two sites. However, the overlap of plant communities by site in the ordination indicates that the sites were generally similar, with the exception of a low density of ferns present only at one of the Harvard Forest plots and a high density of trout lily at one of the McLennan plots, which may contribute to some site separation in the NMDS. Site-to-site variation in community composition could also be attributable to a number of factors not analyzed here, including underlying soil factors (such as pH, which differed among treatments and sites in this study; site effect,  $P < 0.05$ ; Table 1) as well as temporal variation related to successional rate and historic or recent land use. Interestingly, despite divergence between sites, the plant communities in plots without a history of garlic mustard invasion were most similar to each other, whereas there was overlap in composition between invaded and eradicated plots. The treatment effect and the interaction with earthworms suggest that invaded sites with a history of both garlic mustard and earthworms contain a different suite of understory vegetation than uninvaded sites.

#### *Management implications*

Along with recent work showing earthworm declines in response to eradication of other invasive plant species (Madritch and Lindroth 2009, Lobe et al. 2014), our study challenges the general notion that plant invasions are driven by earthworm invasion and suggest instead a positive feedback in which the invasive plants themselves also play a role. Moreover, our results suggest garlic mustard as the primary driver of native forb abundance and overall native plant community composition, though this may not be the case at other sites. From a management perspective, our data suggest that garlic mustard eradication may simultaneously increase the presence of native forbs and help to reduce

the spread of earthworms into forest ecosystems. Outreach activities centering on garlic mustard eradication could thus be potentially combined with public education about limiting earthworm introductions through recreational fishing.

#### *Limitations and future research needs*

We emphasize caution in the extrapolation of our findings to a broader geographic scale. This study reflects the effects of garlic mustard and earthworms three years post-eradication of garlic mustard at two forested sites. Only two other studies have experimentally tested the effects of invasive plant removal on earthworms, and both were conducted at a single location (Madritch and Lindroth 2009, Lobe et al. 2014). Thus, additional studies across a larger number of sites, and longer-term studies following earthworm biomass post-eradication, are warranted for reconciling our data with correlative studies across broader regions (Craven et al. 2016). We note further that, although we found three different functional groups of worms, we did not analyze variation in occurrences of different earthworm species across the treatments; earthworm functional groups exhibit unique ecological effects (Hale and Host 2005), and thus, additional work determining species-specific responses to invasive plant removals would be informative. Finally, earthworms do appear to interact with garlic mustard eradication to increase native plant species diversity, although this may be a transient effect as plant community composition does not fully recover in the three years after initial eradication treatments. Thus, the mechanisms for the earthworm effect on native plant diversity in this study remain unclear and warrant further research.

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