EXAMINING THE RELATIONSHIP BETWEEN GARLIC MUSTARD (*ALLIARIA PETIOLATA*) AND EUROPEAN EARTHWORMS

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By

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ABSTRACT

Zelles, Alexandra M. M.S. Department of Biological Sciences, Wright State University, 2012. Examining the relationship between garlic mustard (*Alliaria petiolata*) and European earthworms.

Our goal was to characterize the interactive feedback between garlic mustard (*Alliaria petiolata*) and European earthworm species in southwest Ohio. Earthworm community composition, abundance and biomass were compared between 0.1 m^2 plots of garlic mustard, cut-leaved toothwort (*Cardamine concatenate*), wild ginger (*Asarum canadense*), or no plant cover. Exotic earthworms were present in the study site. Earthworm abundance and biomass did not correlate with garlic mustard percent cover. There was a greater density of earthworms in the fall than in the spring. Worm abundance differed between garlic mustard and wild ginger plots and cut-leaved toothwort and control plots, suggesting that earthworms may prefer to be located under plants that produce high biomass. Worm biomass did not differ between the plants. There was a greater abundance of endogeic worms below all plants, while anecic worms contributed the most biomass. Our results do not support strong feedback between garlic mustard and European earthworms.

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INTRODUCTION

There is growing awareness within the field of invasive species ecology regarding the positive relationships that may exist between invaders (Simberloff and Von Holle 1999; Heneghan et al. 2007). When the invasion of one species and its effects on the invaded area facilitate the invasion of additional species, a positive feedback loop is created. This relationship defines the 'Invasional Meltdown Hypothesis' (IMH), which depends on positive feedback. The IMH explains that invasive species facilitate each other rather than compete with one another, and, as a result, invasion rates and replacement of native communities should accelerate over time (Simberloff and Von Holle 1999; Heneghan et al. 2007; Madritch and Lindroth 2009). Invasion meltdowns also have the potential to alter basic ecosystem processes (Madritch and Lindroth 2009). Because invasive species may assist one another, investigations on invasive species' interactions and impacts on ecosystem structure and processes are necessary for a mechanistic understanding of invasion consequences (Heneghan et al. 2007).

Invasional meltdown is proposed to occur when there are positive interactions (i.e. mutualism and facilitation) between sequential invaders (Mitchell et al. 2006; Green et al. 2011). The first invasive species to colonize a habitat facilitates the establishment of subsequent invaders by promoting their population growth or magnifying their specific impacts (Green et al. 2011). The introduction of novel species might alter the community of enemies, mutualists and competitors, which in turn could facilitate the colonization by

subsequent invaders and trigger an invasional meltdown (Mitchell et al. 2006). While the IMH has become a popular area of study within the past few years, very little research has been done investigating the mechanisms of invasional meltdown.

Studies have examined the positive relationship that is suggested to exist between Eurasian invasive shrubs and exotic earthworms (Heneghan et al. 2007; Madritch and Lindroth 2009) however, very few have examined if this relationship can also be documented between invasive herbaceous plants, such as garlic mustard (*Alliaria petiolata*), and exotic earthworms. The garlic mustard – earthworm system is one in which we can test the invasional meltdown mechanisms discussed above. Garlic mustard and European earthworms coevolved in their native range, and introductions of these species into North America have resulted in their co-occurrence in their non-native range, as well (Frelich and Reich 2009). Some researchers have recently proposed that invasion of non-native herbaceous plant species, including garlic mustard, is likely heightened due to their adaptations to impacts caused by European earthworms, compared to native species that seem to lack the appropriate adaptations necessary to persist in earthworm invaded areas (Frelich et al. 2006). Nuzzo et al. (2009) supports this thought with their findings that non-native plant cover of garlic mustard, barberry (*Berberis thunbergii*) and browntop (*Microstegium vimineum*) is positively correlated with increasing exotic earthworm biomass. They further hypothesize that the invasion of areas by non-native plants are a consequence of exotic earthworm effects.

We have developed a conceptual model, hypothesizing feedback mechanisms between garlic mustard and European earthworms (Fig. 1). When garlic mustard is actively growing, it releases a variety of N-based metabolites through leaf and root

exudates, such as glucosinolates and cyanide, which are then broken down into varying forms of N within the soil (Fahey et al. 2001). When the plant reaches the end of its growing season and senesces, the dead plant material contributes to the leaf litter layer of the organic soil horizon and also acts as a source of N. Earthworms are active ecosystem engineers that vertically homogenize the soil layers. They increase soil porosity by their burrowing activities and accelerate nutrient cycling, as well as elevate soil N levels. These impacts create a disturbed environment similar to agricultural fields and their soil structure, which can impact native and non-native plant communities. Garlic mustard is considered a nitrophilic species because it accumulates and stores multiple forms of N (Marschner 2002). Its growth has also been shown to positively respond to an increase in ammonium concentration, and its chlorophyll content increases with soil nitrate (Hewins and Hyatt 2009). The impacts of earthworms on ecosystem processes may promote garlic mustard invasion, specifically due to accelerated nutrient cycling and increased N availability. Garlic mustard may facilitate earthworm invasion by contributing high N leaf litter to the organic layer. It has also been suggested, although not directly studied, by Callaway et al. (2008) that European earthworms interact with the biochemical mechanisms of garlic mustard, further facilitating invasion. The environmental impacts of these species have the potential to create a positive feedback mutualism between them, where the effects of European earthworms facilitate the invasion of garlic mustard, which facilitates the continued invasion of earthworms, and so on. This cycle will continue until it reaches an environmental constraint that will either slow or halt the feedback. Based on this model, we expect to find greater earthworm abundance and biomass under garlic mustard than under native herbaceous plants.
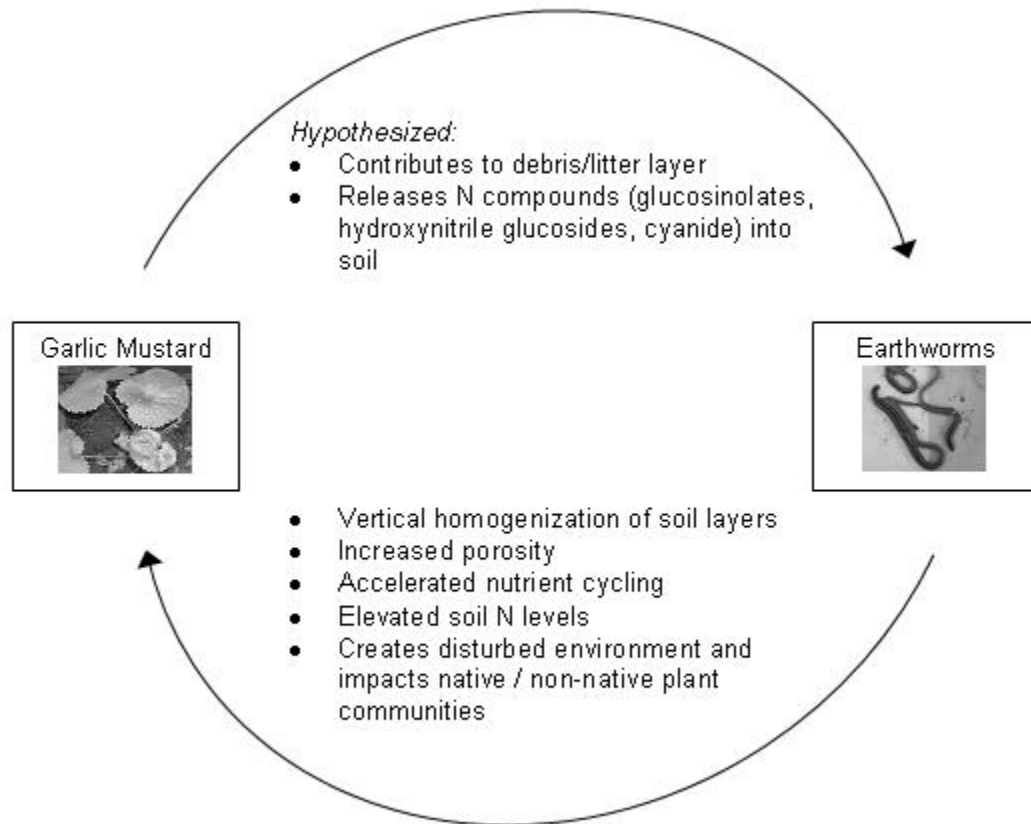
Figure 1. Conceptual model of hypothesized feedback between garlic mustard and

European earthworms. Garlic mustard increases available soil N and adds high quality

organic material to the leaf litter layer, promoting earthworm invasion and population

growth. Earthworms decrease native plant diversity and abundance, opening space for

plant invasion, promoting garlic mustard establishment, increasing garlic mustard whole

plant growth rate, population growth and reproduction.

    The goal of this study was to better understand invasive species interactions and

invasion facilitation, with the specific aim of characterizing the relationship between

garlic mustard and non-native earthworm species in forest understories to develop a mechanistic understanding of garlic mustard's influence on the presence of European earthworms. We hypothesized that greater European earthworm abundance and biomass will be found in plots with high garlic mustard density; lower earthworm abundance and biomass will occur in plots with high density of a native mustard, cut-leaved toothwort (*Cardamine concatenata*), or wild ginger (*Asarum canadense*); and lowest earthworm abundance and biomass will be found in control plots with no plant cover.

BACKGROUND

Definition of an 'Invasive Species'

A biotic invader can be defined as a species that establishes a new range in which it is able to acquire a competitive advantage, proliferate, spread and persist. Non-native invasive species have the potential to significantly alter ecosystem processes, at times to the detriment of the ecosystem and other plant and animal species (Mack et al. 2000; Valéry et al. 2008). Not all non-native species, however, are considered invasive, and their effects on ecosystem processes and individual species can be neutral or fluctuate over time (Davis et al. 2011). Invasions have been occurring throughout Earth's history through a series of biotic exchanges by alterations in physical and climatic barriers that limit the geographical extent of species and biotas. There is often an unequal exchange of biota due to the differences in species resistance and extinctions in the recipient biota (Vermeij 1991). Most species require some type of open space or niche to establish themselves in a new area (Vermeij 1991). Anthropogenic modification of ecosystem processes and species distribution has impacted the ability of recipient biotas to compete against invasion. The movement of invasive species is an important ecological outcome from these modifications, due to increased transport and commerce over the past 100 years (Mack et al. 2000). While many species are transported to new areas, only a small number become naturalized, and of those only a few become invasive (Mack et al. 2000).

Several hypotheses have been developed to explain why non-natives are able to establish populations and persist in a new range. The enemy release hypothesis explains that the escape of a non-native species from its native biological controls can contribute

to its invasibility. Invasive species can also garner assistance from human-caused

disturbance of native communities. The propagule pressure hypothesis is based on the

number of individuals introduced and the number of introduction attempts (Colautti et al.

2006). Typically, invasion is facilitated when both of these variables increase, which can

be a result of cultivation and husbandry in non-native regions, and unintentional

introductions, such as the transport of seeds that are caught in the grooves of hiking boots

or in the treads of tires (Mack et al. 2000). In addition, non-native animals have the

ability to cause extinctions of vulnerable native species through predation, grazing,

competition and habitat alterations; likewise, non-native plants can alter ecosystem

processes in the invaded area, significantly decreasing the abundance and survival of

native species (Mack et al. 2000). The novel weapons hypothesis explains that the

success of some invasives may be due to their release of novel biochemicals that have not

yet been encountered by naïve species (Callaway and Ridenour 2004). This creates a

competitive advantage in favor of the invader and can disrupt ecosystem processes and

the pre-invasion plant community. These are just a few well-known hypotheses within

the field of invasion ecology, highlighting the many mechanisms by which invasive

species establish, proliferate, and interact with other species.

Invasive European earthworms

Invasive exotic earthworms in North America have received attention recently

due to the major changes in patterns of nutrient cycling and loss they have the ability to

cause; yet, they have been overlooked in invasion studies and management (Bohlen et al.

2004 a). Few native earthworm species and populations remain in north temperate

ecosystems due to their elimination during the Pleistocene glaciation approximately 12,000 years ago (Bohlen et al. 2004 a; Heneghan et al. 2007; Hendrix et al. 2008; Madritch and Lindroth 2009). Non-native European earthworm species were first brought to North America by early settlers via plant materials and ship ballast. Presently, they are widely distributed by vermiculture practices and as fish bait, and by the transport of plant and soil materials. The slow recolonization (Terhivuo and Saura 2006) of native earthworms back into North American temperate ecosystems, as well as propagule pressure, has contributed to the establishment of non-native earthworm populations (Hendrix et al. 2008; Madritch and Lindroth 2009),

Three earthworm ecological groups have been identified based on physical characteristics, as well as feeding and burrowing behavior of species (Hale 2007). Each group has varying effects on ecosystem processes. Epigeic, or litter-dwelling, species, such as *Dendrobaena octaedra*, primarily influence the forest floor and do not burrow below the litter layer. These worms feed on leaf litter as well as the fungi and microorganisms found within it and have less impact on soil processes than other groups. They are pigmented and are small in size (1-7 cm long). Endogeic species, such as *Aporrectodea calignosa*, are soil dwelling species that are found between 0 and 50 cm in depth. They create a network of horizontal, branching burrows, affecting mineral soil horizons. This group feeds on a large amount of mineral soil and also digests organic material, soil microorganisms and fungi found there. Endogeic worms contribute to mineral soil mixing and aid in the development of the A soil horizon. They are not pigmented and range in size from small to medium (2-12 cm long). Species such as *Lumbricus rubellus* that live and feed in the litter layer and the top few inches of the

mineral soil layer, but do not form permanent burrow systems, are often referred to as epi-endogeic worms because they exhibit characteristics that fall within both epigeic and endogeic ecological groups. Unlike endogeic worms, epi-endogeics graze in the rhizosphere of plant roots. They also rapidly consume leaf litter and are associated with significant changes in forest soils and plant communities. Deep burrowing anecic species, such as *Lumbricus terrestris*, are large in size (8-15 cm long) and consume a large amount of fresh leaf litter. An established population has the potential to consume all litter produced in a forest each year. They create deep vertical burrows up to two meters in depth with entrances that are flanked with a mound of castings (excrement) and fragmented leaf litter. Anecic species have the potential to significantly reduce the forest floor and mineral soil horizon, impacting soil processes and properties of the forest (Hale 2007; GLWW 2012).

Bohlen et al. (2004 b) addressed the global hypothesis that worm invasion has large consequences for nutrient retention and uptake. They found earthworms' burrowing activities create macropores that affect soil porosity and hydrology, which alters soil horizons, decreasing and possibly eliminating the surface organic horizon. Earthworms also reduce levels of organic matter in the soil, resulting in the shifting distribution of fine roots and the alteration of mycorrhizal interactions (Heneghan et al. 2007). Many studies have documented these effects in cultivated and agricultural soils, but few studies have been conducted on forest soils; regardless, these results suggest that earthworms are important ecosystem engineers and can significantly affect nutrient cycling, retention and loss in forest soils (Bohlen et al. 2004 b). This has potential consequences for soil microbial functioning in invaded areas, suggesting that exotic

earthworm invasion has the potential to alter the structure and function of temperate forest ecosystems.

IMH case studies: European earthworms and European shrubs

Heneghan et al. (2007) examined the invasional meltdown process between European buckthorn (*Rhamnus cathartica*) and European earthworms. Earthworm populations were sampled in three subcommunities, and highest earthworm density and biomass were found in areas dominated by buckthorn, while lowest earthworm density and biomass were found in the un-invaded upland forest subcommunity, suggesting a positive relationship between the species (Heneghan et al. 2007). Buckthorn populations are associated with soils that have high elevated nitrogen (N), modified N cycling, and elevated soil moisture and pH (Heneghan et al. 2006, Heneghan et al. 2007). The presence of earthworms accelerates the mineralization of N, modifying key aspects of soil functioning (Bohlen et al. 2004 b). Therefore, changes in resource availability as a result of worm invasion may result in the invasion of exotic shrub species, forming a positive feedback loop that promotes a synchronized invasion (Heneghan et al. 2007).

Madritch and Lindroth (2009) also examined the relationship between invasive shrubs and invasive earthworms. They hypothesized that if a strong facilitation existed between the species, then controlling aboveground shrubs would reduce earthworm populations. The study was conducted at the University of Wisconsin Arboretum at Madison, Wisconsin where there are no natives. The study took place in two red oak woodland stands, one with a buckthorn understory and one with a honeysuckle (*Lonicera mackii*) understory. Their results show that the removal of invasive shrubs significantly

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reduced the abundance and biomass of exotic worms by approximately 50 percent due to the reduction of high quality leaf litter in the plots. Data also indicated that there was a competitive release and shift in earthworm community composition, resulting in a reduction of smaller non-native worm species. These results illustrate that removing invasive shrubs has direct, negative effects on worm abundance (Madritch and Lindroth 2009). However, the observed decrease in abundance may have simply been due to the removal of a high input of biomass, and similar results may have been seen if native shrubs were removed. These studies support the conclusion by Simberloff and Von Halle (1999) that mutualisms between plants and animals that disperse and/or pollinate them and the modification of habitat by both animals and plants appear to be common and are important in facilitating invasions.

Garlic mustard

Garlic mustard (Brassicaceae) is a non-native, biennial shade-tolerant Eurasian forb that was introduced into North America during the mid 1800s and is now considered a problematic invasive plant species in eastern North American forest understories (Callaway et al. 2008; Rodgers et al. 2008 b; Bauer et al. 2010). Throughout the past century there has been an acceleration of industrial activity, increasing the availability of Nitrogen (N) in the soil, which may be influencing the population growth rate of garlic mustard. Hewins and Hyatt (2010) found that increased levels of available N may facilitate the invasion of garlic mustard by increasing the range of suitable habitats and by also enhancing N uptake that leads to the production of secondary compounds that disturb native species' belowground mutualisms. Because garlic mustard produces a

variety of secondary compounds, such as glucosinolates, flavonoids and cyanide, which can interfere with the growth and reproduction of other plant species, increased N availability is a serious concern (Barto et al. 2010; Hewins and Hyatt 2010).

Garlic mustard also has the potential to impact native plant community composition. Stinson et al. (2007) found that increasing garlic mustard invasion reduces native plant diversity, specifically native graminoid and tree seedling abundance, which may affect the recruitment of tree seedlings. These effects may be a result of the 'Novel Weapons Hypothesis' (NWH).

The NWH states that invasive plants excel in new ranges due to the novel metabolites they produce, which native species possess little resistance to. This theory is supported by the finding that garlic mustard's phytochemical profile differs significantly from four common and closely related Brassicaceaeous species, while the native mustards have the most similar phytochemical profiles. The allelochemicals, cyanide, along with certain glucosinolates and flavonoids, were found only in garlic mustard and not in any of the native mustards studied (Barto et al. 2010). Biological effects of such novel weapons were examined by Callaway et al. (2008). Their study suggests that garlic mustard inhibits mycorrhizal fungal mutualists of North American native plants and also has greater inhibitory effects on mycorrhizas in invaded soils than on native soils. This effect is most likely a result of the novel flavonoid extracts and antifungal phytochemistry exhibited by garlic mustard. North American plant species experience severe inhibition due to the disruption of the mutualistic association between them and belowground arbuscular mychorrhizal fungi while European plants are affected little, supporting the NWH (Stinson et al. 2006; Callaway et al. 2008).

Cut-leaved toothwort

Cut-leaved toothwort (*Cardamine concatenata*) (Brassicaceae) is a native ephemeral herbaceous perennial wildflower commonly found throughout rich temperate deciduous forests (Schemske et al. 1978). It blooms in early spring between the months of March and May (Henn 1998; Voss and Reznicek 2012) and senesces by June.

Cut-leaved toothwort belongs to the tribe Cardamineae and is closely genetically related to the tribe Thlaspideae, of which garlic mustard belongs (Al-Shehbaz et al. 2006); their generas are thought to have diverged within the last 26 million years during the Oligocene (Koch et al. 2000). Although cut-leaved toothwort is closely genetically related to garlic mustard, and native mustards exhibit a distinctly different phytochemical profile from garlic mustard, they do share some chemical features with garlic mustard (Barto et al. 2010). For these reasons, cut-leaved toothwort was used as a native mustard comparative in this study to address if European earthworms do have a preference for or exhibit positive feedback with its native range co-occurring plant, garlic mustard.

Wild ginger

Wild ginger (Aristolochiaceae) is a native herbaceous perennial wildflower found throughout rich deciduous forests and wooded floodplains. The plant blooms in early spring between the months of April and May, and the leaves persist through the summer and early fall (Henn 1998; Voss and Reznicek 2012). It is a clonal, rhizomatous plant (Cain and Damman 1997) and has roots that exude a ginger odor and taste. Like garlic

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mustard, wild ginger has the capability of creating dense mats in the forest understory by its sprawling groundcover habit (Smith and Reynolds 2012).

Wild ginger belongs to the order Piperales, which last shared a common ancestor with the Brassicales approximately 130 mya (Bell et al. 2010). Because of wild ginger's similarity in form and habit and its extremely distant relationship with garlic mustard, it was used as a non-mustard comparative in this study.

Predictions regarding garlic mustard, cut-leaved toothwort and wild ginger

As described above, cut-leaved toothwort and wild ginger were chosen because of their closely-relatedness and distantly-relatedness, respectively. We choose to compare earthworm abundance and biomass under garlic mustard to a closely-related species to examine if earthworms prefer a plant they co-evolved with, or if they prefer a specific family that exudes a specific set of chemicals. We also chose to compare worm abundance and biomass under garlic mustard to a distantly-related species to further examine if there is a similarity in preference for a non-mustard herb as a mustard herb.

Cut-leaved toothwort was selected as the closely-related species comparison because it is a native mustard, and although it does exhibit a different chemical profile from garlic mustard, it also exudes similar allelochemicals. Wild ginger was selected as the distantly-related species comparison because it is not in the same family as garlic mustard, but is still a native herb. It is distinctly different from garlic mustard. We predict earthworm abundance and biomass under cut-leaved toothwort to be more similar to garlic mustard because it is a closely-related species, and we predict worm abundance

and biomass under wild ginger to be different from garlic mustard and cut-leaved toothwort.

MATERIALS AND METHODS

Study Site

The study was conducted at Wright State University (WSU) Biological Preserve in Bath Township, Greene County, Ohio (39º48'N, 84º30'W). The WSU preserve is approximately 80ha with Miamian silt-loam soils over shale and limestone bedrock (Garner et al. 1978). The preserve is composed of two secondary stands and one old growth stand (DeMars and Runkle 1992). The forest canopy is dominated by oak (*Quercus* spp.) and sugar maple (*Acer saccharum*) with an herbaceous spring ephemeral wildflower ground layer (DeMars and Runkle 1992). The understory is also composed of a shrubby layer dominated by honeysuckle (*Lonicera* spp.) and spicebush (*Lindera benzoin*). This study was conducted in the southern extent of the old growth stand in the upland area (Figure 2). Disturbances that may promote the spread of invasive species throughout the preserve include established foot trails, water drainage and associated erosion from paved campus surfaces, as well as disturbance caused by the invasion of other exotic plant and animal species.

Figure 2. Aerial image taken in fall of study site (yellow circle indicates sampling location for cut-leaved toothwort and bareground plots; red circle indicates sampling location for garlic mustard and wild ginger plots). Satellite image from Greene Co, OH GIS (2012).

Field sampling

Field sampling was conducted to compare earthworm abundance, biomass and functional group composition under the focal plants and control. A total of 225 plots were sampled across four treatments in three sampling periods: garlic mustard plots, cut-leaved toothwort plots, wild ginger plots, and plots with no plant cover (bareground). Twenty-five plots of each treatment were sampled during each sampling period, with the exception of bareground plots, which were sampled during spring 2012 only (Table 1).

Plots were chosen haphazardly based on percent cover with the criteria that the dominant

plant within the plot must be the desired focal plant. Plots were located within larger

patches of the desired focal plant. Because of this study design, we were unable to

separate out site or plot effects.

Table 1. Number of plots sampled for each focal plant and control in each season.

Focal Plant	Spring 2011	Fall 2011	Spring 2012
Alliaria	25	25	25
Asarum	25	25	25
Cardamine	25	0	25
Bareground	0	0	25

Earthworms were collected using liquid mustard extraction, which acts as a skin

irritant causing earthworms to surface from the soil (Lawrence and Bowers 2002; Hale

2007). For every plot sample, forty grams of ground yellow mustard seed was mixed

with 100mL of water in a small portable jar in lab prior to sampling. Immediately prior

to sampling, plots were prepared by removing leaf litter and marking the sampling area,

and the 40g mustard mixture was mixed with 3.8 L of water and inverted several times to

mix well. Plots were delimited during sampling with a 0.1 steel quadrat with 12 cm high

sides and an open top and bottom. The quadrat was placed over the sample area and sunk

approximately 5 cm into the ground to trap the earthworms that would surface and to

contain the liquid mustard solution during the extraction. One third of the solution was

poured over the sample area until the area was soaked. Surfaced earthworms were

collected using forceps and placed in a labeled collection bag. Every five minutes the

next third of the solution was poured over the plot until the entire 3.8L solution was used

up. After the final pouring, collection continued for an additional 5 minutes. Removed leaf litter was scattered back over the sample area after sampling was finished.

Plant cover estimates and earthworm sampling were conducted three times throughout the study: spring [May through early July] and fall [October through early November] 2011 and spring [April through early May] 2012 when soil moisture and temperature is considered most favorable for earthworm activity (Lawrence and Bowers 2002; Holdsworth et al. 2007). Unusually high temperatures in the late winter and early spring months of 2012 led to an earlier sampling period in 2012 than in 2011.

Laboratory methods

Collected earthworms were brought back to the lab and stored in the refrigerator for two days to allow the worms to empty their gut content to decrease variability associated with their mass. Hale's field guide (2007) was then used to identify earthworms to genus and to species when possible. Species collected and their abundance in each plot were recorded. Earthworm species were also classified by their functional groups during analysis. Earthworms were anesthetized and stored in the freezer after identification until they were dried. Because the moisture and gut content of worms account for a large degree of variability in biomass measures, earthworm ash-free dry weight was determined. Earthworms were first dried in a drying oven at 65ºC for 48 hours and weighed (Hale et al. 2004). A subsample of worms from 30 plots was then ashed in a muffle furnace at 500ºC for four hours. A regression of the subsample's ash-free dry weights plotted against the dry weights was conducted, and a linear line of best

fit was fitted against the data to calculate an equation to estimate ash-free dry weights of all other samples yielding:

$$\text{ash weight (g)} = 0.263 * \text{dry weight (g)} + 0.0011 \ (R^2=0.84).$$

The R^2 value of the equation is consistent with Hale et al.'s (2004) allometric equations R^2 values, which range from 0.84 to 0.97. Separate equations were not developed for each species because Hale et al. (2004) did not find significant differences between the allometric equations developed for the species found in our study.

The percent plant cover of each plot was estimated using digital image analysis to provide a more accurate estimation of cover than typical sight estimates (Appendix A). This enabled us to examine the possible relationship between percent plant cover and earthworm abundance and biomass. Digital photographs of each plot were taken and were then converted to black and white images using Photoshop© to determine the percent plant cover in each plot.

Statistical analyses

All statistical analyses were conducted in R (R Core Development Team 2009). Data violated assumptions of a normal distribution of residuals and could not be transformed. Therefore, we conducted one-way analysis of variance (ANOVA) permutations with Monte Carlo analysis to compare earthworm abundances and biomasses with and without juveniles included in the models by plant, season, and functional groups. Individual one-way ANOVA permutations were conducted for each pair-wise comparison within the plant, season and functional group variables. Because multiple comparisons were conducted on the data, the Bonferroni correction was applied

to the critical p-values of each test. We conducted Spearman Rank Correlations between

percent plant cover and earthworm abundance and biomass of worm functional groups

under each plant and the control. It should be noted that when analyses were conducted

on earthworm functional groups, data from juveniles was omitted, and biomass refers to

the estimated ash-free dry weights of earthworms. See Appendix B for R code.

RESULTS

We collected 3702 individual earthworms from plots throughout the entire course of the study (Table 2). Eleven thousand and thirty individuals were collected in spring 2011, 1225 individuals were collected in fall 2011, and 1347 individuals were collected in spring 2012. Of these, no native species were collected. When juveniles were excluded from count data, 150 individuals were collected in spring 2011, 199 individuals were collected in fall 2011, and 264 individuals were collected in spring 2012. Five species and three earthworm ecological groups were identified; no strictly epigeic earthworms were found. Juvenile worms were identified only to genus because they did not exhibit sexual features that are used to identify individuals to the species level.

Table 2. Number of earthworms collected by species, categorized by earthworm groups. This table excludes *Lumbricus* juveniles (1738) and *Aporrectodea* juveniles (1351) because they could not be categorized.

Earthworm Functional Group	Earthworm Species	Number
Endogeic	*Aporrectodea calignosa complex*	292
	Aporrectodea longa	2
	Aporrectodea rosea	93
Anecic	*Lumbricus terrestris*	123
Epi-endogeic	*Lumbricus rubellus*	103

Percent plant cover and European earthworm abundance and biomass

Abundance of earthworms in all functional groups under wild ginger and cut-leaved toothwort was not correlated with percent plant cover (Table 3; Fig. 3). Endogeic

earthworm abundance was significantly negatively correlated with increasing percent cover of wild ginger. Biomass of earthworms was not correlated with percent cover of garlic mustard, cut-leaved toothwort. Anecic earthworm biomass was significantly positively correlated with increasing percent cover of wild ginger (Table 3; Fig. 4).

Overall, epi-endogeic earthworms appeared to be the only functional group that was not significantly correlated with increasing plant cover of any focal plant. Worm functional groups that were significantly correlated with plant cover of the focal plants were so only with wild ginger.

Table 3. Spearman rank correlation coefficients for each cover and earthworm functional group abundances and biomasses comparison within each focal plant (bold indicates significant correlation; critical p-value with Bonferroni correction is 0.005). Ap, Ac, Cc and BG indicate *A. petiolata*, *A. canadense*, *C. concatenata*, and bareground, respectively; An, En and Epi indicate anecic, endogeic and epi-endogeic functional groups, respectively.

Cover Comparison	Spearman's rho	p-value
Earthworm abundance		
Ap: An	0.245	0.155
Ap: En	0.164	0.198
Ap: Epi	-0.051	0.825
Ac: An	0.400	0.026
Ac: En	-0.545	**<0.001**
Ac: Epi	-0.073	0.759
Cc: An	0.417	0.059
Cc: En	-0.025	0.886
Cc: Epi	0.195	0.397
Earthworm biomass		
Ap: An	0.181	0.294
Ap: En	-0.039	0.760
Ap: Epi	-0.244	0.284
Ac: An	0.585	**0.001**
Ac: En	-0.331	0.016
Ac: Epi	0.154	0.512
Cc: An	0.307	0.017
Cc: En	-0.046	0.797
Cc: Epi	-0.006	0.977

Figure 3. Spearman rank correlation regressions of earthworm functional group abundances as a function of percent plant cover for focal plants (labeled p-values for significant correlations only). See Table 3 for p-values.
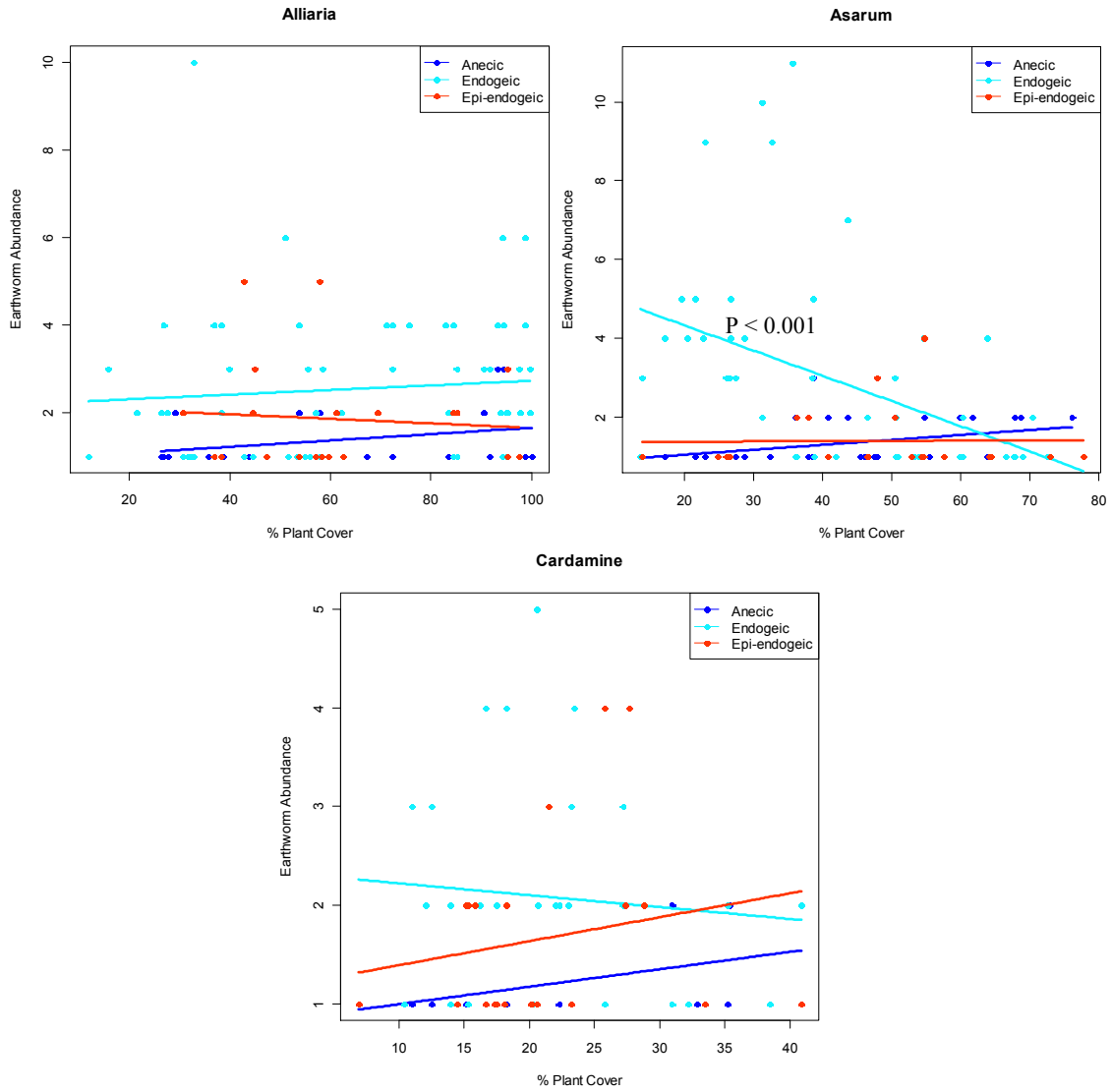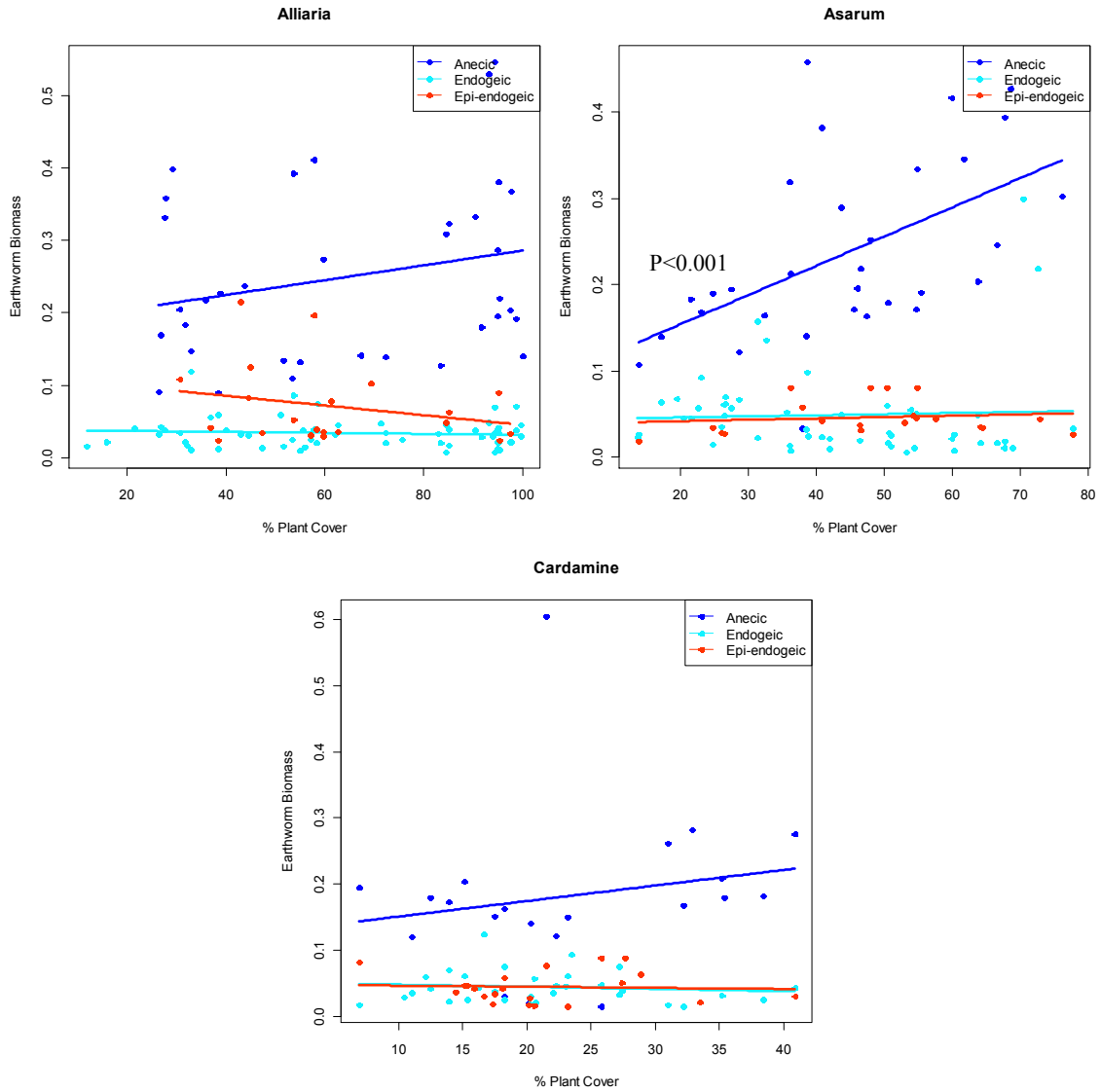
Figure 4. Spearman rank correlation regressions of earthworm functional group biomasses as a function of percent plant cover for focal plants (labeled p-values for significant correlations only). See Table 3 for p-values.

Season / focal plants and European earthworm abundance and biomass

Significant differences were found between plant cover type and between seasons; however, there was no significant interaction between plant cover type and season. Total earthworm abundance per plot did not differ between the spring sampling seasons, but did between the spring seasons and fall season. There were significantly 1.08 times more earthworms per plot in fall 2011 than in spring 2011, and 1.09 times more earthworms in spring 2012 than in fall 2011. Total earthworm biomass per plot in spring 2012 was approximately 28% lower than spring 2011 and fall 2011. When juveniles were excluded from analysis, earthworm abundance per plot did not differ between the spring seasons, but was approximately 25% lower than the fall season. Earthworm biomass per plot did not differ between the sampling seasons (Table 4; Fig. 5).

Total earthworm abundance per plot did not differ between garlic mustard and wild ginger focal plants. Total worm abundance per plot under cut-leaved toothwort was significantly 25% lower than garlic mustard and wild ginger, and abundance per plot under bareground was significantly 50% lower than all three of the focal plants. Total earthworm biomass per plot did not differ between garlic mustard, wild ginger and cut-leaved toothwort focal plants. Total worm biomass per plot under bareground was significantly 41% lower than under the focal plants (Table 5; Fig. 6). When juveniles were excluded from analysis, earthworm abundance per plot under focal plants did not differ. Adult worm abundance per plot under bareground was significantly 50% lower than under garlic mustard and cut-leaved toothwort, but was not different from wild ginger. Adult earthworm biomass per plot did not differ between the focal plants and bareground (Table 5; Fig. 6).

Table 4. One-way ANOVA Monte Carlo analysis results for each season comparison of

differences in earthworm abundance and biomass with and without juveniles (critical p-

value with Bonferroni correction is 0.017).

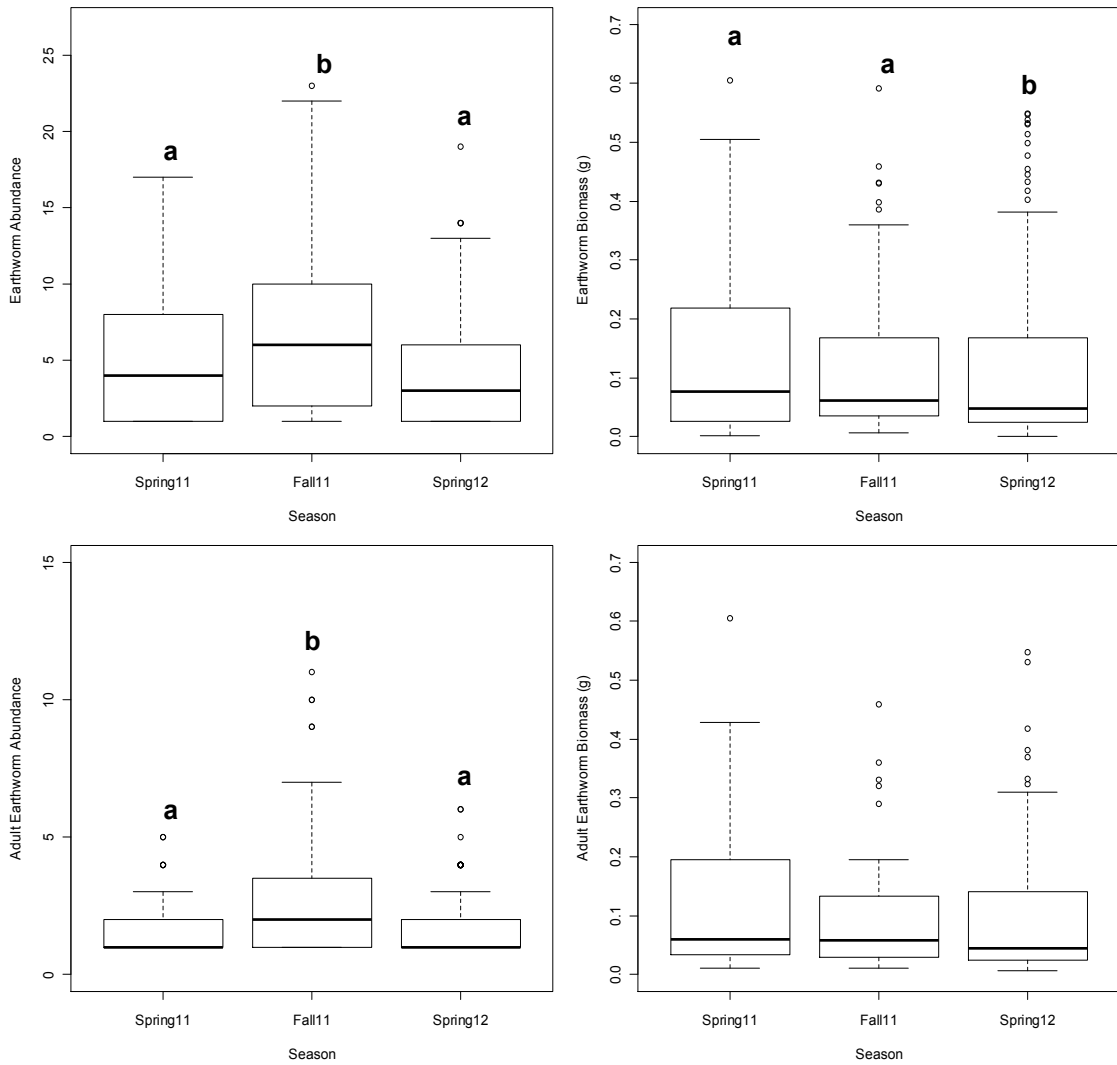| Season Comparison	p-values	
	Earthworm Abundance	Earthworm Biomass
Total		
Spring 2011 – Fall 2011	**<0.001**	0.130
Spring 2011 – Spring 2012	0.019	**0.013**
Fall 2011 – Spring 2012	**<0.001**	0.458
Adults Only		
Spring 2011 – Fall 2011	**<0.001**	0.061
Spring 2011 – Spring 2012	0.548	0.021
Fall 2011 – Spring 2012	**<0.001**	0.875

Figure 5. Boxplots showing earthworm abundance and biomass per plot with juveniles (top row) and without juveniles (bottom row) for each season (different letters indicate significant differences by Monte Carlo analysis with Bonferroni correction). Box plots show the minimum, upper and lower 25% quartiles, median, maximum and outliers. See Table 4 for p-values.

Table 5. One-way ANOVA Monte Carlo analysis results for each focal plant and control comparison of differences in earthworm abundance and biomass with and without juveniles (critical p-value with Bonferroni correction is 0.008). Ap, Cc, Ac and BG indicate *A. petiolata*, *C. concatenata*, *A. canadense* and bareground, respectively.

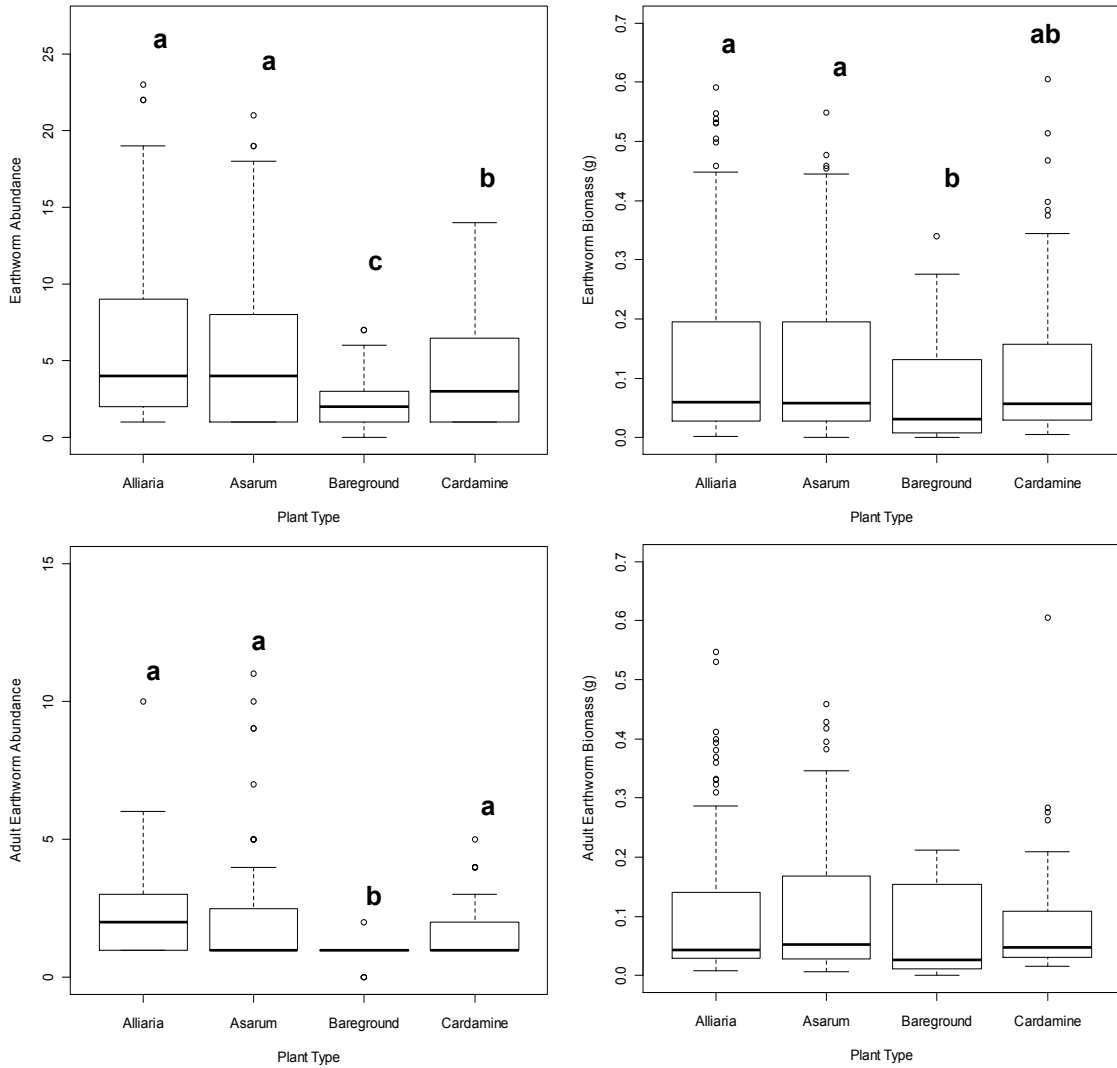| Comparison	p-values	
	Earthworm Abundance	*Earthworm Biomass*
Total		
Ap – Cc	**0.004**	0.073
Ap – Ac	0.988	0.647
Ap – BG	**<0.001**	**0.008**
Cc – Ac	**0.004**	0.154
Cc – BG	**<0.001**	0.070
Ac – BG	**<0.001**	0.010
Adults Only		
Ap – Cc	0.059	0.196
Ap – Ac	0.851	0.942
Ap – BG	**0.001**	0.390
Cc – Ac	0.107	0.162
Cc – BG	**<0.001**	0.820
Ac – BG	0.013	0.343

Figure 6. Boxplots (see Fig. 5 for description) showing earthworm abundance and biomass per plot with juveniles (top row) and without juveniles (bottom row) for each plant type and control (different letters indicate significant differences by Monte Carlo analysis with Bonferroni correction). See Table 5 for p-values.

Earthworm functional group comparisons

Under garlic mustard, wild ginger and cut-leaved toothwort focal plants, the endogeic worms had two times significantly greater abundances than anecic and epi-endogeic worms, which did not differ from each other. Abundances of all functional groups beneath bareground did not differ (Table 6; Fig. 7). Biomass of anecic worms was approximately 3.7 times significantly greater than epi-endogeic worms beneath garlic mustard, and endogeic worms had the least abundance. Under wild ginger and cut-leaved toothwort, biomass of anecic worms was four times greater than endogeic and epi-endogeic worms, which did not differ from each other. Anecic and epi-endogeic worm biomass did not differ from each other and were 7.5 times significantly greater than endogeic worms under bareground (Table 6; Fig. 8).

Although endogeic worms comprise the largest proportion of number of worms in all focal plants, anecic worms contribute the most biomass in all focal plants and the control (bareground), while endogeics contributed the least.

Table 6. One-way ANOVA Monte Carlo analysis results for each earthworm functional group comparison of abundance and biomass beneath each focal plant and control (critical p-value with Bonferroni correction is 0.0042). Ap, Ac, Cc and BG indicate *A. petiolata*, *A. canadense*, *C. concatenata* and bareground, respectively. An, En and Epi indicate anecic, endogeic and epi-endogeic functional groups, respectively.

Comparison	p-values	
	Earthworm abundance	*Earthworm biomass*
Ap: An – En	**<0.001**	**<0.001**
Ap: An – Epi	0.069	**<0.001**
Ap: En – Epi	0.069	**<0.001**
Ac: An – En	**0.001**	**<0.001**
Ac: An – Epi	0.815	**<0.001**
Ac: En – Epi	0.012	0.819
Cc: An – En	**0.001**	**<0.001**
Cc: An – Epi	0.084	**<0.001**
Cc: En – Epi	0.140	0.968
BG: An – En	0.341	**<0.001**
BG: An – Epi	0.721	0.051
BG: En – Epi	0.721	**0.001**

Figure 7. Boxplots (see Fig. 5 for description) showing earthworm abundance per plot for each earthworm functional group under each focal plant and control (different letters indicate significant differences by Monte Carlo analysis with Bonferroni correction). See Table 6 for p-values.
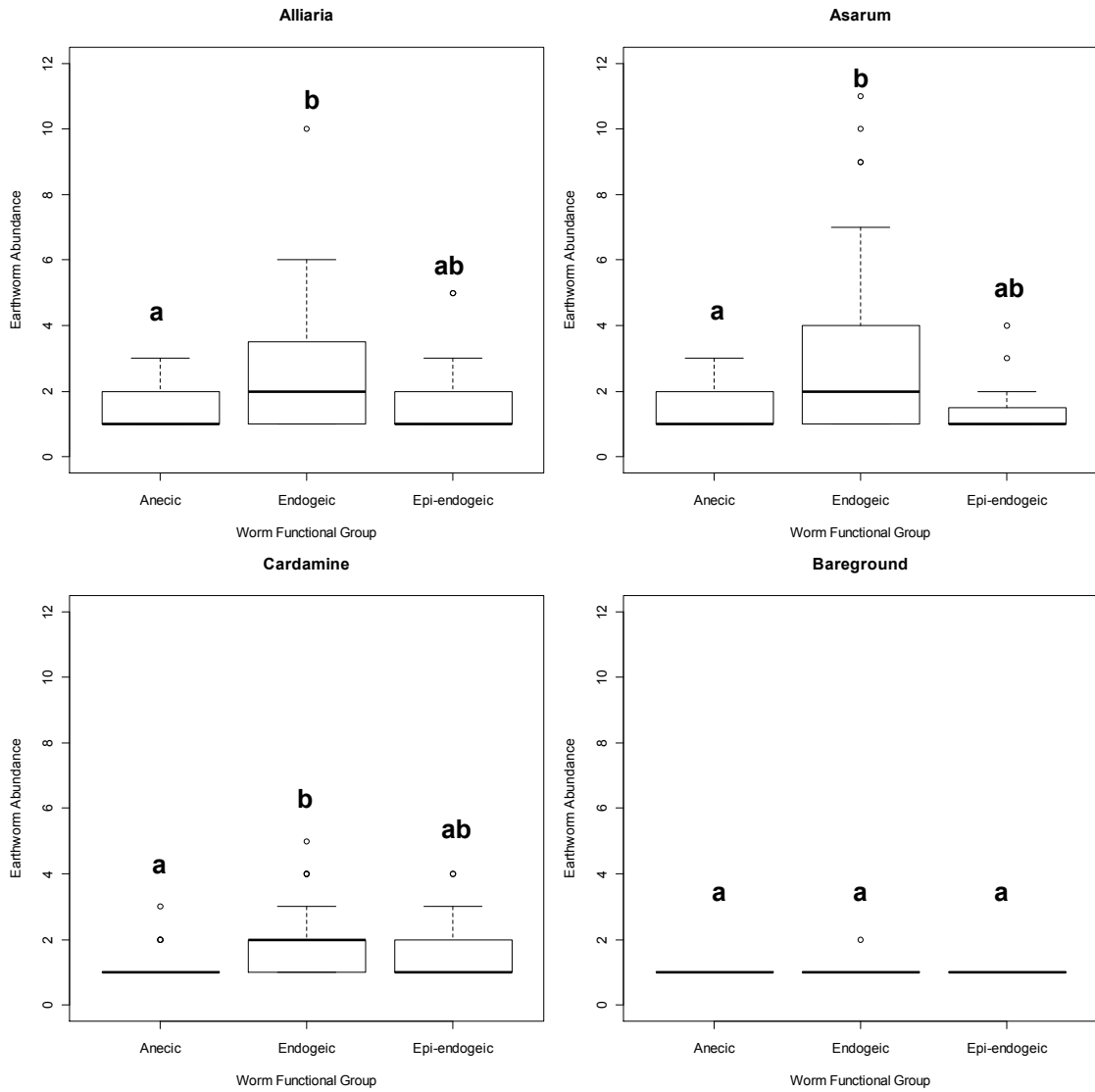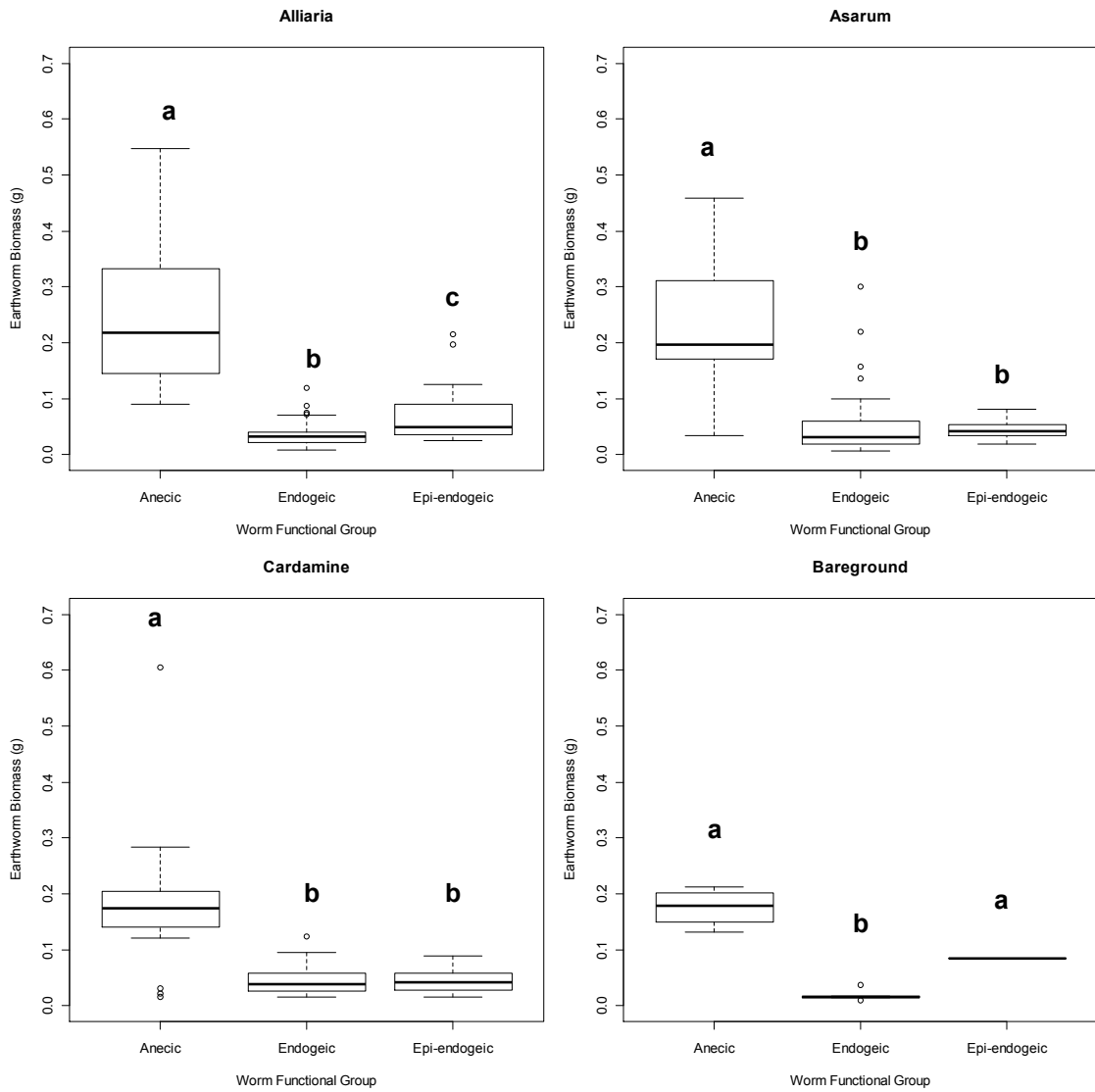
Figure 8. Boxplots (see Fig. 5 for description) showing earthworm biomass per plot for each earthworm functional group under each focal plant and control (different letters indicate significant differences by Monte Carlo analysis with Bonferroni correction). See Table 6 for p-values.

DISCUSSION

The purpose of this study was to characterize the relationship between garlic mustard and European earthworms with the aim of developing a mechanistic understanding of their influences on each other. By comparing invasive earthworm abundance and biomass under the invasive garlic mustard and two native plants, we found differences in earthworm abundance between garlic mustard and cut-leaved toothwort, but not wild ginger. We found no differences in earthworm biomass between the plants. Because we looked at only one half of the feedback cycle and did not find evidence of garlic mustard facilitating invasion of earthworms, we cannot claim that positive feedback or invasion meltdown is occurring within this system. If earthworm facilitation was found in this system, we would expect earthworm abundance and biomass to be positively correlated with increasing garlic mustard percent cover; however, our results do not support our hypothesis. In the few cases where abundance or biomass of earthworm groups were either positively or negatively correlated with plant cover, it did not occur with garlic mustard, but instead with the two native focal plants, wild ginger and cut-leaved toothwort. We also expected garlic mustard to contain greater abundances and biomasses of earthworms than the other two focal plants if positive feedback was acting in this system. Our results show that earthworm populations differed between seasons. Additionally, our results show that worm abundances did not differ between garlic mustard and wild ginger, and worm biomasses did not differ between all three focal plants, suggesting that garlic mustard does not differentially influence the presence of earthworms compared to the other herbs in this study.

Results from the seasonal comparison analyses indicate that more worms were collected per plot in fall than in the spring seasons. These results suggest that, in theory, if our study design had been balanced between seasons a greater number of total earthworms would have been collected in fall than in the spring seasons. A greater density of worms in fall than in spring is most likely observed because of the general ecology of earthworms. Peak worm cocoon production is in spring through early summer and fluctuates with seasonal variation (Edwards and Bohlen 1996). Sampling in the fall allows for the collection of worms that were otherwise uncollectable and still in the cocoon phase of their life cycle; therefore, there is a greater density of earthworms per unit area in fall than in spring.

Our results differ from conclusions drawn from other studies that have investigated similar earthworm and invasive plant systems. Invasion meltdown studies examining earthworm effects and invasive shrubs have suggested that there is a strong association between them and that positive feedback facilitates invasion of both earthworms and shrubs (Heneghan et al. 2007; Madritch and Lindroth 2009). Few studies have been conducted on invasion meltdown between forest herbs and exotic earthworms; however, those that have also suggest the IMH can be applied to an invasive herb-earthworm system. Nuzzo et al. (2009) surveyed plant communities and exotic earthworm biomass in invaded and uninvaded habitats by garlic mustard, barberry (*Berberis thunbergii*), and browntop (*Microstegium vimineum*) to test if increased non-native plant cover was associated with increased exotic earthworm biomass and decreased native plant cover. They found that worm biomass was significantly greater in invaded than uninvaded habitats. Their results suggest that there is a significant positive

association between earthworm biomass and non-native plant cover, as well as native

graminoid cover, all of which are negatively correlated with native woody plant cover.

We argue that differences may also be due to lower overall percent plant cover between

the invaded and uninvaded plots. They further suggest that because earthworm invasion

can negatively impact native species that are mycorrhizal, native sedges, such as

Pennsylvania sedge (*Carex pensylvanica*), and non-native species that are nonobligate or

nonmycorrhizal, such as garlic mustard, are favored and able to establish.

Several other reviews and studies that have not specifically looked at positive

feedback in this study's system have suggested that the effects of earthworms enhance

the establishment of garlic mustard and other non-natives. Bohlen et al. (2004b) suggests

that worms may aid the competitive success of non-mycorrhizal herbs at the expense of

mycorrhizal herbs, based on studies that have found a dominance of non-mycorrhizal

herbs in earthworm invaded sites. Likewise, Frelich et al. (2006) explains that the spread

of exotic shrubs and herbs, such as buckthorn (*Rhamnus* spp.) and garlic mustard may be

enhanced by worm invasion because they are better adapted to the presence of

earthworms and their effects. Hopfensperger et al. (2011) examined the effect of worms

on belowground seed banks and effects on aboveground plant dynamics and soil

characteristics. They found that fewer seeds germinated in plots dominated by multiple

worm species, resulting in lower percent plant cover and less diverse aboveground

vegetation. They suggest that forest floor disturbance caused by earthworms may allow

for more aggressive and competitive plants to invade, specifically, in their study, *Rubus*

spp. and *Carex* spp. We speculate that based on their conclusion, the competitive ability

of garlic mustard to invade would also be enhanced.

The novel results of this study were unexpected because they do not support the idea of garlic mustard directly facilitating earthworm invasion or that worms benefited from garlic mustard. Because of this, we propose that feedback does not occur between these species. While our study does not directly test the somewhat popular hypothesis that earthworms may facilitate the invasion of garlic mustard, our results may provide some insight to indirect facilitation at a highly local level. We have developed several explanations as to why we may have obtained these results.

Our results show that earthworms benefit from any type of plant cover. Bareground plots supported significantly fewer earthworms and less earthworm biomass compared to all the focal plants. However, in general, increasing percent plant cover does not appear to influence earthworm abundance or biomass. When earthworm abundance and biomass is analyzed by functional group, anecic and endogeic earthworms appear to be most affected by wild ginger and only slightly by cut-leaved toothwort. Anecic earthworm abundance and biomass increased under wild ginger, and anecic worm biomass also increased slightly under cut-leaved toothwort, suggesting that these deep-burrowing earthworms may benefit from increased cover of the native herbs. Endogeic worm abundance decreased and biomass slightly increased with increasing wild ginger percent cover. Increasing percent cover of wild ginger appears to be associated with a decreased amount of leaf litter (personal observation). The seemingly negative influence of wild ginger cover on endogeic (litter-dwelling) worms may be due to less available habitat and decreased food source.

Our data also suggest that earthworms may not prefer specific plants to feed on and live under, but rather may be more concerned with available plant biomass to feed

on. Earthworm abundance under cut-leaved toothwort was less than under garlic mustard and wild ginger. This may be due to cut-leaved toothwort contributing less aboveground and belowground biomass and litter to the leaf litter and organic soil layers, compared to garlic mustard and wild ginger which contribute a greater amount of biomass, decreasing the available amount of food for earthworms, resulting in decreased worm abundance. Although leaf litter has been described as the best food source for earthworms, living and dead herbaceous plant roots are also considered an important resource for worms (Lavelle 1988), especially for endogeic and epigeic worms that feed more on organic matter around roots, rather than on surface leaf litter (Edwards and Bohlen 1996). Earthworm biomass, however, does not appear to be affected by the focal plants in this study. Garlic mustard and wild ginger may not differ in earthworm abundance and biomass because of their similar habit and contribution of biomass to the litter layer. Bareground plots supported the least number of earthworms and the least worm biomass. This is most likely attributed to zero aboveground or belowground plant biomass and leaf litter contribution to the litter and organic soil layers. Alternatively, the lack of differences seen in worm abundance and biomass between garlic mustard and the natives may indicate that there was a recent invasion of earthworms into the native dominated plot areas, in which earthworm effects are not yet fully apparent. Therefore, the native herb dominated areas may have not yet experienced enough disturbance to facilitate garlic mustard invasion, which may not be far off from happening.

Of all three earthworm functional groups, endogeic worms were most abundant under all three focal plants, while anecic and epi-endogeic worms were less abundant. It appears that earthworm community structure is similar among the plants, with litter

dwelling earthworms (endogeic) acting as the dominant worm type. Anecic earthworms, on the other hand, contribute the most biomass under all focal plants, probably due to their larger size and greater consumption and incorporation of surface leaf litter, compared to endogeic worms (Lavelle 1988; Edwards and Bohlen 1996). Because of this, anecic worms most likely affect soil characteristics, nutrient cycling and above and belowground plant and animal community structure to a higher degree than endogeic worms (Hale 2007; GLWW 2012); however, the effects of anecic and endogeic worms were enhanced when they inhabited the same space (Edwards and Bohlen 1996).

While this study aimed to examine the mechanisms for and patterns of possible feedback between European earthworms and garlic mustard, there are several caveats to consider. The nature of the study design does not allow us to truly explore mechanisms of the proposed invasion feedback, but rather test if there is an association between worms and garlic mustard. We can then speculate from these associations what possible mechanisms, such as changes to soil horizons and nutrient cycling and habitat disturbance, may be at play in this system. Another limitation of this study is that it was conducted in only one Ohio forest, which may not reflect processes at other sites. There may be differing levels of plant and earthworm invasion, as well as physical characteristics influencing invasions, among different forests, which may result in differing community structure and invasion dynamics. For example, Suárez et al. (2003) looked at effects of worm activity on pools and cycling of soil P in two north temperate deciduous forests. They found that the earthworm effects on soil P are much more complex than originally thought and may depend upon other factors, such as land use history and pre-invasion soil properties. In addition, their data show that soil P fractions

differed between sites, and they suggest that these differences are due to a difference in dominant worm functional groups at the sites. In sites dominated by *L. rubellus* (epi-endogeic), they found a loss of total P, which may be due to increased rates of P cycling and leaching by these worms, whereas in *L. terrestris* (anecic) dominated sites, more available P was found. *L. rubellus* creates more casts per unit of body mass than *L. terrestris* and is also more affective at promoting formation of water-stable soil aggregates, which may, therefore, increase rates of soil P cycling (Suárez et al. 2003), and has the potential to influence plant community composition differently than another earthworm species would. These differences are not accounted for in this study because data were collected from only one site. Additionally, patch size and location varied between the focal plants. Although patch size was not measured, we did note that garlic mustard plots were located within a single large patch, while wild ginger plots were located within a few smaller patches. Cut-leaved toothwort plots, however, were located within several small patches that appeared to exhibit higher plant diversity than the garlic mustard and wild ginger patches. Finally, the advantage to sampling in the fall is that there is a more accurate representation of earthworm community structure because most earthworms have matured and fewer juveniles are present. The use of cut-leaved toothwort as a native mustard comparison in this study limited our sampling of this plant to the spring seasons only; therefore, we had a much larger count of juveniles, limiting our ability to identify many of the collected worms to the species level.

   To test if there is a behavioral response or preference of earthworms for a specific type of plant, we suggest that choice experiments be conducted to examine earthworm preference for garlic mustard and native plants. This type of experiment would enable

researchers to address the question of non-native plant-invasive earthworm facilitation. If feedback does occur between these species, then we would expect earthworms to have a strong preference toward garlic mustard when given a choice of varying species conditioned soil and/or leaf litter. The results of this study, however, suggest that there will be no preference by the earthworms. This type of study may also be better able to address the idea of possible facilitation of earthworms by garlic mustard invasion. We further suggest that studies addressing mechanisms of invasion facilitation and feedback be developed to truly determine if earthworm effects promote garlic mustard establishment.

In conclusion, our study provides no evidence in support of positive feedback between European earthworms and garlic mustard; however, it is possible that earthworms may facilitate garlic mustard invasion and that our data were not able to truly address this issue. Our data do not show greater earthworm abundance and biomass under garlic mustard than native herbs. The data also do not show noteworthy correlations between increasing plant cover and increasing worm abundance and biomass. Reasons for no apparent feedback in this study system may be attributed to the biennial nature of garlic mustard and low annual biomass input, compared to invasive shrubs, which have been utilized as a major area of study in IMH studies. Alternatively, there may simply be no association between earthworms and garlic mustard; thus, there may be no cause for concern. These results will be useful to both research scientists and land managers working with these invasive earthworms and plants on a daily basis. By understanding interactions between non-native and native plant species and mechanisms of invasion, we are better able to manage our natural areas and control invasive species

range expansion and establishment. Further research should be conducted to better

examine the garlic mustard-European earthworm system to expand upon this study.

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APPENDIX A

Determination of Percent Plant Cover

Materials:
Digital images of plots, computer with Adobe Photoshop©

1. Open image in Photoshop. Crop if necessary.

2. Toggle foreground to black.

3. Select the magic wand tool. Set the tolerance to 32. Click on an area of the leaf. From the pull down, click "Select" → "Select Similar". Hit the delete key. The leaf should turn white.

4. Repeat Step 3 until all areas of the plant are white.

5. Toggle foreground to white.

6. Repeat Steps 3 and 4, this time selecting and deleting the background (ground cover) of the image.

7. From the pull down menu, select "Image" → "Adjustments" → "Threshold". Set the threshold level to 128.

8.   Be sure no part of the image is selected. Examine the histogram in the upper right window. The color channel is not important (RGB works fine). Record the mean value.

9. Percent cover = 1 – ((255 – mean value) / 255) *100

Statistical Analyses R Code

Spearman Rank Correlations

```
setwd("G:/WSU/Thesis/Data")

######## Alliaria Cover Regression ########

gm <- read.csv("Alliaria.csv")

  #total
  cor.test(gm$Cover, gm$Abd, method = "spearman")

  lm1 <- lm(Abd~Cover*Funcgroup, data = gm)
  fit.plot(lm1, xlab = "% Plant Cover", ylab = "Earthworm
Abundance", main = "Alliaria")   #Produces correlation graphs

  cor.test(gm$Cover, gm$Ash, method = "spearman")

  lm1.2 <- lm(Ash~Cover*Funcgroup, data = gm)
  fit.plot(lm1.2, xlab = "% Plant Cover", ylab = "Earthworm
Biomass", main = "Alliaria")

  #Anecic
  cor.test(gm$Cover[gm$Funcgroup=="Anecic"],
gm$Abd[gm$Funcgroup=="Anecic"], method = "spearman")
  cor.test(gm$Cover[gm$Funcgroup=="Anecic"],
gm$Ash[gm$Funcgroup=="Anecic"], method = "spearman")

  #Endogeic
  cor.test(gm$Cover[gm$Funcgroup=="Endogeic"],
gm$Abd[gm$Funcgroup=="Endogeic"], method = "spearman")
  cor.test(gm$Cover[gm$Funcgroup=="Endogeic"],
gm$Ash[gm$Funcgroup=="Endogeic"], method = "spearman")

  #Epi-endogeic
  cor.test(gm$Cover[gm$Funcgroup=="Epi-endogeic"],
gm$Abd[gm$Funcgroup=="Epi-endogeic"], method = "spearman")
  cor.test(gm$Cover[gm$Funcgroup=="Epi-endogeic"],
gm$Ash[gm$Funcgroup=="Epi-endogeic"], method = "spearman")
```

```
#Repeat and edit code for mass and remaining comparisons
```

Permutation Analyses

*Conducting one-way ANOVA Monte Carlo analyses to compare earthworm abundance /
biomass between seasons*

```
###### Spring 2011-Spring 2012 Total ######

sp11.sp12 <- read.csv("Sp11~Sp12.csv")

  #Abd
  mod1 <- lm(Abd ~ Season, data = sp11.sp12)
  ANOVA <- summary(aov(mod1))
  cat(" The standard ANOVA for these data follows ","\n")
    ##Saving F values for future use
    Fseason <- ANOVA[[1]]$"F value" [1]
  print(ANOVA, "\n")
  cat( "\n")

  #Now resampling
  nreps <- 5000
    #Set up space to store F values as calculated
    FS <- numeric(nreps)
    #The first F of 5000
    FS[1] <- Fseason
    for (i in 2:nreps) {
      newsp11.sp12 <- sample(sp11.sp12, 561)
      mod2 <- lm(newsp11.sp12 ~ Season)
      b <- summary(aov(mod2))
      FS[i] <- b[[1]]$"F value"[1]
      }
    probP <- length(FS[FS >= Fseason])/nreps

    cat(" The probability value for Season is ", probP, "\n")

  #Repeat and edit code for mass and remaining comparisons
```

*Conducting one-way ANOVA Monte Carlo analyses to compare earthworm abundance /
biomass between focal plants*

```
######## GM-TW ########

  gm.tw<- read.csv("GM-TW.csv")

  #Abd
  mod1 <- lm(Abd ~ Plant, data = gm.tw)
  ANOVA <- summary(aov(mod1))
  cat(" The standard ANOVA for these data follows ","\n")
    ##Saving F values for future use
```

```
    Fplant <- ANOVA[[1]]$"F value" [1]
print(ANOVA, "\n")
cat( "\n")

  #Now resampling
  nreps <- 5000
    #Set up space to store F values as calculated
    FP <- numeric(nreps)
    #The first F of 5000
    FP[1] <- Fplant
    for (i in 2:nreps) {
      newgm.tw <- sample(gm.tw, 438)
      mod2 <- lm(newgm.tw ~ Plant)
      b <- summary(aov(mod2))
      FP[i] <- b[[1]]$"F value"[1]
      }
    probP <- length(FP[FP >= Fplant])/nreps

    cat(" The probability value for Plant is ", probP, "\n")

  #Repeat and edit code for mass and remaining comparisons
```

Conducting one-way ANOVA Monte Carlo analyses to compare earthworm functional group abundance / mass between focal plants

```
######## Alliaria Anecic - Endogeic ########

  gm.an.en<- read.csv("GM_AnEn.csv")

  #Abd
  mod1 <- lm(Abd ~ Funcgroup, data = gm.an.en)
  ANOVA <- summary(aov(mod1))
  cat(" The standard ANOVA for these data follows ","\n")
    ##Saving F values for future use
    Ffungr <- ANOVA[[1]]$"F value" [1]
  print(ANOVA, "\n")
  cat( "\n")

  #Now resampling
  nreps <- 5000
    #Set up space to store F values as calculated
    FF <- numeric(nreps)
    #The first F of 5000
    FF[1] <- Ffungr
    for (i in 2:nreps) {
      newgm.an.en <- sample(gm.an.en, 98)
      mod2 <- lm(newgm.an.en ~ Funcgroup)
      b <- summary(aov(mod2))
      FF[i] <- b[[1]]$"F value"[1]
      }
```

```
    probP <- length(FF[FF >= Ffungr])/nreps

   cat(" The probability value for Functional Group is ", probP,
"\n")

  #Repeat and edit code for mass and remaining comparisons
```

Creating Boxplots

```
setwd("G:/WSU/Thesis/Data")
library(NCStats)

cover<-read.csv("Cover2.csv")
worms<-read.csv("Worms.csv")

######## Boxplots for Overall Worm Abundance and Biomass by
Plants ########

boxplot(Abd~Plant, data=worms, xlab="Plant Type", ylab="Earthworm
Abundance", ylim=c(0,27))
boxplot(Ash~Plant, data=worms, xlab="Plant Type", ylab="Earthworm
Biomass (g)", ylim=c(0,0.7))

######## Boxplots for Overall Worm Abundance and Biomass by
Season ########

seasworms<-read.csv("AllSeason_worms.csv")

print(levels(seasworms$Season))
seasworms$Season<-
factor(seasworms$Season,levels(seasworms$Season)[c(2,1,3)])
print(levels(seasworms$Season)) #Reorders factor levels on x-axis

boxplot(Abd~Season, data=seasworms, xlab="Season",
ylab="Earthworm Abundance", ylim=c(0,27))
boxplot(Ash~Season, data=seasworms, xlab="Season",
ylab="Earthworm Biomass (g)", ylim=c(0,0.7))

#Repeat and edit code for remaining plots
```