UNDERSTANDING ABOVE- AND BELOWGROUND INTERACTIONS WITHIN INVASION BIOLOGY: AN INTEGRATIVE APPROACH ACROSS A FOREST COMMUNITY

By

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Dedication

To my father: Gregory A. Cope

You are greatly missed and all of this would not be possible without all of the love and support you have provided me growing up.

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Understanding Above- And Belowground Interactions Within Invasion Biology: An Integrative Approach Across A Forest Community

Abstract

By

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Above- and belowground interactions play a significant role in forest communities. Individually, white-tailed deer, invasive earthworms, and invasive garlic mustard have all been shown to have a significant influence on forest communities. My dissertation has taken a functional group approach to understand how these three groups interact, and how those interactions effect both the above- and belowground community. Invasive earthworms can be classified into four functional groups on the basis of their feeding/burrowing mode. Anecic are large earthworms that create vertical burrows, epigeic are small litter dwelling earthworms, endogeic are small soil dwelling earthworms, and epi-endogeic are large earthworms that can be found in both the soil and leaf litter. Therefore, I predicted that the functional groups would interact differently with other organisms (e.g. deer, soil microbes).

I found that the presence of white-tailed deer significantly increased the abundance of the endogeic functional group of earthworms, while the presence of deer had no effect on the other functional groups. This suggests the different functional groups respond differently to the presence of deer. Additionally, I found that deer presence and earthworm presence and/or abundance significantly altered bacteria and fungal

community composition. In conjunction, I also found that fungal community richness was positively correlated to plant community richness.

To investigate how interactions among multiple invaders influence plant performance, I conducted a potted experiment involving two invasive species, *Lumbricus terrestris* and garlic mustard (*Alliaria petiolata*), and a native spring ephemeral. In addition, I utilized observational field data collected from Cleveland Metroparks to compare experimental and observation results. In the field I found that garlic mustard significantly decreased mayapple cover, and that plots that also have earthworms reduce this negative effect. However, within the potted experiment I found that the presence of both of these invaders reduced mayapple biomass. Thus interactions with this anecic invader were highly context-dependent. Overall, my dissertation highlights the importance of considering functional feeding groups when studying interactions among multiple species, especially for invasive earthworms.

Chapter 1

Introduction

There has been a recent surge of studies that have focused on above- and belowground interactions involving both plants and animals (Porazinska et al. 2003, Bardgett and Wardle 2003, Wardle et al. 2004, Wardle 2006, Kardol and Wardle 2010, Deyn 2017). However, fewer studies have focused on above- and belowground interactions involving multiple animals (but see Dávalos et al. 2015a, 2015b, Dobson and Blossey 2015) or across multiple trophic levels. Below I outline how three important groups have all been studied individually; however, how these groups interact and how these interactions influence native forest communities is not well understood. Each of these groups influence the forest communities; gaining a better understanding of how these groups interact will provide a foundation in our understanding of how forest communities respond to these stressors. Understanding these interactions will provide useful information for both basic ecology and conservation biology and allow researchers and managers to help restore or maintain native biodiversity within the forest communities.

Earthworms have been introduced into the Great Lakes region from Europe and Asia, and their negative effects on forest ecosystems have been widely studied. These studies have shown that earthworms can have an effect on both the aboveground plant communities and the belowground soil communities. For example, invasive earthworms can influence plant communities through seed predation (Cassin and Kotanen 2016) or hindering rare plant recruitment (Dávalos et al. 2015b). They have also been shown to negatively affect belowground fungal communities by breaking up their hyphal networks

(Lawrence et al. 2003) or alter soil nutrient availability (Hale et al. 2008). However, many studies on earthworms have only focused on a single species or have generalized across all earthworm species. Earthworms are categorized into functional groups based on their feeding and burrowing strategies (Bouche 1977), and here I argue that functional group should be considered when studying how earthworms interact with other components of forest communities because of their differing effects they have on the litter layer and soil horizons.

Earthworms are categorized into four functional groups based on their size as well as their feeding and burrowing traits. One of the most well-known earthworms found in the Great Lakes region is *Lumbricus terrestris*, which belongs to the anecic functional group. These earthworms are fairly large in size (9 – 15cm long) and create long vertical burrows from the soil surface to as deep as a meter belowground. These earthworms have very visible effects on the forest floor and soil column by pulling leaf litter down into the soil from the surface and can rapidly increase nutrient cycling (Fahey et al. 2013). Another functional group that has been shown to have a major influence on the forest floor are the epi-endogeic earthworms. The most common earthworm associated with this functional group are the *Amynthas* sp. which are large (4 – 20cm long) litter and soil dwelling species. Epi-endogeic earthworms have large negative effects on native communities by consuming the soil organic layer and breaking up mycorrhizae hyphal networks (Lawrence and Bowers 2002), which are relied on by many native plants to survive. Epigeic earthworms are small in size (2 - 4cm long) and are found on the soil surface within the leaf litter. They primarily feed within the upper organic layer of soil; however, due to their relatively small size they are thought to have a limited effect on mixing the soil layers. Lastly, endogeic earthworms are non-pigmented earthworms that are found within the top soil horizons and are usually small in size (1 - 7cm long). Unlike the small epigeic earthworms which have not been documented to have significant effects on the forest floor, these earthworms have been shown to significantly alter both the microarthropod community as well as the rate of nutrient cycling (Eisenhauer 2010). Uniquely, endogeic earthworms feed primarily within the soil column, and depend on plant root and soil fungi, unlike the other functional groups, which feed largely on leaf litter.

White-tailed deer (*Odocoileus virginianus*) are largely considered to be ecosystem engineers due to their influence on forest communities and many ecosystem processes (Côté et al. 2004). Throughout much of the Great Lakes and Northeast region of the United States, white-tailed deer have become overabundant due to loss of habitat and lack of a top-level predator to control populations. White-tailed deer have been shown to preferentially browse on vegetation, greatly altering forest plant communities (Bressette et al. 2012). However, white-tailed deer not only affect these aboveground communities but can also influence belowground communities. For example, I predict that the different functional groups of earthworms might respond differently to the presence of deer. Because some of these earthworms feed on the leaf litter at the soil surface, they might be influenced by indirect effects of deer if deer influence plant communities and leaf litter abundance.

Invasive earthworms might also interact with plants, including invasive plants, creating the potential for non-additive interactions among above- and belowground invaders. This is especially true for the anecic functional group which pulls leaf litter down into the soil column from the surface. The invasive plant garlic mustard (*Alliaria petiolata*) has been widely studied due to its ability to effectively invade forest habitats. Garlic mustard is highly invasive and reduces diversity in native plant communities creating monocultures in the forest understory. Garlic mustard has the ability to produce allelopathic chemicals in its leaves and roots, which is thought to provide it with a competitive advantage against native plants. These allelopathic chemicals poison mycorrhizal fungi within the soil and prevent the mutualistic associations from forming between the fungi and many native plants.

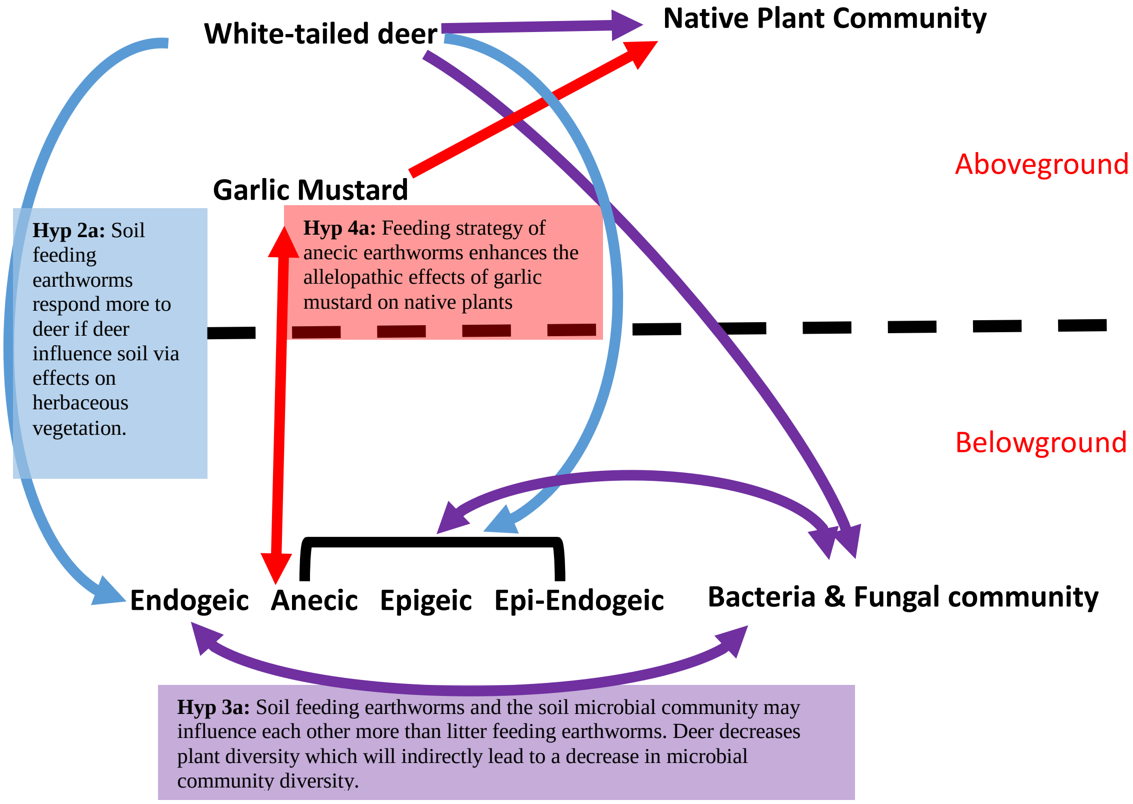
Below, I outline how the interactions among these taxa can have a significant role in forest community composition. I will also show how some of these effects might not have been detected if I had focused solely on individual species. By considering functional feeding groups, I identify a more nuanced understanding of these interactions, with implications for above and below-ground interactions and biological invasions.

In Chapter 2, I take a functional group approach to understand above- and belowground interactions between white-tailed deer and invasive earthworms. I assessed whether the presence/absence and abundances for different functional groups of earthworms are influenced by the presence of white-tailed deer. I hypothesized that soil feeding earthworms (endogeic) would respond more to deer if deer influence soil via effects on herbaceous vegetation (Fig. 1.1). Alternatively, litter feeding/dwelling earthworms could be more responsive to deer presence due to mechanical disturbance of the ground via trampling/scratching, or increases in nutrient deposition from deer defecation (Fig. 1.1).

In Chapter 3, I examine how above- and belowground communities are affected by different functional groups of invasive earthworms and the presence/absence of white-tailed deer. Fungal and bacteria communities are important components of forest ecosystems as they aid in nutrient cycling and decomposition, as well as assisting many native plants in nutrient uptake. I conducted molecular analyses using terminal restriction fragment length polymorphism (TRFLP) to assess how earthworms and deer influenced both bacteria and fungal community composition, as well as field surveys to assess the plant community. I hypothesized that soil feeding earthworms and the soil microbial community may influence each other more than litter feeding earthworms (Fig. 1.1). Alternatively, the large earthworms within the anecic and epi-endogeic functional groups may have a greater influence on the microbial community due to their ability to consume greater quantities of litter and soil. In addition, I also hypothesize that deer will decrease plant diversity which will indirectly lead to a decrease in microbial community diversity.

In Chapter 4, I examine how multiple invaders influence a native plant's performance. I examined how invasive earthworms and invasive garlic mustard (*Alliaria petiolata*) interacted to affected the native plant mayapple (*Podophyllum peltatum*). Because the invasive earthworm, *Lumbricus terrestris,* has an anecic feeding mode, where it feeds by directly pulling leaf litter into the soil, I hypothesized that invasive earthworms would move garlic mustard leaves down into the soil, potentially altering the effects of garlic mustard on a native plant (Fig. 1.1). Alternatively, garlic mustard and anecic earthworms may affect the native plant community independent of each other. I also examined a large scale observational dataset to compare the experimental results with observational data collected from the field. Thus, I explore both mechanisms governing this interaction with a manipulative experiment and the potential for these experimental patterns to hold in local field systems.

In Chapter 5, I conclude by summarizing my results and discussing their importance and relevance to above- and belowground interactions within community ecology and invasion biology. Most importantly, I suggest that considering functional feeding groups, as for invasive earthworms, leads to a deeper and more nuanced understanding of above- and belowground interactions. I also outline areas for future research on this topic.



Hyp 2a: Soil feeding earthworms respond more to deer if deer influence soil via effects on herbaceous vegetation.

Hyp 4a: Feeding strategy of anecic earthworms enhances the allelopathic effects of garlic mustard on native plants

Hyp 3a: Soil feeding earthworms and the soil microbial community may influence each other more than litter feeding earthworms. Deer decreases plant diversity which will indirectly lead to a decrease in microbial community diversity.

Fig 1.1: Hypotheses for each of the three chapters. Colors are associated with each of the chapters (blue = Chapter 2, purple = Chapter 3, red = Chapter 4). Each box outlines main hypothesis for each chapter. Alternative hypotheses for each chapter: Chapter 2: Litter feeding/dwelling earthworms could be more responsive to deer presence due to mechanical disturbance of the ground via trampling/scratching or increases in nutrient deposition from deer defecation. Chapter 3: Large earthworms within the anecic and epi-endogeic functional groups will have a greater influence on the microbial community due to their ability to consume greater quantities of litter and soil. Chapter 4: Garlic mustard and anecic earthworms will affect the native plant community independent of each other.

Chapter 2

A functional group perspective on above- and belowground interactions: invasive earthworms and native deer.

Summary

Above and belowground interactions between animals can influence species abundances, biological invasions, and ecosystem processes. Recent calls in the literature have suggested that a functional traits perspective might enhance our understanding of above and belowground interactions, yet the few studies that take this approach have mostly focused on plant functional traits. Here, I consider the possible role of functional group and body size for interactions between invasive earthworms and deer. Because white-tailed deer are overabundant and change soil disturbance and nutrient availability, I predicted that deer might influence the abundances of invasive earthworms. Because earthworm functional feeding groups differ in their placement in the soil column and feeding mode, I predicted that the feeding groups would respond differently to the presence of deer. I sampled earthworms across 44 paired deer exclosure and control sub-plots across four spatial regions in Ohio, USA. I found that control sub-plots had more than twice as many endogeic, or soil-dwelling earthworms (e.g. *Octolasion tyrtaeum*) than paired deer exclosure sub-plots, in the three regions in which they were found. The experimental effect size of deer exclusion also correlated positively with earthworm body size, including in phylogenetically corrected tests. In other words, smaller earthworms were more likely to have higher abundance in the presence of deer. If deer overpopulation has a positive effect on some functional groups of invasive earthworms, this suggests that managing deer is important, not only for their aboveground effects on plant communities, but also for their belowground effects on invasive earthworms. More generally, studies of above and belowground interactions might benefit from considering

animal functional traits, such as body size, which correlates with important functions such as metabolic rate and desiccation tolerance.

Introduction

There has been a recent surge of studies that have focused on above and belowground interactions involving both plants and animals (Porazinska et al. 2003, Bardgett and Wardle 2003, Wardle et al. 2004, Wardle 2006, Kardol and Wardle 2010, Deyn 2017). While above- and belowground interactions often seem species specific, functional traits hold the promise of potential generalizations. There are thousands of studies that focusing on functional traits (Fig. 2.1a) (Mougi and Kishida 2009, Salgado-Luarte and Gianoli 2012), and plant functional traits have informed our understanding of interactions with herbivores and mutualists (Barber et al. 2012), ecosystem functioning (Diaz et al. 2007, Aguirre-Gutiérrez 2016), and above- and belowground interactions (Deyn 2017). However, there are still few studies of above- and belowground interactions that are aided by functional trait data (Fig. 2.1a), and especially few that focus on animal functional traits, such as those of earthworms.

Invasive earthworms have substantial negative effects on native plant communities and ecosystem processes by altering the nutrient availability (Hale et al. 2008), consuming seeds from the seed bank (Nuzzo et al. 2015), and breaking up root and mycorrhizae hyphal networks (Lawrence et al. 2003). Invasive earthworms are likely to be influenced by deer populations because deer have large effects on soil structure and nutrient profiles (e.g. Murray et al. 2013). There has been recent support of this hypothesis by Dávalos and colleagues, who found greater earthworm abundance in plots where deer had access when compared to plots where deer had been excluded (Dávalos et al. 2015b). Though mechanisms governing this pattern have not been tested directly, some evidence suggests that deer populations could influence invasive earthworm abundance through effects on nutrient availability. For example, deer urine and fecal pellets increased native earthworm biomass and density in a laboratory experiment (Rearick et al. 2011). Further, abundance of a native earthworm (*Eisenoides carolinensis*) was greater in plots where deer were found compared to plots where deer were excluded for three years (Rearick et al. 2011), consistent with the hypothesis that deer may increase earthworm populations.

Earthworms are categorized into four functional groups based on their feeding and burrowing traits as well as their size. The anecic functional group contains large earthworms (9 – 15cm) that create long vertical burrows from the soil surface to as deep as a meter belowground. These earthworms pull leaf litter down into the soil from the surface and can rapidly increase nutrient cycling (Fahey et al. 2013). Endogeic earthworms are usually small (1 - 7cm) non-pigmented worms that are found within the top soil horizons. These worms have been shown to alter both the microarthropod community as well as the rate of nutrient cycling (Eisenhauer 2010). Uniquely, endogeic earthworms feed primarily within the soil column, and depend on plant root and soil fungi, unlike the other functional groups, which feed largely on leaf litter. Epigeic earthworms primarily feed within the upper organic layer of soil; due to their relatively small size (2 - 4cm) they are thought to have a limited effect on mixing the soil layers. Lastly the epi-endogeic earthworms, most commonly *Amynthas* sp., are large (4 – 20cm) soil dwelling species. Epi-endogeic earthworms have large negative effects on native communities by consuming the soil organic layer and breaking up mycorrhizae hyphal networks (Lawrence and Bowers 2002).

While interactions between the belowground biota, such as invasive earthworms, and native deer are possible (Lessard et al. 2012, Bressette et al. 2012), most studies have focused on aboveground interactions. Ungulate overpopulation is a global problem because of increasing urbanization and decreasing predator populations, and their effects on tree regeneration and forest understory plants have been well documented and often dramatic (Côté et al. 2004). There have been numerous long term studies that focus on plant communities in the absence of deer through the use of deer exclosures, tall fences that keep out deer (e.g. McGarvey and Bourg 2013). For example, deer negatively affect native spring ephemerals such as *Trillium grandiflorum* (Knight et al. 2009b). White-tailed deer preferentially feed on the flowering and large non-flowering stages of this plant, thereby stymieing reproduction (Knight et al. 2009b). White-tailed deer also facilitate invasive plant success in forest understories (Knight et al. 2009a), allowing species such as *Alliaria petiolata a*nd *Microstegium vimineum* to exploit the open patches created by reduced native cover (Knight et al. 2009a).

Previous research has suggested that earthworms' responses to environmental change may be species specific (Zicsi et al. 2011, Wandeler et al. 2016), but whether species specific patterns are idiosyncratic, or might be predicted by functional traits, is largely unknown (Karberg and Lilleskov 2009, Zicsi et al. 2011, Dávalos et al. 2015c). Our aim was to put the above and belowground interactions between native white-tailed deer and invasive earthworms into a functional trait context (Fig. 2.1b). I used a long-running (5-15 years) large-scale field experiment with 44 deer exclosures and paired control (deer-access) plots, and sampled invasive earthworm abundance. Rather than considering multivariate trait space (sensu Kraft et al. 2015), I have focused on two specific functional traits, which I expect to be important in invasive earthworms: functional group and body size. Because different functional groups of earthworms use different components of the soil horizon, they might vary in their response to aboveground drivers, such as deer. Earthworm body size correlates with functional group and soil horizon usage, and might be an especially useful trait, because it is very easy to measure for a wide variety of organisms. For example in frogs, smaller body size leads to greater vulnerability to changing environments (Tracy et al. 2010). I use experimental field data to ask whether the effects of deer exclusion differ for earthworms in different functional feeding groups or across body sizes. I expected that the smaller earthworm species (endogeic, epigeic) might be more responsive to changes in the soil environment caused by deer than the larger earthworm species (anecic, epi-endogeic).

Methods

Study System

Our study site in northeastern Ohio, USA, encompassed 44 different plots, each of which contained both a deer exclosure and pre-established paired control sub-plot that were within 5m of each other. The maximum distance between any two given plots was 71 km, and the closest distance between any two plots was 0.1 km. The spatial location of each plot was recorded with a Garmin GPSmap 60 Cx GPS with a resolution of 10 meters. All of the plots have been established between five and twenty years ago (Appendix A: Table S2.1). Soil characteristics consisted of a silt-loam substrate, with the exception of Little Mountain at Holden Arboretum, which consisted of a sandy silt-loam substrate (Web Soil Survey, USDA: 7/12/2016).

Study Design

I collected earthworms in the exclosure and control sub-plots between late August to early October in both 2013 and 2014. This was done so that most of the earthworms would have reached maturity by that point in the season. Two random locations within each sub-plot were sampled for earthworms (Fig. 2.2). The earthworm sampling was conducted using the hot mustard extraction technique (Chan and Munro 2001, Lawrence and Bowers 2002). This method has been demonstrated to be biased towards sampling greater numbers of anecic earthworms compared to endogeic earthworms (Chan and Munro 2001). Thus, absolute differences in abundance among functional groups should be interpreted with caution. For the purpose of this study, I was interested in comparing relative earthworm abundance between control and exclosure sub-plots. Thus, the mustard extraction method is appropriate for answering the key question in this study: does earthworm abundance differ between subplots with and without deer access, including within different functional groups? Leaves were first carefully removed and hand sorted for any earthworms that may be present within the leaf litter from the 0.09 m^2 area within the sample frame. One gallon of water was mixed with 80 mL of hot mustard powder (Penzeys Spices, Wauwatosa, WI) and poured into the 0.09 m^2 frame. Earthworms were collected from within the frame until they no longer surfaced for ten minutes, then preserved in a 15 percent formalin solution until they could be identified and weighed.

I identified each individual adult earthworm to species, while each juvenile earthworm was identified to genus, because juvenile worms cannot typically be identified to species using morphological characteristics. Keying of each earthworm was based on the earthworm identification field guide provided by Great Lakes Worm Watch (Hale 2007). *Amynthas* sp. were only identified to genus as they are all almost indistinguishable from each other without the use of molecular techniques.

To determine the biomass of the earthworms, I aggregated all individuals for each species within each sub-plot and dried them in a 60°C oven for a minimum of 24 hours. Once they were dry, I weighed the aggregated sample to the nearest 0.0001g for each species. Then biomass was placed in a 500°C incinerator for a minimum of 4 hours to ash the earthworms and remove all organic material, leaving only the stomach contents of the earthworms. I then weighed the ash free remains to the nearest 0.0001g and took the difference between the dry and ash-free weight to determine the earthworm biomass (Hale et al. 2004).

Because previous studies have suggested that pH is correlated with the deer-earthworm interaction (Dávalos et al. 2015c), I measured the pH of the soil within both the control and exclosure sub-plots. Soil was collected along three transects that were spaced every 2.5 meters within the sub-plot. Each of the transects contained three sampling points, spaced every two meters, that were then pooled into one sample. The pH was then recorded for each transect within each sub-plot using a Sartorius PP-20 pH meter. I then took the average of the three transects to determine the pH for each sub-plot.

Statistical Analyses

To determine whether the abundance of earthworms differed between deer exclosure and control sub-plots, I compared earthworm count and biomass data across the 44 paired sub-plots. I summed earthworm counts across the two earthworm sampling frames for each sub-plot (Fig. 2.2). Earthworm counts were both spatially autocorrelated and zero-inflated and no single analysis method addressed both of these features of the data. Following the methods of similar studies, I split the earthworm count data into two separate response variables: presence/absence and earthworm count when present (Zuur et al. 2009, Wandeler et al. 2016). For the earthworm count response variable, I retained 37 paired sub-plots that had relevant zeros. For example, if I found an anecic earthworm in the control sub-plot but not its paired exclosure sub-plot, the zero from that exclosure was included to maintain the split-plot design of this experiment.

To test for an effect of deer exclosure treatment on invasive earthworm presence, I used a generalized linear model (glm) with a binomial error distribution on earthworm presence/absence as a function of treatment (control vs. deer exclosure), earthworm functional group, a treatment by functional group interaction, and year the plots were established. To address the possible role of functional groups, I constructed planned contrasts within functional groups to compare the control to the exclosure treatments. While this model does not account for potential spatial autocorrelation in earthworm presence, based on visual inspection of a semivariogram, the residuals showed only a slight correlation when compared to the fitted values.

To test for an effect of deer exclosure treatment on earthworm counts and biomass, I used a generalized least squares (gls) model incorporating spatial autocorrelation in the nlme library (Pinheiro et al. 2017). Square-root transformed earthworm count or untransformed biomass were initially modeled as a function of treatment (control vs. deer exclosure), earthworm functional group, year the plots were established, and average pH, and all possible interactions. I then reduced this model by removing any interactions that were not significant ($p > 0.25$) following Bancroft (1964). I inspected semivariograms, which suggested that there was a significant amount of spatial autocorrelation in earthworm counts. Latitudes and longitudes were jittered by adding a small value (< 0.0000001 seconds), in order to incorporate sub-plots that did not have unique locations. I compared models using different correlation structures (corGaus, corSpher, corExp) with AIC, to determine the best method for modeling spatial autocorrelation. Although this method assumes that residuals are normally distributed, and residuals of this model were right skewed, least squared approaches are highly robust to violations of the distributional assumption (Ives 2015), and diagnostic plots suggested that autocorrelation was well-modeled with this approach.

To determine the robustness of the gls results to alternative methods, I conducted a second analysis on earthworm counts when present. I used a generalized linear mixed effects model (glmer) in the lme4 library (Bates et al. 2015) with a Poisson error structure and untransformed earthworm count as a function of treatment (control vs. deer exclosure), earthworm functional group, a treatment by functional group interaction, and year the plots were established. Plot was also included as a random effect in the model. Diagnostics of this model suggested that not incorporating spatial autocorrelation in this glmer was a more severe violation of model assumptions, compared to the diagnostics of the gls.

To explore a possible correlation between earthworm body size and earthworm response to deer exclusion, I calculated earthworm species and site-specific experimental effect sizes. The effect size of the deer exclusion treatment was calculated for earthworm densities across experimental treatments, following Dávalos et al. 2015c.

Effect size = (exclosure – control)/maximum(exclosure, control)

The resulting effect sizes range from -1 to 1, where 0 indicates no effect of deer exclusion, negative values indicate more earthworms in the control treatments than in the deer exclusion treatments, and positive values indicate greater earthworm densities in exclosure plots. The sample size for this species comparison was 10. I used a linear model to quantify the correlation between deer exclosure effect size and average adult earthworm body size, log transformed. I examined Q-Q plots and standardized residual plots, and model assumptions were well-met.

To determine whether the relationship between body size and experimental deer exclusion effect size was robust to including phylogenetic information (Felsenstein 1985), I conducted a phylogenetic analysis amongst the 11 earthworm species sampled in this study (Supplemental Information, Phylogenetic Methods). I tested for phylogenetic signal on body size using Pagel's λ (Pagel 1999) with the phylosig function in the phytool package in R, with 1000 replicates (Revell 2012). I compared gls models for effect size as the response variable and log transformed body size as the predictor variable, for models with and without phylogeny using AIC and a likelihood ratio test. The phylogenetic gls (PGLS) analysis incorporated phylogeny in the error structure of the model as a variance-covariance matrix (Martins and Hansen 1997) assuming a Brownian motion model of evolution using the corBrownian function in the ape package in R (Paradis et al. 2004).

All analyses were conducted in the R statistics program (version 3.1.1, R Core Development Team 2014).

Results

Earthworm Functional Groups

The presence of invasive earthworms was a function of the year the exclosure was established, soil pH, functional group, and experimental treatment (Table 2.1). There was no interaction between control/exclosure treatment and pH or between control/exclosure treatment and the year the exclosure was established (Appendix A: Table S2.2). Earthworms were more likely present in the control rather than the exclosure sub-plots, and this was true for six out of ten of the adult taxa (Appendix A: Table S2.5). This was also true for eight out of nine juvenile taxa (Appendix A: Table S2.6).

The number of earthworms depended on the control/exclosure treatment by functional group interaction (Table 2.2). Within endogeic earthworms there were over twice as many earthworms within control sub-plots as there were within exclosure sub-plots (Table 2.2, Fig. 2.3a), and this pattern was consistent across spatial regions (Fig. 2.3b). Three out of four of the regions that I sampled had been invaded by endogeic earthworms, the fourth (Cleveland Metroparks) had no endogeic earthworms sampled. The other functional groups had similar numbers of earthworms between control and exclosure sub-plots (Table 2.2, Fig. 2.3a). The different functional groups of earthworms responded differently to variation in soil pH, but this interaction did not depend on the experimental deer exclusion treatment. The year the experimental deer exclusion fence was established was not a significant covariate on the number of earthworms found (Table 2.2).

I found five endogeic species amongst the adults, all of which had more earthworms in the control when compared to the exclosure sub-plots (Appendix A: Table S2.4). Amongst the juveniles, there were four taxa, only two of which had more earthworms within control rather than exclosure sub-plots (Appendix A, Table S2.5). *Lumbricus terrestris* adults were relatively abundant where they were found, on average 1.5 earthworms in control sub-plots and 5.75 earthworms in exclosure sub-plots, but were found across relatively few sub-plots (Appendix A: Table S2.4). *Amynthas* sp. were found in about a quarter of the sub-plots, with an average of 5.4 earthworms per control sub-plot and 7.73 per exclosure sub-plot (Appendix A: Table S2.4).

The amount of earthworm biomass per sub-plot varied across the functional groups (Appendix A: Table S2.2). Amongst adult earthworms, *Lumbricus terrestris* and *Amynthas* sp. contributed the largest amount towards earthworm biomass (Appendix A: Table S2.4). Amongst juveniles *Lumbricus* sp., *Aporrectodea* sp., and the *Amynthas* sp. contributed the largest amount towards earthworm biomass. There was a marginally significant treatment effect on earthworm biomass per sub-plot ($p = 0.09$), and no interaction between treatment and functional group ($p = 0.52$).

Earthworm Body Size

Earthworm adult average body size correlated positively with the experimental effect size of deer exclosure (slope = 0.13, $F_{1,8} = 5.13$, $p = 0.05$) (Fig. 2.4). *Dendrobaena octaedra* was the smallest earthworm in this sample, with an average body size of 0.013 g and a negative experimental effect size (average effect size = -0.11). *Aporrectodea rosea* was similar in size (0.014 g) with a negative effect size (-0.084). The largest earthworms in our sample were *Lumbricus terrestris* (0.30 g) with a positive effect size (0.39) and

Amynthas sp. (0.15 g) with a positive effect size (0.28). Analyses that dropped *Lumbricus terrestris* were no longer significant ($F_{1,7} = 0.88$, p = 0.38).

I found no phylogenetic signal on earthworm body size ($\lambda < 0.01$, P = 1.00; Appendix A: Fig. S2.1). The model without phylogeny had a higher likelihood and lower AIC (log likelihood = 0.56, AIC = 4.88) than a model with phylogeny (log likelihood = -0.39, AIC = 6.77), and the statistical relationship between experimental effect size and body size was robust to incorporating phylogeny ($F_{1,8} = 5.41$, p = 0.049).

Discussion

While above- and belowground interactions for deer are less well studied than the aboveground effects of deer, our study and several others (Frelich et al. 2012, Dávalos et al. 2015a, 2015b, Dobson and Blossey 2015) suggest that deer interact with invasive earthworms. Because these interactions are species-specific (Dobson and Blossey 2015), a consideration of functional groups might enhance our understanding of this species-level variation. Our data suggest that the effects of deer on invasive earthworms depend on earthworm functional group. Other studies have also found that categorizing earthworms by functional group is crucial when understanding both above- and belowground ecological processes (Eisenhauer 2010, Wandeler et al. 2016). Different species of earthworms affect ecological processes such as nutrient cycling (Eisenhauer et al. 2007, Hale et al. 2008, Straube et al. 2009), seed consumption (Nuzzo et al. 2015), mutualistic associations (Lawrence and Bowers 2002, Scheu 2003), and aboveground species (Dávalos et al. 2015b), in diverse ways. Our study suggests that soil-dwelling endogeic earthworms might be more responsive to the effects of deer than other functional groups.

Anecic, epigeic, and epi-endogeic earthworms feed largely on leaf litter, which in these forests is composed mostly of tree litter. Endogeic earthworms exclusively feed in the soil column where the roots of most of the forests herbaceous layer are located. Because the tree community responds so slowly to deer exclusion, and the herbaceous community so quickly, indirect effects of deer mediated by plant community structure are more likely for earthworms that are influenced by the herbaceous layer. This could help explain why endogeic earthworms are more responsive to deer presence then the other functional groups.

Our data also suggest that earthworm body size correlates with the experimental effect size of deer exclusion. Earthworm species with smaller body sizes, such as *Dendrobaena octaedra* and *Aporrectodea rosea*, were more abundant where deer were present. Larger earthworms, such as *Lumbricus terrestris* and *Amynthas* sp. were somewhat more abundant where deer were experimentally excluded, though not significantly so. Body size is an important functional trait in animals, which correlates strongly with metabolic rate across a wide variety of taxa, including invertebrates (Brown et al. 2004). Body size also correlates with other important functional characteristics, such as desiccation tolerance in frogs (Tracy et al. 2010). In invasive earthworms, body size and functional group are not independent of one another (Bouche 1977), and what mechanisms might link body size, functional group and response to deer presence are unknown. However, the strong correlations between body size and metabolic rates (Brown et al. 2004), latitudinal clines (e.g. in birds: Olson et al. 2009), and temperature

(Gardner et al. 2011), suggest that body size is a widely useful functional trait in animals, and our data suggest that body size in earthworms might correlate with the strength and direction of above and belowground interactions.

There are several potential mechanisms that could influence deer and earthworm interactions, and they are not mutually exclusive. First, deer could facilitate earthworm population growth, if deer pellets contain nutrients that are beneficial to earthworms. Deer fecal pellets can lead to an increase in nutrient availability for earthworms, and as a result lead to increases in earthworm mass and population size (Karberg and Lilleskov 2009, Rearick et al. 2011). For example, a laboratory study found that both native earthworms and the invasive earthworm *Lumbricus terrestris* density and biomass increased more in the presence of deer pellets than in just leaf litter alone (Rearick et al. 2011). Deer could also be increasing earthworm populations through soil disturbance, as they have been shown to physically alter the soil column through scrapes (the pawing of the ground to clear a large area of leaf litter) and trampling (Kumbasli et al. 2010). Further studies are needed to test these potential mechanisms influencing the relationship I observed between deer and invasive earthworm populations.

I found that both pH and the year the plots were established affected whether earthworms were present or absent. However, contrary to Dávalos et al. (2015c) I saw no interaction of deer exclusion and pH on the presence of earthworms. Also, I saw no effect of pH on differences in earthworm abundance between control and exclosure treatments. The main effect of year the plots were established could be an artifact of the experimental design. For example, plots at Holden Arboretum were established between 2005-2009, plots at Lake County Metroparks were established between 1994-2001, thus I cannot distinguish between effects of year established and regional variation in earthworm abundances. I also note that there might be variation in deer density across the different sites; however, the paired sub-plot design should control for spatial variation in deer density.

There is currently no known practical way of controlling earthworm invasions into forest ecosystems (Hendrix 2006). Our study shows that managing white-tailed deer populations could have an unexpected additional benefit of reducing endogeic invasive earthworm populations. Managing global ungulate populations has been a major topic of discussion for conservation biologists (Côté et al. 2004), and strategies like controlled hunting, culling, exclusion, and female sterilization have been successfully implemented (Côté et al. 2004). While completely excluding deer is not realistic, reducing overabundant deer populations closer to historical densities could help to control some invasive earthworm populations. Further research needs to be conducted to explore mechanisms governing above- and belowground interactions between deer and earthworms.

This study responds to recent calls in the literature to incorporate functional traits into studies of above- and belowground interactions (Kardol and Wardle 2010, Deyn 2017). Most of this literature has focused on plant functional traits (Deyn 2017). Yet, earthworm functional traits have the potential to help explain some of the seemingly species specific interactions between earthworms and native taxa (Eisenhauer 2010). Future studies are needed to determine whether animal functional traits, such as body size, might be broadly useful for informing our understanding of above- and belowground interactions. Functional traits hold the promise to help explain seemingly idiosyncratic species-specific responses and might lead to a more general theory of above- and belowground interactions.

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Tables

Table 2.1: A generalized linear model with binomial error distribution on the presence/absence of earthworms as a function of control and deer exclosure treatments, earthworm functional group, and the interaction between treatment and functional group with pH and the year of exclosure establishment as covariates.

	DF	Deviance	Residual DF	Residual Deviance	p-value
Control/Exclosure (C/E)	1	6.13	397	522.97	0.01
Functional Group	3	19.59	394	497.25	0.0002
C/E × Functional Group	3	6.29	389	465.38	0.09
Average pH	1	6.55	393	490.70	0.01
Year Exclosure Established	1	19.03	392	471.67	<0.0001

Table 2.2: A generalized least squares model with square root transformed earthworm abundance as a function of control and deer exclosure treatments, earthworm functional group, the interactions between treatment and functional group, and functional group by average pH, with the year of exclosure establishment and pH as covariates. Latitude and longitude were used in a corSper correlation structure to model spatial autocorrelation. Contrasts within functional groups compared control to deer exclosure treatments.

	NumDF	DenDF	F-value	p-value
Control/Exclosure (C/E)	1	81	7.05	0.009
Functional Group	3	81	3.96	0.009
Average pH	1	81	1.44	0.23
Year Exclosure Established	1	81	0.00	0.99
C/E × Functional Group	3	81	3.12	0.03
Functional Group x Average pH	3	81	7.15	0.0002

Contrasts	Estimate	Std Error	z-value	p-value
Anecic	-0.003	0.41	-0.009	1.00
Endogeic	1.08	0.31	3.47	0.002
Epigeic	0.67	0.33	2.02	0.13
Epi-endogeic	0.10	0.36	0.27	1.00

Figures

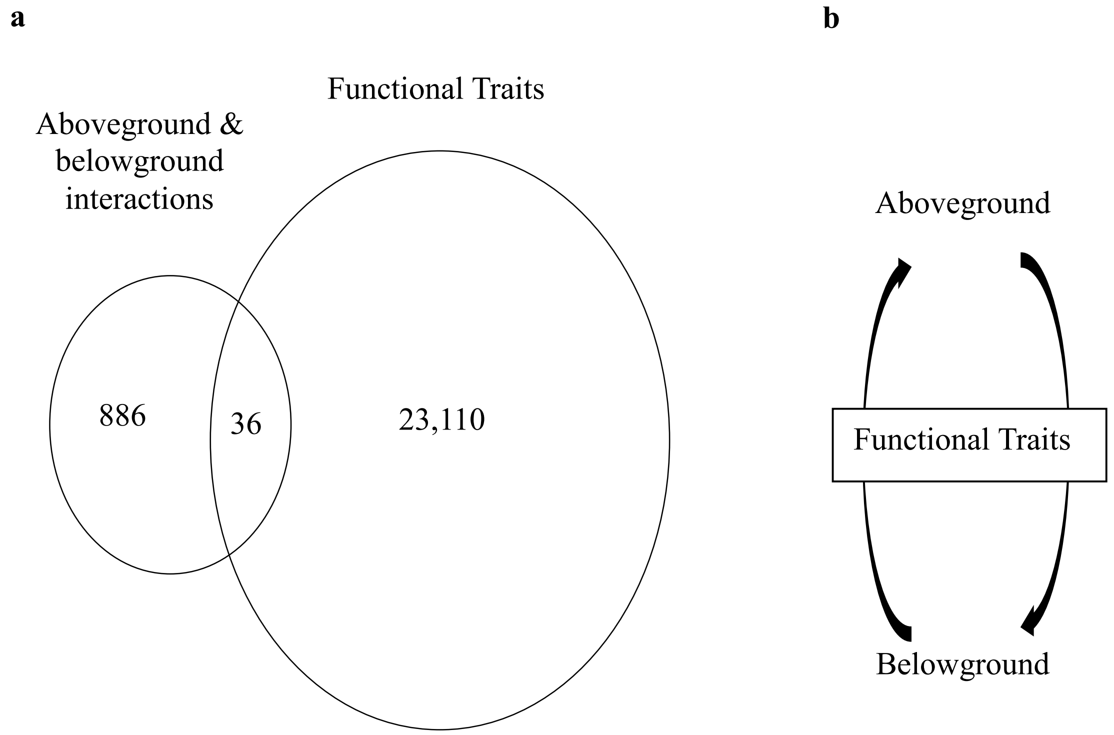

Figure 2.1: (a) Number of publications in Web of Science (24 July 2017) with topic: above and belowground interactions OR aboveground and belowground interactions AND functional trait*, following (Deyn 2017). (b) I suggest that functional traits might enhance our understanding of above- and belowground interactions, such as those between deer and invasive earthworms.

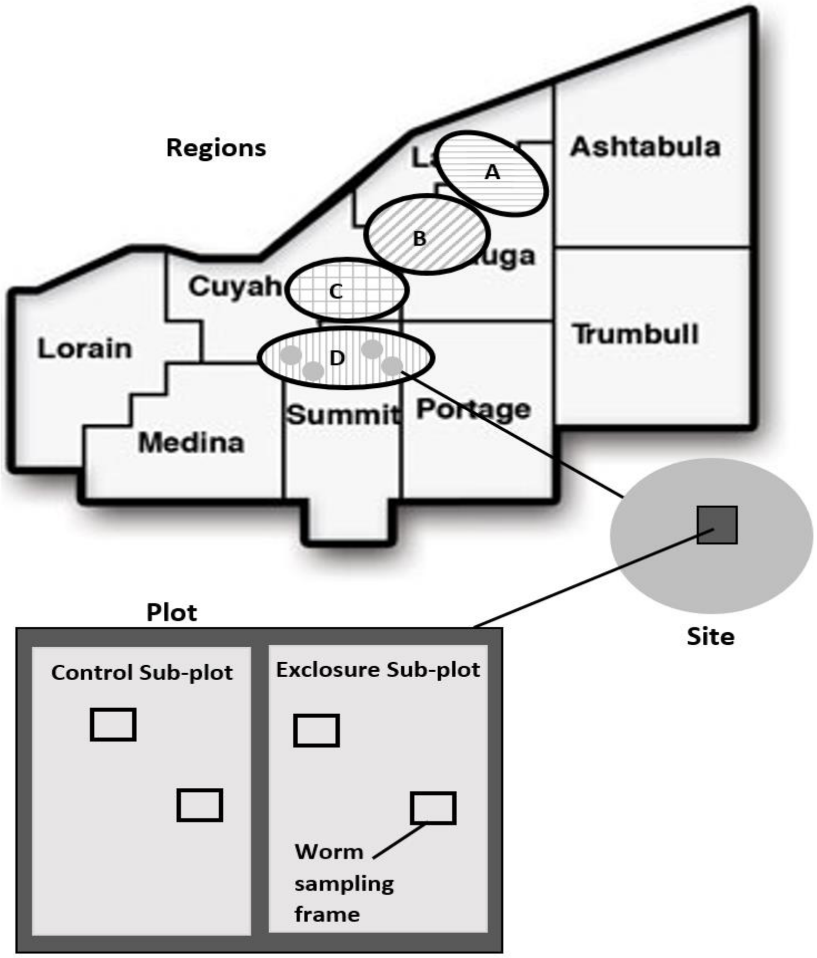

Figure 2.2: The experimental design of the paired control and deer exclosure sub-plots in Northeastern Ohio, USA. Earthworm populations were sampled across 4 regions: Lake Metroparks (A), Holden Arboretum (B), Cleveland Metroparks (C), and Metroparks Serving Summit County (D). Each plot contained two sub-plots: a control sub-plot, which was open to deer, and a fenced deer exclosure sub-plot. Earthworms were randomly sampled in two locations within each sub-plot.

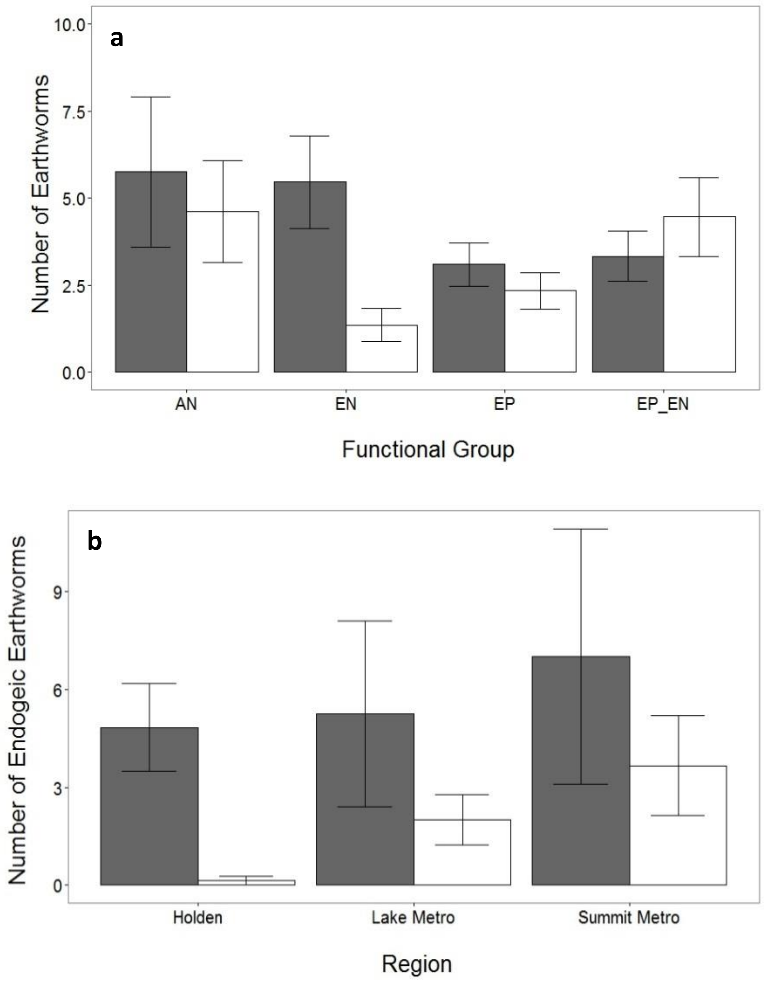

Figure 2.3: (a) The number of invasive earthworms was twice as high in control compared with deer exclosure sub-plots for endogeic (EN) earthworms across 44 plots in northeastern Ohio (p = 0.002). Anecic (AN), epigeic (EP), and epigeic-endogeic (EP_EN) earthworms did not differ in abundance between treatments (Table 2). Grey bars represent control sub-plots and white bars represent deer exclosure sub-plots. (b) The number of endogeic, soil-dwelling, earthworms were greater in the presence of deer (grey bars) than when deer were experimentally excluded (white bars) for three out of four regions sampled, the other region (Cleveland Metroparks) had no endogeic earthworms (means per sub-plot (Fig. 2) ± 1 SE).

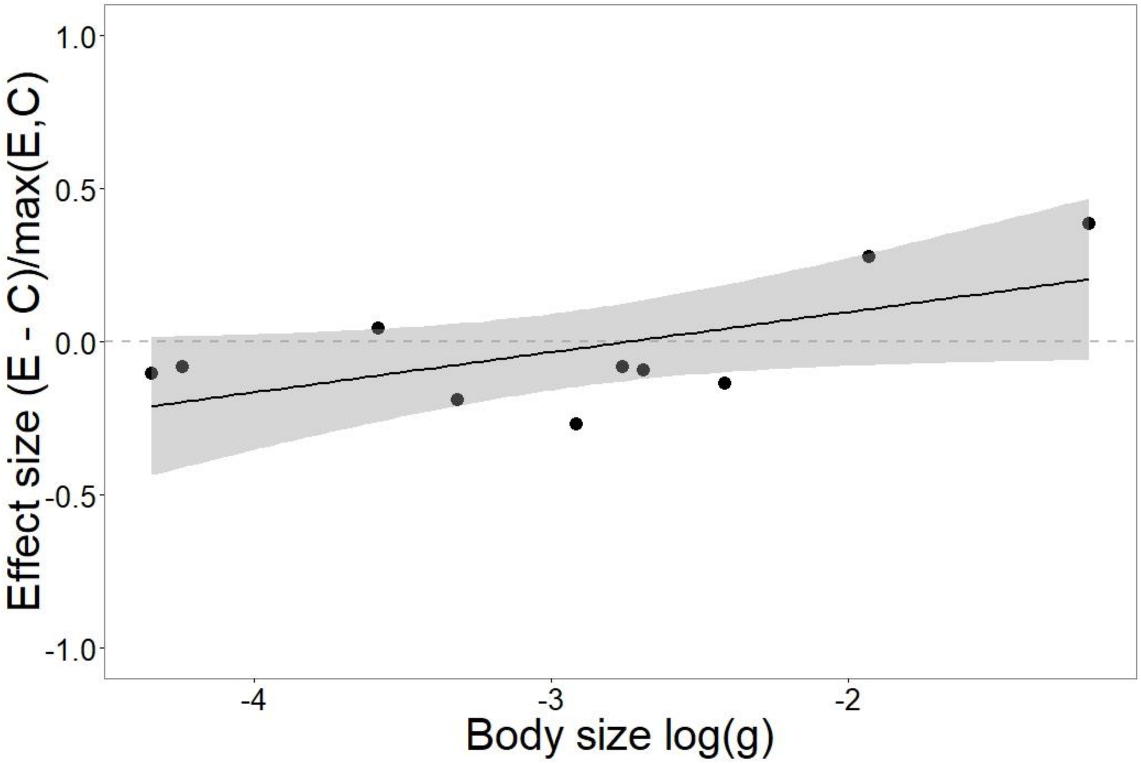

Figure 2.4: The standardized experimental effect size of deer exclosure (E), compared with control (C) sub-plots, correlated with earthworm species average adult body size (note log scale). Effect sizes of zero (dashed line) indicate no effect of deer exclosure on earthworm count; negative values indicate greater earthworm count in control sub-plots, and positive values indicate greater earthworm count in exclosure sub-plots.

Chapter 3

The influence of overabundant white-tailed deer and invasive earthworms on above- and below ground communities.

Summary

Above- and belowground interactions play an important role in shaping forest communities. Recent studies have focused primarily on plant-animal or plant-microbe interactions. Here, I examine how white-tailed deer and invasive earthworms influence both the plant and soil microbial community. I sampled the soil and plant community across 44 paired deer exclosure and control sub-plots across four spatial regions in Ohio, USA. I found invasive earthworms influenced both the bacteria and fungal community composition. However, earthworm functional groups differed in their influence, with the presence/absence or earthworm abundance of the functional groups having a significant effect on bacteria and fungal community structure. Functional groups also differed in how they interacted with deer presence to influence the bacteria and fungal community composition. I also found that fungal OTU richness was positively correlated with plant richness. This study highlights the need to focus on above- and belowground interactions involving multiple animals and across multiple trophic levels, as these interactions can have a significant influence on forest community composition.

Introduction

When studying forest communities, it is important to not just focus solely on aboveground or belowground interactions individually, but to also examine the linkages between both (Wardle et al. 2004, Wardle 2006, Kardol and Wardle 2010). Recent studies on above- and belowground effects have primarily focused on plant-animal interactions (Scheu 2003, Porazinska et al. 2003, Barber et al. 2012) or plant-microbial interactions (Hamilton and Frank 2001, Parniske 2008, Classen et al. 2015). However, fewer studies have focused on above- and belowground interactions involving multiple animals (but see Dávalos et al. 2015a, 2015b, Dobson and Blossey 2015) or across multiple trophic levels. It is through the linkages between the above- and belowground species that I can begin to tease apart interactions that govern forest communities, such as those between ecosystem engineers including invasive earthworms and the white-tailed deer (*Odocoileus virginianus*). Recent evidence also suggests that these two species interact with one another to help shape forest plant communities (Dávalos et al. 2015c, Dobson and Blossey 2015). Deer and earthworms might also be expected to influence plant and soil microbial communities.

Ungulate overpopulation is a major problem throughout much of the world, and studying the effects of it is crucial for forest communities (Côté et al. 2004). In North America, there have been numerous long term studies that focus on plant communities in the absence of deer through the use of deer exclosures, tall fences that keep out deer (e.g. McGarvey and Bourg 2013). Through these experiments I see that deer can alter nutrients within the soil (Frank et al. 2000, Seagle 2003a). Deer can also dramatically alter both tree and understory plant community composition through preferential feeding (Martin et al. 2010, Royo et al. 2010). It is through this preferential feeding that I see reductions in native plant diversity, and deer are generally expected to reduce diversity in native plant communities.

Individually, invasive earthworms have considerable negative effects on both the above- and belowground community, including on native plant diversity. These negative effects can be mediated through facilitation of invasive plants (Nuzzo et al. 2009), selective seed consumption (Eisenhauer et al. 2009, Nuzzo et al. 2015, Cassin and Kotanen 2016), altering mutualistic associations with mycorrhizal fungi and plant roots

(Lawrence et al. 2003), or changing the soil chemistry and nutrients (Reich et al. 2005, Hale et al. 2008), all of which can effect above- and belowground diversity. Not all earthworms have the same effect on forest communities (Burtelow et al. 1998, Marhan and Scheu 2005, Zicsi et al. 2011). Earthworms are categorized into groups by their functional traits, mainly their feeding and burrowing strategies (Bouche 1977). Each of these functional groups has different effects on the above- and belowground community. For example, *Lumbricus terrestris* an anecic earthworm, has the ability to pull leaf litter from the surface deep into the soil, causing litter duff and organic layer of the soil to be greatly reduced or even removed from the forest floor (Zicsi et al. 2011). Other earthworms like the *Amynthas sp.* feed within the organic layer and shallow soil breaking up fungal hyphal networks and reducing the organic layer of the soil (Burtelow et al. 1998).

Soil microbes are also a major component in the forest community, and effects of deer and earthworms on soil microbial communities are poorly understood. Soil microbes are essential for processes such as nutrient cycling (Bever et al. 1997), as well as forming mutualistic associations with the plant community (Parniske 2008). For example, mutualistic associations between plants and mycorrhizal fungi allow for greater intake of water and important nutrients like phosphorus (Parniske 2008), as well as provide greater resistance to soil pathogens that may harm the plant (Wehner et al. 2010). Other fungi found within the soil are considered saprotrophic and play an essential role in decomposition (Hobbie et al. 1999). Soil fungi have also been demonstrated to have an important role in shaping plant community composition through mediating interspecific competition among plants (Lin et al. 2015). It is through these interactions with plants and with each other that the microbial community can shape forest ecosystems (Van der Putten et al. 2001, Classen et al. 2015, Lin et al. 2015).

The goal of our study was to examine the above- and belowground interaction between overabundant white-tailed deer and invasive earthworms to explore possible effects of these species on both the aboveground plant community and the belowground microbial community. Using a long-running (5-15 years), large-scale field experiment that encompassed 44 deer exclosures and paired control plots, I sampled the earthworm, plant, and soil fungal and bacteria communities. I wanted to determine how the plant and soil microbial community would be altered when one or both of them were present. I expected that when both deer and earthworms were present, I would see a reduction in plant diversity and changes in microbial community composition. I also predicted that plant diversity would correlate positively with soil microbial diversity, because plants differentially influence soil microbial communities in their root zones (e.g. Hardoim et al. 2008, Berg and Smalla 2009, Burns et al. 2015), thus plant diversity might in part influence microbial diversity (and vice versa).

Methods

Study System

Our study site in northeastern Ohio, USA, encompassed 44 different plots, each of which contained both a deer exclosure and pre-established paired control sub-plot that were within 5m of each other. The maximum distance between any two given plots was 71 km, and the closest distance between any two plots was 0.1 km. The spatial location of each plot was recorded with a Garmin GPSmap 60 Cx GPS with a resolution of 10 meters. Soil characteristics consisted of a silt-loam substrate, with the exception of Little

Mountain at Holden Arboretum, which consisted of a sandy silt-loam substrate (Web Soil Survey, USDA: 7/12/2016).

Study Design

I collected earthworms in the exclosure and control sub-plots between late August to early October in both 2013 and 2014. This was done so that most of the earthworms would have reached maturity by that point in the season. Two random locations within each sub-plot were sampled for earthworms. The earthworm sampling was conducted using the hot mustard extraction technique (Chan and Munro 2001, Lawrence and Bowers 2002). Leaves were first carefully removed and checked for any earthworms from a 0.09 m^2 area within the sample frame. One gallon of water was mixed with 80 mL of hot mustard powder (Penzeys Spices, Wauwatosa, WI) and poured into a 0.09 square meter frame. Earthworms were collected from within the frame until they no longer surfaced for ten minutes, then preserved in a 15 percent formalin solution until they could be identified and weighed. I identified each individual adult earthworm to species, while each juvenile earthworm was identified to genus, because juvenile worms cannot typically be identified to species using morphological characteristics. Keying of each earthworm was based on the earthworm identification field guide provided by Great Lakes Worm Watch (Hale 2007). *Amynthas* sp. were only identified to genus as they are all almost indistinguishable from each other without the use of molecular techniques.

The soil in each of the sub-plots was sampled to determine what affect the deer exclosures and earthworms were having on the soil biota. Soil cores 2 cm wide by 5 cm deep were taken along 3 transects within each control and exclosure sub-plot. Multiple cores were taken along a single transect and then pooled together to provide a single transect sample. This was done in order to integrate the soil heterogeneity that could be found within transects. All cores were stored in a -80ºC freezer until DNA extractions could be done. DNA was extracted from approximately 500 mg soil wet weight using a CTAB bead-beating protocol and purified using a phenol-chloroform extraction (Baker and Mullin 1994). I targeted the 16S rRNA gene for bacteria using primers 338f and 926r (Muyzer et al. 1993, 1995) following conditions described by Burke et al. (2006). For fungi, I targeted the ITS2 region of the rRNA gene using primers 58A2F and NLB4 (Martin and Rygiewicz 2005) following conditions described in Burke et al. (2005) except the extension step was increased to 90 seconds. PCR was conducted using primers fluorescently labeled with either 6FAM (6-carboxyfluorescein) or HEX (4, 7, 20, 40, 50, 70 -hexachloro-6-carboxyfluorescein) and was carried out in 50-µl reaction volumes using 1-µl of purified DNA and 1 or 2 units of Taq DNA polymerase (Promega, Madison, WI) for bacteria and fungi respectively. Products were confirmed using agarose gel electrophoresis with positive and negative controls.

A restriction enzyme digest was conducted on PCR products using *Msp*I for bacteria and *Hae*III for fungi, with 5 µl PCR product, 0.3 µl restriction digest, 2.0 µl 10x Buffer, 12.7 µl H2), incubated at 37 ºC for 4 hours, followed by 15 min denaturing at 65 ºC. Restriction products were precipitated with 2.5 vol 100% EtOH and 1/10 vol sodium acetate (3 M, pH = 5.2) (Burke et al. 2005, 2006, 2008). Terminal restriction fragment length polymorphisms (TRFLP) were analyzed by the Ohio University Genomics Facility using an Applied BioSystems 3730xl DNA Analyzer (version 1.0, Applied Biosystems 2006). I used Peak Scanner™ software on both the fungal and bacterial datasets to analyze profile peaks. For each dataset, any peak that accounted for greater than 1% of the relative peak area were included in the analysis. Peaks with less than 50 base pairs or greater than 600 base pairs based on a GS600LIZ size standard were excluded from the analysis as well. Peaks were then sorted and binned into operational taxonomic units (OTUs) with an OTU bin width of 0.25 from the center of the bin (Burke et al. 2008). I have found that TRFs less than 1% of total profile area are generally not repeatable between replicate samples, and although excluding these TRFs may provide a more conservative estimate of microbial diversity, it reduces the chance that non-specific TRFs will be included in our analysis (Burke et al. 2008). I present data from the reverse (green channel) primers, because they generated the larger number of TRFs, which are used here in our analysis as operational taxonomic units (OTUs).

Statistical Analysis

To summarize the microbial community structure as quantified by TRFLP, I conducted nonmetric multi-dimensional scaling (NMDS) ordination using Bray-Curtis distances on the fungal and bacteria communities, separately. A stress value of less than 0.2 was chosen as the maximum allowable. I ran a metaMDS (vegan R package, Oksanene et al. 2017) function starting with 2 ordination axes, which produced a stress value of 0.11 for fungi, and 0.09 for bacteria respectively. Thus, a total of two MDS axes were used to minimize the stress of the ordination.

For models exploring the effects of deer presence, earthworms, and the potential interaction between the two, I first tested for spatial autocorrelation using Euclidean distances. For the fungal and bacteria communities, I analyzed ordination axes as response variables, in order to incorporate spatial autocorrelation into the model. Latitude and longitude were combined into a single distance matrix, which was then combined with each of the MDS axes. I then performed Mantel tests using the mantel.rtest with 9,999 randomizations (Dray and Dufour 2007) for each of the MDS axes to determine if there was significant spatial autocorrelation in bacterial and fungal community composition between sub-plots. Each of the Mantel tests showed spatial autocorrelation between our sub-plots. A review conducted by Beale et al. (2010) suggested using a generalized least squares model reduced Type I error, compared to an ordinary least squares model. Thus a generalized least squares model was chosen to best fit the data (Beale et al. 2010). I compared generalized least squares models both with and without spatial autocorrelation in the error structure, and also against a linear mixed effects model with plot as a random effect, and selected the modeling approach that minimized AIC. If the gls with spatial autocorrelation was preferred, I then compared models using different correlation structures (corGaus, corSpher, corExp, corLin) to determine the best method for modeling spatial autocorrelation. Again, I selected the modeling approach that minimized AIC.

In the first chapter I tested whether deer influence earthworm abundances. For three of the four functional groups I found that white-tailed deer did not influence earthworm abundances and thus can be treated as statistically independent of each other. However, within the endogeic functional group I saw that white-tailed deer significantly increased the abundance of endogeic earthworms. Thus I found a non-independence between white-tailed deer and endogeic earthworms. To further test for multi-collinearity between our treatment and functional groups I ran a variance inflation factor (VIF) test on our models. VIF measures how much of the explained variance within our analysis is inflated by the addition of a potential non-independent predictor to the model (Graham

2003). VIF values represent how much larger the standard error is compared to what it would be if the predictor variables were uncorrelated with each other in the model. For example, a VIF value of 3.12 would indicate that the standard error for the coefficient of that predictor variable is 3.12 times as large as it would be if the predictor variables were uncorrelated with each other. Ideally the VIF value should be around 1 however it is believed that any value under 5 is considered acceptable (Ringle et al. 2015).

Because I conducted a large number of tests, creating the possibility of inflated false positive results, I used false discovery rates analysis (Storey et al. 2004). I calculated the false discovery rate corrected q-values for each of the analyses of the fungal and bacteria MDS scores within functional groups using the bootstrap method for π_0 using the qvalue R package (Storey et al. 2004). Q-values indicated that 8 of the 22 significant p-values were no longer significant ($q > 0.05$) when interpreting the more conservative q-values. I interpret results based on q-values were appropriate.

Using this approach, AIC selected a gls with a corSpher correlation structure for each of the bacterial and fungal MDS scores, or microbial OTU richness as the response variables with deer presence and either earthworm presence or functional group abundance, and the interaction between deer and earthworms. Within MDS1 of the bacteria dataset I found a single outlier out of the 88 observations which had an MDS score of 31.13 compared to the rest of the MDS scores which ranged from 0.5 to -2. I thus excluded this point from all analyses of bacteria MDS1. For plant response variables, AIC selected a linear mixed effects model (lme) with either the Shannon's diversity index scores for the plant community or log transformed plant richness as our response variables, control/exclosure, earthworm presence/absence, and the interaction between them as our predictors, with plot included as a random factor. I thus used a linear mixed effects model with plot included as a random factor to test whether log-transformed plant richness influenced fungal OTU richness. Q-Q norm and residual vs. fitted plots were examined for violations of the model assumptions, and model assumptions were well-met for all analyses presented here. All analyses were conducted in the R statistics program (version 3.1.1, R Core Development Team 2014).

Results

The presence of earthworms significantly altered fungal community composition along MDS1 axis (Table 3.1, Fig. 3.1). I found that the presence functional group interacted with our deer exclusion treatment to significantly alter fungal community composition with the exception of the epigeic earthworms where there was only a main effect (Table 3.2). Along the fungal MDS2 axis I found a significant main effect of our treatment within each of the functional groups, albeit marginally significant within the epi-endogeic functional group (Table 3.2). Anecic earthworm abundance interacted with treatment to significantly alter the fungal community (Table 3.3). The abundance of epi-endogeic earthworms also significantly altered fungal community composition (Table 3.3). Neither endogeic or epigeic abundance had any significant effect on fungal communities (Table 3.3). Bacteria community composition was significantly altered by anecic earthworm presence, while the other three functional groups were marginally significant (Table 3.4, Fig. 3.2). Abundance of endogeic and epigeic earthworms both had a marginally significant effect on bacteria community composition (Table 3.5). OTU richness within bacteria or fungi did not significantly differ between treatments or with earthworm presence/absence (Appendix B: Table S3.2). All of the VIF scores were less than 5 with most around 1, which means that I did not have any multi-collinearity between our predictor variables.

I found a significant effect of deer exclusion treatment on the Shannon diversity score of plants, whereby there was an increase in plant diversity inside the deer exclosure (Appendix B: Table S3.1, Fig. 3.3). However, there was no significant difference in Shannon diversity score in sub-plots where earthworms were present versus where they were absent (Appendix B: Table S3.1). I found no difference in plant richness between the control and exclosure sub-plots or with the presence/absence of earthworms (Appendix B: Table S3.1). However, there was a positive correlation between plant richness and fungal richness ($t_{1,84} = 2.59$, $p = 0.01$, Fig. 3.3).

Discussion

Fungal community composition was significantly altered by deer and earthworm presence/absence. There are multiple mechanisms that could explain these patterns. First, deer could compact the soil (Shelton et al. 2014) possibly making it tougher for certain fungal species to create a hyphal network. Alternatively, deer could also directly alter the soil nutrient profile (Seagle 2003b, Moe and Wegge 2008) and thus alter the fungal community. Deer could also indirectly affect the fungal community through their preferential feeding on the plant community. Because different plant species differentially influence fungal community composition (e.g. Burns et al. 2015; reviewed in Ehrenfeld 2010), effects of deer on plant communities could indirectly influence the fungal community. This data further supports this idea because there is a significant correlation between plant richness and fungal OTU richness (Fig. 3.3). Earthworms have also been shown to alter the fungal community (Jusselme et al. 2015). One of the most prominent mechanisms is larger earthworms (e.g. anecic, epi-endogeic) breaking up hyphal networks while feeding within the soil column (Lawrence et al. 2003). One interesting observation is that within anecic earthworms, there was an interaction between earthworm abundance and deer presence on fungal community composition (Table 3.2). Thus whether or not deer are present influences the strength of the earthworm effect on fungal community composition, but only for the highly invasive, anecic *L. terrestris*.

I found that both deer and earthworm presence/absence had no effect on bacteria community structure. I found this surprising as both deer and earthworms have been shown to alter nutrients within the soil (Hale et al. 2005, Moe and Wegge 2008), which might be expected to influence the bacteria community (Wardle et al. 2004). For example, if earthworms increase the amount of available nitrogen in the soil I might expect to see more nitrogen fixing bacteria species within the soil. Deer could also alter these bacterial communities indirectly through herbivory of plants and the reduction of available organic matter being returned to the soil (Bardgett and Wardle 2003).

Earthworm functional groups influenced whether or not earthworms have an effect on microbial communities; each of the functional groups altered both the fungal and bacterial community, but in different ways. For the larger anecic (*L. terrestris*) and epi-endogeic (*Amynthas sp.*) earthworms, their differences in abundances had an effect on the fungal community, while just their presence or absence altered the bacteria community. For the smaller epigeic and endogeic earthworms, differences in abundance influenced bacteria communities while their presence or absence altered fungal communities. This shows that each of the functional groups influenced microbial community composition in a different way. This study highlights the need to incorporate both earthworm functional group and abundance when studying the effects of earthworms on forest communities.

Caution must be used when interpreting any significant result involving endogeic earthworms (Table 3.2, 3.5) as I have shown that white-tailed deer and endogeic earthworms are not independent of one another (Chapter 2). For example, within the endogeic earthworms I found a main effect of deer presence on fungal community composition (Table 3.2). If white-tailed deer and endogeic earthworms are highly confounded, it is possible that this study could have low power to detect abundance effects of these earthworms because the control/exclosure treatment is modeling all of that variance. I encourage experiments that manipulate earthworms independent of deer to help identify whether deer and earthworms influence soil microbial communities independently or have synergistic effects.

Perhaps surprisingly, plant species richness did not differ between the control and exclosure sub-plots. I would have expected there to be greater forest understory regeneration within the exclosures, as they would not be under the same amount of herbivory pressure from deer. One possible explanation is that a long history of multiple stressors such as land use change, deer overpopulation, or earthworm invasions have reduced diversity in the seed bank such that plots that have had deer excluded have not been able to regain their previous richness. For example, earthworms are known seed predators (Cassin and Kotanen 2016); thus there might not be much of a seed bank for forest regeneration to occur within the exclosures.

I found that excluding deer increased the plant diversity (Shannon’s Index) within the exclosures (Appendix B: Table S3.1, Fig. 3.4). This is not surprising given other studies have demonstrated the negative effects deer have on plant community diversity (reviewed in Côté et al. 2004, Martin et al. 2010, Shelton et al. 2014). Because this diversity metric is a function of both richness and evenness, and because there were no effects of deer exclusion on richness, lower diversity in the presence of deer is likely a result of changes in community evenness. Changes in evenness could be a result of preferential browsing leading to shifts toward fewer more dominant species. While most species are still present with deer, the preferred food plants may have become relatively less abundant. If this is true, the rare plant species might become more rare in the future, potentially leading to longer-term shifts in richness.

There was no effect of earthworm presence on plant diversity or richness, which is contrary to other studies that have shown earthworms can significantly reduce plant richness in forest communities (Holdsworth et al. 2007, Dávalos et al. 2015b). However, there was a high degree of variance in diversity among the different sites, which could obscure subtle effects of earthworms on plants. Some of this variance could be caused by the land use history of each of these sites, as some of the sites were agricultural fields while others remained as forests decades ago.

White-tailed deer and invasive earthworms have both been shown to be ecosystem engineers in forests (Scheu 2003, Côté et al. 2004). There have been numerous studies on each of these groups individually that highlight their effects on the forest ecosystem (Rooney and Waller 2003, Szlavecz et al. 2011, Fahey et al. 2013, Shelton et al. 2014), however there are very few that examine their potential interactive effects on forest communities (Dávalos et al. 2014, 2015b, Dobson and Blossey 2015). It is important that future studies on invasive earthworms incorporate functional group; this work highlights the differences across functional groups in earthworm effect on soil microbial communities. Our study adds to the growing body of literature that highlights the need to study above- and belowground interactions, especially those involving white-tailed deer and *L. terrestris*, which had interactive effects on soil microbial community structure. Understanding the interactive effects of invasive earthworms is important because these linkages between above- and belowground biota can potentially influence forest community composition.

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I would like to thank Ryan Trimbath and Gregory A. Smith for their assistance in the design and execution of this project as well as Terry Robison for suggesting the project. I would like to thank the many volunteers especially Erin Berkowitz for their assistance in field collections. Thanks also to Cleveland Metroparks, Metroparks Serving Summit County, Lake Metroparks, and Holden Forests and Gardens for access to their field sites and use of lab space and equipment. Funding for this project was provided by NSF grant to J.H.B. (DEB 1250170), and a Cleveland Metroparks travel grant to C.G.C.

Tables

Table 3.1: Generalized least squares of response variable ordination axes MDS1 and MDS2 for fungal communities with deer exclusion treatment and worm presence/absence as predictors. Latitude and longitude were included in the model as part of the error structure.

Fungal MDS1	NumDF	DenDF	F-value	p-value
Control/Exclosure (C/E)	1	84	0.96	0.33
Earthworm Presence/Absence	1	84	1.01	0.32
C/E x Earthworm Presence/Absence	1	84	0.64	0.43
Fungal MDS2				
Control/Exclosure (C/E)	1	84	3.00	0.08
Earthworm Presence/Absence	1	84	6.00	0.01
C/E x Earthworm Presence/Absence	1	84	0.00	0.64

Table 3.2: Generalized least squares of response variable ordination axes MDS1 and MDS2 for fungal soil communities with deer exclusion treatment and earthworm presence/absence for each functional group as predictors. Latitude and longitude were included in the model as part of the error structure.

	MDS1				**MDS2**			
Anecic	VIF	F-value	p-value	q-value	VIF	F-value	p-value	q-value
Control/Exclosure (C/E)	1.31	2.53	0.12	0.20	1.19	10.00	0.002	0.009
Earthworm Pres/Abs	1.52	0.00	0.98	0.53	2.00	1.00	0.29	0.32
(C/E) x Earthworm Pres/Abs	1.83	19.30	<0.001	<0.001	2.19	2.00	0.12	0.20
Endogeic								
Control/Exclosure	1.66	2.55	0.11	0.20	1.58	10.00	0.002	0.009
Earthworm Pres/Abs	1.48	1.04	0.31	0.32	1.49	1.00	0.46	0.41
(C/E) x Earthworm Pres/Abs	1.30	18.75	<0.001	<0.001	1.35	2.00	0.16	0.23
Epigeic								
Control/Exclosure	1.76	2.49	0.12	0.20	1.70	9.00	0.003	0.01
Earthworm Pres/Abs	1.21	17.10	<0.001	<0.001	1.39	0.00	0.90	0.49
(C/E) x Earthworm Pres/Abs	1.77	0.41	0.52	0.41	1.91	1.00	0.35	0.35
Epi-Endogeic								
Control/Exclosure	1.71	2.22	0.14	0.22	1.52	4.00	0.06	0.12
Earthworm Pres/Abs	1.33	0.02	0.88	0.49	1.94	11.00	0.001	0.006
(C/E) x Earthworm Pres/Abs	1.93	6.41	0.01	0.03	2.39	5.00	0.02	0.06

Degrees of Freedom: MDS1 = (1, 84), MDS2 = (1, 84).

Table 3.3: Generalized least squares of response variable ordination axes MDS1 and MDS2 for fungal soil communities with deer exclusion treatment and earthworm abundance for each functional group as predictors. Latitude and longitude were included in the model as part of the error structure.

	MDS1				**MDS2**			
Anecic	VIF	F-value	p-value	q-value	VIF	F-value	p-value	q-value
Control/Exclosure (C/E)	1.18	1.57	0.21	0.25	1.16	11.00	0.001	0.006
Earthworm Abundance	1.42	25.53	< 0.001	<0.001	1.52	1.00	0.44	0.41
(C/E) x Earthworm Abundance	1.58	31.18	< 0.001	<0.001	1.66	6.00	0.02	0.06
Endogeic								
Control/Exclosure (C/E)	1.18	2.14	0.15	0.23	1.16	9.00	0.003	0.01
Earthworm Abundance	1.10	1.77	0.19	0.24	1.08	0.00	0.53	0.41
(C/E) x Earthworm Abundance	1.09	1.58	0.21	0.25	1.08	1.00	0.40	0.39
Epigeic								
Control/Exclosure (C/E)	1.42	2.10	0.15	0.23	1.37	10.00	0.002	0.009
Earthworm Abundance	1.30	1.74	0.19	0.24	1.39	3.00	0.11	0.20
(C/E) x Earthworm Abundance	1.54	0.02	0.90	0.49	1.61	2.00	0.14	0.22
Epi-Endogeic								
Control/Exclosure (C/E)	1.71	0.40	0.53	0.41	1.52	3.00	0.07	0.14
Earthworm Abundance	1.33	0.00	0.99	0.53	1.94	12.00	< 0.001	<0.001
(C/E) x Earthworm Abundance	1.93	0.25	0.62	0.45	2.39	1.00	0.26	0.30

Degrees of Freedom: MDS1 = (1, 84), MDS2 = (1, 84).

Table 3.4: Generalized least squares of response variable ordination axes MDS1 and MDS2 for bacteria soil communities with deer exclusion treatment and earthworm presence/absence for each functional group as predictors. Latitude and longitude were included in the model as part of the error structure. Bacteria MDS1 had one row removed due to it being an outlier.

	MDS1				**MDS2**			
Anecic	VIF	F-value	p-value	q-value	VIF	F-value	p-value	q-value
Control/Exclosure (C/E)	1.31	0.05	0.81	0.48	1.19	0.10	0.75	0.48
Earthworm Pres/Abs	1.58	0.41	0.52	0.41	2.00	7.67	0.006	0.02
(C/E) x Earthworm Pres/Abs	1.89	1.76	0.19	0.24	2.19	1.00	0.32	0.33
Endogeic								
Control/Exclosure (C/E)	1.43	0.11	0.74	0.48	1.66	0.43	0.52	0.41
Earthworm Pres/Abs	1.62	0.20	0.66	0.46	1.48	0.32	0.58	0.44
(C/E) x Earthworm Pres/Abs	1.51	1.16	0.28	0.32	1.30	4.37	0.04	0.10
Epigeic								
Control/Exclosure (C/E)	1.70	0.06	0.80	0.48	1.76	0.41	0.52	0.41
Earthworm Pres/Abs	1.29	5.91	0.02	0.06	1.21	1.74	0.19	0.24
(C/E) x Earthworm Pres/Abs	1.81	0.77	0.38	0.37	1.77	0.05	0.83	0.48
Epi-Endogeic								
Control/Exclosure (C/E)	1.53	0.11	0.74	0.48	1.71	0.42	0.52	0.41
Earthworm Pres/Abs	1.96	0.21	0.65	0.45	1.33	0.16	0.69	0.47
(C/E) x Earthworm Pres/Abs	2.40	0.02	0.88	0.49	1.93	3.82	0.05	0.11

Degrees of Freedom: MDS1 = (1, 83), MDS2 = (1, 84).

Table 3.5: Generalized least squares of response variable ordination axes MDS1 and MDS2 for bacteria soil communities with deer exclusion treatment and earthworm abundance for each functional group as predictors. Latitude and longitude were included in the model as part of the error structure. Bacteria MDS1 had one row removed due to it being an outlier.

	MDS1				MDS2			
Anecic	VIF	F-value	p-value	q-value	VIF	F-value	p-value	q-value
Control/Exclosure (C/E)	1.17	0.05	0.81	0.48	1.11	0.10	0.76	0.48
Earthworm Abundance	1.42	0.32	0.57	0.43	1.81	1.77	0.19	0.24
(C/E) x Earthworm Abundance	1.58	1.68	0.20	0.25	1.90	0.60	0.44	0.41
Endogeic								
Control/Exclosure (C/E)	1.66	0.06	0.81	0.48	1.16	0.20	0.65	0.46
Earthworm Abundance	1.48	0.53	0.47	0.41	1.09	5.05	0.03	0.08
(C/E) x Earthworm Abundance	1.30	0.02	0.88	0.49	1.08	0.10	0.75	0.48
Epigeic								
Control/Exclosure (C/E)	1.39	0.06	0.81	0.48	1.42	0.43	0.51	0.41
Earthworm Abundance	1.33	3.98	0.05	0.11	1.29	4.80	0.03	0.08
(C/E) x Earthworm Abundance	1.56	1.05	0.31	0.32	1.54	1.14	0.29	0.32
Epi-Endogeic								
Control/Exclosure (C/E)	1.26	0.11	0.74	0.48	1.30	0.40	0.53	0.41
Earthworm Abundance	3.83	0.24	0.62	0.45	2.16	0.00	0.99	0.53
(C/E) x Earthworm Abundance	4.18	0.08	0.78	0.48	2.55	0.25	0.62	0.45

Degrees of Freedom: MDS1 = (1, 83), MDS2 = (1, 84).

Figures

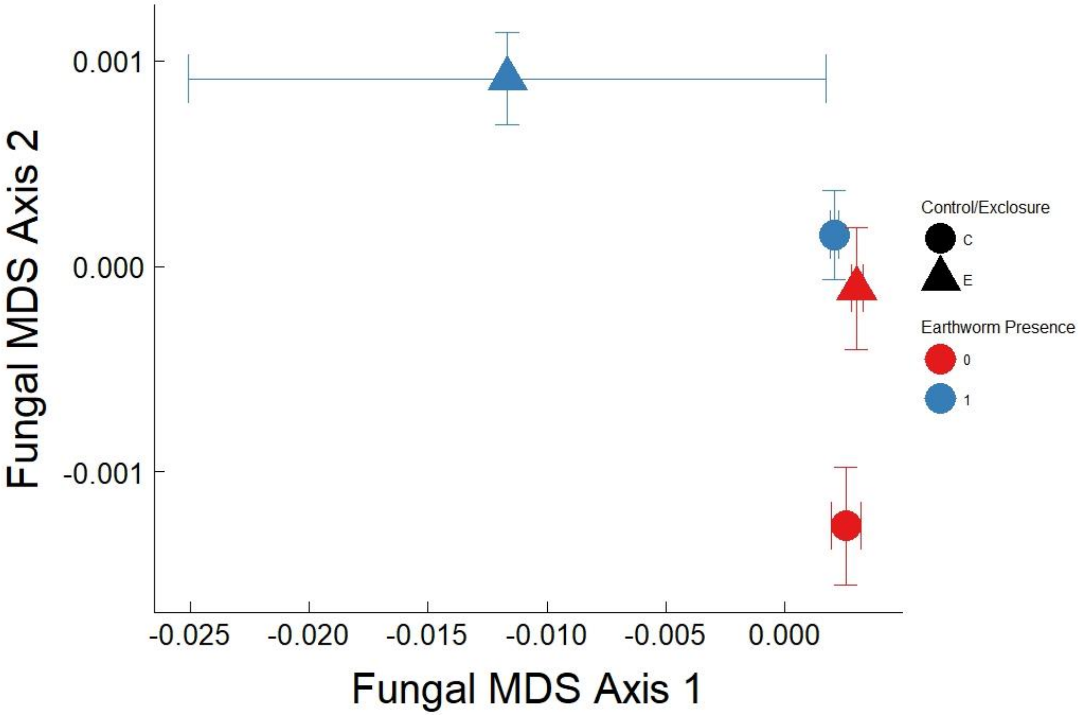

Figure 3.1: Ordination of fungal MDS axes separated out by treatment type and earthworm presence/absence. I found a significant effect of worm presence/absence and a marginally significant effect of treatment along fungal MDS2. Means ± 1 SE.

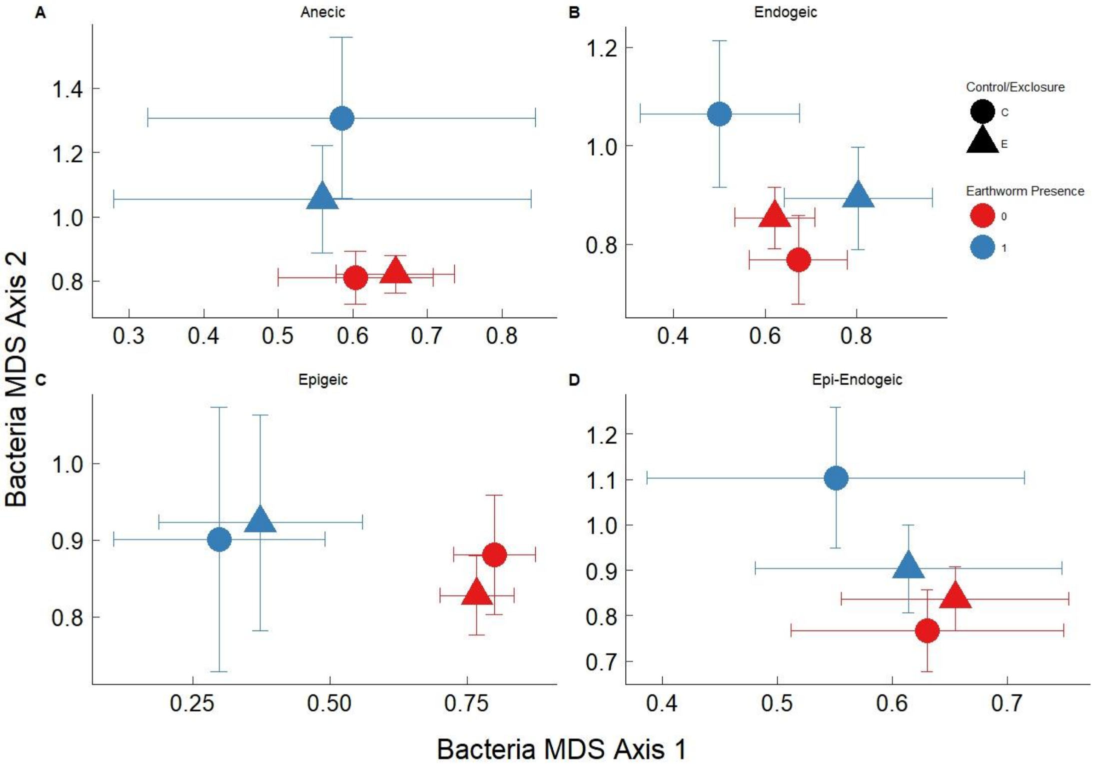

Fig. 3.2: Ordination of bacterial MDS axes separated out by treatment type and earthworm presence/absence for (a) anecic, (b) endogeic, (c) epigeic, (d) epi-endogeic earthworm functional groups. Means ± 1 SE.

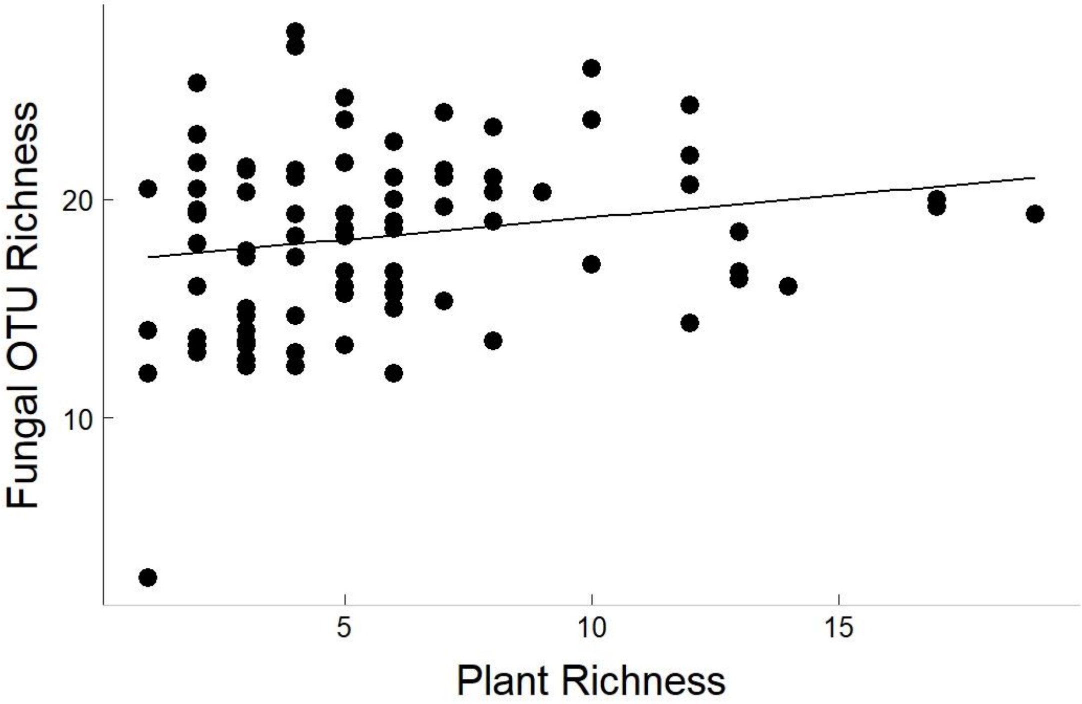

Figure 3.3: The correlation between plant and fungal OTU richness, with points representing either control or exclosure sub-plots. A linear model showed a significant trend between the two ($t_{1,84} = 2.59$, $p = 0.01$).

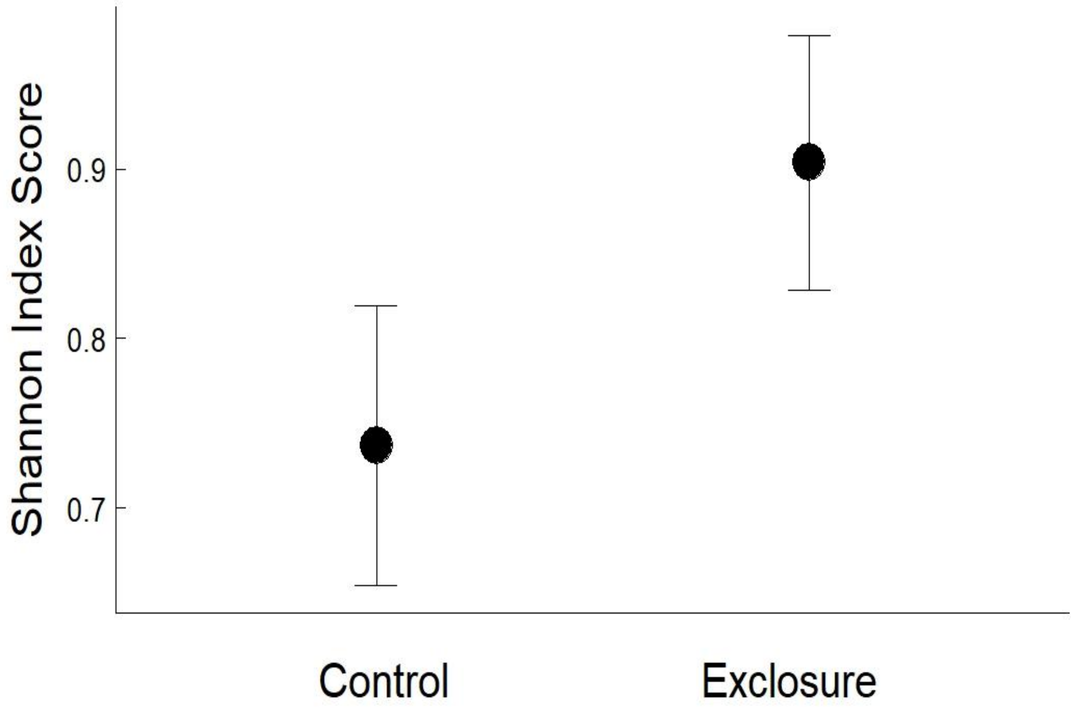

Figure 3.4: The diversity of plants as represented by Shannon's diversity index was significantly higher inside deer exclosure sub-plots than in sub-plots where deer had access. Values are means ± SE.

Chapter 4

Potential interactive effects between invasive *Lumbricus terrestris* and the invasive plant garlic mustard on a native plant.

Summary

Many studies have focused on how single invaders influence other species. However, most ecosystems are not affected by just a single invader. Interactions between species can have additive or non-additive effects. Our study highlights the need to account for interactions amongst multiple invaders, and how these interactions might have additive or non-additive effects. I used observational field data taken from the Cleveland Metroparks to examine how invasive earthworms and garlic mustard interacted to affect the native spring ephemeral mayapple (*Podophyllum peltatum*), and whether the soil environment influences the potential interaction between these invaders. I also used a two-year 2 × 2 × 2 factorial pot experiment with the presence or absence of garlic mustard (*Alliaria petiolata*), *Lumbricus terrestris*, and activated carbon to experimentally test for a potential interaction between these invasive species. Activated carbon was added as a treatment because of its highly absorptive properties, which might absorb allelochemicals produced from the garlic mustard, as well as influencing soil nutrient availability. I measured native plant physiological responses to these experimental treatments, as well as nutrient content within leaf tissues, as a measure of invader effects on nutrient uptake and physiology. Within the field data I found that garlic mustard correlated with reduced mayapple cover within plots and that the presence of worms reduced this negative effect of garlic mustard. Within the factorial experiment, I found that garlic mustard suppressed mayapple growth in the presence of *L. terrestris*, but only when activated carbon was present. The combination of observational and experimental data suggests that the invasive plant, garlic mustard, might interact with the invasive earthworm, *L. terrestris*, to influence plant performance, though the direction of this

effect is likely to be environment-dependent. Further, such effects are mediated by complex nutrient uptake and physiological responses in the native plant. While more physiological studies of invader interactive effects are needed, our study suggests that enhanced nutrient uptake (i.e. in the presence of earthworms) does not always lead to greater maximum photosynthetic rates or greater plant growth rates.

Introduction

Assessment of effects of invaders have generally focused on single invasive species, despite the frequent observation of systems with multiple invaders (Kuebbing et al. 2013). In a recent review, only 6% of studies analyzed interactions among multiple invasive plants (Kuebbing et al. 2013), and most of those did not assess effects on native species, but rather the effects of invaders on one another (Kuebbing and Nuñez 2015). Our ability to predict and perhaps reduce the negative effects of invaders will be enhanced by a deeper understanding of the mechanisms by which multiple invaders influence native species.

Interactions between multiple invaders could be additive, such that they could be predicted from single-species studies (e.g. Milton et al 2007; Cushman and Gaffney 2010; Shaben and Myers 2010). For example, an exotic reed had no effect on other invaders in a field experiment (Cushman and Gaffney 2010). If this is often the case, then management recommendations based on single-species studies may still be useful. However, it is important that studies examine these interactions and not assume that the effect will always be additive.

Interactions between multiple invaders could also be sub-additive, an interaction which is sometimes called "invasional interference" (sensu Yang et al 2011). Competition between invasive plants is the most commonly found result in multi-invader studies (e.g. Kuebbing and Nuñez 2015), though most such studies do not examine effects of these invader interactions on native species (but see Rudgers and Orr 2009). For example, two introduced *Carduus* thistles compete equally with one another (Rauschert and Shea 2012), thus potentially interfering with one another's invasions.

Alternatively, multiple invaders could have super-additive interactions, such that single-species studies may under-predict invasion speed or impacts. This positive interaction among invaders has been called "invasion meltdown" (sensu Simberloff and Holle 1999) or "facilitation" among invaders (Kuebbing et al. 2013) and has been found in some empirical studies (e.g. O'Dowd et al. 2003, Green et al. 2011). For example, an invasive grass is facilitated by the presence of other introduced plants, through reduced native herbivory (Cushman et al., 2011). Plants with mutualistic relationships with nitrogen-fixing bacteria also seem to be more likely to have super-additive interactions with other invasive plants (Kuebbing and Nunez, 2015).

Few studies of multiple invaders examine above- and below-ground interactions, though such interactions are increasing thought to be strong (Dávalos et al. 2015b). However, in one such study, several native woody species were suppressed by the combined effects of an invasive grass and the grass's fungal endophyte (Rudgers and Orr, 2009). This effect was mediated by the influence of the endophyte on soil microbes, suggesting a possible mechanism for this interaction. Another study focused on the interactions between invasive earthworms and multiple invasive plants. They found that earthworms were the main drivers in altering native plant diversity, and that invasive earthworms facilitated plant invasions (Nuzzo et al. 2009).

This study focuses on the interaction between the invasive plant garlic mustard (*Alliaria petiolata)* and another species considered invasive in northeast Ohio, the earthworm *Lumbricus terrestris*. I propose that *L. terrestris* earthworms may amplify the effects of garlic mustard allelopathy through their feeding mechanism of pulling leaf litter into their underground burrows (Bouche 1977). This feeding mechanism may increase the distribution of leaf litter over the roots of native plants, increasing the negative allelopathic effects of garlic mustard. Alternatively, *L. terrestris* moving leaf litter through the soil may break down the nutrients in the leaves faster than decomposition alone, therefore exposing native plants to the allelochemicals for a shorter period of time and lessening their harmful effects or alter nutrient cycling. Garlic mustard is known to have allelopathic effects on arbuscular mycorrhizal fungi (AMF) (Hale and Kalisz 2013), which help plants acquire nutrients from the soil. When garlic mustard allelochemicals kill AMF mutualists, this has been shown to detrimentally affect photosynthetic rates in at least one native plant (Hale et al. 2011).

Photosynthetic rates are limited by phosphorus availability, and phosphorus uptake is mediated by AMF mutualists in many cases. Photosynthetic rates are also limited by nitrogen availability, which is a component of RuBisCO, the enzyme that fixes carbon, in C_3 plants like mayapple. In addition to maximum photosynthetic rates, one measurement that researchers commonly consider is C_i, the carbon concentration in the intercellular part of the leaves. C_i should be mediated by stomatal opening, which influences the rate at which CO_2 enters the leaf, and by the rate at which carbon is shuttled out of the intercellular spaces of the leaf. Therefore, photosynthetic rates in plants are mediated by nutrient limitation, including nitrogen and phosphorus availability in plant tissues, and a complex interplay including light availability, stomatal opening, and carbon fixation rates.

To examine the effects that the *L. terrestris*/garlic mustard interaction might have on native plant growth, I conducted two studies on a common AMF associated spring ephemeral plant native to Ohio: mayapple (*Podophyllum peltatum*). I utilized a large-scale dataset to determine how native mayapple correlates with these invaders in a natural setting and see whether field observations are consistent with these multiple invaders interacting to influence the native plant mayapple. I also conducted a factorial experiment that focused on four main goals. These goals were to determine (1) whether the presence of garlic mustard leaf litter would directly suppress the growth of a native plant, (2) whether the presence of the invasive earthworm *L. terrestris* would interact with garlic mustard to influence native plant growth, (3) whether the conditions of the soil environment influence the suppression of native plant growth by invasive species, and (4) how plant nutrient content and physiology might mediate mayapple response to multiple invaders.

Materials and Methods

Plant Community Assessment Program dataset

I utilized a large (>400 plot) plant community dataset (PCAP) collected over a series of four years between 2010 and 2013 by Cleveland Metroparks to compare our factorial experiment to natural populations of mayapple. The plots that were sampled covered a 20m x 50m area, in which the percent cover of every plant species was recorded, as well as collecting soil samples for nutrient analysis. I focused on plots that contained mayapple and either the presence or absence of garlic mustard and/or earthworms to maintain parallel structure to the potted experiment. There was a total of 184 plots that met these criteria. I then compared the percent cover of mayapple, as well as the percent soil nitrogen and two different measures of inorganic phosphorus in parts per million (ppm) across plots with or without these predictor variables.

Factorial Experiment

The experiment was located at Case Western Reserve University's Squire Valleevue Farm ("University Farm" below) in Hunting Valley, Ohio, USA (41°29' N, 81°25' W) over two years (2014-2015). The experiment was organized in a 2 × 2 × 2 factorial design, with 6 replicates per experimental treatment, for a total of 48 pots. Each treatment included the presence or absence of the three factors of garlic mustard, *L. terrestris*, and activated carbon (See Fig. 4.1). Treatments were randomized within 6 replicate blocks.

To ensure that the mayapple would receive adequate nutrients in the presence of the activated carbon, I added 84 g of nutricote 100-day fertilizer with 18-6-8 N-P-K (#18-6-8NUTRI-100, American Horticultural Supply, Inc.) on 5 June 2014 to all pots, following methods in Hale *et al*. (2011). By adding a small amount of phosphorous, I created conditions where maypple plants should have maintained their AMF mutualism. On 21 May 2014, 40 liters of potting soil (Promix, Quebec, Canada, BX Mycorrhizae general purpose growing medium # 10381) were added to each pot, along with a maypple rhizome obtained from Prairie Moon Nursery in Winona, MN and one cup of live soil from underneath field mayapple to encourage arbuscular mycorrhizal fungi (AMF) colonization.

Activated carbon, a highly absorbent material, was added as a treatment under the assumption that it would absorb both allelochemicals and soil nutrients, to determine whether the effects of these invaders depend on soil conditions. Activated carbon is often used in allelopathy studies to absorb allelochemicals (e.g. Callaway and Aschehoug 2000; Prati and Bossdorf 2004), but has multiple effects on the soil (Lau et al. 2008). Pots with an activated carbon present treatment received 400g of activated carbon pellets incorporated into the soil (following similar methods in Lau et al. 2008).

Pots with a *L. terrestris* present treatment received 16 *L. terrestris* worms. This is consistent with natural populations in our area where I have been finding 12 to 16 individuals per square meter (C.G. Cope, unpublished data). Studies have shown that this species can be found in excess of 100 individuals per square meter (Eisenhauer et al. 2007). Pots with a garlic mustard present treatment were treated weekly with 100g of garlic mustard leaves spread on the surface of the soil during garlic mustard's growing season. This amount allowed the entire surface of the soil within the pot to be covered to provide substantial leaf litter cover for *L. terrestris* feeding in treatments containing worms and make sure that the allelochemicals could leach into all of the soil surface (similar to methods in Hale et al 2011).

Once mayapple began to senesce in the field in mid-July 2014, the pots containing the mayapple rhizomes were stored in an uninsulated barn during the winter to prevent the rhizomes from freezing and dying. The pots were then pulled out of storage in early April 2015. Sixteen worms were again added to *L. terrestris* present pots, and 100g of garlic mustard was again added weekly to garlic mustard present pots in the summer of 2015 until 12 June, when the mayapple plants were harvested. Roots were washed and biomass was separated into separate fractions: leaves, stem, rhizome, roots, and reproductive. Biomass was dried in the drying oven for 24 hours (leaves) or 72 hours (stems, rhizomes, roots and reproductive) at 60ºC, and weighed to 0.01 grams.

Plant nutrient content and physiology

In order to assess the photosynthetic rate of maypple across treatments, I used an LI-6400XT portable photosynthesis system. Ambient conditions were mostly sunny with an average photosynthetic photon flux density (PPFD) of 1500 µmol m^{-2} s^{-1} and ~21^{o}C. Conditions within the chamber were maintained at a flow rate of 400 µmol s^{-1}, a CO_2 concentration of 400 µmol mol^{-1}, a leaf temperature of 21.4 ^{o}C, and between 40 and 50% relative humidity. PPFD was set to 1200 µmol m^{-2} s^{-1} for all individuals measured. Based on previous data, this light level represented saturation for plants without inducing photo inhibition. Once plants stabilized in the chamber I logged 3 data points per plant on 23 May 2015 between 11:00 AM and 1:00 PM before rates began to lose stability.

I also measured leaf nutrients content because I predicted that these experimental treatments might influence nutrient acquisition. If activated carbon binds nutrients from the soil due to its charge, then these nutrients may become less available to the plants and reduce the plant's performance. The alternative approach of measuring soil nutrient availability might thus less directly tie to plant performance, because differences in nutrient concentrations do not necessarily translate into differences in plant nutrient uptake. In order to assess leaf nutrient content, I followed the methods described in Khasanova et al (2013) for CN analysis. Plant biomass was washed with DI water and oven-dried at 65 °C. Subsamples of dried plant tissue were ground with a ball mill, then 2±0.1µg samples were weighed for total N and C concentration by micro Dumas combustion on a CN analyzer (Costech Analytical, Valencia California).

To determine arbuscular mycorrhizal fungi (AMF) colonization I stained roots collected from the mayapple plants at the end of the experiment. This procedure followed root staining protocol put forth by McGonigle and colleagues (1990). Following staining

I examined the stained roots under and microscope at 40x and 100x magnification. I found no traces of any AMF colonization of the roots. I also performed DNA extractions on both ground and unground roots, and the used polymerase chain reaction (PCR) using the AMG1F and AM1 primers to amplify any AMF DNA found within the mayapple roots. I then used gel electrophoresis to determine if there was AMF colonization of our samples. There were no visible bands, indicating that the mayapple roots had no AMF DNA present.

Statistical Analyses

The data were analyzed using generalized linear models (GLM) for both the observational dataset and the potted experiment. For both data sets our main predictor variables were the presence or absence of garlic mustard and/or earthworms. In addition, for the potted experiment the presence or absence of activated carbon was included as a predictor. For the observational dataset, I included either soil nitrogen or soil phosphorus as an additional predictor variable. I also tested whether either invader correlated with or helped to predict nutrient concentrations within the soil. All datasets were examined for potential outliers as well as tested for normality. Within the observational data, percent cover of mayapple and garlic mustard, as well as soil nitrogen and phosphorus were all log transformed to normalize the data. I used a Poisson error structure to test the interaction between garlic mustard and earthworms on maypple cover within the observational dataset, because mayapple cover was best approximated by a Poisson. All other models used a Gaussian error structure. I also conducted planned contrasts of both the earthworm and garlic mustard treatments to compare the means of both treatments with and without activated carbon (Appendix C: Table S4.1-S4.2). Q-Q norm and residual vs. fitted plots were examined for each model to check model assumptions were met. All model assumptions were met. All analyses were conducted in the R statistics program (version 3.1.1, R Core Development Team 2014).

Results

Plant Community Assessment Program Observational data

Prior to answering whether the presence of garlic mustard and earthworms correlate with mayapple percent cover, I first needed to determine if the invasive earthworms and garlic mustard variables were independent from one another. I conducted a contingency table analysis and found that the presence of garlic mustard and earthworms were independent of each other, such that the presence of one did not influence the presence of the other (Chi-Sq = 1.53, P = 0.22). Thus I treated the presence of garlic mustard and earthworms as independent variables in the following analyses.

Following that, I found that mayapple cover was reduced, though only marginally significantly, within plots that just had garlic mustard (Table 4.1, Fig. 4.2). I then included the soil nutrients nitrogen, phosphorus 1 and phosphorus 2 into the model to determine if they interacted with the invaders to influence mayapple cover. The presence of garlic mustard and high concentrations of soil nitrogen interacted to reduce mayapple cover, with the least mayapple cover in garlic mustard sites with high soil nitrogen (Table 4.1, Fig 4.3). Soil phosphorus did not have any significant interaction with either of the invasive species (Table 4.1).

I also asked whether either of the invaders correlated with either of these soil nutrients. I found that garlic mustard abundance increased with higher soil phosphorus ($t = 5.41$, $p < 0.001$) but not higher soil nitrogen ($t = 0.96$, $p = 0.34$), and that earthworm presence significantly correlated with total soil nitrogen (Table 4.2, Fig. 4.4b). There was a significant interaction between garlic mustard and earthworms on phosphorus 1 in the soil (Table 4.2, Fig. 4.4a) but not phosphorus 2 (Table 4.2).

Factorial Experiment

Our focus for this experiment was to determine how mayapple performed under the stress of multiple invaders. I found that the total biomass of mayapple was significantly reduced when earthworms, garlic mustard, and activated carbon were present (Table 4.3, Fig. 4.5). Contrasts examining how garlic mustard influenced mayapple biomass in the presence or absence of earthworms and/or activated carbon showed a significant effect of garlic mustard on mayapple biomass when both earthworms and activated carbon were present (Appendix C: Table S4.1). Conversely, contrasts examining earthworms influence on mayapple biomass in the presence or absence of garlic mustard and/or activated carbon only showed a marginally significant effect when both of the other treatments were present (Appendix C: Table S4.2).

Mayapple leaf nutrient content and physiology were both influenced by experimental treatments. I found plant nutrient content and leaf nitrogen content were significantly increased in the presence of the earthworm treatment (Fig. 4.6a). There was also a significant decrease of internal CO_2 concentration (C_i) within the leaves of mayapple when in the presence of the earthworm treatment (Table 4.5, Fig. 4.6b). There were no significant differences in leaf nitrogen or carbon in any of the other treatments. There was a marginally significant reduction in photosynthetic rate when both garlic mustard and activated charcoal were present (Table 4.4), but no effect of earthworms on photosynthetic rate (Table 4.4, Fig. 4.7).

Discussion

Because relatively few studies have examined effects of multiple invaders on native plants, whether such relationships are largely additive, or sub- or super-additive is still not fully understood. Our data from the field suggest that invasive earthworms appear to soften the negative influence on garlic mustard on native mayapple (Fig. 4.2), and thus these invaders have a sub-additive effect on mayapple. Alternatively, there was a super-additive effect in the potted experiment (Fig. 4.5), possibly as a result of changes in soil nutrient availability. The positive correlation between garlic mustard and soil nutrients in the field could mean either that garlic mustard invades more into nutrient rich sites, or that garlic mustard enhances nutrient availability, e.g. through nutrient cycling and litter deposition effects. Further, mayapple cover was reduced at sites that had both increased soil nitrogen and garlic mustard present (Fig. 4.3), and the presence of earthworms reduces soil nitrogen content in the field (Table 4.2, Fig. 4.4a) and increases leaf nitrogen content in the experiment (Fig. 4.6a). This might help to explain why there was a sub-additive effect of multiple invaders on mayapple cover, as earthworms have been shown to speed up nutrient cycling, therefore there might be less nitrogen in the soil and more within plant tissues. This increase in nutrients within the plant might reduce some of the harmful effects garlic mustard has on mayapple. A sub-additive effect is more likely to occur under natural conditions, as the field observation data supports this idea, though super-additive patterns might still occur under some conditions.

Environmental conditions must be factored into any interpretation of results as both our experiment and field observation data show that changes in nutrient availability or soil conditions can influence the strength and direction of the interactive effect. While adding activated carbon to our experiment was intended to neutralize allelochemicals (but see Lau et al 2008) from garlic mustard, effects of activated carbon on soil nutrient availability and plant performance are complex (Dąbrowski et al. 2005, Lau et al. 2008), and activated carbon may have acted as an additional stressor to the mayapple. This stress then may have caused the mayapple to be more vulnerable to the harmful effects of the worms and garlic mustard, which resulted in reduced mayapple biomass (Fig. 4.5); however, the mechanisms that might govern this effect are unknown. In the field study, both earthworms and garlic mustard influenced soil nutrients (Table 4.2). This suggests that significant decreases in mayapple cover or biomass due to earthworm and garlic mustard invasion may be context dependent and influenced by nutrient availability within the soil.

Plant responses to multiple invaders are likely to be mediated by plant physiology, as others have seen for responses to single invaders, like garlic mustard (Hale et al. 2016). The internal CO_2 concentration in the leaves was reduced in the presence of the invasive earthworm *Lumbricus terrestris*. This suggests that *L. terrestris* alters plant physiology, perhaps by changing nutrient availability. If these invasive earthworms increase nitrogen availability to the plant, this could influence mayapple production or activation of RuBisCO, the enzyme used to fix CO_2 in the leaf (Spreitzer and Salvucci 2002). The higher leaf nitrogen content in the presence of *L. terrestris* is consistent with this explanation and the observation that *L. terrestris* enhances soil nutrient cycling (Hale et al. 2008). This could increase the rate of CO_2 usage, decreasing internal CO_2 concentrations (C_i). Therefore, if stomatal conductance stays the same (and there was no statistical treatment effects on stomatal conductance), lower C_i could indicate greater carbon drawdown within the leaf. This makes sense if worms make nitrogen more available to the plants, increasing leaf RuBisCO content and thus drawdown capacity. However, this faster C_i drawdown does not lead to greater net photosynthetic rates. A possible reason for this could be that photosynthetic rates are limited not just by RuBisCO availability, but also by the rates sugar move through the plant. If there is a backup of sugars near the photosynthetic machinery, there could be a reduction in photosynthetic rates (Brodribb et al. 2007).

This study adds to a growing body of research on effects of multiple invaders (Rauschert and Shea 2012, Kuebbing and Nuñez 2015), which suggest that invader effects are not always super-additive and are likely to be context-dependent. Super-additive effects of multiple invaders might lead to conservation concerns, suggesting that managers should focus, not only on removing single, particularly detrimental invaders, but also on multiple invaders simultaneously. However, sub-additive effects are also possible, and a few studies, including this one, find evidence of sub-additive effects of multiple invaders. While many negative effects of invasive earthworms are known (Lawrence et al. 2003, Eisenhauer 2010, Cassin and Kotanen 2016) invasive earthworms may sometimes benefit native plants, perhaps by enhancing nutrient availability. For above- and below-ground interactors such as plants and earthworms, availability of nutrients for plant uptake and plant physiology might be important constraints, influencing how these interactions play out.

Acknowledgements

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Tables

Table 4.1: Observational field data, modeling the interaction of invasive garlic mustard (GM), invasive earthworms, and soil nitrogen on the percent cover of native mayapple.

	DF	Deviance	AIC	F-value	p-value
GM presence/absence	1	1701	947.45	2.76	0.09
Earthworm presence/absence	1	1686	945.77	1.14	0.29
Total Soil Nitrogen (%)	1	1718	949.29	4.56	0.03
GM x Earthworms	1	1690	946.29	1.65	0.20
GM x Nitrogen	1	1719	949.31	4.58	0.03
Earthworms x Nitrogen	1	1685	945.64	1.02	0.31
GM x Earthworms x Nitrogen	1	1699	947.24	2.57	0.11

Table 4.2: The interaction between invasive garlic mustard (GM) and invasive earthworms on soil nitrogen, phosphorus 1, and phosphorus 2 in observational field data.

Total Soil Nitrogen	DF	Deviance	AIC	F-value	p-value
GM presence/absence	1	15.45	73.68	1.12	0.29
Earthworm presence/absence	1	15.77	77.48	4.90	0.03
GM x Earthworms	1	15.57	75.11	2.53	0.11
Phosphorus 1					
GM presence/absence	1	62.94	332.77	0.01	0.94
Earthworm presence/absence	1	65.03	338.79	6.00	0.02
GM x Earthworms	1	64.49	337.25	4.45	0.04
Phosphorus 2					
GM presence/absence	1	75.86	368.09	1.68	0.20
Earthworm presence/absence	1	76.52	369.69	3.26	0.07
GM x Earthworms	1	75.96	368.32	1.91	0.17

Table 4.3: Total plant biomass of native mayapple in response to experimental treatments.

	DF	Deviance	AIC	F-value	p-value
Garlic Mustard (GM)	1	4090.8	359.30	1.03	0.32
Activated carbon (AC)	1	3989.6	358.12	0.04	0.85
earthworms	1	4103.5	359.44	1.15	0.29
GM x AC	1	4053.9	358.87	0.67	0.42
GM x earthworms	1	4253.1	361.13	2.62	0.11
AC x earthworms	1	4226.2	360.83	2.35	0.13
GM x AC x earthworms	1	4695.3	365.78	6.94	0.01

Table 4.4: Mayapple photosynthesis rate (A_{max}) as a function of garlic mustard and activated carbon experimental treatments. Earthworm presence/absence was dropped from the model as this treatment had no effect on photosynthetic rate.

	DF	Deviance	AIC	F-value	p-value
GM	1	0.75	184.97	1.23	0.27
Activated carbon	1	0.71	184.85	1.12	0.30
GM x activated carbon	1	1.07	187.05	3.23	0.08

Minimal model chosen by AIC.

Table 4.5: C_i, the intercellular CO_2 concentration in leaves, as a function of experimental treatments on native plant mayapple.

	DF	Deviance	AIC	F-value	p-value
Garlic mustard (GM)	1	26255	430.31	0.13	0.72
Activated carbon	1	26339	430.45	0.25	0.62
Earthworms	1	30764	437.44	6.51	0.01
GM x activated carbon	1	26372	430.51	0.30	0.59
GM x earthworms	1	27984	433.18	2.58	0.12
Activated carbon x earthworms	1	27782	432.85	2.29	0.14
GM x activated carbon x earthworms	1	27562	432.49	1.98	0.17

Model was chosen by AIC.

Figures

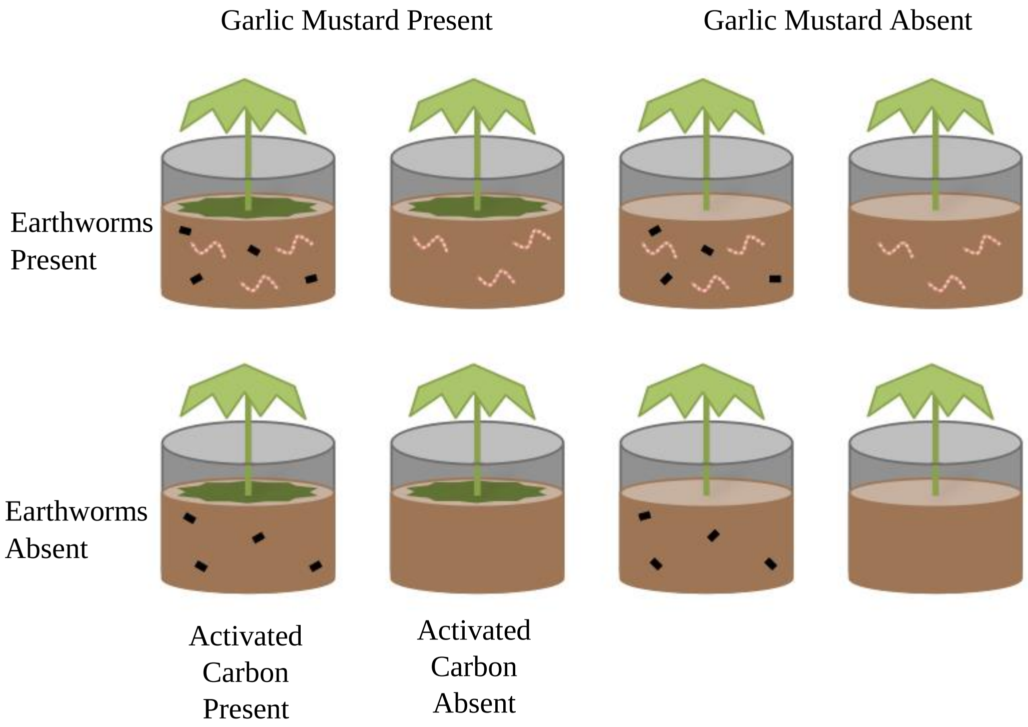

Fig. 4.1: Experimental design assessing the combined effects of invaders on the native plant, mayapple. Garlic mustard leaves were placed on the soil surface in garlic mustard presence treatment, invasive earthworms were placed in pots in the "worms present" treatment, and activated carbon (black rectangles) was placed in the "activated carbon present" treatment.

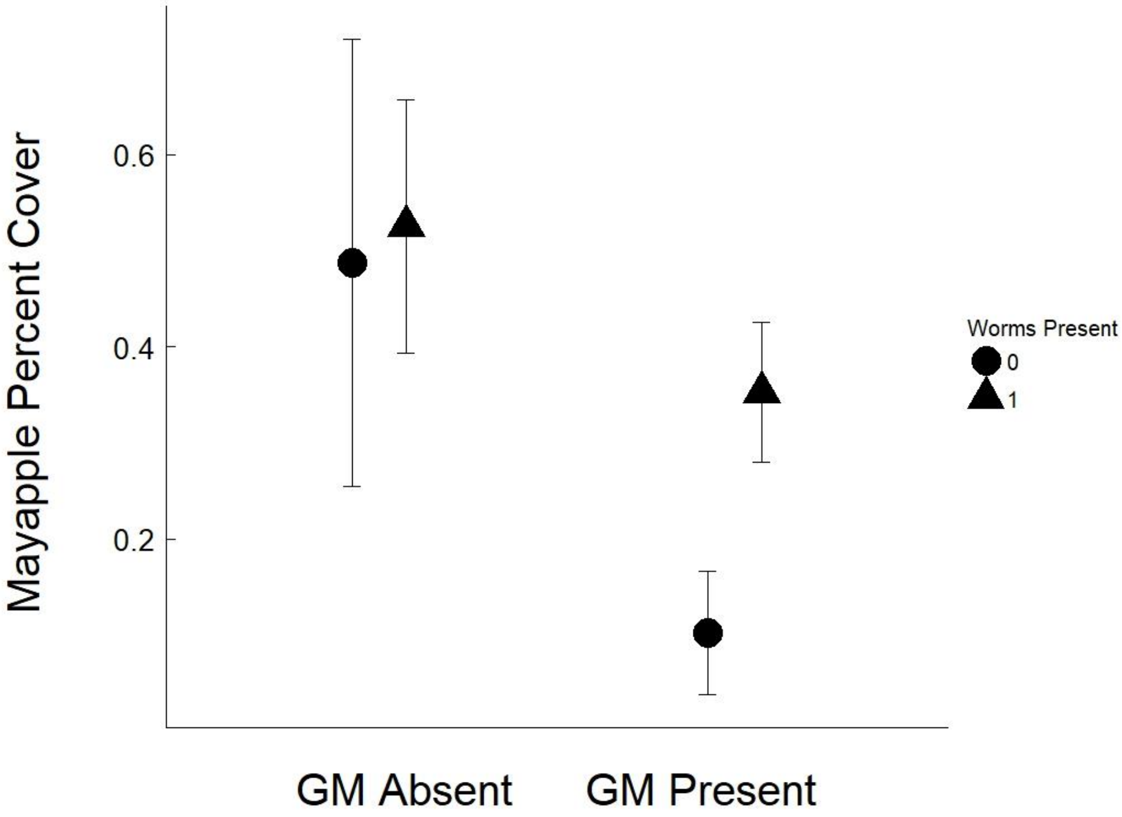

Fig. 4.2: The interaction between the presence or absence of garlic mustard and invasive earthworms on the percent cover of mayapple within 184 observational field plots. Means ± 1 SE.

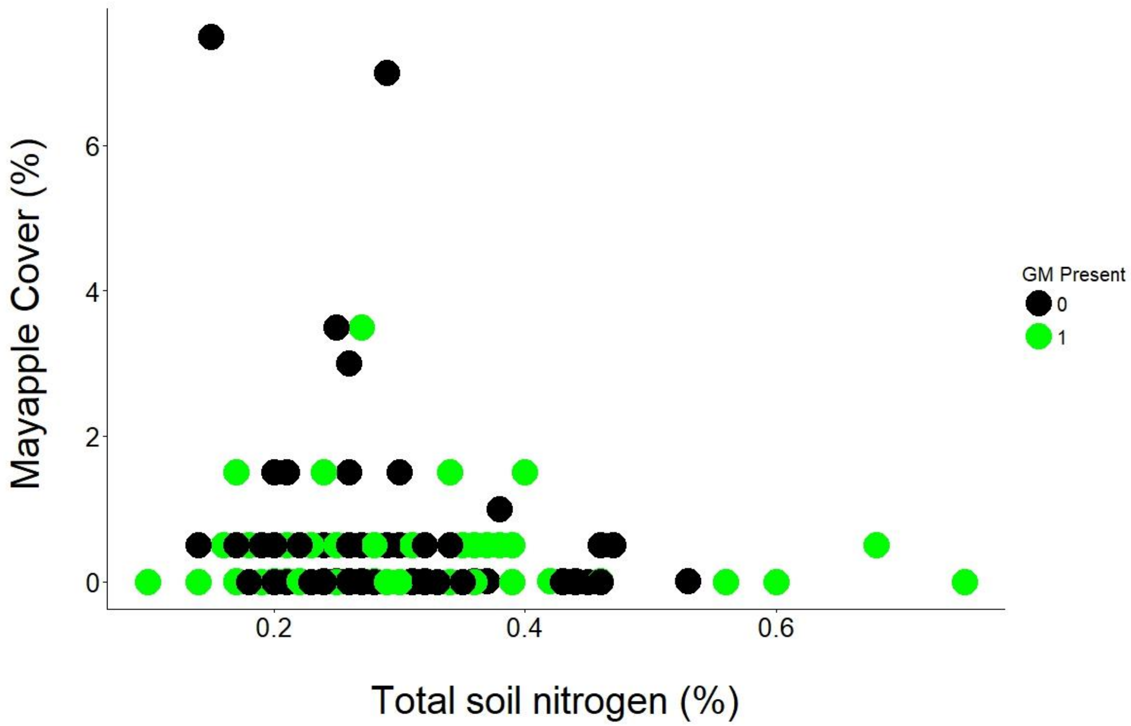

Fig. 4.3: The interaction between soil nitrogen and garlic mustard presence influences maypple cover in the 184 observational field plots (Table 4.1). Mayapple cover was lower in field plots when nitrogen in the soil is higher and when garlic mustard is present (green circles) versus when it was absent (black circles) and was highest for plots with low soil nitrogen and without garlic mustard.

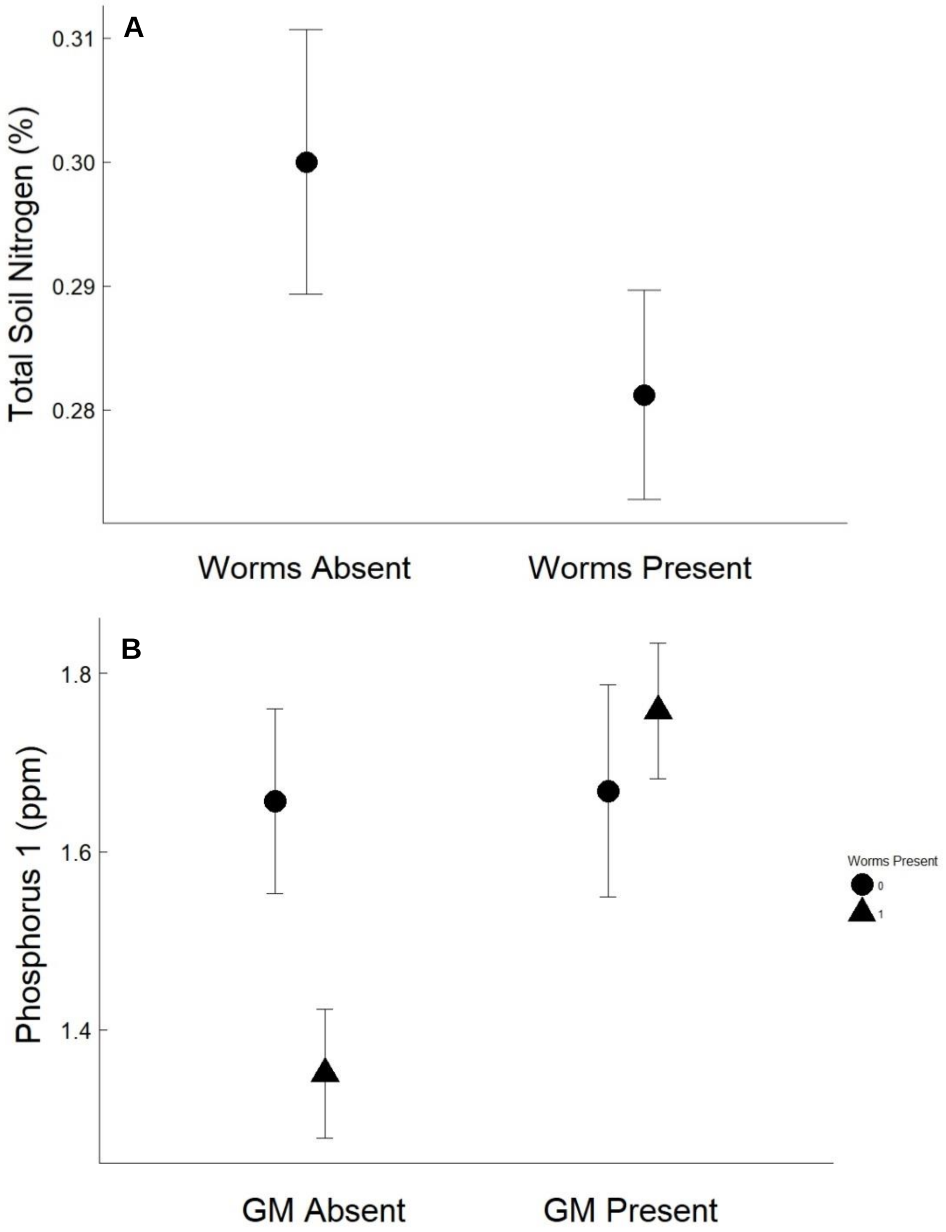

Fig. 4.4: (a) The presence/absence of earthworms on soil nitrogen within observational data in the field. (b) The interaction between earthworms and garlic mustard on the amount of phosphorus within the soil in observational data in the field. Means ± 1 SE.

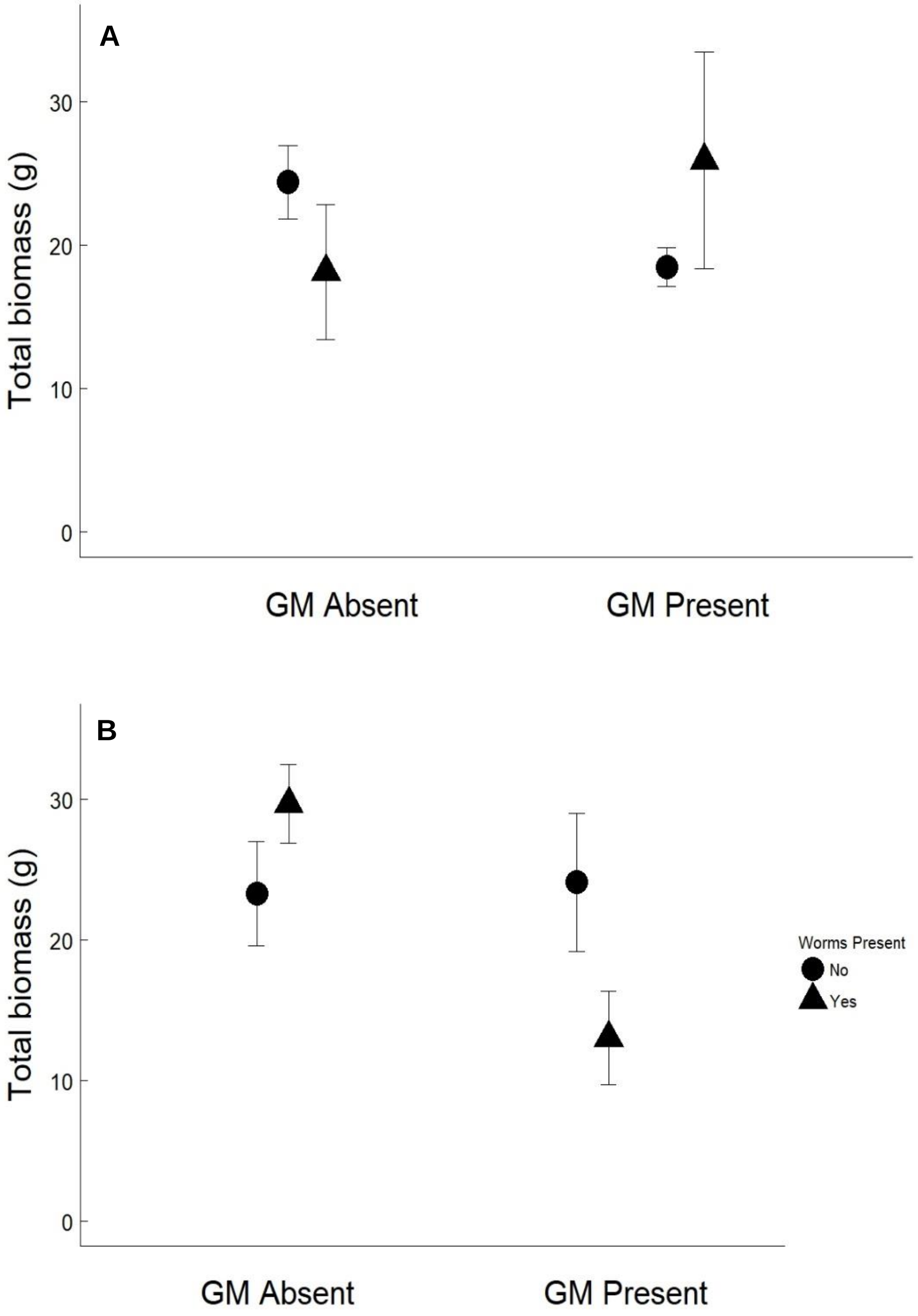

Fig. 4.5: The effect of invasive *L. terrestris* and invasive *Alliaria petiolata* (GM) on native mayapple total biomass. (a) In the absence of activated carbon (AC), there were no differences in mayapple biomass across treatments. (b) In the presence of activated carbon, mayapple biomass was significantly reduced by the presence of both earthworms and garlic mustard (Appendix C: Tables S4.2, S4.3). Means ± 1 SE.

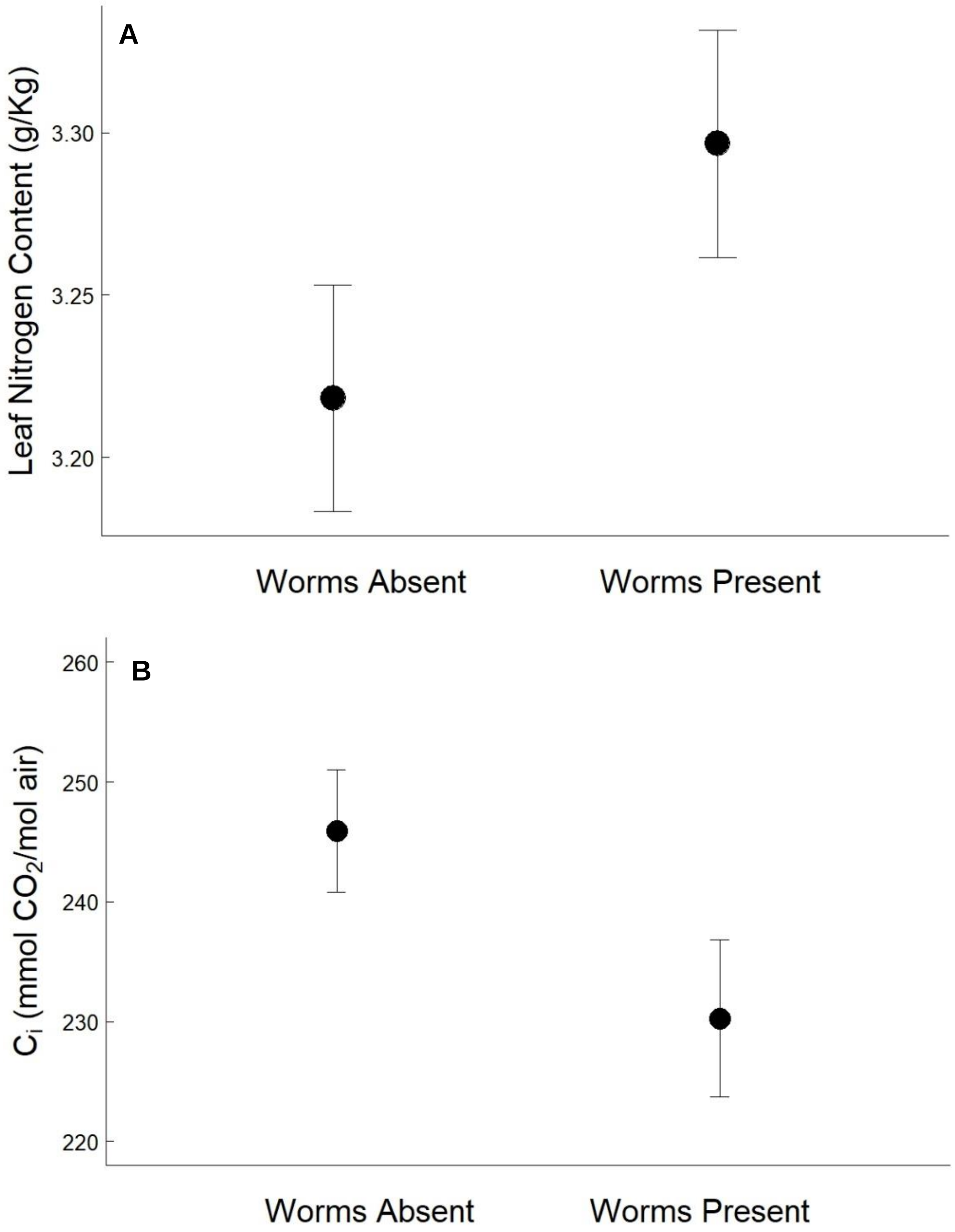

Fig. 4.6: (a) Leaf nitrogen content of mayapple was greater when earthworms was present ($F_{1,1}$=4.16, P=0.05). (b) Internal leaf CO_2 concentration (C_i) of mayapple was reduced when earthworms were present (Table 4.5). Means ± 1 SE.

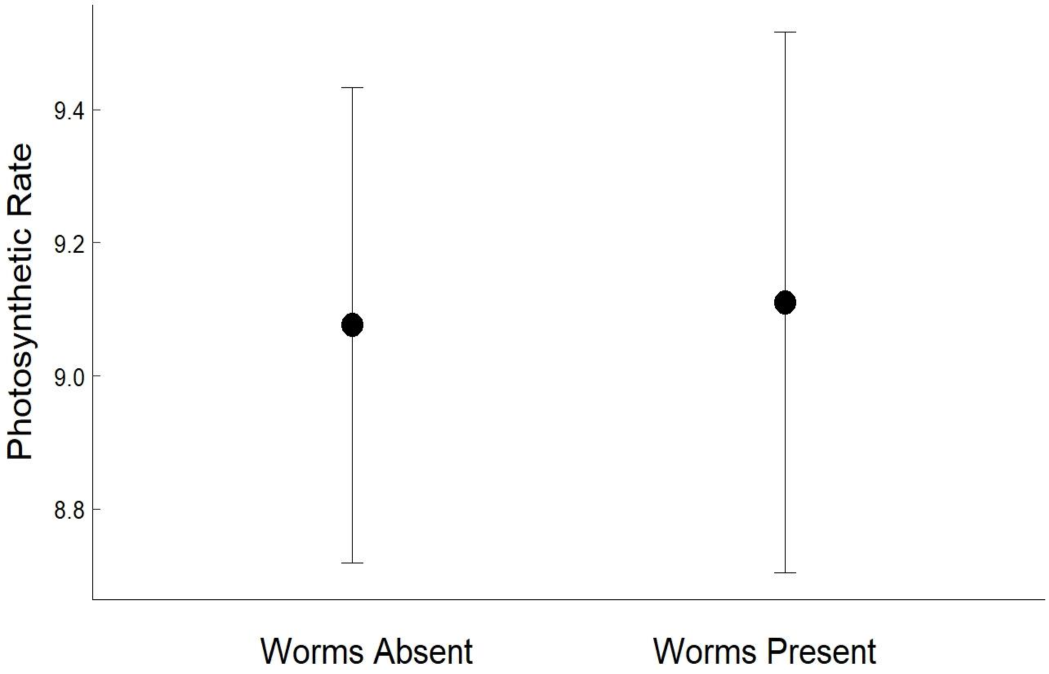

Fig. 4.7: Photosynthetic rate (A_{max}) of maypple leaves in the presence or absence of earthworms. There was no significant difference between treatments ($P > 0.10$). Means ± 1 SE.

Chapter 5

Conclusions

This dissertation provides a deeper understanding of how earthworms interact with above- and belowground organisms and highlights the need to focus on earthworm functional groups rather than generalizing across all earthworms.

In Chapter 2, I highlight the importance of incorporating animal functional groups, and the functional trait body size, into studies of above- and below-ground interactions. There have been numerous studies on above- and belowground interactions (Bardgett et al. 1998, Wardle et al. 2004, Kardol and Wardle 2010), and functional trait studies have primarily focused on plants (Deyn 2017). However, studies that incorporate animal functional traits into above- and belowground interactions are lacking. In this study I focused on white-tailed deer and invasive earthworms, which are both important components of forest communities and individually have a significant influence on forest plant and soil communities and nutrient cycling. I found that white-tailed deer influenced earthworm abundances within the endogeic functional group (Fig 5.1). Endogeic earthworms are unique among the functional groups in feeding primarily on food sources within the soil column, rather than on leaf litter. Thus, endogeic earthworms may depend particularly on plant roots and associated soil fungi for food and respond to indirect effects of deer on plant communities. If I would have pooled earthworm abundances across functional groups instead, I would have missed this important interaction. This study highlights the need for more studies to take a functional group approach when studying above- and belowground interactions involving animals such as invasive earthworms.

In Chapter 3 I show how different functional groups of earthworms interact with deer to influence the aboveground plant community and the belowground microbial community (Fig 5.1). There are numerous studies showing the close associations plant and microbial communities have with each other (e.g. Anacker et al. 2014, Burns et al. 2015, Lemmermeyer et al. 2015). Thus I predicted that both plants and microbial communities might be influenced by deer and earthworms. This study shows that deer and earthworms can independently influence these communities, and that the interaction between the two can also significantly alter these communities. I found that earthworm functional groups varied in how strongly they affected both the fungal and bacteria community. This study once again highlights the need to consider functional groups when conducting studies on above- and belowground interactions involving animals because of their differences in how they altered the microbial communities.

Chapter 4 focused on one functional group with a very unique functional trait, the anecic earthworms. These worms unique feeding strategy of pulling leaf litter into the soil provided the opportunity to test how invaders across multiple trophic level influence native species (Bouche 1977). I predicted that this feeding strategy would enhance the negative effects of garlic mustards allelochemicals, by moving allelochemicals in leaves more deeply into the soil column. Thus, I expected a super-additive effect of these invaders, mediated by allelochemicals. While I found experimental evidence for a super-additive effect, there was no evidence that this effect was mediated by allelochemicals. Instead, I found that nutrients within the soil and plant tissue significantly altered the effects garlic mustard had on mayapple. In addition, garlic mustard and earthworm interactions in the field were not super-additive, further suggesting context-dependence governs multi-invader interactions (Fig 5.1). I found that earthworms influence nutrients within both the soil and plant tissue. Similarly, in previous studies earthworms have been shown to play an integral role in nutrient cycling within the soil (e.g. Hale et al. 2008). I suggest that context-dependence and nutrient uptake large governs whether this multi-invader (earthworms, garlic mustard) interaction is super- or sub-additive.

Throughout this dissertation, I have demonstrated that taking a functional group approach to studying above- and belowground interactions with invasive earthworms is important to teasing apart patterns that might be masked otherwise (Fig 5.1). Studies that generalize across multiple species can miss significant interactions that get masked by increases in variance (i.e. functional groups are very different). Taking a functional group approach allowed me to detect general patterns that can be important to forest communities. Future studies on above- and belowground interactions with invasive earthworms should continue to take a functional group approach. This can be especially important for studies involving multiple invaders or across multiple trophic levels.

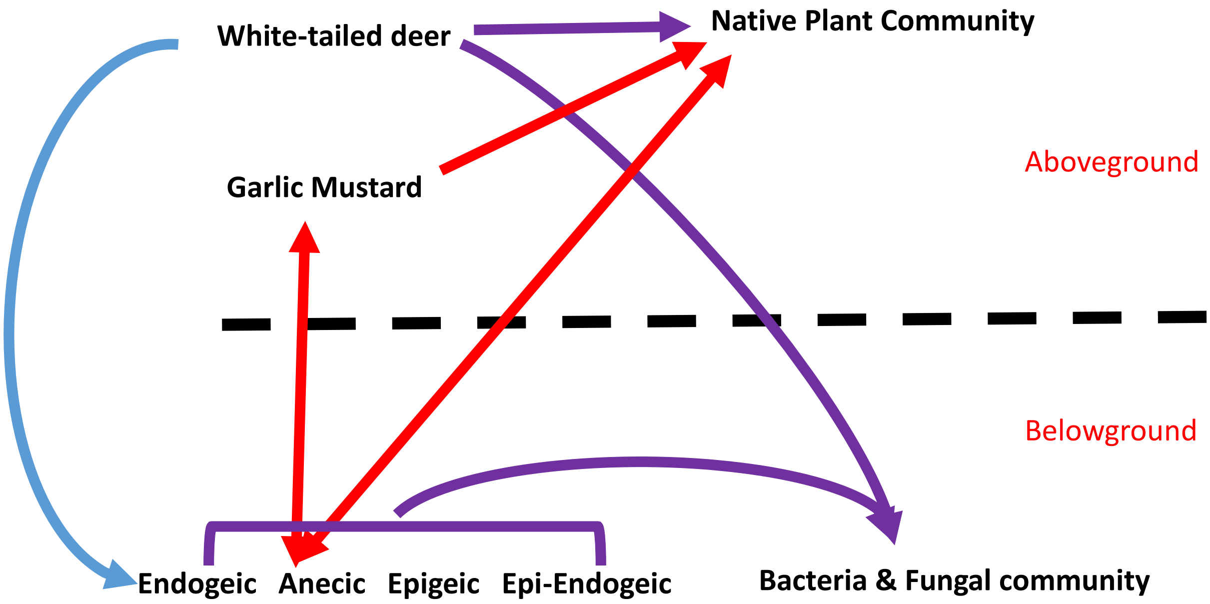

Fig. 5.1. Diagram showing how each focal group interacted with each other to influence the soil microbial and native plant communities. Colors are associated with each of the chapters (blue = Chapter 2, purple = Chapter 3, red = Chapter 4). In Chapter 2 I found that deer influence endogeic earthworm abundance. In Chapter 3 I found that each of the earthworm functional groups and the presence of deer influence the microbial community, and deer influence native plant diversity. In Chapter 4 I found that garlic mustard and anecic earthworms independently influence native mayapple in the observational data and that they interact to influence mayapple within the controlled experiment.

Appendix A: Chapter 2 Supplementary Materials

Table S2.1: The number and dimensions of plots located within each region and the year that they were established.

Region	Sites	Plots	Year Established	Plot dimension (m)
Cleveland Metroparks	2	4	2007	8 x 8
Holden Arboretum	4	32	2005-2009	10 x 10
Lake County Metroparks	3	4	1994-2001	10 x 15
Summit County Metroparks	4	4	2002	8 x 8

Table S2.2: The generalized least squares full model of earthworm count, with latitude and longitude used in a corSper correlation structure to model spatial autocorrelation.

	NumDF	DenDF	F-value	p-value
Control/Exclosure (C/E)	1	167	7.05	0.009
Functional Group	3	167	3.96	0.009
Average pH	1	167	1.44	0.23
Year Exclosure Established	1	167	0.00	0.99
C/E × Functional Group	3	167	3.12	0.03
Functional Group x Average pH	3	167	7.15	0.0002
C/E x Average pH	1	167	0.44	0.51
C/E x Average pH x Functional Group	3	167	1.21	0.31

Table S2.3: Earthworm ash-free biomass as a function of control and deer exclosure treatments, earthworm functional group, average pH, and year the exclosure was established.

	NumDF	DenDF	F-value	p-value
Control/Exclosure	1	177	2.91	0.09
Functional Group	3	177	5.01	0.002
pH	3	177	1.68	0.20
Year Exclosure Established	1	177	0.00	0.99

Table S2.4: The generalized least squares full model of earthworm biomass, with latitude and longitude used in a corSper correlation structure to model spatial autocorrelation.

	NumDF	DenDF	F-value	p-value
Control/Exclosure (C/E)	1	167	2.86	0.09
Functional Group	3	167	4.93	0.002
pH	3	167	1.65	0.20
Year Exclosure Established	1	167	0.00	0.99
Functional Group x C/E	3	167	0.74	0.53
Functional Group x Average pH	3	167	0.55	0.65
C/E x Average pH	1	167	0.15	0.70
Functional Group x Average pH x C/E	3	167	0.98	0.40

Table S2.5: The proportion of sub-plots with earthworms present, the average number of earthworms, and pooled biomass for each adult species within control and exclosure treatments.

		Proportion		Number of earthworms (SE)		Average Biomass (g) (SE)	
Species	Functional Group[a]	Control	Exclosure	Control	Exclosure	Control	Exclosure
Lumbricus terrestris	AN	0.05	0.09	1.50 (0.50)	5.75 (1.70)	0.67 (0.33)	1.20 (0.38)
Aporrectodea caliginosa	EN	0.02	0.00	3.00 (0.00)	0.00 (0.00)	0.16 (0.00)	0.00 (0.00)
Allolobophora chlorotica	EN	0.02	0.02	4.00 (0.00)	2.00 (0.00)	0.17 (0.00)	0.19 (0.00)
Aporrectodea longa	EN	0.02	0.00	1.00 (0.00)	0.00 (0.00)	0.06 (0.00)	0.00 (0.00)
Aporrectodea rosea	EN	0.02	0.00	1.00 (0.00)	0.00 (0.00)	0.01 (0.00)	0.00 (0.00)
Octolasion tyrtaeum	EN	0.16	0.09	5.29 (2.22)	1.50 (0.50)	0.19 (0.08)	0.05 (0.02)
Dendrobaena octaedra	EP	0.18	0.07	1.88 (0.61)	1.33 (0.33)	0.02 (0.01)	0.02 (0.005)
Dendrodrilus rubidus	EP	0.02	0.07	1.00 (0.00)	1.33 (0.33)	0.02 (0.00)	0.03 (0.01)
Amynthas species	EP_EN	0.23	0.25	5.40 (1.38)	7.73 (2.01)	0.61 (0.12)	1.25 (0.56)
Lumbricus rubellus	EP_EN	0.14	0.07	2.83 (0.60)	3.00 (1.00)	0.22 (0.05)	0.33 (0.15)

[a] Anecic (AN), epigeic-endogeic (EP_EN), epigeic (EP), and endogeic (EN) earthworm functional groups.

Table S2.6: The proportion of sub-plots with earthworms present, the average number of earthworms, and pooled biomass for each juvenile species within control and exclosure treatments. Juvenile earthworms cannot always be identified to species, so some taxa are only identified to genus (e.g. *Lumbricus*).

		Proportion		Number of earthworms (SE)		Average Biomass (g) (SE)	
Species	Functional Group[a]	Control	Exclosure	Control	Exclosure	Control	Exclosure
Lumbricus species	AN	0.16	0.14	9.43 (3.02)	6.33 (2.58)	0.37 (0.17)	0.39 (0.16)
Aporrectodea longa	EN	0.00	0.02	0.00 (0.00)	2.00 (0.00)	0.00 (0.00)	0.02 (0.00)
Aporrectodea species	EN	0.23	0.09	7.90 (3.35)	4.25 (0.85)	0.23 (0.13)	0.15 (0.05)
Octolasion species	EN	0.16	0.05	5.43 (1.91)	5.50 (4.50)	0.04 (0.01)	0.07 (0.06)
Octolasion tyrtaeum	EN	0.02	0.00	1.00 (0.00)	0.00 (0.00)	0.01 (0.00)	0.00 (0.00)
Dendrobaena octaedra	EP	0.34	0.27	4.20 (1.04)	4.08 (0.88)	0.02 (0.01)	0.02 (0.01)
Dendrodrilus rubidus	EP	0.07	0.05	4.33 (1.86)	2.00 (0.00)	0.02 (0.01)	0.03 (0.02)
Eudrilus eugeniae	EP	0.05	0.02	2.00 (1.00)	1.00 (0.00)	0.09 (0.01)	0.04 (0.00)
Amynthas species	EP_EN	0.07	0.05	2.00 (1.00)	3.50 (1.50)	0.07 (0.04)	0.15 (0.07)

[a] Anecic (AN), epigeic-endogeic (EP_EN), epigeic (EP), and endogeic (EN) earthworm functional groups.

Phylogenetic methods

To estimate a phylogeny for the 11 species of earthworms found in our sampling, I searched genBank for sequences of 16S, COI, and COII (Appendix S2 TableS1). I aligned each region separately in MUSCLE (version 7) (Edgar 2004a, 2004b) using a gap extension penalty of -400, checked each alignment by eye, conducted preliminary analysis on each separate alignment to check for outliers (diagnosed by long branches), and concatenated the three alignments. I conducted a maximum likelihood phylogenetic analysis on the concatenated alignment with all three DNA regions (version 0.951) (Zwickl 2006) using a GTR + I + Γ model. I also conducted a bootstrap analysis with 100 replicates.

Table S2.7: Genbank accession codes for the 11 species of earthworms sampled in northeastern Ohio, USA.

Species	**16S**	**COI**	**COII**
Allolobophora chlorotica	JN869756.1	HM417955.1	JN869551.1
Amynthas species	JQ904530.1	JX081518.1	JX081530.1
Aporrectodea caliginosa	FJ967624.1	HE611695.1	JQ763498.1
Aporrectodea longa	JN869785.1	JQ908950.1	JQ763504.1
Aporrectodea rosea	JN869784.1	HE611698.1	JN869567.1
Dendrobaena octaedra	JN869794.1	JQ909054.1	JN869576.1
Dendrodrilus rubidus	JN869797.1	JQ909084.1	JN869580.1
Eudrilus eugeniae	KF055857.1	KC122194.1	NA
Lumbricus rubellus	JN869816.1	JN419232.1	JN869599.1
Lumbricus terrestris	JN869833.1	HQ024638.1	JN869614.1
Octolasion tyrtaeum	DQ257299.1	NA	AF381134.1

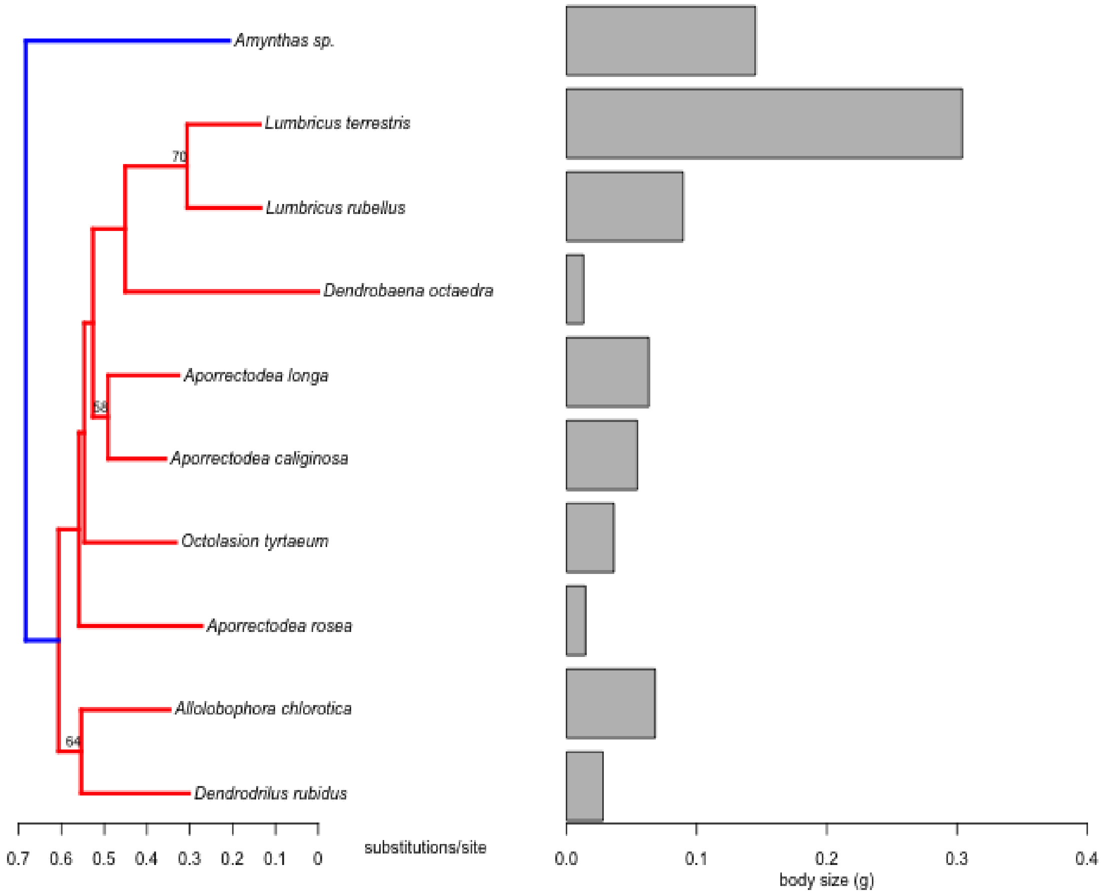

Figure S2.1: A maximum likelihood phylogeny for 10 sampled invasive earthworms in northeastern Ohio, USA, based on three DNA regions: 16S, COI, and COII. Bootstrap values greater than 50 are shown above the nodes. The Megascoledidea family (*Amynthas* sp.) is shown in blue and the Lumbricidae family (all others) is shown in red. The average body size (g) for each of these taxa is shown to the right. *Eudrilus eugeniae* (Megascoledidea) was only found as juveniles (Appendix A: Table S2.7) and was thus not included in the body size analyses.

Appendix B: Chapter 3 Supplementary Materials

Table S3.1: Linear mixed effects model with plot as a random factor and either plant Shannon diversity index score or log transformed plant richness as our response variable.

Shannon Diversity Score	NumDF	DenDF	F-value	p-value
Control/Exclosure (C/E)	1	41	5.40	0.03
Earthworm Pres/Abs	1	41	0.06	0.81
(C/E) x Earthworm Pres/Abs	1	41	3.11	0.09
Plant Richness				
Control/Exclosure (C/E)	1	41	0.45	0.51
Earthworm Pres/Abs	1	41	0.16	0.69
(C/E) x Earthworm Pres/Abs	1	41	0.15	0.70

Table S3.2: Generalized Least Squares model of OTU richness for soil fungi and bacteria as a response to experimental treatment, earthworm presence/absence, and their interaction. Latitude and longitude were included in the model as part of the error structure to account for spatial autocorrelation.

Bacteria OTU Richness	NumDF	DenDF	F-value	p-value
Control/Exclosure (C/E)	1	84	0.13	0.72
Earthworm Pres/Abs	1	84	0.10	0.74
(C/E) x Earthworm Pres/Abs	1	84	0.37	0.55
Fungal OTU Richness				
Control/Exclosure (C/E)	1	84	2.01	0.16
Earthworm Pres/Abs	1	84	0.63	0.43
(C/E) x Earthworm Pres/Abs	1	84	0.16	0.69

Appendix C: Chapter 4 Supplementary Materials

Table S4.1: A linear model testing the effects soil nutrients had on the percent cover of garlic mustard in observational field data.

	DF	Deviance	AIC	F-value	p-value
Total Soil Nitrogen	1	15.45	73.68	1.12	0.29
Phosphorus 1	1	15.77	77.48	4.90	0.03
Phosphorus 2	1	15.57	75.11	2.53	0.11

Table S4.2: Contrasts for garlic mustard effect, contrasting garlic mustard presence to garlic mustard absence treatments, either in the presence or absence of earthworms and Activated Carbon (AC) in the soil.

Within treatment categories:	t-value	p-value
Worms absent; AC absent	1.00	0.32
Worms present; AC absent	-1.27	0.21
Worms absent; AC present	-0.14	0.89
Worms present; AC present	2.85	0.007

Table S4.3: Contrasts for earthworm effect, contrasting earthworm presence to earthworm absence treatments, either in the presence or absence of garlic mustard (GM) and Activated Carbon (AC) in the soil.

Within treatment categories:	t-value	p-value
GM absent; AC absent	1.07	0.29
GM present; AC absent	-1.21	0.23
GM absent; AC present	-1.10	0.28
GM present; AC present	1.90	0.07

Bibliography

Aguirre-Gutiérrez, J. et al. 2016. Functional traits help to explain half-century long shifts in pollinator distributions - unpublished. Nature Publishing Group:1–13.

Anacker, B. L., J. N. Klironomos, H. Maherali, K. O. Reinhart, and Y. S. Sharon. 2014. Phylogenetic conservatism in plant-soil feedback and its implications for plant abundance. Ecology letters.

Baker, D. D., and B. C. Mullin. 1994. Diversity of Frankia nodule endophytes of the actinorhizal shrub Ceanothus as assessed by RFLP patterns from single nodule lobes. Soil Biology and Biochemistry 26:547–552.

Bancroft, T. A. 1964. Analysis and Inference for Incompletely Specified Models Involving the Use of Preliminary Test(s) of Significance. Biometrics 20:427–442.

Barber, N. A., L. S. Adler, N. Theis, R. V Hazzard, E. T. Kiers, S. Adler, and V. Hazzard. 2012. Herbivory reduces plant interactions with above- and belowground antagonists and mutualists. Ecology 93:1560–1570.

Bardgett, R. D., and D. a. Wardle. 2003. Herbivore-mediated linkages between aboveground and belowground communities. Ecology 84:2258–2268.

Bardgett, R. D., D. a Wardle, and G. W. Yeates. 1998. Linking Above-Gound and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biology and Biochemistry 30:1867–1878.

Bates, D., M. Mächler, B. M. Bolker, and S. C. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Beale, C. M., J. J. Lennon, J. M. Yearsley, M. J. Brewer, and D. a Elston. 2010. Regression analysis of spatial data. Ecology Letters 13:246–64.

Berg, G., and K. Smalla. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiology Ecology 68:1–13.

Bever, J. D., K. M. Westover, and J. Antonovics. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. Journal of Ecology 85:561–573.

Bouche, M. B. 1977. Strategies Lombriciennes. Ecological Bullitens 25:122–132.

Bressette, J. W., H. Beck, and V. B. Beauchamp. 2012. Beyond the browse line: complex cascade effects mediated by white-tailed deer. Oikos 121:1749–1760.

Brodribb, T. J., T. S. Feild, and G. J. Jordan. 2007. Leaf Maximum Photosynthetic Rate and Venation Are Linked by Hydraulics. Plant Physiology 144:1890–1898.

Brown, J. H., J. F. Gilloolly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology 85:1771–1789.

Burke, D. J., S. M. Dunham, and A. M. Kretzer. 2008. Molecular analysis of bacterial communities associated with the roots of Douglas fir (Pseudotsuga menziesii) colonized by different ectomycorrhizal fungi. FEMS microbiology ecology 65:299–309.

Burke, D. J., K. J. Martin, P. T. Rygiewicz, and M. a. Topa. 2005. Ectomycorrhizal fungi identification in single and pooled root samples: terminal restriction fragment length polymorphism (TRFLP) and morphotyping compared. Soil Biology and Biochemistry 37:1683–1694.

Burke, D. J., K. J. Martin, P. T. Rygiewicz, and M. a. Topa. 2006. Relative abundance of ectomycorrhizas in a managed loblolly pine (Pinus taeda) genetics plantation as determined through terminal restriction fragment length polymorphism profiles. Canadian Journal of Botany 84:924–932.

Burns, J. H., B. L. Anacker, S. Y. Strauss, and D. J. Burke. 2015. Soil microbial community variation correlates most strongly with plant species identity, followed by soil chemistry, spatial location and plant genus. AoB PLANTS 7:1–10.

Burtelow, A., P. Bohlen, and P. Groffman. 1998. Influence of exotic earthworm invasion on soil organic matter, microbial biomass and denitrification potential in forest soils of the northeastern United States. Applied Soil Ecology 9:197–202.

Callaway, R. M., and E. T. Aschehoug. 2000. Invasive Plants Versus Their New and Old Neighbors: A Mechanism for Exotic Invasion. Science 290:521–523.

Cassin, C. M., and P. M. Kotanen. 2016. Invasive earthworms as seed predators of temperate forest plants. Biological Invasions.

Chan, K., and K. Munro. 2001. Evaluating mustard extracts for earthworm sampling. Pedobiologia 45:272–278.

Classen, A. T., M. K. Sundqvist, J. a. Henning, G. S. Newman, J. a. M. Moore, M. a. Cregger, L. C. Moorhead, and C. M. Patterson. 2015. Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? Ecosphere 6:art130.

Côté, S. D., T. P. Rooney, J.-P. Tremblay, C. Dussault, and D. M. Waller. 2004. Ecological Impacts of Deer Overabundance. Annual Review of Ecology, Evolution, and Systematics 35:113–147.

Cushman, J. H., and K. A. Gaffney. 2010. Community-level consequences of invasion: Impacts of exotic clonal plants on riparian vegetation. Biological Invasions

12:2765–2776.

Dąbrowski, A., P. Podkościelny, Z. Hubicki, and M. Barczak. 2005. Adsorption of phenolic compounds by activated carbon - A critical review. Chemosphere 58:1049–1070.

Dávalos, A., V. Nuzzo, and B. Blossey. 2014. Demographic responses of rare forest plants to multiple stressors: the role of deer, invasive species and nutrients. Journal of Ecology 102:1222–1233.

Dávalos, A., V. Nuzzo, and B. Blossey. 2015a. Single and interactive effects of deer and earthworms on non-native plants. Forest Ecology and Management 351:28–35.

Dávalos, A., V. Nuzzo, and B. Blossey. 2015b. Interactive effects of deer, earthworms and non-native plants on rare forest plant recruitment. Biological Conservation 187:173–181.

Dávalos, A., E. Simpson, V. Nuzzo, and B. Blossey. 2015c. Non-consumptive Effects of Native Deer on Introduced Earthworm Abundance. Ecosystems 18:1029–1042.

Deyn, G. B. D. 2017. Plant life history and above–belowground interactions: missing links. Oikos 126:497–507.

Diaz, S., S. Lavorel, F. de Bello, F. Quetier, K. Grigulis, and T. M. Robson. 2007. Incorporating plant functional diversity effects in ecosystem service assessments. Proceedings of the National Academy of Sciences 104:20684–20689.

Dobson, A., and B. Blossey. 2015. Earthworm invasion, white-tailed deer and seedling establishment in deciduous forests of northeastern North America. Journal of Ecology 103:153–164.

Dray, S., and A.-B. Dufour. 2007. The **ade4** Package: Implementing the Duality Diagram for Ecologists. Journal of Statistical Software 22.

Edgar, R. C. 2004a. MUSCLE: a multiple sequence alignmnet method with reduced time and space complexity. BMC Bioinformatics 5:113.

Edgar, R. C. 2004b. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797.

Ehrenfeld, J. G. 2010. Ecosystem Consequences of Biological Invasions. Annual Review of Ecology, Evolution, and Systematics 41:59–80.

Eisenhauer, N. 2010. The action of an animal ecosystem engineer: Identification of the main mechanisms of earthworm impacts on soil microarthropods. Pedobiologia 53:343–352.

Eisenhauer, N., S. Partsch, D. Parkinson, and S. Scheu. 2007. Invasion of a deciduous forest by earthworms: Changes in soil chemistry, microflora, microarthropods and vegetation. Soil Biology and Biochemistry 39:1099–1110.

Eisenhauer, N., M. Schuy, O. Butenschoen, and S. Scheu. 2009. Direct and indirect effects of endogeic earthworms on plant seeds. Pedobiologia 52:151–162.

Fahey, T. J., J. B. Yavitt, R. E. Sherman, J. C. Maerz, P. M. Groffman, M. C. Fisk, and P. J. Bohlen. 2013. Earthworm effects on the incorporation of litter C and N into soil organic matter in a sugar maple forest. Ecological Applications 23:1185–1201.

Felsenstein, J. 1985. Phylogenies and the Comparative Method. The American Naturalist 125:1–15.

Frank, D. a., P. M. Groffman, R. D. Evans, and B. F. Tracy. 2000. Ungulate stimulation of nitrogen cycling and retention in Yellowstone Park grasslands. Oecologia 123:116–121.

Frelich, L. E., R. O. Peterson, M. Dovčiak, P. B. Reich, J. a Vucetich, and N. Eisenhauer. 2012. Trophic cascades, invasive species and body-size hierarchies interactively modulate climate change responses of ecotonal temperate-boreal forest. Philosophical Transactions of the Royal Society B 367:2955–2961.

Gardner, J. L., A. Peters, M. R. Kearney, L. Joseph, and R. Heinsohn. 2011. Declining body size: A third universal response to warming? Trends in Ecology and Evolution 26:285–291.

Graham, M. H. 2003. Confronting multicollinearity in cological multiple regression. Ecology 84:2809–2815.

Green, P. T., D. J. O'Dowd, K. L. Abbott, M. Jeffery, K. Retallick, and R. Mac Nally. 2011. Invasional meltdown: Invader — invader mutualism facilitates a secondary invasion. Ecology 92:1758–1768.

Hale, A. N., and S. Kalisz. 2013. Perspectives on allelopathic disruption of plant mutualisms: A framework for individual- and population-level fitness consequences. Plant Ecology 213:1991–2006.

Hale, A. N., L. Lapointe, and S. Kalisz. 2016. Invader disruption of belowground plant mutualisms reduces carbon acquisition and alters allocation patterns in a native forest herb. New Phytologist 209:542–549.

Hale, A. N., S. J. Tonsor, and S. Kalisz. 2011. Testing the mutualism disruption hypothesis: physiological mechanisms for invasion of intact perennial plant communities. Ecosphere 2:art110.

Hale, C. M. 2007. Earthworms of the Great Lakes:30–31.

Hale, C. M., L. E. Frelich, P. B. Reich, and J. Pastor. 2005. Effects of European

Earthworm Invasion on Soil Characteristics in Northern Hardwood Forests of Minnesota, USA. Ecosystems 8:911–927.

Hale, C. M., L. E. Frelich, P. B. Reich, and J. Pastor. 2008. Exotic earthworm effects on hardwood forest floor, nutrient availability and native plants: a mesocosm study. Oecologia 155:509–18.

Hale, C. M., P. B. Reich, and L. E. Frelich. 2004. Allometric Equations for Estimation of Ash-free Dry Mass from Length Measurements for Selected European Earthworm Species (Lumbricidae) in the Western Great Lakes Region.

Hamilton, E. W., and D. a. Frank. 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology 82:2397–2402.

Hardoim, P. R., L. S. van Overbeek, and J. D. van Elsas. 2008. Properties of bacterial endophytes and their proposed role in plant growth. Trends in Microbiology 16:463–471.

Hendrix, P. F. 2006. Biological invasions belowground—earthworms as invasive species. Biological Invasions 8:1201–1204.

Hobbie, E. a, S. a Macko, and H. H. Shugart. 1999. Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence. Oecologia 118:353.

Holdsworth, A. R., L. E. Frelich, and P. B. Reich. 2007. Effects of earthworm invasion on plant species richness in northern hardwood forests. Conservation biology : the journal of the Society for Conservation Biology 21:997–1008.

Ives, A. R. 2015. For testing the significance of regression coefficients , go ahead and log-transform count data. Methods in Ecology and Evolution 6:828–835.

Jusselme, M. D., F. Poly, T. Lebeau, C. Rouland-lefèvre, and E. Miambi. 2015. Effects of earthworms on the fungal community and microbial activity in root-adhering soil of Lantana camara during phytoextraction of lead. Applied Soil Ecology 96:151–158.

Karberg, N. J., and E. a. Lilleskov. 2009. White-tailed deer (Odocoileus virginianus) fecal pellet decomposition is accelerated by the invasive earthworm Lumbricus terrestris. Biological Invasions 11:761–767.

Kardol, P., and D. A. Wardle. 2010. How understanding aboveground-belowground linkages can assist restoration ecology. Trends in Ecology and Evolution 25:670–679.

Khasanova, A., J. J. James, and R. E. Drenovsky. 2013. Impacts of drought on plant water relations and nitrogen nutrition in dryland perennial grasses. Plant and Soil 372:541–552.

Knight, T., J. Dunn, L. Smith, J. Davis, and S. Kalisz. 2009a. Deer facilitate invasive plant success in a Pennsylvania forest understory. Natural Areas Journal 29:110–116.

Knight, T. M., H. Caswell, and S. Kalisz. 2009b. Population growth rate of a common understory herb decreases non-linearly across a gradient of deer herbivory. Forest Ecology and Management 257:1095–1103.

Kraft, N. J. B., O. Godoy, and J. M. Levine. 2015. Plant functional traits and the multidimensional nature of species coexistence. Proceedings of the National Academy of Sciences 112:797–802.

Kuebbing, S. E., and M. A. Nuñez. 2015. Negative, neutral, and positive interactions among nonnative plants: Patterns, processes, and management implications. Global

Change Biology 21:926–934.

Kuebbing, S. E., M. A. Nuñez, and D. Simberloff. 2013. Current mismatch between research and conservation efforts: The need to study co-occurring invasive plant species. Biological Conservation 160:121–129.

Kumbasli, M., E. Makineci, and M. Cakir. 2010. Long term effects of red deer (Cervus elaphus) grazing on soil in a breeding area. Journal of Environmental Biology 31:185–188.

Lau, J., K. Puliafico, and J. Kopshever. 2008. Inference of allelopathy is complicated by effects of activated carbon on plant growth. New … 178:412–423.

Lawrence, A., and M. Bowers. 2002. A test of the “hot”mustard extraction method of sampling earthworms. Soil Biology and Biochemistry 34:2000–2003.

Lawrence, B., M. C. Fisk, T. J. Fahey, and E. R. Suarez. 2003. Influence of nonnative earthworms on mycorrhizal colonization of sugar maple (Acer saccharum). New Phytologist 157:145–153.

Lemmermeyer, S., L. Lörcher, M. van Kleunen, and W. Dawson. 2015. Testing the Plant Growth-Defense Hypothesis Belowground: Do Faster-Growing Herbaceous Plant Species Suffer More Negative Effects from Soil Biota than Slower-Growing Ones? The American Naturalist 186:264–271.

Lessard, J.-P., W. N. Reynolds, W. a. Bunn, M. a. Genung, M. a. Cregger, E. Felker-Quinn, M. N. Barrios-Garcia, M. L. Stevenson, R. M. Lawton, C. B. Brown, M. Patrick, J. H. Rock, M. a. Jenkins, J. K. Bailey, and J. a. Schweitzer. 2012. Equivalence in the strength of deer herbivory on above and below ground communities. Basic and Applied Ecology 13:59–66.

Lin, G., M. L. McCormack, and D. Guo. 2015. Arbuscular mycorrhizal fungal effects on plant competition and community structure. Journal of Ecology:n/a-n/a.

Marhan, S., and S. Scheu. 2005. Effects of sand and litter availability on organic matter decomposition in soil and in casts of Lumbricus terrestris L. Geoderma 128:155–166.

Martin, J. L., S. a. Stockton, S. Allombert, and A. J. Gaston. 2010. Top-down and bottom-up consequences of unchecked ungulate browsing on plant and animal diversity in temperate forests: Lessons from a deer introduction. Biological Invasions 12:353–371.

Martin, K. J., and P. T. Rygiewicz. 2005. Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. BMC microbiology 5:28.

Martins, E. P., and T. F. Hansen. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. The American Naturalist 149:646–667.

McGarvey, J., and N. Bourg. 2013. Effects of Twenty Years of Deer Exclusion on Woody Vegetation at Three Life-History Stages in a Mid-Atlantic Temperate Deciduous Forest. Northeastern Naturalist 20:451–468.

McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild, and J. A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 115:495–501.

Milton, S. J., J. R. U. Wilson, D. M. Richardson, C. L. Seymour, W. R. J. Dean, D. M. Iponga, and Ş. Procheş. 2007. Invasive alien plants infiltrate bird-mediated shrub nucleation processes in arid savanna. Journal of Ecology 95:648–661.

Moe, S. R., and P. Wegge. 2008. Effects of deposition of deer dung on nutrient redistribution and on soil and plant nutrients on intensively grazed grasslands in lowland Nepal. Ecological Research 23:227–234.

Mougi, A., and O. Kishida. 2009. Reciprocal phenotypic plasticity can lead to stable predator-prey interaction. Journal of Animal Ecology 78:1172–1181.

Murray, B., C. Webster, and J. Bump. 2013. Broadening the ecological context of ungulate-ecosystem interactions: the importance of space, seasonality, and nitrogen. Ecology 94:1317–1326.

Muyzer, G., A. Teske, C. O. Wirsen, and H. W. Jannasch. 1995. Phylogenetic relationships of Thiomicrospira species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel-electrophoresis of 16S rDNA fragments. Archives of Microbiology 164:165–172.

Muyzer, G., E. De Waal, and A. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microb 59:695–700.

Nuzzo, V. A., J. C. Maerz, and B. Blossey. 2009. Earthworm invasion as the driving force behind plant invasion and community change in northeastern North American forests. Conservation Biology 23:966–974.

Nuzzo, V., A. Dávalos, and B. Blossey. 2015. Invasive earthworms shape forest seed bank composition. Diversity and Distributions:n/a-n/a.

O’Dowd, D. J., P. T. Green, and P. S. Lake. 2003. Invasional “meltdown” on an oceanic island. Ecology Letters 6:812–817.

Oksanene, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R.

Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2017. vegan: Community Ecology Package. R package version 2.4-5.

Olson, V. A., R. G. Davies, C. D. L. Orme, G. H. Thomas, S. Meiri, T. M. Blackburn, K. J. Gaston, I. P. F. Owens, and P. M. Bennett. 2009. Global biogeography and ecology of body size in birds. Ecology Letters 12:249–259.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. Nature 401:877–884.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: Analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290.

Parniske, M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nature reviews. Microbiology 6:763–775.

Pinheiro, J., D. Bates, S. DebRoy, and D. Sarkar. 2017. Linear and Nonlinear Mixed Effects Models.

Porazinska, D. L., R. D. Bardgett, M. B. Blaauw, H. W. Hunt, A. N. Parsons, T. R. Seastedt, and D. H. Wall. 2003. Relationships at the aboveground-belowground interface: Plants, Soil Biota, and Soil Processes. Ecological Monographs 73:377–395.

Prati, D., and O. Bossdorf. 2004. Allelopathic inhibition of germination by Alliaria petiolata (Brassicaceae). American Journal of Botany 91:285–288.

Van der Putten, W. H., L. E. M. Vet, J. a. Harvey, and F. L. Wäckers. 2001. Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. Trends in Ecology & Evolution 16:547–554.

Rauschert, E. S. J., and K. Shea. 2012. Invasional interference due to similar inter-and intraspecific competition between invaders may affect management. Ecological Applications 22:1413–1420.

Rearick, D., L. Kintz, K. L. Burke, and T. S. Ransom. 2011. Effects of white-tailed deer on the native earthworm, Eisenoides carolinensis, in the southern Appalachian Mountains, USA. Pedobiologia 54:173–180.

Reich, P. B., J. Oleksyn, J. Modrzynski, P. Mrozinski, S. E. Hobbie, D. M. Eissenstat, J. Chorover, O. a. Chadwick, C. M. Hale, and M. G. Tjoelker. 2005. Linking litter calcium, earthworms and soil properties: a common garden test with 14 tree species. Ecology Letters 8:811–818.

Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution 3:217–223.

Ringle, C. M., S. Wende, and J.-M. Becker. 2015. Boenningstedt: SmartPLS GmbH.

Rooney, T. P., and D. M. Waller. 2003. Direct and indirect effects of white-tailed deer in forest ecosystems. Forest Ecology and Management 181:165–176.

Royo, A. a., R. Collins, M. B. Adams, C. Kirschbaum, and W. P. Carson. 2010. Pervasive interactions between ungulate browsers and disturbance regimes promote temperate forest herbaceous diversity. Ecology 91:93–105.

Rudgers, J. A., and S. Orr. 2009. Non-native grass alters growth of native tree species via leaf and soil microbes. Journal of Ecology 97:247–255.

Salgado-Luarte, C., and E. Gianoli. 2012. Herbivores Modify Selection on Plant Functional Traits in a Temperate Rainforest Understory. The American Naturalist 180:E42–E53.

Scheu, S. 2003. Effects of earthworms on plant growth: patterns and perspectives. Pedobiologia 47:846–856.

Seagle, S. 2003a. Can ungulates foraging in a multiple-use landscape alter forest nitrogen budgets? Oikos 103:230–234.

Seagle, S. W. 2003b. Can ungulates foraging in a multiple-use landscape alter forest nitrogen budgets?

Seki, Y., and M. Koganezawa. 2013. Does sika deer overabundance exert cascading effects on the raccoon dog population? Journal of Forest Research 18:121–127.

Shaben, J., and J. H. Myers. 2010. Relationships between Scotch broom (Cytisus scoparius), soil nutrients, and plant diversity in the Garry oak savannah ecosystem. Plant Ecology 207:81–91.

Shelton, A. L., J. A. Henning, P. Schultz, and K. Clay. 2014. Effects of abundant white-tailed deer on vegetation, animals, mycorrhizal fungi, and soils. Forest Ecology and Management 320:39–49.

Simberloff, D., and B. Von Holle. 1999. Positive interactions of nonindigenous species: invasional meltdown? Biological invasions 1:21–32.

Spreitzer, R. J., and M. E. Salvucci. 2002. RUBISCO: Structure, Regulatory Interactions, and Possibilities for a Better Enzyme. Annual Review of Plant Biology 53:449–475.

Storey, J. D., J. E. Taylor, and D. Siegmund. 2004. Strong control, conservative point estimation and simultaneous conservative consistency of false discovery rates: a unified approach. Journal of the Royal Statistical Society: Series B (Statistical Methodology) 66:187–205.

Straube, D., E. A. Johnson, D. Parkinson, S. Scheu, and N. Eisenhauer. 2009.

Nonlinearity of effects of invasive ecosystem engineers on abiotic soil properties and soil biota. Oikos 118:885–896.

Szlavecz, K., M. McCormick, L. Xia, J. Saunders, T. Morcol, D. Whigham, T. Filley, and C. Csuzdi. 2011. Ecosystem effects of non-native earthworms in Mid-Atlantic deciduous forests. Biological Invasions 13:1165–1182.

Tracy, C. R., K. A. Christian And, and C. Richard Tracy. 2010. Not just small, wet, and cold: Effects of body size and skin resistance on thermoregulation and arboreality of frogs. Ecology 91:1477–1484.

Wandeler, H. De, R. Sousa-silva, E. Ampoorter, H. Bruelheide, L. Finer, M. Carnol, S. M. Dawud, D. Gabriel, S. Müller, M. Pollastrini, S. Ratcliffe, K. Raulund-rasmussen, F. Selvi, F. Valladares, K. Van Meerbeek, K. Verheyen, L. Vesterdal, and B. Muys. 2016. Drivers of earthworm incidence and abundance across European forests. Soil Biology and Biochemistry 99:167–178.

Wardle, D. a. 2006. The influence of biotic interactions on soil biodiversity. Ecology Letters 9:870–886.

Wardle, D. a, R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science 304:1629–1633.

Wehner, J., P. M. Antunes, J. R. Powell, J. Mazukatow, and M. C. Rillig. 2010. Plant pathogen protection by arbuscular mycorrhizas: A role for fungal diversity? Pedobiologia 53:197–201.

Yang, S., M. J. Ferrari, and K. Shea. 2011. Pollinator Behavior Mediates Negative Interactions between Two Congeneric Invasive Plant Species. The American

Naturalist 177:110–118.

Zicsi, A., K. Szlavecz, and C. Csuzdi. 2011. Leaf litter acceptance and cast deposition by peregrine and endemic European lumbricids (Oligochaeta: Lumbricidae). Pedobiologia 54:S145–S152.

Zuur, A. F., E. N. Ieno, N. Walker, A. a. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Page Springer. Springer New York, New York, NY.

Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion.