Common buckthorn (*Rhamnus cathartica*), European earthworms, and ecosystem management: Invasion and restoration in Minnesota's deciduous forests

A DISSERTATION
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

Alexander MacEachern Roth

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Dr. Lee Frelich, Adviser
Dr. Peter Reich, Co-Adviser

May 2015

Acknowledgements

I feel an extreme sense of gratitude to the many people who have in various ways helped guide me through graduate school. I was lucky to work with two exceedingly smart and driven advisers, Lee Frelich and Peter Reich. Thank you to Lee for always being available to talk shop and for trusting me to pursue the research topics of my choice, and to Peter for encouraging me to think about the whole picture, both big and small. Without their shared approach to mentoring, I would never have felt comfortable enough to take on a variety of new projects and topics, let alone complete a dissertation. I would also like to thank my other committee members, George Heimpel and Laura Van Riper, for their support and insight throughout the entire process, and for bringing diverse perspectives and important ideas to my research.

None of my actual research would have been possible without the help of a number of collaborators and field assistants. While specific contributions are acknowledged at the end of each chapter, a few people deserve particular attention. Thank you to both Tim Whitfeld and Kevin Mueller for their endless enthusiasm and for showing that life after graduate school isn't all that bad. Thank you to Rachael Nicoll and the rest of our field and lab assistants for help with data collection and processing, and for braving the field in any weather. Thank you to the various land managers and site contacts for allowing us to conduct research on their properties. Thank you to Cindy Buschena, who was endlessly helpful in sorting out the nitty gritty details and made sure we always had what we needed to do our work. Thank you to the Forest Ecology Lab

Group for support, ideas and feedback. Thank you to Jane Cowles, whose help with statistics and support for my ideas meant more than I can describe. And last but certainly not least, thank you to Sascha Lodge for being a tireless sounding board and endless source of support and encouragement. I couldn’t think of a better person to work alongside for four-plus years.

Finally, a large cast of characters made it repeatedly and abundantly clear that I have one of the best support groups around. Thank you to my Ohio crew, who never fails to keep me grounded or lets me forget where I come from. Thank you to friends from undergraduate and beyond whose humor is always appreciated. Thank you to all the amazing friends I’ve made in the Twin Cities, and especially to the Scudder House for becoming my partners in crime over the last few years. Finally, thank you to my parents, Randy and Allison. Your love and support mean the world to me, and I know how lucky I am to have grown up with you two as my guides.

Dedication

This dissertation is dedicated to my parents, Randy and Allison, whom I love very much.

Abstract

Common buckthorn (*Rhamnus cathartica*) and European earthworms are problematic invasive species in forests of the upper Midwest United States, and it is hypothesized that these two species may have a facilitative relationship. To better understand their invasion, it is necessary to understand how they interact with biotic and abiotic filters, as well as with each other. We established a greenhouse microcosm experiment to investigate the effects of important biotic and abiotic factors on buckthorn establishment and further explored the relationship between buckthorn and earthworms using a 24-plot field study. Using insights from our greenhouse results, we manipulated factors affecting plant colonization in a buckthorn removal experiment in order to improve buckthorn removal and ecosystem restoration efforts. Greenhouse results showed that the presence of earthworms increased buckthorn abundance and biomass across all light and leaf litter treatment levels, supporting the hypothesis that earthworms facilitate buckthorn invasion in upper Midwest forests. Results from the field study, conducted across a naturally-occurring gradient of buckthorn abundance, suggest that buckthorn, in turn, facilitates earthworms in this study system. Plots with higher buckthorn abundance had higher earthworm biomass, with linear regression, mixed model, and path analysis results supporting the directionality of the relationship. Together, these results lend support to a co-facilitative relationship between the two organisms. Co-facilitation my increase the success of both species and strengthen their negative impacts on native species and forest ecosystems.

Finally, we tested three buckthorn removal methods (weed-wrenching, cut and paint, and basal bark herbicide application) chosen to differentially affect conditions controlling plant establishment. Removal plots differed in the subsequent cover and diversity of plant regeneration, with methods that disturbed soil and increased available light resulting in the highest species cover and diversity. Non-metric multidimensional scaling ordinations and indicator species analysis demonstrated that the resulting plant communities differed significantly in their species composition, with weed-wrench plots associated with more early-successional community assemblages. Ultimately, removal methods can differentially affect the regeneration of understory vegetation and affect future community succession. Understanding why and how a species invades can encourage a more scientific approach to invasive species management, potentially resulting in improved management outcomes.

Table of Contents

List of Tables

List of Figures

Introduction

Biological invasions have become one of the most important global environmental concerns. Invasive species are spreading at alarming rates and are now the second-leading cause of human-mediated species extinction, behind only habitat loss (Heneghan et al. 2007). The United States alone incurs at least $120 billion annually in costs related to the effects and control of invasive species, much of which is due to the loss of ecosystem services (Pimentel et al. 2005). While charismatic invaders, like zebra mussels (*Dreissena polymorpha*) and Burmese pythons (*Python bivittatus*) garner much of the national spotlight due to their immediate and highly visible negative environmental and economic impacts, many other species are at least as pervasive and damaging. For example, the invasive shrub common buckthorn (*Rhamnus cathartica* L.) has the potential to decrease native species diversity and alter forest ecosystem characteristics, changing the course of future succession toward novel environments (Knight et al. 2007). While the negative effects of buckthorn and other invaders are not necessarily immediate, they can become visible over time, and the potential for interactions with factors like global climate change and other invasive organisms means that the future of many ecosystems is in jeopardy (Frelich and Reich 2009).

Minnesota is celebrated not only for its lakes but for its forests. The state contains a variety of forest types, from deciduous hardwoods to boreal conifers. However, these forests are increasingly threatened by invasive plants. The Minnesota Department of Natural Resources (MNDNR) has identified at least 37 invasive plant species present in a variety of ecosystems across the state (MNDNR 2012). The effects of buckthorn—one of the better-established invasive species in the state—are well documented (Knight et al. 2007). Buckthorn is a woody invasive shrub from Eurasia that was brought to the United States in the 1800s as an ornamental plant (Heneghan et al. 2007). It has since escaped cultivation and has come to dominate many forested areas, fallow farm fields, prairies, and riparian areas (Kurylo and Endress 2012). It now occurs in 34 U.S. states and eight Canadian provinces (USDA NRCS 2014). Buckthorn has characteristics common to ruderal species; it is able to take advantage of excess nutrients in the soil, use light efficiently, and vigorously compete with native vegetation. It has also been shown to take advantage of increased bare soil and changes in light availability within forest systems, often caused by disturbance (Knight et al. 2007).

Buckthorn has harmful effects on native plant and animal species in part through competition for resources and shading of the understory (Fagan and Peart 2004; Knight et al. 2007; McKinney and Goodell 2010), allelopathy (Seltzner and Eddy 2003; Klionsky et al. 2011; Sacerdote and King 2014; but see Knight 2006), and by changing forest structure (Schmidt and Whelan 1999). Buckthorn is also a host for multiple agricultural pests, increasing damage to and costs associated with agricultural production (Heimpel et al. 2010). In general, buckthorn control is costly, with much time and effort spent every year to control and eradicate populations, but with limited success (Invasive Species Program 2012; Gassman and Tosevsky 2014). It has been proposed that plants like buckthorn, in tandem with the activities of invasive earthworms and climate change, are preventing forest regeneration and hastening the decline of many U.S. forests (Hartman and McCarthy 2008).

In the previously glaciated forests of northern North America, European earthworms have also become an invasive species of serious concern. Earthworms cause a variety of changes to the soil and leaf litter layer and negatively affect many species of flora and fauna (Maerz et al. 2009; Loss and Blair 2011, and see a review in Frelich et al. 2006). For example, earthworms increase the bulk density of soils and mix organic material into deeper soil horizons. This leads to changes in the composition and abundance of soil microfauna (Frelich et al. 2006). Earthworms also reduce the overall availability of nutrients like nitrogen and phosphorus and increase leaching of these nutrients from forests systems (Frelich et al. 2006). Aside from the changes in available nutrients and soil structure, earthworms also disrupt beneficial mycorrhizal interactions, further inhibiting the growth and survival of many native plants (Frelich et al. 2006). These effects may compound the effects of buckthorn (Knight et al. 2007; Heneghan et al. 2007), leading to severely degraded systems (Frelich and Reich 2010). Indeed, it is proposed that buckthorn and earthworms may facilitate one another in forests of the upper Midwest (Heneghan et al. 2007; Heimpel et al. 2010), potentially strengthening their negative effects on forest ecosystems.

Loss of native plant diversity in the understory, changes in nutrient cycling, and suppression of forest regeneration all make these invaders a serious concern (Frelich et al. 2006). Furthermore, they have the potential to interact with other invasive species (Heimpel et al. 2010) and compound the effects of climate change, leading to rapid, wholesale changes in forest environments (Frelich and Reich 2009). Therefore, there is a growing focus on restoration of areas degraded by these invaders. Early studies have focused on the success of different invasive plant eradication methods (Nyober 1992; Archibold et al. 1997), with few studies addressing the re-establishment of plant communities post-removal (Love and Anderson 2009; Guido and Pillar 2014). Once invasive plants have been removed, restoration projects often suffer from a lack of native species recruitment, often attributable in part to depleted seedbanks (Collier et al. 2002), and reinvasion by the same or new non-native and invasive species (Vidra et al. 2007).

The problem under consideration is multifaceted: first, it is necessary to understand the precise ways in which these species invade ecosystems. In other words, what do we know about the biotic and abiotic controls on their invasion, and do these species benefit from direct or indirect facilitation by other organisms? Second, it is necessary to determine the best way to manage invaded areas. What are the best methods to remove these species and encourage the recovery of the native plants and ecosystem processes? We explored these topics using both observational and experimental studies of the concurrent invasions of buckthorn and earthworms in Minnesota’s deciduous forests.

Chapter one investigated biotic and abiotic controls on the germination of common buckthorn. Using a greenhouse microcosm experiment, we manipulated light levels, leaf litter depth and earthworm presence to investigate the independent and interactive effects of these treatments on buckthorn establishment. Specifically, we measured buckthorn abundance and biomass in response to the different light and leaf litter treatments to address whether shade and thick leaf litter provide resistance to buckthorn germination and growth, as well as whether the presence of earthworms positively influenced buckthorn germination and growth throughout the seedling establishment stage.

Chapter two explored the relationship between buckthorn and earthworms using a 24-plot field study across a gradient of buckthorn invasion in a mesic, oak-dominated forest. Over the course of two growing seasons, we examined the relationships between buckthorn abundance, earthworm biomass and other environmental characteristics. Specifically, we aimed to test the hypothesis that there is a positive relationship between common buckthorn and European earthworms, and that buckthorn facilitates earthworms in forests of the upper Midwest US.

In chapter three we applied ecological principles governing plant colonization and establishment to a buckthorn removal experiment in order to improve restoration of ecosystems invaded by common buckthorn. To investigate how different buckthorn removal methods affect the regeneration of understory vegetation, we established a four-site buckthorn removal experiment in upland, mesic oak forests in east central Minnesota. By using the knowledge gained from the greenhouse experiment in the first chapter, we applied ideas about controls on germination and establishment in order to better implement removal strategies that also promote plant regeneration.

Overall, an enhanced understanding of the invasion process can help improve management strategies. In this dissertation, we explored controls on buckthorn establishment as well as mechanisms of inter-trophic facilitation between buckthorn and European earthworms. Understanding the mechanisms behind how and why a species invades and establishes is an important part – though only the first part – of understanding and responding to the invader. Ultimately, we can use the knowledge gained through these endeavors to implement successful removal strategies while simultaneously promoting the regeneration of native species.

CHAPTER 1

Invasive earthworms interact with abiotic conditions to influence the invasion of common buckthorn (*Rhamnus cathartica* L.)

Summary

Common buckthorn (*Rhamnus cathartica* L.) is one of the most abundant and ecologically harmful non-native plants in forests of the upper Midwest United States. At the same time, European earthworms are invading previously glaciated areas in this region, with largely anecdotal evidence suggesting that they compound the negative effects of buckthorn and influence the invasibility of these forests. Germination and seedling establishment are important control points for colonization by any species, and manipulation of the conditions influencing these life history stages may provide insight into why invasive species are successful in some environments and not others. Using a greenhouse microcosm experiment, we examined the effects of important biotic and abiotic factors on the germination and seedling establishment of common buckthorn. We manipulated light levels, leaf litter depth and earthworm presence to investigate the independent and interactive effects of these treatments on buckthorn establishment. We found that light and leaf litter depth were significant predictors of buckthorn germination but that the presence of earthworms was the most important factor; earthworms interacted with light and leaf litter to increase the number and biomass of buckthorn across all treatments. Path analysis suggested both direct and moisture-mediated indirect mechanisms controlled these processes. The results suggest that the action of earthworms may provide a pathway through which buckthorn invades forests of the upper Midwest United States. Hence, researchers and managers should consider co-invasion of plants and earthworms when investigating invasibility and creating preemptive or post-invasion management plans.

Introduction

Biological invasions are one of the most important global environmental problems (Vitousek 1996; Pimentel et al. 2005). With increased global connectivity and trade, invasive species are now the second-leading cause of human-mediated species extinction (Heneghan et al. 2007), and northern temperate forests are especially at risk (Murphy and Romanuk 2014). The United States alone incurs roughly $120 billion annually in costs related to the effects and control of invasive species, much of which is due to the loss of ecosystem services (Pimentel et al. 2005). While a few high profile invasive species, such as zebra mussels (Dreissena polymorpha) and Burmese pythons (*Python bivittatus*), garner much attention, many other less well-known species are at least as pervasive and often as damaging. For example, plant species like common buckthorn (*Rhamnus cathartica*) are linked to losses of native plant diversity, changes in nutrient cycling, and suppression of forest regeneration, making these invaders a serious concern (Fagan and Peart 2004; Knight et al. 2007; Klionsky et al. 2011). While the negative effects of invasive species are not necessarily immediate, they can become visible over time (Mack et al. 2000), and the potential for interactions with factors like global climate change and other invasive organisms means that the future health and diversity of many ecosystems is threatened (Theoharides and Dukes 2007; Frelich & Reich, 2009; Bellard et al. 2013; Polgar et al. 2014). Researchers propose that invasive plants such as common buckthorn, in tandem with the activities of ecosystem engineers (such as invasive earthworms and deer) and the influence of climate change, are negatively impacting forest regeneration and biological diversity in many U.S. forests (Hartman and McCarthy 2004; Frelich and Reich 2009).

Buckthorn is a tall shrub or small tree native to Europe and Asia, and was introduced into the U.S. as an ornamental and medicinal plant before the turn of the 18th century (Kurylo and Endress 2012). Buckthorn has since spread throughout many forested areas, where it can form dense monospecific stands (Knight et al. 2007). Buckthorn germinates well across a range of light levels, though deep shade can reduce germination, growth and survival (Knight 2006). Evidence suggests that buckthorn also germinates best on bare mineral soil and that its germination decreases in the presence of leaf litter or herbaceous plant cover (Gourley and Howell 1984; Gill and Marks 1991; Bisikwa 2005). It is currently found in at least 34 US states and five Canadian provinces and has been linked to losses in species diversity and changes in ecosystem characteristics such as nutrient and light availability (Prati and Bossdorf 2004; Stinson et al. 2006; Knight et al. 2007; Klionsky et al. 2011; USDA NRCS 2014). The effects of buckthorn on decomposition and soil processes have been widely studied (Kollman and Grubb 1999; Heneghan et al. 2002; Heneghan et al. 2004; Heneghan et al. 2006; Knight 2006), may play a role in sustaining its own dominance (Heneghan et al. 2002), and are likely to affect future plant succession (Heneghan et al. 2006). Additionally, the ubiquitous presence of buckthorn in forests may reduce native plant and animal diversity and abundance in part through shading of the understory (Fagan and Peart 2004; K. S. Knight et al., 2007; McKinney and Goodell 2010), allelopathy (Seltzner and Eddy 2003; Klionsky et al. 2011; Sacerdote and King 2014; but see Knight 2006), and replacing native species (Schmidt and Whelan 1999). Buckthorn is also a host for multiple agricultural pests, increasing damage to and costs associated with agricultural production (Heimpel et al. 2010). In general, buckthorn control is costly, with much time and effort spent every year to control and eradicate populations, but with limited success (Invasive Species Program 2012; Gassman and Tosevsky 2014).

Common buckthorn may also facilitate other invasive species and be a catalyst for a cascade of negative effects on ecosystem processes. For example, buckthorn has been proposed to facilitate invasion of European earthworms into North American forests. Buckthorn creates ideal conditions for invading European earthworms by providing nutrient-rich leaf litter and creating high-shade conditions that cool soils (Heneghan et al. 2007; Holdsworth et al. 2008; Heimpel et al. 2010). The presence of earthworms in previously earthworm-free forests has many effects on soil properties and nutrient cycling (Bohlen et al. 2004; Groffman et al. 2004; Frelich et al. 2006), reducing the overall availability of nutrients like nitrogen and phosphorus (Frelich et al. 2006; Costello and Lamberti 2008; Eisenhauer et al. 2011; Sackett et al. 2013), inhibiting the growth and survival of many native plants (Gundale 2002; Lawrence et al. 2003; Frelich et al. 2006; Larson et al. 2010; McCormick et al. 2013) and leading to simplified vegetation layers and reduced tree recruitment (Lawrence et al. 2003; Frelich et al. 2006; Hale et al. 2006; Drouin et al. 2014). Once earthworms are established, forest ecosystems may reach a new steady state with novel soil and plant communities and functions (Eisenhauer et al. 2011).

While some largely circumstantial evidence suggests that common buckthorn facilitates earthworm invasion, earthworms may also facilitate invasion by buckthorn and other invasive plants (Eisenhauer et al. 2012). This facilitation may be especially influential at the germination and establishment stage, where invaders must overcome various abiotic and biotic obstacles in order to colonize and establish in an area (Williamson and Fitter 1996;

Richardson et al. 2000; Levine et al. 2004). Because earthworms rapidly consume leaf litter, they expose bare soil on which many invasive species – including buckthorn – preferentially germinate (Heneghan et al. 2007; Knight et al. 2007). The bare soil may give an advantage to invaders over native species adapted to the thick organic horizon present in previously earthworm-free northern forests (Gundale 2002; Hale et al. 2006). However, little is known about how earthworms and litter interact to affect buckthorn germination. Earthworms also increase decomposition and can provide temporary pulses of nutrients available to plants (Heneghan et al. 2006; Hale et al. 2008; Heimpel et al. 2010). Invaders are often able to better utilize these nutrient pulses (Huenneke et al. 1990; Davis et al. 2000; Gilliam 2006; Funk and Vitousek 2007), possibly aiding germination and growth of their seedlings. These processes may undermine the resistance to invasion that is potentially provided by abiotic factors like low light and deep leaf litter. It is also unknown whether earthworm facilitation of invasive plants may differ with variation in light and litter.

To explore these issues, we conducted a greenhouse experiment to investigate the effects of abiotic (light and leaf litter depth) and biotic (earthworms) controls on the germination and early establishment of common buckthorn. We addressed the following questions: (1) Do shade and thick leaf litter provide resistance to buckthorn germination and growth? (2) Does the presence of earthworms positively influence buckthorn germination and growth, and do earthworms change the effects of shade and leaf litter? (3) Does the importance of these variables change throughout the seedling establishment stage?

Methods

Experimental design

In a temperature-controlled greenhouse, we established 126 microcosms each containing the same six native plant species but varying levels of litter depth and light availability. We also manipulated exotic earthworm presence/absence. Each microcosm was made from a 25 cm length of PVC pipe with 10 cm diameter. We taped a 5 mm mesh screen to the bottom of each microcosm and added 5 cm of perlite to aid in water drainage. To represent local temperate deciduous forests, soil was collected from the upper layer of a mesic hardwood forest in central Minnesota (DeMontreville loamy fine sand; texture: 69% sand, 23% silt, 8% clay). The soil was sifted to remove roots, rocks, and other organic matter, and then thoroughly mixed before being added to each microcosm. We kept the pots moist for eight weeks, and removed any germinating seeds from the existing seedbank. To simulate conditions typical of the local growing season, we set the day/night greenhouse light regime to 16/8 hours and the temperature to 20/16 °C.

Native plant species were germinated in planting trays using soil collected from the same location. Native species included *Desmodium glutinosum, Elymus hystrix, Carex blanda, Eurybia macrophylla, Asclepias exaltata,* and *Galium boreale*. Each of these native species is commonly found in local mesic hardwood forests and was among the most common species in a survey of 67 deciduous forest sites in central and southeastern Minnesota (Whitfeld et al. 2014a). Seeds of the native species were purchased from Prairie Moon Nursery in Winona, Minnesota. Once they germinated and established in the trays, one seedling of each of the six native species was transplanted into each of the microcosms (equally spaced) and allowed to grow for four weeks. Seedlings that did not survive the transplant were replaced.

Once the native plant community was established in all microcosms, we applied the abiotic treatments. Native leaf litter, collected at the same site as the soil and composed of red oak (*Quercus rubra)*, white oak (*Quercus alba)*, and sugar maple (*Acer saccharum*), was cut into 2-3 cm strips and applied to a subset of microcosms for the different leaf litter treatments. One-third of the microcosms received no leaf litter (no litter treatment), while one-third received 2.5 g (low litter treatment), and the last third received 5.0 g (high litter treatment). Litter amounts were chosen to represent low and high litterfall values in earthworm-invaded sites in Minnesota (Holdsworth et al. 2012).

Shade enclosures were constructed from PVC piping and layered combinations of 85% and 68% shadecloth so that one-third of the microcosms received 15% of full outside sunlight to simulate light levels under an oak canopy (medium light treatment) and one-third received 3% of full light to simulate light levels under a dense buckthorn canopy (low light treatment). We also included an unshaded treatment that received ambient light inside the greenhouse (high light treatment). Light levels in the greenhouse were roughly 8% lower than full sunlight, and our shade treatments were designed to incorporate this difference when arriving at our final treatment light levels.

Finally, one European nightcrawler (*Lumbricus terrestris*) was added to half of the microcosms in each treatment. This equates to a density of roughly 128 earthworms per square meter, which is a high density for anecic earthworms. However, Eisenhauer et al. (2007) found that at the peak of invasion, densities of *Lumbricus terrestris* in the Canadian

Rockies reached 109 individuals per square meter; thus the density in our study is similar to a heavily invaded forest. Earthworms were purchased from Blue Ribbon Bait and Tackle Shop in Hugo, Minnesota, and kept in the experimental soil with ample leaf litter in order to acclimate them to experimental conditions. Once all treatments were established, clear plastic "worm screens" were applied around the rim of each pot to prevent earthworm escape. Six control microcosms under ambient light with no native species or leaf litter were also established to measure baseline invasive seed germination. Overall, the experimental design included three litter treatments, three light treatments and two worm treatments for a total of 18 different treatment combinations. Each treatment combination was replicated in seven pots for a total of 126 experimental microcosms and six control pots.

Ten seeds each of four invasive species (buckthorn, barberry (*Berberis thumbergii*), garlic mustard (*Alliaria petiolata*), and dandelion (*Taraxacum officinale*)) were then added on top of the leaf litter in each microcosm to simulate natural seed rain. Pots were watered every other day for the eight-week duration of the experiment. As biomass accumulated, we increased the amount of water from 50 ml to 150 ml for each pot to minimize any limitation from lack of water. Finally, the location of the pots on the greenhouse benches was randomized each week to avoid any effects due to uneven light intensity in the greenhouse.

Data collection

Each week, we recorded the number of germinated invasive seedlings by species. Because there was very low mortality of germinating invasive seeds – and the mortality we did see was due to direct earthworm predation – we define germination success as the cumulative number of seeds that germinated at any time during the experiment, and use final biomass as an estimate of their early establishment success. Percent cover of native plants was also estimated, as well as the percent cover of remaining leaf litter. Using a LICOR LI-250A light meter, percent light transmittance at the soil surface was measured just prior to harvest. Once the experiment had run for eight weeks – long enough for the invasive species to germinate and establish but with minimal microcosm-induced limitations on native species' root growth – aboveground biomass was harvested and separated into native and invasive categories and any remaining litter was collected. All plant material was dried at 70°C for three days before being weighed. Approximately sixty grams of soil was taken from the upper 5 cm of each pot to measure treatment effects on soil moisture. After being dried for three days at 70°C, we calculated the soil moisture of the samples. Earthworm activity was documented by the presence of burrows in the soil column or middens at the soil surface. Finally, invasive and native roots were separated and washed before being dried and weighed for root biomass, though it was not possible to separate the roots to the species level. None of the garlic mustard seeds (invader species) germinated despite following cold stratification guidelines prior to the experiment (Baskin and Baskin 1992).

Data analysis

Germination of the other invasive seeds in control pots (pots not part of the experiment and without other plants) was high; common buckthorn, barberry, and dandelion germinated at rates of 90%, 80%, and 64% respectively. However in the experimental treatment pots, buckthorn was the only invader to consistently germinate; barberry and dandelion germinated in only 1% of pots on average, whereas at least one buckthorn seedling was present in 119 out of 132 pots. Therefore, we limited our data analysis to buckthorn. Data analysis was conducted with number of buckthorn seedlings and buckthorn aboveground biomass – per microcosm – as response variables, and was performed in JMP ver. 9.0.2 (SAS Institute, Inc., Cary, NC, USA). We treated number of buckthorn seedlings as count data and conducted any related analyses using a generalized linear model approach with a Poisson distribution. Overdispersion did not occur in the resulting models (<1.17). We used analyses of variance (ANOVA) to investigate differences in the final number and biomass of buckthorn across the different treatments. We created general and generalized linear models using the buckthorn biomass and number of buckthorn seedlings as the response variables. Light, leaf litter and earthworm presence/absence were all included as predictor variables. Because we were interested in how earthworms might influence the effects of light and litter treatments, interactions between earthworms and the abiotic treatments were also included in the models. While it was not a treatment – and was assumed to be similar in each pot at the outset of the experiment – we felt it important to investigate the potential effect of native plant biomass. Thus, as an additional test, we ran each model with and without native species biomass in order to examine its potential effects.

In order to explore the direct and indirect relationships between earthworm presence, light and litter, and their effects on buckthorn biomass, we performed path analysis using AMOS 5 (Amos Development Corporation, Crawfordsville, FL, USA). Using only paths that were ecologically relevant, we constructed an initial model based on prior knowledge. The treatments served as exogenous variables, and final buckthorn biomass was the response, or endogenous, variable. Earthworm presence/absence was included in the analysis as a predictor variable with a value of 0 when earthworms were absent and 1 when they were present. Light transmittance and litter cover were included in the analysis as continuous variables. We used the specification search tool in AMOS to compare related models and used non-significant chi-squared tests ($p>0.05$), AICc scores, and goodness of fit metrics (Grace 2006; Arbuckle 2012) to select the best-fit model. Because we also had weekly invasive seedling counts, we used a generalized regression approach with pot as a random effect to explore the relationships between treatments, time, and the number of buckthorn seedlings throughout the experiment.

Results

Native species biomass did not differ between earthworm ($F_{(1,124)} = 0.12$, $p=0.73$) or litter ($F_{(2,123)} = 0.23$, $p=0.80$) treatments, but was significantly positively related to light level ($F_{(2,123)} = 219.63$, $p<0.0001$). However, pre-harvest light availability at the soil surface did not differ between earthworm treatments due to the similarity in native species biomass ($F_{(1,124)} = 0.35$, $p=0.55$). A heat wave during the last three days of the experiment resulted in 45% earthworm mortality, as the greenhouse cooling system was unable to completely buffer the outside temperature. However, earthworms remained active until the final days of the experiment, as indicated by steady leaf litter decline and the appearance of fresh castings throughout the experiment (Dávalos et al. 2013). Due to the timing of earthworm mortality – partially decomposed earthworms were still found during harvest – we are confident that any potential pulse of nutrient released by their decomposition had little effect on the outcome of the experiment. At the end of the experiment, litter mass and soil moisture were 57% ($F_{(1,124)}$ = 16.53, p=0.001) and 19% ($F_{(1,124)}$ = 5.64, p=0.019) lower in microcosms with earthworms, respectively, supporting our observation that earthworms were active until the final days of the experiment.

Our linear models (Number of seedlings: χ^2 = 88.31, df =17, p<0.0001; Biomass: R^2 =0.40, $F_{(17,108)}$ = 4.22, p<0.0001) showed that the abiotic light and leaf litter depth treatments significantly affected buckthorn seedling number and biomass. Litter positively affected both number of seedlings and biomass of buckthorn, while the negative effect of light was only significant for the number of buckthorn seedlings (Fig. 1; Table 1). The independent effect of earthworms was positive and significant for both number of seedlings and biomass of buckthorn (Fig. 1; Table 1). When native plant biomass was added to the models as a covariate, it had a significant negative effect on seedling number (χ^2 = 12.96, P=0.0002) and buckthorn biomass ($F_{(1,107)}$ = 14.93, P=0.0002). Moreover, the addition of native plant biomass changed the overall effect of light to positive and made the effect of light on buckthorn seedlings significant, though it did not change the significance or effect of any of the other treatment variables.

Across all treatments, buckthorn establishment (measured as number of seedlings that germinated and survived) was 34% higher in microcosms with earthworms than microcosms without earthworms ($F_{(1,124)}$ = 14.56, p=0.0002). Final buckthorn biomass was 33% higher in microcosms where earthworms were present ($F_{(1,124)}$ = 12.52, p=0.0006). As biomass and seedling numbers were similarly increased by earthworms, the effect on biomass was largely a result of the numbers of buckthorn seedlings that germinated and survived, and minimally influenced by their average growth rate, except in one case. When earthworms were present, buckthorn seedlings in the high light treatment had 25 percent higher average biomass than those in the other light treatments.

The effect of earthworms differed markedly depending on litter level (Fig. 1; Table 1). The presence of earthworms had no effect when no litter was present, boosted buckthorn success by roughly 38% at the intermediate litter level, and more than doubled buckthorn numbers and biomass at the highest litter treatment (Figure 1, Table 1). In turn, litter effects depended on the presence of earthworms; when earthworms were absent, litter level had no effect on buckthorn performance, but when earthworms were present, greater litter levels resulted in greater buckthorn success (Fig. 1).

The effect of earthworms also differed markedly depending on light level (Fig. 1; Table 1). The presence of earthworms had a generally positive effect on buckthorn, but the effect was largest at high light levels (Figure 1). Buckthorn success was only modestly dependent on light, except in the high light, no earthworm treatment, where buckthorn performed poorly (Fig. 1). Compared to a model with the aforementioned interactions, a model without the interaction terms was only able to explain 18% of the variance in buckthorn biomass, demonstrating the importance of the interactions between earthworms and the other variables in explaining the observed results.

Path analysis supported results from our linear models. It also provided further insight into the mechanisms behind the observed relationships and revealed indirect effects that helped to explain our observed results. The initial model fit the data, but included non-significant paths and could be improved ($\chi^2_4 = 1.23$, p=0.54; AICc=27.23) (Supplementary

Material). The final model improved the fit ($\chi^2_4 = 1.45$, p=0.84; AICc=23.45) and fit better than the saturated and independence models (NFI = 0.98; CMIN = 0.36; RMSEA = 0). The model indicated that light had an indirect negative effect on buckthorn biomass through its negative effect on soil moisture (Figure 2). By contrast, litter cover positively influenced buckthorn biomass. Earthworms had a direct positive effect on buckthorn biomass, as well as smaller indirect negative effects by decreasing soil moisture and litter cover. Earthworm activity in the field is often affected by soil moisture (Hale et al. 2005), but the continuous activity of earthworms in our experiment was likely to affect soil moisture, which was a potential impact of particular interest (Larson et al. 2010; Eisenhauer et al. 2012). While explaining more of the variation in soil moisture, a model including a native plant biomass term was a poorer fit and explained less variation in buckthorn biomass.

We also visually investigated the temporal changes in the number of germinated buckthorn seedlings throughout the experiment. Treatment effects on the number of buckthorn seedlings manifested at different points throughout the experiment. The different levels of the earthworm, light, and litter treatments began to diverge in the second, third and fourth weeks, respectively, providing insight into the dynamics of these processes (Fig. 3a-c). For example, the greater cumulative buckthorn germination from week two onward demonstrated the positive effect of earthworms on buckthorn, but incorporating time into the analysis provided a better understanding of when this process was most important (Fig. 3c). Our generalized linear mixed effects model provided statistical support for these observed trends and showed that date, light, litter and earthworms were all significant predictors of number of buckthorn seedlings (Table 2).

Discussion

Common buckthorn is an important invasive species in forests of the upper Midwest United States; its negative effects on soils, flora and fauna make it a concern for researchers and managers (Heneghan et al. 2006; Knight et al. 2007; Heimpel et al. 2010; Invasive Species Annual Program Report MNDNR 2013). However, an understanding of the controls on the germination and initial establishment of buckthorn remains limited. It has been proposed that earthworms facilitate the invasion of buckthorn, but to our knowledge, there is little experimental evidence to support this prediction (Whitfeld et al. 2014b). In the following sections we discuss how the results contribute to a better understanding of our three main questions. Because forests of the upper Midwest developed without earthworms, we feel that it is helpful to begin by briefly focusing on the effects of light and litter on buckthorn success without earthworms before considering the effects of earthworms and their interactions in our statistical models. This can help us frame our understanding of how earthworms influence the current invasion process in forests of the upper Midwest. While we have presented data for both buckthorn number and biomass, because of the similarity in responses we subsequently refer to buckthorn success when discussing treatment effects on buckthorn.

Question 1: Do shade and litter depth provide resistance to buckthorn germination and growth when earthworms are not present?

Light and litter had significant but differing effects on buckthorn success that reflect their influence on key limiting resources. In the absence of earthworms, buckthorn performed best in the low and medium light treatments. Buckthorn's diminished success at high light levels was likely due in part to seed desiccation and low soil moisture. The inclusion of native species biomass as a covariate in our models was also helpful in explaining this result. In the high light treatment, native species biomass was highest, which reduced light at the soil surface and also reduced soil moisture. The poor performance of buckthorn in the high light treatment was then not necessarily due to direct effects of high light itself, but potentially to indirect effects on moisture conditions. This corroborates results of previous studies demonstrating that buckthorn performs better with more light only if moisture levels are adequate (Wyckoff et al. 2005; Wyckoff et al. 2012), and may actually germinate and grow best at medium light levels (Gourley 1985). However, while the overall effect of light on buckthorn success was negative, the seedlings that were able to survive in the high light treatment had 25% higher average biomass than those in the other light treatments. This is the only case where average biomass per seedling differed across treatments. Moreover, buckthorn was able to establish in considerable numbers in the low and high light treatments, demonstrating its adaptability to different environmental conditions (Grubb et al. 1996; Knight 2006; Kurylo and Knight 2007).

In the absence of earthworms, leaf litter levels had negligible effects on buckthorn establishment. Buckthorn performed poorly in the high litter treatment, though this was not statistically different from the other treatments. In this case, the limited effect of litter may be due to the design of our experiment, as the 2-3 cm strips of leaf material are less representative of conditions in the field and may have allowed more seeds to reach the soil surface. While our experiment does not provide conclusive evidence that leaf litter provides resistance to buckthorn invasion, the observed trend is in line with previous evidence that leaf litter can reduce buckthorn germination and growth by providing a physical barrier to seed and seedling penetration and decreasing light availability to seeds at the soil surface (Gourley and Howell 1984; Bisikwa 2005).

Question 2: Do earthworms positively influence buckthorn establishment, and does this depend on light and litter conditions?

Overall, microcosms with earthworms had about a one-third increase in both the number of established buckthorn seedlings (34%) and their total biomass (33%), compared to those without earthworms. The importance of earthworms was corroborated in all of our analyses, and while the mechanisms behind this simple relationship are not clear, previous studies suggest the positive effect could be due in part to earthworms mixing litter and helping seeds reach the soil, protecting seeds from desiccation through burial, and concentrating seeds near middens with moist, high nutrient castings (Eisenhauer and Scheu 2008; Regnier et al. 2008). Throughout the experiment, we observed earthworms burying and concentrating seeds near their middens, and germination of buckthorn was often clustered near midden openings. However, as demonstrated by our path analysis results, earthworms also had negative effects on buckthorn biomass through their negative effects on litter cover and soil moisture, which were supported by our observations of seed desiccation in both the litter and no-litter treatments. This negative effect was clearly swamped by an overall positive influence of earthworms, and was likely attributable in part to our specific experiment, as the small pot size exacerbated the effects of soil drying. In the field, buckthorn is able to tolerate a wide range of moisture conditions, including a degree of drought (Stewart and Graves 2004), and is unlikely to be as strongly affected by earthworm-induced soil moisture losses.

Our experiment not only demonstrated the roles of light and leaf litter in influencing the germination and biomass of buckthorn, but also emphasized the importance of the interactions between earthworms and these variables in affecting this important stage of plant growth. Ultimately, the positive impacts of earthworms on buckthorn were much larger at higher light and higher litter levels. For example, the presence of earthworms resulted in greater buckthorn success in the high light treatment, which, when earthworms were absent, was associated with poor buckthorn performance. In high light, earthworms likely increased buckthorn success by mixing seeds into the soil and caching them in their burrows, which has been shown to prevent desiccation of seeds and protect against seed predation in natural environments (Azcarate and Peco 2006; Regnier et al. 2008). This assumption is supported by our field and experimental observations of concentrated germination of buckthorn seeds in and around earthworm middens (Roth, *personal observations*), where castings likely provide higher soil moisture and nutrients (Eisenhauer and Scheu 2008; Regnier et al. 2008). There were also treatment-dependent interactions between earthworms and leaf litter that affected buckthorn performance. In the no-litter treatment, earthworms perhaps contributed to a slight reduction in buckthorn establishment by burying seeds beyond a critical depth (Traba et al. 1998; Milcu et al. 2006; Regnier et al. 2008). We also noted previously healthy buckthorn seedlings that were subsequently bent over and whose cotyledons were pulled down into earthworm burrows, leaving only the bare stalk and suggesting seedling predation by earthworms (Eisenhauer et al. 2010; Griffith et al. 2013). By contrast, in the high litter treatment where there was more food for the earthworms, the presence of earthworms increased buckthorn success, likely by consuming and mixing the litter barrier and providing pathways for the seeds to reach the soil. This suggests that in otherwise undisturbed forests where light and litter conditions may influence plant invasion, earthworms may interact with these conditions to facilitate buckthorn invasion.

Question 3: Does the importance of these variables change throughout the seedling establishment process?

Our generalized regression model confirmed the importance of light, litter and earthworms in influencing buckthorn germination and also highlighted the overall importance of time in the process. This allowed us to pinpoint when buckthorn experienced higher germination and provided insights into mechanistic explanations of the observed results. For example, time had a significant interaction with earthworms in our model; between the second and fourth weeks, the presence of earthworms accelerated the number of germinating buckthorn seedlings compared to pots without earthworms. We consistently observed earthworms removing and mixing the litter barrier, which has been shown to benefit invaders like buckthorn (Bisikwa 2005). This is also supported by the significant earthworm by litter interaction term in our model, as explained in the previous section. We also observed earthworms burying and concentrating seeds near their moist and nutrient-rich middens. As long as seeds are buried above critical germination depths, the effects of earthworms on germination can be positive (Regnier et al. 2008).

Investigating the other treatments, the positive effect of litter was not evident until week four, when the medium and high litter treatments first showed significantly higher buckthorn numbers than the no litter treatment; this difference remained throughout the experiment, though the interaction between litter and time was not significant in our statistical model. One potential explanation for this trend can again be found in litter's interaction with earthworms. In microcosms with earthworms, we saw a steady decline in litter cover throughout the experiment as earthworms consumed and moved litter into their burrows. By week four, it is possible that earthworms had mixed and removed enough litter for seeds to germinate, but that enough litter remained to provide protection from desiccation. In the field, deep litter may inhibit buckthorn germination (Bisikwa 2005) and has been shown to inhibit other invaders (Schramm and Ehrenfeld 2010; Yeo et al. 2014), but seeds deposited on otherwise bare soil may also find temporary protection against predation, frost heaving, and desiccation from a thin layer of leaf litter (Gill and Marks, 1991; Cintra 1997).

Light effects on buckthorn performance also seemed to vary with time, though the interaction between light and time was not significant in our model. By week three, pots in the high light treatment had significantly lower numbers of buckthorn than pots in the medium and low light treatments. While light is instrumental in helping seeds germinate, in this experiment it negatively affected soil moisture and likely caused seed desiccation, reducing the number of germinants (Gill and Marks 1991). Native species biomass was also highest in the high light treatment, exacerbating the negative effect of light on soil moisture.

Conclusions and implications

Consistent with prior work documenting the effects of earthworms in forests of the upper Midwest (Hale 2005; Holdsworth 2007), our results suggest that earthworms may provide a pathway for buckthorn's invasion in these forests. Coupled with earthworms' ability to negatively affect resident plant species richness and abundance in forests (Holdsworth 2007), these results suggest that earthworms may also facilitate invasive plants such as common buckthorn. These processes will likely affect the trajectory of forest regeneration, and may interact with other processes, such as insect and herbivore damage and climate change, to shape our future forests (Frelich et al. 2012). Using a greenhouse microcosm experiment, we were able to establish that earthworms interact with light and leaf litter conditions to increase buckthorn germination and initial establishment. Microcosm experiments are an important and widely used tool for exploring the ecological processes but they do have limitations. Interpretations and extrapolation to natural systems should, therefore, be made with caution (Drake et al. 1996; Benton et al. 2007; but see Carpenter 1996; Schindler 1998).

If our results are representative of natural systems, managing deciduous forests of the upper Midwest for other conditions that have been shown to decrease invasibility may prove more successful than managing for light and litter conditions. For example, managing forests for native plant diversity may help resist invasion (Elton 1958; Lodge 1993; Kennedy et al. 2002; Davis et al. 2005; Fridley et al. 2007; Whitfeld et al. 2014a). Managing surrounding lands to decrease available propagules will also keep buckthorn and other invasive plants from arriving at the site, and may be the most important step in preventing invasion

(Eschtruth and Battles 2009; Warren et al. 2012). Finally, our data suggest that preventing the spread of earthworms will also be valuable for reducing the spread of buckthorn and potentially other invasive plants as well. Given these results, future studies of plant invasion and forest dynamics in northern North America, particularly in the upper Midwest, should take into consideration the effects of earthworms.

Acknowledgements

We thank the staff at Warner Nature Center for allowing us to collect soil and leaf litter for the experiment. We thank Cindy Buschena and Susan Barrott for their help throughout the experiment. We also thank the undergraduate student workers in the Reich lab for help planting and harvesting the experiment. This project was supported by a grant from the Legislative Citizen Commission on Minnesota Resources (M.L. 2010, Chp. 362, Sec. 2, Subd. 6c "Healthy Forests to Resist Invasion," to PR). Support was also provided by the Integrative Graduate Education and Research Traineeship: Risk Analysis for Introduced Species and Genotypes (NSF DGE-0653827); University of Minnesota Graduate School; the Wilderness Research Foundation; and the Dayton Fund of the Bell Museum of Natural History. NE gratefully acknowledges funding by the Deutsche Forschungsgemeinschaft (DFG; Ei 862/1, Ei 862/2).

Table 1.1. Generalized and general linear model results for the effects of litter, light, earthworms and their interactions on the number of buckthorn seedlings and buckthorn biomass in experimental microcosms.

Response: Buckthorn Seedlings (#)

Source	DF	L-R χ^2	Prob > χ^2
Litter Level	2	9.3445	**
Light Level	2	26.5917	***
Litter Level*Light Level	4	2.5032	0.6441
Earthworms	1	19.9612	***
Litter Level*Earthworms	2	14.5291	***
Light Level*Earthworms	2	10.6430	**
Litter Level*Light Level*Earthworms	4	4.3850	0.3564

Significant effects: *** ($P < 0.001$), ** ($0.001 \leq P < 0.01$), * ($0.01 \leq P < 0.05$). Pearson goodness of fit: χ^2=126.45, df=108, p=0.12

Response: Buckthorn Biomass (g)

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Litter Level	2	2	0.00946050	5.5057	**
Light Level	2	2	0.00369137	2.1483	0.1216
Litter Level*Light Level	4	4	0.00373724	1.0875	0.3665
Earthworms	1	1	0.01416796	16.4906	***
Litter Level*Earthworms	2	2	0.01582325	9.2086	***
Light Level*Earthworms	2	2	0.01105472	6.4335	**
Litter Level*Light Level*Earthworms	4	4	0.00372508	1.0839	0.3692

Significant effects: *** ($P < 0.001$), ** ($0.001 \leq P < 0.01$), * ($0.01 \leq P < 0.05$). Total R^2 = 0.40

Table 1.2. Generalized regression results for the effects of treatments, time, and their interactions on buckthorn germination in experimental microcosms

Source	DF	Wald χ^2	Prob > χ^2
Date	7	272.52	***
Litter	1	4.98	*
Light	1	20.66	***
Earthworms	1	19.92	***
Litter Level*Earthworms	1	30.03	***
Light Level*Earthworms	1	28.15	***
Litter Level*Light Level	1	0.35	0.5527
Date*Litter Level	7	7.59	0.3698
Date*Light Level	7	4.59	0.7095
Date*Earthworms	7	40.05	***

Significant effects: *** ($P < 0.001$), ** ($0.001 \leq P < 0.01$), * ($0.01 \leq P < 0.05$).

Figure 1.1 Effects of light, litter, earthworms, and their interactions on mean number of buckthorn (*Rhamnus cathartica*) seedlings and biomass in experimental microcosms. P-values denote general linear model results. NS, not significant for treatment effect. n=42 for light and litter treatments. n=63 for earthworm treatments. Error bars are standard error

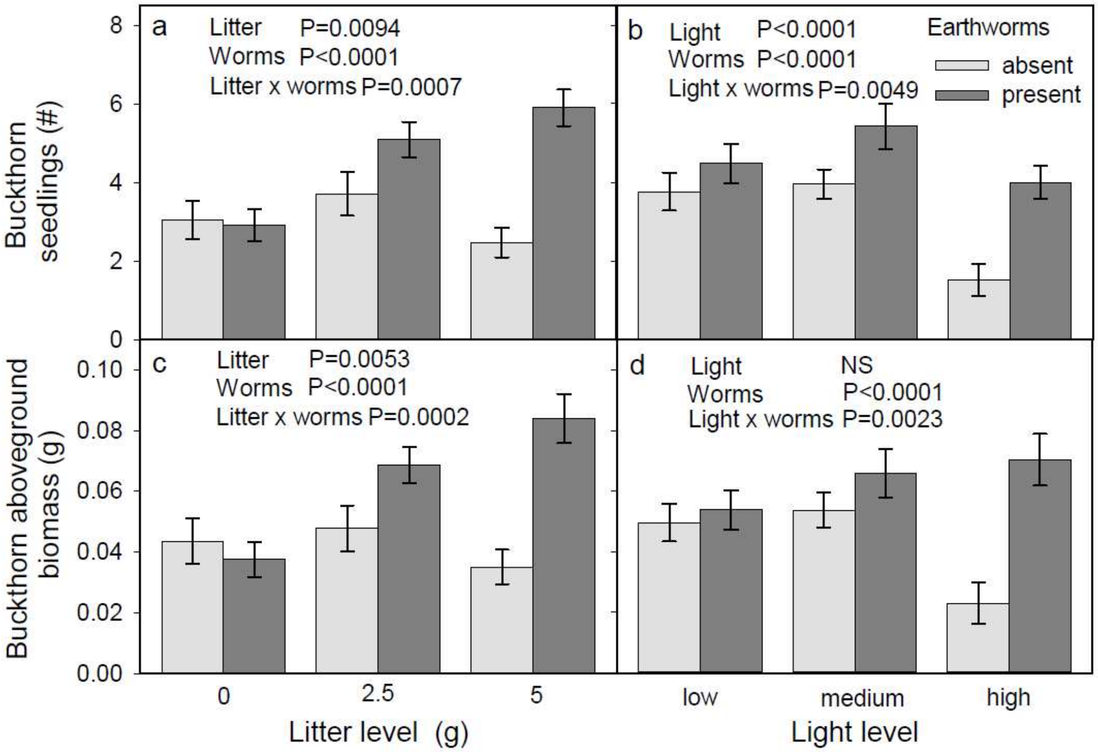


Figure 1.2. Path analysis model displaying causal influences of experimental treatments on buckthorn biomass. Numbers on arrows are standardized path coefficients. Solid lines indicate negative relationships and dashed lines indicate positive relationships. All paths are significant ($P < 0.05$). Circles indicate error terms (E1-E3). The overall model fit the data: Chi-square = 1.45, probability level = 0.84, AIC = 23.45

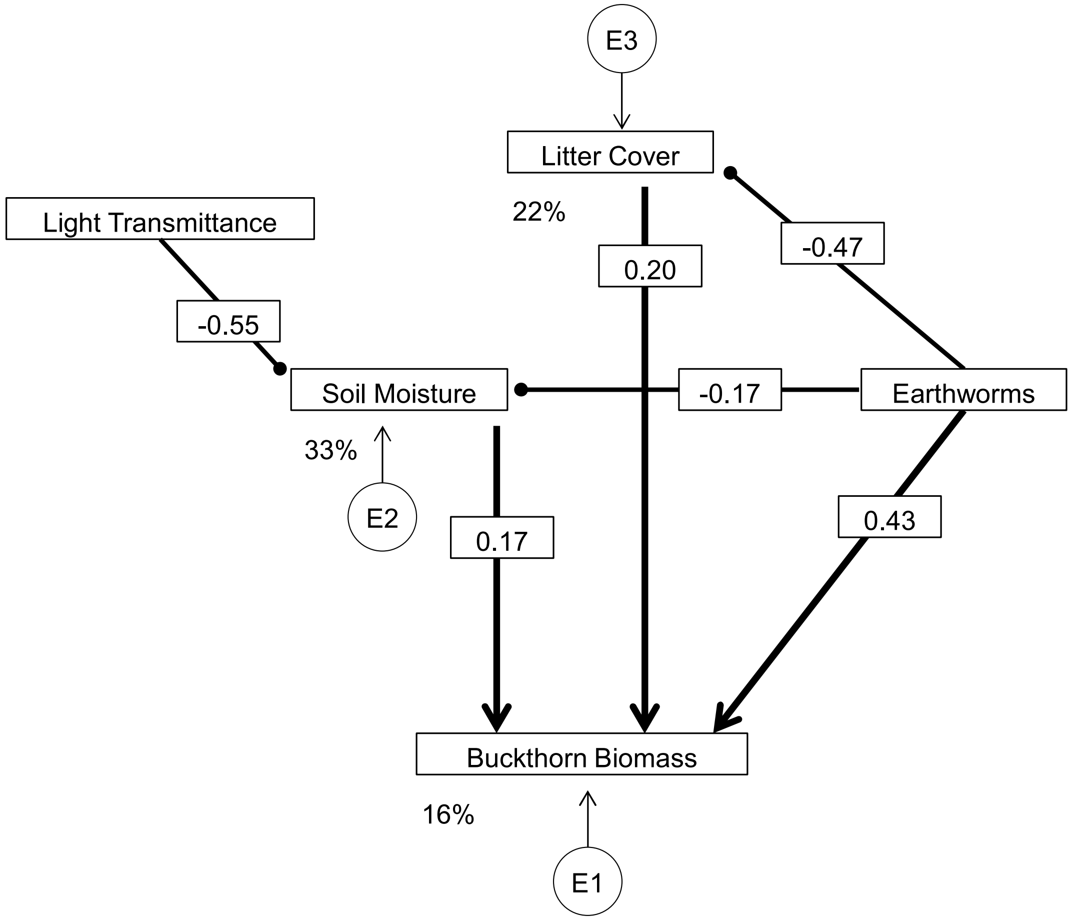
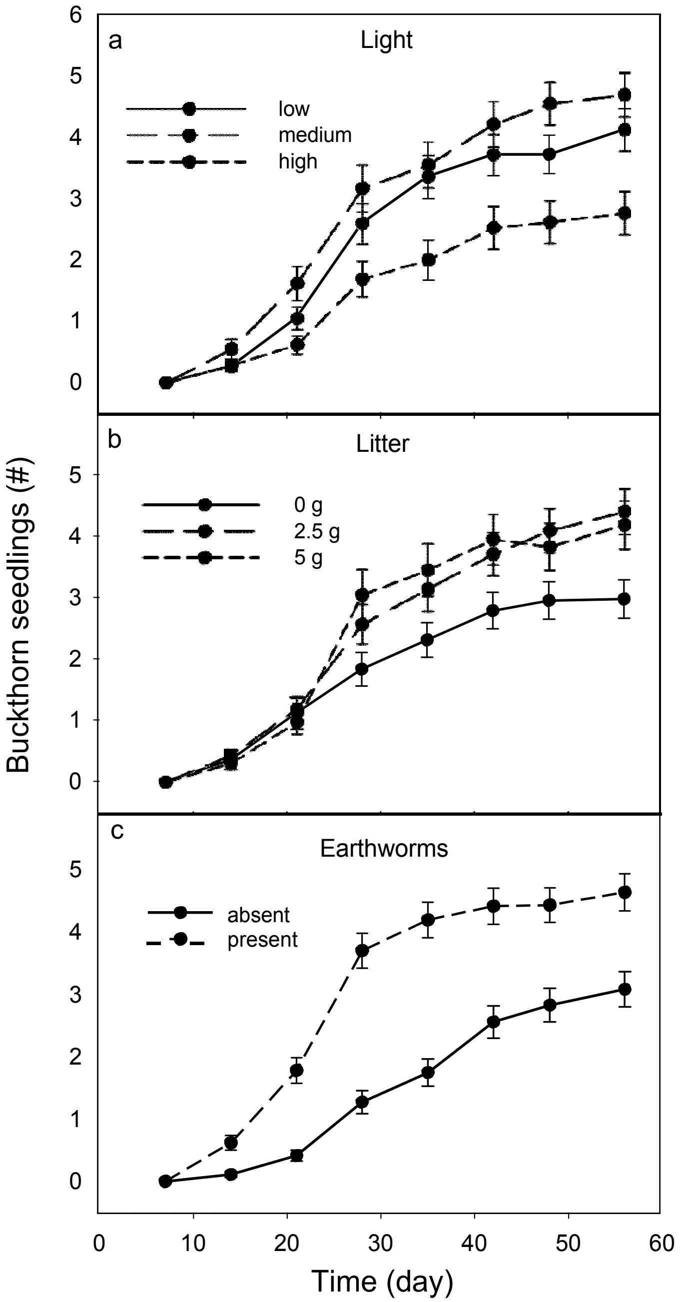


Figure 1.3. Visual depiction of the effects of earthworms, light and litter on the mean number of buckthorn germinants over the duration of the experiment. n=42 for light and litter treatments. n=63 for earthworm treatments. Error bars are standard error



CHAPTER 2

An invasive one-two punch: Inter trophic facilitation by a non-native shrub increases the success of invasive earthworms in a North American temperate forest

Summary

Common buckthorn (*Rhamnus cathartica* L.) is one of the most abundant and ecologically harmful non-native plants in forests of the upper Midwest United States. European earthworms are also invading previously glaciated areas in this region, leading to changes in soil structure, simplifying vegetative communities, and impeding forest regeneration. Together, these organisms are negatively influencing the future health and stability of these forests. Largely anecdotal evidence suggests a potential facilitative relationship between buckthorn and earthworms, though direct evidence for a relationship is sparse. In order to address the hypothesis that buckthorn facilitates earthworms in forests of the upper Midwest US, we sampled earthworm populations across a gradient of buckthorn abundance in a mesic oak forest in east central Minnesota. Over the course of two growing seasons, we examined the relationships between buckthorn abundance, earthworm biomass and other plot characteristics. Earthworm biomass was higher in patches with greater buckthorn cover in two seasons in both years. We found that soil type, soil moisture, and percent cover of large buckthorn – individuals greater than 1.3m tall – had significant positive relationships with earthworm biomass when included in multiple regressions and mixed effects models. Path analyses for each sampling period showed that buckthorn positively influenced earthworm biomass directly and indirectly through its positive effect on soil moisture. We conclude that the presence of buckthorn in deciduous forests of the upper Midwest likely increases the success of European earthworms. Coupled with evidence from previous studies demonstrating the facilitation of buckthorn by earthworms, these results support the hypothesis of co-facilitation

between the two species. Furthermore, researchers and managers should consider co-invasion of introduced plants and earthworms when investigating invasibility and creating preemptive or post-invasion management plans.

Introduction

In the last few decades, biological invasions have received increasing attention as researchers gain a better understanding of the ecological impacts of invasion (Vila et al. 2011; Ricciardi et al. 2013). While it is still debated whether invasive species are a significant cause of human-mediated species extinction (Heneghan et al. 2007; Gilbert and Levine 2013; but see Davis 2011), they can decrease the abundance of native species (Huenneke et al. 1990), alter ecosystem processes and functioning (Vitousek 1990; Lodge 1993), and cost countries billions of dollars in prevention and control measures (Pimentel et al. 2005). Northern temperate forests are particularly at risk as invasive species continue to spread, and invaders have led to large declines in producer and endotherm species richness and ecosystem function (Murphy and Romanuk 2014).

The question remains: how have invasive species become so successful in so many parts of the world? Increased human activity has no doubt played a large role in their spread, but there are many other theories that attempt to explain invasive success once they arrive, from enemy release and the evolution of increased competitive ability (EICA), to facilitation by other invaders (Thuiller et al. 2006; Gavier-Pizarro et al. 2010; Lamarque et al. 2011; Flory and Bauer 2014). Despite some evidence underlying these hypotheses, a universal theory of invasive species success remains elusive (Eschtruth and

Battles 2009; but see Fridley and Sax 2014). In recent years, there has been growing attention given to the role of facilitation in ecological systems, where one species benefits directly or indirectly from another (Brooker et al. 2008). Until recently, facilitation had been a common but under-appreciated concept in invasion biology (Simberloff and Von Holle 1999), but it has recently been implicated in the success of certain invasive species (Heimpel et al. 2010; Flory and Bauer 2014; Travaset and Richardson 2014). Here we investigate facilitation between two invasive species – common buckthorn (*Rhamnus cathartica L.*) and European earthworms – in forests of the upper Midwest United States.

Common buckthorn is a tall shrub or small tree native to Europe and Asia, and was introduced into the U.S. as an ornamental and medicinal plant before the turn of the 18th century (Kurylo and Endress 2012). Buckthorn has since spread throughout many forested areas, where it can form dense monospecific stands (Knight et al. 2007) and has myriad effects on soils (Heneghan et al. 2006), flora (Knight et al. 2007; Klionsky et al. 2011) and fauna (Schmidt and Whelan 1999; Sacerdote and King 2014). In the previously glaciated forests of northern North America, European earthworms have invaded many of the same areas. Originally transported to the US with European settlement, earthworms continue to spread, largely due to their use as bait for recreational fishing (Hale et al. 2005). In newly invaded areas, earthworm cause a variety of changes to the soil and litter layer, negatively affecting many species of flora and fauna (Maerz et al. 2009; Loss and Blair 2011, and see a review in Frelich et al. 2006). These effects may compound the effects of buckthorn (Knight et al. 2007; Heneghan et al. 2007), leading to severely degraded systems (Frelich and Reich 2010).

Anecdotal evidence suggests that invasive earthworms may facilitate the invasion of buckthorn (Heneghan et al. 2007), and recent experimental evidence has added support to this hypothesis (Whitfeld et al. 2014; Roth et al. 2014). In mesic, deciduous forests, earthworms rapidly consume leaf litter during the growing season, creating abundant bare soil on which many invasive species – including buckthorn – preferentially germinate (Heneghan et al. 2007; Knight et al. 2007). The bare soil may give an advantage to invaders over native species adapted to the thick litter and duff layers historically present in previously glaciated northern forests (Gundale 2002; Hale et al. 2006). Earthworms also increase decomposition and can provide pulses of nutrients (Heimpel et al. 2010; Heneghan et al. 2006; Hale et al. 2008) that some invaders are able to exploit more effectively than native plants (Huenneke et al. 1990; Davis et al. 2000; Gilliam 2006; Funk and Vitousek 2007). Thus, the presence of earthworms may aid in the germination and growth of buckthorn seedlings. Field observations (Nuzzo et al. 2009) suggest that earthworms are correlated with invasive species presence and native plant decline in northeastern US forests. However, direct causal evidence for this relationship is difficult to find.

There is also sparse evidence for the facilitation of earthworms by buckthorn. It has been proposed that buckthorn provides conditions conducive to European earthworms by providing nutrient-rich leaf litter and creating high-shade conditions that cool soils (Heneghan et al. 2007; Holdsworth et al. 2008; Heimpel et al. 2010). Furthermore, Madritch and Lindroth (2009) observed that removing buckthorn temporarily reduced earthworm populations by as much as 50%, suggesting a link between the two species.

However, earthworm populations began to recover just months after removal. Buckthorn and earthworms have also been implicated in a complex "invasional meltdown" in North American forests (Heimpel et al. 2010) that catalyzes a facilitative chain reaction passing from one invasive species to another. In such invasional meltdowns, the reciprocal facilitation by each species can cause greater abundance of both species than would have otherwise occurred, increasing each species' ability to survive and impact the surrounding community (Simberloff and Von Holle 1999).

Given the paucity of evidence for facilitation between invasive species, let alone at different trophic levels, here we test the hypothesis that there is a positive relationship between common buckthorn and European earthworms in deciduous forests of Minnesota. We collected buckthorn and earthworm abundance data from across a local gradient of buckthorn abundance in a mesic oak forest in east central Minnesota, USA. Over the course of two growing seasons, we examined the relationships between buckthorn abundance, earthworm biomass and other plot characteristics. Specifically, we propose that common buckthorn increases the success of European earthworms in our forest system, and, coupled with recent evidence supporting facilitation in the reverse direction (Whitfeld et al. 2014; Roth et al. 2014), we propose that co-facilitation may be aiding the success of both species in forests of the upper Midwest.

Methods

Plot set-up

In the spring of 2012, we established 24 plots situated along two naturally occurring gradients of buckthorn abundance in mixed oak-maple forests at Warner Nature Center in Marine on St. Croix, MN (45.172830°, -92.832019°). Average yearly precipitation at the site is 71-91 cm and mean annual temperature is 6.1-7.8 °C. Twelve plots were established in an area with soils consisting of 40-50% sand, 20% clay and 20% silt ("sandy plots") and twelve in an area with soils consisting of 40% clay, 40% silt, and 20% sand ("silty plots"). The dominant overstory species by basal area in both soil types were white oak (*Quercus alba*), red oak (*Q. rubra*), and red maple (*Acer rubrum*), respectively. Buckthorn was the dominant shrub species, with chokecherry (*Prunus virginiana*), gooseberry (*Ribes cynosbati*), and nannyberry (*Viburnum lentago*) composing a smaller proportion of the shrub layer. Within each soil category, plots were located across a gradient of buckthorn abundance as measured by buckthorn percent cover. This naturally occurring gradient is caused by an invasion front as buckthorn has been spreading across the site for the past 30 years (Ron Lawrenz, personal communication). Plots differed widely in the amount of buckthorn present; buckthorn cover ranged from zero to 80%. While the average basal area fraction was low (2.6%), the proportion of buckthorn stems was much higher. Buckthorn accounted for an average of 57.6% of all woody stems in the 24 plots (0 to 93%). When only the shrub layer is considered, buckthorn accounted for an average of 97% of the stems and 85% of the basal area, illustrating its dominance of the understory woody vegetation. Sampling plots were concentric circles of three and twelve meter diameters. The three meter circle was divided into six equal wedges, each with an area of 1.78m^2 (Fig. 1). Prior to earthworm sampling, we documented the abundance and composition of the herbaceous and low shrub (<1.3 m tall) communities within each 3 m plot and took soil cores to characterize the soil types and determine soil moisture and texture. Species were identified according to the Flora of North America (Flora of North America Editorial Committee 1993) and Gleason and Cronquist (1991). In the lab, we used the hydrometer method to determine soil texture (Gee and Bauder 1986). Within the 6 m plot, we identified and measured all woody plants >1.3 m tall and estimated each species' percent cover. We established two litter traps per plot, one at the plot center and one just outside the 3m ring, to obtain an estimate of each species' contribution to total litterfall. Traps consisted of large planter buckets (top diameter = 48.3 cm, height = 41.9 cm) staked into the ground. Beginning September 7, 2012, litter was collected from these traps once every two weeks until December 3rd and dried at 70°C for three days in the lab before being sorted to species and weighed.

Earthworm sampling

Earthworm sampling occurred in July and November (before the first frost) of 2012, and July and September of 2013. Sampling dates were chosen to ensure a representative sample of earthworm populations at different points in the growing season. The July samples captured mid-summer earthworm activity while ensuring that enough adult earthworms were present to allow for species identification. In Minnesota, peak earthworm abundance and maturity usually occurs in September, and sampling during this time was designed to capture the peak earthworm activity during the growing season (Hale et al. 2005). However, in 2012, the second sampling date was delayed due to an extended drought that reduced earthworm activity; sampling occurred in early November after rains returned soil moisture to normal levels. Drought can kill many litter-dwelling (epigeic) earthworm species and cause soil-dwelling (endogeic) and burrowing (anecic) species to estivate deep in the soil; thus, sampling during a drought will underrepresent true earthworm abundances (Hale et al. 2005). At each of the 24 plots, earthworms were sampled using two different methods. First, earthworm extractions were conducted using a liquid mustard solution (Lawrence and Bowers 2002). During each sampling period, extractions were performed at two locations in each plot. These extraction locations were determined by randomly assigning earthworm sample plots to one of three available areas in each worm sampling wedge (Fig. 1). At each location, a 35cm by 35cm metal frame was placed on the ground and a solution of 40 g ground yellow mustard powder dissolved in four liters of water was slowly poured inside the frame. The solution was mixed just prior to application, and applied one-third at a time. After five minutes, or when earthworm activity had slowed, more solution was poured. Each sampling period lasted roughly fifteen minutes. As earthworms emerged, they were collected and placed in containers containing 95% ethanol.

Data analysis

Data were analyzed using JMP ver. 9.0.2 (SAS Institute, Inc., Cary, NC, USA) and R ver. 3.0.1 (R Core Development Team 2014). Earthworm ash-free dry biomass was estimated from earthworm lengths using the allometric regression equations developed by Hale et al. (2004). When necessary, data were log transformed to fit the assumptions of normality. We separated buckthorn cover into large (height >1.3m) and small (height <1.3m) classes and used large buckthorn cover for the bulk of our analyses. We assume that large buckthorn have been present on the site long enough to affect earthworm populations through shade, moisture, and provision of leaf litter, while small buckthorn stems – including newly emerged seedlings – are unlikely to measurably affect the physical environment experienced by earthworms. Total *Lumbricus* spp. (*L. terrestris* and *L. rubellus*) biomass was used as the response variable for the analyses; both species feed on leaf litter and organic matter and are associated with a rapid loss of the forest floor in areas where they invade. Therefore, we assume that the proposed mechanism of buckthorn facilitation associated with *L. terrestris* – provision of high quality leaf litter – applies to *L. rubellus* as well (Hale et al. 2006; Hale et al 2008; Greiner et al. 2012), though it does not apply as readily to the other species collected in this study. To investigate whether all earthworms were affected similarly, we also ran our analyses using the genera *Dendrobaena* and *Aporrectodea*. The results of these analyses included both weakly positive and negative relationships, though none were ultimately significant. We used simple analysis of variance (ANOVA) and regression to compare earthworm number, biomass and species composition between soil types and across the buckthorn abundance gradient. Tukey's HSD test was used to make post-hoc comparisons of means.

Ordinary least squares regressions were used to investigate the relationships between buckthorn percent cover and the earthworm variables. When heteroscedasticity of the residuals was encountered, we compared the regression results to those obtained using generalized linear regression conducted in R 3.0.1 (R Development Core Team 2013). Generalized linear regression is robust to heteroscedasticity, and comparable results from both methods lend support to the validity of the ordinary least squares results.

We also created separate general linear models to explain earthworm biomass in each sampling period. Potential explanatory variables encompassed a variety of soil and vegetation variables. Soil variables included textural measures, as well as soil nutrients, moisture and pH. Vegetative variables included cover of herbaceous and woody species, as well as basal area, stem counts and leaf litter mass of woody species. When collinearity was detected, we selected the most ecologically relevant variable from the correlated pair. The best-fit model was selected from all possible models containing the candidate variables using AIC criteria; ecologically relevant two-way interactions between significant predictor variables were also investigated as potential predictor variables in the models. In order to examine all sampling periods together, we used a univariate mixed effects analysis to examine the effect of sampling period and the aforementioned plot characteristics on earthworm biomass. Earthworm biomass data were stacked across the four sampling periods in order to incorporate time into the model, and plot was included as a random effect. Finally, in order to explore the direct and indirect relationships between buckthorn abundance, earthworm biomass, and related environmental variables, we performed path analyses using AMOS 5 (Amos

Development Corporation, Crawfordville, FL, USA). Using only paths that were ecologically relevant, we constructed an overall model incorporating time, as well as one separate model for each sampling period. Initial explanatory variables included all those selected for use in our linear models. Soil moisture, large buckthorn percent cover, and maple litter were expected to positively affect earthworm biomass, while low pH, overstory basal area, and oak litter were expected to negatively affect earthworm biomass. Earthworms have been shown to prefer the leaf litter of species like maple over that of oak due to higher nutrient contents and lower structural compounds and tannins (Holdsworth et al. 2008; 2012). The overall model was based on prior knowledge and the previous analyses, and the separate sampling period models were constructed based on the overall model. Buckthorn abundance and environmental conditions served as exogenous variables, and earthworm biomass was the response, or endogenous, variable. We used non-significant chi-squared tests ($p>0.05$) and AICc scores (Grace 2006; Arbuckle 2012) to determine the model fit, and used the specification search feature to select the best model using model fit criteria. When necessary, Bollen-Stine bootstrapping was used to account for non-normality in the data (Bollen and Stine 1993).

Results

Extent of buckthorn invasion

Buckthorn averaged 4.4% of total leaf litter weight, and accounted for between zero and 24% of the leaf litter in these plots. In plots where buckthorn had greater than 50% cover, buckthorn made up an average of only 6.8% of the total basal area, but accounted for 9.5% of the total leaf litter collected in our traps. Moreover, much of the buckthorn in these plots was <1.3m in height, meaning that our litter traps (0.42 m tall) likely underestimated the buckthorn leaf litter inputs in our plots. The timing of buckthorn litterfall also differed from the other woody species: maples had dropped the majority of their leaves by October 18th, oaks by November 1st, and buckthorn by November 14th. Large buckthorn was not correlated with any of the measured soil variables (pH, sand, moisture etc.). However, small buckthorn did have a positive correlation with soil moisture ($F_{(1,22)}$ = 7.48, p=0.0121). There was also, as expected, a significant, positive relationship between small buckthorn and large buckthorn in our plots. Plots with higher large buckthorn percent cover had significantly higher small buckthorn stem counts ($F_{(1,22)}$ = 16.41, p=0.0005), basal area ($F_{(1,22)}$ = 8.45, p=0.0082), and percent cover ($F_{(1,22)}$ = 11.4, p=0.0028).

Extent of earthworm invasion

In total we collected 4,683 earthworms, with abundances ranging from one to 139 in individual plots during a given sampling period. Across sampling periods, we collected the fewest worms in November 2012 (325) and the most in September 2013 (1704). Individual plots ranged in species richness from one to five species and contained earthworms from up to three different genera. Soil type significantly affected earthworm abundance and richness; plots in the silty soil group had greater earthworm abundance in all four sampling periods (Table 1). However, the total earthworm biomass did not differ between soil types due to the abundance of larger *L. terrestris* in the sandy soils. Species richness was significantly higher in silty than sandy plots in July 2012, but the difference was marginally significant or non-significant for the remaining sampling periods. At the genus level, richness was significantly higher in the silty plots in three of four sampling periods, with only marginal significance in July 2013 (Table 1). Simple ANOVA results also demonstrated that there was a significant difference between total earthworm biomass across sampling periods; in November 2012, earthworm biomass was significantly lower than during the other sampling periods ($F_{(3,89)}$ = 5.56, p=0.0015).

Buckthorn-earthworm relationships

Simple linear regressions showed that during each of the four sampling periods there was a significant, positive relationship between large buckthorn percent cover and the total biomass of *Lumbricus* species (Fig 2). The amount of variance in earthworm biomass explained by this relationship differed for each sampling period, ranging from an R^2 of 0.15 in the fall of 2013 to an R^2 of 0.30 in the fall of 2012 (Fig 2).

Our best fit mixed effects model established that sampling period, large buckthorn percent cover and soil moisture were significant predictors of earthworm biomass throughout the entire experiment. Using sampling period as a fixed effect and plot as a random effect, this model was able to explain 66 percent of the variation in earthworm biomass (Table 2). These relationships were also supported by running separate multiple regression models for each sampling date. Buckthorn percent cover was a significant, positive predictor of earthworm biomass during all four sampling periods (Fig 3). No other predictor was significant in all four models, though soil moisture was significant in three of the four. Two of the models also included significant interactions between maple leaf litter and cover of woody species other than buckthorn, and one included the interaction between buckthorn cover and maple litter.

Path analyses supported these results and highlighted both direct and indirect mechanisms driving the observed relationship between large buckthorn cover and earthworms. The initial overall model incorporating sampling period fit the data well, but could be improved (χ^2_3 = 0.54, p=0.91; AICc=24.54; Supplementary material). The final model improved the fit (χ^2_3 = 0.95, p=0.97; AICc=20.95) and fit better than both the saturated and independence models (NFI = 0.979; CMIN = 0.949; RMSEA = 0), with specification search criteria determining the best fit final model. Large buckthorn cover and soil moisture had significant, direct positive effects on earthworm biomass (Fig 4). Sampling period had a marginally significant indirect effect on earthworm biomass through its effect on soil moisture. In this case, the positive effect of buckthorn on soil moisture was not significant, hinting at the importance of buckthorn's direct effect on earthworm biomass in this system. These effects were supported by our independent models for each sampling period (e.g., November 2012: χ^2_3 = 0.44, p=0.93; AICc=22.44). Large buckthorn percent cover had a significant, positive effect on earthworm biomass in all four sampling periods; its effect was direct in three out of four sampling periods and was indirect – through soil moisture – in two out of the four sampling periods (Supplementary Material). Soil moisture had a direct positive effect on earthworm biomass in all four sampling periods, with wetter sites supporting more earthworm biomass (Supplementary Material).

In contrast, there were few significant relationships between small buckthorn (stems <1.3m) and earthworm biomass in the four sampling periods. Small buckthorn percent cover was only significantly positively correlated with earthworm biomass in July of 2013, and the number of newly emerging buckthorn seedlings was only significantly positively correlated with the biomass of *L. terrestris* in November of 2012. Separate multiple regression models also investigated whether earthworm biomass was a predictor of small buckthorn abundance in each of the four sampling periods; however, earthworm biomass was not significant in any of the models.

Discussion

Common buckthorn and European earthworms are arguably two of the most abundant and harmful invaders in forests of the upper Midwest United States (Bohlen et al. 2004; Frelich et al. 2006; Hale et al. 2006; Heneghan et al. 2006; Knight et al. 2007; Heimpel et al. 2010; Larson et al. 2010; Invasive Species Program 2012) and facilitation may play an important part in the success of these two species (Heimpel et al. 2010). The aim of this observational field study was to investigate the potential relationship between the two organisms. We provide novel evidence to support the prediction that buckthorn increases the success of earthworms in deciduous forest systems, especially during later stages of earthworm invasion where *Lumbricus* species dominate. Coupled with previous evidence for the facilitation of buckthorn by earthworms, these results support the hypothesis that the two species engage in co-facilitation in forests of the upper Midwest United States.

Some previous evidence exists for the facilitation of buckthorn by earthworms, but until recently that evidence has been largely anecdotal. For example, earthworms are known to rapidly consume forest floor litter and duff layers, providing bare soil on which invaders like buckthorn have been shown to preferentially germinate (Bisikwa 2005; Heneghan et al. 2007; Knight et al. 2007). Earthworms also quickly recycle nutrients and make them available to invaders like buckthorn, potentially aiding buckthorn growth (Heimpel et al. 2010). Recent experimental evidence shows that buckthorn realizes higher abundance and biomass in the presence of earthworms, regardless of light or litter conditions, providing important support for the facilitation of buckthorn by earthworms (Whitfeld et al. 2014; Roth et al. 2014).

The current study provides novel evidence for facilitation in the reverse direction. Earthworms have previously been shown to be more abundant in areas of high buckthorn density versus in buckthorn-free oak woodlands (Heneghan et al. 2006), and more concrete evidence to support this facilitation comes from Madritch and Lindroth (2009), who showed that removal of buckthorn aboveground biomass led to large declines – albeit temporary – in the earthworm population. Across our buckthorn gradient, areas with greater buckthorn percent cover had higher earthworm biomass in all four sampling periods. Moreover, there was a three-fold difference in mean *Lumbricus* spp. biomass across the range of buckthorn abundance (0-80% cover) in our plots.

On the surface this evidence is correlational and cannot fully support the proposed directionality of the relationship. However, by including these and other variables in mixed and multiple regression models, we can better establish whether buckthorn is an important predictor of earthworm biomass. Our multiple regression models showed that large buckthorn abundance was important in explaining earthworm biomass in all four sampling periods. Soil moisture was another important variable in three of these models, suggesting that both species may prefer moister areas. This is supported by previous studies that show that buckthorn is able to tolerate a wide range of moisture conditions (Stuart and Graves 2004) while earthworms have been shown to prefer moister soils (Frelich et al. 2006). We also collected the lowest number and biomass of earthworms in our November 2012 sampling, which was at the end of a long drought. Low soil moisture negatively affects survival of epigeic and endogeic earthworm species that depend on moist litter and mineral and organic soil layers, and can force anecic species to deeper depths and negatively affect their survival (Hale et al. 2005; Frelich et al. 2006). We recognize that environmental conditions such as soil moisture may jointly influence the distribution of these two species, but have done our best to control for these variables in our models.

Even controlling for soil moisture, the influence of large buckthorn on earthworms was significant in each sampling period. Furthermore by including plot as a random variable, our mixed model accounted for any potential variation in earthworm biomass due to unmeasured plot differences. Buckthorn remained significant in this model, lending further support to the proposed directionality of the relationship.

Two of our multiple regression models also included significant interactions between woody species cover and maple litter. These estimates were negative, suggesting that at higher levels of maple litter, the effect of woody cover becomes more negative, potentially due to the increase in stems and roots, leaving less area for earthworms to freely access. The November 2012 model also included a significant positive interaction between large buckthorn cover and maple litter. This may be due to the simple fact that in areas of high maple litter where earthworms are already doing well, an increase in buckthorn abundance – and leaf litter – increases the amount of palatable food and the overall palatability of the litter mixture (Heneghan et al. 2002; 2007).

To better explore the directional relationships between large buckthorn, earthworms and soil moisture, we generated path analysis models based on our multiple regression models. Path analysis tests direct and indirect relationships between variables to help establish causality (Grace 2006). Our path analyses established that buckthorn cover had both direct and indirect positive effects on earthworm biomass. Buckthorn's direct positive effect on earthworms was significant in the overall model and in three of four separate sampling period models. While direct paths are unexplained, a likely mechanism may be buckthorn's provision of high quality leaf litter as a food source for earthworms (Heneghan et al. 2007; Heimpel et al. 2010). Earthworms prefer buckthorn leaf litter over that of many other species, as buckthorn has high calcium and nitrogen and is low in tannins and other hard to digest compounds (Heneghan et al. 2007; Holdsworth et al. 2008; Heimpel et al. 2010; Holdsworth et al. 2012). On average, buckthorn litter in our plots had 60% and 64% higher nitrogen and calcium concentrations, respectively, than maple leaf litter, the species with the next highest values (unpublished data). That buckthorn litter makes up a higher percentage of total leaf litter relative to its basal area in our plots supports this hypothesis. In plots where buckthorn leaf litter accounts for a larger proportion of the total leaf litter, earthworms may benefit from this abundant, nutritious food source and reach higher population sizes and biomass.

We also found that buckthorn had a positive indirect effect on earthworms by increasing soil moisture in the July 2012 and September 2013 sampling periods, although this path was not significant in the overall model. This supports results from our previous analyses and provides more mechanistic evidence to support the hypothesis that buckthorn increases the success of earthworms in our study site. Buckthorn has been shown to shade and cool soils (Heneghan et al. 2007; Heimpel et al. 2010), and may prevent soil desiccation enough to benefit earthworm populations in the surrounding soils. While we did not assess soil temperature in our study, it may nevertheless help explain the observed relationships. While buckthorn's positive effect on soil moisture was only significant in two of the four sampling periods, larger changes in overall soil moisture may have muted the positive effect of buckthorn on soil moisture throughout this study.

Conclusions

Our results suggest that common buckthorn likely increases the success of European earthworms in forests of the upper Midwest. Together with previous experimental evidence for facilitation of buckthorn establishment by earthworms (Whitfeld et al. 2013; Roth et al. 2014), these new data lend support to the hypothesis of co-facilitation between the two organisms. While we did not find evidence of facilitation of buckthorn by earthworms in our site, this may be due in part to our study design. This observational study was designed to investigate buckthorn's effects on earthworms using a naturally occurring buckthorn gradient. There was no leading edge of earthworm invasion at our site, so any effects of earthworms on buckthorn establishment may have been occluded. Where earthworms have been implicated in buckthorn success, this may be due in part to earthworms' ability to remove leaf litter and create bare soil on which buckthorn has been shown to preferentially germinate (Heneghan et al. 2007; Knight et al. 2007). In our site, there was little large-scale variation in litter availability, as each plot at the site was located in an area of heavy earthworm invasion (IERAT = 5; Loss et al. 2013). Co-facilitation may bolster populations of both common buckthorn and European earthworms in forests of the upper Midwest. It may also increase the success of both species and strengthen their negative impacts on native species and forest ecosystems (Simberloff and Von Holle 1999). Along with the effects of climate change and deer (Frelich and Reich 2010; Fisichelli et al. 2013), buckthorn and earthworms may negatively influence forest regeneration and have the potential to accelerate a fundamental change to the structure of Midwest forests (Frelich and Reich 2009; Eisenhauer et al. 2011).

Management of buckthorn will be necessary to diminish its effects on forests, while preventing the movement of both organisms – and especially earthworms – into new areas should remain a top priority. Future experimental research is needed to further elucidate the mechanisms behind this facilitative relationship; manipulation of buckthorn and earthworm populations in the field (sensu Madritch and Lindroth 2009) could help solidify the link between the two organisms, and in situ earthworm feeding trials with buckthorn leaf litter could provide more concrete mechanistic evidence to explain the relationship. Researchers and managers should also consider co-invasion of plants and earthworms when investigating invasibility and creating preemptive or post-invasion management plans.

Acknowledgements

We thank the staff at Warner Nature Center for allowing us to conduct research on their property. We also thank the graduate students and undergraduate student workers in the Reich lab for help with earthworm sampling and processing. Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR). Support was also provided by the Integrative Graduate Education and Research Traineeship: Risk Analysis for Introduced Species and Genotypes (NSF DGE-0653827); University of Minnesota Graduate School; the Wilderness Research Foundation; the Dayton Fund of the Bell Museum of Natural History; and the Wood-Rill Fellowship.

Table 2.1. Simple ANOVA results for the effects of soil type (silty vs. sandy) on earthworm abundance, species and genus richness in field plots.

		Soil Type					
		Silty		Sandy			
	Sampling Period	Mean # / Biomass	SE	Mean # / Biomass	SE	F Ratio	Prob > F
Abundance	July 2012	83.08	5.63	25.58	5.63	52.2425	<0.0001*
	November 2012	21.58	7.63	5.42	3.78	43.2417	<0.0001*
	July 2013	77.17	17.9	35.33	14.9	37.7308	<0.0001*
	September 2013	89.42	20.4	50.83	15.8	26.9016	<0.0001*
Species Richness	July 2012	3.42	0.24	2.58	0.24	5.7895	0.025*
	November 2012	2.33	0.78	1.75	0.87	3.0112	0.0967
	July 2013	3.75	0.87	3.42	0.9	0.8544	0.3653
	September 2013	3.42	0.51	3.17	0.58	1.2532	0.275
Genus Richness	July 2012	2.92	0.16	2.25	0.16	8.1860	0.0091*
	November 2012	2.25	0.75	1.58	0.67	5.2537	0.0318*
	July 2013	3.0	0.0	2.75	0.45	3.6667	0.0686
	September 2013	3.0	0.0	2.58	0.51	7.8571	0.0104*
Biomass (g)	July 2012	1.1	0.45	0.98	0.68	.2674	0.6102
	November 2012	0.5	0.29	0.38	0.38	0.7055	0.4100
	July 2013	1.86	0.75	1.58	0.57	1.0563	0.3152
	September 2013	3.02	1.14	2.89	1.35	0.0595	0.8096

Table 2.2. Linear mixed effects model results showing predictors of earthworm biomass throughout the experiment. Model $R^2 = 0.66$

Variable	DFDen	F Ratio	Prob > F
Buckthorn Percent Cover	19.5	5.5239	0.030*
Soil Moisture	20.28	12.5018	0.002**
Sampling Period	66.56	21.35	<0.0001***
Woody Cover	18.55	3.87	0.064
Maple Litter	18.63	3.30	0.085

Figure 2.1. Schematic diagram of one of the 24 sampling plots. Empty wedges were used for a separate study.

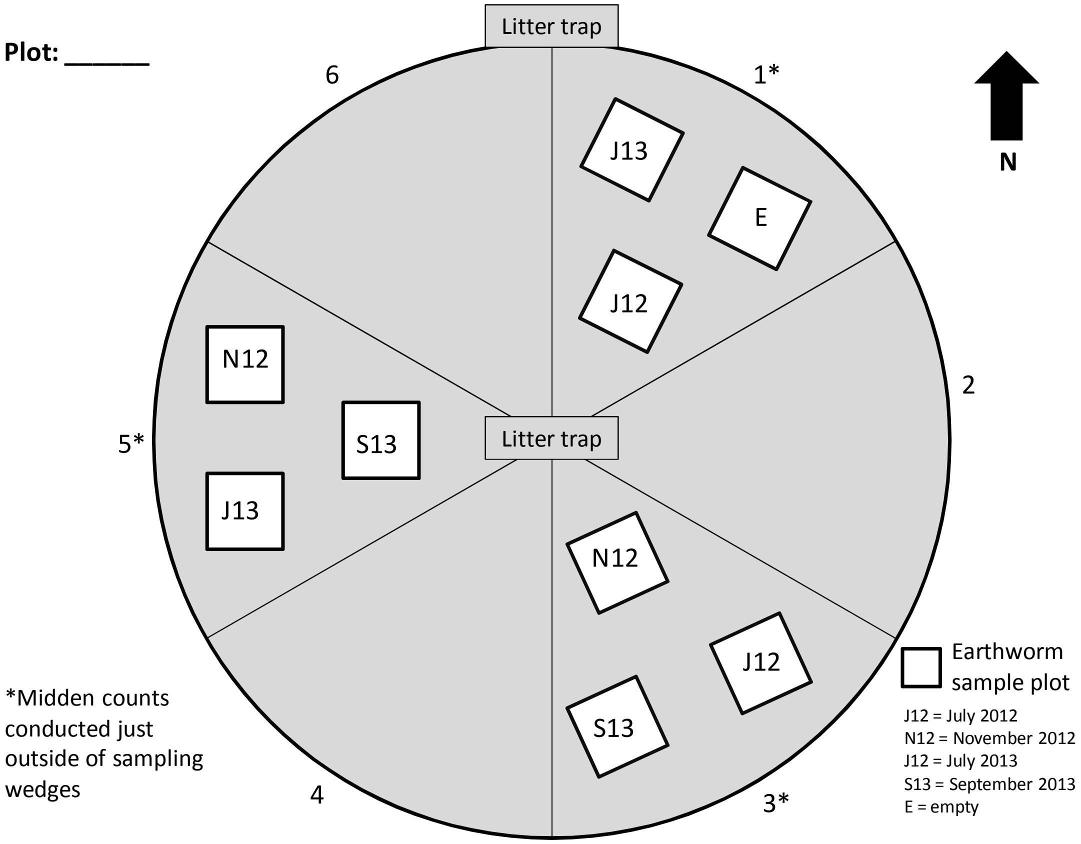


Figure 2.2. Simple linear regressions depicting the relationship between buckthorn percent cover and earthworm biomass for each sampling period. A) July 2012 B) November 2012 C) July 2013 D) September 2013.

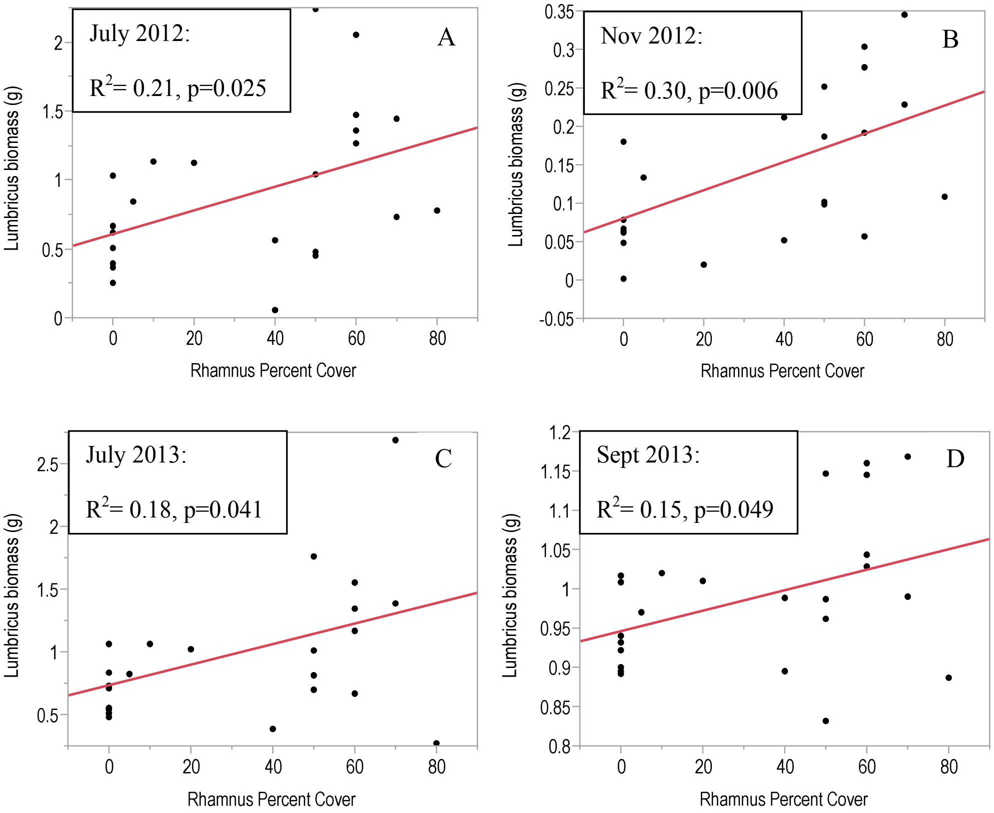


Figure 2.3. Effect sizes for buckthorn when included in separate multiple regression models explaining earthworm biomass in each sampling period. All bars are significantly different from zero. Error bars are standard errors.

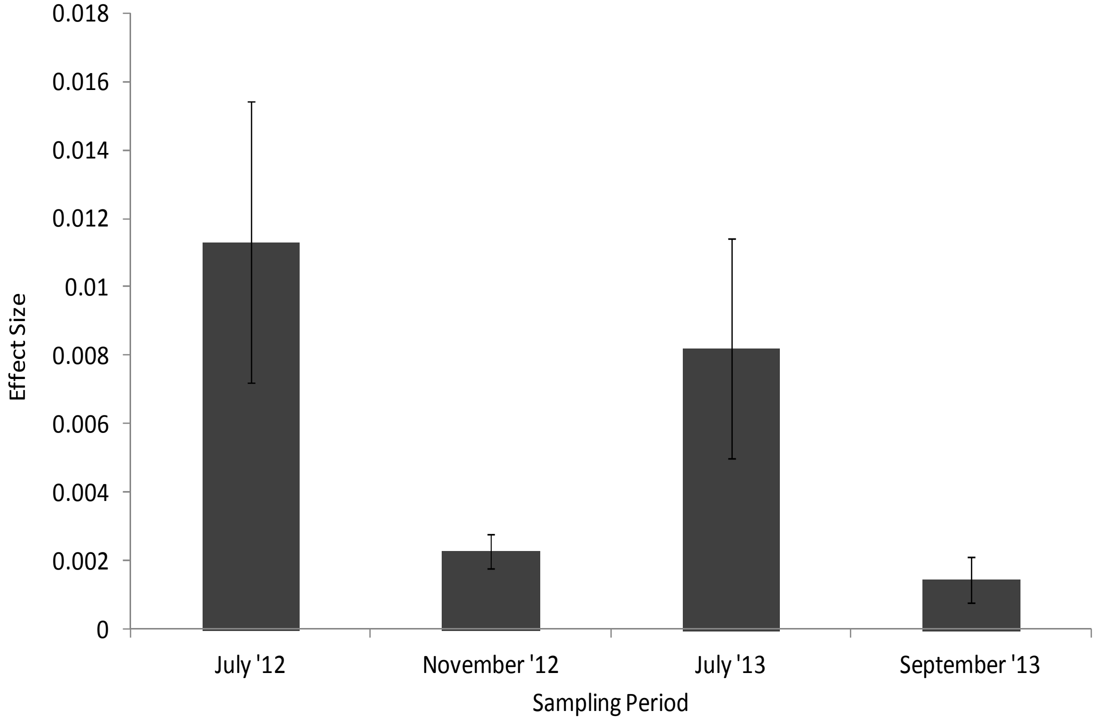
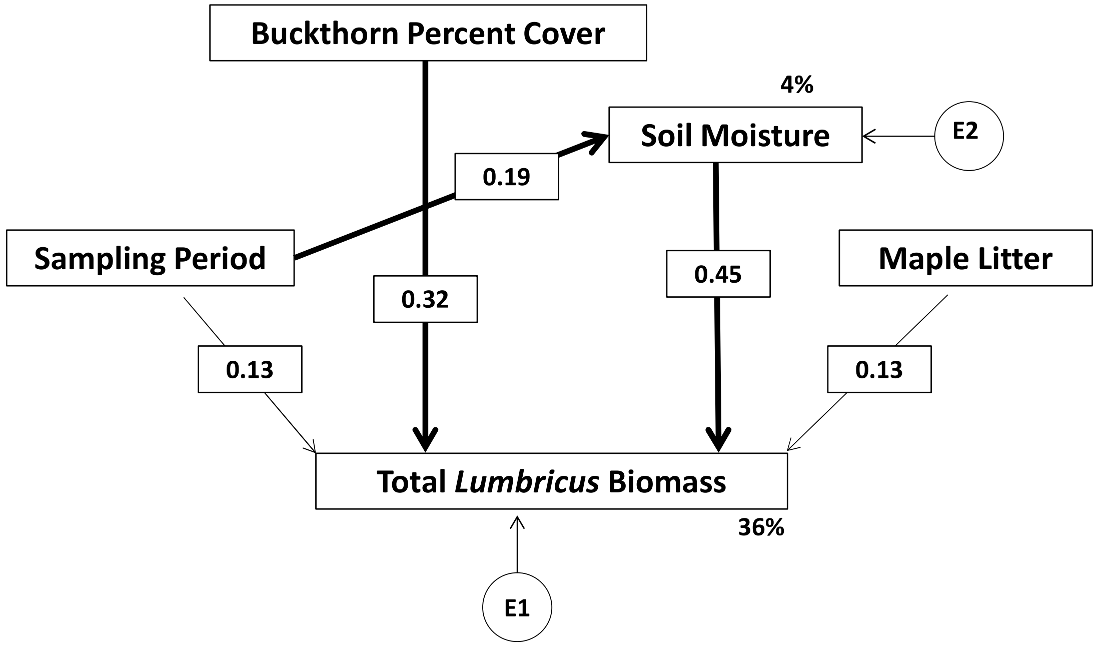


Figure 2.4. Path analysis model displaying causal influences of buckthorn and other environmental variables on earthworm biomass for all sampling periods. Separate models for each sampling period are included in the supplementary material. Numbers on arrows are standardized path coefficients. Lines ending in arrows indicate positive relationships. Bold lines are significant (P < 0.05), while non-bolded lines are not significant (0.062<P<0.162). Circles indicate error terms (E1-E2). The final model fit the data: Chi-square = 0.95, probability level = 0.97, AIC = 20.95.



CHAPTER 3

A restoration experiment: Effects of three buckthorn (*Rhamnus cathartica* L.) removal techniques on the regeneration of understory vegetation

Summary

Common buckthorn (*Rhamnus cathartica* L.) is one of the most abundant and ecologically harmful non-native plants in forests of the upper Midwest United States, changing nutrient cycling, decreasing native plant abundance and diversity, and impeding forest regeneration. Removal of buckthorn and restoration of invaded systems are time-intensive and costly processes, and few studies have moved past studying the immediate efficacy of removal projects to examine how buckthorn removal affects subsequent community regeneration. In order to investigate how different buckthorn removal methods affect the regeneration of understory vegetation, we established a four-site buckthorn removal experiment in upland, mesic oak forests in east central Minnesota. The goal of this project was to explore invasive species removal through an experimental lens, manipulating controls on plant establishment to understand the results of current management practices. Buckthorn was removed using three methods: weed wrenching (WW), manual removal of the above and belowground biomass; cut-and-paint (CP), removal of the aboveground biomass and application of herbicide to the cut stump; and basal bark removal (BB), application of herbicide to the stem, leaving the standing dead biomass. Methods were selected because they are among the most common techniques applied by managers; moreover, they have differing effects on light and leaf litter availability, which are important controls on plant germination and establishment. We examined the relationships between removal treatments, environmental conditions, and regenerating vegetation in twelve 6 x 6 m removal plots at each site. Buckthorn was removed in the fall of 2011, and vegetation and plot characteristics were surveyed twice in 2012 and twice in 2013 to monitor the regeneration of the plant community. Treatments varied in their ability to promote more cover and diversity than the control plots; the WW treatment resulted in significantly higher herbaceous and woody species cover than all other treatments, while only some plots where herbicide was applied surpassed cover levels in the control plots. Nonmetric multidimensional scaling ordinations demonstrated that treatments significantly differed in the composition of both herbaceous and woody vegetation that regenerated post-removal. Indicator species analysis corroborated that result, suggesting that specific species associated with different treatments, and together, these results demonstrate that the WW treatment was associated with more early successional and non-native species while the CP and BB treatments were associated with a mix of early and later successional species. Thus, WW leads to faster recovery of plant cover and diversity, but of a typically less desirable mix of species. We conclude that the specific buckthorn removal method used at a site can differentially affect the regeneration of understory vegetation. Ultimately, careful consideration of the effects of different removal methods on both environmental conditions and subsequent vegetation may improve the success of invasive species removal projects and ecosystem restoration efforts.

Introduction

Invasive plant species are among the most pressing ecological concerns of the 21st century, with countries spending billions of dollars on invasive species control and ecosystem restoration (Pimentel et al. 2005). The field of restoration ecology is relatively young, but research into the restoration of ecosystems affected by invasive plant species is becoming increasingly common. Early studies have focused on the success of different eradication methods (Nyboer 1992; Archibold et al. 1997), with few studies addressing the re-establishment of communities post-removal (Bisikwa 2005; Love and Anderson 2009; Guido and Pillar 2014). However, there is still no consensus on the best way to eradicate invasive plant species, even within a particular functional group. Much of this debate arises from environmental variation: invaded sites often have different characteristics and management must be geared to the specific site, and even at a single site changing environmental conditions can affect the success of a particular strategy (Dornbos and Pruim 2012). For example, Luken and Mattimiro (1991) found that after repeated management, an invasive woody shrub re-sprouted more often when growing in clearings than in forest habitats. When focusing on invasive shrubs in particular, some broadly successful but often labor-intensive methods of removal have been documented, though their success still depends in part on the site and environmental conditions (Dornbos and Pruim 2012). Mechanical removal by saw, lopper, or hand-pulling, followed by herbicide application to the remaining stump or roots, is generally thought to be effective at eliminating and preventing the re-growth of certain shrub species (Hartman and McCarthy 2004; Pergams and Norton 2006; Love and Anderson 2009; Dornbos and Pruim 2012), though not necessarily their re-colonization.

There are also less labor intensive foliar herbicide sprays and basal bark herbicide applications. However, the timing of the treatment and the herbicide type and concentration are important variables. For example, foliar application of glyphosate was found to be more effective at controlling an invasive bush honeysuckle (*Lonicera morrowii*) in the spring (Love and Anderson 2009), and application of glyphosate to cut stumps of common buckthorn (*Rhamnus cathartica* L.) was more effective at preventing re-sprouting in dry versus moist soils (Dornbos and Pruim 2012). In the same study, glyphosate concentrations of 20% or more were required to prevent re-sprouting in a majority of treated buckthorn stumps, while a 41% concentration saw even better control. Although increasing the amount of active ingredient may result in marginally improved control, it is also more expensive and potentially harmful to native plants. The costs and benefits of any restoration method must be weighed carefully and must match the goals and means of the restoration organization (Love and Anderson 2009).

While the hope is that with the invaders gone, natural succession can take place and native plants will re-colonize the area, this is usually not the case (Holmes 2001). Swab et al. (2008) showed that a year after honeysuckle removal with the cut-and-paint treatment, there was no correlation between honeysuckle cover and understory species abundance. A lack of native species recruitment following removal is often due in part to depleted seedbanks (Collier et al. 2002), and eventual colonization may depend on seed sources outside the site. However, Runkle et al. (2007) showed that eight years after honeysuckle removal at a different site, removal plots had higher herbaceous species richness, percent cover, and tree seedling density than did non-removal plots. While time may be an important factor in colonization by native species, re-colonization by invasives may also present a problem and depend on the seed bank and seed rain from neighboring infestations (Vidra et al. 2007). To prevent this, studies are now examining the efficacy of strategies such as native species planting immediately following shrub removal (Ghersa et al. 2002; Hartman and McCarthy 2004). Hartman and McCarthy (2004) showed that planted native seedlings exhibited greater survival in honeysuckle removal plots versus control plots. Moreover, different eradication methods yielded different seedling survival percentages due to treatment effects on micro-environmental conditions (Hartman and McCarthy 2004). Aside from re-establishing the native community, post-removal plantings can also prevent future invasion, as plants will take up space, light and other resources that invaders would otherwise be able to use (Shea and Chesson 2002). For similar reasons, diverse communities have often been shown to better resist invasion (Frankow-Lindberg 2012; Whitfeld et al. 2014; but see review in Levine and D'Antonio 1999).

Even with initial planted seedling survival, restoration may not yield a return to the original system. Additional plantings may be necessary for native vegetation to take hold in invaded areas (Hartman and McCarthy 2004; Vidra et al. 2007). Furthermore, underground processes may affect the success of restoration. For example, in northern temperate forests, invasive earthworms negatively impact many native forest plant species (Frelich et al. 2006) and have been shown to facilitate invasive plants including buckthorn (Heneghan et al. 2007; Whitfeld et al. 2014; Roth et al. 2014). Earthworms can disrupt nutrient cycling and mycorrhizal interactions in these invaded areas, and without attention to these underground processes, forest regeneration may not proceed as planned (Frelich et al. 2006). Some hope is provided by recent research demonstrating that the removal of invasive shrubs decreases earthworm populations, though the study period lasted only two growing seasons (Madritch and Lindroth 2009). Ultimately, there is still little research to assess whether restoration after invasive shrub removal will be effective in the long run. It is possible that the changes caused by invasive shrubs create a barrier between the current and original states that cannot be surmounted. Likewise, restoration may move any ecosystem to an unforeseen, alternative stable state that may persist indefinitely (Beisner et al. 2003).

Common buckthorn is a particularly pervasive introduced shrub that is the focus of much management attention in North American forests. It is currently present in at least 34 states and eight Canadian provinces (USDA NRCS 2014), and can dominate forests and natural areas throughout its invasive range (Knight et al. 2007). Its ability to form monospecific stands (Knight et al. 2007), its effects on decomposition (Heneghan et al. 2004, 2006), its negative effects on plant and animal diversity (Schmidt and Whelan 1999; McKinney and Goodell 2010; Klionsky et al. 2011; Sacerdote and King 2014) and its role as a host for major agricultural pests (Heimpel et al. 2010) make buckthorn a serious concern for states, counties and local land managers. Efforts to control buckthorn are notoriously costly and have been frequently ineffective (Invasive Species Program 2012; Gassman and Tosevsky 2014). Managers continually deal with re-invasion post-removal, and restoration to pre-buckthorn conditions is rarely achieved. Since post-removal restoration strategies are not often logistically or financially feasible, removal strategies that encourage re-vegetation may provide an important improvement in restoration efforts. The use of removal methods that affect environmental conditions controlling plant germination and establishment – namely light, leaf litter, and diversity –

may provide insight into why certain removal methods may be more successful at encouraging re-vegetation. Here we manipulated these controls on plant germination and establishment in order to improve restoration of ecosystems invaded by common buckthorn. Specifically, we conducted a four-site buckthorn removal experiment using removal methods that differentially affected light availability, soil disturbance and diversity. We selected three commonly used removal methods: weed wrench (WW), cut and paint (CP), and basal bark (BB) treatments. WW is the physical removal of above and belowground biomass by hand pulling or the use of a specialized weed wrench tool, CP is the removal of aboveground biomass and application of herbicide to the cut stump, and BB involves application of an herbicide to the standing stem. We addressed the following questions:

1. Do different removal methods differ in their ability to reduce buckthorn abundance?

2. Do different removal methods differ in the cover and diversity of plant regeneration post-removal?

3. Does post-removal plant community composition (both herbaceous and woody) differ depending on the removal method used?

As it leaves standing dead buckthorn biomass, we expected the BB treatment to result in the lowest available light of the three removal treatments, though still significantly higher than the control plots. We expected the WW treatment to result in the lowest available leaf litter, as removal of belowground buckthorn biomass would disturb the soil and create bare patches where root balls had once been. We hypothesized that removal methods where herbicide was used – CP and BB – would be most successful at reducing buckthorn abundance by preventing re-sprouting. We also hypothesized that the WW treatment would result in the highest cover and diversity of plant species due to the combination of increased available light, bare soil, and an exposed soil seedbank. Finally, we hypothesized that the three treatments would result in different post-removal species compositions depending on the specific treatment. We expected that all treatments would be different from the control, while the BB and CP treatments were expected to be the most similar to each other due to their effects on light availability alone.

Methods

Site selection and plot set-up

In the summer of 2011, we established four buckthorn removal sites in the eastern broadleaf forest province of east central Minnesota. Sites were selected in order to minimize environmental variation among sites, and were located in upland, mesic oak-dominated forests with a dominant buckthorn shrub layer. Sites were located at Afton State Park (44.845186, -92.789102), Hyland Lake Park Reserve (44.840596, -93.367861), Warner Nature Center (45.171252, -92.826218), and St. Benedict's Monastery (45.555348, -94.328778). Sites were all located in east central Minnesota, where the average yearly precipitation is 71-91 cm and mean annual temperature is 6.1-7.8 °C. The dominant overstory species at each site included red and white oak (*Quercus rubra* and *Q. alba*), red maple (*Acer rubrum*) and green ash (*Fraxinus pennsylvanica*). Within each site, plots were located in areas of relatively uniform buckthorn density and were established at least 10 m from any forest edges or trails. Plots were 6 m by 6 m squares with 5 m buffers between plots, and three types of invasive species removal treatments were assigned to the plots. Each treatment was replicated three times, including control plots with no buckthorn removal, for a total of 12 plots at each site and 48 plots total. Randomization was used to determine the arrangement of the treatments within each site. Before the treatments were applied, we established three 1 m-radius circular subplots in each plot for herbaceous plant surveys. Subplots were located approximately 1.5 m from the plot center, and were positioned at 0, 120 and 240 degrees. Plots were permanently marked with rebar stakes.

Buckthorn removal and vegetation surveys

In August 2011, we surveyed all sites to record baseline light, litter and vegetation conditions. Within each 1 m circular subplot, we identified all vegetation to species; percent cover was documented for herbaceous species, and percent cover, stem counts, and diameter-at-breast height (dbh) were documented for woody plants. Species were identified according to the Flora of North America (Flora of North America Editorial Committee 1993) and Gleason and Cronquist (1991). Once the vegetation was characterized, environmental measurements were taken, including leaf litter and organic matter depth, percent bare ground in a 1 m square to the north of each subplot, canopy openness as a proxy for light levels – using a densiometer – and the slope and aspect of the entire 6 m by 6 m plot. Invasive earthworm presence was also documented at the site level using a rapid visual assessment method (Loss et al. 2013). All sites were heavily invaded by European earthworm species, and ranked as a 5 on the invasive earthworm rapid assessment tool (IERAT) scale (Loss et al. 2013).

Buckthorn removal was conducted in November of 2011 to ensure that all native plants had fully senesced prior to herbicide application. Removal was conducted according to the methods randomly assigned to each plot. In the WW treatment, all buckthorn individuals were removed by physically pulling the plants from the ground using either hand pulling for smaller individuals or a weed wrench for larger individuals. In both cases, care was taken to remove as much of the root as possible in order to prevent re-sprouting. In plots where herbicide was used, we applied a 20% solution of Garlon 4 (triclopyr; Dow AgroSciences, Indiana, USA) to either the cut stump (CP) or around the base of the live stems using a hand sprayer (BB). We were careful to avoid any overspray; however, it likely occurred, especially during basal bark application. Plots were not re-treated after the initial intervention. Plots were re-surveyed with the same methodology in June and August of both 2012 and 2013 to allow for two full growing seasons of data to document the changes in the vegetation community and the environmental characteristics. Overall efficacy of each eradication method was recorded through counts of buckthorn stems that survived or re-sprouted during subsequent surveys.

Data analysis

Data were analyzed using JMP ver. 9.0.2 (SAS Institute, Inc., Cary, NC, USA) and R ver. 3.0.1 (R Core Development Team 2014). We used analysis of variance

(ANOVA) to compare treatment effects on herbaceous and woody cover and richness. Post-hoc Tukey's HSD tests were used to compare average cover and diversity of the different treatments at each site. We calculated Shannon diversity in order to incorporate species abundance into our diversity metrics. When necessary, data were transformed to achieve normality; when transformations did not result in normal data, nonparametric tests (Kruskal-Wallis and Steel-Dwass) were used. We also created linear mixed effects models to examine the effects of site, treatment, time, and the interaction between treatment and time on vegetative cover and diversity. Plot was included in these models as a random effect. Ordinations were conducted in R ver. 3.0.3 (R Core Team 2014) using the Vegan package (Oksanen et al. 2013). Non-metric multidimensional scaling (NMS) was used to accommodate non-normal species occurrence data as NMS is able to handle non-parametric data. We also conducted indicator species analysis in R using the labdsv package (Roberts 2013) in order to better determine whether specific species associated with the different treatments at each site.

Results

All three removal methods were equally effective at removing buckthorn; each removal method attained at least 99% efficacy in terms of the number of buckthorn stems removed. Few stems in any size class were missed when applying treatments, and re-sprouting happened only occasionally and was not associated with any specific treatment type. All removal methods significantly increased available light at the forest floor over the control, though the order of the treatments varied across sites ($F_{(3,185)} = 21.9$,

$p<0.0001$; Fig 1). Overall, the BB treatment had the highest available light, though the differences between the three removal treatments were not significant. Treatments also differentially affected litter depth at the four sites ($F_{(3,137)} = 4.74$, $p=0.0035$; Fig1). The three removal treatments did not differ significantly from each other, but as expected, WW plots had the lowest leaf litter and were significantly lower than the control plots ($p=0.02$).

Removal treatments differed significantly in their effects on post-removal herbaceous cover ($F_{(3,137)} = 4.74$, $p=0.0035$), and were mixed in their ability to encourage more cover than control plots. Plots in the WW treatment had significantly higher average herbaceous species cover than all other treatments, while BB plots had significantly lower average cover than all other treatments (Fig 2). There were also strong interactions between treatment and time since removal (Fig 3). Plots in the CP and BB treatments tended to experience temporary declines in cover in the spring following treatment, recovering to either approximate or surpass the cover in the control plots by the fall of the next year (Supplementary Material). While woody species cover was always highest in the control plots, owing to the dense cover of buckthorn, the trends in woody species cover between plots in the three removal treatments were similar to those in herbaceous cover. However, the cover of woody species was much lower than that of herbaceous species due to slower woody species growth over the study period.

WW plots had significantly higher levels of herbaceous and woody richness than all other treatments, while BB plots had the lowest species richness (Fig 2). When examining total species richness, the same trends remained and were statistically stronger than when either herbaceous or woody richness alone was used as the response variable. WW plots also attainted the highest Shannon diversity, while plots in the BB treatment had the lowest.

Our linear mixed effects models demonstrated the importance of treatment, site, time since treatment, and the interaction between treatment and time in explaining herbaceous and woody species cover. These models were able to explain over 75 percent of the variance in herbaceous ($r^2=0.76$) and woody ($r^2=0.83$) species cover (Table 1). Treatments significantly impacted both herbaceous (df=3, F=46.71, p=<0.0001) and woody (df=3, F=228.33, p=<0.0001) cover. While the BB treatment had a slightly negative effect on cover, the CP treatment had a slightly positive effect and the WW a much larger positive effect on cover. Site (herbaceous: df=3, F=6.87, p=0.0002; woody: df=3, F=2.70, p=0.04) and time period (herbaceous: df=3, F=68.12, p=<0.0001; woody: df=3, F=5.96, p=0.0007) were also important determinants of herbaceous and woody cover, with cover differing across sites and increasing with each sampling period. Finally, the interaction between treatment and time was significant for herbaceous cover (df=9, F=4.93, p=<0.0001). Plots in the WW treatment gained more species cover in the spring immediately following buckthorn removal, and gained less cover in subsequent sampling periods. In the CP and BB treatments, plots gained relatively little species cover in the first year, but saw increasingly larger gains in cover in the spring and fall of the second year (Fig 3). For woody cover, the interaction between treatment and time was not significant.

Our mixed effects models were also able to explain at least 65 percent of the variance in average herbaceous (r^2=0.72), woody (r^2=0.65), and total (r^2=0.75) species richness (Table 1). The models contained the same significant trends as the cover models, demonstrating the importance of the explanatory variables in influencing both cover and richness. Treatments significantly affected both herbaceous (df=3, F=52, p=<0.0001) and woody (df=3, F=49.68, p=<0.0001) richness, with the WW and CP treatment having positive effects and the BB treatment having a slight negative effect. Herbaceous and woody richness differed across sites (herbaceous: df=3, F=30.74, p=<0.0001; woody: df=3, F=20.10, p=<0.0001) and increased with each sampling period (herbaceous: df=3, F=10.19, p=<0.0001; woody: df=3, F=10.43, p=<0.0001). The interaction between treatment and time was again significant for herbaceous richness (df=9, F=6.36, p=<0.0001), but not for woody richness (df=9, F=1.66, p=0.10), with WW plots gaining more species immediately following buckthorn removal while BB and CP plots gained progressively more species in the later sampling periods.

Ordinations indicated that the plant communities resulting from the treatments were different from one another (Fig 4; Supplementary Material). Unsurprisingly, the sites differed significantly in their respective species make-ups. However, within sites, plant communities separated out in the three dimensional ordination space by treatment type, demonstrating the importance of the treatments in affecting the specific suites of species that colonized post-buckthorn removal (Warner: p=0.001; St. Benedict's: p=0.023; Afton: p=0.001; Hyland: p=0.001). We were also able to fit species vectors to ordinations, allowing us to visualize which species caused the majority of separation between treatments. Species vectors often pointed in the direction of the WW plots, owing to the more abundant vegetation in those plots. Across sites, plots in the CP treatment associated with later successional forest species such as Canada mayflower (*Maianthemum canadense*), large-leaf aster (*Eurybia macrophyllus*), rosy sedge (*Carex rosea*), bedstraw (*Galium triflorum*), and enchanter's nightshade (*Circaea lutetiana*), all associated with axis two of the ordination. Plots in the BB treatment separated from plots in the other treatments based on a higher frequency of lady fern (*Athyrium filix-femina*) and Jack-in-the-pulpit (*Arisaema triphyllum*), both of which showed evidence of herbicide damage but were among the most common species regenerating in these plots. Conversely, plots in the WW treatment were characterized by more early successional, disturbance tolerant, and often non-native species such as stickseed (*Hackelia virginiana*), Canada thistle (*Cirsium arvense*) motherwort (*Leonurus cardiaca*), and dandelion (*Taraxacum officinale*), associated with axis one of the ordination.

Indicator species analysis demonstrated that, in some cases, certain species were significantly associated with specific treatments, and most commonly with the WW treatment (Table 2). These species included climbing buckwheat (*Fallopia scandens*), northern bedstraw (*Gallium boreale*) and garlic mustard (*Alliaria petiolata*). These specific species demonstrate the more disturbed nature of the WW plots, where species range from typical forest understory natives (bedstraw), to disturbance-loving natives (climbing buckwheat) and disturbance-loving non-natives (garlic mustard).

For woody species in the shrub layer and seedling layers, communities resulting from the treatments were less significantly different from each other than the herbaceous communities, but often very different from the control plots, where buckthorn dominated (Fig 5; Supplementary Material). At two sites, treatment plots separated out in the three dimensional ordination space due to the presence of seedlings of woodbine (*Parthenocissus vitacea*) and hackberry (*Celtis occidentalis*), while at the remaining sites, plots separated mainly by the presence of buckthorn in the control plots. However, there was no significant difference in the number of first year buckthorn seedlings present in the treatment plots at any site or when all sites were investigated together ($F_{(3,140)}=0.769$, $p=0.5132$), highlighting the often clustered and localized nature of buckthorn dispersal and seedbank presence. In most cases, significant indicator species associated with either the control or WW plots; CP and BB plots had few significant indicator species at any site. Buckthorn was associated only with the control treatment at all sites, while WW plots had a mix of early and later successional associated species, including gooseberry (*Ribes cynosbati*), exotic bush honeysuckles (*Lonicera spp.*), chokecherry (*Prunus virginiana*), elm (*Ulmus rubra*), and box elder (*Acer negundo*).

Discussion

Common buckthorn is one of the most prolific and potentially harmful forest invaders in the upper Midwest United States (Heneghan et al. 2006; Knight et al. 2007; Klionsky et al. 2011), and removal of this species is a concern for many local and state land management agencies (Invasive Species Program 2012; Gassman and Tosevsky 2014). This experiment moves past previous removal studies to investigate whether and how different buckthorn removal techniques affect post-removal vegetation regeneration.

Post-removal ecosystem restoration is often plagued with difficulties, including lack of viable seedbanks (Collier et al. 2002) and difficulty attaining high diversity (Sinclair et al. 1995) and native species composition (Harrington 1999). Using a four-site buckthorn removal study, we provide novel evidence to show that both within and across sites, the type of removal treatment used will affect subsequent plant regeneration and may influence the success of subsequent restoration.

All treatments used in this experiment were extremely successful at removing buckthorn. This high efficacy is likely due to the focused nature of the experiment and the direct, careful application of the different treatments over a relatively small area. While such attention to detail was important for our experiment, this degree of success may not be realistically attainable on large properties or infestations, or with workers whose experience, skills, focus, or time is limited.

The three removal treatments differed in the subsequent environmental conditions they created. All three removal treatments resulted in higher light levels at the forest floor than in the control plots, and although the differences between the three removal treatments were not significant, the BB treatment resulted in the highest overall available light. The lack of significant differences may be due in part to the random placement of treatment plots in areas of lower or higher canopy cover, which can vary considerably in forests, and may ultimately control understory vegetation dynamics (Figueroa-Rangel and Olvera-Vargas 2000). While the treatments were successful at increasing available light at the forest floor, the WW treatment was unique in its added soil disturbance, as the other treatments left the leaf litter and soil layers relatively intact. Although all three removal treatments had slightly less leaf litter than the control plots, only the WW treatment had statistically lower average leaf litter levels than the control plots. Thus, the resulting effects on vegetation may have to do less with leaf litter depth and its effects on germination and more to changes in light, creation of bare soil patches, and disturbance of the soil seedbank (Putz 1983).

The different treatments showed similar patterns across sites in terms of how they affected cover, richness and diversity of post-removal vegetation. The weed wrench treatment was generally the most successful at bringing back cover and diversity of both herbaceous and woody plant species. While these herbaceous and woody patterns were dynamic throughout the sampling dates, WW was consistently the most successful treatment in terms of increasing plant cover and richness. That WW often resulted in higher cover and diversity of plant species is likely due to changes in environmental factors attributable to the nature of the treatment. WW increased available light at the forest floor by removing buckthorn's aboveground biomass. It also removed the majority of belowground biomass, disturbing the soil and creating bare soil patches conducive to early successional plant colonization (Battles et al. 2001; Knight et al. 2007). This soil disturbance is also likely to uncover some of the soil seedbank, potentially jump-starting germination by plants already present at the site (Putz 1983).

The CP and BB treatments were often similar in their effects on the cover and diversity of both herbaceous and woody plants. CP and BB plots experienced temporary declines in both cover and richness following buckthorn removal. While cover and diversity often rebounded, in many cases surpassing that of the control plots, the BB and

CP plots nonetheless lagged behind cover and richness levels attained in the WW plots. BB plots frequently had the lowest cover and richness of the treated plots, with levels lower than those in the control plots.

While these trends are likely due in part to the lack of soil disturbance in the plots where WW was not used, given that both the CP and BB plots saw similar patterns of species decline and resurgence, these results suggest a potential temporary suppressive effect of herbicide on both herbaceous and woody vegetation. Triclopyr has been reported to persist in the soil for various durations and at various depths depending on the soil texture and moisture levels, and subsequent precipitation in the weeks following application can aid movement in the soil column and off target (SERA 1996). While all treatments were applied in late fall after leaf senescence, it is possible that overspray, especially in BB plots, could have resulted in damage to plant roots and tissues (SERA 1996). In multiple BB plots, we noticed damaged or deformed leaves on regenerating Jack-in-the-pulpit and lady fern. Triclopyr has also been shown to decrease germination when persisting in soils (SERA 1996), providing another potential explanation for the lower cover and richness in BB plots. As with any herbicide, care should be taken to avoid applying more than necessary and to limit overspray.

We also noticed some control plots gaining species in later sampling periods. While this trend did not significantly change the percent cover in those plots, this increased richness may have been due in part to the species colonizing the treatment plots, and in many cases specifically the WW plots. Once the early successional species colonized the WW plots, they may have dispersed into neighboring plots, even though these plots were buffered with areas of standing buckthorn.

Ordinations showed that in many cases the treatments resulted in communities composed of species that were different from those of the control plots and often statistically different from each other. While at three sites the treatments often resulted in higher cover and diversity, their species composition tended to contain more early successional and disturbance-tolerant species than the control plots. In contrast, at the fourth site, where cover and diversity were the lowest of any site, treatment plots often contained many of the later successional species found in the control plots, though the exact make-up of species in each treatment differed. The pattern in the first three sites is likely due in part to an increase in available resources once buckthorn was removed (Davis et al. 2000). With the buckthorn gone, there were higher light levels, potentially larger untapped nutrient pools, and in the case of the WW plots, disturbed soil areas on which to germinate. These newly available resources are often readily taken advantage of by disturbance-tolerant and sometimes invasive species (Huenneke et al. 1990; Davis et al. 2000; Gilliam 2006; Funk and Vitousek 2007). This was evidenced by the significant indicator species at these sites; indicator species, especially in the WW plots, were often early successional and in many cases non-native species such as garlic mustard and exotic bush honeysuckles. At the fourth site (St. Benedict’s Monastery), which was the most isolated forest fragment and located in a more agricultural landscape, the propagule sources for colonization by new species may not have been available, resulting in only limited colonization by species present at the site.

Conclusions

Our results suggest that the specific method used for removing buckthorn and other invasive shrub species is important for influencing future plant colonization. This can have implications for the vegetation trajectory and successful restoration of invaded systems. Although treatments that disturb the soil are better able to encourage initial cover and diversity, this cover may be skewed toward a more early successional, weedy, or non-native community. While the increased cover and diversity may help resist future invasion by buckthorn and other non-native species, the species make-up may include other problematic invaders that could come to dominate the site, or may lack specific desirable native forest plants. Some species associated with the WW treatment, such as garlic mustard, may necessitate their own future management interventions. Treatments where soil is not disturbed may experience less initial cover than treatments like WW, but may also reach higher diversity over time and contain more late-successional forest species. While these trends were consistent across the four sites examined, ultimately, the exact species involved will depend on the specific site and the local propagule availability. In any case, the increased cover and diversity in treated areas may help to limit buckthorn re-invasion, as these species may be able to pre-empt buckthorn's use of available resources (Davis et al. 2000; Shea and Chesson 2002; Whitfeld et al. 2014a; Whitfeld et al. 2014b). However, buckthorn has a long-lived seedbank, and seed sources persisted around all of the removal sites, so the time-scale of the experiment was too short to fully test this. Also, it is possible that the trends in cover, diversity and composition could change in the long-term; a longer study duration might reveal different trends (Runkle et al. 2007), and may be especially important for tracking trends in woody species cover and diversity.

The goal of this experiment was to view invasive species removal through an experimental lens, using controls on plant establishment to understand the results of current management practices. By investigating how different treatments affect the conditions that govern plant colonization and growth, we will be better able to understand the results of management and potentially tailor management to the specific management goals at each site. It is important to note that this experiment did not take into account the cost of the various treatments; ultimately, the feasibility of these treatments will depend on the scale of the project, the specific site, and the resources of the managing agency. Moreover, these results do not suggest that any of the methods used will lead to successful regeneration of communities on their own. While some treatments did result in high cover and diversity over a two year period, restoration plantings may still be needed to attain the specific mixture of species desired at a particular site (Ghersa et al. 2002; Vidra et al. 2007). Future research should focus on long term monitoring of plant succession in removal projects, whether plant community differences associated with different treatments will persist in the long term, and whether they may differentially affect the community's long term susceptibility to re-invasion or its ability to prevent buckthorn establishment from the existing seedbank. While such research will be important for further improving restoration outcomes, resistance to future invasions will ultimately depend on concurrent management of local propagule sources.

Acknowledgements

We thank the Minnesota DNR, Three Rivers Park District, St. Benedict's Monastery and Warner Nature Center for granting us permission to conduct research on their properties. In conjunction, we thank staff at their respective sites for helping with site selection. We also thank the Minnesota Conservation Corps, as well as graduate and undergraduate students in the Reich lab for help with buckthorn removal and vegetation surveys. Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (M.L. 2010, Chp. 362, Sec. 2, Subd. 6c 'Healthy Forests to Resist Invasion', to P.B.R.). Support was also provided by the Integrative Graduate Education and Research Traineeship: Risk Analysis for Introduced Species and Genotypes (NSF DGE-0653827); University of Minnesota Graduate School; the Wilderness Research Foundation; the Dayton Fund of the Bell Museum of Natural History; the Wood-Rill Fellowship; and the Catherine S. Hill Fellowship.

Table 3.1. Linear mixed model results for the effects of treatment, site, time period, and the interaction between treatment and time on the cover and richness of herbaceous and woody species in buckthorn removal plots. ** $0.001 \leq p < 0.01$, *** $p < 0.001$

Herbaceous Species Cover

	Nparm	DF	F Ratio	Prob > F
Treatment	3	3	46.71	***
Site	3	3	68.12	***
Time Period	3	3	6.87	***
Treatment x Time Period	9	9	4.93	***

Total $R^2 = 0.76$.

Woody Species Cover

	Nparm	DF	F Ratio	Prob > F
Treatment	3	3	228.33	***
Site	3	3	5.96	***
Time Period	3	3	2.70	*
Treatment x Time Period	9	9	0.5314	0.85

Total $R^2 = 0.83$.

Herbaceous Species Richness

	Nparm	DF	F Ratio	Prob > F
Treatment	3	3	51.99	***
Site	3	3	30.74	***
Time Period	3	3	10.20	***
Treatment x Time Period	9	9	6.36	***

Total $R^2 = 0.72$

Woody Species Richness

	Nparm	DF	F Ratio	Prob > F
Treatment	3	3	49.67	***
Site	3	3	20.11	***
Time Period	3	3	10.43	***
Treatment x Time Period	9	9	1.66	0.10

Total $R^2 = 0.65$

Total Species Richness

	Nparm	DF	F Ratio	Prob > F
Treatment	3	3	77.48	***
Site	3	3	17.09	***
Time Period	3	3	13.15	***
Treatment x Time Period	9	9	6.12	***

Total $R^2 = 0.75$

Table 3.2. Selected indicator species for each of the four buckthorn removal sites.

	Species	Treatment	IndVal	Pvalue
Hyland	*Alliaria petiolata*	WW	0.9929	**
	Fallopia scandens	WW	0.9358	*
	Galium boreale	WW	0.8706	*
St. Benedict's	*Sanguinaria canadensis*	C	0.2667	*
	Trifolium pretense	WW	0.2333	*
	Carex gracillima	WW	0.3319	*
Warner	*Geranium maculatum*	C	0.3971	**
	Eurybia macrophylla	CP	0.3976	**
	Athyrium filix-femina	BB	0.3916	**
	Hackelia virginiana	WW	0.7596	***
	Leonurus cardiaca	WW	0.5619	***
Afton	*Carex pensylvanica*	C	0.6552	**
	Maianthemum canadense	C	0.2536	*
	Aralia nudicaulis	CP	0.3333	**
	Athyrium filix-femina	CP	0.2564	*
	Plantago rugelii	WW	0.4667	***
	Taraxacum officinale	WW	0.4657	***
	Conyza canadensis	WW	0.4465	**
	Hackelia virginiana	WW	0.3967	**

** $0.001 \le p < 0.01$, *** $p < 0.001$

Figure 3.1. Treatment effects on forest floor light (canopy openness measured using densiometer) and leaf litter depth (cm) at each of the four removal sites. Letters denote significant differences. Error bars are standard error

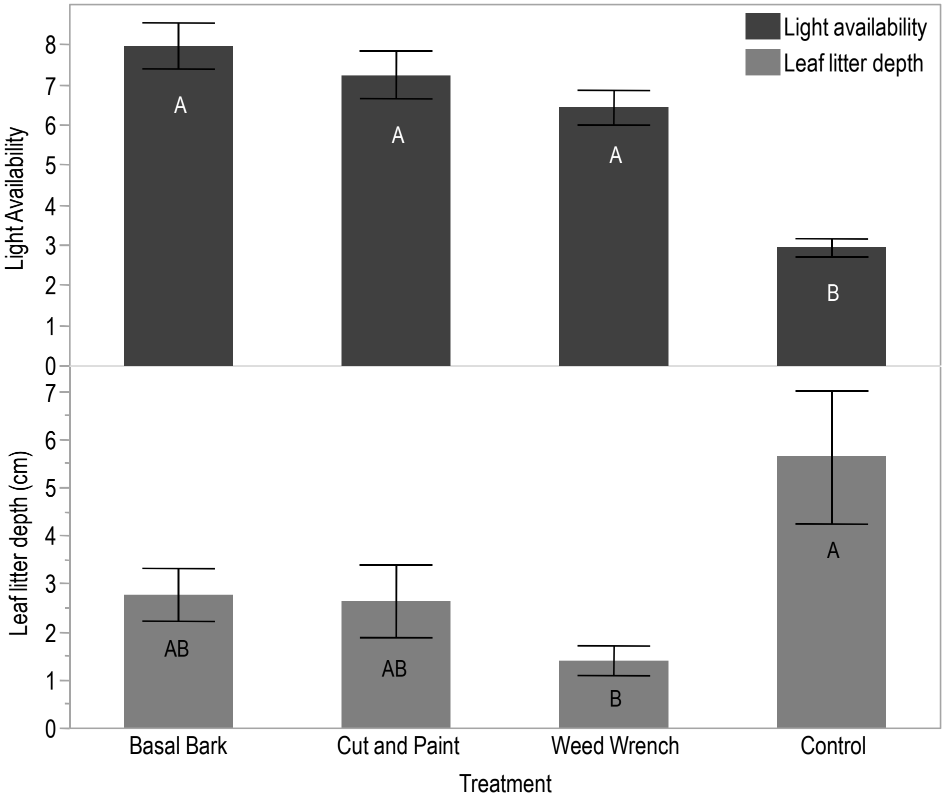


Figure 3.2. Treatment effects on herbaceous and woody species cover and richness. Letters denote significant differences. Error bars are standard error.

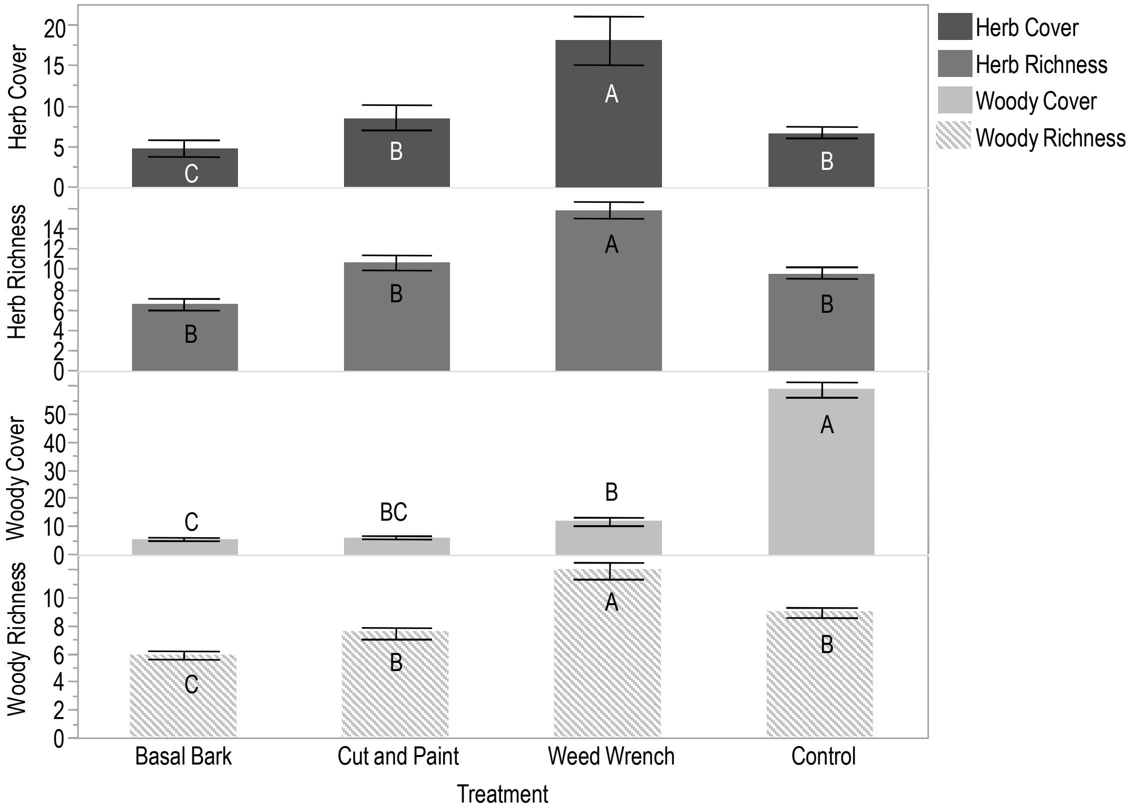


Figure 3.3. LSmeans plots for the interaction between treatment and time since buckthorn removal in models for herbaceous cover and richness. Buckthorn (*Rhamnus cathartica* L.) removal occurred after the fall 2011 survey.

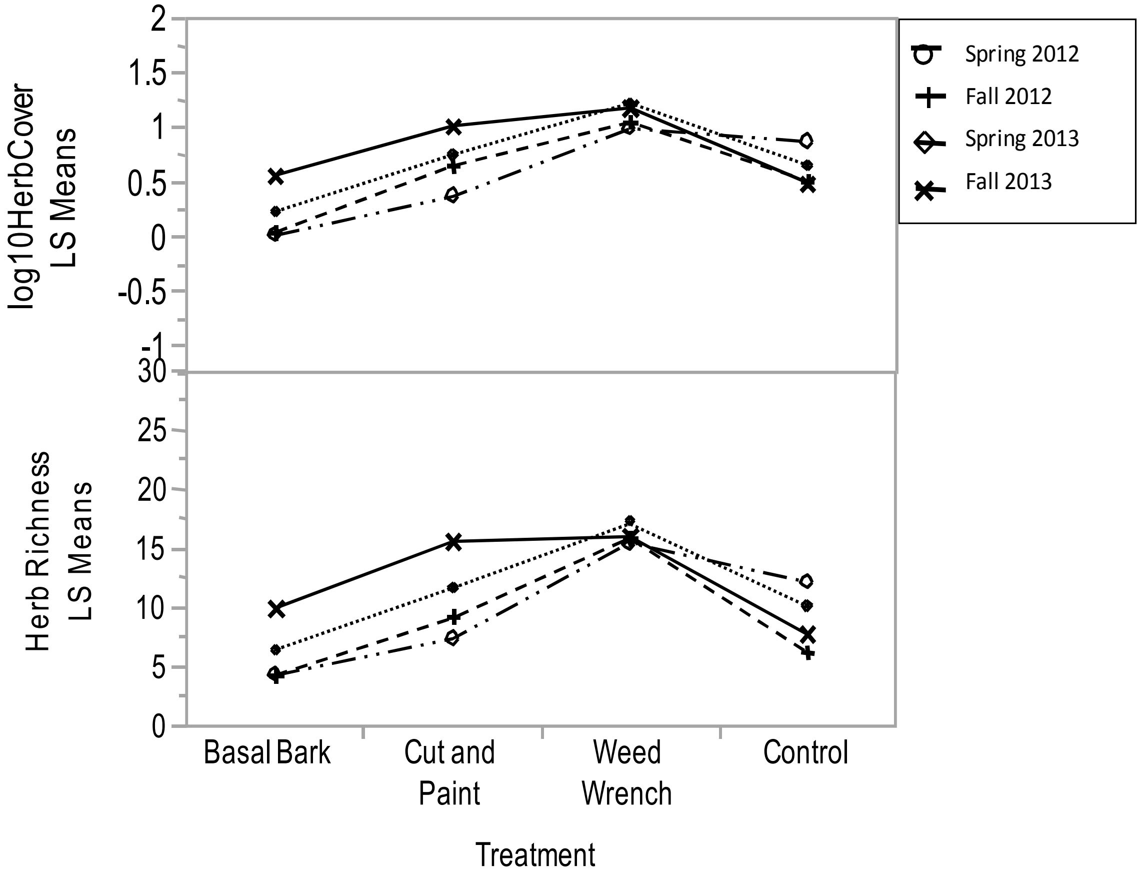


Figure 3.4. NMDS ordination solution of herbaceous species in buckthorn removal plots at Warner Nature Center (Stress=0.11). The treatments occupy statistically different areas of the 3D species space (p=0.001). C = Control, CP = Cut and Paint, BB = Basal Bark, WW = Weed Wrench.

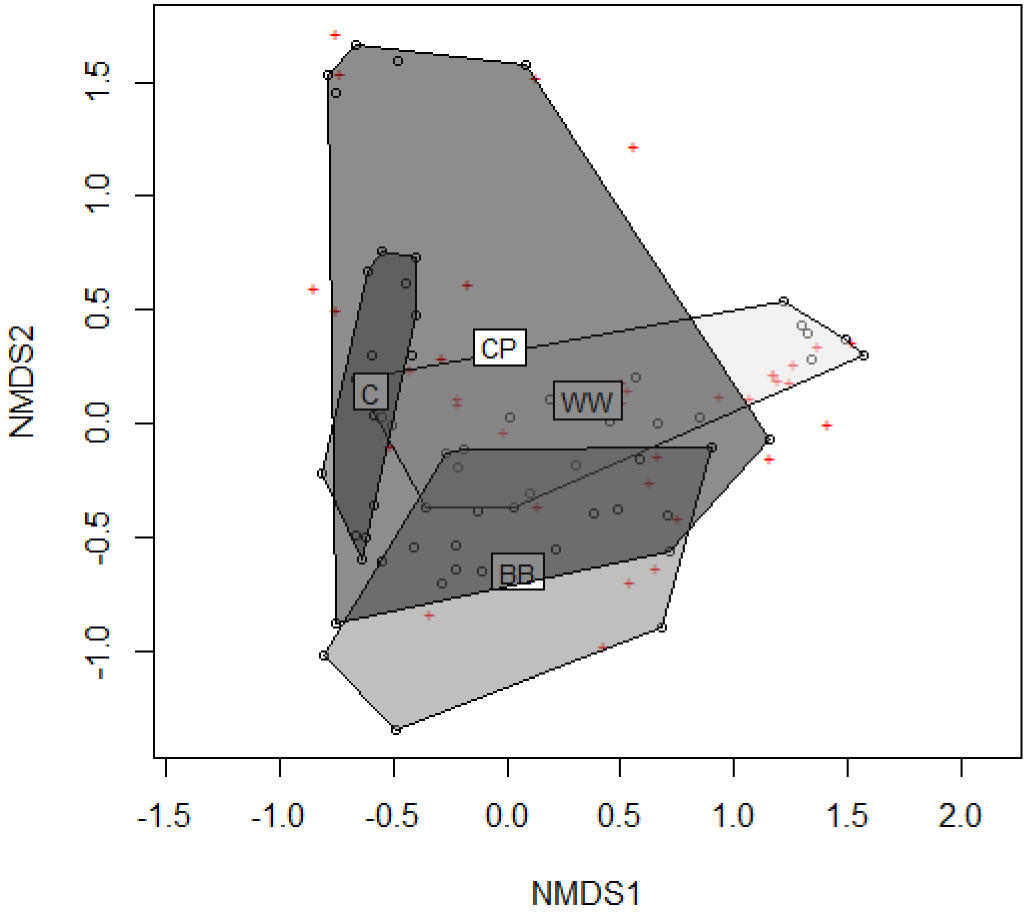
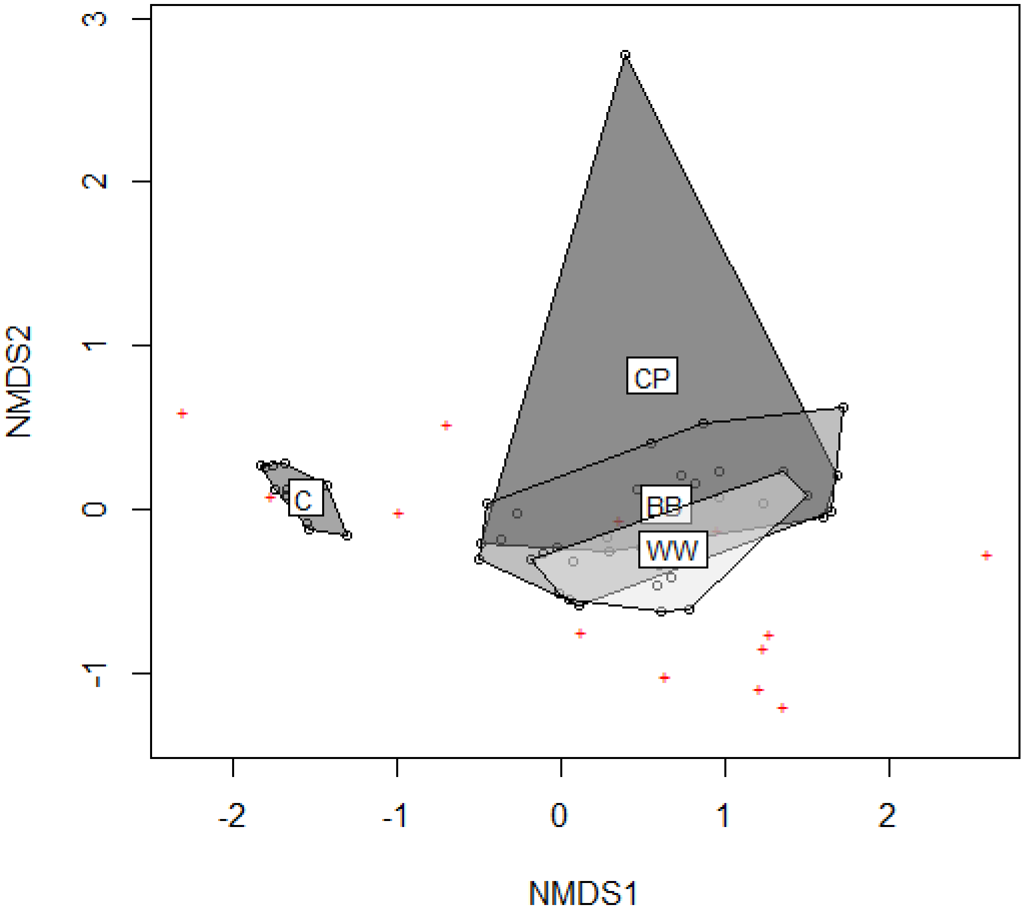


Figure 3.5. NMDS ordination solution of woody species in buckthorn removal plots at Warner Nature Center (Stress=0.07). The treatments occupy statistically different areas of the 3D species space (p=0.001). C = Control, CP = Cut and Paint, BB = Basal Bark, WW = Weed Wrench.



Conclusion

Common buckthorn and European earthworms are two of the most abundant and harmful invaders in forests of the upper Midwest United States (Bohlen et al. 2004; Frelich et al. 2006; Hale et al. 2006; Heneghan et al. 2006; Knight et al. 2007; Heimpel et al. 2010; Larson et al. 2010; Invasive Species Program 2012). Their effects on soils (Frelich et al. 2006; Heneghan et al. 2006), flora (Knight et al. 2007; Klionsky et al. 2011) and fauna (Schmidt and Whelan 1999; Maerz et al. 2009; Loss and Blair 2011; Sacerdote and King 2014) make them a concern for researchers and managers. Results of this dissertation provide information about the invasion biology of these species, as well as insights into restoration of forests invaded by buckthorn.

Results from chapter one revealed that earthworms increased buckthorn abundance and biomass in an experimental microcosm setting, lending support to the hypothesis that earthworms facilitate buckthorn germination and establishment in forests of the upper Midwest. The degree of impact depended in part on specific light and leaf litter levels, demonstrating the interactions between earthworms and abiotic conditions in affecting buckthorn establishment.

In chapter two we found evidence that buckthorn, in turn, may increase the success of earthworms in upper Midwest deciduous forests. Using a naturally occurring gradient of buckthorn invasion, we observed that earthworm biomass was highest in plots with abundant buckthorn, and that buckthorn facilitated earthworms through increasing soil moisture and by providing an abundant palatable food source via its leaf litter.

Finally, in chapter three we found that incorporating information about controls on plant germination and establishment into invasive species management can help us to better understand the results of current management practices and tailor management strategies to site-specific restoration goals. Using insights gained from chapter one, we used a four-site buckthorn removal experiment to explore the idea that by choosing removal methods that modify conditions controlling plant establishment and growth, we may be able to affect how plant communities regenerate post-removal. We found that weed wrenching, cut-and-paint herbicide application, and basal bark herbicide application all differentially affected environmental conditions and the resulting plant communities, with the weed wrench treatment associated with a more early-successional community.

Results from these three studies provide novel evidence for the co-facilitation of buckthorn and earthworms in forests of the upper Midwest United States. Furthermore, these results show that an improved understanding of the controls on the invasion process can provide important insights to explain and influence the results of invasive species management. Understanding how and why a species invades can encourage a more scientific approach to invasive plant management, potentially resulting in improved management outcomes.

Future research should further address the mechanisms behind the facilitative relationships between buckthorn and earthworms. Additional studies should also focus on whether plant community differences associated with different removal treatments will persist in the long term and whether they may differentially affect the communities' long term susceptibility to re-invasion or its ability to prevent buckthorn re-establishment from the existing seedbank. Ultimately, researchers and managers should be cognizant of the relationship between earthworms and buckthorn when studying the invasion of either species or implementing forest management or restoration plans.

Bibliography

Arbuckle JL (2012) IMB SPSS Amos 19 user's guide. Amos Development Corporation, Crawfordville, FL USA

Archibold OW, Brooks D, Delanoy L (1997) An investigation of the invasive shrub European buckthorn, *Rhamnus cathartica* L., near Saskatoon, Saskatchewan. Can Field Nat 111:617-621

Azcarate FM, Peco B (2006) Effects of seed predation by ants on Mediterranean grassland related to seed size. J Veg Sci 17:353-360

Baskin JM, Baskin CC (1992) Seed germination biology of the weedy biennial *Alliaria petiolata*. Nat Area J 12:191–197

Battles, J. J., A. J. Shlisky, R. H. Barrett, R. C. Heald, and B. H. Allen-Diaz (2001) The effects of forest management on plant species diversity in a Sierran conifer forest. Forest Ecol Manag146:211–222

Beisner BE, Haydon DT, Cuddington K (2003) Alternative stable states in ecology. Front Ecol Environ 1:376-382

Bellard C, Thuiller W, Leroy B, Genovesi P, Bakkenes M, Courchamp F (2013) Will climate change promote future invasions? Glob Change Biol 19:3740-3748

Benton TG, Solan M, Travis JMJ Sait SM (2007) Microcosm experiments can inform global ecological problems. Trends Ecol Evol 22:516-21

Bisikwa J (2005) Establishment and management of European buckthorn (*Rhamnus cathartica* L.). Dissertation, University of Minnesota, 117 pp

Bohlen PJ, Groffman PM, Fahey TJ, Fisk MC, Suarez E, Pelletier DM, Fahey RT (2004) Ecosystem consequences of exotic earthworm invasion of north temperate forests. Ecosystems 7:1-12

Bollen KA, Stine RA (1993) Bootstrapping goodness-of-fit measures in structural equation models. In K. A. Bollen and J. S. Long (Eds.) Testing structural equation models. Newbury Park, CA: Sage Publications

Brooker RW, Maestre FT, Callaway RM, Lortie CL, Cavieres LA, Kunstler G, Liancout P, Tielborger K, Travis JMJ, Anthelme F, Armas C, Coll L, Corcket E, Delzon S, Forey E, Kikvidze Z, Olofsson J, Pugnaire F, Quiroz CL, Saccone P, Schiffers K, Seifan M, Touzard B, Michalet R (2008) Facilitation in plant communitites: the past, the present, and the future. J Ecol 96:18-34

Carpenter SR (1996) Microcosm experiments have limited relevance for community and ecosystem ecology. Ecology 77:677-680

Cintra R (1997) Leaf litter effects on seed and seedling predation of the palm *Astrocaryum murumuru* and the legume tree *Dipteryx micrantha* in Amazonian forest. J Trop Ecol 13:709-725

Collier, M. H., J. L. Vankat, and M. R. Hughes. 2002. Diminished plant richness and abundance below Lonicera maackii, an invasive shrub.American Midland Naturalist 147:60–71

Costello, DM, Lamberti GA (2008) Biological and physical effects of non-native earthworms on nitrogen cycling in riparian soils. Soil Biol Biochem 41:2230-2235.

Dávalos A, Nuzzo V, Stark J, Blossey B (2013) Unexpected earthworm effects on forest understory plants. BMC Ecol 13:48. doi: 10.1186/1472-6785-13-48

Davis, M.A., Grime, J,P., Thompson, K (2000) Fluctuating resources in plant communities: A general theory of invasibility. J Ecol 88:528-534.

Davis, MA, Thompson, K, Grime, JP (2005) Invasibility: the local mechanism driving community assembly and species diversity. Ecography 28:696-704.

Davis MA (2011) Researching invasive species 50 years after Elton: A cautionary tale. In: Richardson, DM (ed). Fifty years of invasion ecology: The legacy of Charles Elton. 269-276

Dornbos DL, Pruim J (2012) Moist soils reduce the effectiveness of Glyphosate on cut stumpsof buckthorn. Nat Area J 32:240-246

Drake JA, Huxel GR, Hewitt CL (1996) Microcosms as models for generating and testing community theory. Ecology 77:670-677

Drouin M, Bradley R, Lapointe L, Whalen J (2014) Non-native anecic earthworms (*Lumbricus terrestris* L.) reduce seed germination and seedling survival of temperate and boreal trees species. App Soil Ecol 75:145-149

Eisenhauer N, Scheu S (2008) Invasibility of experimental grassland communities: the role of earthworms, plant functional group identity and seed size. Oikos 117:1026–1036. doi: 10.1111/j.2008.0030-1299.16812.x

Eisenhauer N, Butenschoen O, Radsick S, Scheu S (2010) Earthworms as seedling predators: Importance of seeds and seedlings for earthworm nutrition. Soil Biol Biochem 42:1245-1252

Eisenhauer N, Schlaghamersky J, Reich PB, Frelich LE (2011) The wave towards a new steady state: effects of earthworm invasion on soil microbial functions. Biol Invasions 13:2191-2196

Elton CS (1958) The ecology of invasions by animals and plants. Metheun and Co., London

Eschtruth AK, Battles JJ (2009) Assessing the relative importance of disturbance, herbivory, diversity, and propagule pressure in exotic plant invasion. Ecol Monogr 79:265-280

Fagan M, Peart D (2004) Impact of the invasive shrub glossy buckthorn (Rhamnus frangula L.) on juvenile recruitment by canopy trees. Forest Ecol Manag 194:95–107. doi: 10.1016/j.foreco.2004.02.015

Figueroa-Rangel BL, Olvera-Vargas M (2000) Regeneration patterns in relation to canopy species composition and site variables in mixed oak forests in the Sierra de Manantlan Biosphere Reserve, Mexico. Ecol Res 15:249-261

Fisichelli NA, Frelich LE, Reich PB, Eisenhauer N (2013) Linking direct and indirect pathways mediating earthworms, deer, and understory composition in Great Lakes forests. Biol Invasions 15:1057-1066

Flora of North America Editorial Committee (1993) Flora of North America: North of Mexico. Vols 16+. New York: Oxford

Flory SL, Bauer JT (2014) Experimental evidence for indirect facilitation among invasive plants. J Ecol 102:12-18

Frankow-Lindberg BE (2012) Grassland plant species diversity decreases invasion by increasing resource use. Oecologia 169:793-802

Frelich L, Hale C, Scheu S, et al. (2006) Earthworm invasion into previously earthworm-free temperate and boreal forests. Biol Invasions 8:1235–1245. doi: 10.1007/s10530-006-9019-3

Frelich L, Reich P (2009) Wilderness Conservation in an Era of Global Warming and Invasive Species: A Case Study from Minnesota's Boundary Waters Canoe Area Wilderness. Nat Area J 29:385–393.

Frelich LE, Reich PB (2010) Will environmental changes reinforce the impact of global warming on the prairie–forest border of central North America? Front Ecol Environ 8:371-378.

Frelich LE, Peterson RO, Dovciak M, Reich PB, Vucetich JA, Eisenhauer N (2012) Trophic cascades, invasive species and body-size hierarchies interactively modulate climate change responses of ecotonal temperate-boral forest. PNAS 367: 2295-2261

Fridley JD, Sax DF (2014) The imbalance of nature: revisiting a Darwinian framework for invasion biology. *Global Ecology and Biogeography* 23:1157-1166

Fridley JD, Stachowicz JJ, Naeem S, Sax DF, Seabloom EW, Smith MD, Stohlgren TJ, Tilman DE, Von Holle B (2007) The invasion paradox: reconciling pattern and process in species invasions. Ecology 88:3-17

Funk JL, Vitousek PM (2007) Resource-use efficiency and plant invasion in low-resource systems. Nature 446:1079-1081

Gassman A, Tosevsky I (2014) Biological control of *Rhamnus cathartica*: is it feasible? A revies of work done in 2002-2012. J Appl Ento 138:1-13

Gavier-Pizarro GI, Radeloff VC, Stewart SI, Huebner CD, Keuler NS (2010) Housing is positively associated with invasive exotic plant species richness in New England, USA. Ecol App 20:1913-1925

Ghersa C M, de la Fuenta E, Suarez S, Leon RJC (2002) Woody species invasion in the Rolling Pampa grasslands, Argentina. Agr Ecosyst Environ 88:271-278

Gill D, Marks P (1991) Tree and shrub seedling colonization of old fields in central New York. Ecol Monogr 61:183–205.

Gilliam FS (2006) Response of the herbaceous layer of forest ecosystems to excess nitrogen deposition. J Ecol 94:1176-1191

Gee GW, Bauder JM (1986) Particle size analysis. In Klute A (ed). Methods of Soil Analysis, Part 1: Physical and Mineralogical Methods. Agronomy Monograph No. 9, 2nd edn. Madison, WI: American Society of Agronomy 383–411

Gilbert B, Levine JM (2013) Plant invasions and extinction debts. P Natl Acad Sci USA 110:1744-1749

Gilliam FS (2006) Response of the herbaceous layer of forest ecosystems to excess nitrogen deposition. J Ecol 94:1176-1191

Gleason HA, Cronquist A (1991) Manual of Vascular Plants of Northeastern United States and Adjacent Canada. New York: The New York Botanical Garden.

Gourley LC, Howell E (1984) Factors in Buckthorn invasion documented; control measures checked (Wisconsin). Restor Manag Notes 2:87

Gourley LC (1985) A study of the ecology and spread of Buckthorn (*Rhamnus cathartica* L.) with particular reference to the University of Wisconsin Arboretum. Dissertation, University of Wisconsin, Madison, 166 pp

Grace JB (2006) Structural equation modeling and natural systems. Cambridge University Press, Cambridge

Greiner HG, Kashain DR, Tiegs SD (2012) Impacts of invasive Asian (*Amynthas hilgendorfi*) and European (*Lumbricus rubellus*) earthworms in a North American temperate deciduous forest. Biol Invasions 14:2017-2027

Griffith B, Turke M, Weisser WW, Eisenhauer N (2013) Herbivore behavior in the anecic earthworm species *Lumbricus terrestris L.*? Eur J Soil Biol 55:62-65

Groffman PM, Bohlen PJ, Fisk MC, Fahey TJ (2004) Exotic earthworm invasion and microbial biomass in temperate forest soils. Ecosystems 7:45-54

Grubb P, Lee W, Kollmann J, Wilson J (1996) Interaction of irradiance and soil nutrient supply on growth of seedlings of ten European tall-shrub species and Fagus sylvatica. J Ecol 84:827–840.

Guido A, Pillar VD (2014) Are removal experiments effective tools for assessing plant community resistance and recovery from invasion? J Veg Sci DOI: 10.1111/jvs.12248

Gundale MJ (2002) Influence of exotic earthworms on the soil organic horizon and the rare fern *Botrychium mormo*. Conserv Bio 16:1555-1561

Hale CM, Reich PB, Frelich LE (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. Am Mid Nat 151:179-185

Hale CM, Frelich LE, Reich PB (2005) Exotic European earthworm invasion dynamics in northern hardwood forests of Minnesota, USA. Ecol Appl 15:848-860

Hale CM, Frelich LE, Reich PB (2006) Changes in hardwood forest understory plant communities in response to Euopean earthworm invasion. Ecology 87: 1637-1649

Hale CM, Frelich LE, Reich PB (2008) Exotic earthworm effects on hardwood forest floor, nutrient availability and native plants: a mesocosm study. Oecologia 155:509-518

Harrington CA (1999) Forests planted for ecosystem restoration or conservation. New Forest 17:175–190.

Hartman KM, McCarthy BC (2004) Restoration of a Forest Understory After the Removal of an Invasive Shrub, Amur Honeysuckle (Lonicera maackii). Restor Ecol 12:154–165. doi: 10.1111/j.1061-2971.2004.00368.x

Heimpel GE, Frelich LE, Landis DA, et al. (2010) European buckthorn and Asian soybean aphid as components of an extensive invasional meltdown in North America. Biol Invasions 12:2913–2931. doi: 10.1007/s10530-010-9736-5

Heneghan L, Clay C, Brundage C (2002) Rapid decomposition of buckthorn litter may change soil nutrient levels. Ecol Restor 20:108–111.

Heneghan L, Rauschenberg C, Fatemi F, Workman M (2004) European buckthorn and its effects on some ecosystem properties in an urban woodland. Ecol Restor 22:275-280.

Heneghan L, Fatemi F, Umek L, et al. (2006) The invasive shrub European buckthorn (Rhamnus cathartica, L.) alters soil properties in Midwestern U.S. woodlands. Appl Soil Ecol 32:142–148. doi: 10.1016/j.apsoil.2005.03.009

Heneghan L, Steffen J, Fagen K (2007) Interactions of an introduced shrub and introduced earthworms in an Illinois urban woodland: Impact on leaf litter decomposition. Pedobiologia 50:543–551. doi: 10.1016/j.pedobi.2006.10.002

Holdsworth AR, Frelich LE, Reich PB (2007) Effects of earthworm invasion on plant species richness in northern hardwood forests. Conserv Bio 21:997-1008

Holdsworth A, Frelich L, Reich P (2008) Litter decomposition in earthworm-invaded northern hardwood forests: Role of invasion degree and litter chemistry. Ecoscience 15:536–544. doi: 10.2980/15-4-3151

Holmes P M (2001) Shrubland restoration following woody alien invasion and mining: effects of topsoil depth, seed source, and fertilizer addition. Rest Ecol 9:71-83

Hooper DU, Chapin FS, Ewel JJ, Hector A, Inchuasti P et al. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge and needs for future research. Ecological Monographs 75:3–35

Huenneke LF, Hamburg SP, Koide R, Mooney HA, Vitousek PM (1990) Effects on soil resources on plant invasion and community structure in Californian serpentine grassland. Ecology 71:478-491

Invasive Species Program (2012) Invasive species of aquatic plants and wild animals in Minnesota: Annual report for 2012. Minnesota Department of Natural Resources, St. Paul, MN USA

Kennedy TA, Naeem S, Howe KM, Knops JMH, Tilman D, Reich P (2002) Biodiversity as a barrier to ecological invasion. Nature 417:636-638

Klionsky SM, Amatangelo KL, Waller DM (2011) Above- and Belowground Impacts of European Buckthorn (Rhamnus cathartica) on Four Native Forbs. Restor Ecol 19:728–737. doi: 10.1111/j.1526-100X.2010.00727.x

Kollman J, Grubb PJ (1999) Recruitment of fleshy-fruited species under different shrub species: Control by under-canopy environment. Ecol Res 14:9-21

Knight KS (2006) Factors that influence invasion success of two woody invaders of forest understories. Dissertation, University of Minnesota, 117 pp

Knight KS, Kurylo JS, Endress AG, et al. (2007) Ecology and ecosystem impacts of common buckthorn (Rhamnus cathartica): a review. Biol Invasions 9:925–937. doi: 10.1007/s10530-007-9091-3

Kurylo J, Knight K (2007) Rhamnus cathartica: Native and naturalized distribution and habitat preferences. J Torrey Bot Soc 134:420–430.

Kurylo J, Endress A (2012) Rhamnus cathartica: Notes on Its Early History in North America. Northeast Nat 19:601–610.

Lamarque LJ, Delzon S, Lortie CJ (2011) Tree invaions: a comparative test of the dominant hypotheses and functional traits. Biol Invasions 13:1969-1989

Larson ER, Kipfmueller KF, Hale CM, Frelich LE, Reich PB (2010) Tree rings detect earthworm invasions and their effects in northern Hardwood forests. Biol Invasions 12:1053-1066

Lawrence AP, Bowers MA (2002) A test of the ‘hot’ mustard extraction method of sampling earthworms. Soil Biol Biochem 34:549-552

Lawrence B, Fisk MC, Fahey TJ, Suarez ER (2003) Influence of nonnative earthworms on mycorrhizal colonization of sugar maple (*Acer saccharum*). New Phytol 157:145-153

Levine JM, D'Antonio CM (1999) Elton revised: a review of evidence linking diversity and invasibility. Oikos 87:15-26

Levine JM, Adler PB, Yelenik SG (2004) A meta-analysis of biotic resistance to exotic plant invasions. Ecol Lett 7:975-989

Lodge DM (1993) Biological invasions - lessons for ecology. Trends Ecol Evol 8:133-137

Loss SR, Blair RB (2011) Reduced density and nest survival of ground-nesting songbirds relative to earhtowmr invasions in northern hardwood forests. Conserv Biol 25:983-992

Loss SR, Hueffmeier RM, Hale CM, Host GE, Sjerven G, Frelich LE (2013) Earthworm invasions in northern hardwood forests: a rapid assessment method. Nat Area J 33:21-30

Love JP, Anderson JT (2009) Seasonal Effects of Four Control Methods on the Invasive Morrow's Honeysuckle (*Lonicera morrowii*) and Initial Responses of Understory Plants in a Southwestern Pennsylvania Old Field. Rest Ecol 14:549-559

Luken JO, Mattimiro DT (1991) Habitat-specific resilience of the invasive shrub amur honeysuckle (*Lonicera maackii*) during repeated clipping. Ecol Appl 1:104-109

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: Causes, epidemiology, global consequences, and control. Ecol App 10:689-710

Madritch MD, Lindroth RL (2009) Removal of invasive shrubs reduces exotic earthworm populations. Biol Invasions 11:663-671.

Maerz JC, Nuzzo VA, Blossey B (2009) Declines in woodland salamander abundance associates with non-native earthworm and plant invasions. Conserv Biol 23:975-981

McCormick MK, Parker KL, Szlavecz K, Whigham DF (2013) Native and exotic earthworms affect orchid seed loss. AoB Plants DOI:10.1093/aobpla/plt018

McKinney AM, Goodell K (2010) Shading by invasive shrub reduces seed production and pollinator services in a native herb. Biol Invasions 12:2751–2763. doi: 10.1007/s10530-009-9680-4

Milcu A, Schumacher J, Scheu S (2006) Earthworms (*Lumbricus terrestris*) affects plant seedling recruitment and microhabitat heterogeneity. Func Ecol 20:261-268

Murphy GEP, Romanuk TN (2014) A meta-analysis of declines in local species richness from human disturbances. Ecol Evol 4:91-103

Nuzzo VA, Maerz JC, Blossey B (2009) Earthworm Invasion as the driving force behind plant invasion and community change in Northeastern North American Forests. Conserv Biol 23:966–974

Nyboer R (1992) Vegetation management guideline: bush honey-suckles—tatarian, Morrow's, Belle, and Amur honeysuckle (*Lonicera tatarica* L., *L. morrowii* Gray, *L. x bella* Zabel, and *L. maackii* [Rupr.] Maxim.). Nat Area J 12:218–219

Oksanen J, Guillaume Blanchet F, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Henry M, Stevens H, Wagner H (2013). vegan: Community Ecology Package. R package version 2.0-10. http://CRAN.R-project.org/package=vegan

Pergams ORW, Norton JE (2006) Treating a single stem can kill the whole shrub: a scientific assessment of buckthorn control methods. Nat Area J 26:300-309

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol Econ 52:273–288. doi: 10.1016/j.ecolecon.2004.10.002

Prati D, Bossdorf O (2004) Allelopathic inhibition of germination by Alliaria petiolata (Brassicaceae). Am J Bot 91:285–288.

Polgar C, Gallinat A, Primack RB (2014) Drivers of leaf-out phenology and their implications for species invasions: insights from Thoreau's Concord. New Phytol 202:106-115

Putz FR (1983) Treefall pits and mounds, buried seeds, and the importance of soil disturbance to pioneed trees on Barro Colorado Island, Panama. Ecology 64:1069-1074

Regnier E, Harrison SK, Liu J, et al. (2008) Impact of an exotic earthworm on seed dispersal of an indigenous US weed. J Appl Ecol 45:1621–1629. doi: 10.1111/j.1365-2664.2008.01489.x

Ricciardi A, Hoopes MF, Marchetti MP, Lockwood JL (2013) Progress toward understanding the ecological impacts of nonnative species. Ecol Monogr 83:263-282

Richardson DM, Pysek P, Rejmanek M, Barbour MG, Panetta FD, West CJ (2000) Naturalization and invasion of alien plants: concepts and definitions. Divers Distrib 6:93-107

Roberts DW (2013) labdsv: Ordination and Multivariate Analysis for Ecology. R package version 1.6-1. http://CRAN.R-project.org/package=labdsv

Roth AR, Whitfeld TJS, Lodge AG, Eisenhauer N, Frelich LE, Reich PB. (2014) Invasive earthworms interact with abiotic conditions to influence the invasion of common buckthorn (*Rhamnus cathartica*). Oecologia DOI 10.1007/s00442-014-3175-4

Runkle JR, DiSalvo A, Graham-Gibson Y (2007) Vegetation release eight years after removal of Lonicera maackii in West-Central Ohio. Ohio J Sci 105:125-129

Sacerdote AB, King RB (2014) Direct effects of an invasive European buckthorn metabolite on embryo survival and development in *Xenopus laevis* and *Pseudacris triseriata*. J Herpetol 48:51-58

Sackett TE, Smith SM, Basiliko N (2013) Indirect and direct effects of exotic earthworms on soil nutrient and carbon pools in North American temperate forests. Siol Biol Biochem 57:459-476

Schindler DW (1998) Replication versus realism: The need for ecosystem-scale experiments. Ecosystems 1:323-334

Schmidt K, Whelan C (1999) Effects of exotic Lonicera and Rhamnus on songbird nest predation. Conserv Biol 13:1502–1506.

Schramm JW, Ehrenfeld JG (2010) Leaf litter and understory canopy shade limit the establishment, growth and reproduction of *Microstegium vimineum*. Biol Inva 12:3195-3204

Seltzner S, Eddy TL (2003) Allelopathy in *Rhamnus cathartica*, European Buckthorn. The Michigan Botanist 42:51-61

Shartell LM, Lilleskov EA, Storer AJ (2013) Predicting exotic earthworm distribution in the northern Great Lakes region. Biol Invasions 15:1665-1675

Shea K, Chesson P (2002) Community ecology theory as a frame-work for biological invasions. Trends Ecol Evol 17:170-176

Simberloff D, Von Holle B (1999) Positive interactions of nonindigenous species: invasional meltdown? Biol Invasions 1:21-32

Sinclair ARE, Hik DS, Schmitz OJ, Scudder GGE, Turpin DH, Larter NC (1995) Biodiversity and the need for habitat renewal. Ecol Appl 5:579–587

Stewart JR, Graves WR (2004) Photosynthesis and Growth of Rhamnus caroliniana during Drought and Flooding : Comparisons to the Invasive Rhamnus cathartica. Hortscience 39:1280–1284.

Stinson KA, Campbell SA, Powell JR, Wolfe BR, Callaway RM, Thelen GC, Hallett SG, Prati D, Klironomos JN (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLOS Biol DOI: 10.1371/journal.pbio.0040140

Swab RM, Zhang L, Mitsch WJ (2008) Effect of hydrologic restoration and Lonicera maackii removal on herbaceous understory vegetation in a bottomland hardwood forest. Rest Ecol 16:453-463

Syracuse Environmental Research Associates (SERA) (1996) Selected commercial formulations of tyiclopyr – Garlon 3a and Garlon 4 risk assessment, Final report. Prepared for USDA Forest Service. Report # SERA TR 95-22-02-02a. http://www.fs.fed.us/r5/hfqlg/publications/herbicide_info/1996b_triclopyr.pdf

Theoharides KA, Dukes JS (2007) Plant invasion across space and time: Factors affecting nonindigenous species success during four stages of invasion. New Phytol 176:256-273

Thuiller W, Richardson DM, Rouget M, Proches S, Wilson JRU (2006) Interactions between environment, species traits, and human uses describe patterns of plant invasions. Ecology 87:1755-1769

Traba J, Azcarate FM, Peco B (2004) From what depth do seeds emerge? A soil seed bank experiment with Mediterranean grassland species. Seed Sci Res 14:297-303

Travaset A, Richardson DM (2014) Mutualistic interactions and biological invasions. Annu Rev Ecol Evol Syst 45:89-113

USDA, NRCS (2014) The PLANTS Database (http://plants.usda.gov, 4 June 2014). National Plant Data Team, Greensboro, NC USA

Vidra RL, Shear TH, Stucky JM (2007) Effects of vegetation removal on native understory recovery in an exotic-rich urban forest. J Torrey Bot Soc 134:410-419

Vila M, Espinar JL, Hejda M, Hulme PE, Jarosik V, Maron JL, Pergl J, Schaffner U, Sun Y, Pysek P (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities, and ecosystems. Ecol Lett 14:702-708

Vitousek PM, D'Antonio CM, Loope LL, Westbrooks R (1996) Biological invasions as global environmental change. Ame Sc 84:468-478

Warren RJ, Bahn V, Bradford MA (2012) The interaction between propagule pressure, habitat suitability and density-dependent reproduction in species invasion. Oikos 121:874-881

Whitfeld TJS, Lodge AG, Roth AM, Reich PB (2014a) Community phylogenetic diversity and abiotic site characteristics influence abundance of the invasive plant *Rhamnus cathartica* L. J Plant Ecol 7:202-209

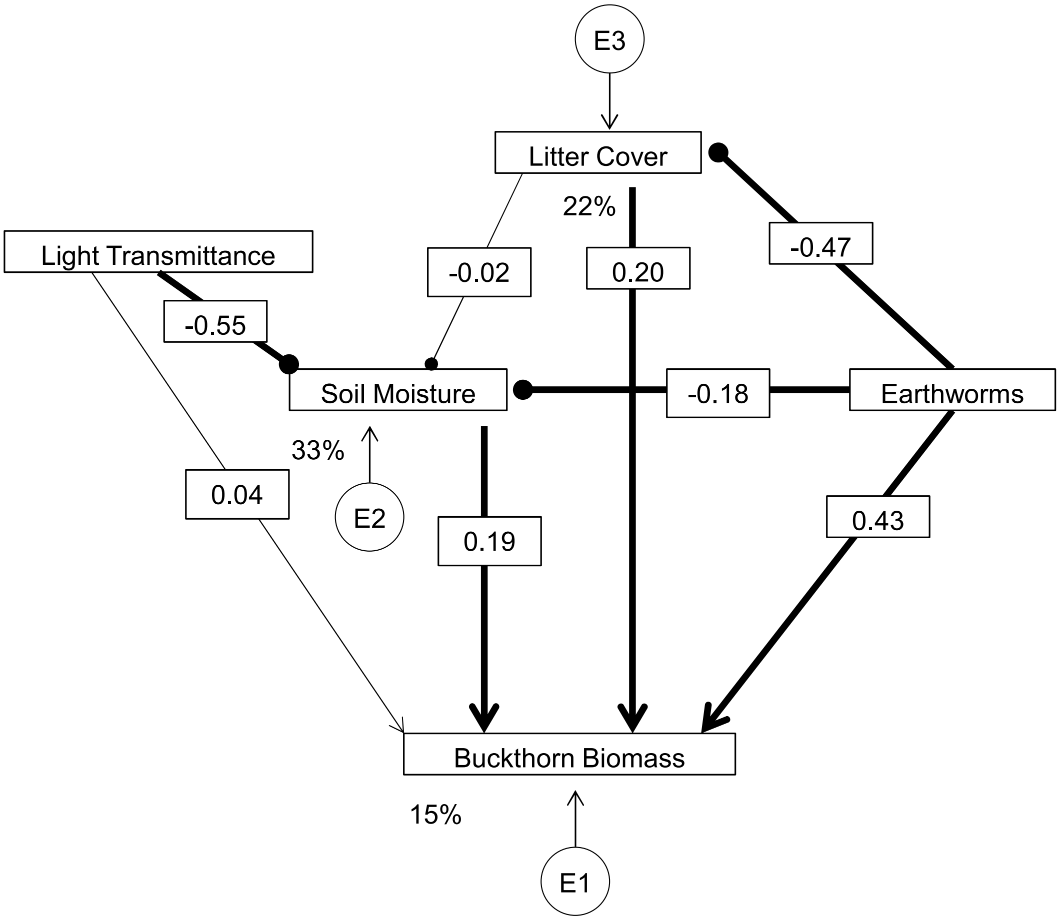
Whitfeld TJS, Roth AM, Lodge AG, Eisenhauer N, Frelich LE, Reich PB (2014b) Resident plant diversity and introduced earthworms have contrasting effects on the success of invasive plants. Biol Invasions DOI:10.1007/s10530-014-0657-6

Williamson M, Fitter A (1996) The varying success of invaders. Ecol 77:1661-1666

Yeo HHT, Chong KY, Yee ATK, Giam X, Corlett RT, Tan HTW (2014) Leaf litter depth as an important factor inhibiting seedlings establishment of an exotic palm in tropical secondary forest patches. Biol Invasions 16:381-392

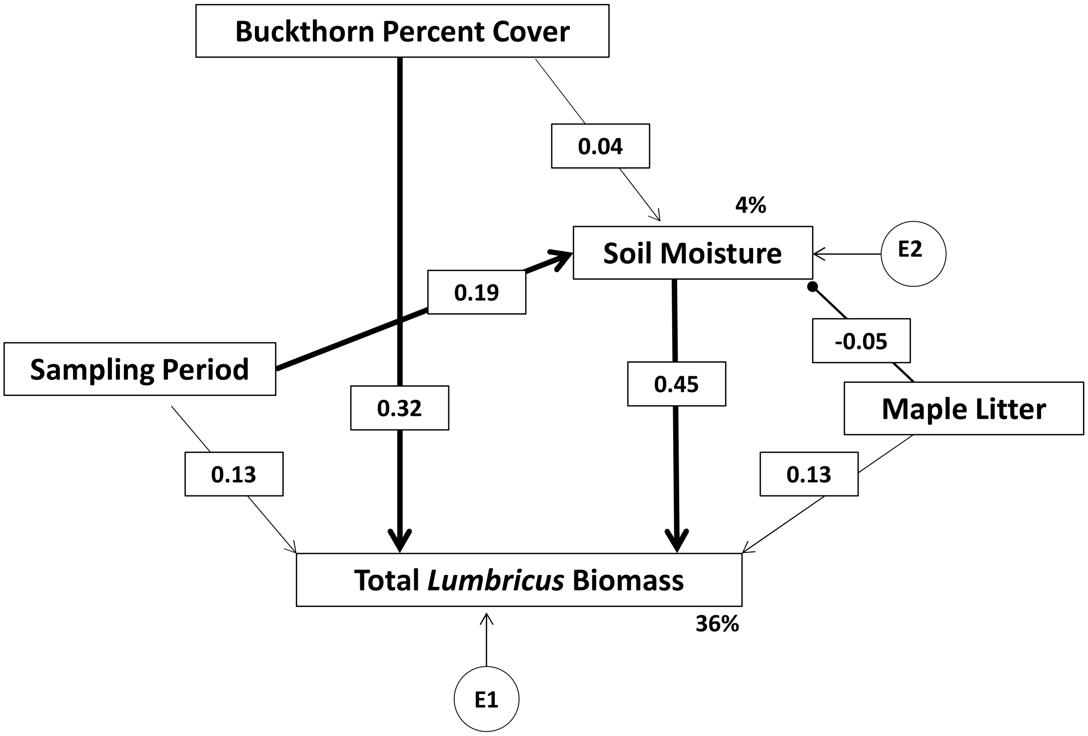
Appendix 1: Supplementary material for chapter 1

Appendix. S1. Initial path analysis model displaying causal influences of experimental treatments on buckthorn biomass. Numbers on arrows are standardized path coefficients. Lines ending in arrows indicate positive relationships and lines ending in dots indicate negative relationships. Bold lines are significant ($P < 0.05$), while non-bolded lines are not significant ($0.72 < P < 0.77$). Circles indicate error terms (E1-E3). The overall model fit the data ($\chi^2_4 = 1.23$, probability level = 0.54, AIC = 27.23, NFI = 0.98; CMIN = 1.24; RMSEA = 0) but was improved upon by the final model.

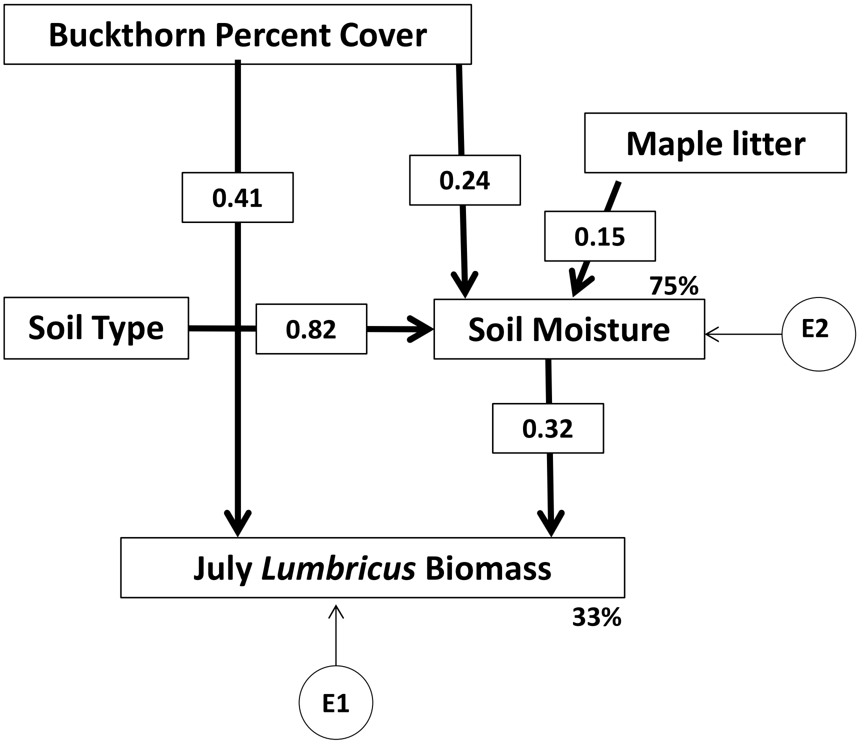


Appendix 2: Supplementary material for chapter 2

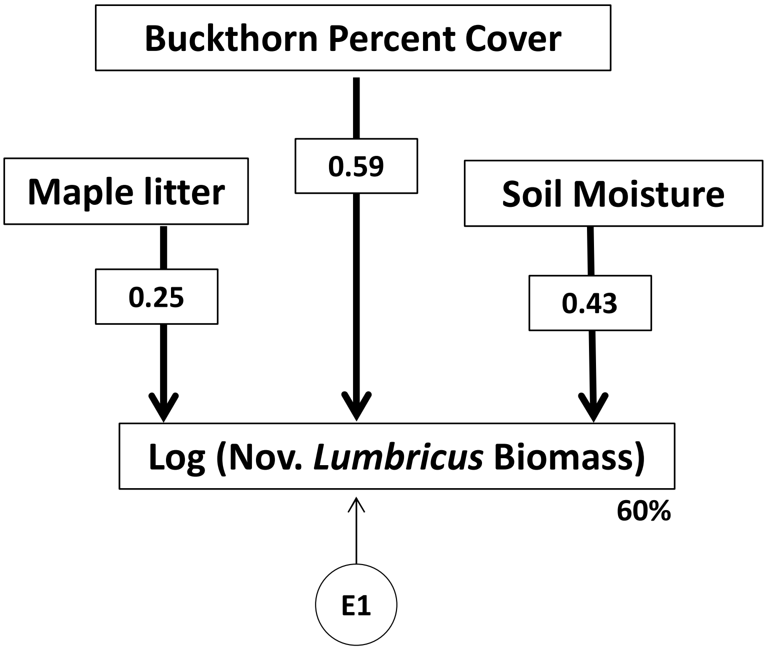
Appendix. S2. A. Initial path analysis model displaying causal influences of experimental treatments on buckthorn biomass. Numbers on arrows are standardized path coefficients. Lines ending in arrows indicate positive relationships and lines ending in dots indicate negative relationships. Bold lines are significant ($P < 0.05$), while non-bolded lines are not significant ($0.06 < P < 0.72$). Circles indicate error terms (E1-E3). The overall model fit the data ($\chi^2_3 = 0.54$, $p=0.91$; AICc=24.54) but was improved upon by the final model.



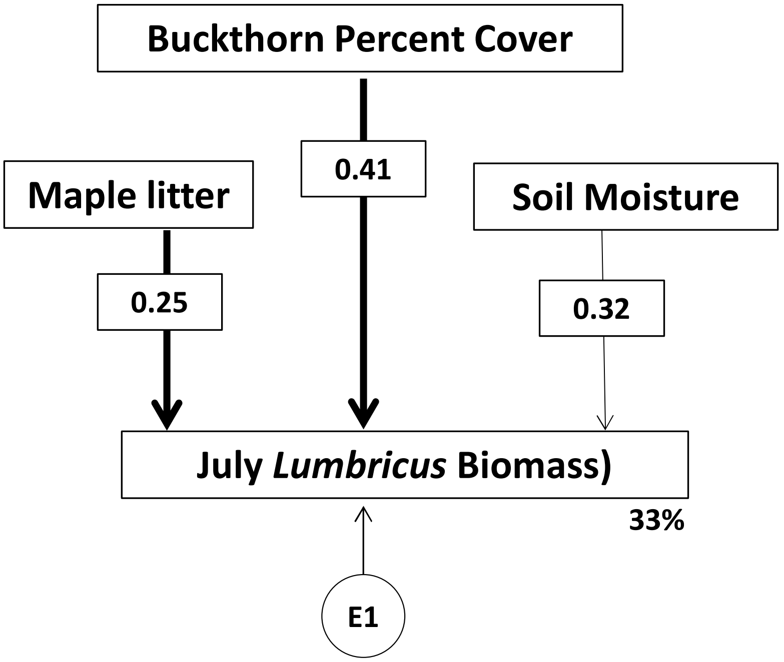
Appendix. S2. B. Path analysis model displaying causal influences of buckthorn and other environmental variables on earthworm biomass for the July 2012 sampling period. Numbers on arrows are standardized path coefficients. Lines ending in arrows indicate positive relationships and lines ending in dots indicate negative relationships. Bold lines are significant ($P < 0.05$). Circles indicate error terms (E1-E2). The final model fit the data: Chi-square = 6.18, probability level = 0.24, AIC = 36.18.



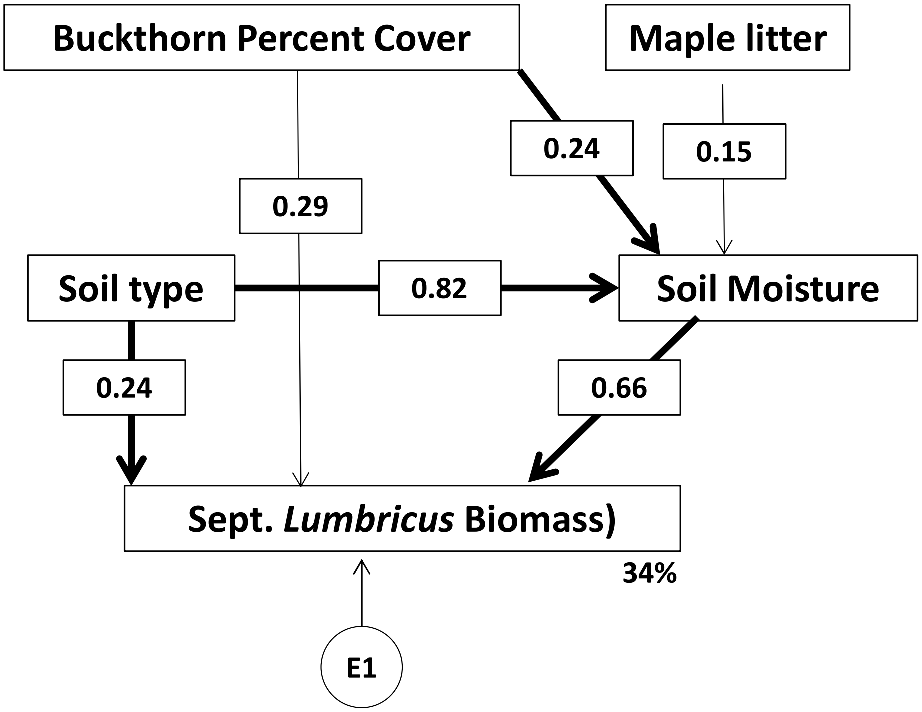
Appendix. S2. C. Path analysis model displaying causal influences of buckthorn and other environmental variables on earthworm biomass for the November 2012 sampling period. Numbers on arrows are standardized path coefficients. Lines ending in arrows indicate positive relationships and lines ending in dots indicate negative relationships. Bold lines are significant ($P < 0.05$). Circles indicate error terms (E1-E2). The final model fit the data: Chi-square = 0.44, probability level = 0.93, AIC = 22.44.



Appendix. S2. D. Path analysis model displaying causal influences of buckthorn and other environmental variables on earthworm biomass for the July 2013 sampling period. Numbers on arrows are standardized path coefficients. Lines ending in arrows indicate positive relationships and lines ending in dots indicate negative relationships. Bold lines are significant ($P < 0.05$), while non-bolded lines are not significant ($P=0.134$). Circles indicate error terms (E1-E2). The final model fit the data: Chi-square = 0.30, probability level = 0.96, AIC = 22.30.

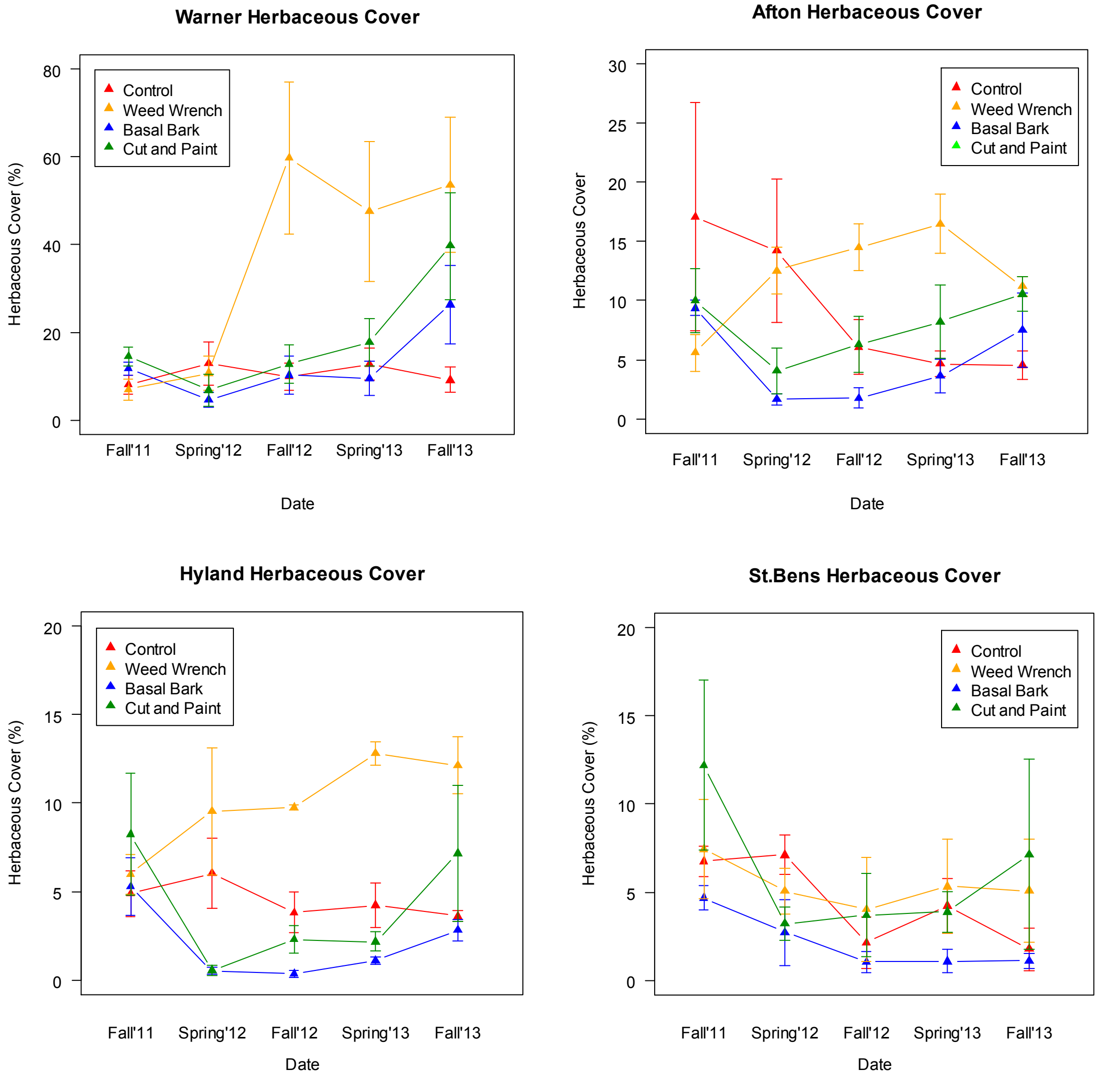


Appendix. S2. E. Path analysis model displaying causal influences of buckthorn and other environmental variables on earthworm biomass for the September 2013 sampling period. Numbers on arrows are standardized path coefficients. Lines ending in arrows indicate positive relationships and lines ending in dots indicate negative relationships. Bold lines are significant ($P < 0.05$), while non-bolded lines are not significant ($0.112<P<0.138$). Circles indicate error terms (E1-E2). The final model fit the data: Chi-square = 2.43, probability level = 0.66, AIC = 34.43.

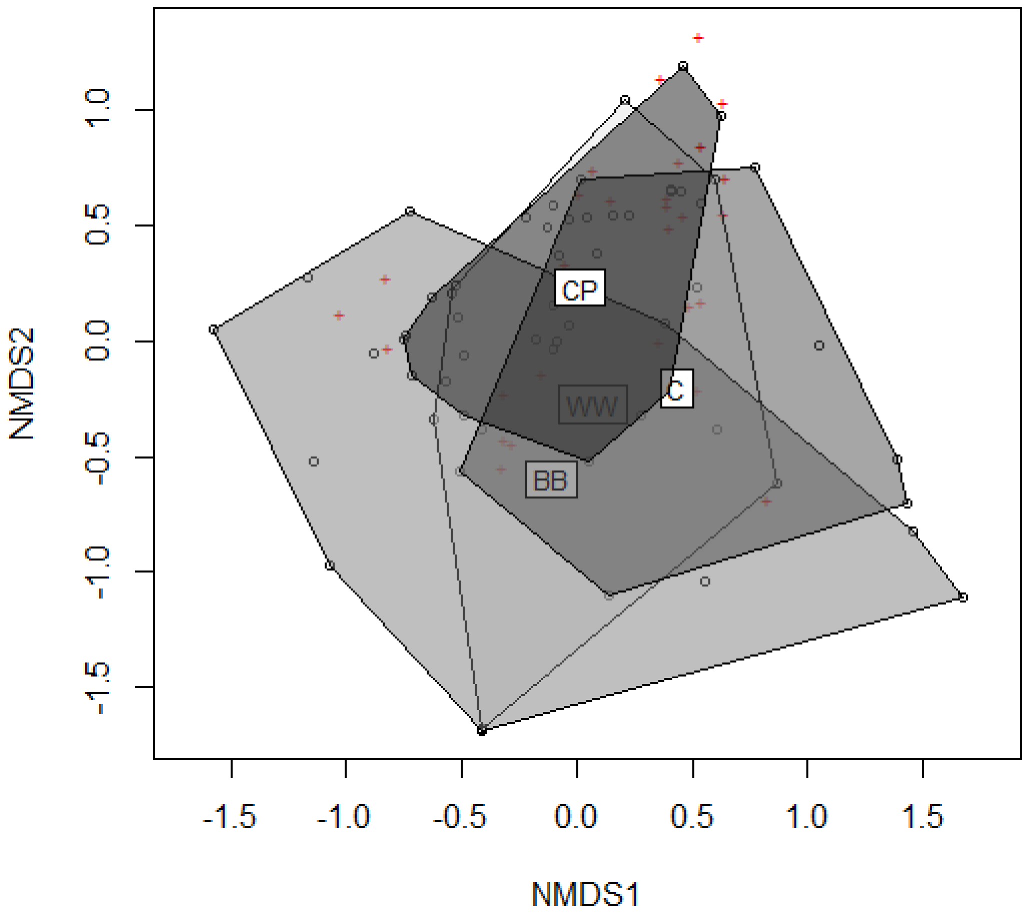


Appendix 3: Supplementary material for chapter 3.

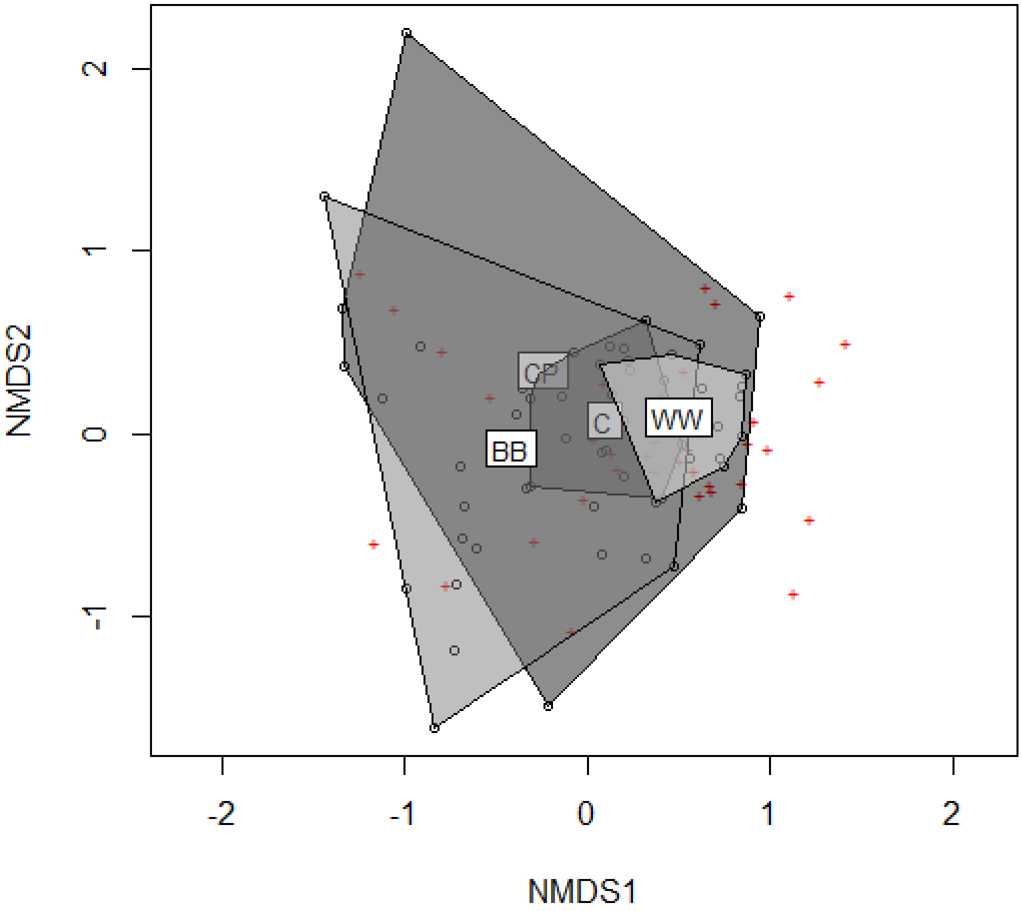
Appendix S3.A. Treatment effects on herbaceous species cover over time at all four removal sites. Buckthorn (*Rhamnus cathartica*) removal occurred after the fall 2011 survey. Error bars are standard error.



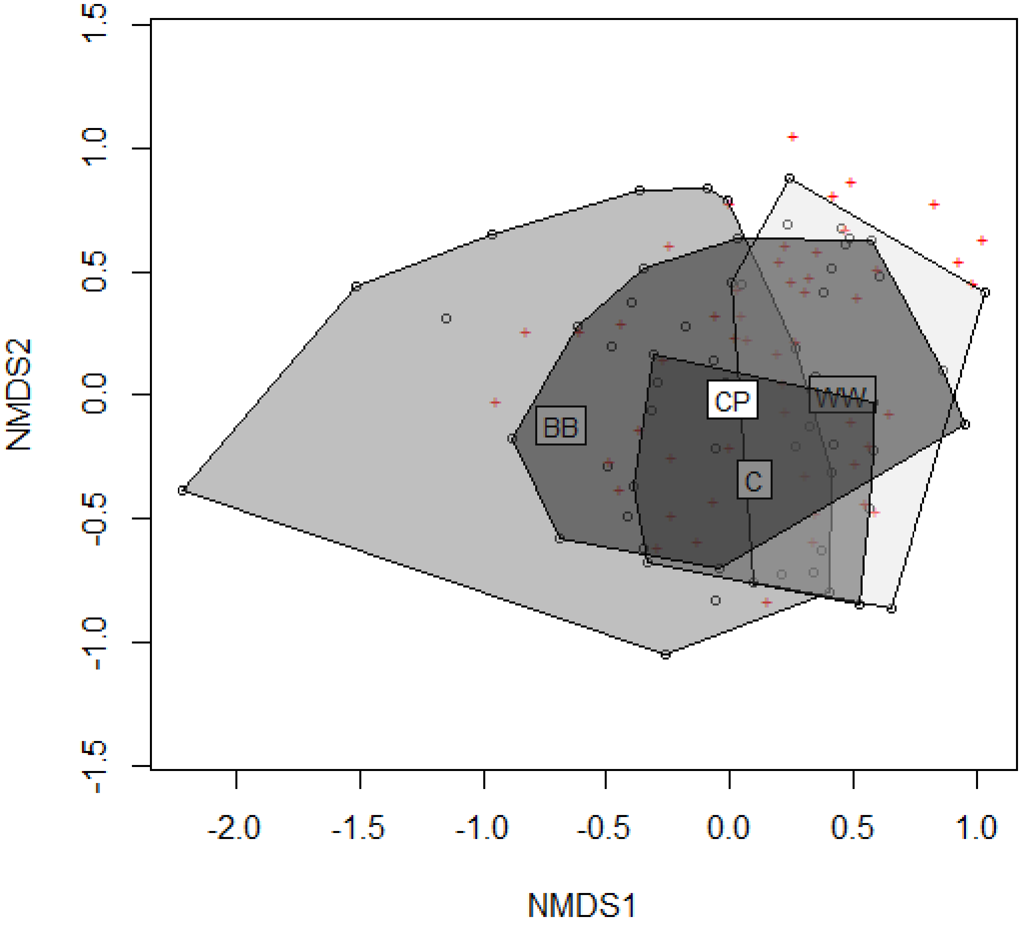
Appendix S3.B. NMDS ordination solution of herbaceous species in buckthorn removal plots at St. Benedict's Monastery (Stress=0.14). The treatments occupy statistically different areas of the 3D species space (p=0.028). C = Control, CP = Cut and Paint, BB = Basal Bark, WW = Weed Wrench



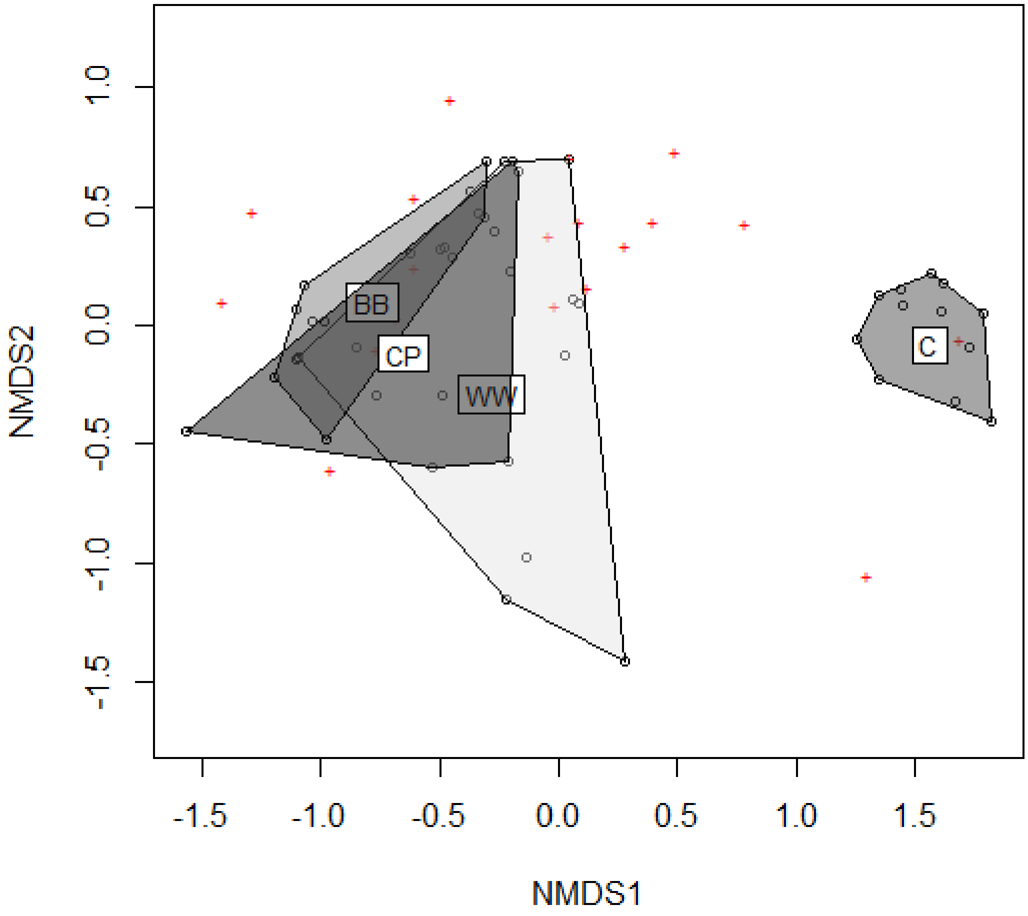
Appendix S3.C. NMDS ordination solution of herbaceous species in buckthorn removal plots at Hyland Lake Park Reserve (Stress=0.14). The treatments occupy statistically different areas of the 3D species space (p=0.001). C = Control, CP = Cut and Paint, BB = Basal Bark, WW = Weed Wrench



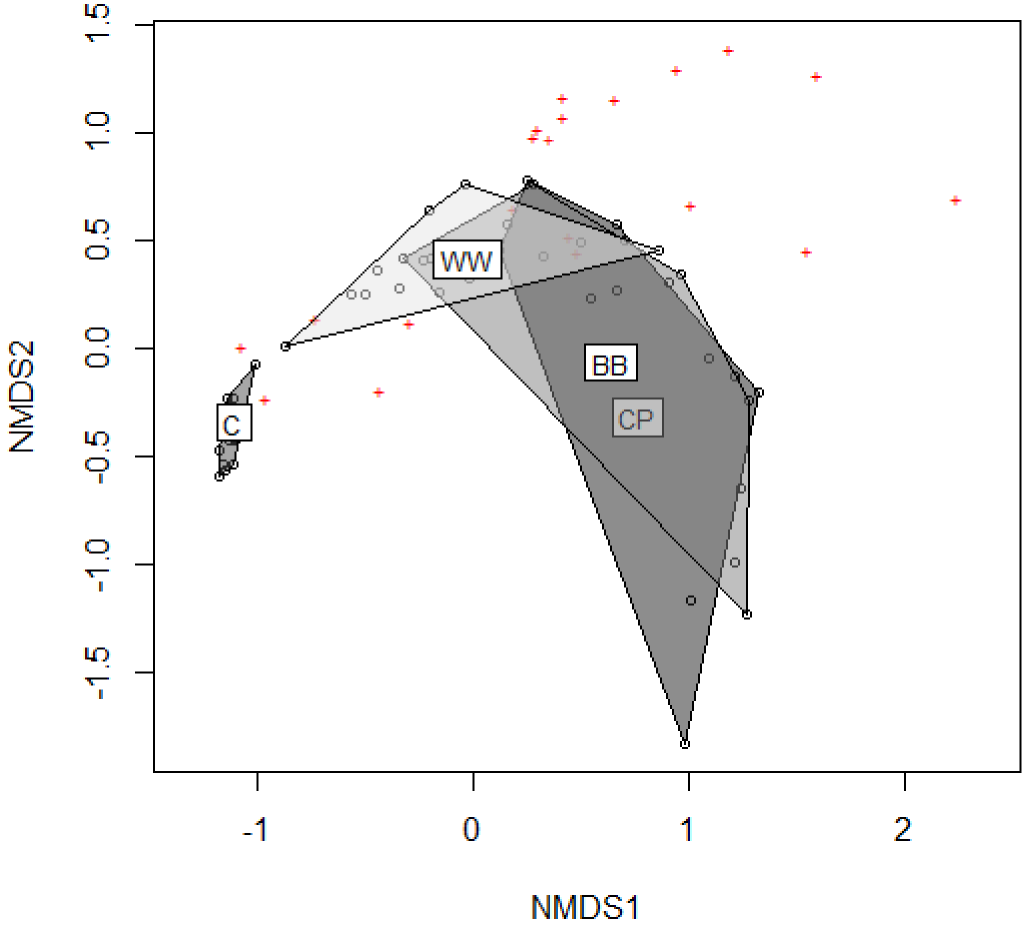
Appendix S3.D. NMDS ordination solution of herbaceous species in buckthorn removal plots at Afton State Park (Stress=0.16). The treatments occupy statistically different areas of the 3D species space (p=0.001). C = Control, CP = Cut and Paint, BB = Basal Bark, WW = Weed Wrench



Appendix S3.E. NMDS ordination solution of woody species in buckthorn removal plots at St. Benedict's Monastery (Stress=0.07). The treatments occupy statistically different areas of the 3D species space (p=0.001). C = Control, CP = Cut and Paint, BB = Basal Bark, WW = Weed Wrench



Appendix S3.F. NMDS ordination solution of woody species in buckthorn removal plots at Hyland Lake Park Reserve (Stress=0.06). The treatments occupy statistically different areas of the 3D species space (p=0.001). C = Control, CP = Cut and Paint, BB = Basal Bark, WW = Weed Wrench



Appendix S3.G. NMDS ordination solution of woody species in buckthorn removal plots at Afton State Park (Stress=0.06). The treatments occupy statistically different areas of the 3D species space (p=0.001). C = Control, CP = Cut and Paint, BB = Basal Bark, WW = Weed Wrench