M.L. 2010, Chp. 362, Sec. 2, Subd. 6c - Healthy Forests Project Abstract
For the Period Ending June 30, 2012

PROJECT TITLE: Healthy Forests to Resist Invasion
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2010, Chp. 362, Sec. 2, Subd. 6c - Healthy Forests

APPROPRIATION AMOUNT: $359,000

Overall Project Outcome and Results

The primary project goal was to identify forest characteristics effective as deterrents to invasive plants. Healthy forests are likely more resistant to invaders, so management to enhance these key characteristics might slow the spread of invaders.

Invasive plants sometimes form dense thickets that affect recreation and wildlife and exclude native plant species. To determine how various site characteristics affected the abundance of common buckthorn and other invaders, we surveyed plant diversity in 67 sites in central and southern Minnesota. At each site, we measured environmental characteristics to simultaneously account for other factors that might influence invasibility. Buckthorn was most abundant in sites with sparse leaf litter, where seed availability was high, and where native plant diversity was low. Both a greenhouse experiment and a second field study indicated that introduced earthworms also benefit germinating invasive plants by eliminating leaf litter.

We propose the idea of "preventive environmental care" that, like preventative medicine, manages forests to maintain "wellness." Although not a panacea for reducing invasion, it is worth considering given the challenges of controlling established invasive species. We suggest managers enhance the competitive challenge to invaders by increasing the diversity of native species by seeding natives and/or reducing the density of white-tailed deer, a species that severely impacts native forest plants. Furthermore, timber harvests should be limited to the winter season and trail maintenance should be done in a way that limits disturbance. This will help maintain intact native understory plants and litter layers, important deterrents to invasive plant establishment. However, none of these approaches are likely to be successful without a strong effort to control landscape level seed availability. Collaborative management with neighboring landowners is crucial to any effort that hopes to reduce invasibility.

Project Results Use and Dissemination

To summarize results from the project and provide guidelines for management, we prepared a pamphlet that included all aspects of the research, as it pertains to the invasion of buckthorn. The pamphlet also provides suggestions for pre-invasion management to reduce invasibility, the main focus of the "Healthy Forests" research project. We distributed the pamphlet to all participants at a symposium held on August 14, 2013. The pamphlet is available as a pdf from the project website, http://forestecology.cfans.umn.edu/Research/Buckthorn/index.htm

We presented talks at the Upper Midwest Invasive Species conference (a regional meeting focused on invasive species) and the Ecological Society of America conference (an international conference focusing on all aspects of ecology) in 2012 and 2013. The talks focused on measuring propagule pressure, the greenhouse study, the relationship between earthworm and buckthorn buckthorn, and the effects of native species diversity on buckthorn abundance.

On August 14, we hosted a symposium on the St. Paul campus that brought together managers, researchers, and private landowners to share the latest information on invasive plants in Minnesota forests. In addition to talks based on this LCCMR project, other speakers presented information about buckthorn invasion on the prairie-forest border in west central Minnesota, garlic mustard (another common plant invader in Minnesota's forests) as a driver of species invasion, management of buckthorn from a forester's perspective, and management efforts to control other common invasive plants. The symposium was attended by 100 people. The project website has links to recordings of all the symposium talks, as well as links to the MS Access database, species lists from all survey sites, and a photo gallery.

We have published one paper ("Community phylogenetic diversity and abiotic site characteristics influence abundance of the invasive plant *Rhamnus cathartica* L.") in the *Journal of Plant Ecology*. A second paper based on results from our greenhouse experiment ("Native plant diversity and introduced earthworms have contrasting effects on the success of invasive plants") has been submitted to the peer-reviewed journal *Biological Invasions*. More papers are in preparation including one focusing on propagule pressure and another that documents the relationship between earthworms and buckthorn abundance.

Environment and Natural Resources Trust Fund (ENRTF) 2010 Work Program Final Report

Date of Progress Report: August 15, 2013
Final Report
Date of Work Program Approval: June 6, 2012
Project Completion Date: June 30, 2013

I. PROJECT TITLE: Healthy Forests to Resist Invasion

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Location: Regions: Northeast, Central, Southeast **Counties:** Aitkin, Anoka, Becker, Beltrami, Benton, Carlton, Carver, Cass, Chisago, Clearwater, Cook, Crow Wing, Dakota, Dodge, Douglas, Faribault, Freeborn, Goodhue, Hennepin, Houston, Hubbard, Isanti, Itasca, Kanabec, Kandiyohi, Koochiching, Lake, Lake of the Woods, Le Sueur, Meeker, Mille Lacs, Morrison, Mower, Nicollet, Olmsted, Otter Tail, Pine, Ramsey, Rice, Roseau, Scott, Sherburne, Sibley, St. Louis, Steele, Todd, Wabasha, Wadena, Waseca, Washington, Winona, Wright

See Map (Figure 1.)

Total ENRTF Project Budget:	**ENRTF Appropriation**	**$** 359,000
	Minus Amount Spent:	**$** 335,664
	Equal Balance:	**$** 23,336

Legal Citation: M.L. 2010, Chp. 362, Sec. 2, Subd. 6c

Appropriation Language:
$359,000 is from the trust fund to the Board of Regents of the University of Minnesota to assess the role of forest health management in resisting infestation of invasive species. This appropriation is available until June 30, 2013, by which time the project must be completed and final products delivered.

II. FINAL SUMMARY AND RESULTS:

The primary goal of this project was to identify which forest characteristics are effective deterrents to invasive plants. Healthy forests are likely to be more resistant to invaders so management to enhance these key characteristics might slow the spread of forest invaders. This pre-invasion strategy is likely to be more effective and economical than post-invasion removal of invasive species.

Invasive plants form dense thickets that affect recreation and wildlife, exclude many native plant species, and reduce overall diversity. To determine how various site characteristics affected the abundance of common buckthorn, an abundant invasive plant in Minnesota’s forests, we surveyed plant diversity in 67 sites in central and southern Minnesota. At each site, we also measured environmental characteristics to simultaneously account for a wide range of factors that might influence invasibility. Buckthorn was most abundant in sites with a sparse layer of leaf litter, where seed availability was high, and where native plant diversity was low. A greenhouse experiment indicated that the presence of introduced earthworms also benefited germinating invasive plants. A second field study reinforced this possible facilitation between buckthorn and invasive earthworms and also suggested that earthworms decrease leaf litter.

We propose the idea of “preventive environmental care” that, like preventative health care, manages forests to maintain “wellness.” Although not a panacea for reducing invasion, it is worth considering given the challenges of controlling established invasive species. Our results suggest managing for enhanced diversity is an important pre-invasion technique. Examples of management techniques to achieve this include controlling the density of white-tailed deer, a species that severely decreases plant growth when populations are high, and limiting disturbance by using winter timber harvest and low impact trail construction techniques. An intact litter layer also appears to be particularly important and reducing landscape-scale seed availability of invasive plants through collaborative management is also critical.

III. PROGRESS SUMMARY AS OF April 19, 2011

In the summer of 2010, ten study sites were selected after consulting with regional DNR foresters and other landowners. At each of these sites, plots were established and data on vegetation, light levels, and disturbance history were collected. Additionally, soil samples were taken from each plot for analysis in the laboratory. In spring 2011, additional candidate sites are being selected and will be visited beginning in May. One new graduate student began working on the project in early 2011 (Alex Roth). Alex will be participating in the broad project and will also be focusing on assessing how different invasive species removal methods affect the environmental conditions that regulate invasive plant colonization. Alexandra Lodge, the graduate student who has been working on this project since June 2010, is continuing to participate in the broad project as well as focusing her research on how white-tailed deer may facilitate plant invasions. Each of these students plans to follow the currently-established project protocol as well as conduct additional data collection at a subset of the research sites that will further contribute to determining the links between forest attributes and plant invasion. An advertisement for a post-doc was posted in March 2011 and a candidate will be selected soon to join the team working on this project.

PROGRESS SUMMARY AS OF September 21, 2011

During the summer of 2011, 31 study sites were selected and surveyed after consulting with regional DNR foresters, wildlife managers, and other landowners. Twenty-two of these sites have experienced no recent disturbance; six sites have been harvested for timber; and three sites have been managed for invasive plants. At each of these sites, sixteen plots were established and detailed data on vegetation, light levels and disturbance history were collected. Additionally, soil samples were taken from each plot for pH and soil texture analysis in the laboratory. An additional 10 sites have been identified for future survey, with more sites planned to be selected in winter 2011. We will begin analyzing data collected in 2010 and 2011 (a total of 41 sites surveyed thus far) during the fall/winter 2011.

Four sites have been identified from general surveys for a manipulative experiment investigating how different invasive plant removal methods affect the environmental conditions that regulate invasive plant colonization. At all of these sites, we have set up plots and surveyed vegetation. In late September/early October, buckthorn will be removed from the study plots using a variety of common management methods. Vegetation response and changes in environmental characteristics will be monitored at three different times during the growing season for the next three years.

As part of a study comparing the functional plant traits (such as growth form, specific leaf area, seed size, etc.) of buckthorn and other invasive plants to those of native plants, we have also collected over 350 leaf samples from a number of our study sites (with another 150 or so anticipated in the next two weeks). We have measured leaf area on these samples and will weigh them and measure foliar nitrogen. We are also in the process of acquiring additional plant trait information on these species from international plant trait databases.

A post-doc, Timothy Whitfeld, joined the project team in June 2011. His expertise in Minnesota flora identification has been a great addition to the project. Two field assistants were hired for the 2011 field season. They assisted in data collection over the summer. Undergraduate research assistants will be employed during the academic year to assist in soil and leaf processing and analysis, and two more field assistants in the summer of 2012.

PROGRESS SUMMARY AS OF January 10, 2012

Considerable progress (see laboratory analyses and data analyses paragraphs below) has been made in fall/winter 2011/2012 from field data collection. We have preliminary evidence that suggests that higher native plant diversity does reduce the abundance of European buckthorn. We have also realized that sites of the appropriate stand age, soil type, climate zone, and with high numbers of invasive species propagules that would be most useful in testing effects of certain management practices and natural disturbances are rare. Thus, we concluded that our research objectives could be better met by establishing a lower total number of descriptive sites than originally envisioned and instead also use a number of manipulative studies and sites. In such direct manipulative studies we can employ specific management practices ourselves and also directly add propagules of numerous species rather than rely on natural dispersal. During the fall/spring of 2011/2012 period, three additional components were (or will be) added to the project: buckthorn removal, a greenhouse study of invasive establishment, and a field study of invasive establishment. All of the new components are outlined and justified below.

Buckthorn removal

In order to more fully examine whether disturbed communities have characteristics that make them more susceptible to invasion than the non-disturbed systems, we decided to add another disturbance 'treatment'. In addition to blowdown and timber harvest sites, we are including invasive species removal as a disturbance type. Invasive removal as a disturbance has received little attention, but as it is a management strategy employed by a variety of public and private agencies, it deserves investigation. In particular, considerable public and private funds are spent on such management, with little documented evidence as to the success or failure of these approaches.

We set up a total of 48 invasive species removal plots in buckthorn-dominated forest stands and used three common removal techniques to remove the invasive biomass (plus a control treatment where nothing was done). Tracking the changes in abiotic conditions such as light, bare soil availability, nutrient availability as well as changes in the earthworm and plant community, will allow us to mechanistically see how each removal technique changes the environment and whether those changes lead to re-invasion by buckthorn or regeneration of native species.

The three buckthorn removal treatments were weed-wrenching, basal bark herbicide application, and biomass removal using the cut-and-paint technique. Treatments were applied at four sites during October and early November, 2011. Each site contained twelve 6x6 meter plots, and each plot was randomly assigned to a different treatment type. At each site, three plots had no removal, three had removal using the cut-and-paint technique, three had removal using manual pulling/weed wrenching, and three left dead standing biomass using a basal bark application.

Greenhouse experiment

Several generations of ecologists have tested the hypothesis that high diversity of native plants decreases the likelihood of invasion by non-native plants (Elton 1958). Some studies support the hypothesis (Case 1990, Tilman 1997) whereas others do not (Robinson et al. 1995, Palmer and Maurer 1997). Given this lack of consensus, it is hard to predict whether native plant diversity will be an important factor in determining the invasibility of Minnesota's forests. Our field study addresses the question by examining the relationship between site level diversity and invasive species' abundance. However, the potential confounding effects of other environmental factors such as soil nutrients and propagule pressure make it difficult to directly answer this question.

A controlled greenhouse experiment, that holds constant environmental conditions while varying plant diversity, directly addresses the question of whether more native plant species leads to lower invasibility. This dual approach that uses manipulative experiments to test hypotheses based on patterns seen in the field is a robust way to investigate potential mechanisms. Our greenhouse experiment will be unique because we use forest species, where most similar studies include only grassland plants. We also will test the effects of earthworms and light levels on invasion success. We include Minnesota plants that were common in our field surveys which will make the results directly relevant to management recommendations for local forests.

Soil for the experiment was collected from Warner Nature Center, buckthorn seeds were locally collected, and the seeds of native species were purchased from Prairie Moon Nursery. All seeds were planted in November and the experimental treatments will begin in mid January.

Field experiment

In addition to the role of species diversity, many other factors influence the success of invasive propagules in establishing new juveniles in forests. Among these are environmental factors such as light, nutrient supply, temperature, rainfall, and soil moisture; as well as competition, facilitation, or herbivory from neighboring plants and animals. We will make use of an existing experiment in northern Minnesota, that both monitors and manipulates (directly or indirectly) many of these factors to test the ability of a number of different non-native species to colonize forests from seed, and how each of the environmental and biotic factors listed above influences that success. The experiment is located in native forest and includes a total of 72 plots. This research will help us characterize how different management strategies may exacerbate or ameliorate the invasion process. As an illustration, we might hypothesize that microsites with higher light availability, warmer temperatures, higher soil moisture, modest competition from a low diversity set of neighbors lacking in conifers will be most advantageous for invasives. If such a scenario turns out to be true, sites could be managed to ensure the best species mix and right canopy closure to minimize invasive success, with invasive control strategies particularly emphasized under conditions most conducive to invasives. For example, if planting dense conifer canopies lowers soil pH, reduces earthworms, light and water availability, and temperatures at the forest floor, and in the process reduces invasive success, such strategies could be more widely recommended. Note: the above is merely given as an example of the kind of outcomes, and not a specific prediction of what we will find.

Laboratory analyses

Soil analyses were performed on samples collected from 41 sites surveyed in 2010 and 2011. Composited samples from the 16 plots at each site were analyzed for pH and soil texture.

Additionally, we received requested records from the TRY plant traits database (try-db.org) and have been compiling trait information on the 230 plant species identified in our sites. In order to use locally-measured values for certain traits that have been shown to vary regionally, we plan to measure specific leaf area, leaf carbon, and leaf nitrogen in leaves collected from species in our sites. One hundred forty four leaf samples were ground and sent to the Ecosystem Analysis Laboratory at the University of Nebraska for carbon and nitrogen analyses this fall. We received these results recently and have added them to our traits database. We plan to collect leaves from the remaining 130 species next summer for analysis.

Data analysis
We generated a phylogenetic tree summarizing the evolutionary relationships between all 275 plant species growing in our survey plots. This tree forms the basis of analysis to investigate the effect of plant diversity on the abundance of invasive species in Minnesota's forests. Preliminary analysis suggests that higher native plant diversity does reduce the abundance of European buckthorn. There is also a strong positive effect of propagule pressure on the abundance of buckthorn. These results will be included in a paper that is in preparation for publication in a peer review journal highlighting site level characteristics that influence buckthorn abundance.

PROGRESS SUMMARY AS OF May 31, 2012

Amendment Request (05/31/2012):

Our original research proposal focused on identifying the causes of buckthorn invasion into Minnesota forests. Through this work amendment proposal, we also aim to study some of the consequences of buckthorn invasion. Specifically we aim to determine how the presence and abundance of buckthorn influences the nitrogen cycle in Minnesota forests.

Our interest in the nitrogen cycle stems primarily from two common scientific observations. First, the productivity, i.e. growth, of forests is often positively correlated with rates of nitrogen input and nitrogen recycling in soil (Zak et al. 1989, Reich et al. 1997). Second, nitrogen outputs from forest ecosystems, including nitrate in groundwater and surface water as well as nitrous oxide in the air, can have important consequences for water quality, atmospheric chemistry, and climate (Galloway et al. 2004). Due to the unusually high concentrations of nitrogen that are present in buckthorn leaves (Knight et al. 2007), we expect that forest sites with abundant buckthorn will have greater nitrogen inputs through falling leaves (i.e. litterfall), have greater rates of nitrogen recycling in soil, and, at least seasonally, have greater nitrogen exports into water and air. We will test these hypotheses by measuring inputs, recycling, and outputs of nitrogen at multiple forest sites in Minnesota, including the Lee and Rose Warner Nature Center near Marine on St. Croix. By including the Warner Nature Center, which has education as a primary mission, our results will be readily accessible to the public.

Buckthorn invasion into forests is also associated with invasion of exotic earthworms (Heimpel *et al.* 2010), which can also influence nitrogen cycling. To understand how earthworms and buckthorn interact to influence soil nitrogen recycling and potential nitrogen exports, we will also use forest sites at the Warner Nature Center to identify how buckthorn presence and abundance influences the composition of earthworm communities. In addition, controlled experiments will be conducted at the Department of Forest Resources at the U of M to evaluate if earthworms mitigate or exacerbate the effects of buckthorn on soil nitrogen cycling.

Since the effects of buckthorn on the nitrogen cycle have not been well studied (Knight *et al.* 2007), this proposed research will add substantially to our knowledge of the impacts of buckthorn invasion. Moreover, teasing apart the effects of buckthorn and invasive earthworms on nitrogen cycling will provide information to land managers regarding potential restoration practices.

This research will be accomplished with current personnel and by partially funding a post doc, Kevin Mueller, who has expertise in nitrogen cycling.

References

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Amendment Approved: June 6, 2012

PROGRESS SUMMARY AS OF July 10, 2012

Field surveys

Since the beginning of the field season, we have surveyed 10 additional sites in central and southern Minnesota. In each site, we documented the abundance of native and invasive species; measured abiotic factors such as light level, slope, aspect, earthworm abundance, and percent bare soil; and collected soil for texture and pH analysis in the lab.

These new surveys add to the 41 sites already surveyed in 2010 and 2011. Two of the new sites are in recently harvested stands and two others in stands with recent invasive species management. We plan to complete surveys in more managed sites to increase our ability to assess the effects of forest management on invasive species abundance.

This summer, we will complete 10-15 more field surveys across the central and southern parts of Minnesota's eastern deciduous forest. This will increase the total number of survey sites to 60-65.

Buckthorn removal

In May 2012, we completed the first post-removal vegetation survey in each of the buckthorn removal experimental plots. We identified all species in each plot and estimated their abundance and percent cover to evaluate the effects of buckthorn removal on native species establishment. We also counted the number of germinating buckthorn seedlings. We will complete additional vegetation surveys later in the growing season and in 2013.

This experiment allows us to document the effects of buckthorn removal on native vegetation. Whether or not these removal techniques lead to long term suppression of buckthorn or create conditions conducive to further invasion is not clear. To assess the effectiveness of a post-removal management technique on buckthorn suppression, we initiated an additional element to the buckthorn removal experiment. In each of the 48 removal plots, we established six 1 m^2 subplots. We seeded rye grass, a common native woodland species, in two of these plots; oats, a commonly used cover crop in two plots; and left the final two plots as untreated controls. We will measure buckthorn regeneration in each of these plots over the next two growing seasons and assess the effectiveness of using cover crops to suppress buckthorn regeneration after removal.

Greenhouse experiment

High native plant diversity is predicted to decreases the likelihood of invasion by non-native plants. However, observational and experimental tests of this prediction are inconclusive. Given this lack of consensus, it is hard to predict whether native plant diversity will be an important factor in determining the invasibility of Minnesota's forests. Our field study, described above *("Field surveys")*, addresses the question by examining the relationship between site level diversity and invasive species' abundance. However, the potential confounding effects of other environmental factors difficult to directly answer this question.

As a result, we established a controlled greenhouse experiment with 264 microcosms containing various combinations of native species to investigate whether native plant diversity affects the germination and short-term survival of invasive species. We also tested whether different light levels and leaf litter affected the establishment of invasive species and introduced earthworms to a subset of the microcosms. We began the establishment of microcosms containing native plant communities January 12, 2012, added worms to a subset of the microcosms on February 15, and added seeds of invasive plants on February 17. The invasive species we included were buckthorn, barberry, garlic mustard, and dandelion. These are all common, non-native species found in Minnesota's forest communities. After nine weeks of monitoring the experimental microcosms, we harvested all biomass. Roots were separated from the above ground biomass and will be weighed separately. We also separated above and below ground biomass of the germinated invasive seedlings; collected soil for nutrient analysis and water content; and weighed the worms before and after the experiment. The processing of samples from the microcosms will be complete by the end of August and we will begin analysis in September.

Seedbank study

Propagule pressure, i.e., the number of individuals/seeds of a species released into an area where they are not native, is an important component of invasion potential. Estimating the difference between actual propagule pressure, i.e., the numbers of seeds in the seedbank, and realized abundance of an invasive species, i.e., the actual number of stems of an invasive species in a given area, is a possible way to estimate whether environmental characteristics of a site are suppressing invasion. To investigate whether characteristics of Minnesota's deciduous forest play a role in suppressing invasion by buckthorn we collected soil from 12 forest stands with a gradient of buckthorn abundance from low to high. We exposed this soil to 'ideal conditions' in the green house (high light, adequate moisture, and low competition) to germinate as many invasive species as possible from the seed bank. The soil was collected in May and we have monitored the germinating seeds since then. At the end of the experiment, we will investigate the correlation between site characteristics and propagule pressure to assess whether certain environmental variables affect the level of buckthorn invasion.

Nitrogen cycling

The nitrogen rich leaves of buckthorn are predicted to affect nitrogen mineralization and nitrification. To test this prediction, we identified 28 study plots within our focal site, Warner Nature Center near Marine on St. Croix. On two different soil types, including a sandy and a silty soil, there are two replicate plots for 6 different levels of buckthorn invasion, ranging from plots with no buckthorn present to plots that contain dense and mature buckthorn stands (12 plots along the buckthorn gradient on each soil type). Additionally, on the silty soil type, we have located 4 plots where buckthorn has been removed: 2 plots where buckthorn was removed twice in the last 6 years and two plots where buckthorn was removed once three years ago. Each of these 28 plots has been surveyed for vegetation characteristics, such as the number and stem size of each buckthorn individual. In early July (2012) soil cores were removed from each plot to measure concentrations of soil nutrients, including ammonium, nitrate, and total carbon and nitrogen. At the same time (early July 2012), we also placed incubation tubes and resin bags in each plot; these will be harvested in early August (2012) for estimating nitrogen availability in soil and rates of soil nitrogen recycling. We have also surveyed earthworms to investigate their potential interaction with buckthorn and the resulting affects on soil nitrogen.

A part time post-doc, Kevin Mueller, joined the project team in May 2012. Kevin has expertise in nitrogen cycling. Two field assistants were hired for the 2012 field season. They will assist in field data collection over the summer. A temporary technician was also hired in May; she will assist the project in the field as well as in the lab.

III. PROGRESS SUMMARY AS OF 09/21/2012:

Amendment Request (09/21/2012):

This amendment is to request that some Result 3 travel funds in be used for travel and lodging for two nights in La Crosse, Wisconsin to attend the Upper Midwest Invasive Species Conference for Timothy Whitfeld, Alex Roth and Sascha Lodge on October 29-31, 2012. See paragraph under Result 3.
Amendment Approved: September 21, 2012

PROGRESS SUMMARY AS OF January 10, 2013
Field surveys
Since the start of the project, we have surveyed a total of 67 sites in central and southern Minnesota. Each of these sites is located in Minnesota’s eastern deciduous forest province. In each site, we documented the abundance of native and invasive species; measured abiotic factors such as light level, slope, aspect, earthworm abundance, and percent bare soil; and collected soil for texture and pH analysis in the lab. The surveyed sites include 17 that were recently (within the past 10 years) harvested for timber, 12 that were managed for invasive plants, and 38 that had no major disturbance in the past 10 years.

Buckthorn removal
In August 2012 we completed the second post-removal vegetation in each of the buckthorn removal experimental plots. We identified all species in each plot and estimated their abundance and percent cover to evaluate the effects of buckthorn removal on native species establishment. We also counted the number of germinating buckthorn seedlings. In addition, we estimated the percent cover of the cover crops (rye and oats) that had been seeded into subplots in May 2012. After one more post-removal survey in late May 2013, data will be analyzed to determine how each removal treatment and the combinations of different removal and planting treatments affected buckthorn germination and regeneration of native plants.

Greenhouse study
All samples from the greenhouse study were processed during the summer of 2012. This included biomass of native species in each of the treatments (each treatment had equal numbers of pots with and without an invasive earthworm):

Light levels: low, medium, and ambient
Litter levels: zero litter, 2.5 g, 5 g
Native plant diversity: 1 species, 2 species, 6 species

Biomass of all invasive species:
Buckthorn
Barberry
Dandelion
Garlic mustard (failed to germinate in any pots)

We also measured soil moisture, light transmittance at the soil surface, and remaining leaf litter in each pot at the end of the experiment and weighed the surviving earthworms.

Our analysis indicated higher native diversity lead to lower invasive biomass. Also, invasive earthworms had a significant effect on invasive biomass such that pots with earthworms had more invasive plant biomass. Furthermore, when worms were absent from pots, light and litter had simple effects on buckthorn biomass. Across all light treatments, buckthorn had the lowest biomass in high light due to seed dessication (unexpected). Across all litter treatments, buckthorn experienced the lowest biomass in high litter (expected) due to separation from the soil and the shading effects of litter. However, when worms were present in pots, the effects were reversed. Across light treatments, buckthorn saw the highest biomass when light was high. This was due to the action of earthworms mixing seeds into the soil to prevent dessication. Moreover, across all litter treatments, buckthorn had the highest biomass in the high litter treatment due to initial effects of litter preventing dessication, but delayed effects of earthworms removing the litter to allow growth. Overall, forest invaders experienced higher germination in the presence of earthworms.

We are currently preparing a manuscript for publication in a peer-reviewed journal with results from the diversity manipulation part of the greenhouse experiment.

Nitrogen cycling
On September 7, 2012 we installed two 10 gallon pots in each plot for collection of leaf litterfall. Litter was collected biweekly until December 3, 2012, when the pots were removed. On October 2, 2012 we installed resin bags in each plot to measure nitrate and ammonium availability during leaf senescence of the dominant tree species present along the buckthorn gradient. We removed these bags in mid November for analysis and replaced them at that time to measure nitrate and ammonium availability from late fall through early spring. On November 26, a second round of incubation tubes was installed to assess winter nitrogen cycling. An additional set of soil cores was also removed on November 26 to assess the amount of N in soil present as nitrate, ammonium, and microbial biomass.

IV. OUTLINE OF PROJECT RESULTS*:*

RESULT 1: Finalize research plans, select 80 sites, and establish 16 research plots in each site

Description: Information such as regional and state-wide forest inventories (i.e. FIM and MCBS native plant community data) will be used to select candidate sites.

Summary Budget Information for Result 1:	**ENRTF Budget:**	**$** 43,206
	Amount Spent:	**$** 43,206
	Balance:	**$** 0

Deliverable	**Completion Date**	**Budget**
1. Identify, locate 80 forest sites	12/15/2010	$16,000

2. Establish 16 plots at each site	6/30/2011	$21,000
3. Selection of four buckthorn removal sites from overall site pool	09/30/2011	$1,000
4. Set up 12 6x6 meter plots per buckthorn removal site and conduct vegetation survey in subplots	09/30/2011	$1,700
5. Complete buckthorn removal	11/30/2011	$1,300

Result 1 Completion Date: 6/30/2012

Result Status as of April 19, 2011:
1. Identify, locate 80 forest sites
Ten sites were identified in summer 2010 by using a combination of GIS data (including FIM and MCBS native plant community data) and discussions with DNR area foresters and private landowners (Science Museum of Minnesota). Additional candidate sites are currently being identified. These will be visited beginning in May to determine if they will be selected as research sites. Instead of selecting all sites, establishing the plots, and then censusing forest attributes as discrete successive steps (as is stated in the grant proposal), we have been selecting a group of sites then establishing plots and sampling sites simultaneously. We hope to identify most or all of the remaining sites by the end of summer 2011.

2. Establish 16 plots at each site
Sixteen plots were established at each of the ten sites identified in summer 2010. Within each plot, data was collected on vegetation species and cover, light level, evidence of disturbance, slope, aspect, and earthworm presence. Soil samples were also collected for analysis in the laboratory for pH and soil texture. We anticipate establishing plots and censusing forest characteristics in at least 40 additional sites during the summer of 2011, with the remaining plots being censused in summer 2012.

Result Status as of September 21, 2011:
1. Identify, locate 80 forest sites
Forty one sites have been identified to date by using a combination of GIS data (including FIM and MCBS native plant community data) and discussions with DNR area foresters and private landowners (Science Museum of Minnesota). We are still in the process of identifying additional candidate sites. We continue to select a group of sites then establish plots and sample sites simultaneously (i.e. Results 1 and 2 are being done simultaneously).

2. Establish 16 plots at each site
Sixteen plots were established at each of the new sites identified in summer 2011. Within each plot, data was collected on vegetation species and cover, light level, evidence of disturbance, slope, aspect, and earthworm presence. Soil samples were also collected for analysis in the laboratory for pH and soil texture. Remaining plots will be censused in summer 2012 and we hope to get to approximately 80 sites.

Result Status as of January 10, 2012**:**
1. Identify, locate 80 forest sites
No field surveys of potential sites were done between September 2011 and March 2012 but approximately 32 sites were preliminary identified based on previous scouting trips and discussions with DNR managers and others. As described above, our research to date suggests that our objectives (to assess the role of forest health management in resisting infestation of invasive species) can be better met by establishing fewer descriptive sites and instead adding a number of manipulative sites. Thus instead of filling out the remainder of the 80 survey with sites that provide the rest of the originally intended contrasts (which are difficult to find), we may establish a small number of descriptive sites if needed, and instead use the other studies described above to address these issues.

2. Establish 16 plots at each site
At four sites, buckthorn removal treatments including weed-wrenching, basal bark herbicide application, and biomass removal using the cut-and-paint technique were completed. This experimental procedure will assess how common methods for the management of invasive species might influence the environmental conditions that regulate invasive plant colonization.

Result Status as of July 10, 2012**:**
1. Identify, locate 80 forest sites
Since the beginning of the field season, we have surveyed 10 additional sites in central and southern Minnesota. This brings the total to 51 sites since the start of the project. We hope to bring the total number to 60-65 by the end of this field season.

2. Establish 16 plots at each site
Within each plot, data was collected on the abundance of native vegetation and invasive species. We measured abiotic factors such as light level, slope, aspect, earthworm abundance, and percent bare soil. Soil samples were also collected for analysis in the laboratory for pH and soil texture.

3. Selection of four buckthorn removal sites from overall site pool
Done

4. Set up 12 6x6 meter plots per buckthorn removal site and conduct vegetation survey in subplots
Done

5. Complete buckthorn removal
Done

Result Status as of January 10, 2013**:**
1. Identify, locate 80 forest sites

Done. A total of 67 sites were identified and surveyed. Seventeen sites were harvested for timber within the past ten years, 12 were previously managed for invasive plant removal, and the remaining 38 had no major disturbance in the previous decade.

2. Establish 16 plots at each site
Done. Within each plot, data was collected on the abundance of native vegetation and invasive species. We measured abiotic factors such as light level, slope, aspect, earthworm abundance, and percent bare soil. Soil samples were also collected for analysis in the laboratory for pH and soil texture.

3. Selection of four buckthorn removal sites from overall site pool
Done

4. Set up 12 6x6 meter plots per buckthorn removal site and conduct vegetation survey in subplots
Done

5. Complete buckthorn removal
Done

Final Report Summary (August 15, 2013)**:**

1. Identify, locate 80 forest sites

During the first field season of the project (summer 2010), we were able to identify ten sites using GIS data from the Minnesota DNR (Forest Inventory Module and Minnesota Biological Survey) and discussions with DNR area foresters and private landowners. We determined the most efficient way to approach site selection and survey was to do the two simultaneously rather than as discrete steps as described in the original proposal. This method allowed us to adjust the focus of our site selection as we moved forward since we knew where the geographic and habitat "gaps" were in the completed sites and could then target our selection effort accordingly to ensure we had good coverage in the region. By the end of the second field season (summer 2011) we completed surveys in 41 sites. At this point in the project, we determined that in order to assess the role of forest health management in resisting infestation of invasive species we should establish fewer sites in relatively undisturbed forest stands and instead add a number of manipulative sites to investigate whether ongoing forest management influenced a site's invasibility. By the end of the final field season, we surveyed a total of 67 sites with a balance between stands with no recent disturbance (38 sites), sites with recent timber harvest (17 sites), and sites with recent invasive species management (12 sites). This total was less than in the original proposal (80 sites) but still gave us a substantial number of data points from which we could examine the potential effects of site characteristics on invasibility.

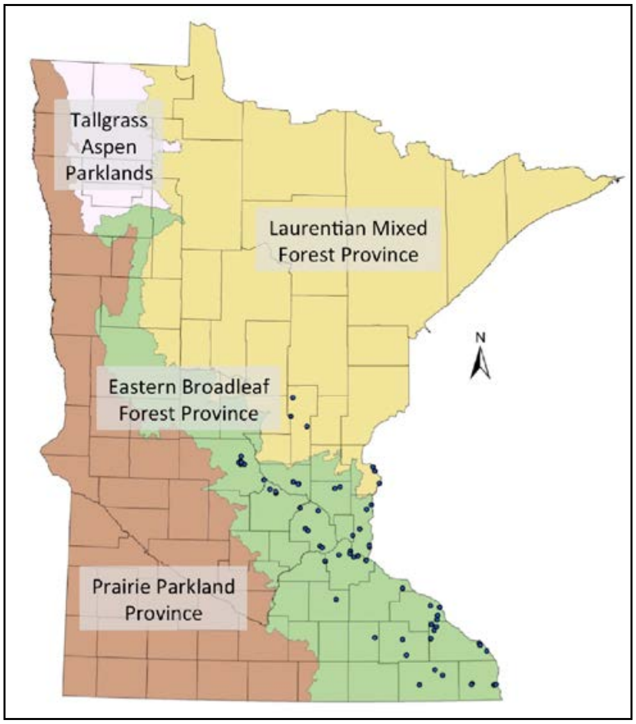


Figure 1. Map of Minnesota showing the location of our 67 study sites (dots)

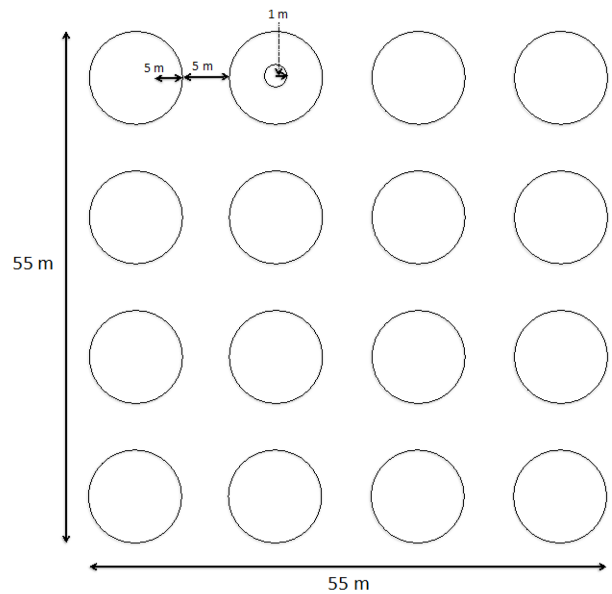
After the field surveys were complete, it was obvious that the most frequent and abundant non-native plant species in the oak forests we focused on was common buckthorn *(Rhamnus cathartica).* As a result, we focused our analyses on common buckthorn since we consider this to be the most pernicious invader of forests in southern Minnesota at this time. Introduced honeysuckle (*Lonicera* sp.), garlic mustard *(Alliaria petiolate)*, barberry, *(Berberis thunbergii),* and multiflora rose *(Rosa multiflora)* were present in a few sites but they were rarely abundant.

Figure 2. Common buckthorn *(Rhamnus cathartica* L.), the most frequent and abundant invasive plant in central and southeastern Minnesota forests

2. Establish 16 plots at each site

At each of the 67 survey sites we established 16 sample plots in a four by four grid. Each sample plot had a 5 m radiu and the plot centers were 15 m apart. Within each 5 m plot we identified and measured the diameter at breast height (dbh) of all woody species (trees and shrubs) that were at least 1.3 m tall. At the center of each plot, we established a subplot with a radius of 1 m in which we counted stems and measured the dbh of all smaller trees and shrubs (<1.3 m tall). In addition, in the 1 m subplots, we estimated cover of all herbaceous plants.

Figure 3. Sample plot layout. Within each 5 m plot all trees and shrubs taller than 1.3 m were identified. All of the herbaceous plants and shorter woody plants (<1.3 m) were identified in each of the 1 m subplots located at the center of each 5 m plot.



Within each forest stand, the 16 sample plots were set up in the first area encountered that had a relatively homogenous topography and was large enough to encompass the four by four grid. In each plot we collected data on plant species identity and cover for all native and introduced trees, shrubs, and herbaceous plants. We also collected voucher specimens for all species encountered and deposited these in the Bell Museum Herbarium at the University of Minnesota. In addition, we measured light levels, duff layer thickness, the amount of bare soil, slope, aspect, and earthworm presence. Soil samples were also collected for analysis in the laboratory for pH, soil texture, and nutrients. Overall, we used this survey design to sample 67 sites throughout central and southern Minnesota.

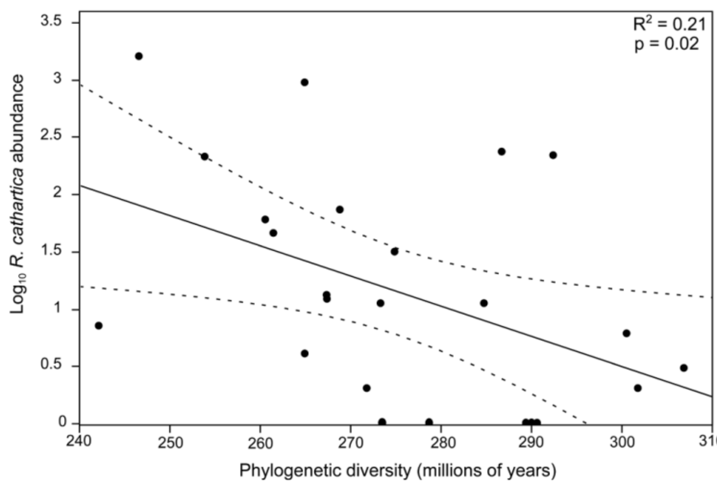
Figure 4. Field crews surveying plots in Minnesota's oak forests

Across all of our survey sites, we documented 354 plant species. The table below summarizes the range of taxonomic diversity in each site.

	Range of species diversity per survey site
Herbaceous plants	8-63
Trees and shrubs	11-39
All species	23-99

The field surveys suggested that the most important site characteristics in determining the abundance of buckthorn were light levels, leaf litter, and evolutionary diversity of the resident plant species. Buckthorn abundance was lowest in sites with lower light, more leaf litter, and higher diversity.

Figure 5. As the evolutionary diversity of native species increases in a forest stand, the abundance of buckthorn goes down.



Given these findings, pre-invasion management to reduce invasibility should maximize diversity to leave less space for invaders. One way to do this is by controlling the density of white-tailed deer, a species that has severe impacts on plant growth when populations are high. Another is to limit disturbance by using winter timber harvest and low impact trail construction techniques that minimize soil disturbance and keep sunlight relatively low. Maintaining an intact litter layer also appears to be particularly important.

Seed availability is another important factor in determining the spread of invasive species. We documented a strong positive relationship between landscape scale “propagule pressure” and local buckthorn abundance.

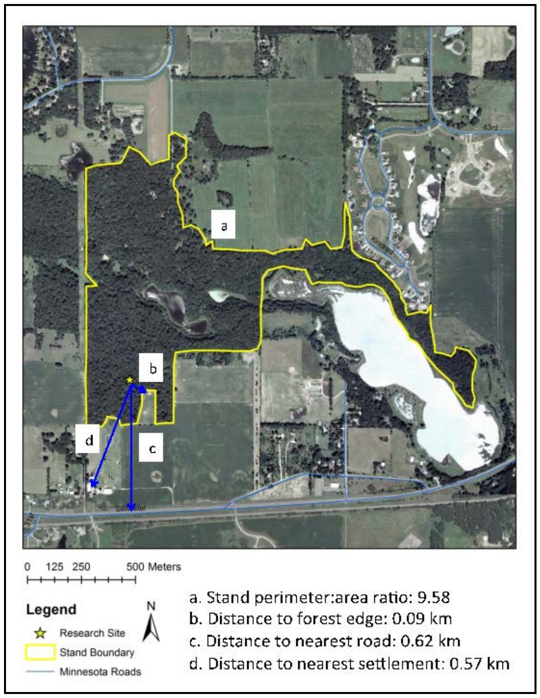


Figure 6. To estimate an index of seed availability, we used GIS to measure several site characteristics that previous research suggested would influence how many seeds could reach a given area within a stand. Some examples are shown here. These were combined in our analysis to estimate an index of “propagule pressure.”

Reducing common buckthorn seed sources is a promising first step in successful pre- and post-invasion management. Pre-invasion, reducing seed availability is particularly important where common buckthorn is not yet abundant in natural areas but present in nearby towns. A community effort to remove common buckthorn from residential areas would be one of the most effective ways to slow its spread into surrounding forests. Post-invasion, focusing first on removal of mature adult plants before removing smaller individuals will improve the success of a management program. Collaboration with neighboring landowners is necessary since any attempt to remove buckthorn from an individual forest stand will ultimately fail without reducing seed availability in the surrounding area.

3. Selection of four buckthorn removal sites from overall site pool.
To examine whether disturbed communities have characteristics that make them more susceptible to invasion than the non-disturbed survey sites we included stands disturbed by natural blowdown and also timber harvest sites. In the summer of 2011, we decided to add an experimental aspect to four of the survey sites that contained large areas of dense buckthorn infestation (College of St. Benedict, St. Joseph; Afton State Park, Afton; Warner Nature Center, Marine on St. Croix; and Hyland Lake Park

Reserve, Bloomington). This allowed us to investigate invasive species removal management as another disturbance 'treatment'. Invasive removal as a type of disturbance has received little attention, but since it is a management strategy employed by a variety of public and private agencies, the efficacy of the approach deserves attention.

4. Set up 12 6x6 meter plots per buckthorn removal site and conduct vegetation survey in subplots.

5. Complete buckthorn removal

We are combining summary of deliverables 4 and 5 for Results 1 in the final report since they are part of the same buckthorn removal project. At each of the four selected sites we established grid consisting of 12 six by six meter invasive species removal plots (in a four by three arrangement) with a six-meter buffer around each plot. Within each plot, we established three one-meter radius subplots at 0°, 120°, and 240° from the center of the main plot (1.5 m from the center). In each of these subplots, we identified and estimated percent cover of all herbaceous species. In addition, we identified, measured the diameter, and counted all the stems of the tree and shrub species. Environmental variables were also recorded for each six-meter square plot (slope, aspect, leaf litter depth, percent bare soil, light levels).

Figure 7. Design of the buckthorn removal experiment. We used this plot layout for each of the four survey sites

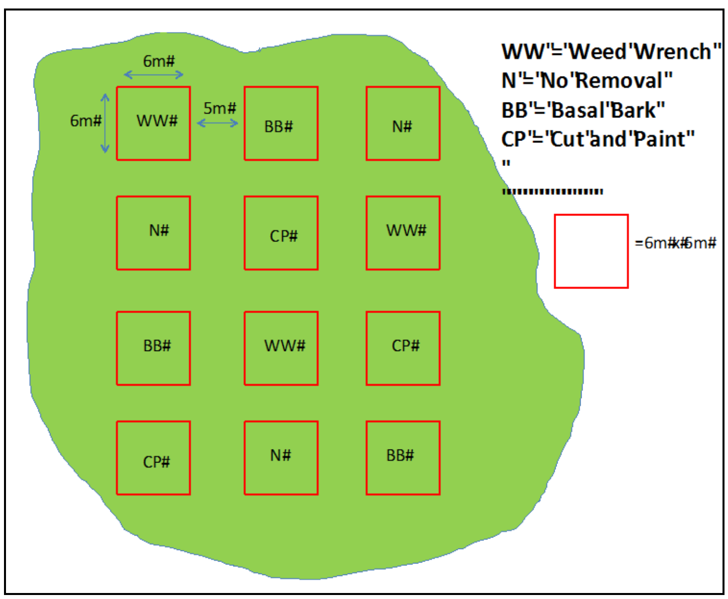
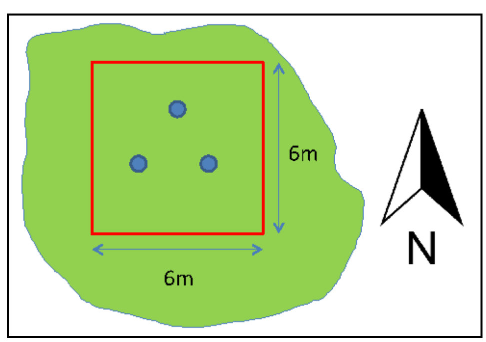


Figure 8. Close-up of one plot showing arrangement of the one-meter radius subplots.



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In each plot, we used three common techniques to remove the invasive biomass (plus a control treatment where nothing was done). Plots were randomly assigned to a different treatment types. The three buckthorn removal treatments were weed-wrenching, basal bark herbicide application, and biomass removal using the cut-and-paint technique. Treatments were applied at each of the four sites during October and early November, 2011 after surveying all vegetation in each plot.

Figure 9. Buckthorn removal techniques: left panel = manual removal, middle panel = basal bark application of herbicide, right panel = cut and paint application of herbicide.

Figure 10. One of the 6 x 6 m plots with all buckthorn manually removed.

Figure 11. Stems of dead buckthorn after a basal bark application of herbicide (on the right side of the picture the buckthorn with green leaves are outside the research plot and have not been treated with herbicide).

In addition to the buckthorn removal study, we also initiated a seed addition pilot study to investigate the possibility of using covers crops to reduce buckthorn regeneration after removal. These post-removal strategies to improve native species regeneration have to deal not only with getting the native species back, but also with preventing the germination of buckthorn. A cover crop could provide shade and take up available space, thus preventing germination of buckthorn from the seedbank. However, it is unclear how planting will interact with the variety of buckthorn removal methods. We expected that a high cover of seeded species would prevent the germination of buckthorn relative to control conditions (i.e., no cover crop planted) Also we expected a perennial cover crop to be more successful at resisting buckthorn germination compared to an annual species. Within each six by six meter plot, six 1m square subplots were set up, three in one randomly determined corner and three in another corner. Two different species were selected for planting: Virginia wild rye *(Elymus virginicus)*, a perennial species and oats *(Avena sativa)* an annual. Within each set of three plots, the plots were randomly assigned to three cover crop treatments: control (no cover crop), oats, or rye and seeding was carried out six months after buckthorn removal. Each planting plot was surveyed before seed application for percent cover of vegetation, percent bare soil, and number of buckthorn seedlings and resurveyed in August 2012, May 2013, and August 2013 (with funding from the University of Minnesota). Analysis of recent results are ongoing for the next six- twelve months.

Figure 12. One-meter square planting plot (delineated by blue flags). This plot was seeded with wild rye, a perennial species

RESULT 2: Assess degree of plant invasion, disturbance history, and health and structural integrity of native plant communities.

Description: Over the course of two years, all plots in all sites will be censused for ecosystem attributes and the native and invasive plant community. Other data on climate and distance from development will be obtained and maintained in a geographical information system.

Summary Budget Information for Result 2:	**ENRTF Budget:**	**$** 229,794
	Amount Spent:	**$** 223,356
	Balance:	**$** 6,438

Deliverable	Completion Date	Budget
1. Field data collection completed on forest health and invasion status sites	9/30/2012	$170,000
2. Final data base on plant invasion, forest health and integrity	12/31/2012	$ 32,000
3.Obtain native and non-native seeds	10/31/2011	$ 600
4. Germinate seeds of native species in the greenhouse	12/31/2011	$ 400
5. Establish experimental communities of native species in pots and add non-native seeds	02/29/2012	$ 1,000
6**.** Greenhouse experiment completed	06/30/2012	$ 3,000
7. Resurvey buckthorn removal subplots	10/30/2012	$ 5,000
8. Add non-native seeds to experimental field plots	06/30/2012	$ 2,000
9.Complete surveys of non-native establishment success in experimental field plots	10/30/2012	$ 8,000
10. Selection of 8-15 plots with variable buckthorn presence within Warner Nature center and one other forest site	06/15/2012	$ 1,000
11. Characterize plots with respect to buckthorn abundance, canopy basal area, soil texture, slope, elevation, and aspect	08/01/2012	$ 2,000
12. Measure nitrogen inputs, recycling, and outputs at each plot	06/30/2012	$ 3,500
13. Measure earthworm composition and abundance at each plot twice	09/01/2012 05/31/2013	$ 2,000
14. Spray ^{15}N labeled urea on buckthorn leaves to produce litter for controlled experiment; collect leaves	09/01/2012	$ 1,500
15. Conduct controlled experiment on earthworm-buckthorn interactions using ^{15}N labeled leaf litter	05/31/2013	$3,000

Result 2 Completion Date: 05/31/2013

Result Status as of September 21, 2011**:** Within each of the descriptive experimental plots, data has been collected on vegetation species and cover, light level, evidence of disturbance, slope, aspect, and earthworm presence. Soil samples were also collected for analysis in the laboratory for pH and soil texture. All collected soils have been sieved in preparation of pH and textural analyses. Soil pH analysis is currently underway on 480 soil samples (180 completed). As stated in Result 1, site selection is still underway. We will continue to select sites, then establish plots and sample simultaneously (i.e. Results 1 and 2 are being done simultaneously).

Result Status as of January 10, 2012**:** Analysis of soil pH was completed for the 41 sites that have been surveyed. Analysis of soil texture for all these sites is currently underway. GIS software was used to measure the forest stand area within which is survey site is located, the perimeter to area ratio of each stand, the distance from the survey site to the forest edge, the distance to the nearest road, and the distance to the nearest settlement. These variables have been combined in a principle components analysis to generate an index of invasive species propagule pressure that is included in analyses of site level characteristics affecting the abundance of non-native species.

Result Status as of July 10, 2012**:**

1. Field data collection completed on forest health and invasion status sites
Ten more sites surveyed. We will continue to collect data during the field season of 2012.

2. Final data base on plant invasion, forest health and integrity
Ongoing

3. Obtain native and non-native seeds
Done

4. Germinate seeds of native species in the greenhouse
Done

5. Establish experimental communities of native species in pot and add non-native seeds
Done

6. Greenhouse experiment
We established a greenhouse experiment with 264 microcosms containing combinations of native species. We tested whether different light levels and leaf litter affected the establishment of invasive species and introduced earthworms to a subset of the microcosms. After nine weeks of monitoring the experimental microcosms, we harvested all biomass between April 23 and April 27, 2012.

Seedbank greenhouse study still in progress.

7. Resurvey buckthorn removal subplots
We completed the first post-removal vegetation survey in each of the buckthorn removal experimental plots. We identified all species in each plot and estimated their abundance and percent cover to evaluate the effects of buckthorn removal on native species establishment. We also counted the number of germinating buckthorn seedlings.

8. Add non-native seeds to experimental field plots.
This has been accomplished at two sites in northern Minnesota (near Cloquet and Ely). The non-native species are buckthorn, barberry and honeysuckle.

9. Complete surveys of non-native establishment
In progress

10. Selection of 8-15 plots with variable buckthorn presence within Warner Nature center and one other forest site
We identified 28 study plots within our focal site, Warner Nature Center near Marine on St. Croix.

11. Characterize plots with respect to buckthorn abundance, canopy basal area, soil texture, slope, elevation, and aspect
Each of the 28 plots has been characterized and surveyed for vegetation characteristics, such as the number and stem size of each buckthorn individual. Canopy species were identified and their basal area measured. Soil was collected and texture/pH were measured in the lab. Slope, aspect, and elevation were all measured in the field.

12. Measure nitrogen inputs, recycling, and outputs at each plot
On July 5 and 6, 2012 soil cores were removed from each plot to measure concentrations of soil nutrients, including ammonium, nitrate, and total carbon and nitrogen. At the same time, we also placed incubation tubes and resin bags in each plot.

13. Measure earthworm composition and abundance at each plot twice
First sampling is being done during the week of July 9, 2012

Result Status as of January 10, 2013**:**
1. Field data collection completed on forest health and invasion status sites
Completed. Data has been collected on vegetation species and cover, light level, evidence of disturbance, slope, aspect, and earthworm presence at all 67 sites. Data entry was completed in December 2012, and analysis will proceed during the winter of 2013.

2. Final database on plant invasion, forest health and integrity
Ongoing. Soil pH was measured on composite samples from each site in December 2012. Soil texture analysis on the 24 sites surveyed in 2012 will be completed in January 2013. Measurement of variables to be used to create an index of propagule pressure is currently being conducted using GIS software.

3. Obtain native and non-native seeds
Done

4. Germinate seeds of native species in the greenhouse
Done

5. Establish experimental communities of native species in pot and add non-native seeds
Done

6. Greenhouse experiments

The seedbank greenhouse study concluded in July 2012. Far fewer buckthorn seeds germinated from the collected soils than expected, causing it to be unlikely that we will have sufficient statistical power to analyze these results. Due to the spatial variability of buckthorn seeds in the soil, future studies would need to collect far more soil to determine a representative available seedbank.

The greenhouse experiment that investigated the effects of native plant diversity, light, and litter on the success of invasive plants finished and all samples from the study were processed during the summer of 2012. Analyses indicated higher native diversity lead to lower invasive biomass. Also, invasive earthworms had a significant effect on invasive biomass such that pots with earthworms had more invasive plant biomass. Furthermore, when worms were absent from pots buckthorn had the lowest biomass in high light due to seed dessication (unexpected). Across all litter treatments, buckthorn experienced the lowest biomass in high litter (expected). However, when worms were present in pots, the effects were reversed. Overall, forest invaders experienced higher germination in the presence of earthworms. We are currently preparing a manuscript for publication in a peer-reviewed journal with results from the diversity manipulation part of the greenhouse experiment.

7. Resurvey buckthorn removal subplots
We completed the second post-removal vegetation survey (in late summer 2012) in each of the buckthorn removal experimental plots. We identified all species in each plot and estimated their abundance and percent cover to evaluate the effects of buckthorn removal on native species establishment. We also counted the number of germinating buckthorn seedlings. Additionally, we estimated the percent cover of the cover crops (rye and oats) that had been seeded into subplots in May 2012. After one more post-removal survey in late May 2013, data will be analyzed to determine how each removal treatment and the combinations of different removal and planting treatments affected both the germination of buckthorn and the regeneration of native plant species. In August 2013 we will conduct one final survey in order to obtain two full years of data, though this will not be included in the June final report.

8. Add non-native seeds to experimental field plots.
Done

9. Complete surveys of non-native establishment
In progress

10. Selection of 8-15 plots with variable buckthorn presence within Warner Nature center and one other forest site
Done

11. Characterize plots with respect to buckthorn abundance, canopy basal area, soil texture, slope, elevation, and aspect
Done

12. On September 7, 2012, two 10 gallon pots were installed in each plot for collection of leaf litterfall. Litter from the pots was collected biweekly until December 3, 2012, when the pots were removed. Leaf litter was sorted to species, dried, weighed, and then ground in preparation for analysis of N concentrations. On October 2, resin bags were installed in each plot to measure nitrate and ammonium availability during leaf senescence of the dominant tree species present along the buckthorn gradient. On November 13, those resin bags were removed and replaced in order to measure nitrate and ammonium availability from late fall through early spring. On November 26, a second round of incubation tubes was installed. These will remain in the ground until spring and will be used to assess winter nitrogen cycling. An additional set of soil cores was also removed on November 26 and then extracted with various salt solutions to assess the amount of N in soil present as nitrate, ammonium, and microbial biomass.

13. Measure earthworm composition and abundance at each plot twice
Earthworms were sampled on July 9, 2012, and again on November 5. The second earthworm sampling was planned for September, but due to the drought it was necessary to push it back until the ground was moist enough for sampling. Analysis will determine whether the composition and abundance of earthworms differs along a gradient of buckthorn invasion.

Final Report Summary August 15, 2013**:**

Amendment Request (08/15/2013)
<u>We request that $2566 be moved from the field supplies budget in Results 2 to the chemical analyses budget in Results 2, to remedy a negative balance of that amount in the chemical analyses budget.</u>

<u>Ammendent approved:</u>

RESULT 2: Assess degree of plant invasion, disturbance history, and health and structural integrity of native plant communities.

1. Field data collection completed on forest health and invasion status sites

Site selection, plot establishment, and sampling occurred simultaneously (i.e. Results 1 and 2 were completed simultaneously). As stated in the final report of Results 1, Deliverable 1, sixty-seven sites were surveyed.

2. Final data base on plant invasion, forest health and integrity

All data from the 67 field surveys are in a relational database (Microsoft Access). The database has a table for site descriptions that includes latitude/longitude coordinate, disturbance history, stand size, earthworm status, soil pH, soil texture, the amount of bare soil, leaf litter depth, duff depth, and light levels. There is also a table with survey plot descriptions (that includes abundance data for each species of tree, shrub, and herbaceous plant) and another with abundance of invasive plants across the landscape around the survey site (estimated from driving surveys). Plant taxonomy follows the Bell Museum of the University of Minnesota Herbarium and is accessed via a drop down menu. This eliminates spelling errors during data entry. Other drop down menus access tables for other aspects of site and plot level descriptions to further reduce the chance of data entry error. The structure of the database allows for custom queries that can extract data in various forms for analysis. This database is downloadable from our website and could be used by managers to determine the abundance of buckthorn in survey sites close to a particular forest stand of interest. We will also provide species lists (in Microsoft Excel spreadsheets) for each of our survey sites that can be downloaded from the Healthy Forests website.

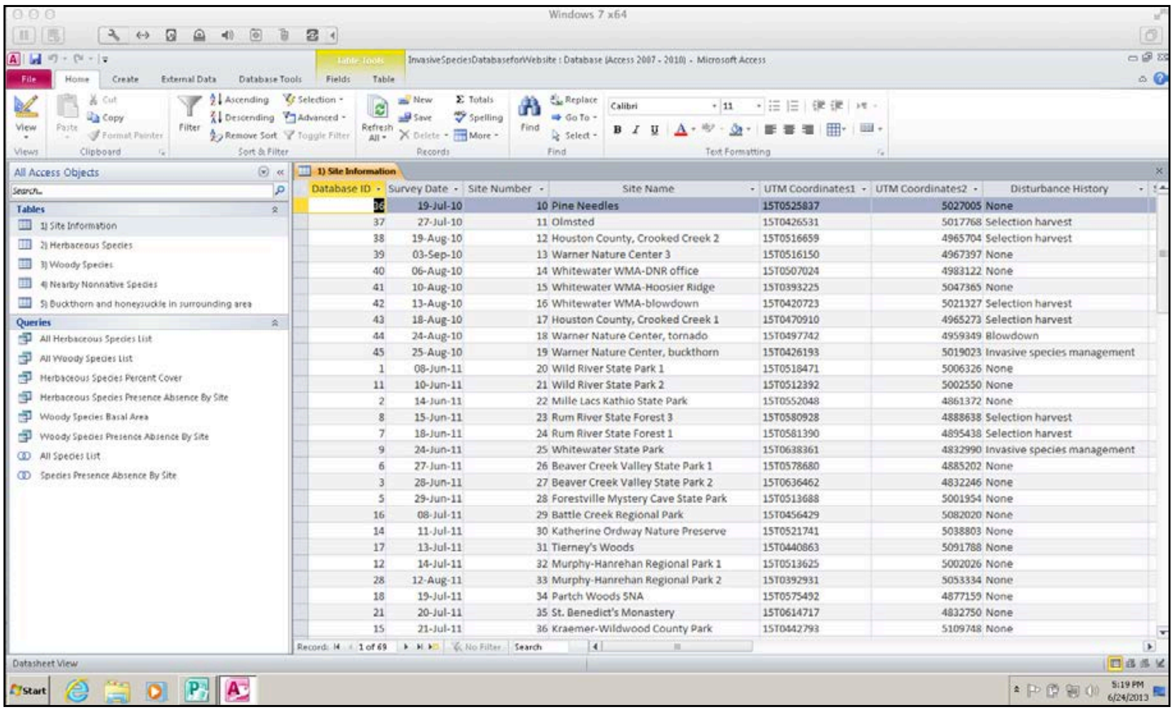


Figure 13. Screen shot of the relational database for the Healthy Forests project. This database includes all aspects of the research project and is available for download from our website.

3. Obtain native and non-native seeds

4. Germinate seeds of native species in the greenhouse

5. Establish experimental communities of native species in pots and add non-native seeds

6. Greenhouse experiment completed

We are combining summary of deliverables 3, 4, 5, and 6 for Results 2 in the final report since they are all part of the same greenhouse experiment. We established this experiment to test the hypothesis that high diversity of native plants would decrease the likelihood of invasion by non-native plants. Previous studies provide mixed support for this hypothesis. The patterns we observed in our field study suggested there was a negative relationship between the diversity of native plants and the abundance of buckthorn but whether native plant diversity is driving the relationship or whether common buckthorn invasion is responsible for the lower diversity of native plants is not clear. As a result, it is hard to predict whether native plant diversity will be an important factor in determining the invasibility of Minnesota's forests. The potential confounding effects of other environmental factors such as soil nutrients and propagule pressure also make it difficult to directly answer this question based on the field surveys alone. We also wanted to account for the effects of other environmental variables such as light levels and leaf litter depth that our field study suggested were important in determining invasibility. The best way to combine these goals was a controlled greenhouse experiment that held constant environmental conditions while varying plant diversity, light, and litter directly. This dual approach that used manipulative experiments to test hypotheses based on patterns seen in the field is a robust way to investigate potential mechanisms of invasibility. We used native plant species that were abundant in our field surveys and also decided that the effects of earthworms should be part of the experiment since they are a ubiquitous presence in Minnesota's forests and have been shown to have profound effects on native plant diversity.

Our greenhouse experiment included 264 pots with different combinations of native species. We also manipulated light levels and leaf litter and added earthworms to a half of the microcosms in each treatment. Each pot had an established community of native plants before we added seeds of invasive species (common buckthorn, barberry, garlic mustard, and dandelion) so we could directly test the effects of our treatments on the success of the invasives. After nine weeks of monitoring the experimental microcosms, we harvested all biomass of the native and invasive species.

Figure 14. We germinated seeds of eight native species for use in the greenhouse experiment

Figure 15. Before the experiment started, we transferred the seedlings into pots to create experimental communities of native species. Once these communities were established, we added seeds of invasive species.

Figure 16. The bench on the right has pots that differ in their native plant diversity. The bench on the left has different shade and leaf litter treatments.

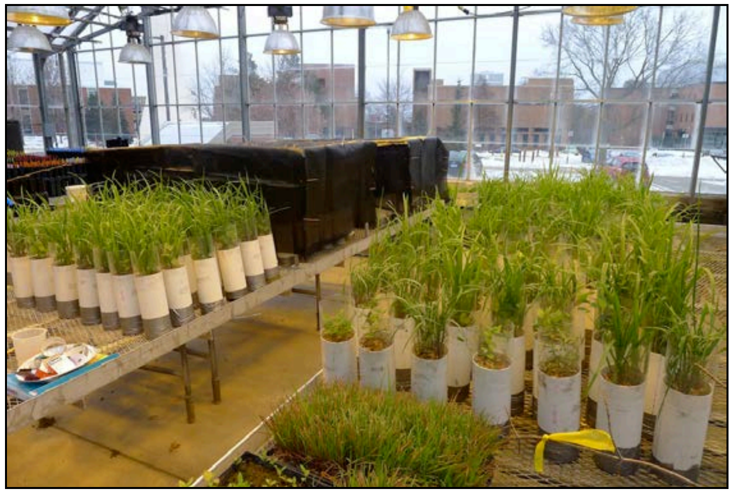


Figure 17. Individual pot showing emerging buckthorn seedlings

Figure 18. At the end of the experiment, we removed the plants and soil from the pot to harvest the biomass of native and invasive species. In this pot, that contained an earthworm, the worm burrow is clearly visible

Figure 19. In many cases, buckthorn seedlings germinated on the earthworm casting suggesting that the presence of earthworms is beneficial to buckthorn

As we found in the field study, common buckthorn was the most successful invader. Barberry and dandelion germinated in some of the pots whereas garlic mustard did not germinate in any experimental pots even though we followed published protocols for pre-germination scarification treatment. After the experiment concluded our analyses indicated higher native diversity did lead to lower invasive biomass. Also, invasive earthworms had a significant effect on invasive biomass so that pots with earthworms had more invasive plant biomass regardless of the native species diversity.

Figure 20. When earthworms were present (gray bars), increased native plant diversity was associated with lower biomass of invasive species. Across all diversity treatments, invasive species biomass was higher in the presence of earthworms.

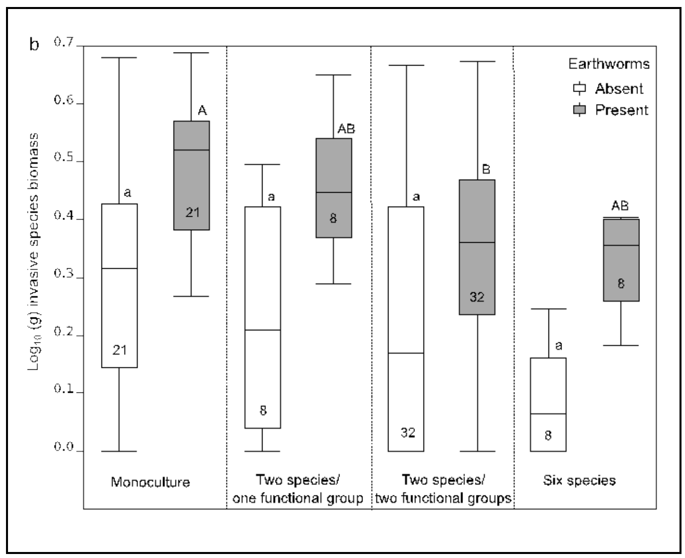
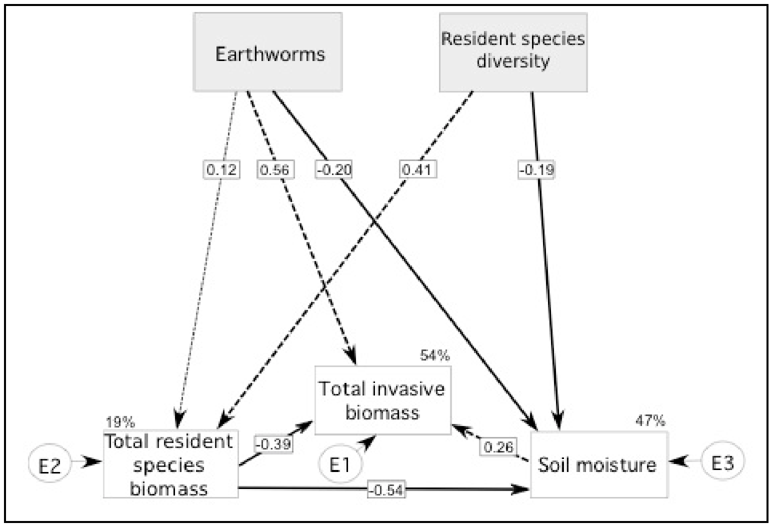


Figure 21. Resident species diversity and the presence of earthworms both affected the biomass of invasive species. The relationship was mediated through soil moisture and native species biomass.



Furthermore, when worms were absent invasive species biomass was lower in high light due to seed desiccation. This was an unexpected result since most previous studies suggest that buckthorn grows best in high light. Across the litter treatments, invasive plants had lower biomass in high litter as we expected based on field observation and previous studies. However, when worms were present in pots, the effects were reversed and there was high germination of invasive in the pots with more litter. Overall, forest invaders experienced higher germination in the presence of earthworms and these belowground invaders appear to be an important variable determining a site's invasibility.

Figure 22. When worms were absent invasive species biomass was low in high light due to seed desiccation

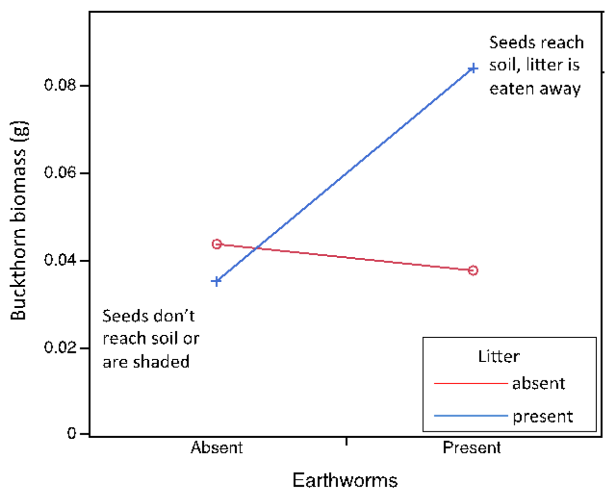
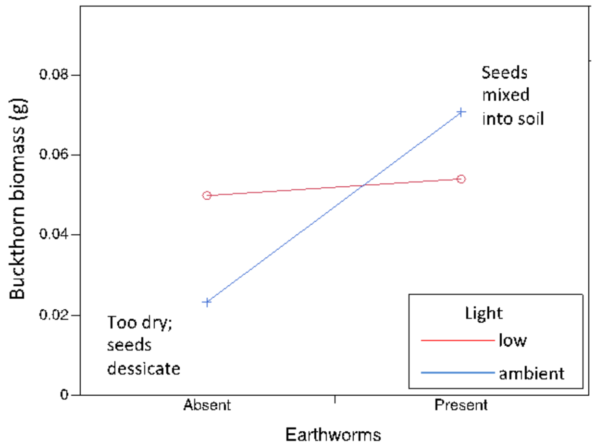


Figure 23. In the presence of earthworms, invasive species biomass was higher when leaf litter was present. Without worms, leaf litter reduced the success of invasive species



We also ran a seedbank study in the greenhouse to investigate propagule pressure, i.e., the number of seeds of a given species released into an area. This is an important component of invasion potential and something our field study suggested was an important predictor buckthorn abundance in our study sites. Estimating the difference between actual propagule pressure, i.e., the numbers of seeds in the seedbank, and realized abundance of an invasive species, i.e., the actual number of stems of the invasive species in a given area, is a way to estimate whether environmental characteristics of a site are suppressing invasion. To investigate whether characteristics of Minnesota's deciduous forest play a role in suppressing invasion by buckthorn we collected soil from 12 forest stands across a low to high gradient of buckthorn abundance. We exposed this soil to 'ideal conditions' in the green house (high light, adequate moisture, and low competition) to germinate as many invasive species as possible from the seed bank. The seedbank greenhouse study concluded in July 2012. Far fewer buckthorn seeds germinated from the collected soils than expected so we did not have sufficient statistical power to analyze these results. Due to the spatial variability of buckthorn seeds in the soil, future studies would need to collect far more soil to determine a representative available seedbank.

Figure 24. After collecting soil from several sites with differing amounts of buckthorn, we monitored germination from the seed bank to assess the difference between seed availability in the seed bank and the actual abundance of buckthorn at a site.

8. Add non-native seeds to experimental field plots
9. Complete surveys of non-native establishment success in experimental field plots

We are combining the summary of deliverables 8 and 9, Results 2 since they are part of the same field experiment. We were able to make use of an existing experiment in northern Minnesota, which monitors and manipulates light, heat and precipitation, to test the ability of several non-native species (buckthorn, barberry, and honeysuckle) to colonize forests from seed. The two experimental sites are located in Cloquet and Ely and include a total of 72 plots. This experiment should help us characterize how different management strategies may exacerbate or ameliorate the invasion process. For example, sites with higher light availability, warmer temperatures, higher soil moisture, modest competition from a low diversity set of neighbors that lacks conifers might be most advantageous for invasives. If this is the case, sites could be managed to ensure the best species mix and right canopy closure to minimize invasive success. Seeds of the three invasive species were sown at both sites

Preliminary data from a smaller scale growth chamber experiment suggests that buckthorn seeds were larger when grown at elevated temperature (+3°C and +6°C above ambient temperature). In the larger scale field experiment, significantly more invasive species germinated in the experimental plots across all temperature treatments but further analysis is needed to establish whether the temperature treatments had an impact on germination rate. With University funding we will continue to monitor the seeds during the summer of 2013 to establish if invasive species respond in a different way to changes in temperature, precipitation, or light.

10. Selection of 8-15 plots with variable buckthorn presence within Warner Nature center and one other forest site
11. Characterize plots with respect to buckthorn abundance, canopy basal area, soil texture, slope, elevation, and aspect
12. Measure nitrogen inputs, recycling, and outputs at each plot

We are combining the summary of deliverables 10, 11, and 12 for Results 2 since they are part of the same field experiment. We are interested in the nitrogen cycle because the productivity of forests is positively correlated with rates of nitrogen input and recycling in soil. Also, nitrogen outputs from forest ecosystems, including nitrate in groundwater and surface water and nitrous oxide in the air, can have important consequences for water quality and climate. Buckthorn leaves have unusually high concentrations of nitrogen so we expect that forest sites with abundant buckthorn will have greater nitrogen inputs through falling leaves, greater rates of nitrogen recycling in soil, and, at least seasonally, greater nitrogen exports into water and air. We tested these hypotheses by measuring inputs and recycling of nitrogen at the Lee and Rose Warner Nature Center near Marine on St. Croix. Since we were able to find plot locations on two different soil types (sandy and silty) within one large study area it was not necessary to set up the experiment at two site as original proposed. This made the experimental setup more efficient and logistically easier.

We identified 28 study plots at our study site. On each soil, the plots were located across a gradient of buckthorn invasion from low to high abundance. For each level of buckthorn invasion on each soil type, there were two replicate plots for each of the six different levels of buckthorn abundance (12 plots along the buckthorn gradient on each soil type). Additionally, on the silty soil type, we located four plots where buckthorn has been removed: two of these where buckthorn was removed twice in the last 6 years and two where buckthorn was removed once three years ago.

At the start of the project we surveyed each plot for vegetation characteristics including the number and stem size of each buckthorn individual, canopy species identity and basal area. We also measured slope, aspect, and elevation in each plot. In early July (2012) we initiated soil sampling from each plot to measure concentrations of soil nutrients, including ammonium, nitrate, and total carbon and nitrogen. Nitrates and ammonia represent mineralized nitrogen that is usable by plants (compared to non-mineralized organic nitrogen that is not available to plants). We used two methods to measure in situ levels of nitrogen across the gradient of buckthorn abundance. First, we installed soil incubation tubes consisting of two inch diameter PVC tubes, each 20 cm long. These were hammered into the soil, capped, and left for around 28 days. On the same day that the PVC tubes were installed, we also collected soil close to the incubation tubes and analyzed this soil for concentrations of nitrates and ammonia to serve as an initial value for mineralized nitrogen available to plants. After 28 days we removed the PVC tubes and analyzed the soil inside each one for nitrate and ammonia in the same way as for the initial soil samples. Since the soil in the incubation tubes had been isolated from plant roots and further nitrogen input we were able to subtract the amount of nitrates and ammonia in the initial soil sample from the amount in the PVC tubes and use this value as an index for the recycling of N in organic matter by soil microbes (i.e. net nitrogen mineralization). In plots with more abundant buckthorn, we would expect nitrogen mineralization to be higher since buckthorn has nitrogen-rich leaves.

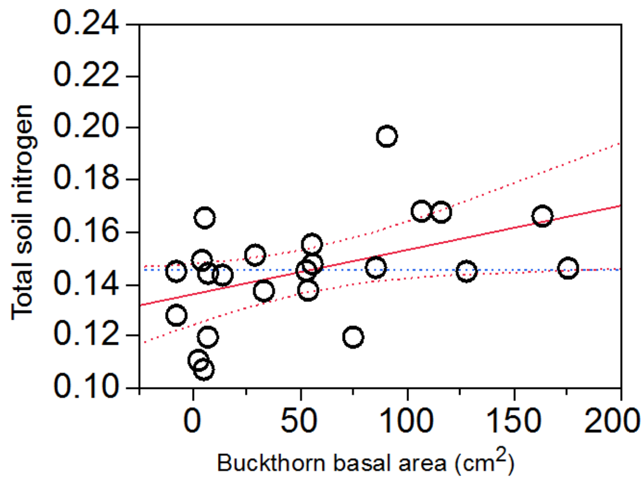
Figure 25. Incubation tubes (short lengths of PVC tube) were hammered into the ground, capped and left in place for 28 days.

The second method of estimating nitrogen mineralization was by using buried bags of ion-exchange resin, a granular material onto which nitrate is adsorbed. These bags were buried 10 cm under an undisturbed soil profile so nitrate that was leached out above the bags is caught in the bag. We removed the resin bags after about 28 days and then analyzed them for nitrate and ammonia in the same manner as for the soil extracts. We performed this procedure (incubation tube and resin bag deployment) during the summer and fall of 2012, and also over the winter of 2012/2013 and spring of 2013. In this way, we were able to estimate the amount of soil mineralization during each season.

Figure 26. We also buried small bags of ion-exchange resin as another way to measure the amount of nitrates in the soil. These were also buried for 28 days.

Overall, the total amount of nitrogen in the soil increased as the abundance of buckthorn increased. Whether or not this translates into higher amounts of mineralized nitrogen (ammonia and nitrate) available for plant growth will be established when we have analyzed all the data from the incubation tubes and resin bags. This analysis is ongoing, with funding from the University of Minnesota and will allow us to assess whether buckthorn alters soil nutrients after invasion. This has implications for productivity and growth rates of native species and also might influence invasibility by other introduced species.

Figure 27. Total soil nitrogen as a function of buckthorn basal area.

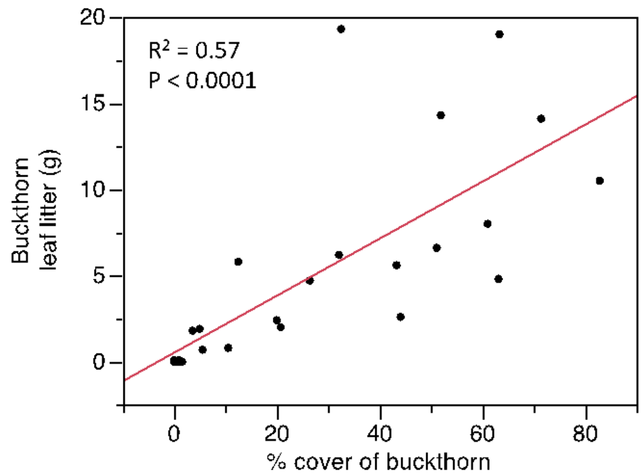


On September 7, 2012 we installed two 10 gallon pots in each survey plot for collection of leaf litterfall. This allowed us to estimate the proportion each of the major tree and shrub species (including buckthorn) contribute to total leaf litter. Every two weeks, until December 3, 2012, we collected all the leaves that had fallen into each buckets. We sorted the litter to species in order to estimate the percentage of litter each species contributed. After sorting, we dried, weighed, and ground a sample of leaves for analysis of nitrogen concentrations so we could estimate nitrogen inputs into the soil.

Figure 28. We collected falling leaves in large buckets from in the fall of 2012 (September to December) to measure the relative amount each tree species contributed to the overall litter layer

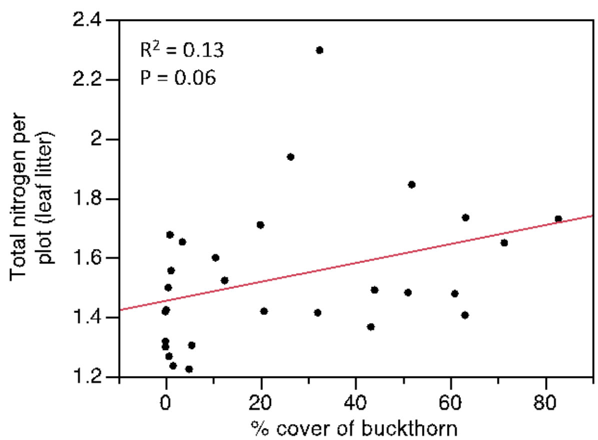
As expected, plots with a higher abundance of buckthorn stems, also had more buckthorn leaf litter.

Figure 29. The amount of buckthorn leaf litter increased in plots with more buckthorn



The total nitrogen in all leaf litter combined was also higher in plots where buckthorn was more abundant indicating that heavy buckthorn cover does influence nitrogen inputs.

Figure 30. Total nitrogen in all leaf litter versus the abundance (% cover) of buckthorn



13. Measure earthworm composition and abundance at each plot twice

While beneficial to gardeners, as they mix and aerate soil and increase nutrients, earthworms are determental to hardwood forests. Forests in Minnesota developed during the thousands of years since glacial retreat without earthworms. Hardwood trees produce nutrient rich leaf litter faster than it decomposes, so a thick organic layer of decomposing leaf litter develops. As bacteria and fungi decompose the forest floor litter, nutrients are made available for understory plants and tree seedlings. The thick forest floor of earthworm-free hardwood forests is where most nutrient cycling occurs and where almost all understory plants and tree seedling germinate and grow. When earthworms invade, the result is homogenization of top layers of soil and and removal of litter and organic material, resulting in loss of habitat for germinanting native trees and wildflowers.

The fallen leaves of buckthorn have unusually high concentrations of nitrogen, which makes them a preferred food for many soil invertebrates including invasive European earthworms that are present in nearly all forested sites across Minnesota. As a result, buckthorn invasion is also associated with invasion of exotic earthworms, which can have further influences on nitrogen cycling. To understand how earthworms and buckthorn interact to influence soil nitrogen recycling and potential nitrogen exports into ground water and the atmosphere, we sampled the same plots at Warner Nature Center described above for soil nitrogen to investigate the relationship between buckthorn and earthworm abundance. In the early and later summer of 2012 and 2013, we sampled earthworm communities four times to quantify their abundance and community composition. Preliminary analysis indicates that earthworms were more diverse and abundant in silty versus sandy soil. Also, total earthworm biomass increased with increasing buckthorn. An ecological model that included buckthorn abundance light levels, soil moisture, and pH as predictors of earthworm abundance indicated that buckthorn facilitates worms through increased soil moisture.

Figure 31. Number of individual earthworms on the two main soil types at the study area (Warner Nature Center, Marine on St. Croix, MN)

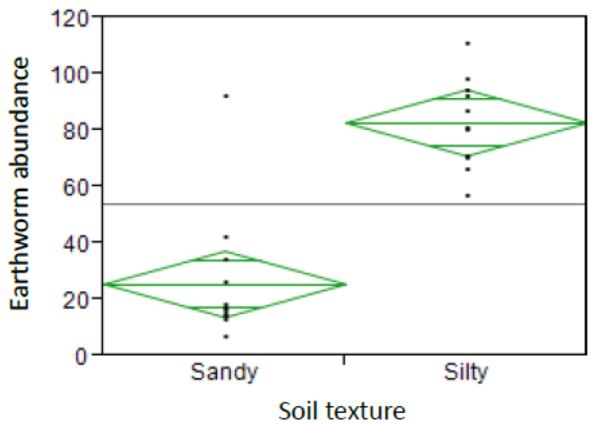
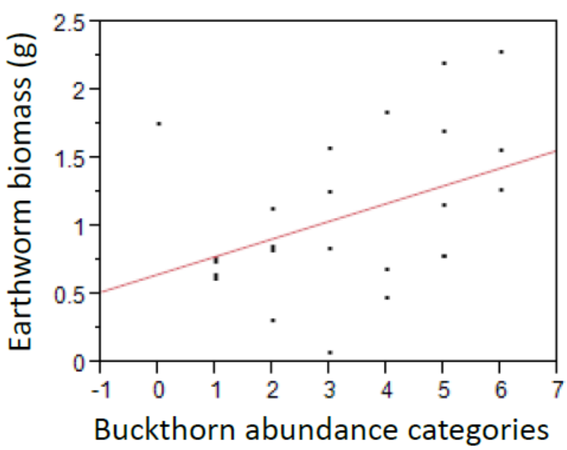


Figure 32. Earthworm biomass versus buckthorn abundance at the study area in Warner Nature Center, Marine on St. Croix, MN



14. Spray ^{15}N labeled urea on buckthorn leaves to produce litter for controlled experiment; collect leaves
15. Conduct controlled experiment on earthworm-buckthorn interactions using ^{15}N labeled leaf litter

We were unable to complete these two deliverables related to Results 2. We did not have time to collect the buckthorn leaf litter in the fall of 2012 so were unable to conduct this experiment. Because of projected salary cost savings due to graduate student fellowships and scholarships, items #10-15 were added to the original work program. But since items #14 and 15 were not completed, money will be returned to the Trust Fund. Additional cost savings were realized on this project because we were able to hire undergraduate students with work-study funding over the course of the academic year.

RESULT 3: Analyze data, develop management guidelines, disseminate results via outreach presentations, workshops, and reports, DNR/UM web site, scientific publications.

Description: Guidelines for forest management to resist invasion will be developed. These will be provided to resource managers and the public through a series of presentations and workshops as well as via an interactive web site.

Summary Budget Information for Result 3: **ENRTF Budget:** **$** 86,000
Amount Spent: **$** 69,101
Balance: **$** 16,899

Deliverable	**Completion Date**	**Budget**
1. Final report, “Do Healthy Forests Resist Invasion?”	6/30/2013	$26,000
2. Forest management guidelines	6/30/2013	$25,000
3. Outreach via presentations, workshops, web site	6/30/2013	$10,000
4. Scientific publications written	6/30/2013	$25,000

Result 3 Completion Date: 6/30/2013

Result Status as of September 21, 2012: Work has not yet begun.

Result Status as of January 10, 2012: Work has not yet begun.

Result Status as of July 10, 2012: Work has not yet begun.

Result Status as of September 21, 2012:
With this amendment, we are requesting permission to use some travel funds within Result 3 be used for travel to the Upper Midwest Invasive Species Conference for three members of our research team. The Upper Midwest Invasive Species Conference in the premier regional forum for sharing information related to invasive species. Stated goals of the meeting are to strengthen awareness of invasive species management and prevention and to facilitate information sharing. These are also goals of our LCCMR funded "Healthy forests to resist invasion" project and our presence at the meeting will allow us report findings from this study to land managers, natural resource environmental and forestry professionals, landowners, and governmental agencies many of whom will be from Minnesota. This is one of our best opportunities to communicate results from this LCCMR funded study to the largest possible audience of Minnesota stakeholders. Despite being just across the border in Wisconsin, the meeting is strongly relevant to invasive species issues in Minnesota and many of the attendees will be from Minnesota. This conference is co-sponsored by Minnesota Invasive Species Advisory Council (MISAC) and the Midwest Invasive Plant Network (MIPN), both of which have high number of members from Minnesota, the people who are most interested in our research. No change in funding or budget lines.

Result Status as of January 10, 2013**:**

1. Final report
Ongoing

2. Forest management guidelines
Ongoing

3. Outreach via presentations, workshops, website.
Presentation of preliminary results at the Upper Midwest Invasive Species Conference, La Crosse, WI. We presented talks focused on measuring propagule pressure, the greenhouse study, and the effects of native species on buckthorn abundance.

4. Scientific publications written
We submitted a manuscript (“Community phylogenetic diversity and abiotic site characteristics influence abundance of the invasive plant *Rhamnus cathartica* L.”) to the *Journal of Plant Ecology*. The paper is currently in review. We are also preparing a second manuscript for submission to the journal *Oecologia* (“Native plant functional diversity and introduced earthworms affect the success of invasive plants”) based on results from the greenhouse experiment.

Final Report Summary August 15, 2013**:**

1. Final report, “Do Healthy Forests Resist Invasion?”

The long-term goal is to provide information to land managers so they can develop an understanding of the potential invasibility of their forests. This can be incorporated into their planning efforts to ensure the ongoing health and integrity of the ecosystem. We will work to ensure that the results of the study are widely disseminated and available to the widest possible audience.

2. Forest management guidelines

To summarize results from the project and provide guidelines for management, we put together a pamphlet that included all aspects of the research, as it pertains to the invasion of buckthorn. The pamphlet also provides suggestions for pre-invasion management to reduce invasibility, the main focus of the “Healthy Forests” research project. We distributed the pamphlet to all participants at a symposium held on the St. Paul campus on August 14, 2013, and it is available as a pdf from the project website. See appendix A for a pdf of the pamphlet.

Preventing and managing common buckthorn invasion: recent research and recommendations

Tim Whitfeld, Peter Reich, Sascha Lodge, Alex Roth, and Cindy Buschena
Department of Forest Resources, University of Minnesota
Email: cbuschen@umn.edu Website: http://forestecology.cfans.umn.edu/Research/Buckthorn/index.htm

Figure 33. Pamphlet summarizes the results of the Healthy Forests project

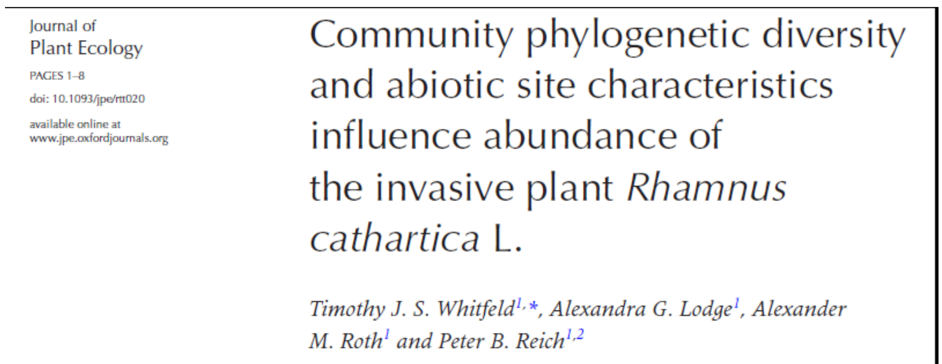
3. Outreach via presentations, workshops, web site

We presented talks at the Upper Midwest Invasive Species conference (a regional meeting focused on invasive species) and the Ecological Society of America conference (an international conference focusing on all aspects of ecology) in 2012 and 2013. See appendix B for the ESA poster presentation. The talks focused on measuring propagule pressure, the greenhouse study, the relationship between earthworm and buckthorn buckthorn, and the effects of native species diversity on buckthorn abundance.

On August 14, 2013, we hosted a symposium that brought together managers and researchers to share the latest information on invasive plants in Minnesota forests. See Appendix C for a list of speakers and presentation titles. The symposium was attended by 100 participants from across the state (see Appendix D for a list of the attendees and their affiliations). In addition to talks based on this LCCMR project, other speakers presented information about buckthorn invasion on the prairie-forest border in west central Minnesota, garlic mustard (another common plant invader in Minnesota’s forests) as a driver of species invasion, management of buckthorn from a foresters perspective, and management efforts to control other common invasive plants. We also summarized results from all aspects of the research project in the pamphlet that was distributed at the symposium and is available as a pdf from our website: (http://forestecology.cfans.umn.edu/Research/Buckthorn/index.htm). The site also includes links to recordings of all the symposium talks, as well as a links to the MS Access database, species lists from all survey sites, and a photo gallery.

4. Scientific publications written

We published one paper ("Community phylogenetic diversity and abiotic site characteristics influence abundance of the invasive plant *Rhamnus cathartica* L.") in the *Journal of Plant Ecology*. In addition, we submitted a second paper based on results from our greenhouse experiment ("Native plant diversity and introduced earthworms have contrasting effects on the success of invasive plants") to the peer-reviewed journal *Biological Invasions*. See appendix E for pdf of the *Journal of Plant Ecology* publication. More papers are in preparation including one focusing on propagule pressure and another that documents the relationship between earthworms and buckthorn abundance. Additional publications will be provided to LCCMR as they are published.


Journal of
Plant Ecology
PAGES 1–8
doi: 10.1093/jpe/rtt020
available online at
www.jpe.oxfordjournals.org

Community phylogenetic diversity and abiotic site characteristics influence abundance of the invasive plant *Rhamnus cathartica* L.

Timothy J. S. Whitfeld[1,], Alexandra G. Lodge[1], Alexander M. Roth[1] and Peter B. Reich[1,2]*

Figure 34. Publication based on results from the Healthy Forests project

Because of graduate student salary savings (students obtained scholarships), money is being returned to the Environmental and Natural Resources Trust Fund.

V. TOTAL ORIGINAL ENRTF PROJECT BUDGET:

BUDGET ITEM	**AMOUNT**
Personnel:	
1 Research associate, 100%, coordination of day to day project activities ($44,596 salary + $14,405 fringe) for 2 years	$118,002
1 Graduate student, 50%, develop dissertation research project from some aspect of project research ($21,000 salary + $3,536 health insurance + $ 11,170 tuition for 2 years	$71,412
1 Project assistant, 50% ($36,000 salary + $6,660 fringe) for 2 years	$49,320
4 undergrad students (summer, 100%) 2000 hours @ $11/hour + $1,795 fringe) for 2 summers	$47,590

3 undergrad students (academic year, 25%) 8 hrs/week, 960 hours @ $11/hour for 2 academic years	$21,120
Equipment/Tools/Supplies: Misc. field supplies and tools (data sheets, labels, bags, vials, etc.) for 2 years; and laser range finders (2) and light sensors (2)	$15,976
Travel: Intensive in-state travel to 80 scattered and remote field sites, for 2 years, includes lodging and mileage on personal vehicles	$21,500
Chemical analyses of plants and soils: cost based on one vegetation and one soil sample per plot (16 plots x 80 sites, at a total cost of $11 for the two analyses), for 2 years	$14,080
TOTAL ENRTF PROJECT BUDGET	**$359,000**

See Final Attachment A: Budget Detail

This is proposed as a three-year project. Budget is for 2 years, but to be spent over 3 years.

Explanation of Capital Expenditures Greater Than $3,500: None

VI. PROJECT STRATEGY:

A. Project Partners:

Ann Pierce, Conservation Management and Rare Resources Unit, Ecological Resources, MNDNR
Kathleen Knight, U.S. Forest Service

All funds will be administered through the University of Minnesota. Hence, each partner will receive none of the funds from the appropriation.

B. Project Impact and Long-term Strategy: In the long term this information can be used to help land managers develop management prescriptions that incorporate the current invasive status of the plant community and the health and integrity of the ecosystem, which will serve as an indicator of vulnerability to invasion. Results of this project can be used to inform silvicultural interpretations being developed based on the Ecological Classification System. This information is critical to maintaining a resilient forest system in the face of future climate change coupled with invasive species.

C. Other Funds Proposed to be Spent during the Project Period: None

D. Spending History: No funds will be spent prior to the start of this project.

VII. DISSEMINATION:

We will work to ensure that the results of the study are widely disseminated and used. The third deliverable of our project is by definition the translation of our work to relevant public and private organizations and groups. This includes a variety of means, including workshops, reports available on the web, presented seminars, and the like. Additionally, we will work through relevant units within and outside management agencies (e.g., DNR Forestry, the Minnesota Forest Resources Council) to make recommendations widely known, and as appropriate, we will urge their adoption and implementation. Research results will be published in peer reviewed journals and other outlets. Publications resulting from this work will be posted on http://forestecology.cfans.umn.edu/ as they become available, as well as information relating to this project.

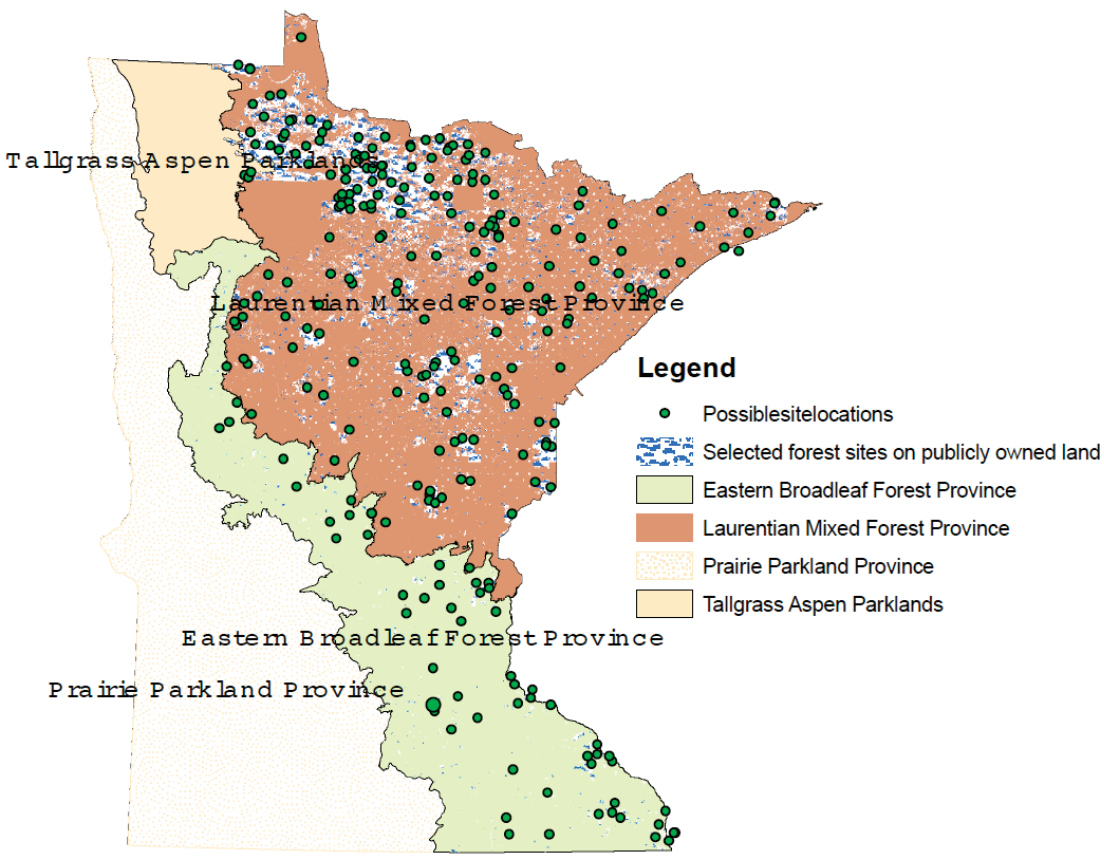
VIII. REPORTING REQUIREMENTS:

Periodic work program progress reports will be submitted not later than July 10, 2010, January 10, 2011, July 10, 2011, January 10, 2012 and January 10, 2013. A final work program report and associated products will be submitted between June 30 and August 15, 2013 as requested by the LCCMR.

IX. RESEARCH PROJECTS:

See attached research addendum

Figure 1.



Appendices:

A Pamphlet: Preventing and managing common buckthorn invasion: recent research and recommendations

B Poster: Ecological Society of America

C List of symposium speakers and presentation titles

D List of symposium attendees and their affiliations

E Whitfeld, T. *et al.* 2013. Community phylogenetic diversity and abiotic site characteristics influence abundance of the invasive plant *Rhamnus cathartica* L. *Journal of Plant Ecology*

Final Attachment A: Budget Detail for 2010 Projects

Project Title: Healthy Forests to Resist Invasion - M.L. 2010, Chp. 362, Sec. 2, Subd. 6c

Project Manager Name: Peter Reich

Trust Fund Appropriation: $ 359,000

2010 Trust Fund Budget	**Result 1 Budget** *(June 2013)*	**Amount Spent** *(June 2013)*	**Balance** *(June 2013)*	**Result 2 Budget** (*June 2013*)	**Revised Result 2 Budget** (June 2013)	**Amount Spent** *(June 2013)*	**Balance** *(June 2013)*	**Result 3 Budget** *(June 2013)*	**Amount Spent** *(June 2013)*	**Balance** *(June 2013)*	**TOTAL BUDGET**	**TOTAL BALANCE**
	Finalize research plans, select 80 sites, and establish 16 research plots in each site			Assess degree of plant invasion, disturbance history, and health and structural integrity of native plant communities.	Assess degree of plant invasion, disturbance history, and health and structural integrity of native plant communities.			Analyze data, develop management guidelines, disseminate results via outreach presentations, workshops, and reports, DNR/UM web site, scientific publications.				
BUDGET ITEM												
TOTAL PERSONNEL	37,706	37,706	0	184,201	184,201	184,201	0	81,150	67,431	13,719	303,057	13,719
PERSONNEL: 1 Research associate, 100%, coordination of day to day project activities												
PERSONNEL: 1 Graduate student, 50%, develop dissertation research project from some aspect of project research ($21,000 salary + $3,536 health insurance + $ 11,170 tuition for 2 years												
1 Project assistant, 50% ($36,000 salary + $6,660 fringe) for 2 years												
4 undergrad students (summer, 100%) 2000 hours @ $11/hour + $1,795 fringe) for 2 summers												
3 undergrad students (academic year, 25%) 8 hrs/week, 960 hours @ $11/hour for 2 academic years												
Non-capital Equipment / Tools: laser range finders (2) @ $450 each and light sensors (2) @ $1,107 each				3,114	3,114	2,758	356				3,114	356
Supplies: Misc. field supplies (data sheets, labels, bags, vials, etc.) for 2 years				~~12,862~~	10,296	5,025	5,271				10,296	5,271
Travel expenses in Minnesota: Intensive in-state travel to 80 scattered and remote field sites, for 2 years, includes lodging and mileage on personal vehicles	5,500	5,500	0	13,500	13,500	12,689	811	4,100	1,021	3,079	23,100	3,890
Travel expenses outside of Minnesota: travel to and from La Crosse, Wisconsin to attend the Upper Midwest Invasive Species Conference							0	400	347	53	400	53
Other: Chemical analyses of plants and soils: cost based on one vegetation and one soil sample per plot (16 plots x 80 sites, at a total cost of $11 for the two analyses), for 2 years				~~13,436~~	16,002	16,002	0				16,002	0
Other: Printing charges							0	350	302	48	350	48
Other: Greenhouse space charges				1,394	1,394	1,394	0				1,394	0
Other: Professional services (buckthorn removal)				1,287	1,287	1,287	0				1,287	0
COLUMN TOTAL	**$43,206**	**$43,206**	$0	**$229,794**	**$229,794**	**$223,356**	**$6,438**	**$86,000**	**$69,101**	**$16,899**	**$359,000**	**23,336**

Preventing and managing common buckthorn invasion: recent research and recommendations

Tim Whitfeld, Peter Reich, Sascha Lodge, Alex Roth, and Cindy Buschena

Department of Forest Resources, University of Minnesota

Email: cbuschen@umn.edu Website: http://forestecology.cfans.umn.edu/Research/Buckthorn/index.htm

What is buckthorn? Common buckthorn *(Rhamnus cathartica)*, sometimes called European buckthorn, is a shrub or small tree native to Europe and western Asia. It was brought to North America as an ornamental and medicinal plant in the late 18th century and has since spread across northern parts of the continent. The first record in Minnesota was from Hennepin County in 1937. Common buckthorn grows in a wide range of habitats including forests, savannas, wetland edges, open areas, and disturbed areas. It tolerates shade and can also grow in the open so is an effective colonizer of forest gaps. Two other species of buckthorn grow in Minnesota: glossy buckthorn *(R. frangula)*, an introduced shrub that has become a significant invader in parts of eastern North America, and alder buckthorn *(R. alnifolia),* a native species.

What are its effects? In North America, common buckthorn often forms thickets that are much denser than observed in its native range. These thickets can impede hunters, hikers, and wildlife moving through the forest. Furthermore, the highly nutritious common buckthorn leaves are attractive food for soil invertebrates and thus decompose rapidly. This results in large areas of bare soil that are ideal for subsequent germination of more buckthorn and other invasive plants. Common buckthorn leaves are also a preferred food for invasive European earthworms that further increase litter decomposition. The combined effects of buckthorn and earthworms often exclude many native plant species (especially those sensitive to disturbance) and reduce overall diversity. Dense stands of common buckthorn also increase shade

Leaves and ripening fruit of common buckthorn

(which reduces tree seedling growth and survival) and increase competition for water and nutrients. Buckthorn thickets can also reduce bird diversity since birds are more likely to be eaten by predators when they nest in non-native shrubs.

Why is common buckthorn such a successful invader? The leaves of buckthorn have high levels of nitrogen so it can photosynthesize and grow quickly, giving it an advantage over other plants. This benefit is enhanced by an early leaf flush and late leaf drop, characteristics not observed in its native range, that give common buckthorn a longer growing season than native plants. Furthermore, it produces many seeds with high germination rates and low mortality. It also has secondary compounds (e.g., emodin) that may deter herbivores (deer and insects) and limit neighboring plant growth. Since common buckthorn seeds are bird dispersed, it can easily establish new populations far from existing infestations.

This work 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”

Negative impacts of buckthorn:

- Forms dense, tangled thickets that impede hunters, hikers, and wildlife
- Increases shade and reduces growth of native plant species
- Nutritious leaves are rapidly decomposed leaving large areas of bare soil to be colonized by other invasive plants
- Provides nutritious leaf litter for invasive earthworms

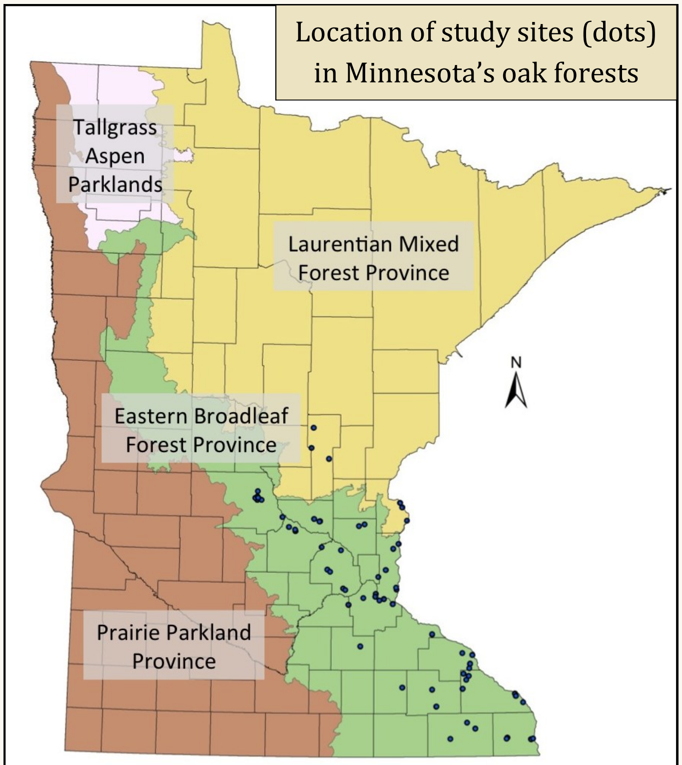
Recent LCCMR-funded research findings: The primary goal of this project was to understand which forest characteristics, especially those amenable to management, are effective deterrents to common buckthorn invasion. Undisturbed, healthy forests are likely to be more resistant to invaders so management aimed at maintaining or enhancing these key characteristics could be a useful way to slow the spread of forest invaders. This pre-invasion strategy is also likely to be more effective and economical than post-invasion removal of invasive species.

Field survey: To determine how various site characteristics affected common buckthorn invasion in Minnesota's forests, we surveyed plant diversity and abundance in 55 sites in central and southern Minnesota. At each site, we also measured light levels, amount of leaf litter on the forest floor, soil quality, availability of nearby buckthorn seeds, duff layer thickness, and slope. By doing this, we were able to simultaneously account for a wide range of factors that previous studies suggested might influence invasibility.

Common buckthorn forms dense thickets and holds its leaves longer in the fall than native trees and shrubs

Common buckthorn was most abundant in sites with more openings in the canopy and a sparser layer of leaf litter on the forest floor. Surprisingly, the number of native species did not influence the abundance of buckthorn. We had expected there to be a negative relationship because sites with more native species would limit the space for invaders.

Location of study sites (dots) in Minnesota's oak forests



However, when we tallied the diversity of plant types (grasses, wildflowers, shrubs, trees) and plant families (oaks, maples, dogwoods, sunflowers, etc.), forests with more overall diversity did resist buckthorn invasion better. This is because different types and families of plants acquire light and nutrients for growth in many different ways, leaving less ecological space for invaders.

Buckthorn seed availability study: The presence of buckthorn seed sources in the survey area was also, not surprisingly, an important part of its invasion success. When there were many

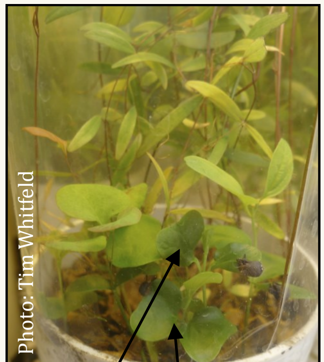
We documented plant diversity and site characteristics in forests across central and southeastern Minnesota

mature buckthorn near the forest stand of interest, buckthorn was more abundant within the site. To characterize local seed availability, we developed an easy-to-use driving survey technique that can be implemented by land managers to better estimate buckthorn seed sources surrounding a site of interest. This will be especially useful in parts of the state where buckthorn is not yet common in natural areas but present in people's yards. Observing buckthorn populations along easily

Experimental communities showing shade and diversity treatments in the greenhouse

accessible roadsides could be a signal for managers to look out for buckthorn populations within more remote forested areas.

GREENHOUSE EXPERIMENT: Our field study suggested that light levels, leaf litter, and native plant diversity all affected common buckthorn abundance. To test these observations in a controlled setting, we manipulated light, leaf litter, plant diversity, and the presence of earthworms in a greenhouse experiment. As we observed in the field study, common buckthorn was less abundant when native species diversity was high. However, it was always more successful in the presence of earthworms, regardless of plant diversity. Furthermore, buckthorn was most abundant when litter levels were low and light levels were intermediate. Overall, it appears that native plant diversity, light levels, and litter depth do impact the success of common buckthorn. However, invasive earthworms, an almost ubiquitous presence in Minnesota's forests, can undermine the benefits of manipulating these factors.



Emerging buckthorn seedlings

Buckthorn does best...

...in areas where leaf litter depth is low

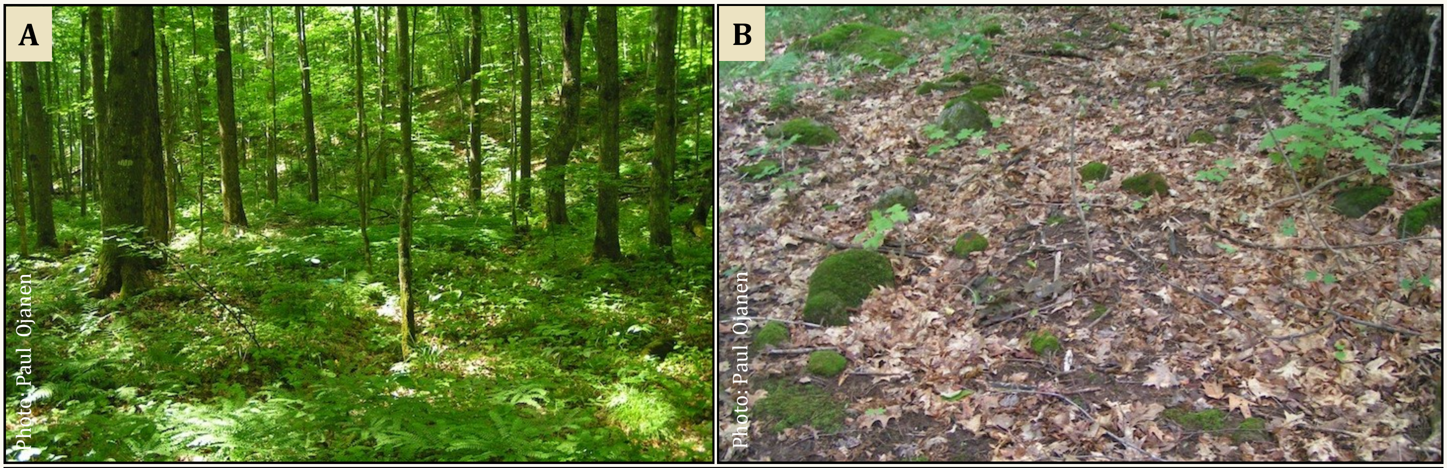
...at intermediate light levels

...when diversity of native plant species is low

...in the presence of earthworms

BUCKTHORN AND EARTHWORMS STUDY: Given the strong evidence to suggest that invasive earthworms increase common buckthorn abundance, we also investigated the combined effects of these above and belowground invaders in the forest. When buckthorn became more abundant, its dense shade increased soil moisture, which led to more earthworms.

Diverse, minimally disturbed forests are less susceptible to common buckthorn invasion



(A) Before earthworm invasion, herbaceous plants form a continuous cover. (B) After invasion, plant cover and leaf litter are reduced perpetuating an invasion cycle with common buckthorn.

Management recommendations: The best opportunity to decrease forest susceptibility exists before common buckthorn invades. This can be done by enhancing native species diversity or minimizing disturbance. Alternatively, post-invasion management focuses on buckthorn removal and subsequent treatments to prevent reinvasion. In either case, management should involve manipulation of environmental conditions to enhance forest characteristics that help resist invasion (e.g., shade, diversity, leaf litter).

Common buckthorn hedgerows provide seed sources for invasion of natural areas

Pre-invasion management: Although not immune to invasion, "healthy forests" are more resistant than disturbed forests; thus enhancing key characteristics might slow the spread of common buckthorn. The degree of disturbance, the ecological integrity of an ecosystem, and the diversity of forest species (characteristics amenable to management) all likely influence how easily forests are colonized by invasive plant species. We propose the idea of "preventive environmental care" that, like preventative health care, manages forests for"wellness" and "illness." Although not a panacea for reducing invasion, it is a tool worth considering given the challenges of controlling established invasive species.

Managing seed availability:

Seed availability is one of the most important factors in determining the spread of invasive species. Reducing common buckthorn seed sources is a promising first step in successful pre- and post-invasion management. Pre-invasion, reducing seed availability is particularly important where common buckthorn is not yet abundant in natural areas but is present in nearby towns. Community efforts to remove common buckthorn from residential areas are one of the most effective ways to slow its spread into surrounding forests. Post-invasion, focusing first on removal of mature adult plants before removing smaller individuals will improve the success of a management program. Collaboration with neighboring landowners is necessary since any attempt to remove buckthorn from an individual forest stand will ultimately fail without first reducing seed availability in the surrounding area.

Our results suggest diverse communities reduce invasion by leaving less space for invaders. Therefore, managing for enhanced diversity is an important pre-invasion technique. One way to do this is by controlling the density of white-tailed deer, a species that severely decreases plant growth when populations are high. Another is to limit disturbance by using winter timber harvest and low impact trail construction techniques that minimize soil disturbance and keep the forest floor as shaded as possible. Maintaining an intact litter layer also appears to be particularly important.

Post-invasion strategies: Strategies for forest management following common buckthorn establishment have been described in various publications (see *Other resources),* and include methods like mechanical removal, chemical application, and controlled burning. Since buckthorn seeds can survive for up to five years, several follow-up treatments are required to remove emerging seedlings. Ongoing removal of buckthorn, by whatever method, is the first step and should be combined with replanting/reseeding native trees, shrubs, and herbs. Our results suggest an intact litter layer is also an important barrier to invasion. Following removal, treatments that replicate this, such as mulching, may prevent reinvasion. Also, planting a temporary cover crop following removal will take up valuable space that would otherwise be available for invasive plants. Diverse mixtures may have even greater success.

Examples of management strategies

Pre-invasion	**Post-invasion**
• Maintain forest wellness to reduce invasion	• Remove invasive plants or burn after infestation
• Low impact timber harvest and trail maintenance to maintain dense leaf litter & canopy layer	• Apply mulch, plant cover crop, and/or reseed to reduce buckthorn reinvasion
• Improve plant diversity by minimizing disturbance and managing white-tailed deer	• Improve diversity by planting native trees, shrubs, and herbaceous species
• Ongoing monitoring to detect new invasions	• Ongoing removal of new seedlings

A

B

C

Post-invasion removal techniques: (A) hand pulling, (B) basal bark herbicide application, and (C) cut and paint herbicide application

Future directions: Overall, our results suggest significant roles for native plant diversity, leaf litter, and light levels in determining the invasibility of Minnesota's forests by common buckthorn. Large-scale manipulative experiments to test whether these preventative measures are effective in reducing invasion of common buckthorn are the obvious extension of the "healthy forests" concept. Investigating other potential interactions with invasive earthworms is also a key component of long-term forest health, as is better post-removal monitoring to see if native species re-establish or whether invasive species continue to dominate.

Ongoing research questions:

- How do common buckthorn management techniques affect post-removal forest recovery & what is the best way to minimize reinvasion?
- How do earthworms and buckthorn facilitate each other's invasion? Does control of one reduce abundance of the other?
- Do high nitrogen buckthorn leaves affect how soil nutrients are recycled in invaded forests? If so, this could influence the growth of native species and have implications for future invasions.

Other resources:

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Native plant diversity and introduced earthworms have contrasting effects on the success of invasive plants

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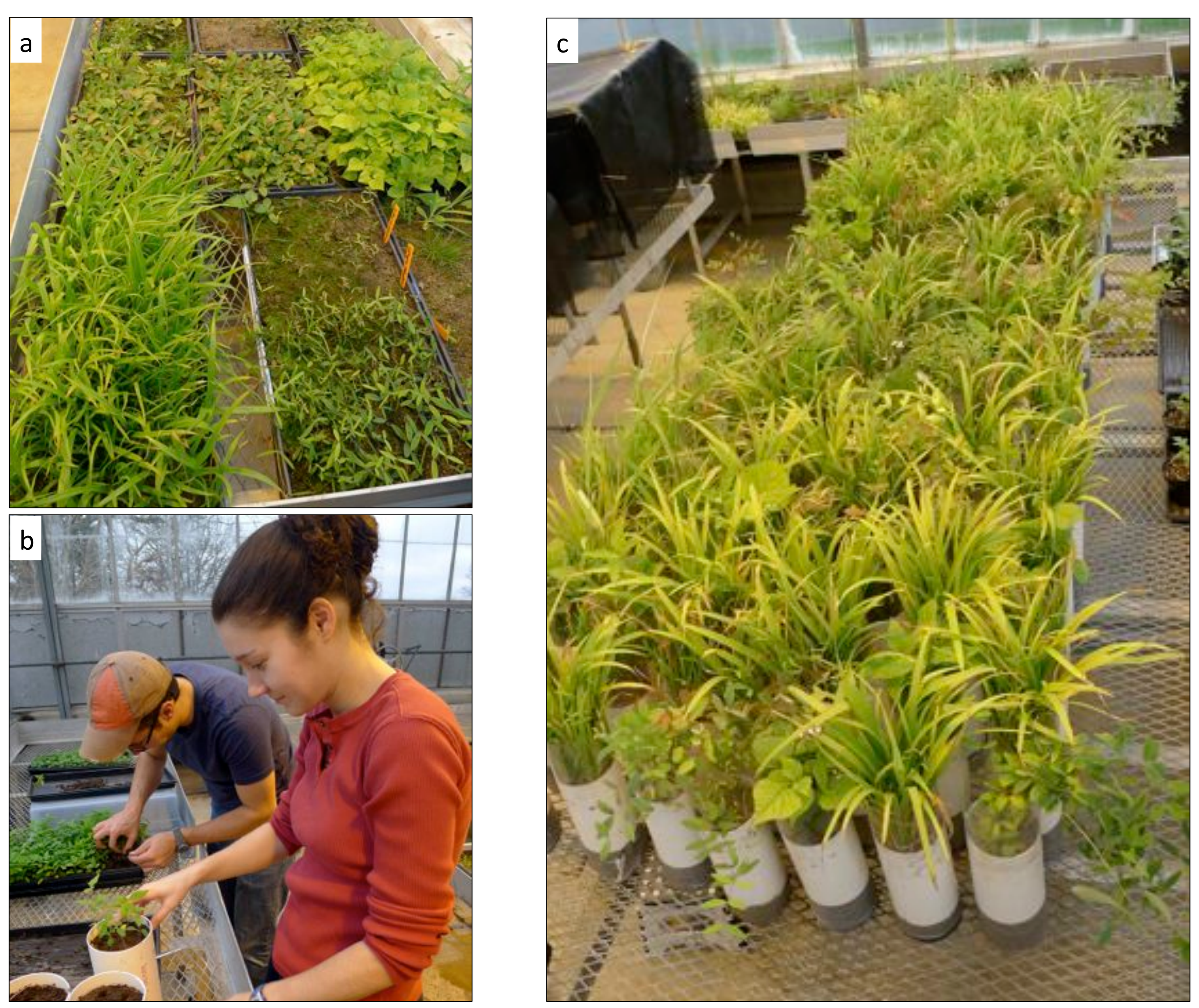
1. Introduction

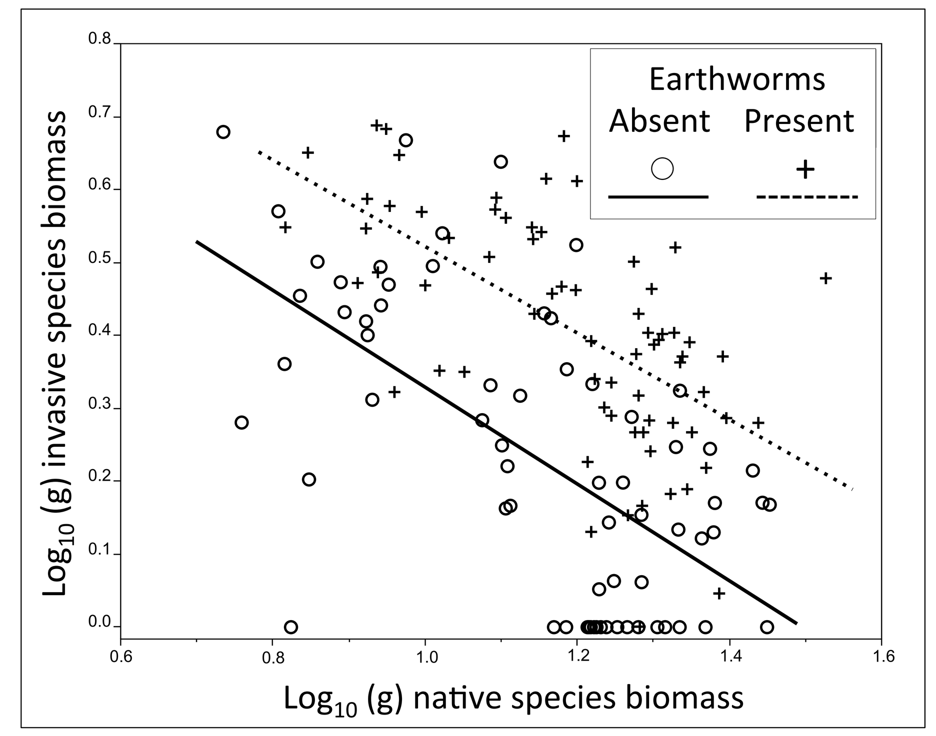
THEORETICAL PREDICTIONS AND EMPIRICAL STUDIES SUGGEST THAT SPECIES DIVERSITY is an important driver of community invasibility. Communities with high resident species diversity occupy a wider range of ecological niches and are more productive, reducing the opportunities for invasion. Ecosystem engineers, such as earthworms, can also affect invasibility by reducing leaf litter and changing soil conditions. In a greenhouse experiment, we simultaneously manipulated species diversity and earthworm presence to investigate how these biotic variables influenced the success of invasive plants.



(a) We germinated seeds of native species and (b) transplanted these into microcosms with one, two, or six native species (half containing an earthworm) and seeds of invasives, (c) after nine weeks we harvested all above and below ground biomass

2. Methods

WE SIMULATED MINNESOTA FOREST COMMUNITIES BY ESTABLISHING 138 MICROCOSMS OF NATIVE HERBACEOUS SPECIES. Each contained one, two, or six species from up to three functional groups (legumes, graminoids, forbs). We also added a single invasive earthworm *(Lumbricus terrestris)* to half the microcosms in each diversity treatment. At the start of the experiment, we added 10 seeds each of four common invasive plants: common buckthorn *(Rhamnus cathartica)*, Japanese barberry *(Berberis thunbergii)*, garlic mustard *(Alliaria petiolata)*, and dandelion (*Taraxacum officinale)* and ran the experiment for nine weeks.

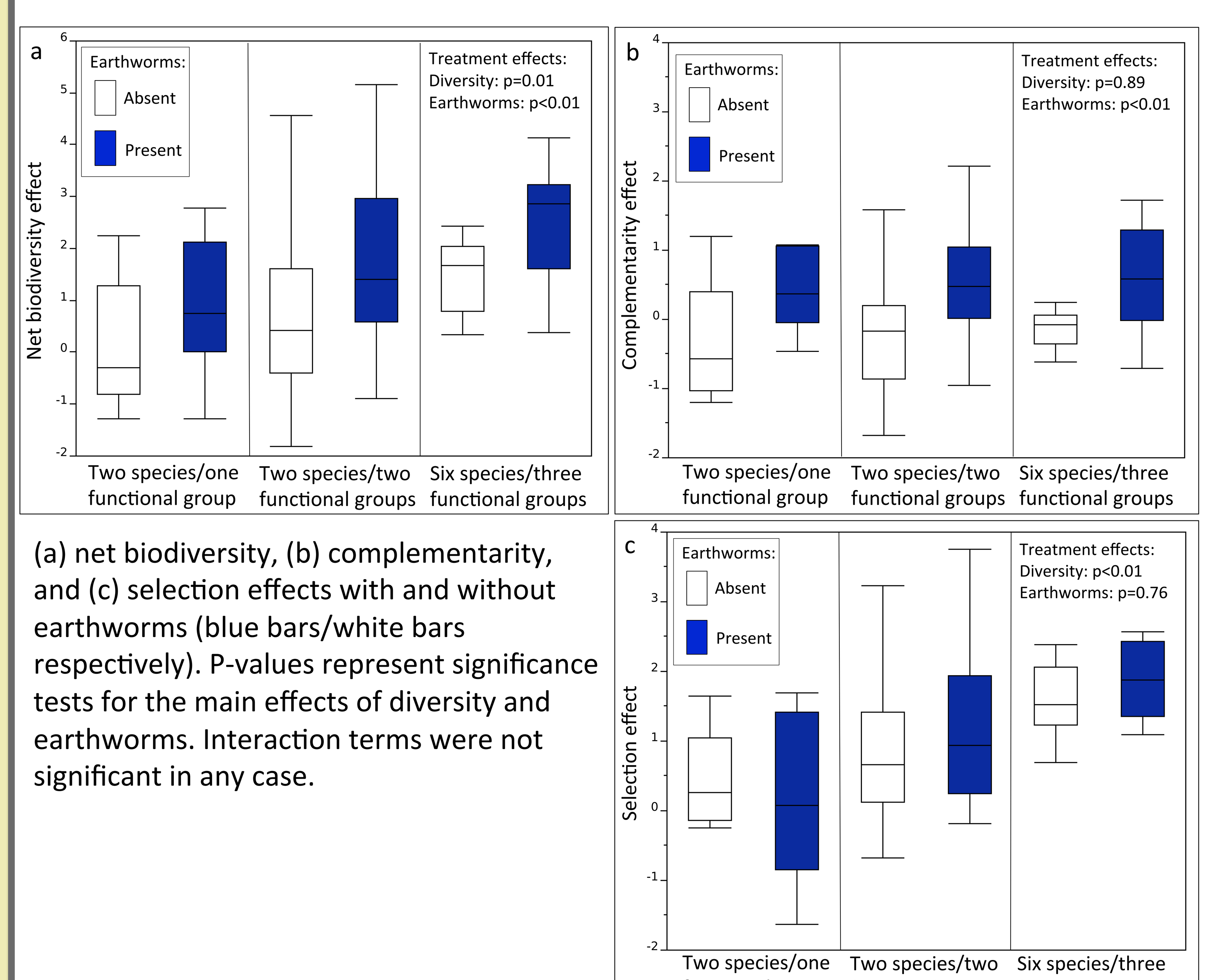


Invasive species biomass was higher when earthworms were present irrespective of native species biomass (R^2 = 0.39/0.37 for worms absent/present; $p < 0.01$)

Emerging buckthorn seedlings

3. Results

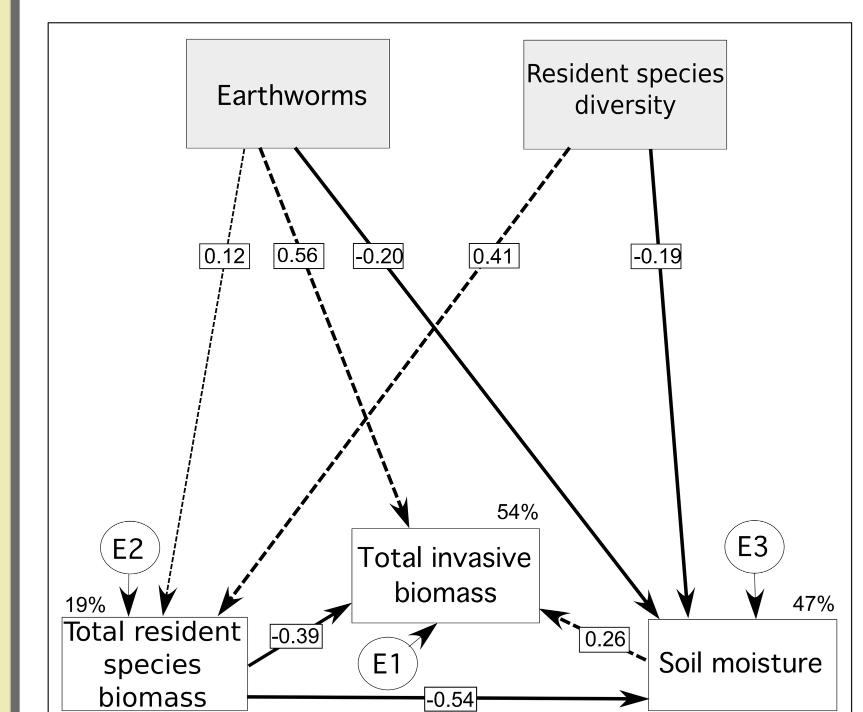
INVASIVE SPECIES BIOMASS WAS LOWER WHEN NATIVE SPECIES BIOMASS WAS HIGHER. Earthworms directly increased the biomass of invasive species at all levels of resident species diversity but also suppressed invasives indirectly via decreased soil moisture. Partitioning the net biodiversity effect indicated that selection effects increased with native species diversity whereas complementarity effects did not although complementarity effects were higher when earthworms were present.



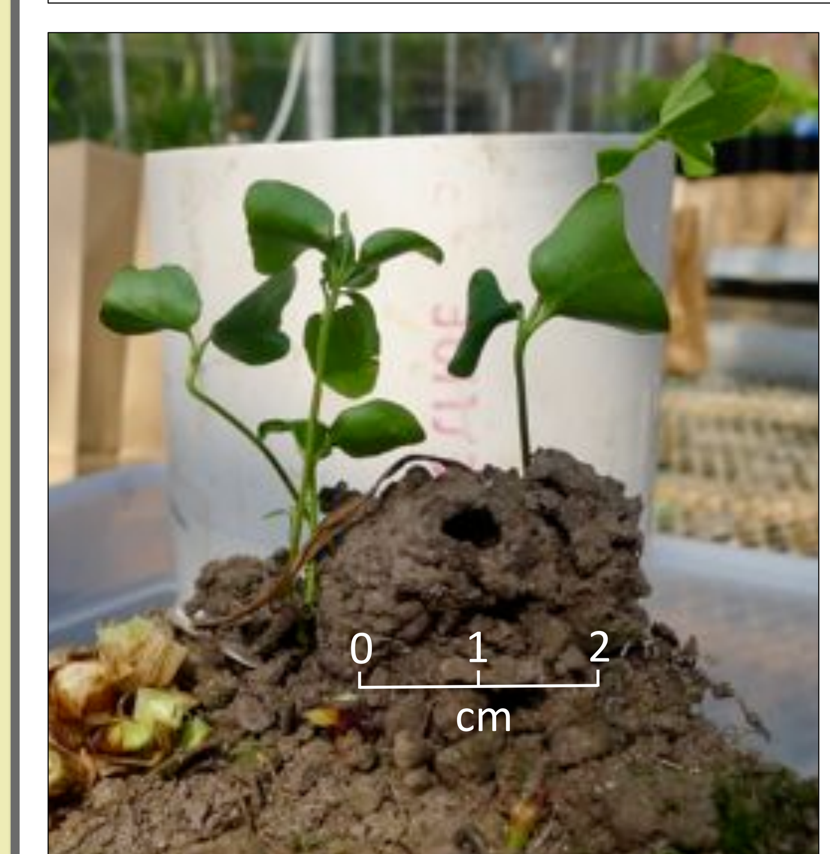
(a) net biodiversity, (b) complementarity, and (c) selection effects with and without earthworms (blue bars/white bars respectively). P-values represent significance tests for the main effects of diversity and earthworms. Interaction terms were not significant in any case.

4. Conclusions

- THE UBIQUITOUS PRESENCE OF EARTHWORMS may undermine reductions in invasibility conferred by native species diversity
- DIVERSITY AND EARTHWORMS have opposing influences on a bottleneck stage of invasive plant establishment
- STUDIES OF PLANT COMMUNITY INVASIBILITY in previously earthworm free regions should account for the effects of invasive earthworms.



Path analysis showed that resident plant diversity and earthworms had opposing effects on invasive plant biomass. Solid and dashed lines indicate negative and positive relationships respectively (fine dashed line indicates a non-significant path coefficient)



Buckthorn seeds often germinated on worm castings showing how one introduced species can benefit another

Earthworm burrows were clearly visible indicating their activity during the experiment

Acknowledgements

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UNIVERSITY OF MINNESOTA

Invasive plants in Minnesota's forests: knowledge, action, and challenges

Symposium

August 14, 2013

8:30-noon

105 Cargill

University of Minnesota

Free and open to the public

8:30am	Coffee and refreshments
8:55 am	**Peter Reich**, *Regents Professor, U of M* Welcoming remarks
9:00 am	**Laura Van Riper**, *Terrestrial Invasive Species Coordinator, MNDNR* The Minnesota DNR's long-term perspective on garlic mustard and buckthorn prevention and management
9:20 am	**Peter Wyckoff**, *Associate Professor of Biology, U of M - Morris* The European buckthorn invasion: insights (and incitement?) from west central Minnesota
9:40 am	**Alex Roth**, *PhD candidate, U of M* It takes two: explaining the success of buckthorn and earthworms in Minnesota's deciduous forests
10:00 am	**Sascha Lodge**, *PhD candidate, U of M* From the hedgerows to the forests: Seed availability is more important than disturbance history
10:20 am	Coffee break
10:35 am	**Laura Phillips-Mao**, *Postdoctoral Research Associate, U of M* Garlic mustard as a "back-seat driver" of change in Minnesota's woodlands – implications for restoration and management
10:55 am	**Paul Kortebein**, *Senior Manager for Forestry & Horticulture, Three Rivers Park District* Keeping Murphy-Hanrehan Park Reserve wild (but not that wild)
11:15 am	**John J. Moriarty**, *Senior Manager of Wildlife, Three Rivers Park District* Buckthorn battle at Battle Creek
11:35 am	Panel Discussion (Moderator: Tim Whitfeld, *Postdoctoral Research Associate, U of M*)

Department of FOREST RESOURCES
UNIVERSITY OF MINNESOTA

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Community phylogenetic diversity and abiotic site characteristics influence abundance of the invasive plant *Rhamnus cathartica* L.

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Abstract

Aims

Theory predicts that the success of introduced species is related to the diversity of native species through trait-based processes. Abiotic site characteristics may also affect a site's susceptibility to invasion. We quantified resident plant species richness, phylogenetic diversity and several abiotic site characteristics for 24 oak forests in Minnesota, USA, to assess their impact on the abundance of a widespread, introduced terrestrial plant species, common buckthorn (*Rhamnus cathartica* L.). Specifically, we asked (1) whether resident species richness and phylogenetic diversity affected the abundance of *R. cathartica* and (2) what site characteristics explained the overall abundance of *R. cathartica*.

Methods

Our survey included 24 oak-dominated stands in Minnesota's deciduous forests. In each stand, we identified all species in 16 plots. We also measured a series of environmental site characteristics, including canopy openness (a proxy for light availability), percent bare soil, soil pH, percent sand, an index of propagule availability, duff layer thickness (a proxy for earthworm activity), an index of insolation and slope. For all species present in at least one site, we estimated a community phylogeny. We combined all site-level characteristics, including phylogenetic diversity of the resident plant species, in a multiple regression model to examine site level drivers of community invasibility.

Important Findings

Results indicate that sites with higher overall plant phylogenetic diversity harbor less *R. cathartica*, even though native species richness was not significantly related to *R. cathartica* abundance. Regression analyses indicated that, in addition to resident species phylogenetic diversity, the most important predictors of *R. cathartica* abundance were canopy openness and the amount of bare soil, both positively related to the abundance of the invader. By combining the effects of abiotic site characteristics and resident species phylogenetic diversity in a model that predicted the abundance of *R. cathartica*, we were able to simultaneously account for a wide range of factors that might influence invasibility. Overall, our results suggest that management strategies aimed at reducing disturbances that lead to increased bare soil and light levels may be more successful if they also maximize phylogenetic diversity of the resident plant community.

Keywords: invasibility, *R. cathartica* L., phylogenetic diversity, site characteristics

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INTRODUCTION

Invasive plant species are known to cause considerable ecological and economic damage (Mack *et al*. 2000; Pimentel *et al*. 2000; Vitousek *et al*. 1996). The uneven density of these invaders in natural communities could be partially explained by heterogeneous propagule pressure but may also suggest that some communities are more receptive to invasives than others. Because post-invasion control of these species is difficult and expensive, understanding site characteristics that make some communities more susceptible to invasion could help inform management decisions that preempt or reduce the level of invasion at a given site.

One important site characteristic predicted to affect invasibility is resident plant diversity. The potential relationship between resident species diversity and susceptibility to invasion has been a focus for several generations of ecologists (Elton 1958; Fridley *et al*. 2007; Kennedy *et al*. 2002; Levine and D'Antonio 1999; Lodge 1993). Diverse communities are predicted to have higher competition for light, space and nutrients and the theory of fluctuating resources suggests this should make them less susceptible to invasion (Davis *et al.* 2000). However, evidence from experimental and observational studies investigating these predictions remains equivocal, with previous studies reporting both negative (Brown and Peet 2003; Frankow-Lindberg 2012) and positive relationships (Cleland *et al*. 2004; Stohlgren *et al*. 2006) between resident species diversity and invasive species abundance. In addition, landscape level environmental heterogeneity means the relationship is also dependent on the spatial scale under investigation (Eschtruth and Battles 2009; Kennedy *et al*. 2002; Knight and Reich 2005; Levine 2000; Naeem *et al.* 2000; Tilman 1997). Despite several decades of research, no clear consensus has been reached and the role of native plant diversity in resisting invasive species is unclear.

Species richness reflects one aspect of diversity, but not all groups of species are evolutionary equivalents. Consequently, tallying the number of species may account for only a portion of the biological variation in a community (Srivastava *et al*. 2012). Communities may be composed of close relatives, distant relatives or both, so an estimation of the mean phylogenetic distance between coexisting plants provides a measure of diversity that incorporates evolutionary and functional diversity (Flynn *et al*. 2011; Srivastava *et al*. 2012). In theory, a community with high phylogenetic diversity is likely to be more resistant to invasion because it can use resources more effectively and will have fewer unfilled 'trait niches' (Cavender-Bares *et al*. 2004; Davis *et al*. 2000). Few studies have attempted to quantify the effects of resident species phylogenetic diversity on community susceptibility to invasion, but one that did (Gerhold *et al*. 2011) concluded that higher phylogenetic diversity was associated with lower receptiveness to introduced species.

Whatever method is used to measure diversity, it is unlikely to be the only factor determining the success of invaders. Abiotic factors such as soil, topography and light also affect the success of species whether or not they are introduced (Huston 2004). Previous studies have documented positive relationships between invasive species and disturbance (Mack *et al*. 2000), particularly in more productive environments (Huston 2004), and soil moisture is also known to affect the success of invasives (Larson *et al*. 2001). The fluctuating resource hypothesis predicts that a plant community will be more susceptible to invasion when there is an increase in the amount of unused resources. This could result from disturbances that reduce the abundance of native species or additions from outside the system (Davis *et al*. 2000).

This study investigated how resident species richness and phylogenetic diversity, together with abiotic site characteristics (canopy openness, percent bare soil, soil pH, percent sand, thickness of the duff layer, insolation and slope) and propagule availability affected the abundance of a non-native species common in temperate deciduous forests in mid-North America. We surveyed plant diversity and abiotic site characteristics in 24 oak-dominated stands in Minnesota's upland forests. Common buckthorn (*R. cathartica* L.) was the most frequent and abundant invader, so we focus solely on it hereafter. *R. cathartica* is a species that can tolerate a wide range of habitats and is a pernicious invasive plant across much of North America. Its myriad effects include changes to nutrient cycling, forest floor conditions (Knight *et al*. 2007), light availability and native species cover (Alsum 2003). In addition, it is the overwintering host of the soybean aphid (a major agricultural pest) and an alternate host for oat crown rust (Heimpel *et al*. 2010). *R. cathartica* was introduced to North America as an ornamental and medicinal shrub in the early 1800s and was first recorded in the Midwest in the 1850s (Kurylo and Endress 2012; Torrey 1824).

We used a community phylogeny of 275 plant species to ask whether species richness and phylogenetic diversity of resident plants affected the abundance of *R. cathartica*. We then included resident species phylogenetic diversity in a multiple regression model with measurements of abiotic site characteristics and an estimation of propagule availability. This simultaneous analysis allowed us to examine which factors are most predictive of *R. cathartica* abundance.

MATERIALS AND METHODS

Field sampling

This study focused on the Eastern Broadleaf Forest Province (MNDNR 2005) in central and southeastern Minnesota, USA, from 41°34′12″N to 46°10′28″N latitude and from 94°27′38″W to 91°13′39″W longitude. Within this area, we sampled in upland, dry-mesic to mesic hardwood forests with at least 40% oak cover (*Quercus rubra*, *Q. ellipsoidalis*, *Q. alba*) and total canopy cover of at least 75%. This community comprises a large proportion of Minnesota forestland (MNDNR 2005), is both economically and ecologically important and is the favored habitat of *R. cathartica* (MNDNR 2003). Within these stands, we established sites in the first area encountered that had a relatively homogenous topography and was large enough to encompass our sample grid (described below). Because we were interested in the effects of resident species diversity and abiotic site characteristics on the invasion success of *R. cathartica*, not the other way around, we did not sample forests where *R. cathartica* was already the overwhelmingly dominant understory shrub (>75% of the shrub layer basal area). Overall, we sampled 24 sites between June and September in 2010 and 2011. All sites were located at least 40 m from the forest edge in stands that varied in size from 8 to 1060 ha. These stands were within a landscape mosaic of woodlands, wetlands and agricultural fields. At each site, we established 16 circular plots, each with a radius of 5 m, in a 4×4 grid. Plot centers were 15 m apart. Within each plot, we identified all trees, shrubs and woody vines that were >1.3 m tall. In a 1-m radius plot located in the center of each 5-m plot, we identified all herbaceous species and woody species <1.3 m tall. Taxonomy followed the Flora of North America (FNA 1993+) and Gleason and Cronquist (1991). Voucher specimens were deposited in the Herbarium of the J. F. Bell Museum of Natural History at the University of Minnesota (MIN).

Site characteristics

In each plot, we used a densiometer to record canopy openness above 1 m as a proxy for light availability. We also estimated the amount of soil without leaf litter in two 1-m^2 plots located 3–5 m from the plot center at 120° and 240° as a proxy for invasive earthworm activity (Holdsworth *et al.* 2008). In addition, we collected mineral soil in three locations to 20 cm depth (three cores per plot). These soil samples were pooled across the 16 plots per site, then air-dried and passed through a 2-mm sieve. Soil pH was measured using a Corning pH meter 240 with soils resuspended in $CaCl_2$ solution. Soil texture was measured using the hydrometer method (Gee and Bauder 1986). At each location where a soil core was collected, we also measured the thickness of the duff layer to the nearest 0.5 cm. The duff layer consists of partially decayed leaf material and is reduced in the presence of invasive earthworms. The slope of each plot was estimated using a clinometer and an index of insolation (I) was calculated based on each plot's aspect using the equation I = cos(22.5 − aspect in radians) + 1 (Kuhman *et al.* 2010).

To estimate landscape-scale propagule availability, we counted all mature *R. cathartica* individuals at 1-mile intervals on a driving loop around each site. The loops were centered on each survey site and ranged in length from 5 to 22 miles, always on the roads closest to each site. The smallest loops were, on average, 0.6 km from the survey site, whereas the largest were no more than 3.6 km distant. This falls within the known home range of several bird species known to eat *R. cathartica* fruit (Lindsey 1939; Minderman *et al.* 2010). We calculated the mean point count number of *R. cathartica* individuals as an estimate of landscape-level propagule availability for each site.

Community phylogeny

Using Phylomatic (Webb and Donoghue 2005), we estimated a backbone phylogenetic hypothesis for all 275 species recorded in the survey plots based on a published supertree (APG 2009). Where possible, within-family phylogenetic relationships were resolved based on published phylogenetic treatments (Fig. S1, see online supplementary material). Preference was given to recent molecular phylogenies, particularly those using Maximum Likelihood or Bayesian estimation. When no satisfactory resolution could be found, nodes were left as polytomies. Branch lengths in the resulting topology were adjusted according to 39 minimum age estimates based on fossil evidence (Wikström *et al.* 2001) using the branch length adjustment algorithm implemented in Phylocom (Webb *et al.* 2008). This method of estimating a community phylogeny allowed us to synthesize the collective phylogenetic knowledge present in the published literature (Beaulieu *et al.* 2012) without incurring the expense of developing a molecular phylogeny from scratch. A sensitivity analysis was used to investigate the robustness of the results to phylogenetic uncertainty (described in *Sensitivity analysis*).

Community phylogeny metrics

We used the Picante package (Kembel *et al.* 2010) implemented in R (R Development Core Team 2009) to calculate mean pair-wise phylogenetic distance. This metric takes into account pair-wise phylogenetic distances between all species in a sample and provides an overall measure of phylogenetic diversity (Webb 2000). Many of our samples contained ferns and gymnosperms that are distant relatives of the dominant angiosperm species. Patterns of inclusion or exclusion of these basal groups would strongly influence phylogenetic diversity; consequently, they were removed from the analysis.

Sensitivity analysis

Despite constantly improving phylogenetic knowledge, the evolutionary relationships among some members of our samples were still uncertain, so some nodes on our phylogeny were unresolved. Phylogenetic distance metrics could potentially be affected by incomplete phylogenetic resolution particularly if a community contains species from a few diverse clades (Srivastava *et al.* 2012). To account for this possibility, we investigated the impact of phylogenetic uncertainty on the power to detect relationships between community phylogenetic diversity and *R. cathartica* abundance. To do this, we used Mesquite ver. 2.72 (Maddison and Maddison 2011) to randomly resolve our phylogeny 100 times and then calculated phylogenetic diversity for all survey sites using each of these randomly resolved trees. This analysis allowed us to examine whether phylogenetic uncertainty affected the observed relationship between phylogenetic diversity and *R. cathartica* abundance.

Multiple regression analysis

Community phylogenetic diversity, abiotic site characteristics and propagule availability were combined in a general linear model to predict site level *R. cathartica* abundance, defined as the sum of stems in all plots at each site. Stem counts of *R. cathartica* were strongly correlated with *R. cathartica* basal area at each site (R^2 = 0.84, $F_{(1,22)}$ = 78.61, P < 0.0001) and so provided a reliable estimate of site level *R. cathartica* cover.

The best-fit model was chosen based on the minimum Akaike information criteria (AICc) score. All analyses were performed using JMP ver. 9.0.2 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Diversity

Across all survey sites, we recorded 275 species of trees, shrubs, vines and herbaceous plants. Total species richness per site ranged from 32 to 86. Herbaceous species richness per site varied from 12 to 61 and woody species richness ranged from 11 to 38. Resident species richness was not significantly correlated with the abundance of *R. cathartica* (R^2 = 0.02, $F_{(1,22)}$ = 0.38, P = 0.52).

We estimated the phylogenetic relationships between these species (Fig. S1, see online supplementary material) and found that resident species phylogenetic diversity was negatively correlated with total *R. cathartica* abundance (Fig. 1) (R^2 = 0.21, $F_{(1,22)}$ = 5.88, P = 0.02). Phylogenetic diversity of resident herbaceous species alone was also correlated with *R. cathartica* abundance (R^2 = 0.24, $F_{(1,22)}$ = 6.83, P = 0.02) but no such relationship was observed for the diversity of resident woody plants (R^2 = 0.12, $F_{(1,22)}$ = 2.69, P = 0.09). In addition, as the mean phylogenetic distance between *R. cathartica* and all resident species at a site decreased, the abundance of *R. cathartica* increased (R^2 = 0.21, $F_{(1,22)}$ = 5.74, P = 0.03). However, this estimate of phylogenetic distance was strongly correlated with overall phylogenetic diversity (R^2 = 0.97, $F_{(1,21)}$ = 373.25, P < 0.0001) and the two appear to be related, possibly due to an overlap in species composition between sites. As a result, we focus on the relationship between overall phylogenetic diversity and *R. cathartica* hereafter. We observed significant relationships between *R. cathartica* abundance and resident species phylogenetic diversity for each of the 100 randomly resolved trees (R^2 = 0.21–0.29, P < 0.01), indicating that our results were robust to phylogenetic uncertainty.

Site characteristics

Combining phylogenetic diversity, abiotic site characteristics and propagule availability indicated the best-fit multiple regression model predicting total *R. cathartica* abundance, based on minimum AICc scores, included canopy openness, percent bare soil and resident species phylogenetic diversity (Table 1, Fig. 2). Adding resident species richness to the model marginally increased the total R^2 value from 0.63 to 0.64 but also increased the AICc score from 55.18 to 58.66. *R. cathartica* abundance was highest where bare soil and canopy openness were high (Fig. 2A–C) and lowest in sites with high phylogenetic diversity of resident species (Fig. 2B and C). There was little evidence of multicolinearity among predictors based on variance inflation factors that ranged from 1.5 to 2.7.

Simple regression analysis indicated that propagule availability was also an important predictor of *R. cathartica* abundance (R^2 = 0.52, P < 0.001; Table S1, see online supplementary material for results of all univariate analyses), even though this environmental characteristic was not part of the best-fit multiple regression model. Covariance between

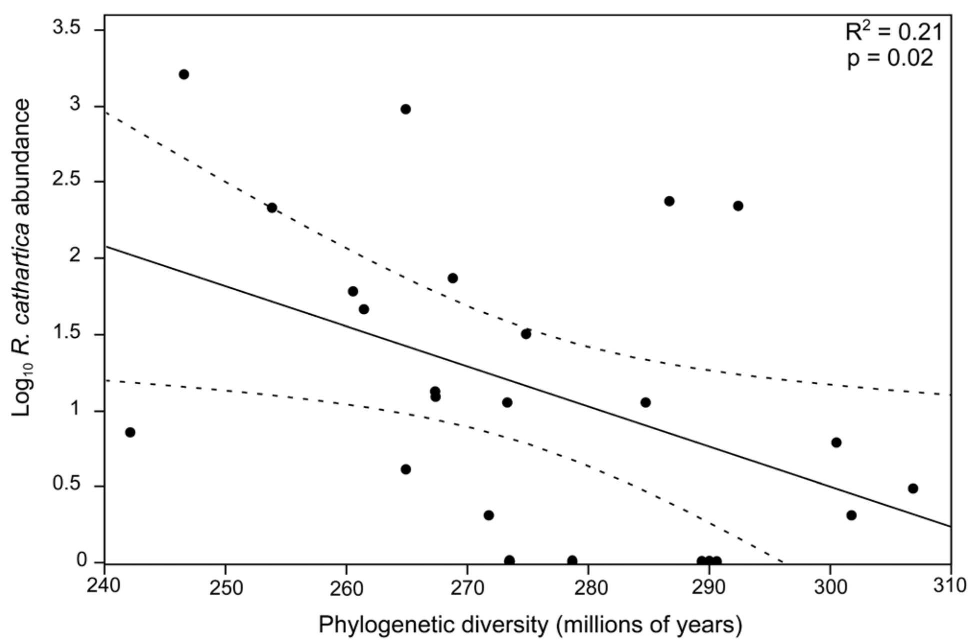

Figure 1: site level *R. cathartica* abundance ($\log_{10}$ transformed) as a function of resident species phylogenetic diversity, based on the mean pairwise phylogenetic distance. Dotted lines represent 95% confidence intervals. Samples represent mean values from 24 survey sites, each with 16 individual plots of 5-m radius located in mesic, upland, oak-dominated, deciduous forests in central and southeastern Minnesota, USA. *R. cathartica* abundance included stem counts for seedlings (<1.3 m tall) and mature individuals (>1.3 m tall). Resident species phylogenetic diversity included all herbaceous plants plus, vines, shrubs and trees.

Table 1: results of best-fit multiple regression for *R. cathartica* abundance in 24 survey plots (number of individual stems, log-transformed) located in mesic, upland, oak-dominated, deciduous forests in central and southeastern Minnesota, USA

	Sum of Squares	*F*-ratio	*P* value	R^2
Canopy openness	5.71	14.21	0.001	0.26
Percent bare soil	4.69	11.68	0.002	0.22
Phylogenetic diversity	1.37	3.413	0.08	0.16

The complete model included mean canopy openness, percent bare soil, soil pH, percent sand, duff layer thickness, index of insolation, propagule availability, resident species phylogenetic diversity and slope. The best-fitting model was chosen based on the minimum AICc score (total R^2 = 0.64).

all site characteristics that showed significant univariate relationships with *R. cathartica* abundance is shown in Fig. S2, see online supplementary material.

DISCUSSION

Introduced invasive plants often have significant economic and ecological impacts. Once established, control or elimination of these species is difficult and expensive. Identifying factors that make some communities more susceptible to invasion would help guide management decisions designed to preempt or reduce the level of invasion. Our study integrated measures of native plant diversity and abiotic site characteristics in an attempt to understand broad-scale factors influencing the abundance of *R. cathartica*, an invasive plant now common across much of North America.

That we observed no significant relationship between resident species richness and *R. cathartica* abundance, but a significant negative relationship between resident species phylogenetic diversity and the abundance of this invasive species highlights the ecological differences between a community composed of many close relatives and one composed of an equal number of distant relatives (Srivastava *et al*. 2012). Additionally, this may help to explain why investigations into the effects of species diversity on invasibility often come to different conclusions.

Estimating community phylogenetic diversity accounts for evolutionary relationships among community members and provides a measure of diversity that in this instance is independent of species richness. In our study, there was no significant relationship between total site level species richness and phylogenetic diversity, R^2 = 0.06, P = 0.26. Surprisingly, few studies have accounted for community phylogenetic diversity in the context of invasibility—one such study found communities with high phylogenetic diversity were less receptive to introduced species than communities composed of close relatives (Gerhold *et al*. 2011). Our analysis, which documented the relationship between phylogenetic diversity and the abundance of one invasive species, *R. cathartica*, suggested a similar negative relationship. However, the mechanisms behind these observed patterns remain uncertain.

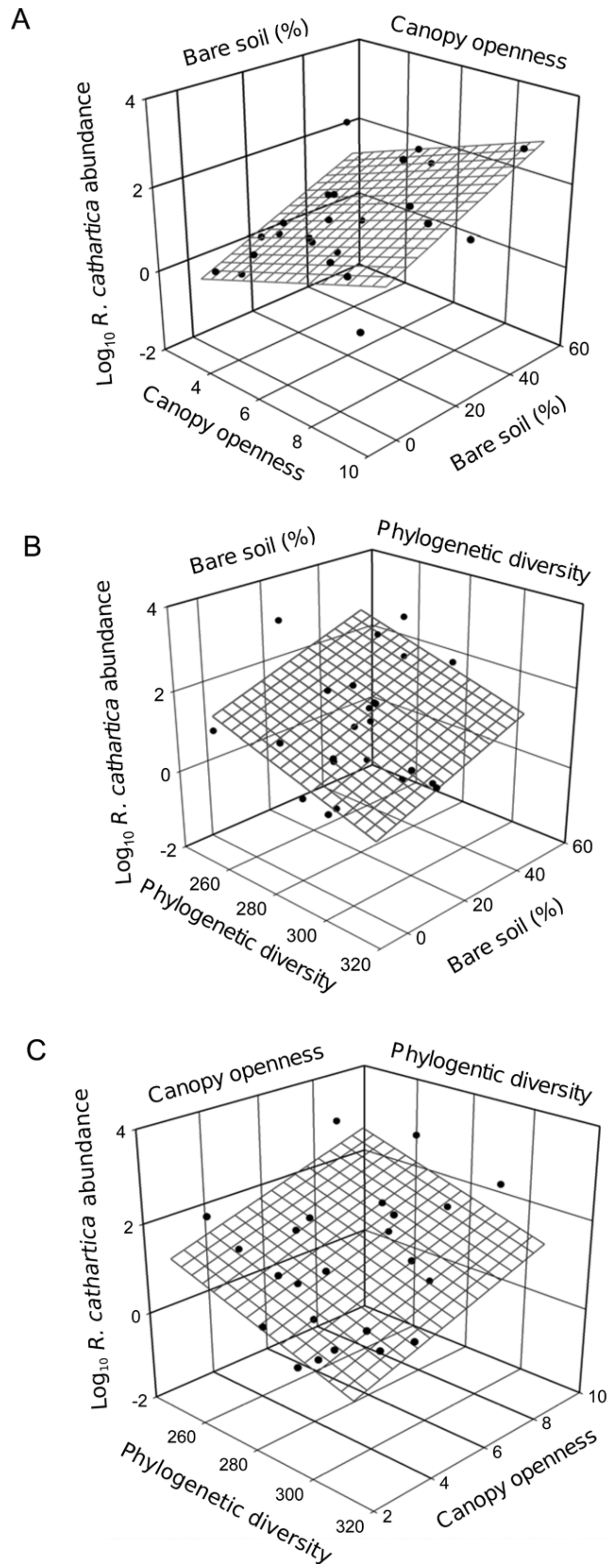

Figure 2: surface plots showing relationships between *R. cathartica* abundance ($\log_{10}$ transformed) and (A) bare soil and canopy openness, (B) bare soil and resident species phylogenetic diversity and (C) resident species phylogenetic diversity and canopy openness. Measurements were taken at 24 sites in oak-dominated

A community composed of distant relatives maximizes resource acquisition (Cadotte *et al*. 2008; Cavender-Bares *et al*. 2009), provides fewer opportunities for invaders and is predicted to be more resistant to invasion. Furthermore, high phylogenetic diversity is a good predictor of productivity (Cadotte *et al*. 2009), so increased shading of germinating *R. cathartica* seedlings could explain the observed relationship (per Knight and Reich 2005). However, although we did observe a significant positive relationship between resident herbaceous species richness and percent cover ($R^2 = 0.30$, $P = 0.005$) and resident shrub species richness and cover ($R^2 = 0.21$, $P = 0.02$), no such relationship existed between phylogenetic diversity and cover of herbs or shrubs. Therefore, the lower abundance of *R. cathartica* in sites with high native phylogenetic diversity appears to be the result of something other than increased productivity leading to more shade. It is also worth noting that the relationship between herbaceous species phylogenetic diversity and *R. cathartica* abundance ($R^2 = 0.24$, $P = 0.02$) was slightly stronger than when all species were considered together (Fig. 1), suggesting a potential role for herbaceous species diversity in reducing a site's invasibility through niche-based processes and environmental stability (Gilliam 2007; Levine and D'Antonio 1999). We did not observe any direct relationships between our measured environmental characteristics and site level phylogenetic diversity, but the observed pattern of *R. cathartica* abundance may be related to unmeasured environmental characteristic (Srivastava and Vellend 2005) or other factors connected to each site's history such as disturbance.

Phylogenetic diversity is one aspect of a community's susceptibility to invaders. Abiotic site characteristics can also be influential (Knight *et al*. 2007) so we measured several environmental variables that previous studies have suggested are important drivers of *R. cathartica* abundance in isolation. We combined these variables with an estimate of phylogenetic diversity in a multiple regression analysis that simultaneously modeled all measured independent variables. The model indicated that canopy openness, percent bare soil and resident species phylogenetic diversity were all significant predictors of *R. cathartica* abundance. The explanatory power of this model accounted for two-thirds of the variation in *R. cathartica* abundance, indicating the combined importance of these variables in driving site level susceptibility to invasion by *R. cathartica*.

The inclusion of bare soil in the best-fit model is in line with predictions based on previous studies suggesting *R. cathartica* has high germination rates where leaf litter is sparse (Biswiki 2005; Knight *et al*. 2007). Exotic earthworms can have a strong effect on the amount of bare soil in upland deciduous forests (Frelich *et al*. 2006; Holdsworth *et al*. 2008). In our survey, analysis of variance indicated sites with high earthworm abundance, based on a visual assessment (Loss *et al*. 2013), had significantly more bare soil than sites with fewer earthworms ($F_{(2,21)} = 5.80$, $P = 0.02$). The relationship between these two introduced species is likely to be an important mechanism driving *R. cathartica* abundance (Heimpel *et al*. 2010; Heneghan *et al*. 2007). It also helps explain the relationship between propagule availability and bare soil (Fig. S2, see online supplementary material) because *R. cathartica* abundance was highest where propagule availability was high and it was also positively related to earthworm abundance.

Canopy openness was also part of the best-fit model. *R. cathartica* can tolerate a wide range of light conditions but has its greatest growth rate at higher light levels (Gourley and Howell 1984; Wyckoff *et al*. 2005), suggesting that undisturbed forests where light levels are low are less susceptible to invasion.

Given these observations, site level management to preempt buckthorn invasion should consider the amount of bare soil, light levels and resident species phylogenetic diversity. Minimizing disturbances that increase light or bare soil and managing to maintain phylogenetic diversity, e.g. maintaining structural heterogeneity or reducing white-tailed deer (*Odocoileus virginianus*) browse pressure (Goetsch *et al*. 2011), are high priorities. When a site is already heavily infested, complete removal of all *R. cathartica* stems is a short-term solution that could fail in the long run because of the resulting increased light levels and bare soil. Removal of all large, fruit-bearing female plants in a site followed by selective removal of smaller stems and replacement with native shrub species might be a more effective approach. Previous studies using manipulative experiments indicated that propagule availability is also an important determinant of habitat invasibility (Eschtruth and Battles 2009; Öster and Eriksson 2012; Von Holle and Simberloff 2005). Therefore, accounting for propagule availability and collaborating with surrounding landowners to reduce propagule pressure should also be part of efforts to reduce *R. cathartica* abundance at any given site. Phylogenetically diverse communities are predicted to incorporate greater functional trait diversity and reduce opportunities for introduced species to be successful. High phylogenetic diversity at one trophic level is also predicted to support greater diversity at higher trophic levels (Dinnage *et al*. 2012) leading to higher overall biodiversity and a more stable community.

By taking a phylogenetic approach, our study is one of the first to investigate the role of resident species evolutionary history on invasibility. We found (1) no relationship between species richness and the abundance of *R. cathartica*, but a significant negative correlation with resident species phylogenetic diversity. This finding underscores the importance of accounting for evolutionary relationships among species when assessing how diversity affects invasibility. (2) Multiple regression analysis suggested that light levels, the amount of bare soil and resident species phylogenetic diversity combined in the best-fit

mesic hardwood forests in central and southeastern Minnesota, USA. Bare soil represents the mean value of estimates in two 1-m^2 quadrats in each of 16 plots at each survey site. Canopy openness is the mean value estimated in the middle of each plot with a densiometer. Resident species phylogenetic diversity included all herbaceous plants plus, vines, shrubs and trees.

model to predict stand level *R. cathartica* abundance. Therefore, a successful plan for reducing *R. cathartica* should include management strategies to minimize disturbances and maximize phylogenetic diversity of the resident plant community.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

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