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

Alexander M. Roth · Timothy J. S. Whitfeld · Alexandra G. Lodge · Nico Eisenhauer · Lee E. Frelich · Peter B. Reich

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

**Keywords** Light · Leaf litter · Invasibility · *Lumbricus terrestris* · *Rhamnus cathartica*

**Introduction**

Biological invasions are one of the most important global environmental problems (Vitousek 1996; Pimentel et al. 1994).
With increased global connectivity and trade, invasive species are now the second-leading cause of human-mediated species extinction (Heneghan et al. 2007), and northern temperate forests are especially at risk (Murphy and Romanuk 2014). The United States alone incurs roughly $120 billion annually in costs related to the effects and control of invasive species, much of which is due to the loss of ecosystem services (Pimentel et al. 2005). While a few high-profile invasive species, such as zebra mussels (Dreissena polymorpha) and Burmese pythons (Python molurus bivittatus), garner much attention, many other less well-known species are at least as pervasive and often as damaging. For example, plant species like common buckthorn (Rhamnus cathartica) are linked to losses of native plant diversity, changes in nutrient cycling, and suppression of forest regeneration, making these invaders a serious concern (Fagan and Peart 2004; Knight et al. 2007; Klionsky et al. 2011). While the negative effects of invasive species are not necessarily immediate, they can become visible over time (Mack et al. 2000), and the potential for interactions with factors like global climate change and other invasive organisms means that the future health and diversity of many ecosystems is threatened (Theoharides and Dukes 2007; Frelich and Reich 2009; Bellard et al. 2013; Polgar et al. 2014). Researchers propose that invasive plants such as common buckthorn, in tandem with the activities of ecosystem engineers (such as invasive earthworms and deer) and the influence of climate change, are negatively impacting forest regeneration and biological diversity in many US forests (Hartman and McCarthy 2004; Frelich and Reich 2009).

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

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

While some largely circumstantial evidence suggests that common buckthorn facilitates earthworm invasion, earthworms may also facilitate invasion by buckthorn and other invasive plants (Eisenhauer et al. 2012). This facilitation may be especially influential at the germination and establishment stage, where invaders must overcome various abiotic and biotic obstacles in order to colonize and establish in an area (Williamson and Fitter 1996; Richardson et al. 2000; Levine et al. 2004). Because earthworms rapidly consume leaf litter, they expose bare soil on which many invasive species—including buckthorn—preferentially germinate (Heneghan et al. 2007; Knight et al. 2007). The bare soil may give an advantage to invaders over native species adapted to the thick organic horizon present in previously earthworm-free northern forests (Gundale 2002;
Hale et al. 2006). However, little is known about how earthworms and litter interact to affect buckthorn germination. Earthworms also increase decomposition and can provide temporary pulses of nutrients available to plants (Heneghan et al. 2006; Hale et al. 2008; Heimpel et al. 2010). Invaders are often able to better utilize these nutrient pulses (Hueneke et al. 1990; Davis et al. 2000; Gilliam 2006; Funk and Vitousek 2007), possibly aiding germination and growth of their seedlings. These processes may undermine the resistance to invasion that is potentially provided by abiotic factors like low light and deep leaf litter. It is also unknown whether earthworm facilitation of invasive plants may differ with variation in light and litter.

To explore these issues, we conducted a greenhouse experiment to investigate the effects of abiotic (light and leaf litter depth) and biotic (earthworms) controls on the germination and early establishment of common buckthorn. We addressed the following questions:

1. Do shade and thick leaf litter provide resistance to buckthorn germination and growth?
2. Does the presence of earthworms positively influence buckthorn germination and growth, and do earthworms change the effects of shade and leaf litter?
3. Does the importance of these variables change throughout the seedling establishment stage?

Materials and methods

Experimental design

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

Native plant species were germinated in planting trays using soil collected from the same location. Native species included Desmodium glutinosum, Elymus hystrix, Carex blanda, Eurybia macrophylla, Asclepias exaltata, and Galium boreale. Each of these native species is commonly found in local mesic hardwood forests and was among the most common species in a survey of 67 deciduous forest sites in central and southeastern Minnesota (Whitfeld et al. 2014a). Seeds of the native species were purchased from Prairie Moon Nursery in Winona, Minnesota. Once they germinated and established in the trays, one seedling of each of the six native species was transplanted into each of the microcosms (equally spaced) and allowed to grow for 4 weeks. Seedlings that did not survive the transplant were replaced.

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

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

Finally, one European nightcrawler (Lumbricus terrestris) was added to half of the microcosms in each treatment. This equates to a density of roughly 128 earthworms per square meter, which is a high density for anecic earthworms. However, Eisenhauer et al. (2007) found that at the peak of invasion, densities of Lumbricus terrestris in the Canadian Rockies reached 109 individuals per square meter; thus the density in our study is similar to a heavily invaded forest. Earthworms were purchased from Blue Ribbon Bait and Tackle Shop in Hugo, Minnesota, and kept in the experimental soil with ample leaf litter in order to acclimate them to experimental conditions. Once all treatments were established, clear plastic “worm screens” were applied around the rim of each pot to prevent earthworm escape. Six control microcosms under ambient light with no native species, leaf litter or earthworms were also established to measure baseline invasive seed germination.
Overall, the experimental design included three litter treatments, three light treatments and two worm treatments for a total of 18 different treatment combinations. Each treatment combination was replicated in seven pots for a total of 126 experimental microcosms and six control pots.

Ten seeds each of four invasive species [buckthorn, barberry (Berberis thunbergii), garlic mustard (Alliaria petiolata), and dandelion (Taraxacum officinale)] were then added on top of the leaf litter in each microcosm to simulate natural seed rain. Pots were watered every other day for the 8-week duration of the experiment. As biomass accumulated, we increased the amount of water from 50 to 150 ml for each pot to minimize any limitation from lack of water. Finally, the location of the pots on the greenhouse benches was randomized weekly to avoid any effects due to uneven light intensity in the greenhouse.

Data collection

Each week, we recorded the number of germinated invasive seedlings by species. Because there was very low mortality of germinating invasive seeds—and the mortality we did see was due to direct earthworm predation—we define germination success as the cumulative number of seeds that germinated at any time during the experiment, and use final biomass as an estimate of their early establishment success. Percent cover of native plants was also estimated, as well as the percent cover of remaining leaf litter. Using a LI-COR LI-250A light meter, percent light transmittance at the soil surface was measured just prior to harvest. Once the experiment had run for 8 weeks—long enough for the invasive species to germinate and establish but with minimal microcosm-induced limitations on native species’ root growth—aboveground biomass was harvested and separated into native and invasive categories and any remaining litter was collected. All plant material was dried at 70 °C for 3 days before being weighed. Approximately 60 g of soil was taken from the upper 5 cm of each pot to measure treatment effects on soil moisture. After being dried for 3 days at 70 °C, we calculated the soil moisture of the samples. Earthworm activity was documented by the presence of burrows in the soil column and midden at the soil surface. Finally, invasive and native roots were separated and washed before being dried and weighed for root biomass, though it was not possible to separate the roots to the species level. None of the garlic mustard seeds (invader species) germinated despite following cold stratification guidelines prior to the experiment (Baskin and Baskin 1992).

Analysis

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

In order to explore the direct and indirect relationships between earthworm presence, light and litter, and their effects on buckthorn biomass, we performed path analysis using AMOS 5 (Amos Development Corporation, Cary, NC). Using only paths that were ecologically relevant, we constructed an initial model based on prior knowledge. The treatments served as exogenous variables, and final buckthorn biomass was the response, or endogenous, variable. Earthworm presence/absence was included in the analysis as a predictor variable with a value of 0 when earthworms were absent and 1 when they were present. Light transmittance and litter cover were included in the analysis as continuous variables. We used the specification search tool in AMOS to compare related models and used non-significant χ²-tests (p > 0.05), corrected Akaike’s information criterion (AICc) scores, and goodness of fit metrics (Grace 2006; Arbuckle 2012) to select the best-fit model. Because we also had weekly invasive seedling counts, we used a generalized regression approach with pot as a random effect to explore the relationships between treatments, time, and the number of buckthorn seedlings throughout the experiment.
**Results**

Native species biomass did not differ between earthworm \[ F_{(1,124)} = 0.12, p = 0.73 \] or litter \[ F_{(2,123)} = 0.23, p = 0.80 \] treatments, but was significantly positively related to light level \[ F_{(2,123)} = 219.63, p < 0.0001 \]. However, pre-harvest light availability at the soil surface did not differ between earthworm treatments due to the similarity in native species biomass \[ F_{(1,124)} = 0.35, p = 0.55 \]. A heat wave during the last 3 days of the experiment resulted in 45 % earthworm mortality, as the greenhouse cooling system was unable to completely buffer the outside temperature. However, earthworms remained active until the final days of the experiment, as indicated by steady leaf litter decline and the appearance of fresh castings throughout the experiment (Dávalos et al. 2013). Due to the timing of earthworm mortality—partially decomposed earthworms were still found during harvest—we are confident that any potential pulse of nutrients released by their decomposition had little effect on the outcome of the experiment. At the end of the experiment, litter mass and soil moisture were 57 % \[ F_{(1,124)} = 16.53, p = 0.001 \] and 19 % \[ F_{(1,124)} = 5.64, p = 0.019 \] lower in microcosms with earthworms, respectively, supporting our observation that earthworms were active until the final days of the experiment.

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

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

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

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

Path analysis supported results from our linear models. It also provided further insight into the mechanisms behind the observed relationships and revealed indirect effects that helped to explain our observed results. The initial model fit the data, but included non-significant paths and could be improved ($\chi^2 = 1.45$, $p = 0.84$; AICc = 23.45) and fit better than the saturated and independence models (NFI = 0.98; CMIN = 0.36; RMSEA = 0). The model indicated that light had an indirect negative effect on buckthorn biomass through its negative effect on soil moisture (Fig. 2). By contrast, litter cover positively influenced buckthorn biomass. Earthworms had a direct positive effect on buckthorn biomass, as well as smaller indirect negative effects by decreasing soil moisture and litter cover. Earthworm activity in the field is often affected by soil moisture (Hale et al. 2005), but the continuous activity of earthworms in our experiment was likely to affect soil moisture, which was a potential impact of particular interest (Larson et al. 2010; Eisenhauer et al. 2012). While explaining more of the variation in soil moisture, a model including a native plant biomass term was a poorer fit and explained less variation in buckthorn biomass.

We also visually investigated the temporal changes in the number of germinated buckthorn seedlings throughout the experiment. Treatment effects on the number of buckthorn seedlings manifested at different points throughout the experiment. The different levels of the earthworm, light, and litter treatments began to diverge in the second, third and fourth weeks, respectively, providing insight into the dynamics of these processes (Fig. 3a–c). For example, the greater cumulative buckthorn germination from week

Table 1 Generalized and general linear model results for the effects of litter, light, earthworms and their interactions on the number of buckthorn seedlings and buckthorn biomass in experimental microcosms

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>L-R $\chi^2$</th>
<th>Prob. &gt; $\chi^2$</th>
</tr>
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<tr>
<td>Response: buckthorn seedlings (no.)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Litter level</td>
<td>2</td>
<td>9.3445</td>
<td>**</td>
</tr>
<tr>
<td>Light level</td>
<td>2</td>
<td>26.5917</td>
<td>***</td>
</tr>
<tr>
<td>Litter level $\times$ light level</td>
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<tr>
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<td>1</td>
<td>19.9612</td>
<td>***</td>
</tr>
<tr>
<td>Litter level $\times$ earthworms</td>
<td>2</td>
<td>14.5291</td>
<td>***</td>
</tr>
<tr>
<td>Light level $\times$ earthworms</td>
<td>2</td>
<td>10.6430</td>
<td>**</td>
</tr>
<tr>
<td>Litter level $\times$ light level $\times$ earthworms</td>
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<td>4.3850</td>
<td>0.3564</td>
</tr>
<tr>
<td>Source</td>
<td>nparm</td>
<td>df</td>
<td>Sum of squares</td>
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<tr>
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<td>---</td>
</tr>
<tr>
<td>Response: buckthorn biomass (g)</td>
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<td></td>
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<td>2</td>
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<td>4</td>
<td>0.00372508</td>
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</table>

Total $R^2 = 0.40$. Pearson goodness of fit $\chi^2 = 126.45$, df = 108 and $p = 0.12$

L-R Likelihood ratio, Prob. probability, nparm number of parameters

** 0.001 $\leq p < 0.01$, *** $p < 0.001$
Fig. 2 Path analysis model displaying causal influences of experimental treatments on buckthorn biomass. Numbers on arrows are standardized path coefficients. Solid lines indicate negative relationships and dashed lines indicate positive relationships. All paths are significant \( p < 0.05 \). Circles indicate error terms \( (E1-E3) \). The overall model fit the data: \( \chi^2 = 1.45 \), probability level \( = 0.84 \), Akaike information criterion \( = 23.45 \).

Discussion

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

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

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

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

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

Overall, microcosms with earthworms had about a one-third increase in both the number of established buckthorn seedlings (34%) and their total biomass (33%), compared to those without earthworms. The importance of earthworms was corroborated in all our analyses, and while the mechanisms behind this simple relationship are not clear, previous studies suggest the positive effect could be due in part to earthworms mixing litter and helping seeds reach the soil, protecting seeds from desiccation through burial, and concentrating seeds near middens with moist, high nutrient castings (Eisenhauer and Schuler 2008; Regnier

Table 2 Generalized regression results for the effects of treatments, time, and their interactions on buckthorn germination in experimental microcosms

<table>
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<th>Source</th>
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</tr>
<tr>
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</tr>
<tr>
<td>Light</td>
<td>1</td>
<td>20.66 ***</td>
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</tr>
<tr>
<td>Earthworms</td>
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<td>19.92 ***</td>
<td></td>
</tr>
<tr>
<td>Litter level × earthworms</td>
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<td>30.03 ***</td>
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</tr>
<tr>
<td>Light level × earthworms</td>
<td>1</td>
<td>28.15 ***</td>
<td></td>
</tr>
<tr>
<td>Litter level × light level</td>
<td>1</td>
<td>0.35</td>
<td>0.5527</td>
</tr>
<tr>
<td>Date × litter level</td>
<td>7</td>
<td>7.59</td>
<td>0.3698</td>
</tr>
<tr>
<td>Date × light level</td>
<td>7</td>
<td>4.59</td>
<td>0.7095</td>
</tr>
<tr>
<td>Date × earthworms</td>
<td>7</td>
<td>40.05 ***</td>
<td></td>
</tr>
</tbody>
</table>

* 0.01 ≤ p < 0.05, *** p < 0.001
et al. 2008). Throughout the experiment, we observed earthworms burying and concentrating seeds near their middens, and germination of buckthorn was often clustered near midden openings. However, as demonstrated by our path analysis results, earthworms also had negative effects on buckthorn biomass through their negative effects on litter cover and soil moisture, which were supported by our observations of seed desiccation in both the litter and no-litter treatments. This negative effect was clearly swamped by an overall positive influence of earthworms, and was likely attributable in part to our specific experiment, as the small pot size exacerbated the effects of soil drying. In the field, buckthorn is able to tolerate a wide range of moisture conditions, including a degree of drought (Stewart and Graves 2004), and is unlikely to be as strongly affected by earthworm-induced soil moisture losses.

Our experiment not only demonstrated the roles of light and leaf litter in influencing the germination and biomass of buckthorn, but also emphasized the importance of the interactions between earthworms and these variables in affecting this important stage of plant growth. Ultimately, the positive impacts of earthworms on buckthorn were much larger at higher light and higher litter levels. For example, the presence of earthworms resulted in greater buckthorn success in the high light treatment, which, when earthworms were absent, was associated with poor buckthorn performance. In high light, earthworms likely increased buckthorn success by mixing seeds into the soil and caching them in their burrows, which has been shown to prevent desiccation of seeds and protect against seed predation in natural environments (Azcarate and Peco 2006; Regnier et al. 2008). This assumption is supported by our field and experimental observations of concentrated germination of buckthorn seeds in and around earthworm middens (Roth, personal observations), where castings likely provide higher soil moisture and nutrients (Eisenhauer and Scheu 2008; Regnier et al. 2008). There were also treatment-dependent interactions between earthworms and leaf litter that affected buckthorn performance. In the no-litter treatment, earthworms perhaps contributed to a slight reduction in buckthorn establishment by burying seeds beyond a critical depth (Traba et al. 2004; Milcu et al. 2006; Regnier et al. 2008). We also noted previously healthy buckthorn seedlings that were subsequently bent over and whose cotyledons were pulled down into earthworm burrows, leaving only the bare stalk and suggesting seedling predation by earthworms (Eisenhauer et al. 2010; Griffith et al. 2013). By contrast, in the high litter treatment where there was more food for the earthworms, the presence of earthworms increased buckthorn success, likely by consuming and mixing the litter barrier and providing pathways for the seeds to reach the soil. This suggests that in otherwise undisturbed forests where light and litter conditions may influence plant establishment, earthworms may interact with these conditions to facilitate buckthorn invasion.

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

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

Investigating the other treatments, the positive effect of litter was not evident until week 4, when the medium and high litter treatments first showed significantly higher buckthorn numbers than the no-litter treatment; this difference remained throughout the experiment, though the interaction between litter and time was not significant in our statistical model. One potential explanation for this trend can again be found in litter’s interaction with earthworms. In microcosms with earthworms, we saw a steady decline in litter cover throughout the experiment as earthworms consumed and moved litter into their burrows. By week 4, it is possible that earthworms had mixed and removed enough litter for seeds to germinate, but that enough litter remained to provide protection from desiccation. In the field, deep litter may inhibit buckthorn germination (Bisikwa 2005) and has been shown to inhibit other invaders (Schramm and Ehrenfeld 2010; Yeo et al. 2014), but seeds deposited on otherwise bare soil may also find temporary protection against predation, frost heaving, and desiccation from a thin layer of leaf litter (Gill and Marks 1991; Cintra 1997).

Light effects on buckthorn performance also seemed to vary with time, though the interaction between light and time was not significant in our model. By week 3, pots in the high light treatment had significantly lower numbers of buckthorn than pots in the medium and low light treatments. While light is instrumental in helping seeds germinate, in this experiment it negatively affected soil moisture
and likely caused seed desiccation, reducing the number of germinants (Gill and Marks 1991). Native species biomass was also highest in the high light treatment, exacerbating the negative effect of light on soil moisture.

Conclusions and implications

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

If our results are representative of natural systems, managing deciduous forests of the Upper Midwest for other conditions that have been shown to decrease invasibility may prove more successful than managing for light and litter conditions. For example, managing forests for native plant diversity may help them resist invasion (Elton 1958; Lodge 1993; Kennedy et al. 2002; Davis et al. 2005; Fridley et al. 2007; Whitfeld et al. 2014a). Managing surrounding lands to decrease available propagules will also keep buckthorn and other invasive plants from arriving at the site, and may be the most important step in preventing invasion (Eschtruth and Battles 2009; Warren et al. 2012). Finally, our data suggest that preventing the spread of earthworms will also be valuable for reducing the spread of buckthorn and potentially other invasive plants as well. Given these results, future studies of plant invasion and forest dynamics in northern North America, particularly in the Upper Midwest, should take into consideration the effects of earthworms.

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