Restoration Ecology
THE JOURNAL OF THE SOCIETY FOR ECOLOGICAL RESTORATION INTERNATIONAL

RESEARCH ARTICLE

Above- and Belowground Impacts of European Buckthorn (*Rhamnus cathartica*) on Four Native Forbs

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Abstract

Fast-growing European buckthorn (*Rhamnus cathartica*) shrubs are aggressively invading woodlands in eastern and midwestern North America. Buckthorn casts dense shade, alters soil conditions, and may be allelopathic. We used greenhouse and field experiments to investigate above- and belowground effects of *Rhamnus* on four herbaceous species native to southern Wisconsin. In the greenhouse, we assessed how *Rhamnus* leaves and fruit affected seed germination and seedling growth relative to sugar maple (*Acer saccharum*) leaves. Fewer seeds of *Eurybia macrophylla* and *Thalictrum dasycarpum* germinated under *Rhamnus* leaves, and those that germinated were slowed. We planted seedlings of the four natives into four treatments at three sites in the field to assess how their survival, growth, and flowering respond to the separate and combined effects of light levels, buckthorn canopies, and buckthorn soils. Buckthorn consistently reduced native plant performance by diminishing survival, flowering, and growth in *Thalictrum*, survival and flowering in *Eurybia*, and flowering and growth in *Symphyotrichum lateriflorum*. Removing buckthorn canopies enhanced growth of these native species, but buckthorn soils separately inhibited growth at least as much (despite being more fertile). Buckthorn's impacts on native plants exceed effects attributable to light levels and soil fertility, suggesting allelopathy. Buckthorn reduced performance more in the uncommon species (*Thalictrum* and *Eurybia*) than the common species (*Geranium maculatum* and *Symphyotrichum*). As we do not yet know how long these inhibitory soil effects persist, we need additional research to learn how best to control buckthorn's impacts on native plant communities.

Key words: **allelopathy, buckthorn, forest understory, invasive shrub, *Rhamnus*.**

Introduction

Invasive species alter ecosystem functioning, community composition, and structure (Vitousek 1990; Mack et al. 2000). They also threaten biodiversity, forest production, and many native species (Pimentel et al. 2005). As exotic species do not all pose equal threats, managers need to prioritize efforts to control these invaders (Webster et al. 2006). Although several studies of invasibility have examined the relationship between native species richness and invasion (Hutchinson & Vankat 1997; Stohlgren et al. 1999; Brown & Peet 2003; Knight & Reich 2005), fewer use experiments to examine the physical and chemical effects of a specific invader on elements of the native community (Frappier et al. 2003; Gorchov & Trisel 2003; Stinson et al. 2007).

Woody exotics strongly affect the structure and function of forest ecosystems (Webster et al. 2006). Experimental studies have found negative effects of the invasive shrubs *Lonicera maackii* (Amur honeysuckle) and *Rhamnus frangula* (glossy buckthorn) on survival, fitness, and fecundity of native species (Gould & Gorchov 2000; Gorchov & Trisel 2003; Fagan & Peart 2004; Miller & Gorchov 2004). *Rhamnus cathartica*, or European buckthorn (hereafter *Rhamnus*), represents the most common woody invader in southern Wisconsin (Rogers 2006). It is native to Europe and western Asia (Godwin 1943), but was introduced to North America by 1849 and appeared in Wisconsin before 1900 (Warner 2007). It thrives in a variety of ecosystems from swamps to upland forests (Mascaro & Schnitzer 2007). Only a few studies have investigated *Rhamnus'* effects on forest understory species. These suggest that *Rhamnus* may negatively affect other species primarily via its effects on the light or soil environments (Leitner 1985; Seltzner & Eddy 2003; Heneghan et al. 2006; Vincent 2006; Knight et al. 2007; Madritch & Lindroth 2009). *Rhamnus* casts dense shade, expands leaves early in the Spring, and retains its leaves late into the Fall (Harrington et al. 1989), reducing light levels for forest herbs (Leitner 1985). *Rhamnus* also affects soil chemistry by acidifying soil, increasing nitrogen content, and lowering soil C:N (Heneghan et al. 2006). These changes are, in turn, associated with high populations of exotic earthworms and soil arthropods, faster decomposition, and potential "invasional meltdowns" (Simberloff & Von Holle 1999; Heneghan et al. 2007; Madritch & Lindroth 2009).

Hypotheses to explain the success of invaders include exploiting empty niches, superior competitive abilities, lack

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doi: 10.1111/j.1526-100X.2010.00727.x

of coevolved competitors and predators, and the recent "novel weapons" hypothesis (Davis et al. 2000; Davis & Pelsor 2001; Keane & Crawley 2002). The latter proposes that non-native plants produce allelochemicals from leaves, roots, or fruit that asymmetrically suppress the growth of native plants (Callaway & Aschehoug 2000). While it is unlikely that allelopathy solely causes invasions (Inderjit & Weston 2000), it may interact with resource-based mechanisms to affect how plant communities respond to invasion (Inderjit & Callaway 2003). There is some evidence for allelopathy in *Rhamnus* (Archibold et al. 1997; Seltzner & Eddy 2003; Vincent 2006). Emodin, a secondary compound with adverse effects on some plants, has been isolated from *Rhamnus*. Emodin is most prevalent in the bird-dispersed berries, but is also found in other parts including leaves (Inderjit & Nishimura 1999; Izhaki 2002; Tsahar et al. 2002). Evidence for allelopathy in *Rhamnus* derives from laboratory bioassays (Archibold et al. 1997; Seltzner & Eddy 2003; Vincent 2006) rather than field experiments (Knight 2006), and Knight et al. (2007) concluded that *Rhamnus's* allelopathic effects on native plants remain "unsubstantiated."

Given the prevalence and impacts of *Rhamnus* invasions, in addition to the cost of control, we should learn more about *Rhamnus'* effects on native plants. Impacts may include competition for light and soil water and nutrients as well as interference competition in the form of allelopathy. Separating above- and belowground effects requires manipulative experiments. As species of different stature, shade tolerance, and sensitivity to soil conditions respond differently to *Rhamnus*-induced changes in the environment, we should also test these impacts across multiple species.

Here, we examine the above- and belowground effects of *R. cathartica* on four native, herbaceous perennials native to the forests of southern Wisconsin. Light is the primary aboveground effect, while belowground effects include root competition for water and nutrients and shifts in the litter layer and soil chemistry. We assess potential allelopathic effects of *Rhamnus* leaves and fruit on germination and seedling growth in four native herbs in a greenhouse experiment. We also report results from a field experiment designed to test whether (1) *Rhamnus* canopies reduce the light available for native understory herbs; (2) removing a *Rhamnus* canopy enhances the survival, growth, or reproduction of native forest herbs; (3) local microsites occupied by *Rhamnus* differ in soil fertility from nearby sites lacking *Rhamnus*; (4) differences in soil conditions under buckthorn affect the growth of understory herbs; and (5) belowground effects of *Rhamnus* reflect simple differences in soil fertility or additional negative effects as expected with allelopathy.

Methods

Species

We assayed effects on four understory herbs (*Geranium maculatum*, *Thalictrum dasycarpum*, and two asters, *Symphyotrichum lateriflorum* and *Eurybia macrophylla*) that vary in their recent demographic trajectories across southern WI (Rogers et al. 2008). Between 1950 and 2002, *Geranium* has remained common, *Thalictrum* has remained uncommon, *Symphyotrichum* has increased in abundance, and *Eurybia* has decreased. We obtained seeds and seedlings of each species from nearby nurseries that use local seed sources: Prairie Moon Nursery in Winona, MN; Prairie Restorations Inc. in Princeton, MN; and Kinnicinnic Natives in River Falls, WI.

Greenhouse Experiment

As *Rhamnus cathartica* leaves and fruit have the most allelopathic potential (Seltzner & Eddy 2003; Vincent 2006), we focused on these parts. We compared the effects of *Rhamnus* foliage to those of leaves of *Acer saccharum*, a tree native to southern WI with similarly high foliar nitrogen content (Heneghan et al. 2002; Hunt et al. 2008). We also compared leaves of *Acer* and *Rhamnus* for thickness, penetrability, and specific leaf area (SLA) to quantify possible structural differences. We measured leaf thickness with an Ames pocket thickness measure to 0.01 mm. To estimate penetrability, we secured a leaf between plexiglas blocks and punched a hole through the lamina with a penetrometer attached to a force gauge. We scanned fresh leaves to estimate their area using ImageJ (National Institutes of Health, Bethesda, MD, U.S.A.) and then massed dried leaves to estimate SLA as the ratio of area to mass. We averaged measurements on five healthy leaves from each species.

We cold-stratified all seeds in moist Jiffy mix (Ferry Morse Seed Company—sphagnum peat moss, perlite, and lime) for 90 days at 2°C. Following stratification, we sowed one seed per plug into 96-plug greenhouse flats filled with a soilless mix (three parts sand:one part compost:one part peat) previously pasteurized at 80°C. We used a randomized block design containing 12 blocks of four flats each. Flats within each block each received one of four treatments: control (no addition of leaves or fruit), *Rhamnus* leaves, *Acer* leaves, or *Rhamnus* fruit. Fresh leaves were oven-dried for the litter treatments and berries were frozen until use. Within flats, each species occupied 24 plugs. We randomized the order of species within flats and the order of treatments within blocks, which allowed us to account for greenhouse environmental variability. After seeds were planted on 19–20 March 2008, we distributed the litter treatments by spreading 10 g (a single layer) of dried *Acer* or *Rhamnus* foliage on the foliage flats and 25 g (one berry) of *Rhamnus* fruit on the fruit flats.

We recorded germination every 1–2 days for the first 8 weeks, adding 500 mL water per flat each time. We measured the length and width of the cotyledons and plant height at 8 weeks. We then began to water and record germination more frequently (6–7×/week). We supplemented daylight with artificial lights to provide a 14-hour photoperiod from March to May and recorded temperature and relative humidity every 30 minutes using a Hobo pro V2 data logger. On 25 June 2008, we counted each plant's leaf number, harvested aboveground parts, oven-dried these at 60°C for 72 hours, and determined dry mass.

We used mixed linear models to assess the effects of treatment, block, and their interaction on germination and plant size treating block as a random effect. As *G. maculatum* (L.) did not germinate successfully, we do not discuss it further. No *T. dasycarpum* seeds germinated in half the blocks, forcing us to eliminate plants from those blocks from the analysis. To achieve normality and uniform variance, we log-transformed germination and size variables and square-root transformed proportion germination where necessary. Block significantly affected the number of days to germination in both *Symphyotrichum* ($F_{[11,77]} = 4.55$, $p = 0.00003$) and *Thalictrum* ($F_{[5,42]} = 2.71$, $p = 0.032$), but there were no significant block × treatment interaction effects.

Field Experiment

The field experiment compared above- and belowground effects of *R. cathartica* on the growth and survival of the same four native forest herbs across three Southern Wisconsin sites. These sites had 4–566 ha of mature forest cover and distinct zones with and without *Rhamnus.* The forest canopy at the Aldo Leopold Foundation (ALF) property site in Sauk County, WI is dominated by *Quercus rubra* and *Fraxinus americana*. The zone lacking buckthorn had few small subcanopy trees and a dense understory (approximately 75–100% cover) with *Polygonum virginianum*, *Eupatorium rugosum*, and several *Rubus* sp. The buckthorn zone had a somewhat more open canopy, scattered small trees, and a sparser herb layer (approximately 25–50% cover) that included weedy species like *Pilea nummulariifolia* and *Glechoma hederacea. Rhamnus* has occurred at ALF since the 1940s, but most stands date to the 1970s–1980s (Warner 2007). Although our site was treated in 2003, many resprouts reproduced again by 2006.

The canopy of Observatory Woods (OBS) in western Dane County is dominated by *Quercus alba* and *Quercus rubra*. The zone lacking buckthorn contained few shrubs or small trees and a sparse understory (approximately 5–25% cover) with *Toxicodendron radicans*, *Parthenocissus quinquefolia*, *Circea quadrisculata*, and *Rubus allegheniensis*. Where buckthorn was present, the herb layer was sparse (approximately 5–25% cover) and included *Hepatica acutiloba*, *Caulophllyum thalictroides*, *Thalictrum dioicum*, and *Asarum canadense.* In 2007, *Rhamnus* covered greater than 2.4 ha of the site.

Gallistel Woods in the UW-Madison Arboretum (ARB) in Madison, WI consists of an oak forest with sugar maple, basswood, and beech planted in the understory. The zone lacking buckthorn had a dense canopy dominated by *Acer saccharum* with some *Carya ovata*, *Prunus serotina*, and *Q. rubra* and a sparse understory (approximately 5–25% cover). The buckthorn zone occurred under a slightly more open canopy dominated by *Q. alba* and *C. ovata* with small trees, shrubs, and a very sparse herb layer (approximately 1–5% cover) in the understory. *Rhamnus* has occurred here since the 1950s with intermittent control efforts since 1980 (Warner 2007).

Within each site, we distinguished adjacent zones with and without a buckthorn canopy (termed “native” and “buckthorn”). Within each zone, we placed a pair of plots 10 m from each other and less than 200 m from a second pair, resulting in four plots (treatments) per site. In the native soil zone, we covered one plot with 30% shade cloth to mimic the shade cast by buckthorn and left the other unmanipulated (“native+shade” and “control,” respectively). Within the buckthorn zone, we cut and removed all aboveground buckthorn biomass in one plot (“buckthorn removed”). In the other, we left the buckthorn undisturbed (“buckthorn canopy”). We also erected deer fences around the plots to prevent herbivory.

Each plot contained four quadrats into which we planted eight to nine seedlings of the four forb species in late June 2007, leaving buffers greater than 25 cm between species. In mid-July, we planted an additional 50 cold-stratified seeds of the species into adjacent areas in each quadrat. We revisited all sites every 2 weeks from July to October 2007 and May to October 2008, scoring survival, other characteristics, and germinants where seeds were sowed. We estimated germination as the maximum number of germinants per quadrat in 2008. For *Geranium*, we measured height and the numbers of stems, leaves, buds, flowers, and fruit. For *Thalictrum*, we measured height, the numbers of leaves and leaflets, and the presence of buds, flowers, and fruit. For *Symphyotrichum*, we measured height, the numbers of flowers and stems, and the presence of buds and fruit. For *Eurybia*, we measured the length of the longest leaf and the numbers of leaves, buds, flowers, and fruits.

To assess how buckthorn affects earthworm abundance, we sampled the earthworm community twice in each zone using the mustard liquid extraction method (Bouché & Gardner 1984). Mustard powder (10 g/L) is suspended in water then slowly poured into a 35 × 35–cm frame inserted into the ground. We counted and collected all worms coming to the surface within 10 minutes. We then preserved the worms in formalin until they were identified to species and functional group.

To characterize light environments, we measured light at ground level in each plot three times during the summers of 2007 and 2008 on the same days at all sites. We used an Extech 401025 light meter taking five measurements (in lux) every 30 seconds from each plot, completing the four plots within 15 minutes. We averaged and normalized these data in two ways. To minimize the effects of sunflecks, we first averaged and log-transformed the five successive lux values producing a normally distributed average “log lux” value for each plot on each day. We then corrected for variation due to the time of day and Julian day of the year by fitting a quadratic regression model to these means (both factors were highly significant). The residuals from this model provide time- and day-corrected values for comparing site and treatment effects. Finally, we computed light levels for each treatment relative to the native control plots at each site as the percent differences (in log lux) among the plots. This corrects for any differences among sites in overall light availability.

To compare soil conditions among sites and treatments, we took 3 × 30–cm soil cores from each plot in June 2007. We pooled plot cores within each zone providing combined samples for the native and buckthorn soil zones at each site. Samples were analyzed at the University of Wisconsin Soil and Plant Analysis lab for pH, organic matter, nitrogen, phosphorus, potassium, calcium, magnesium, and structure (% sand, silt, and clay). To reduce dimensionality, we used principal components analysis to summarize variation in the measured soil variables. The primary axis (PCA1) accounted for most (89%) of the variation present in these variables across samples. Sites with lower scores had sandy, nutrient poor, acid soil with low organic matter. We calculated a "norm soil PCA1" variable by subtracting the native soil score from the buckthorn soil zone axis score at each site.

Field Experiment Analyses

We first compared how site, canopy treatment, and their interaction affected raw light levels (log lux) and soil fertility (soil PCA1) using analysis of variance (ANOVA). This allowed us to assess how the sites compared in absolute terms and how the soil zones and canopy treatments affected these variables. We then explored how the zones and treatments affected the germination, survival, and growth of the native herbs. For the continuous variables (earthworm density, germination, plant height, and leaf number), we applied linear models. To ensure uniform variances, linearity of effects, and reduce skew, we log-transformed the number of *Eurybia* leaves and the number of earthworms. We square-root transformed the heights of *Symphyotrichum* and *Thalictrum* (but not *Geranium*). For the binary response variables (survival and flowering), we used logistic regression. By including the site-adjusted (norm lux and norm soil PCA1) variables, we assessed how light and soil fertility affected plant responses as well as how site and the canopy treatments affected plant responses once environmental effects were accounted for. Such analyses are conservative in that they attribute much of the variation in plant performance to these light and soil effects before assessing any additional effects of *Rhamnus* canopies and soils. We lacked enough degrees of freedom to evaluate interactions so we discuss them qualitatively. We used the Tukey–Kramer honestly significant differences (HSD) tests to make post hoc comparisons. All analyses were completed in JMP 8.0 (SAS Institute, Cary, NC, U.S.A.) or R (R Foundation for Statistical Computing, Vienna, Austria).

Results

Greenhouse Experiment

Rhamnus and *Acer* leaves hardly differed in thickness ($p = 0.11$), but *Rhamnus* leaves were heavier and tougher, taking 250% more force to penetrate ($p = 0.0009$). *Acer* leaves supported 210% more leaf area per gram (SLA, $p = 0.0002$).

Overall, levels of seed germination were low (<26%). Germination rates in the exposed control and fruit treatments were too low (4.4 and 2.7%, respectively) for statistical analyses. After 8 weeks, more seeds had germinated under *Acer* leaves (86) than under *Rhamnus* leaves (47, $F_{[1,47]} = 3.89$, $p = 0.015$). By the end of the 14-week study, more *Eurybia macrophylla* (25.0 vs. 4.2%, $F_{[1,11]} = 9.79$, $p = 0.01$) and *Thalictrum dasycarpum* (37.5 vs. 20.8%, $F_{[1,6]} = 6.30$, $p = 0.046$) seeds had germinated under the *Acer* foliage (Fig. 1a). An initially significant block effect ($F_{[11,47]} = 3.62$, $p = 0.001$) disappeared by 14 weeks. All three species germinated

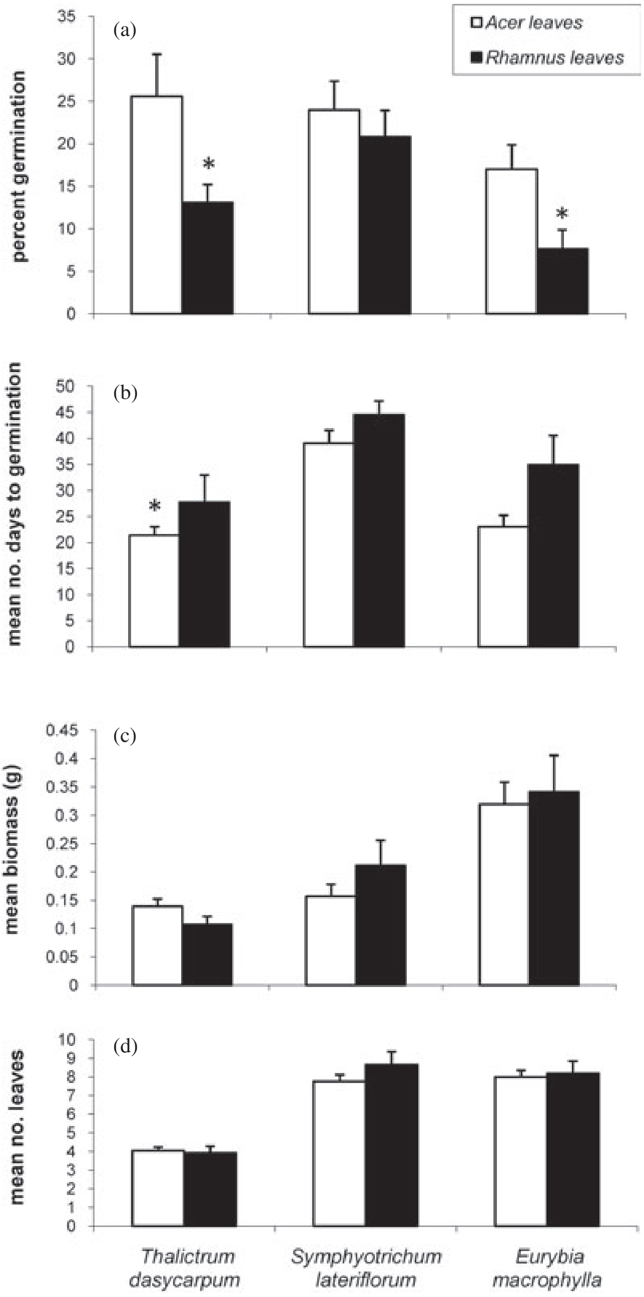

Figure 1. Effects of leaf litter on the germination and growth of three native herbs ($n = 288$ per species per treatment). Bars show means and SE for percent germination at 14 weeks (a), mean number of days to germination (b), mean aboveground biomass at 14 weeks (c), and mean number of leaves at 14 weeks (d) comparing the two litter treatments (*Rhamnus cathartica* and *Acer saccharum*). Asterisks represent significance at $p < 0.05$.

more quickly under *Acer* leaves than *Rhamnus* leaves (Fig. 1b), and this litter effect was significant for *Thalictrum* (21.4 vs. 27.8 days, $F_{[1,42]} = 6.04$, $p = 0.018$). After germination, litter type did not affect height, width of cotyledons, length of cotyledons, biomass (Fig. 1c), or number of leaves (Fig. 1d) in any species.

Field Experiment

Variation in Light Levels and Soil Fertility. Light levels were highest at ALF, lowest at ARB, and varied among the canopy treatments. *Rhamnus*-removed plots consistently received the most light (Fig. 2a). The native+shade (shadecloth) treatment successfully mimicked light levels under *Rhamnus* at ALF and OBS. At ARB, light levels under *Rhamnus* were best matched by the native control treatment, so shadecloth reduced light to even lower levels. The norm lux variable (adjusted for time of day and date) showed consistent patterns of variation among the treatments (Fig. 2b). Removing *Rhamnus* canopy increased light levels about 3× relative to the buckthorn plots, 1.25× relative to the native plots, and 3.65× relative to the native+shade plots (all significant by Tukey−Kramer HSD, $p = 0.05$). As with raw light values, differences among the native control, native+shade, and buckthorn treatments varied over sites ($F_{[6,56]} = 3.31$, $p = 0.007$) reflecting the low light under shadecloth at ARB.

Soil conditions differed between the native cover and *Rhamnus* zones (Fig. 3a & 3b). Under *Rhamnus*, the organic layer was twice as thick as under native vegetation (2.58 vs. 0.92 cm, $t = -2.7$, $p = 0.02$), and organic matter and nitrogen levels were greater than 2× as high (Fig. 3a). Soil at ALF was least fertile with more sand and lower nutrients reflected in low soil PCA1 values (Fig. 3b). OBS native cover soils had higher cation (calcium, potassium, and magnesium) concentrations than the other sites. *Rhamnus* generally increased soil fertility, with the greatest effect at ARB and the least effect in the naturally fertile soils at OBS.

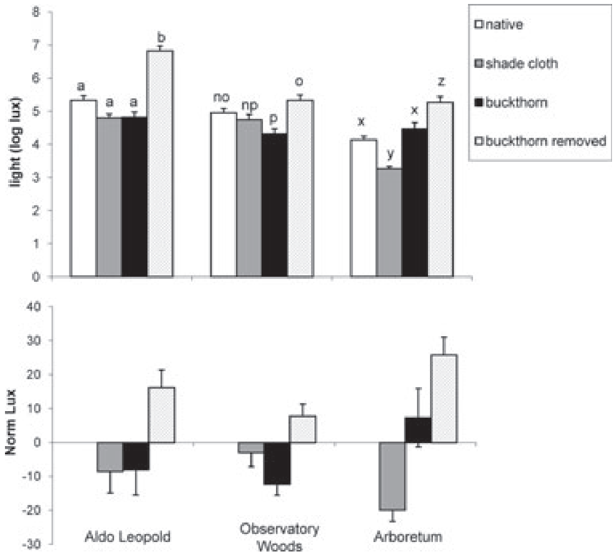

Figure 2. Light levels (mean ± standard error) among the three sites. (a) Light as mean log lux levels in each of the four treatments, averaged across the 2007 and 2008 growing seasons and corrected for day of year and time of day. Bars with different letters differ significantly by Tukey's HSD ($\alpha = 0.05$). (b) Light levels normalized to the "native" plot levels at each site.

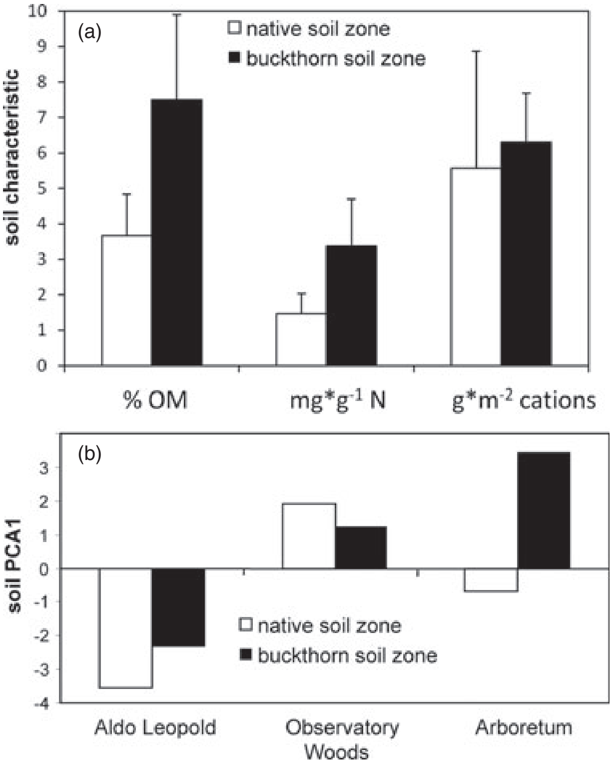

Figure 3. Comparison of soil characteristics (a) and soil PCA1 (b) between zones with and without buckthorn. Means and standard errors were calculated based on single samples from the three sites. Soil PCA1 values are scores from a principal components analysis based on all soil variables.

Effects on Germination, Height, Survival, and Flowering. Field germination was low (<20%), preventing us from finding any statistical relations to factors or covariates. Observed seed movements among plots suggest rodent activity.

Higher light levels enhanced seedling survival and height in *Thalictrum* and flowering in *Geranium* (Table 1). Soil fertility also affected survival and height in *Thalictrum* and flowering in *Geranium*, but these effects were always negative. Richer soils (higher PCA1) depressed leaf number in *Eurybia*. Thus, the more nutrient-rich soils under *Rhamnus* appear to inhibit plant growth and survival. This unexpected result makes our multivariate tests conservative in that any negative effects of buckthorn soil fertility are accounted for (if significant) before testing for other *Rhamnus* soil effects.

Seedling survival in all four species differed significantly among sites and was lowest at ARB, except for *Thalictrum* where survival was lowest at ALF (Table 1). Seedlings of *Geranium*, *Symphyotrichum*, and *Eurybia* survived best at

Table 1. Performance of four native herbs in the field in response to site conditions and experimental treatments.

Species	*Fitness Variable*	r^2	*Factor*	*Effect*	*F- or X^2-Value*
Thalictrum dasycarpum	Survival ($n = 96$)	0.37	Light	+	7.67^{**}
			Soil fertility	−	7.68^{**}
			Site	ALF lowest	13.2^{**}
			Treatment	*Rhamnus* removed lowest	10.71^{*}
	Flowering ($n = 85$)	0.59	Site	ARB lowest	24.96^{***}
			Treatment	*Rhamnus* lowest	12.04^{**}
	Height ($n = 71$)	0.46	Light	+	6.34^{*}
			Soil fertility	−	5.14^{*}
			Site	ALF lowest	7.21^{*}
			Treatment	*Rhamnus* removed lowest	4.91^{**}
Eurybia macrophylla	Survival ($n = 96$)	0.27	Site	ARB lowest	22.6^{***}
			Treatment	*Rhamnus* lowest	14.69^{**}
	Flowering ($n = 59$)	0.16	Site	OBS lowest	4.67 AS
			Treatment	*Rhamnus* lowest	7.01 AS
Geranium maculatum	Survival ($n = 108$)	0.13	Site	ARB lowest	11.12^{**}
			Treatment	*Rhamnus* lowest	5.22
	Flowering ($n = 80$)		Norm light	+	3.14 AS
			Soil fertility	−	$4.55*$
			Site	ALF lowest	7.42^{*}
			Treatment	*Rhamnus* removed lowest	3.91
Symphyotrichum lateriflorum	Survival ($n = 96$)	0.22	Site	ARB lowest	19.68^{***}
			Treatment	*Rhamnus* lowest	2.61
	Flowering ($n = 69$)	0.20	Site	ARB lowest	4.94 AS
			Treatment	*Rhamnus* lowest	15.82^{*}
	Height ($n = 69$)	0.17	Site	OBS lowest	2.18
			Treatment	*Rhamnus* lowest	2.91^{*}

Analyses show how predictor variables affected the performance of seedlings added to all plots as measured by height (square-root transformed, analyzed using analysis of covariance [ANCOVA]) or by survival and flowering (binary response variables analyzed using logistic regression). Covariate effects (mean light levels and soil fertility) that were significant ($p < 0.1$) were included in these models and thus incorporated before the effects of site and treatment were estimated. Significance levels: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; AS $0.05 < p < 0.10$.

ALF. Seedlings of all species also varied across sites in their propensity to flower ($p < 0.1$; Table 1).

The soil/canopy combination treatments affected some aspect of plant performance in all species except *Geranium* (Table 1). *Rhamnus* canopies and soils consistently reduced native plant performance. *Rhamnus* plots had the lowest plant performance in all 10 cases where we tested for treatment effects and all 7 that were significant. In two of the seven significant cases, plants performed worst in the *Rhamnus* canopy-removed treatment rather than the *Rhamnus* canopy-intact treatment, suggesting that the inhibitory effects of *Rhamnus* soils are of the same magnitude as its shading effects. Still, removing *Rhamnus* canopies appeared to enhance seedling survival (*Eurybia*), height (*Thalictrum*), and possibly flowering (*Thalictrum*, *Symphyotrichum*, and *Eurybia*; Fig. 4). In a combined mixed model analyzing plant height in *Thalictrum* and *Symphyotrichum*, treatment was significant ($F = 7.43$, $p < 0.0001$) with the best native forb growth in the native (control) treatment, intermediate growth in the native+shade and buckthorn-removed treatments, and worst growth in the buckthorn treatment. This distribution contrasts strongly with the rank orders of both soil fertility (buckthorn > native) and light levels (buckthorn removed > control ∼ buckthorn > native+shade). Following back transforms of adjusted cell means, the intact *Rhamnus* canopy decreased forb height by more than twice as much (50 vs. 21%) as the native+shade treatment (relative to the *Rhamnus*-removed and control treatments, respectively). In addition to these conspicuous light effects, heights declined by 59.4% in the *Rhamnus* treatment relative to the control treatment despite having only slightly less light.

Exotic Earthworms. Soil zones overlapped in the species of earthworm they supported with eight of the nine species found in the buckthorn soil zone also occurring in native soils. However, almost twice as many exotic earthworms occurred in the more fertile buckthorn soils compared to otherwise similar soils only a few meters away (15.2 ± 4.28 vs. 8.67 ± 2.54, $F_{[1,6]} = 6.77$, $p = 0.04$). This difference was particularly dramatic at site OBS, where the buckthorn soil zone had many more worms.

Discussion

Rhamnus cathartica affected these four native forbs in several distinct ways including inhibition of germination in the greenhouse and reduced survival, growth, and reproduction in the field. In no cases did *Rhamnus* litter, canopies, or soils act to enhance any of these fitness characters at any life-history stage, despite the fact that *Rhamnus* litter and soils had higher levels of most nutrients. *Rhamnus* casts

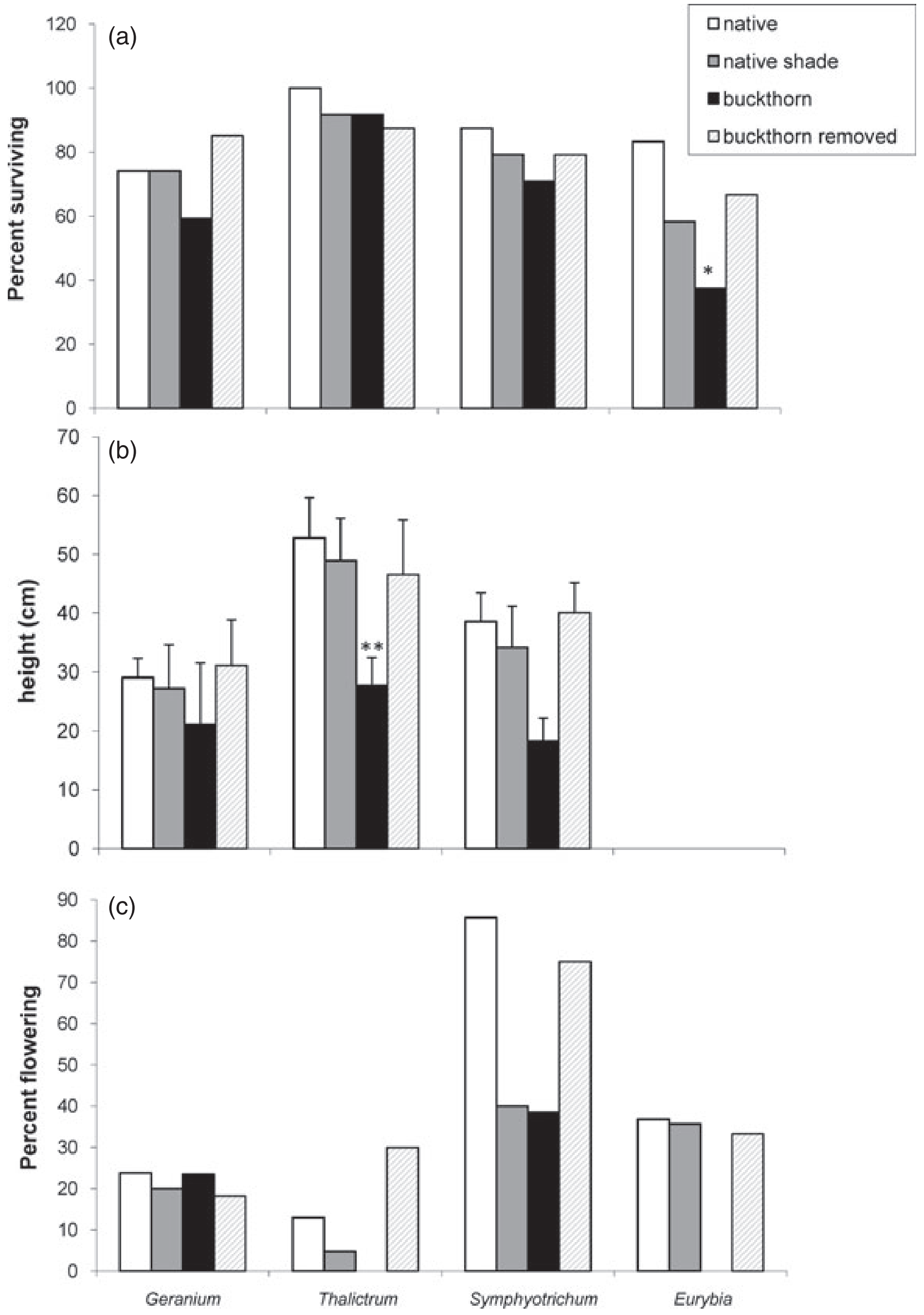

Figure 4. Performance of the four native herb species across the four canopy treatments averaged across sites. Performance was estimated as: (a) seedling survival, (b) seedling height, and (c) flowering percentage in *Geranium maculatum* ($n = 81$ per treatment), *Thalictrum dasycarpum*, *Symphyotrichum lateriflorum*, and *Eurybia macrophylla* (all $n = 72$ per treatment). Plotted values show mean values ± standard errors. Height values for *E. macrophylla* are not presented as the number of leaves was used to estimate growth. Significance levels as in Table 1.

deep shade conspicuously reducing the light available to support the growth of native plants over the entire growing season (Leitner 1985; Harrington et al. 1989). The impacts of *Rhamnus* on these native herbs, however, extend beyond what can be attributed to these obvious light effects. The results presented here support observations that *R. cathartica* changes the soil environment in ways that often depress the germination, growth, and flowering of forest herbs (Heneghan et al. 2002, 2006).

In the greenhouse experiment, *Rhamnus* litter had conspicuous effects on seed germination reducing it to half the levels observed under an equivalent amount of maple leaf litter and slowing seedling emergence. Both *Acer* and *Rhamnus* leaves are high in nitrogen (1.8 and 2.2%, respectively) relative to native trees such as oak (1.4%) and cherry (0.6%) (Heneghan et al. 2002; Hunt et al. 2008). *Acer* leaves were thinner and less tough, however, perhaps allowing seedlings to penetrate the litter more easily (although small-seeded *Symphyotrichum* seedlings emerged equally well in both treatments). Maple leaves may also have favored germination by maintaining higher soil moisture as they covered more area.

Because *Rhamnus* shrubs also alter light, soil nutrients, and alleolochemicals that were not part of the greenhouse experiment, the greenhouse results underestimate the effects of these shrubs in the field. Although seedlings had similar growth after emergence in the greenhouse, our field-grown native seedlings usually suffered reduced growth, survival, and flowering when growing under buckthorn or in buckthorn soils.

In the field, the buckthorn-intact and buckthorn-removed treatments reduced native plant performance the most, consistently reducing survival, growth, and/or flowering. *Rhamnus* appears to invade initially lighter areas of the forest floor, but then casts deeper shade than usually found elsewhere in these forests. Removing buckthorn canopies often enhanced survival, growth, and/or reproduction in these forbs. If buckthorn canopies alone account for its impacts on native understory plants, clearing away its dense foliage should have allowed these native herbs to thrive. Instead, the buckthorn-removed treatment also reduced native plant success, with three of the four native seedling species doing worse than expected based on available light. Thus, the inhibitory effects of *Rhamnus* soils are at least as large as its effects via shading on these native forbs.

Given the increase in soil fertility we observed under *Rhamnus*, these impacts presumably reflect allelopathy. Previous studies of allelopathic effects in *Rhamnus* have shown mixed results (Archibold et al. 1997; Seltzner & Eddy 2003; Vincent 2006). Knight's (2006) field study found no evidence for negative belowground effects like allelopathy. This could reflect the fact that she studied an expanding invasion edge where negative soil changes may not yet have accumulated. In contrast, our sites within mature buckthorn stands had many years to accumulate buckthorn litter and soil allelochemicals. The impacts of emodin on intra- and interplant interactions in *Rhamnus* have not yet been studied (Izhaki 2002) nor has anyone yet isolated potential allelochemicals like emodin from soils growing under *Rhamnus* (Blum 1999). We see, however, evidence for the "novel weapons" hypothesis of invader success in both *Alliaria petiolata* (Prati & Bossdorf 2004; Stinson et al. 2007; Cipollini et al. 2008) and *Centaurea maculosa* (Callaway & Aschehoug 2000). The soil effects showed here deserve additional study to characterize their chemical basis, mode of action, and how they might be neutralized. Managers will also be keen to learn how quickly allelochemicals from *R. cathartica* accumulate and how long their effects persist.

Differences in *Rhamnus* litter unrelated to allelochemicals could also contribute to its inhibitory effects, e.g. by enhancing exotic earthworm populations. Worms alter forest ecosystems by reducing the O horizon, shifting soil nutrient dynamics, and reducing plant diversity (Bohlen et al. 2004; Hale et al. 2006). Buckthorn and earthworms may interact synergistically to facilitate each others' invasions (Heneghan et al. 2007; Madritch & Lindroth 2009; Nuzzo et al. 2009). We actually found an increased O horizon under buckthorn, and exotic earthworms were twice as abundant there as under nearby native vegetation. Buckthorn may be producing enough nutrient-rich litter to increase rather than decrease the O horizon despite larger earthworm populations.

Although our transplanted seedlings survived in a variety of light and soil conditions, they did not thrive or reproduce well under buckthorn canopies or in buckthorn soil. Miller and Gorchav (2004) found the shrub *Lonicera maackii* to similarly affect transplanted perennial herbs. In cases like these where herb species persist but fail to thrive in the presence of shrubby invaders, the invader can substantially shift community composition and structure despite not excluding species (Parker et al. 1999).

Negative above- and belowground effects of *Rhamnus* occurred at all sites, but the specific effects varied by site and species. In general, native plants performed best at ALF and worst at ARB, where *Rhamnus* most affected light and soil fertility. The higher survival and growth of herbs at the ALF site may reflect its higher light levels and sandier, more acidic soils. Including these soil and light effects in the linear model enhanced the positive effects of site ALF, however, suggesting that the benefits of this site extend to other differences. Survival and growth were lowest at the ARB, a wet site, despite the fact that all four herbs are said to favor moist sites (Voss 1985). The ARB site also occurred within an urban matrix with anthropogenic disturbance. Other studies also describe differences among sites in the effects of invaders (Woods 1993; Gould & Gorchov 2000; Miller & Gorchov 2004; Heneghan et al. 2006). *Lonicera maackii*'s effects on native annuals and perennials were also greater at more disturbed sites (Gould & Gorchov 2000; Miller & Gorchov 2004).

All four forbs tended to decline in the presence of *Rhamnus* but differed in their responses. The two more common species (*Geranium* and *Symphyotrichum*) grew well across a variety of field conditions. In contrast, the two less common species (*Eurybia* and *Thalictrum*) grew best in native soils and under the native canopy, suffering greater declines in germination, growth, and reproduction in the presence of *Rhamnus*. Other field (Gould & Gorchov 2000; Gorchov & Trisel 2003; Hartman & McCarthy 2004) and greenhouse studies (Grant et al. 2003; Skulman et al. 2004) also found the impacts of invasive litter on native plants to vary over species and sites. Whether *Rhamnus* generally has greater impacts on less common plant species should be studied further.

Conclusions

These experiments allowed us to separate above- and belowground effects of buckthorn in natural field settings, perhaps for the first time. Both effects are substantial, and it was surprising to find that buckthorn soils inhibit native forbs as much as, or more than, their canopies. The number, consistency, and persistence of these effects make it clear that *Rhamnus* significantly alters native understory plant communities.

Although it was known that *Rhamnus* alters soil nutrients and pH (Heneghan et al. 2002, 2006, 2007; Madritch & Lindroth 2009), the impacts of buckthorn soils on native plants exceed those attributable to soil fertility and structure. This makes it important to understand more about the allelochemicals that *Rhamnus* excretes including their quantities, mode of action, and persistence. Such belowground effects can persist and should be accounted for in devising restoration strategies (Conser & Connor 2009). If *Rhamnus* allelochemicals are confirmed to have residual effects, they will need to be ameliorated or compensated for in future management plans.

Much money and effort is spent controlling *Rhamnus* aboveground by cutting and herbicide treatments (Bondreau & Wilson 1992). These may do little to ameliorate allelochemicals and their legacy, however. Mechanical or chemical removals of invasive shrubs from heavily invaded areas often fail to enhance native species richness and density (Borgmann & Rodewald 2005; Vidra et al. 2007; Love & Anderson 2009). Land managers sometimes use fire as a tool to control buckthorn, first cutting and burning invaded areas then applying herbicide to buckthorn stems. As fires also change the amounts of available light, nutrients, organic matter, and soil organisms (Covington & Sackett 1984), they may mitigate some belowground effects of buckthorn. The surrounding landscape (Borgmann & Rodewald 2005), available propagules (Luken et al. 1997; Love & Anderson 2009; Pavlovic et al. 2009), and seedbank (Vidra et al. 2007) also affect the success of restoration projects. More research is needed to determine how fire, cutting, and herbicides should be combined to most effectively reduce the aboveground and belowground effects of *Rhamnus* and restore forest ecosystems.

Implications for Practice

- The invasive exotic shrub, *Rhamnus cathartica*, inhibits the germination of some forbs native to North America.
- *Rhamnus* restricts the performance of native plants via both shading from its dense canopy and by modifying conditions below ground. These belowground effects often exceed the impacts of shading.
- Belowground effects of *Rhamnus* are complex. Its rich litter may favor dense populations of exotic earthworms, which increases soil organic matter and fertility. *Rhamnus* also appears to deploy “novel weapons” (allelochemicals) depressing native plant germination, growth, survival, and flowering.
- The inhibitory effects of *Rhamnus* soils persist after the aboveground parts of this shrub have been eliminated. We do not know the exact composition of these substances, how long they persist, or how the measures we use to control *Rhamnus* affect their levels or longevity.

Acknowledgments

We are grateful for funding from the USDA NRI-CSREES program in weedy and invasive species (2007-02902) and the University of Wisconsin-Madison Department of Botany. We would also like to thank the UW Arboretum and Aldo Leopold Foundation for allowing us to work on their property. P. Zedler provided valuable guidance and suggestions.

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