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Recovery of forest floor diversity after removal of the nonnative, invasive plant *Euonymus fortunei*¹

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Abstract. The vine *Euonymus fortunei* (Turcz.) Hand.-Mazz. is invading forests of the eastern United States; as a result, removal of *E. fortunei* has become a priority of resource managers. This study examined the effectiveness of five techniques for eliminating *E. fortunei*, restoring plant species richness, and enhancing recolonization by woody species. In 2003, the following five treatments were applied: burn with a propane torch, light exclusion by plastic tarp, burn and glyphosate application, cut (simulated grazing) and glyphosate application, mow and glyphosate application, plus an untreated control. Each treatment was replicated four times in a randomized block design located in a heavily *E. fortunei*-invaded forest remnant in Lexington, KY. Vegetation was surveyed in 2004, 2005, 2006, 2007, and 2013. Across years, most treatments were associated with reduced *E. fortunei* cover and increased total species richness. Over time, *E. fortunei* cover increased across treatments, such that by 2013, no difference in *E. fortunei* cover was detectible among treatments. Some differences in total and native species richness among treatments were still perceptible by 2013. Increased *E. fortunei* cover was correlated with decreased ground-layer species richness, native species richness, sapling richness, and sapling density. Light exclusion by plastic tarp, a method absent from many management recommendations, was unique in its long-term reduction of *E. fortunei* cover and its association with increased total species richness, but use of plastic tarps may have drawbacks. This study quantified the long-term community effects of removing an established invasive species from a mature, urban forest. Removal allowed native plants, notably woody species, to reestablish.

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Because richness continues to decline as *E. fortunei* reinhabits plots, land managers seeking to conserve biodiversity under conditions similar to those within our study site should maintain proactive *E. fortunei* removal plans.

Key words: *Euonymus fortunei*, invasive species, purple wintercreeper, restoration, species diversity

Nonnative, invasive species threaten the diversity and function of native ecosystems (Zavala 2002), and their removal poses an increasing and expensive challenge (Pimental *et al.* 2000). A need exists for refined restoration and adaptive management plans that control invasive plants and promote native plant recovery, and the study of these activities is an important scientific goal (Webster *et al.* 2006). Invasive plants possess a suite of traits that may facilitate their invasion and may negatively affect native species (McNeish *et al.* 2012, Luken 2014), but these traits and their effects vary across spatiotemporal scales (Theoharides and Dukes 2007). Studies at various scales, over time, and of different species provide insights that contribute to our understanding of invasion ecology.

Wintercreeper (*Euonymus fortunei* (Turcz.) Hand.-Mazz.) (winter creeper) is an invasive woody vine that was first introduced to the United States from Asia in 1907 (Remaley 2009). This species has been described as invasive in 11 states (Invasive Plant Atlas of the United States 2013), and currently, the invasion is most severe in central Kentucky, the site of the present study, where *E. fortunei* affected an estimated 2,593 ha (6,644 ac) of forest in 2008 (Miller *et al.* 2008). The current extent of invasion by *E. fortunei* is patchily distributed, confined to disturbed sites, such as roadsides, parks, and urban woodlands (Zouhar 2009). *Euonymus fortunei* is still available from many nurseries and may continue to colonize urban and suburban forests. Ongoing urbanization fragments forests, and fragments may conserve biodiversity by providing refugia for plants and wildlife (Campbell 1981, Miller and Hobbs 2002). Proximity of urban forests to cultivated landscapes and further anthropogenic disturbance may allow nonnative, invasive plants to displace native plants in these ecosystems (McKinney 2002), increasingly so, toward urban centers (Kowarik 1990). *Euonymus fortunei* seriously threatens the forests it currently inhabits, and increased penetration into forests of the eastern United States could have devastating effects on biodiversity. Others have recognized the threat of *E. fortunei* to

native species ecology; a collaboration of Chinese and American researchers (Ding *et al.* 2006) recognized *E. fortunei* as a “top 10 concern” among invasive plants of Asian origin.

Relatively little research has explored the ecology of *E. fortunei*, and most knowledge of the life history of *E. fortunei* comes from horticultural literature and anecdotal observations. The plant is a popular ground cover in the United States. Its features include a fast growth rate and rapid spread enabling it to achieve nearly 100% ground cover quickly, and it is available in many ornamental cultivars, including ‘Emerald and Gold,’ ‘Coloratus,’ and ‘Variegata’ (Dirr 1998, Zouhar 2009). *Euonymus fortunei* is shade tolerant and has thick-cuticled leaves that resist drought (Zouhar 2009). These traits make *E. fortunei* not only a hardy ornamental but also a competitive understory plant, which may suppress less-competitive native understory species (Randall and Marinelli 1996, Swearingen *et al.* 2002). *Euonymus fortunei* propagates either through seed or vegetatively (Zouhar 2009). Its vegetative spread along the forest floor is thought to contribute to its invasion; it forms a thick mat of vegetation that may suppress other plants (Randall and Marinelli 1996, Swearingen *et al.* 2002, T. J. Rounsaville, University of Kentucky Arboretum, pers. comm.).

To our knowledge, no published study has examined the community ecology of recovering forests that have undergone removal of *E. fortunei*, although many have investigated responses to removal of other invasive plants. A meta-analysis by Kettenring *et al.* (2011) suggested that different invasive species control methods produced different native and invasive revegetation outcomes based on a review of 355 invasive species control studies. They found that herbicide reduced invasive species most effectively overall, whereas removal by cutting decreased invasive species biomass and cover less effectively, and burning increased invasive species biomass and density. For native species across studies, no treatments were associated with strongly positive gains, and burning actually reduced native biomass (Kettenring *et al.* 2011). The authors also highlight

differences in methodology among studies, especially concerning duration and scale. Interestingly, only 6% of the 355 studies included in this meta-analysis monitored treatment plots for longer than 5 yr (Kettenring *et al.* 2011).

The structure of a community, consisting of both biotic and abiotic factors, may predict its vulnerability to invasion or reinvasion after treatment (Souza *et al.* 2011, Wilson *et al.* 2013), and invasive species can create permanent changes in affected ecosystems (Bakker and Wilson 2004, Bradford *et al.* 2012). The success of many invasive plants is associated with tolerance of disturbance, and some invasive plants have been shown to promote additional disturbance in sites they invade (Buckley *et al.* 2007). Smith and Reynolds (2011) found evidence that *E. fortunei* conditions the soil in which it grows, likely by affecting microbial communities. *Euonymus fortunei* is known to produce compounds that repel insects (Jinbo *et al.* 2002), but the allelopathic effects on other plants have not been examined. It remains to be determined whether, after *E. fortunei* removal, native plant communities can recover and maintain plant species diversity. In general, reinvasion, whether by the originally treated species or novel invaders, is common in treated sites even after effective initial control (Kettenring *et al.* 2011, Webster *et al.* 2006).

It is crucial that land managers create effective plans for *E. fortunei* removal, given the plant's potential threat to forests of the eastern United States. The US Department of Agriculture, Forest Service, currently recommends several *E. fortunei* removal strategies. The most-effective method for eliminating *E. fortunei* is manually removing the whole plant, including roots (Zouhar 2009). Unfortunately, this technique is labor intensive and often impractical, so systemic herbicides, such as glyphosate, are commonly employed. Cutting or mowing without additional application of systemic herbicide has been shown to increase growth of *E. fortunei* and other invasive plants (Sink *et al.* 2005), so the supplementation of systemic herbicide after disturbance is essential. Some land managers have used plastic tarps to suppress *E. fortunei* by light exclusion, with anecdotal success (Zouhar 2009). Other removal techniques tested in the past have been shown to be ineffective. For example, burning cannot remove belowground biomass, whereas the thick cuticle of *E. fortunei* resists damage

aboveground (Zouhar 2009). Often, a variety of removal techniques are used in combination.

The present study evaluated the effectiveness of five removal techniques for eliminating *E. fortunei* and examined the plant community responses to removal over 10 yr. The specific research questions addressed in this study were (a) which removal treatments provide the greatest long-term control of *E. fortunei*, (b) which treatments most effectively increased native plant species cover and richness, and (c) how does removal of *E. fortunei* affect sapling density. Based on the results of this study, recommendations can be made for *E. fortunei* control, pertaining to the restoration of plant species diversity and forest community structure.

Materials and Methods. **STUDY SITE AND BACKGROUND.** This study was conducted in the University of Kentucky's Arboretum Woods, located in Lexington, KY (38°0'54.87"N, 84°30'38.37"W). Measuring 5.8 ha, the Arboretum Woods is one of the largest fragments of eastern deciduous forest located in the Inner Bluegrass physiographic region of central Kentucky (Campbell 1981). At the time of this study, the most important species in the overstory of the woodland included *Celtis occidentalis* L. (common hackberry), *Juglans nigra* L. (black walnut), *Fraxinus americana* L. (white ash), *Acer negundo* L. (boxelder), *Prunus serotina* Ehrh. (black cherry), and *Quercus macrocarpa* Michx. (bur oak). Shrubs and saplings near the treatment plots included *Euonymus atropurpureus* Jacq. (eastern wahoo), *Carya laciniosa* (Michx. f.) G. Don (shellbark hickory), *Gymnocladus dioica* (L.) K. Koch (Kentucky coffeetree), *Fraxinus quadrangulata* Michx. (blue ash), and *Ulmus americana* L. (American elm). The soils of treatment plots were phosphate-rich silt loams that were deep and well-drained (Wharton and Barbour 1991). Regional climate was continental, and mean annual temperature and precipitation were 12.8 °C and 111.8 cm, respectively (Wharton and Barbour 1991). The location of the study site in an urban/suburban area has made it particularly vulnerable to disturbance and to the encroachment of invasive plants. *Euonymus fortunei* cover approaches 100% in most areas of this woods and has been established for many years (Campbell 1981). The site for this study was chosen based on its uniform 100% *E. fortunei* cover, level

Table 1. Treatment abbreviations and details of *E. fortunei* removal treatments in an urban forest, Lexington, KY.

Abbreviation	Treatment	Description
CONT	Control	Retained ~100% <i>E. fortunei</i> cover with no additional disturbance, approximating pretreatment condition.
TARP	Plastic tarp and herbicide	Covered April 2003 to October 2003 with a plastic tarp typically used to protect athletic turf. Covered from October 2003 to fall 2004 with 6-mil black plastic sheeting. In fall 2004, remaining green stems near the perimeter of the black tarp were sprayed with herbicide.
MHRB	Mow and herbicide	Plots mowed in April 2003, then cut stems were sprayed on the same day with herbicide. After 7 mo, in November 2003, this process was repeated.
XHRB	Cut and herbicide	For cuttings meant to approximate the effects of goat grazing, vegetation was mowed in two plots and cut with hedge trimmers in the two other plots in April 2003. After 1 mo, this treatment was intended to experience a controlled burn, but weather prevented a burn, and herbicide was used to treat each plot instead.
XBRN	Cut and burn and herbicide	In April 2003, vegetation was cut with hedge trimmers. In June 2003, plots were lightly burned with a propane torch enough to penetrate cuticle and were covered with herbicide on the same day.
BURN	Burn	Burned with a propane torch in April 2003 until stems and leaves were no longer green, then new growth was burned again in June 2003 and once again burned in July 2003. In February 2004, three of the four plots were burned again.

topography, and adequate drainage after abundant rainfall.

Understanding the plant community's response to *E. fortunei* removal, the focus of this study, requires understanding the history of invasion and recovery in the study area. In the past, the area existed as woodland underlain by mowed understory. Mowing ceased in 1980 (Campbell 1981). Since then, the area has been in succession to forest understory, but invasive species, such as *Lonicera maackii* (Rupr.) Herder (Amur honeysuckle), *Lonicera japonica* Thunb. (Japanese honeysuckle), *Alliaria petiolata* (M. Bieb.) Cavara & Grande (garlic mustard), *Euonymus alatus* (Thunb.) Siebold (burningbush), and *E. fortunei* have impeded reestablishment of native herbs and shrubs. This study began in 2003 as part of an adaptive management plan to determine an effective means for *E. fortunei* removal.

EXPERIMENTAL DESIGN AND RESTORATION TREATMENTS. Beginning in April 2003, Arboretum staff established an experiment to examine the effectiveness of five different *E. fortunei*-removal treatments (Table 1). All herbicide treatments were foliar applications of Roundup Concentrate Plus (Monsanto Company, St. Louis, MO; 18% glyphosate and 0.73% diquat) diluted to a final concentration of 1.6% glyphosate. A sponge-like nozzle minimized herbicide contamination of other plots. All burn treatments were performed with a "Go Devil" propane torch (500,000 BTU rating) until the leaf

cuticles visibly cracked. Each plot measured 6.096 m × 6.096 m (20 ft × 20 ft), and each treatment was replicated four times in a randomized block design (Fig. 1). Treatment plots directly bordered each other, and the total treatment area was surrounded by untreated vegetation. Control (CONT) plots were not included in the design in 2003 but were added in 2004 within the untreated vegetation directly bordering the treatment area.

VEGETATION SURVEY. Vegetation surveys were performed in 2004, 2005, 2006, and 2013 using the following sampling technique. Percentage of cover by *E. fortunei* and other species was estimated within 1-m² subplots within each larger treatment plot. In 2004, 2005, 2006, and 2007 we used two 1-m² subplots in fixed locations in each plot, and in 2013, we used four evenly spaced 1-m² subplots. Within-plot samples were averaged for analysis. To supplement the 1-m² samples and compile more exhaustive species lists that could better characterize species communities, in summer 2005 and summer 2007, all plant species present in the whole block were inventoried (Table 2).

Percentage of cover for each ground-layer species was estimated according to the guidelines of Kent and Coker (1995). Class A (almost absent) was defined as a percentage of cover of <1%, and class B (barely present) was defined as 1%–5% cover. These two classes were estimated using cardboard squares measuring 10 cm × 10 cm for class A and 22.36

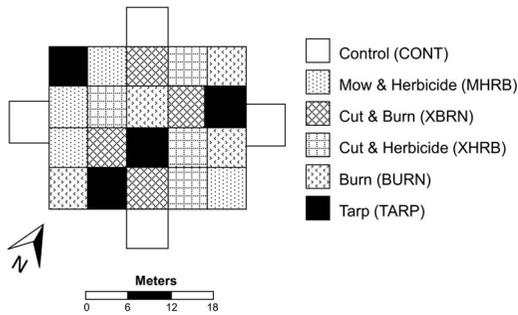


FIG. 1. Spatial layout of treatment plots created in 2003 in a randomized block design; each row is a block. Controls were added to treatment area perimeter in 2004.

cm \times 22.36 cm for class B. The remaining classes were visually estimated, and include class R (rare) of 6%–25%, class P (patches) of 25%–49%, class I (interrupted) of 50%–74%, and class C (continuous) of \geq 75%. This scheme was used for all herbaceous species and woody species $<$ 50 cm tall, with the exception of *E. fortunei*, which was not estimated categorically. Because *E. fortunei* was our focus species, we estimated cover for this species to 1% cover. Botanical nomenclature follows Jones (2005).

Data from 2004, 2005, 2006, and 2007 were collected in late summer or early fall. In 2013, data were collected twice, in summer (between June 10 and July 2) and early fall (September 21–23). For 2013, summer data were used in statistical analyses and all graphs depicted here because there were no statistical differences in *E. fortunei* cover ($F = 0.327$, $P = 0.569$) between midsummer and early fall data.

By 2013, a sapling layer had developed where previously it was absent, so woody plants were quantified for the first time in the 2013 survey. All woody species $>$ 50 cm tall but $<$ 10 cm diameter at breast height (DBH; 1.37 m) were inventoried within each 6-m \times 6-m plot and are henceforth called *saplings*. Woody species with DBH $>$ 10 cm were not recorded but were assumed to have predated the treatments.

STATISTICAL ANALYSIS. Analyses were conducted using JMP 10 Statistical Discovery Software (SAS Institute Inc. 2013), IBM SPSS Statistics version 19 (SPSS Inc. 2010), and R version 3.1.3 (R Core Team 2015). Figures were made using SigmaPlot (Systat Software 2011). None of the data were normally distributed after log or square-root transformation,

so nonparametric analyses were used to test for trends in response variables.

Kruskal-Wallis tests were employed to examine effects of treatment on vegetative characteristics (*E. fortunei* cover, species richness, native species richness, sapling density, seedling cover) in 2013. Mann-Whitney U-test pairwise comparisons were performed if Kruskal-Wallis tests indicated significance. Only treatment-control pairwise comparisons were performed (e.g., cut, burn, and herbicide [XBRN]-CONT, not XBRN-plastic tarp and herbicide [TARP]) to minimize type I error. A Bonferroni-corrected alpha was computed ($\alpha = 0.05/\text{number comparisons} = 0.01$). P -values reported were exact 2-tailed significance values, except in cases in which exact significance could not be computed. In such cases, asymptotic significance was reported.

Friedman tests were used to compare effects of treatment and year on vegetative characteristics measured in 2004, 2005, 2006, 2007, and 2013. If Friedman tests indicated significance, Wilcoxon pairwise comparisons were performed. For treatments, only treatment-control pairwise comparisons were performed, and each year was compared with 2004, the first year after treatment, at which time *E. fortunei* cover was presumably lowest. Bonferroni corrected alpha was computed for treatments ($\alpha = 0.01$) and years ($\alpha = 0.0125$), and P -values reported were exact 2-tailed significance values.

We calculated Spearman's rank correlations to examine relationships between *E. fortunei* cover and 2013 vegetative characteristics (average herbaceous richness, average native herbaceous richness, sapling richness, and saplings density).

To test for effect of plot location, we performed a series of Kruskal-Wallis tests comparing vegetative characteristics measured in 2013 to block number. The CONT plots were excluded from this analysis because they were not located within the blocks but on the exterior (Fig. 1).

To characterize differences in plant communities developing in response to different treatments, nonmetric multidimensional scaling (NMS) ordinations were conducted. For species community data compiled in 2013, PC-ORD version 6 software was used to create NMS ordinations describing the plant communities associated with each treatment type (McCune and Mettford 2011). The main matrix was composed of the 37 species found in

Table 2. Species present in years 2005, 2007, and 2013; NMS ordination *r*-values. Species lists from 2005 and 2007 were compiled by surveying every plant in each 6-m × 6-m plot. In 2013, four 1-m² subplots were inventoried within each larger 6-m × 6-m plot. Nomenclature follows Jones (2005). Nonnative status indicated with an asterisk (*). If only identified to genus, native/nonnative status could sometimes not be ascertained. For ground layer species present in summer 2013, Pearson correlation *r*-values for NMS axes are included (for ordination graph of axes, Fig. 4); *r*-values > 0.4 are shown in bold.

Species	Years observed			r-values		
	2005	2007	2013	Axis 1	Axis 2	Axis 3
<i>Acer negundo</i> L.			x			
<i>Ageratina altissima</i> (L.) R.M. King and H. Rob		x				
<i>Alliaria petiolata</i> (M. Bieb.) Cavara and Grande*	x					
<i>Ambrosia trifida</i> L.	x	x	x	-0.074	-0.003	-0.011
<i>Asclepias</i> L.	x					
<i>Barbarea</i> R. Br.	x					
<i>Bidens</i> L.	x					
Bryophyta	x		x			
<i>Calystegia</i> (L.) R. Br.	x					
<i>Cardamine hirsuta</i> L.*	x					
<i>Carduus nutans</i> L. subsp. <i>nutans</i> *	x					
<i>Carex blanda</i> Dewey			x	0.089	-0.067	0.060
<i>Carex granularis</i> Muhl. ex Willd.			x	0.154	0.130	0.069
<i>Carex grisea</i> Wahlenb.			x	-0.035	0.027	0.086
<i>Carex</i> spp. L.	x	x	x	0.048	-0.023	-0.002
<i>Carya cordiformis</i> (Wangenh.) K. Koch	x	x	x			
<i>Carya laciniosa</i> (F. Michx.) Loudon		x	x			
<i>Celtis occidentalis</i> L.	x		x	0.101	-0.149	0.069
<i>Cercis canadensis</i> L.			x			
<i>Cirsium arvense</i> (L.) Scop. var. <i>arvense</i> *	x					
<i>Cirsium vulgare</i> (Savi) Ten.*	x					
<i>Conyza canadensis</i> (L.) Cronquist	x					
<i>Cornus drummondii</i> C.A. Mey.	x	x	x	0.094	-0.057	0.014
<i>Cornus foemina</i> Mill.			x			
<i>Crataegus</i> L.		x				
<i>Dichanthelium clandestinum</i> (L.) Gould		x				
<i>Digitaria</i> Haller	x					
<i>Duchesnea indica</i> (Andr.) Focke*	x	x	x	0.212	-0.013	-0.060
<i>Elephantopus carolinianus</i> Raeusch.	x	x				
<i>Elymus canadensis</i> L.	x					
<i>Elymus virginicus</i> L.	x					
<i>Elymus villosus</i> Muhl.			x	-0.006	-0.101	0.132
<i>Erechtites hieraciifolia</i> (L.) Raf.	x					
<i>Erigeron annuus</i> (L.) Pers.	x	x	x			
<i>Euonymus alatus</i> (Thunb.) Siebold*			x			
<i>Euonymus atropurpureus</i> Jacq.	x	x				
<i>Euonymus fortunei</i> (Turcz.) Hand.-Mazz.*	x	x	x			
<i>Fraxinus americana</i> L.	x	x	x	0.003	-0.235	-0.476
<i>Fraxinus quadrangulata</i> Michx.	x					
<i>Geranium carolinianum</i> L.	x					
<i>Geum canadense</i> Jacq.	x	x	x	0.422	0.137	-0.436
<i>Geum vernum</i> (Raf.) Torr. and A. Gray	x	x	x	0.603	0.156	-0.466
<i>Glechoma hederacea</i> L.*	x		x	0.016	-0.234	-0.049
<i>Glyceria</i> R. Br.	x	x				
<i>Gymnocladus dioica</i> (L.) K. Koch	x	x	x			
<i>Hackelia virginiana</i> (L.) I.M. Johnst.	x					
<i>Hypericum perforatum</i> L.*	x					
<i>Ilex</i> L.		x				
<i>Impatiens capensis</i> Meerb.			x	0.104	0.159	0.178
<i>Juglans nigra</i> L.		x	x			
<i>Juncus</i> L.	x	x	x	-0.006	-0.106	-0.092
<i>Juniperus virginiana</i> L.			x	-0.050	-0.162	0.097
<i>Lactuca canadensis</i> L.	x					
<i>Lactuca serriola</i> L.*	x					
<i>Lamium</i> L.*		x				

Table 2. Continued.

Species	Years observed			r-values		
	2005	2007	2013	Axis 1	Axis 2	Axis 3
<i>Leersia virginica</i> Willd.	x					
<i>Ligustrum</i> sp. L.*		x	x	0.203	0.019	0.053
<i>Liriodendron tulipifera</i> L.	x					
<i>Lobelia inflata</i> L.	x					
<i>Lobelia siphilitica</i> L.	x	x				
<i>Lonicera japonica</i> Thunb.*	x		x	0.263	0.539	0.065
<i>Lonicera maackii</i> (Rupr.) Maxim.*	x	x	x	0.050	0.110	-0.010
<i>Morus alba</i> L.*	x	x	x	-0.058	0.115	0.023
<i>Morus rubra</i> L.	x	x	x	-0.083	-0.049	-0.127
<i>Muhlenbergia schreberi</i> J.F. Gmel.	x	x	x	0.131	0.122	-0.160
<i>Nyssa sylvatica</i> Marshall	x					
<i>Oxalis</i> L.	x	x	x	0.028	-0.087	0.078
<i>Packera</i> Á. Löve and D. Löve	x					
<i>Parthenocissus quinquefolia</i> (L.) Planch.	x	x	x	-0.265	-0.282	0.537
<i>Phacelia purshii</i> Buckley	x					
<i>Phytolacca americana</i> L.	x	x				
<i>Plantago major</i> L.*	x					
<i>Polygonum caespitosum</i> Blume var. <i>longisetum</i> (Bruijn) A.N. Steward*	x		x			
<i>Polygonum punctatum</i> Elliot		x				
<i>Polygonum pensylvanicum</i> (L.) Small	x					
<i>Polygonum virginianum</i> L.	x	x	x	0.367	-0.091	-0.248
<i>Prunella vulgaris</i> L.			x	0.003	-0.106	0.007
<i>Prunus serotina</i> Ehrh.	x	x	x	0.069	-0.073	0.241
<i>Quercus</i> L.		x				
<i>Quercus macrocarpa</i> Michx.	x		x			
<i>Quercus palustris</i> Münchh.		x				
<i>Rosa multiflora</i> Thunb.*		x	x	-0.036	0.122	-0.064
<i>Rubus occidentalis</i> L.	x	x	x	0.445	0.430	-0.127
<i>Sambucus canadensis</i> L.	x	x	x	0.177	-0.135	0.092
<i>Sanicula</i> L.	x					
<i>Sanicula canadensis</i> L.	x					
<i>Setaria</i> P. Beauv.*		x				
<i>Sisyrinchium atlanticum</i> E.P. Bicknell	x					
<i>Smilax glauca</i> Walter			x	0.022	0.094	-0.113
<i>Solanum</i> L.	x					
<i>Solidago</i> L.	x	x	x	0.024	0.234	-0.147
<i>Sonchus arvensis</i> L. subsp. <i>arvensis</i> *	x					
<i>Stellaria media</i> (L.) Vill.*	x					
<i>Stylophorum diphyllum</i> (Michx.) Nutt.			x	0.004	0.096	-0.002
<i>Symphoricarpos orbiculatus</i> Moench.	x	x	x	0.142	-0.076	-0.144
<i>Symphytotrichum</i> Nees.	x					
<i>Taraxacum officinale</i> (L.) Weber*	x					
<i>Toxicodendron radicans</i> (L.) Kuntze	x	x	x	-0.362	0.414	-0.100
<i>Trifolium repens</i> L.*	x	x				
<i>Ulmus americana</i> L.		x				
<i>Ulmus rubra</i> Muhl.			x	0.185	0.057	-0.007
<i>Vernonia gigantea</i> (Walter) Trel.	x					
<i>Veronica agrestis</i> L.*	x					
<i>Veronica hederifolia</i> L.*	x					
<i>Vitis vulpina</i> L.	x	x	x	-0.130	0.491	-0.365

the plots in 2013, but did not include *E. fortunei*, the most obvious driver of difference among treatments. The final NMS configuration was reached by analyzing a relativized Sorenson's distance, stepping down in dimensionality, from six-axis to one-axis solution using 25 runs each of real and Monte Carlo

randomized data, with a maximum of 300 iterations and a final instability of 0.0005. The best solution was a three-axis solution. Coefficients of determination for the correlations between ordination distances and distances in the original n -dimensional space were examined to determine the amount of variation described by

each axis. Differences in species composition among treatments were examined with multiresponse permutation procedures (MRPPs) using the relative Sorenson distance measure of the species-composition matrix. The MRPP is a nonparametric procedure in which the A-value describes within-group homogeneity and the P -value ($P \leq 0.01$ to reject null) evaluates how likely an observed difference is due to chance (McCune and Grace 2002).

Results. TREATMENT EFFECTIVENESS. Changes in *E. fortunei* were considered by treatment and over time (Fig. 2A). We found a significant treatment-by-year interaction effect ($\chi^2 = 0.842$, 20 d.f., $P < 0.001$), indicating that *E. fortunei* cover changed over time and varied by treatment. All treatments had significantly lower mean ranks for *E. fortunei* cover compared with CONT ($P < 0.001$, all comparisons). For year, a significantly higher richness for *E. fortunei* cover was detected for 2004 compared with 2013 ($P < 0.001$), indicating that *E. fortunei* had increased across treatments by 2013. By 2013, difference among treatments was not statistically significant ($\chi^2 = 10.250$, 5 d.f., $P = 0.068$), but *E. fortunei* cover in TARP averaged 45.625%, whereas the CONT and burning (BURN) approached 100% cover (Fig. 2A). For 2013, plot location was not associated with *E. fortunei* cover ($\chi^2 = 4.055$, 20 d.f., $P = 0.256$).

GROUND LAYER SPECIES RICHNESS AND SAPLINGS. Total species richness in the ground layer (including herbaceous plants and woody plants <50 cm tall) showed a significant treatment-by-year interaction ($\chi^2 = 0.738$, 20 d.f., $P < 0.001$) (Fig. 2B), as did ground layer total native richness ($\chi^2 = 0.726$, 20 d.f., $P < 0.001$). These results indicated that species richness changed over time and that these changes varied among treatments. For total species richness across years, all treatments, with the exception of TARP ($P = 0.153$), had significantly higher mean ranks compared with the CONT ($P < 0.001$, all other comparisons). Although TARP had high total species richness in later years, TARP was associated with a longer lag in posttreatment response, whereas other treatments supported faster but less sustained increases in total species richness (Fig. 2B). For year, a significantly higher richness was detected for 2006 and 2013 ($P < 0.001$, both comparisons), as compared with 2004. Using

a noncorrected $\alpha = 0.05$, the other two years in which data were collected also had higher richness, compared with 2004 ($P = 0.024$ for 2005, $P = 0.040$ for 2007), indicating a trend of increased richness in later years compared with the first year posttreatment. Differences in richness were dynamic over time with the highest values for total species richness recorded in the intermediate years of the study (2005, 2006), whereas richness decreased and stabilized in later years (2007, 2013).

By 2013, there were significant differences in total species richness among all treatments ($\chi^2 = 11.438$, 5 d.f., $P = 0.043$) (Fig. 2B). Mean total species richness in 2013 ranged from <4 for CONT to >10 for TARP. Using a Bonferroni correction ($\alpha = 0.01$), no treatment had significantly different total species richness compared with the CONT. Using a noncorrected $\alpha = 0.05$, TARP had significantly higher total species richness compared with the CONT ($P = 0.029$), indicating marginally greater 2013 species richness in TARP vs. CONT.

For sapling density recorded in 2013, there were significant differences among treatments ($\chi^2 = 16.167$, 5 d.f., $P = 0.006$). Using a Bonferroni-corrected $\alpha = 0.01$, none of the treatments had significantly different sapling densities compared with the CONT. A noncorrected $\alpha = 0.05$ indicated marginally higher sapling densities than the CONT for the treatments cut and herbicide plots (XHRB) ($P = 0.029$), XBRN ($P = 0.029$), and TARP ($P = 0.029$). For 2013 seedling cover, there were no significant differences among treatments ($\chi^2 = 5.699$, 5 d.f., $P = 0.337$).

Species richness and density were negatively correlated with *E. fortunei* cover (Fig. 3). A scatter plot (Fig. 3A) indicated greater ground layer species richness in treatments with low *E. fortunei* cover in 2013 ($\rho = -0.797$, $n = 24$, $P < 0.001$), and the four CONT plots were clustered at high *E. fortunei* cover and low herbaceous richness. Most mow and herbicide (MHRB) plots and XBRN plots were clustered nearer to the CONT, whereas most TARP and XHRB plots diverged from the CONT, reflecting greater richness and lower *E. fortunei* cover (Fig. 3A). Similarly, increasing *E. fortunei* cover was negatively correlated with total native species richness in the ground layer ($\rho = -0.770$, $n = 24$, $P < 0.001$) (Fig. 3B), sapling richness ($\rho = -0.620$, $n = 24$, $P = 0.001$) (Fig. 3C), and sapling density ($\rho = -0.621$, $n = 24$, $P = 0.001$) (Fig. 3D).

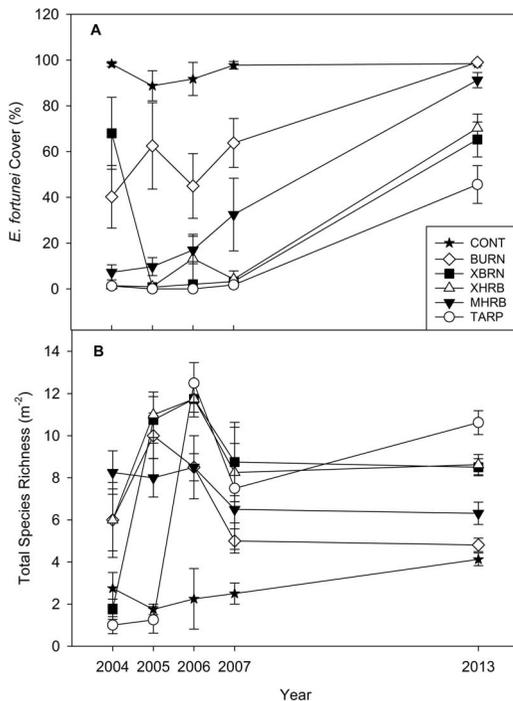


FIG. 2. (A) Mean percentage of cover of *E. fortunei* (\pm SE) by treatment and year. For descriptions of abbreviated treatments, see Table 1. Friedman's test, $\chi^2 = 0.842$, 20 d.f., $P < 0.001$. (B) Mean total species richness per square meter (\pm SE) by treatment and year. For descriptions of abbreviated treatments, see Table 1. Friedman's test, $\chi^2 = 0.738$, 20 d.f., $P < 0.001$.

In 2013, plot location within the study site was not a predictor of ground layer total species richness ($\chi^2 = 1.321$, 20 d.f., $P = 0.748$), ground layer native species richness ($\chi^2 = 1.244$, 20 d.f., $P = 0.766$), sapling richness ($\chi^2 = 1.988$, 20 d.f., $P = 0.603$), or sapling density ($\chi^2 = 0.670$, 20 d.f., $P = 0.880$). These findings indicated that experimental design did not significantly affect any of these vegetative characteristics.

NMS ORDINATION OF GROUND LAYER COMMUNITY. The NMS ordination revealed variation among the treatment plots in overall community composition in the 2013 survey (Fig. 4). The NMS ordination yielded a three-axis solution that explained a large amount of variation in the overall plant community ($R^2_{total} = 0.777$), and the final stress (16.322) and final instability (<0.001) were within acceptable ranges (McCune and Grace 2002). The CONT was distinct from all other treatments, which were spread mostly along axis 1

with TARP on the far right (Fig. 4A, B). The MRPP analysis indicated that plant community composition varied among treatments ($A = 0.106$, $P < 0.001$). The MRPPs revealed that three of the treatments varied significantly from the CONT: TARP ($P < 0.001$), XBRN ($P < 0.001$), and XHRB ($P = 0.002$). The MHRB was almost significantly different from the control ($P = 0.014$), but $P \leq 0.01$ was required to reject the null based on a Bonferroni correction for multiple comparisons. The other treatment, BURN, had plant communities that were not significantly different from the CONT ($P = 0.320$).

We explored the source of these differences by considering the correlation between individual species abundances and the NMS axes (Table 2). *Euonymus fortunei* was an obvious source of variation among communities and was not included in the ordination matrix, allowing for consideration of other species associated with divergence among treatments. Some species showed strong correlations with axes, and this correlation information allowed for further interpretation of the NMS ordination (Fig. 4). Native species more commonly found in TARP plots as compared with CONT plots (positively correlated with axis 1) included *Geum vernum* (Raf.) Torr. & A. Gray (spring avens) ($r_1 = 0.603$, $r_3 = -0.466$) and *Geum canadense* Jacq. (white avens) ($r_1 = 0.422$, $r_3 = -0.436$), and the woody understory species *Rubus occidentalis* L. (black raspberry) ($r_1 = 0.445$). Several vines were more frequent in TARP (positively correlated with axis 2) as compared with other treatments, including *Vitis vulpina* L. (frost grape) ($r_2 = 0.491$), *Toxicodendron radicans* (L.) Kuntze (eastern poison ivy) ($r_2 = 0.414$), and nonnative *L. japonica* ($r_2 = 0.539$). The TARP treatment was also more likely (negatively correlated with axis 3) to contain *F. americana* seedlings ($r_3 = -0.476$) as compared with the CONT and the BURN treatment. Species more frequent in the CONT and the BURN treatment (positively correlated with axis 3) included *Parthenocissus quinquefolia* (L.) Planch. (Virginia creeper) ($r_3 = 0.537$) and *R. occidentalis* ($r_3 = 0.430$). Other species in the matrix contributed relatively less to the spatial configuration.

Discussion. TREATMENT AND REINVASION. Across years, treatments reduced *E. fortunei* cover and increased total and native species richness in the ground layer. However, 10 yr

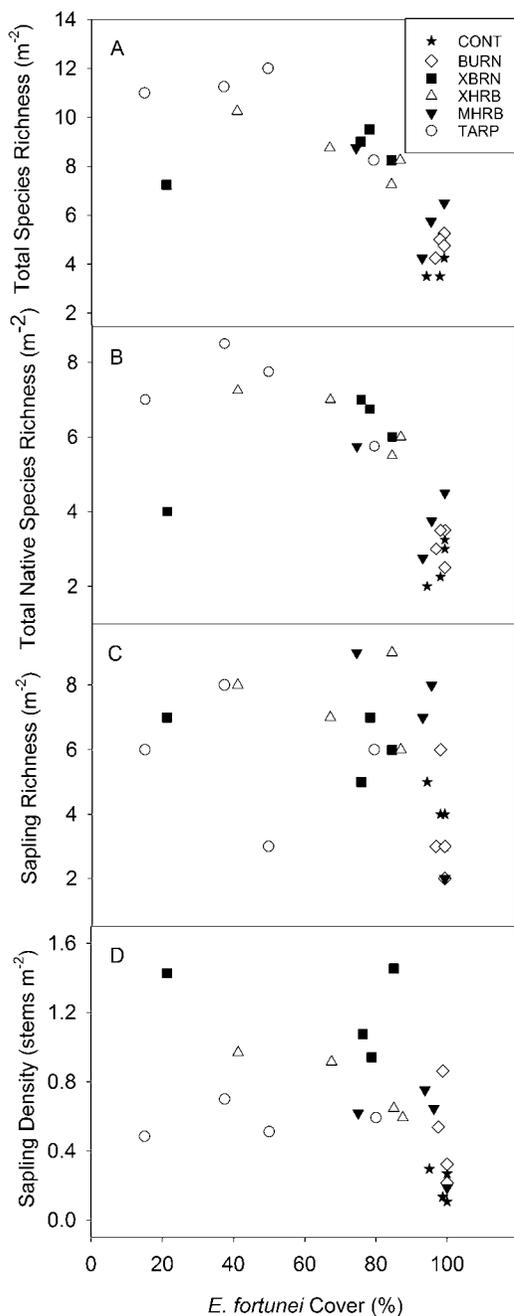


FIG. 3. Scatter plots of vegetative characteristics vs. mean *E. fortunei* percentage of cover in 2013. For descriptions of abbreviated treatments, see Table 1. (A) Total species richness m^{-2} in 2013 vs. mean *E. fortunei* percentage of cover; $\rho = -0.797$, $n = 24$, $P < 0.001$. (B) Total native species richness per square meter in 2013 vs. mean *E. fortunei* percentage of cover; $\rho = -0.770$, $n = 24$, $P < 0.001$. (C) Total sapling richness per square meter in 2013, adjusted from 6-m \times 6-m plot surveys vs. mean *E. fortunei* percentage of cover; $\rho = -0.620$, $n = 24$, $P = 0.001$.

after *E. fortunei* removal, *E. fortunei* cover and ground layer species richness had mostly converged among treatments, and a similar trend appeared for woody species. Still, in 2013, treated plots contained herbaceous and woody species not present in the CONT plots, which typically had nearly 100% *E. fortunei* cover. A few of the most common herbaceous species included *P. quinquefolia*, *V. vulpina*, *R. occidentalis*, *G. canadense*, and *G. vernum*, whereas common woody species included *Symphoricarpos orbiculatus* Moench (coralberry), *F. americana*, *P. serotina*, and *Cornus drummondii* C.A. Mey. (roughleaf dogwood). Different communities emerged in response to different treatments, and NMS ordination and MRPP distinguished TARP as a unique treatment in terms of community composition. Considering plots and sampling units were small, the fact that these results showed treatment communities that differed significantly from the CONT, as well as each other, strengthened our findings. Overall, these results suggest that, although treatment only suppressed *E. fortunei* in the short term, in the long term, treatments facilitated establishment of several woody and herbaceous species not found in untreated plots.

Over time, we observed interesting trends in total species richness in the ground layer. Species richness increased posttreatment, then reached maximums, decreased, and remained relatively stable in the later years of the study. Our long-term study allowed us to perceive this temporal trend, whereas other, shorter studies may not observe these dynamics (Kettinger *et al.* 2011). Species richness may have increased in early years as species took advantage of newly cleared areas created by treatments. Establishment of these species may have been via seed rain or from the existing seed bank. In later years, we observed increasing *E. fortunei* cover along with decreases in species richness. *Euonymus fortunei* may have decreased species richness by preventing the germination, establishment, or both of plants from recently fallen seeds or seeds present in the seed bank. Decreases in species richness may have also reflected depletion of the existing

←

(D) Total density of sapling stems per square meter in 2013, adjusted from 6-m \times 6-m plot surveys vs. mean *E. fortunei* percentage of cover; $\rho = -0.621$, $n = 24$, $P = 0.001$.

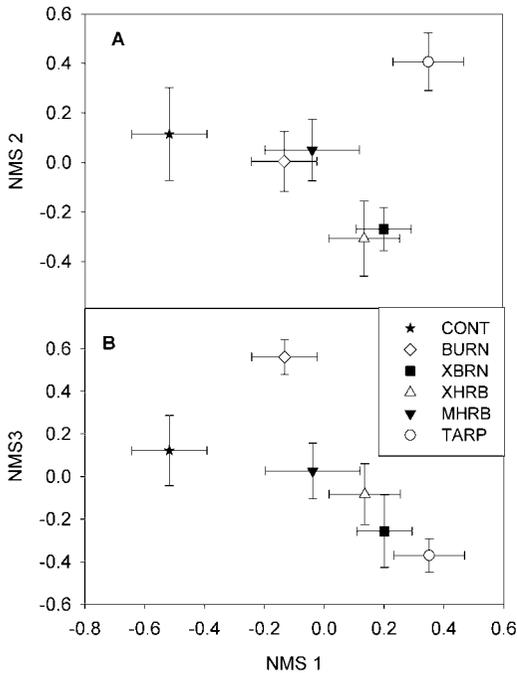


FIG. 4. Nonmetric multidimensional scaling (NMS) ordination depicting average plant community composition (\pm SE) in 2013 for each treatment type. For descriptions of abbreviated treatments, see Table 1. (A) Three-dimensional model best described the solution; $R^2_{total} = 0.777$ ($R^2_{axis1} = 0.255$, $R^2_{axis2} = 0.252$, $R^2_{axis3} = 0.270$), final instability < 0.001 , final stress = 16.322. For MRPPs, $A = 0.106$, $P < 0.001$. (A) Depicts axis 1 vs. axis 2. (B) Depicts axis 1 vs. axis 3.

seed bank over time (a null hypothesis, wherein impacts to the species community were not directly due to our treatments) or a combination of these factors. Our study did not investigate species propagule limitation, as Kettenring *et al.* (2011) recommended for studies of revegetation after removal of invasive species.

Others have described the ability of *E. fortunei* to outcompete other plants (Randall and Marinelli 1996, Swearingen *et al.* 2002). Increases in *E. fortunei* are likely attributable to both vegetative spread among plots and seed dispersal from untreated locations in the surrounding woods (Randall and Marinelli 1996, Swearingen *et al.* 2002, Remaley 2009), where the plant was still very well established (T.J. Rounsaville, University of Kentucky Arboretum, pers. comm.). We consider vegetative spread to be the primary means of the infiltration of *E. fortunei* into treated plots because, anecdotally, a relative scarcity of fruiting *E.*

fortunei plants has been observed in the area. Vegetative reproduction is often associated with rapid invasions (Booth *et al.* 2010). Regardless of the mechanism of spread, the ongoing increase in *E. fortunei* cover indicated the need for long-term management of treated sites.

Other studies have illustrated the role of plant invasions in decreasing species richness and the phenomenon of reinvasion (Hejda *et al.* 2009). When plots are cleared of invasive plants, the act of management itself creates disturbance (Buckley *et al.* 2007). Likewise, in this study, treatments removed existing vegetation and opened establishment sites to be occupied by other plants. Because many invasive plants, and perhaps *E. fortunei*, tolerate and even thrive under disturbed conditions, active management could exacerbate invasions or make way for new invaders (Reid *et al.* 2009). We inventoried several nonnative species in treatment plots, some of which are considered invasive, such as *L. maackii* and *A. petiolata*. Reported values for ground layer total species richness, sapling richness, and sapling density include nonnative species. In our study and in general, increased species richness is not always a positive outcome if new invasions are facilitated. Although invasive species removal may not fully achieve goals, our treatments did restore a site from a near monoculture of *E. fortunei* to a more-diverse plant community.

EXPERIMENTAL DESIGN. The experimental design of this study may have influenced our ability to interpret results. No buffers were established between individual plots or between the study site and surrounding vegetation. Still, we did not find significant differences in any vegetative characteristic based on plot location, including *E. fortunei* cover. Other limitations include our low sample sizes and omission of control plots from the original 2003 randomized block design. Despite limitations, our study revealed different impacts of treatments, including long-term changes in plant species richness and composition due to *E. fortunei* removal and recolonization.

Our findings may not be independent of the study area's history of invasive species management. Before and during this study, the study area experienced treatment of *L. maackii* via cutting and direct application of glyphosate. Under this management regime, the study area forest was a dynamic system undergoing structural adjustment and succession in

response to removal of both *E. fortunei* and *L. maackii*. Changes in forest structure reflected in this study may be due to the combined effects of removing both species. We chose to focus on *E. fortunei* because of the apparent vigor with which it recolonized the study area and because it is an understudied invasive species.

Indeed, this study revealed a forest whose species composition and structure changed following treatment to reduce *E. fortunei*. Increased richness and an emerging sapling layer demonstrate that, whereas tree regeneration had been virtually halted under an *E. fortunei*-dominated system, treatment was associated with increased woody species regeneration. Tree regeneration was probably accomplished through a combination of seed rain and establishment of seeds from the seed bank.

MANAGEMENT IMPLICATIONS. These results reinforced the importance of the management of invasive species, including *E. fortunei*, in restoring forest biodiversity and structure. The TARP treatment stood out among treatments, a notable finding given that TARP has not been widely reported as an *E. fortunei* control method. By 2013, TARP contained the highest total species richness and lowest *E. fortunei* cover, although these differences were not statistically significant. Plant communities present in TARP were also spatially separated by NMS ordination. The TARP treatment appeared to operate differently in *E. fortunei* suppression compared with other treatments. Other treatments were associated with increased total species richness in the year immediately following treatment, whereas the plant community in TARP took longer to develop but was more persistent over time, likely because of TARP's ability to kill belowground biomass of all species present. Given enough treatment time (in this case 6 mo), TARP may have killed *E. fortunei* rootstock by combined light-exclusion and systemic herbicide addition. After this lag in species richness, plants were able to recolonize, and species richness increased in TARP. Other treatments not experiencing this temporal lag may not eliminate rootstock as fully as the TARP treatment did. The TARP treatment was unique but has drawbacks: it is inefficient to apply TARP to large areas, all vegetation is killed indiscriminately (including newly established native herbaceous and woody species), and plastic coverings are unsightly

and collect standing water. The Forest Park Forever Nature Reserve in St. Louis, MO, has attempted to overcome both aesthetic and water retention issues by using quilts made of canvas and nylon as alternatives to plastic tarps (Schenkenberg 2014).

Within our study site, an urban forest fragment with a history of disturbance, vegetative spread of *E. fortunei* into treatment plots from surrounding areas was a significant long-term factor that resulted in a loss of species richness and sapling density. Land managers facing field conditions similar to those present at our study site may find our results relevant. Of course, management choices should be site specific. For follow-up treatments in places where propagule pressure is high, we suggest methods that control spread of *E. fortunei* from untreated areas. Treatment at these zones must avoid disturbing newly established species. If the affected area is small, removal might be best accomplished manually or through use of plastic tarps despite their drawbacks. If the area to be treated is larger, immediate application of glyphosate to stems cut close to the ground (Remaley 2009), as in our XHRB treatment condition, may be more easily implemented. Winter application of herbicide to evergreen *E. fortunei* foliage may be one way to avoid negatively affecting newly established native species that are dormant during winter. Overall, our results suggest that active retreatment of sites is critical for native species colonization and overall restoration success.

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