

Article

Short-Term Response of Native Flora to the Removal of Non-Native Shrubs in Mixed-Hardwood Forests of Indiana, USA

Joshua M. Shields ^{1,2}, Michael R. Saunders ^{1,2}, Kevin D. Gibson ³, Patrick A. Zollner ¹, John B. Dunning, Jr. ¹ and Michael A. Jenkins ^{1,2,*}

¹ Department of Forestry and Natural Resources, Purdue University, 715 West State Street, West Lafayette, IN 47907, USA; E-Mails: joshua_shlds@yahoo.com (J.M.S.); msaunder@purdue.edu (M.R.S.); pzollner@purdue.edu (P.A.Z.); jdunning@purdue.edu (J.B.D.J.)

² Hardwood Tree Improvement and Regeneration Center (HTIRC), Purdue University, 715 West State Street, West Lafayette, IN 47907, USA

³ Department of Botany and Plant Pathology, Purdue University, 915 West State Street, West Lafayette, IN 47907, USA; E-Mail: kgibson@purdue.edu

* Author to whom correspondence should be addressed; E-Mail: jenkinsma@purdue.edu; Tel.: +(765)-494-3602.

Academic Editors: John Innes and Eric J. Jokela

Received: 6 March 2015 / Accepted: 20 May 2015 / Published: 29 May 2015

Abstract: While negative impacts of invasive species on native communities are well documented, less is known about how these communities respond to the removal of established populations of invasive species. With regard to invasive shrubs, studies examining native community response to removal at scales greater than experimental plots are lacking. We examined short-term effects of removing *Lonicera maackii* (Amur honeysuckle) and other non-native shrubs on native plant taxa in six mixed-hardwood forests. Each study site contained two 0.64 ha sample areas—an area where all non-native shrubs were removed and a reference area where no treatment was implemented. We sampled vegetation in the spring and summer before and after non-native shrubs were removed. Cover and diversity of native species, and densities of native woody seedlings, increased after shrub removal. However, we also observed significant increases in *L. maackii* seedling densities and *Alliaria petiolata* (garlic mustard) cover in removal areas. Changes in reference areas were less pronounced and mostly non-significant. Our results suggest that removing non-native shrubs allows short-term recovery of native communities across a range of invasion intensities. However,

successful restoration will likely depend on renewed competition with invasive species that re-colonize treatment areas, the influence of herbivores, and subsequent control efforts.

Keywords: Amur honeysuckle; spring ephemerals; Indiana; invasive plants; *Lonicera maackii*; Wilcoxon tests

1. Introduction

As land managers with limited resources strive to counteract the negative effects of invasive species, context-dependent information about the invader and the invaded ecosystem becomes increasingly critical. Specifically, an understanding of effective control strategies, spatial and temporal processes, and biotic and abiotic factors contributing to successful invasion are all important when prioritizing management actions. In the case of invasive plants, restoration efforts often focus upon the removal of invasives with the goal of allowing native species to re-establish. Evaluating the effectiveness of these efforts depends upon scientifically-valid before-and-after studies as opposed to relying on anecdotal evidence of suspected impacts [1]. While some investigators have examined the effects of invasive plant management, a more comprehensive body of work is needed given the gap between scientific research and management programs [2].

Within forest ecosystems, woody invasive shrubs are particularly problematic because their longevity and persistence in the understory exerts considerable influence over herbaceous-layer processes, including forest regeneration [3]. These species are often able to establish in forest understories in the absence of overstory disturbance [4]. For example, *Lonicera maackii* (Amur honeysuckle), an invasive shrub introduced from Asia [5], has become a serious management concern in forests across much of eastern North America. Numerous studies have shown that this shrub negatively affects native plant communities by reducing the survival, reproduction, and growth of native species [6–10], inducing apparent competition [11], and driving decreased abundance and diversity of native flora [12]. The control of this and other invasive shrubs has become a high priority for restoration, highlighting the need to evaluate the response of native plants to the removal of this widespread species.

Although the effects of *L. maackii* removal on native vegetation have been examined [13–16], most of these studies have employed small experimental removals; only two studies [15,17] treated areas larger than 40 m². However, because invasions are typically treated at the forest or woodlot scale, examinations of larger-scale removal provide a more accurate evaluation of real-world environmental and dispersal conditions. For example, research has shown that immature *L. maackii* seedlings are typically clustered around mature shrubs [18], suggesting that residual shrubs that remain next to small treatment plots may hasten recolonization by serving as local sources of propagules or as perch sites for birds. Additionally, invasive shrub removal affects the abundance of mice (*Peromyscus* spp.; [19,20]), whose foraging for seeds has been shown to negatively affect the recruitment of tree seedling [21]. The size of a removal area relative to the home range of an individual mouse could affect protective cover and foraging behavior. In addition, plant community diversity can vary spatially due to a multitude of biotic and abiotic factors [22]; therefore, pre- and post-treatment data are critical to understanding treatment effects. However, with the exception of [23], studies have lacked pre-removal data.

The primary objective of this study was to determine the response of native and non-native herbaceous and woody plants to the removal of *L. maackii* and other non-native shrubs across a gradient of invasion intensities and overstory compositions. Specifically, we examined before-and-after changes in the species diversity of native spring and summer herbaceous flora and woody seedlings in 0.64 ha areas where *L. maackii* and other non-native shrubs were removed and reference areas where non-native shrubs were left intact. In removal areas, we used a combination of mechanical and chemical treatments and removed all slash so that aboveground biomass of woody invaders was absent. We hypothesized that species diversity of native herbaceous plants and tree seedlings would increase following removal of non-native shrubs. We predicted a particularly dramatic increase for the vernal flora (herbaceous plants that flower primarily in the spring) given that *L. maackii* expands its leaves earlier than overstory trees, thus, directly competing with vernal herbs [6,24,25]. We also hypothesized that the cover of *Alliaria petiolata* (garlic mustard) would increase following removal treatments given the presence of this species at our study areas prior to removing non-native shrubs.

2. Materials and Methods

2.1. Study Area

We collected data in six mature, second-growth mixed-hardwood forests in central Indiana (39°20' N to 40°26' N, and 86°57' W to 87°26' W; Figure 1—(1) Hawthorn Park; (2) Fowler Park; (3) Rifle Range woodlot (hereafter RR); (4) Department of Forestry and Natural Resources Farm (FNR Farm); (5) Ross Biological Reserve (Ross); and (6) Pursell Woodlot (Pursell). Study sites were on glacial landforms with out-wash and alluvial parent materials resulting in soil textures and drainage classes ranging from very poorly drained loams to excessively drained sandy loams (Table 1). Deer hunting was not allowed at four of our study sites; Fowler Park, Hawthorne Park, RR, and Ross. Sites were generally level with slopes of 5% or less.

Canopies across all study sites consisted of deciduous trees with $\geq 85\%$ canopy closure. *Lonicera maackii* comprised $>88\%$ of non-native shrubs/ha across all sites and the last major canopy disturbance pre-dated *L. maackii* invasion. Our six study sites were chosen to represent a range of overstory species composition and a gradient of *L. maackii* density (Table 1). Honeysuckle density (stems ≥ 1.37 m height) ranged from 1042 stems·ha⁻¹ at Ross to 3135 stems·ha⁻¹ at FNR Farm (Table 1).

2.2. Experimental Design

We sampled vegetation in two, 80-m × 80-m areas (extending from the forest edge into interior) at each site. We placed one boundary of a given sample area along the forest edge, with the other three boundaries located ≥ 10 m from any edge. In one of the areas at each study site (chosen randomly), we removed *L. maackii* and all other non-native shrubs (hereafter removal area). While *L. maackii* was the dominant invasive species at all sites, we also removed all other non-native shrubs, which represents a more realistic management practice. In the other sample area, non-native shrubs were left intact (hereafter reference area). From November 2010 through March 2011, within removal areas we hand-pulled or cut all non-native shrubs at ground level using a gas-powered clearing saw and treated stumps with herbicide

(20% Garlon 4[®]-triclopyr, 1% Stalker[®]-imazapyr, and 79% Ax-it[®]-basal oil). Large slash (diameter ≥ 5 cm) was removed from each site. Slash <5 cm diameter was scattered on the forest floor, but noticeable piles were not created. The purpose of removing the large slash was to create a condition reflective of future condition in the most effective restoration programs, where the largest individuals are cut during the first entry, re-sprouts and new seedlings are killed in successive years, and the slash from the first entry decays, eventually resulting in little or no invasive shrub biomass.

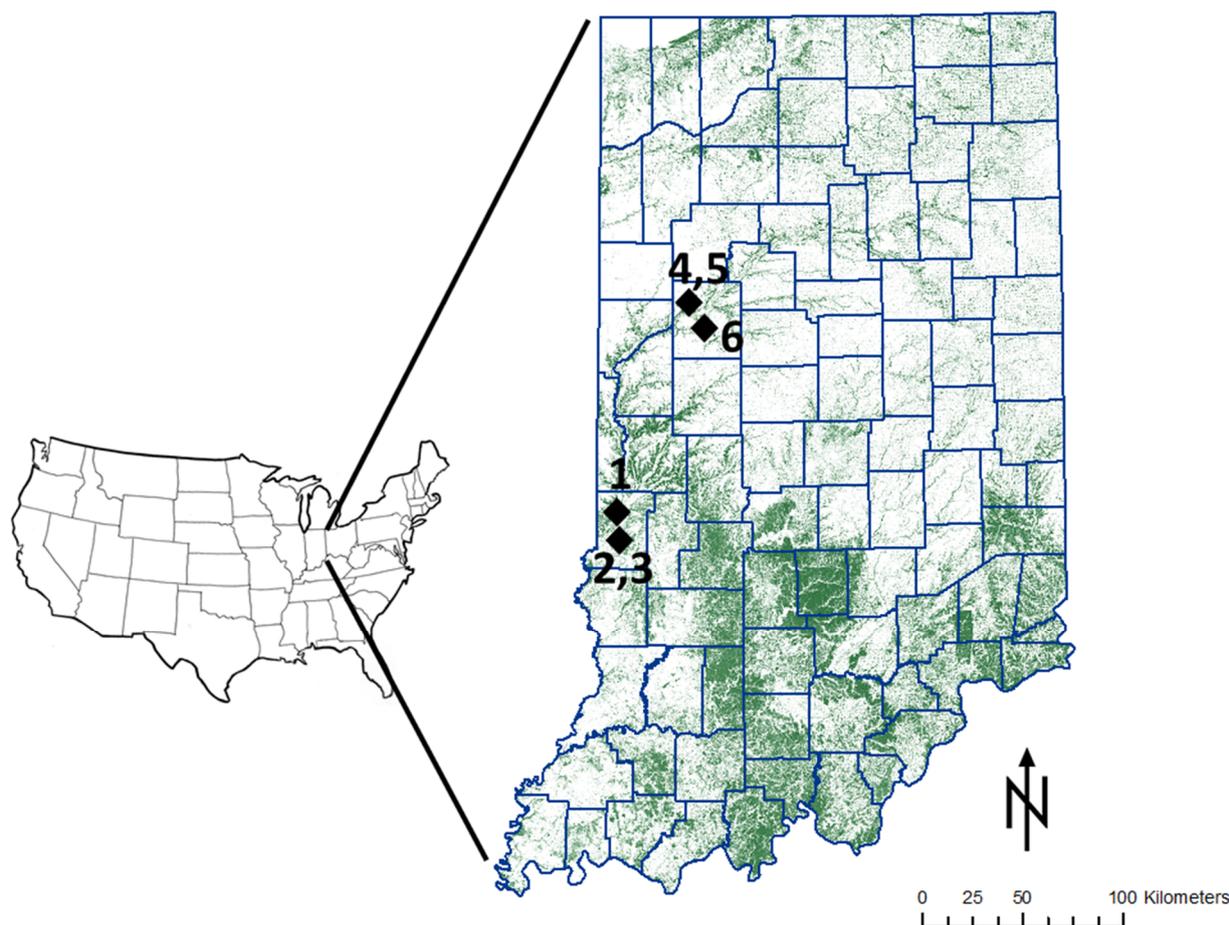


Figure 1. Location of study sites within Indiana, USA. 1 = Hawthorn Park, 2 = Fowler Park, 3 = Rifle Range, 4 = FNR Farm, 5 = Ross Biological Reserve, 6 = Pursell Woodlot. Study sites represented by a single symbol were too close together to separate at a state-wide scale. Shading on map represents forest canopy cover as derived from the National Land Cover Database [26].

2.3. Data Collection

At each site, vegetation data were collected within fixed-area plots located along transects. Each removal or reference area contained three transects (spaced 20 m apart) extending from the forest edge into the interior. Along each transect, we placed four, 40-m² circular plots and four 2-m \times 2-m square quadrats. Circular plots were spaced 20 m apart along each transect with the first plot 5–10 m from the forest edge. Each quadrat was randomly placed to the upper right, upper left, lower right, or lower left

within the circular plot and oriented parallel to the transect. This design resulted in 12 circular plots and 12 quadrats per sample area.

Table 1. Dominant cohort species (woody stems ≥ 10 cm dbh), soil types, and mean (± 1 SE) densities of *L. maackii* (stems ≥ 1.37 m height; defined as a group of basal stems originating from the same rootstock) in six mixed hardwood forests in Indiana. Overstory and *L. maackii* data were collected as described in [17]. Overstory species listed in descending order of importance value (IV = [relative basal area + relative density]/2). Soil information was obtained from the USDA Web Soil Survey [27].

Study Site	Dominant Overstory Cohort	Soil Type(s)	<i>L. maackii</i> Saplings/ha
Fowler Park	<i>Fraxinus americana</i> , <i>Juglans nigra</i> , <i>Prunus serotina</i> , <i>Ulmus americana</i> , <i>Sassafras albidum</i>	Silt loam	2500 \pm 459
FNR Farm	<i>Robinia pseudoacacia</i> , <i>U. americana</i>	Silt loam	3135 \pm 176
Hawthorn Park	<i>S. albidum</i> , <i>Nyssa sylvatica</i> , <i>Liriodendron tulipifera</i> , <i>P. serotina</i> , <i>Quercus bicolor</i>	Silt loam	1708 \pm 256
Pursell	<i>Maclura pomifera</i> , <i>P. serotina</i>	Loam, loamy sand, sandy loams, silt loams	1354 \pm 255
Rifle Range	<i>L. tulipifera</i> , <i>P. serotina</i> , <i>S. albidum</i>	Fine sandy loams	2375 \pm 158

In the circular plots, we recorded the number of woody individuals by species in the sapling layer (stems < 10 cm diameter at breast height (dbh) and ≥ 1.37 m tall). An individual was defined as a single stem or a clump of stems originating from the same point at ground level. In each quadrat, data were recorded in lower (≤ 1 m) and upper (1.01–5 m) strata. In the lower stratum, we recorded percent cover of native and non-native herbaceous and woody species, coarse woody debris (CWD; midpoint diameter ≥ 10 cm), fine woody debris (FWD; midpoint diameter < 10 cm), dead leaves/dead herbaceous stems, and bare soil. We also recorded number of seedlings and shrubs (< 1.37 m tall). In the upper stratum, we recorded percent cover of native and non-native trees, shrubs, and vines. All percent cover estimates were based upon categories modified from [28]: 0%–1%, 1.1%–2%, 2.1%–5%, 5.1%–10%, 10.1%–25%, 25.1%–50%, 50.1%–75%, 75.1%–95%, and $> 95\%$. Cover classes were estimated by a single observer to reduce bias.

Quadrats in removal and reference areas were sampled in the spring (April) and summer (July–August) before (2010) and after removal (2011). Seedlings and shrub densities were tallied during the summer of each year. Circular plots were sampled in September of both years. Taxa not identifiable to species were categorized into multi-species groups or identified to genus. Nomenclature follows the United States Department of Agriculture Plants Database [29].

2.4. Data Analyses

For herbaceous-layer vegetation and substrate variables, we calculated percent cover by taxon using cover-class midpoints. Mean percent cover was also calculated by stratum, sample area (removal, reference), study site, season (spring, summer), and year (2010, 2011). For woody saplings, and for seedlings and shrubs, we calculated mean stems/ha by taxon within a plot, as well by sample area, study site, and year.

Quadrat-level taxonomic richness (S, number of taxa), Pielou's Evenness Index (J'), and Shannon's Diversity Index (H') were calculated based on the percent covers of native herbaceous and woody plants in the lower stratum. We also calculated mean H', S, and J' by sample area, study site, season, and year. For summer data, any native taxa recorded in the quadrats were considered. However, spring data calculations only included herbaceous species that flower primarily between March and June [30].

We used Wilcoxon signed rank tests [31] to examine differences between 2010 and 2011 in removal and reference areas for the following response variables: density of *L. maackii* shrubs in the sapling layer, density of *L. maackii* shrubs <1.37 m tall, percent cover of *Alliaria petiolata* in spring and summer, spring and summer native S, J', and H', density of native seedlings (species were combined as a group), percent cover of vegetation groups based on nativity and growth habit, and percent cover of substrate variables (CWD, FWD, dead leaves/herbaceous stems, bare soil). The USDA Plants Database [29] was used to create the following groups based upon nativity and growth habit: native forbs, native grasses, native sedges, native ferns, native vines, native trees and shrubs, non-native forbs, non-native grasses, non-native vines, and non-native shrubs. Important assumptions of the Wilcoxon signed rank test are (1) data are paired and pairs come from the same population; (2) each pair is chosen randomly and is independent of another pair; and (3) data need not be normally distributed, but must be ordinal. For data that do not adhere to parametric assumptions of normality (we used histograms to determine that data and differences were not normally distributed), such as those in this and many other ecological studies, it is more appropriate to use non-parametric procedures instead of parametric procedures, such as paired *t*-tests or ANOVA [32]. The program R was used to perform Wilcoxon signed rank tests [33]. We used the R package vegan [34] to calculate H', S, and J'.

Because of the large number tests performed, we used a graphically-sharpened procedure based upon control of the false discovery rate [FDR]; [35,36] to adjust *p*-values [37]. Because they retain statistical power while keeping the proportion of false discoveries small relative to all significant results [37,38], multiple comparison techniques based upon FDR have become more widely used in ecological studies as an alternative to traditional controls of family-wise error rate. *P*-values of reference and removal areas were pooled separately for analysis and adjustment.

3. Results

3.1. Response of *Lonicera maackii*

In 2010 (pre-removal), mean *L. maackii* density was 2372 ± 480 shrubs·ha⁻¹ in the sapling stratum in removal areas; however, we found no *L. maackii* shrubs ≥ 1.37 m tall in removal areas in 2011 (Figure 2). Conversely, sapling-layer *L. maackii* densities in reference areas changed little from 2010–2011 (Figure 2; *p* = 0.71). However, removal areas contained 7743 ± 1593 seedlings/ha in 2010, but increased to $28,299 \pm 7708$ seedlings·ha⁻¹ by 2011 (*p* value = 0.04), largely due to changes at Pursell and Ross

(Figure 2). In reference areas, mean *L. maackii* seedling density increased from 5382 ± 1389 shrubs/ha to only 6424 ± 1480 shrubs/ha ($p = 0.17$).

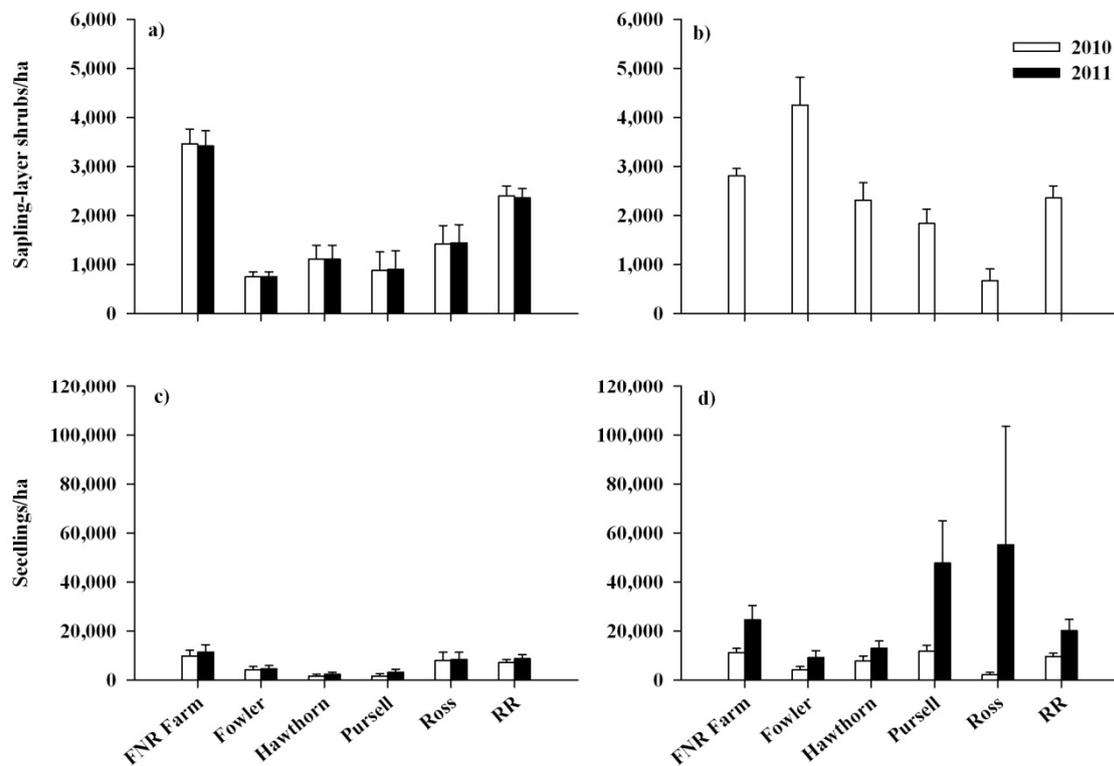


Figure 2. Mean *Lonicera maackii* sapling-layer densities (shrubs/ha for shrubs ≥ 1.37 m tall) in reference areas (a) and removal areas (b) and seedlings/ha (shrubs < 1.37 m tall) in reference areas (c) and removal areas (d), in 2010 and 2011, at six mixed-hardwood forests in central Indiana. Error bars are ± 1 SE.

3.2. Response of Native Flora and *Alliaria petiolata*

Between 2010 and 2011, changes in cover of native vegetation groups in the lower-vertical stratum were generally greater and more positive in removal than reference areas in spring (Table 2) and summer (Table 3). In spring, cover of forbs in the lower stratum in reference areas did not change significantly (20.6 ± 6.5 in 2010 vs. 22.6 ± 6.9 in 2011; $p = 0.22$), but increased from 17.2 ± 3.8 to 31.0 ± 6.3 in removal areas ($p = 0.04$; Table 2). Summer forb cover was largely unchanged (20.9 ± 6.6 in 2010, 20.1 ± 6.3 in 2011; $p = 0.78$) in reference areas, but increased from 22.7 ± 5.2 to 37.8 ± 6.8 in removal areas ($p = 0.04$; Table 3). Changes in the upper stratum for native trees/shrubs and vines were less pronounced than those in the lower stratum (Table 2).

Table 2. Mean percent cover (± 1 SE) and Wilcoxon signed rank test results for native and non-native plants (grouped according to growth habit) and substrate variables in reference and removal areas, during the spring seasons of 2010 and 2011 at six mixed-hardwood forests in central Indiana. Although mean values are presented, Wilcoxon p values were calculated to examine significance of differences between 2010 and 2011, based on ranks. For each group/substrate variable, mean values were calculated from the six study sites ($n = 6$ for each species in a given treatment type, year, and season). Plant data were collected in the lower stratum (≤ 1 m) and upper stratum (1.01–5 m) whereas substrate data were collected only in the lower stratum. p -values were adjusted for multiple comparisons with a graphically-sharpened procedure to control the false discovery rate [35]. Adjustments were made separately for reference and removal areas. T = trace ($<0.1\%$ cover).

Vegetation Group	Reference			Removal		
	2010	2011	p	2010	2011	p
Lower stratum (≤ 1 m)						
Native forbs	20.6 \pm 6.5	22.6 \pm 6.9	0.19	17.2 \pm 3.8	31.0 \pm 6.3	0.04
Native grasses	0.6 \pm 0.4	0.7 \pm 0.5	0.78	0.5 \pm 0.2	0.6 \pm 0.3	0.23
Native sedges	0.2 \pm 0.1	0.2 \pm 0.1	---	0.5 \pm 0.3	0.9 \pm 0.3	0.08
Native ferns	0.2 \pm 0.1	0.4 \pm 0.2	0.22	0.2 \pm 0.1	0.4 \pm 0.1	0.08
Native vines	0.7 \pm 0.3	1.06 \pm 0.31	0.32	0.8 \pm 0.5	1.4 \pm 0.7	0.08
Native trees and shrubs	2.8 \pm 1.1	4.6 \pm 1.6	0.17	3.8 \pm 1.7	5.3 \pm 1.5	0.06
Non-native forbs	0.5 \pm 0.4	0.7 \pm 0.4	0.19	1.0 \pm 0.5	2.3 \pm 0.9	0.06
Non-native grasses	T	T	0.78	---	---	---
Non-native shrubs	12.0 \pm 2.6	12.3 \pm 2.9	0.71	18.7 \pm 4.0	3.3 \pm 0.6	0.04
Non-native vines	0.3 \pm 0.3	0.3 \pm 0.3	---	0.1 \pm 0.1	0.2 \pm 0.1	0.23
Upper stratum (1.01–5 m)						
Native trees and shrubs	9.8 \pm 3.2	10.9 \pm 3.4	0.19	9.3 \pm 2.7	9.7 \pm 2.8	0.20
Native vines	0.2 \pm 0.1	0.1 \pm 0.1	0.78	0.1 \pm 0.1	0.1 \pm 0.1	0.30
Non-native shrubs	35.5 \pm 9.3	35.6 \pm 10.5	0.71	40.6 \pm 7.0	0.1 \pm 0.1	0.04
Non-native vines	---	---	---	0.1 \pm 0.1	---	0.49
Substrate variables						
Bare soil	15.6 \pm 6.9	14.7 \pm 5.8	0.78	13.6 \pm 4.0	10.1 \pm 2.9	0.06
Coarse woody debris	5.2 \pm 2.1	5.1 \pm 2.1	0.78	3.0 \pm 0.7	3.2 \pm 0.8	0.24
Fine woody debris	7.9 \pm 1.1	8.1 \pm 1.0	0.56	8.6 \pm 1.4	10.6 \pm 1.3	0.04
Dead leaves/dead herbaceous stems	35.1 \pm 8.3	36.7 \pm 7.9	0.56	27.8 \pm 8.8	26.7 \pm 8.2	0.24

Table 3. Mean percent cover (± 1 SE) and Wilcoxon signed rank test results for native and non-native plants (grouped according to growth habit) and substrate variables in reference and removal areas, during the summer seasons of 2010 and 2011 at six mixed-hardwood forests in central Indiana. Although mean values are presented, Wilcoxon p values were calculated to examine significance of differences between 2010 and 2011, based on ranks. For each group/substrate variable, mean values were calculated from the six study sites ($n = 6$ for each species in a given treatment type, year, and season). Plant data were collected in the lower stratum (≤ 1 m) and upper stratum (1.01–5 m) whereas substrate data were collected only in the lower stratum. p -values were adjusted for multiple comparisons with a graphically-sharpened procedure to control the false discovery rate [35]. Adjustments were made separately for reference and removal areas. T = trace ($<0.1\%$ cover).

Vegetation Group	Reference			Removal		
	2010	2011	p	2010	2011	p
Lower stratum (≤ 1 m)						
Native forbs	20.9 \pm 6.6	20.1 \pm 6.3	0.78	22.7 \pm 5.2	37.8 \pm 6.8	0.04
Native grasses	0.9 \pm 0.6	0.8 \pm 0.5	0.56	0.7 \pm 0.3	0.8 \pm 0.4	0.24
Native sedges	0.2 \pm 0.1	0.2 \pm 0.1	0.78	0.9 \pm 0.3	1.0 \pm 0.4	0.13
Native ferns	0.4 \pm 0.2	0.4 \pm 0.2	0.78	0.3 \pm 0.1	0.4 \pm 0.1	0.08
Native vines	1.4 \pm 0.5	1.4 \pm 0.4	0.78	2.1 \pm 1.1	2.7 \pm 0.8	0.15
Native trees and shrubs	5.7 \pm 2.0	5.0 \pm 1.6	0.22	6.4 \pm 2.5	8.7 \pm 2.1	0.06
Non-native forbs	0.6 \pm 0.4	1.0 \pm 0.5	0.19	1.1 \pm 0.6	3.3 \pm 1.5	0.06
Non-native grasses	T	T	---	---	---	---
Non-native shrubs	12.0 \pm 2.6	12.7 \pm 2.9	0.22	19.9 \pm 3.7	4.7 \pm 0.7	0.04
Non-native vines	0.3 \pm 0.3	0.4 \pm 0.4	0.78	0.2 \pm 0.1	0.3 \pm 0.2	0.49
Upper stratum (1.01–5 m)						
Native trees and shrubs	13.2 \pm 4.4	12.6 \pm 4.1	0.32	12.4 \pm 3.4	11.6 \pm 3.0	0.24
Native vines	0.2 \pm 0.1	0.2 \pm 0.1	---	0.3 \pm 0.1	0.2 \pm 0.1	0.15
Non-native shrubs	37.2 \pm 10.3	37.3 \pm 10.3	0.56	42.4 \pm 6.9	0.33 \pm 0.31	0.04
Non-native vines	---	---	---	0.1 \pm 0.1	---	0.49
Substrate variables						
Bare soil	17.8 \pm 7.4	15.0 \pm 6.0	0.19	15.1 \pm 4.0	8.4 \pm 2.9	0.04
Coarse woody debris	5.2 \pm 2.1	5.3 \pm 2.1	0.78	3.3 \pm 0.9	3.4 \pm 1.0	0.23
Fine woody debris	8.3 \pm 1.1	8.3 \pm 1.2	---	8.9 \pm 1.6	12.5 \pm 3.0	0.04
Dead leaves/dead herbaceous stems	30.0 \pm 8.8	31.6 \pm 7.1	0.56	22.2 \pm 8.4	19.4 \pm 7.7	0.15

Native H' , S , and J' generally increased from 2010 to 2011 in both spring and summer, but changes were greater in removal areas (Figures 3 and 4). During spring, H' (0.61 ± 0.41 in 2010 vs. 0.55 ± 0.15 in 2011; $p = 0.17$), S (2.46 ± 0.48 vs. 2.68 ± 0.52 ; $p = 0.17$), and J' (0.54 ± 0.10 vs. 0.58 ± 0.11 ; $p = 0.19$) changed little in reference areas. In removal areas during spring, H' increased from 0.45 ± 0.13 in 2010 to 0.88 ± 0.10 in 2011 ($p = 0.04$). Species richness increased from 1.86 ± 0.37 to 3.43 ± 0.44 ($p = 0.04$), and J' increased from 0.43 ± 0.11 to 0.71 ± 0.03 ($p = 0.04$). Trends in the summer were similar to those of spring; H' , S , and J' changed little in reference areas (Figure 4), but generally increased in removal areas; H' increased from 1.68 ± 0.19 in 2010 to 2.08 ± 0.13 in 2011 ($p = 0.04$), S increased from 8.43 ± 1.47 to 13.71 ± 1.04 ($p = 0.04$), but J' did not change significantly ($p = 0.20$).

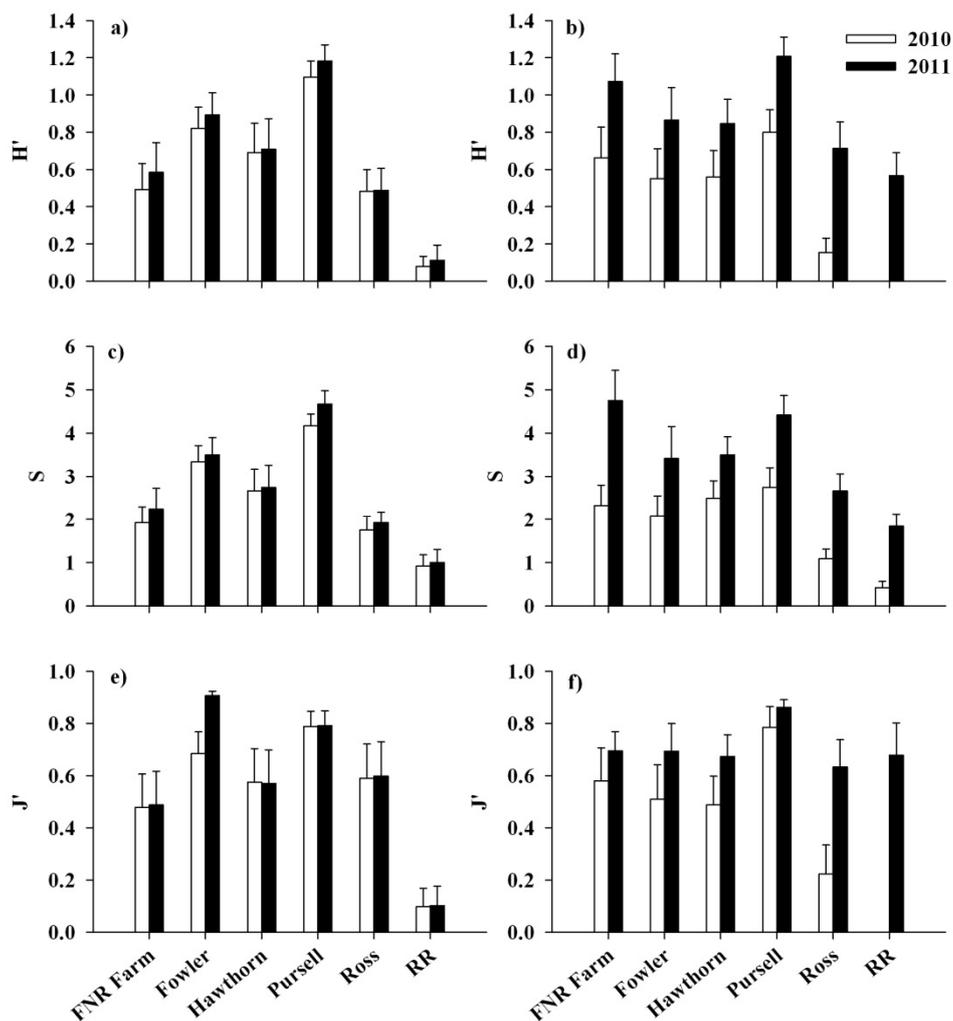


Figure 3. Mean Shannon’s Diversity Index (H') in reference areas (a) and removal areas (b); mean taxonomic richness (S) in reference areas (c) and removal areas (d), and mean Pielou’s Evenness Index (J') in reference areas (e) and removal areas (f) during the spring seasons of 2010 and 2011 at six mixed-hardwood forests in central Indiana. Error bars are ± 1 SE. Indices were based on percent cover data from native herbaceous species that flower primarily during the spring.

At all study sites, native seedlings as a group increased in response to the removal of non-native shrubs, whereas in reference areas, seedling densities changed little (Figure 5). In removal areas, seedling density increased from $12,847 \pm 4361$ stems·ha⁻¹ in 2010 to $33,021 \pm 8504$ stems·ha⁻¹ in 2011 ($p = 0.04$), whereas in reference areas, seedling density was $15,069 \pm 5719$ stems·ha⁻¹ in 2010 vs. $14,653 \pm 5625$ stems·ha⁻¹ in 2011 ($p = 0.32$). In reference areas, the largest changes in individual taxa were for *Fraxinus americana/pennsylvanica* (white/green ash; change in mean seedlings/ha = -139) and *Liriodendron tulipifera* (tuliptree; change = 104.2). Changes in removal areas were greater than in reference areas, where the largest changes were observed for *Prunus serotina* (black cherry; change = 8611) and *L. tulipifera* (change = 5660).

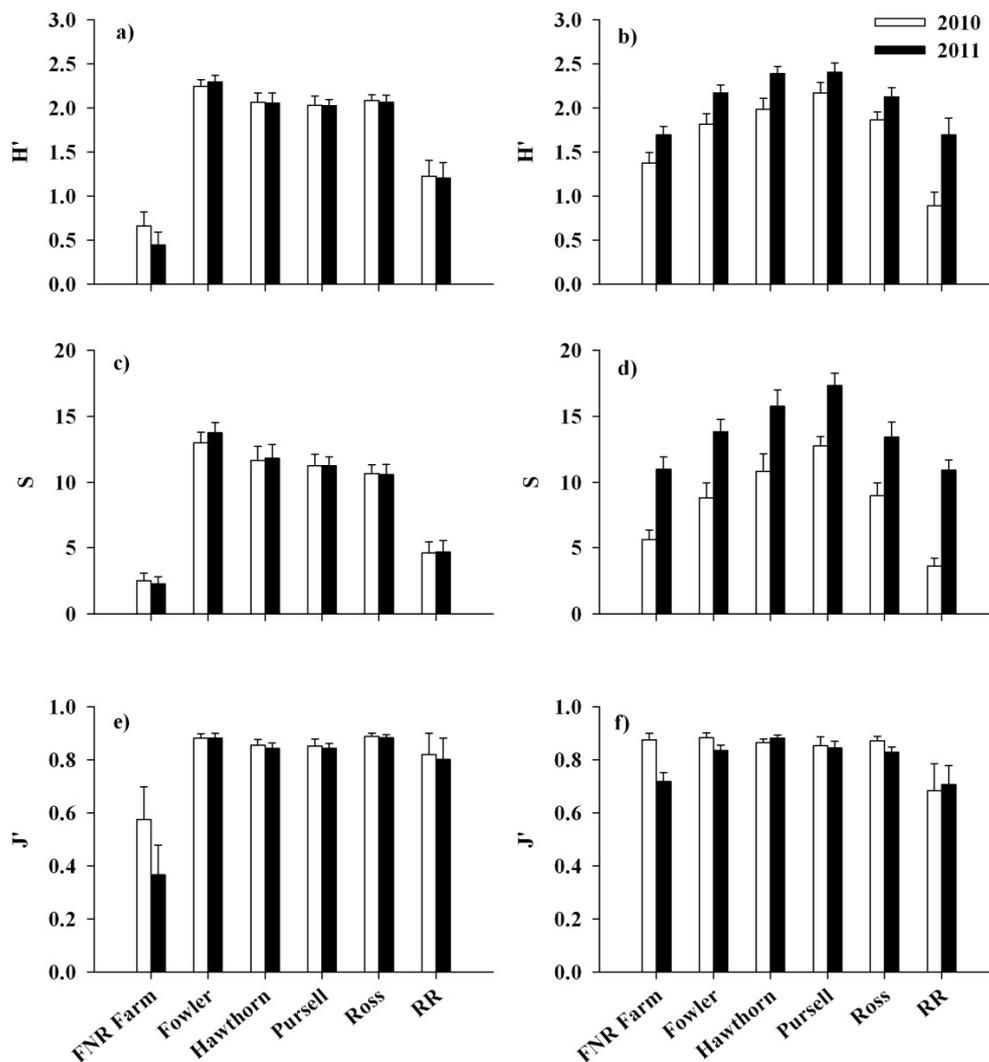


Figure 4. Mean Shannon's Diversity Index (H') in reference areas (a) and removal areas (b); mean taxonomic richness (S) in reference areas (c) and removal areas (d); and mean Pielou's Evenness Index (J') in reference areas (e) and removal areas (f) during the summer seasons of 2010 and 2011 at six mixed-hardwood forests in central Indiana. Error bars are ± 1 SE. Indices were based on percent covers of all native herbaceous and woody taxa encountered during the summer.

In the lower stratum, changes in abundance of native flora were generally positive and greater in removal areas than reference areas, indicating a positive response to the removal of non-native shrubs (Figure 6, Appendix A1). Between 2011 and 2010 during the spring seasons, 54 taxa increased and 10 taxa decreased in reference areas whereas in removal areas, 88 taxa increased and seven taxa decreased (Appendix A1). The largest changes in reference areas during spring were observed for *Ageratina altissima* var. *altissima* (white snakeroot; change in cover = 0.7) and *Polygonum virginianum* (jumpseed; change = 0.61). Changes in removal areas were greater than in reference areas during spring, with the largest changes observed for *Sanicula* spp. (sanicle; change = 2.8) and *Podophyllum peltatum* (mayapple; change = 1.5). Changes in percent cover during the summer seasons were similar to those observed in spring. In reference areas, 26 taxa exhibited a positive change and 37 taxa exhibited a negative change whereas in removal areas, 72 taxa exhibited a positive change and 26 taxa exhibited a negative change. The largest change in reference areas in the summer was observed for *P. virginianum* (change in mean percent cover from 2010 to 2011 = -0.5). As with spring flora, changes in removal areas were greater than in reference areas during the summer, where the largest changes in removal areas were observed for *Pilea pumila* (clearweed; change = 4.2) and *Sanicula* spp. (change = 2.2).

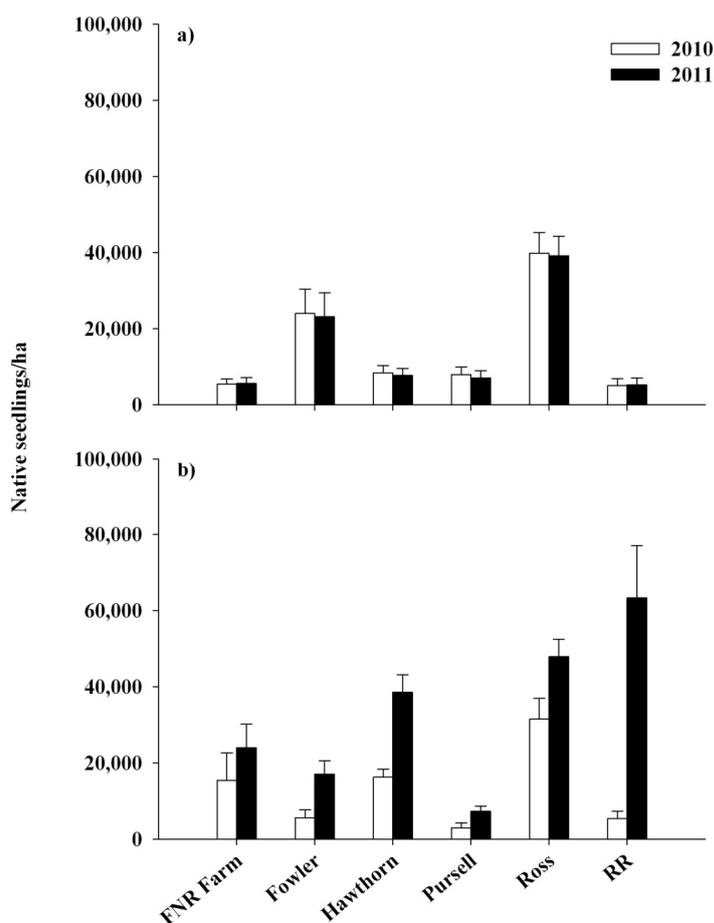


Figure 5. Mean (± 1 SE) densities (stems/ha) of native seedlings (woody stems < 1.37 m tall) in reference (a) and removal areas (b), in 2010 and 2011, at six mixed-hardwood forests in central Indiana.

Changes in abundance of taxa in the upper stratum were more variable than those observed in the lower stratum (Appendix A2). During the spring in reference areas, 14 taxa increased in cover from 2010 to 2011, and four taxa decreased in reference areas. In removal areas, 10 taxa exhibited a positive change and 12 taxa exhibited a negative change. The largest changes in reference areas in the spring were observed for *Cornus florida* (flowering dogwood; change in percent cover = 0.3), *L. tulipifera* (change = 0.3), and *C. occidentalis* (change = -0.3). Changes in removal areas were similar to those observed in reference areas for spring, with the largest change observed for *L. benzoin* (change = -1.0). In the summer, fewer taxa exhibited changes compared to spring. Specifically, three taxa exhibited a positive change and five exhibited a negative change in reference areas, whereas in removal areas, three taxa exhibited a positive change and 10 exhibited a negative change. The largest change in reference areas in the summer was observed for *C. occidentalis* (change = -0.4). In summer, changes in removal areas were similar to changes in reference areas. The largest changes in removal areas were observed for *Acer saccharum* (sugar maple; change = -0.3) and *C. occidentalis* (change = -0.3).

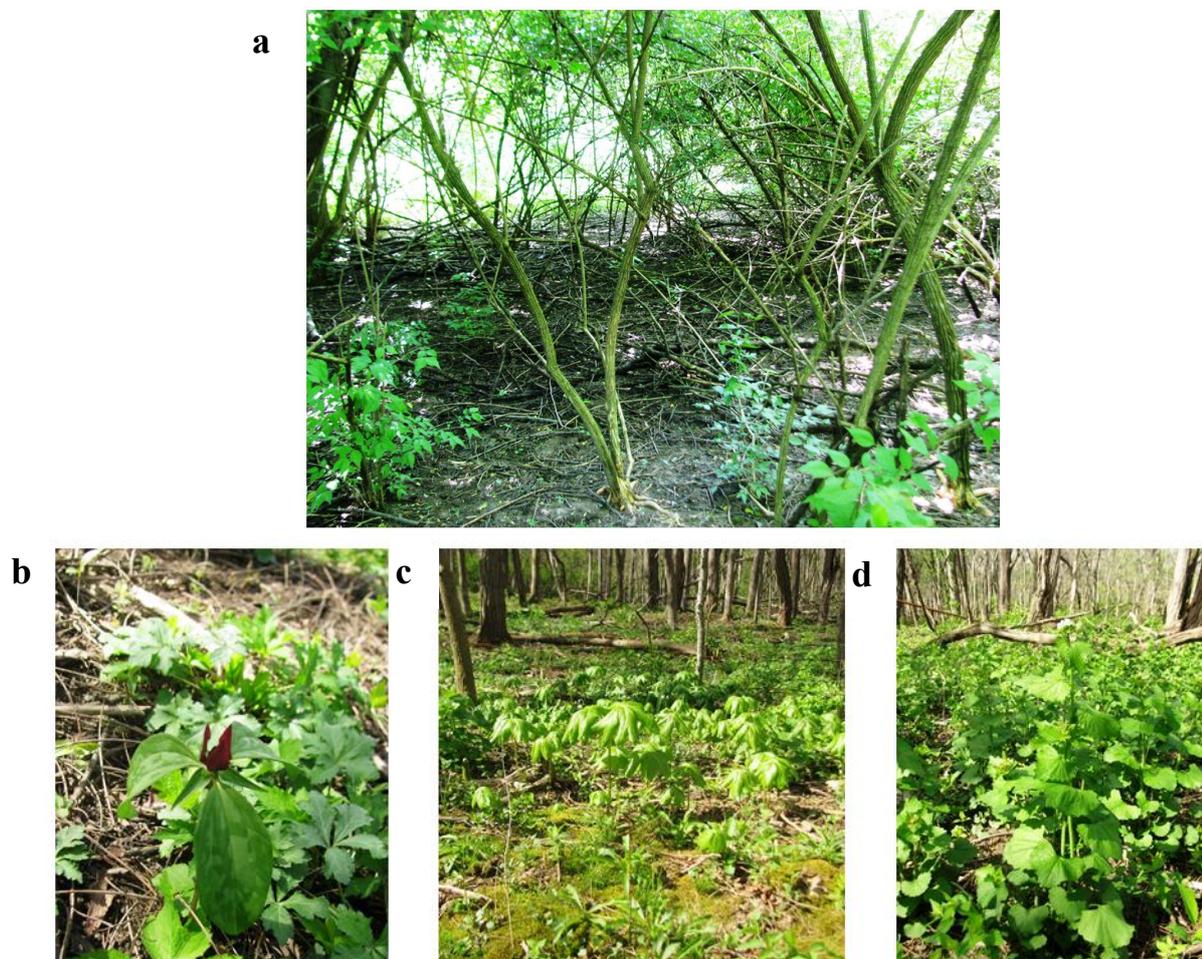


Figure 6. (a) *Lonicera maackii* thicket showing no native plant regeneration on the forest floor; (b) *Trillium recurvatum* (bloody butcher); (c) *Podophyllum peltatum*, and (d) *Alliaria petiolata* displayed positive responses to the removal of non-native shrubs. All photos were taken at Purdue University, Department of Forestry and Natural Resources Farm (FNR Farm), by Michael Jenkins.

Alliaria petiolata cover also increased following the removal of non-native shrubs (Figure 7), but this varied with pre-treatment percent cover of the species across study sites. *Alliaria petiolata* was not detected in either sample area at RR or Hawthorn before or after removal treatments and was not detected in the reference area during spring or summer at Fowler (Figure 7). *Alliaria petiolata* did occur in all other sample areas, and changes in mean percent cover from 2010 to 2011 were greater in removal areas than reference areas (Figure 7). Cover in reference areas did not change significantly in spring ($p = 0.77$) or summer ($p = 0.54$). Conversely, in removal areas during the spring, cover increased from 1.4 ± 0.6 in 2010 to 3.0 ± 0.8 in 2011 ($p = 0.09$). During the summer, cover increased from 1.0 ± 0.4 to 4.1 ± 1.5 ($p = 0.09$) in removal areas.

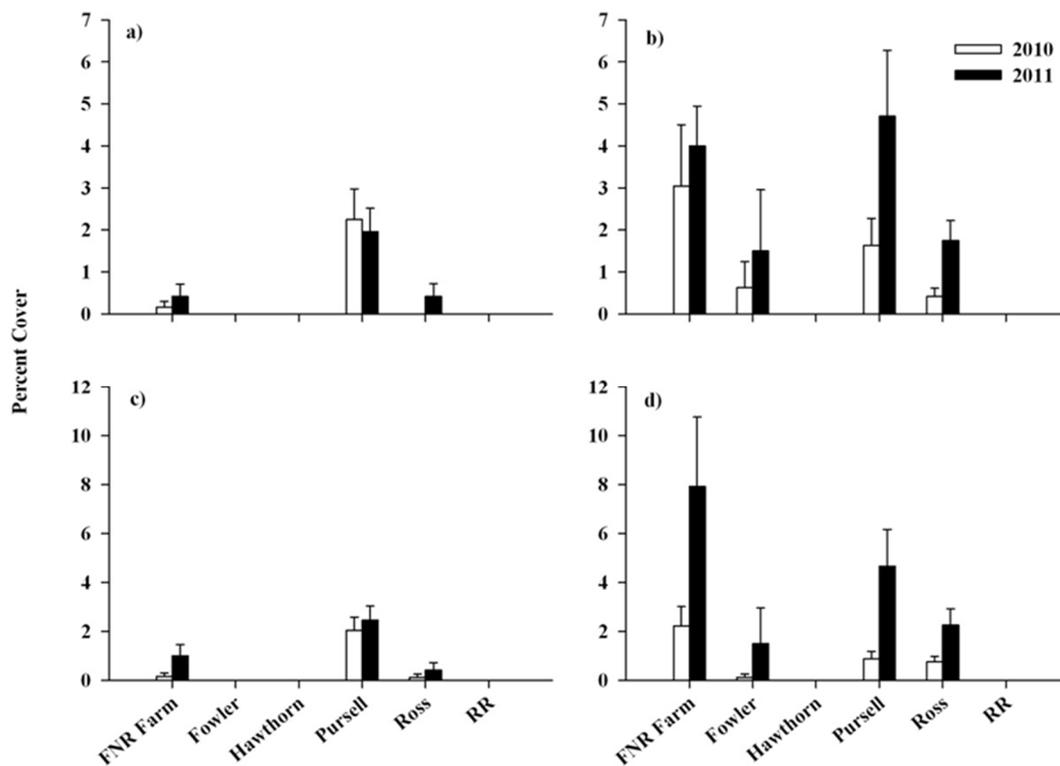


Figure 7. Mean percent cover (± 1 SE) of *Alliaria petiolata* during the spring in reference areas (a) and removal areas (b); and during the summer in reference areas (c) and removal areas (d); in 2010 and 2011, at six mixed-hardwood forests in central Indiana.

We also observed changes in some substrate variables. Mean cover of bare soil decreased in removal areas during both spring and summer (Tables 2 and 3). Conversely, mean percent cover of FWD increased from 2010 to 2011 in removal areas ($p = 0.04$ for both seasons; Tables 1 and 2).

4. Discussion

Our results suggest that removing *Lonicera maackii* and other non-native shrubs allows the recovery of native herbaceous and woody plant communities, at least in the short term. Removal areas exhibited significant increases in the cover and diversity of native species in both spring and summer, as well as increased densities of native seedlings, compared to reference areas. Furthermore, we observed significant decreases in the percent cover of bare soil in removal areas in spring and summer but no significant

changes in the cover of dead leaves/dead herbaceous stems, indicating that growing space was being filled by native vegetation. These subsequent increases in native flora were documented at all six study sites, which represented a wide range of pre-treatment *L. maackii* densities. Thus, our results suggest that even at sites with invasions in the advanced expansion or saturation phases [3], control efforts can allow the recovery of native taxa. The depauperate ground layer (Figure 6a) that occurs during the saturation stage could lead a manager to conclude that the area is completely void of native vegetation with little hope for recovery in response to restoration efforts. However, removing *L. maackii* and other non-native shrubs at our sites caused dramatic increases in the abundance of native flora, particularly vernal herbs. Based upon a review of the literature, our study is the first to identify before-and-after effects across a range of forest compositions and invasion intensities in forests that contained little to no aboveground biomass from non-native shrubs following removal treatments. As such, our study adds to a limited, but growing, body of work that documents the recovery potential of post-treatment forests (e.g., [15,23]).

The major environmental change resulting from removing non-native shrubs was likely an increase in light availability near the forest floor. Increased light may have played a particularly important role for the recovery of native vernal herbs [6,9]. Because *L. maackii* expands its leaves earlier in the year than other woody plants in its invaded range [5], it competes with spring-flowering herbs that utilize light available prior to canopy leaf out [39]. This response is illustrated by the dramatic increases in H' and S we observed for vernal herbs after removal.

For herbaceous-layer taxa that increased in post-removal cover, dispersal and re-establishment undoubtedly occurred via a combination of sexual and vegetative reproduction [40]. The most dramatic increases were observed for taxa that exhibited high fecundity and effective dispersal mechanisms. For example, during the summer, generalist species such as *P. pumila*, *Sanicula* spp., and *P. quinquefolia* (Appendix A1) exhibited the greatest increases in cover. While this response by generalists is not surprising, their cover was quite low prior to removal, suggesting that invasions by *L. maackii* and other shrubs suppressed even the most resilient native herbs. Although *L. maackii* removal also allowed woody seedlings to establish, subsequent canopy disturbance will be necessary for most of these seedlings to recruit into the overstory.

Following removal of invasive shrubs, long-term recovery of native taxa will be largely influenced by competition with non-native species and herbivory by white-tailed deer (*Odocoileus virginianus*). As originally hypothesized, we observed a post-harvest influx of *L. maackii* seedlings and garlic mustard, indicating that undesirable outcomes may also result from control efforts. Given that *L. maackii* seeds are readily dispersed by birds [41], white-tailed deer [42], and possibly mice [43], effective management will require the continual control of new seedlings in treatment areas. *Alliaria petiolata*, like native taxa, responded to the increased light levels and possibly the soil disturbance associated with cutting non-native shrubs and dragging slash [44]. Increased cover of *A. petiolata* following removal of *L. maackii* has been observed elsewhere when the species co-occur [13,15,16].

It is widely acknowledged that overabundant deer populations can cause declines in native taxa through intense herbivory [45], and help perpetuate the dominance of invasive species by preferentially browsing on native plants [46,47]. While we observed a strong positive response of native plant species to *L. maackii* removal, this increased herbaceous cover, in combination with the removal of a thick shrub layer that reduced access, may lead to increased herbivory of native species and increased dominance of

A. petiolata [16,48,49]. This negative interaction between deer herbivory and invasive plant species could be particularly problematic following shrub removal in natural areas where hunting is prohibited.

5. Conclusions

Our results suggest that implementing control of invasive shrubs may allow for the recovery of native plant species, even in forests with heavy densities resulting from long-term invasions. However, managers implementing invasive control in forests should understand that removal treatments may result in increased cover of both native and exotic plant species. Given the increased density of *L. maackii* seedlings after removal, as well as increased *A. petiolata* cover, long-term control efforts will be necessary to sustain the recovery of native communities. Long-term efforts to control invasive plants, such as *L. maackii*, should balance reclaiming heavily invaded areas with preventing areas with more recent invasions from reaching the saturation phase of population growth [18].

Acknowledgments

Thanks to Gene Rhodes, Rob Quackenbush, Kyle Leffel, Lindsay Jenkins, Amanda Parks, Michael Loesch-Fries, Brian Bailey, Caleb Brown, Justin Schmal, Brian Beheler, Oriana Rueda Krauss, Seth Howard, Amy Wetzels, Lindsey Purcell, Jeff Clark, Rick Meilan, Burk Thompson, Andy Meier, Don Carlson, Brent Pursell, and Kerry Rabenold, Matt Kraushar, Zach Lowe, Keith Ruble, Greg Ressler, Mike Homoya, and Sally Weeks for their assistance. The Department of Forestry and Natural Resources at Purdue, the Hardwood Tree Improvement and Regeneration Center, and the Purdue Doctoral Fellowship and Frederick N. Andrews and Russell O. Blosser Environmental Travel Grant programs supported this research.

Author Contributions

Joshua Shields was responsible for conceptualizing the study, data collection and analysis, and writing the manuscript. Michael Jenkins, Michael Saunders, Kevin Gibson, Patrick Zollner, and John Dunning were responsible for conceptualizing and advising the study and assisting with writing and editing the manuscript.

Supporting Information

Table S1. Mean percent cover (± 1 SE) of native plants, non-native plants, and substrate variables in the lower stratum (≤ 1 m) in reference and removal areas.

Table S2. Mean percent cover (± 1 SE) of native and non-native vines, trees, and shrubs in the upper stratum (1.01–5 m) in reference and removal areas.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Blossey, B. Before, during and after: The need for long-term monitoring in invasive plant species management. *Biol. Invasions* **1999**, *1*, 301–311.
2. Reid, A.M.; Morin, L.; Downey, P.O.; French, K.; Virtue, J.G. Does invasive plant management aid the restoration of natural ecosystems? *Biol. Conserv.* **2009**, *142*, 2342–2349.
3. Webster, C.R.; Jenkins, M.A.; Jose, S. Woody invaders and the challenges they pose to forest ecosystems in the eastern United States. *J. For.* **2006**, *104*, 366–374.
4. Uddin, M.B.; Steinbauer, M.J.; Jentsch, A.; Mukul, S.A.; Beierkuhnlein, C. Do environmental attributes, disturbances, and protection regimes determine the distribution of exotic plant species in Bangladesh forest ecosystem? *For. Ecol. Manag.* **2013**, *303*, 72–80.
5. Luken, J.O.; Thieret, J.W. Amur honeysuckle, its fall from grace: Lessons from the introduction and spread of a shrub species may guide future plant introductions. *BioScience* **1996**, *46*, 18–24.
6. Gould, A.M.; Gorchov, D.L. Effects of the exotic invasive shrub *Lonicera maackii* on the survival and fecundity of three species of native annuals. *Am. Midl. Nat.* **2000**, *144*, 36–50.
7. Collier, M.H.; Vankat, J.L.; Hughes, M.R. Diminished plant richness and abundance below *Lonicera maackii*, an invasive shrub. *Am. Midl. Nat.* **2002**, *147*, 60–71.
8. Gorchov, D.L.; Trisel, D.E. Competitive effects of the invasive shrub *Lonicera maackii*. *Plant Ecol.* **2003**, *166*, 13–24.
9. Miller, K.E.; Gorchov, D.L. The invasive shrub, *Lonicera maackii*, reduces growth and fecundity of perennial forest herbs. *Oecologia* **2004**, *139*, 359–375.
10. Hartman, K.M.; McCarthy, B.C. Changes in forest structure and species composition following invasion by a non-indigenous shrub, Amur honeysuckle (*Lonicera maackii*). *J. Torrey Bot. Soc.* **2008**, *135*, 245–259.
11. Meiners, S.J. Apparent competition: An impact of exotic shrub invasion on tree regeneration. *Biol. Invasions* **2007**, *9*, 849–855.
12. Hutchinson, T.F.; Vankat, J.L. Invasibility and effects of Amur honeysuckle in Southwestern Ohio forests. *Conserv. Biol.* **1997**, *11*, 1117–1124.
13. Luken, J.O.; Kuddes, L.M.; Tholemeier, T.C. Response of understory species to gap formation and soil disturbance in *Lonicera maackii* thickets. *Restor. Ecol.* **1997**, *5*, 229–235.
14. Hartman, K.M.; McCarthy, B.C. Restoration of a forest understory after the removal of an invasive shrub, Amur honeysuckle (*Lonicera maackii*). *Restor. Ecol.* **2004**, *12*, 154–165.
15. Runkle, J.R.; diSalvo, A.; Graham-Gibson, Y.; Dorning, M. Vegetation release eight years after removal of *Lonicera maackii* in west-central Ohio. *Ohio J. Sci.* **2007**, *107*, 125–129.
16. Cipollini, K.; Ames, E.; Cipollini, D. Amur honeysuckle (*Lonicera maackii*) management method impacts restoration of understory plants in the presence of white-tailed deer (*Odocoileus virginiana*). *Invasive Plant Sci. Manag.* **2009**, *2*, 45–54.
17. Swab, R.M.; Zhang, L.; Mitsch, W.J. Effect of hydrologic restoration and *Lonicera maackii* removal on herbaceous understory vegetation in a bottomland hardwood forest. *Restor. Ecol.* **2008**, *16*, 453–463.
18. Shields, J.M.; Jenkins, M.A.; Saunders, M.R.; Zhang, H.; Jenkins, L.H.; Parks, A.M. Age distribution and spatial patterning of an invasive shrub in secondary hardwood forests. *For. Sci.* **2014**, *60*, 830–840.

19. Dutra, H.P.; Barnett, K.; Reinhardt, J.R.; Marquis, R.J.; Orrock, J.L. Invasive plant species alters consumer behavior by providing refuge from predation. *Oecologia* **2011**, *166*, 649–657.
20. Shields, J.M.; Jenkins, M.A.; Zollner, P.A.; Saunders, M.R. Effects of Amur honeysuckle invasion and removal on white-footed mice. *J. Wildlife Manag.* **2014**, *78*, 867–880.
21. Ostfeld, R.S.; Manson, R.H.; Canham, C.D. Effects of rodents on survival of tree seeds and seedlings invading old fields. *Ecology* **1997**, *78*, 1531–1542.
22. Whigham, D.F. Ecology of woodland herbs in temperate deciduous forests. *Annu. Rev. Ecol. Evol. Syst.* **2004**, *35*, 583–621.
23. Owen, H.R.; McDonnell, A.L.; Mounter, A.M.; Todd, B.L. Influence of stem cutting and glyphosate treatment of *Lonicera maackii*, an exotic and invasive species, on stem regrowth and native species richness. *Trans. Illinois State Acad. Sci.* **2005**, *98*, 1–17.
24. Harrington, R.A.; Brown, B.J.; Reich, P.B. Ecophysiology of exotic and native shrubs in southern Wisconsin. I. Relationship of leaf characteristics, resource availability, and phenology to seasonal patterns of carbon gain. *Oecologia* **1989**, *80*, 356–367.
25. Harrington, R.A.; Brown, B.J.; Reich, P.B.; Fownes, J.H. Ecophysiology of exotic and native shrubs in southern Wisconsin. II. Annual growth and carbon gain. *Oecologia* **1989**, *80*, 368–373.
26. Homer, C.; Dewitz, J.; Fry, J.; Coan, M.; Hossain, N.; Larson, C.; Herold, N.; McKerrow, A.; VanDriel, J.N.; Wickham, J. Completion of the 2001 National Land Cover Database for the conterminous United States. *Photogramm. Eng. Remote Sens.* **2007**, *73*, 337–341.
27. Soil Survey Staff; Natural Resources Conservation Service; United States Department of Agriculture. Web Soil Survey. Available online: <http://websoilsurvey.nrcs.usda.gov/> (accessed on 20 March 2013).
28. Peet, R.K.; Wentworth, T.R.; White, P.S. A flexible, multipurpose method for recording vegetation composition and structure. *Castanea* **1998**, *63*, 262–274.
29. US Department of Agriculture. The PLANTS Database USDA. 2013. Available online: <http://plants.usda.gov> (accessed on 29 October 2013).
30. Yatskievych, K. *Field Guide to Indiana Wildflowers*; Indiana University Press: Bloomington, IN, USA, 2000.
31. Hollander, M.; Wolf, D.A. *Nonparametric Statistical Methods*, 2nd ed.; John Wiley & Sons: New York, NY, USA, 1999.
32. Carter, D.L.; Blair, J.M. Recovery of native plant community characteristics on a chronosequence of restored prairies seeded into pastures in west-central Iowa. *Restor. Ecol.* **2012**, *20*, 170–179.
33. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available online: <http://www.R-project.org> (accessed on 8 July 2013).
34. Oksanen, J.; Blanchet, G.F.; Kindt, R.; Legendre, P.; Minchin, P.R.; O’Hara, R.B.; Simpson, G.L.; Solymos, P.; Henry, M.; Stevens, H.; Wagner, H. *Vegan: Community Ecology*. Available online: <http://CRAN.R-project.org/package=vegan> (accessed on 20 February 2013).
35. Benjamini, Y.; Hochberg, Y. Controlling the false discovery rate—A practical and powerful approach to multiple testing. *J. Royal. Stat. Soc. B* **1995**, *57*, 289–300.
36. Benjamini, Y.; Hochberg, Y. On the adaptive control of the false discovery rate in multiple testing with independent statistics. *J. Educ. Behav. Stat.* **2000**, *25*, 60–83.

37. Pike, N. Using false discovery rates for multiple comparisons in ecology and evolution. *Meth. Ecol. Evol.* **2011**, *2*, 278–282.
38. Verhoeven, K.J.F.; Simonsen, K.L.; McIntyre, L.M. Implementing false discovery rate control: Increasing your power. *Oikos* **2005**, *108*, 643–647.
39. Schemske, D.W.; Willson, M.F.; Melampy, M.N.; Miller, L.J.; Verner, L.; Schemske, K.M.; Best, L.B. Flowering ecology of some spring woodland herbs. *Ecology* **1978**, *59*, 351–366.
40. Bierzychudek, P. Life histories and demography of shade-tolerant temperate forest herbs: A review. *New Phytol.* **1982**, *90*, 757–776.
41. Bartuszevige, A.M.; Gorchoy, D.L.; Raab, L. The relative importance of landscape and community features in the invasion of an exotic shrub in a fragmented landscape. *Ecography* **2006**, *29*, 213–222.
42. Castellano, S.M.; Gorchoy, D.L. White-tailed deer (*Odocoileus virginianus*) disperse seeds of the invasive shrub, Amur honeysuckle (*Lonicera maackii*). *Nat. Areas J.* **2013**, *33*, 78–80.
43. Williams, C.E.; Ralley, J.J.; Taylor, D.H. Consumption of seeds of the invasive Amur honeysuckle, *Lonicera maackii* (Rupr.) Maxim. by small mammals. *Nat. Areas J.* **1992**, *12*, 86–89.
44. Bartuszevige, A.M.; Hrenko, R.L.; Gorchoy, D.L. Effects of leaf litter on establishment, growth and survival of invasive plant seedlings in a deciduous forest. *Am. Midl. Nat.* **2007**, *158*, 472–477.
45. Rooney, T.P. Deer impacts on forest ecosystems: A North American perspective. *Forestry* **2001**, *74*, 201–208.
46. Webster, C.R.; Rock, J.R.; Froese, R.E.; Jenkins, M.A. Drought-herbivory interaction disrupts competitive displacement of native plants by *Microstegium vimineum*, 10 year results. *Oecologia* **2008**, *157*, 497–508.
47. Knight, T.M.; Dunn, J.L.; Smith, L.A.; Davis, J.; Kalisz, S. Deer facilitate invasive plant success in a Pennsylvania forest understory. *Nat. Areas J.* **2009**, *29*, 110–116.
48. Dornbush, M.E.; Hahn, P.G. Consumer and establishment limitation contribute more than competitive interactions in sustaining dominance of the exotic herb garlic mustard in a Wisconsin, USA forest. *Biol. Invasions* **2013**, *15*, 2691–2705.
49. Waller, D.M.; Maas, L.I. Do white-tailed deer and the exotic plant garlic mustard interact to affect the growth and persistence of native forest plants? *For. Ecol. Manag.* **2013**, *304*, 296–302.

© 2015 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).