

Invasive plant removal method determines native plant community responses

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Summary

1. Restoration of habitats invaded by non-native plants should include both the removal of invasive plants and re-establishment of native plant communities. To develop appropriate restoration strategies and quantify the effects of invasions, experiments that evaluate multiple removal methods and native community responses to those removal methods are needed.

2. We evaluated the response of native plant communities to removal of the invasive grass *Microstegium vimineum* (Japanese stiltgrass) in eastern forests in the USA. At eight field sites in southern Indiana, we applied three common removal treatments and compared native community responses among treatments and to untreated reference plots.

3. After 2 years of treatment, native community responses to *Microstegium* removal varied significantly among methods and plant functional groups in autumn 2006. Graminoid richness was greater when the invader was removed with hand-weeding, while graminoid biomass was lower in plots treated with post-emergent herbicide compared to reference plots. Forb richness was greater with hand-weeding and post-emergent herbicide compared to plots treated with post-emergent plus pre-emergent herbicides or untreated plots. Forb biomass was greater across all removal treatments. Overall native community diversity was 24% greater when the invasion was removed with hand-weeding and 21% greater with post-emergent herbicide compared to reference plots. No positive response in plant diversity occurred with post-emergent plus pre-emergent herbicide.

4. By spring 2007, graminoid percentage cover was greater with hand-weeding but not with herbicide treatments compared to untreated plots. However, forb cover was greater across all removal treatments compared to plots where the invader was not removed. The density of native tree seedlings was 123% greater in post-emergent herbicide treated plots than in untreated plots, indicating that the invasion was inhibiting tree recruitment.

5. *Synthesis and applications.* Our results demonstrate that multiple techniques can be used to control invasive plants but that the responses of native plant communities vary among removal methods. Further, greater native plant diversity and biomass following removal shows that invasions were suppressing native plant communities. Management of plant invasions should consider not only the effectiveness of removal methods but also how different methods influence native plant responses.

Key-words: diversity, evenness, grass-specific post-emergent herbicide, hand-weeding, Japanese stiltgrass, *Microstegium vimineum*, non-native grass, pre-emergent herbicide, richness

Introduction

The negative effects of plant invasions on natural and managed systems (e.g. Vitousek *et al.* 1997; Mack *et al.* 2000) have driven extensive research on restoration of affected communities (e.g. Carlson & Gorchoff 2004; Hartman & McCarthy 2004; Mason & French 2007). Restoration requires both the

removal of invasive plants and re-establishment of native plant communities (Diaz *et al.* 2003; Hulme 2006). Much research has focused on developing effective chemical, mechanical, and biological removal methods (e.g. Paynter & Flanagan 2004; Judge, Neal & Derr 2005a; Simmons *et al.* 2007), but the response of native communities to invasive plant removal has received less attention (reviewed by Zavaleta, Hobbs & Mooney 2001). Further, few studies have quantified native community responses to different removal

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methods (Holmes *et al.* 2000; Biggerstaff & Beck 2007; Mason & French 2007). Experiments that test the efficiency of multiple removal methods and their effects on native communities can aid in the development of appropriate restoration strategies and help to quantify the impacts of plant invaders on native communities (D'Antonio *et al.* 1998; Diaz *et al.* 2003; Hulme & Bremner 2006).

Although different invasive plant removal methods such as hand-weeding or herbicide application can affect native communities through soil disturbance or non-target herbicide effects for example (Aarssen & Epp 1990; Campbell *et al.* 1991; McLellan, Fitter & Law 1995), previous experiments explicitly evaluating native community responses have typically focused on a single removal method (D'Antonio *et al.* 1998; Alvarez & Cushman 2002; Hulme & Bremner 2006). Removal treatments such as hand-weeding often disturb the soil, which can disrupt root systems and mycorrhizal networks of non-target plants (McLellan, Fitter & Law 1995), but can also facilitate native (Biggerstaff & Beck 2007) and non-native (Mack & Lonsdale 2002; Ogden & Rejmanek 2005; Mau-Crimmins 2007) plant establishment. Chemical treatments may have strong effects on a particular plant functional group if the herbicide is designed to kill only monocotyledonous or dicotyledonous plants, for example (Pavlik, Nickrent & Howald 1993; Cione, Padgett & Allen 2002), and may leave toxic residues that inhibit native plant recruitment. Further, pre-emergent herbicides may inhibit seed germination of non-target species. Other control methods such as grazing, fire, mowing or shading may also have unexpected or unintended consequences. To develop effective invasive plant management techniques that promote native community recovery, studies that test multiple removal methods for specific plant invaders are needed.

In addition to the direct application of restoring invaded communities, invasive plant removal experiments provide information on the effects of the invader on native communities (Gould & Gorchoff 2000; Alvarez & Cushman 2002; Diaz *et al.* 2003). The impacts of plant invasions can be evaluated through both introduction (e.g. Robinson, Quinn & Stanton 1995) and removal (e.g. Alvarez & Cushman 2002) experiments. Experimental introductions may provide the best measure of the effects of plant invasions, but small-scale invasion experiments in containers and common gardens yield limited insights for natural systems. However, adding noxious species to natural areas can present obvious management and ethical problems. In contrast, long-term removal experiments in natural areas can help to quantify the effects of plant invasions on native communities (Diaz *et al.* 2003). Because different removal methods could have different effects on native communities, studies designed to evaluate the effects of invasions through removal experiments should test multiple removal methods.

We evaluated the response of native plant communities to removal of *Microstegium vimineum* (Trin.) A. Camus (Japanese stiltgrass), a C-4 non-native annual grass that is rapidly invading eastern USA forests (Winter, Schmitt & Edwards 1982; Horton & Neufeld 1998). *Microstegium* was

introduced to the USA from south-east Asia in the early 1900s (Fairbrothers & Gray 1972) and is currently invasive in more than 20 states (USDA & NRCS 2005). Invasions by *Microstegium* and other species threaten to reduce the diversity of woodland herbaceous communities and inhibit forest regeneration and succession (Winter, Schmitt & Edwards 1982; Barden 1987; Oswalt, Oswalt & Clatterbuck 2007). *Microstegium* is a prolific seed producer (Tu 2000) and is dispersed through water, animals, and anthropogenic activities. It most commonly invades forest openings, riparian areas, and along roads, streams and trails (Redman 1995; Tu 2000). *Microstegium* is highly shade tolerant and can reproduce under deeply shaded conditions (Winter, Schmitt & Edwards 1982; Horton & Neufeld 1998). Multiple methods have been used to control *Microstegium*, including mowing, fire, selective (grass-specific) and non-selective (glyphosate) herbicides, and hand-pulling (Tu 2000; Czarapata 2005), but studies testing the effectiveness of different treatments for removing invasions and the specific responses of native communities to removal treatments are needed (Judge *et al.* 2005a,b; Flory 2008).

Previously, we reported on the effectiveness of *Microstegium* removal methods over multiple growing seasons (Flory 2008). We found that two herbicide-based removal methods each reduced *Microstegium* biomass by 99%, while a hand-weeding treatment was slightly less effective (87% reduction, Flory 2008). Here, we use the same system to evaluate the specific responses of native plant communities to multiple invasive plant removal methods. After two seasons of treatment, we quantified the species richness, biomass, and cover of specific native plant functional groups, the number of colonizing tree seedlings, and overall native plant community richness, evenness, and diversity. We evaluated specific native plant functional groups separately because *Microstegium* and other invaders may be having disproportionate effects on different native plant groups. In addition, the method used to remove invasions may differentially affect functional group responses to removal. Specifically, we asked the following questions: (i) Do the responses of native plant communities depend on the method used to remove invasive *Microstegium*? (ii) Do the responses of native plant functional groups differ among removal methods? (iii) Does removal result in greater native plant diversity and biomass? Answers to these questions have applications for management of this specific invader, but are also broadly relevant to native plant community responses to removal methods of any plant invader.

Methods

STUDY SITES

Our goal with this experiment was to evaluate both the effectiveness of treatments for removing an important invasive species (Flory 2008) and the specific responses of native plants to removal (this study) across the wide range of habitats where invasions occur. To provide generality across research sites, we deliberately chose sites that varied in species composition, light availability, soil moisture, forest successional ages, and land-use history. Eight *Microstegium*-

Table 1. Locations, properties, land-use histories, forest successional ages, and light availability of the eight research sites

Location		Property	Land-use history	Forest successional age	Percentage light availability
Latitude (W)	Longitude (N)				
85°38'82"	39°04'98"	BONWR	Walnut-dominated forest	Late	13
85°43'72"	39°01'81"	BONWR	Walnut-dominated forest, former home site	Mid	27
85°45'31"	38°98'43"	BONWR	Agricultural land, prescribed burns every 2–3 years	Mid	14
86°63'98"	38°19'84"	HNF	Former logging road, colonized by tree saplings	Surrounded by mid	6
86°65'86"	38°05'95"	HNF	Undisturbed bottomland forest	Late	5
86°05'00"	38°84'57"	JWSF	Wildlife opening on ridge top	Surrounded by late	64
86°41'47"	39°32'46"	MMSF	~15 year old log yard	Early	21
86°42'24"	39°33'35"	MMSF	Power line right-of-way	Surrounded by late	60

BONWR, Big Oaks National Wildlife Refuge; HNF, Hoosier National Forest; JWSF, Jackson–Washington State Forest; MMSF, Morgan–Monroe State Forest. Successional ages: early, < 30 years; mid, 30–60 years; late, > 60 years. Percentage light availability is the average percentage of ambient light reaching the plots at each site in June 2006 (AccuPAR Linear PAR/LAI ceptometer, Decagon Devices, Inc., Pullman, WA, USA).

invaded sites were selected at four public properties in southern Indiana including two state forests, a national forest, and a national wildlife refuge (Table 1). Study sites within properties were located at least 1 km apart. All sites were historically covered by forest but have been subjected to various anthropogenic disturbances including agriculture, prescribed fires, and timber harvests (Table 1).

The local flora at each site varied widely. Study sites were mixed oak–hickory (*Quercus–Carya*) or beech–maple (*Fagus–Acer*) forests (Woodall *et al.* 2005). Canopy trees at the sites also included tulip poplar *Liriodendron tulipifera*, black walnut *Juglans nigra*, and elm (*Ulmus* spp.) and understory woody species included *Rubus* spp., *Sassafras albidum*, greenbriar (*Smilax* spp.), spicebush *Lindera benzoin*, sumac (*Rhus* spp.), and *Viburnum* spp. In general, most of the native herbaceous species were perennials so the response of those species to invasive removal could include resprouting, new growth, or recruitment from seed. The dominant graminoid species at the sites often included *Dicanthelium clandestinum*, *Elymus* spp., *Cinna arundinacea*, *Uniola latifolia*, *Carex* spp., and *Leersia* spp., and the dominant forbs were often *Eupatorium rugosum*, *Solidago* spp., *Polygonum* spp., *Packera* spp., *Impatiens capensis*, *Geum* spp., and *Viola* spp. The most common fern species were *Polystichum acrostichoides*, *Onoclea sensibilis*, and *Cystopteris* spp.

Precipitation in southern Indiana averages 102.1 cm year⁻¹ and the average daily maximum temperature during summer months is 29.4 °C (Noble, Wingard & Ziegler 1990). Average gravimetric soil moisture at the eight study sites was 10.5 to 22.3% in late July 2005 (data not shown) and the average percentage of ambient light ranged from 5 to 64% among the sites in June 2006 (Table 1).

EXPERIMENTAL DESIGN

We established 40 × 2 × 2 m plots at each of the eight sites (320 plots total) in June 2005. Plots of similar size have been used successfully in previous studies to evaluate the responses of native plant communities to the removal of invasive species (Alvarez & Cushman 2002; Carlson & Gorchoy 2004; Hulme & Bremner 2006). Plots were located in dense stands of *Microstegium* and positioned to avoid the confounding effects of large trees and logs, and standing water. At least 2 m separated adjacent plots. At each site, plots were randomly assigned to one of four groups (10 replicates per treatment per site): reference plots (REF), hand-weeded (HW), grass-specific post-emergent herbicide (POST), or post-emergent herbicide plus pre-emergent herbicide (POST + PRE).

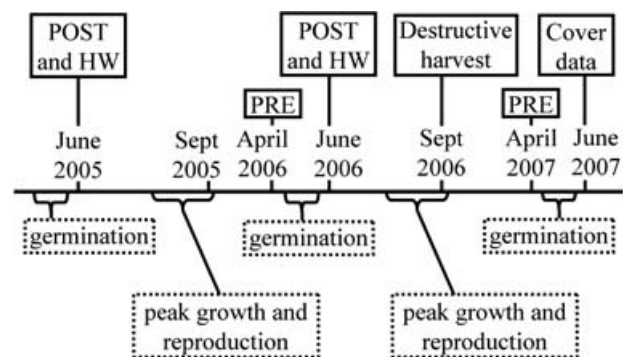


Fig. 1. Timeline showing when *Microstegium* removal treatments were applied and when the destructive harvest and percentage cover data were collected (boxes with solid lines) and the periods of *Microstegium* germination and peak growth (boxes with dotted lines). POST refers to the grass-specific post-emergent herbicide, HW is hand-weeding, and PRE is the application of the pre-emergent herbicide for the POST + PRE treatment.

For the hand-weeding treatment, all *Microstegium* were pulled and placed outside of the plots while avoiding other species. Hand-weeding was used because it is a common control method for many invasive plants, does not require equipment, is relatively low-cost, and is non-chemical. The post-emergent grass-specific herbicide treatment was 0.21 kg a.i. ha⁻¹ (active ingredient per hectare) of fluzafop-P-butyl (12 oz ac⁻¹ Fusilade DX; Syngenta Crop Protection, Inc., Greensboro, NC) mixed with 14.8 ml of nonionic surfactant (Surf Plus 584, Townsend Chemical Division, Muncie, IN). This herbicide is known to effectively remove *Microstegium* (Judge, Neal & Derr 2005a) and other invasive grasses and because it is grass-specific, we hypothesized that it would have fewer negative effects on native plant communities compared to non-selective herbicides. For the post-emergent plus pre-emergent herbicide treatment, the pre-emergent herbicide was 1.34 kg a.i. ha⁻¹ of pendimethalin (19.2 oz ac⁻¹ Pendulum AquaCap; BASF, Research Triangle Park, NC), which was applied in the spring following the application of post-emergent herbicide (Fig. 1). The pre-emergent herbicide was tested in addition to post-emergent herbicide because *Microstegium* and many other plant invaders produce abundant seed that can remain viable in the seed bank for years (Gibson, Spyreas & Benedict 2002). The pre-emergent herbicide is designed to kill seeds as they germinate, regardless of

functional group. Herbicides were applied with backpack sprayers at 40 psi. Hand-weeding and post-emergent herbicide treatments were conducted in the fourth week of July 2005 and in the third week of June 2006. At the time of treatment, *Microstegium* seeds had germinated and seedlings were approximately 10–20 cm in height. Pre-emergent applications were done in the first week of April 2006 and in the second week of April 2007, 2 to 4 weeks prior to *Microstegium* seed germination (Fig. 1).

Overall, this experimental design included three highly replicated treatments for removing *Microstegium* applied across three growing seasons and a wide range of invaded sites, and untreated control plots for reference. No uninvaded areas with similar environmental conditions existed at our research sites, and thus, uninvaded plant community comparisons would have been confounded with substantial differences in environmental conditions. Therefore, we could not determine if native species composition following removal was similar to communities that would have occupied the sites prior to the invasion.

These results should provide general information on the response of invaded communities to invasive plant removal. However, the time frame of this study does not confirm how plant communities respond to removal of invasions over many years. We focused our sampling efforts on specific plant groups (e.g. graminoids, forbs, spring ephemerals) that may respond differently to invasive plant removal methods. This provides an important first step in understanding the responses of communities to *Microstegium* removal and baseline data for further study on this and other invasive plant species.

DATA COLLECTION

To quantify the responses of native plant communities to *Microstegium* removal after two growing seasons, we conducted a destructive harvest during the third week of August 2006 (Fig. 1). The centre 1 m² area of each plot was divided into four 0.5 × 0.5-m quadrats and one quadrat was randomly selected for harvest. All vegetation except for trees larger than 2 cm basal diameter was cut at ground level, sorted to species, dried at 60 °C to constant weight, and weighed (± 0.01 g).

Destructive sampling in autumn 2006 may have missed species that senesced earlier in the year. To evaluate the responses of spring ephemeral species and to further assess the colonization and growth of other native species, we also collected cover data in spring 2007 (Fig. 1). We quantified graminoid and forb percentage cover within each plot in June 2007 using a 0.5 × 0.5-m PVC frame divided into 100 5 × 5 cm squares. The frame was placed over the next quadrat clockwise from the quadrat harvested in 2006. The frame had 40 cm legs so we could quantify cover while standing directly overhead. We also counted tree seedlings in each quadrat.

DATA ANALYSIS

We used ANOVA to analyse the fixed effects of site and treatment (REF, HW, POST, and POST + PRE) on multiple response variables: (i) biomass and species richness of four functional groups (graminoids, forbs, woody species, and ferns); (ii) overall native plant community richness, evenness, and diversity; (iii) spring graminoid and forb percentage cover; and (iv) number of tree seedlings (PROC GLM, SAS Institute Inc. 2002). The Shannon diversity index of each plot was calculated based on the proportional biomass of each species (Magurran 1988): $H' = -\sum [(p_i)(\ln p_i)]$, where p_i is the proportion

of the total plot biomass belonging to the i th species (Krebs 1989). Because comparing Shannon values across treatments can provide an inaccurate estimate of the magnitude of differences among treatments (Hill 1973; Jost 2006), Shannon values were converted to the 'effective number of species ($e^{H'}$), the number of species if all were equally common (MacArthur 1965; Jost 2006) for analysis and presentation. Evenness was calculated as $J' = H'/(\ln S)$, where S = species richness (Pielou 1975). *Post hoc* Tukey tests were used to evaluate differences among treatments within responses.

Results

The responses of the four functional groups (graminoids, forbs, woody species, and ferns) of native species to *Microstegium* removal differed significantly based on the removal method (Table 2, Fig. 2). For graminoids, there was greater species richness when *Microstegium* was removed with hand-weeding (HW) but lower richness with POST + PRE compared to REF plots (Fig. 2a). In contrast, forb richness was greater with both the HW and POST treatments compared to REF plots (Fig. 2c). Although there were trends for greater woody species richness with HW and POST (Fig. 2e), there were no statistically significant differences among treatments. There were also non-significant trends for greater fern species richness with the POST treatment (Fig. 2g). There was a significant site × treatment interaction for forb biomass (Table 2) due to greater positive responses of forb species to removal at some sites than others.

Graminoid biomass was lower when *Microstegium* was removed with POST compared to REF plots (Fig. 2b) but forb biomass was greater across all removal treatments (Fig. 2d). Woody species biomass was also greater across all removal treatments but the trends were not significant (Fig. 2f). Likewise, there was a non-significant trend for greater fern biomass with POST (Fig. 2h).

Overall native community diversity was greater with the HW and POST, but not POST + PRE, compared to REF plots (Table 3, Fig. 3). Native species richness was greater with HW and POST (Fig. 3a) but native species evenness was unaffected by the treatments (Fig. 3b), indicating that changes in diversity were driven by changes in species richness. There were greater positive responses in overall native community richness at some sites than others resulting in a significant site × treatment interaction (Table 3). There was 24% and 21% greater native community diversity when *Microstegium* was removed using HW and POST respectively compared to REF plots.

After 2 years of treatment, spring graminoid percentage cover was greater with the HW treatment compared to REF plots but not with POST or POST + PRE (Table 4, Fig. 4a). However, forb cover was greater across all removal treatments compared to REF plots. The greatest difference in forb cover (43%) was observed in POST-treated plots (Fig. 4b). However, there were greater positive responses in forb cover at some sites than others, resulting in a site × treatment interaction (Table 4). Tree seedling density was 123% greater with POST than in REF plots, but tree seedling colonization was no greater with HW or POST + PRE (Fig. 4c).

Table 2. Results of ANOVA tests for the effects of treatment, site, and their interaction on graminoid, forb, woody species, and fern richness and biomass in fall 2006

Source	df	Graminoid			Forb			Woody species			Fern						
		Richness		Biomass	Richness		Biomass	Richness		Biomass	Richness		Biomass				
		F	P	F	P	F	P	F	P	F	P	F	P				
Treatment	3	14.52	< 0.0001	3.39	0.02	21.27	< 0.0001	3.75	0.01	2.81	0.04	1.75	0.16	3.19	0.02	2.30	0.08
Site	7	27.68	< 0.0001	8.51	< 0.0001	9.27	< 0.0001	13.68	< 0.0001	3.02	0.0045	6.32	< 0.0001	8.30	< 0.0001	4.50	< 0.0001
Trt × Site	21	1.17	0.28	1.49	0.08	1.6	0.05	3.23	< 0.0001	1.36	0.14	1.03	0.43	0.93	0.56	1.39	0.12

Values in bold indicate significance at $P < 0.05$, d.f., degrees of freedom.

Discussion

Our results show that the method used to remove a widespread invasive grass significantly influenced the response of native plant communities. Species richness and biomass responses varied among functional groups, with forb species exhibiting the greatest positive responses and graminoid species showing few responses compared to untreated plots. Overall native species diversity was greater when *Microstegium* was removed with hand-weeding and post-emergent herbicide, but not post-emergent plus pre-emergent herbicide, compared to untreated plots. These results indicate that when invaded habitats are treated with hand-weeding or post-emergent herbicide to remove invasions, native plant community biomass and diversity can be at least partially restored. Moreover, the greater native plant biomass and diversity we observed following invasive plant removal demonstrates that invasions are having significant negative effects on native plant communities.

FUNCTIONAL GROUP RESPONSES

The three removal methods resulted in different responses among native plant functional groups. Treatments including the grass-specific herbicide probably killed native grasses such as *Dichanthelium clandestinum*, *Elymus* spp., and *Leersia* spp., in addition to *Microstegium*, resulting in no positive responses in graminoid species richness or biomass. However, graminoid species richness was greater with the hand-weeding treatment compared to reference plots, suggesting that *Microstegium* was inhibiting the growth of native graminoids. Alternatively, or possibly in addition, soil disturbance generated by the hand-weeding may have enhanced native graminoid recruitment (Thomson 2005; Biggerstaff & Beck 2007). However, post-emergent herbicide alone did not result in soil disturbance but forb richness was still greater than in untreated plots. Thus, we conclude that release from competition with *Microstegium* was a more important factor driving overall native species responses than soil disturbance. For example, Hulme & Bremner (2006) removed the invasive forb *Impatiens glandulifera* from riparian areas in the UK by above-ground clipping and found that it resulted in greater species recruitment even though the removal treatment did not disturb the soil. However, they found that a substantial portion of the colonizing species were undesirable non-natives, a trend observed in many invasive plant removal experiments (Holmes *et al.* 2000; Ogden & Rejmanek 2005; Mason & French 2007; Mau-Crimmins 2007; Truscott *et al.* 2008). In contrast, we occasionally found only two non-native invasive species in our removal plots (*Lonicera japonica* and *Rosa multiflora*), and both species also occurred in untreated reference plots.

Removal of invasions over two growing seasons resulted in higher densities of tree seedlings, demonstrating that invasions were suppressing tree seedling establishment. Our experimental results confirm the observations of Oswalt *et al.* (2007), who observed that *Microstegium* cover was negatively correlated with tree seedling density and diversity. Because *Microstegium*

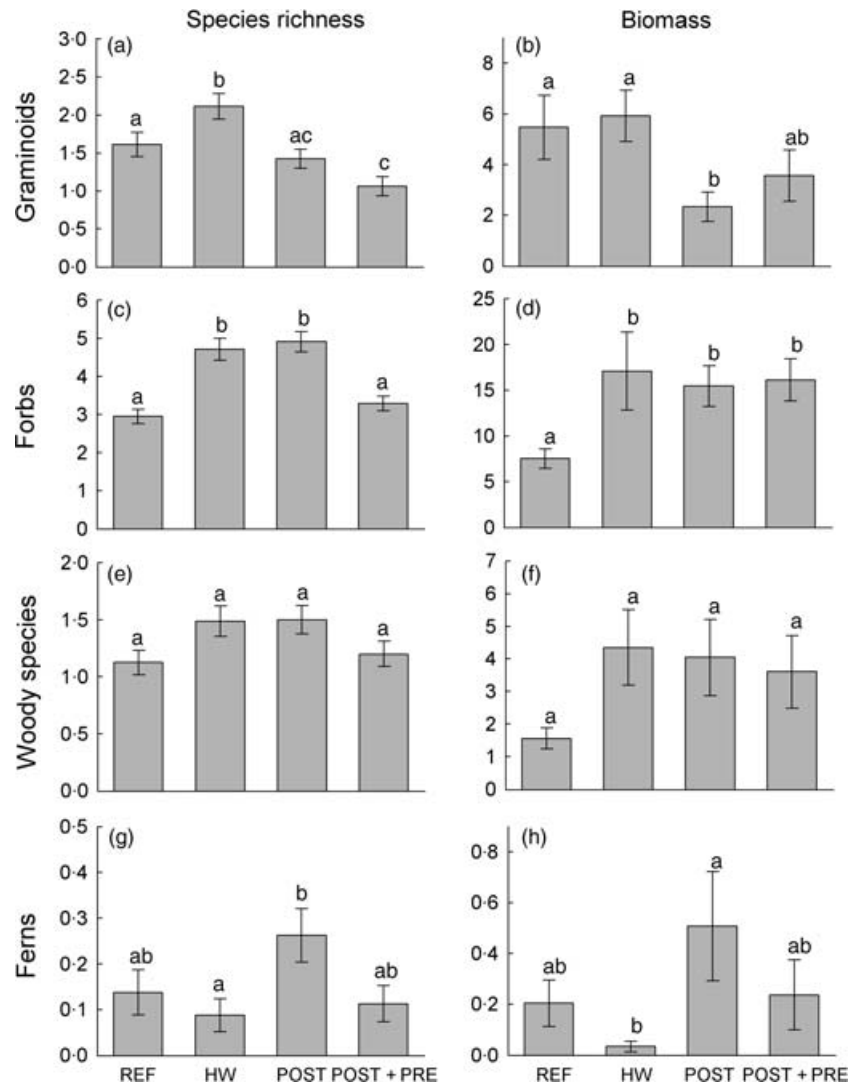


Fig. 2. Average (\pm SE) species richness and biomass of four functional groups per 0.25 m² quadrat, graminoids (a,b), forbs (c,d), woody species (e,f), and ferns (g,h) in REF, HW, POST, and POST + PRE plots for fall 2006. Different letters indicate significant differences at $P < 0.05$.

Table 3. Results of ANOVA tests for the effects of treatment, site, and their interaction on native community richness, evenness, and diversity in fall 2006

Source	d.f.	Native community					
		Richness		Evenness		Diversity	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Treatment	3	25.91	< 0.0001	0.55	0.65	8.94	< 0.0001
Site	7	20.18	< 0.0001	2.26	0.03	7.50	< 0.0001
Treatment \times site	21	1.72	0.03	1.33	0.16	1.33	0.16

Values in bold indicate significance at $P < 0.05$. d.f., degrees of freedom.

aggressively colonizes early successional habitats (Redman 1995), invasions have the potential to alter forest community dynamics, particularly if the effects of invasion differ among tree species. However, removal method significantly affected tree recruitment. Tree seedling density was greater in plots treated with post-emergent herbicide, but not in hand-weeded or post-emergent plus pre-emergent herbicide treated plots, compared to untreated plots. Although previous

studies have found that disturbance due to invasive plant removal can increase native plant colonization rates (Biggerstaff & Beck 2007), the lack of colonization with the hand-weeded treatment suggests that the process of pulling *Microstegium* inhibited tree seedling establishment or survival. Tree seeds could have been disturbed when *Microstegium* was uprooted or seedlings may have accidentally been pulled during the weeding process. For plots treated with both

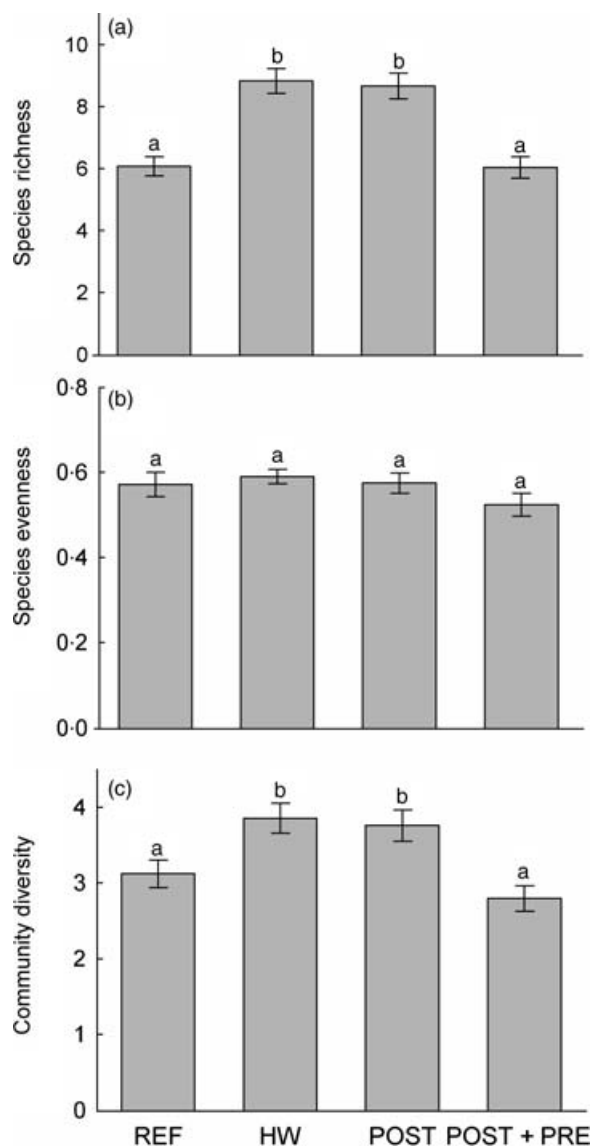


Fig. 3. Average (\pm SE) native species richness (a), evenness (b), and diversity (c) 0.25 m^{-2} quadrat for fall 2006 in REF, HW, POST, and POST + PRE plots. Different letters indicate significant differences at $P < 0.05$.

post-emergent and pre-emergent herbicides, the pre-emergent herbicide probably killed tree seeds as they germinated. Overall, removal of *Microstegium* using the grass-specific post-emergent herbicide alone best alleviates the effects of invasions on tree recruitment.

Table 4. Results of ANOVA tests for the effects of treatment, site, and their interaction on graminoid and forb cover and the number of tree seedlings per plot in spring 2007

Source	d.f.	Graminoid cover		Forb cover		Tree seedlings	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Treatment	3	4.55	0.0039	21.68	< 0.0001	3.5	0.02
Site	7	27.94	< 0.0001	15.39	< 0.0001	22.27	< 0.0001
Treatment \times site	21	1.5	0.08	1.67	0.04	1.4	0.12

Values in bold indicate significance at $P < 0.05$. d.f., degrees of freedom.

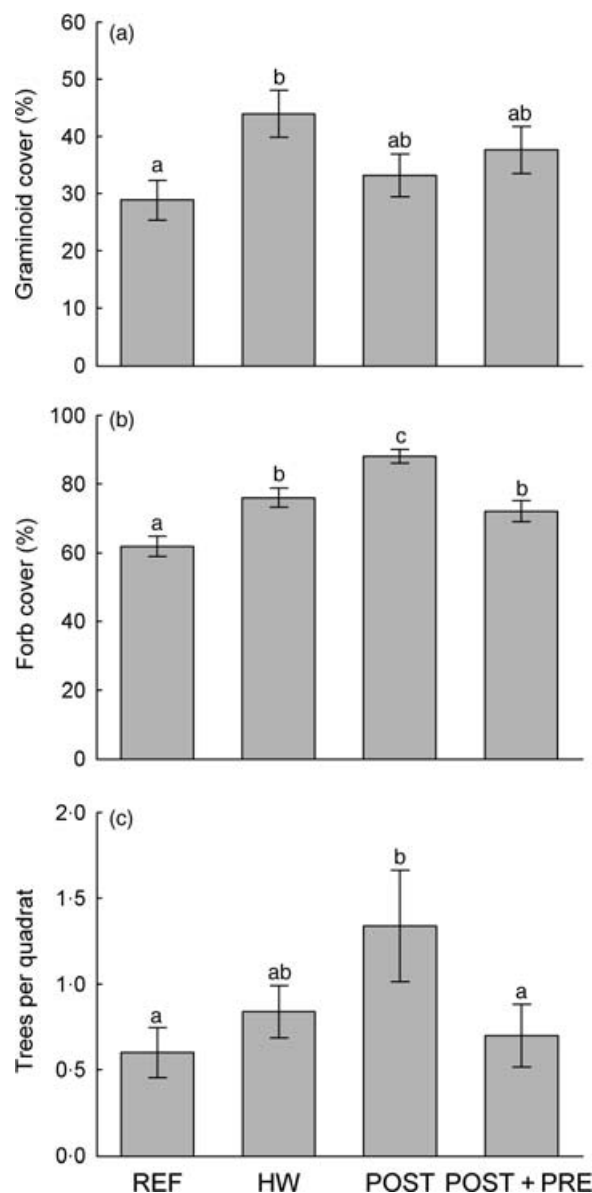


Fig. 4. Average (\pm SE) graminoid (a) and forb (b) percentage cover and the number of tree seedlings (c) 0.25 m^{-2} quadrat for spring 2007 in REF, HW, POST, and POST + PRE plots. Different letters indicate significant differences at $P < 0.05$.

Ferns are an important component of the herbaceous layer in eastern deciduous forests, particularly in moist, shaded habitats where *Microstegium* occurs (Winter, Schmitt & Edwards 1982; Cole & Weltzin 2004). Although not statistically

significant, there were trends toward greater fern richness and biomass with post-emergent herbicide but not with post-emergent plus pre-emergent herbicide compared to untreated reference plots. In addition, disturbance generated by hand-weeding may have prevented fern re-establishment following removal and may have damaged extant ferns, thereby affecting fern richness and biomass. Additional experiments are needed to more specifically evaluate the effects of *Microstegium* on ferns. Our results suggest that invasions in fern-rich communities could be managed using grass-specific post-emergent herbicide to reduce invasive impacts and restore fern richness and biomass.

POSSIBLE MECHANISMS

The positive responses of native species to invasive plant removal indicate that invasions are having significant negative effects on native communities by several possible mechanisms. The inhibition of native species could be due to competition for resources such as light, nutrients, or water (Ehrenfeld, Kourtev & Huang 2001; Belote & Weltzin 2006). For example, invasions of non-native annual grasses in California reduce light availability in early spring and suppress native bunchgrasses (Dyer & Rice 1999). Similarly, *Microstegium* grows at very high densities and produces abundant biomass, which can reduce light at the soil surface (W.B. Mattingly and S.L. Flory, unpublished). In addition, senesced plants produce a dense mat that is slow to decompose and may inhibit the growth of native plant species. The suppressive effects of invasive grasses have been documented previously by D'Antonio *et al.* (1998) who found that removing invasive grasses over 3 years in a Hawaiian forest increased the growth of two shrub species and altered the architecture of a third. Soil surface light availability and soil nitrogen were also greater where the invasive grasses were removed (D'Antonio *et al.* 1998). Recent work suggests that plant invasions can also have indirect effects by altering the behaviour of vertebrate and invertebrate species that feed on native plants (S.L. Flory and K. Clay, unpublished; Sessions & Kelly 2002; Meiners 2007).

VARIATION AMONG SITES

To obtain the broadest possible results regarding the restoration of invaded habitats and impacts on native species, we intentionally chose research sites that spanned a wide range of environmental characteristics, land-use histories, and plant community types including upland forests, bottomland riparian forests, and a rich undisturbed interior forest. Despite the variation in site characteristics, only forb biomass and native community richness in the autumn of 2006 and forb cover in the spring of 2007 showed site \times treatment interactions. All of these interactions were due to much larger positive responses of native species to removal at some sites than others, not because of responses in opposite directions. In general, there were significant positive responses of native species when *Microstegium* was removed with hand-weeding and post-emergent herbicide regardless of the site conditions.

CONCLUSIONS AND APPLICATIONS

The results of our removal experiment confirm other observational (Redman 1995; Oswalt, Oswalt & Clatterbuck 2007) and experimental (Leicht, Silander & Greenwood 2005; Flory, Rudgers & Clay 2007) reports that suggest *Microstegium* has substantial negative effects on native plant communities. Removing invasions by hand-weeding or grass-specific post-emergent herbicide resulted in greater native plant biomass, species richness, and tree regeneration, demonstrating that invasions were suppressing native species.

More importantly, by using multiple removal methods in an experimental framework, we show that the response of native plant communities to invasive plant removal depends on the removal method. Previous studies using only a single removal method may have found different native community responses if a different removal method had been used. Conclusions about the impacts of invasive species may therefore be contingent on removal methods. Future studies on invasive species should incorporate multiple removal methods to more accurately determine the response of native communities. Furthermore, studies should not only evaluate overall community diversity and biomass, but also partition community responses among functional groups since removal methods may differentially affect plant functional groups.

Of the management methods evaluated in this study, the best technique for controlling *Microstegium* invasions, considering time and costs of labour and herbicide, is the grass-specific, post-emergent herbicide. The hand-weeding treatment resulted in significant positive responses in native plant communities but it is extremely labour-intensive and failed to prevent re-invasion of the sites the following year (Flory 2008). The pre-emergent herbicide was very effective at removing the invader but inhibited the return of native species. Grass-specific post-emergent herbicide was affordable and easy to apply, effectively removed invasions and prevented re-invasion the next season (Flory 2008), and resulted in greater native species biomass and diversity. Our results should be applicable to a wide range of invasive grass species. Overall, our results show that *Microstegium* has a consistent negative effect on native plant communities and that removing invasions using the techniques recommended here should result in greater native plant biomass and diversity.

Acknowledgements

We thank the many undergraduate students who assisted with this project and Karen Haubensak, Vicky Meretsky, and Heather Reynolds who reviewed earlier drafts of this manuscript. We also thank the staff at the properties where we worked who provided access and logistical support. Financial support was provided by Townsend Chemical Division, The National Wild Turkey Federation, The Nature Conservancy, and by a partnership between Indiana University and the USDA Forest Service Hoosier National Forest.

References

- Aarssen, L.W. & Epp, G.A. (1990) Neighbor manipulations in natural vegetation – a review. *Journal of Vegetation Science*, **1**, 13–30.
- Alvarez, M.E. & Cushman, J.H. (2002) Community-level consequences of a plant invasion: effects on three habitats in coastal California. *Ecological Applications*, **12**, 1434–1444.

- Barden, L.S. (1987) Invasion of *Microstegium vimineum* (Poaceae), an exotic, annual, shade-tolerant, C-4 grass, into a North-Carolina floodplain. *American Midland Naturalist*, **118**, 40–45.
- Belote, R.T. & Weltzin, J.F. (2006) Interactions between two co-dominant, invasive plants in the understorey of a temperate deciduous forest. *Biological Invasions*, **8**, 1629–1641.
- Biggerstaff, M.S. & Beck, C.W. (2007) Effects of method of English ivy removal and seed addition on regeneration of vegetation in a southeastern piedmont forest. *American Midland Naturalist*, **158**, 206–220.
- Campbell, B.D., Grime, J.P., Mackey, J.M.L. & Jalili, A. (1991) The quest for a mechanistic understanding of resource competition in plant communities – the role of experiments. *Functional Ecology*, **5**, 241–253.
- Carlson, A.M. & Gorchov, D.L. (2004) Effects of herbicide on the invasive biennial *Alliaria petiolata* (garlic mustard) and initial responses of native plants in a southwestern Ohio forest. *Restoration Ecology*, **12**, 559–567.
- Cione, N.K., Padgett, P.E. & Allen, E.B. (2002) Restoration of a native shrubland impacted by exotic grasses, frequent fire, and nitrogen deposition in southern California. *Restoration Ecology*, **10**, 376–384.
- Cole, P.G. & Weltzin, J.F. (2004) Environmental correlates of the distribution and abundance of *Microstegium vimineum*, in east Tennessee. *Southeastern Naturalist*, **3**, 545–562.
- Czarapata, E. (2005) *Invasive Plants of the Upper Midwest: An Illustrated Guide to Their Identification and Control*. The University of Wisconsin Press, Madison, WI, USA.
- D'Antonio, C.M., Hughes, R.F., Mack, M., Hitchcock, D. & Vitousek, P.M. (1998) The response of native species to removal of invasive exotic grasses in a seasonally dry Hawaiian woodland. *Journal of Vegetation Science*, **9**, 699–712.
- Diaz, S., Symstad, A.J., Chapin, F.S., Wardle, D.A. & Huenneke, L.F. (2003) Functional diversity revealed by removal experiments. *Trends in Ecology & Evolution*, **18**, 140–146.
- Dyer, A.R. & Rice, K.J. (1999) Effects of competition on resource availability and growth of a California bunchgrass. *Ecology*, **80**, 2697–2710.
- Ehrenfeld, J.G., Kourtev, P. & Huang, W.Z. (2001) Changes in soil functions following invasions of exotic understorey plants in deciduous forests. *Ecological Applications*, **11**, 1287–1300.
- Fairbrothers, D.E. & Gray, J.R. (1972) *Microstegium vimineum* (Trin.) A. Camus (Gramineae) in the United States. *Journal of the Torrey Botanical Society*, **99**, 97–100.
- Flory, S.L. (2008) Management of *Microstegium vimineum* invasions and recovery of resident plant communities. *Restoration Ecology*, DOI: 10.1111/j.1526-100X.2008.00425.x.
- Flory, S.L., Rudgers, J.A. & Clay, K. (2007) Experimental light treatments affect invasion success and the impact of *Microstegium vimineum* on the resident community. *Natural Areas Journal*, **27**, 124–132.
- Gibson, D.J., Spyreas, G. & Benedict, J. (2002) Life history of *Microstegium vimineum* (Poaceae), an invasive grass in southern Illinois. *Journal of the Torrey Botanical Society*, **129**, 207–219.
- Gould, A.M.A. & Gorchov, D.L. (2000) Effects of the exotic invasive shrub *Lonicera maackii* on the survival and fecundity of three species of native annuals. *American Midland Naturalist*, **144**, 36–50.
- Hartman, K.M. & McCarthy, B.C. (2004) Restoration of a forest understorey after the removal of an invasive shrub, Amur honeysuckle (*Lonicera maackii*). *Restoration Ecology*, **12**, 154–165.
- Hill, M.O. (1973) Diversity and evenness – unifying notation and its consequences. *Ecology*, **54**, 427–432.
- Holmes, P.M., Richardson, D.M., Van Wilgen, B.W. & Gelderblom, C. (2000) Recovery of South African fynbos vegetation following alien woody plant clearing and fire: implications for restoration. *Austral Ecology*, **25**, 631–639.
- Horton, J.L. & Neufeld, H.S. (1998) Photosynthetic responses of *Microstegium vimineum* (Trin.) A. Camus, a shade-tolerant, C-4 grass, to variable light environments. *Oecologia*, **114**, 11–19.
- Hulme, P.E. (2006) Beyond control: wider implications for the management of biological invasions. *Journal of Applied Ecology*, **43**, 835–847.
- Hulme, P.E. & Bremner, E.T. (2006) Assessing the impact of *Impatiens glandulifera* on riparian habitats: partitioning diversity components following species removal. *Journal of Applied Ecology*, **43**, 43–50.
- Jost, L. (2006) Entropy and diversity. *Oikos*, **113**, 363–375.
- Judge, C.A., Neal, J.C. & Derr, J.E. (2005a) Preemergence and postemergence control of Japanese stiltgrass (*Microstegium vimineum*). *Weed Technology*, **19**, 183–189.
- Judge, C.A., Neal, J.C. & Derr, J.F. (2005b) Response of Japanese stiltgrass (*Microstegium vimineum*) to application timing, rate, and frequency of postemergence herbicides. *Weed Technology*, **19**, 912–917.
- Krebs, C.J. (1989) *Ecological Methodology*. Harper Row, New York.
- Leicht, S.A., Silander, J.A. & Greenwood, K. (2005) Assessing the competitive ability of Japanese stilt grass, *Microstegium vimineum* (Trin.) A. Camus. *Journal of the Torrey Botanical Society*, **132**, 573–580.
- MacArthur, R.H. (1965) Patterns of species diversity. *Biological Reviews of the Cambridge Philosophical Society*, **40**, 510–533.
- Mack, R.N. & Lonsdale, W.M. (2002) Eradicating invasive plants: hard-won lessons for islands. *Turning the Tide: The Eradication of Invasive Species* (eds C.R. Veitch & M.N. Clout), pp. 164–172. IUCN SSC Invasive Species Specialist Group, Gland, Switzerland.
- Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. & Bazzaz, F.A. (2000) Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications*, **10**, 689–710.
- Magurran, A.E. (1988) *Ecological Diversity and Its Measurement*. Princeton University Press, Princeton, NJ, USA.
- Mason, T.J. & French, K. (2007) Management regimes for a plant invader differentially impact resident communities. *Biological Conservation*, **136**, 246–259.
- Mau-Crimmins, T.M. (2007) Effects of removing *Cynodon dactylon* from a recently abandoned agricultural field. *Weed Research*, **47**, 212–221.
- McLellan, A.J., Fitter, A.H. & Law, R. (1995) On decaying roots, mycorrhizal colonization and the design of removal experiments. *The Journal of Ecology*, **83**, 225–230.
- Meiners, S.J. (2007) Apparent competition: an impact of exotic shrub invasion on tree regeneration. *Biological Invasions*, **9**, 849–855.
- Noble, R.A., Wingard, R.C. & Ziegler, T.R. (1990). *Soil Survey of Brown County and Part of Bartholomew County, Indiana*. United States Department of Agriculture, Soil Conservation Service and Forest Service, Washington, D.C.
- Ogden, J.A.E. & Rejmanek, M. (2005) Recovery of native plant communities after the control of a dominant invasive plant species, *Foeniculum vulgare*: Implications for management. *Biological Conservation*, **125**, 427–439.
- Oswalt, C.M., Oswalt, S.N. & Clatterbuck, W.K. (2007) Effects of *Microstegium vimineum* (Trin.) A. Camus on native woody species density and diversity in a productive mixed-hardwood forest in Tennessee. *Forest Ecology and Management*, **242**, 727–732.
- Pavlik, B.M., Nickrent, D.L. & Howald, A.M. (1993) The recovery of an endangered plant. 1. Creating a new population of *Amsinckia grandiflora*. *Conservation Biology*, **7**, 510–526.
- Paynter, Q. & Flanagan, G.J. (2004) Integrating herbicide and mechanical control treatments with fire and biological control to manage an invasive wetland shrub, *Mimosa pigra*. *Journal of Applied Ecology*, **41**, 615–629.
- Pielou, E.C. (1975) *Ecological Diversity*. Wiley & Sons, New York.
- Redman, D.E. (1995) Distribution and habitat types for Nepal *Microstegium* [(*Microstegium vimineum* (Trin.) Camus)] in Maryland and the District of Columbia. *Castanea*, **60**, 270–275.
- Robinson, G.R., Quinn, J.F. & Stanton, M.L. (1995) Invasibility of experimental habitat islands in a California winter annual grassland. *Ecology*, **76**, 786–794.
- SAS Institute Inc. (2002). *Statistical Analysis System*. Cary, NC, USA.
- Sessions, L. & Kelly, D. (2002) Predator-mediated apparent competition between an introduced grass, *Agrostis capillaris*, and a native fern, *Botrychium australe* (Ophioglossaceae), in New Zealand. *Oikos*, **96**, 102–109.
- Simmons, M.T., Windhager, S., Power, P., Lott, J., Lyons, R.K. & Schwowe, C. (2007) Selective and non-selective control of invasive plants: The short-term effects of growing-season prescribed fire, herbicide, and mowing in two Texas prairies. *Restoration Ecology*, **15**, 662–669.
- Thomson, D. (2005) Measuring the effects of invasive species on the demography of a rare endemic plant. *Biological Invasions*, **7**, 615–624.
- Truscott, A.-M., Palmer, S.C., Soulsby, C., Westaway, S. & Hulme, P.E. (2008) Consequences of invasion by the alien plant *Mimulus guttatus* on the species composition and soil properties of riparian plant communities in Scotland. *Perspectives in Plant Ecology, Evolution and Systematics*, **10**, 231–240.
- Tu, M. (2000). *Element Stewardship Abstract for Microstegium vimineum*. The Nature Conservancy, Arlington, VA, USA.
- USDA & NRCS. (2005). The PLANTS Database. Data compiled from various sources by Mark W. Skinner. Vol. 2005, Version 3-5. National Plant Data Center, Baton Rouge, LA, USA.
- Vitousek, P.M., D'Antonio, C.M., Loope, L.L., Rejmanek, M. & Westbrooks, R. (1997) Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology*, **21**, 1–16.
- Winter, K., Schmitt, M.R. & Edwards, G.E. (1982) *Microstegium vimineum*, a shade adapted C-4 grass. *Plant Science Letters*, **24**, 311–318.
- Woodall, C., Johnson, D., Gallion, J., Perry, C., Butler, B., Piva, R., Jepsen, E., Nowak, D. & Marshall, P. (2005). Indiana's forests 1999–2003 (Part A). *Resource Bulletin*, NC-253A, US Department of Agriculture, Forest Service, North Central Research Station, St Paul, MN, USA.
- Zavaleta, E.S., Hobbs, R.J. & Mooney, H.A. (2001) Viewing invasive species removal in a whole-ecosystem context. *Trends in Ecology & Evolution*, **16**, 454–459.

Received 18 July 2008; accepted 29 December 2008

Handling Editor: Phil Hulme