



## **Restoring Invaded Pacific Northwest Prairies: Management Recommendations from a Region-Wide Experiment**

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## Restoring Invaded Pacific Northwest Prairies: Management Recommendations from a Region-Wide Experiment

### Abstract

We conducted a 5-year study at 10 sites from British Columbia to the Willamette Valley aimed at improving methods for restoring degraded prairies and oak savannas. Our manager-recommended treatment combinations were applied over 4 years and included the following components: spring and fall mowing, grass-specific and broad-spectrum herbicide, and fall burning. All treatment combinations were crossed with native seed addition. As expected, we found there was no ‘silver bullet’; while some treatment combinations led to large improvements in weed control and native diversity and abundance, the optimum combination and degree of success varied across sites. Where non-native grasses are the most pressing problem, we recommend the use of grass-specific herbicides as highly effective with minimal non-target effects on native forbs and some native grasses. Fire is a useful tool for preparing a site for seeding and can be followed closely with a broad spectrum herbicide to control rapidly resprouting weeds. Careful timing of post-fire herbicide application avoids impacting later-sprouting natives. At all sites, we recommend seed addition to enhance native diversity and abundance, as our data show even relatively high quality sites are strongly seed-limited. Repeat mowing is ineffective at reducing herbaceous weed abundance. Additionally, mowing did not increase bare soil, resulting in poor seedling establishment. If fire is not an option, we recommend testing additional treatments to increase bare soil and seeding success. At all sites, we conclude that enhancing natives and controlling invasives are likely to be most successful through repeated applications of treatment combinations.

### Introduction

The native prairies and oak savannas in the Willamette Valley/Puget Trough/Georgia Basin ecoregion (WPG—Figure 1) are among the most endangered ecosystems in North America (Noss et al. 1995). These areas were historically burned frequently by Native Americans (Boyd 1986, Kruckeberg 1991). Today fire suppression, habitat conversion, fragmentation, species invasion, and loss of native diversity contribute to the large scale degradation of these habitats (Floberg et al. 2004, Dunwiddie et al. 2006). Remaining sites are often small and highly fragmented and have been heavily invaded by non-native plant species, especially perennial grasses (Dunn and Ewing 1997). These invasive plants reduce native diversity and alter vegetation structure, fire regimes, soil characteristics, and faunal diversity (Haubensak and Parker 2004, Dunwiddie et al. 2006, Sinclair et al. 2006). Nonetheless, many remnants support imperiled species and are important conservation sites (Floberg et al. 2004).

Frequently, native and invasive species share many traits (e.g., phenology, degree of susceptibility to grazing or fire) and are often extensively intermixed with one another at a particular location. These conditions pose a particularly difficult restoration challenge: how can we selectively remove invasive plant species without causing damage to natives? Standard methods for controlling invasive plants, such as burning, mowing, and herbicide application, often impact many native species (Smith and Knapp 1999, Sheley and Krueger-Mangold 2003). Additionally, our knowledge regarding the effectiveness of restoration techniques in this region is largely anecdotal, or based on studies testing single treatments over a limited geographic range (Schuller 1997, Tveten 1997, Wilson and Clark 2001).

To address these problems, we worked with numerous partners from 2005 – 2010 to test multifaceted restoration techniques for reducing invasive species and enhancing natives at selected upland prairie and oak savanna sites across the WPG ecoregion (Figure 1; Noss et al. 1995; Dunn and Ewing 1997; Stanley et al. 2008, 2010, 2011). We focused on reducing non-native herbaceous species, as our collaborating land managers

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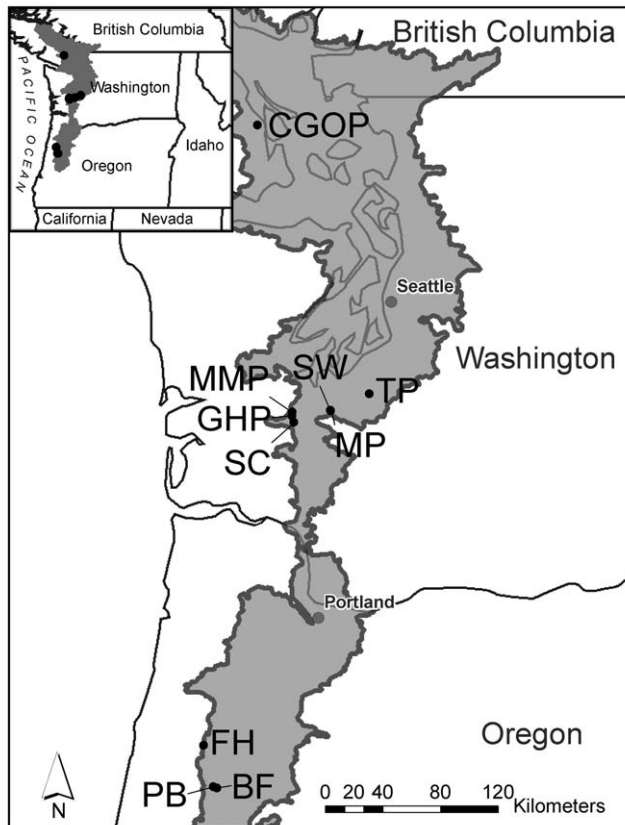


Figure 1. Map of study sites and WPG ecoregion (shaded area). See Table 1 for explanation of site abbreviations, ownership, and site quality.

felt methods for controlling woody plant encroachment were well-tested and reliable.

The non-native perennial grasses succeed by their rapid growth, structural dominance, and thatch accumulation (Sinclair et al. 2006). Prescribed fire often

benefits these fire-tolerant invaders as much as native species (Anzinger and Radosevich 2008). Grass-specific herbicides, such as sethoxydim (Poast) or fluazifop (Fusilade), can target non-native grasses, but most native grasses are also susceptible. Roemer's fescue (*Festuca roemeri*), one of the most common native grasses in the region, is resistant to both sethoxydim (Dunwiddie and Delvin 2006) and fluazifop (Blakeley-Smith 2006).

Previous analyses of our experimental results focused on the responses of broadly defined functional groups (e.g., perennial native and non-native grasses) to management treatments (Stanley et al. 2008, 2011). These analyses showed several treatments were promising in their ability to reduce non-natives without reducing the abundance of native species. While a functional group as a whole may have a consistent response to restoration treatments, the individual species that comprise the group may vary. Information about the responses of key species of management concern is essential to appropriate decision-making. Also of interest is the relative importance of native seed addition versus weed control on the diversity and abundance of native species. In this paper, we present how key native and non-native species responded to management treatments, examine how seeding affected native richness and cover of sown species, and combine this information with our previous analyses to provide recommendations for management.

## Study Area

We selected ten research sites in natural areas managed by various agencies and organizations (Figure 1; Table 1). Although there is considerable overlap in species composition among these prairies, they vary widely in terms of soils, climate, land use history, and degree of

TABLE 1. Study site ownerships and pre-treatment diversity and cover, measured in sampling quadrats prior to treatment in spring 2005. We used richness, the number of plant species, as our metric of diversity. Relative native cover (RNC) was calculated as the percentage of total vegetative cover comprised of native species.

Site	Site Code	Ownership	No. Non-native Spp.	No. Native Spp.	RNC
Cowichan Garry Oak Preserve	CGOP	Nature Conservancy of Canada	19	31	40%
Triangle Prairie	TP	Joint Base Lewis-McChord, US Army	23	30	48%
South Weir Prairie	SW	Joint Base Lewis-McChord, US Army	18	21	12%
Mima Mounds Natural Areas Preserve	MMP	WA Dept. Natural Resources	17	24	32%
Glacial Heritage Preserve	GHP	Thurston County Parks	15	19	23%
Scatter Creek Wildlife Area	SC	WA Dept. Fish and Wildlife	19	28	63%
Morgan Property (aka Tenalquot)	MP	The Nature Conservancy	12	22	28%
Fort Hoskins Historical Park	FH	Benton County Natural Areas and Parks Dept.	24	13	36%
Bellfountain Road	BF	Finley Wildlife Refuge, USFWS	34	34	40%
Pigeon Butte	PB	Finley Wildlife Refuge, USFWS	32	28	43%

TABLE 2. Treatment combinations applied to 5 × 5 m plots. Treatment elements include 1.5% sethoxydim (S), mowing (M) in spring, fall burning (B), and glyphosate treatment (G). See methods section for additional details.

Year	Season	Treatments				
		SBG	MBG	MM	SM	Control
2005	Spring	sethoxydim	Mow	Mow	sethoxydim	
	Fall			Mow	Mow	
2006	Spring	sethoxydim		Mow	sethoxydim	
	Fall	burn + glyphosate	burn + glyphosate	Mow	Mow	
2007	Spring	sethoxydim		Mow	sethoxydim	
	Fall			Mow	Mow	
2008	Spring			Mow		
	Fall	burn + glyphosate	burn + glyphosate	Mow	Mow	

TABLE 3. Amount of native seed (g m<sup>-2</sup>) added to subplots at each site in each year. See Table 1 for explanation of site codes.

Species	Common name	2006	2007	Sites
<i>Achillea millefolium</i>	common yarrow	0.029	0.035	All
<i>Balsamorhiza deltoidea</i>	arrowleaf balsamroot	--	0.528-0.568	WA sites only
<i>Danthonia californica</i>	California oatgrass	0.392-0.698	0.344-0.698	All except GHP and SC
<i>Danthonia spicata</i>	poverty oatgrass	0.544	0.288	GHP, SC
<i>Eriophyllum lanatum</i>	Oregon sunshine	0.038-0.065	0.065	All
<i>Festuca roemerii</i>	Roemer's fescue	0.234	0.234	All
<i>Lomatium nudicaule</i>	barestem biscuitroot	1.285	0.692	TP
<i>Lomatium utriculatum</i>	common lomatium	0.146	0.073-0.146	All except TP
<i>Plectritis congesta</i>	seablush	0.040	0.040	All
<i>Ranunculus occidentalis</i>	western buttercup	0.168-0.220	0.219	All

invasion (Floberg et al. 2004, Dunwiddie et al. 2006). Each of these sites retained at least some native prairie vegetation (Table 1; Stanley et al. 2010), and shrubs were largely absent.

## Methods

### Experimental Design and Data Collection

In many studies, treatments are often tested alone or in limited combinations in a factorial design. In our study, we tested sets of treatment combinations (hereafter referred to as “treatments”) because this is more consistent with the on-the-ground practices of land managers. The treatments were applied over 4 years (Table 2) and included the following components: spring application of 1.5% sethoxydim (S) to reduce abundance of exotic perennial grasses; mowing (M) in spring to prevent seed set and reduce stored reserves of exotic perennial grasses or in fall to reduce thatch accumulation and cut back fall-regrowing grasses; fall burning (B) to reduce biomass and thatch accumulation and prepare sites for re-seeding; and post-burn application of 1.5% glyphosate (G) (a broad-spectrum

herbicide) to reduce abundance of broadleaf weeds. This last component was developed based on observations that non-native species resprout more quickly after fire than do most native species. Mowing height was 3-10 cm and biomass was left in place (Stanley et al. 2010). Because the focus of this study was herbaceous vegetation, plots were initially free of shrubs and any shrub seedlings were pulled.

Each of our ten sites contained twenty 25 m<sup>2</sup> experimental plots (5 treatments × 4 replicates = 20 plots per site), with treatments applied to randomly assigned plots. Plots were divided into four equal 6.25 m<sup>2</sup> subplots and received one of four native seeding treatments (not seeded, seeded in 2006, seeded in 2007, or seeded in both years). To reduce variability caused by species identity, we selected a common suite of 7-8 species that occurred throughout the region with sufficient available seed, with a total seeding rate of approximately 13.5 kg ha<sup>-1</sup> and a ratio of 40% grasses to 60% forbs by seed number (Table 3). This ratio was developed in consultation with land managers and review of the literature (e.g., Diboll 2005). Managers suggested a few congeneric substitutions to match species to sites. Seeds

were purchased from local growers or hand collected on or near each site. Actual seed amounts varied due to seed availability (Table 3). Seeds were broadcast in late fall, after all fall treatments, and plots were lightly raked to improve seed-soil contact. Unseeded portions of the plots were also raked.

Data were collected from four 1-m<sup>2</sup> permanent sampling quadrats per plot – one in each subplot – in late April to early June 2005 (pre-treatment) and each following spring 2006-2009. Percent cover was visually estimated to the nearest 1% for all vascular plant species. Total cover for a plot was at least 100%, and often exceeded that when many layers of vegetation were present. Species nomenclature and information followed the most recent local flora (Kozloff 2005).

### Data Analysis

We analyzed the treatment responses of 23 key species of management concern (nine grasses and fourteen forbs) that occurred in ≥ 66% of the sampling quadrats in ≥ 1 site (species listed in Tables 4 and 5). We included both problematic non-native taxa and native taxa of

concern due to rarity, use by wildlife, or other restoration goals. Initial exploration of the data found no effect of seeding on the non-sown (resident) species. Only one sown species, *F. roemerii*, was included in our analysis of individual species responses, and initial analysis showed that seeding had no significant effect on the cover of this species due to poor establishment and slow growth of seedlings. Therefore, for all key species, we used the average of the four sampling quadrats per plot. Cover data for 2009 and 2005 were arcsine-square root transformed ( $Y = \sin^{-1}(\sqrt{(0.01 * \text{Cover})})$ ) prior to analysis to meet assumptions of normality.

We tested for treatment effects on the 2009 cover of each species using ANCOVA, accounting for pre-treatment differences by using the 2005 cover as a covariate. This allowed us to report 2009 cover values, and where applicable their significant differences between treatments, rather than percent or absolute changes in cover during the study period. We also used a model simplification process, starting with Site, Treatment, and Site × Treatment interaction as fixed effects. Terms were dropped from the model until the

TABLE 4. Effects of site, treatments, and pre-treatment cover (2005) on 2009 cover of native and exotic grasses using ANCOVA. Non-significant (n.s.) terms were dropped from the analysis. If significant site × treatment interactions were found, sites were analyzed separately or grouped by common response. Species provenance and duration are indicated (E = Exotic; N = Native; P = Perennial; A = Annual). See Table 1 for explanation of site codes.

Species	Common name	Sites analyzed	Site	P-values Treatment	Cover 05
<i>Agrostis</i> spp. (EP) <sup>1</sup>	bentgrass	BF	--	<0.0001	n.s.
		GHP	--	<0.0001	n.s.
		MP	--	<0.0001	n.s.
		TP	--	<0.0001	n.s.
		SW	--	<0.0001	n.s.
<i>Anthoxanthum odoratum</i> (EP)	sweet vernal grass	CGOP	--	<0.0001	<0.0001
		GHP	--	0.006	n.s.
<i>Arrhenatherum elatius</i> (EP)	tall oatgrass	MMP	--	0.003	0.008
		PB	--	<0.0001	n.s.
		SC	--	<0.0001	n.s.
<i>Bromus hordeaceus</i> (EA)	soft brome	FH	--	<0.001	0.004
<i>Dactylis glomerata</i> (EP)	orchard grass	CGOP	--	<0.001	n.s.
<i>Holcus lanatus</i> (EP)	velvet grass	FH & PB	n.s.	<0.001	0.002
<i>Poa pratensis</i> (EP)	Kentucky bluegrass	CGOP, MMP, SC	<0.001	0.003	0.020
<i>Carex inops</i> (NP)	long-stolon sedge	CGOP	--	<0.001	0.084
		GHP & SW	n.s.	0.001	0.002
		MP, SC, & TP	0.051	0.431	0.007
		MMP, MP, & TP	<0.0001	<0.0001	<0.0001
<i>Festuca roemerii</i> (NP)	Roemer's fescue	SC	--	<0.0001	n.s.
		SW	--	0.009	<0.001

<sup>1</sup>*Agrostis* spp. is mostly *A. capillaris*, a non-native turf grass, but may include some of the native perennial grass *A. pallens*.

TABLE 5. Effects of site, treatments, and pre-treatment cover (2005) on 2009 cover of native and exotic forbs using ANCOVA. Non-significant (n.s.) terms were dropped from the analysis. If significant Site  $\times$  Treatment interactions were found, sites were analyzed separately or grouped by common response. Species provenance and duration are indicated (E = Exotic; N = Native; P = Perennial; B=Biennial; A = Annual). See Table 1 for explanation of site codes.

Species	Common name	Sites analyzed	Site	P-values Treatment	Cover 05
<i>Daucus carota</i> (EB)	wild carrot	PB	--	0.002	0.012
<i>Hypochaeris radicata</i> (EP)	hairy cat's ear	BF & MMP	<0.0001	<0.0001	n.s.
		FH & TP	0.086	<0.0001	n.s.
		MP & SW	0.006	<0.0001	n.s.
		GHP	--	<0.0001	n.s.
<i>Leucanthemum vulgare</i> (EP)	ox-eye daisy	BF, FH, GHP, MMP, MP, TP, SW	<0.0001	<0.0001	<0.0001
<i>Galium divaricatum</i> (EA)	Lamarck's bedstraw	BF, FH, PB	0.006	<0.0001	n.s.
<i>Calystegia atriplicifolia</i> (NP)	nightblooming false bindweed	PB	--	0.12	<0.001
<i>Camassia quamash</i> (NP)	small camas	CGOP, GHP, MMP, MP,	<0.0001	0.010	<0.0001
		SC, TP, SW			
<i>Campanula rotundifolia</i> (NP)	bluebell bellflower	MMP	--	0.08	n.s.
<i>Dodecatheon hendersonii</i> (NP)	shooting star	CGOP	--	<0.001	<0.001
<i>Fragaria virginiana</i> (NP)	wild strawberry	BF & PB	--	<0.0001	<0.0001
<i>Prunella vulgaris</i> (NP)	common self-heal	MMP	--	0.002	0.034
<i>Viola adunca</i> (NP)	hookedspur violet	SC	--	0.43	n.s.
<i>Lotus micranthus</i> (NA)	desert deervetch	BF, GHP, FH, PB	<0.0001	0.884	n.s.
<i>Microsteris gracilis</i> (NA)	slender phlox	BF	--	0.51	n.s.
<i>Triphysaria pusilla</i> (NA)	dwarf owllover	BF	--	0.39	n.s.

minimal adequate model was obtained, using deletion tests with  $P < 0.05$  as the rejection criteria (Crawley 2009). If a significant Site  $\times$  Treatment interaction was found, we analyzed sites separately or grouped sites with similar responses. Where treatment was significant, we examined all pair-wise comparisons using Tukey's HSD. For some species, we aggregated non-significant levels of treatment in a stepwise *a posteriori* procedure to improve statistical power (Crawley 2009), as long as such aggregation did not significantly worsen the model fit to the data.

We also analyzed the effects of treatments and seeding on native richness (number of native species  $m^{-2}$ ) and cover of seeded forbs as a group (seeded grasses were excluded because of poor establishment at most sites). Native richness in 2009 was analyzed with a generalized linear mixed-effects model (GLMM) with Poisson errors, with Site, Treatment, and Seeding as fixed effects, Seeding within plot as the random effect to account for the split-plot design, and 2005 richness as a covariate to account for pre-treatment differences. Cover of seeded forbs in 2009 was analyzed with a linear mixed-effects model (LME) on arcsine-square

root transformed data, with the same fixed and random effects. As above, if a significant Site  $\times$  Treatment interaction was found, we analyzed sites separately or grouped by common response. All data analyses were performed in R 2.11 (R core development team, www.cran-r.org), using package lme4 (D. Bates and M. Maechler), for LME and GLMM.

## Results

### Non-native Grasses

Treatments had a significant effect on all non-native grass species examined, with SBG, SM, and MBG having the largest effects (Table 4, Figure 2). Some non-native grasses (*Agrostis* spp., *Anthoxanthum odoratum*, and *Arrhenatherum elatius*) varied among sites in their responses to the treatments, and thus each site was analyzed separately. *Arrhenatherum elatius* was the only non-native grass to show a consistent decline with MM, although this decline was significant at only 2 out of 3 sites, and the decline was not as large as the SBG, MBG, or SM treatments (Figure 2a). *Agrostis* spp. were reduced most substantially by the SBG treatment

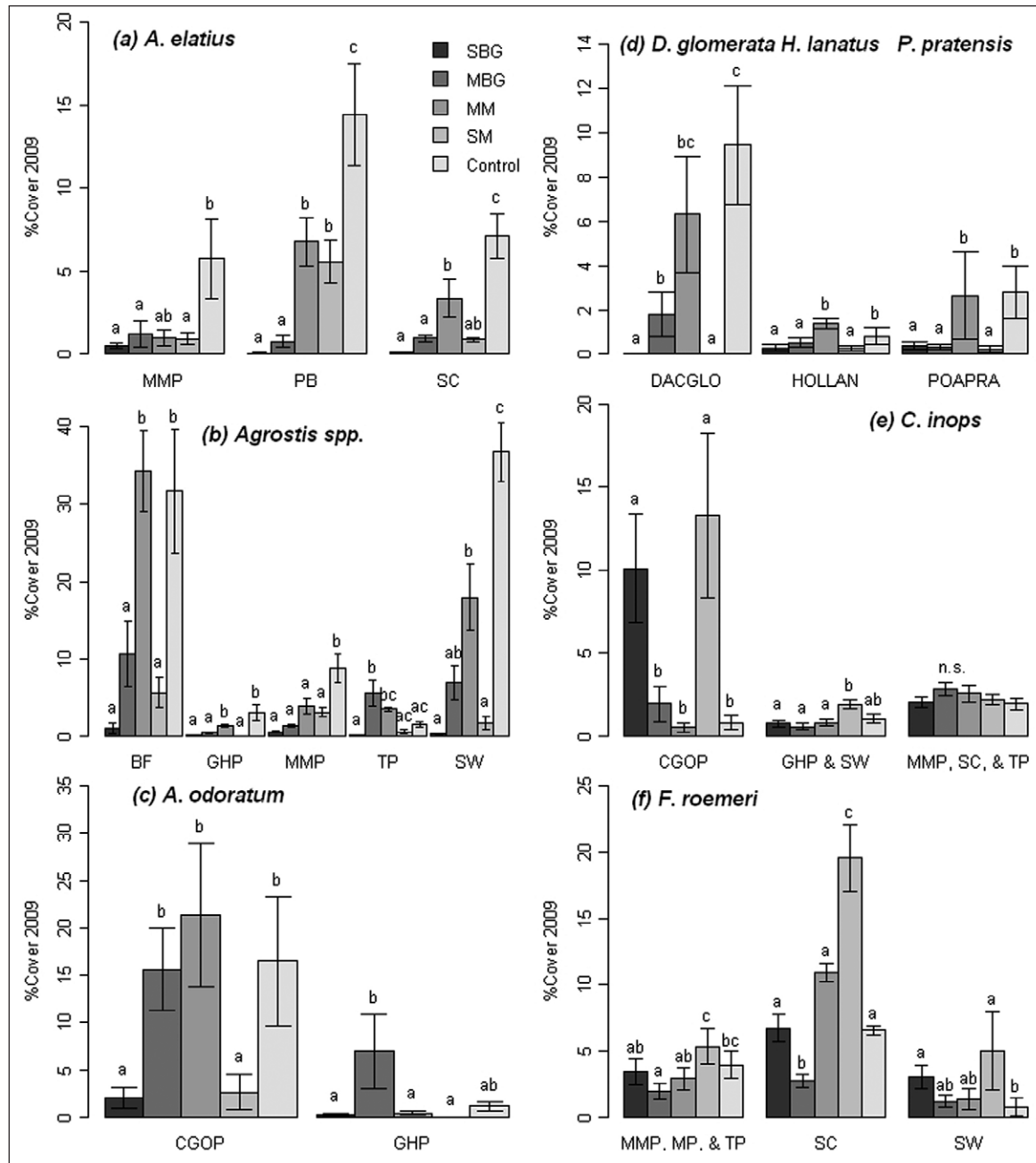


Figure 2. Percentage cover in 2009 of key grass and sedge species. Treatments with different letters indicate significant difference at  $P < 0.05$ . For each species, sites were grouped by common response to avoid significant Site  $\times$  Treatment interactions. Site name abbreviations and treatment codes are explained in Tables 1 and 2, respectively. Data represent means  $\pm$  1 SE.

(Figure 2b). MBG and SM also resulted in significant but generally smaller reductions in *Agrostis* spp. when compared with the controls, but MM had little or no effect on this species at 3 out of 5 sites (Figure 2b).

Treatment effects on *A. odoratum* could only be analyzed at two sites. At CGOP, where this species was abundant, the two sethoxydim treatments (SBG, SM) significantly reduced its mean cover (Figure 2c).

At GHP, where *A. odoratum* was much less abundant, cover in MBG was higher than all treatments but the controls.

*Poa pratensis* and *Holcus lanatus* both significantly decreased with SBG, MBG, and SM treatments, but not with MM (Figure 2d). *Dactylis glomerata* also exhibited this pattern, only differing in that it was significantly higher in MBG as compared to SBG and SM (Figure

2d). The only non-native annual grass analyzed, *Bromus hordeaceus*, was sufficiently abundant for analysis at only one site (FH). Non-significant treatment levels were aggregated to improve statistical power; we found that the cover in SBG, MBG, and SM (2009 mean  $0.05\% \pm 0.02$  SE) was significantly lower than in the MM treatment and controls (2009 mean  $3.64\% \pm 1.73$  SE; Table 4).

#### Native grasses and sedges

Only one native sedge (*Carex inops*) and one native grass (*F. roemerii*) were sufficiently common for statistical analyses, and both are resistant to sethoxydim. We grouped sites for both species by common responses to eliminate Site  $\times$  Treatment interactions (Table 4). *Carex inops* responded strongly to treatments at CGOP, where it increased from 0.8% in the controls to 10% in the SBG and 13% in the SM treatments (Figure 2e). At all other sites, none of the treatments led to changes in *C. inops* that were significantly different from controls. *Festuca roemerii* increased significantly in the SM treatment compared to controls at both SC and SW but not at the other sites (Figure 2f). At SW, *F. roemerii* also increased with the SBG treatment. At all sites except SW, *F. roemerii* declined in the MBG treatment. Mowing had no effect on *F. roemerii*.

#### Non-native Forbs

We analyzed the responses of four non-native forb species (Table 5). For *Hypochaeris radicata*, we divided the sites into 4 groups because of significant Site  $\times$  Treatment interactions (Figure 3a, Table 5). Cover of *H. radicata* (at all sites except BF and MMP) was substantially lower in the 2 burn treatments (SBG and MBG) than controls in 2009 (Figure 3a) and higher in the SM treatment at 4 of 7 sites. Mowing (MM) had a positive effect on *H. radicata* at 5 sites.

We aggregated non-significant treatment levels for *Leucanthemum vulgare* to increase statistical power. All 7 sites could be analyzed together as the Site  $\times$  Treatment interaction was non-significant (Table 5). Cover of *L. vulgare* showed a striking pattern over the study period (Figure 4), declining sharply after burning + glyphosate in 2006, rebounding quickly, and then declining again after the second burn in 2008. In 2009, *L. vulgare* was lowest in SBG and MBG and highest in the SM treatment (Figure 4).

*Daucus carota* increased in the MM and SM treatments (Figure 3b, Table 5). The non-native annual *Galium divaricatum* increased with SBG and MBG (Figure 3c), exhibiting a short-term response to fire

that is typical of annuals. Cover of *G. divaricatum* increased to  $10.8\% \pm 1.7$  SE following the 2006 burn and then declined to only  $2\% \pm 0.5$  SE the following year (2008), while the unburned plots never exceeded 2.6% cover.

#### Native Forbs

We analyzed the responses of ten native forb species. We found significant treatment effects for four perennial native forbs (*Camassia quamash*, *Prunella vulgaris* var. *lanceolata*, *Fragaria virginiana*, and *Dodecatheon hendersonii*; Table 5). Three other native perennial forbs (*Calystegia atriplicifolia*, *Campanula rotundifolia*, and *Viola adunca*) showed no significant treatment effect (Table 5). The three native annuals analyzed (*Lotus micranthus*, *Microsteris gracilis*, and *Triphysaria pusilla*) were present at very low amounts (typically  $< 1\%$  cover) and none of them showed a significant treatment effect in 2009.

We were able to analyze all 7 sites together for *C. quamash*, as there was no significant Site  $\times$  Treatment interaction, and non-significant treatment levels were aggregated to increase power. *Camassia quamash* increased slightly in the burn treatments (mean cover of SBG and MBG: 2005,  $2.5\% \pm 0.28$  SE; 2009,  $2.9\% \pm 0.37$  SE) but declined in the other treatments (mean cover of SM, MM, and controls: 2005,  $2.7\% \pm 0.23$  SE; 2009,  $2.2\% \pm 0.23$  SE; Table 5).

*Prunella vulgaris* increased slightly but significantly in the burned plots (mean 2009 cover in SBG and MBG:  $0.6\% \pm 0.14$  SE) compared to MM, SM, and control (mean 2009 cover:  $0.2\% \pm 0.04$  SE; Table 5). *Dodecatheon hendersonii* was initially high in the SM and SBG plots, and did not change over the study. Cover of *D. hendersonii* also stayed constant over time in the MBG and MM treatments, but declined in the controls. Taking the 2005 pre-treatment differences into account, in 2009 all treatments were higher than the controls, but not different from each other (Figure 3d, Table 5). *Fragaria virginiana* was lower in the two burn treatments in 2009 (Figure 3e) because of a sharp decline after the 2008 burn (from  $17.9\% \pm 4.7$  SE cover in SBG and MBG in 2008 to  $2.4\% \pm 0.7$  SE in 2009). However, *F. virginiana* did not decline after the 2006 burn ( $13.8\% \pm 3.3$  SE cover in SBG and MBG in 2006 and  $13.4\% \pm 3.4$  SE in 2007).

#### Seed Addition

We analyzed native richness in 3 groups of sites because there were significant Site  $\times$  Treatment interactions (Table 6). Also, Treatment was collapsed to two levels



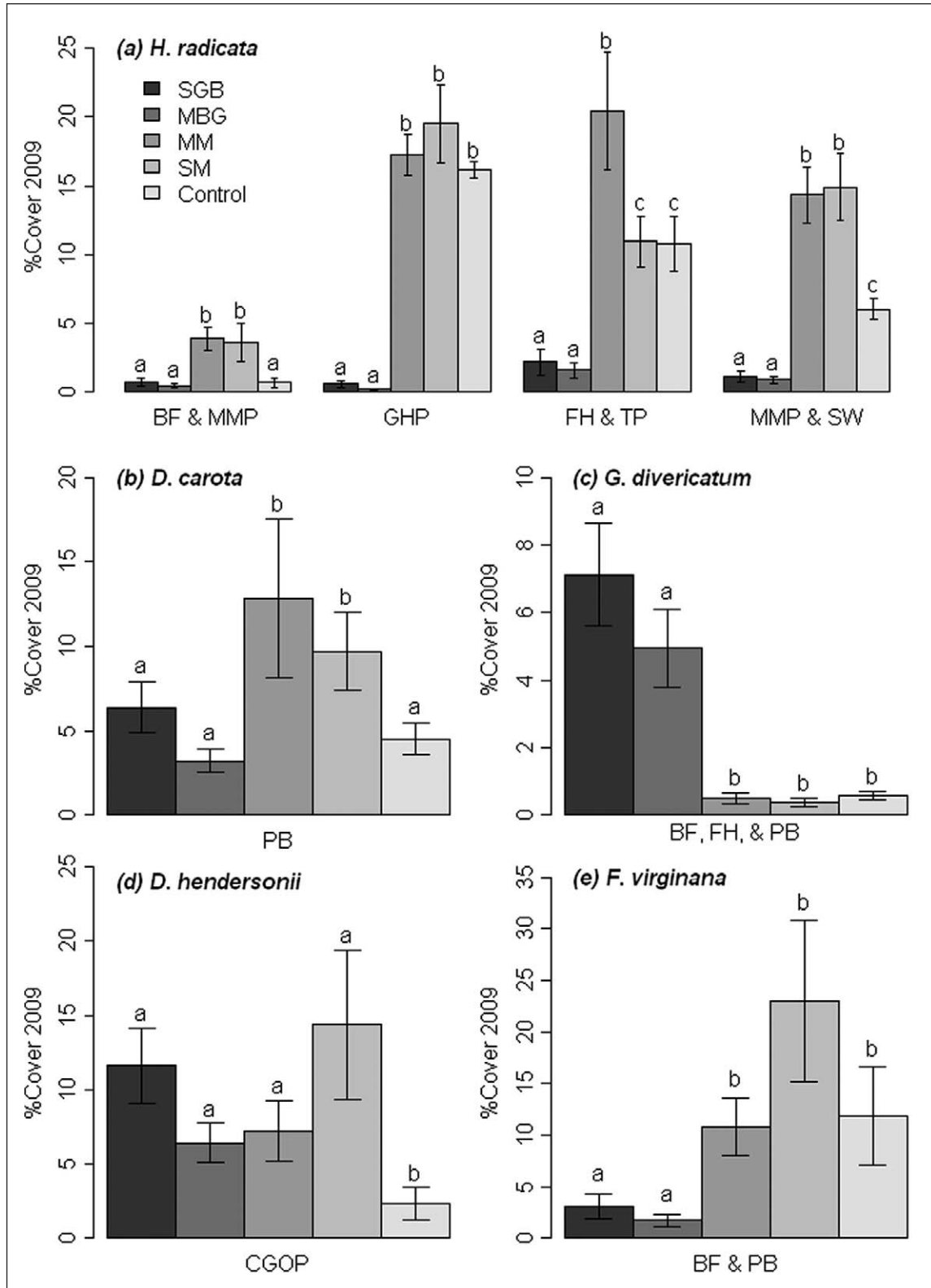


Figure 3. Percentage cover in 2009 of selected forb species. Treatments with different letters indicate significant difference in 2009 at  $P < 0.05$ . For *H. radicata*, sites were grouped by common response to avoid significant Site  $\times$  Treatment interactions. Site name abbreviations and treatment codes are explained in Tables 1 and 2, respectively. Data represent means  $\pm$  1 SE.

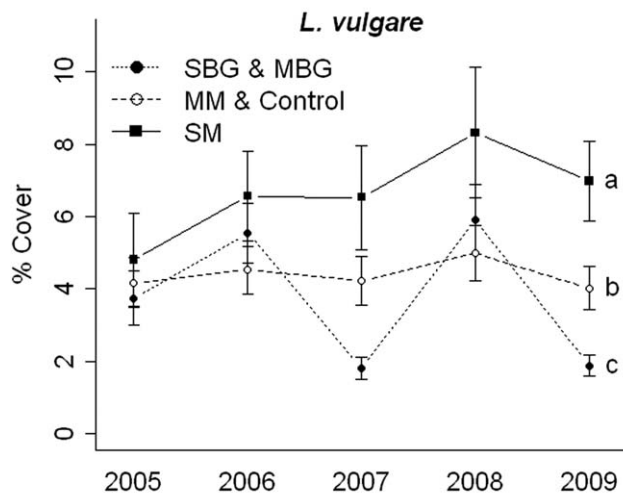


Figure 4. Percentage cover of *Leucanthemum vulgare* over the study period. All 7 sites can be summarized together as no site  $\times$  treatment interactions were found. Treatments were aggregated to improve statistical power. Letters indicate significant differences between aggregated treatments in 2009. Data represent means  $\pm$  1 SE.

(treatments with or without burning) as was Seeding (seeded in 2006, 2007, and both years vs. not seeded), as these were the only significant differences. While effects of treatments and seeding on native richness varied among sites, two common patterns emerge. First, we found that treatments alone did not lead to a substantial increase in native richness at any site (Figure 5a, 5b, Table 6). While Treatment had a significant effect on native richness at 3 sites (Figure 5b), this increase was small compared to the increase caused by seeding. Second, sites varied in their response to seeding without burning. At 5 sites (Figure 5a), the increase in native richness with seeding was the same across all treat-

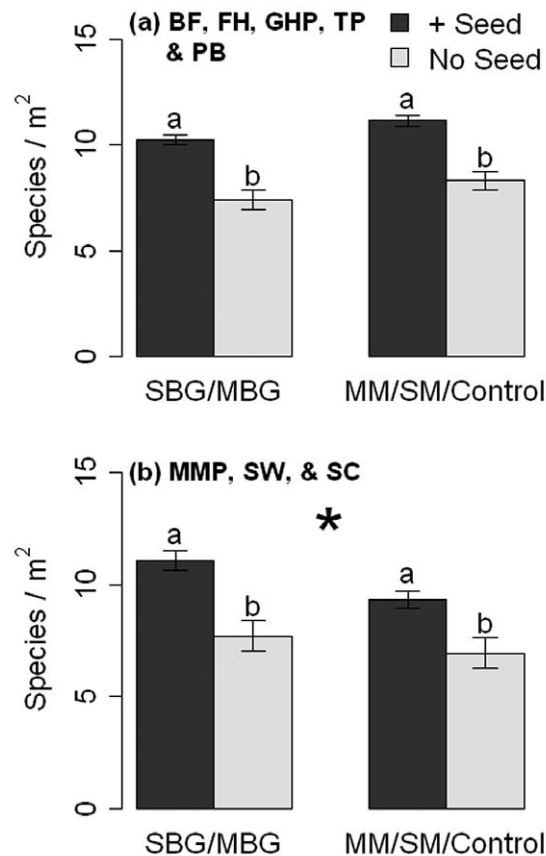


Figure 5. Number of native species per sampling quadrat (native richness) at (a) BF, FH, GHP, TP, and PB and (b) MMP, SW, and SC. Black bars = seeded in 2006, 2007, or both years; Grey bars = Not seeded. Letters indicate significant difference between seeding levels within a treatment; asterisk (\*) indicates significant difference between treatments. No Treatment  $\times$  Seeding interactions were found. Site name abbreviations and treatment codes are explained in Tables 1 and 2, respectively. Data represent means  $\pm$  1 SE.

TABLE 6. Effects of site, treatments (TRT)<sup>1</sup>, seeding (SD)<sup>2</sup>, and pre-treatment values (Cover 05) on native richness and cover of seeded forbs in 2009. Non-significant (n.s.) terms were dropped from the analysis. To eliminate Site  $\times$  Treatment interactions, sites were grouped by common response. Site abbreviations are described in Table 1.

Response	Sites analyzed	Site	TRT	SD	P-values		Cover 05
					Site $\times$ SD	TRT $\times$ SD	
Native richness	BF, FH, GHP, PB, TP	<0.0001	0.089	<0.0001	n.s.	n.s.	<0.0001
	MMP, SW, SC	0.045	<0.0001	<0.0001	n.s.	n.s.	<0.0001
	MP	--	0.53	0.168	--	n.s.	<0.0001
Seeded spp. cover	BF, FH	<0.0001	<0.0001	<0.0001	0.001	<0.0001	<0.0001
	GH, MMP, MP, SC, TP	<0.0001	<0.0001	<0.0001	n.s.	<0.0001	<0.0001
	PB	--	0.69	<0.0001	--	n.s.	<0.0001
	SW	--	<0.0001	<0.0001	--	0.041	0.038

<sup>1</sup> grouped as with (SBG and MBG) or without burning (SM, MM, and Control)

<sup>2</sup> For richness tests, SD was grouped as seeded in 2006, 2007, or both years vs. not seeded; for cover tests, SD was grouped as seeded in 2006 or both years vs. 2007 only or not seeded

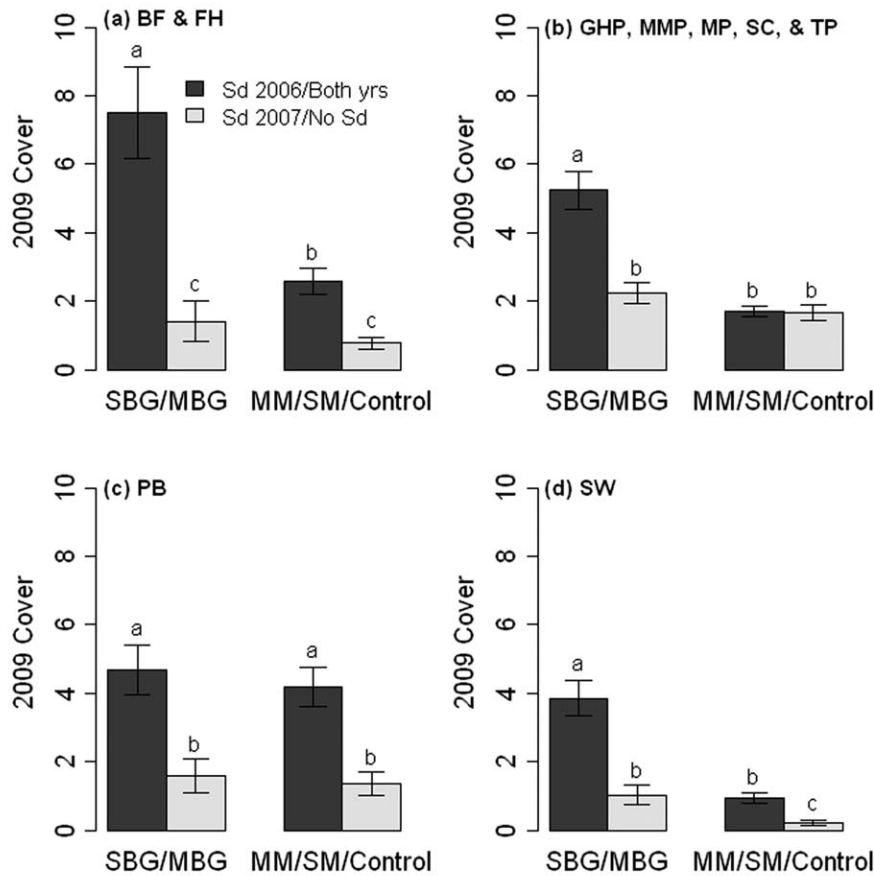


Figure 6. Percentage cover in 2009 of seeded forbs at (a) BF and FH; (b) GHP, MMP, MP, SC, and TP; (c) PB; and (d) SW. Black bars = seeded in 2006 or both years; Grey bars = seeded in 2007 or not seeded. Treatment  $\times$  Seeding interactions were significant at all sites except (c) PB, and thus letters indicate differences between both Treatment and Seeding levels. Site name abbreviations and treatment codes are explained in Tables 1 and 2, respectively. Data represent means  $\pm$  1 SE.

ments. At 3 sites (Figure 5b), seeding in the unburned treatments did not yield as large of an increase as in the burn treatments, although the increase was significant in all treatments. At one site, MP, neither seeding nor treatment affected native richness (Table 6).

As with native richness, we found significant Site  $\times$  Treatment interactions in the response of cover of seeded forbs, so we analyzed sites in 4 groups (Table 6). Similarly, Treatment was collapsed to two levels (with or without burning) as was Seeding (2006 or both years vs. 2007 or not seeded). The cover of seeded forbs increased with both treatment and seeding, although the responses were complex and varied between sites (Figure 6a through 6d, Table 6).

However, these complexities can be distilled down to a key conclusion: the cover of seeded forbs was greatest with the two burn treatments (MBG and SBG), but only

when seed was added immediately following the burn (the 2006 seeding). The sole exception to this is PB, the only site where seeding success was not affected by treatment (Figure 6c). As with the effects of seeding on native richness, treatments alone did not cause a substantial change in the cover of seeded forbs, and sites varied most in their response to seeding in the absence of fire. South Weir (SW) was the only site where the cover of seeded forbs increased significantly in response to burning treatments in the absence of seeding (Figure 6d, light grey bars), but this change was small compared to the effect of seeding.

## Discussion

### Non-Native Grasses

Three of our treatment combinations, SBG, SM, and MBG, proved highly effective at reducing cover of both perennial and annual non-native grasses, although there was substantial variation between species and sites. The grass-specific herbicide sethoxydim worked as anticipated in both the SBG and SM combinations by killing susceptible grasses. More surprising was the reduction in many non-native grasses by the MBG treat-

ment; we noted that many non-native grasses greened up rapidly after fire and were thus susceptible to the post-fire glyphosate application. The one exception to this was *A. odoratum*, which increased with MBG at one site (Figure 2c), indicating that this fire tolerant species (Tveten 1997, Anzinger and Radosevich 2008) may increase after burning without sethoxydim.

Mowing alone (MM) did not affect most of the non-native grasses we examined, with the exception that it reduced *A. elatius* and *Agrostis* spp. at some sites (Figure 2a,b). While mowing has been shown to reduce *A. elatius* at least temporarily (Wilson and Clark 2001), the turf grass *Agrostis* is typically considered resistant to mowing. The presence of the native *A. pallens*, which at the time of sampling was indistinguishable from the non-native *A. capillaris*, may be confounding our results. If *A. pallens* declines with mowing, that could explain the pattern we observed. The two sites that showed a

decline with mowing (MP and SW) had both species present. *Agrostis pallens* was not present at BF or TP, neither of which showed a decline with mowing.

#### Native Grasses and Sedges

*Carex inops* showed little response to treatment at most sites, similar to the neutral response to burning reported by Schuller (1997) (Table 4). The different response at CGOP (Figure 2e) could be because *C. inops* is better able to respond to removal of dominant grasses in CGOP's relatively cool and shady climate. *Festuca roemerii* was most abundant at SC, and here we saw it increase in the SM treatment (Figure 2f), suggesting that the release from competition by *A. elatius* (the dominant species prior to treatment) allowed this native fescue to increase. Because *F. roemerii* is known to be temporarily set back by burning (Tveten 1997, Schuller 1997, Dunwiddie 2002), we were not surprised to see it reduced in the MBG treatment at 4 out of 5 sites. The cover of *F. roemerii* in SBG was the same (4 sites) or higher (SW) than controls in 2009, suggesting that an increase in cover from removal of non-native grasses was offset by a reduction following burning.

#### Non-Native Forbs

Non-native perennial forbs were often the species that had the strongest short-term increase following removal of non-native grasses. All three perennial forbs we examined – *H. radicata*, *L. vulgare*, and *D. carota* – increased in the SM treatment at all or most sites (Figure 3, 4). Both *H. radicata* and *D. carota* also increased with mowing at some sites, indicating that the reduction in thatch (Stanley et al. 2011) as well as invasive grasses (Figure 2) is beneficial to these species. In contrast, both the SBG and MBG treatments were effective at most sites in reducing *H. radicata* and *L. vulgare*, the most abundant non-native forbs in our study. These results suggest that the broad-spectrum herbicide glyphosate can carefully target newly emerging non-native forbs when applied after burning.

While the full time series for *H. radicata* is not shown, both *H. radicata* and *L. vulgare* (Figure 4) exhibited large declines in SBG and MBG treatments in the spring following each burn. However, this decrease lasted for only one year after the first burn (Figure 4). Adult plants were killed, but a flush of seedlings of both species, likely arising from the seedbank or dispersing into the plots from large infestations just outside, resulted in rapid reinvasion. We believe the burn-glyphosate combination will be more effective when applied over

larger scales to reduce edge effects, and with repeated treatments that reduce seedbanks.

*Daucus carota* was not reduced by SBG and MBG (Figure 3c) compared to the control, perhaps because the species emerged after glyphosate application. Alternatively, this species may have some resistance to glyphosate, a possibility suggested by local managers. *D. carota* is known to develop resistance to other herbicides (Heap 1997).

The non-native annual *G. divaricatum* exhibited short-term increases after fire (Figure 3d). This mirrors the pattern we saw for non-native annuals as a whole – we saw large increases in this functional group following burning, but their abundance did not persist (Stanley et al. 2011, Stanley et al. 2010). This pattern of short-term annual dominance following burning has been seen elsewhere in this ecoregion (Dunwiddie 2002). Post-fire glyphosate treatment probably does not impact non-native annual plants because they germinate later in fall or winter after the glyphosate application.

#### Native Forbs

As a group, native forbs showed little response to treatments (Stanley et al. 2010, 2011). Six of the species analyzed here showed no response to treatment and the rest showed mostly modest changes. For example, we found a significant but very small increase in *C. quash* in SBG and MBG. Other authors have reported an increase in frequency (Schuller 1997, Storm and Shebitz 2006) or cover (Dunwiddie 2002) of *Camassia* species after a single fall burn, but results appear more mixed following repeated burns (Schuller 1997, Dunwiddie 2002, Beckwith 2004).

This poor response of native forbs after successful reduction of dominant non-native grasses was surprising. Several factors may account for this result. First, many native species appear to be seed limited (see following section). Second, because the cover of many native forbs was initially low, detecting a significant change may be difficult. Third, native forbs may respond with increased flowering, not with increased foliar cover (e.g., Dunwiddie 2002). And fourth, it may take multiple burns or herbicide applications over many years to result in enough new recruitment and growth to be reflected in a significant increase in native forb cover. While Tveten (1997) and Tveten and Fonda (1999) concluded that most native species in Washington prairies are fire neutral based on data from a single burn, Dunwiddie (2002) concluded that many native forbs increased in cover with repeated burning.

We found a negative effect of SBG and MBG on *Fragaria virginiana* (Figure 3e), which declined following the 2008 but not the 2006 burn. *F. virginiana* was still green at the time of burning, so it is unclear whether it was harmed more by variations in fire intensity or differences in timing of glyphosate application.

### Seed Addition

In our study, we found that treatment combinations alone caused no increase, or a very small increase, in the number of native species; only seeding substantially increased native richness (Figure 5). New native species did not appear after weed removal, confirming studies showing the native seed bank is completely lacking at most of the existing prairie remnants (Andreu 2005). Seeding also led to an increase in the cover of seeded forbs in combination with the burn treatments (Figure 6). Seeding without burning provided some gains at a few sites, but the increase in cover was small compared to seeding with burning. One possible mechanism for this is that burning leads to large reductions in litter and moss and an increase in bare soil, which creates sites suitable for seed establishment (Romo 2010; Stanley et al. 2010, 2011).

We conclude that most native species in our study failed to produce seed in sufficient quantities to readily fill open microsites when they are created, a common finding in many systems (Foster and Tilman 2003, Martin and Wilsey 2006, Clark et al. 2007). Instead, these gaps tended to be rapidly filled by non-native species. For example, at most sites we saw a flush of non-native annuals following burning (Figure 3c, Stanley et al. 2011). While native annuals can show a similar response when locally abundant (Dunwiddie 2002) or seeded (Stanley et al. 2011), this group is currently largely lacking from the flora in these prairie remnants (Dunwiddie et al. 2006).

### Management Recommendations

Treatments that include grass specific herbicide and/or burning followed by glyphosate application (SBG, MBG, and SM) show particular promise for restoration. Although burning has a unique set of challenges, when done at large scales, it can be very cost-effective (Stanley 2010). Which treatment combination is the most suitable for a particular site depends to a large extent on initial conditions and ecological goals.

Grass-specific herbicide combined with mowing (SM) reduced non-native grasses, although non-native forbs increased and native seed establishment was poor. This combination may be useful at sites with burning

restrictions. However, susceptible native grasses may be lost and will be difficult to re-establish from seed without additional treatments to increase bare soil. It may be most appropriate for sites that retain good native diversity and abundance, have a low abundance of exotic forbs, and where the most pressing problem is the dominance of exotic perennial grasses.

The SBG and MBG treatments worked well to control both non-native grasses and forbs and prepare a seed bed. Seeding success was highest in these two treatment combinations, and led to an increase in both native richness and native cover. The MBG treatment works nearly as well as SBG as long as *A. odoratum* is not present, and has a cost savings over SBG in that 3 fewer herbicide applications are required (Stanley 2010). One burn followed by glyphosate provided some gains, but the non-native perennial grasses still had a significant presence and we saw a flush of non-native forb seedlings. The second burn + glyphosate reduced the perennial grasses substantially as well as the non-native forbs (Stanley 2010). Because of this, if managers are unsure whether they will be able to successfully implement 2 burns, we would recommend using sethoxydim as well.

We do not recommend mowing alone, except as a method of controlling woody plants (Ussery and Krannitz 1998), as our mowing treatment (MM) did not control most herbaceous weeds, did not increase seeding success, and was costly compared to other treatments (Stanley 2010). If other management options are not available, mowing can reduce *A. elatius*, albeit temporarily. For this purpose, we recommend a high mow in mid-season to cut off flowering culms of this species and avoid most native plants (Wilson and Clark 2001).

We strongly recommend that measures to control non-native species include seeding of native plants. Seeding, in turn, will be more successful following prescribed fire, a finding substantiated elsewhere (Maret and Wilson, 2000, Maret and Wilson 2005). Because most native species are dormant in the fall or are slow to green up after fire, the burn + glyphosate combination has minimal impacts to seeded species. If seed availability is limited or if there is concern about harming seeded species, seeding after the second burn would likely be adequate. If burning is not possible, mowing is not an effective substitute to improve seeding success because it does not increase bare soil (Stanley et al. 2011). We recommend exploring other alternatives to burning, such as grazing or dethatching, to increase bare soil for seedling establishment (Gibson et al. 1987, Tix and Charvat 2005).

## Future Research Needs

Our observations indicate that some of these treatments could be further refined; for example, we believe the initial mow could be left out of the MBG treatment. The use of grass-specific herbicides could be improved by exploring application timing, frequency, and active ingredient. Limited testing on *A. elatius* suggests fluazifop may be more effective than sethoxydim (The Nature Conservancy 2008). However, alternating between several herbicides may be advantageous to prevent inadvertent selection of resistance to a single chemical (Diggle et al. 2003).

More investigation is needed on developing effective seed mixes, determining seeding rate, and improving methods for seedling establishment. Our experiment used a very small selection of potential species, and did not test different sowing methods; for example, drill seeding may significantly enhance germination as compared to the broadcast seeding used in our study. Our results do underscore the importance of seed addition for increasing native diversity and abundance. Since native seed is expensive and in limited supply, refining seeding rate and method could help improve the allocation of resources to restoration.

This project has shown that large-scale collaboration between scientists and managers can result in innovative treatment combinations backed by experimental

rigor. Relying on any one treatment is likely to produce disappointing results. For example, widespread efforts to reintroduce fire into WPG prairie systems (Hammann et al. 2011) may succeed in controlling invasive shrubs, but may not achieve goals of increased native diversity unless accompanied by glyphosate application and native seeding. Only when prescriptions employ strategically selected treatment combinations which are repeatedly applied over time, is the likelihood of success significantly enhanced.

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