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# Integrated Management of Cheatgrass (*Bromus tectorum*) with Sheep Grazing and Herbicide

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**Abstract:** Cheatgrass (*Bromus tectorum* L.) is one of the most problematic weeds in western United States rangelands and sagebrush steppe. It responds positively to different forms of disturbance, and its management has proven difficult. Herbicide or targeted grazing alone often fail to provide adequate long-term control. Integrating both may afford better control by providing multiple stressors to the weed. We assessed herbicide application, targeted sheep grazing and integrated herbicide and grazing on *B. tectorum* and the plant community in rangeland in southwestern Montana from 2015 until 2017. Herbicide treatments included spring-applied (May 2015 and 2016) glyphosate, fall-applied (October 2015) glyphosate, imazapic and rimsulfuron, and spring-applied glyphosate plus fall-applied imazapic. Targeted grazing, consisting of four sheep/0.01 ha for a day in 5 m × 20 m plots (all vegetation removed to the ground surface), occurred twice (May 2015 and 2016). While no treatments reduced *B. tectorum* biomass or seed production, grazing integrated with fall-applied imazapic or rimsulfuron reduced *B. tectorum* cover from approximately 26% to 14% in 2016 and from 33% to 16% in 2017, compared to ungrazed control plots, and by an even greater amount compared to these herbicides applied without grazing. By 2017, all treatments except spring-applied glyphosate increased total plant cover (excluding *B. tectorum*) by 8%–12% compared to the control plots, and forbs were generally responsible for this increase. *Bromus tectorum* management is difficult and our results point to a potential management paradox: Integrating grazing and fall-applied herbicide decreased *B. tectorum* cover but did not increase native grass cover, while some herbicides without grazing increased native grass cover, but failed to control *B. tectorum*. Additional research is necessary to determine grazing strategies that will complement herbicide control of *B. tectorum* while also stimulating native grass recovery, but this initial study demonstrates the potential of integrated management of *B. tectorum* compared to grazing or herbicide alone.

**Keywords:** downy brome; integrated weed management; novel ecosystem; restoration; soil disturbance

## 1. Introduction

Disturbance alters plant communities by removing existing vegetation or changing abiotic conditions, facilitating plant invasions [1,2]. After disturbance, some communities require intervention to alter the trajectory toward a desired state, although returning to the original plant community may not be possible [3,4]. The most common method to alter the trajectory of plant communities and remove undesired weeds is via herbicide application [5–7]. However, mortality of targeted weeds can vary

based on abiotic conditions, growth rate, or life stage. Herbicides can also harm desired plants growing in the same community, especially closely related plants with the same life form as the weeds, such as rangeland grasses [8]. One approach to reduce undesired consequences of herbicide applications is to use herbicides that are selective. For example, the herbicide imazapic can control some weedy annual grass species with minimal impact to desired grass species [9,10]. Another approach is timing herbicide application to take advantage of different phenological stages of the weedy and desired species; ideally herbicides are applied when the desired species are dormant reducing off-target damage. For example, Morris et al. [11] determined that a spring application of glyphosate reduced the cover of cheatgrass (*Bromus tectorum* L., also known as downy brome) and had no effect on native grasses because of differences in growth stages or dormancy. Typically, managing weed populations to restore desired species is extremely difficult, and many populations persist despite repeated control efforts [12].

Another approach to alter the trajectory of a community and manage undesired species is targeted grazing with livestock. Kleppel and LaBarge [13] demonstrated that sheep grazing reduced purple loosestrife (*Lythrum salicaria* L.) cover by 41 percent and prevented it from flowering. Goats reduced yellow starthistle (*Centaurea solstitialis* L.) cover by 75% and seedheads by 94% with three years of grazing [14]. The efficacy and conservation benefits of targeted grazing can vary based on timing, duration, and intensity of grazing as well as the grazing species. High intensity spring grazing for short durations was most effective at reducing the abundance of the non-native annual grass medusahead (*Taeniatherum caput-medusae* L.), while native plant abundance increased for both sheep and cow grazing [15]. In a study on leafy spurge (*Euphorbia esula* L.), Rinella and Hileman [16] demonstrated that the overall effect of grazing on *E. esula* and native plants varied greatly depending on the timing, intensity, and frequency. Results suggested that removing small quantities of both native and invader biomass at early growth stages reduced the invader while increasing the resident species over time. These studies demonstrate the value of targeted grazing but also highlight that grazing timing, intensity, and frequency need to be defined for specific species and habitats.

Integrated weed management, designed to stress target plants at multiple life stages, is another approach to manage weeds [17] and achieve desired communities. Livestock grazing and herbicide can potentially be combined to provide multiple stressors and consequently improve weed management. Livestock can graze weeds in the spring, and then herbicide can be used to manage weeds that regrow later in the summer or fall when desired species are dormant. Alternatively, herbicide can be used to control early spring growth, and after an appropriate exclusion time (herbicide specific), livestock can graze the regrowth. Spring grazing combined with a fall application of 2,4-D reduced leafy spurge density more than either grazing or herbicide alone [18]. Similarly, spotted knapweed (*Centaurea stoebe* L.) rosette density was greatly reduced in plots with sheep grazing plus 2,4-D compared to individual treatments [19], and sheep grazing followed with fall imazapic application to leafy spurge sustained the productivity of desired plant biomass and prevented an increase of the leafy spurge seedbank [20].

Adopting an integrated weed management approach that uses grazing and herbicide application could improve the management of *B. tectorum*, one of the most problematic weeds in western North America. *Bromus tectorum* has a positive feedback with fire leading to its dominance in some areas [21–23], and the result is invasions covering millions of hectares of rangeland in the western USA [24]. Furthermore, *B. tectorum* is one of a few winter annual grasses in the western USA, a region that is dominated by shrubs and perennial grasses. The annual life history of *B. tectorum* allows it to utilize soil water early in the season, grow rapidly as temperatures warm, and complete its lifecycle while other species are still growing [25].

The dominance of *B. tectorum* across large areas of western USA has led to a plethora of research on *B. tectorum* management, yet this research has yielded little success in terms of controlling and reducing the extent and abundance of the species. A recent review of 119 journal articles by Monaco et al. [7] showed that while numerous control methods reduced *B. tectorum* biomass and cover in the short term (one year after treatment), only herbicide and revegetation were successful two years after

treatment. Further, some methods such as burning increased *B. tectorum* cover long-term. Thus, despite considerable research throughout the western USA, reliable and affordable long-term control measures have not been developed.

Glyphosate, imazapic, and rimsulfuron are commonly used herbicides for managing *B. tectorum* [26], and while these herbicides have varying levels of selectivity, applying them when native plants are dormant minimizes their impact on desired vegetation [11]. Espeland et al. [27] determined that a low dose of glyphosate applied in spring and fall did not harm native grasses and reduced *B. tectorum* cover in the first year after spraying, but a second fall glyphosate application failed to reduce *B. tectorum* cover or seed bank. Morris et al. [11], however, determined that spring-applied glyphosate alone reduced *B. tectorum* cover for two years, and combining with imazapic provided even greater control. Imazapic has been widely used [28–31] because it provides residual soil activity, which is important for controlling annual plants such as *B. tectorum*, with early post-emergent fall application recommended [29].

Similar to applying herbicide at optimum times to limit off-target impacts, targeted grazing of *B. tectorum* infestations is best performed in the spring when native plants are still dormant, though research is scant. For example, cattle grazing for two consecutive years greatly reduced *B. tectorum* biomass and its presence in the seedbank, however, this approach had spatial and temporal limits because of the logistical issues with managing the cattle (83 cow-calf pairs ha<sup>-1</sup>) [32]. These results highlight the need for research on integrated management techniques for *B. tectorum*.

The lack of consistent success with *B. tectorum* management, especially where it has become dominant after a disturbance, indicates additional research on integrated management of *B. tectorum* with a longer period of monitoring is warranted. Our research took advantage of a site where *B. tectorum* had recently become the dominant species as the result of a mechanical soil disturbance in sagebrush steppe rangeland in southwestern Montana, USA. We used targeted sheep grazing, with or without herbicide, to assess the impacts of these management practices on *B. tectorum* abundance and seed production, native species abundance, and changes to plant community structure. Specifically, we expected that integrated management with sheep grazing and herbicide would (1) suppress *B. tectorum* cover, biomass, and seed production more than grazing or herbicide applications alone, and (2) facilitate an increase in forbs and native grasses. We also expected that (3) grazing and herbicide treatments would begin to shift plant communities dependent upon grazing, herbicide mode of action, and timing of application.

## 2. Materials and Methods

### 2.1. Study Site

Research was conducted at the Montana State University Red Bluff Research Ranch (45.593° N, 111.628° W; hereafter Red Bluff), from 2015 to 2017. The soil was of the Nuley Rock outcrop complex, with a typical profile of sandy loam (0–10 cm), sandy clay loam (10–28 cm), gravelly sandy loam (28–61 cm), and gravelly coarse sand (61–107 cm) over unweathered bedrock [33]. Vegetation at the site was historically classified as *Festuca idahoensis* Elmer—*Agropyron spicatum* (i.e., *Pseudoroegneria spicata* (Pursh) Á. Löve) [34]. A moderate severity wildfire burned a portion of Red Bluff on 7 July 2012, and a bulldozer was used to create a fire-break to prevent the fire from spreading uphill. *Bromus tectorum* did not increase in canopy cover in the burned area compared to the unburned areas uphill; however, on the fire-break *B. tectorum* cover increased to approximately 18%, 33%, and 38% in 2013, 2014, and 2015, respectively, more than double the amount in the burned or unburned plots [35]. During the same time, native grasses in the burned and unburned plots averaged ~27% cover while on the fire break native grass cover was approximately one third of this [35]. Thus, at the beginning of our study in 2015, the fire-break was dominated by *B. tectorum*, but native grasses including *Hesperostipa comata* (Trin. and Rupr.) Barkworth, *Pascopyrum smithii* (Rydb.) Á. Löve and *P. spicata* were also present in small amounts [35].

## 2.2. Experimental Design

A nested design (herbicide nested within grazing) was used to assess effects of grazing, herbicide application and the combination of herbicide and grazing (Table 1). Sixteen plots (4 m wide by 20 m long) were established along a 500 m portion of the fire-break. Plots were randomly assigned to be either grazed by sheep or ungrazed.

**Table 1.** List of treatments applied to manage cheatgrass at Red Bluff, Montana.

Main Plot Treatment	Subplot Treatment	Treatment Code <sup>a</sup>	Date Applied	Number of Replicates
Ungrazed	None	U_C	NA	8
Ungrazed	Spring glyphosate	U_Sg	5/1/15; 5/3/16	4
Ungrazed	Spring glyphosate + fall imazapic	U_Sg_Fi	5/1/15; 10/15/15; 5/3/16	4
Ungrazed	Fall glyphosate	U_Fg	10/15/15	4
Ungrazed	Fall imazapic	U_Fi	10/15/15	8
Ungrazed	Fall rimsulfuron	U_Fr	10/15/15	4
Grazed	None	G_C	5/4/15; 5/19/16	8
Grazed	Fall glyphosate	G_Fg	10/15/15	8
Grazed	Fall imazapic	G_Fi	10/15/15	8
Grazed	Fall rimsulfuron	G_Fr	10/15/15	8

<sup>a</sup> C = control; U = ungrazed; S = spring; g = glyphosate; F = fall; i = imazapic; r = rimsulfuron; G = grazed.

## 2.3. Sheep Grazing

Targeted, high intensity grazing occurred twice for ~24 hours, first on 4–5 May 2015 and secondly on 19–20 May 2016, with four western whiteface sheep per plot (0.01 ha) until all forage was consumed (all vegetation removed to the ground surface). In 2015 sheep were non-pregnant, non-lactating mature ewes, approximately 62 kg, and in 2016 they were non-pregnant, non-lactating 12 month yearlings, weighing approximately 43 kg. Prior to being used in the experiment, sheep had been fed approximately 2.3 kg and 1.8 kg of second cutting grass/alfalfa hay per day, in 2015 and 2016, respectively. Portable electric fencing operated by a battery with a solar charger was installed around each grazed plot, with a 0.5 m buffer on all sides. After grazing in 2015, plots were divided into four subplots (4 m wide by 5 m long): Three subplots received one of three herbicide treatments and one subplot was not treated with herbicide.

## 2.4. Herbicide Application

Herbicide treatments included spring-applied glyphosate (Glyphomate®, 199.4 g ai ha<sup>-1</sup>, PBI/Gordon Corporation, 1217 West 12th St., Kansas City, MO, USA), fall-applied glyphosate (same rate as spring), fall-applied imazapic (Panoramic 2SL, 104.9 g ai ha<sup>-1</sup>, Alligare, LLC, 13 N. 8th St., Opelika, AL), fall-applied rimsulfuron (Matrix SG®, 48.3 g ai ha<sup>-1</sup>, du Pont de Nemours and Company, 1007 Market Street, Wilmington, DE, USA), and spring-applied glyphosate + fall-applied imazapic (same rates as above; Table 1). Herbicide was applied at a total volume of 178 liters ha<sup>-1</sup> with a CO<sub>2</sub> powered backpack sprayer at 45 psi, using TeeJet nozzles. A non-ionic surfactant was added to the tank at 0.01% volume/volume. Relative humidity and temperature at the time of spraying were 43% and 13.3 °C in the spring and 40% and 13.9 °C in the fall. Wind speeds averaged less than 5 km hr<sup>-1</sup> each year. Our original design was modified after high fall emergence of *B. tectorum* in 2015; in the ungrazed treatments we added a fall imazapic application to one half of the spring glyphosate subplots, and a fall-applied rimsulfuron treatment to one half of the subplots slated to receive glyphosate in the fall. This resulted in four replications for each of these treatments and fall glyphosate, while all other treatments had eight replications (Table 1). Herbicide application dates were 1 May 2015 (4–5 leaf stage) and 3 May 2016 (4–5 leaf stage) for the spring-applied glyphosate, and 16 October 2015 (2–4 leaf stage) for the fall-applied glyphosate, imazapic, and rimsulfuron. The sparse vegetation in fall 2016 did not warrant a second application of the fall herbicide treatments.

## 2.5. Data Collection

Within each subplot, four 1 m<sup>2</sup> frames were established for data collection. The four frames were situated such that there were 1 m and 0.5 m buffers from the short and long edges of the subplots, respectively. Canopy cover (%) of each individual grass species was estimated ocularly on 1 May 2015 (1st year pre-treatment), 24 June 2015 (1st year post-treatment), 18 May 2016 (2nd year pre-treatment), and 14 June 2016 and 15 June 2017 (2nd and 3rd years post-treatment). Additionally, after senescence each year, *B. tectorum* was harvested from two 1 m<sup>2</sup> areas within the subplots, with different areas being harvested each year to avoid sampling previously harvested areas. Biomass was dried at 40 °C for one week, weighed, and seeds were then separated from other plant material, weighed, and counted.

## 2.6. Statistical Analysis

Biomass, plant canopy cover (%) and seed weight were analyzed using linear mixed effects models using the package “lme4” [36] in the R statistical environment [37]. Residual versus fitted value plots from an analysis of non-transformed data revealed heteroscedasticity, and data were corrected/improved with a log transformation. The models included treatment (see Table 1, column 3) as the fixed effect, and the random effect terms were frame, nested within replicate, and location of the plot across the site. This latter term was included to account for heterogeneity of the slope and aspect across the site. For analysis of June 2015 and 2016 *B. tectorum* biomass, cover, and seed production, the cover of *B. tectorum* recorded during the preceding May sampling was included as a random effect in the model. Means separation was via the package “lmerTest” and function “diffsmeans”, which uses the Satterthwaite’s approximation for degrees of freedom and calculates the differences of the least squares means. Non-transformed means are presented in figures for ease of interpretation.

Differences in plant communities among the treatments were assessed by non-metric multidimensional scaling (NMDS) with the package “vegan” [38]. The function “adonis” was used for permutational multivariate analysis of variance of the distance matrices for the different treatments, using 1000 permutations. Species rank-abundance (based on cover) was calculated with the package BiodiversityR [39].

## 3. Results

### 3.1. Grazing

Grazing alone did not reduce *B. tectorum* biomass, cover, or seed production in 2015, 2016, or 2017 compared to the ungrazed controls (Tables 2–4, Figures 1–3). The only exception to this was during the initial year of the study when grazed plots averaged 34% cover compared to 49% cover in ungrazed plots (Figure 2). The overall effects of grazing are similar to the results from Bates and Davies [40] who determined that even long-term grazing did not alter cover and production of *B. tectorum* compared to ungrazed plots following wildfire. Our results differ, however, from those of Diamond et al. [32] who determined that *B. tectorum* seed production was decreased in grazed compared to ungrazed plots: We determined no differences, but grazing occurred twice within a season in their study and only once in ours. A second grazing of our plots, or grazing later in the season, could potentially have reduced *B. tectorum* seed production, but this could have been at the expense of native plant growth [16]. The window of opportunity at our site for grazing *B. tectorum* while desired species are dormant is minimal, emphasizing the difficulty in implementing prescribed grazing in this northern sagebrush steppe system.

**Table 2.** The response of *Bromus tectorum* biomass to grazing and/or herbicide treatments, analyzed using a linear mixed effects model. All treatments (parameters) are with respect to the ungrazed control treatment (U\_C), which is the intercept in the model. In 2015 only the grazed (G) and ungrazed control, and the spring applied herbicide treatment results are shown, because fall herbicide application had not yet occurred. Results are based on log transformed *B. tectorum* biomass.

Fixed Effects						Random Effects	
Year	Parameter <sup>a</sup>	Estimate	SE	t Value	Pr (> t ) <sup>b</sup>	Group	Variance
2015	Intercept	2.27	0.18	12.3	<0.001	pBROTE <sup>c</sup>	0.82
	G_C	-0.15	0.11	-1.35	0.181	Frame/Rep	0.07
	U_Sg	-0.76	0.18	-4.27	<0.001	Rep	0.00
						Location	0.01
						Residual	0.53
2016	Intercept	2.42	0.38	6.43	<0.001	pBROTE	0.88
	G_C	0.27	0.38	0.71	0.481	Frame/Rep	0.00
	G_Fg	0.20	0.34	0.58	0.564	Rep	0.11
	G_Fi	0.04	0.35	0.11	0.911	Location	0.49
	G_Fr	0.19	0.36	0.54	0.592	Residual	0.86
	U_Fg	-0.02	0.41	-0.05	0.962		
	U_Fi	0.33	0.35	0.95	0.345		
	U_Fr	0.91	0.44	2.06	<b>0.042</b>		
	U_Sg	0.11	0.44	0.25	0.800		
	U_Sg_Fi	0.41	0.43	0.96	0.341		
2017	Intercept	3.04	0.39	7.34	<0.001	Frame/Rep	0.00
	G_C	-0.36	0.32	-1.13	0.260	Rep	0.18
	G_Fg	-0.33	0.32	-1.03	0.305	Location	0.64
	G_Fi	-0.59	0.32	-1.88	0.063 *	Residual	0.89
	G_Fr	-0.14	0.32	-0.45	0.657		
	U_Fg	-0.67	0.39	-1.71	0.091 *		
	U_Fi	0.36	0.31	1.16	0.250		
	U_Fr	0.66	0.39	1.70	0.092 *		
	U_Sg	-0.11	0.39	-0.28	0.780		
	U_Sg_Fi	-0.09	0.39	-0.24	0.811		

<sup>a</sup> G\_C = grazed control, G\_Fg = grazed + fall glyphosate, G\_Fi = grazed + fall imazapic, U\_Fg = ungrazed + fall glyphosate, U\_Fi = ungrazed + fall imazapic, U\_Fr = ungrazed + fall rimsulfuron, U\_Sg = ungrazed + spring glyphosate, U\_Sg\_Fi = ungrazed + spring glyphosate + fall imazapic. <sup>b</sup> Bold values indicate significance at a *p* value of < 0.05. \* = significance at *p* value of < 0.10. <sup>c</sup> Pre-treatment amount of *Bromus tectorum* from May sampling in the respective year.

**Table 3.** The response of *Bromus tectorum* cover to grazing and/or herbicide treatments, analyzed using a linear mixed effects model. All treatments (parameters) are with respect to the ungrazed control treatment (U\_C), which is the intercept in the model. In 2015 only the grazed (G) and ungrazed control, and the spring applied herbicide treatment results are shown, because fall herbicide application had not yet occurred. Results are based on log transformed *B. tectorum* cover.

Fixed Effects						Random Effects	
Year	Parameter <sup>a</sup>	Estimate	SE	t Value	Pr (> t ) <sup>b</sup>	Group	Variance
2015	Intercept	3.67	0.14	25.4	<0.001	pBROTE <sup>c</sup>	0.65
	G_C	-0.32	0.04	-4.42	<0.001	Frame/Rep	0.22
	U_Sg	-0.25	0.11	-2.20	<b>0.029</b>	Rep	0.09
						Location	0.00
						Residual	0.50
2016	Intercept	2.97	0.17	17.5	<0.001	pBROTE	0.82
	G_C	-0.00	0.16	-0.02	0.987	Frame/Rep	0.00
	G_Fg	0.05	0.14	0.35	0.728	Rep	0.19
	G_Fi	-0.26	0.15	-1.70	0.090 *	Location	0.03



Table 3. Cont.

Fixed Effects						Random Effects	
Year	Parameter <sup>a</sup>	Estimate	SE	t Value	Pr (> t ) <sup>b</sup>	Group	Variance
2016	G_Fr	−0.21	0.15	−1.35	0.179	Residual	0.54
	U_Fg	0.03	0.18	0.16	0.875		
	U_Fi	0.10	0.15	0.60	0.551		
	U_Fr	0.35	0.19	1.87	0.063 *		
	U_Sg	−0.04	0.19	−0.19	0.848		
	U_Sg_Fi	−0.29	0.19	−1.55	0.123		
2017	Intercept	2.84	0.35	8.13	<0.001	Frame/Rep	0.00
	G_C	−0.34	0.21	−1.59	0.11	Rep	0.37
	G_Fg	−0.39	0.21	−1.84	0.07 *	Location	0.58
	G_Fi	−0.61	0.21	−2.91	<b>0.004</b>	Residual	0.854
	G_Fr	−0.47	0.21	−2.21	<b>0.028</b>		
	U_Fg	−0.32	0.27	−1.15	0.252		
	U_Fi	0.32	0.21	1.54	0.126		
	U_Fr	0.37	0.27	1.35	0.179		
	U_Sg	−0.16	0.27	−0.60	0.552		
	U_Sg_Fi	−0.38	0.27	−1.38	0.168		

<sup>a</sup> G\_C = grazed control, G\_Fg = grazed + fall glyphosate, G\_Fi = grazed + fall imazapic, U\_Fg = ungrazed + fall glyphosate, U\_Fi = ungrazed + fall imazapic, U\_Fr = ungrazed + fall rimsulfuron, U\_Sg = ungrazed + spring glyphosate, U\_Sg\_Fi = ungrazed + spring glyphosate + fall imazapic. <sup>b</sup> Bold values indicate significance at a *p* value of < 0.05. \* = significance at *p* value of < 0.10. <sup>c</sup> Pre-treatment amount of *Bromus tectorum* from May sampling in the respective year.

**Table 4.** The response of *Bromus tectorum* seed production to grazing and/or herbicide treatments, analyzed using a linear mixed effects model. All treatments (parameters) are with respect to the ungrazed control treatment (U\_C), which is the intercept in the model. In 2015 only the grazed (G) and ungrazed control, and the spring applied herbicide treatment results are shown, because fall herbicide application had not yet occurred. Results are based on log transformed *B. tectorum* seed production.

Fixed Effects						Random Effects	
Year	Parameter <sup>a</sup>	Estimate	SE	t Value	Pr (> t ) <sup>b</sup>	Group	Variance
2015	Intercept	0.67	0.19	3.49	<b>0.002</b>	pBROTE <sup>c</sup>	0.72
	G_C	0.31	0.16	1.92	0.057 *	Frame/Rep	0.00
	U_Sg	−0.87	0.26	−3.40	<b>0.001</b>	Rep	0.00
						Location	0.05
	Residual	0.78					
2016	Intercept	1.59	0.44	3.61	<b>0.003</b>	pBROTE	1.0
	G_C	0.37	0.46	0.80	0.43	Frame/Rep	0.00
	G_Fg	0.50	0.41	1.21	0.23	Rep	0.24
	G_Fi	−0.08	0.43	−0.19	0.85	Location	0.54
	G_Fr	0.16	0.44	0.37	0.72	Residual	1.05
	U_Fg	−0.00	0.51	−0.01	0.99		
	U_Fi	0.20	0.42	0.47	0.64		
	U_Fr	0.75	0.54	1.41	0.16		
	U_Sg	0.09	0.54	0.16	0.87		
	U_Sg_Fi	0.43	0.53	0.81	0.42		
2017	Intercept	2.30	0.43	5.38	<0.001	Frame/Rep	0.00
	G_C	−0.38	0.33	−1.15	0.251	Rep	0.20
	G_Fg	−0.28	0.33	−0.86	0.394	Location	0.70
	G_Fi	−0.62	0.33	−1.87	0.065 *	Residual	0.93
	G_Fr	−0.12	0.33	−0.37	0.715		
	U_Fg	−0.68	0.41	−1.65	0.102		

Table 4. Cont.

Fixed Effects						Random Effects	
Year	Parameter <sup>a</sup>	Estimate	SE	t Value	Pr (> t ) <sup>b</sup>	Group	Variance
2017	U_Fi	0.42	0.33	1.28	0.204		
	U_Fr	0.62	0.41	1.51	0.133		
	U_Sg	-0.09	0.41	-0.21	0.831		
	U_Sg_Fi	-0.09	0.41	-0.22	0.829		

<sup>a</sup> G\_C = grazed control, G\_Fg = grazed + fall glyphosate, G\_Fi = grazed + fall imazapic, U\_Fg = ungrazed + fall glyphosate, U\_Fi = ungrazed + fall imazapic, U\_Fr = ungrazed + fall rimsulfuron, U\_Sg = ungrazed + spring glyphosate, U\_Sg\_Fi = ungrazed + spring glyphosate + fall imazapic. <sup>b</sup> Bold values indicate significance at a p value of < 0.05. \* = significance at p value of < 0.10. <sup>c</sup> Pre-treatment amount of *Bromus tectorum* from May sampling in the respective year.

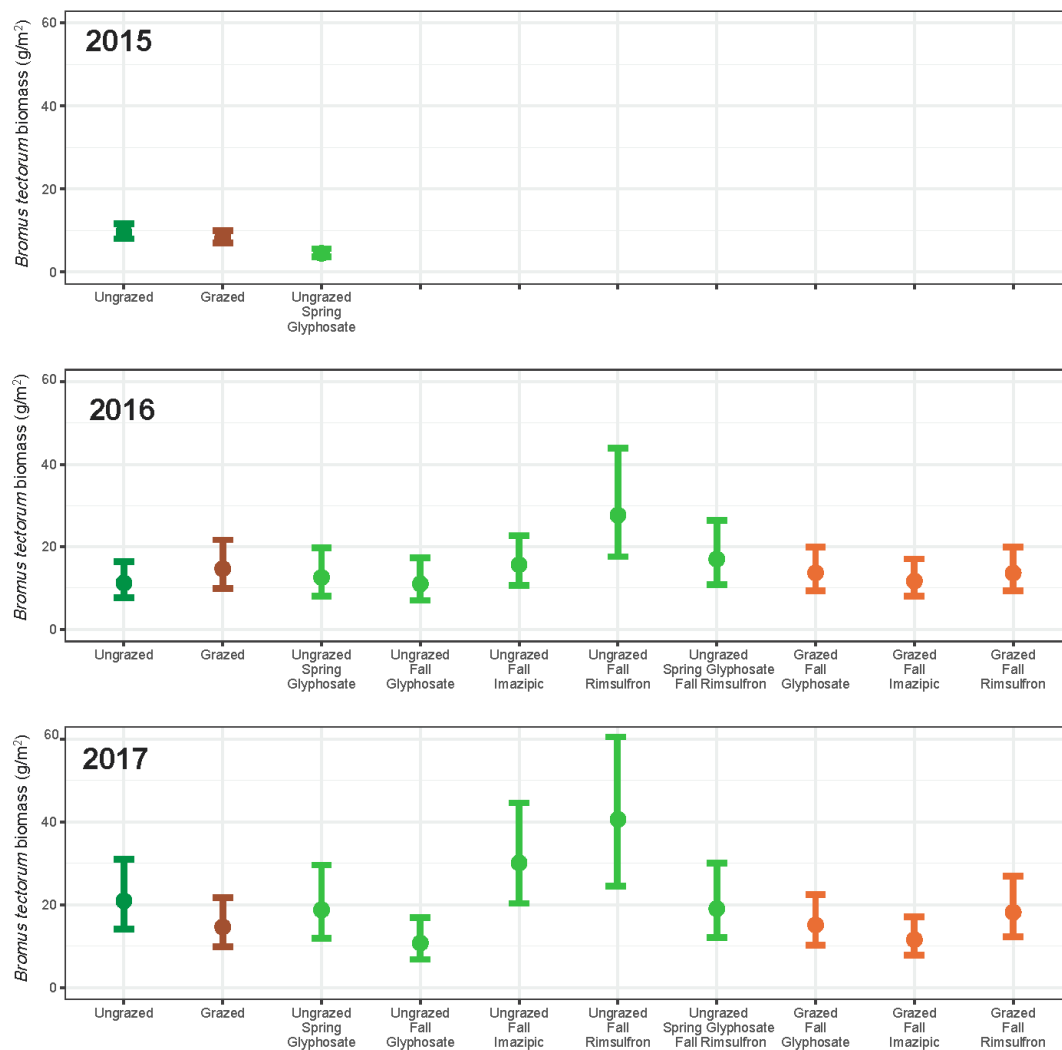
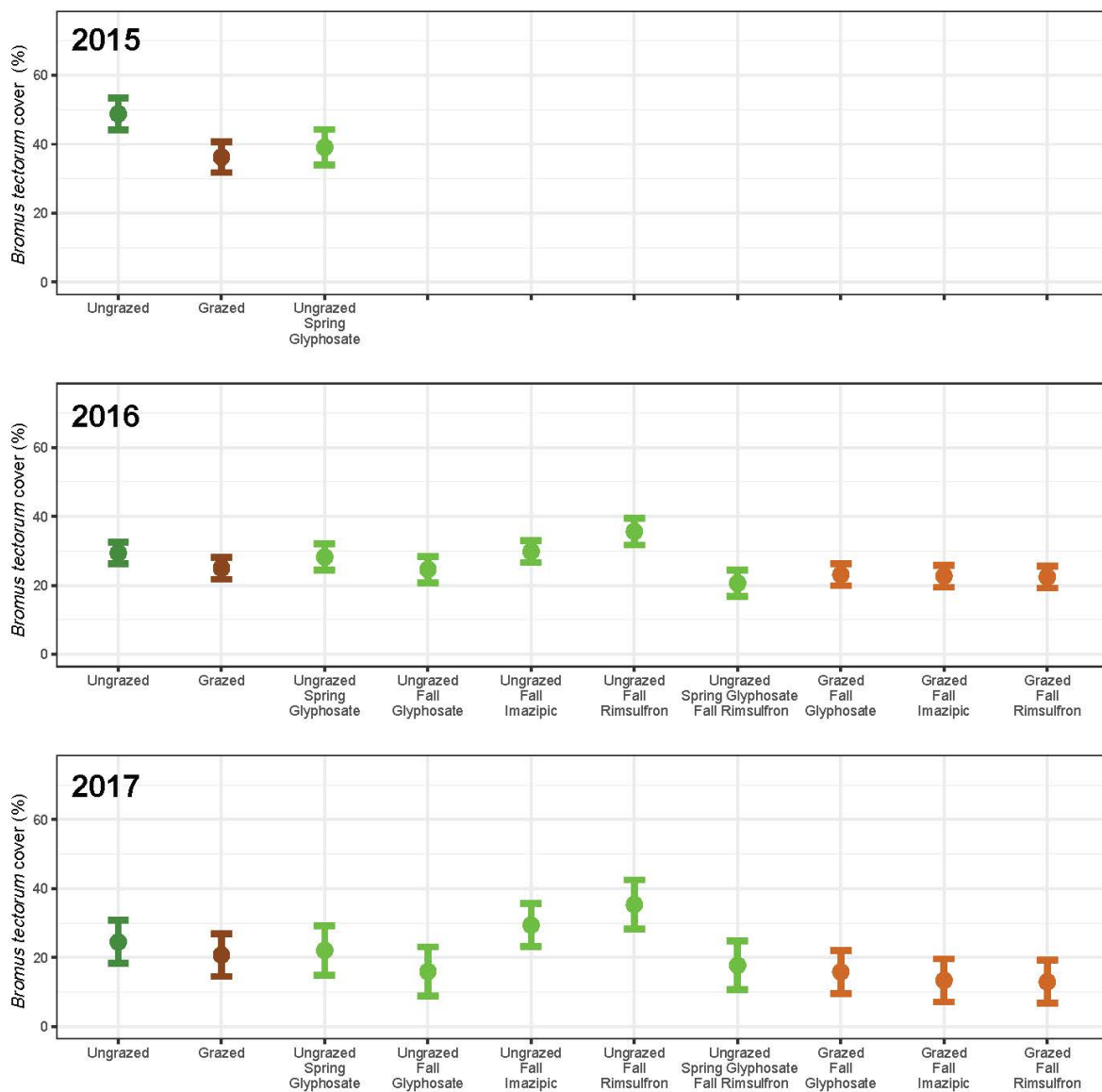
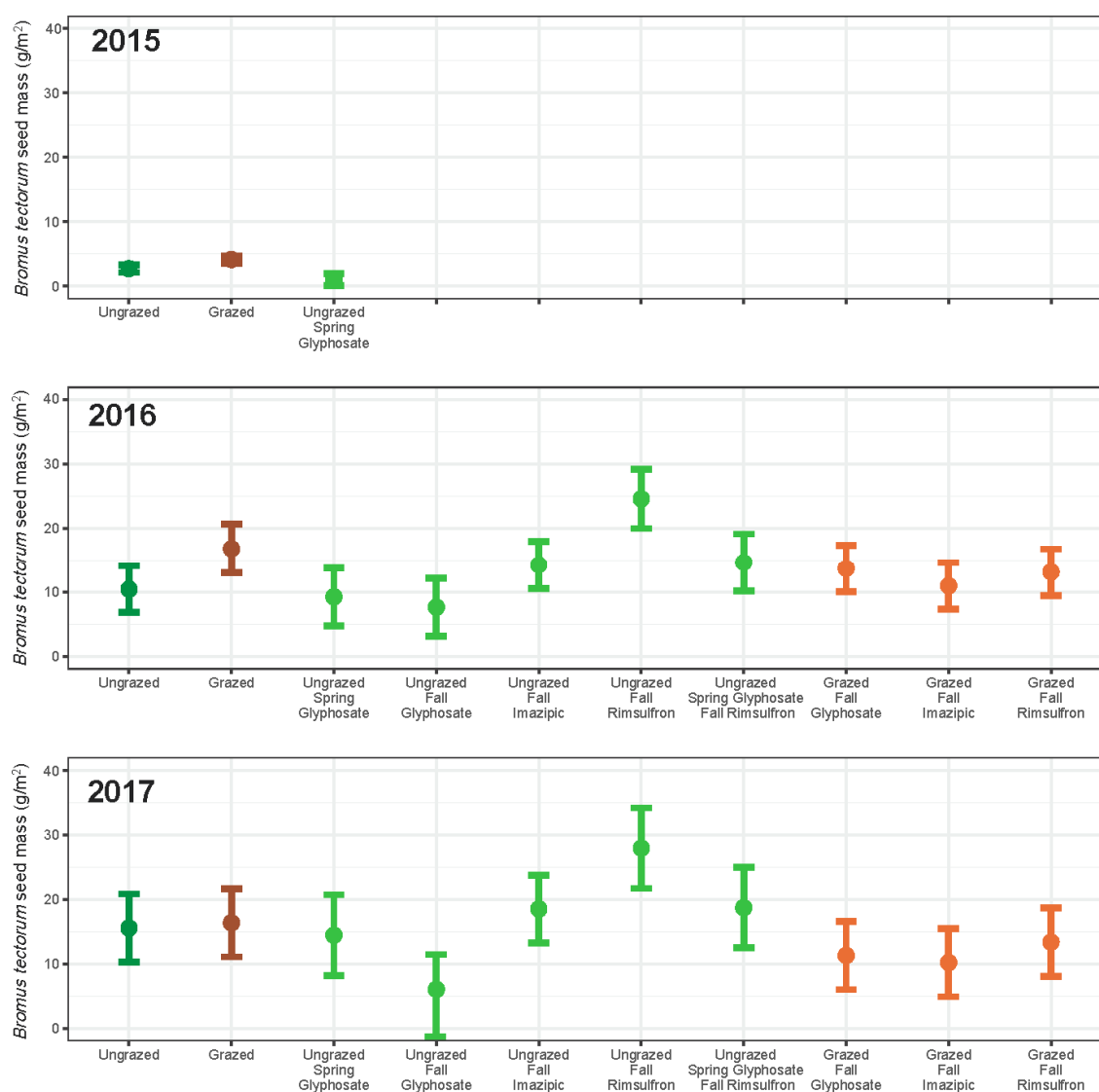


Figure 1. Fitted model results for *Bromus tectorum* biomass (g; mean ± SE) response to grazing, herbicide and integrated treatments at Red Bluff, Montana in 2015, 2016, and 2017. Fall herbicide treatments were implemented after the summer data collection in 2015, so these treatments do not show results in 2015. Furthermore, fall herbicide results in 2016 and 2017 represent treatments implemented in the prior years. Data shown are predicted values from linear mixed effects models, and thus the means are not identical to means from the raw data.





**Figure 2.** Fitted model results for *Bromus tectorum* cover (%; mean  $\pm$  SE) response to grazing, herbicide and integrated treatments at Red Bluff, Montana in 2015, 2016, and 2017. Fall herbicide treatments were implemented after summer data collection in 2015, so these treatments do not show results in 2015. Furthermore, fall herbicide results in 2016 and 2017 represent treatments implemented in the prior years. Data shown are predicted values from linear mixed effects models, and thus the means are not identical to means from the raw data.



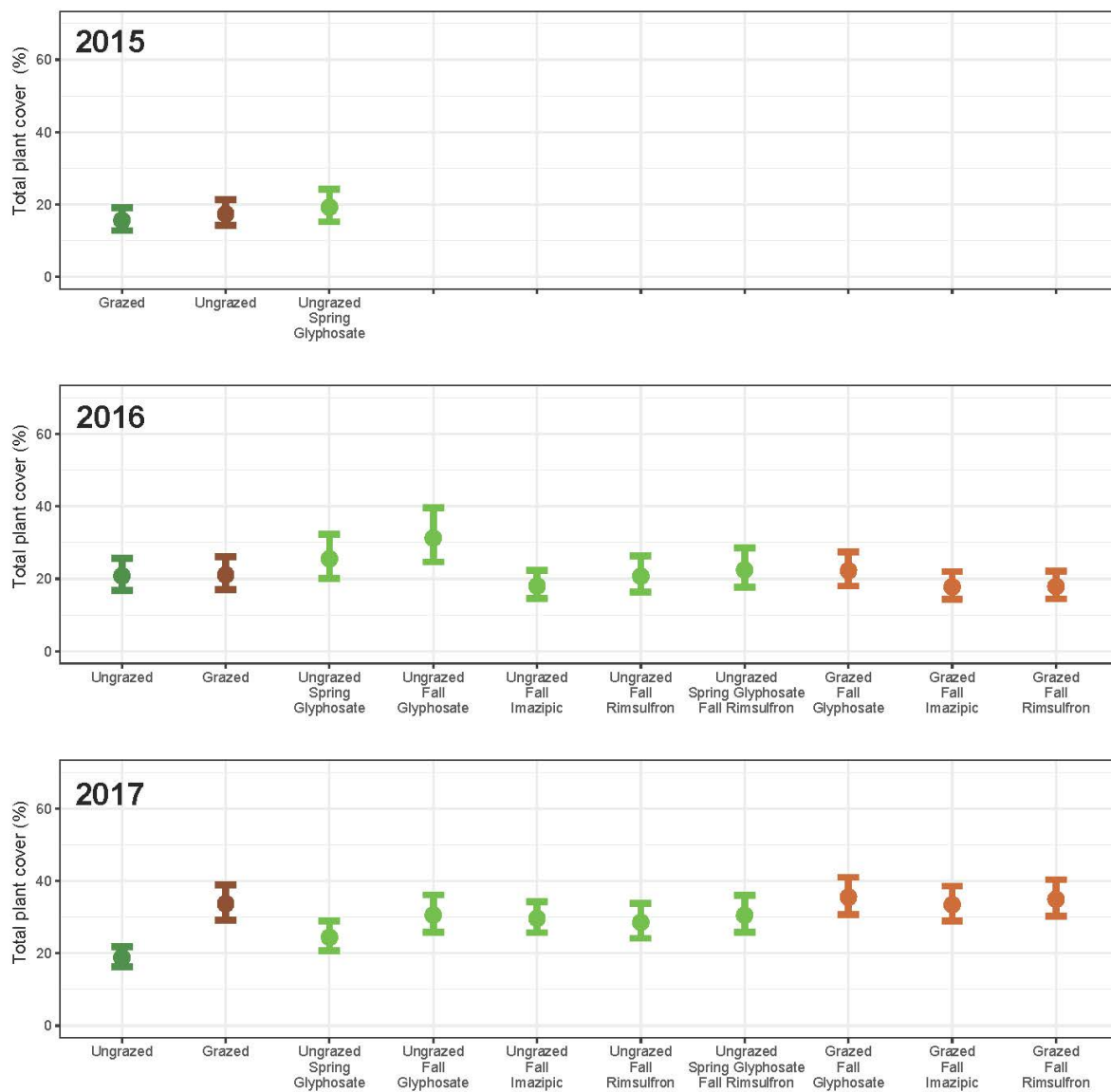
**Figure 3.** Fitted model results for *Bromus tectorum* seed weight (g; mean  $\pm$  SE) response to grazing, herbicide and integrated treatments at Red Bluff, Montana in 2015, 2016, and 2017. Fall herbicide treatments were implemented after the summer data collection in 2015, so these treatments do not show results in 2015. Furthermore, fall herbicide results in 2016 and 2017 represent treatments implemented in the prior years. Data shown are predicted values from linear mixed effects models, and thus the means are not identical to means from the raw data.

Total plant cover, excluding *B. tectorum*, did not differ between grazed controls (i.e., grazed but no herbicide treatment) and ungrazed controls in years when grazing occurred (2015 and 2016); however, total plant cover increased to 32% in 2017 in previously grazed plots compared with 22% cover in ungrazed control plots (Table 5; Figure 4). Forb cover was greater in grazed only plots each year (Table 6; Figure 5), and generally accounted for the increase in total plant cover. Native grass cover was reduced from 11% to 7% and 11% to 6% in 2015 and 2016, respectively, in the plots that were grazed and had no herbicide application compared to the ungrazed control, but in 2017 native grass cover in grazed plots increased and was similar to the ungrazed plots with no herbicide application (Table 7; Figure 6). Short duration, intensive grazing may reduce perennial grass cover and potentially favor forbs in semi-arid systems [41,42], while light grazing, even for several years may not reduce the cover of perennial grasses in the sagebrush steppe [40]. Our results indicate that native grass cover rebounded within a year after intensive, short duration, grazing.

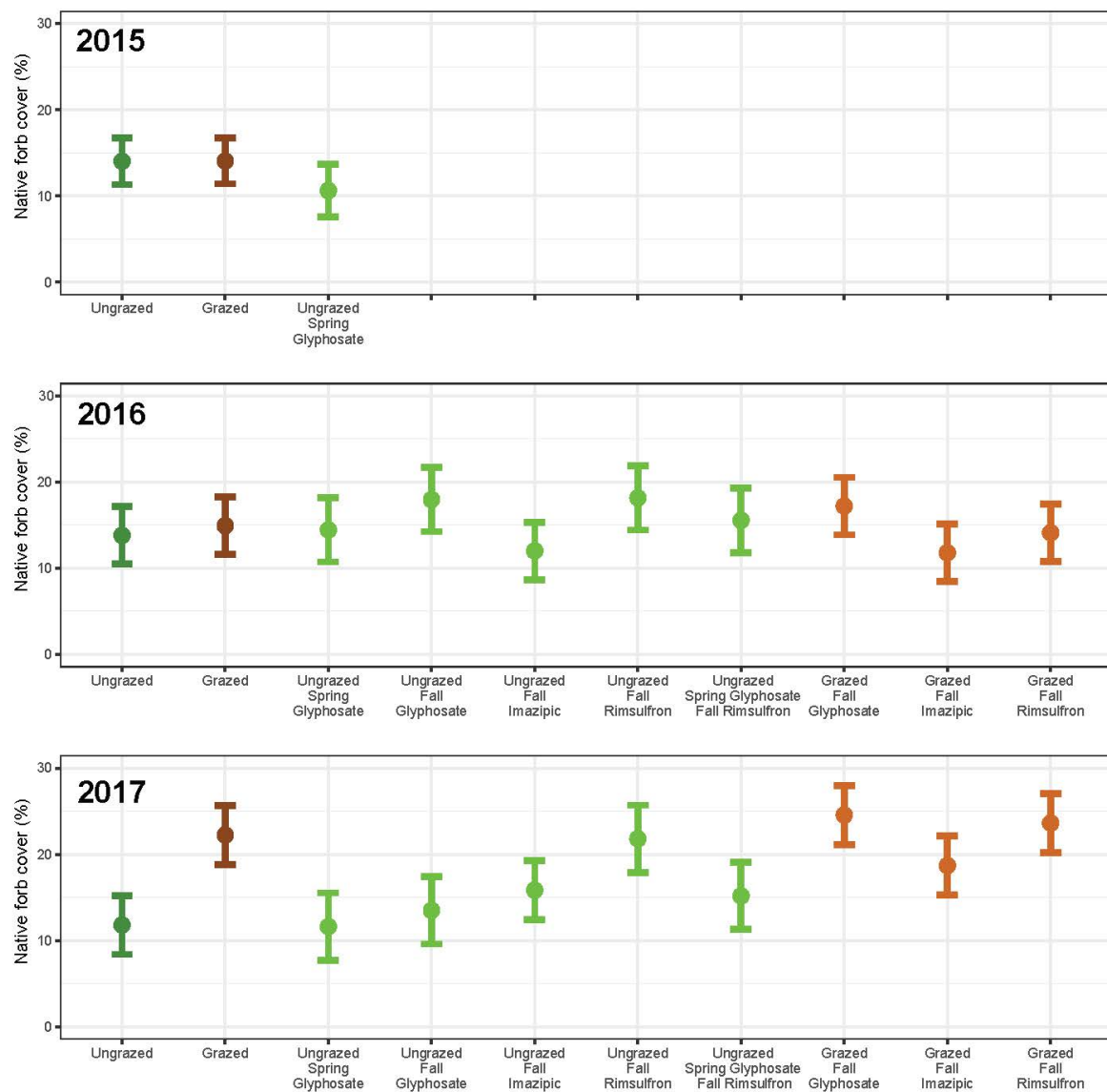
**Table 5.** The response of total plant cover to grazing and/or herbicide treatments, analyzed using a linear mixed effects model. All treatments (parameters) are with respect to the ungrazed control treatment (U\_C), which is the intercept in the model. In 2015 only the grazed (G) and ungrazed control, and the spring applied herbicide treatment results are shown, because fall herbicide application had not yet occurred. Results are based on log transformed total plant cover.

Fixed Effects						Random Effects	
Year	Predictor <sup>a</sup>	Estimate	SE	t Value	Pr (> t ) <sup>b</sup>	Group	Variance
2015	Intercept	2.92	0.20	14.7	<0.001	Frame/Rep	0.00
	G_C	0.00	0.11	0.01	0.993	Rep	0.34
	U_Sg	−0.07	0.17	−0.43	0.665	Location	0.26
						Residual	0.79
2016	Intercept	3.00	0.23	13.2	<0.001	Frame/Rep	0.00
	G_C	0.10	0.14	0.70	0.485	Rep	0.17
	G_Fg	0.09	0.14	0.61	0.542	Location	0.39
	G_Fi	−0.02	0.14	−0.13	0.896	Residual	0.58
	G_Fr	−0.07	0.14	−0.47	0.637		
	U_Fg	0.43	0.18	2.35	<b>0.020</b>		
	U_Fi	−0.07	0.14	−0.50	0.616		
	U_Fr	0.14	0.18	0.79	0.432		
	U_Sg	0.25	0.18	1.34	0.180		
	U_Sg_Fi	0.23	0.18	1.24	0.216		
	2017	Intercept	2.94	0.14	20.4	<0.001	Frame/Rep
G_C		0.58	0.12	5.03	<0.001	Rep	0.10
G_Fg		0.63	0.12	5.48	<0.001	Location	0.23
G_Fi		0.57	0.12	4.95	<0.001	Residual	0.46
G_Fr		0.62	0.12	5.34	<0.001		
U_Fg		0.48	0.14	3.35	<0.001		
U_Fi		0.45	0.12	3.95	<0.001		
U_Fr		0.41	0.14	2.88	<0.001		
U_Sg		0.26	0.14	1.80	0.073 *		
U_Sg_Fi		0.48	0.14	3.34	<0.001		

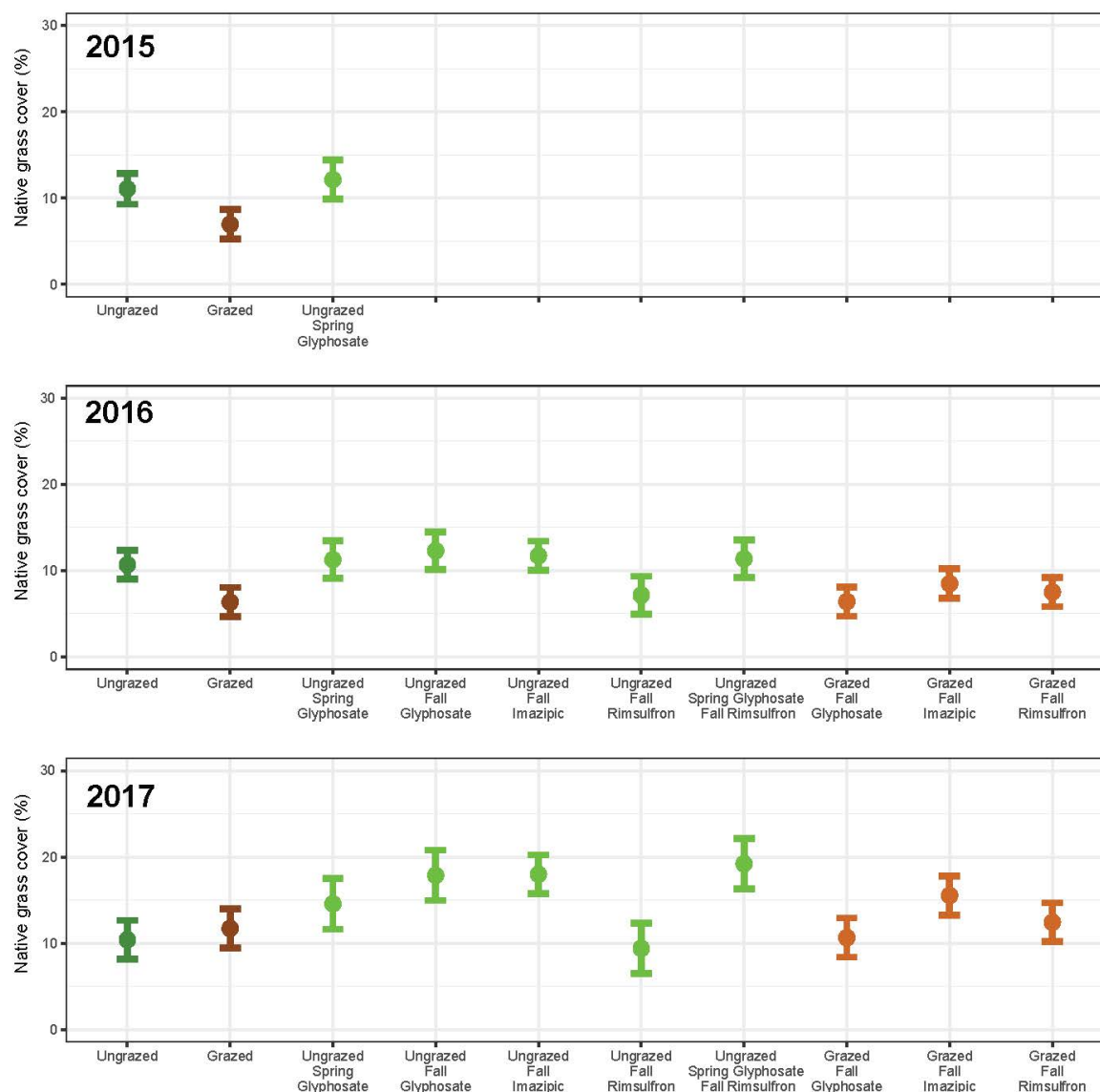
<sup>a</sup> G\_C = grazed control, G\_Fg = grazed + fall glyphosate, G\_Fi = grazed + fall imazapic, U\_Fg = ungrazed + fall glyphosate, U\_Fi = ungrazed + fall imazapic, U\_Fr = ungrazed + fall rimsulfuron, U\_Sg = ungrazed + spring glyphosate, U\_Sg\_Fi = ungrazed + spring glyphosate + fall imazapic. <sup>b</sup> Bold values indicate significance at a *p* value of < 0.05. \* = significance at *p* value of < 0.10.



**Figure 4.** Fitted model results for total plant cover (excluding *Bromus tectorum*; %; mean  $\pm$  SE) response to grazing, herbicide, and integrated treatments at Red Bluff, Montana in 2015, 2016, and 2017. Fall herbicide treatments were implemented after summer data collection in 2015, so these treatments do not show results in 2015. Furthermore, fall herbicide results in 2016 and 2017 represent treatments implemented in the prior years. Data shown are predicted values from linear mixed effects models, and thus the means are not identical to means from the raw data.



**Figure 5.** Fitted model results for forb cover (%; mean ± SE) response to grazing, herbicide, and integrated treatments at Red Bluff, Montana in 2015, 2016, and 2017. Fall herbicide treatments were implemented after summer data collection in 2015, so these treatments do not show results in 2015. Furthermore, fall herbicide results in 2016 and 2017 represent treatments implemented in the prior years. Data shown are predicted values from linear mixed effects models, and thus the means are not identical to means from the raw data.



**Figure 6.** Fitted model results for native grass cover (%; mean  $\pm$  SE) response to grazing, herbicide, and integrated treatments at Red Bluff, Montana in 2015, 2016, and 2017. Fall herbicide treatments were implemented after summer data collection in 2015, so these treatments do not show results in 2015. Furthermore, fall herbicide results in 2016 and 2017 represent treatments implemented in the prior years. Data shown are predicted values from linear mixed effects models, and thus the means are not identical to means from the raw data.

### 3.2. Herbicide

Herbicide application in ungrazed plots had minimal impact on *B. tectorum* biomass, cover or seed production in any year compared to ungrazed plots that did not receive any herbicide treatment (Tables 2–4, Figures 1–3). Spring glyphosate application in 2015 reduced biomass and cover of *B. tectorum* by 3% and 15%, respectively. Neither glyphosate nor imazapic applied in the fall reduced *B. tectorum* biomass, cover, or seed production in any subsequent year, except in 2016 when ungrazed plots treated with glyphosate in the spring and imazapic the previous fall (2015) had lower *B. tectorum* cover (9%) than ungrazed control plots (28%). One notable undesired result of herbicide treatment was that ungrazed plots treated with fall rimsulfuron tended to have higher *B. tectorum* biomass (14 g and 28 g higher in 2016 and 2017, respectively) than untreated control plots, although the 2017 result was not significant ( $p = 0.091$ , Table 2). These results, showing that herbicides had minimal effect on *B. tectorum*,

highlight the inconsistency in the ability of herbicides to reduce *B. tectorum* abundance, especially with only limited herbicide application (two years for spring glyphosate but only one year for fall applications due to low fall germination in 2016). Previous studies have measured the effective control of *B. tectorum* via imazapic [28,29,43,44] and rimsulfuron [30,45], although a review of imazapic efficacy studies ( $n = 24$ ) in Montana showed effective but variable results, with one year of application [29].

Glyphosate was not effective in our study in contrast to Espeland [27] and Morris [11], which may be attributed to the abundant *B. tectorum* seedbank at our site, where *B. tectorum* had been the dominant species since the fire break was established in 2012 [35] and no control action had been implemented. *Bromus tectorum*, which is capable of both fall and spring germination [46], may have emerged after herbicide application. Spring precipitation is vital for *B. tectorum* growth [47], and abundant spring precipitation at the site (mean March–May for study period = 21.6 cm) [48] could have promoted *B. tectorum* germination, and provided ample moisture for growth of plants surviving either the spring or the previous fall herbicide treatments. Further, late spring recruits could offset earlier losses [46], such that treated *B. tectorum* populations could produce similar biomass, cover, and seeds to the untreated population even after the spring glyphosate application.

Glyphosate applied in the fall, but not the spring, increased total plant cover (excluding *B. tectorum*) in 2016. Furthermore, all fall-applied herbicides (glyphosate, imazapic, and rimsulfuron), as well as spring glyphosate + fall imazapic, increased total plant cover in 2017, two years after herbicide treatment, compared to ungrazed controls (Table 5; Figure 4). Similarly, fall-applied herbicides generally increased forb cover, with the exception of fall imazapic in 2016 and fall glyphosate in 2017, which did not alter forb cover (Table 6; Figure 5). Spring glyphosate alone did not alter forb cover, but forb cover was slightly higher in spring glyphosate + fall imazapic plots in 2017.

**Table 6.** The response of forb cover to grazing and/or herbicide treatments, analyzed using a linear mixed effects model. All treatments (parameters) are with respect to the ungrazed control treatment (U\_C), which is the intercept in the model. In 2015 only the grazed (G) and ungrazed control, and the spring applied herbicide treatment results are shown, because fall herbicide application had not yet occurred. Results are based on log transformed forb cover.

Fixed Effects						Random Effects	
Year	Parameter <sup>a</sup>	Estimate	SE	t Value	Pr (> t ) <sup>b</sup>	Group	Variance
2015	Intercept	2.18	0.30	7.17	<b>0.002</b>	Frame/Rep	0.00
	G_C	0.35	0.12	2.86	0.005	Rep	0.33
	U_Sg	−0.18	0.19	−0.98	0.330	Location	0.53
						Residual	0.88
2016	Intercept	2.29	0.38	6.10	<b>0.004</b>	Frame/Rep	0.00
	G_C	0.47	0.19	2.51	<b>0.013</b>	Rep	0.28
	G_Fg	0.47	0.19	2.52	<b>0.012</b>	Location	0.67
	G_Fi	−0.11	0.19	−0.59	0.555	Residual	0.74
	G_Fr	0.08	0.19	0.40	0.688		
	U_Fg	0.45	0.24	1.86	0.064 *		
	U_Fi	−0.23	0.19	−1.23	0.222		
	U_Fr	0.51	0.24	2.13	<b>0.035</b>		
	U_Sg	0.40	0.24	1.67	0.096 *		
	U_Sg_Fi	0.25	0.24	1.04	0.301		
2017	Intercept	2.06	0.27	7.57	<b>0.001</b>	Frame/Rep	0.00
	G_C	1.05	0.17	6.19	<b>&lt;0.001</b>	Rep	0.17
	G_Fg	1.18	0.17	6.95	<b>&lt;0.001</b>	Location	0.47
	G_Fi	0.65	0.17	3.83	<b>0.002</b>	Residual	0.68
	G_Fr	0.96	0.17	5.65	<b>&lt;0.001</b>		
	U_Fg	0.36	0.21	1.69	0.092 *		
	U_Fi	0.35	0.17	2.05	<b>0.042</b>		
	U_Fr	0.95	0.21	4.46	<b>&lt;0.001</b>		



Table 6. Cont.

Fixed Effects						Random Effects	
Year	Parameter <sup>a</sup>	Estimate	SE	t Value	Pr (> t ) <sup>b</sup>	Group	Variance
2017	U_Sg	0.27	0.21	1.27	0.207		
	U_Sg_Fi	0.44	0.21	2.06	<b>0.041</b>		

<sup>a</sup> G\_C = grazed control, G\_Fg = grazed + fall glyphosate, G\_Fi = grazed + fall imazapic, U\_Fg = ungrazed + fall glyphosate, U\_Fi = ungrazed + fall imazapic, U\_Fr = ungrazed + fall rimsulfuron, U\_Sg = ungrazed + spring glyphosate, U\_Sg\_Fi = ungrazed + spring glyphosate + fall imazapic. <sup>b</sup> Bold values indicate significance at a *p* value of < 0.05. \* = significance at *p* value of < 0.10.

Native grass cover was not affected by any herbicide treatment on the ungrazed plots during treatment years (2015 or 2016), but plots treated with fall glyphosate, fall imazapic, and spring glyphosate + fall imazapic increased native grass cover by 7%, 8%, and 10%, respectively, compared with the control in 2017 (Table 7; Figure 6). These results differ from those of Baker et al. [44] who determined that imazapic (175 g a.i. ha<sup>-1</sup>) reduced both forbs and native grasses in Wyoming big sagebrush habitat. However, our results are supported by Wallace et al. [49] who determined that imazapic and rimsulfuron had negligible effects on *Pseudoroegneria spicata* and *Thinopyrum intermedium* when used to treat *Ventenata dubia*. Nonetheless, while native grasses were not damaged in our study, similar to Elseroad et al. [43], our results indicate that herbicide application alone does not effectively increase native perennial grasses through the reduction of annual grasses. Multiple years of herbicide application may be needed to measure positive changes in perennial grass cover. However, at our site fall application was not advisable in the second year due to sparse vegetation cover in late fall.

**Table 7.** The response of native grass cover to grazing and/or herbicide treatments, analyzed using a linear mixed effects model. All treatments (parameters) are with respect to the ungrazed control treatment (U\_C), which is the intercept in the model. In 2015 only the grazed (G) and ungrazed control, and the spring applied herbicide treatment results are shown, because fall herbicide application had not yet occurred. Results are based on log transformed native grass cover.

Fixed Effects						Random Effects	
Year	Parameter <sup>a</sup>	Estimate	SE	t Value	Pr (> t ) <sup>b</sup>	Group	Variance
2015	Intercept	11.2	1.62	6.90	<0.001	Frame/Rep	0.00
	G_C	-4.18	1.29	-3.25	<b>0.001</b>	Rep	2.62
	U_Sg	0.05	1.95	0.02	0.981	Location	1.81
						Residual	9.35
2016	Intercept	10.7	1.69	6.32	<0.001	Frame/Rep	0.00
	G_C	-4.16	1.93	-2.15	<b>0.032</b>	Rep	0.88
	G_Fg	-4.37	1.93	-2.26	<b>0.025</b>	Location	1.91
	G_Fi	-1.65	1.93	-0.85	0.395	Residual	7.70
	G_Fr	-3.12	1.93	-1.61	0.108		
	U_Fg	1.39	2.38	0.59	0.559		
	U_Fi	1.31	1.93	0.68	0.496		
	U_Fr	-2.92	2.38	-1.23	0.220		
	U_Sg	0.58	2.38	0.24	0.808		
	U_Sg_Fi	1.14	2.38	0.48	0.632		
2017	Intercept	10.4	2.26	4.61	<0.001	Frame/Rep	0.00
	G_C	1.31	2.45	0.54	0.593	Rep	2.47
	G_Fg	0.25	2.45	0.10	0.920	Location	2.32
	G_Fi	5.14	2.45	2.10	<b>0.037</b>	Residual	9.76
	G_Fr	2.03	2.45	0.83	0.408		
	U_Fg	7.47	3.06	2.44	<b>0.015</b>		
	U_Fi	7.59	2.44	3.11	<b>0.002</b>		
	U_Fr	-1.0	3.06	-0.33	0.745		

Table 7. Cont.

Fixed Effects						Random Effects	
Year	Parameter <sup>a</sup>	Estimate	SE	t Value	Pr (> t ) <sup>b</sup>	Group	Variance
2017	U_Sg	4.16	3.06	1.36	0.176		
	U_Sg_Fi	8.81	3.06	2.88	<b>0.004</b>		

<sup>a</sup> G\_C = grazed control, G\_Fg = grazed + fall glyphosate, G\_Fi = grazed + fall imazapic, U\_Fg = ungrazed + fall glyphosate, U\_Fi = ungrazed + fall imazapic, U\_Fr = ungrazed + fall rimsulfuron, U\_Sg = ungrazed + spring glyphosate, U\_Sg\_Fi = ungrazed + spring glyphosate + fall imazapic. <sup>b</sup> Bold values indicate significance at a *p* value of < 0.05. \* = significance at *p* value of < 0.10.

### 3.3. Integrated Grazing and Herbicide

Integrating grazing and herbicide did not improve *B. tectorum* management during treatment years compared to the ungrazed control plots; no integrated treatments reduced *B. tectorum* biomass, cover, or seed production in 2016, presumably due to a large annual grass seedbank, which takes several seasons to diminish. However, by 2017 some positive impacts were observed, and *B. tectorum* cover was less in grazed plots treated with imazapic or rimsulfuron than in ungrazed control plots (Table 3; Figure 2). Furthermore, grazed plots treated in the fall of 2015, and grazed plus imazapic or rimsulfuron plots had lower *B. tectorum* biomass in 2017 and cover in both 2016 and 2017 (Tables 2 and 3, Figures 1 and 2) than the respective herbicide treated but ungrazed plots. Similarly, in 2017, one year after the last grazing treatments were applied, *B. tectorum* seed production was less in grazed (11.2 g) plots treated with fall imazapic compared to ungrazed (18.5 g) fall imazapic plots (Table 4, Figure 3). These results indicate that integrated management of *B. tectorum* with herbicide and grazing could provide a level of control greater than herbicide or grazing alone. Diamond et al. [32] determined that the combination of intensive, short-term grazing, and prescribed burning reduced *B. tectorum* cover more than either treatment independently. Success was attributed to intensive grazing reducing *B. tectorum* biomass and reducing reproductive potential while prescribed burning killed seeds in the litter. The benefit of integrated management was also noted by Whitson and Koch [50] who determined that glyphosate (0.55 kg ha<sup>-1</sup>) provided better control of *B. tectorum* when combined with intensive cattle grazing. Similarly, our integrated treatments were more successful than individual treatments likely because grazing removed biomass and then fall-applied herbicides not only limited growth but also minimized further seedling emergence.

The combination of grazing and herbicide had minimal impact on total plant, forb, or native grass cover in 2016: A few grazed treatments had slightly less cover than their respective ungrazed treatments (Tables 5–7; Figures 4–6). All treatments had higher total plant cover (excluding *B. tectorum*) than the ungrazed control in 2017 except ungrazed spring glyphosate, and there were no differences between grazed and ungrazed herbicide plots. For native grasses, cover was lower in grazed fall glyphosate plots compared to the respective ungrazed plots in both 2016 (6% vs. 12%) and 2017 (10% vs. 18%), but there were no other differences (Figure 6). Forb cover differences were minimal between ungrazed and grazed herbicide treatments: Forb cover in 2016 was lower in grazed fall rimsulfuron plots than respective ungrazed plots (13% vs. 19%) and conversely in 2017 when it was lower in ungrazed fall glyphosate plots compared to grazed ones (14% vs 23%; Figure 5). Overall, these results indicate that integrating grazing with some herbicides (imazapic and rimsulfuron) can have a positive effect on *B. tectorum* management and is not detrimental to native grasses or forbs, with the exception of integration of grazing and fall glyphosate, which reduced native grasses and increased forbs.

### 3.4. Community Change

Despite the slight improvements of integrated management over either grazing or herbicide alone, no treatments provided adequate control of *B. tectorum*, nor did they substantially change the plant community. Non-metric multidimensional scaling analysis indicated that treatments led to different

plant communities, although the  $R^2$  values were low and NMDS stress values were moderately high (Table 8), suggesting no strong conclusions can be made. While plant communities may have changed slightly within three years, the dominant species generally did not (Supplemental Table S1). *Bromus tectorum* was most often the dominant species (18 out of 23 treatment  $\times$  year combinations) and was never lower in rank than second. Combined forbs were typically the second most abundant, and became the highest abundance in years when *B. tectorum* was second. The native grasses *Pascopyrum smithii*, *Festuca idahoensis*, *Pseudoroegneria spicata*, *Hesperostipa comata*, and *Bouteloua gracilis* were generally the next most abundant species. These results highlight not only the fact that *B. tectorum* is an incredibly challenging weed to manage, but also the difficulty of altering the plant community in an area that has transitioned to a novel state dominated by *B. tectorum*. The lack of substantial community change is not surprising over the short time frame of our study because, while we did repeat most treatments over two years, more years of treatment may be necessary to change the plant community [51,52] from an altered state.

**Table 8.** Treatment effects on plant community structure. Results based on non-metric multidimensional scaling (NMDS) and the distance matrix of plant species abundances. Model summary is from the package “adonis” in R and stress is provided by the NMDS analysis.

Year		DF	Sum of Squares	Mean Squares	F Model	$R^2$	Pr (>F)	NMDS Stress
2015	Treatment	2	1.35	0.67	5.6	0.04	1	0.24
	Residuals	253	30.7	0.12		0.96		
	Total	255	32.1			1.00		
2016	Treatment	9	3.13	0.35	2.27	0.08	<0.001	0.27
	Residuals	246	37.8	0.15		0.92		
	Total	255	40.9			1.00		
2017	Treatment	9	3.64	0.37	2.56	0.09	<0.001	0.27
	Residuals	246	35.9	0.15		0.91		
	Total	255	39.2			1.00		

#### 4. Conclusions

Overall, none of the single treatments adequately reduced *B. tectorum* or increased other vegetation. Two combinations of integrated grazing and herbicide, specifically grazing with fall-applied imazapic or rimsulfuron, provided better results than herbicide alone; however, there is only weak support for our hypothesis that integrated management would be superior to individual treatment methods. Our second hypothesis that total plant cover, forbs, and grasses would differ between treatments was partially supported, as grazing alone or with fall-applied herbicides tended to reduce native grass cover while increasing forb cover in the second year of the study. A positive result from this research is that the intensive treatments of grazing plus herbicide did not reduce cover of the desired species in the final year (one year after the last treatments had been implemented), indicating that continued management of *B. tectorum* may facilitate the increase other plant cover. The final hypothesis that different treatments would shift plant communities was weakly supported statistically, but *B. tectorum* was still the most or second most dominant species. These results highlight the difficulties in managing dense *B. tectorum* infestations with either traditional or integrated management but do provide some encouragement for the integrated grazing and herbicide approach. Future research should incorporate different timing, frequency and intensity of grazing [16], possibly also new herbicide chemistries such as indaziflam in areas not actively grazed [53], and longer-term application and monitoring of treatments. Effective, consistent, and long-term control of *B. tectorum* once it becomes abundant, often due to disturbances, is notoriously difficult. Overall, integrating grazing and herbicide shows some promise, but further research is needed to establish reliable practices across a range of sites.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2073-4395/9/6/315/s1>, Table S1: Cumulative abundance, proportion and accumulated frequency of plant species in plots at Red Bluff, Montana for 2015, 2016 and 2017.

**Author Contributions:** Conceptualization and methodology, E.A.L., L.J.R., J.M.M. and D.R.; Investigation, E.A.L., L.J.R., J.M.M., and T.S.; Data analysis, E.A.L. and L.J.R.; Writing, E.A.L., L.J.R., T.S., J.M.M. and D.R.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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