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Novel Approaches to Invasive Annual Grass Management: Assessing Efficacy and Impacts on Target Species and Plant Diversity

Erin B. Teichroew¹  | Jane M. Mangold¹  | Lilly Sencenbaugh^{1,2}  | Lisa J. Rew¹ 

¹Land Resources and Environmental Sciences, Montana State University, Bozeman, Montana, USA | ²Department of Plant Science, The Pennsylvania State University, State College, Pennsylvania, USA

Correspondence: Erin B. Teichroew (erin.teichroew@student.montana.edu)

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ABSTRACT

Aims: The management of invasive species is a global concern that requires a paradigm shift away from only assessing control efficacy to also including impacts to the diversity of the surrounding plant community. Many rangelands in the western United States are invaded by annual grasses like *Bromus tectorum* (cheatgrass). Current management of invasive annual grasses (IAGs) relies heavily on herbicides and their efficacy in targeting IAGs. However, this approach often does not consider the response of the surrounding plant community. Evaluations of alternative management options and the efficacy of all approaches on target and desired vegetation are needed. We assessed four management options and a restoration seeding split treatment to control *B. tectorum* and restore the desired plant community.

Location: Semi-arid grasslands, southwestern Montana, United States.

Methods: We monitored vegetation for three growing seasons after management treatment application (non-treated control, mustard seed meal (MSM), NutraFix, and indaziflam) and restoration seeding (native grasses) with spatial (3) and temporal (2) replication, assessing changes in *B. tectorum* cover, species richness, alpha and beta diversity, and rank abundance.

Results: The MSM provided control for 1 year but also reduced species richness, and *B. tectorum* was highly dominant 2 and 3 years after treatment. NutraFix provided control for 2 years and had no meaningful impacts on species richness or alpha diversity. Indaziflam provided 3 years of *B. tectorum* control, reduced species richness, and facilitated a shift toward a *Pascopyrum smithii* (western wheatgrass) dominated community. Restoration seeding did not affect *B. tectorum* cover nor beta diversity but did increase richness and alpha diversity.

Conclusions: This study demonstrates the importance of a paradigm shift away from evaluating only management efficacy of the target species to include other species and metrics that support recovery of the desired plant community.

1 | Introduction

Non-native invasive plant species are a global issue in both agricultural production and wildlands (Pysek et al. 2012; Paini et al. 2016). They can cause a variety of impacts, from reducing

species richness on a local scale to altering whole ecosystem functioning (Vila et al. 2011; Pysek et al. 2012). Upward of \$9 billion has been spent attempting to manage non-native and invasive plants globally over the past 50 years (Diagne et al. 2021).

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Management success is typically measured as the level of control achieved of the target species (e.g., percent reduction in the species' cover or biomass) (Shackelford et al. 2013; Reaser et al. 2020). However, management actions can also impact other plants in the community. Overlooking these non-target impacts drastically limits our ability to fully understand the influence of management. Even though many practitioners focus on control of a target species, others have a variety of goals and have coalesced toward a more ecosystem-focused approach to invasive plant management, with the aim of returning the land to a productive and functioning system (Skurski et al. 2013). Therefore, the evaluation of invasive plant management practices should also include assessments of the entire plant community. One way to do this is to examine biodiversity metrics such as species richness, alpha diversity, and community composition (i.e., beta diversity and rank abundance). Metrics of biodiversity have long been used to estimate the health and functioning of plant communities (Tilman et al. 1997; Balvanera et al. 2014; van der Plas 2019). Within each biodiversity metric (e.g., alpha diversity), there are numerous indices that can be employed, and while there are limitations to some, the current recommendation is to perform multiple different indices for each metric to determine if the patterns in the results match each other (e.g., diversity is higher in community A than community B for each index) (Magurran and McGill 2011).

Biodiversity is also extremely important in supporting sustainable agriculture (Kazemi et al. 2018; Ortiz et al. 2021). Both traditional row cropping systems and rangelands experience increased productivity and stability when biodiversity is increased due to heterogeneity in plant performance across time and space (Kazemi et al. 2018; Yang et al. 2021). Sustainably managing agroecosystems is an essential task producers are facing, made harder due to growing human population sizes and a changing climate (Ortiz et al. 2021). Reframing the paradigm of invasive plant management away from focusing predominately on control of the target species toward the response of the desired community, concurrently improving the functioning of the entire agroecosystem, is essential if we are going to meet global sustainability goals.

Rangelands, an important grassland agroecosystem, depend on stable forage production and therefore benefit from diverse plant assemblages. In grassland systems, the relationship between biodiversity and increased production is relatively consistent across studies (van der Plas 2019). However, many rangelands in the western United States are degraded and have been invaded by annual grasses, particularly *Bromus tectorum* L. (cheatgrass) (De Stefano et al. 2024). These rangelands are in danger of transitioning to invasive annual grass (IAG)-dominated systems with lower quality and less predictable forage, lower biodiversity, and lower ecosystem functionality (Bagchi et al. 2013; Chambers et al. 2014). As these reductions have become more apparent, managers are shifting their goals toward restoring resilience instead of simply focusing on eliminating IAGs. To this end, new management tactics balancing the control of IAGs with improving the abundance and diversity of native plant species are required.

Herbicides are the most common management method for IAGs (Monaco et al. 2017). Herbicide applications in western rangelands often aim to exploit the winter annual life history of IAGs

because most of the native species are dormant in the fall when winter annuals are germinating. The use of pre-emergent or early post-emergent herbicides in the summer (pre-) or fall (post-) can selectively target these fall germinating species (Sebastian et al. 2016; Koby et al. 2019; Mangold et al. 2024). However, residual impacts can affect plant community dynamics over time, leading to reduced species richness and abundance (Skurski et al. 2013; Pearson et al. 2016; Meyer-Morey et al. 2021). Additionally, most of these herbicides only provide 1–2 years of control (Sebastian et al. 2016; Koby et al. 2019; Mangold et al. 2024). A new herbicide, indaziflam (Rejuvra), was approved for use in rangelands in 2020 (Environmental Protection Agency [EPA] 2020). Indaziflam is a pre-emergent, non-selective herbicide that reduces seed germination for multiple years (EPA 2020). It has garnered much interest from managers due to its long soil residual activity and lack of damage to established plants (Clark et al. 2019). However, there are concerns with the potential negative effects to native annual forbs (Meyer-Morey et al. 2021; Courkamp et al. 2022), though there is some evidence that these effects may not be long term (Sebastian et al. 2025). While annual forbs may seem unimportant to grazing practices, forbs make up the majority of species richness in rangeland systems and increases in their diversity contribute to overall community stability and productivity (Pokorny et al. 2004).

Alternative methods to control IAGs are needed, and some managers are interested in approaches not centered on synthetic herbicides. One novel method that could target the ecological life history of *B. tectorum* and other IAGs is biofumigants. Mustard seed meal is a biofumigant that has historically been used in cropping systems to manage soil pathogens (Matthiessen and Kirkegaard 2006), but recent research has shown that it can also inhibit seed germination and be used in weed management (De Cauwer et al. 2019). The selectivity of mustard seed meal (MSM) varies by species (Sencenbaugh et al. 2024a), so the potential impact to germinating native species is uncertain. The biofumigant molecules are volatiles and quickly dissipate into the air after the meal contacts water (Gimsing and Kirkegaard 2009), so with careful application timing, MSM could be used to target IAG seeds while mitigating harm to dormant or established perennial bunchgrasses (Sencenbaugh et al. 2024a, 2024b), but will likely only provide short-term IAG control.

Another novel, under-researched approach is altering soil composition through soil amendments. NutraFix (Edaphix LLC) is a new micronutrient soil amendment designed to restore rangeland soils and is comprised of the micronutrients boron, copper, manganese, molybdenum, chloride, and zinc (Jennings 2020). It has been proposed that perennial grasses have a higher tolerance for micronutrients than annual species, so IAG abundance could be reduced with low risk to perennials (Jennings 2020). However, micronutrients, specifically boron, can be phytotoxic, and the range of dosages which are beneficial to plant health to where damage occurs is small (Landi et al. 2019). Vulnerable life stages such as germinating seedlings may be susceptible to damage if the application rates of this product are too high. This amendment is designed to be long acting and is expected to provide multiple years of effective management (Jennings 2020).

Severely degraded rangelands with very high cover of IAGs and low cover of desired species may require management plans that

TABLE 1 | Hypothesized effects of treatments on efficacy metrics relative to the non-treated controls over 3 years. “Additional effect” refers to any effect of the seeding split treatment on top of effects from the original management approach.

Treatment	<i>Bromus tectorum</i> cover	Richness	Alpha diversity	Community composition
MSM	Reduced, only the 1st year post application	No effect	No effect	No effect
NutraFix	Reduced	Reduced	Reduced	Altered evenness
Indaziflam	Reduced	Reduced	Reduced	Altered evenness
Control + Seeding	Reduced	Higher	Higher	Higher abundance of seeded species
MSM + Seeding	Reduced	Higher	Higher	Higher abundance of seeded species
NutraFix + Seeding	No additional effect	No additional effect	No additional effect	No additional effect
Indaziflam + Seeding	No additional effect	No additional effect	No additional effect	No additional effect

incorporate restoration seeding (Nyamai et al. 2011). A review of studies on common management tactics found that herbicide application and revegetation were the only two methods to reduce *B. tectorum* abundance long term (2+ years) (Monaco et al. 2017). Other studies have shown that semi-arid grasslands with high levels of perennial grasses (> 20% cover) have lower levels of *B. tectorum* (Reisner et al. 2013; Chambers et al. 2016; Larson et al. 2017). Therefore, the aim of restoration seeding is to increase the levels of perennial grasses, improving resistance and lowering the probability of reinvasion.

To assess these novel approaches to IAG management and their impacts on plant diversity, we applied treatments to semi-arid rangelands invaded by *B. tectorum*. We compared the efficacy of these novel management practices (MSM and NutraFix) to the efficacy of the herbicide indaziflam and to no management (i.e., non-treated control). We assessed the impacts of these methods alone and in combination with a broadcast restoration seeding treatment. The metrics we examined were *B. tectorum* cover, species richness, alpha diversity, and community composition (Table 1). We hypothesized that MSM would initially reduce *B. tectorum* cover and have little impact on diversity metrics (Table 1), and that NutraFix and indaziflam would reduce *B. tectorum* cover for all 3 years but would also have negative impacts on the surrounding plant community (Table 1). We also hypothesized that the addition of restoration seeding would benefit the native community diversity by further reducing *B. tectorum* cover over time in the non-treated control and MSM treatments, but we expected no changes in the NutraFix and indaziflam treatments due to their predicted inhibition of seed germination (Table 1).

2 | Methods

2.1 | Sites

The study was established at three locations and replicated over 2 years. Two sites (Red Bluff & Highland) were established in fall 2020, and three sites were established in fall 2021 (Red Bluff, Highland, & Emigrant) (Appendix S1). Sites were semi-arid grasslands located in the Middle Rockies of southwestern Montana, close to Bozeman where average annual precipitation is 412 mm, with

warm summers (14.1°C) and cold winters (−0.7°C); values for our sites during the study can be found in the supporting information (Appendix S2; PRISM Climate Group n.d.). Dominant grass species of the region and our sites include *Pseudoroegneria spicata* (Pursh) Å. Löve (bluebunch wheatgrass), *Pascopyrum smithii* Å. Löve (western wheatgrass), *Hesperostipa comata* (Trin. & Rupr.) (needle and thread grass), and *Festuca idahoensis* Elmer (Idaho fescue). A complete list of species, including their nativity, observed at our sites can be found in the supplemental materials (Appendix S3).

2.2 | Experimental Design

Each site consisted of five replicates of four treatments: control (non-treated), mustard seed meal (*Brassica juncea* L. Czern.; 15,680 kg/ha; \$148,176/ha; Farm Fuel, Watsonville CA), NutraFix (415 kg/ha; \$1245–\$2280/ha; Edaphix, Bozeman MT), and indaziflam (72.9 g ai/ha; \$20/ha; Rejuvra; Envu). These rates were recommended by the manufacturers or informed by previous studies (Sencenbaugh et al. 2024b). A split-plot restoration seeding treatment was also applied, and half of each plot was broadcast seeded by hand with important forage species observed in the region (*F. idahoensis*, *Koeleria macrantha* (Ledeb.) Schult. (prairie Junegrass), *Nassella viridula* (Trin.) Barkworth (green needlegrass), and *P. spicata*) at the Natural Resources Conservation Service recommended broadcast rate of 20.2 kg/ha (\$152/ha; Pokorny 2020; Bruce Seed Farm, Townsend MT). In the year of establishment (2020 or 2021), the management treatments were applied in late summer, and the restoration seeding was applied the following spring (Appendix S1). Treatment placement was fully randomized within each replication. Individual plots were 1.25 × 2.5 m with buffers of 1.5 × 2.5 m between each plot to prevent contamination across treatments.

2.3 | Sampling

Ocular canopy cover was estimated to the nearest 1% for all individual species in two 0.75 m² quadrats per plot (one each for the seeded and not seeded split treatment). Sampling was performed each summer at peak vegetation (late June) for 3 years following the fall management treatment. The study established

in 2020 at Highland was inadvertently grazed by cattle directly prior to sampling; thus, no data are available for that site 1 year post application, and cattle were excluded with fencing in subsequent years. Soil samples were collected in the spring of 2023 and analyzed for a variety of soil characteristics (Appendix S4; Agvise Laboratories, Northwood ND). Three plots of each treatment were randomly sampled from each study. Six soil cores (1 cm diameter) of the top 10 cm were collected from outside the sampling frame and pooled from each plot for analysis.

2.4 | Data Analysis

Our response variables were *B. tectorum* cover, species richness, and alpha and beta diversity. We performed linear mixed effects models with analysis of variance (ANOVA) or linear models with permutation-based analysis of variance (Adonis) for the beta analysis (Appendix S5). Fixed effects included year of establishment (2020 vs. 2021), site (Red Bluff, Highland, & Emigrant), seeding treatment (seeded/not), and the interaction between management treatment (control, MSM, NutraFix, & indaziflam) and year post application (1–3 years). If the interaction was not significant, we removed it from the model. *Bromus tectorum* was not included in richness or diversity metrics to avoid conflating successful management with decreased diversity, but *B. tectorum* cover was included as a fixed effect in these models. Random effects of plot ID accounted for the split-treatment design and repeated measures. We assessed diagnostic plots of all models for violations of equal variance and linearity assumptions. We performed a natural log transformation (ln) on the responses *B. tectorum* cover and species richness, as diagnostic plots revealed violations to linear model assumptions (Appendix S5). Dunnett's tests from the emmeans package (Lenth et al. 2022) compared treatment means to non-treated control means within and between years for all relevant models.

We calculated species accumulation curves for the control plots at each site using the specaccum function in the vegan package (Oksanen et al. 2022). Plots were randomly selected without replacement from the total pool of sampled plots 1000 times. We calculated Shannon's and Inverse Simpson's alpha diversity indices (Appendices S6 & S7) and Bray–Curtis and Morisita–Horn beta diversity indices (Appendices S8 & S9) with the vegan package (Oksanen et al. 2022). Alpha diversity combines the species richness and species evenness of a community. There is variation in how alpha diversity metrics are calculated (Magurran and McGill 2011), so we compared two common metrics, Shannon's and Inverse Simpson's, and confirmed the general patterns agreed with each other (e.g., that treatments caused similar changes in alpha diversity). Diagnostic plots more closely followed the patterns expected for linear models for the Shannon's analysis, so those results are presented. The Inverse Simpson's results can be found in the supporting information (Appendices S10–S12). Beta diversity measures the dissimilarity among or between communities. Similar to alpha diversity, there is variation in the calculation of beta diversity metrics (Magurran and McGill 2011), so we calculated two common abundance-based metrics, Bray–Curtis and Morisita–Horn and confirmed the general patterns agreed with each other. The Morisita–Horn results are presented, as this index was derived from the Shannon's index; the Bray–Curtis results can be found

in the supporting information (Appendix S13). We used dissimilarity matrices to create non-metric multidimensional scaling plots using the labdsv package (Roberts 2023) to visually assess how dissimilarity related to site, management treatment, seeding treatment, year post application, and year of establishment (Appendices S14 & S15). We calculated the proportional rank abundance of species composition in each treatment and year using the BiodiversityR package (Kindt and Coe 2005).

To assess the impacts of management treatment on soil characteristics, we performed linear mixed effects models for 14 soil characteristics (nitrate, phosphorous, potassium, sulfur, boron, zinc, iron, manganese, copper, magnesium, calcium, sodium, organic matter, and pH). Management treatment and year of establishment were included as fixed effects. Random effects accounted for site differences. All analyses were performed in R version 4.4.1 (R Core Team 2024).

3 | Results

3.1 | *Bromus tectorum* Cover

Bromus tectorum cover was impacted by management treatment ($p < 0.001$; Appendix S16), year of establishment ($p = 0.018$; Appendix S16), and site ($p < 0.001$; Appendix S16). There was also strong evidence of an interaction between management treatment and year post application indicating the effects of the treatments on *B. tectorum* cover changed over time ($p < 0.001$; Appendix S16). The addition of restoration seeding had no impact on *B. tectorum* cover ($p = 0.250$; Table 2; Appendix S16).

Mean *B. tectorum* cover in the non-managed control plots was 25%, ranging from 1% to 88%, and did not change over the 3 years of the study ($SD = 21$; $p = 0.463$; Figure 1). The MSM treatment reduced *B. tectorum* cover by 92% (to 1% cover ± 0.5 SE) the first year post application ($p < 0.001$; Appendix S17) but did not differ from the control in the second year ($p = 0.703$; Appendix S17) or third year ($p = 0.327$; Figure 1; Table 2; Appendix S17). The NutraFix treatment reduced *B. tectorum* cover by 83% (to 3% cover ± 1.2 SE) the first year ($p < 0.001$; Appendix S17) and 72% (to 5% cover ± 1.4 SE) the second year post application ($p = 0.004$; Appendix S17), but did not differ from the control in the third year ($p = 0.361$; Figure 1; Table 2; Appendix S17). Indaziflam was the only treatment that reduced *B. tectorum* cover all 3 years, by 75% (to 6% cover ± 2.1 SE), 89% (to 2% cover ± 0.5 SE), and 92% (to 1% cover ± 0.4 SE), respectively (all $p < 0.010$; Figure 1; Table 2; Appendix S17). The Highland site tended to have 5% (± 2.9 SE) more *B. tectorum* than the other two sites ($p < 0.001$; Figure 1).

3.2 | Community Response

A total of 57 unique species were found across our three sites, with 32 occurring at two or more locations (Appendix S3). A total of 33 species were observed at Emigrant, 40 at Highland, and 42 at Red Bluff. These consisted of 12 native perennial grasses, one non-native perennial grass, 28 native perennial forbs, three non-native perennial forbs, one native and one invasive annual grass, four native annual/biennial forbs, and seven non-native annual/biennial forbs (Appendix S3).

TABLE 2 | Summarized results of hypothesized effects of treatments on efficacy metrics relative to the non-treated controls, with *p*-values taken from relevant analysis. “Additional effect” refers to any effect of the seeding split treatment on top of effects from the original management approach.

Treatment	<i>Bromus tectorum</i> cover	Richness	Alpha diversity	Community composition
MSM	Reduced 1st year ($p < 0.001$), not 2nd ($p = 0.703$) or 3rd ($p = 0.327$)	Reduced all years ($p = 0.026$)	No effect ($p = 0.127$)	Increased dominance of <i>B. tectorum</i>
NutraFix	Reduced 1st ($p < 0.001$) and 2nd year ($p = 0.004$), not 3rd ($p = 0.361$)	No effect ($p = 0.805$)	No effect ($p = 0.127$)	Increased dominance of <i>B. tectorum</i>
Indaziflam	Reduced all years ($p = 0.001$)	Reduced all years ($p = 0.015$)	No effect ($p = 0.127$)	Increased dominance of <i>P. smithii</i>
Control + Seeding	No effect ($p = 0.742$)	Increased ($p = 0.033$)	Increased ($p = 0.020$)	No additional effect
MSM + Seeding	No additional effect ($p = 0.513$)	No additional effect ($p = 0.790$)	No additional effect ($p = 0.607$)	No additional effect
NutraFix + Seeding	No additional effect ($p = 0.401$)	No additional effect ($p = 0.660$)	No additional effect ($p = 0.405$)	No additional effect
Indaziflam + Seeding	No additional effect ($p = 0.201$)	No additional effect ($p = 0.305$)	No additional effect ($p = 0.496$)	No additional effect

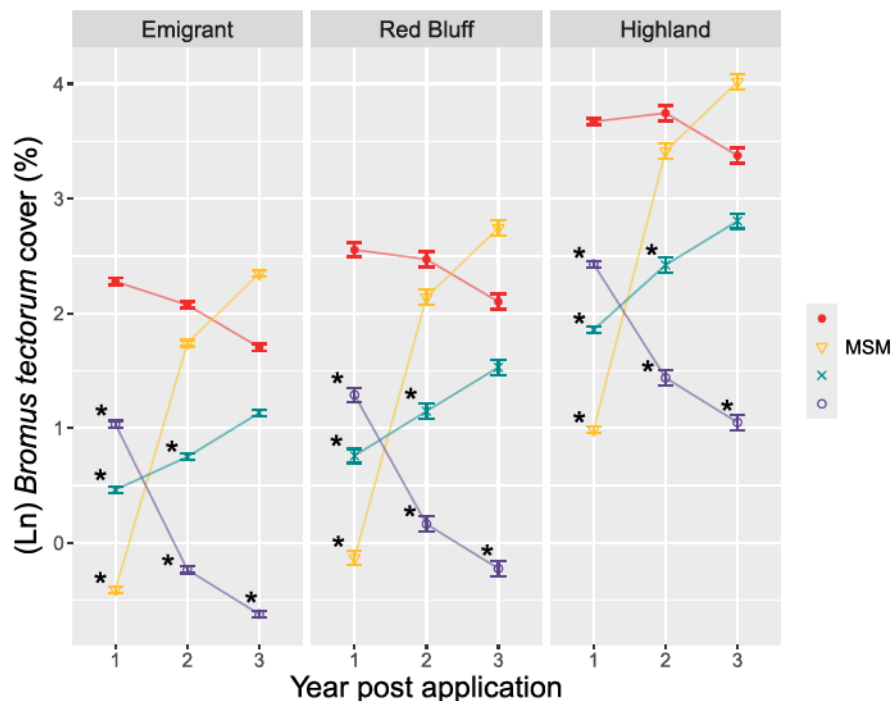


FIGURE 1 | Change in *Bromus tectorum* cover (%) (natural log (Ln)) by treatment at three sites. Treatments were applied in fall 2020 or 2021 to semi-arid grasslands in southwestern Montana. Emigrant contains data from only one temporal replicate (2021), Red Bluff and Highland contain two (2020 & 2021). There were no interactions between year of application and treatment effect. Ocular cover of all species present was recorded to the nearest 1% annually during peak biomass for 3 years following application. Ln-mean *B. tectorum* cover and the corresponding standard error were predicted from the final mixed effect linear model estimating *B. tectorum* cover. *Indicates a significant difference ($\alpha = 0.05$) from the control within year of observation (Appendix S17).

An average of 3.9 species ± 0.3 SE (per 0.75 m² quadrat) were present in non-managed controls, and this did not change over the 3 years of the study ($p = 0.187$; Figure 2). Additionally, the

species accumulation calculation predicted 20–32 unique species in non-managed control plots across the different sites (Figure 3).

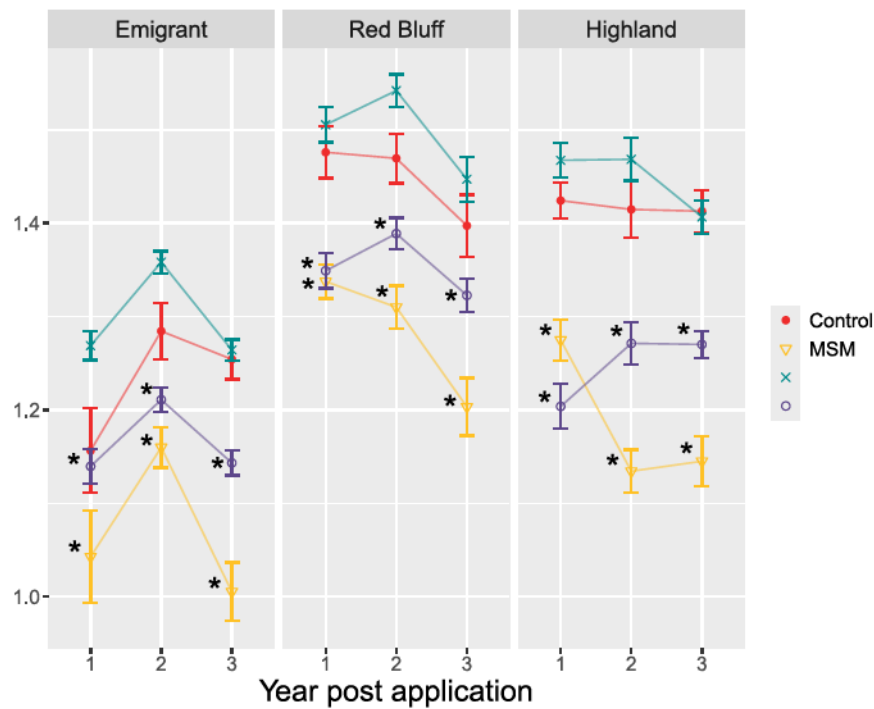


FIGURE 2 | Change in species richness (natural log (Ln)) by treatment at three sites. Treatments were applied in fall 2020 or 2021 to semi-arid grasslands in southwestern Montana. Emigrant contains data from only one temporal replicate (2021), Red Bluff and Highland contain two (2020 & 2021). There were no interactions between year of application and treatment effect. Species presence was recorded annually during peak biomass for 3 years following application. *Bromus tectorum* cover was excluded from the analysis. Ln-mean species richness and the corresponding standard error were predicted from the final mixed effect linear model estimating species richness. *Indicates a significant difference ($\alpha = 0.05$) from the control within year of observation (Appendix S19).

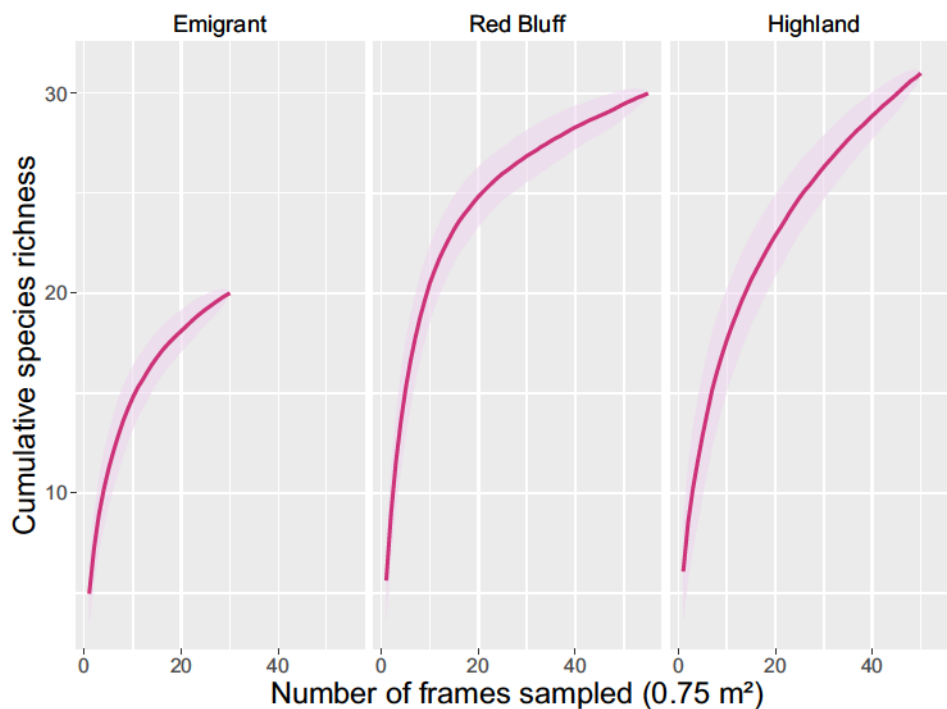


FIGURE 3 | Differences in species accumulation in control plots at three sites. Plots were established in fall 2020 or 2021 to semi-arid grasslands in southwestern Montana. Emigrant contains data from only one temporal replicate (2021), Red Bluff and Highland contain two (2020 & 2021). Species presence was recorded annually during peak biomass for 3 years following application. Species accumulation and the corresponding standard deviation were predicted from 1000 random quadrat selections using the specaccum function in the vegan package (Oksanen et al. 2022).

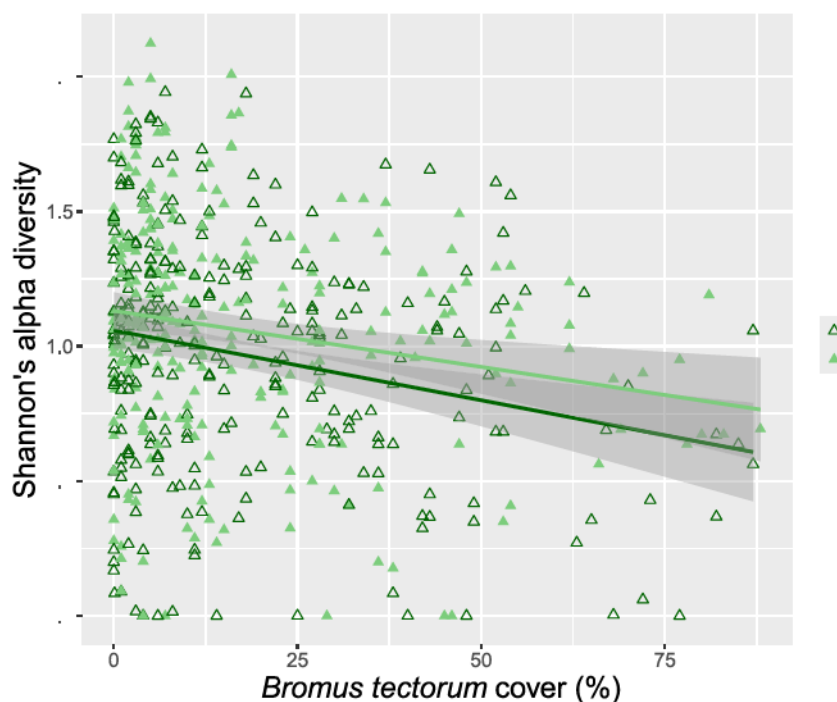


FIGURE 4 | Change in Shannon's alpha diversity by *Bromus tectorum* cover and restoration seeding treatment. Treatments were applied in fall 2020 or 2021 to semi-arid grasslands in southwestern Montana. Ocular cover of all species present was recorded to the nearest 1% annually during peak biomass for 3 years following application. Higher values represent more diverse and even communities. *Bromus tectorum* cover was excluded from the analysis. Both *B. tectorum* cover and restoration seeding treatment significantly impacted alpha diversity ($\alpha=0.05$) (Appendix S22).

Species richness was impacted by management treatment ($p=0.006$; Appendix S18), restoration seeding ($p=0.030$; Appendix S18), *B. tectorum* cover ($p<0.001$; Appendix S18), and site ($p=0.002$; Appendix S18), but not time since application ($p=0.188$; Appendix S18) nor year of establishment ($p=0.214$; Appendix S18). Species richness was reduced by 0.8 species ± 0.2 SE in the MSM ($p=0.026$; Figure 2; Table 2; Appendix S19) and indaziflam treatments ($p=0.015$; Figure 2; Table 2; Appendix S19) in all years. Annual forbs only made up around a quarter of the species per quadrat (mean of 1.2 species in control) but were reduced to 0.3 species ± 0.2 SE in both the MSM and indaziflam treatments ($p<0.001$). The NutraFix treatment had no impact on species richness ($p=0.805$; Figure 2; Table 2; Appendix S19). For every 10% increase in *B. tectorum* cover, species richness decreased by 4% ± 1.0 SE, 0.2 species from non-treated controls ($p<0.001$; Appendix S20). Plots that received restoration seeding had 8% higher species richness ± 1.4 SE than plots not seeded, 0.3 species in non-treated controls ($p=0.031$; Table 2; Appendix S20). The Highland ($p=0.002$; Appendix S20) and Red Bluff sites ($p<0.001$; Appendix S20) tended to have 1.1 more species ± 0.2 SE per quadrat (0.75 m²) than Emigrant (Figure 2).

Alpha diversity was impacted by restoration seeding ($p=0.003$; Appendix S21) and *B. tectorum* cover ($p=0.014$; Appendix S21), but was not impacted by management treatment ($p=0.127$; Appendix S21), time since application ($p=0.747$; Appendix S21), year of establishment ($p=0.141$; Appendix S21), nor site ($p=0.205$; Appendix S21). For every 10% increase in *B. tectorum* cover, alpha diversity decreased by 3% ± 3.0 SE ($p<0.001$; Figure 4; Appendix S22) regardless of treatment or site. Plots that received restoration seeding had 9% higher alpha diversity

± 3.6 SE than plots not seeded ($p=0.007$; Figure 4; Table 2; Appendix S22).

An Adonis analysis on the differences in community composition (species presence and abundance) found beta diversity was influenced the most by site ($R^2=22.0\%$; $p<0.001$; Appendix S23), followed by year post application ($R^2=4.8\%$; $p<0.001$; Appendix S23), management treatment ($R^2=2.5\%$; $p<0.001$; Appendix S23), year of establishment ($R^2=2.0\%$; $p<0.001$; Appendix S23), *B. tectorum* cover ($R^2=0.8\%$; $p<0.001$; Appendix S23), and the interaction between management treatment and time since application ($R^2=1.2\%$; $p=0.030$; Appendix S23); this interaction indicates the impact of treatment on community dissimilarity changed over time. Together, the above factors explained 33% of the variation in community composition (Appendix S23). Restoration seeding had no impact on community composition ($p=0.973$; Appendix S23). Although several factors contributed to differences in community composition, clustering in the NMDS ordination space was not clear (Appendix S14).

Rank abundance analysis revealed that the most abundant species changed over time in all treatments except the control plots (Table 3; Appendix S24). The control plots were dominated by *B. tectorum* all 3 years along with moderate amounts of perennial grasses—*H. comata*, *Poa secunda* J. Presl (Sandberg bluegrass), and *P. smithii*—and non-native *Alyssum* spp. (Table 3; Appendix S24). *Bromus tectorum* dominated the indaziflam plots the first 2 years post treatment, the MSM plots in the final 2 years, and NutraFix plots in the third year (Table 3; Appendix S24). When *B. tectorum* was not the most abundant species, it was the second (Table 3; Appendix S24). Evenness also shifted across treatments and over time (Figure 5). The first

year after treatments were applied, the non-treated control plots had the most uneven distribution of species abundance (Figure 5). The second year, the MSM plots were highly dominated by *B. tectorum* (Figure 5; Table 2). In the third year, dominance of the most abundant species increased in all treated plots, with *B. tectorum* being the most abundant in MSM and NutraFix plots and *P. smithii* dominating indaziflam plots (Figure 5; Table 2).

3.3 | Soil Analysis

Treatments influenced soil micronutrients in the second- and third-year post-treatment. The MSM increased levels of phosphorus, sulfur, iron, manganese, copper, and organic matter (all $p < 0.05$; Appendix S4). The NutraFix increased both boron and sodium levels (both $p < 0.05$; Appendix S4). Finally, plots

TABLE 3 | Community composition metrics of the top three most abundant species, with relative percent cover in parenthesis. Rank abundance was calculated for each management treatment for all 3 years post-treatment. Red shading highlights *Bromus tectorum*.

Treatment	Rank abundance	Year post-treatment		
		1	2	3
Control	1	<i>Bromus tectorum</i> (41.9%)	<i>B. tectorum</i> (50.3%)	<i>B. tectorum</i> (46.7%)
	2	<i>Hesperostipa comata</i> (14.5%)	<i>H. comata</i> (10.2%)	<i>P. smithii</i> (8.2%)
	3	<i>Poa secunda</i> (6.7%)	<i>Alyssum</i> spp. (5.4%)	<i>H. comata</i> (8.0%)
MSM	1	<i>H. comata</i> (26.0%)	<i>B. tectorum</i> (44.7%)	<i>B. tectorum</i> (62.8%)
	2	<i>B. tectorum</i> (24.1%)	<i>Sisymbrium altissimum</i> (11.7%)	<i>P. smithii</i> (8.1%)
	3	<i>P. smithii</i> (13.9%)	<i>H. comata</i> (8.2%)	<i>H. comata</i> (6.4%)
NutraFix	1	<i>H. comata</i> (28.3%)	<i>H. comata</i> (20.2%)	<i>B. tectorum</i> (28.9%)
	2	<i>B. tectorum</i> (20.0%)	<i>B. tectorum</i> (18.5%)	<i>H. comata</i> (16.1%)
	3	<i>P. smithii</i> (10.9%)	<i>S. altissimum</i> (9.8%)	<i>Alyssum</i> spp. (10.0%)
Indaziflam	1	<i>B. tectorum</i> (27.3%)	<i>B. tectorum</i> (25.3%)	<i>P. smithii</i> (28.2%)
	2	<i>H. comata</i> (23.3%)	<i>P. smithii</i> (18.9%)	<i>B. tectorum</i> (13.9%)
	3	<i>P. smithii</i> (10.8%)	<i>H. comata</i> (15.3%)	<i>P. spicata</i> (9.7%)

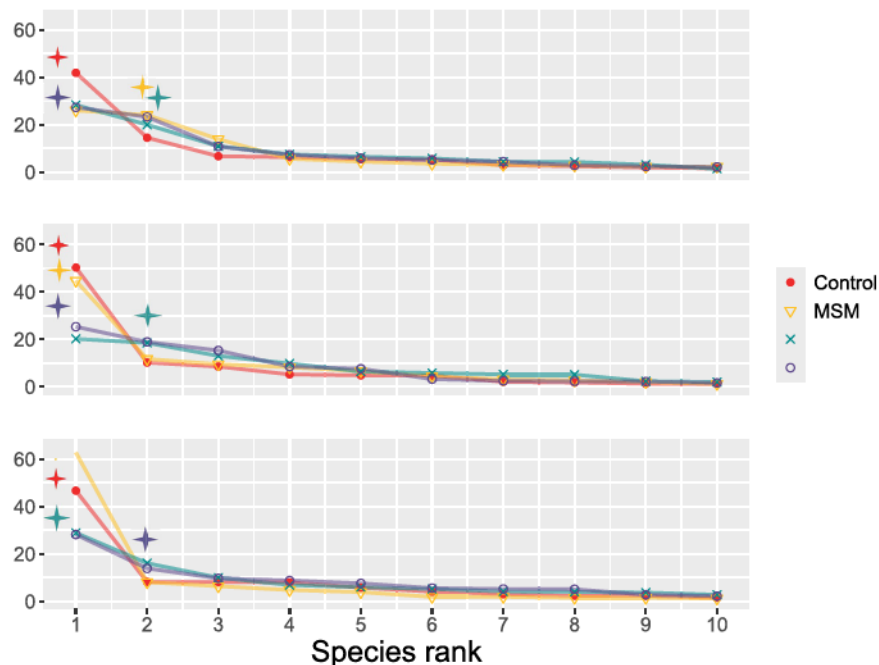


FIGURE 5 | Change in proportional species rank abundance by treatment. Treatments were applied in fall 2020 or 2021 to semi-arid grasslands in southwestern Montana. Ocular cover of all species present was recorded to the nearest 1% annually during peak biomass for 3 years following application. Total species richness varied from 28 to 38 across all treatments and years, but only the top 10 ranked species are presented for visualization (Appendix S24). Position of *Bromus tectorum* is marked by the star in the color that corresponds to treatment applied.

treated with indaziflam had elevated nitrate levels ($p=0.004$; Appendix S4).

4 | Discussion

Non-native species management has traditionally focused only on the effective control of targeted species. This has often proven ineffective to meet long-term, sustainable land management goals (Krueger-Mangold et al. 2006). For example, management may remove the target species but then lead to a secondary invasion (Pearson et al. 2016) or damage other plants in the community (Meyer-Morey et al. 2021). Management strategies need to evolve to consider both the impacts to the target species and the remaining community (Rew et al. 2007). This is especially true in arid and semi-arid annual grass invaded systems where low precipitation can lower ecosystem resilience and management success (Chambers et al. 2014). The objectives of this study were to compare the impacts of novel management approaches on both the target IAG, *B. tectorum*, and the surrounding plant community.

Mustard seed meal has been shown to reduce germination and emergence of weed seeds in cropping systems (De Cauwer et al. 2019) but using MSM to manage IAGs in rangelands is just starting to be explored (Sencenbaugh et al. 2024a, 2024b). In our study, the MSM performed in accordance with our hypothesis providing strong, but short-lived *B. tectorum* control, followed by unexpected increases in the following years. Additionally, species richness was decreased, contrary to our initial hypothesis. These results could be explained by the high levels of nitrogen in *Brassica* seed meals (Lewis et al. 2019). Mustard amendments have been shown to alter soil characteristics like carbon, nitrogen, phosphorous, sulfur, soil organic matter, and pH (Lewis et al. 2019; Temmen et al. 2023). While our seed meal was not incorporated into the soils, we believe the addition could still explain the quick rebound of *B. tectorum* levels—after the first year the MSM decomposed, potentially releasing nitrates into the soil that were utilized by the next season's population of *B. tectorum*. This provided *B. tectorum* with a competitive advantage over our native species because annual grasses are known to cycle resources more quickly than established perennial species and seedlings (Ehrenfeld 2003), resulting in the observed reduced species richness. Another possible explanation for the reduced species richness could be that the MSM directly reduced germination of some native seeds as demonstrated by Sencenbaugh et al. (2024a).

Surprisingly, we found strong evidence across our sites that NutraFix reduced *B. tectorum* cover for only 2 years, which is contrary to the advertised long-term control (Jennings 2020). Boron can cause phytotoxicity at relatively low doses, particularly in semi-arid regions where limited rainfall leads to higher natural accumulation near the soil surface (Herrera-Rodriguez et al. 2010; Ashagre et al. 2014). We found elevated levels of boron in plots treated with NutraFix, and this toxicity could be contributing to the control of *B. tectorum*. In contrast, the NutraFix did not negatively impact our predominantly perennial plant community, as neither species richness nor alpha diversity declined. This is encouraging, but negative impacts from boron are known to vary by species (Wallander 1993; Nable et al. 1997). Given that this is the first study to evaluate the efficacy of NutraFix, the role

of boron in rangeland soils and plants must be better understood before any recommendations can be made.

In accordance with our hypothesis, indaziflam controlled *B. tectorum* and reduced species richness for all 3 years. The continued control of *B. tectorum* over time suggests indaziflam could be depleting *B. tectorum*'s seed bank. This is encouraging for long-term management because seeds of *B. tectorum* and other IAGs tend to retain viability for only 1–3 years in the seed bank, with most germinating the season they are produced (Smith et al. 2008; Wallace et al. 2015). However, indaziflam is a non-selective herbicide, meaning timing of application is essential to minimize effects to germination of non-target species. Here, we found a reduction in species richness and annual forbs, although changes in abundance were less apparent. Indaziflam purportedly has less impact on dicotyledonous species (EPA 2020), but recent studies evaluating the impact of indaziflam on native annual dicots found reductions in annual species richness both in seed banks (Meyer-Morey et al. 2021; Courkamp et al. 2022) and aboveground communities (Meyer-Morey et al. 2021; Mangold et al. 2024). Additionally, the composition of our indaziflam-treated plots shifted over time to be heavily dominated by the perennial rhizomatous grass *P. smithii*. While increases in native perennial grasses are often a desired goal of IAG management, the loss of species richness or evenness, and dominance of one perennial grass species would not meet other ecosystem level goals such as temporal stability of forage. This is a tradeoff that managers need to carefully consider when approaching IAG management.

In partial accordance with our hypothesis, our restoration seeding treatment slightly improved measures of species richness and alpha diversity but did not have any impact on *B. tectorum* control. While the increases in richness and diversity were statistically significant, practically they were small changes. Though small changes on a 0.75-m² area may seem minor, they can translate to larger impacts at broader scales, as quadrat-level richness was much lower than site richness and our species accumulation estimates. Restoration seeding projects in semi-arid regions are notoriously difficult, and seedling establishment is often low due to harsh conditions and low precipitation (Havrilla et al. 2020). However, there is evidence that combining restoration seeding with herbicide applications can improve IAG control efforts in the western United States (Bennion et al. 2025). It should be noted that the selectivity of our treatments is not fully understood, and our native grass seed may have been negatively impacted by our treatments. The seed was broadcast the spring after treatment application occurred the previous fall. Due to the volatility of the MSM, we expect that it likely did not impact seed viability. However, the longer term activity of NutraFix and indaziflam could have negatively impacted seedling recruitment. This highlights that incorporation of restoration seeding along with IAG management could be beneficial but should be carefully considered.

Similar to restoration seeding, the negative impact of *B. tectorum* cover on species richness and alpha diversity was small yet statistically significant. The suggestion that the presence of *B. tectorum* is having little effect could appear counterintuitive considering the negative impacts *B. tectorum* and other IAGs can have on rangelands are widely stated (De Stefano et al. 2024). However, there is evidence that the impacts of *B. tectorum* are dependent on its abundance—which our study supported, the corresponding abundance

of native vegetation, and climatic factors like the seasonality of precipitation and soil temperature (Chambers et al. 2016). Chambers et al. (2014) hypothesized that cooler regions that receive more precipitation have higher levels of resilience to *B. tectorum* invasions. Our sites occur in the cooler/wetter end of the proposed spectrum indicating higher levels of resilience to *B. tectorum* in our region, which could be contributing to the limited negative impacts on species richness and alpha diversity. Furthermore, our sites had cover of native grasses ranging from 10% to 30% per plot: levels above 20% are suggested to support higher resilience (Chambers et al. 2014). Consequently, our abundant, established perennial grasses could also be contributing to the significant but low impact of restoration seeding, indicating that restoration seeding may be more beneficial in depauperate systems.

Overall, our sites experienced slightly warmer and wetter years than average, with the exception of 2021 which was slightly drier. While these variations likely impacted our results, especially with 2021 being one of the years we applied treatments, our replication over time and space allows us to be confident in our results. That being said, there is ample space for future work into the interactions between precipitation, temperature, and the timing of treatment application. Additionally, the possibility of combining these management approaches either with each other or with other existing approaches in integrated weed management plans warrants further research as well. This could advance our knowledge of non-synthetic approaches and how they may be utilized alongside traditional management options.

Many practitioners and researchers attempting to advance the sustainable management of IAGs are exploring novel tools that have little to no research behind them. In these cases, it is especially imperative that the impacts to the entire plant community are considered before large-scale recommendations can be made. Consideration of how each approach performs across a greater range of sites within and across ecosystems is also a crucial next step in this research. In our study, we found that all three of our control methods were able to reduce *B. tectorum* cover, albeit to differing amounts and longevity. They also had differing impacts on species richness, alpha diversity, and composition of the surrounding plant community. Analyzing these diversity metrics allowed us to track community level changes that resulted from management actions suggesting these assessments could be applied to and benefit other studies in this field. A deeper understanding of how plant communities respond to management actions, and not just how effective the management actions are, will help advance both the efficacy and sustainability of invasive species management.

Author Contributions

L.J.R. conceived of the research idea and acquired funding, J.M.M. provided logistical support in the field, E.B.T. and L.S. collected the data, E.B.T. performed all data analysis and wrote the original draft, and all authors reviewed and edited the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data and code are available on Mendeley <https://doi.org/10.17632/jzngzjymhj.2>.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Appendix S1:** Study locations and characteristics. **Appendix S2:** Seasonal precipitation and temperature. **Appendix S3:** Complete species list. **Appendix S4:** Soil analysis results. **Appendix S5:** Models used and their response and predictor variables. **Appendix S6:** Shannon's alpha diversity equation. **Appendix S7:** Inverse Simpson's alpha diversity equation. **Appendix S8:** Bray–Curtis dissimilarity equation. **Appendix S9:** Morisita–Horn dissimilarity equation. **Appendix S10:** Linear model output predicting Inverse Simpson's alpha diversity. **Appendix S11:** Analysis of variance (ANOVA) output of Inverse Simpson's alpha diversity model. **Appendix S12:** Change in Inverse Simpson's alpha diversity given *Bromus tectorum* cover. **Appendix S13:** Permutational multivariate analysis of variance (Adonis) of Bray–Curtis distance matrix. **Appendix S14:** Non-metric multidimensional scaling (NMDS) of Morisita–Horn distance matrix. **Appendix S15:** NMDS of Bray–Curtis distance matrix. **Appendix S16:** ANOVA output of *Bromus tectorum* model. **Appendix S17:** Differences in *Bromus tectorum* cover between treated and control plots. **Appendix S18:** ANOVA output of species richness model. **Appendix S19:** Differences in species richness between treated and control plots. **Appendix S20:** Linear model output predicting species richness. **Appendix S21:** ANOVA output of Shannon's alpha diversity model. **Appendix S22:** Linear model output predicting Shannon's alpha diversity. **Appendix S23:** Adonis of Morisita–Horn distance matrix. **Appendix S24:** Complete rank abundance for each treatment over 3 years.