Responses of Flowering Plant and Butterfly Communities to Experimental Herbicide and Seeding Treatments for Native Grassland Restoration

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ABSTRACT

Globally, grasslands are among the most degraded habitats, and within that category tallgrass prairies are especially threatened. To restore native species in tallgrass prairie, it is often necessary to concurrently remove exotic plant species while restoring the disturbance processes that many prairie taxa depend upon. Here, we coupled one herbicide application and seed mixture addition with tri-annual prescribed fire and annual cattle grazing to explore the consequences for floral resource (i.e., nectar-producing) plant communities and butterfly communities up to four years later. Each site was divided into three equally sized patches which were placed into either control, "spray-only", or "spray-and-seed" treatment groups. We quantified both floral resource plant and butterfly communities in the year prior to herbicide application and the four years following that treatment. In the four years post-treatment, we found that floral resource abundance, richness, and diversity increased over time but did not significantly vary among treatments. No response to treatment was observed in butterflies, but butterfly abundance decreased while richness was stable and diversity increased over time, which may indicate that the changes to floral resources at the patch scale supported more speciose and diverse butterfly communities at the site scale. Butterfly abundance decreasing over time could be an effect of baseline management or unrelated regional factors. Regardless of whether either treatment benefited butterflies, we find support for a one-time herbicide application changing the floral community in desirable ways and at least not harming butterflies.

Keywords: community, pasture, pollinator, prairie, pyric-herbivory

Restoration Recap

- We measured plant and butterfly response to herbicide and seed mixture treatments in restored Midwestern prairies dominated by an exotic cool-season grass species.
- Sites were divided into three equal "patches" for treatment application.
- Treatments included herbicide alone, herbicide and a seed mixture added, and a control.
- Floral richness and diversity increased in treatment patches relative to the control while butterfly diversity improved across whole sites but not among patches.
- Herbicide application—in situations like these—may prove an effective restoration tool in combatting invasive cool-season grasses but further action is likely necessary for the restoration of more environmentally sensitive animal groups.

Grasslands around the world are among the most imperiled ecosystems and this is particularly true of tallgrass prairie. It has been estimated that total land cover of tallgrass prairie has fallen by 99.9% since the expansion of Euro-American settlers over the last two centuries (Samson and Knopf 1994). An ecosystem that once covered much of North America was relatively rapidly reduced to a patchwork of small, isolated remnants that currently dot the landscape (Fuhlendorf and Engle 2001). One of the contributors to this decline has been the massive expansion of agricultural practices into the tallgrass prairie region,

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as these areas are extensively converted into crop fields and grazing lands for livestock. Agricultural practices also have hindered vital processes that tallgrass prairies rely on, namely semi-frequent wildfires and grazing by native bison and elk (Fuhlendorf and Engle 2004). Historically, fires reduced woody plant colonization and increased plant community heterogeneity (Copeland et al. 2002). Grazing provided another key disturbance to prairies through continual stress by herbivory, leading to a higher diversity of forb species (Joern 2005). Moderate stocking by cattle in combination with burning can return some of these historic disturbances to grassland sites (Fuhlendorf and Engle 2001) and community composition can shift greatly in as few as three years after management (Camill et al. 2004, Delaney et al. 2016).

However, even in grasslands that are currently managed with moderate grazing and fire, the legacy of disturbances from past agricultural practices (Isbell et al. 2019) and prevalence of dominant exotic plant species (Wilsey et al. 2009) can leave tallgrass prairie in a state of low biodiversity that can be difficult to restore. From an economic perspective, exotic and invasive plant species contribute to a loss of ecosystem services valued at $2 billion annually on rangelands (DiTomaso 2000). The invasive grass *Schedonorus arudinaceus* (tall fescue; hereafter "fescue") is one such costly invader that covers 14 million hectares in the United States (Ball et al. 1991).

Ironically given that fescue was introduced as a forage species for cattle (Ball et al. 1991), fescue associated with a fungal endophyte can cause a condition known as "fescue toxicosis" where beef cattle are less able to regulate their internal body temperature, gain less weight, produce less milk, and have lower conception rates, all of which can cost cattle producers hundreds of millions of dollars (Paterson et al. 1995). While this economic argument is important, fescue also poses an ecological threat (Rudgers and Clay 2007, Severns and Warren 2008, McGranahan et al. 2012). Fescue has also been shown to have dramatic implications for plant community structure via altered succession pathways, diversity, and plant-plant competitive interactions (Rudgers and Clay 2007) as well as reduced amount and evenness of distribution of litter accumulation and reduced spatial extent of fire via changed fire regimes (McGranahan et al. 2012). Perhaps unsurprisingly given the impacts fescue has on other plants, this species can also indirectly change plant-herbivore interactions (Rudgers and Clay 2007) and modify pollinator communities by changing the availability of host plants for oviposition (Severns and Warren 2008).

Given the breadth of the suite of taxa that fescue can impact, it is desirable to select a subset of those taxa to serve as indicators that can be more feasibly assessed. Ideal indicator species are groups that are ecologically linked with other taxa of interest and are more easily measured than taxa they are associated with. Given the intuitively tight ecological bonds between nectar-producing flowering plants and pollinators (Koh et al. 2004, Westgate et al. 2017), butterflies and their resource plants are logical indicator species. For instance, *Danaus plexippus* (monarch butterfly) are known to be strongly affected by the presence of their obligate larval host plant (Oberhauser et al. 2017) so measurements of that host plant can be one useful indicator of habitat suitability for that butterfly species. Additionally for butterflies in this context, the primary tool used in managing fescue—the responsible use of herbicides (Washburn et al. 1999, 2000)—has previously been shown to increase the abundance of native plants (Washburn et al. 1999, 2000) and benefit pollinators (Madison et al. 2001). Given some butterfly species' dependence upon specific larval host plants (Koh et al. 2004), it would be reasonable to be concerned about herbicide spray indirectly affecting butterflies via their larval resource requirements, but previous work found that herbicides had little to no effect on larval survival and oviposition preference in native butterflies (LaBar and Schultz 2012, Glaeser and Schultz 2014).

In the Grand River Grasslands region of southern Iowa and northern Missouri, U.S.A., exotic grasses including fescue are a significant ecological threat to prairie remnants (Wilsey et al. 2009) and an economic threat to the local cattle industry, the dominant agricultural activity in the area (Fuhlendorf and Engle 2004). Previous work in the Grand River Grasslands has found that prescribed fire and grazing initiated a trajectory of slow recovery in reducing exotic dominance (Delaney et al. 2015), but given fescue's aforementioned ecological (Rudgers and Clay 2007) and economic consequences (Ball et al. 1991), further management intervention was deemed necessary. We decided to test the efficacy of application of a non-selective herbicide in reducing fescue and reaching restoration targets. Given the prevalence of propagule limitation and degraded seedbanks in systems in need of restoration (Kettenring and Adams 2011), we also included a one-time seed mixture addition following the herbicide application to facilitate native plant recruitment. These two treatments were proposed to reduce exotic dominance which could allow native plants—and their associated butterflies—to increase in abundance and diversity (Wilsey et al. 2009).

We quantified the abundance, richness, diversity, and community composition of butterfly and nectar-producing flowering plants (hereafter "floral resources") for one year prior to treatment and for four years following herbicide application and herbicide application plus interseeding of native plants on sites managed by annual moderate grazing and a prescribed fire once every three years in the Grand River Grasslands. Each site was split into three equally sized "patches" and these patches were then allocated to one of three treatments: application of glyphosate ("spray-only"), application of glyphosate followed by drilling of a diverse seeding mixture ("spray-and-seed"), and no treatment ("control"). We hypothesized the following:

Table 1. Experimental sites. While stocking rate apparently differed, this was done to ensure that grazing pressure was consistent across sites of different levels of forage production. Stocking rate is measured in AUM, animal unit months. IES = intensive early stocking, SLS = season long stocking.

Site	Data Code	Site Size (ha)	Stocking Rate (AUM/ha) *	Stocking Type	Stocking Duration
Gilliland	GIL	31	2.47	IES	Early April–Early July
Lee Trail	LTR	34	3.51	IES	Early April–Early July
Pyland West	PYW	18	1.83	SLS	Early April–Early September

1. Floral resource abundance, richness, and diversity would be higher in the two sprayed treatments than in the control.
2. Butterfly abundance, richness and diversity will also be higher in the two sprayed treatments than in the control as revegetation of native plant species promotes the re-establishment of associated butterflies (Washburn et al. 2000, Madison et al. 2001).
3. Community composition of nectar resource plants and butterflies would be similar between spray-only and spray-and-seed patches but different between those two patches and the control patch.
4. Both the abundance and species richness of flowering plants in the seed mixture would be highest in the spray-and-seed patches following treatment due to the difficulty of native plants revegetating without management (Kettenring and Adams 2011).
5. The percent of the flowering stems that are from native plants would be greatest in spray-and-seed patches following treatment as the seed mixture establishes in those patches.

Implicit in these hypotheses is the assumption that fescue cover would be reduced in the two both treatments including glyphosate. This paper focuses on the effect of the treatments on floral resources and butterflies; we reference the work of Coon et al. (2021) where the response of fescue and other plant community metrics is fully described and analyzed to provide needed context.

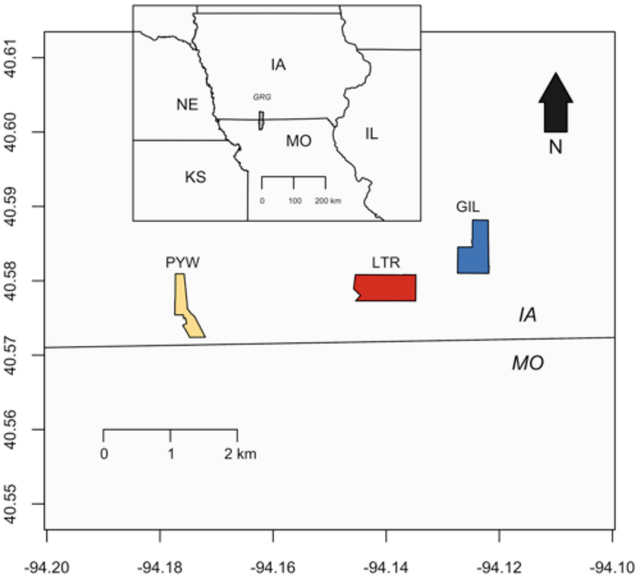

Figure 1. Map of the three study sites in relation to one another with an inset showing the larger region around the sites. Latitude and longitude are provided on the vertical and horizontal axes of the map, respectively. In the site-level map, sites are given different colors, and abbreviations are as follows: PYW = Pyland West, LTR = Lee Trail, GIL = Gilliland, IA = Iowa, MO = Missouri, IL = Illinois, NE = Nebraska, KS = Kansas. In the inset map, the gray rectangle labeled "GRG" indicates the location of the Grand River Grasslands. Site-level management information is provided elsewhere (Table 1). Patch identity is not shown on this map, but sites are divided as follows: GIL and PYW are divided evenly into north, center, and south patches while LTR is divided evenly into east, center, and west. Patches within a site immediately abut one another without separation. Location is shown in UTMs.

Materials and Methods

Experimental Design

We selected three sites within the Grand River Grasslands of southern Iowa and northern Missouri for this study (2014–18; Table 1; Figure 1). These sites ranged from 18 to 34 ha in size and all had a relatively high level of invasion by fescue, with average percent cover exceeding 30%. The three sites used in this study were part of a larger suite of sites previously used to assess butterfly and floral response to combinations of grazing and prescribed fire in a multi-year experiment (Moranz et al. 2012, Delaney et al. 2016). However, our study takes place on the three sites used for only one treatment from those studies: annual grazing by cattle from April to September and burning in March every third year. Two of the three sites—Gilliland and Lee Trail—were grazed via intensive early stocking (IES) while the remaining site—Pyland West—was grazed with season long stocking (SLS; Table 1). These approaches have the same stocking rate across a season, but in IES there are twice as many cattle for half the time. We applied the first prescribed burn before the inter-seeding on spray-and-seed patches (described below) and the second one three years later (before the final season of data collection).

We divided each site into equal thirds (hereafter "patches") for treatment application. Each site included one of the three treatments such that each patch was placed into either control, spray-only, or spray-and-seed treatment groups. We did not include a seed-only treatment because the dominance of fescue makes establishment of seeded species unlikely without anti-fescue management. In spray-only patches we applied glyphosate (a non-selective, translocated herbicide) once in the fall. We treated spray-and-seed patches with the same glyphosate treatment and an interseeding of native prairie plants in the subsequent spring. We left control patches untreated beyond the site-wide grazing and prescribed fire. We used visual cover estimates of fescue differences among patches to allocate treatments. We selected the patch with the highest average cover of fescue (43.0%) for spray-and-seed, the intermediate average fescue cover (35.7%) for spray-only, and the lowest average (30.3%) for the control. However, these putative differences in fescue cover were not statistically significant pre-treatment.

We applied glyphosate on the spray-only and spray-and-seed patches at a rate of 1.3 kg ae/ha on 4 or 5 November 2014. Our goal in applying the herbicide in November was to reduce the fescue cover while minimizing direct negative effects on native vegetation. As a C_3 grass, fescue was still green and photosynthesizing in early November 2014, while C_4 plants (the vast majority of the native grasses in the system) were dormant due to several days of below-freezing temperatures prior to 1 November. Dormant plants do not take up glyphosate (Martin and Moomaw 1974, Waller and Schmidt 1983). The spray-and-seed patches were inter-seeded using a drill with a seeding mixture of native grasses and flowering plants in early May 2015 (Supplemental Table S1).

Floral Resource Sampling

We sampled floral resources four times annually, from late May to mid-August, from 2014 to 2018. We established two permanent 100 m long sampling transects in each patch of the experimental sites, for a total of six transects per site (Moranz et al. 2012). We divided each transect into five 20 m long sections, and counted all reproductive ramets (flowering shoots) of floral resource plants (i.e., nectar-producing flowering plants) within 1 m of a randomly-selected side of the transect in each section (Moranz et al. 2012, Delaney et al. 2015). Transect visits occurred twice between late May and June 30th, and two more times between July 1st and mid-August. A complete list of the floral resource species we observed, their scientific names, whether they are native or exotic in the contiguous United States (according to the Natural Resources Conservation Service of the United States Department of Agriculture), and their total abundance across this study is provided in the supplemental materials (Supplemental Table S2).

Butterfly Sampling

We also sampled butterflies annually from 2014 to 2018 during the same sampling season as the floral resources and on the same transects during the same site visits. To account for the two butterfly emergence periods, which occured from June–July and July–August (Schlicht et al. 2007, Moranz et al. 2012), we sampled butterflies on each transect twice during the field season, once in May–June and once in July–early August, using a transect method modified from Thomas (1983). We used a modified Pollard walk method (Pollard 1977). Observers walked from one end of the transect to the other over a 10-minute period at a constant pace (10 m/min) while simultaneously identifying each butterfly species observed. Butterflies that could not be identified were captured using a net and identified post hoc. Butterfly sampling was conducted between 0900–1830 hours Central Standard Time when temperatures were between 21 and 35°C and wind speed was below 16 kph. The full details of this sampling approach can be found in Moranz et al. (2012) and Delaney (2014). The common and scientific names of the butterfly species we observed and their total abundance in each year are included elsewhere (Supplemental Table S3).

Statistical Analysis

We used species-specific sums across both visits to both transects on each patch within a given sampling year to calculate butterfly and floral resource abundance, species richness, and Shannon diversity (H'). Because adult butterfly lifespans are generally 2–3 weeks (Schlicht et al. 2007), we assumed that butterflies surveyed on separate survey dates were in fact different individuals. We summed the total number of reproductive ramets observed in each section of each transect in the same way. We split the data into pre-treatment (2014) and post-treatment (2015–18) to determine whether patches differed with respect to any response variable prior to the application of treatments.

We used the R statistical environment (R Core Team 2016) for all statistical analyses and figure creation. We ran permutational analyses of variance (perMANOVAs) with a residual randomization using the “lm.rrpp” function in the RRPP package (Collyer and Adams 2018). Permutational approaches generate a distribution of F statistics from permutations of the data and as such are non-parametric (Collyer and Adams 2019). We used perANOVA rather than the parametric equivalent (i.e., ANOVA) because our sample size was low ($N = 3$), and permutation approaches are less sensitive to sample size than traditional ANOVA. We included both treatment and year as explanatory variables with an interaction term and refit the model without the interaction term where it was not significant ($p > 0.05$). We used abundance, species richness, and Shannon diversity (separately for floral and butterfly communities), abundance and richness of flowering plant species included in the seed mixture, and the percent of flowering stems that were native as our response variables in separate perANOVAs. We ran pairwise comparisons among treatments with a sequential Bonferroni multiple comparison adjustment to the critical point (Holland and Copenhaver

1987). To facilitate interpretation of the results of this multiple comparison method, both *p*-value and critical point are reported together. Model assumptions and fit were checked with the "resid_panel" function (Goode and Rey 2019). All univariate figures were created in the ggplot2 (Wickham et al. 2018) and cowplot (Wilke 2019) R packages.

We conducted multivariate perANOVAs (perMANOVAs) using Jaccard distances (Jaccard 1912) of community matrices as our response variable with the RRPP function (Collyer and Adams 2018) for both butterfly and floral resource communities. We performed these tests within each of the study years (including the pre-treatment year) and each used only treatment as an explanatory variable. A second set of perMANOVAs was run for both butterfly and floral resource communities using Bray-Curtis dissimilarities (Bray and Curtis 1957) of the same community datasets. This allowed us to test both whether the species presence-absence community composition (i.e., Jaccard distance) or the community composition considering species abundances (i.e., Bray-Curtis dissimilarity) differed among our treatments. Pairwise comparisons were run as described for the univariate analyses when treatment was significant. When significant, we used Principle Coordinates Analysis (PCoA) to visualize community composition data (Gower 1966) using the "pcoa" function in the ape package in R (Paradis et al. 2020). All R code and data are available via Git (Torvalds and Hamano 2021) in a public repository (Lyon 2021).

Results

To review, we hypothesized that, relative to control patches, spray-and-seed and spray-only patches would have 1) more abundant, speciose, and diverse communities of floral resource plants and 2) more abundant, speciose, and diverse communities of butterflies, and that 3) the community composition of butterflies and floral resources on spray-only and spray-and-seed patches would be similar to one another but differ from the control. Additionally, we hypothesized that 4) spray-and-seed patches would have higher abundance and species richness of plants included in the seed mixture than the other patches and that 5) spray-and-seed patches would also have a higher percentage of native flowering stems than the treatment patches.

Abundance, Richness, and Diversity

There were no differences in pre-treatment floral abundance, richness, or diversity among treatments (Figure 2, Table 2). Following treatment application, floral abundance did not vary with any of our explanatory variables (Figure 2A, Table 2). Floral richness increased over time regardless of patch-level treatment (Figure 2B, Table 2). There is weak evidence that floral richness was higher in spray-and-seed patches than in control or spray-only patches, but this putative effect is nonsignificant after accounting for multiple comparisons (Table 3). Floral diversity followed a pattern similar to richness: diversity increased over time without respect to treatment (Figure 2C, Table 2) and Shannon diversity was slightly higher in spray-only patches than in control patches though this difference was again nonsignificant after accounting for multiple comparisons (Table 3).

As with floral resources, there were no pre-treatment differences in butterfly abundance, species richness, or diversity among patches (Figure 3, Table 2). Butterfly abundance decreased over the post-treatment years across all treatments, but this decline was not affected by treatment (Figure 3A, Table 2). Despite the weak (i.e., nonsignificant) differences in floral richness and diversity among treatments, butterfly richness did not change, and Shannon diversity increased post-treatment across all patches but did not show even a weak treatment effect (Figure 3B–C; Table 2).

Community Composition

Floral community composition did not differ among any of the three treatments (Table 4) within any study year. Unsurprisingly given the lack of difference among floral communities, butterfly community composition also did not significantly differ among the treatments in any study year (Table 4). Analysis of Jaccard distances and Bray-Curtis dissimilarities were consistent in this lack of significant differences.

Seed Mixture Establishment and Percent Native Flowers

The abundance of flowering stems from plants included in the seed mixture did not differ among the treatments or across sampling years post-treatment (Figure 4A, Table 2). The species richness of seed mixture plants was also unaffected by treatment or sampling year (Figure 4B; Table 2). Finally, the percent of flowering stems from native plants did not vary among treatments or sampling years (Figure 5, Table 2) though there was dramatic among-site variation among patches at different sites (see standard error bars in Figure 5).

Discussion

A substantial caveat to our hypotheses is our assumption that fescue cover would decline in both treatments using glyphosate. While we do not analyze fescue cover changes in this paper, Coon et al. (2021) found that fescue was significantly reduced in both spray-only and spray-and-seed patches on these sites. In addition, there was no evidence of fescue recovery over the four post-treatment years.

We do not find support for our first hypothesis; floral resource abundance, richness, and diversity do not

Table 2. Results of perANOVA analyses of post-treatment among-treatment abundance (#), species richness (S) and Shannon diversity (H′) over time. Significant *p* values ($p < 0.05$) are followed by an * for ease of visualization.

Figure Panel	Year(s)	Response	Model Term	F	DF	Z	*p*
1a	2014	Floral #	Treatment	0.26	2, 8	–0.54	0.7230
			Interaction	0.67	2, 35	0.11	0.5186
1a	2015–18	Floral #	Treatment	2.16	2, 35	1.02	0.1300
			Year	0.64	1, 35	0.36	0.4274
1b	2014	Floral S	Treatment	0.11	2, 8	–1.45	0.9343
			Interaction	0.14	2, 35	–1.05	0.8643
1b	2015–18	Floral S	Treatment	4.15	2, 35	1.51	0.0260*
			Year	11.99	1, 35	—	0.0011*
1c	2014	Floral H′	Treatment	0.25	2, 8	–1.03	0.8331
			Interaction	2.19	2, 35	1.03	0.1293
1c	2015–18	Floral H′	Treatment	3.06	2, 35	1.41	0.0353*
			Year	7.41	1, 35	1.44	0.0108*
2a	2014	Butterfly #	Treatment	0.96	2, 8	0.27	0.4131
			Interaction	0.31	2, 35	–0.48	0.7388
2a	2015–18	Butterfly #	Treatment	2.08	2, 35	0.97	0.1437
			Year	5.90	1, 35	1.34	0.0233*
2b	2014	Butterfly S	Treatment	0.28	2, 8	–0.60	0.7414
			Interaction	0.78	2, 35	0.22	0.4649
2b	2015–18	Butterfly S	Treatment	0.04	2, 35	–2.16	0.9585
			Year	2.95	1, 35	—	0.0907
2c	2014	Butterfly H′	Treatment	0.26	2, 8	–0.63	0.7507
			Interaction	1.21	2, 35	0.56	0.3167
2c	2015–18	Butterfly H′	Treatment	0.35	2, 35	–0.47	0.7435
			Year	16.33	1, 35	1.80	0.0002*
3a	2014	Seed Mixture #	Treatment	0.28	2, 8	–0.76	0.7993
			Interaction	0.03	2, 33	–2.23	0.9686
3a	2015–18	Seed Mixture #	Treatment	0.40	2, 33	0.14	0.5356
			Year	0.40	1, 33	0.14	0.5365
3b	2014	Seed Mixture S	Treatment	0.24	2, 8	–0.92	0.8655
			Interaction	0.003	2, 33	–3.85	0.9960
3b	2015–18	Seed Mixture S	Treatment	2.45	2, 33	1.07	0.1033
			Year	2.13	1, 33	—	0.1590
4	2014	% Native	Treatment	0.39	2, 8	–1.07	0.8207
			Interaction	0.44	2, 35	–0.22	0.6493
4	2015–18	% Native	Treatment	0.002	2, 35	–2.36	0.9696
			Year	0.21	1, 35	–0.74	0.7978

Table 3. Summary statistics from pairwise comparisons for richness (S) and Shannon diversity (H′) due to significant treatment effects in 2015–2018. While no *p* values were significant (*p* < Alpha), *p* values that approached significance are preceded by an * for ease of visualization.

Figure Panel	Response	Con–Spr		Con–SnS		Spr–SnS	
		Alpha (α)	*p*	Alpha (α)	*p*	Alpha (α)	*p*
1b	Floral S	0.0250	0.1015	0.0170	*0.0225	0.0500	0.5467
1c	Floral H′	0.0170	*0.0182	0.0500	0.3458	0.0250	0.1825

significantly differ among treatments though all three metrics increase over time. The floral resource results indicate that the baseline prescribed fire and grazing may benefit flowering plant communities and potentially the nectar resources available to pollinators regardless of patch-level treatment. These increases could also be due to some combination of establishment of forbs from the seed bank and establishment of the seed mixture plants during that interval following the reduction of fescue dominance. The lack of differences among the treatments may be due to cool-season grasses (i.e., C_3 grasses) becoming significantly more abundant in spray-and-seed patches than control and somewhat more abundant in spray-only patches (Coon et al. 2021). Most of the other cool-season grasses in this system are also exotic species (e.g., *Bromus inermis* [smooth bromegrass] and *Poa pratensis* [Kentucky

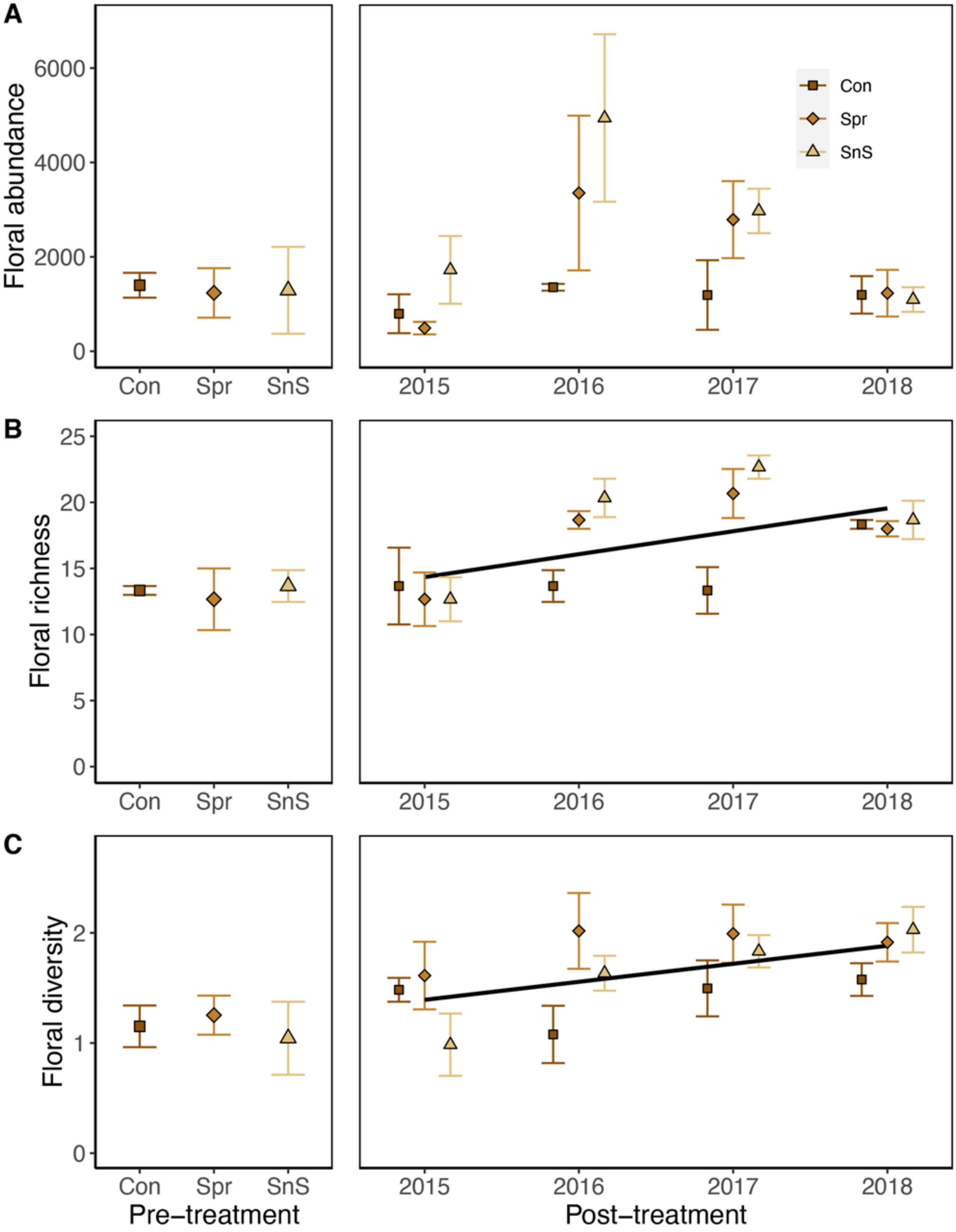

Figure 2. Differences in floral resource (A) abundance, (B) species richness, and (C) Shannon diversity across sampling years. Points are means ± SE. The left panel indicates pre-treatment (2014) comparisons among patches while the right panel shows post-treatment response (2015–18). Con refers to the control treatment, Spr refers to the spray-only treatment, and SnS refers to the spray-and-seed treatment. Solid lines indicate significant change over time. The black line is included where there was significant change over time, but that change was not treatment-dependent (i.e., year was significant, but not the interaction of year and treatment).

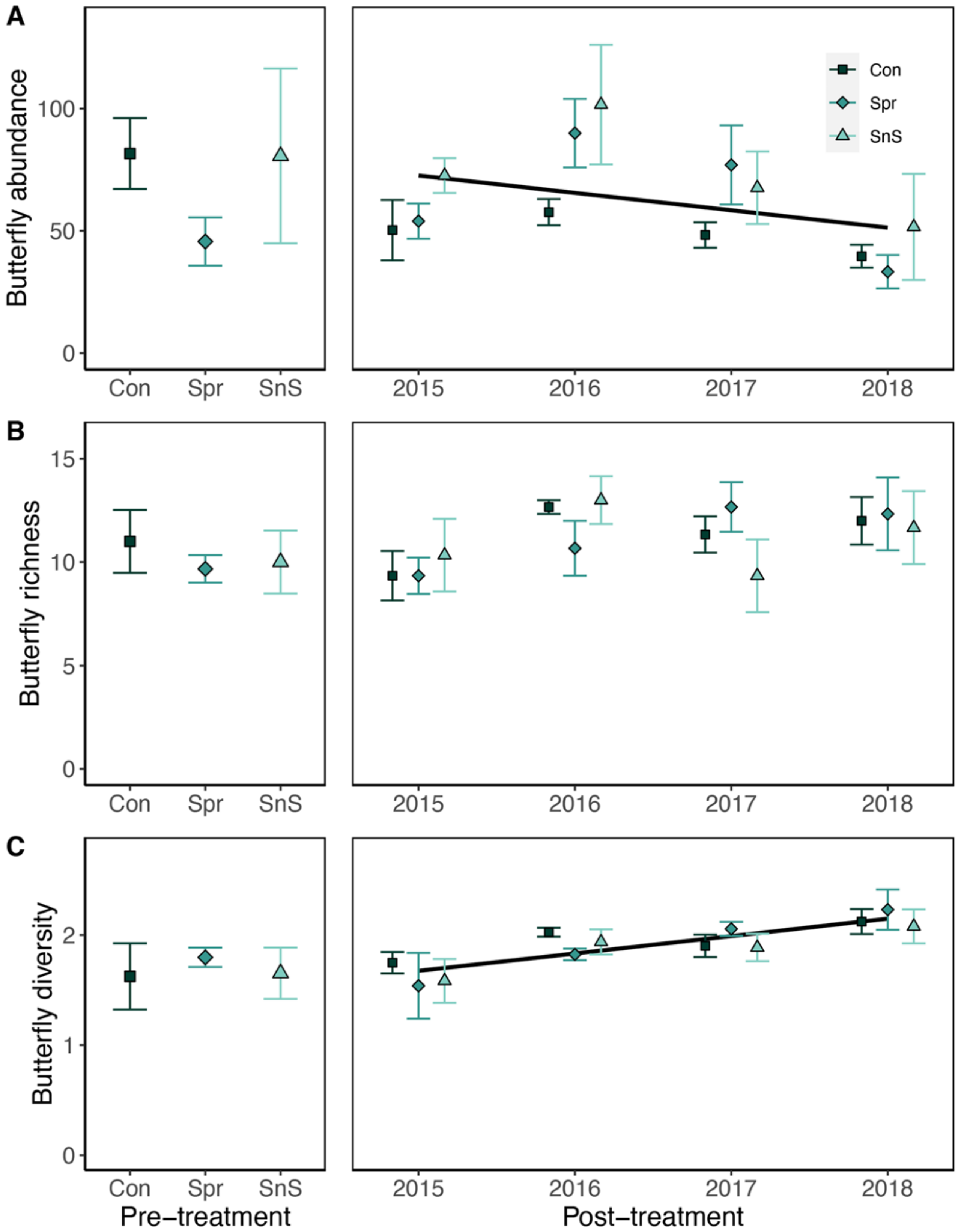

Figure 3. Differences in butterfly (A) abundance, (B) species richness and (C) Shannon diversity across sampling years. Points are means ± SE. The left panel indicates pre-treatment (2014) comparisons among patches while the right panel shows post-treatment response (2015–18). Con refers to the control treatment, Spr refers to the spray-only treatment, and SnS refers to the spray-and-seed treatment. Solid lines indicate significant change over time. The black line is included where there was significant change over time, but that change was not treatment-dependent (i.e., year was significant, but not the interaction of year and treatment).

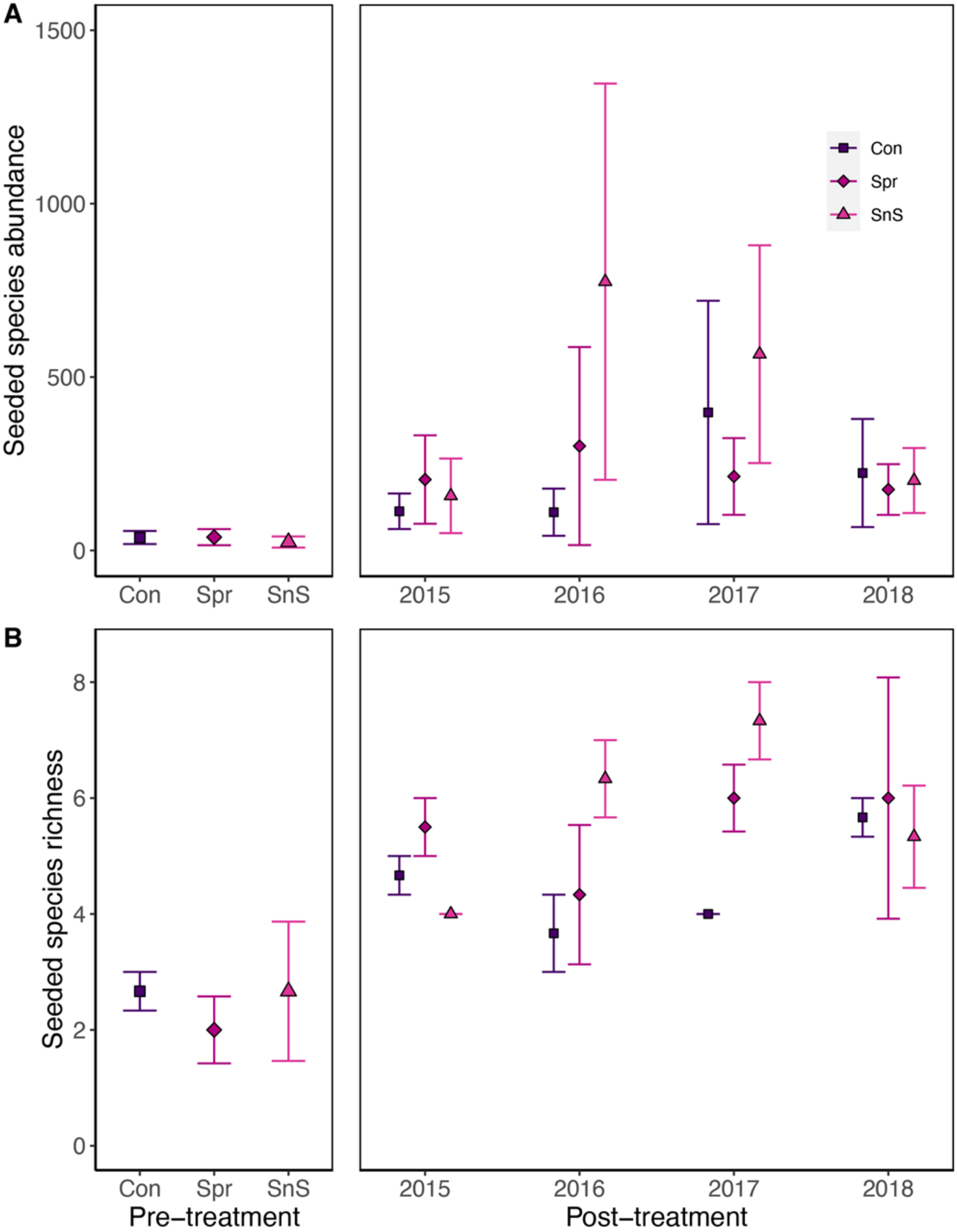

Figure 4. Difference in seeded species' (A) floral abundance and (B) richness over the study years. Points are means ± SE. The left panel indicates pre-treatment (2014) comparisons among patches while the right panel shows post-treatment response (2015–18). All three treatments are included because the plants in the seed mixture are native to the area and may recruit from the seed bank with management rather than only from the seed mixture. Con refers to the control treatment, Spr refers to the spray-only treatment, and SnS refers to the spray-and-seed treatment.

Table 4. PerMANOVA summary statistics of analyses of among-treatment community composition differences within each study year.

Taxon	Year	F	DF	Z	*p*
Floral Community Jaccard Distance	2014	0.61	2, 8	–1.29	0.920
	2015	0.89	2, 8	–0.54	0.683
	2016	0.98	2, 8	–0.01	0.460
	2017	0.86	2, 8	–0.72	0.753
	2018	0.59	2, 8	–1.81	0.980
Butterfly Community Jaccard Distance	2014	0.89	2, 8	–0.15	0.571
	2015	0.96	2, 8	–0.02	0.506
	2016	1.27	2, 8	0.73	0.223
	2017	0.70	2, 8	–1.03	0.866
	2018	0.86	2, 8	–0.74	0.767
Floral Community Bray-Curtis Dissimilarity	2014	0.45	2, 8	–1.43	0.936
	2015	0.85	2, 8	–0.42	0.650
	2016	1.01	2, 8	0.10	0.448
	2017	0.82	2, 8	–0.58	0.720
	2018	0.37	2, 8	–2.16	0.981
Butterfly Community Bray-Curtis Dissimilarity	2014	0.82	2, 8	–0.18	0.570
	2015	0.91	2, 8	–0.09	0.515
	2016	1.29	2, 8	0.70	0.235
	2017	0.58	2, 8	–1.22	0.908
	2018	0.78	2, 8	–0.74	0.792

bluegrass]), indicating that as fescue was removed, other aggressive non-native grasses at least partially filled that competitive gap. We suspect that this factor may have reduced the impact of our treatments and contributed to the often small or variable differences in floral abundance between control patches and treatment patches.

We also found no support for our second hypothesis as there was no evidence of butterflies responding to the treatments. While the baseline pyric-herbivory management appears to increase butterfly diversity, the decline in butterfly abundance over time has worrying implications for this restoration effort. Butterfly abundance declining over time may indicate that either our herbicide treatments or pyric-herbivory baseline management may be affecting larval resource requirements despite prior findings that herbicides have little effect on oviposition preference or larval survival (LaBar and Schultz 2012, Glaeser and Schultz 2014). The decline of two of the most abundant butterfly species (*Colias philodice* [clouded sulphur] and *Cupido comyntas* [Eastern tailed-blue]) could also explain the broader community trend. Of the total 2,856 observed butterflies, *C. philodice* was the second most abundant (18.4%) while *C. comyntas* is the third most (18.3%). While we did not analyze the response of individual butterfly species, both of these species seem to decline markedly over the study years (Supplemental Table S3). Such a decline could be due to the drivers mentioned above or unrelated climatic changes but the decline of such generalist non-threatened species without corresponding declines in sensitive or rare species has less grim implications for unintended consequences of management. However, without an outgroup using a different baseline management, it is challenging to identify precise drivers for site-level declines at either the community or species-level.

Post-treatment increases in butterfly diversity could potentially be result from the increase in floral resource richness and diversity over the same time period. This change in diversity is consistent with the observations of a study using mechanical and herbicide methods to remove *Ligustrum sinense* (an invasive shrub) that found butterfly diversity increased following management (Hanula and Horn 2011). However, that study also observed an increase in butterfly abundance while we observed a decrease, but this could be an artifact of their study being conducted in a riparian forest. Similarly, a study conducted in the sagebrush steppe using fire and herbicide to control *Bromus tectorum* (an invasive grass) found increases in both butterfly abundance and species richness, which the authors suggested may be related to increased floral resource availability (McIver and Macke 2014). Our results may differ due to the different floral resource plant and butterfly communities under consideration in that paper, but it is difficult to argue either side with a reasonable degree of certainty.

Butterflies' insensitivity to treatments (Figure 3) may simply be due to the spatial scale at which butterflies use the landscape. *Junonia coenia* (common buckeye; found in our study) and *Euptoieta claudia* (variegated fritillary; absent in this study, but confamilials were observed) have been found to use corridors between suitable habitats up to 150 meters long (Haddad and Tewksbury 2005) which provides strong implicit support that they could disperse

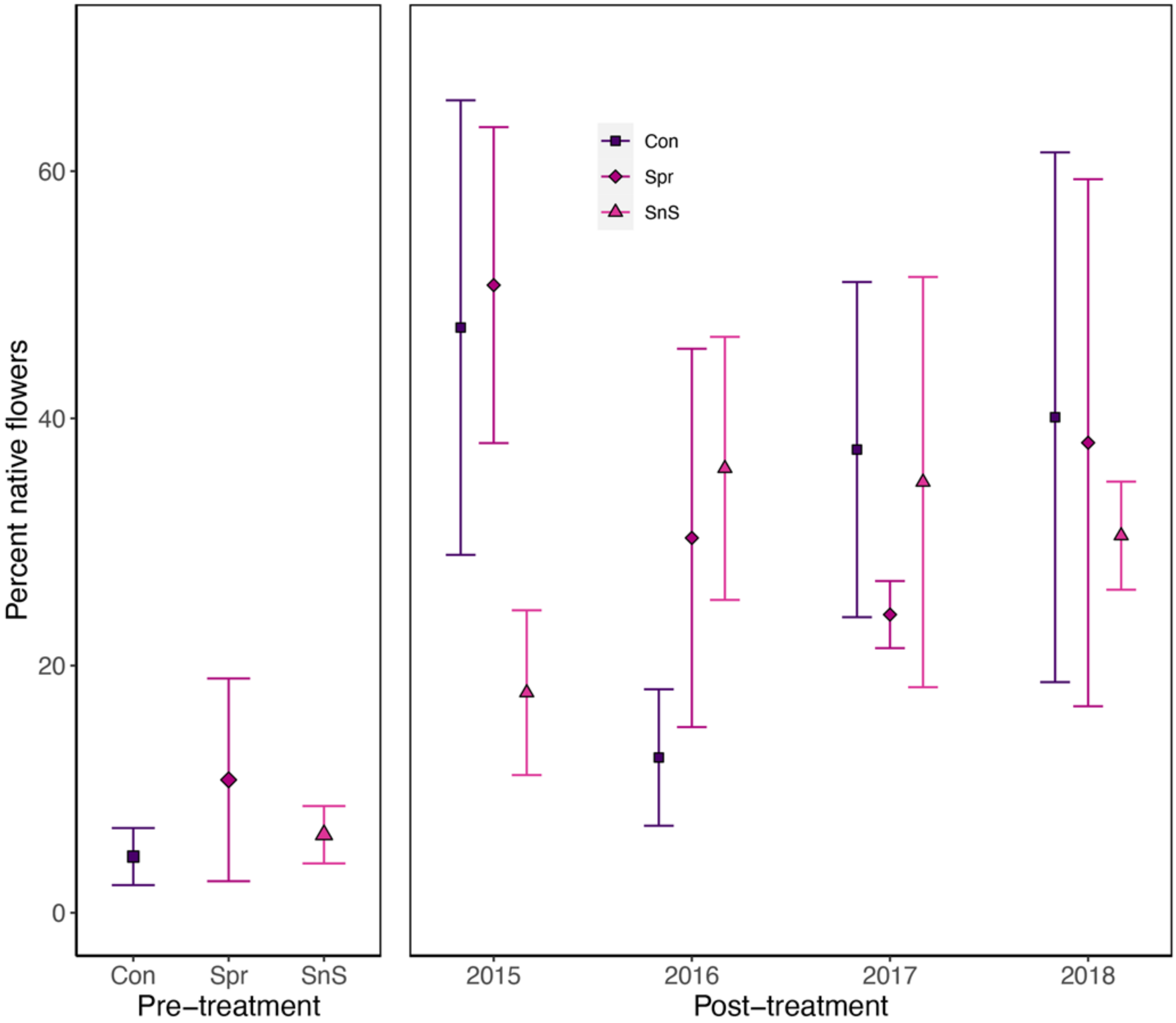

Figure 5. The percent of floral resources that was native did not vary with sampling year or treatment. Points are means ± SE. The left panel indicates pre-treatment (2014) comparisons among patches while the right panel shows post-treatment response (2015–18). Con refers to the control treatment, Spr refers to the spray-only treatment, and SnS refers to the spray-and-seed treatment.

within a suitable site up to at least that distance. Such a range, while perhaps relatively short, would be more than sufficient for a single adult butterfly to cross from a patch of one treatment to a patch of another. Many other butterflies are quite mobile and can even disperse on the scale of kilometers (Pullin 1994). Given that our patches were entirely contiguous with one another within each site, individual butterflies observed on different patches in our study could easily have been able to benefit from flowers anywhere within our 18–34 ha sites (Figure 1). Indeed, it is possible that butterfly species could disperse not just among patches within a site but potentially among the three sites in our study. That said, the study sites are separated by land containing many different habitats—ranging from riparian forest to heavily stocked conventional cattle pastures—which likely decreases the ease of among-site dispersal. It is more likely that butterflies recorded on different patches were actually part of a site-wide community and were neither specializing on a single patch nor part of one contiguous region-wide community. Although butterfly metrics did not differ at the patch level, the application of herbicide and seeding may have altered some aspects of the site-level butterfly community over time. However, without data from sites managed in the same way (i.e., same combination of prescribed fire and grazing) but without the spray-only and spray-and-seed treatments to serve as a comparison group, it is difficult to determine whether the spray-only and spray-and-seed treatments caused butterfly communities to change across whole sites as a result of patch-level treatments; such a possibility may be a fruitful avenue for future research.

We did not find any significant floral resource community composition differences among treatments (Table 4) which runs counter to our third hypothesis. It may be that our species pool is biased towards only those species that can tolerate the combination of prescribed fire and grazing that formed our baseline management. Some legumes (Fabaceae) have been shown to tolerate fire (Towne and Knapp 1996) and some of our most abundant species, though exotic, are legumes (e.g., *Lotus corniculatus* [birdsfoot trefoil], *Trifolium repens* [white clover], etc.). This limitation in species with the potential to colonize our treatment patches may constrain how much these communities can vary in response to treatment. To facilitate diagnosing differences in community composition, we used both a presence-absence distance measure (Jaccard 1912) and a dissimilarity measure that considers relative abundances (Bray and Curtis 1957). The former is robust against fluctuations in species' abundances but also does not differentiate between a single individual of a particular species versus many individuals. The latter does consider species abundances but may reduce the emphasis on ecologically interesting but rare species. There were no significant differences using either metric (Table 4) which could indicate either that communities did not differ among treatments or that we lacked sufficient replication to identify a weak but significant effect.

Consistent with our previous three hypotheses, we found no support for our fourth hypothesis: we found no significant differences among treatments or changes over time in the abundance or richness of floral resource species in the seed mixture (Figure 4; Table 2). This suggests that if establishment of seeded species was one of the mechanisms driving these community-wide increases, it was not the major driver of such change. A comparison of the list of floral species in the seed mixture (Supplemental Table S1) with the list of the floral resources we observed across our study (Supplemental Table S2) seems to support this idea. Previous work has found that combining glyphosate and a post-emergence herbicide with no-till seeding resulted in seeded species establishment (Masters and Sheley 2001). Our study lacked a post-emergence herbicide, which may explain our failure to demonstrate consistent seeded species colonization. That said, it is possible that our study did not continue sampling for long enough to document seeded species' establishment. Many of the seed mixture floral resources are perennial and some may require cold stratification; either of these factors coupled with the intense competition likely in this system could delay flowering from appearing even in our fourth year of sampling. Experiments including repetitive seedings may see greater establishment of seeded species or projects that identified non-flowering plants as well to assess whether the seed mixture did establish but had not yet reached reproductive maturity.

Our final hypothesis was also unsupported by the data: the percent of flowering stems of native plant species did not differ with treatment (Figure 5; Table 2). While we did not statistically analyze the response of particular species to our treatments, just over half of the 81,116 flowering stems we counted came from two exotic species: *Lotus corniculatus* (30.5%) and *Trifolium repens* (21.1%). This is particularly striking given that the next most abundant species (*Erigeron strigosus*), though native, was less than a quarter as abundant (6.9%; 5,563 flowers). It may be that exotic nectar-producing species are not as valuable to pollinators as are natives (Morandin and Kremen 2013), and this may mean that the increase in richness or diversity of floral resources writ large (Figure 1) may overstate the change in resources available to pollinators on these sites. This result may also highlight the limitation of a single herbicide treatment and the difficulty of establishing new plants from seed in this system (Figure 5).

As mentioned earlier, treatment allocation was non-random—more heavily invaded patches received the treatments aimed at reducing fescue cover—which may make fescue cover a confounding variable in our analyses. Instead of creating a gradient of low to high fescue cover (as would be expected if fescue cover were equal pre-treatment among patches) we may have instead made fescue cover more similar across the patches. This could have the effect of reducing among-treatment differences which would explain our total lack of significant among-treatment differences. Further complicating this issue is the fact that one site was grazed under season-long stocking while the other two were managed with intensive early stocking (Table 1) making our putative "baseline management" somewhat inconsistent. Both of these issues likely contributed to the high level of variability we found in most of our study metrics, particularly in our seed mixture efficacy (Figure 4) and percent native (Figure 5) results. While our results are valuable in demonstrating a lack of strong treatment effects, repetition of this experiment with a less convoluted backdrop would be better suited to identifying weak though potentially biologically relevant responses.

A one-time fall application of glyphosate and a spring seeding of a diverse native plant mixture resulted in an increase in floral resource richness and diversity in some treatments relative to our control. The butterfly response in terms of diversity was not evident at the patch level, but it was manifested across treatments over time. Site conditions following treatments did not consistently favor the establishment of native or seeded forbs, and changes may have been driven by non-native (i.e., exotic or invasive) floral resource species. Our results suggest that a one-time application of glyphosate and seeding is unlikely to be enough to restore these communities but may complement other management approaches. The lack of larger community composition shifts may be a function of all project sites being grazed and burned in roughly the same way. Future studies should include a wider breadth of "baseline" management conditions onto which herbicide and seed mixture additions are added as the frequency, intensity, and extent of prescribed fire and grazing are all likely to modify the results of within-site management.

Despite the likelihood of publication bias against nonsignificant results in ecology (Jennions and Møller 2002) we argue that the general lack of support for our hypotheses has unexpectedly positive implications for the specifics of studies like ours. Our results suggest that the responsible one-time use of an herbicide to control fescue has little negative effect on either floral resource or butterfly communities even up to four years following treatment. This finding suggests that we as restoration ecologists may have a freer hand in what methods are available to us in battling even the most pernicious of invaders, though of course replication of our findings with larger sample sizes and more taxa is vital in supporting this claim.

Restoration of prairie habitats, particularly those already heavily invaded by exotic plant species such as fescue, is a challenge for managers and researchers working in these areas. The results of this study suggest that iterative management approaches are likely necessary to favor native-dominated communities that are stable in the long term.

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