University of Nevada, Reno

Restoration and evolution in the Great Basin: non-target herbicide effects and potential for adaptive responses to fire

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Abstract

Cheatgrass (*Bromus tectorum*) has dramatically transformed the Great Basin and increased the incidence of fire to the detriment of native plants. In Chapter 1, we assessed the potential for rapid evolution in Great Basin native plants in response to fire and associated changes. Through a resurrection study, we examined how populations with or without a recent wildfire have changed over time by comparing past collections (collected 3-11 years ago through the Bureau of Land Management's Seeds of Success program) and contemporary re-collections from the same populations. We grew seeds in a common greenhouse environment and assessed how burn status and collection time influenced plant responses. We found evidence consistent with rapid evolution for at least one trait in every species, and temporal changes were concentrated in early seed and seedling traits. We saw potential evidence of rapid evolutionary response to fire in *Elymus elymoides* with temporal changes in burned populations towards potentially adaptive traits, including higher seed weight, higher emergence, and earlier emergence relative to the past collections. Our results support the effort of seed banking programs, as genotypes may shift over time within a population. In Chapter 2, we assessed the effects of the herbicides imazapic and indaziflam on target and non-target plants in 1) a natural, invaded field setting, 2) an agricultural field used to grow native annual forbs, and 3) on seeds of the perennial grass *E. elymoides* at various planting depths. In the natural field experiment, the density of non-native plants declined or remained low in all herbicide treated plots, while increasing dramatically in control plots. The combination and imazapic treatments also benefited native plants, the majority of which were perennial. All herbicides harmed at least some native annual forbs in the agricultural field experiment, although there were offsets at lower application rates, and some species were less susceptible than others. Herbicides reduced *E. elymoides* emergence, but planting seeds at 2-3 cm instead of the recommended 1 cm helped offset these negative effects, particularly for imazapic.

We suggest that land managers make efforts to survey and protect native annual plants before applying herbicides, and that the combination of these two herbicides with potentially lower application rates may help balance weed control with promoting native diversity in sagebrush systems.

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Thesis introduction

The Great Basin has undergone extensive degradation over the past century, and invasive annual grasses are one of the primary drivers behind community change and ecosystem service loss. Cheatgrass (*Bromus tectorum*) and other invasive annual grasses fuel a grass-fire cycle by producing copious amounts of flammable litter then recovering quicker than native plants post-fire (Bradley et al., 2018; Knapp, 1996; Whisenant, 1990). With repeated wildfires, ecosystems can be turned into near monocultures of cheatgrass and other invasive species, to the detriment of native plants and wildlife (Crawford et al., 2004; Knapp, 1996; Melgoza et al., 1990). Many of the native plants of the Great Basin are not fire-tolerant, and thus can have difficulty surviving, resprouting, or reestablishing from seed after fire (Knapp, 1996). However, there may be points where managers can break this cycle, and tip the scales towards native plants. When native plants co-exist with cheatgrass, they may evolve the ability to tolerate or even compete with cheatgrass (Goergen et al., 2011; Leger, 2008; Rowe & Leger, 2010). This means that disturbed landscapes may produce plants with genotypes that could be adaptive in restoration settings. While competitive genotypes alone may not be able to substantially reduce cheatgrass density, herbicides with pre-emergent qualities are a promising and effective tool to reduce cheatgrass presence (Clark, 2020). There is evidence that when an area is heavily invaded with invasive annual grasses, applying herbicides can benefit native plants through competitive release (Clark, 2020; Clark et al., 2020). Further, pre-emergent herbicides theoretically shouldn’t harm established perennial vegetation, as they primarily target seeds and seedlings (Sebastian et al., 2017). However, herbicides do have the potential to cause non-target harm to native plants, so land managers must decide what’s worse for an ecosystem: invasive annual plants or herbicides.

In Chapter 1 we asked if native plants are evolving in response to fire and associated changes in the Great Basin. We assessed potential rapid evolutionary response through a resurrection study, by growing past and contemporary seeds in a common environment. The past seeds in our study were collected 3-11 years ago through the Bureau of Land Management's Seeds of Success program (Haiet & Olwell, 2015), and the contemporary seeds were collected from the same populations in 2020 and 2021. We used a paired framework to compare how plant traits and competitive abilities changed over time in a population that had burned between collection dates to a nearby control pair that hadn't burned. Specifically, each of our six species had two pairs (burned and unburned) matched as well as possible (similar ecoregions and collection histories) and two generations of seed (past and contemporary). We also grew plants from all collections with and without *B. tectorum* to assess their competitive abilities with this common invader after fire. We asked how plant traits have changed over time in past vs. contemporary collections, and if there are differences in trait shifts and competitive abilities between the burned vs. unburned populations. We hypothesized that fire would alter the evolutionary trajectory of native plants through natural selection, specifically by reducing the abundance or genotypes that are not favored in the post-ubn landscape, and increasing the frequency of more successful genotypes. We expected to see shifts in plant traits in contemporary burned groups relative to other groups towards traits that are potentially adaptive in the invaded Great Basin. We also expected that contemporary burned populations would be the most tolerant of cheatgrass.

In Chapter 2 we examined the effects of two herbicides, imazapic and indaziflam, on cheatgrass or other non-native species and non-target native vegetation. We conducted three experiments to investigate these effects, including: (1) imazapic and indaziflam applied to a cheatgrass-invaded site in Elko County, Nevada, using large-scale aerial applications. We collected pre-application seed bank samples to uncover the "potential" plant community and compared these results in treated and untreated sites after application to determine how herbicides affect site potential in real-world treatment conditions. (2) In a small-scale experiment, we applied imazapic and indaziflam to an agricultural field in Reno, Nevada that had been used to grow a wide diversity of native annual forbs (de Queiroz et al., 2021) and thus has a native seed bank density and diversity of annual forbs that is difficult to find in natural settings. Because this field lacks the litter and living plants typically found in field sites, we applied both full and reduced rates to approximate the amount of herbicide that would reach the soil if it was intercepted by annual grass litter (Clark et al., 2019). (3) In the same field, we tested the effects of imazapic and indaziflam on seed depth by planting seeds of *Elymus elymoides* (squirreltail), a desirable perennial grass, at five depths. We asked how each treatment affected the emergence of target non-native and non-target native species emergence in a natural field setting and in the agricultural field, and how planting depth of *E. elymoides* affected emergence in each herbicide treatment. We hypothesized that the herbicides would reduce the density of cheatgrass and other invasive annual species in the natural field setting, and that this would benefit existing non-target perennial vegetation. Further, we expected that both herbicides would harm native annual plants in our annual forb experiment, but that the reduced rates may offset some of those changes, and that species tolerance may vary. Lastly, the recommended planting depth for *E. elymoides* is 0.6-1.3 cm, but we hypothesized that seeds planted at 1 and 2 cm depths would be affected by herbicides and unable to emerge in plots that receive full treatment. We expected that seeds in the shallowest depths (1 and 2 cm) would be able to emerge in the reduced application plots and in the control plots, while moderate depths (3 and 5 cm) may help seeds avoid being damaged by the herbicides, but they would still be able to emerge despite being deeper than the recommended planting depth.

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Chapter 1:

Assessing potential for rapid evolutionary response to fire through a resurrection study

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Abstract

Rapid evolutionary change that affects the distribution and abundance of species can occur over one or a few generations, especially in response to anthropogenic disturbance. We assessed the potential for rapid evolution in Great Basin native plants in response to fire and associated changes. In this region, the cheatgrass (*Bromus tectorum*) invasion has dramatically increased the risk and incidence of fire, and native plants did not evolve with frequent fires. We assessed change over time through a resurrection study, examining how populations with or without a recent wildfire have changed in phenotype, relative to past collections (collected 3-11 years ago through the Bureau of Land Management's Seeds of Success program). We used past samples from seed banks and re-collected from the same populations in 2020-2021, then grew seeds in a common greenhouse environment. We assessed how burn status and collection time influenced plant responses including seed and seedling traits, mortality, reproductive timing and output, morphological traits, and biomass. We also asked if competitive ability changes with burn status and time by growing plants in competition with *B. tectorum*. Our study included six species: *Chaenactis douglasii, Elymus elymoides, Erigeron linearis, Poa secunda, Pseudoroegneria spicata*, and *Sphaeralcea grossulariifolia*. We found evidence consistent with rapid evolution for at least one trait in every species, in both burned and unburned populations, however the direction of change was not consistent across species, possibly because burning did not have the same impact on every plant community. Across species and collections, changes were concentrated in early seed and seedling traits, as well as height. We saw the greatest evidence of rapid evolutionary response to fire in *E. elymoides* despite the short time between collection dates for this species, with temporal changes in burned populations towards potentially adaptive traits, including higher seed weight, higher emergence, and earlier emergence relative to the unburned populations. We also saw a shift towards lower height in the burned *P. secunda* population relative to the unburned, and some evidence that burned *E. elymoides* and *P. secunda* populations had increased tolerance to *B. tectorum*, through increased survival, though the *B. tectorm* x burn x time interactions were not significant. Our results demonstrate change over time in at least some traits for all species, some of which were suggestive of rapid adaptive evolution, and support the continued effort of seed banking programs, as genotypes may shift over time within a population and historical seed collections may be a valuable resource for research and conservation.

Introduction

Rapid evolutionary change is often associated with anthropogenic environmental changes, like pollution (Cook & Saccheri, 2013; Klerks & Levinton, 1989; Wu & Bradshaw, 1972), climate change (Anstett et al., 2021; Franks & Weis, 2008; Lustenhouwer et al., 2017), and invasive species (Carroll, 2007; Oduor, 2013; Strauss et al., 2006). Plant invasions are a prime system to study rapid evolution, as one can often compare invaded to non-invaded environments, and because they can cause cascades of environmental effects that can influence the evolutionary trajectory of a population (Strauss et al., 2006). One of the most devastating plant invasions of the past century is the cheatgrass (*Bromus tectorum*) invasion of the Great Basin. *Bromus tectorum* is an invasive annual grass that has taken over vast swaths of the Great Basin, replacing native plants through high fecundity and germination rates, by being highly competitive with plants for soil moisture, by producing large amounts of flammable litter, and by dramatically increasing the incidence of wildfire (Bradley et al., 2018; Knapp, 1996; Whisenant, 1990). This increase in wildfire is particularly problematic for native Great Basin plants, as they did not evolve with frequent fires, and can have difficulty re-establishing from seed after fire, especially when fires are repeated and sites are invaded (Knapp, 1996). However, many ecosystems have remnant natives that are co-existing with cheatgrass, and there is evidence that these plants have evolved unique adaptations to persist in the cheatgrass-invaded landscape (Goergen et al., 2011; Leger, 2008; Rowe & Leger, 2010). Here, we ask if the native plants of the Great Basin are also evolving in response to fire and fire-induced landscape changes.

Most studies of rapid evolution substitute "space-for-time" to infer evolutionary dynamics, meaning they involve the comparison of populations with and without the selective agent (Weider et al., 2018). Studies can look at the genetic sequences of each population (Dolan et al., 2008), or assess genetic differences by growing plants in a common environment, which is often called a common garden experiment. For example, the space-for-time approach has been used to study evolutionary response to invasion, by collecting plants from cheatgrass-invaded and nearby uninvaded sites in the Great Basin and growing them in a common garden (Goergen et al., 2011; Leger, 2008; Rowe & Leger, 2010). In this system, there is evidence that native plants, including big squirreltail (*Elymus multisetus*) and sandberg bluegrass (*Poa secunda*), are evolving in response to cheatgrass pressure. When grown alongside cheatgrass in competition experiments, plants from invaded sites lost less biomass (Leger, 2008), had a greater ability to suppress cheatgrass (Rowe & Leger, 2010), and in two of four sites were more tolerant of cheatgrass competition (Goergen et al., 2011). Plants from invaded areas also had earlier phenology than those from uninvaded areas (Goergen et al. 2011), and smaller size and increased allocation to root biomass were associated with invaded populations of native plants (Rowe & Leger 2010). These results are not confined to cheatgrass-ecosystems; there is evidence that native plants are evolving in response to other North American invasive plants including *Centaurea maculosa* (Callaway et al., 2005), *Acroptilon repens* (Ferrero-Serrano et al., 2011; Mealor & Hild, 2007), and *Alliaria petiolata* (Huang et al., 2018; Lankau, 2012).

Substituting space for time is often necessary for studying rapid evolution in plants because there is a lack of historical seed collections available for research. However, when historical collections are available and are grown with contemporary collections, they can be a powerful tool to directly assess evolutionary change over time (Etterson et al., 2016; Franks et al., 2008, 2018). The approach of growing past seeds with contemporary seeds in a common garden is called a resurrection study, and this method has been used to detect evolutionary change within populations of invasive plants in their new ranges (Hernández et al., 2019; Sultan et al., 2013; Whitney & Gabler, 2008), changes in flowering phenology, size, and mating system (Cheptou et al., 2022), changes floral morphology and phenology, possibly in response to climate change and pollinator decline (Thomann et al., 2015), and leaf morphology in response to drought (Anstett et al., 2021). None, it appears, have been used to compare seeds collected before and after fires.

For our resurrection study, we took advantage of the Bureau of Land Management's (BLM) Seeds of Success (SOS) program, which has been collecting seeds from the Great Basin since 2000 (Haiet & Olwell, 2015). Over 600 of these seed collection sites have burned since collection, creating a unique opportunity to re-collect seeds from these populations and examine change over time with a clear before-and-after framework (S. C. Barga et al., 2020). Using these valuable seed resources, we grew past (collected 3-11 years ago) and contemporary (collected in 2020 or 2021) seeds from six species in a common environment, from both burned and unburned sites. We compared plant traits and competitive abilities of plants collected before and after fires with those of plants collected in the same timeframe in nearby areas that had not burned, using a paired framework. Specifically, each of our six species had two pairs (burned and unburned) matched as well as possible (similar ecoregions and collection histories) and two generations of seed (past and contemporary). We also grew plants from all collections with and without *B. tectorum* to assess their competitive abilities with this common invader after fire. We asked the following questions:

1. How have plant traits changed between past and contemporary collections?
2. Are there differences in trait shifts in burned vs. unburned populations?
3. How does the ability to compete with *B. tectorum* differ between burned vs. unburned populations, or past vs. contemporary groups?

We hypothesized that fire has altered the evolutionary trajectory of native plants through natural selection, specifically by reducing the abundance of or eliminating genotypes that are not favored in burned and invaded landscapes and increasing the frequency of more successful genotypes. We also expected to see shifts in plant traits in contemporary burned groups relative to unburned groups and pre-burn collections towards traits that are potentially adaptive in the invaded Great Basin, including higher seed weight (Atwater et al., 2015; Leger et al., 2019; Leger & Goergen, 2017), earlier emergence (Agneray et al., 2022; Goergen et al., 2011; Kulpa & Leger, 2013; Leger et al., 2019; Leger & Baughman, 2015), earlier flowering phenology (Goergen et al., 2011; Kulpa & Leger, 2013; Leger & Baughman, 2015), smaller plant size (Fergosun et al., 2015; Kulpa & Leger, 2013; Leger & Baughman, 2015; Rowe & Leger, 2010), and greater belowground biomass allocation (Fergosun et al., 2015; Leger et al., 2019; Leger & Baughman, 2015; Leger & Goergen, 2017; Rowe & Leger, 2010). We also expected that the burned contemporary groups, relative to other groups, would have higher emergence, lower mortality, and higher reproductive output because these traits are often associated with plant resilience and success, and we expect that only the most resilient plants will survive, reproduce, and germinate in the post-burn landscape. Lastly, we expected seed weight to be lower in past populations, as seed weight can decline with age (De Vitis et al., 2020; Franks et al., 2019). Overall, we believe that these trait shifts will confer a competitive advantage to burned populations, relative to unburned populations, when grown in competition with cheatgrass. Specifically, we hypothesized that, in line with past studies of native plants from invaded and uninvaded sites, plants from burned populations would be more tolerant of cheatgrass, measured through changes in mortality, morphology, and biomass, than plants from unburned populations.

At a basic level, this project will expand our knowledge of plant evolution, fire ecology, and invasion ecology in the Great Basin, including documenting change over time in wild populations with divergent fire histories. On an applied level, we hope to inform seed collection strategies for land managers in the Great Basin. The Plant Conservation Alliance's Native Seed Strategy urges land managers to use the "right seed" for restoration (Oldfield & Olwell, 2015), which means seed that has the best chance of surviving and persistent at restoration sites. If burned populations are superior competitors with cheatgrass, then it is imperative to target these populations for seed collection so land managers can use seed that is adapted to the invaded, burned, and degraded ecosystems of the Great Basin.

Methods

Source populations

Our populations consisted of six species with three forbs and three grasses: *Chaenactis douglasii* (Hook. Hook. & Arn.*, Elymus elymoides* (Raf.) Swezey*, Erigeron linearis* (Hook.) Piper*, Poa secunda* J. Presl*, Pseudoroegneria spicata* (Pursh) Á. Löve*,* and *Sphaeralcea grossulariifolia* (Hook. & Arn.) Rydb. The populations were located in Nevada, Oregon, and Idaho (Figure 1), and they were selected because they are common components of sagebrush steppe ecosystems, and because they met our criteria for availability of burned/unburned populations with historic SOS collections. For each of these six species, we collected seeds from paired burned and unburned sites, and had two generations of seed (past and contemporary). With this design, we had four groups of plants: burned past, burned contemporary, unburned past, and unburned contemporary. The past seeds were obtained through the Germplasm Resources Information Network (GRIN, ars-grin.gov). The contemporary seeds were collected in 2020 and 2021. The burned-unburned pairs were matched based on physical distance and the requirement that they were in the same Environmental Protection Agency Level 3 Ecoregion (Omernik, 1987), and similarity of past collection dates (Table 1). Sites almost certainly differed in other ways, including grazing or other land use history along with intrinsic environmental factors, but as past seed collections are limited, we were not able to fully account for all differences between sites when selecting burned and unburned pairs.

At each collection site, we collected plant community data with a step-point method along three 50 m transects radiating out from the GPS point associated with each collection site at 0, 120, and 240 degrees. We recorded all vegetation and the soil surface intersecting a pin flag approximately every meter along each transect. We recorded vegetation in the following categories: *Artemisia tridentata*, native shrub, non-native shrub, native perennial grass, non-native perennial grass, native annual grass, non-native annual grass, *B. tectorum*, *Taeniatherum caput-medusae,* native perennial forb, non-native perennial forb, native annual forb, and non-native annual forb. We recorded *A. tridentata*, *B. tectorum*, and *T. caput-medusae* to species because we were particularly interested in how these species differed at burned and unburned sites, *A. tridentata* because it is a useful verification of the burn status of a particular site (*A. tridentata* does not resprout following fire, and frequently fails to recruit from seed) and the two annual grass species because they are highly competitive species that would likely affect any remnant natives. For the soil surface, we recorded litter, moss, cryptobiotic crust, rock, and soil.

Seed collection

We collected contemporary seeds in the summer of 2020 and 2021 (Table 1) by hand into paper bags. We collected small amounts of seed from at least 25 plants, and as many plants as possible from the entire population when feasible, or from all plants with seeds within 500 feet of the GPS point associated with the population, for larger populations, with most collections from 50-100 plants. The seeds were cleaned with an air separator for larger collections, or hand-cleaned for smaller collections.

Greenhouse planting

We grew the seeds in a common greenhouse environment with a randomized design. Each species was grouped together because they had different planting times and watering needs, but the source population order was random within each species group. The greenhouse is located at the Valley Road Greenhouse Complex in Reno, Nevada. We watered the plants daily when seedlings were emerging and establishing, then bi-weekly once established. The greenhouse temperatures were allowed to fluctuate with ambient conditions, with the greenhouse controls set to bound the temperatures between 2 and 20 degrees C in the winter and early spring, and up to 25 degrees C in daytime the late spring and summer. We had more contemporary seed than past seed, so we planted two to three contemporary seeds in each individual pot, and one past seed in an individual pot. Once three days had passed since the last seedling emerged, we thinned any pots with multiple seedlings to one randomly chosen individual. The grasses and *E. linearis* were directly seeded into pots without treatment. To germinate *S. grossulariifolia*, we scarified each seed by scratching each seed once on a #320-grit aluminum oxide Emory cloth, then we stratified the seeds in a freezer at 3.8ºC for 15 days, at which point the majority had germinated (Dunn, 2011). To germinate *C. douglasii*, we stratified in a freezer at 3.8ºC for 30 days (Gucker & Shaw, 2018).

We conducted two greenhouse trials: in Trial 1 we planted *E. elymoides, P. spicata,* and *S. grossulariifolia* in January 2020, with 100 pots planted for each past population and 75 planted for each contemporary. We harvested *E. elymoides* and *P. spicata* after they finished flowering in August 2021, and harvested *S. grossulariifolia* in August 2022 to give them a chance to flower. The Trial 1 plants were planted in short Stuewe & Sons, Inc. TP49 4 x 9.5” “Short One” mini treepots. We planted in topsoil and decomposed granite from Moana Nursery in Reno, Nevada with 20% decomposed granite and 80% topsoil. In Trial 2 we grew all six species from January 2021 (or March 2021 for *C. douglasii*) until harvesting them in September 2022. We adjusted growing conditions slightly, after our first year’s experience, and grew this second round of plants in smaller Stuewe & Sons, Inc. D40H 2.5 x 10” Heavyweight Deepot Cells, and planted 100 pots per population. We planted in topsoil obtained from Red Rock Landscaping in Reno, Nevada with 33% decomposed granite and 67% topsoil. In the second trial, half of the plants in each group were planted with two cheatgrass seeds, randomly assigned to pots, to quantify competitive ability. The cheatgrass seeds were sown at the same time as the native seeds. If the cheatgrass did not emerge after 10 days, we replanted it, repeating this process until both cheatgrass seedlings had emerged.

Data collection

We recorded a breadth of responses that are potentially adaptive for growth, survival, and reproduction in the Great Basin. Before planting, we weighed five randomly chosen seeds ten times to calculate seed weight. We recorded data on emergence (percent emergence, emergence phenology) (*S. grossulariifolia* emergence phenology was not included because it germinated in the growth chamber before we could record phenology), mortality, reproductive output (percent flowered, flowering phenology, number of inflorescences produced), morphology (height, number of leaves produced, leaf width), and biomass (aboveground biomass, belowground biomass, root mass ratio, and total biomass). Percent emergence and days to emergence were calculated using all seeds that were planted, including the seeds that were pulled if more than one native seed germinated in a pot. The rest of the data were recorded only for the plants that were not pulled. Percent emergence was calculated by dividing the number of seeds that were planted by the number that emerged and emergence phenology was the number of days a plant took to emerge since planting. Flowering and mortality percentages were calculated only using plants that emerged, and flowering phenology was the number of days until flower formation since the date of emergence. Height was measured from soil to the tallest part of any structure of the plant, leaf width was the widest part of any leaf, and we only counted leaves if they had some amount of green on them. Leaf width was only measured for *C. douglasii, E. elymoides,* and *S. grossulariifolia* because they were the only species with noticeable variation in leaf widths. Biomass was harvested once all plants had stopped producing seeds for *E. elymoides* and *P. spicata*, or after the plants had been growing for at least six months. Aboveground biomass was snipped at the soil surface. To extract the roots for belowground biomass in the non-competition pots, we used our lab's established method of soaking the pots in water, removing the plant from its container, and gently rubbing the dirt of the roots while they are submerged in water (Leger et al., 2021). For plants grown with cheatgrass, only above-ground biomass of the native plant was harvested. After harvest, biomass was dried in an oven at 40 degrees Celcius for at least 5 days. Total dry above and belowground biomass were weighed with a Mettler Toledo XS105 DualRange scale. The root mass ratio was calculated by dividing the root biomass by the total plant biomass.

We will mainly present results from Trial 2 (larger sample size, all species included in the same growing medium and environment, included a competition experiment), which included all traits except for reproductive data and belowground biomass, as no plants flowered during Trial

2. We will present only biomass and reproductive data for the three species that were also included in Trial 1.

Data analysis

All data were analyzed with linear models with R version 4.1.3 (R Core Team, 2021) with the "tidyverse" package (Wickham et al., 2019) and figures were made with the R package "ggplot2" (Wickham, 2016). We used the glm function to specify a distribution, with a poisson distribution with a log link for count data (days to phenological event, number of structure), a Gaussian distribution for positive continuous data (height, leaf width, biomass), and a binomial distribution for binary data (emergence, flowering, and mortality), and assessed the distribution of residuals visually through Q-Q plots and histograms. We used log transformations to improve residuals for responses with Gaussian distributions if they were non-normally distributed in continuous positive datasets. If there were 0's in the dataset we wanted to transform, then we added 0.5 to all the variables. The test statistics and p values were calculated using the R package "car" (Weisberg, 2019) and significance was based on a $P < 0.05$ criteria for analyses, but we also present and discuss non-significant data for interpretation for some metrics.

First we asked whether the burned and unburned sites differed in plant community composition. We used linear models with burn status (burned vs. unburned), species, and the interaction between burn status and species as fixed effects and specified a Gaussian data distribution. We analyzed the percent cover of non-native plants, non-native annual grasses, *B. tectorum*, native plants, native shrubs. We converted the 50 stops on our transects to percent cover by multiplying the values by two, or by 3.125 for one transect that ended at 32 stops due to a rattlesnake den on the transect.

Next we asked how characteristics of plants from burned vs. unburned populations were changing over time, using linear models with burn status (burned vs. unburned), collection time (past vs. contemporary), and the interaction between burn status and collection time as fixed effects. Several of the *E. elymoides* and *P. spicata* plants from Trial 1 were infected with the stripe smut fungus *Ustilago striiformis* on their inflorescences, which can affect plant traits (Hodges, 1970). For all traits collected during and after the plants produced inflorescences, we used linear models with smut presence as an additional fixed effect to account for any effects. The traits we analyzed with smut as a fixed effect included flowering percentage, reproductive phenology, number of inflorescences, aboveground biomass, belowground biomass, total biomass, and root mass ratio. For the Trial 2 plants grown with cheatgrass, we also analyzed how cheatgrass status affected each response, using a model that included each factor and interactions between burn status, time, and cheatgrass presence. The traits we assessed with cheatgrass presence included mortality, height, number of leaves, leaf width, and aboveground biomass.

Results

Source population community composition

The percent cover of all plant species and plant groups was significantly affected by burn status, site pairing, and had significant burn by sight pairing interactions (Table 2). Specifically, the percent cover of non-native species (Figure 2a), invasive annual grasses, and *Bromus tectorum* was significantly higher in burned than unburned sites (Table 2), though this was stronger in some burned/unburned pairs than in others (significant site x burn interaction). Specifically, the burned sites had more non-native species than the unburned sites for *C. douglasii, E. elymoides, P. spicata,* and *S. grossulariifolia*. Specifically, *C. douglasii* burned had 4124% higher cover of non-natives than unburned, burned *E. elymoides* had 233% higher cover, and *P. spicata* and *S.*

grossularfiifolia had 0% cover of non-natives in the unburned sites and 20-33% non-natives in the burned sites. *Erigeron linearis* and *P. secunda*, on the other hand, had more non-natives in the unburned sites than burned. *Erigeron linearis* had low percent cover of non-natives in both sites (<2%), and the burned site had 67% lower cover of non-natives than the unburned. The *P. secunda* sites had moderate cover of non-natives (6-11%), and the burned site had 44% lower cover of non-natives than the unburned. The percent cover of invasive annual grasses followed the same trend as overall non-native plants, with all species except for *E. linearis* and *P. secunda* having higher cover of invasive annual grasses in burned sites than the unburned sites. As with non-native plants overall, the *C. douglasii* burned site had dramatically higher cover of invasive annual grasses than unburned (3356%), *E. elymoides* had 40% higher, and the *P. spicata* and *S. grossulariifolia* had 0% invasive annual grass cover. On the other hand, *E. linearis* and *P. secunda* burned sites had 62-67% lower percent cover of invasive annual grasses than the unburned sites.

Overall, the unburned sites had significantly higher cover of native plants (Figure 2b) and native shrubs than the burned sites (Table 2), with again, some differences among pairs. The unburned sites for all species had more native plants than burned except for *E. linearis*, where the burned sites actually had 147% more native plant cover than the unburned site. The remaining species had between 51 to 97% lower native plant cover in the burned than unburned sites, *P. secunda* with 51%, and other species at 88-97%. Additionally, all burned sites had lower percent native shrub cover than unburned, except for *E. linearis*, where the burned site had 300% higher native shrub cover than the unburned site. The other species all had at least 64% lower native shrub cover in burned than unburned, and the *E. elymoides* and *P. spicata* burned site (the burned site is the same for both species) had 0% native shrub cover. Litter and bare ground had no differences among sites (data not shown).

Overview of change over time

Overall, the majority of significant differences between past and contemporary collections (a significant time response, or a burn by time interaction) occurred in the seed and seedling stages (Table 3), with some changes in height, mortality and flowering phenology. We also observed that *E. elymoides* had the most significant time and burn by time responses, despite having the shortest time between past and contemporary collections, followed by *P. secunda* (Table 3), and that every species had at least one, if not more, traits differ over time as well as at least one significant burn x time interaction.

Seed weight

We analyzed seed weight before the seeds were grown in the greenhouse and found that the seeds of all species except *C. douglasii* had significant responses to burn status, time, or the interaction between burn status and time (Table 4), though the direction of change was not consistent across species. Specifically, *E. linearis* had a significant main effect response to time, and a significant burn by time interaction (Table 4). Contrary to our prediction, the contemporary *E. linearis* seeds had lower weight than the past seeds, and this drop in weight was strongest in the burned population (Figure 3). *Elymus elymoides* had significant responses to burn status and time, and there was a significant burn by time interaction (Table 4). The unburned *E. elymoides* group had higher seed weight than the burned, and the unburned seed weight declined from the past to contemporary groups while the burned population slightly increased (Figure 3). Both *P. spicata* and *S. grossulariifolia* had significant responses to burn status and time (Table 4), with the contemporary seeds being heavier than the past seeds (Figure 3). Additionally, the burned *P. spicata* population had higher seed weights than the unburned population, and the past unburned *S. grossulariifolia* group had higher seed weight than the past burned population, while the contemporary groups had similar seed weight (Figure 3). Lastly, the contemporary *P. secunda* groups had significantly lower seed weight than the past *P. secunda* groups (Table 4, Figure 3).

Emergence

Percent emergence

Four of six species had a significant effect of time or a time x burn status interaction on percent emergence, and two (*P. spicata* and *E. linearis*) had significant differences between burned and unburned sites, but no response to time or its interaction (Table 5, Figure 4). As was true for seed weight, changes in emergence were not in a consistent direction either over time (i.e. not all contemporary populations had higher emergence than past) or consistent response to burning. There was a significant interaction between burn status and time for *E. elymoides* (Table 5), with the emergence percentage increasing by 11% in the contemporary burned population and decreasing by 4% in the contemporary unburned population (Figure 4). Burn status, time, and their interaction was also significant for *P. secunda* (Table 5), with the percent emergence decreasing in both burned and unburned sites in contemporary collections, but more dramatically so in the burned population (Figure 4). *Sphaeralcea grossulariifolia* germination also responded significantly to burn status and time, and a significant burn by time interaction, with the unburned contemporary group having 69% lower emergence than the past group, and the burned group changing less over time (Figure 4). *Chaenactis douglasii* had a significant main effect of time on emergence, with more seeds emerging in the past group than the contemporary group (Table 5), and the reduction in emergence of contemporary collections were between 50 and 51% in both the burned and unburned population (Figure 4). There was no significant effect of time on emergence percentage for *P. spicata* or *E. linearis,* though for both species, burned populations (both past and contemporary collections) had slightly lower emergence than the unburned population (Figure 4).

Emergence phenology

Of the five species we analyzed for emergence phenology, three had a significant burn by time interaction, and all responded significantly to at least one factor (Table 6), though responses were not in a consistent direction, either over time or across burn status. Specifically, *C. douglasii* had a significant main effect of burn status on emergence timing and showed significant interactions between time and burn status (Table 6), with the burned population emerging 2.1 days later in the contemporary group while the contemporary unburned population emerged 2.3 days earlier (Figure 5). *Elymus elymoides* also had a significant interaction between burn status and time, and significantly responded to burn status and time, and was the only collection that had changes in emergence time that fully conformed with our predictions (Table 6). Specifically, the burned *E. elymoides* group emerged 4.7 days earlier in the contemporary group, while the unburned population had the same mean emergence timing in the past and contemporary groups (Table 6). *Pseudoroegneria spicata* also had a significant burn by time interaction, and a significant response to time (Table 6). Specifically, both burned and unburned populations emerged earlier in the contemporary group, but there was a difference in degree, with a 0.9 day change in the burned population and 2.5 days in the unburned population (Figure 5). For *E. linearis* both time and burn significantly affected days to emergence (Table 6), with both the burned and unburned population emerging 2.1 to 1.5 days later in the contemporary group than the past group, respectively (Figure 5). Lastly, the contemporary *P. secunda* groups emerged significantly later than the past groups, with the burned population emerging 2.2 days later and the unburned emerging 1.8 later (Table 6, Figure 5).

Mortality

Mortality was assessed for all six species. Mortality after emergence of *E. linearis* and *S. grossulariifolia* was significantly higher in the past groups than the contemporary groups, but no other species or factors significantly affected mortality (Table 7). In general, the forbs tended to have higher mortality than the grasses, with *C. douglasii* having the highest mortality rates of any species (Figure 6).

Reproduction

Percent flowered

Of the two species that flowered, the percent of *E. elymoides* that flowered was significantly affected by burn status and time, but there was no significant burn by time interaction, and no factors were significant for *P. spicata* (Table 8). The number of *E. elymoides* that flowered was57% lower in the contemporary burned population, and 64% lower in the unburned population, as only one unburned contemporary individual flowered (Figure 7). All *P. spicata* groups had similar flowering percentages (Figure 7).

Flowering phenology/number of inflorescences

The flowering phenology of *E. elymoides* differed significantly between past and contemporary populations (significant time effect) and was nearly significant for *P. spicata* ($p<0.07$) (Table 9). Though there was no significant time x burn status interaction, the burned *E. elymoides* group flowered 8.9 days later in the contemporary group than in the past group while the unburned population flowered an average of 3.5 days earlier, though only one unburned contemporary individual flowered (Figure 8). Although it was not significant, both *P. spicata* contemporary groups flowered later than the past groups, but this change was strongest in the unburned population, which flowered 8.3 days later while the burned population flowered 0.6 days later (Figure 8). The number of inflorescences produced was not significantly affected by any factor for either species (Appendix 1).

Morphology

Height

We analyzed height for all six species, and found one had a significant time by burn interaction, one had a significant time effect, and one had significant differences between the burned and unburned population. Specifically, the interaction between burn status and time was significant for *P. secunda* (Table 10), as the burned population became 26% shorter over time while the unburned population became 16% taller (Figure 9). For *E. elymoides,* height decreased significantly over time for both the burned and unburned populations (Table 10, Figure 9). Lastly the burned *P. spicata* group was significantly shorter than the unburned populations in both the past and contemporary groups (Table 10, Figure 9).

Leaf number and width

The number of leaves and leaf width did not significantly vary by burn status or time, and there were no significant interactions between burn and time (Appendix 2).

Biomass

We analyzed aboveground biomass, belowground biomass, total biomass, and the root to shoot ratio for the three Trial 1 species, and no species showed significant responses to time or significant burn by time interactions (Appendix 3). We analyzed only aboveground biomass for the six Trial 2 species, and found only one significant time response (Appendix 3 Table 3.2): in

Trial 2, *P. spicata* responded significantly to time, with both the burned and unburned group having higher biomass in the contemporary group than the past group (Appendix 3 Table 3.2). However, this trend with *P. spicata* was not observed in Trial 1.

Cheatgrass competition

We modeled the interaction between burn status, time, and whether or not a plant was grown with *B. tectorum* (referred to as cheatgrass in this section) for all Trial 2 species to see if burn status and time affect a group's ability to compete with cheatgrass. Cheatgrass presence significantly affected all responses for all species, regardless of burn status or time, but the magnitude of responses differed by species (Table 11), and there were several significant two-way interactions between cheatgrass presence and burn or time for mortality (Table 12) and plant height (Table 14).

One species showed a significant interaction between burn status and cheatgrass presence. Specifically, *C. douglasii* mortality had a significant interaction with burn status and cheatgrass (Table 12), with the unburned population having higher mortality when grown with cheatgrass than the burned population (Table 13), in both past and contemporary collections. Additionally, the interaction between burn status and cheatgrass approached significance for *P. spicata* ($p<0.095$) (Table 12), as the unburned population past group had much higher mortality than any other groups (Table 13). In addition to interactions, plants grown with cheatgrass tended to have increased mortality compared to plants grown without cheatgrass (Table 13), and mortality was significantly higher when grown with cheatgrass for *C. douglasii, P. secunda,* and *P. spicata* (Table 12).

Three species had significant height interactions, including time by cheatgrass, burn status by cheatgrass, and time by burn status. *Erigeron linearis* showed a significant interaction between time and cheatgrass presence on height (Table 13), with the past groups losing less height when grown with cheatgrass than the contemporary groups (Table 15). The time by cheatgrass presence was almost significant for *E. elymoides* (<p<0.09) (Table 14), with the past groups losing more height when grown with cheatgrass than the contemporary groups, in a trend opposite to *E. linearis* (Table 15). *Pseudoroegneria spicata* height had a significant interaction between burn status and cheatgrass presence (Table 14), with the burned populations losing less height when grown with cheatgrass than the unburned populations (Table 15). Lastly, *P. secunda* had a significant burn by time interaction, which was the case with the non-cheatgrass data as well, as the burned population became shorter over time while the unburned population became taller (Table 15). Additionally, cheatgrass significantly affected the height of all species, with the plants grown with cheatgrass being significantly shorter than the plants grown without (Table 14, Figure 17, Table 15).

Discussion

Seed banks preserve important plant diversity, and they also provide unique opportunities to assess change in native plant populations over time (Barga et al., 2020). Through our resurrection approach, we were able to use the efforts of the BLM's Seeds of Success program to assess change over time by directly comparing seeds collected before and after fires with control unburned pairs. We found evidence of temporal changes for every species in traits, especially in the early seed and seedling stages, which often predict survival and performance in the invaded Great Basin (Leger et al., 2021). Notably, much of this change occurred in both burned and unburned populations, and while fire increased invasion in many sites, this was not the case for all populations. Burn status may have been the selective agent for change over time in a few species or traits, particularly for *E. elymoides,* which showed greater invasion in burned sites, along with the most change overall and patterns in burned populations that were in line with our predictions of rapid evolutionary response to fire and invasion. However, as we only grew one generation of plants, we cannot rule out the role that maternal effects may have played in these temporal changes (Bischoff & Müller-Schärer, 2010). Additionally, the strength and direction of temporal change varies for individual responses and across species, indicating either that species and populations may have highly individualized responses to their environments, or that genetic drift is common in this system. Some of the responses shifted in ways that may be potentially adaptive in the Great Basin, while others changed in new and unexpected directions.

The majority of temporal changes occurred in the early seed and seedling stages, including seed weight, percent emergence, and emergence phenology. We expected that seed weight and emergence percentage would be lower in past populations, as seed weight and viability can decline in storage and with age as seeds metabolize stored resources (De Vitis et al., 2020; Franks et al., 2019). We also expected that the contemporary burned groups, relative to other groups, would have higher percent emergence due to selection for the most resilient genotypes, and that we might observe higher seed weights in contemporary burned sites, as this can increase competitive ability in some scenarios (Atwater et al., 2015; Leger et al., 2019; Leger & Goergen, 2017). Seed weight did increase over time for *P. spicata* and *S. grossulariifolia*, but this was true for both burned and unburned populations, and we were surprised to find that seed weight decreased over time for *E. linearis* and *P. secunda*. For *E. elymoides*, seed weight changes matched our predictions, as seed weights declined in the unburned population while increasing in the burned population, which may be adaptive, though seed weight can have a complicated effect on survival in these systems (Appendix 4). It is difficult to disentangle evolutionary change from the potential effects of seed storage and maternal effects, which tend to have an outsized effect on early stages of plant development (Bischoff & Müller-Schärer, 2010). However, the shift towards lighter seeds in *E. linearis* and *P. secunda* likely is not a storage effect, since the past seeds would be unlikely to gain weight in storage. Despite many changes in seed size, we saw no results that suggest negative effects of storage for percent emergence, as the majority of populations changed little over time or had lower emergence in the contemporary group than the past group. This is promising for the field of seed banking, as it appears that the past seeds did not lose viability after being stored for 3-11 years. Several populations, including both *P. secunda* and *C. douglasii* populations as well as the burned *S. grossulariifolia* populations, had lower contemporary germination, which could be due to seed dormancy. The *E. elymoides* again had different responses than other species, and more in line with our predictions, with the burned population having higher contemporary emergence and the unburned population having lower contemporary emergence. Although high germination is often associated with plant success, reduced germination is not necessarily a sign of maladaptation; it could signal that these populations are exhibiting increased dormancy. *Poa secunda* is known to exhibit dormancy (Chen et al., 2022), and dormancy is the norm for forbs *C. douglasii* (Kildisheva et al., 2019) and most *Sphaeralcea* species (Dunn, 2011; Kildisheva et al., 2011). Dormancy can help plants bet-hedge in systems with infrequent favorable germination and unpredictable climates (Willis et al., 2014). Dormancy is common and can be adaptive in dryland systems (Baskin & Baskin, 2014), and dormancy appears to be widespread in the forbs of the Great Basin, where favorable germination conditions are rare (Kildisheva et al., 2019).

Emergence phenology is another early trait that can predict adult plant traits and success (Donohue et al., 2010). We expected to see earlier emergence in contemporary groups, particularly in the contemporary burned groups, since early emergence phenology is potentially adaptive in disturbed areas of the Great Basin (Leger et al., 2019, 2020), and further may be associated with an increased ability to compete with *B. tectorum* (Barak et al., 2015; Goergen et al., 2011). This could be because *B. tectorum* germinates more rapidly than many native species, giving it a competitive advantage (Hardegree et al., 2010). Emergence phenology changed over time for all species, but again, these changes were not in a consistent direction. In line with our hypothesis, both *P. spicata* populations and the burned *E. elymoides* populations showed significant temporal shifts towards early emergence. This was particularly striking in *E. elymoides*, as the contemporary burned population emerged almost five days earlier than the past burned population, while the unburned population changed little over time. The burned *E. elymoides* site was one of our most invaded sites, so this change suggests that the burned *E. elymoides* population may have undergone selection for earlier emergence in the invaded post-burn landscape. This would be in line with observations of rapid evolution towards earlier emergence of *E. elymoides* or congener *E. multisetus* from *B. tectorum*-invaded sites (Agneray et al., 2022; Goergen et al., 2011; Kulpa & Leger, 2013; Leger et al., 2019). In contrast to our expectations, the contemporary *P. secunda* and *E. linearis* groups actually had later emergence than their past groups. For *E. linearis*, this may be because both the burned and unburned sites had low invasive annual grass cover, so there may not be strong selective pressure for earlier emergence in these systems. Both *P. secunda* sites, on the other hand, have invasive-annual grass pressure, so this same rationale does not apply.

In contrast to seed and seedling traits, we saw less temporal change in mortality and adult reproductive and morphological traits. As with early traits, however, responses continued to vary across species. Mortality was similar in past and contemporary groups for most species, except for *E. linearis* and *S. grossulariifolia,* which had higher mortality in the past groups than the contemporary groups. This may have been a function of seed age as mortality after germination can increase with seed age (Franks et al., 2019). Ideally, we would have grown these plants for a second generation to account for maternal and seed storage effects, but this was not possible in our time frame as the majority of species did not reproduce. Only *E. elymoides* and *P. spicata* reproduced, and they only reproduced during Trial 1, though issues with smut precluded the use of seeds produced. We expected that the burned contemporary populations would exhibit increased fecundity and earlier flowering phenology than other groups, assuming that natural selection would result in greater representation of plants with these characteristics after disturbance. However, we were surprised to see that flowering was lower in the contemporary burned *E. elymoides* group than the past group. Very few unburned *E. elymoides* individuals flowered in both the past and contemporary groups, so we don't have an adequate comparison point to see if burn status was the driving force behind the temporal changes in the burned *E. elymoides* population. However, since the burned *E. elymoides* site was highly invaded and degraded, it is possible that the plants may have shifted towards allocating energy to survival and growth instead of reproduction. Notably, the contemporary *E. elymoides* seeds for our experiment were collected in 2020, and when we revisited the population in 2021 no *E. elymoides* individuals in the burned population were flowering. Evolution of lower fecundity is not consistent with known responses to invasion, as generally native plants that have evolved with non-natives have higher reproductive performance than plants that have not evolved with non-natives (Oduor, 2013). In contrast to *E. elymoides*, all *P. spicata* groups had similar flowering percentages. *Pseudoroegneria spicata* shared a burned site with *E. elymoides*, so their different flowering responses show that these species have individualized responses in their common environment. We also saw an unexpected shift in flowering phenology in the burned *E. elymoides* population, as well as a trend towards this same response in the unburned *P. spicata* population, with contemporary plants flowering later than past plants. Again, this is not consistent with other systems, as rapid evolution for earlier flowering phenology has been observed after disturbances, including cheatgrass invasion (Goergen et al., 2011) and drought (Franks et al., 2007; Franks & Weis, 2008), and may be associated with positive restoration outcomes in the Great Basin (Kulpa & Leger, 2013; Leger & Baughman, 2015).

We measured a suite of morphological traits, including height, leaf width, leaf number, aboveground biomass, as well as belowground biomass and root to shoot ratio for Trial 1 species. Surprisingly, height was the only of these traits in which we found significant changes over time. Specifically, both *E. elymoides* and the burned *P. secunda* populations were shorter in the contemporary group. In contrast to the burned *P. secunda* population, the unburned population was slightly taller in the contemporary group. Smaller plant size may be adaptive in the Great Basin, especially for *E. elymoides,* as numerous studies have demonstrated that smaller plant size for this species or its congener *E. multisetus* is associated with cheatgrass invasion and restoration success (Fergosun et al., 2015; Kulpa & Leger, 2013; Leger & Baughman, 2015; Rowe & Leger, 2010). The shift towards shorter plants in only the burned *P. secunda* population may indicate that burn status was the selective agent for this change. However, this shift may have been driven by burn associated changes other than invasive annual grass invasion, as the burned *P. secunda* population actually had less non-native plants than the unburned site. Beyond height we saw very little temporal change in other morphological traits, which may be due to the relatively short duration of our study. We were surprised that we did not see a change in biomass allocation, as increased belowground biomass is likely adaptive in the Great Basin (Agneray et al., 2022; Fergosun et al., 2015; Leger et al., 2019; Leger & Baughman, 2015; Leger & Goergen, 2017, p. 201; Rowe & Leger, 2010).

Elymus elymoides showed more change than other species, and several changes were consistent with rapid evolution in response to fire. The burned and unburned *E. elymoides* populations may not have been a perfect match as the burned *E. elymoides* population had five years to change between collection dates, while the unburned pair only had three years. However, evolution can happen on this time scale (Leger & Goergen, 2017), either through selective mortality, increased reproduction and germination by the best-adapted genotypes, or drift removing certain genotypes from the population. The *E. elymoides* burned site had much more invasive annual grasses than the unburned, as well as fewer native plants, and these changes associated with burn status may have been the selective agent for increased contemporary seed weight, higher emergence, and earlier emergence in *E. elymoides,* along with reduced flowering and later flowering phenology.

While all species were negatively affected by *B. tectorum*, there were differences in how much each species was negatively affected by cheatgrass presence. Specifically, *E. linearis* experienced the least negative effects of cheatgrass, especially in terms of mortality, as the species overall only had 3% higher mortality when grown with *B. tectorum* than without. This is surprising, because both *E. linearis* populations had low invasive annual grass cover, and because, at least in terms of height, the populations may have become less tolerant of *B. tectorum* over time. However, lower plant stature is not necessarily maladaptive, and the relative tolerance of this species to *B. tectorum* may make it a promising forb to include in seed mixes for distribed sites. *Elymus elymoides* and *S. grossulariifolia* were less tolerant of *B. tectorum* than *E. linearis*, but slightly more tolerant than the remaining species. *Sphaeralcea grossulariifolia* has also been labeled a "native winner" in a study of several forbs that may be able to grow in cheatgrass-degraded sites, though challenges with germination can make it difficult to use for restoration (Barak et al., 2015). *Elymus elymoides* is one of the most common species used for restoration in the Great Basin as it germinates quickly and reliably and is a strong competitor with cheatgrass (Leger et al., 2021; Stevens et al., 2014). *Pseudoroegneria spicata* and *Poa secunda* had the highest increases in mortality when grown with *B. tectorum* than the other species, particularly the unburned past *P. spicata* population, though they had moderate tolerance in morphology and biomass traits. Both of these perennial grasses are often used for restoration in the Great Basin (Leger & Baughman, 2015), and both have the capacity to compete with *B. tectorum* (Goergen et al., 2011; Larson et al., 2018), so we believe their continued use is certainly warranted. *Chaenactis douglasii* was one of the most negatively affected by *B. tectorum* of all the species for survival, morphology, and biomass measures.

In our study, the burned *E. elymoides* and population showed some signs that it was more comeptitive with cheatgrass than unburned population, though the burn x time x cheatgrass interaction was not significant. Specifically, the burned *E. elymoides* saw a temporal shift towards increased survival when grown with *B. tectorum* that did not occur in the unburned population. While our results are far from concrete, they are consistent with studies that show that *E. elymoides,* and congener *E. multisetus* have likely evolved to compete with *B. tectorum* in the Great Basin (Fergosun et al., 2015; Goergen et al., 2011; Leger et al., 2019; Leger & Goergen, 2017; Rowe & Leger, 2010). Here, we suspect that surviving a wildfire and associated changes has the potential to cause rapid evolutionary change in *E. elymoides* and may be associated with increased performance in the invaded Great Basin. Our study examined the effects of burn status on change over time, but sites also differed in their level of invasion, and had differences in mean annual temperature and precipitation, soil characteristics, and grazing history. Further analyses could examine how site differences beyond burn status affected plant responses.

Conclusion

Overall, we observed change over time for every species in all populations, with at least one burn x time interaction for every species, with early seed and seedling traits and *E. elymoides* showing the most change. These changes could be due to rapid evolution, through either selection or drift, and in a few cases it appears that burn status may have been the selective agent for change. However, these changes could also be due to maternal effects, especially possible for seed traits. Researchers conducting common garden studies strive to grow multiple generations of plants to control for maternal effects, but this is unfortunately not always feasible. Whether the changes were rooted in maternal effects or a result of evolutionary change, our observation that seed bank and contemporary collections are likely to differ in potentially adaptive traits is noteworthy, and relevant for the use of these seeds for restoration or research. Specifically, one cannot expect that seed banked seeds are a mirror image of contemporary populations, which may continue to change while the seed bank gene pool remains static. These historic collections are a valuable resource, because they may harbor alleles that are no longer present in a given population, and could potentially be used to relieve inbreeding depression and loss of diversity through re-introduction. Our conclusions support the continued effort of programs like Seeds of Success (Haiet & Olwell, 2015), that collect wild seed for restoration and long-term storage, and further suggest that revisiting collected populations could be beneficial for capturing rapid evolutionary changes.

While all species changed in some way, we did notice several interesting trends, including the fact that the direction and strength of change was not consistent across species for any trait. This suggests that if natural selection is responsible for observed changes, there is not consistent selection across all of these species, despite living in similar habitats. Our expectations for adaptive traits are heavily based on previous work with perennial grasses, especially *Elymus* species, and while this species showed shifts towards traits that we suspect are adaptive in invaded Great Basin communities, others surprised us by changing in opposite directions. Strategies that are adaptive for one species may be maladaptive for another based on life history, pollinator and dispersal interactions, and seed dispersal method. It is likely that at least some of these changes are genetically-based rather than due to maternal effects, and combining common garden efforts with sequencing may be helpful for disentangling the relative contribution of genetic change vs. maternal effects. We also are excited by the growing use of resurrection studies to assess rapid evolution, and encourage other researchers to take advantage of the Germplasm Resource Information Network (Volk & Richards, 2008) and Project Baseline (Etterson et al., 2016) to request historic seed collections for resurrection studies.

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Tables

Table 1. Collection location information for each species/burn status, including mean annual precipitation (MAP; 30-year normals, 1990-2020, mm), mean annual temperature (MAT, degrees C) (*PRISM Climate Group, Oregon State University*, 2014), past and contemporary (contemp.) collections years, the distance between paired sites, and the difference in past/contemporary collection years.

Species	**Burn status**	**MAP**	**MAT**	**Past collection year**	**Contemp. collection year**	**Distance apart (km)**	**Years between collections**
C. douglasii	Burned	228.8	11.6	2011	2021	88.8	10
C. douglasii	Unburned	398.9	10	2013	2021	-	8
E. elymoides	Burned	253.1	10	2015	2020	201.8	5
E. elymoides	Unburned	361.5	8.9	2017	2020	-	3
E. linearis	Burned	410.5	8.5	2012	2021	53.5	9
E. linearis	Unburned	469.2	8.3	2016	2021	-	5
P. secunda	Burned	346.3	8.5	2011	2021	94.4	10
P. secunda	Unburned	300.5	9.1	2011	2021	-	10
P. spicata	Burned	253.1	10	2015	2020	194.9	5
P. spicata	Unburned	318.6	9.8	2016	2020	-	4
S. grossulariifolia	Burned	244.1	10.3	2015	2020 & 2021	24.9	3 to 4
S. grossulariifolia	Unburned	232.0	10.3	2017	2020 & 2021	-	5 to 6

Table 2. Results from linear models showing the effect of burn status, species, and the interaction between burn status and species on percent cover. The data distribution was specified as Gaussian. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Response variable	**Factor**	**X^2 value**	**Degrees of freedom**	**P value**
Density of non-native species	Burn	53.4	1	**<0.0001**
	Species	33.6	5	**<0.0001**
	Burn*Species	43.5	5	**<0.0001**
Density of invasive annual grasses$_1$	Burn	15.8	1	**<0.0001**
	Species	57.4	5	**<0.0001**
	Burn*Species	48.6	5	**<0.0001**
Density of *Bromus tectorum*$_1$	Burn	40.1	1	**<0.0001**
	Species	41.2	5	**<0.0001**
	Burn*Species	63.8	5	**<0.0001**
Density of native species	Burn	43.7	1	**<0.0001**
	Species	96.9	5	**<0.0001**
	Burn*Species	122.6	5	**<0.0001**
Density of native shrubs	Burn	9.1	1	**<0.01**
	Species	26.7	5	**<0.0001**
	Burn*Species	25.7	5	**<0.001**

No subscript indicates that the variable was not transformed.
1 indicates that the variable was log transformed.

Table 3. Summary of significant responses to burn (B), time (T), and time by burn interaction (BxT) for our six study species; full statistical results are reported elsewhere. Seed and seedling responses include seed weight (Seed wt), whether or not a species emerged (Emer), emergence phenology (Time emer). Mort stands for mortality, and reproductive responses include percent flowered (% flow), flowering phenology (Time flow), and number of inflorescences (# flow). Morphology (Morph) traits include height (Ht), number of leaves (# lvs), and leaf width (Lw). Lastly, biomass traits include aboveground biomass from Trial 1 (AB T1), aboveground biomass for Trial 2 (AB T2), and belowground biomass (BB T1), total biomass (TB T1), and root mass ratio (RMR T1) from Trial 1. A dash (-) indicates that that response was not measured for that species.

	Seed and seedling			**Mort**	**Reproduction**			**Morph**		
Species	**Seed wt**	**Emer**	**Time emer**	**Mort**	**% flow**	**Time flow**	**# flow**	**Ht**	**# lvs**	**Lw**
C. douglasii		T	B BxT		-	-	-			
E. elymoides	B T BxT	BxT	B T BxT		B T	T		T		
E. linearis	T BxT	B	B T	T	-	-	-			-
P. secunda	T	B T BxT	T		-	-	-	BxT		-
P. spicata	B T	B	T BxT					B		-
S. grossulariifolia	B T	B T BxT	-	T	-	-	-			

	Biomass				
Species	**AB T1**	**AB T2**	**BB T1**	**TB T1**	**RMS T1**
C. douglasii	-		-	-	-
E. elymoides	B	B			
E. linearis	-		-	-	-
P. secunda	-		-	-	-

P. spicata	T	B
S. grossulariifolia		B

Table 4. Results from linear models showing the effect of burn status, time, and the interaction between burn status and time on the weight of one seed. The data distribution was specified as Gaussian. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Species	**Factor**	**X2 value**	**Degrees of freedom**	**P value**
Chaenactis douglasii	Burn	2.2	1	0.1419
	Time	0.5	1	0.5026
	Burn*Time	0.4	1	0.5124
Elymus elymoides	Burn	140.7	1	**<0.0001**
	Time	4.2	1	**<0.05**
	Burn*Time	9.3	1	**<0.01**
Erigeron linearis	Burn	0.0	1	0.8495
	Time	10.1	1	**<0.01**
	Burn*Time	5.6	1	**<0.05**
Poa secunda	Burn	1.3	1	0.2577
	Time	66.2	1	**<0.0001**
	Burn*Time	2.4	1	0.1245
Pseudoroegneria spicata	Burn	9.1	1	**<0.01**
	Time	38.2	1	**<0.0001**
	Burn*Time	1.9	1	0.1741
Sphaeralcea grossulariifolia	Burn	4.0	1	**<0.05**
	Time	9.2	1	**<0.01**
	Burn*Time	1.8	1	0.1847

Table 5. Results from linear models showing the effect of burn status, time, and the interaction between burn status and time on emergence percentage. The data distribution was specified as binomial. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Species	**Factor**	**X2 value**	**Degrees of freedom**	**P value**
Chaenactis douglasii	Burn	1.1	1	0.2946
	Time	23.6	1	**<0.0001**
	Burn*Time	0.0	1	0.9789
Elymus elymoides$_1$	Burn	0.1	1	0.7545
	Time	0.9	1	0.34
	Burn*Time	5.5	1	**<0.05**
Erigeron linearis	Burn	16.0	1	**<0.0001**
	Time	0.0	1	0.8932
	Burn*Time	0.4	1	0.5076
Poa secunda	Burn	17.5	1	**<0.0001**
	Time	118.7	1	**<0.0001**
	Burn*Time	24	1	**<0.0001**
Pseudoroegneria spicata	Burn	5.9	1	**<0.05**
	Time	2.8	1	0.0946
	Burn*Time	0.0	1	0.9534
Sphaeralcea grossulariifolia	Burn	12.4	1	**<0.001**
	Time	18.0	1	**<0.0001**
	Burn*Time	18.5	1	**<0.0001**

Table 6. Results from linear models showing the effect of burn status, time, and the interaction between burn status and time for days to emergence. The data distribution was specified as Poisson. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Species	**Factor**	**X2 value**	**Degrees of freedom**	**P value**
Chaenactis douglasii	Burn	13.5	1	**<0.001**
	Time	0.0	1	0.8596
	Burn*Time	22.3	1	**<0.0001**
Elymus elymoides	Burn	8.9	1	**<0.01**
	Time	36.9	1	**<0.0001**
	Burn*Time	38	1	**<0.0001**
Erigeron linearis	Burn	5.0	1	**<0.05**
	Time	5.6	1	**<0.05**
	Burn*Time	0.1	1	0.7412
Poa secunda	Burn	2.2	1	0.1414
	Time	15.5	1	**<0.001**
	Burn*Time	0.1	1	0.7718
Pseudoroegneria spicata	Burn	1.0	1	0.3077
	Time	22.9	1	**<0.0001**
	Burn*Time	4.8	1	**<0.05**

Table 7. Results from linear models showing the effect of burn status, time, and the interaction between burn status and time on mortality after emergence. The data distribution was specified as binomial. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Response variable	**Factor**	**X2 value**	**Degrees of freedom**	**P value**
Chaenactis douglasii	Burn	2.7	1	0.1029
	Time	0.4	1	0.5256
	Burn*Time	1.3	1	0.2593
Elymus elymoides	Burn	0.6	1	0.4217
	Time	0.2	1	0.6985
	Burn*Time	0.2	1	0.7034
Erigeron linearis	Burn	0.6	1	0.4487
	Time	5.8	1	**<0.05**
	Burn*Time	1.3	1	0.2575
Poa secunda	Burn	1.5	1	0.2279
	Time	0.6	1	0.437
	Burn*Time	1.4	1	0.2288
Pseudoroegneria spicata	Burn	0.1	1	0.753
	Time	0.2	1	0.6895
	Burn*Time	1.6	1	0.2103
Sphaeralcea grossulariifolia	Burn	1.4	1	0.2344
	Time	3.9	1	**<0.05**
	Burn*Time	0.2	1	0.6315

Table 8. Results from linear models showing the effect of burn status, time, and the interaction between burn status and time on the percent of plants that flowered. Smut was included as a fixed factor (results not shown) and the data distribution was specified as binomial. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Response variable	**Factor**	**X2 value**	**Degrees of freedom**	**P value**
Elymus elymoides	Burn	42.6	1	**<0.0001**
	Time	10.2	1	**<0.01**
	Burn*Time	111.9	1	0.5773
Pseudoroegneria spicata	Burn	0.7	1	0.4073
	Time	0.1	1	0.7506
	Burn*Time	0	1	0.9747

Table 9. Results from linear models showing the effect of burn status, time, and the interaction between burn status and time on flowering phenology. Smut was included as a fixed factor (data not shown) and the data distribution was specified as Poisson. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Species	**Factor**	**X2 value**	**Degrees of freedom**	**P value**
Elymus elymoides	Burn	1.2	1	0.2814
	Time	7.6	1	**<0.01**
	Burn*Time	1.2	1	0.2819
Pseudoroegneria spicata	Burn	0.3	1	0.6011
	Time	3.4	1	0.0670
	Burn*Time	1.8	1	0.1755

Table 10. Results from linear models showing the effect of burn status, time, and the interaction between burn status and time on plant height (mm). The data distribution was specified as Gaussian. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Species	**Factor**	**X2 value**	**Degrees of freedom**	**P value**
Chaenactis douglasii	Burn	2.3	1	0.1268
	Time	0.0	1	0.9408
	Burn*Time	0.3	1	0.5917
Elymus elymoides	Burn	0.8	1	0.3858
	Time	4.9	1	**<0.05**
	Burn*Time	1.5	1	0.2139
Erigeron linearis	Burn	1.6	1	0.2073
	Time	0.2	1	0.6636
	Burn*Time	1.1	1	0.2979
Poa secunda	Burn	0.8	1	0.3852
	Time	1.3	1	0.2482
	Burn*Time	3.9	1	**<0.05**
Pseudoroegneria spicata	Burn	7.3	1	**<0.01**
	Time	0.0	1	0.6895
	Burn*Time	0.1	1	0.2103
Sphaeralcea grossulariifolia	Burn	0.1	1	0.7961
	Time	1.2	1	0.271
	Burn*Time	1.9	1	0.169

Table 11. Percent change of each response from pretreatment from plants grown without cheatgrass to plants grown with cheatgrass for each species (regardless of bun status and time). Red colors indicate harmful responses, with each response type broken down into four colors, the darkest being the most extreme change. The lightest colors indicate a 1-33% change, second lightest is 33-66% change, second darkest is 66-99% change, and the darkest is 100% or greater change. Note that mortality values are positive while other values are negative, because increased mortality was a harmful response while decreased morphology and biomass were harmful responses. Asterisks indicate that the response was significantly affected by cheatgrass for a given species.

Species	**Mortality**	**Height**	**Leaf number**	**Leaf width**	**Biomass**
Chaenactis douglasii	44*	-51*	-36*	-58	-68*
Elymus elymoides	38	-10*	-37*	-21*	-45*
Erigeron linearis	3	-11*	-24*	-	-52*
Poa secunda	259*	-18*	-22*	-	-63*
Pseudoroegneria spicata	210*	-18*	-37*	-	-39*
Sphaeralcea grossulariifolia	38	-20*	-18	-45*	-56*

Table 12. Results from linear models showing the effects of cheatgrass, and two- and three-way interactions between cheatgrass presence, burn status, and time on mortality after emergence. The data distribution was specified as binomial. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Species	**Factor**	**X^2 value**	**Degrees of freedom**	**P value**
Chaenactis douglasii	Cheatgrass	6.1	1	**<0.05**
	Burn*Time	0.0	1	0.9513
	Burn*Cheatgrass	4.5	1	**<0.05**
	Time*Cheatgrass	0.7	1	0.4048
	Burn*Time*Cheat	3.0	1	0.0829
Elymus elymoides	Cheatgrass	0.9	1	0.3330
	Burn*Time	0.3	1	0.5961
	Burn*Cheatgrass	0.4	1	0.5570
	Time*Cheatgrass	1.0	1	0.3122
	Burn*Time*Cheat	1.2	1	0.2754
Erigeron linearis	Cheatgrass	0.0	1	0.8669
	Burn*Time	0.4	1	0.5047
	Burn*Cheatgrass	1.6	1	0.2004
	Time*Cheatgrass	1.4	1	0.2422
	Burn*Time*Cheat	0.9	1	0.3541
Poa secunda	Cheatgrass	34.5	1	**<0.0001**
	Burn*Time	1.4	1	0.2452
	Burn*Cheatgrass	2.1	1	0.1509
	Time*Cheatgrass	0.1	1	0.7401

	Burn*Time*Cheat	0.5	1	0.4984
Pseudoroegneria spicata	Cheatgrass	8.3	1	**<0.01**
	Burn*Time	0.6	1	0.4604
	Burn*Cheatgrass	2.8	1	0.0921
	Time*Cheatgrass	2.4	1	0.1235
	Burn*Time*Cheat	1.0	1	0.3070
Sphaeralcea grossulariifolia	Cheatgrass	1.2	1	0.2638
	Burn*Time	0.2	1	0.7031
	Burn*Cheatgrass	0.2	1	0.6291
	Time*Cheatgrass	0.0	1	0.9439
	Burn*Time*Cheat	0.1	1	0.7657

Table 13. Percent mortality of plants grown with and without cheatgrass and the change in percent mortality with cheatgrass for each species and burn status collection time group, with negative numbers indicating greater mortality with cheatgrass competition. Statistical results are shown in Table 12.

Species	**Grou**	**Mortality without cheatgrass (%)**	**Mortality with cheatgrass (%)**	**Change in mortality**
Chaenactis douglasii	Unburned Past	41	65	-24
Chaenactis douglasii	Unburned Contemporary	33	93	-60
Chaenactis douglasii	Burn Past	50	59	-9
Chaenactis douglasii	Burn Contemporary	69	67	2
Elymus elymoides	Unburned Past	7	6	1
Elymus elymoides	Unburned Contemporary	4	6	-1
Elymus elymoides	Burn Past	9	23	-14
Elymus elymoides	Burn Contemporary	9	6	2
Erigeron linearis	Unburned Past	40	24	16
Erigeron linearis	Unburned Contemporary	14	21	-7
Erigeron linearis	Burn Past	26	36	-10
Erigeron linearis	Burn Contemporary	19	29	-10
Poa secunda	Unburned Past	17	36	-19
Poa secunda	Unburned Contemporary	13	47	-34
Poa secunda	Burn Past	5	38	-33
Poa secunda	Burn Contemporary	15	61	-46

Pseudoroegneria spicata	Unburned Past	2	27	-25
Pseudoroegneria spicata	Unburned Contemporary	7	12	-5
Pseudoroegneria spicata	Burn Past	5	5	-1
Pseudoroegneria spicata	Burn Contemporary	2	2	0
Sphaeralcea grossulariifolia	Unburned Past	52	63	-11
Sphaeralcea grossulariifolia	Unburned Contemporary	25	40	-15
Sphaeralcea grossulariifolia	Burn Past	33	40	-7
Sphaeralcea grossulariifolia	Burn Contemporary	19	22	-2

Table 14. Results from linear models showing the effects of cheatgrass, and two- and three-way interactions between cheatgrass presence, burn status, and time on plant height (mm). The data distribution was specified as binomial. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Species	**Factor**	**X2 value**	**Degrees of freedom**	**P value**
Chaenactis douglasii	Cheatgrass	18.4	1	**<0.0001**
	Burn*Time	0.4	1	0.5252
	Burn*Cheatgrass	2.1	1	0.1469
	Time*Cheatgrass	0.0	1	0.92
	Burn*Time*Cheat	0.0	1	0.93
Elymus elymoides	Cheatgrass	8.8	1	**<0.01**
	Burn*Time	0.6	1	0.4262
	Burn*Cheatgrass	0.0	1	0.9312
	Time*Cheatgrass	3.0	1	0.0838
	Burn*Time*Cheat	0.9	1	0.3402
Erigeron linearis$_1$	Cheatgrass	6.6	1	**<0.05**
	Burn*Time	1.1	1	0.2785
	Burn*Cheatgrass	0.7	1	0.3945
	Time*Cheatgrass	6.3	1	**<0.05**
	Burn*Time*Cheat	0.1	1	0.7398
Poa secunda$_1$	Cheatgrass	4.1	1	<0.05*
	Burn*Time	8.5	1	<0.01*
	Burn*Cheatgrass	0.3	1	0.6061

	Time*Cheatgrass	0.0	1	0.9317
	Burn*Time*Cheat	0.0	1	0.9525
Pseudoroegneria spicata	Cheatgrass	20.4	1	<0.0001*
	Burn*Time	1.2	1	0.2791
	Burn*Cheatgrass	14.1	1	**<0.001**
	Time*Cheatgrass	0.0	1	0.8897
	Burn*Time*Cheat	0.5	1	0.4674
Sphaeralcea grossulariifolia$_1$	Cheatgrass	4.2	1	**<0.05**
	Burn*Time	0.1	1	0.8083
	Burn*Cheatgrass	0.8	1	0.3772
	Time*Cheatgrass	0.0	1	0.9947
	Burn*Time*Cheat	2.6	1	0.1074

No subscript indicates that the variable was not transformed.
1 indicates that the variable was log transformed.

Table 15. Height (mm) of plants grown with and without cheatgrass and their difference for each species and burn status collection time group. Statistical results are shown in Table 14.

Species	**Group**	**Height without cheatgrass**	**Height with cheatgrass**	**Difference without to with**
Chaenactis douglasii	Unburned Past	1.8	0.6	1.1
Chaenactis douglasii	Unburned Contemporary	1.6	0.6	1.0
Chaenactis douglasii	Burn Past	1.3	0.8	0.6
Chaenactis douglasii	Burn Contemporary	1.5	0.9	0.5
Elymus elymoides	Unburned Past	89.0	78.1	10.9
Elymus elymoides	Unburned Contemporary	85.2	78.8	6.3
Elymus elymoides	Burn Past	90.7	73.5	17.2
Elymus elymoides	Burn Contemporary	77.0	75.5	1.5
Erigeron linearis	Unburned Past	2.0	2.0	-0.1
Erigeron linearis	Unburned Contemporary	1.9	1.4	0.5
Erigeron linearis	Burn Past	1.6	1.6	0.0
Erigeron linearis	Burn Contemporary	1.9	1.4	0.5
Poa secunda	Unburned Past	41.0	33.1	7.9
Poa secunda	Unburned Contemporary	47.6	37.2	10.4
Poa secunda	Burn Past	51.9	42.9	9.0
Poa secunda	Burn Contemporary	38.6	33.1	5.5
Pseudoroegneria spicata	Unburned Past	93.6	67.5	26.1

Pseudoroegneria spicata	Unburned Contemporary	93.2	63.1	30.1
Pseudoroegneria spicata	Burn Past	78.9	73.2	5.6
Pseudoroegneria spicata	Burn Contemporary	81.1	81.3	-0.2
Sphaeralcea grossulariifolia	Unburned Past	1.4	1.1	0.3
Sphaeralcea grossulariifolia	Unburned Contemporary	1.3	1.4	-0.2
Sphaeralcea grossulariifolia	Burn Past	1.1	1.0	0.1
Sphaeralcea grossulariifolia	Burn Contemporary	1.5	1.0	0.5

Figures

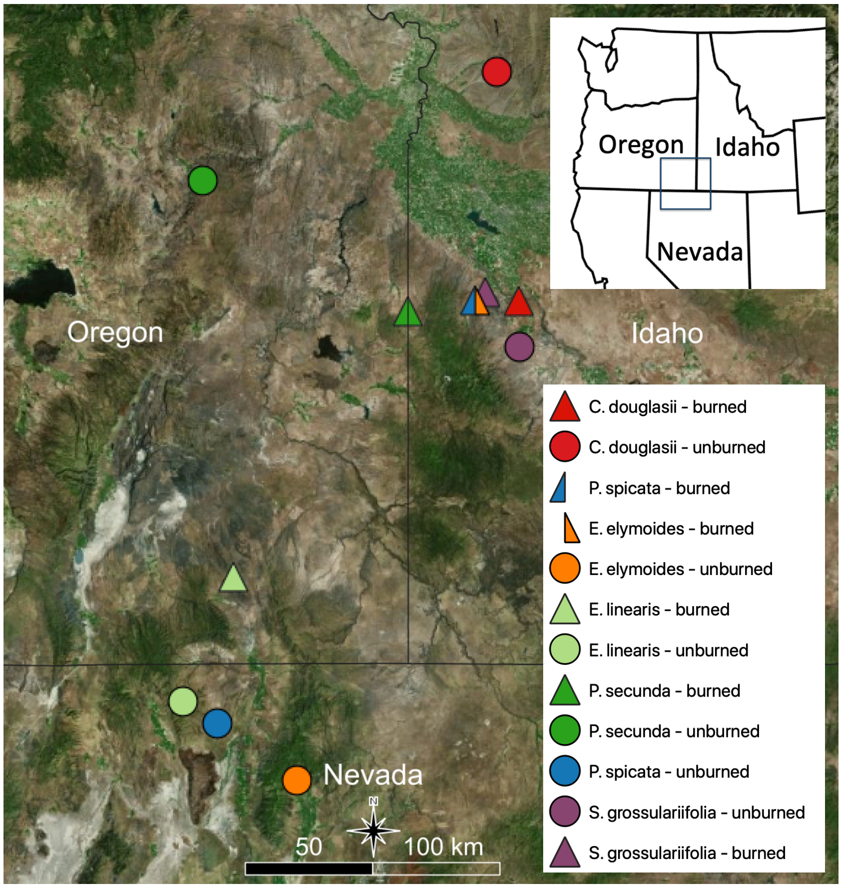

Figure 1. Collection site for the six study species in Oregon, Idaho, and Nevada. The points are color-coded for each species, and the triangles represent burned populations and circles represent unburned populations. The triangle that is blue on the left and orange on the right represents the *P. spicata* and *E. elymoides* burned populations that were collected from the same location.

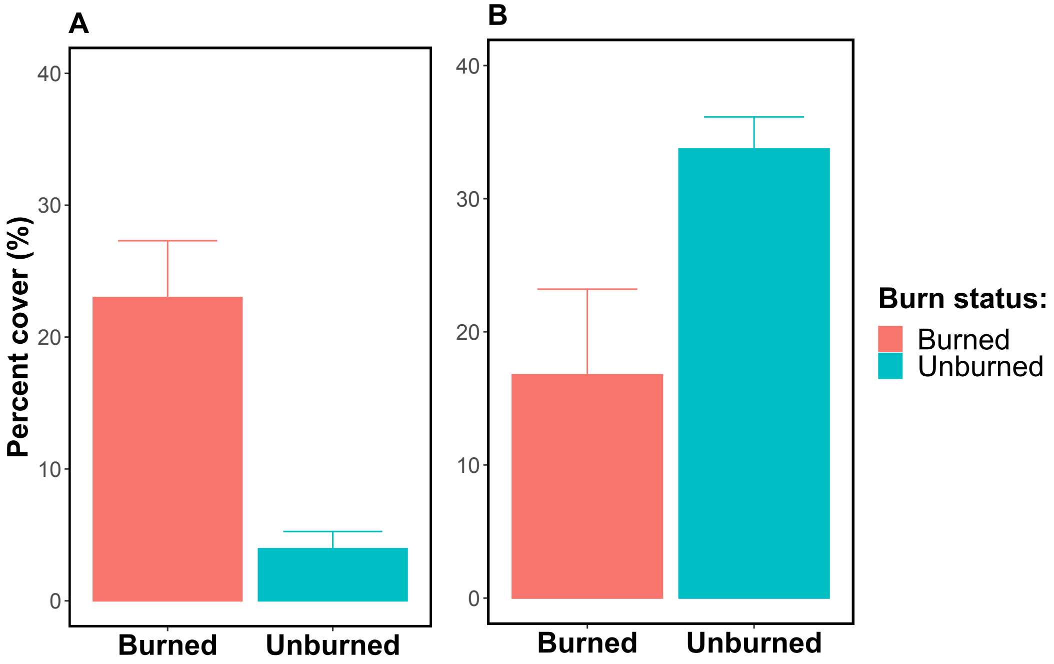

Figure 2. A) Density of non-native plants by site burn status and B) native plants by site burn status. Values are total percent cover of non-native of native plants, averaged across all sites, and error bars represent standard error. The y axis is the same for both plots.

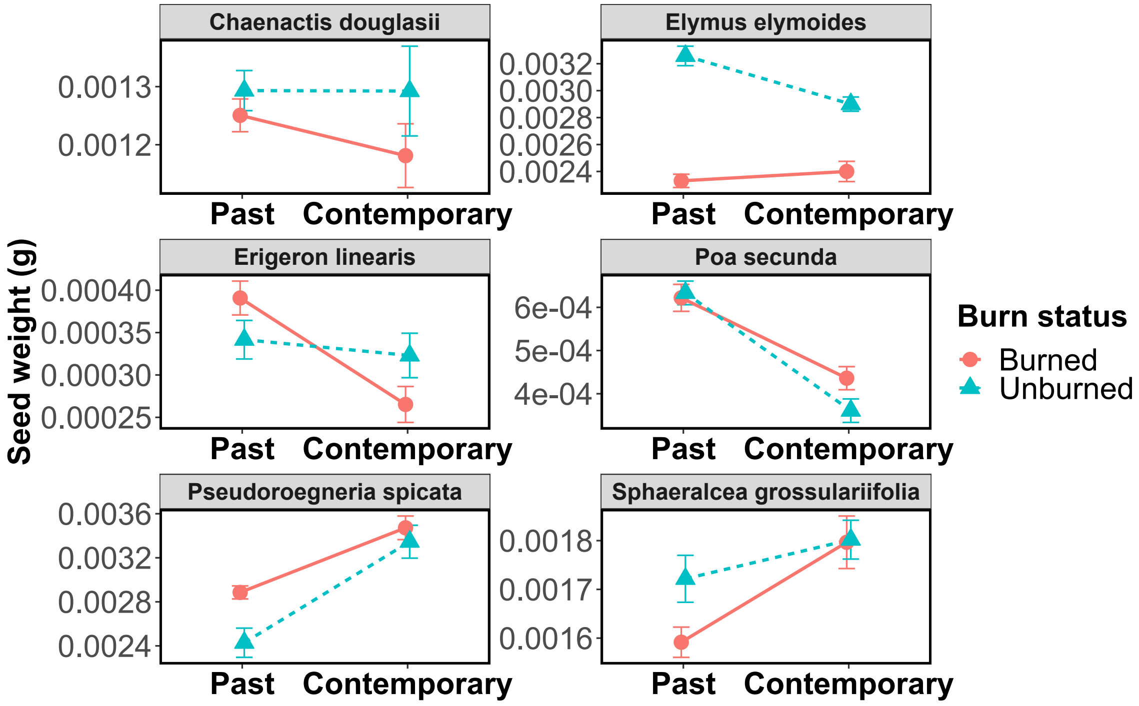

Figure 3. Seed weight (of one seed) in g in past or contemporary groups for burned and unburned populations. Points are means and error bars represent standard error. Note the y axis differs for each species because seed weight and the range of values differ substantially between species.

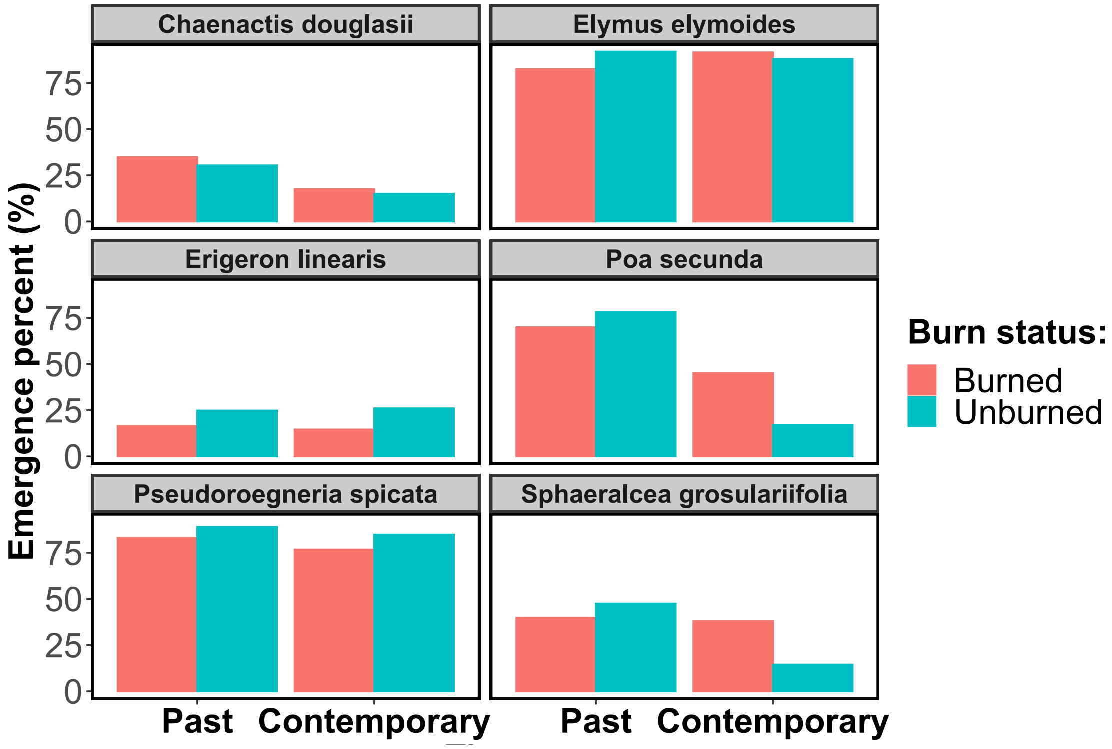

Figure 4. Percent emergence of each species by burn status with past and contemporary groups. Values are total germination of all seeds planted, including any seedlings that were thinned in a random selection after germination. Burned populations are in red to the left of blue unburned populations on the x axis. The y axis is the same for all plots.

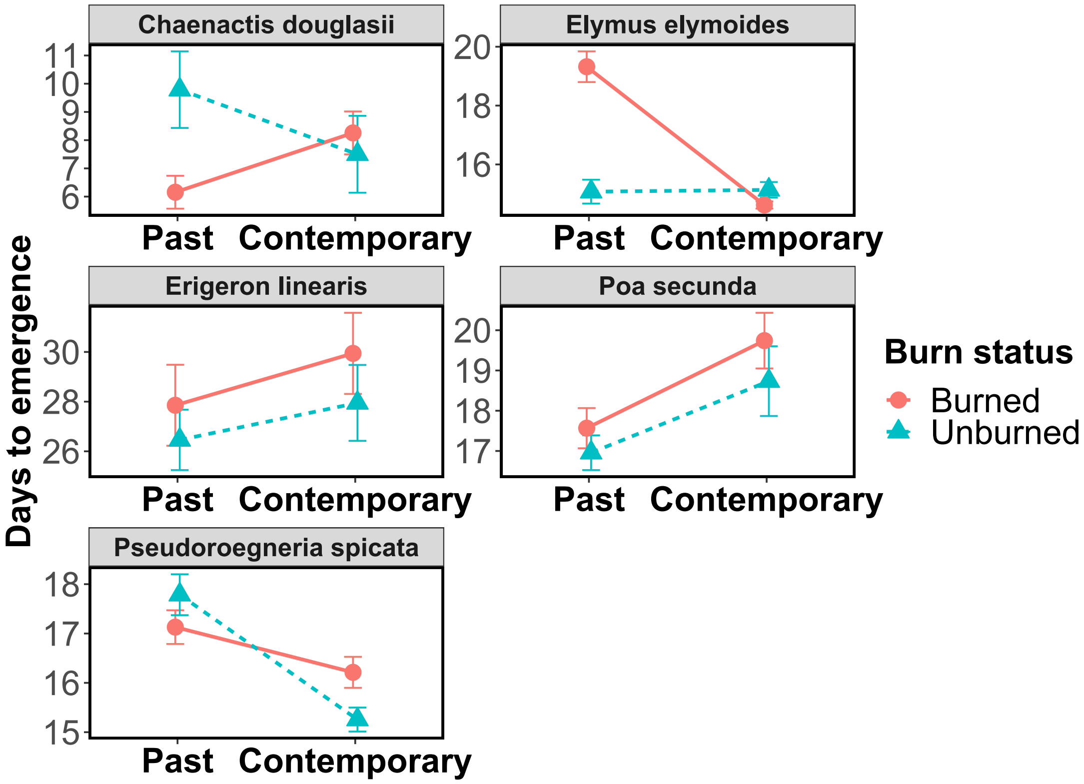

Figure 5. Changes in days to emergence from past to contemporary groups for burned and unburned populations. Points are means and error bars represent standard error. Note that phenology scales are different.

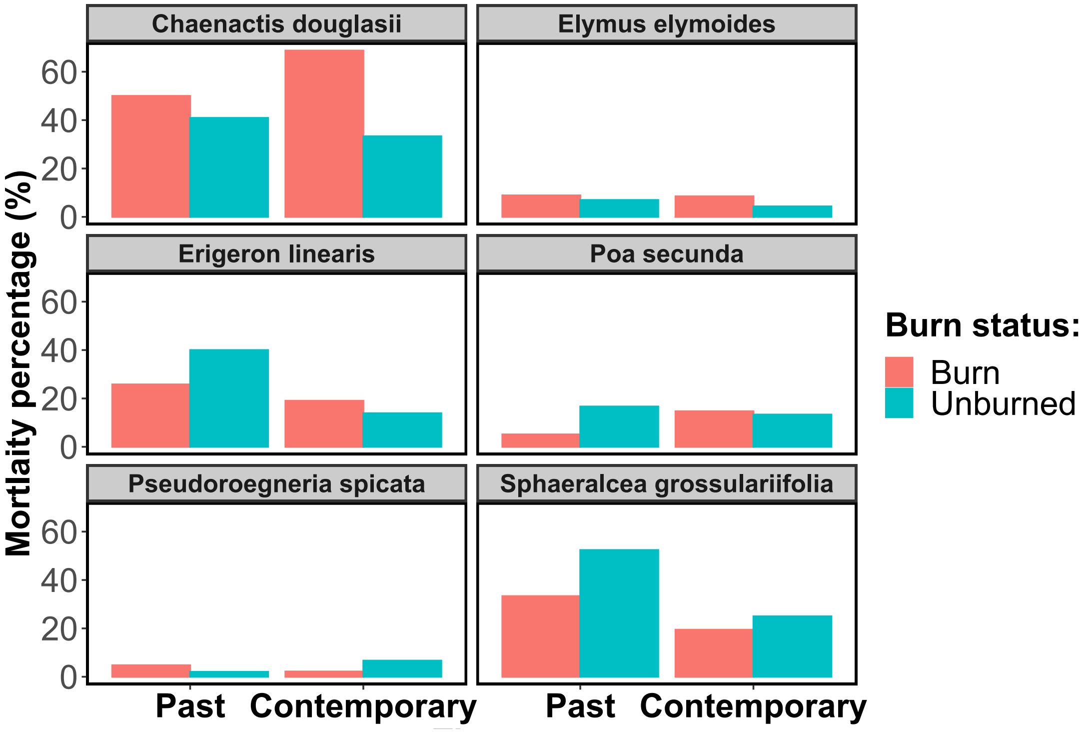

Figure 6. Percent mortality of each species by burn status with past and contemporary groups. Burned populations are in red to the left of blue unburned populations on the x axis. Values are total mortality and the y axis is the same for all the plots.

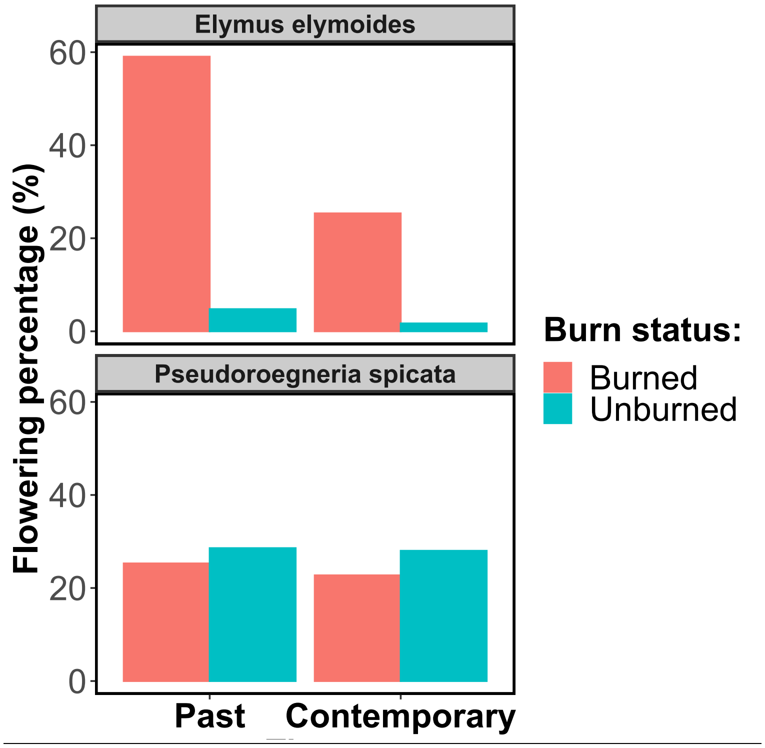

Figure 7. Percent emergence of each species by burn status with past and contemporary groups. Burned populations are in red to the left of blue unburned populations on the x axis. Values are the total percent of plants that flowered and the y axis is the same for both plots.

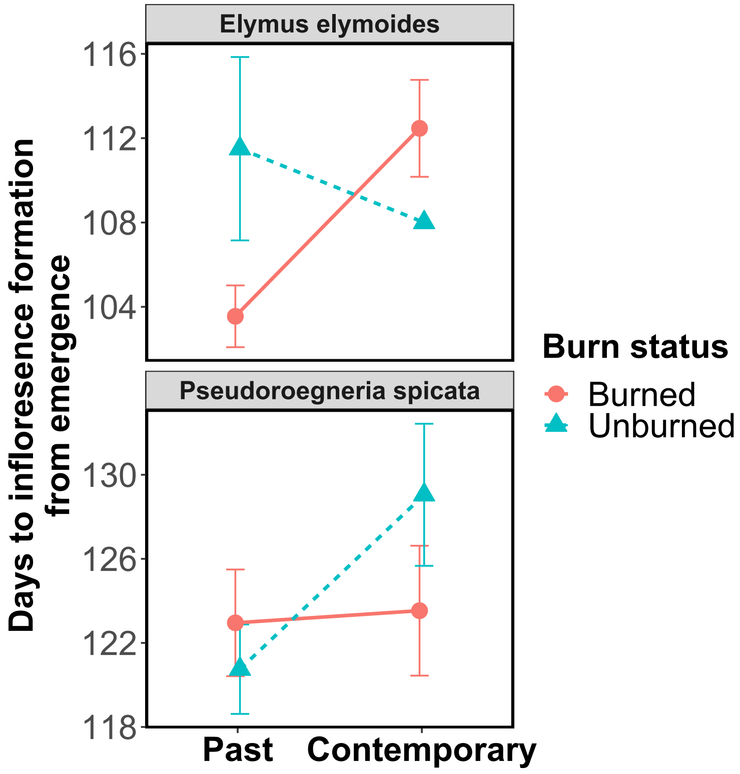

Figure 8. Changes in days to inflorescence formation since emergence from past to contemporary groups for burned and unburned populations. Points are means and error bars represent standard error. Note that phenology scales are different but each covers 12 days.

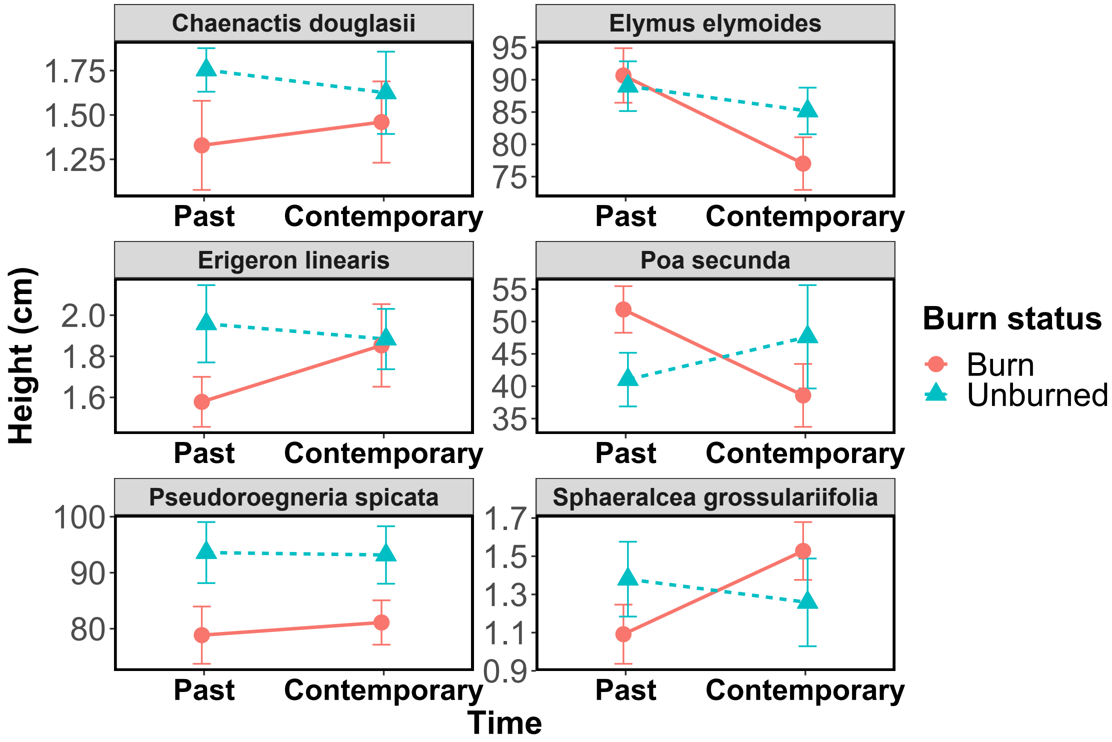

Figure 9. Changes in height (cm) from past to contemporary groups for burned and unburned populations. Points are means and error bars represent standard error. Note the y axis varies for each species because the plant height and the range of values differ substantially between species.

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Appendices

Appendix 1: Number of inflorescences

Appendix Table 1.1 Results from linear models showing the effect of burn status, time, and the interaction between burn status and time on the number of inflorescences. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Response variable	**Factor**	**X2 value**	**Degrees of freedom**	**P value**
Elymus elymoides	Burn	0.7	1	0.3826
	Time	1.9	1	0.1704
	Burn*Time	0.1	1	0.7362
Pseudoroegneria spicata	Burn	0.0	1	0.9446
	Time	0.2	1	0.6863
	Burn*Time	1.7	1	0.1935

Appendix 2: Leaf number and width

Appendix Table 2.1 Results from linear models showing the effect of burn status, time, and the interaction between burn status and time on the number of leaves. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Response variable	**Factor**	**X2 value**	**Degrees of freedom**	**P value**
Chaenactis douglasii	Burn	0.0	1	0.8811
	Time	0.7	1	0.4154
	Burn*Time	0.6	1	0.4258
Elymus elymoides	Burn	0.0	1	0.8998
	Time	0.9	1	0.3529

	Burn*Time	0.4	1	0.5336
Erigeron linearis	Burn	1.3	1	0.2555
	Time	0.5	1	0.4643
	Burn*Time	0.0	1	0.9891
Poa secunda	Burn	0.3	1	0.5792
	Time	2.0	1	0.1583
	Burn*Time	1.7	1	0.1946
Pseudoroegneria spicata	Burn	0.3	1	0.5571
	Time	1.7	1	0.1937
	Burn*Time	0.0	1	0.8649
Sphaeralcea grossulariifolia	Burn	1.2	1	0.2722
	Time	0.7	1	0.3944
	Burn*Time	0.0	1	0.9559

Appendix Table 2.2 Results from linear models showing the effect of burn status, time, and the interaction between burn status and time on leaf width (mm). Values are X^2 values, degrees of freedom, and P values, with asterisks indicating significance at alpha = 0.05.

Response variable	**Factor**	**X2 value**	**Degrees of freedom**	**P value**
Chaenactis douglasii	Burn	0.7	1	0.3995
	Time	0.9	1	0.3305
	Burn*Time	0.4	1	0.5138
Elymus elymoides	Burn	0.4	1	0.5403

	Time	0.0	1	0.9301
	Burn*Time	0.7	1	0.4187
Sphaeralcea grossulariifolia	Burn	2.8	1	0.0919
	Time	3.0	1	0.0802
	Burn*Time	1.3	1	0.2412

Appendix 3: Biomass

Appendix Table 3.1. Results from linear models showing the effect of burn status, time, and the interaction between burn status and time on aboveground biomass (g) from Trial 1 plants. Smut was included as a fixed factor for *E. elymoides* and *P. spicata* and the data distribution was specified as Gaussian. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Species	**Factor**	**X^2 value**	**Degrees of freedom**	**P value**
Elymus elymoides	Burn	9.7	1	**<0.01**
	Time	0.1	1	0.784
	Burn*Time	2	1	0.1493
Pseudoroegneria spicata$_1$	Burn	0.5	1	0.4764
	Time	1	1	0.3148
	Burn*Time	3.6	1	0.0567
Sphaeralcea grossulariifolia$_1$	Burn		1, 164	0.108
	Time		1, 164	0.0694
	Burn*Time		1, 164	0.1271

No subscript indicates that the variable was not transformed.

1 indicates that the variable was log transformed.

Appendix Table 3.2 Results from linear models showing the effect of burn status, time, and the interaction between burn status and time on aboveground biomass (g) from Trial 2 plants. The data distribution was specified as Gaussian. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Response variable	**Factor**	**X2 value**	**Degrees of freedom**	**P value**
Chaenactis douglasii	Burn	0.1	1	0.7197
	Time	0.4	1	0.5053
	Burn*Time	0.1	1	0.7776
Elymus elymoides	Burn	5.9	1	**<0.05**
	Time	0.4	1	0.5402
	Burn*Time	0.2	1	0.6677
Erigeron linearis	Burn	3.4	1	0.0643
	Time	2.1	1	0.1498
	Burn*Time	2.0	1	0.16
Poa secunda$_1$	Burn	1.2	1	0.2796
	Time	0.7	1	0.4189
	Burn*Time	3.0	1	0.0814
Pseudoroegneria spicata$_1$	Burn	0.1	1	0.7043
	Time	5.6	1	**<0.05**
	Burn*Time	0.1	1	0.7396
Sphaeralcea grossulariifolia$_1$	Burn	0.1	1	0.7371
	Time	1.0	1	0.9034

	Burn*Time	0.5	1	0.4676

No subscript indicates that the variable was not transformed.
1 indicates that the variable was log transformed.

Appendix Table 3.3 Results from linear models showing the effect of burn status, time, and the interaction between burn status and time on belowground biomass (g) from Trial 1 plants. The data distribution was specified as Gaussian. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Species	**Factor**	**X2 value**	**Degrees of freedom**	**P value**
Elymus elymoides	Burn	1.5	1	0.2231
	Time	0.3	1	0.5575
	Burn*Time	0.9	1	0.3368
Pseudoroegneria spicata$_1$	Burn	3	1	0.0817
	Time	0.1	1	0.7441
	Burn*Time	1.6	1	0.2063
Sphaeralcea grossulariifolia	Burn	1.1	1	0.2993
	Time	0.1	1	0.8088
	Burn*Time	0.5	1	0.4606

No subscript indicates that the variable was not transformed; 1 indicates that the variable was log transformed.

Appendix Table 3.4 Results from linear models showing the effect of burn status, time, and the interaction between burn status and time on total biomass (g) from Trial 1 plants. The data distribution was specified as Gaussian. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Response variable	**Factor**	**X^2 value**	**Degrees of freedom**	**P value**
Elymus elymoides	Burn	3.2	1	0.0721

	Time	0.2	1	0.6285
	Burn*Time	1.4	1	0.2298
Pseudoroegneria spicata$_1$	Burn	1.7	1	0.1985
	Time	0.3	1	0.569
	Burn*Time	2.1	1	0.1463
Sphaeralcea grossulariifolia	Burn	0.2	1	0.6645
	Time	0.5	1	0.4771
	Burn*Time	0	1	0.8434

No subscript indicates that the variable was not transformed.

1 indicates that the variable was log transformed.

Appendix Table 3.5. Results from linear models showing the effect of burn status, time, and the interaction between burn status and time on root mass ratio from Trial 1 plants. The data distribution was specified as Gaussian. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05

Species	**Factor**	**X2 value**	**Degrees of freedom**	**P value**
Elymus elymoides	Burn	2.6	1	0.105
	Time	1.7	1	0.188
	Burn*Time	0	1	0.9472
Pseudoroegneria spicata	Burn	7.3	1	**<0.05**
	Time	1.3	1	0.2583
	Burn*Time	0.7	1	0.412
Sphaeralcea grossulariifolia	Burn	4.7	1	**<0.05**
	Time	2.4	1	0.1187

Burn*Time	2.7	1	0.1003

Appendix 4: Seed weight discussion

The adaptive value of seed weight is highly debated, and results are mixed within the Great Basin, with some suggesting that larger seed size is beneficial for survival (Atwater et al., 2015), some suggesting that smaller are better (Kulpa & Leger, 2013), and others showing mixed effects across taxa (Agneray et al., 2022; Leger & Goergen, 2017). Seed size has sometimes been linked to germination speed with smaller seeds germinating faster (Norden et al., 2009), though this is not always the case (Barak et al., 2018). We did not see a predictive pattern of seed weight on germination across all species, but for two species, P. secunda and P. spicata, seed weight was negatively correlated with emergence phenology. Specifically, both contemporary P. secunda groups had lower seed weight, later emergence, and a lower percent emergence than the past group. Pseudoroegneria spicata, on the other hand, had higher seed weight and earlier emergence in the contemporary groups relative to the past groups.

Chapter 2:

Detecting non-target herbicide effects on native Great Basin plants

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Abstract

The Great Basin has seen rapid transformation over the past century, with extensive degradation from invasive species, particularly invasive annual grasses. Herbicides with pre-emergent capabilities can be an effective tool to reduce the abundance of invasive annual plants. However, they also have the potential to harm the native plant community, particularly native annual plants or perennial seedlings. In this study, we assessed the effects of imazapic and indaziflam on target and non-target plants in 1) a natural, invaded field setting, 2) an agricultural field used to grow native annual forbs, and 3) on perennial grass *Elymus elymoides* at various planting depths. The natural field study involved seed bank and plant surveys in aerially treated plots (each herbicide alone or in combination) and controls, and the agricultural field study monitored above-ground responses of the seed bank and planted *E. elymoides* seeds to individual and combined herbicide treatments at two application rates. Imazapic and combination treatment reduced non-native plants, including *Bromus tectorum* and *Ceratocephala testiculata* in our natural field experiment, which is notable as these species increased greatly in control plots. Indaziflam treatments prevented non-native plants from increasing, but did not substantially reduce their density. The combination and imazapic treatments also benefited the native plant community, the majority of which was perennial, with 85% and 49% increases in density, respectively. Combination treatments were especially beneficial for native perennial grasses. Indaziflam treatments on their own did not appear to benefit the native plant community, and may have reduced growth in native

perennial forbs, likely because it did not provide as strong non-native control as imazapic and combination. The seed bank experiment revealed suppression of non-native species that were present in the seed bank but not aboveground. However, the agricultural field experiment revealed extensive harm to native annual forbs, although there were offsets at lower application rates, and some species were less susceptible than others. We also saw that herbicides, particularly indaziflam, reduced *E. elymoides* emergence, but that planting seeds at 2-3 cm instead of the recommended 1 cm helped offset these negative effects, particularly for imazapic, with seeds planted at 2 or 3 cm having 15-68% greater emergence than seeds planted at 1 cm. We suggest that land managers survey for native annual forbs before applying herbicides, and make efforts to reduce harm to this functional group, as they are important components of Great Basin ecosystems. We also suggest planting *E. elymoides* at 2-3 cm when applying herbicides, and this could also be effective for other perennial grasses, and note that the combination of these two herbicides with potentially lower application rates may have promise for balancing weed control with promoting native diversity in sagebrush systems.

Introduction

The Great Basin has been dramatically transformed by cheatgrass (*Bromus tectorum*)—an invasive annual grass that is replacing native plants by prolifically producing seeds with high germination rates, resulting in many highly competitive plants that can reduce soil moisture, and copious litter than can increase the incidence of wildfire (Bradley et al., 2018; Knapp, 1996; Whisenant, 1990). Great Basin historically has had an infrequent fire return interval of 60-100 years, but the cheatgrass invasion has increased it, to less than five years in the most extreme cases (Whisenant, 1990). With repeated wildfires, native shrublands and grasslands are converted into near monocultures of cheatgrass and other invasive species to the detriment of native plants and the wildlife that depend on them (Crawford et al., 2004; Knapp, 1996; Melgoza et al., 1990). This ecosystem degradation is especially problematic for wildlife, including the greater sage-grouse (*Centrocercus urophasianus*), a candidate for protection under the Endangered Species Act (Crawford et al., 2004). Greater sage-grouse rely on native grasses and shrubs for nesting, and native forbs are an essential food source, especially during the breeding season (Crawford et al., 2004; Smith et al., 2019). Given the devastating effects of cheatgrass invasion, it is imperative to find methods to reduce its presence and increase the abundance of native plants, including forbs.

Herbicides are one of the best tools land managers have to combat cheatgrass (Brisbin et al., 2013; Mangold et al., 2013). Imazapic can be applied as a pre- or post-emergent herbicide and inhibits the acetohydroxyacid synthase enzyme that is responsible for producing several amino acids that are necessary for cellular development in plants (Stidham, 1991; Stidham & Shaner, 1990). Imazapic has been the primary herbicide used for cheatgrass treatment since its release in 1996, but it does not stay in the soil long enough to provide more than one year of cheatgrass control (Mangold et al., 2013; Owen et al., 2011; Sebastian et al., 2016; Sheley et al., 2007;

Wallace & Prather, 2016). Indaziflam is a pre-emergent herbicide and cellulose biosynthesis inhibitor that targets seeds and seedlings by inhibiting root elongation (Sebastian, Fleming, et al., 2017). Indaziflam was approved for wildland use in 2016 and rangeland use in 2020 and may be a promising way to suppress invasive annual species for longer periods of time than imazapic. Indaziflam can suppress cheatgrass germination for three or more years, which is needed for long-term cheatgrass control (Clark et al., 2020; Sebastian et al., 2016; Sebastian, Nissen, Sebastian, & Beck, 2017; Sebastian, Nissen, Sebastian, Meiman, et al., 2017). While indaziflam may be superior to imazapic in controlling cheatgrass for long periods of time, land managers must also consider that this longevity in the soil may also have negative, non-target effects on desirable species (Olszyk et al., 2004; Strandberg et al., 2017).

Studies on the non-target effects of imazapic and indaziflam have found mixed and sometimes contradictory effects, and their capacity to help or harm non-target plants may depend on life history and life stage. Imazapic can benefit non-target established perennial plants (Bahm et al., 2011; Barnes, 2007; Davies et al., 2022; Ehlert et al., 2019; Kyser et al., 2007, 2013; Link & Hill, n.d.; Monaco et al., 2005; Sheley et al., 2012) and annual or seeded plants (Ehlert et al., 2019; Sheley et al., 2012) through reduced competition with non-native species. However, imazapic can also cause injury to perennials (Baker et al., 2009; Pyke et al., 2014) and annuals or seeded plants (Brisbin et al., 2013; Burnett & Mealor, 2015; Pyke et al., 2014). Indaziflam can also benefit non-target established perennial plants (Clark, 2020; Clark et al., 2020; J. S. Courkamp et al., 2022; Koby et al., 2019; Sebastian, Fleming, et al., 2017) in addition to seeded plants (Clark et al., 2020). However, indaziflam also can harm non-target vegetation, especially annual plants that germinate and establish in indaziflam's most active zone (J. S. Courkamp et al., 2022; Meyer-Morey et al., 2021; Terry et al., 2021). Given the range of effects imazapic and indaziflam have on non-target vegetation, it is important to evaluate how various native species respond to each herbicide on different functional groups and under different environmental conditions.

In addition to herbicide applications, land managers often seed native species because existing seed banks can be sparse in disturbed areas in the Great Basin (S. Barga & Leger, 2018; Humphrey & Schupp, 2001). However, imazapic and indaziflam can harm seeded species in addition to cheatgrass, as pre-emergents prevent seeds from germinating (Sbatella et al., 2011; Shinn & Thill, 2004; Terry et al., 2021). The concentration of imazapic (Neto et al., 2017) and indaziflam (Clark, 2020; J. Courkamp & Meiman, 2021; González-Delgado & Shukla, 2020) decreases with soil depth after it is applied on the surface, so deeper seeds may be less affected by the herbicides than shallower seeds. For example, indaziflam has been found to reduce the density of native annual seeds and overall seed bank richness, but the seed bank deeper than 1 cm is affected to a lesser degree (J. Courkamp & Meiman, 2021). In Great Basin restoration, desirable species are usually seeded within 0-2.5 cm (e.g. Ott et al. 2016), which may place some seeds within the most active zone of herbicides. If seeds planted deeper are less affected by herbicides than shallow seeds, then land managers could potentially plant desirable species deeper than recommended to offset non-target herbicide effects, though experiments should be conducted to identify the maximum planting depth for different species.

Here, our objectives are to examine the impacts of imazapic and indaziflam on cheatgrass and non-target vegetation and to search for a “Goldilocks” depth at which seeds of native perennial grasses can escape from herbicides but still emerge from the soil. We conducted three experiments to investigate these effects, including: (1) imazapic and indaziflam applied to a cheatgrass-invaded site in Elko County, Nevada, using large-scale aerial applications. We collected pre-application seed bank samples to uncover the “potential” plant community and compared these results in treated and untreated sites after application to determine how herbicides affect site potential in real-world treatment conditions. (2) In a small-scale experiment, we applied imazapic and indaziflam to an agricultural field in Reno, Nevada that had been used to grow a wide diversity of native annual forbs (de Queiroz et al. 2021) and thus has a native seed bank density and diversity of annual forbs that is difficult to find in natural settings. Several of these species are food sources for the greater sage-grouse (Luna et al., 2018), and several of the species in this seed bank have never been observed after application of imazapic or indaziflam (Table 1). Because this field lacks the litter and living plants typically found in field sites, we applied both full and reduced rates to approximate the amount of herbicide that would reach the soil if it was intercepted by annual grass litter (Clark et al., 2019). (3) In the same field, we tested the effects of imazapic and indaziflam on seed depth by planting seeds of *Elymus elymoides* (squirreltail), a desirable perennial grass, at five depths.

We asked the following questions:

1. What are the effects of imazapic, indaziflam, and their combination on the emergence of target non-native species and non-target native species in a natural field setting?
2. What are the effects of imazapic, indaziflam, and their combination on the emergence of native annual forbs and non-native weeds in an agricultural field setting?
    a) How do the effects of these herbicide applications differ at full and reduced rates?
3. How does planting depth of *E. elymoides* affect emergence in each of our herbicide treatments?

We expected that both herbicides would benefit existing non-target vegetation in the natural field site by reducing cheatgrass levels. The reduced cheatgrass would benefit already established perennial plants and may allow some species to emerge from the seed bank that are not present in pre-sprayed or control plots, but are part of the potential plant community represented in the seed bank. Further, we expected that both herbicides would harm native annual plants in our annual forb experiment, but that the reduced rates may offset some of those changes, and that species tolerance may vary. To inform our hypotheses on individual species tolerance, we searched Google Scholar for (imazapic + species name) or (indaziflam + species name) and reviewed all papers that mentioned both the herbicide and the plant species known to grow in the annual forb agricultural field. If herbicide application has been found to increase a species or does not affect it, then we assume that it has some degree of tolerance to that herbicide, but if a species decreases after application, we predict that it will also decline in our study (Table 1). Lastly, the recommended planting depth for *E. elymoides* is 0.6-1.3 cm, but we hypothesized that seeds planted at 1 and 2 cm depths would be affected by herbicides and unable to emerge in plots that receive full treatment. We expected that seeds in the shallowest depths (1 and 2 cm) would be able to emerge in the reduced application plots and in the control plots, while moderate depths (3 and 5 cm) may help seeds avoid being damaged by the herbicides, but they would still be able to emerge despite being deeper than the recommended planting depth. We included an extreme depth (10 cm) but did not expect many of the seeds to emerge, since it is much deeper than the recommended depth for planting.

This project is being conducted in partnership with the U.S. Fish and Wildlife Service (USFWS) and addresses issues of extreme management importance. Specifically, USFWS and other land managers need to understand the impacts of imazapic and indaziflam on non-target vegetation, particularly native annual forbs to protect biodiversity, pollinators, and the greater sage-grouse. Additionally, if we find a “Goldilocks depth” where native seeds can germinate but are not impacted by surface herbicide applications, it could allow for cost and time effective restoration efforts in cheatgrass invaded areas. Lastly, our study will increase our knowledge of the understudied seed banks of the Great Basin.

Methods

Experiment 1: Natural field experiment

This experiment was conducted in a natural field setting in Elko County, Nevada (40.94183, -116.20893). The site is a sagebrush-dominated community with a rich native forb and grass community, but is also heavily invaded with *Bromus tectorum* and *Ceratocephala testiculata*. The site is in the Central Basin and Range Omernik Level III Ecoregion at an elevation of 1700 m with 4 to 15 percent slopes. The 30 year normal (1991-2020) minimum and maximum temperatures at the site are 2.8 º C and 20.5 º C, respectively, and the 30 year normal of total annual precipitation is 128.36 mm (*PRISM Climate Group, Oregon State University*, 2014). The soil is in the Stampete-Short Creek association with 65% Stampeded and similar soils, 20% Short Creek and similar soils, and 15% minor components. Plants in this region typically germinate and/or grow during the cooler, wetter fall, winter, and spring seasons, with senescence or dormancy common over the warm, dry summer months.

Twelve 290 x 560 m plots were arranged in a randomized complete block design, with three replicates per treatment (Figure 1). Herbicides were applied aerially on September 29, 2021. Our treatments included imazapic at 5oz/acre, indaziflam at 6oz/acre, the combination of imazapic and indaziflam (also at 5oz/acre and 6oz/acre, respectively), and unsprayed control plots. The imazapic rate is the maximum allowed for rangeland applications and the indaziflam rate is what is typically used in Nevada (6 oz/acre, Kutosky personal communication 2020). To evaluate the treatments for this study (additional above-ground surveys are ongoing), we established a single transect through the center of each plot before treatment and created six evenly spaced quadrat locations along the transect. Corners of each quadrat were marked with nails, so we could revisit the exact same area pre-and post-treatment. In each quadrat, we recorded percent cover of each functional group and soil surface type (native shrubs, native grasses, native forbs, non-native grasses, non-native forbs, litter, rock, and bare ground), and estimated density with species counts for every species in the quadrat. We collect pre-application data from July 26th-27th in 2021 and post-application data from May 25th-26th in 2022. Despite the monitoring in different months, we were able to identify plants to species in both years and accurately count them for a direct comparison between years; we may, however, have missed the growing season for any early-growing annual species, in both years, as they senesce early and litter does not always remain on-site.

In addition to pre-application data, we collected soil seed bank samples in 2021, to quantify site potential. We took 3 cm-deep soil cores from the center and four corners of each quadrat, including the litter layer, then bulked them together to represent the seed bank in that 1 m^2 quadrat. We processed our soil samples and set up the experiment on January 28th, 2022 in the Valley Road Greenhouse Complex at the University of Nevada, Reno. We set up the seed bank trays using the methods described in Espeland et al., 2010. Briefly, we used a layered approach to keep the soil evenly moist, by laying quilt batting on top of the tables, filling trays (Garland Products mini seed trays) with 4 mm of perlite, then placing a layer of landscaping fabric between the perlite and soil sample. We sieved samples through a ½ cm screen to remove large rocks, then evenly spread 60 mL of soil on top of the perlite and landscape fabric. The trays were arranged in a randomized design on two tables. We watered the samples every other day to keep the soil consistently moist, in a greenhouse that was allowed to fluctuate with ambient conditions, with the greenhouse controls set to bound the temperatures between 2 and 20 degrees C in the winter and early spring, and up to 25 degrees C in daytime the late spring and summer. We recorded the emergence of all vascular plants within each tray. We assigned all plants that emerged a number ID, then once they were large enough to identify genus or species, we recorded which taxa corresponded with which number ID. Several species did not survive these transplants, and a few never developed enough to identify to species, leaving us with five unknown forbs. Once plants were identified to species, we removed them from the soil sample. We recorded emergence five times in the first 30 days (January 6th-22nd, 2022), then bimonthly in the following months. Once there had been two weeks without any new seedling emergence (June 2nd, 2022), we allowed the sample to dry out for 30 days, then began watering the samples again. After two weeks into the second wetting treatment no plants had emerged, so we ended the experiment.

Experiment 2: Annual forb experiment

We applied herbicides to a tilled agricultural field at the University of Nevada, Reno Valley Road Field Station on October 19th, 2020, with treatments similar to the field experiment. Annual forbs have been grown in this field since 2016 (de Queiroz et al., 2021), creating a seed bank of native and introduced species that emerge annually. We applied six herbicide treatments and one non-treated control in 12m by 1m plots in a complete randomized design with four replications each (Figure 2). Our six treatments included imazapic (5 oz/acre), imazapic (2.5 oz/acre), indaziflam (6 oz/acre), indaziflam (4.5 oz/acre), imazapic + indaziflam (full rates of each), imazapic + indaziflam (reduced rates of each). The full rates match the treatments in the field design (Experiment 1), and reduced rates approximate the amount of herbicide that reached the soil in an experiment where herbicides were applied with annual grass litter in Colorado, allowing us to mimic likely conditions in natural field settings (Clark et al., 2019). We recorded whole-plot presence absence data for all plant species that emerged, and identified and counted seedlings in four half-meter square quadrats per plot to measure density. We recorded these data monthly and grouped them into one year after treatment and two years after treatment. The first year runs from March 2020 through August 2020, and the second year runs from November 2021 to August

2022. Year two monitoring started earlier than year 1 because the fields were plowed in October before herbicide treatments, so it took longer for plants to re-establish in year 1 than year 2.

Experiment 3: Planting depth

We planted seeds of a desirable perennial grass, *E. elymoides*, at 10 cm, 5 cm, 3 cm, 2 cm, and 1 cm to examine emergence after herbicide application. We placed three 0.3 m^2 plots in each of the plots in our annual forb experiment before herbicide application (Experiment 2, Figure 2). In the depth plots, four seeds per depth arranged in rows from 10 cm to 1 cm, and the aspect of the plot was randomly chosen from the four cardinal directions. Following modified protocols (Espeland & Richardson, 2015) to achieve precision depths, we glued seeds to toothpicks with Elmer's glue at standard depths, drilled into the soil to make a hole for the toothpick, then placed the toothpick into the soil with 1 cm showing on the top. Since Great Basin soils have been known to push out toothpicks due to the freeze thaw cycle, especially without litter cover (Elizabeth Leger, personal communication 2020), we attached mesh on top of the toothpicks with ground staples to prevent them from being pushed out of the soil. When the seeds began germinating in late February, the mesh was removed. Several mesh treatments were disturbed by the wind during the experiment, and some toothpicks heaved out of the ground despite the mesh, so these plots and individual seeds were not included in the analysis of emergence and survival. We recorded emergence data weekly from February through April, then stopped after no plants had emerged for over two weeks. We then recorded mortality data in August 2021.

Overall data analysis

All data were analyzed with linear models or linear mixed models with R version 4.1.3 (R Core Team, 2021) with the "tidyverse" package (Wickham et al., 2019) and figures were made with the R package "ggplot2" (Wickham, 2016). We used the glm function to specify a poisson distribution with a log link for count data, a gaussian distribution for positive continuous data, and a binomial distribution for binary data, and assessed the distribution of residuals visually through Q-Q plots and histograms. The test statistics and P values were calculated using the R package "car" (Weisberg, 2019) and significance was based on a $P<0.05$ criteria for analyses, but we also present non-significant data for interpretation for some metrics. For example, several of the individual species had patchy distributions among sites, and we could not achieve normality of residuals, so instead of presenting the results of analysis for these species, we simply present values of change over time in each treatment type.

Analysis for experiment 1: Natural field experiment

For above-ground data, linear models were used to assess differences in a diversity metric, percent cover, or density among treatments, years, and the interaction between treatment and year, with block included as a random factor. We also included observer as a random factor for density and percent cover analyses as a random factor to account for differences in how each person collected data. We used the R package "lme4" to run the mixed models (Bates et al., 2015). We calculated diversity metrics (richness, Shannon's Diversity, and Simpson's diversity) for each treatment with the R package "HillR" (Li, 2018). We also visualized community dynamics before and after herbicide treatment with an NMDS analysis on presence/absence data using the R package vegan (Oksanen et al., 2022), but as there were no clear clusters of plant groups by treatment, we don't present that analysis. For density, we grouped and analyzed data by habit (grass, shrub, etc.), life history (annual, perennial) and native status, and our groups included: non-native species, native species, native shrubs, native grasses, native annual grasses, native perennial grasses, native forbs, native annual forbs, and native perennial forbs. We also analyzed the density of individual species if there were individuals present in all plots before treatment, as well as the density of two families that had abundant species. Specifically, we analyzed density of non-natives (*B. tectorum* and *C. testiculata),* natives grasses (*E. elymoides, Poa secunda,* and *Vulpia microstachys),* native forbs (*Crepis accuminata, Ionactis alpina, Phlox longifolia*)*,* and the families Asteraceae and Polemoniaceae. For percent cover, we analyzed native shrubs, non-native species, non-native grasses (the only non-native grass at the sites was *B. tectorum*), non-native forbs, native species, native grass, native forb, litter, and bare ground. We counted *Crepis occidentalis* and *Mertensia oblongifolia* in both our annual and perennial analysis since their life history can vary.

To describe the differences between site potential (seed bank) and the above-ground plant community after treatment, we calculated the number of "mismatches" for each quadrat, which is when a species was present in the seed bank for a given quadrat, but was not in the aboveground community of that quadrat after treatment. We analyzed these mismatches with generalized mixed linear models with treatment as a fixed factor, using a poisson distribution. We included the treatment block as a random effect (Figure 1). We analyzed the sum of the mismatches for all species present, all native species, non-native species, and for the most abundant individual species. The only individual species that had mismatches that were significantly related to treatment was *C. testiculata*, so we only present results for this species on its own.

Analysis for experiment 2: Annual forb experiment

Using the data from the agricultural field treatments, linear models were used to assess differences in diversity metric or density among treatments, years, and the interaction between treatment and year. For species that were present only in one year, we did not analyze year or the year by treatment interaction. To visualize community dynamics of each herbicide treatment, we performed an NMDS analysis on total plot presence/absence data for each species, using the R packages vegan (Oksanen et al., 2022), but there were no clear clusters of plant groups by treatments so we don’t present this analysis. We also calculated diversity metrics (richness, Shannon’s Diversity, and Simpson’s diversity) for each of the treatments. We analyzed the effects of treatment on the maximum density of plants seen at any point of time in each plot (hereafter referred to as maximum density). We analyzed the effects of treatment, year, and their interaction on the maximum density and persistence of native annual forbs and non-native species, as well as the most abundant individual species. We analyzed only native annual forbs because they comprised most of the native species in the quadrats, and they are the focus of this experiment. The results of these persistence measures were nearly identical to maximum density, so we do not present these analyses here. For individual species, we analyzed *Collinsia parviflora, Amsinckia tessellata, Mentzelia veatchiana, Microsteris gracilis, Salsola tragus, Malva neglecta, Descurainia sophia,* and *Lactuca serriola*.

Analysis for experiment 3: Planting depth

We used generalized linear models with a binomial distribution to ask how treatment type (imazapic full, imazapic reduced, indaziflam full, indaziflam reduced, combination full, combination reduced, and control), planting depth (1 cm, 2 cm, 3 cm, 5 cm, and 10 cm) and their interaction affected emergence and mortality of *E. elymoides* seeds, on a per seed basis (0/1). We ran a generalized mixed linear model with a binomial distribution with depth plot within the treatment plot as a fixed effect. We initially ran a model with depth plot as a random effect, but the model failed to converge, likely due to missing plots and missing depths in some plots.

Results

Experiment 1: Natural field experiment

Diversity metrics

We recorded a total of 41 species in our plots in Elko County, Nevada (Appendix 1). Treatment significantly affected species richness (P<0.05), but neither treatment, year, nor the interaction between these factors significantly affected any other diversity metrics (all P>0.071, Appendix 2), however, there were several trends in the diversity indices (Figure 3). Species richness significantly increased in the control, but qualitatively, diversity in the control decreased by the other two metrics. The combination of indaziflam and imazapic was the only treatment that qualitatively increased diversity for all three metrics—species richness, Shannon's Diversity, and Simpson's diversity (Figure 3). In contrast, imazapic was the only treatment to qualitatively reduce richness and diversity across all three metrics.

Non-native species

There were multiple non-native plants in the natural field plots, including *B. tectorum, C. testiculata, Alyssum desertorum, Descurainia sophia, Erodium cicutarium* and *Tragopogon dubius* (Appendix 1). The density and percent cover of non-native species (considered as a whole) responded significantly to treatment and the interaction between treatment and year, and density also differed significantly between years (Table 2). In general, combination and imazapic were slightly more effective at controlling non-native plants than indaziflam alone (Figure 4). Specifically, the density of non-native plants decreased in all herbicide treatments, ranging from a 11% decrease in indaziflam plots, a 51% decrease in imazapic plots, and a 76% decrease in plots with the combination of imazapic and indaziflam (Figure 4a). Similarly, the percent cover of non-native plants decreased in all herbicide treatments, with a 5% decrease in indaziflam plots, 80% decrease in combination plots, and a 89% decrease in imazapic plots (Figure 4b). These decreases were particularly notable because the density of non-native plants increased in the control plots by 472%, and the percent cover of non-native plants increased by 414%. *Bromus tectorum* and *C. testiculata* accounted for most of the increase in non-native plants in the control plots and decrease in treatment plots (Figure 5).

When considering single-species responses in their own analysis, the density and percent cover of *B. tectorum* was significantly affected by treatment and showed significant interactions between treatment and year, and density also differed significantly between years (Table 2). The density of *B. tectorum* decreased by 38% in imazapic plots, and 60% in the combination plots, while actually increasing by 32% in indaziflam plots (Table 3, Figure 5). In contrast, in the control plots, *B. tectorum* increased by 388%. The percent cover of *B. tectorum* also decreased in all treatment plots (Table 3), by 39% in indaziflam plots, 85% in imazapic plots, and 73% in combination plots (Figure 5). Similar to density results, in control plots, the percent cover of *B. tectorum* increased by 294%. The density of *C. testiculata* and the percent cover of all non-native forbs were also significantly affected by treatment and had significant interactions between treatment and year (Table 2). Specifically, the density of *C. testiculata* decreased in all treatment plots, by 78% in indaziflam plots, 61% in imazapic plots, and 100% in the combination plots. In the control plots, the density of *C. testiculata* increased by 582% (Table 3, Figure 5). The percent cover of all non-native forbs followed a similar trend, with combination and indaziflam decreasing by 100%, and imazapic decreasing by 91%, while control plots increased by 100% (Table 3).

Native species

There was a diverse native plant community at our natural field plots, with 31 native species, including 3 native shrubs, 5 perennial grasses, 1 annual grass, 17 perennial forbs, 3 annual forbs, and 2 forbs that can be annual or perennial (Appendix 1). We report results first for all native plants combined, then consider functional groups and individual species to understand which taxa were driving overall responses. The density of native plants as a whole responded significantly to treatment and year, and there was a significant treatment by year interaction (Table 4, Figure 6a). The density of native plants increased in all herbicide treatments, with a 13% increase in indaziflam plots, 49% increase in imazapic plots, and 85% in combination plots, while the density of native plants changed very little in density in control plots, increasing by only 1%. The percent cover of all native plants did not respond significantly to any factors (Table 4), but there were noticeable differences in how percent cover changed over time in each treatment (Table 4, Figure 6b). Qualitatively, the percent cover of native plants increased most in combination plots at 41%, followed by imazapic plots at 39%. The increase in percent cover of native plants was more modest in indaziflam plots and control plots, at 7 and 6%, respectively.

The density of native grasses as a whole (including native annuals and perennials together) responded significantly to treatment, year, and there were significant interactions between treatment and year. The percent cover of native grasses, on the other hand, did not respond significantly to any factors. (Table 4). The density of native grasses increased in all herbicide treatments, by 9% in indaziflam plots, 74% in imazapic plots, and 103% in combination plots. Conversely, the density of native grasses decreased by 31% in control plots (Figure 7a). There were non-significant qualitative differences between treatments, with cover changing by less than 3% for all plots while increasing by 44% in combination plots (Figure 7b). This decrease in the density of native grasses in control plots was mainly due to a decrease in native annual grass *V. microstachys*, which was unevenly distributed across plots, with the majority of individuals in control plots (Figure 8). Treatment and year significantly affected *V. microstachys* density, but there were no significant interactions (Table 4). *Vulpia microstachys* density declined by 75% in control plots, and by 100% in indaziflam and combination plots, although there were very few individuals in the pre-treatment indaziflam and combination plots (Figure 8). Imazapic plots gained *V. microstachys* density, as there were no individuals in the pre-treatment surveys (Figure 8).

The density of native perennial grasses on their own also responded significantly to treatment and year, and there were significant interactions between treatment and year (Table 4). Native perennial grass density increased over time in all plots, and this trend was strongest in the combination plots (Figure 9). Specifically, native perennial grasses increased by 18% in imazapic plots, 21% in indaziflam plots, 38% in control plots, and 109% in combination plots. The large increase in combination plots was driven by increases in *P. secunda, E. elymoides,* and *P. spicata;* the latter was only present in combination plots (Figure 8).

We examined changes in density of the two most common perennial grasses, *E. elymoides* and *P. secunda,* individually. The most abundant grass, *P. secunda,* responded significantly to treatment and year, and there was a significant treatment by year interaction (Table 4). The density of *P. secunda* increased in all treatments, especially in combination plots. Specifically, *P. secunda* density increased by 20% in imazapic, 35% in indaziflam, 58% in control, and 139% in combination plots (Table 3). *Elymus elymoides* did not respond significantly to any factors (Table 4), but it is notable that *E. elymoides* density declined in all plots except for the combination plots (Table 3).

The density and percent cover of native forbs were both significantly affected by treatment, and density also responded significantly to year and had a significant interaction between treatment and year (Table 5). The density of native forbs increased in all plots, and this trend was strongest in the combination plots (Figure 10a) and weakest in the indaziflam plots.

The density of native forbs increased by 17% in indaziflam plots, 32% in imazapic plots, 52% in control plots, and 74% in combination plots. The percent cover of native forbs also increased in all plots, and this trend was strongest in imazapic plots at 97%, followed by combination at 38%, indaziflam at 17%, and control at 12% (Figure 10b). Breaking these trends down by habit, native annual forbs had a significant main effect of treatment, but did not respond significantly to year and there was not a significant treatment by year interaction (Table 5). The density of native perennial forbs responded significantly to treatment, year, and there were significant treatment by year interactions (Table 5). The changes in the density of perennial native forbs over time were essentially the same as the changes for overall native forbs (Figure 11), with again the smallest increase in density in indaziflam plots.

We examined the most common perennial forb species, which included *P. longifolia, I. alpina,* and *C. accuminata* (Figure 12). The density of the most abundant native perennial forb, *P. longifolia*, responded significantly to treatment and there was a significant treatment by year interaction (Table 5, Figure 12). *Phlox longifolia* density increased in all plots, except for control plots, which saw a 32% decrease (Table 3, Figure 12). In contrast, *P. longifolia* density increased in combination plots by 30%, increased by 8% in indaziflam plots, and changed little in imazapic plots (Table 3, Figure 12). *Ionactis alpina* also responded significantly to treatment and year, and had a significant treatment by year interaction (Table 5). This species increased in density in all treatments, by 37% in indaziflam plots, 65% in control, 72% in combination, and 264% in imazapic plots (Table 3, Figure 12). *Crepis accuminata* responded significantly to treatment and year, but did not have a significant treatment by year interaction (Table 5). The density of *C. accuminata* also increased in all treatments, by 27% in indaziflam plots, 38% in combination, 76% in imazapic, and 136% in control plots (Table 3, Figure 12).

Lastly, the herbicides had no effect on the density or percent cover of native shrubs, which included *Artemisia tridentata* and *Chrysothamnus viscidiflorus* (Table 5). All individuals that were present in 2021 were also alive and present in 2022 and percent cover changed by less than 8% in all treatments (data not shown).

Plant families

We also examined the effects of herbicides on the two most common forb families: Asteraceae and Polemoniaceae. Asteraceae was the most common non-grass family, and included *A. tridentata, C. viscidiflorus, Agoseris glauca, Antennaria sp., B. hookeri, B. sagittata, C. accuminata, C. occidentalis, I. alpina, Lactuca serriola, Nothocalais troximoides, Tragopogon dubius*, and an unknown Asteraceae individual, probably in the Lactucae tribe. Asteraceae had significant main effects of treatment and year, and the treatment by year interaction was nearly significant (P<0.06) (Table 5). The density of individuals in Asteraceae increased in all treatments, and this trend was strongest in combination plots and weakest in indaziflam plots (Figure 13a). Specifically, Asteraceae density increased in indaziflam treatments by 25%, by 51% in controls, 77% in imazapic, and by 144% in combination plots. Polemoniaceae included individuals from *Lianthus pungens, M. gracilis,* an unknown *Phlox* species, *P. longifolia,* and *P. hoodii*. The density of Polemoniaceae differed significantly by year, but did not respond significantly to treatment, and there was not a significant treatment by year interaction (Table 5). However, there were potential biological differences in how density changed before and after treatment, with less than a 6% change in imazapic and indaziflam plots, a 30% increase in combination plots, and a 38% increase in control plots (Figure 13b).

Litter and bare ground

Lastly, in addition to plant cover, we also recorded the percent cover of litter and bare ground. The percent of both litter and bare ground was not significantly affected by treatment, year, or the treatment year interaction (data not shown).

Seed bank experiment

Nineteen species emerged in our seed bank experiment in the natural field site (Appendix 3). Five of them were forbs we could not identify to species. Of the identifiable species, there were two species that were present in the seed bank but not in the plots when we monitored them: *Draba verna* and *Juncus sp*. The number of identifiable species that were in the seed bank but not the aboveground community did not significantly differ among plots (Table 6). However, there were differences between treatments, with imazapic plots having 18% more species in the seed bank but not aboveground than control plots, indaziflam 27% more, and combination having 6% less than control plots. The number of native species in the seed bank that weren't above ground also did not significantly differ among treatments (Table 6), with about 1.5- 2 native species present in the seed bank but not above ground for all treatments, including the control (Figure 14). In contrast, when looking at just non-native species, there were significantly more non-native species in the seed bank than the aboveground community (Table 6). Specifically, there was ~1 species in the seed bank that was not above ground for all herbicide plots, but this was not true for control plots (Figure 14). Most of the mismatch between the seed bank and aboveground community for non-native plants was caused by *C. testiculata*, which was over-represented in the seed bank of herbicide plots (Table 6). Specifically, in comparison to control plots, there were 1317% more *C. testiculata* that were in the seed bank but not aboveground in imazapic plots, 1100% in indaziflam plots, and 1236% in combination plots.

Experiment 2: Annual forb experiment

Diversity metrics

Density of native annual forbs can be low in wild seed banks, so we were fortunate to have a former agricultural field to work in where densities of annual forbs were high enough to detect species responses. There were 24 total species that emerged in the plots in the annual forb experiment at Valley Road in Reno, Nevada; 10 native annual forbs (including *Grindelia squarrosa*, whose life history can vary), 1 native perennial grass, 8 non-native annual forbs (including *Malva neglecta* and *Polygonum aviculare* whose life histories can vary), two unknown forbs, and one unknown grass (Appendix 4). For whole plot presence and absence measurements, Richness, Shannon's diversity, and Simpson's diversity all responded significantly to treatment (Table 7), but not to year or the year by treatment interaction. In comparison to control plots, all treatments had 18-51% lower diversity, with the combination plots always having the lowest diversity, and imazapic and indaziflam plots having similar diversity (Figure 15).

Non-native species

There were ten non-native plants growing in the agricultural field, including two annual grasses *(B. tectorum* and *Hordeum murinum*)*,* six annual forbs *(Tragopogon dubius, Descurainia sophia, Lactuca serriola, Salsola tragus, Erodium cicutarium* and *Kochia scoparia,* and*,* and two forbs whose life history can vary but behaved as annuals in our study (*Malva neglecta* and *Polygonum aviculare*) (Appendix 4, Figure 16). We report results first for all non-native plants combined, then describe individual species responses to understand which taxa are driving overall responses. The maximum density (highest number ever observed) responded significantly to treatment and year, and there were significant interactions between treatment and year (Table 8). The control plots had the highest maximum density of non-native species, and all treatment plots had at least 50% fewer non-native plants than the control plots (Figure 17). Specifically, imazapic full had 53% non-native plants than control, imazapic half had 73% less, indaziflam full had 73% less, indaziflam half had 63% less, combination full had 50% less, and combination half had 82% less (Figure 17).

We also analyzed the response of individual non-native species *Salsola tragus, Malva neglecta, Descurainia sophia,* and *Lactuca serriola* (Table 9). The maximum density of *Salsola tragus* was significantly affected by treatment and year and there were significant interactions between treatment and year (Table 8). Compared to control plots, treatment plots had 24-88% lower maximum density of *Salsola tragus*, with imazapic full plots 62% lower, imazapic half 24% lower, indaziflam full 83% lower, indaziflam half 51% lower, combination full 88% lower, and combination half 74% lower (Table 8, Figure 16). The maximum density and persistence of *Malva neglecta* was significantly affected by treatment, and *M. neglecta* was only present in the second year (Table 5). All treatment plots had 29-80% lower maximum density of *M. neglecta* than control plots (Table 9, Figure 16). Specifically, imazapic full had 44% lower, imazapic half had 80% lower, indaziflam full and half had 65% lower, combination full had 29% lower, and combination half had 80% lower density than control (Table 9, Figure 16). The maximum density and persistence of *Descurainia sophia* was significantly affected by treatment and year, and further there were significant interactions between treatment and year (Table 8). All herbicide treatments strongly reduced the amount of *D. sophia* in the plots across both years, with treatment plots having 90-100% lower maximum density than control plots, and effects being stronger in year 2 when *D. sophia* was more abundant (Table 9, Figure 16). Specifically, imazapic full had 92% lower maximum density, imazapic half had 100%, indaziflam full had 90%, indaziflam half had 95%, and the combination plots both had 100% less (Table 9, Figure 16). Treatment significantly affected the maximum density of *Lactuca serriola,* and it was only observed in the second year (Table 8). The maximum density of *L. serriola* was always lower in herbicide treatment plots than in control plots, with treatment plots having 75-100% lower maximum density (Table 9, Figure 16). Imazapic full plots had 95% lower maximum density, imazapic half had 100% lower, indaziflam full had 100% lower, indaziflam half had 75% lower, combination full had 100% lower, and combination half had 95% lower (Table 9, Figure 16).

Annual forbs

There were several native plants growing in the fields (Appendix 4), 7 of which were native annual forbs growing in areas sampled with our survey quadrats (two other annual forb species were present in full plot surveys only; Appendix 4). First we will report results for all native annual forbs combined, then describe individual species responses. The maximum density of all native annual forbs combined responded significantly to treatment, and there were significant interactions between treatment and year (Table 10). The maximum density of native annual forbs in all herbicide treatments was 44 to 94% lower than the control plots (Figure 19). Specifically, imazapic full had 93% lower maximum density, imazapic half had 44% lower, indaziflam full had 84% lower, indaziflam half had 55% lower, combination full had 94% lower, and combination half had 87% lower maximum density (Figure 19).

For individual native species, we analyzed *A. tessellata, C. parviflora, M. veatchiana,* and *M. gracilis*. The maximum density of *A. tessellata* was significantly affected by treatment and year, and there was a significant treatment by year interaction (Table 10). The maximum density of *A. tessellata* was lower in all treatment plots than in control plots averaged across years, except for in imazapic half plots, where it was 159% higher (Table 9, Figure 18). In the other plots, the maximum density of *A. tessellata* was 68% lower than control in imazapic full plots, 88% lower in indaziflam full, 24% lower in indaziflam half, 65% lower in combination full, and 29% lower in combination half (Table 9, Figure 18). Further, these effects were stronger during year 2 when *A. tessellata* was more abundant. The maximum density of *C. parviflora* was significantly affected by treatment (Table 10). The maximum density of *C. parviflora* was 100% lower than control plots for all herbicide treatments except for indaziflam full, which had 95% lower maximum density (Table 9, Figure 18).

The maximum density of *M. veatchiana* was significantly affected by treatment and year, and further there were significant interactions between treatment and year (Table 10). The maximum density of *M. veatchiana* was lower in all treatment plots than in control plots by 94-100% averaged across both years, with the effects strongest during year 1 when it was most abundant (Table 9, Figure 18, Figure 20). The maximum density of *M. gracilis* was significantly affected by treatment and year (Table 10). The maximum density of *M. gracilis* was lower in all treatment plots than in control plots, except for imazapic and indaziflam half-rate plots, which had 87 and 107% higher maximum density, respectively (Table 9, Figure 18). For the other treatments, the maximum density of *M. gracilis* was 87% lower in imazapic full plots,, 60% lower in indaziflam full plots, 100% lower in combination full plots, and 93% lower in combination half plots (Table 9, Figure 18).

Experiment 3: Depth trial

Elymus elymoides emergence in our depth experiment was significantly affected by treatment and depth, and the interaction between treatment and depth was significant (Table 11). The control plots had similar emergence percentages at the 1cm, 2cm, and 3cm depths (Figure 21). For several herbicide treatments, there was greater emergence when seeds were planted at depths slightly deeper than 1cm, specifically at the 2cm or 3cm depths, depending on treatment (Figure 21). Specifically, there were improvements in emergence in depths slightly deeper than 1cm in the full imazapic treatment: the 2cm depths in imazapic full had 54% greater emergence than 1cm, and in imazapic half, there was 20% greater emeregence at 2cm than 1cm. Indaziflam also had offsets at 2-3 cm, with the indaziflam full plots having 32% higher emergence at 3cm than 1cm, and the indaziflam half having 68% greater emergence at 2cm than 1cm. The combination half plots also showed an offset at 3cm greater than the control offset with the 3cm depth having

32% greater emergence than 1cm. Lastly, the combination full plots had a more modest offset that was similar to control plots, specifically, with the 3cm plots having 15% higher emergence than the 1cm plots.

Even in the best-performing depths, emergence was reduced relative to the control for all treatments that contained indaziflam (full, half, and both combinations). This was not the case for imazapic only treatments. Specifically, we observed ~72-76% emergence in control and both imazapic treatments at three shallowest depths (1-3cm) (though the 1cm depths had lower emergence in imazapic than control plots), but full treatments with indaziflam (indaziflam full and combination full) had only 13-16% emergence in the shallowest depths. There were slight improvements in the half treatments that contained indaziflam (indaziflam half and combination half), with emergence in the shallowest depths at 34-35% and indaziflam half 2 cm approaching 50% emergence, but emergence at all depths was consistently lower in plots that contained indaziflam.

Discussion

Herbicides are one of our best tools to help recover the invaded and degraded ecosystems of the Great Basin. Numerous studies have demonstrated the effectiveness of imazapic (Baker et al., 2009; Burnett & Mealor, 2015; Davies et al., 2022; Ehlert et al., 2015; Kyser et al., 2013) and indaziflam (Clark, 2020; Clark et al., 2020; Donaldson & Germino, 2022; Sebastian et al., 2016; Sebastian, Fleming, et al., 2017; Seedorf et al., 2022) at reducing invasive annual grasses, while sometimes also benefiting the native plant community (Bahm et al., 2011; Barnes, 2007; Clark, 2020; Clark et al., 2020; J. S. Courkamp et al., 2022; Davies et al., 2022; Ehlert et al., 2019; Koby et al., 2019; Kyser et al., 2007, 2013; Link & Hill, n.d.; Monaco et al., 2005; Sebastian et al., 2016; Sebastian, Fleming, et al., 2017; Sheley et al., 2012). However, herbicides also have the potential to harm non-target vegetation in the native plant community, and due to their pre-emergent effects on seeds, particularly annual plants (Brisbin et al., 2013; Davies & Sheley, 2011; Ehlert et al., 2019; Elseroad & Rudd, 2011; Meyer-Morey et al., 2021; Pyke et al., 2014) and species seeded near the time of application (Brisbin et al., 2013; Burnett & Mealor, 2015; Terry et al., 2021). We examined the effects of imazapic, indaziflam, and their combination on non-native plants and non-target native plants through 1) a natural field experiment in a *B. tectorum*-invaded site, 2) an annual forb experiment in an agricultural field used to grow native annual forbs, and 3) a planting depth experiment with seeded *E. elymoides*. In our natural field experiment, we found that imazapic and indaziflam were highly effective at reducing invasive annual weeds, including *B. tectorum* and *C. testiculata*. Combination and imazapic treatments also benefited the native plant community, with the combination treatments particularly benefiting native perennial grasses, and while no treatments caused reductions in native perennial forb density and cover, the indaziflam alone treatments reduced perennial forb growth, relative to controls and other treatments. Further, our native annual forb experiment revealed detrimental effects to native annual forbs. These reductions were slightly offset with lower herbicide rates for some species, which may help land managers mitigate effects to native annual forbs with herbicide application. We also saw that herbicides, particularly indaziflam, can reduce the emergence of *E. elymoides* seeds through a depth experiment. However, the negative effects of the herbicides on plant emergence was offset by planting at a slightly deeper than recommended depth (2-3 cm), especially when treated with imazapic. Further, the half-rate plots had better emergence than full-rate, so applying herbicides at a reduced rate may also help the establishment of species seeded alongside herbicide application.

These two herbicides were designed to be effective at suppressing annual weeds, and they did not disappoint. In the natural field experiment, all herbicide treatments were effective at reducing invasive species or keeping them at low numbers, while control plots saw dramatic increases in non-native species in the same timeframe. These increases were driven by *B. tectorum, C. testiculata*, and small amounts of *A. desertorum*, and it appears that imazapic and indaziflam can control these species well. The effectiveness of imazapic and indaziflam against *B. tectorum* has been documented (J. S. Courkamp et al., 2022; Davies et al., 2022; Elseroad & Rudd, 2011; Sebastian et al., 2016; Sebastian, Fleming, et al., 2017) along with the genus *Alyssum* (Meyer-Morey et al., 2021), but this appears to be the first demonstration that these herbicides can reduce *C. testiculata.* The combination plots had the greatest reductions in non-native species density, and indaziflam plots showed the least reductions. Notably, the combination treatment was the only treatment to 100% eradicate *C. testiculata* after treatment. Our seed bank experiment revealed that *C. testiculata* is extremely abundant in the seed bank, so the reduction in *C. testiculata* in the aboveground community after herbicide application is promising for controlling this species. Imazapic and combination plots were more effective at reducing the density and percent cover of all non-native plants and *B. tectorum* than indaziflam, which is surprising given previous results showing that indaziflam tends to provide more invasive species control than imazapic (J. S. Courkamp et al., 2022, p. 2022; Sebastian et al., 2016; Sebastian, Nissen, Sebastian, Meiman, et al., 2017; Terry et al., 2021).

In addition to reducing non-native species, the combination and imazapic treatments in the natural site benefited the native plant community, which was primarily perennial plants. Specifically, the pre- to post-treatment density of native plants increased by 49% for imazapic and 85% for combination treatments. These benefits to the native plant community could be related to how well each herbicide treatment controlled non-native species—combination treatments reduced non-natives the most, followed by imazapic, and these are also the two treatments that had the most positive effects on native perennial plants. Our seed bank experiment revealed that native species observed in the seed bank were also present above ground in all herbicide treatments, which is promising for the long-term diversity of these sites. Maintaining biodiversity after herbicide application is important, as systems with high biodiveristy may be more resilient to disturbances, including invasive species (Folke et al. 2004). In general, treatments affected native plant density, but not percent cover, suggesting that there were greater increases in the number of plants rather than the existing plants becoming larger. Native grasses saw the most benefits from imazapic and combination plots, with their density doubling in combination plots. This is consistent with other studies that have found imazapic can benefit native perennial grasses (Barnes, 2007; Davies, 2010; Davies et al., 2022; Davies & Sheley, 2011; Ehlert et al., 2019; Monaco et al., 2005). Indaziflam treatments alone had mostly neutral effects on native grasses, though the highly effective combination plots contained indaziflam, and there is evidence that under other conditions, indaziflam can benefit native perennial grasses (Clark et al., 2020; J. S. Courkamp et al., 2022; Koby et al., 2019; Sebastian, Fleming, et al., 2017). The combination treatments resulted in the largest increases in the most common perennial grass, *P. secunda*. *Poa secunda* density more than doubled in combination plots but increased more in control plots than imazapic and indaziflam only treatments. This suggests that that *P. secunda* has the potential to be harmed by these herbicides, but that harm may be outweighed by non-native weed control, which was highest in combination plots. Indeed, the effects of imazapic on *P. secunda* in previous studies are mixed, with *P. secunda* increasing after application in some studies (Link & Hill, n.d.; Monaco et al., 2005) while declining in others (Baker et al., 2009; Pyke et al., 2014). *Poa secunda* is known to tolerate disturbance, and can compete with *B. tectorum* (Goergen et al., 2011; Leger et al., 2021), so it is promising that it may have benefited from reduced competition in combination treatments.

While the herbicides benefited native grasses, their effects on native annual grasses were hard to determine. There was only one native annual grass in our study, *V. microstachys*, and unfortunately it was distributed unevenly across plot types, with the majority concentrated in control plots. We did find that *V. microstachys* was abundant in the seed bank in all plots, but the conditions and timing may not have been right for it to be above ground during our surveys. Over the course of our study, *V. microstachys* declined steeply in control plots, while increasing in imazapic plots. Although the treatment x time interaction was not significant for this species, it could be that *V. microstachys* benefited from the reduction of *B. tectorum* and other invasive species in imazapic plots. However, it is difficult to draw conclusions about the effects of imazapic and indaziflam on *V. microstachys* in our study due to low sample sizes and uneven distributions, and there is evidence that it can be negatively affected by imazapic (Ehlert et al., 2019; Elseroad & Rudd, 2011).

In addition to native perennial grasses, we also examined the effects of herbicides on native forbs in our natural field site, the majority of which were perennial. Perennial forbs are important components of sagebrush systems, and studies have found mixed effects on their tolerance to herbicides. Indaziflam has been shown to benefit native perennial forbs in some studies (Clark, 2020; Sebastian, Fleming, et al., 2017) while harming them in others (Meyer-Morey et al., 2021). Similarly, imazapic can benefit (Kyser et al., 2007, 2013) or alternatively harm native perennial forbs (Baker et al., 2009). In our study, native forbs increased in combination and imazapic treatments, with increases similar to those in control treatments. On the other hand, native forbs may have been held back by indaziflam application, as they didn't decrease in density or cover, but did not show growth responses observed in other treatments. *Phlox longifolia* was the most abundant forb in our study, and it increased in combination plots while declining in control plots and persisted in all treatment plots. This is consistent with the observation that congener *P. drummondii* is tolerant to imazapic (Norcini et al., 2003). Asteraceae as a whole, as well as the two most common Asteraceae, *I. alpina* and *C. accuminata*, increased in all treatment types, including control. The increases were strongest in control for *C. accuminata*, in imazapic for *I. alpina*, and combination for the Asteraceae, while indaziflam treatments consistently had the lower pre- to post-treatment increases in density. These results provide tentative support to the hypothesis that members of the Asteraceae may be tolerant of imazapic (Bahm et al., 2011; Barnes, 2007), though mechanisms are unknown. Plants in the Asteraceae may be slightly less tolerant of indaziflam, although indaziflam was present in the combination treatments where they showed the greatest positive responses, possibly because the reduction in annual weeds offset any potential negative effects. Overall, it appears that the native perennial forbs were not reduced in density or cover by any herbicide treatments, and that combination treatments were the most beneficial.

One critical group missing in our field experiment was annual forbs. Annual forbs are an important part of Great Basin plant communities, as they provide food for wildlife, including the Greater sage-grouse (Drut et al., 1994; Luna et al., 2018). Further, they can be strong competitors against *B. tectorum* and are often disturbance-tolerant (Barak et al., 2015; Leger et al., 2014). To help illuminate the effects of imazapic and indaziflam on native annual forbs, we conducted an experiment in a field of native annual forbs that had been grown for seed increase (de Queiroz et al., 2021). There is evidence that imazapic (Davies & Sheley, 2011; Pyke et al., 2014) and indaziflam (Meyer-Morey et al., 2021) can harm native annual forbs, which is concerning given their importance in Great Basin ecosystems. In some cases, however, herbicides may be less harmful to native annual forbs than invasive plants, as they can benefit from herbicide application (Kyser et al., 2013), or recover after a few years (Davies, 2010; Elseroad & Rudd, 2011) in invaded systems. Both herbicides significantly harmed the native annual forbs in our experiments, with herbicide treatments having 44-94% lower native annual forb density than control plots. However, there was variation in susceptibility among species, years, and treatments. The negative effects of herbicides on native plants were less intense in half-rate treatments than full-rate treatments, particularly for imazapic and indaziflam alone. This suggests that lower rates of herbicide application may ameliorate the non-target effects to native annual forbs, although the half-rate treatments still resulted in significant damage to the native plant community.

Several native annual forbs seemed particularly susceptible to imazapic and indaziflam, including *C. parviflora,* which was reduced by at least 95% in all treatment plots, and the half-rate plots did not appear to offset this harm. This is in line with our hypothesis that *C. parviflora* would decline, and with observations that *C. parviflora* is highly susceptible to indaziflam (Meyer-Morey et al., 2021). Also in line with our hypothesis, *M. veatchiana* was reduced in all plots, though there was a slight offset in half-rate plots, especially for indaziflam half-rate plots. This appears to be the first documentation of the effects of imazapic and indaziflam on a *Mentzelia* species. Our other most abundant forbs, *A. tessellata* and *M. gracilis*, were intolerant of full-rate plots, but more tolerant of half-rate plots. Both species actually had higher density in imazapic half-rate plots relative to controls, and *M. gracilis* density was also higher in indaziflam half-rate plots. This is consistent with observations that *A. tessellata* can increase after imazapic application in an invaded field setting (Kyser et al., 2013), and that *M. gracilis* can persist after indaziflam application, although in lower numbers than in control treatments (Meyer-Morey et al., 2021). This further supports our observation that members of the Polemoniaceae family, which includes the genus *Microsteris*, may have some degree of tolerance to imazapic and indaziflam. Overall, our results suggest that native annual forbs are susceptible to imazapic and indaziflam, although there may be offsets at reduced rates for some species, though mechanisms for these differences remain unknown.

Providing further motivation for reduced-rate application, all herbicide treatments had similar effects on non-native plant density, suggesting that half-rate applications were just as effective as full-rate, and in some cases the half-rate plots actually provided superior control to the full-rate plots. Looking at the most abundant non-native species, all herbicide treatments provided strong control of *D. sophia,* and *L. serriola*. This is consistent with observations that *D. sophia* is harmed by, though can persist in low numbers after indaziflam application (Meyer-Morey et al., 2021). On the other hand, the decreases in *L. serriola* were not consistent with existing literature, as Donaldson & Germino observed increases in this species after imazapic and indaziflam application (2022). The herbicide treatments also reduced *M. neglecta* and *S. tragus*, but to a lesser degree, and the level of control varied by treatment. Specifically, half-rate plots surprisingly provided equal or superior control of *M. neglecta*. *Salsola tragus*, on the other hand, was better controlled in full-rate plots, suggesting that it may have a higher degree of tolerance to imazapic and indaziflam than other species, which is supported in the literature for *S. kali* (Elseroad & Rudd, 2011).

Finally, while herbicides can be effective at reducing weeds, active restoration through revegetation and seeding is an important step for preventing their recolonization (Pilliod et al., 2017). Thus, as part of this study on herbicide effects, we included an experiment that allowed us to simultaneously measure the effects of planting depth and herbicide treatment on emergence of an ecologically important restoration species, *E. elymoides*. Overall, the indaziflam and combination plots reduced emergence of *E. elymoides* more than imazapic, with poor emergence in all treatments that contained any amount of indaziflam. This is consistent with observations that *E. elymoides* may be relatively tolerant of imazapic, as individuals can tolerate or benefit from imazapic application in invaded areas (Davies, 2010), and that individuals in the Hordeae tribe may be more tolerant of imazapic than other grasses (Kyser et al., 2007). The half rate plots of indaziflam and combination did have slightly better emergence averaged across all depths (24%) than full rates (9%), but emergence was still considerably lower than optimal rates in control plots (~53%). We observed that planting seeds slightly deeper than the recommended 1 cm, specifically at 2 or 3 cm, may offset the negative effects of imazapic, and of indaziflam at its half rate. This is consistent with the observations that seeds deeper than 1 cm are affected by indaziflam to a lesser degree than shallower seeds (J. Courkamp & Meiman, 2021) and that furrow treatments can mitigate the effects of imazapic with *Pseudoroegneria spicata*, another native perennial grass, though they didn’t mitigate the effects of indaziflam (Terry et al., 2021).

Combining a deeper planting with a treatment like activated carbon protection pods (Clenet et al., 2019, 2020) may allow for even greater success. Our results indicate that there may be ways to seed native species at the same time of herbicide application, and other studies should examine the interaction between depth and herbicide treatment with other commonly seeded native species to see if this trend is consistent across species. If so, then land managers may be able to improve restoration outcomes with a simple fix of planting seeds a little deeper, and possibly at a higher seeding rate to offset any effects of reduced emergence from lower planting depths.

Conclusions

In restoration projects, it is essential to do more good than harm. However, it can be difficult to strike a balance between removing invasive species with herbicide treatments while not damaging the native plant community. Our natural field study reveals that applying herbicides in heavily-invaded areas can benefit perennial native plants, likely through reduced competition with invasive plants, and that a combination of herbicides provided the most consistently positive outcomes. However, our annual forb experiment revealed that imazapic and indaziflam can substantially reduce native annual forbs, which are essential components of Great Basin ecosystems. It is possible that native annual forbs could benefit from reduced competition with invasive species if herbicides were applied in an invaded natural field setting, so future studies should make an effort to survey wild sites while native annual forbs are present, and ideally over multiple years to capture true annual forb diversity. We suggest that land managers also survey with native annual forbs in mind when deciding whether or not to apply herbicides, and if there is an abundant and rich native annual forb community, that they consider methods other than herbicides, consider using reduced rates, or make efforts to increase native annual forbs after spraying. This could be accomplished by collecting seed from native annual forbs on site pre-treatment, as they may already be adapted to competing with the non-native plants that are already at that site. We also suggest that further studies investigate the possibility that planting deeper seeds could offset the negative effects of herbicides, and that land managers planting *E. elymoides* alongside imazapic or indazialam application plant seeds between 2 or 3 cm.

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Tables

Table 1. Species that emerged from the seed bank at the University of Nevada, Reno Valley Road Field Station agricultural fields, what is known about each species in relation to imazapic and indaziflam tolerance, and predictions of their tolerance in our study. Bold species with an asterisk are non-native.

Species	Family	Reactions to imazapic and indaziflam	Predicted reaction to imazapic and indaziflam
Amsinckia tessellata	Boraginaceae	Plots treated with imazapic had 2.5 times more native forb cover, primarily from A. tessellata, three years after spraying (Kyser et al. 2013).	Imazapic and indaziflam will not affect *A. tessellata,* or it may increase.
Blepharipappus scaber	Asteraceae	There are no studies mentioning B. scaber, but plants in Asteraceae may have resistance to imazapic (Barnes 2007).	Imazapic and indaziflam will not affect *B. scaber.*
Collinsia parviflora	Plantaginaceae	*C. parviflora* was observed only in control plots and was not present in indaziflam plots (Meyer-Morey 2021).	Imazapic and indaziflam will reduce *C. parviflora*.
Descurainia sophia*	Brassicaceae	Imazapic treatment reduced cover of introduced forbs, including *D. sophia* (Kyser et al. 2013)	Imazapic and indaziflam will reduce *D. sophia*.
Gilia inconspicua	Polemoniaceae	Unknown	Imazapic and indaziflam will reduce *Gilia inconspicua*.

Layia glandulosa	Asteraceae	There are no studies mentioning *L. glandulosa*, but plants in Asteraceae may have resistance to imazapic (Barnes 2007).	Imazapic and indaziflam will not affect *L. glandulosa* .
Mentzelia veatchiana	Loasaceae	Unknown	Imazapic and indaziflam will reduce *M. veatchiana*.
Microsteris gracilis	Polemoniaceae	*M. gracilis* was present in indaziflam plots as well as control plots (Meyer-Morey 2021).	Imazapic and indaziflam will not affect *M. gracilis*.
Nicotiana attenuata	Solanaceae	Unknown	Imazapic and indaziflam will reduce *N. attenuata*.
Salsola tragus*	Chenopodiaceae	The percent cover of *S. tragus* was similar in control plots and plots treated with imazapic (Barnes et al. 2009)	Imazapic and indaziflam will not affect *S. tragus*.

Table 2. Results from linear mixed models showing the effect of treatment, year, and treatment by year interaction on all non-native species. Block and observer were included as random factors, and data distribution was specified as Poisson for density, or a Guassian distribution with logged values for percent cover. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Response variable	**Factor**	**X^2 value**	**Degrees of freedom**	**P value**
Density of non-native species	Treatment	2597.4	3	**<0.0001**
	Year	371.5	1	**<0.0001**
	Treatment*Year	1714.5	3	**<0.0001**
Percent cover of non-native species[1]	Treatment	34.1	3	**<0.0001**
	Year	0.2	1	0.6642
	Treatment*Year	48.5	3	**<0.0001**
Density of *Bromus tectorum*	Treatment	926.85	3	**<0.0001**
	Year	141.0	1	**<0.0001**
	Treatment*Year	623.8	3	**<0.0001**
Percent cover of *Bromus tectorum*[1]	Treatment	19.8	3	**<0.0001**
	Year	0.2	1	0.6337
	Treatment*Year	27.1	3	**<0.0001**
Density of *Ceratocephala testiculata*	Treatment	594.5	3	**<0.0001**
	Year	312.0	1	**<0.0001**
	Treatment*Year	620.7	3	**<0.0001**
Percent cover of non-native forbs[1]	Treatment	43.1	3	**<0.0001**
	Year	1.7	1	0.1923
	Treatment*Year	43.2	3	**<0.0001**

No subscript indicates that the variable was not transformed.
1 indicates that the variable was log transformed.

Table 3. Percent change in density from pre-treatment (2021) to post-treatment (2022) surveys for individuals species in each treatment. Blue colors indicate positive responses and red are negative responses, with each response type broken down into four colors, the darkest being the most extreme change. An asterisk indicates that the interaction between treatment and year was significant for a given species. The lightest colors indicate a 1-33% change, second lightest is 33-66% change, second darkest is 66-99% change, and the darkest is 100% or greater change, and white is no change.

Species	**Control**	**Imazapic**	**Indaziflam**	**Combination**
*Bromus tectorum**	388	-38	32	-60
*Ceratocephala testiculata**	582	-61	-78	-100
Crepis accuminata	136	76	27	38
Elymus elymoides	-19	-19	-43	32
*Ionactis alpina**	65	264	37	72
*Phlox longifolia**	-32	1	8	30
*Poa secunda**	58	20	35	139

Table 4. Results from linear mixed models showing the effect of treatment, year, and treatment by year interaction on all native species and native grasses. Block and observer were included as random factors, and data distribution was specified as Poisson for density, or a Guassian distribution. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Response variable	**Factor**	**X^2 value**	**Degrees of freedom**	**P value**
Density of all native species	Treatment	63.6	3	**<0.0001**
	Year	45.9	1	**<0.0001**
	Treatment*Year	54.6	3	**<0.0001**
Percent cover of all native species	Treatment	3.7	3	0.3014
	Year	2.8	1	0.0964
	Treatment*Year	1.8	3	0.6165
Density of all native grasses	Treatment	77.5	3	**<0.0001**
	Year	9.9	1	**<0.01**
	Treatment*Year	91.4	3	**<0.0001**
Percent cover of all native grasses	Treatment	6.0	3	0.1114
	Year	0.4	1	0.526
	Treatment*Year	1.5	3	0.6824
Density of *Vulpia microstachys*	Treatment	211.1	3	**<0.0001**
	Year	90.3	1	**<0.0001**
	Treatment*Year	0.0	3	1.0
Density of native perennial grasses	Treatment	34.6	3	**<0.0001**
	Year	35.8	1	**<0.0001**
	Treatment*Year	20.3	3	**<0.0001**

Density of *Elymus elymoides*	Treatment	4.2	3	0.2421
	Year	0.5	1	0.4783
	Treatment*Year	4.0	3	0.2596
Density of *Poa secunda*	Treatment	15.2	3	**<0.01**
	Year	41.3	1	**<0.0001**
	Treatment*Year	24.9	3	**<0.0001**

Table 5. Results from linear mixed models showing the effect of treatment, year, and treatment by year interaction on native forbs and shrubs. Block and observer were included as random factors, and data distribution was specified as Poisson for density, or a Guassian distribution with some variables log transformed for percent cover. Values are X^2 values, degrees of freedom, and P values, with asterisks indicating significance at alpha = 0.05.

Response variable	**Factor**	**X^2 value**	**Degrees of freedom**	**P value**
Density of native forbs	Treatment	13.8	3	**<0.01**
	Year	41.0	1	**<0.0001**
	Treatment*Year	16.9	3	**<0.001**
Percent cover of native forbs$_1$	Treatment	8.6	3	**<0.05**
	Year	2.2	1	0.1388
	Treatment*Year	0.6	3	0.8945
Density of native annual forbs	Treatment	34.7	3	**<0.0001**
	Year	1.5	1	0.2249
	Treatment*Year	5.9	3	0.1152
Density of native perennial forbs	Treatment	11.6	3	**<0.01**
	Year	63	1	**<0.0001**
	Treatment*Year	9.5	3	**<0.05**
Density of *Phlox longifolia*	Treatment	17.8	3	**<0.001**
	Year	0.9	1	0.3500
	Treatment*Year	14.3	3	**<0.01**
Density of *Ionactis alpina*	Treatment	17.8	3	**<0.001**
	Year	29.8	1	**<0.0001**
	Treatment*Year	8.7	3	**<0.05**

Density of *Crepis accuminata*	Treatment	22.8	3	**<0.0001**
	Year	12.7	1	**<0.001**
	Treatment*Year	5.0	3	0.1727
Density of native shrubs	Treatment	1.8	3	0.6260
	Year	0.0	1	0.9855
	Treatment*Year	0.5	3	0.9332
Percent cover of native shrubs$_1$	Treatment	2.9	3	0.4074
	Year	0.0	1	0.9060
	Treatment*Year	0.1	3	0.9861
Density of all Asteraceae species	Treatment	13.4	3	**<0.01**
	Year	52.8	1	**<0.0001**
	Treatment*Year	7.5	3	0.05703
Density of all Polemoniaceae species	Treatment	0.6	3	0.8890
	Year	6.6	1	**<0.01**
	Treatment*Year	5.5	3	0.1378

No subscript indicates that the variable was not transformed.
1 indicates that the variable was log transformed.

Table 6. Results from generalized linear models showing the effect of treatment on the number of species that were present in the seed bank but not aboveground. Block was included as a random factor, and data distribution was specified as Poisson. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Response variable	**Factor**	**X^2 value**	**Degrees of freedom**	**P value**
All species	Treatment	2.6	3	0.4612
Native species	Treatment	3.5	3	0.3227
Non-native species	Treatment	8.9	3	**<0.05**
Ceratocephala testiculata$_2$	Treatment	6.7	3	0.0829

Table 7. Results from linear models showing the effect of treatment, year, and treatment by year interaction on diversity metrics. The data distribution was specified as Gaussian. Values are X^2 values, degrees of freedom with numerator (n) and denominator (d) for F statistics, and P values, with asterisks indicating significance at alpha = 0.05.

Response variable	**Factor**	**X^2 value**	**Degrees of freedom**	**P value**
Richness	Treatment	49.1	6	**<0.0001**
	Year	0.2	1	0.6505
	Treatment*Year	11.5	6	0.0733
Shannon's	Treatment	49.0	6	**<0.0001**
	Year	0.0	1	0.9137
	Treatment*Year	11.6	66, 42	0.0727
Simpson's	Treatment	48.9	66, 42	**<0.0001**
	Year	0.6	11, 42	0.4282
	Treatment*Year	12.0	66, 42	0.0717

Table 8. Results from linear models showing the effect of treatment, year, and treatment by year interaction on the maximum density of non-native species. The data distribution was specified as Poisson. Values are f X^2 values, degrees of freedom, , and P values, with bold font indicating significance at alpha = 0.05.

Maximum density	**Factor**	**Test statistic**	**Degrees of freedom**	**P value**
Non-native species	Treatment	386.9	6	**<0.0001**
	Year	1631.9	1	**<0.0001**
	Treatment*Year	52.3	6	**<0.0001**
Salsola tragus	Treatment	103	6	**<0.0001**
	Year	312.3	1	**<0.0001**
	Treatment*Year	6.5	6	0.3669
Malva neglecta	Treatment	308.3	6	**<0.0001**
Descurainia sophia	Treatment	124.9	6	**<0.0001**
	Year	45.4	1	**<0.0001**
	Treatment*Year	13.2	6	**<0.01**
Lactuca serriola	Treatment	63.0	6	**<0.0001**

Table 9. Percent difference of maximum density between treatment plots and control plots. Blue colors indicate positive responses and red are negative responses, with each response type broken down into four colors, the darkest being the most extreme change. An asterisk indicates that treatment significantly affected the density of each species. The lightest colors indicate a 1-33% difference, second lightest is 33-66% difference, second darkest is 66-99% difference, and the darkest is 100% or greater difference. Note that *M. neglecta* and *S. serriola* do not have analyses for year or year x treatment because they were only present in year 2.

Species	**Imazapic full**	**Imazapic half**	**Indaziflam full**	**Indaziflam half**	**Combination full**	**Combination half**
*Amsinckia tessellata**	-68	159	-88	-24	-65	-29
*Collinsia parviflora**	-100	-100	-95	-100	-100	-100
*Descurania sophia**	-92	-100	-90	-95	-100	-100
*Lactuca serriola**	-95	-100	-100	-75	-100	-95
*Malva neglecta**	-44	-80	-65	-65	-29	-80
*Mentzelia veatchiana**	-98	-95	-82	-63	-99	-96
*Microsteris gracilis**	-87	87	-60	107	-100	-93
*Salsola tragus**	-62	-24	-83	-51	-88	-74

Table 10. Results from linear models showing the effect of treatment, year, and treatment by year interaction on the maximum density and persistence of native annual forbs. The data distribution was specified as Poisson. Values are X^2 values, degrees of freedom, and P values, with bold font indicating significance at alpha = 0.05.

Maximum density	**Factor**	**X^2 value**	**Degrees of freedom**	**P value**
Native annual forbs	Treatment	419.8	6	**<0.0001**
	Year	0.4	1	0.5423
	Treatment*Year	98.2	6	**<0.0001**
Amsinckia tessellata	Treatment	141.0	6	**<0.0001**
	Year	114.2	1	**<0.0001**
	Treatment*Year	20.8	6	**<0.01**
Collinsia parviflora	Treatment	217.3	6	**<0.0001**
	Year	0.6	1	0.4457
	Treatment*Year	5.0	6	0.5495
Mentzelia veatchiana[1]	Treatment	274.6	6	**<0.0001**
	Year	65.6	1	**<0.0001**
	Treatment*Year	15.4	6	**<0.05**
Microsteris gracilis	Treatment	94.6	6	**<0.0001**
	Year	21.2	1	**<0.0001**
	Treatment*Year	9.8	6	0.1358

Table 11. Results from a generalized linear model showing the effect of treatment, depth, and treatment by depth interaction on the number of *E. elymoides* that emerged. The location of the depth plot was specified as a fixed factor and the data distribution was specified as binomial.Values are X^2 values, degrees of freedom, and P values, with asterisks indicating significance at alpha = 0.05.

Response variable	**Factor**	**X^2 value**	**Degrees of freedom (n,d)**	**P value**
Emergence	Depth	409.43	4	**<0.0001**
	Treatment	11.75	6	**<0.0001**
	Treatment*Depth	41.33	24	**<0.05**

Figures

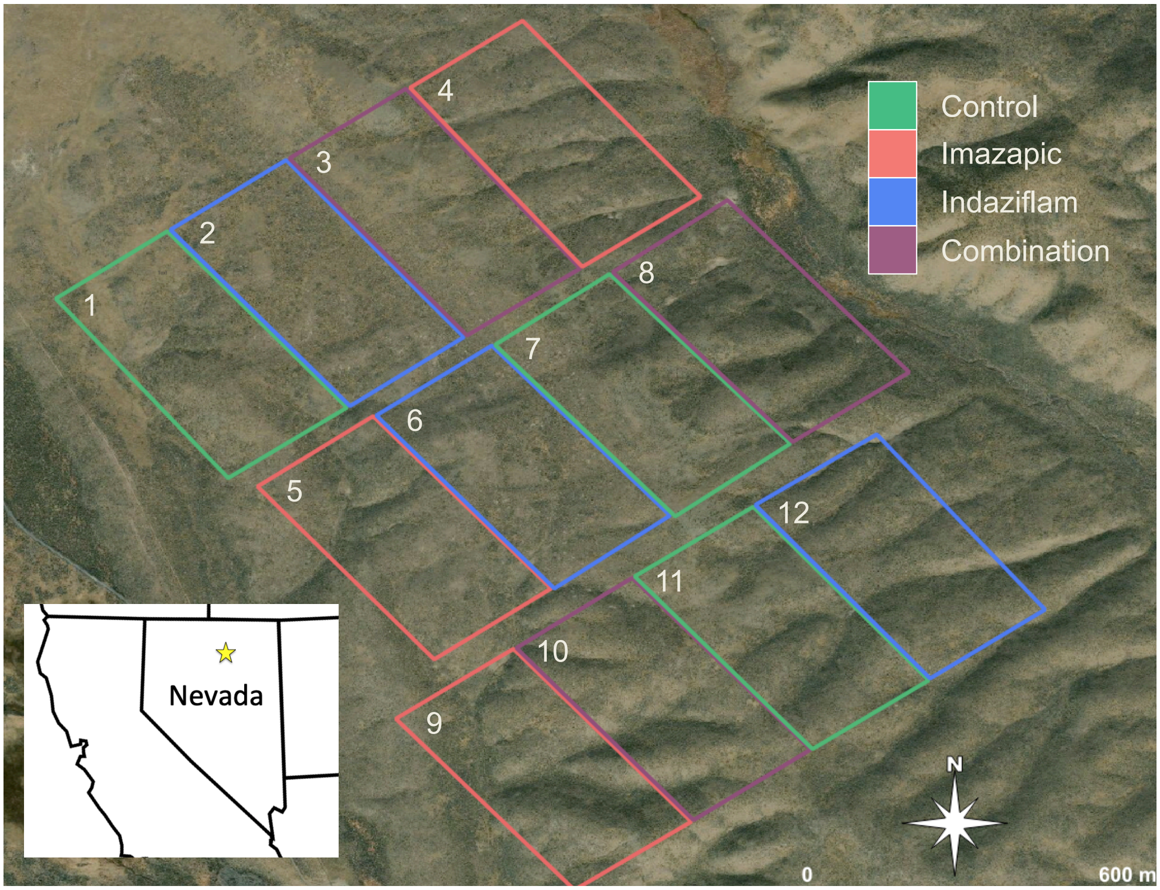

Figure 1. Map of the field plots in Elko County, Nevada. Plots (290m x 560m) were arranged in three blocks, with the northernmost blocks consisting of plots 1-4, the middle 5-8, and the southernmost 9-12. Each treatment was applied once per block: plots 1, 7, and 11 are control plots, plots 4, 5, and 9 are imazapic, plots 2, 6, and 12 are indaziflam, and plots 3, 8, and 10 are indaziflam.

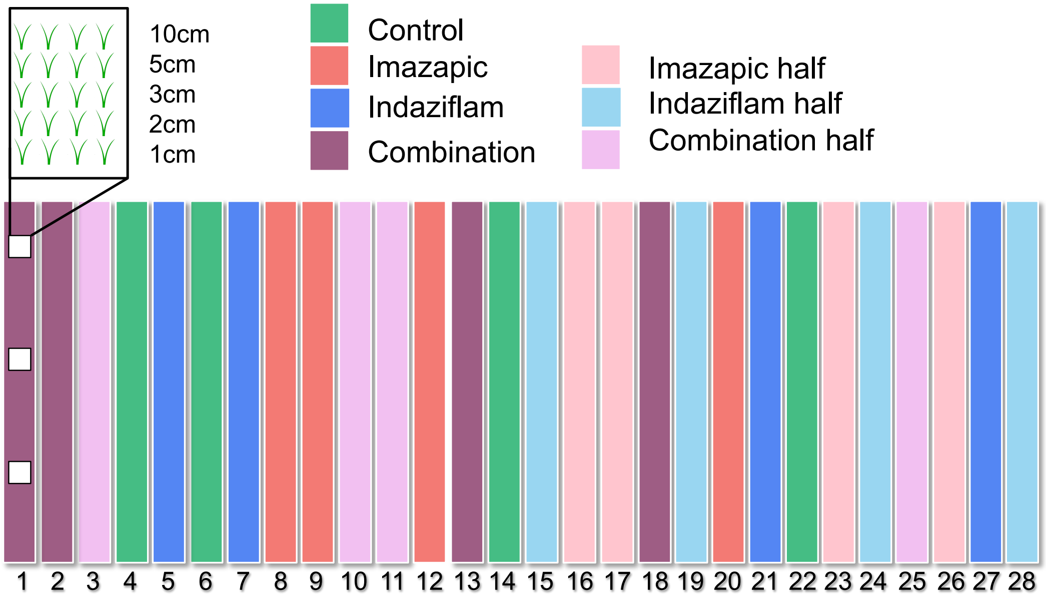

Figure 2. A visual diagram of the herbicide treatments within our experiment. We had 28 plots, 7 treatments, and four replications of each treatment, randomly assigned to a field position. The white rectangles in the left plot represent the three *E. elymoides* seeding depth plots included in every plot. The zoomed-in section shows that each depth plot has five rows of four seeds planted at each depth from deepest to shallowest (10 cm, 5 cm, 3 cm, 2 cm, 1 cm).

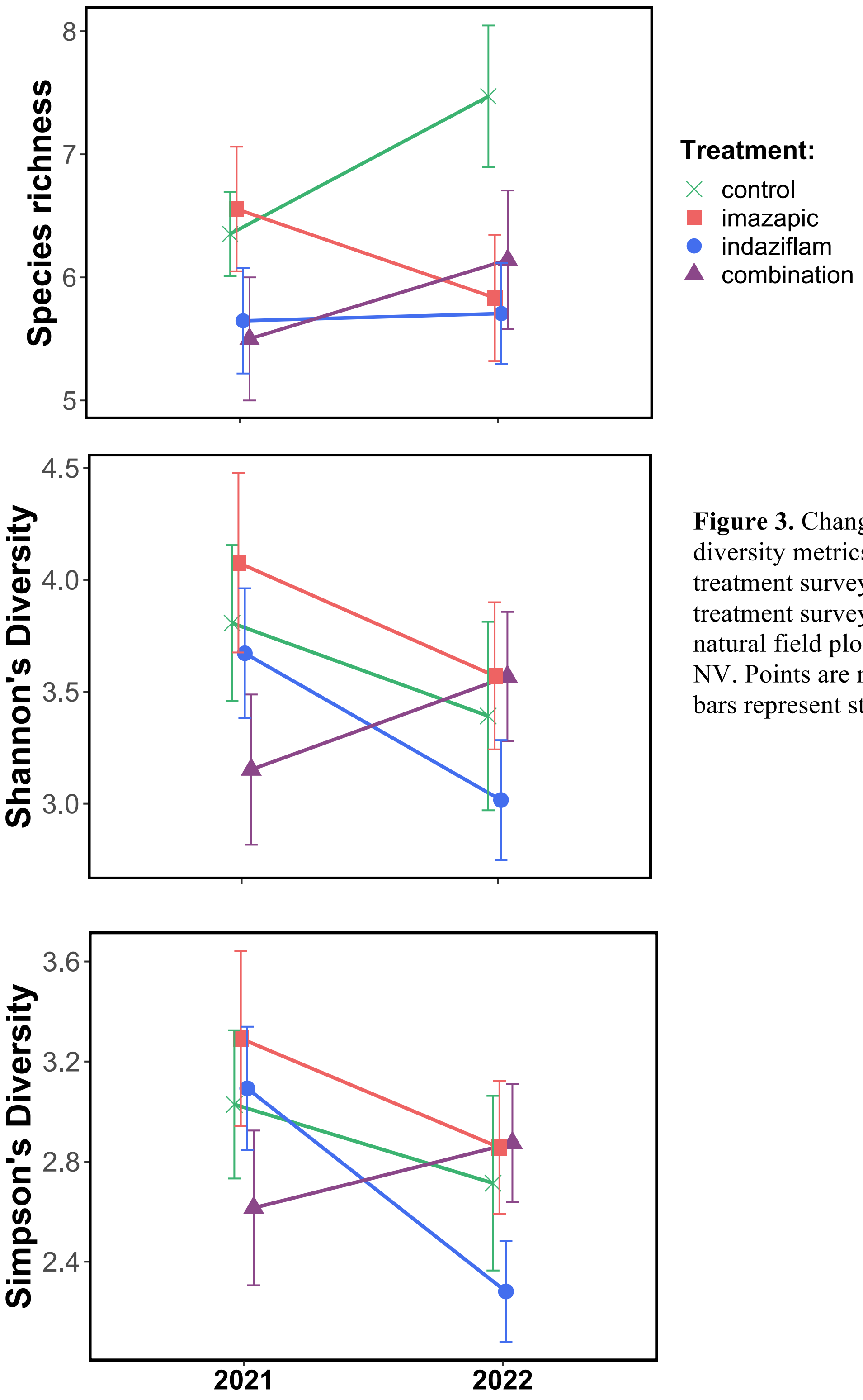

Figure 3. Changes in three diversity metrics from pre-treatment surveys (2021) to post-treatment surveys (2022) in natural field plots in Elko County, NV. Points are means and error bars represent standard error.

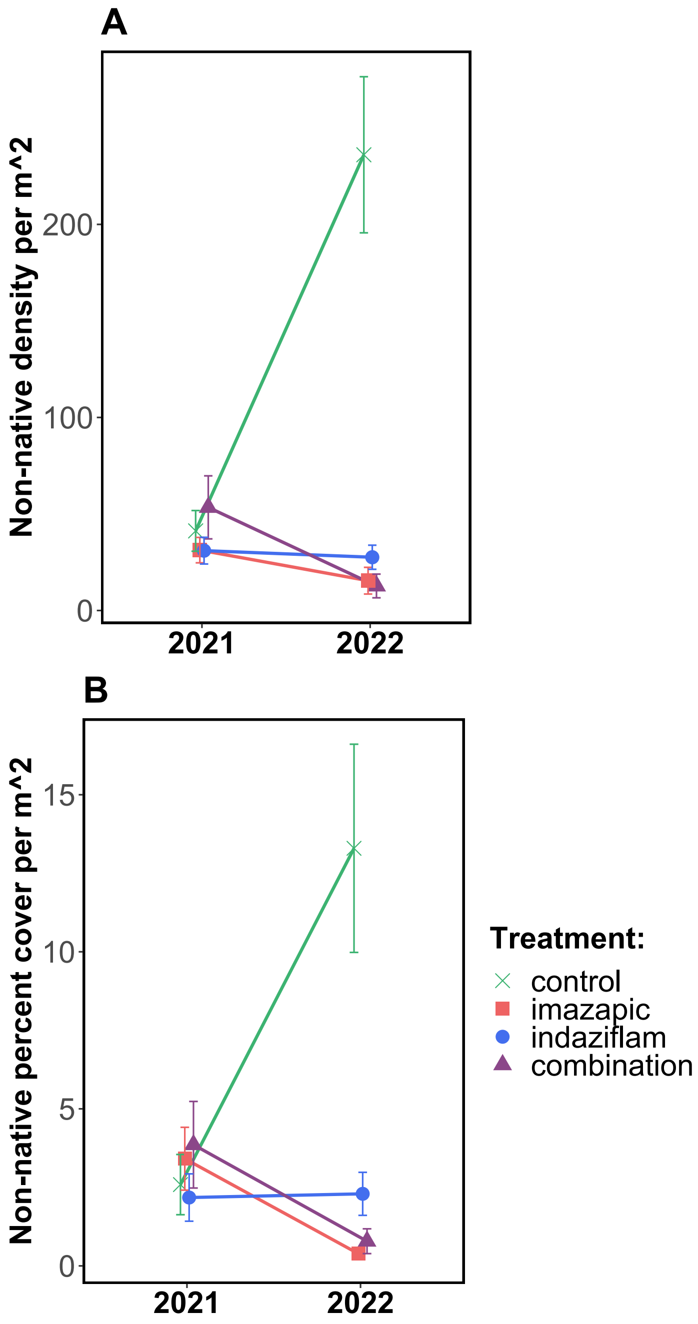

Figure 4. Changes in density (A) and percent cover (B) of all non-native species (considered for all species together) from pretreatment surveys (2021) to post-treatment surveys (2022). Points are means and error bars represent standard error.

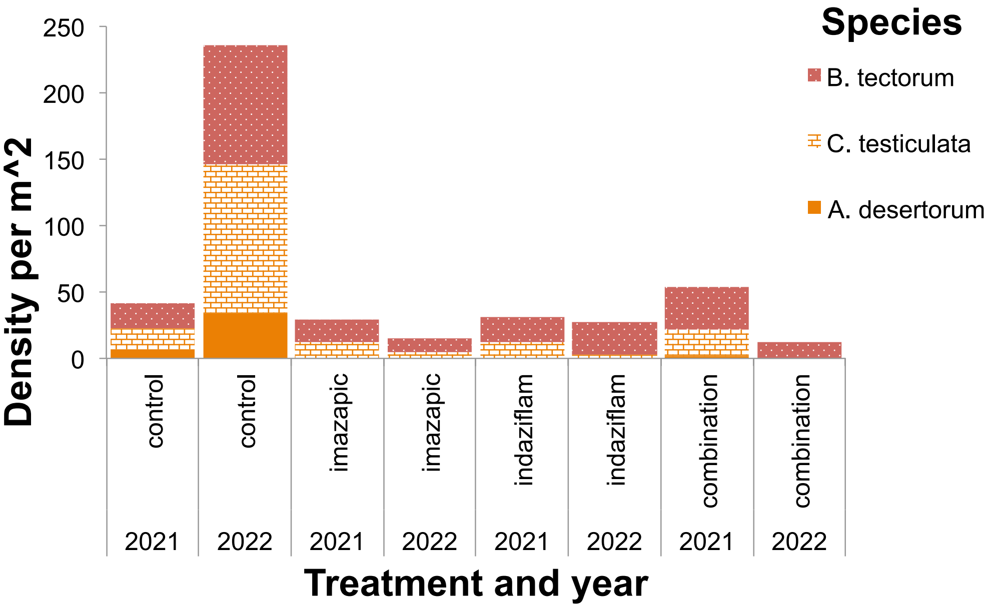

Figure 5. Values are the average density of the three most common non-native species in 1 m^2 quadrat placed within each treatment (control, imazapic, indaziflam, and combination). Each treatment has values for two years: pre-treatment surveys (2021) and post-treatment surveys (2022). The species represented, from top to bottom of the bars, are *Bromus tectorum*, *Ceratocephala testiculata*, and *Alyssum desertorum*. *Descurainia sophia, Tragopogon dubius, and Lactuca serriola* were present as well, but are too small to be visible in the graph, so are not shown.

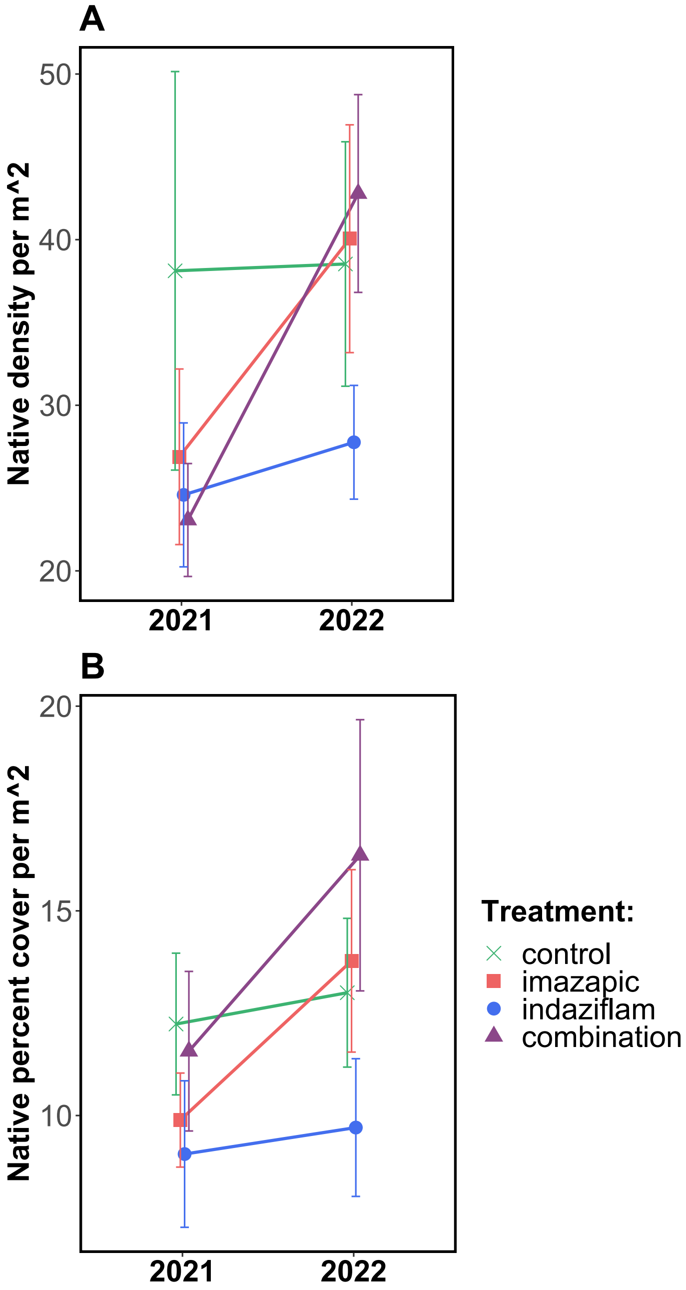

Figure 6. Changes in density (A) and percent cover (B) of all native species (considered for all species together) from pretreatment surveys (2021) to post-treatment surveys (2022). Points are means and error bars represent standard error.

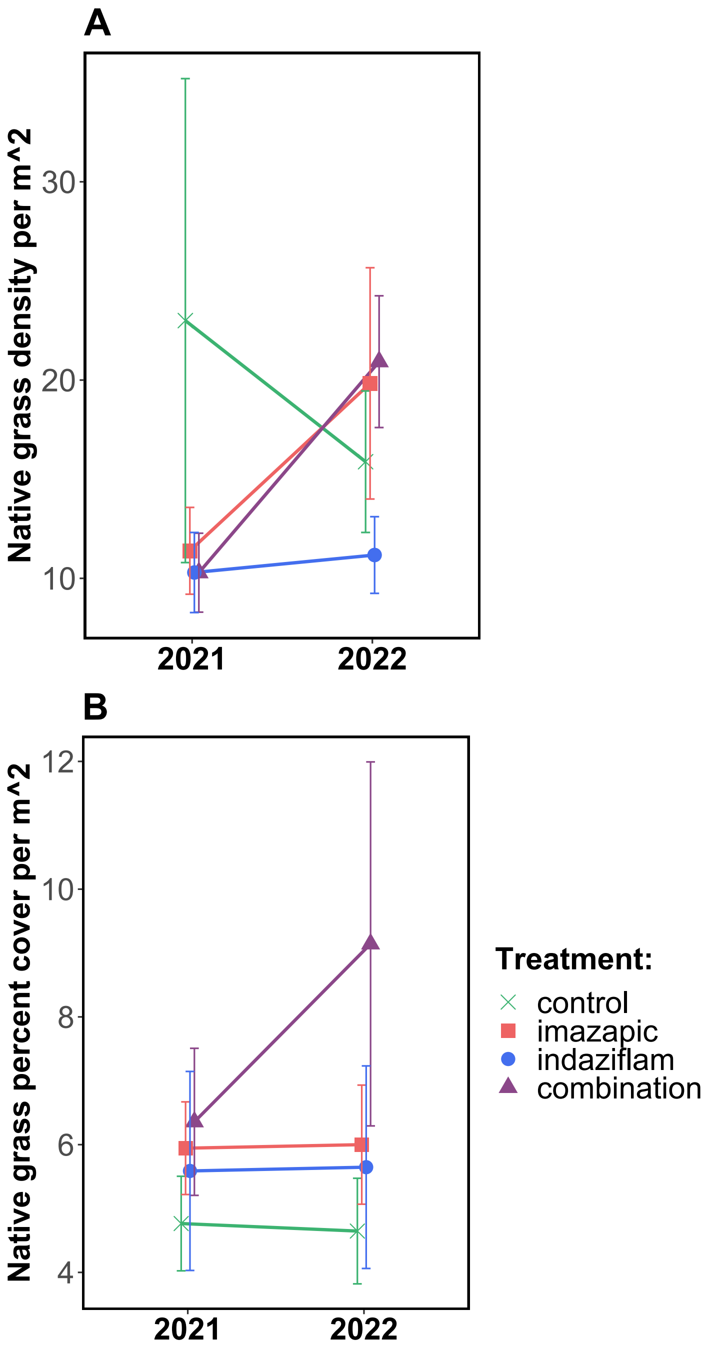

Figure 7. Changes in density (A) and percent cover (B) of all native grasses (considered for all species together) from pretreatment surveys (2021) to post-treatment surveys (2022). Points are means and error bars represent standard error.

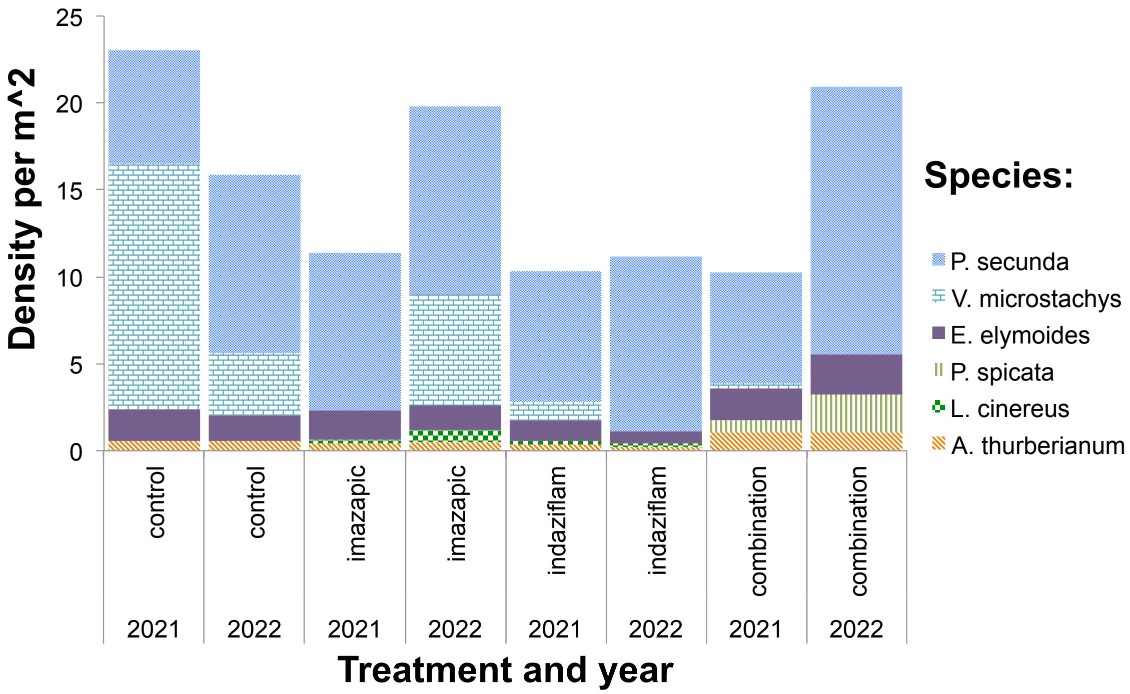

Figure 8. Values are the average density of native grasses in 1 m^2 quadrat placed within each treatment (control, imazapic, indaziflam, and combination). Each treatment has bars for two years: pretreatment surveys (2021) and posttreatment surveys (2022). The species represented, from top to bottom of the bars, are *Poa secunda, Vulpia microstachys, Elymus elymoides, Pseudoroegneria spicata, Leymus cinereus,* and *Achnatherum thuberianum*. Note that *Pseudoroegneria spicata* was only found in combination plots.

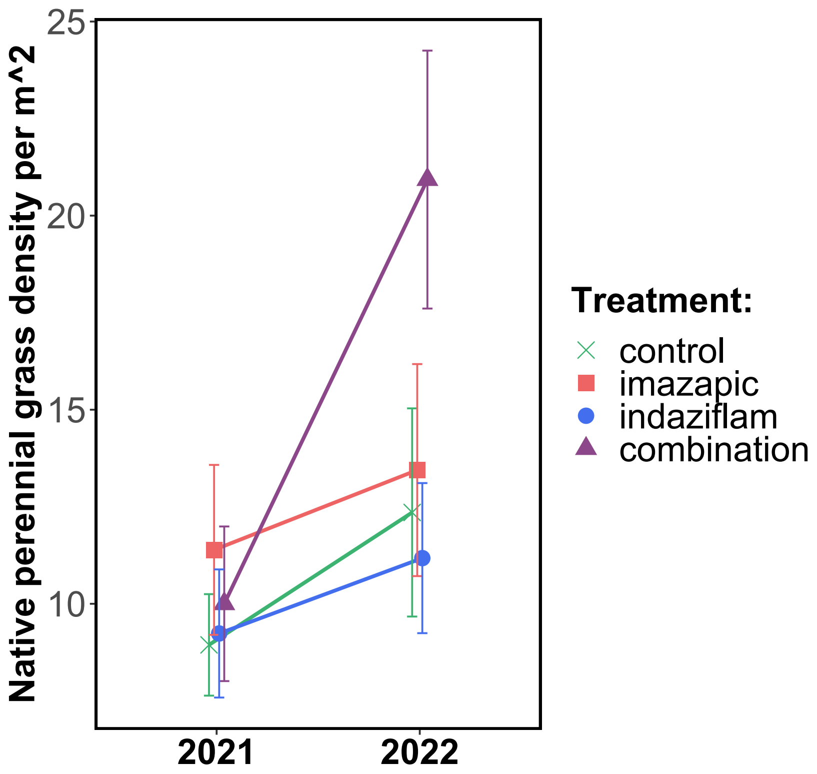

Figure 9. Changes in density of native perennial grasses from pretreatment surveys (2021) to post-treatment surveys (2022). Points are means and error bars represent standard error.

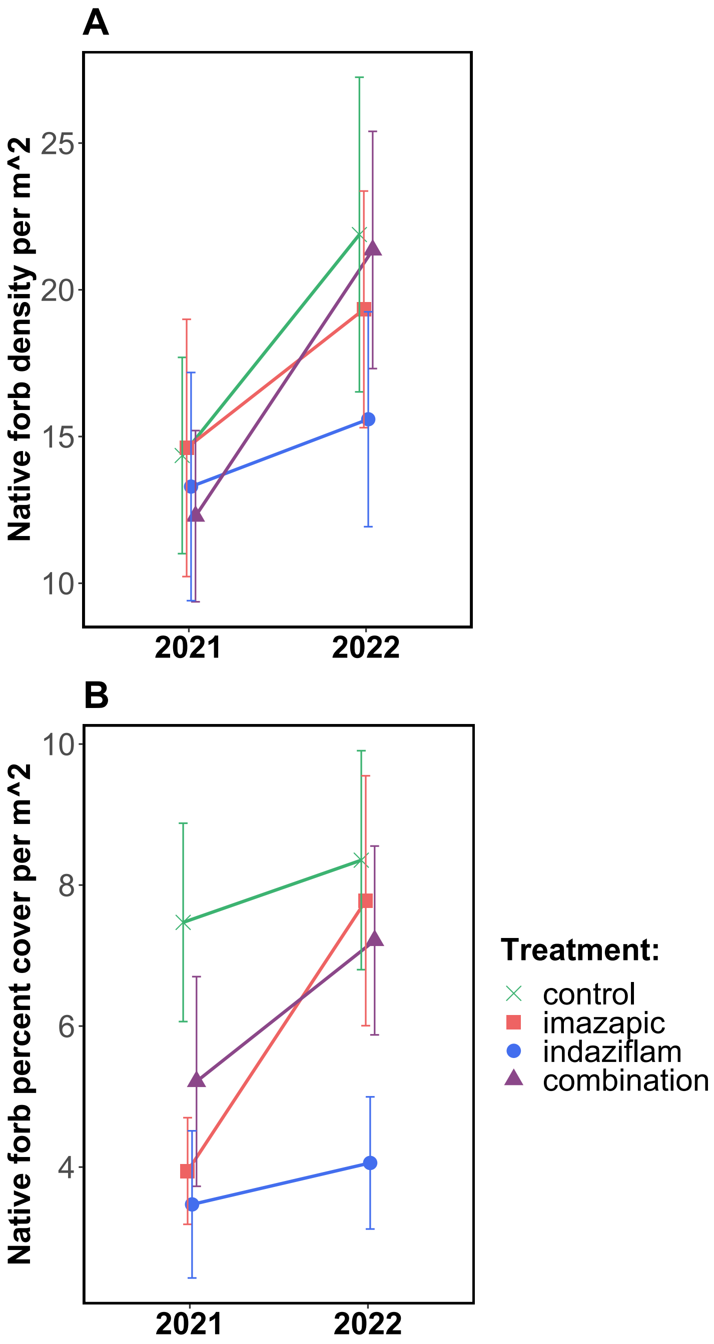

Figure 10. Changes in density (A) and percent cover (B) of all native forbs (considered for all species together) from pretreatment surveys (2021) to post-treatment surveys (2022). Points are means and error bars represent standard error.

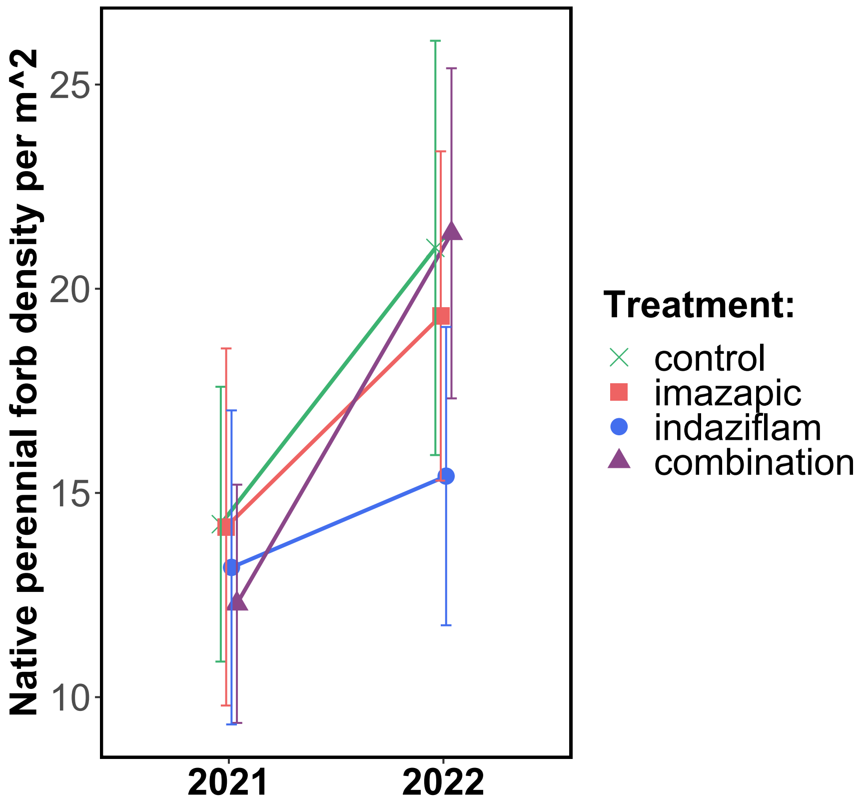

Figure 11. Changes in density of native perennial forbs from pretreatment surveys (2021) to post-treatment surveys (2022). Points are means and error bars represent standard error.

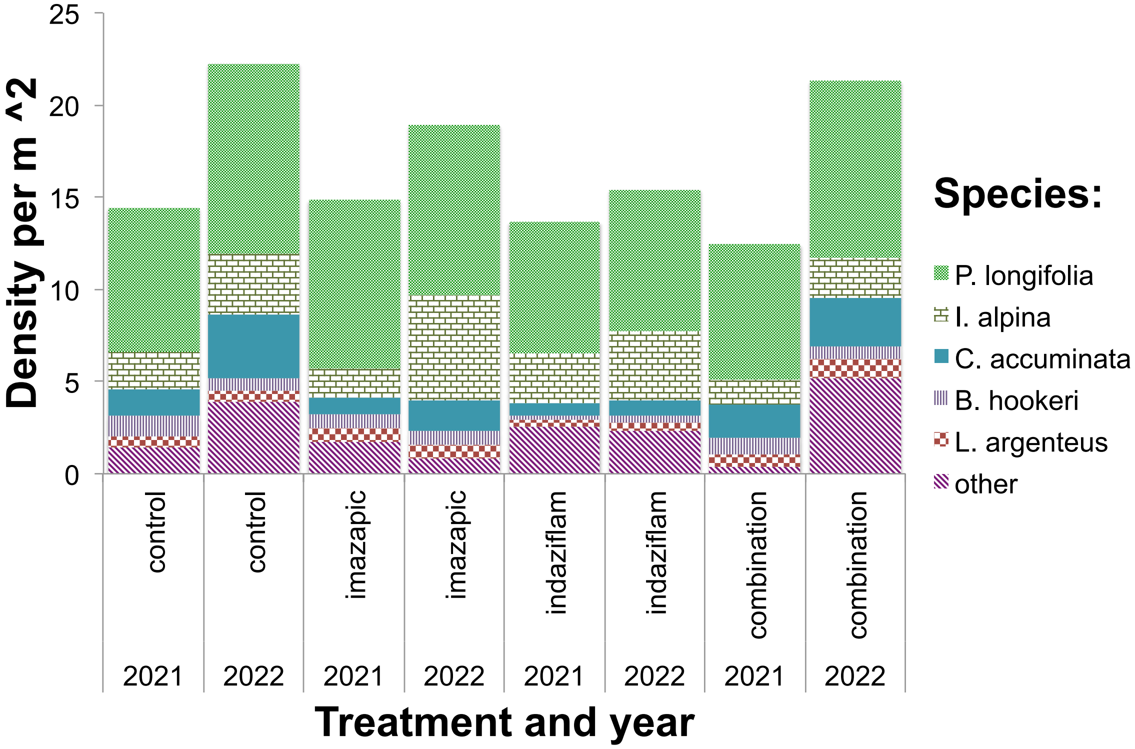

Figure 12. Values are the average density of native forbs in 1 m^2 quadrat placed within each treatment (control, imazapic, indaziflam, and combination). Each treatment has bars for two years: pretreatment surveys (2021) and posttreatment surveys (2022). The species represented, from top to bottom of the bars, are *Phlox longifolia, Ionactis alpina, Crepis accuminata, Balsamhoriza hookeri, Lupinus argenteus*, and a category for other native forbs. The 14 native forbs in the other category can be found in Appendix 1.

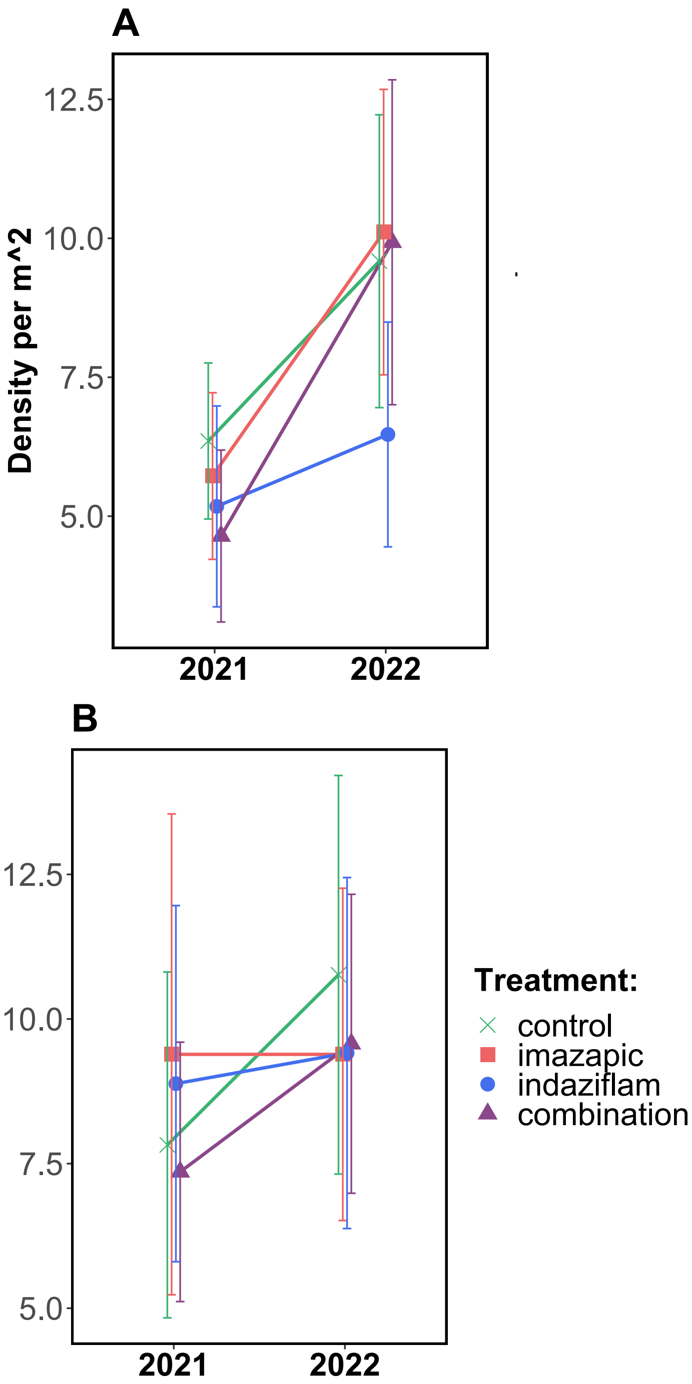

Figure 13. Changes in density of all Asteraceae species (A) and all Polemoniaceae species (B) of all from pretreatment surveys (2021) to post-treatment surveys (2022). Points are means and error bars represent standard error.

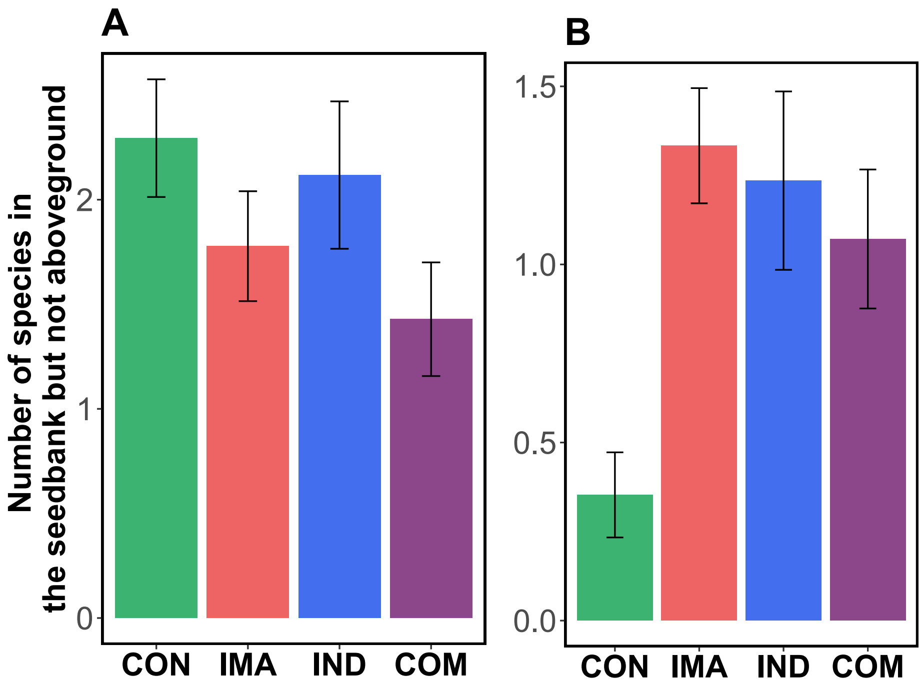

Figure 14. Number of native species (A) and non-native species (B) that were present in the seed bank, but weren't present aboveground, per treatment. Error bars represent standard error. CON stands for control, IMA stands for imazapic, IND stands for indaziflam, and COM stands for combination.

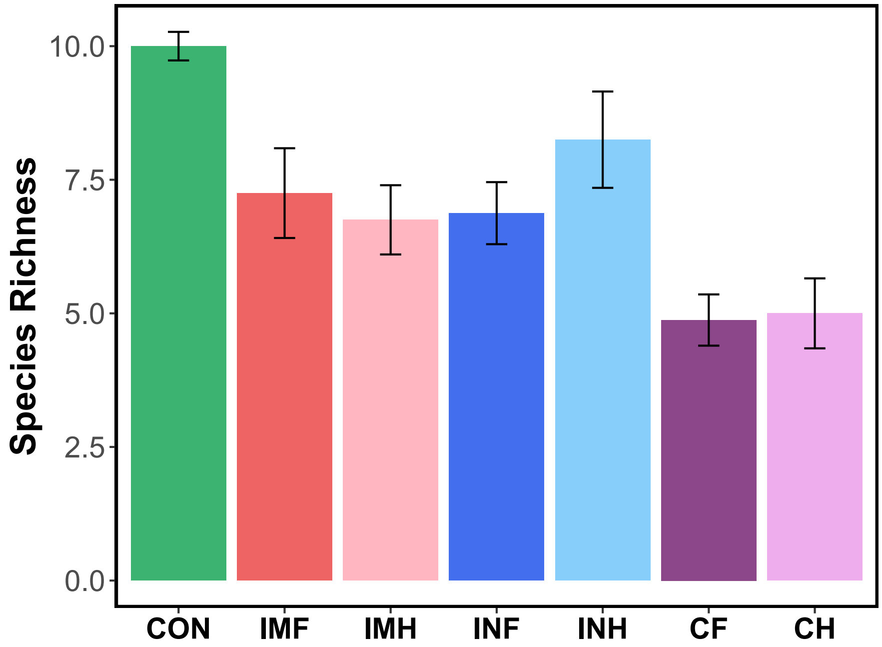

Figure 15. Species richness averaged across both years. Note that only species richness is shown, because results were very similar for species richness, Shannon's diversity, and Simpson's diversity. Error bars represent standard error. CON standard for control, IMF imazapic ful, IMH imazapic half, INF indaziflam full, INH indaziflam half, CF combination full, and CH combination half.

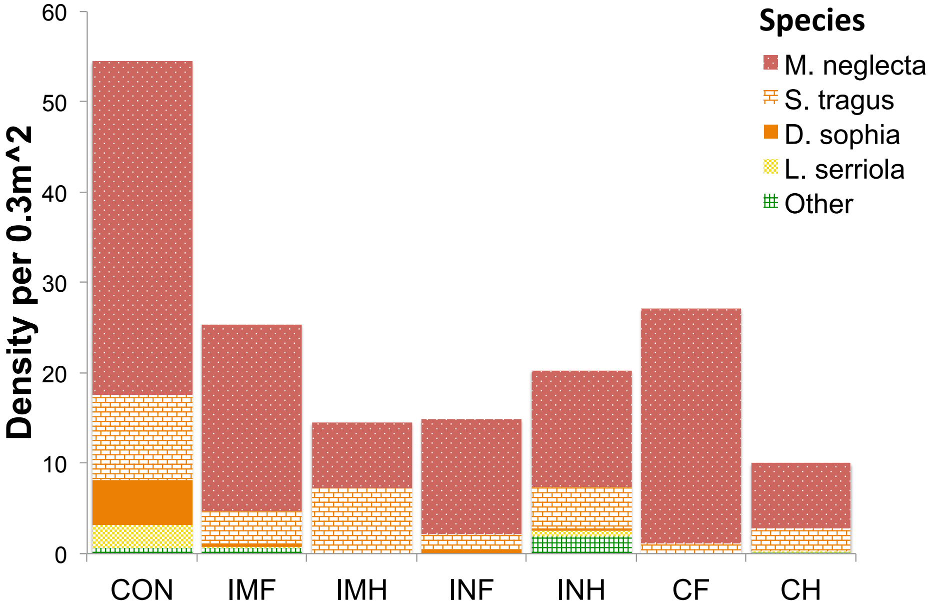

Figure 16. Values are the average maximum density observed in either year of each non-native species in each quadrat placed within each treatment (control, imazapic full, imazapic half, indaziflam full, indaziflam half, combination full, and combination half). The species represented, from top to bottom of the bars, are *Malva neglecta, Salsola tragus, Descurainia sophia, Lactuca serriola,* and a category for other non-native species. The 6 non-native species in the other category can be found in Appendix 4. CON standard for control, IMF imazapic ful, IMH imazapic half, INF indaziflam full, INH indaziflam half, CF combination full, and CH combination half.

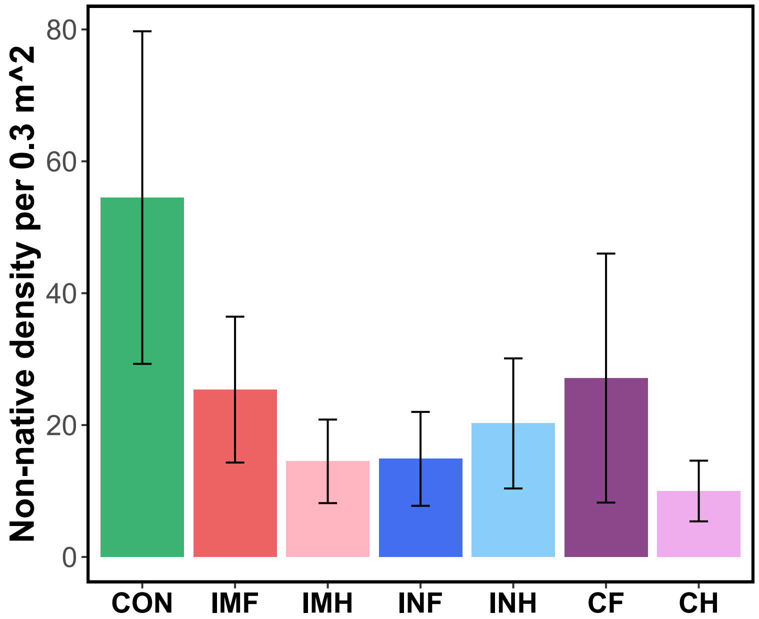

Figure 17. Maximum density of non-native species observed in either year. Error bars represent standard error. CON standard for control, IMF imazapic ful, IMH imazapic half, INF indaziflam full, INH indaziflam half, CF combination full, and CH combination half.

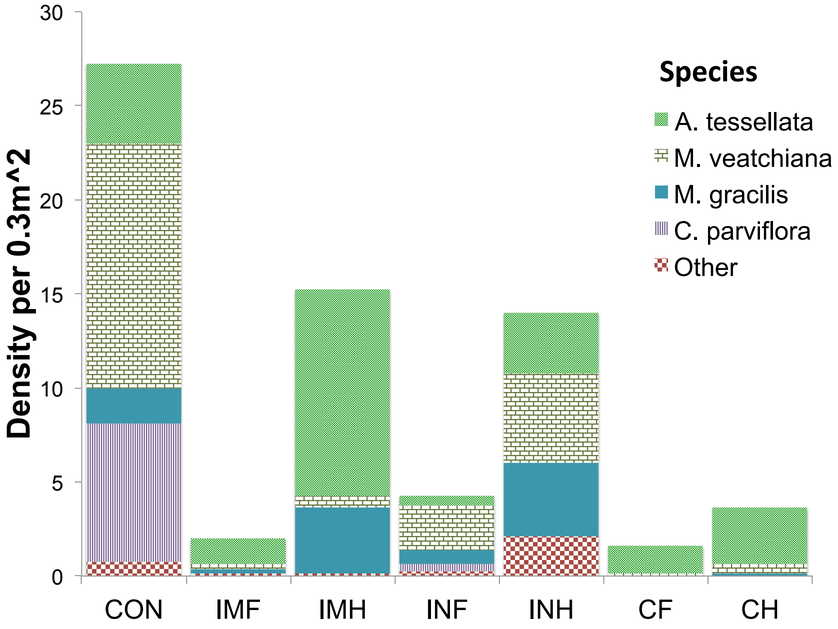

Figure 18. Values are the average maximum density observed in either year of each native species in each quadrat placed within each treatment (control, imazapic full, imazapic half, indaziflam full, indaziflam half, combination full, and combination half). The species represented, from top to bottom of the bars, are *Amsinckia tessellata, Mentzelia veatchiana, Microsteris gracilis, Collinsia parviflora,* and a category for other native species that includes *B. scaber, G. inconspicua,* and *L. glandulosa*. CON standard for control, IMF imazapic ful, IMH imazapic half, INF indaziflam full, INH indaziflam half, CF combination full, and CH combination half.

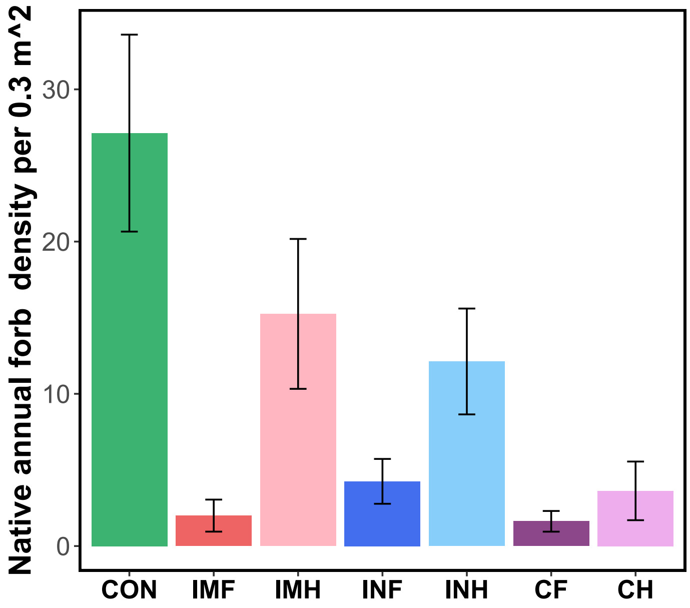

Figure 19. Maximum density of native species observed in either year. Error bars represent standard error. CON standard for control, IMF imazapic ful, IMH imazapic half, INF indaziflam full, INH indaziflam half, CF combination full, and CH combination half.

Figure 20. Photo taken at the annual forb experiment field at Valley Road in Reno, Nevada in April 2021. The control plots had high densities of *Mentzelia veatchiana* in 2021, demonstrated by the control plot on the left, while the herbicide plots had strong suppressive effects, illustrated by the indaziflam full plot on the right.

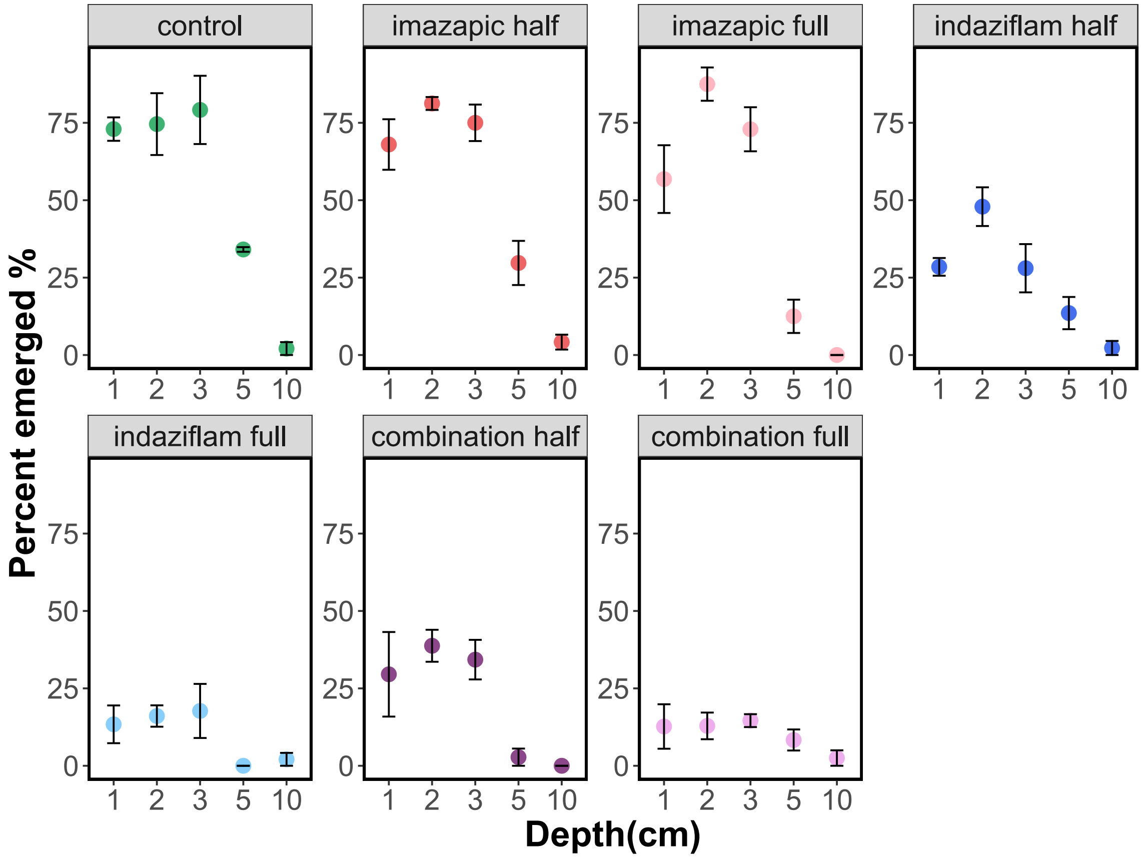

Figure 21. Changes in percent emergence at different depths on the x axis for each of the seven treatments. Points are means and error bars represent standard error.

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Appendices

Appendix Table 1: Species in natural field experiment

Appendix Table 1 Species found in natural field experiment plots. Values are the number of quadrats with each species (out of a total of 66 possible quadrats) present in above ground surveys in 2021, which was pre-treatment, and 2022, which was post-treatment. Values are combined for all treatments, as an indication of relative abundance of each species at the site.

Species	Family	Native to continental U.S.?	Number of quadrats found pre-treatment (2021)	Number of quadrats found post-treatment (2022)
Achnatherum thurberianum	Poaceae	Yes	15	15
Agoseris glauca	Asteraceae	Yes	0	1
Allium sp.	Amaryllidaceae	Yes	2	1
Alyssum desertorum	Brassicaceae	No	6	7
Antennaria sp.	Asteraceae	Yes	0	5
Artemisia tridentata	Asteraceae	Yes	18	18
Astragalus purshii	Fabaceae	Yes	8	9
Astragalus sp.	Fabaceae	Yes	1	3
Balsamorhiza hookeri	Asteraceae	Yes	19	18
Balsamorhiza sagittata	Asteraceae	Yes	1	1
Bromus tectorum	Poaceae	No	59	49
Ceratocephala testiculata	Ranunculaceae	No	34	23
Chrysothamnus viscidiflorus	Asteraceae	Yes	13	13
Collinsia parviflora	Plantaginaceae	Yes	0	2
Crepis accuminata	Asteraceae	Yes	17	27

Crepis occidentalis	Asteraceae	Yes	7	3
Descurainia sophia	Brassicaceae	No	0	1
Elymus elymoides	Poaceae	Yes	38	35
Epilobium brachycarpum	Onagraceae	Yes	6	1
Erodium cicutarium	Geraniaceae	No	1	0
Ionactis alpina	Asteraceae	Yes	16	24
Lactuca serriola	Asteraceae	No	0	1
Leymus cinereus	Poaceae	Yes	3	3
Lianthus pungens	Polemoniaceae	Yes	1	1
Lomatium sp.	Apiaceae	Yes	0	2
Lupinus argenteus	Fabaceae	Yes	23	27
Mertensia oblongifolia	Boraginaceae	Yes	0	4
Microsteris gracilis	Polemoniaceae	Yes	0	1
Nothocalais troximoides	Asteraceae	Yes	1	7
Penstemon kingii	Plantaginaceae	Yes	1	3
Phlox hoodii	Polemoniaceae	Yes	1	3
Phlox longifolia	Polemoniaceae	Yes	39	39
Phlox sp.	Polemoniaceae	Yes	3	1
Poa secunda	Poaceae	Yes	52	54
Pseudoroegneria spicata	Poaceae	Yes	1	1

Tragopogon dubius	Asteraceae	No	1	0
Vulpia microstachys	Poaceae	Yes	5	4
Unknown forb 1	Apiaceae	Unknown	5	3
Unknown forb 2	Asteraceae	Unknown	2	3
Unknown forb 3 forb	Unknown	Unknown	0	1
Unknown forb 4 forb	Unknown	Unknown	0	1

Appendix Table 2: Diversity metrics from natural field experiment

Appendix Table 2 Results from linear models showing the effect of treatment, year, and treatment by year interaction on diversity metrics in the natural field experiment. Values are X2 values, degrees of freedom, and P values. No p values are significant at alpha = 0.05.

Response variable	**Factor**	**X^2 value**	**Degrees of freedom**	**P value**
Richness	Treatment	7.4	1	0.0603
	Year	0.5	3	0.5016
	Treatment*Year	3.4	1	0.3300
Shannon’s	Treatment	2.1	1	0.5470
	Year	1.8	3	0.1764
	Treatment*Year	3.0	1	0.3973
Simpson’s	Treatment	1.8	1	0.6096
	Year	3.0	3	0.0856
	Treatment*Year	3.8	1	0.2791

Appendix Table 3: Species in seed bank experiment

Appendix Table 3 Species found in seed bank experiment, for samples collected from the natural field experiment site. Values are the number of quadrats with each species (out of a total of 72 possible quadrats) present in the seed bank experiment, which was collected from the plots pre-treatment. Values are combined for all treatments, as an indication of relative abundance of each species at the site.

Species	Family	Native to continental U.S.?	Number of plots found in
Agoseris glauca	Asteraceae	Yes	3
Allium acuminatum	Amaryllidaceae	Yes	2
Bromus tectorum	Poaceae	No	23
Ceratocephala testiculata	Ranunculaceae	No	39
Collinsia parviflora	Plantaginaceae	Yes	4
Draba verna	Brassicaceae	No	31
Epilobium brachycarpum	Onagraceae	Yes	2
Erodium cicutarium	Geraniaceae	No	2
Juncus sp.	Juncaceae	Yes	4
Lactuca serriola	Asteraceae	No	2
Microsteris gracilis	Polemoniaceae	Yes	7
Poa secunda	Poaceae	Yes	50
Pseudoroegneria spicata	Poaceae	Yes	9
Vulpia microstachys	Poaceae	Yes	15
Unknown forb 1	Unknown	Unknown	42
Unknown forb 2	Unknown	Unknown	10
Unknown forb 3	Unknown	Unknown	18
Unknown forb 4	Unknown	Unknown	18

Unknown forb 5	Unknown	Unknown	1

Appendix 4: Species in annual forb experiment

Appendix Table 4: Species found in annual forb experiment plots. Values are the number of plots with each species (out of a total of 28 possible quadrats) present in surveys in 2021 and 2022. Values are combined for all treatments, as an indication of relative abundance of each species at the site. An asterisk means that the plant was growing in the survey quadrats, while other species were growing in the full plots and used to calculate diversity metrics.

Species	Family	Native to continental U.S.?	Number of plots found in (2021)	Number of plots found in (2022)
Amsinckia tessellata	Boraginaceae	Yes	23	25
Blepharipappus scaber*	Asteraceae	Yes	1	0
Bromus tectorum*	Poaceae	No	6	1
Collinsia parviflora*	Plantaginaceae	Yes	19	1
Descurainia sophia*	Brassicaceae	No	10	8
Elymus elymoides*	Poaceae	Yes	8	9
Epilobium brachycarpum	Onagraceae	Yes	0	1
Erodium cicutarium*	Geraniaceae	No	0	7
Gilia inconspicua*	Polemoniaceae	Yes	0	6
Grindelia squarrosa	Asteraceae	Yes	5	2
Hordeum murinum *	Poaceae	No	9	2
Kochia scoparia*	Amaranthaceae	No	2	6
Lactuca serriola*	Asteraceae	No	5	10
Layia glandulosa*	Asteraceae	Yes	4	10

Malva neglecta*	Malvaceae	No	0	26
Mentzelia veatchiana*	Loasaceae	Yes	24	13
Microsteris gracilis*	Polemoniaceae	Yes	8	9
Nicotiana attenuata	Solanaceae	Yes	0	6
Polygonum aviculare*	Polygonaceae	No	3	0
Salsola tragus*	Amaranthaceae	No	20	28
Tragopogon dubius*	Asteraceae	No	0	1
Unknown forb 1	Brassicaceae	Unknown	0	1
Unknown forb 2	Unknown	Unknown	0	1
Unknown grass 1*	Poaceae	Unknown	18	8

Summary, conclusions, and reccomendations

Our results have implications for improving restoration in the Great Basin. In Chapter 1, we observed change over time for every species in all populations, with at least one burn x time interaction for every species, with early seed and seedling traits and *E. elymoides* showing the most change. These changes could be due to rapid evolution, through either selection or drift, and in a few cases it appears that burn status may have been the selective agent for change. However, these changes could also be due to maternal effects, especially possible for seed traits. Researchers conducting common garden studies strive to grow multiple generations of plants to control for maternal effects, but this is unfortunately not always feasible. Whether the changes were rooted in maternal effects or a result of evolutionary change, our observation that seed bank and contemporary collections are likely to differ in potentially adaptive traits is noteworthy, and relevant for the use of these seeds for restoration or research. Specifically, one cannot expect that seed banked seeds are a mirror image of contemporary populations, which may continue to change while the seed bank gene pool remains static. These historic collections are a valuable resource, because they may harbor alleles that are no longer present in a given population, and could potentially be used to relieve inbreeding depression and loss of diversity through re-introduction. Our conclusions support the continued effort of programs like Seeds of Success (Haiet & Olwell, 2015) that collect wild seed for restoration and long-term storage, and further suggest that revisiting collected populations could be beneficial for capturing rapid evolutionary changes. We also are excited by the growing use of resurrection studies to assess rapid evolution, and encourage other researchers to take advantage of the Germplasm Resource Information Network (Volk & Richards, 2008) and Project Baseline (Etterson et al., 2016) to request historic seed collections for resurrection studies.

In Chapter 2, we explored the ways that land managers try to strike a balance between removing invasive species with herbicide treatments while not damaging the native plant community. Our natural field study reveals that applying herbicides in heavily-invaded areas can benefit perennial native plants, likely through reduced competition with invasive plants, and that a combination of herbicides provided the most consistently positive outcomes. However, our annual forb experiment revealed that imazapic and indaziflam can substantially reduce native annual forbs, which are essential components of Great Basin ecosystems. It is possible that native annual forbs could benefit from reduced competition with invasive species if herbicides were applied in an invaded natural field setting, so future studies should make an effort to survey wild sites while native annual forbs are present, and ideally over multiple years to capture true annual forb diversity. We suggest that land managers also survey with native annual forbs in mind when deciding whether or not to apply herbicides, which might require seed bank sampling. If there is an abundant and rich native annual forb community, managers may consider methods other than herbicides, use reduced rates, or make efforts to increase native annual forbs after spraying. This could be accomplished by collecting seed from native annual forbs on site pre-treatment, as they may already be adapted to competing with the non-native plants that are already at that site. We also suggest that further studies investigate the possibility that planting deeper seeds could offset the negative effects of herbicides, and that land managers planting *E. elymoides* alongside imazapic or indazialam application plant seeds between 2 or 3 cm.

Restoration in the Great Basin is challenging, however, we believe that thoughtful and appropriate use of herbicides has the potential to combat invasive species while benefting the native plant community. Further, native plants may be evolving in response to distubances like fire and increased cheatgrass pressure, and using seeds with potentially adaptive traits for restoration may help land managers restore degraded sites with the most tolerant and competitive seeds possible.