# **THESIS**

# CHEATGRASS (*BROMUS TECTORUM* L.) MANAGEMENT AND NATIVE PLANT COMMUNITY RECOVERY ON SITES SELECTIVELY TREATED WITH IMAZAPIC IN ROCKY MOUNTAIN NATIONAL PARK

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### **ABSTRACT**

CHEATGRASS (*BROMUS TECTORUM* L.) MANAGEMENT AND NATIVE PLANT

COMMUNITY RECOVERY ON SITES SELECTIVELY TREATED WITH IMAZAPIC IN ROCKY

MOUNTAIN NATIONAL PARK

Cheatgrass, a winter annual grass introduced to North America from Eurasia, has invaded much of the Western United States over the last century. Recently, cheatgrass has become a threat to the montane and subalpine plant communities and ecosystems of Rocky Mountain National Park (RMNP). Cheatgrass aggressively invades disturbed sites and competes with native plant species by rapidly establishing a root system capable of depleting soil moisture and available nitrogen, making cheatgrass control a priority when restoring disturbed areas within RMNP.

The purposes of this study were to determine the effectiveness of imazapic for cheatgrass control, its effects on non-target native species, and how the plant community recovers following cheatgrass control. In 2008, 12 permanent monitoring plots were established in six sites in RMNP, each with one reference and one imazapic treatment plot. Reference plots were chosen to represent the desired final condition for each imazapic treatment site. Imazapic (23.6% a.i.) was applied to cheatgrass infestations post-emergence in 2008 (105 g a.i./ha) and pre-emergence in 2009 (105 g a.i./ha) and 2010 (70 g a.i./ha). Imazapic was applied to cheatgrass patches selectively, avoiding application to native species as much as possible. Cheatgrass cover was reduced more than fourfold to approximately 5% in 2013, and there was no decrease in cover of native forbs, grasses, or shrubs. There was no subsequent increase in native species abundance following cheatgrass removal, suggesting further action is needed if the ultimate management goal is to encourage native species recovery in treatment plots after satisfactory cheatgrass control is achieved.

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#### INTRODUCTION

Non-native invasive species have caused large-scale ecological and economic impacts across the globe (Mack et al. 2000; Pimentel et al. 2001), resulting in native species displacement and extinction, significant changes in ecosystem composition and function (D'Antonio and Vitousek 1992), and necessitating the use of costly control measures (DiTomaso 2000). The impacts of invasive species coupled with changes in land use, increases in pollution, and the effects of climate change, threaten biodiversity worldwide (Wilcove et al 1998; Dukes and Mooney 1999). Invasive plant species have been identified in the majority of United States National Park units, with more than 3,700 unique invasive species observed on park lands (Allen et al. 2009). The National Park Service (NPS), whose mission is to conserve the biological integrity and function of park resources (NPS 1916), employs exotic plant management crews in individual parks and 16 regional exotic plant management teams to manage invasive plant species (NPS 2006). In 2003, Rocky Mountain National Park (RMNP) introduced an exotic plant management plan outlining the threats of invasive plant species to the park's resources and identifying priority species for control using integrated pest management techniques (RMNP 2003).

One invasive plant species identified as a significant threat in RMNP is the Eurasian winter annual grass *Bromus tectorum* L. (hereafter, cheatgrass); one of the most widespread (Hulburt 1955; Mack 1981) and aggressively invasive non-native plants of Western North America (Knapp 1996). The initial introduction of cheatgrass to the Intermountain West region of the United States likely occurred in the late 1800's as a result of contaminated seed stock and intentional seeding in overgrazed grassland areas (Mack 1981). Since its introduction it has expanded its introduced range to approximately 40,000,000 hectares (Mack 1981) and has become the dominant species in many of the semi-arid systems in this region (Klemmedson and Smith 1964; Morrow and Stahlman 1984). Cheatgrass rapidly invades regularly disturbed sites

(Hulbert 1955) and competes directly with native plant species by germinating in autumn, maturing in early spring, and rapidly establishing a root system (Arrendondo et al. 1998) capable of depleting soil moisture and nitrogen (Link et al. 1995). Cheatgrass matures before many native plant species, breaking dormancy in late winter or early spring (Klemmedson and Smith 1964), resulting in a fall cohort capable of producing large amounts of seed (Young et al. 1969). If fall or winter conditions are unfavorable, cheatgrass can also produce a spring cohort, ensuring continued propagule pressure (Young et al. 1969; Mack and Pyke 1983). Cheatgrass also displays a high degree of phenotypic plasticity allowing it to rapidly invade a wide variety of habitats (Rice and Mack 1991; Kao et al. 2008; Griffith et al. 2014).

Increased fire frequency due to cheatgrass infestation can suppress post-fire recovery of native species adapted to longer fire return intervals (Knapp 1996), creating a positive feedback loop favoring further invasion and dominance by the fire-tolerant grass (Brooks 2004). This alteration of fire regimes allows cheatgrass to indirectly compete with large, deeply rooted woody species that would otherwise be minimally affected by its presence (D'Antonio and Vitousek 1992). High nitrogen availability has been shown to favor cheatgrass invasion and increase its competitive pressure on native species (Lowe et al. 2003; Gao et al. 2014; Leffler et al. 2014; Vasquez et al. 2008) while reduced available nitrogen reduces cheatgrass abundance and invasion success (Paschke et al. 2000; Rowe et al. 2009). In invaded communities, cheatgrass has been shown to alter nitrogen dynamics by creating a nitrogen feedback loop between nitrogen-rich cheatgrass litter and cheatgrass seedlings, depleting surrounding soil nitrogen and favoring cheatgrass growth over native perennials (Booth et al. 2003b; Sperry et al. 2006). Cheatgrass may also increase the rate of nitrogen loss from systems through volatilization resulting from increased fire frequency (Evans et al. 2001).

Although typically limited to lower elevation habitat with milder winters (Chambers 2007), cheatgrass has been increasing in abundance in the montane and subalpine regions of RMNP (Bromberg et al. 2011; J. Connor, personal communication), and has been observed growing at

elevations as high as 2,900 m (personal observation). In RMNP cheatgrass is typically more abundant near roads and developed areas of the park where frequent disturbance is common (Bromberg et al. 2011, Banks and Baker 2011). Recent research suggests that reduced winter snowpack, increased winter rains (Griffith and Loik 2010; Concilio et al. 2012), and nitrogen inputs in the form of atmospheric deposition (Concilio et al. 2012, Concilio and Loik 2013) have contributed to increased cheatgrass invasion at high elevations. Changing climate is also predicted to result in increased distribution and elevation range in the Rocky Mountains (Mealor et al. 2012) and RMNP (West et al. 2015) in coming years.

In response to growing cheatgrass accessions in RMNP, park managers made its management a priority for the exotics control program beginning in 2008. In addition to manual control methods, like hand-pulling, that are used to remove small cheatgrass patches, the herbicide imazapic is used to manage large cheatgrass infestations in the park. Imazapic requires a relatively low application volume to control cheatgrass and exhibits low-to-moderate environmental toxicity and moderate soil persistence (BASF 2011; Tu et al. 2001). Imazapic is an effective tool for reducing cheatgrass abundance, but has been shown to have variable effects on non-target plants (Shinn and Thill 2002; Shinn and Thill 2004; Elseroad and Rudd 2011; Baker et al. 2009; Kyser et al. 2013, BASF 2011). Several steps are taken at RMNP to avoid damage to non-target plant species in imazapic treatment sites, including (1) targeted cheatgrass spraying using backpack sprayers, (2) spraying when many native species are dormant, (3) forgoing the use of herbicide adjuvants, and (4) avoiding spraying when winds may cause spray drift. Little information is currently available regarding the effects of imazapic on non-target native species or the effectiveness of targeted imazapic application in controlling cheatgrass in a natural resources management context.

In 2008, permanent monitoring plots were established in sites that were infested with cheatgrass (~25% mean absolute cover) and scheduled to be treated with imazapic later that year. Each imazapic treatment plot was paired with an adjacent reference plot that had no

cheatgrass present at the time of establishment. Reference plot plant communities were representative of the desired post-treatment condition of the invaded treatment plots. Treatment plots were compared to reference plots to assess the effects of imazapic treatments and the trajectory of plant community recovery in treatment plots following imazapic application.

The objectives of this study were to assess (1) the effectiveness of selective imazapic application for cheatgrass control, (2) non-target plant community responses to proximate imazapic treatments, and (3) treatment plot plant community similarity to reference plot communities following cheatgrass removal.

#### **MATERIALS AND METHODS**

# **Study Site**

Rocky Mountain National Park is located in the north-central region of Colorado on the Front Range of the Rocky Mountain chain and is one of the most visited parks in the National Parks system, with visitation rates averaging nearly 3 million people a year over the past decade (NPS 2015). All monitoring sites were located on the northeastern slope of RMNP at elevations of approximately 2,450-2,700 meters, and were near roads, facilities, and trailheads that experience heavy seasonal use by visitors and park personnel. Average annual precipitation in this part of the park is 620 mm, with most precipitation occurring in fall and early spring (NRCS 2015). Treatment monitoring sites were chosen on the basis that there was an existing cheatgrass infestation scheduled to be treated with imazapic after pre-treatment data were collected. Adjacent to the treatment site, reference monitoring sites that were free of any cheatgrass and not being treated with imazapic were selected to represent the desired posttreatment plant community for the treatment plots following cheatgrass removal. Plant communities in reference plots were not similar to treatment plots before herbicide treatment, and were not true control plots. This study was conducted on actively-managed sites in the park, and untreated cheatgrass control plots were not included in the design of this study to avoid reinvasion of treatment sites from adjacent untreated plots. Treatment and reference plot pairs were installed in forest, shrubland, and grassland sites for a total of six monitoring sites, each with a treatment/reference plot pair.

# **Vegetation Monitoring**

In June 2008, prior to herbicide application, two permanent circular nested vegetation monitoring plots (CNP), each with an area of approximately 168.2 m2 (0.01682 ha or 1/24 of an acre), were installed at each of the six sites. Each CNP had three 7.2 m transects radiating from

the center stake at 30, 150 and 270 degrees. A 1 m2 quadrat was sampled on the right side of each transect at 4.9 m when viewed from the center stake of the plot (see Fig. 1). Coordinates for the center point of each CNP were recorded using a handheld GPS unit and a photograph of each quadrat viewed from the center of the CNP was taken every year data were collected.

Data were collected annually from 2008-2013, and included one pre-treatment collection, three treatment year collections, and two post-treatment year collections. In 2012, one plot pair was destroyed as a result of road-widening construction along Bear Lake road. In 2013 additional transects, quadrats, and nutrient manipulations were added to the existing CNPs where species richness data were collected in past years, resulting in only five years of richness data.

Vegetation, bare soil, litter, rock, and moss and lichen percent cover was visually estimated within each 1 m2 quadrat of the CNP. Any living plant species within the quadrat was identified and an estimation of percent cover for each individual species was made. Tree, shrub, and forb percent cover were estimated using canopy cover and graminoid (grass, sedge, and rush species) cover was estimated using basal, or ground level cover. Percent cover was estimated using modified Daubenmire cover class ranges (Daubenmire 1959). Plant species that were within the CNP but not observed within the three quadrats were identified and recorded to evaluate changes in species richness over time.

# **Herbicide Application**

Each year from 2008 to 2010, RMNP's exotic plant management crew treated cheatgrass infested sites with imazapic (23.6% a.i.; Plateau®, BASF, Research Triangle Park, USA) using Solo 425 backpack sprayers (Solo, Newport News, USA). In the early Summer of 2008, cheatgrass patches were treated post-emergence at a maximum plant height of five cm and at the maximum recommended rate of 105 g a.i./ha. In mid-Fall of 2009 and 2010 all cheatgrass patches were treated pre-emergence as a soil application to comply with revised Plateau label standards that no longer recommended post-emergence treatment. Imazapic was

applied at a rate of 105 g a.i./ha in 2009 and at a rate of 70 g a.i./ha in 2010. The application rate was decreased to the minimum recommended rate in 2010 to further reduce the potential of damage to adjacent non-target plants because satisfactory cheatgrass control was being achieved. Several treatment techniques were employed to avoid non-target species injury or mortality. Cheatgrass was selectively spot-sprayed to minimize damage to native plant species, though non-target species growing in or around cheatgrass patches were difficult to avoid partially spraying. Post-emergent applications were applied directly to cheatgrass plants, and pre-emergent applications were applied to soils below senescent cheatgrass plants that had dropped their seed. No-spray-days were enforced, which called for the stoppage of any herbicide application when wind speeds exceeded 2.7 m/s. Surfactants or adjuvants were not included in the imazapic herbicide solution, making it less likely that any small particles of drifting herbicide spray would penetrate non-target plant tissue. Finally, spraying cheatgrass pre-emergence limits imazapic applications to the autumn months, when many native species are going dormant and may be less susceptible to foliar imazapic exposure, due to imazapic's activity in actively growing meristematic tissue (Roberts et al. 1998).

## **Data Analysis**

Percent cover for each species in each quadrat was calculated as the mean of the maximum and minimum Daubenmire cover-class values (e.g. a cover-class of 10-25% has a mean value of 17.5%). Using these midpoint cover values, the mean species cover was calculated across all three quadrats in a CNP. The species in each CNP were grouped according to growth habit and their status as either a native, non-native invasive or non-native, non-invasive species. The species richness data from the survey of the entire CNP were also grouped according to these criteria. The possible growth habit categories were tree, shrub, graminoid, forb, or moss and lichen. Species were classified as having a particular growth habit based on their classification in the USDA PLANTS profile database (USDA 2015) and were

classified as native or non-native based upon their designation as such in Weber and Wittman (2001). Species were classified as invasive if they were listed on RMNP's list of invasive species or the Colorado Department of Agriculture's list of noxious weeds (CDA 2014). Species richness values were summarized using these same designations. Cheatgrass presence and cover were also summarized separately to evaluate imazapic's effects on the target species. Shannon-Weiner diversity index values and Sorenson similarity index values were also calculated to provide another measure of plant community change over time.

Cover, richness, and diversity data were analyzed using SAS PROC MIXED repeated measures ANOVA with treatment type and vegetation type as independent variables, and similarity data were analyzed using PROC GLM repeated measures ANOVA (SAS 9.3, SAS Institute, Cary, NC, USA). Differences between specific treatment types or years were analyzed using t-tests of least square means. Cover data were log or square root transformed as needed to meet the model assumptions of equal variances using Studentized residual plots. Significant interactions or main effects were graphed using untransformed data (Sigma Plot 10, Systat Software Inc., San Jose, CA, USA).

## **RESULTS**

## Cheatgrass

There was a four-fold reduction in absolute cheatgrass cover from 2008 to 2013 following imazapic application in treatment plots (Table 1, Absolute Cover, time x treatment). Cheatgrass cover was reduced from 24% to 5% absolute cover in 2011 after three annual imazapic treatments, and this reduction in cover persisted through two non-treatment years (Fig. 2A). By 2012 and 2013 absolute cheatgrass cover was not significantly different between treated and untreated reference plots (2012:  $t_{1,29}$  = -1.60, P = 0.12; 2013:  $t_{1,29}$  = -1.47, P = 0.15). Cheatgrass absolute cover also decreased more rapidly in forest and grassland plots than in shrubland plots over time (Table 1, Absolute Cover, time x veg type).

Relative cover of cheatgrass in treatment plots was reduced from 43% in 2008 to 13% in 2013 (Table 1, Relative Cover time x treatment), and this reduction in cover persisted through two non-treatment years (Fig. 2B). Although relative cover of cheatgrass was significantly reduced in both forest and grassland plots through 2013, cheatgrass relative cover in shrublands was initially reduced following treatment, but returned to pre-treatment levels after 2011 (Table 1, time x veg type x treatment, Relative Cover; Fig. 2C).

# **Native Species**

#### Total Native Species

There was no significant change in absolute native species cover over time in response to herbicide treatment (Table 2, Absolute Cover, time x treatment; Fig. 3A). Absolute native species cover was greater in reference plots in all study years (Table 2, Absolute Cover, time) and although there was a decrease in the magnitude of the difference in absolute cover between treatment types, this was due to a 50% loss in native species cover in untreated reference plots from 2011 to 2013 ( $t_{5.29}$  = 3.49, P = <0.01). Total absolute native species cover

decreased over time independent of treatment type (Table 2, Absolute Cover, time).

Relative cover of native species changed in response to treatment, approximately doubling from 2008 to 2013 in plots treated with imazapic, while remaining unchanged in reference plots (Table 2, Relative Cover, time x treatment; Fig. 3B). Relative native species cover also increased independent of treatment type in grassland and forest plots (Table 2, Relative Cover, time x veg. type).

Total native species richness did not change in response to treatment, but was greater in reference plots when averaged over time (Table 2, Species Richness, treatment; Fig. 3C).

#### Native Forbs

There was no change in absolute native forb cover over time in plots treated with imazapic (Table 3, Absolute Cover, time x treatment; Fig. 4A). Absolute native forb cover was greater in untreated reference plots when averaged over time (Table 3, Absolute Cover, treatment). Absolute native forb cover was greatest in reference shrubland plots and lowest in treatment shrubland plots, but did not change over time in response to herbicide application (Table 3, absolute cover, veg. type x treatment; Fig. 4B). Total native forb absolute cover averaged over both treatment types varied over time, likely due to significant annual variation in reference plot native forb cover (Table 3, absolute cover, time). Absolute native forb cover was similar in reference and treatment plots by 2013 ( $t_{1,32}$  = -0.62, P = 0.54), but this was due to a large decrease in cover in reference plots rather than an increase of cover in treatment plots.

Relative native forb cover in treatment plots increased by more than 25% cover over time and remained unchanged in reference plots (Table 3, Relative Cover, time x treatment; Fig 4C).

Relative native forb cover also depended on treatment and vegetation type and was greatest in forest and grassland treatment plots and lowest in shrubland treatment plots when averaged over time (Table 3, Relative Cover, veg. type x treatment; Fig 4D).

Native forb species richness was greater in reference plots when averaged over time (Table 3, Species Richness, treatment; Fig. 4E) and varied yearly, independent of treatment type (Table 3, species richness, time).

# Native graminoids

Absolute cover of native graminoids did not change over time in response to treatment (Table 4, Absolute Cover, time x treatment). Native graminoid absolute cover was greater in treatment plots prior to treatment in 2008 and remained greater than cover in reference plots over time (Table 4, Absolute Cover, treatment; Fig. 5A). Relative cover of native graminoids did not change in response to treatment, but was greater in treatment plots before and after treatment (Table 4, Relative Cover, treatment; Fig. 5B). Species richness of native graminoids declined over time in shrubland plots independent of treatment type (Table 4, Species Richness, time x veg. type) Richness fell from a mean of 5.75 graminoid species in 2008 to 3.25 species in 2012 in shrubland plots (Fig. 5C).

#### Native Shrubs

There was no change in absolute or relative native shrub cover over time in plots treated with imazapic (Table 5, Absolute & Relative Cover, time x treatment; Fig. 6A). Native shrub species richness did not change over time and was greater in forest and shrubland plots independent of treatment type, though not significantly so (Table 5, Species Richness, veg. type; Fig. 6B).

## **Non-Native Species**

## Invasive Forbs

Absolute cover of non-native forbs did not change in response to treatment, but was greater in forested treatment plots (1% in forest plots compared with %0 in grassland and shrubland), and was likely due to musk thistle encroachment from the adjacent meadow (Table

6, Absolute Cover, veg type x treatment). Relative cover of invasive forbs was also greatest in forest treatment plots independent of time, again due to musk thistle presence (Table 6, Relative Cover, veg type x treatment). Average invasive forb species richness was very low and did not change in response to treatment (Table 6, Species Richness), time x treatment).

Invasive Graminoids (excluding cheatgrass)

Non-native graminoid absolute cover decreased over time in all plots (Table 7, Absolute Cover, time; Fig. 7A). Relative cover also decreased over time in all plots (Table 7, Relative Cover, time) and was also dependent on vegetation and treatment type independent of time (Table 7, Relative Cover, veg type x treatment; Fig. 7B). Species richness was greater in treatment plots averaged over time, and decreased over time independent of treatment and vegetation type (Table 5, Species Richness, treatment and time, respectively; Fig. 7C). These differences are most likely due to early misidentification of a native *Poa* species as an invasive.

## Non-Invasive Non-Native Species

Non-invasive, non-native (NI) species were uncommon in all plots, with average absolute cover around 1% across all years. Absolute cover of NI species decreased from 7% to 0.1% in treatment plots between 2008 and 2013 (Table 8, Absolute Cover, time x treatment). Non-native spurge species are controlled in RMNP using imazapic, and this reduction of cover in treatment plots is likely a result of intended imazapic application to these species. Relative cover of NI species decreased over time in shrubland and grassland plots in response to treatment (Table X, Relative Cover, time x veg type x treatment). Shrubland and grassland plots had greater pre-treatment NI species relative cover (22% and 13%, respectively) than forest plots (0.1%) and NI relative cover was reduced to 0.6% in all treatment plots by 2013. The number of NI species changes over time dependent on treatment and vegetation type (Table 8, Species Richness, time x veg type x treatment). Richness declined in all plots except for grassland reference plots, where NI richness increased from 0 to 3 between 2008 and 2013.

#### **Ground Cover**

#### Moss and Lichen

There was no change in absolute cover ( $F_{5,29} = 0.54$ , P = 0.74) or relative cover ( $F_{5,29} = 0.41$ , P = 0.84) of moss and lichen species in treatment plots over time.

Litter

Absolute cover of litter increased in all plots between 2008 and 2013 independent of treatment type (Table 9, time; Fig. 8).

#### Bare Ground

There was no change over time in bare ground cover in treatment plots (Table 10, time x treatment; Fig. 9A). Bare ground cover was two times greater in treatment plots than reference plots when averaged over all years (Table 10, treatment) and there was a two-fold increase of bare ground cover in all plots between 2008 and 2013 (Table 10, time). In grassland plots bare ground was seven times greater in treatment plots than reference plots, and twice as great in shrubland treatment plots than reference plots averaged over all years (Table 10, veg type x treatment; Fig 9B).

## **Total Plant Community Diversity and Species Similarity**

Shannon-Weiner plant species diversity decreased by 32% between 2011 and 2012 in reference plots, but did not change in treatment plots (Table 11, time x treatment; Fig. 10)

Plant species similarity between treatment and reference plots averaged approximately 50% similarity when calculated using the Sorenson similarity index, and species similarity between treatment types did not change over time (Table 12; Fig 11).

#### DISCUSSION

My results suggest that targeted imazapic application is an effective method for reducing cheatgrass abundance. This finding agrees with previous studies that have shown imazapic reduces annual grass emergence, biomass (Hirsch et al. 2012), frequency (Elseroad and Rudd 2011), and cover (Mangold et al. 2013; Kyser et al. 2007). Three consecutive years of imazapic application resulted in an 80% reduction in cheatgrass cover in treatment plots, and this reduced cover persisted for two years after herbicide application. There is a large degree of variation in residual control of annual grasses with imazapic shown in the literature, with residual control lasting from one (Morris et al. 2009) to two years (Brisbin et al. 2013; Kyser et al. 2013) to as long as four years (Elseroad and Rudd 2011), and can depend on factors like soil type, precipitation, and litter cover (Morris et al. 2009). A significant rebound in cheatgrass cover was observed in shrubland plots after imazapic treatments stopped. These shrubland plots were located in areas surrounded by roads, park housing and stables, which are typically associated with a higher risk of cheatgrass invasion in RMNP (Bromberg et al. 2011; Banks and Baker 2011).

Native species absolute cover and species richness did not change over time in treatment plots, indicating that native plant species did not experience decreased vigor and were not killed by adjacent imazapic treatments. Relative cover of all native species in treatment plots increased as a result of the removal of a large percentage of cheatgrass in treatment sites. Additionally, there were no changes in absolute cover or species richness in native forb, graminoid, and shrub functional groups in response to imazapic treatments. Relative cover for these three functional groups increased over time in treatment plots, again the result of cheatgrass removal. Native forb cover experienced an approximate 2/3 decline in absolute cover from 2012 and 2013 in untreated reference plots, probably due to a dry growing season that preceded 2013 data collection (NRCS 2015) and there was no concurrent drop in native

forb cover in treatment plots. I attempted to determine if this dramatic drop in native forb abundance was due to the loss of certain types of forbs (e.g. annual or drought-intolerant species) during this dry period, but did not find any clear pattern in the data.

A decrease in absolute and relative cover and species richness of invasive graminoids (excluding cheatgrass) was observed in treatment plots over time. This was most likely due to several native *Poa* species misidentified as the invasive *Poa pratensis* early in the study being correctly identified in later years, and not a response to herbicide treatments. Invasive forb absolute and relative cover and species richness did not change in treatment plots over time. Total non-invasive non-native species absolute and relative cover was reduced in treatment plots due to the removal of non-native spurge species from these plots. Non-native spurges are controlled in RMNP using imazapic, and this reduction of cover in treatment plots is likely the result of intended imazapic application to these species concurrent with cheatgrass treatment. Previous studies have shown that non-target plant responses to imazapic are variable (Sheley et. al 2007), and exposure can result in reduced vigor and increased mortality in forb, graminoid, and shrub species (Baker et al. 2009, Kyser et al. 2007; Owen et al. 2011, Shinn and Till 2004). Additionally, the imazapic specimen label identifies several native forb, graminoid, and shrub species of RMNP that are susceptible to suppression or mortality if exposed to imazapic (BASF 2011). The absence of any observable native species suppression or mortality in this study is likely due to the targeted application methods employed by the RMNP management to achieve imazapic specificity for cheatgrass control. Previous studies that detected damage to non-target plants (Kyser et al. 2007; Owen et al. 2011, Shinn and Till 2004; Sheley et al. 2007) typically used imazapic mixes that included adjuvants like methylated seed oil (MSO) that increase the spread and penetration of herbicide into plant tissue and enhance herbicide effectiveness. Avoiding the use of adjuvants may reduce the likelihood that drifting herbicide will penetrate non-target plant tissue. Treating annual grasses with imazapic at the seed and early-seedling stage has been shown to be more effective than treatments applied to more mature plants

(Kyser et al. 2007; Mangold et al. 2013), and during this study imazapic was applied at the seed or early germination growth stage for the final two years of imazapic treatments.

Although I expected to see an increase in native vegetation abundance and diversity following cheatgrass control, I did not observe any measureable recovery of the native plant community after cheatgrass abundance decreased. In spite of an 80% reduction in cheatgrass cover in treatment plots, absolute native species cover and plant species diversity did not increase, species similarity between treatment and reference plots remained at the pretreatment level of 50%, and bare ground did not decrease in treatment plots. This suggests that there was no increase in abundance of plants in treatment plots in response to reduced cheatgrass competition. Studies have shown that removal of invasive species does not necessarily guarantee an increase in native plant recruitment in those sites (Dela Cruz et al. 2014; Elseroad et al. 2011).

There are several potential explanations for this lack of native plant community recovery, including reduced native soil seed banks resulting from cheatgrass invasion, residual imazapic suppression of the native soil seed bank, soils that only support cheatgrass establishment, and climate conditions that did not encourage native seedling establishment. Annual-grass dominated habitat has been shown to have less abundant and less diverse native soil seed banks than uninvaded soils (McLaughlin and Bowers 2007; Humphrey and Schupp 2001) which hinders post-invasion recovery of native plant species on these sites. There is evidence that imazapic inhibits germination of non-target grass, forb, and shrub seed in soils (Brisbin et al. 2013, Owen et al. 2011). Seeded perennial grass species also experience reduced germination rates when sown from zero to 90 days after imazapic application, while seed sown 90 days to a year after imazapic application does not (Davies et al. 2014; Sbatella et al. 2011). Regular disturbance of soils can result in altered soil microbial communities (Martenson et al. 2012), which can favor cheatgrass invasion and competition by preventing the establishment of native perennial species that thrive when soil biota is intact (Owen et al. 2013; Rowe et al. 2008; Rowe

et al. 2009). Changes in annual precipitation, particularly drought conditions during spring and summer, negatively affect perennial plant establishment and persistence (Robins et al. 2013; Prevey and Seastedt 2014). In the 2012 spring growing season (March-May), the first year imazapic was not applied after three consecutive applications, precipitation was far below average (10.7 cm in 2012 compared to the average 22.5 cm) (NRCS 2015) possibly contributing to the lack of native species reestablishment in treatment plots.

I recommend further investigation to determine the most effective approach to improving native species reestablishment in cheatgrass infestations following imazapic treatment. An important component of the long-term control of annual grass infestations can be the reestablishment of native perennial flora that may have been displaced by cheatgrass infestations (Owen et al. 2011; Elseroad and Rudd 2011; Davies and Sheley 2009). Bare soil is typically correlated with increased cheatgrass presence in RMNP and was observed to be greater in treatment plots. Active management actions, like seeding, aimed at increasing plant cover in these bare spaces may reduce the likelihood of cheatgrass reinvasion following imazapic treatment (Reisner et al. 2013; Rayburn et al. 2014). Mature native perennial plants like Elymus elymoides and Pascopyrum smithii have been shown to successfully compete with and exclude cheatgrass seedlings, and can provide resilience against reinvasion in restoration sites (McGlone et al. 2012; Humphrey and Schupp 2004). Carefully selected seed mixes can influence ecosystem succession and promote species heterogeneity in restoration sites (Hoelzle et al 2012), which improves resistance to reinvasion at the local (Allen and Meyer 2014) and landscape scale (Anderson and Inouye 2001) and encourages recruitment of native species (Booth et al. 2003a; Brown et al. 2008).

Reduced cheatgrass abundance following imazapic application may provide a window of opportunity for native species to regain a foothold in these invaded sites, but further intervention is likely needed to allow for successful plant community recovery. Follow-up treatments may be needed in sites that are at greater risk for reinvasion as evidenced by the rapid return of

cheatgrass in some shrubland plots located in areas of high human activity. Since cheatgrass seeds typically remain viable for two years in semi-arid sites (Smith et al. 2008), continuing a treatment regime of two or three concurrent years of imazapic application is recommended to ensure the cheatgrass seedbank is effectively suppressed. When planning post-treatment management action, resource managers should assess whether the short-term objective of cheatgrass management is to restore the plant community to a pre-invasion state or to a community that will provide resistance to cheatgrass reinvasion. Finally, continued monitoring and early detection efforts will be critical in locating new accessions of cheatgrass as well as identifying expansion of existing cheatgrass populations into wilderness and high-elevation habitat within the park.

# **TABLES**

Table 1 Results of repeated measures ANOVA to assess changes in absolute and relative cheatgrass cover in response to imazapic treatments. Statistically significant P values in bold ( $\alpha$ =0.05). (Treatment: herbicide treatment and untreated reference plots; Veg Type: forest, grassland, and shrubland habitat).

Source of Variation	DF	F	Р
Absolute cover			
Between subjects			
Veg Type	2	0.75	0.55
Treatment	1	109.69	<0.01
Veg Type x Treatment	2	1.14	0.33
Within subjects			
Time	5	9.97	<0.01
Time x Treatment	5	12.74	<0.01
Time x Veg Type	10	2.53	0.03
Time x Veg Type x Treatment	10	2.07	0.06
Relative cover			
Between subjects			
Veg Type	2	0.27	0.78
Treatment	1	186.60	<0.01
Veg Type x Treatment	2	0.18	0.84
Within subjects			
Time	5	6.85	<0.01
Time x Treatment	5	9.50	<0.01
Time x Veg Type	10	3.65	<0.01
Time x Veg Type x Treatment	10	2.52	0.03

Table 2. Results of repeated measures ANOVA to assess changes in native plant species absolute and relative cover and species richness. Statistically significant P values in bold ( $\alpha$ =0.05). (Treatment: herbicide treatment and untreated reference plots; Veg Type: forest, grassland, and shrubland habitat).

Source of Variation	DF	F	Р
Absolute cover			
Between subjects			
Veg Type	2	0.45	0.67
Treatment	1	57.76	<0.01
Veg Type x Treatment	2	0.50	0.61
Within subjects			
Time	5	4.27	0.00
Time x Treatment	5	1.84	0.14
Time x Veg Type	10	1.10	0.40
Time x Veg Type x Treatment	10	0.86	0.58
Relative cover			
Between subjects			
Veg Type	2	0.05	0.95
Treatment	1	27.15	<0.01
Veg Type x Treatment	2	0.63	0.54
Within subjects			
Time	5	6.27	0.00
Time x Treatment	5	4.97	0.00
Time x Veg Type	10	2.54	0.02
Time x Veg Type x Treatment	10	0.84	0.59
Species richness			
Between subjects			
Veg Type	2	5.05	0.11
Treatment	1	30.35	<0.01
Veg Type x Treatment	2	0.95	0.40
Within subjects			
Time	4	1.99	0.13
Time x Treatment	4	1.09	0.38
Time x Veg Type	8	1.98	0.09
Time x Veg Type x Treatment	8	1.68	0.15

Table 3. Results of repeated measures ANOVA for native forb species absolute and relative cover and species richness. Statistically significant P values in bold ( $\alpha$ =0.05). (Treatment: herbicide treatment and untreated reference plots; Veg Type: forest, grassland, and shrubland habitat).

Source of Variation	DF	F	Р
Absolute cover			
Between subjects			
Veg Type	2	2.06	0.14
Treatment	1	7.67	<0.01
Veg Type x Treatment	2	7.41	<0.01
Within subjects			
Time	5	3.79	0.01
Time x Treatment	5	2.35	0.06
Time x Veg Type	10	0.65	0.76
Time x Veg Type x Treatment	10	1.08	0.41
Relative cover			
Between subjects			
Veg Type	2	10.99	<0.01
Treatment	1	0.89	0.35
Veg Type x Treatment	2	10.32	<0.01
Within subjects			
Time	5	3.10	0.02
Time x Treatment	5	2.54	0.05
Time x Veg Type	10	1.01	0.46
Time x Veg Type x Treatment	10	0.50	0.88
Species richness			
Between subjects			
Veg Type	2	0.36	0.73
Treatment	1	31.31	<0.01
Veg Type x Treatment	2	1.97	0.16
Within subjects			
Time	4	3.92	0.01
Time x Treatment	4	0.64	0.64
Time x Veg Type	8	0.78	0.62
Time x Veg Type x Treatment	8	1.12	0.38

Table 4. Results of repeated measures ANOVA for native graminoid species absolute and relative cover and species richness. Statistically significant P values in bold ( $\alpha$ =0.05). (Treatment: herbicide treatment and untreated reference plots; Veg Type: forest, grassland, and shrubland habitat).

Source of Variation	DF	F	Р
Absolute cover			
Between subjects			
Veg Type	2	1.28	0.41
Treatment	1	31.06	<0.01
Veg Type x Treatment	2	2.82	0.08
Within subjects			
Time	5	3.51	0.01
Time x Treatment	5	0.96	0.46
Time x Veg Type	10	1.11	0.38
Time x Veg Type x Treatment	10	0.93	0.52
Relative cover			
Between subjects			
Veg Type	2	2.01	0.29
Treatment	1	8.75	<0.01
Veg Type x Treatment	2	2.22	0.13
Within subjects			
Time	5	0.37	0.86
Time x Treatment	5	1.02	0.42
Time x Veg Type	10	1.50	0.19
Time x Veg Type x Treatment	10	0.78	0.64
Species richness			
Between subjects			
Veg Type	2	0.19	0.84
Treatment	1	0.83	0.37
Veg Type x Treatment	2	0.17	0.84
Within subjects			
Time	4	0.87	0.50
Time x Treatment	4	1.11	0.37
Time x Veg Type	8	0.33	0.94
Time x Veg Type x Treatment	8	0.12	1.00

Table 5. Results of repeated measures ANOVA for native shrub species absolute and relative cover and species richness. Statistically significant P values in bold ( $\alpha$ =0.05). (Treatment: herbicide treatment and untreated reference plots; Veg Type: forest, grassland, and shrubland habitat).

Source of Variation	DF	F	Р
Absolute cover			
Between subjects			
Veg Type	2	3.16	0.19
Treatment	1	1.96	0.17
Veg Type x Treatment	2	3.91	0.03
Within subjects			
Time	5	0.04	1.00
Time x Treatment	5	0.16	0.97
Time x Veg Type	10	0.13	1.00
Time x Veg Type x Treatment	10	0.02	1.00
Relative cover			
Between subjects			
Veg Type	2	5.09	0.12
Treatment	1	0.53	0.47
Veg Type x Treatment	2	3.10	0.06
Within subjects			
Time	5	0.32	0.90
Time x Treatment	5	0.28	0.92
Time x Veg Type	10	0.09	1.00
Time x Veg Type x Treatment	10	0.06	1.00
Species richness			
Between subjects			
Veg Type	2	8.22	0.06
Treatment	1	1.84	0.19
Veg Type x Treatment	2	2.33	0.12
Within subjects			
Time	4	1.44	0.25
Time x Treatment	4	0.43	0.79
Time x Veg Type	8	0.48	0.86
Time x Veg Type x Treatment	8	0.37	0.93

Table 6. Results of repeated measures ANOVA for invasive forb species absolute and relative cover and species richness. Statistically significant P values in bold ( $\alpha$ =0.05). (Treatment: herbicide treatment and untreated reference plots; Veg Type: forest, grassland, and shrubland habitat).

Source of Variation	DF	F	Р
Absolute cover			
Between subjects			
Veg Type	2	0.87	0.50
Treatment	1	4.79	0.04
Veg Type x Treatment	2	8.33	<0.01
Within subjects			
Time	5	0.24	0.94
Time x Treatment	5	0.08	1.00
Time x Veg Type	10	0.25	0.99
Time x Veg Type x Treatment	10	0.24	0.99
Relative cover			
Between subjects			
Veg Type	2	0.92	0.49
Treatment	1	5.60	0.02
Veg Type x Treatment	2	7.76	<0.01
Within subjects			
Time	5	0.15	0.98
Time x Treatment	5	0.13	0.99
Time x Veg Type	10	0.16	1.00
Time x Veg Type x Treatment	10	0.19	1.00
Species richness			
Between subjects			
Veg Type	2	0.13	0.88
Treatment	1	2.22	0.15
Veg Type x Treatment	2	2.16	0.14
Within subjects			
Time	4	1.82	0.16
Time x Treatment	4	0.43	0.79
Time x Veg Type	8	0.87	0.55
Time x Veg Type x Treatment	8	1.01	0.45

Table 7. Results of repeated measures ANOVA for invasive graminoid species absolute and relative cover and species richness. Statistically significant P values in bold ( $\alpha$ =0.05). (Treatment: herbicide treatment and untreated reference plots; Veg Type: forest, grassland, and shrubland habitat).

Source of Variation	DF	F	Р
Absolute cover			
Between subjects			
Veg Type	2	1.36	0.52
Treatment	1	1.87	0.26
Veg Type x Treatment	1	0.28	0.63
Within subjects			
Time	4	12.00	0.04
Time x Treatment	4	0.97	0.47
Time x Veg Type	7	3.43	0.09
Time x Veg Type x Treatment	3	0.40	0.47
Relative cover			
Between subjects			
Veg Type	2	1.35	0.38
Treatment	1	8.82	<0.01
Veg Type x Treatment	2	6.13	<0.01
Within subjects			
Time	5	10.15	<0.01
Time x Treatment	5	1.82	0.08
Time x Veg Type	10	2.21	0.10
Time x Veg Type x Treatment	10	2.09	0.06
Species richness			
Between subjects			
Veg Type	2	0.52	0.64
Treatment	1	9.60	<0.01
Veg Type x Treatment	2	2.38	0.11
Within subjects			
Time	4	10.76	<0.01
Time x Treatment	4	1.14	0.36
Time x Veg Type	8	2.29	0.05
Time x Veg Type x Treatment	8	0.64	0.74

Table 8. Results of repeated measures ANOVA for non-invasive non-native species absolute and relative cover and species richness. Statistically significant P values in bold ( $\alpha$ =0.05). (Treatment: herbicide treatment and untreated reference plots; Veg Type: forest, grassland, and shrubland habitat).

Source of Variation	DF	F	Р
Absolute cover			
Between subjects			
Veg Type	2	0.06	0.94
Treatment	1	0.01	0.91
Veg Type x Treatment	2	1.79	0.19
Within subjects			
Time	5	1.68	0.17
Time x Treatment	5	4.54	<0.01
Time x Veg Type	10	1.25	0.30
Time x Veg Type x Treatment	10	0.72	0.70
Relative cover			
Between subjects			
Veg Type	2	0.12	0.89
Treatment	1	0.44	0.52
Veg Type x Treatment	2	6.53	0.03
Within subjects			
Time	5	1.89	0.20
Time x Treatment	5	3.10	0.07
Time x Veg Type	10	5.18	0.02
Time x Veg Type x Treatment	10	4.85	0.03
Species richness			
Between subjects			
Veg Type	2	0.02	0.98
Treatment	1	8.03	0.01
Veg Type x Treatment	2	0.98	0.39
Within subjects			
Time	4	0.84	0.51
Time x Treatment	4	4.98	<0.01
Time x Veg Type	8	0.62	0.75
Time x Veg Type x Treatment	8	2.51	0.04

Table 9. Results of repeated measures ANOVA for litter absolute cover. Statistically significant P values in bold ( $\alpha$ =0.05). (Treatment:herbicide treatment and untreated reference plots; Veg Type: forest, grassland, and shrubland habitat).

Source of Variation	DF	F	Р
Absolute cover			
Between Subjects			
Veg Type	2	1.16	0.43
Treatment	1	0.76	0.39
Veg Type x Treatment	2	2.50	0.10
Within Subjects			
Time	5	4.18	0.01
Time x Treatment	5	2.39	0.07
Time x Veg Type	9	1.04	0.44
Time x Veg Type x	9	0.60	0.78

Table 10. Results of repeated measures ANOVA for absolute bare soil cover. Statistically significant P values in bold ( $\alpha$ =0.05). (Treatment: herbicide treatment and untreated reference plots; Veg Type: forest, grassland, and shrubland habitat).

Source of Variation	DF	F	Р
Absolute cover			
Between subjects			
Veg Type	2	0.09	0.92
Treatment	1	30.31	<0.01
Veg Type x Treatment	2	6.05	<0.01
Within subjects			
Time	5	2.91	0.03
Time x Treatment	5	1.20	0.33
Time x Veg Type	10	1.11	0.39
Time x Veg Type x Treatment	10	0.40	0.93

Table 11. Results of repeated measures ANOVA for Shannon-Weiner diversity index. Statistically significant P values in bold ( $\alpha$ =0.05). (Treatment: herbicide treatment and untreated reference plots; Veg Type: forest, grassland, and shrubland habitat).

Source of Variation	DF	F	
Source or variation	DF	Г	Р
Absolute cover			
Between subjects			
Veg Type	2	1.93	0.30
Treatment	1	29.82	<0.01
Veg Type x Treatment	2	0.93	0.41
Within subjects			
Time	4	4.05	0.01
Time x Treatment	4	3.60	0.02
Time x Veg Type	8	1.48	0.22
Time x Veg Type x Treatment	8	1.71	0.14

Table 12. Repeated measures ANOVA to assess changes in the Sorenson species similarity index over time. Statistically significant P values in bold ( $\alpha$ =0.05).

Source of Variation	DF	Sum of squares	Mean square	F	Р	
Sorenson Index						
Year	4	0.00822	0.0021	0.13	0.97	
Error	24	0.38707	0.0161			
Total	28	0.39529				

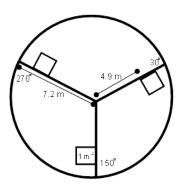


Fig. 1. Circular nested plot design for monitoring vegetation at the treatment and reference sites.

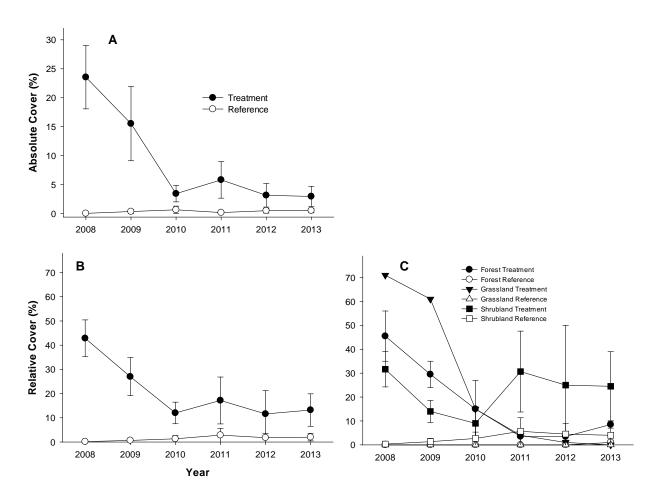
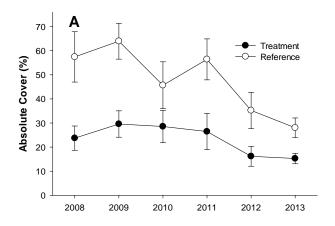
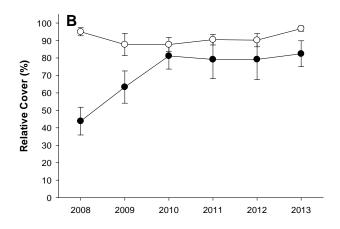


Fig. 2. Changes in absolute (A) and relative (B & C) cheatgrass cover over time in plots treated with imazapic (mean ± one standard error of the mean). (Treatment: herbicide treatment plots treated in 2009, 2010, & 2011; Reference: untreated and uninvaded reference plots)





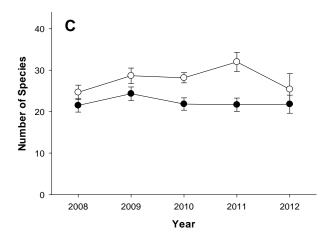


Fig. 3. Total native species absolute (A) and relative (B) cover and species richness (C) response to imazapic application (mean  $\pm$  one standard error of the mean). Tree cover data were omitted to eliminate effects caused by the lack of tree canopy cover data for the 2010 season. (Treatment: herbicide treatment plots treated in 2009, 2010, & 2011; Reference: untreated and uninvaded reference plots)

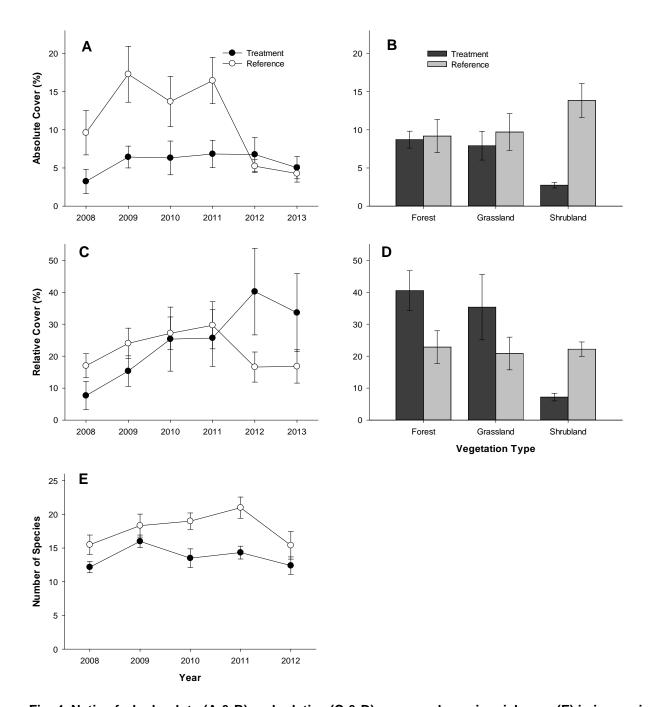
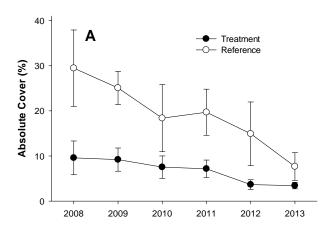
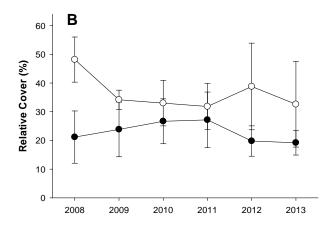


Fig. 4. Native forb absolute (A & B) and relative (C & D) cover and species richness (E) in imazapic treatment and reference plots (mean  $\pm$  one standard error of the mean). (Treatment: herbicide treatment plots treated in 2009, 2010, & 2011; Reference: untreated and uninvaded reference plots)





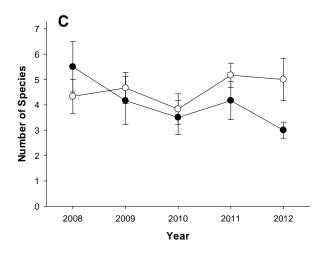


Fig. 5. Native graminoid absolute (A) and relative (B) cover and species richness (C) response in imazapic treatment plots and untreated reference plots (mean ± one standard error of the mean). (Treatment: herbicide treatment plots treated in 2009, 2010, & 2011; Reference: untreated and uninvaded reference plots)

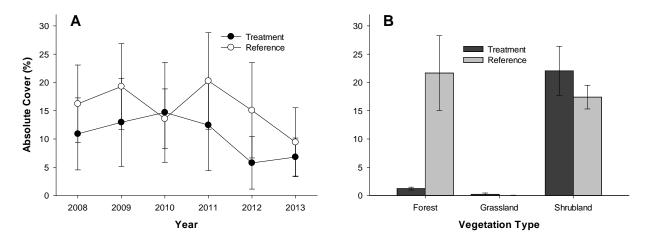


Fig. 6. Native shrub absolute cover (A) and species richness (B) in treated and untreated plots (mean ± one standard error of the mean). (Treatment: herbicide treatment plots treated in 2009, 2010, & 2011; Reference: untreated and uninvaded reference plots)

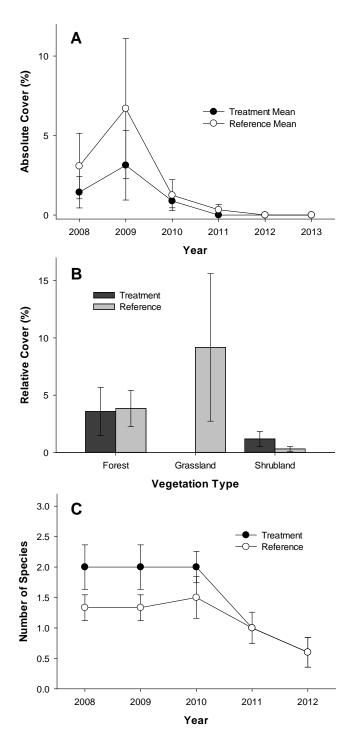


Fig. 7. Invasive graminoid absolute (A) and relative (B) cover and species richness (C) response in treated and untreated plots (mean ± one standard error of the mean). (Treatment: herbicide treatment plots treated in 2009, 2010, & 2011; Reference: untreated and uninvaded reference plots)

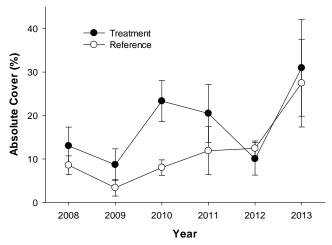


Fig. 8. Change in litter cover in treated and untreated plots (mean  $\pm$  one standard error of the mean). (Treatment: herbicide treatment plots treated in 2009, 2010, & 2011; Reference: untreated and uninvaded reference plots)

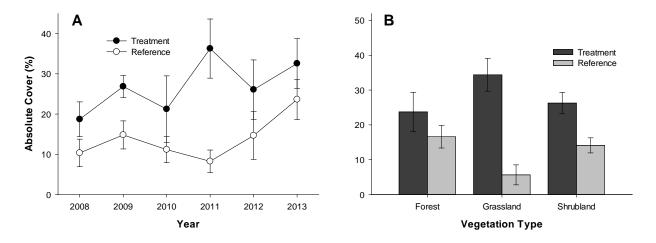


Fig. 9. Bare soil absolute cover in treated and untreated plots over time (A) and by vegetation type (B) (mean ± one standard error of the mean). (Treatment: herbicide treatment plots treated in 2009, 2010, & 2011; Reference: untreated and uninvaded reference plots)

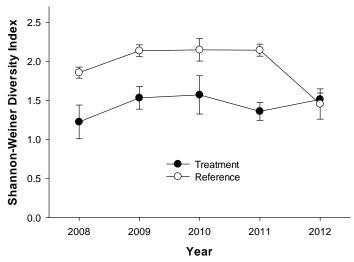


Fig. 10. Shannon-Weiner diversity index comparing plant community evenness between imazapic treatment plots and untreated reference plots over time (mean  $\pm$  one standard error of the mean). (Treatment: herbicide treatment plots treated in 2009, 2010, & 2011; Reference: untreated and uninvaded reference plots)

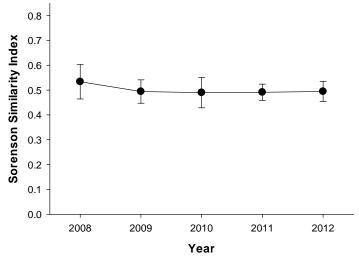


Fig. 11. Sorenson similarity indices comparing plant community similarity in imazapic treatment and untreated reference plots over time (mean ± one standard error of the mean).

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