

# ROCKY MOUNTAIN NATIONAL PARK 2013 REVEGETATION AND EXOTIC SPECIES MANAGEMENT MONITORING REPORT



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# EFFECTS OF AMINOPYRALID APPLICATION ON CANADA THISTLE (*Cirsium arvense*) AND NATIVE PLANT SPECIES IN ROCKY MOUNTAIN NATIONAL PARK

## ABSTRACT

Canada thistle is an aggressive invasive weed that readily colonizes disturbances resulting in species diversity losses among native plant species and reduced forage for grazing animals. Aminopyralid is an herbicide used in Rocky Mountain National Park for the control of Canada thistle infestations. While aminopyralid is an effective method of Canada thistle control, studies have shown that some native plant species can be injured by broadcast application of this herbicide. The purpose of this study is to determine the effectiveness of aminopyralid in controlling Canada thistle infestations in montane meadows of Rocky Mountain National Park, as well as what effects it has on non-target species. In the summer of 2011, five treatment and five control plots were established in areas of Upper Beaver Meadows that were infested with Canada thistle and treatment plots were treated with aminopyralid (40.6% a.i.). As of 2013, aminopyralid has significantly reduced Canada thistle density and there have been no non-target effects.

## INTRODUCTION

Canada thistle (*Cirsium arvense*) is a perennial invasive forb in the family Asteraceae that is common throughout much of North America and reproduces by seed as well as clonally by vegetative budding (Beck 2008). It is listed as a List B noxious weed by the state of Colorado, meaning its control and eradication is a priority in the state. The control of this exotic species has been a focus of the resources stewardship division of Rocky Mountain National Park (RMNP) since the 1960s (NPS 1987). Canada thistle is highly competitive with native plant species and can result in reduced species diversity in grasslands, though ecological impacts of Canada thistle are less understood in most natural, non-crop areas (Enloe et al. 2007).

Application of the herbicide aminopyralid is included in the methods approved for control of Canada thistle in RMNP. Aminopyralid has been shown to be a moderately selective and cost-effective means of controlling Canada thistle infestations in restored tallgrass prairie and other rangeland sites and is comparable to other herbicides used for the same purpose (Almquist et al. 2010, Enloe et al. 2007). Aminopyralid is particularly effective against broadleaf species in the families Asteraceae, Fabaceae and Solanaceae (Dow 2005). While many non-target native grass and forb species are tolerant to aminopyralid treatment, studies have shown that aminopyralid can cause changes in species richness and diversity as well as changes in decomposition rates and soil microbial communities (Almquist et al. 2010, Enloe et al. 2007, Prietkel et al. 2006). Some species of the Rosaceae family have been shown to be significantly impacted by non-target effects of aminopyralid application, with injury rates as high as 77% (Duncan 2009).

The purpose of this study is to determine the effectiveness of aminopyralid in controlling Canada thistle and its effects on non-target native species in the Upper Beaver Meadows area of RMNP.

## MATERIALS AND METHODS

### *Herbicide Application*

In September of 2011 and 2012, RMNP's exotic plant management crew treated Canada thistle infested sites in Upper Beaver Meadows with aminopyralid (Milestone, 40.6% a.i., DOW, Indianapolis, USA) using a boomless sprayer attachment on a 2010 Bobcat 2300 utility vehicle (Bobcat, West Fargo, USA). Aminopyralid was broadcast at a rate of 208 ml a.i./ha (2.8 oz a.i./ac).

### *Monitoring Site Selection*

Canada thistle infestations in the Upper Beaver Meadows (UBM) area of RMNP were selected for inclusion in the pool of potential monitoring plots if an infestation was dense enough to warrant broadcast application of aminopyralid. After surveying UBM, 26 potential infestations were chosen as candidates for herbicide treatment. From this pool of 26 infestation sites, 10 sites were randomly chosen for inclusion in this study. From those 10 sites, five sites were randomly assigned to be treated with aminopyralid and five sites were chosen as untreated controls. Sites were chosen using a random number table to ensure randomization.

In the fall of 2012, control plots and treatment plots were all treated with aminopyralid using the previously described methods.

### *Vegetation Monitoring*

In 2011, permanent circular nested vegetation monitoring plots (CNP), each with an area of approximately 168.2 m<sup>2</sup> (1810 ft<sup>2</sup>), or 0.01682 ha (1/24 of an acre), were installed at each site. The design of these plots consists of a circular plot with three 7.2 m (24 ft) spokes at 30, 150 and 270 degrees. A 1 m<sup>2</sup> quadrat is located on each of these spokes 4.9 m (16 ft) from the center of the plot (see Fig. 1). Detailed plot installation and monitoring instructions can be found in Appendix 1.

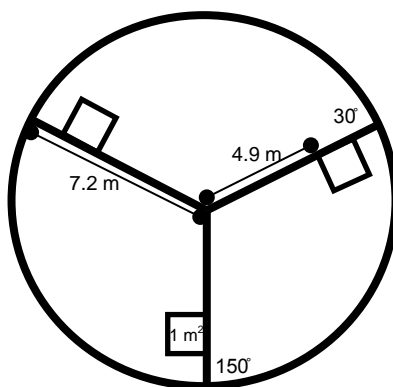


Fig. 1 Circular nested plots used to monitor vegetation at the treatment and reference sites.

Percent cover was estimated within each of the three 1 m<sup>2</sup> quadrats. Each species present in the quadrat was recorded and an ocular estimation of percent cover for the individual species

was made. Percent cover was also estimated for bare ground, litter, rock, moss and lichen and animal scat. Tree, shrub and forb percent cover were estimated using canopy cover while graminoid cover was estimated using cover at ground level (basal cover). Percent cover was estimated using modified Daubenmire cover classes (Daubenmire 1959) as ranges of percent cover. All species within the CNP but not observed within the three quadrats were recorded. This was done by performing a time-constrained survey of the entire CNP.

The total number of individual Canada thistle plants within each CNP was also counted to determine Canada thistle densities. A Canada thistle plant was considered to be inside the plot if it was rooted inside the perimeter of the CNP.

### *Data Analysis*

To calculate cover of a species in a plot, the midpoint percent cover for each species in each quadrat was calculated as the mean of the maximum and minimum cover range values and the average for each species across all three quadrats was calculated. Density of Canada thistle was calculated by dividing the total number of plants observed by the area of a CNP, 168.2 m to get the number of plants per square meter.

For cover analyses, the species in each CNP and their corresponding mean percent cover were then 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 habits were tree, shrub, graminoids (grass, rush and sedge species), forb, or moss and lichen and species were classified as having a particular growth habit based on their designation in the USDA PLANTS profile database. Species were classified as native or non-native based upon their designation as such in Weber and Wittmann (2001). Species were classified as invasive if they were listed on Rocky Mountain National Park's list of invasive species or the Colorado Department of Agriculture's Noxious Weed List. Species richness was also categorized using these same criteria. Cover and species richness values of species in the plant family asteraceae were also isolated and analyzed separately, as these plants are particularly sensitive to the herbicide aminopyralid.

Data were analyzed using the JMP 7 statistical analysis software package (SAS Institute, Cary, NC, USA). The data were analyzed for each of the species categories using analysis of variance (ANOVA). For each species category, single-factor ANOVA was used to determine if there were differences in species cover between plots assigned to control and herbicide treatments before the treatments were applied. If a significant ( $\alpha=0.05$ ) main effect or interaction between variables was found, this interaction was graphed using Microsoft Excel.

## **RESULTS/DISCUSSION**

### ***Canada thistle density***

Canada thistle density decreased over time in response to treatment with aminopyralid (Table 1, time x treatment). In 2011, prior to treatment, Canada thistle density averaged 5.2 plants/m<sup>2</sup> in both treatment and control plots. In 2012, following the first year's treatment, Canada thistle

density decreased to 1.1 plants/m<sup>2</sup> in treatment plots while Canada thistle densities did not change in untreated control plots (Fig. 2). In the fall of 2012 both treatment plots and control plots were treated with herbicide resulting in a reduction of Canada thistle to 0.03 plants/m<sup>2</sup> and 0.003 plants/m<sup>2</sup> in control and treatment plots, respectively.

Table 1. ANOVA for Canada thistle density. Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Between Subjects			
Treatment	1	1.10	0.3250
Within Subjects			
Time	2	6.69	<b>0.0237</b>
Time x Treatment	2	6.89	<b>0.0222</b>

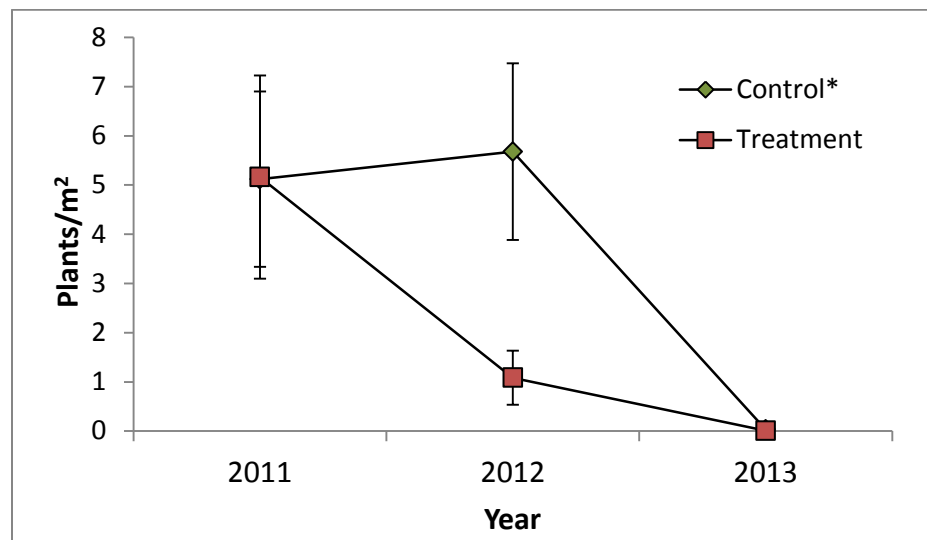


Fig. 2. Change in Canada thistle density over time (mean  $\pm$  one standard error of the mean).

\*Control plots were treated with herbicide between 2012 and 2013 data collection.

### ***Native species functional groups***

Native aster species cover was greater in control plots averaged over all study years (Table 2, treatment; Fig. 2). Native aster cover in control plots exhibited an upward trend in cover in 2013, while native aster cover in treatment plots remained at the previous year's lower level. These results suggest that after one and two years of herbicide application, native aster species did not experiencing significant negative effects from broadcast aminopyralid application. There was no change in native aster species richness in response to treatment (Table 3).

Table 2. ANOVA for native *Asteraceae* species cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	5.35	<b>0.0495</b>
Within Subjects			
Time	2	3.19	0.1036
Time x Treatment	2	1.90	0.2198

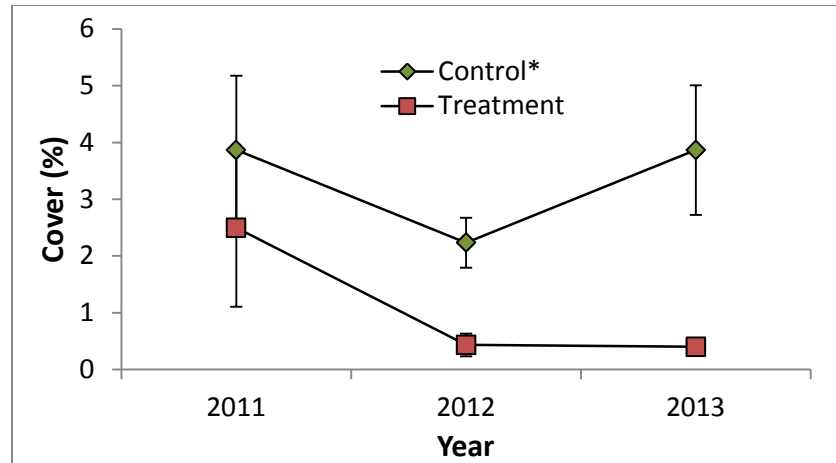


Fig. 2. Change in native *Asteraceae* species cover over time (mean  $\pm$  one standard error of the mean). \*Control plots were treated with herbicide between 2012 and 2013 data collection.

Table 3. ANOVA for native *Asteraceae* species richness. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	0.51	0.4970
Within Subjects			
Time	2	3.93	0.0716
Time x Treatment	2	4.02	0.0687

Native forb cover and richness for forbs of all families including asters did not change over time or in response to herbicide treatments (Table 4 & 5), indicating no negative effects of broadcast aminopyralid application.

Table 4. ANOVA for native forb cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	0.02	0.8808
Within Subjects			
Time	2	3.02	0.1136
Time x Treatment	2	2.58	0.1452

Table 5. ANOVA for native forb richness . Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	2.19	0.1771
Within Subjects			
Time	2	1.09	0.3867
Time x Treatment	2	0.50	0.6250

Native graminoid cover and richness did not change over time or in response to herbicide treatments (Table 6 & 7), indicating no negative effects of broadcast aminopyralid application. There was a marginally significant time by treatment effect mostly due to an increase from 47% to 74% native graminoid cover in control plots between 2012 and 2013.

Table 6. ANOVA for native graminoid cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	3.75	0.0887
Within Subjects			
Time	2	4.09	0.0667
Time x Treatment	2	2.24	0.1768

Table 7. ANOVA for native graminoid richness . Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	0.42	0.5358
Within Subjects			
Time	2	1.93	0.2148
Time x Treatment	2	0.21	0.8152

Native shrub cover and richness for shrubs of all families including asters did not change over time or in response to herbicide treatments (Table 8 & 9), indicating no negative effects of broadcast aminopyralid application.

Table 8. ANOVA for native shrub cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	0.78	0.4027
Within Subjects			
Time	2	2.03	0.2018
Time x Treatment	2	1.20	0.3553

Table 9. ANOVA for native shrub richness. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	1.83	0.2126
Within Subjects			
Time	2	2.08	0.1953
Time x Treatment	2	0.13	0.8822

Total cover for all native species did not change over time or in response to herbicide treatments (Table 10 & 11), indicating no negative effects of broadcast aminopyralid application. There was a marginally significant time by treatment effect mostly due to an increase from 53% to 86% native species cover in control plots between 2012 and 2013. This increase was due to an increase in native grass species as described above.

Table 10. ANOVA for total native species cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	2.91	0.1263
Within Subjects			
Time	2	3.38	0.0937
Time x Treatment	2	3.78	0.0769

Table 11. ANOVA for total native spp richness. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	2.61	0.1451
Within Subjects			
Time	2	1.92	0.2169
Time x Treatment	2	0.35	0.7135

### ***Invasive Species Functional Groups***

Invasive aster species cover decreased in both control and treatment plots over time, though the rate of this decrease depended on treatment type (Table 12, time x treatment). Invasive aster cover was more than five times greater in control plots in 2012, but this difference was not observed in 2013 (Fig. 3). This functional group is primary comprised of Canada thistle cover, and in 2012 cover was significantly lower in treatment plots, indicating successful reduction of Canada in treatment plots. In 2013 all plots had been treated with herbicide, and



cover of invasive asters was the same in both plot types. This indicates that Canada thistle cover was successfully reduced in all plots with aminopyralid application. There was no change in invasive aster species richness (Table 13).

Table 12. ANOVA for invasive *Asteraceae* species cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	1.81	0.2149
Within Subjects			
Time	2	4.70	0.0508
Time x Treatment	2	4.75	<b>0.0497</b>

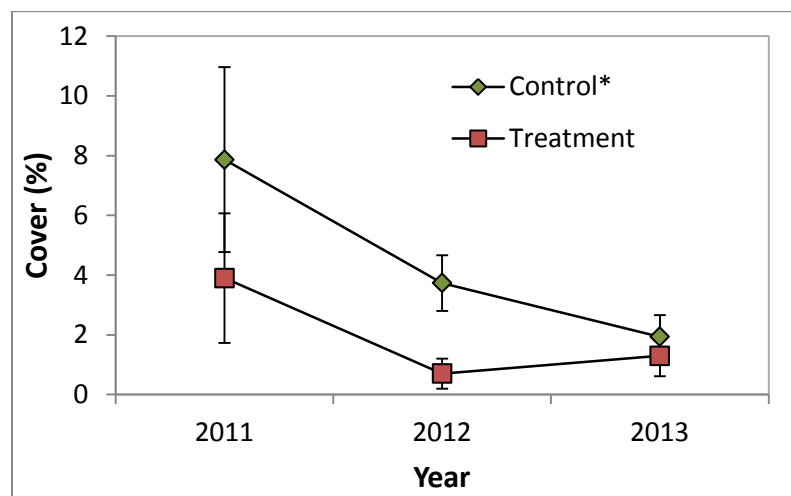


Fig. 3. Change in invasive *Asteraceae* species cover over time (mean  $\pm$  one standard error of the mean). \*Control plots were treated with herbicide between 2012 and 2013 data collection.

Table 13. ANOVA for invasive *Asteraceae* species richness. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	2.25	0.1720
Within Subjects			
Time	2	1.17	0.3654
Time x Treatment	2	1.17	0.3654

Invasive forb cover and richness for all families including asters did not change over time in response to herbicide treatments (Table 14 & 15). There was a marginally significant reduction in average invasive forb cover over time (Table 14, time). The lack of a significant reduction in invasive forb cover, which is mostly comprised of Canada thistle, is most likely due to the inclusion of non-target invasive forb species in this functional group.

Table 14. ANOVA for invasive forb cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	0.58	0.4678
Within Subjects			
Time	2	4.56	0.0539
Time x Treatment	2	3.14	0.1064

Table 15. ANOVA for invasive forb richness. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	0.17	0.6938
Within Subjects			
Time	2	1.17	0.3654
Time x Treatment	2	1.17	0.3654

Invasive graminoid cover averaged over both treatment types decreased over time (Table 16, time). This reduced cover was mainly due to the nearly three-fold decline of timothy (*Phleum pratense*) in these plots (Fig. 4). The 2012 growing season was very dry on these sites, and timothy has been shown to be sensitive to drought conditions as well as being slower to recover from drought stress when compared to other grasses and forbs (Signarbieux and Feller 2011, Okomato et al. 2011). Invasive graminoid richness did not change over time (Table 17).

Table 16. ANOVA for invasive graminoid cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	0.52	0.4933
Within Subjects			
Time	2	10.17	<b>0.0085</b>
Time x Treatment	2	0.91	0.4450

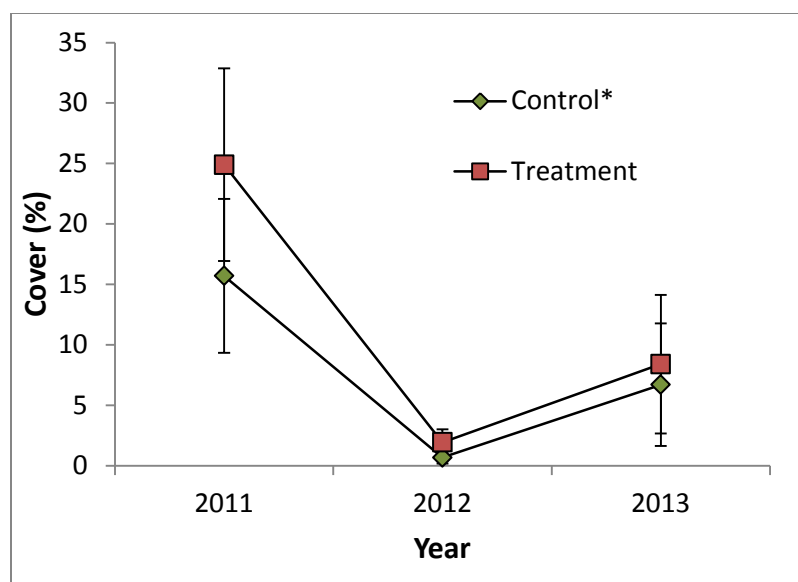


Fig. 4. Change in invasive graminoid cover over time (mean  $\pm$  one standard error of the mean). \*Control plots were treated with herbicide between 2012 and 2013 data collection.

Table 17. ANOVA for invasive graminoid species richness. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	0.04	0.8383
Within Subjects			
Time	2	3.79	0.0766
Time x Treatment	2	3.79	0.0766

Total invasive species cover averaged over both treatment types decreased over time (Table 18, time, Fig. 5). This reduced cover was mainly due to the decline of timothy (*Phleum pratense*) in these plots (see invasive graminoid cover above). Total invasive species richness averaged over both treatment types also decreased over time (Table 18, time, Fig. 5). This change was the result of losing an average of one type of invasive species per plot in the 2012 season (Fig. 6). As 2012 was a dry year and many of the exotic species in RMNP are annual species, this small drop in the number of invasive species is likely explained by this, as evidenced by the recovery of invasive richness in the wetter 2013 season.

Table 18. ANOVA for total invasive species cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	0.17	0.6901
Within Subjects			
Time	2	9.40	<b>0.0104</b>
Time x Treatment	2	0.25	0.7841

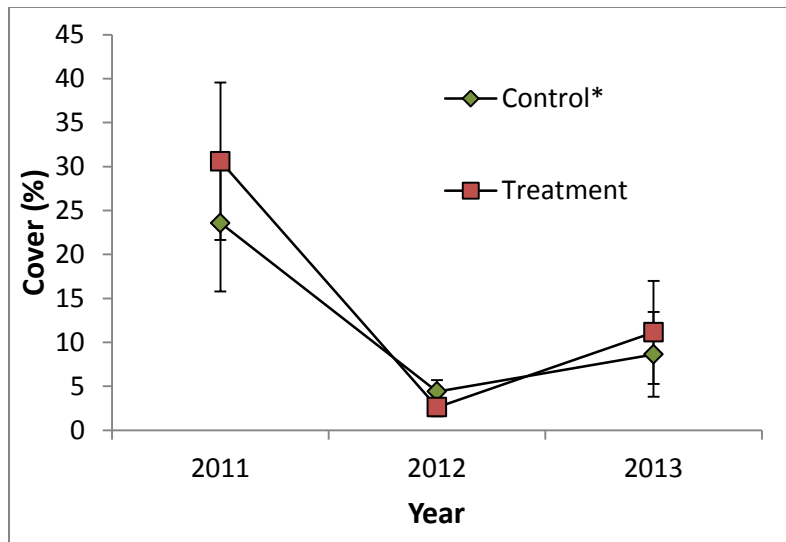


Fig. 5. Change in total invasive species cover over time (mean  $\pm$  one standard error of the mean). \*Control plots were treated with herbicide between 2012 and 2013 data collection.

Table 19. ANOVA for invasive species richness. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	0.00	1.0000
Within Subjects			
Time	2	6.10	<b>0.0293</b>
Time x Treatment	2	0.27	0.7729

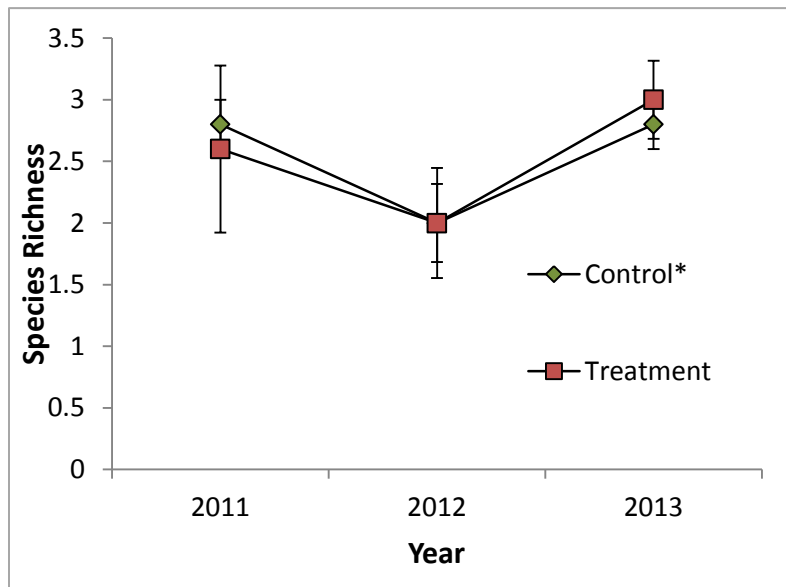


Fig. 6. Change in invasive species richness over time (mean  $\pm$  one standard error of the mean). \*Control plots were treated with herbicide between 2012 and 2013 data collection.

### **Non-native non-invasive species**

Non-native non-invasive (NNNI) aster species cover and richness did not change over time in response to herbicide treatments (Table 20 & 21).

Table 20. ANOVA for non-native non-invasive *Asteraceae* cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	1.07	0.3308
Within Subjects			
Time	2	2.78	0.1294
Time x Treatment	2	0.64	0.5545

Table 21. ANOVA for non-native non-invasive *Asteraceae* species richness. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	0.91	0.3670
Within Subjects			
Time	2	2.86	0.1234
Time x Treatment	2	2.51	0.1507

There was no change in NNNI forb cover over time (Table 22). Species richness of NNNI forbs averaged over both treatment types increased over time from approximately two species in 2011 and 2012 to three species in 2013 (Table 23, Fig. 7). This was mostly due to an increase in the number of observations of field pennycress (*Thlaspi arvense*) in control plots in 2013.

Table 22. ANOVA for non-native non-invasive forb cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	0.57	0.4705
Within Subjects			
Time	2	3.53	0.0869
Time x Treatment	2	0.13	0.8837

Table 23. ANOVA for non-native non-invasive forb species richness. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	1.29	0.2882
Within Subjects			
Time	2	9.63	<b>0.0098</b>
Time x Treatment	2	3.54	0.0867

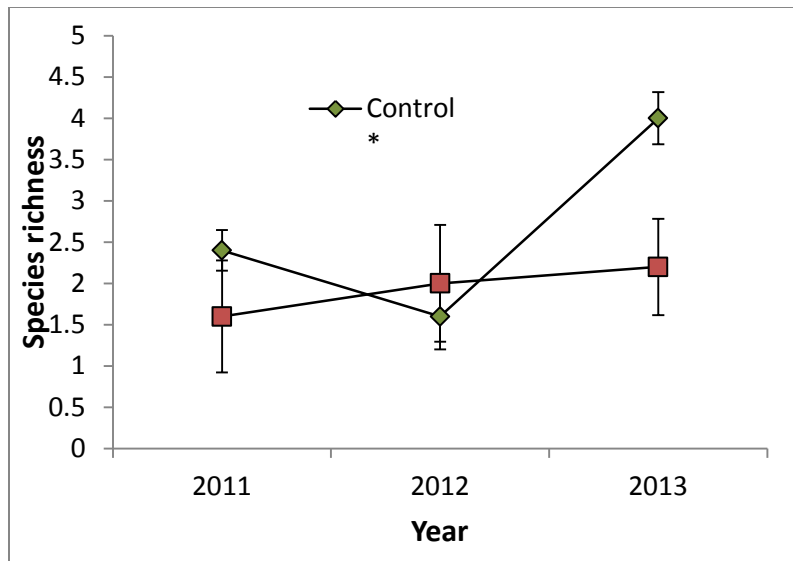


Fig. 7. Change in non-native non-invasive species richness over time (mean  $\pm$  one standard error of the mean).  
 \*Control plots were treated with herbicide between 2012 and 2013 data collection.

There was no change in NNNI graminoid species cover or richness over time (Table 24 & 25).

Table 24. ANOVA for non-native non-invasive graminoid cover . Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	0.33	0.5798
Within Subjects			
Time	2	1.01	0.4121
Time x Treatment	2	0.15	0.8670

Table 25. ANOVA for non-native non-invasive graminoid species richness. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	0.33	0.5796
Within Subjects			
Time	2	3.65	0.0822
Time x Treatment	2	0.15	0.8669

There was no change in total NNNI species cover over time (Table 26). Total species richness of NNNI species averaged over both treatment types increased over time from approximately two

species in 2011 and 2012 to three species in 2013 (Table 27, time; Fig. 8). Again, this was mostly due to an increase in the number of observations of field pennycress (*Thlaspi arvense*) in control plots in 2013.

Table 26. ANOVA for total non-native non-invasive species cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	0.88	0.3747
Within Subjects			
Time	2	0.93	0.4368
Time x Treatment	2	0.27	0.7735

Table 27. ANOVA for total non-native non-invasive species richness. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	0.97	0.3533
Within Subjects			
Time	2	10.42	<b>0.0080</b>
Time x Treatment	2	3.16	0.1050

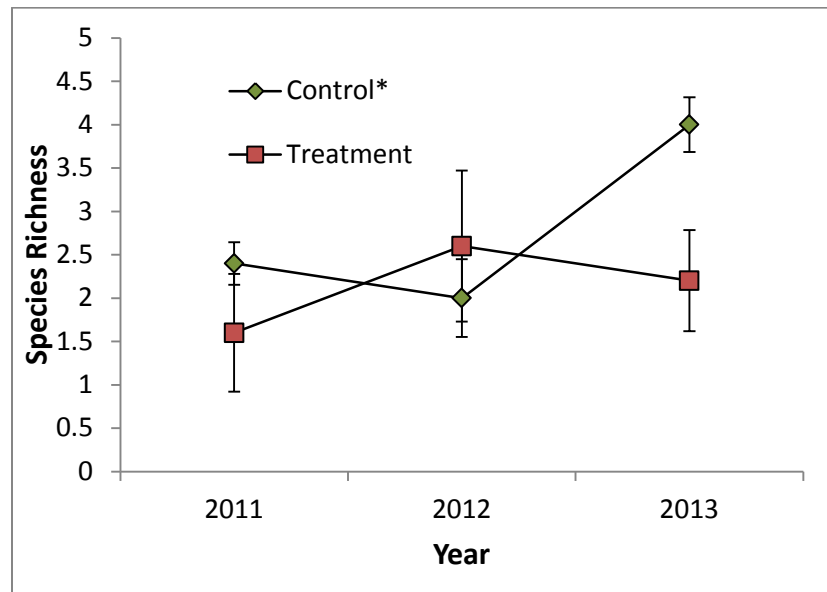


Fig. 8. Change in total non-native non-invasive species richness over time (mean  $\pm$  one standard error of the mean). \*Control plots were treated with herbicide between 2012 and 2013 data collection.

### Bare ground and Litter

Bare ground cover did not change over time, indicating there has been no denuding effect resulting from herbicide use (Table 28).

Table 28. ANOVA for bare ground cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	0.08	0.7872
Within Subjects			
Time	2	3.35	0.0954
Time x Treatment	2	0.95	0.4330

Litter cover increased in all plots from approximately 2% in 2011 to 14% in 2012, which was a drought year, likely resulting in the death and litter production of plants that year (Table 29,time; Fig. 9)

Table 29. ANOVA for litter cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	0.24	0.6371
Within Subjects			
Time	2	18.20	<b>0.0017</b>
Time x Treatment	2	1.26	0.3406

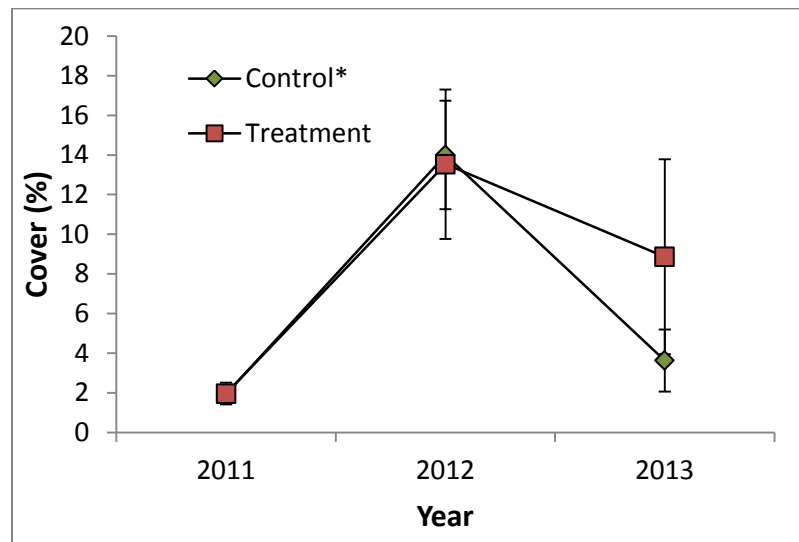


Fig. 9. Change in litter cover over time (mean  $\pm$  one standard error of the mean). \*Control plots were treated with herbicide between 2012 and 2013 data collection.



### All Plant Species

Total vegetation cover of all species averaged over treatment type changed over time from 102% in 2011 to 72% in 2012 and back up to 104% in 2013. (Table 29, time; Fig. 10). Again, this is likely due to the extremely dry conditions in 2012. There was no change in species richness for all plant species (Table 30).

Table 29. ANOVA for total vegetation cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	2.69	0.1397
Within Subjects			
Time	2	45.28	<b>&lt;.0001</b>
Time x Treatment	2	2.25	0.1765

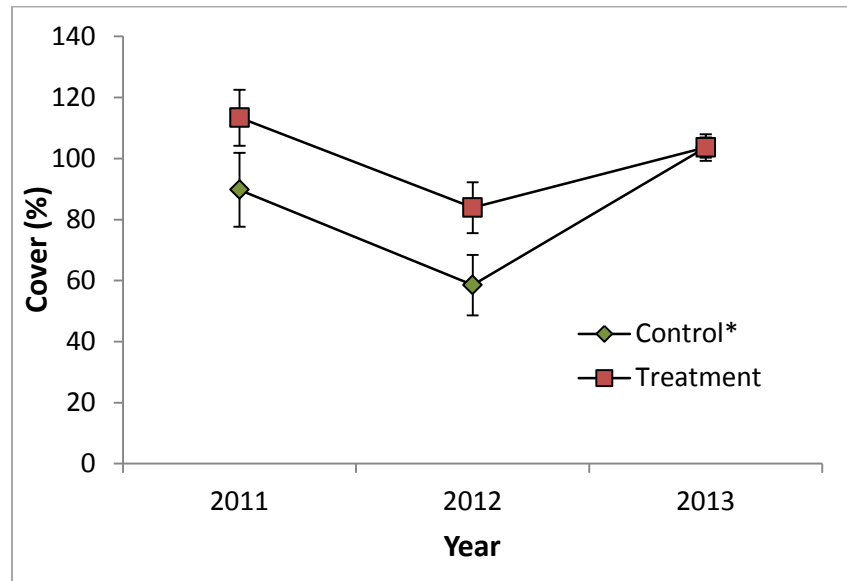


Fig. 10. Change in total vegetation cover over time (mean  $\pm$  one standard error of the mean). \*Control plots were treated with herbicide between 2012 and 2013 data collection.

Table 30. ANOVA for total vegetation cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	2.19	0.1773
Within Subjects			
Time	2	2.86	0.1235
Time x Treatment	2	0.00	0.9971

*ANOVA tables and figures for other more general plant groups not included in this results section can be found in the appendices.*

## **DISCUSSION**

After single and repeated broadcast applications, aminopyralid successfully reduced Canada thistle densities in Upper Beaver Meadows treatment plots. This supports the findings of several other studies that demonstrate that aminopyralid is a highly effective herbicide for use in controlling Canada thistle (Almqvist et al. 2010, Samuel and Lym 2008, Enloe et al. 2007).

The absence of any significant reductions in native species cover or richness following herbicide treatment suggests that non-target species were not affected by broadcast aminopyralid application in our study site. Of particular note is that the potentially sensitive native species of the plant family *Asteraceae* did not experience any changes in cover or richness following herbicide treatment.

Other studies have returned inconclusive results on the effects of aminopyralid on native plant communities, and research on the effects of aminopyralid on Canada thistle and native plant communities is limited. It appears that initial plant community composition may influence how systems respond to aminopyralid treatment. Almqvist and Lym found that aminopyralid treatment resulted in reduced species richness and cover in tallgrass prairies ten months after fall herbicide application (2010). Samuel and Lym observed an initial reduction and subsequent increase in vegetation cover and no reduction in total species richness when mixed-grass prairie sites were similarly treated with aminopyralid (2008). Samuel and Lym also detected a small reduction in native species richness in response to herbicide treatment (2008). Comparison of the species lists provided by the authors of the two previously discussed studies shows that the species observed in our study site were more similar to the Samuel and Lym study. Average annual precipitation of our study site was more like that of the Almqvist and Lym study than the Samuel and Lym study (38 cm/y and 55 cm/y, respectively) (Fig 13). The results of these studies as well as ours indicate that several factors together may influence the effects of aminopyralid on non-target plant species in treatment sites.

The most common change in vegetation we observed in all plant groups was an overall decline in vegetation cover in many functional groups independent of treatment type in 2012. This is most likely due to the droughty conditions that were experienced during what is typically the wettest part of the growing season for many plant communities at RMNP (Fig. 11).

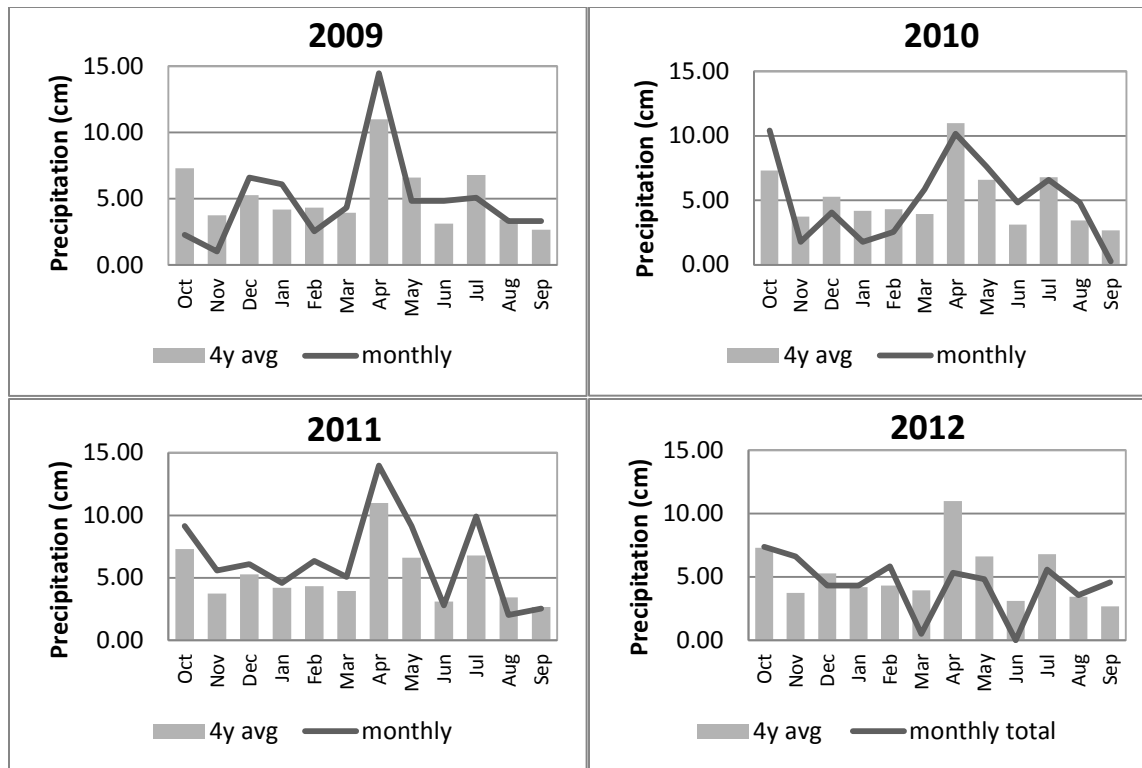


Fig 11.

Yearly accumulated precipitation at NRCS Hourglass Lake climate monitoring station in RMNP (NRCS 2012). Years indicated in the legend are water years (e.g. water year 2012 is Oct. 1, 2011 - Sep. 30, 2012). The four year average is for water years 2009-2012.

### Recommendations:

We recommend that in future seasons control plots (left untreated in 2011 and 2013 and inadvertently treated in 2012) be treated with aminopyralid along with treatment plots to discourage spread of these dense Canada thistle patches into previously treated areas. Post-treatment results from 2012 sufficiently demonstrated that aminopyralid effectively controlled Canada thistle, while most other vegetation attributes were not affected (see RMNP 2012 Vegetation Monitoring Report).

While continued treatment and monitoring of these plots is recommended to determine the effects of aminopyralid use, we think this project is a good candidate for a one, two, three, five and ten year (2011, 2012, 2013, 2015, 2020) data collection schedule. This will allow for an examination of the long-term effects of aminopyralid effects on target and non-target plants.

## LITERATURE CITED

- Almquist, T.L. and R.G. Lym. 2010. Effect of aminopyralid on Canada thistle (*Cirsium arvense*) and the native plant community in a restored tallgrass prairie. *Invasive Plant Science and Management*. **3**:155–168.
- Beck, K.G. 2008. Colorado state university extension Canada thistle fact sheet. No. 3.016.
- Daubenmire, R.F. 1959. Canopy coverage method of vegetation analysis. *Northwest Science*. **33**:43-64.
- Dow Agrosiences. 2005. Milestone specialty herbicide specimen label. D-02-879-001.
- Duncan, C., A. Kulla, M. Halstvedt, and R.A. Masters. 2009. Effect of aminopyralid on non-target vegetation after aerial application. *Proc. Soc. Range Management Annual Meeting*. **62**:70–75. Available from <[http://www.srmmeetings.org/pdf\\_Abstracts/tech70\\_invasiveSpecies/70\\_5.pdf](http://www.srmmeetings.org/pdf_Abstracts/tech70_invasiveSpecies/70_5.pdf)>
- Enloe, S.F., R.G. Lym, R. Wilson, P. Westra, S. Nissen, G. Beck, M. Moechnig, V. Peterson, R.A. Masters and M. Halstvedt. 2007. Canada thistle (*Cirsium arvense*) control with aminopyralid in range, pasture, and noncrop areas. *Weed Technology*. **21**:890–894.
- Okamoto, H., K. Ishii and P. An. 2011. Effects of soil moisture deficit and subsequent watering on the growth of four temperate grasses. *Grassland Science*. **57**:192-197.
- [NPS] National Park Service. 1987. 27 years of exotic plant control in Rocky Mountain National Park: Summary and recommendations. *Resources Management Report No. 1*.
- Prietkel, C., N.S. Whitmore-Olson and J.C. Moore. 2006. Impacts from invasive plant species and their control on the plant community and belowground ecosystem at Rocky Mountain National Park, USA. *Applied Soil Ecology*. **32**:132–141.
- Samuel, L.W. and R.G. Lym. 2008. Aminopyralid effects on Canada thistle (*Cirsium arvense*) and native plant species. *Invasive Plant Science and Management*. **1**:265-278.
- Signarbieux, C. and U. Feller. 2011 Non-stomatal limitations of photosynthesis in grassland species under artificial drought in the field. *Environmental and Experimental Botany*. **71**:192-197.
- Weber, W.A and R.C. Wittmann. 2001. *Colorado flora eastern slope* 3<sup>rd</sup> ed. University Press of Colorado, Boulder, Colorado, USA.

# THE EFFECTS OF INCREASED NITROGEN AVAILABILITY ON *BROMUS TECTORUM* AND SEEDED GRASS ESTABLISHMENT IN RESTORATION SITES

## ABSTRACT

The current rate of atmospheric nitrogen deposition in Rocky Mountain National Park (RMNP) is nearly 20 times greater than pre-industrial background N deposition rates and it is projected that atmospheric nitrogen deposition rates may double in the next century. Exotic plant species dominance in RMNP is positively associated with increased nitrogen availability and decreased overstory cover. Herbicide treatments to control *Bromus tectorum* in RMNP have been shown to greatly reduce *B. tectorum* cover while also resulting in an increase in cover of bare ground. Immobilization of available soil nitrogen by adding sucrose soil amendments has also been shown to reduce *B. tectorum* cover and stimulate perennial plant growth in old-fields as well as restoration sites in RMNP. The purpose of this study will be to determine the effects of both increased and decreased available soil nitrogen on native plant species recruitment, seeded native perennial grass success and *B. tectorum* reinvasion of herbicide-treated sites.

## INTRODUCTION

Nitrogen (N) is a critical component to life on earth and is needed to synthesize organic compounds such as amino acids, nucleic acids and chlorophyll. In spite of this universal need, N is unavailable to most organisms in its most abundant state, atmospheric N or N<sup>2</sup> and most N assimilated by plants and their consumers has been fixed to a biologically reactive form by bacteria or archaea. This severe limitation of biologically accessible N results in most ecosystems around the world being limited by N availability to at least some degree (Galloway et al. 2004). Following the industrial revolution, increased combustion of fossil fuels and the discovery of the Haber-Bosch process of N fixation in the early 20<sup>th</sup> century drastically increased anthropogenic inputs of N to ecosystems around the world (Fenn et al. 2003). This N input comes in the form of N fertilizers added directly to agricultural soils, dust and particulates blown from N enriched agricultural fields, and atmospheric deposition of N formed from the burning of fossil fuels. It is estimated that anthropogenic inputs of reactive N to the biosphere now equals or exceeds the input of reactive N by natural processes (Fenn et al. 2003).

Consequences of increased N deposition in ecosystems include changes in plant community composition, decreases in species richness, increases in plant biomass and changes to soil microbial communities (Bechtold and Inouye 2007). Elevated soil N may also increase a system's vulnerability to invasion by exotic plant species that can take advantage of increased N levels, though this relationship is not true for all invasive species (Brooks 2003; Leffler et al. 2011; Ross et al. 2010). Cheatgrass invasion in particular has been shown to be positively associated with increased levels of available soil nitrogen, though this response may be co-dependent on water availability (Lowe et al. 2003, Monaco et al. 2003, Chambers et al. 2007, Adair et al. 2008).

Decreased available soil N has been shown to reduce *B. tectorum* cover and stimulate perennial plant growth in old-fields as well as restoration sites in RMNP (Paschke et al. 2000, Rowe et al. 2009). Studies have shown that some perennial grass species, like *Elymus elymoides* (hereafter, squirreltail), are resistant to annual grass invasion and are even capable of invading sites occupied by the invasive annual grass *Bromus japonicus* (Hironaka and Sindelar 1973; Hironaka and Sindelar 1975). While cheatgrass competes with squirreltail seedlings, mature stands of squirreltail are strongly resistant to competition from cheatgrass (McGlone et al. 2011, Humphrey and Schupp 2004) and integrating herbicide treatment with seeding of native perennial grasses has been shown to be more effective at revegetating annual grass-invaded sites than herbicide treatment alone (Davies 2010).

Exotic plant species dominance in RMNP is positively associated with increased N availability and decreased overstory cover (Kalkhan et al. 2007). As discussed earlier, cheatgrass, a focus of exotic species management in RMNP, responds positively to available soil N. The current rate of atmospheric N deposition in Rocky Mountain National Park (RMNP) is approximately  $3.2 \text{ kg ha}^{-1} \text{ y}^{-1}$ , or nearly 20 times greater than the pre-industrial background N deposition rate of  $0.2 \text{ kg ha}^{-1} \text{ y}^{-1}$  (Beem et al. 2010). It has been projected that atmospheric N deposition rates may double in the next century (Galloway et al. 2004).

Herbicide (imazapic) treatments to control *B. tectorum* in RMNP have been shown to greatly reduce *B. tectorum* cover while also resulting in an increase in cover of bare ground (RMNP 2011). While the use of imazapic has been shown to be effective in reducing the cover of cheatgrass infestations, cheatgrass can readily reinvade areas that have been treated with imazapic after a period of one to two years (Hirsch et al. 2012, Kyser et al. 2007 and Beck 2003).

Increases in bare ground cover in herbicide treatment sites in concurrence with increasing atmospheric N deposition may result in aggressive reinvansion of these restoration sites once herbicide treatments are stopped.

The purpose of this study is to demonstrate the effects of different levels of available soil N on cheatgrass management and site restoration in RMNP. This study will specifically address these research questions: What effect will increased and decreased available soil N have on cheatgrass reinvansion in herbicide treatment sites and what effect will increased and decreased available soil N have on native species recruitment (seeded and volunteer species) in herbicide treatment areas? We predict that increased soil N will result in increased cover of both cheatgrass and native plant species and decreased soil N will result in decreased cheatgrass cover which will allow greater establishment success of native plant species.

## **MATERIALS AND METHODS**

### *Monitoring Site Selection*

All monitoring sites are located on the eastern slope within Rocky Mountain National Park in the montane life zone. Monitoring sites were chosen on the basis that there was a cheatgrass

infestation scheduled to be treated with imazapic and a nearby reference plot that was free of any cheatgrass and not scheduled for herbicide treatment. These reference plots were judged to represent the desired final, post-treatment stage of succession for these plots.

### *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). Cheatgrass was selectively spot-sprayed to minimize damage to native plant species. In 2008 cheatgrass plants were treated post-emergence at a maximum plant height of two inches at the time of application. Starting in 2009, all cheatgrass infestations were treated pre-emergence (soil application) to comply with the revised Plateau label. Imazapic was applied at a rate of 103 ml a.i./ha (~1.4 oz a.i./ac) in 2008 and 2009 and at a rate of 69 ml a.i./ha (~1 oz a.i./ac) in 2010. Application rate was decreased in 2010 to avoid damage to adjacent non-target plants, and both application rates were within label recommended rates of imazapic application for cheatgrass control. In addition to selective spot-spraying of cheatgrass, several other techniques were employed to avoid non-target species injury or mortality. These methods included the implementation of no-spray-days, where herbicide application was stopped when wind speeds exceeded 2.7 m/s. Surfactants or adjuvants were not included in the imazapic backpack sprayer solution, making it less likely that any drifting herbicide spray would penetrate non-target plant tissue.

### *Vegetation Monitoring*

In 2008, permanent circular nested vegetation monitoring plots (CNP), each with an area of approximately 168.2 m<sup>2</sup> (1810 ft<sup>2</sup>), or 0.01682 ha (1/24 of an acre), were installed at each site. The design of these plots consists of a circular plot with three 7.2 m (24 ft) spokes at 30, 150 and 270 degrees. A 1 m<sup>2</sup> quadrat is located on each of these spokes 4.9 m (16 ft) from the center of the plot (Fig. 1).

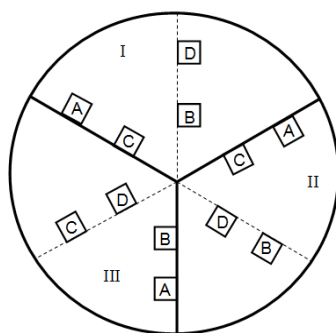


Fig. 2 Circular nested plot design used to monitor vegetation. A: ambient N deposition, B: intermediate N, C: high N, D: N immobilization (sugar addition).

Percent cover was estimated within each of the three 1 m<sup>2</sup> quadrats of the CNP. Each species present in the quadrat was recorded and an ocular estimation of percent cover for the individual species was made. Percent cover was also estimated for bare ground, litter, rock,

moss and lichen and animal scat. Tree, shrub and forb percent cover were estimated using canopy cover while graminoid (grass, sedge and rush species) cover was estimated using cover at ground level (basal cover). Percent cover was estimated using modified Daubenmire cover classes (Daubenmire 1959) as ranges of percent cover. All species within the CNP but not observed within the three quadrats were recorded.

#### *Seeding Plot Installation*

In 2011, seeding plots were installed at five sites that had been treated with imazapic (23.6% a.i.) as previously described. The high seeding rate (HSR) plot design was a 168 m<sup>2</sup> circular nested plot (described below). In addition to these seeded plots, one unseeded control plot was established at each herbicide treatment site for a total of six unseeded plots and six high-rate seeding plots. The unseeded control plots were plots established four years ago for an earlier imazapic study.

#### *Nitrogen Manipulation*

In addition to ambient soil N subplots, intermediate and high N addition subplots (6 kg/ha 32 kg/ha, respectively) were installed to increase soil N availability (Fig. 1). These levels of N addition are double and ten times the current rate of ambient atmospheric N deposition in RMNP. Nitrogen was added in the form of reagent-grade ammonium nitrate combined with a small volume of sand to facilitate uniform N application. The total volume of ammonium nitrate added to the intermediate and high N addition subplots was 0.92 g m<sup>-2</sup> and 8.24 g m<sup>-2</sup>, respectively.

Sucrose treatment subplots were also installed to decrease soil N availability. The total volume of sucrose added to the low N availability plots was 379 g m<sup>-2</sup>. Both N and sucrose additions were divided into three applications and subplots were treated in the months of June, July and September.

#### *Data Analysis*

The midpoint percent cover for each species in each quadrat was calculated as the mean of the maximum and minimum cover range values. Using these midpoint cover values, the average for each species across all three quadrats was calculated.

The species in each CNP and their corresponding mean percent cover was 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 was also grouped according to these criteria. The possible growth habit categories are tree, shrub, graminoids (grass, rush, and sedge species), forb, or moss and lichen. Species were classified as having a particular growth habit based on their designation in the USDA PLANTS profile database 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 will be listed on RMNP's list of invasive species or the Colorado Department of Agriculture's schedule of noxious weeds. Since the focus of this study is cheatgrass management, cheatgrass presence and its percent cover was also summarized individually.



Data were analyzed using the JMP 7 statistical analysis software package (SAS Institute, Cary, NC, USA). The data were analyzed for each of the species categories using the multivariate analysis of variance (MANOVA) with time as a repeated measure. The between subjects factors were treatment and vegetation type, and the within subjects factor was time. For each species category, MANOVA was used to test for main effects of and interactions between treatment and vegetation type over time. If an interaction between variables was detected, this interaction was graphed using Microsoft Excel. Exact *F*-tests or Wilk's Lambda approximate *F*-tests will be reported as appropriate.

## RESULTS/DISCUSSION

### *Cheatgrass*

Cheatgrass cover was greater in shrubland plots in 2013, independent of seeding or nitrogen treatments (Table 1, veg type). In shrublands cheatgrass cover averaged 4.6% while in grasslands in forests cover was below 1% (Fig. 2). Cheatgrass has been more abundant in in these shrubland sites in the past, and may experience greater propagule pressure than other sites due to their close proximity to campgrounds, horse stables and park housing.

Table 1. ANOVA for cheatgrass cover. Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	1.09	0.3744
Seeding	1	0.24	0.6311
Veg Type	2	23.85	<b>&lt;.0001</b>
Nitrogen level x Veg Type	6	0.60	0.7267
Veg Type x Seeding	2	1.56	0.2351
Nitrogen level x Seeding	3	0.36	0.7809
Nitrogen level x Veg Type x Seeding	6	0.40	0.8675

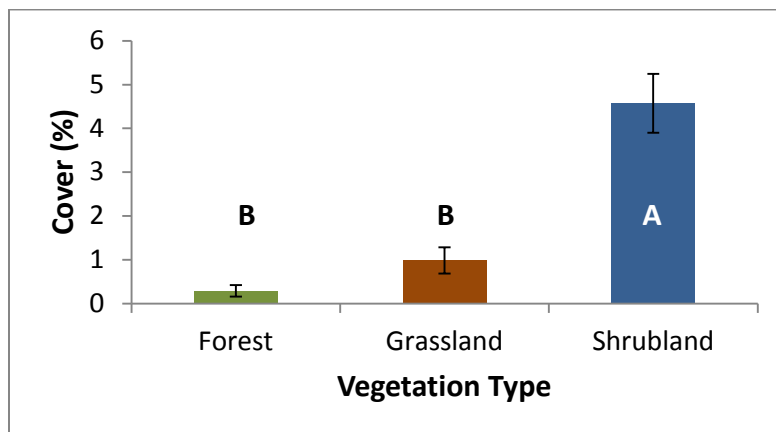


Fig 2. Cheatgrass cover by vegetation type (mean  $\pm$  1 standard error of the mean). Levels not connected by the same letter are significantly different.

## Native Species Functional Groups

Native graminoid cover was lower in some plots dependent on vegetation type and seeding treatment (Table 2, veg type x seeding). In forest sites cover was lower in high seeding rate plots while in shrubland sites cover was lower in unseeded plots (Fig. 3). There was no difference in native graminoid richness between plots (Table 3). Data from a seeding trial study being conducted in these plots have shown that there has been no increase in native graminoid cover following seeding in 2011. It is unlikely these differences in native graminoid cover are due to seeding in these plots (see Seeding Trial chapter of this report).

Table 2. ANOVA for native graminoid cover. Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	2.58	0.0822
Seeding	1	0.93	0.3458
Veg Type	2	2.02	0.1591
Nitrogen level x Veg Type	6	1.25	0.3221
Veg Type x Seeding	2	6.25	<b>0.0078</b>
Nitrogen level x Seeding	3	2.22	0.1175
Nitrogen level x Veg Type x Seeding	6	1.34	0.2850

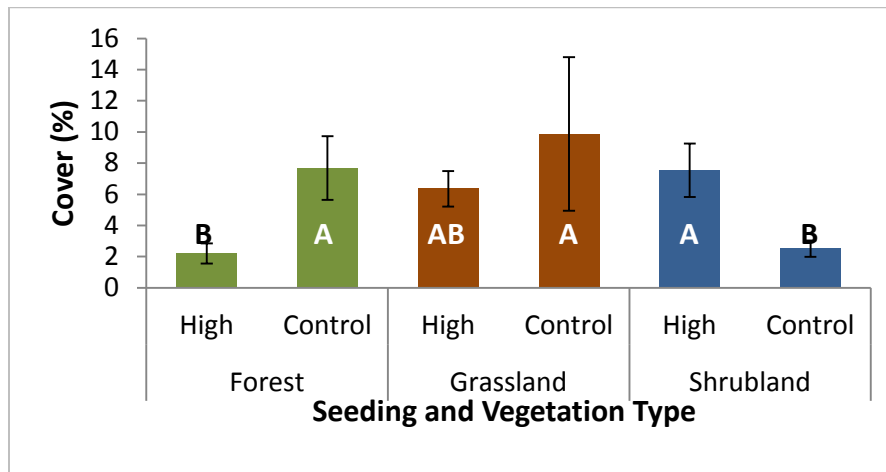


Fig 3. Native graminoid cover by seeding rate and vegetation type (mean  $\pm$  1 standard error of the mean). Levels not connected by the same letter are significantly different.

Table 3. ANOVA for native graminoid species richness. Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	0.31	0.8206
Seeding	1	1.12	0.3020
Veg Type	2	1.00	0.3854
Nitrogen level x Veg type	6	0.24	0.9567
Veg Type x Seeding	2	3.03	0.0709
Nitrogen level x Seeding	3	0.88	0.4705
Nitrogen level x Veg Type X Seeding	6	0.45	0.8337

Native forb cover was more than two times greater in grassland plots independent of nitrogen or seeding treatments (Table 4, veg type). Forest and shrubland plots both had similar native forb cover in 2013 (Fig. 4). Native forb species richness varied depending on vegetation type and seeding treatment (Table 5, veg type x seeding). It is unlikely these differences in native forb cover and richness are due to effects of seeding in these plots (see Seeding Trial chapter of this report).

Table 4. ANOVA for native forb cover. Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	0.37	0.7728
Seeding	1	0.05	0.8194
Veg Type	2	11.30	<b>0.0005</b>
Nitrogen level x Veg Type	6	0.27	0.9446
Veg Type x Seeding	2	2.44	0.1130
Nitrogen level x Seeding	3	0.21	0.8885
Nitrogen level x Veg Type x Seeding	6	0.52	0.7881

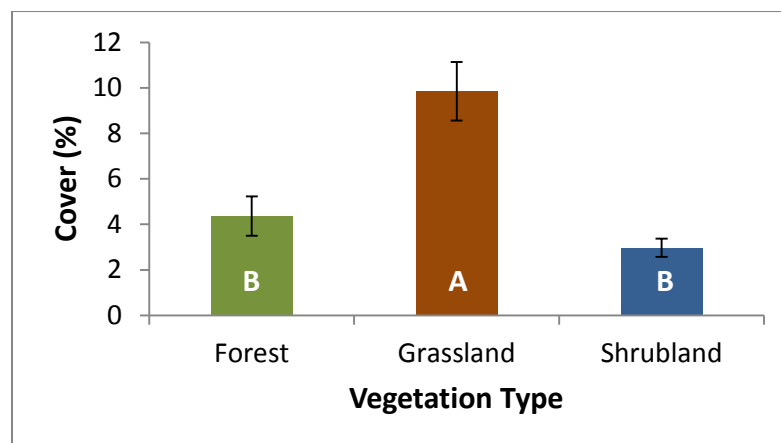


Fig 4. Native forb cover by vegetation type (mean  $\pm$  1 standard error of the mean).

Table 5. ANOVA for native forb species richness. Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	11.74	<b>0.0001</b>
Seeding	1	0.31	0.5840
Veg Type	2	0.63	0.5431
Nitrogen level x Veg type	6	0.91	0.5104
Veg Type x Seeding	2	5.52	<b>0.0123</b>
Nitrogen level x Seeding	3	1.03	0.3991
Nitrogen level x Veg Type X Seeding	6	0.40	0.8676

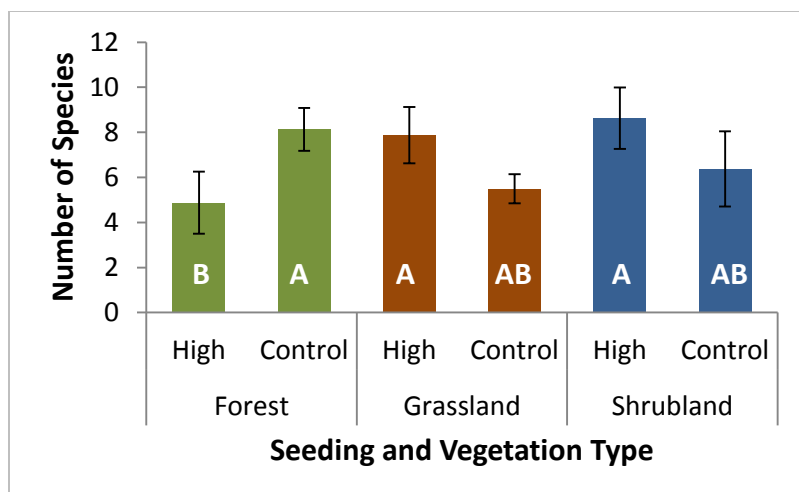


Fig 5. Native forb richness by seeding rate and vegetation type (mean  $\pm$  1 standard error of the mean). Levels not connected by the same letter are significantly different.

Native shrub cover and species richness was greatest in shrubland plots when averaged over nitrogen and seeding treatment types (Table 6, veg type; Table 7, veg type). Native shrub richness was also twice as great in plots that were not treated with any nitrogen or sucrose amendments averaged over seeding treatment and vegetation types (Table 7, nitrogen level, Fig. 6).

Table 6. ANOVA for native shrub cover. Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	0.19	0.8990
Seeding	1	0.32	0.5753
Veg Type	2	14.19	<b>0.0001</b>
Nitrogen level x Veg Type	6	0.24	0.9586
Veg Type x Seeding	2	0.51	0.6107
Nitrogen level x Seeding	3	0.12	0.9486
Nitrogen level x Veg Type x Seeding	6	0.15	0.9859

Table 7. ANOVA for native shrub species richness. Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	3.75	<b>0.0276</b>
Seeding	1	0.01	0.9261
Veg Type	2	15.86	<b>&lt;.0001</b>
Nitrogen level x Veg type	6	0.65	0.6873
Veg Type x Seeding	2	0.31	0.7379
Nitrogen level x Seeding	3	0.10	0.9574
Nitrogen level x Veg Type X Seeding	6	0.24	0.9571

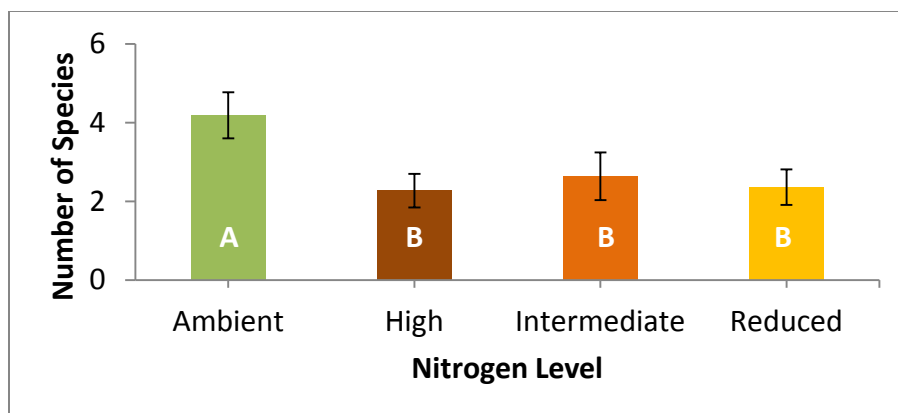


Fig. 6. Native shrub richness by nitrogen treatment (mean  $\pm$  1 standard error of the mean). Levels not connected by the same letter are significantly different.

Native tree cover and species richness was greatest in forest plots independent of nitrogen treatment and seeding treatments (Table 8, veg type; Table 9, veg type).

Table 8. ANOVA for native tree cover. Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	0.49	0.6907
Seeding	1	0.00	0.9587
Veg Type	2	14.39	<b>0.0001</b>
Nitrogen level x Veg Type	6	0.64	0.6978
Veg Type x Seeding	2	0.16	0.8526
Nitrogen level x Seeding	3	0.05	0.9835
Nitrogen level x Veg Type x Seeding	6	0.12	0.9919

Table 9. ANOVA for native tree species richness. Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	0.24	0.8687
Seeding	1	0.01	0.9070
Veg Type	2	15.00	<b>0.0001</b>
Nitrogen level x Veg type	6	0.11	0.9947
Veg Type x Seeding	2	0.21	0.8137
Nitrogen level x Seeding	3	0.24	0.8687
Nitrogen level x Veg Type X Seeding	6	0.07	0.9984

### ***Invasive Species Functional Groups***

There was no difference in invasive graminoid cover, which did not include cheatgrass cover, averaged over all treatment and vegetation types (Table 10). Invasive graminoid species richness, which does include cheatgrass, was greater in forest plots that were not treated with

any nitrogen or sucrose amendments (Table 11, nitrogen level x veg type). This difference was approximately one species more than other plot types, mostly due to the presence of smooth brome (*Bromus inermis*) in those plots.

Table 10. ANOVA for invasive graminoid cover (excluding cheatgrass). Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	0.60	0.6220
Seeding	1	1.05	0.3167
Veg Type	2	1.15	0.3356
Nitrogen level x Veg Type	6	0.66	0.6845
Veg Type x Seeding	2	1.15	0.3356
Nitrogen level x Seeding	3	0.60	0.6220
Nitrogen level x Veg Type x Seeding	6	0.66	0.6845

Table 11. ANOVA for invasive graminoid species richness (including cheatgrass). Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	0.57	0.6403
Seeding	1	0.29	0.5989
Veg Type	2	0.31	0.7351
Nitrogen level x Veg type	6	2.60	<b>0.0497</b>
Veg Type x Seeding	2	0.25	0.7812
Nitrogen level x Seeding	3	0.86	0.4793
Nitrogen level x Veg Type X Seeding	6	0.29	0.9339

There was no difference in invasive forb cover or species richness averaged over all treatment and vegetation types (Table 12, Table 13).

Table 12. ANOVA for invasive forb cover. Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	0.52	0.6705
Seeding	1	1.28	0.2706
Veg Type	2	1.40	0.2688
Nitrogen level x Veg Type	6	0.57	0.7467
Veg Type x Seeding	2	1.40	0.2688
Nitrogen level x Seeding	3	0.52	0.6705
Nitrogen level x Veg Type x Seeding	6	0.57	0.7467

Table 13. ANOVA for invasive forb species richness. Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	0.48	0.7024
Seeding	1	0.71	0.4080
Veg Type	2	1.45	0.2593
Nitrogen level x Veg type	6	0.30	0.9299
Veg Type x Seeding	2	2.38	0.1180
Nitrogen level x Seeding	3	0.24	0.8687
Nitrogen level x Veg Type X Seeding	6	0.40	0.8678

### ***Non-native Non-invasive Functional Groups***

We did not observe any non-native non-invasive graminoids in these plots in 2013.

Non-native non-invasive forb cover varied depending on vegetation type and seeding treatment, though the differences in cover were less than 0.5% (Table 14, Fig. 7). Non-native non-invasive forb species richness was approximately one species greater in plots that were not treated with any nitrogen or sucrose amendments (Table 15).

Table 14. ANOVA for non-native non-invasive forb cover. Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	0.18	0.9106
Seeding	1	0.00	0.9958
Veg Type	2	1.94	0.1692
Nitrogen level x Veg Type	6	0.22	0.9655
Veg Type x Seeding	2	7.61	<b>0.0035</b>
Nitrogen level x Seeding	3	0.15	0.9299
Nitrogen level x Veg Type x Seeding	6	0.32	0.9174

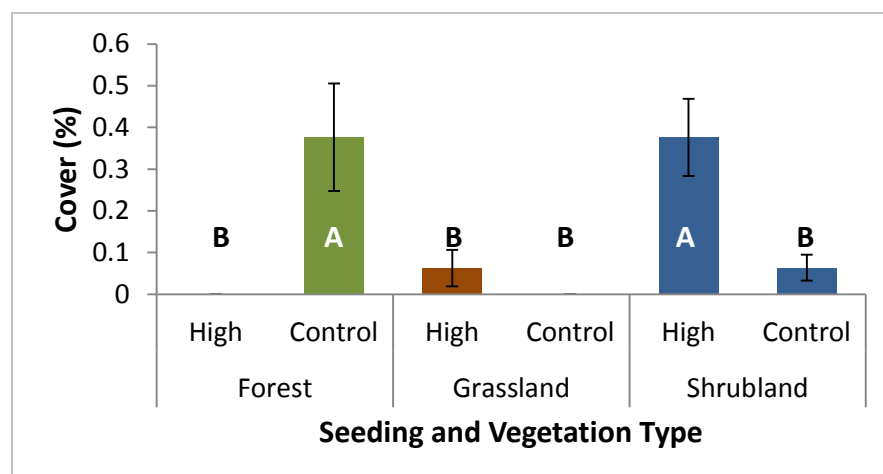


Fig 7. Non-native non-invasive forb cover by seeding rate and vegetation type (mean  $\pm$  1 standard error of the mean). Levels not connected by the same letter are significantly different.

Table 15. ANOVA for non-native non-invasive forb species richness. Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	6.31	<b>0.0034</b>
Seeding	1	1.06	0.3154
Veg Type	2	0.15	0.8604
Nitrogen level x Veg type	6	0.81	0.5757
Veg Type x Seeding	2	1.97	0.1656
Nitrogen level x Seeding	3	0.83	0.4931
Nitrogen level x Veg Type X Seeding	6	0.30	0.9280

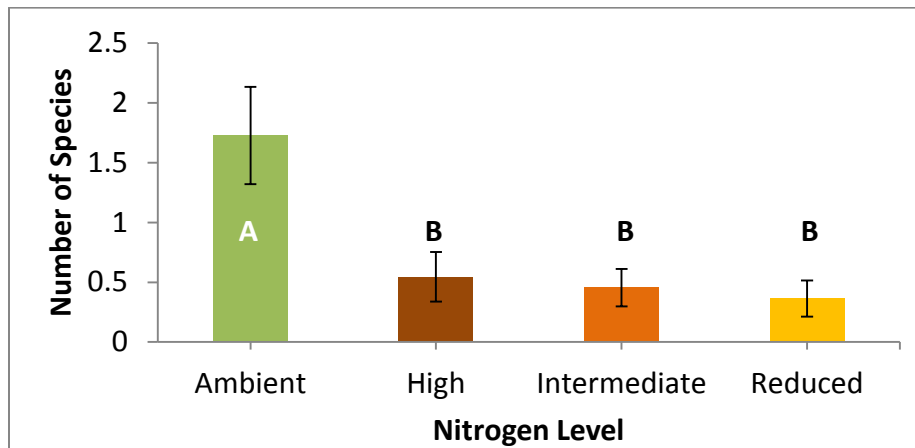


Fig 7. Non-native non-invasive forb richness by nitrogen treatment (mean  $\pm$  1 standard error of the mean). Levels not connected by the same letter are significantly different.

## Bare Soil

Bare soil cover was twice as great in grassland plots independent of nitrogen and seeding treatments (Table 16, veg type; Figure 8). Most grassland plots were located on road cuts that have been disturbed recently and are still in the process of recovery, possibly explaining the high level of bare ground.

Table 16. ANOVA for bare soil cover. Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	0.21	0.8851
Seeding	1	0.46	0.5049
Veg Type	2	6.34	<b>0.0074</b>
Nitrogen level x Veg Type	6	0.27	0.9439
Veg Type x Seeding	2	2.12	0.1467
Nitrogen level x Seeding	3	0.12	0.9495
Nitrogen level x Veg Type x Seeding	6	0.49	0.8079



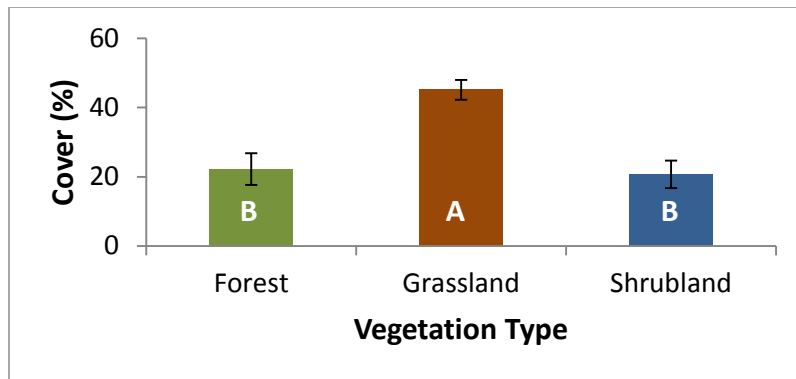


Fig 8. Bare soil cover by vegetation type (mean  $\pm$  1 standard error of the mean). Levels not connected by the same letter are significantly different.

*Annotated ANOVA tables and figures for other general functional groups not discussed in this results section can be found in the appendix.*

## CONCLUSIONS

There were no consistent or compelling differences in vegetation when comparing nitrogen and seeding treatments in the first year of data collection. Nearly all of the significant differences we detected were due to effects of seeding treatment, vegetation type, or an interaction between these two factors. As discussed in the seeding trial chapter of this report, there has been no significant increase in seeded grass species in these plots as of 2013. This makes it highly likely that any differences due to a seeding effect or a seeding and vegetation type interaction are not truly the result of seeding treatments.

This is the first year of data collection for this study, and further monitoring of these plots will indicate what effects long-term nitrogen manipulation will have on these plant communities.

Additionally, treatment plot data from the 2008-2012 Cheatgrass/Imazapic study used the same plots and quadrat placements as the unseeded control plots in the Nitrogen Manipulation study. These data are analogous to having 5 years of pre-manipulation data for use in future analyses.

## LITERATURE CITED

- Adair, E.C., I.C. Burke and W.K. Lauenroth. 2008. Contrasting effects of resource availability and plant mortality on plant community invasion by *Bromus tectorum* L. *Plant Soil*. 304:103-115
- Bechtold, H. A., and R.S. Inouye. 2007. Distribution of carbon and nitrogen in sagebrush steppe after six years of nitrogen addition and shrub removal. *Journal of Arid Environments*. 71:122-132.
- Beck, K.G. 2003. Downy brome (*Bromus tectorum*) and Japanese brome (*Bromus japonicus*): Biology, ecology, and management. Colorado State University Department of Bioagricultural Sciences and Pest Management.
- Beem, K.B., S. Raja, F.M. Schwandner, C. Taylor, T. Lee, A.P. Sullivan, C.M. Carrico, G.R. McMeeking, D. Day, E. Levin, J. Hand, S.M. Kreidenweis, B. Schichtel, W.C. Malm and J.L. Collett Jr. 2010. Deposition of reactive nitrogen during the Rocky Mountain Airborne Nitrogen and Sulfur (RoMANS) study. *Environmental Pollution*. 158:862-872.
- Brooks, M.L. 2003. Effects of increased soil nitrogen on the dominance of alien annual plants in the Mojave Desert. *Journal of Applied Ecology*. 40:344-353.
- Chambers, J.C., B.A. Roundy, R.R. Blank, S.E. Meyer, and A. Whittaker. 2007. What makes the Great Basin sagebrush ecosystems invisable by *Bromus tectorum*? *Ecological Monographs*. 77:117-145.
- Davies, K.W. 2010. Revegetation of medusahead-invaded sagebrush steppe. *Rangeland Ecology and Management*. 63:564-571.
- Daubenmire, R.F. 1959. Canopy coverage method of vegetation analysis. *Northwest Science*. 33:43-64.
- Fenn, M.E., J.S. Baron, E.B. Allen, H.M. Rueth, K.R. Nydick, L. Geiser, W.D. Bowman, J.O. Sickman, T. Meixner, D.W. Johnson, and P. Neitlich. 2003. Ecological effects of nitrogen deposition in the Western United States. *BioScience*. 53:404-420.
- Galloway, J. N., F. J. Dentener, D. G. Capone, E. W. Boyer, R. W. Howarth, S. P. Seitzinger, G. P. Asner, C. C. Cleveland, P. A. Green, E. A. Holland, D. M. Karl, A. F. Michaels, J. H. Porter, A. R. Townsend, and C. J. Vörösmarty. 2004. Nitrogen cycles: Past, present, and future. *Biogeochemistry*. 70:153-226.
- Hironaka, M., B.W. Sindelar. 1973. Reproductive success of squirreltail in medusahead infested ranges. *Journal of Range Management*. 26:219-221
- Hironaka, M., B.W. Sindelar. 1975. Growth characteristics of squirreltail seedlings in competition with medusahead. *Journal of Range Management*. 28:283-285.
- Hirsch, M.C., T.A. Monaco, C.A. Call and C.V. Ransom. 2012. Comparison of herbicides for reducing annual grass emergence in two Great Basin soils. *Rangeland Ecological Management*. 65:66-75.
- Humphrey, L.D. & E.W. Schupp. 2004. Competition as a barrier to establishment of a native perennial grass (*Elymus elymoides*) in alien annual grass (*Bromus tectorum*) communities. *Journal of Arid Environments*. 58:405-422.
- Kalkhan, M.A., E.J. Stafford, P.J. Woodyly and T.J. Stohlgren. 2007. Assessing exotic plant species invasions and associated soil characteristics: A case study in eastern Rocky Mountain National Park, Colorado, USA, using the pixel nested plot design. *Applied Soil Ecology*. 35:622-634.

- Kyser, G.B., J.M. DiTomaso, M.P. Doran, S.B. Orloff, R.G. Wilson, D.L. Lancaster, D.F. Lile, and M.L. Porath. 2007. Control of medusahead (*Taeniatherum caput-medusae*) and other annual grasses with imazapic. *Weed Technology*. 21:66-75.
- Leffler, A. J., T. A. Monaco and J. J. James. 2011. Nitrogen acquisition by annual and perennial grass seedlings: testing the roles of performance and plasticity to explain plant invasion. *Plant Ecology*. 212:1601-1611.
- Lowe, P.N, W.K. Lauenroth, and I.C. Burke. 2003. Effects of nitrogen availability on competition between *Bromus tectorum* and *Bouteloua gracilis*. *Plant Ecology*. 167:247-254.
- McGlone, C.M., C.H. Sieg & T.E. Kolb. 2011. Invasion resistance and persistence: Established plants win, even with disturbance and high propagule pressure. *Biological Invasions*. 13:291-304.
- Monaco, T.A., D.A. Johnson, J.M. Norton, T.A. Jones, K.J. Connors, J.B. Norton, and M.B. Redinbaugh. 2003. Contrasting responses of Intermountain West grasses to soil nitrogen. *Journal of Range Management*. 56:282-290
- Paschke, M.W., T. McLendon, E.F. Redente. Nitrogen availability and old-field succession in a shortgrass steppe. *Ecosystems*. 3:144-158.
- [RMNP] Rocky Mountain National Park. 2011. Rocky Mountain National Park 2011 Vegetation Monitoring Report.
- Ross, K.A., J.G. Ehrenfeld, and M.V. Patel. 2011. The effects of nitrogen addition on the growth of two exotic and two native forest understory plants. *Biological Invasions*. 13:2203-2216.
- Rowe, H.I., C.S. Brown and M.W. Paschke. 2009. The Influence of soil inoculum and nitrogen availability on restoration of high-elevation steppe communities invaded by *Bromus tectorum*. *Restoration Ecology*. 17:686-694.

# Effectiveness of Combined Imazapic Treatment and Native Grass Seeding in Preventing Cheatgrass (*Bromus tectorum*) Reinvasion of Restoration Sites

## ABSTRACT

Cheatgrass, a winter annual grass introduced from Eurasia, has invaded much of the Western United States over the last century. In recent decades, 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 maturing in early spring and rapidly establishing a root system capable of depleting soil moisture and available nitrogen. These invasion characteristics make control of cheatgrass of primary importance when restoring disturbances within RMNP. This study examines the effect of imazapic treatment followed by seeding with competitive native grass species on controlling cheatgrass reinvasion in forest, shrubland and grassland habitats. In 2011, seeding plots were installed at six sites that had been treated with imazapic (23.6% a.i.) for three consecutive years to control cheatgrass infestations. Plots were seeded with a native seed mix containing *Elymus elymoides* and *Elymus canadensis* at rates of 200 pure live seed (PLS)/ft<sup>2</sup> (2150 PLS/m<sup>2</sup>) or 70 PLS/ft<sup>2</sup> (750 PLS/m<sup>2</sup>). No seed was added to a third plot, which served as the unseeded control. *Elymus elymoides* and *Elymus canadensis* are vigorous native grass species that are expected to be able to competitively exclude cheatgrass. Seeding occurred in autumn of 2011 and baseline data before seeding treatment are reported here. Results of this study will provide site-specific information regarding the effectiveness of herbicide treatment combined with different rates of reseeding in controlling cheatgrass invasion.

## INTRODUCTION

The Eurasian winter annual grass *Bromus tectorum* (hereafter, cheatgrass) is one of the most widespread and invasive exotic weeds of Western North America (Mack 1981). The introduction of cheatgrass to the Intermountain West of the United States is believed to have occurred in the late 1800's as a result of contaminated seed stock and intentional seeding in overgrazed grassland areas (Mack 1981). Cheatgrass aggressively invades disturbed sites (Baker et al. 2009) and competes intensely with native plant species by maturing in early spring and rapidly establishing a root system capable of depleting soil moisture and nitrogen content (Hulbert 1955). Cheatgrass also alters natural fire regimes and can shorten fire return intervals, suppressing re-establishment of native species adapted to longer intervals and favoring further invasion by the fire-tolerant grass (Knapp 1996).

Cheatgrass infestation an increasing problem in the montane and subalpine regions of Rocky Mountain National Park (RMNP), and the plant is listed as a noxious weed by both RMNP and the state of Colorado. In 2008, park management made the reduction of cheatgrass infestations in the park a priority, and the Park's exotic plant management and restoration teams have used mechanical, cultural, and chemical means for this purpose.

Included in the methods employed for the control of cheatgrass is application of the herbicide imazapic, which has been approved for limited use in RMNP for treatment of cheatgrass

infestations. Each year from 2008 to 2010, five sites in forest, grassland and shrubland habitats that were infested with cheatgrass were treated with imazapic and the effects of this herbicide application were monitored. Imazapic application effectively reduced total cheatgrass cover sixfold in treatment plots by 2011.

While the use of imazapic has been shown to be effective in reducing the cover of cheatgrass infestations, cheatgrass can readily reinvade areas that have been treated with imazapic after a period of one to two years (Beck 2003). Imazapic has a soil half life of 120 days to two years depending on soil moisture, light and microbial conditions and loses its effectiveness in controlling cheatgrass as chemical degradation advances (Tu et al. 2001; ACC 2000). Studies have shown that some perennial grass species, like *Elymus elymoides* (hereafter, squirreltail), are resistant to annual grass invasion and are even capable of invading sites occupied by the invasive annual grass *Bromus japonicus* (Hironaka and Sindelar 1973; Hironaka and Sindelar 1975). While cheatgrass competes with squirreltail seedlings, mature stands of squirreltail are strongly resistant to competition from cheatgrass (McGlone et al. 2011, Humphrey and Schupp 2004). Integrating cheatgrass control techniques such as herbicide treatment with seeding of native perennial grasses like squirreltail has been shown to be more effective at successfully revegetating annual grass-invaded sites than herbicide treatment alone (Davies 2010). Squirreltail has also been shown to be moderately resistant to imazapic, and can become established in disturbed sites more readily than other native grass species following imazapic treatment (Sheley et al. 2007).

Establishment of native plant species that can effectively compete with cheatgrass in areas of imazapic treatment represents a potential long-term strategy in preventing the spread of cheatgrass infestations. Using such integrated pest management strategies is useful in both reducing the amount of herbicide deployed within the park, and allowing for improvement of aesthetics in areas being treated for cheatgrass control.

The purpose of this study is to monitor sites treated with imazapic and seeded at a high and low seeding rate with a native grass seed mix to determine whether seeding in combination with imazapic treatment is more effective than imazapic treatment alone in preventing reinvasion by cheatgrass.

## **MATERIALS AND METHODS**

### *Herbicide Application*

Each year from 2008 to 2010, RMNP's exotic plant management crew treated cheatgrass infested sites with imazapic (Plateau, BASF, Research Triangle Park, USA) using backpack sprayers (23.6% a.i.). Cheatgrass was selectively spot sprayed to minimize damage to native plant species. The timing of imazapic application was changed in 2009 to comply with the revised Plateau label. In 2008 and 2009 cheatgrass plants were treated post-emergence at a maximum plant height of two inches at the time of application. Starting in 2009, all cheatgrass infestations were treated pre-emergence (soil application) to comply with the revised Plateau label. Imazapic was applied at a rate of 103 ml a.i./ha (~1.4 oz a.i./ac) in 2008 and 2009 and at a

rate of 69 ml a.i./ha (~1 oz a.i./ac) in 2010. Application rate was decreased in 2010 to avoid damage to non-target plants.

#### *Monitoring Site and Seeding Site Selection*

Monitoring sites were chosen on the basis that they were treated with imazapic to control cheatgrass. These sites were treated with imazapic as part of an older study examining the effectiveness of imazapic in controlling cheatgrass and its effects on non-target species.

#### *Seeding Plot Installation*

In 2011, seeding plots were installed at six sites that had been treated with imazapic (23.6% a.i.) for three consecutive years to control cheatgrass infestations. At each site, two seeding plots were installed; one plot seeded at a high rate (HSR) and one plot seeded at a low rate (LSR). The HSR plot design was a 168.2 m<sup>2</sup> (1810 ft<sup>2</sup>) circular nested plot and the LSR plot design was a 9 m<sup>2</sup> (96.9 ft<sup>2</sup>) square plot. Because the area of imazapic treatment was limited at each site, the smaller plot size for the LSR plots was necessary to allow for the inclusion of a test of the effectiveness of a lower seeding rate in addition to the large HSR plots. In addition to these seeded plots, one unseeded CNP control plot was established at each herbicide treatment site for a total of six unseeded plots, six high-rate seeding plots and six low-rate seeding plots. The unseeded control plots are plots that were established four years ago for an earlier imazapic study. In the interest of monitoring the success of cheatgrass control and revegetation in different plant communities, representative treatment and seeding plot groups were chosen from forest, shrubland and grassland sites for a total of 6 monitoring sites, each with a treatment/seeding/control plot group.

#### *Seeding*

A native seed mix containing 84% *Elymus elymoides* and 16% *Elymus canadensis* (hereafter, Canada wildrye) was used to seed the herbicide treatment areas in an effort to establish native vegetation that could competitively exclude cheatgrass. Seed was obtained from the Natural Resources Conservation Service (NRCS) Upper Colorado Environmental Plant Center (Meeker, CO) where a native seed expansion program has been contracted by RMNP. This allows for the use of propagules derived from seed collected from within RMNP, preserving the native genetic profile of the plants used for revegetation. Proportions of each species in the final mix were based on proportions of available seed at the NRCS seedbank.

Prior to seeding a site, the soil was raked to a depth of approximately 1 cm. Seed was hand-broadcast and raked into the soil to ensure sufficient soil coverage. Seeded sites were mulched using coarse wood fiber mulch at a cover rate of 50-75%. HSR plots were seeded at a rate of 2150 pure live seed (PLS)/m<sup>2</sup> (200 PLS/ft<sup>2</sup>) and low seeding rate plots were seeded at a rate of 750 PLS/m<sup>2</sup> (70 PLS/ft<sup>2</sup>). All plots were seeded in mid-September and were covered by snowfall within three weeks of seeding.

#### *Vegetation Monitoring*

In 2011, permanent circular nested vegetation monitoring plots (CNP) were installed at each control and HSR monitoring site and square nested vegetation plots (SNP) were installed at each LSR monitoring site.

The design of the CNPs consists of a circular plot with three 24 foot spokes at 30, 150 and 270 degrees. A 1 m<sup>2</sup> vegetation sampling quadrat is then placed along each of these spokes (see Fig. 1). Detailed plot installation and monitoring directions for this study can be found in Appendices 1 and 2.

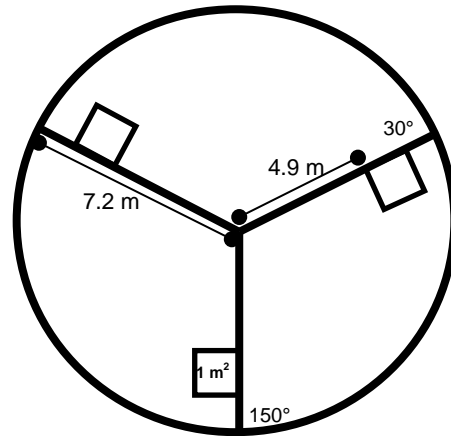


Fig. 3 Circular nested plot used to monitor vegetation at the HSR and control sites.

The design of the small LSR plots consists of a SNP with sides measuring 3 m in length (Fig. 2). A 1 m<sup>2</sup> quadrat is placed directly in the center of the SNP.

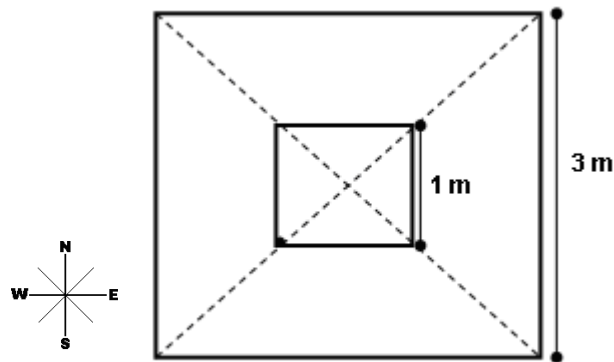


Fig. 2. Square nested plot used to monitor vegetation at the LSR sites.

To survey the plant species in all plots, both percent cover and species presence were recorded. Percent cover was estimated within a 1 m<sup>2</sup> quadrat placed at the 30, 150 and 270 degree spokes of the CNP and at the center of the SNP. Each species present in a quadrat was recorded and an ocular estimation of percent cover for the individual species was made. Percent cover was also estimated for bare ground, litter, rock, moss and lichen. Tree, shrub and forb percent cover were estimated using canopy cover while graminoid (grass, sedge and rush species) cover was estimated using cover at ground level (basal cover). Percent cover was estimated using modified Daubenmire cover classes (Daubenmire 1959) as ranges of percent cover. All species observed within the CNP or SNP but not observed within the three quadrats, were recorded by performing a time-constrained survey of the entire CNP.

### *Data Analysis*

The mid-point of the cover class was used as the cover value for each species in a quadrat. This was obtained by calculating the mean of the minimum and maximum values of the cover class range. When summarizing data for entire CNPs, the average percent cover for each species in each quadrat was averaged across all three quadrats in the CNP. Summarizing data for SNPs required only calculating the mid-point of the cover class for the single 1 m<sup>2</sup> quadrat in each SNP.

The species in each CNP and SNP and their corresponding mean percent cover were then grouped according to growth habit and 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 and SNP 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 designation in the USDA PLANTS profile database and were classified as native or non-native based upon their designation as such in Weber and Wittmann (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. Cheatgrass presence and its percent cover were also summarized separately from other invasive grass species because the focus of this study is cheatgrass management.

Data were analyzed using the JMP 7 statistical analysis software package (SAS Institute, Cary, NC, USA). For analysis in JMP, the data were formatted so a single row contained all of the data for one CNP. This included plot name, treatment type (treated or reference), vegetation type (forest, grassland or shrubland), and mean percent cover for each of the species categories (native shrubs, non-native forbs, etc.). The data were analyzed for each of the species categories using an analysis of variance (ANOVA) *F*-test to determine whether there were differences in functional group cover between treatment and control plots and vegetation types before seeding. If a significant difference ( $\alpha=0.05$ ) between plots or an interaction between treatment and vegetation type was detected, this was graphed using Microsoft Excel.



## RESULTS

### *Cheatgrass*

Changes in cheatgrass cover over time were dependent on seeding and vegetation type (Table 1, time x seeding x vegetation type). Overall cheatgrass cover averaged across all seeding and vegetation types exhibited a downward trend from 4.5% cover in 2011 to 2.2% in 2013.

Table 1. ANOVA for cheatgrass cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Seeding	2	1.21	0.3467
Veg Type	2	5.74	<b>0.0284</b>
Seeding x Veg Type	4	0.77	0.5745
Within Subjects			
Time	2	4.24	0.0621
Time x Seeding	4	1.17	0.3662
Time x Veg Type	4	3.81	<b>0.0269</b>
Time x Seeding x Veg Type	8	3.11	<b>0.0305</b>

On average, cheatgrass cover was lowest in forested plots, and increased by nearly 1% in unseeded plots between 2012 and 2013 (Fig. 3).

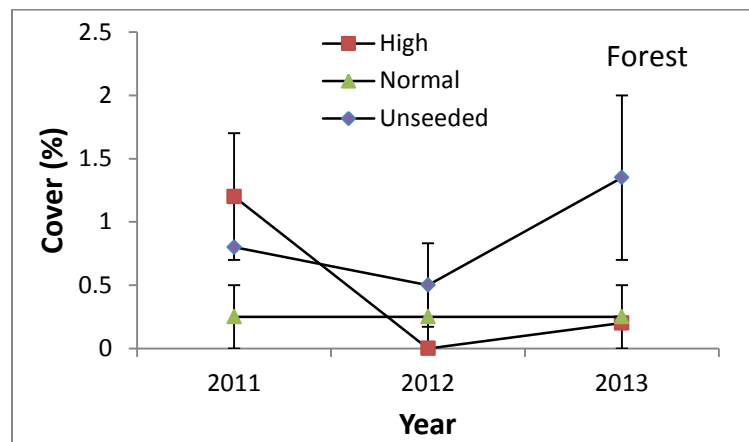


Fig 3. Cheatgrass cover in forest plots by native grass seeding rate (mean  $\pm$  1 standard error of the mean).

Cheatgrass cover in grassland plots was greatest in high seeding rate plots in 2011, but a 4.5% drop in cover eliminated this difference between plot types by 2013 (Fig. 4).

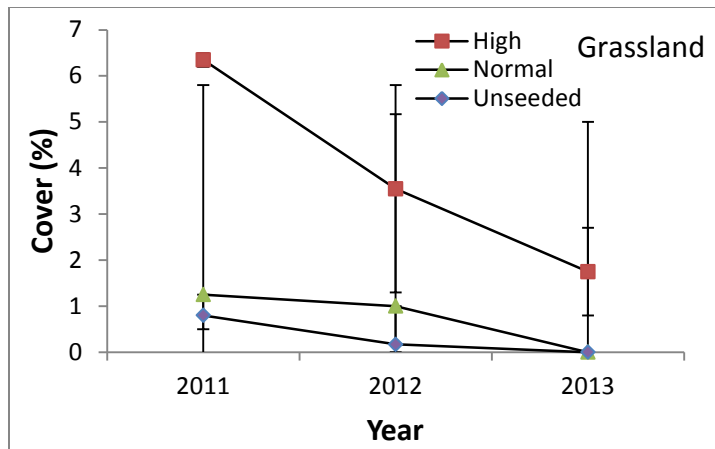


Fig 4. Cheatgrass cover in grassland plots by native grass seeding rate (mean  $\pm$  1 standard error of the mean).

Averaged over seeding treatments, cheatgrass cover was greatest in shrubland plots and has exhibited a downward trend between 2011 and 2013; being reduced by nearly half (Fig. 5).

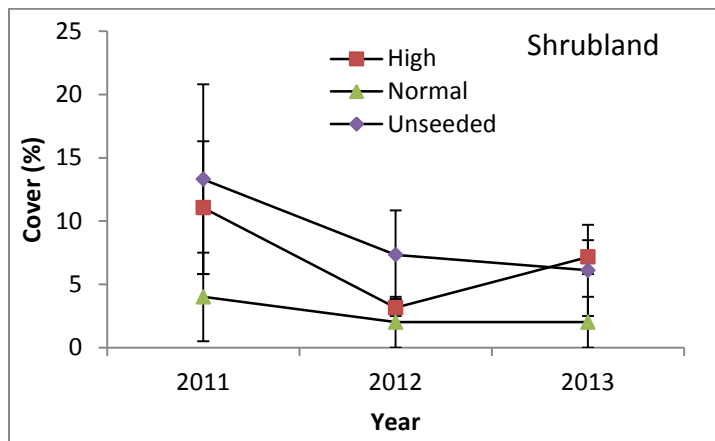


Fig 5. Cheatgrass cover in shrubland plots by seeding rate (mean  $\pm$  1 standard error of the mean).

### ***Native Species Functional Groups***

There was no change in native graminoid cover or species richness over time or in response to seeding (Table 2, Table 3). Average graminoid cover was half as great in 2012 and 2013 than in 2011, which was a marginally significant change over time (Table 2, time; Fig. 6). The native grass species (*E. elymoides* and *E. canadensis*) used to seed these plots were only observed in trace amounts in any plot, with only one or two individual plants observed in a few instances in all plots.

Table 2. ANOVA for native graminoid cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Seeding	2	0.21	0.8174
Veg Type	2	0.37	0.7032
Seeding x Veg Type	4	1.36	0.3297
Within Subjects			
Time	2	4.50	0.0555
Time x Seeding	4	0.48	0.7476
Time x Veg Type	4	0.22	0.9255
Time x Seeding x Veg Type	8	1.74	0.1734

Table 3. ANOVA for native graminoid species richness. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Seeding	3	2.60	0.1103
Veg Type	2	0.21	0.8159
Seeding x Veg Type	6	0.14	0.9863
Within Subjects			
Time	2	0.86	0.4546
Time x Seeding	6	0.81	0.5745
Time x Veg Type	4	1.25	0.3266
Time x Seeding x Veg Type	12	0.29	0.9826

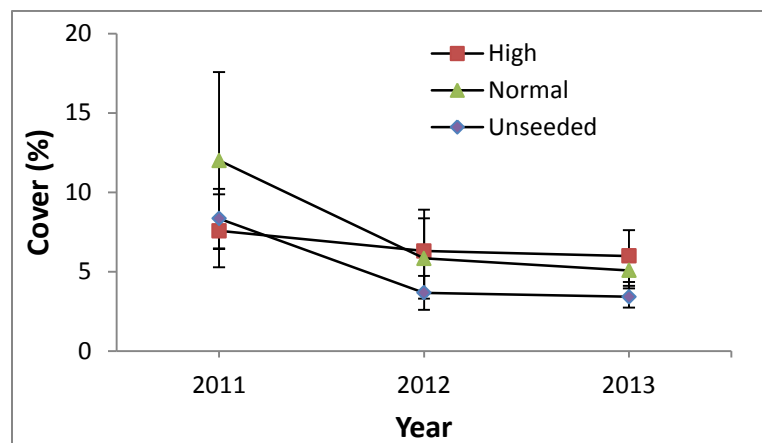


Fig 6. Native graminoid cover by seeding rate (mean  $\pm$  1 standard error of the mean).

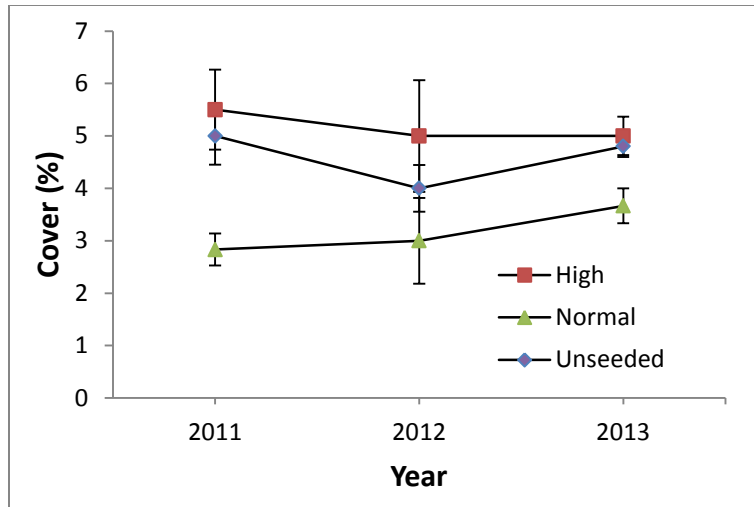


Fig 7. Native graminoid species richness by seeding rate (mean  $\pm$  1 standard error of the mean).

Native forb cover changed over time dependent on vegetation type and seeding type (Table 4, time x seeding x veg type).

In forest plots native forb cover fell from 12% to 6% between 2011 and 2012 in high seed plots, while in normal seeding rate plots cover increased from 2% to 6% (Fig. 8). Average native forb cover was greatest in grassland plots and declined from a high of 30% in 2011 to 12.5% in 2013, mostly due to changes in cover of the most common forb species: *Heterotheca villosa*, *Erigeron flagellaris* and *Thermopsis divaricarpa* (Fig. 9). In shrubland plots forb cover remained relatively unchanged over time (Fig. 10).

Table 4. ANOVA for native forb cover. Statistically significant p-values in bold.

Source of Variation	df	F-statistic	P-value
Between Subjects			
Seeding	2	2.15	0.1791
Veg Type	2	17.33	<b>0.0012</b>
Seeding x Veg Type	4	4.57	<b>0.0325</b>
Within Subjects			
Time	2	7.55	<b>0.0179</b>
Time x Seeding	4	3.49	<b>0.0354</b>
Time x Veg Type	4	2.26	0.1141
Time x Seeding x Veg Type	8	3.03	<b>0.0334</b>

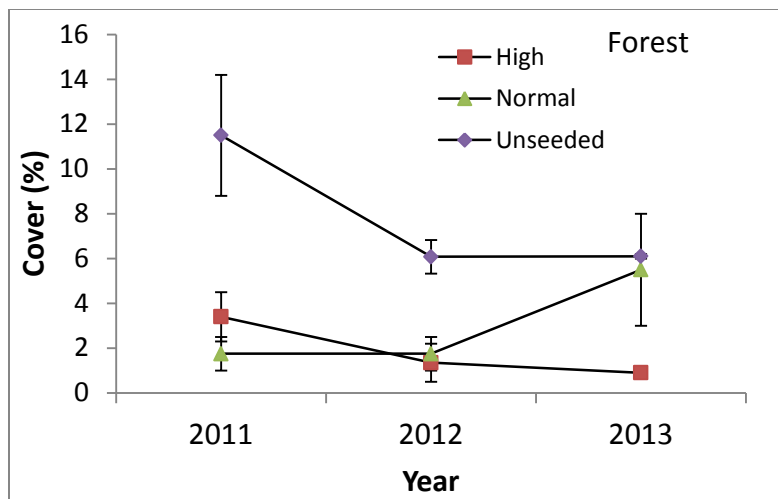


Fig 8. Native cover in forest plots by seeding rate (mean  $\pm$  1 standard error of the mean).

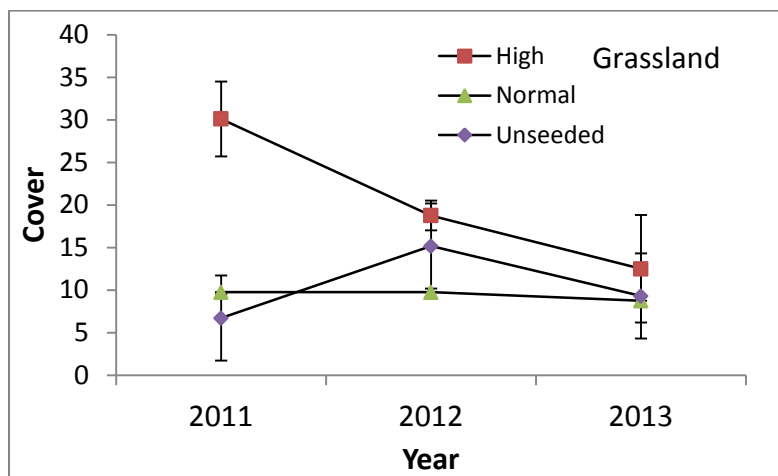


Fig 9. Native cover in forest plots by seeding rate (mean  $\pm$  1 standard error of the mean).

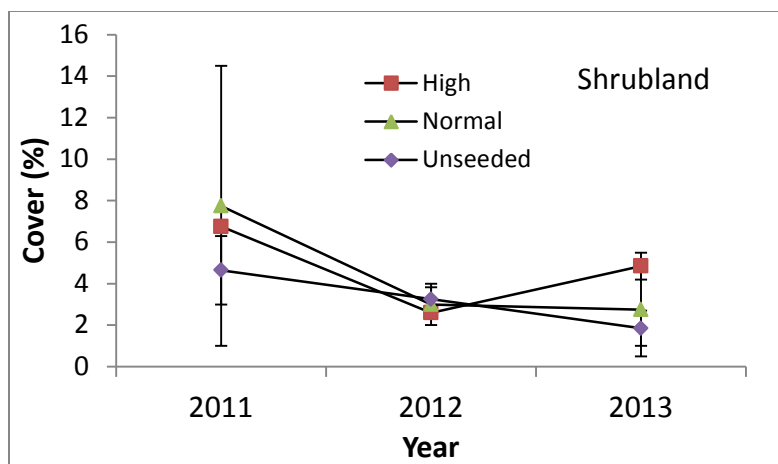


Fig 10. Native cover in forest plots by seeding rate (mean  $\pm$  1 standard error of the mean).

Native shrub cover did not change in response to seeding over time (Table 5).

Table 5. ANOVA for native shrub cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Seeding	2	2.59	0.1356
Veg Type	2	2.13	0.1812
Seeding x Veg Type	4	0.26	0.8965
Within Subjects			
Time	2	0.57	0.5875
Time x Seeding	4	0.84	0.5209
Time x Veg Type	4	0.62	0.6580
Time x Seeding x Veg Type	8	0.34	0.9378

Native tree cover was greatest in forest plots in all years and did not change over time (Table 6, veg type).

Table 6. ANOVA for native tree cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Seeding	2	0.12	0.8923
Veg Type	2	7.57	<b>0.0143</b>
Seeding x Veg Type	4	0.12	0.9732
Within Subjects			
Time	2	0.62	0.5665
Time x Seeding	4	1.00	0.4389
Time x Veg Type	4	0.60	0.6671
Time x Seeding x Veg Type	8	1.06	0.4403

### ***Invasive Species Functional Groups***

Invasive graminoid cover, not including cheatgrass cover, did not change in response to seeding over time (Table 7). Invasive grasses other than cheatgrass were very uncommon in these plots and were only observed in trace amounts in 2011 and not observed in 2012 or 2013.

Table 7. ANOVA for invasive graminoid cover (excluding cheatgrass). Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Seeding	2	0.85	0.4633
Veg Type	2	0.73	0.5126
Seeding x Veg Type	4	0.82	0.5470
Within Subjects			
Time	2	0.35	0.7164
Time x Seeding	4	0.35	0.8374
Time x Veg Type	4	0.30	0.8699
Time x Seeding x Veg Type	8	0.33	0.9411

Invasive forb cover did not change in response to seeding over time (Table 8). Invasive forbs were typically uncommon in these plots, though musk thistle (*Carduus nutans*) has been gradually encroaching upon the Moraine Park forest plots from an adjacent infestation.

Table 8. ANOVA for invasive forb cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Seeding	2	0.64	0.5514
Veg Type	2	0.73	0.5108
Seeding x Veg Type	4	0.88	0.5181
Within Subjects			
Time	2	0.37	0.7037
Time x Seeding	4	0.47	0.7599
Time x Veg Type	4	0.88	0.5001
Time x Seeding x Veg Type	8	0.83	0.5938

### ***Non-native Non-invasive Functional Groups***

There was no change in non-native, non-invasive graminoid cover in response to seeding over time (Table 9). Cover for this functional group was only observed in trace amounts in all years.

Table 8. ANOVA for non-native non-invasive graminoid cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Seeding	2	0.73	0.5126
Veg Type	2	0.85	0.4633
Seeding x Veg Type	4	0.82	0.5470
Within Subjects			
Time	2	0.35	0.7164
Time x Seeding	4	0.30	0.8699
Time x Veg Type	4	0.35	0.8374
Time x Seeding x Veg Type	8	0.33	0.9411

Non-native non-invasive forb cover decreased in high seed shrubland plots from 0.7% in 2011 to 0.2% in 2013 (Table 9, time x seeding x veg type). This was mainly due to changes in cover of *Alyssum alyssoides*, a common species in those plots.

Table 9. ANOVA for non-native non-invasive forb cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Seeding	2	7.43	<b>0.0150</b>
Veg Type	2	8.84	<b>0.0094</b>
Seeding x Veg Type	4	6.27	<b>0.0138</b>
Within Subjects			
Time	2	17.91	<b>0.0018</b>
Time x Seeding	4	5.28	<b>0.0083</b>
Time x Veg Type	4	5.72	<b>0.0061</b>
Time x Seeding x Veg Type	8	3.72	<b>0.0155</b>



## Bare Soil

Bare soil cover did not change over time in response to seeding (Table 10).

Table 10. ANOVA for bare soil cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Seeding	2	0.56	0.5915
Veg Type	2	2.07	0.1881
Seeding x Veg Type	4	0.28	0.8803
Within Subjects			
Time	2	1.66	0.2578
Time x Seeding	4	0.74	0.5809
Time x Veg Type	4	0.63	0.6476
Time x Seeding x Veg Type	8	1.31	0.3133

*Annotated ANOVA tables for other general functional groups not discussed in this results section can be found in the appendix.*

## DISCUSSION

Seeded grasses did not successfully germinate and become established between 2011 and 2013, and any changes in vegetation cover observed thus far are not likely due to seeding treatments.

A common trend in many functional groups across all vegetation and seeding types was a reduction of vegetation cover in 2012 due to drought conditions experienced that year (Fig. 11). Previous studies have shown that available soil moisture greatly influences perennial grass seedling survival, especially during the germination stage of development (Eissenstat and Caldwell 1988, Chambers 2000, James et al. 2012). Studies have specifically shown that droughty summer conditions can result in significant seedling mortality of perennial grasses (Pyke 1990, Salahi and Norton 1987). Also, perennial grass species may require up to two growing seasons to become established following seeding (Winslow 2002).

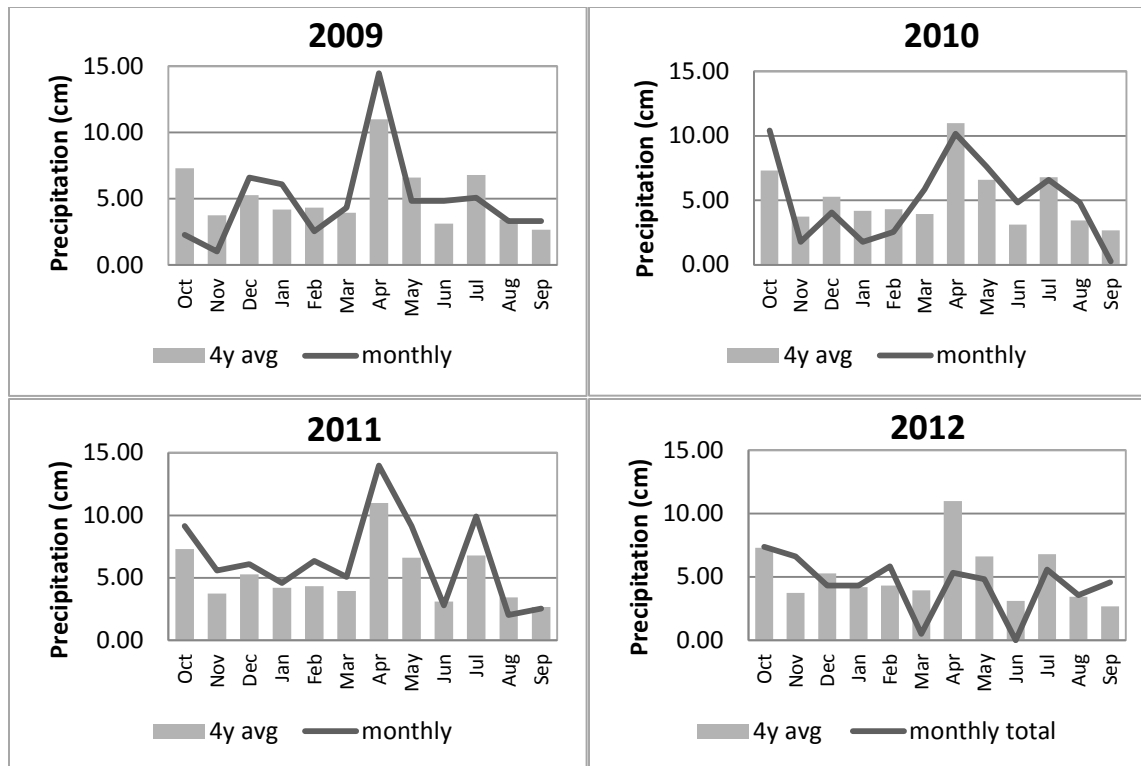


Fig 11. Yearly accumulated precipitation at NRCS Hourglass Lake climate monitoring station in RMNP (NRCS 2012). Years indicated in the legend are water years (e.g. water year 2012 is Oct. 1, 2011 - Sep. 30, 2012). The four year average is for water years 2009-2012.

The dry spring and summer of 2012 may have affected germination rates of seed in our plots the first growing season following seeding and effectively ‘reset’ the two year establishment period, causing a lag in the increase in cover of those seeded species. If this assumption is correct, we would expect to observe greater seeded species cover in the 2014 monitoring season.

Interestingly, cheatgrass cover has not increased from the levels achieved after three consecutive years of imazapic treatment. It has also been three years since imazapic treatments were last applied and far beyond the typically observed period of soil activity of imazapic (Tu et al. 2001; ACC 2000). In 2013, many disturbed and untreated sites around the park experienced noticeable increases in cheatgrass abundance and vigor (personal observation). This suggests that three consecutive years of imazapic treatment has effectively inhibited reestablishment of cheatgrass in treatment sites, possibly through a reduction of cheatgrass seed availability in the treated soils.

This project is ongoing and data collected in the upcoming field seasons will allow us to better understand what mechanisms are influencing seeding success in these plots and to monitor changes in cheatgrass infestation at these sites.

## LITERATURE CITED

- [ACC] American Cyanamid Company. 2000. Plateau herbicide, for weed control, native grass establishment and turf growth suppression on roadsides and other noncrop areas. PE-47015.
- Baker, W.L., J. Garner and P. Lyon. 2009. Effects of imazapic on cheatgrass and native plants in Wyoming big sagebrush restoration for Gunnison sage-grouse. *Natural Areas Journal*. **29**:204-209.
- Beck, K.G. 2003. Downy brome (*Bromus tectorum*) and Japanese brome (*Bromus japonicus*): Biology, ecology, and management. Department of Bioagricultural Sciences and Pest Management, Colorado State University. p 1-60.
- Chambers, J.C. 2000. Seed movements and seedling fates in disturbed sagebrush steppe ecosystems: Implications for restoration. *Ecological Applications*. **10**:1400-1413.
- Daubenmire, R.F. 1959. Canopy coverage method of vegetation analysis. *Northwest Science*. **33**:43-64.
- Davies, K.W. 2010. Revegetation of medusahead-invaded sagebrush steppe. *Rangeland Ecology and Management*. **63**:564-571.
- Eissenstat, D.M. and M. M. Caldwell. 1988. Competitive ability is linked to rates of water extraction. A field study of two aridland tussock grasses. *Oecologia*, **75**:1-7
- James, J.J., M. J. Rinella and T. Svejcar. 2012. Grass seedling demography and sagebrush steppe restoration. *Rangeland Ecology & Management*. **65**:409-417.
- Hironaka, M. and B.W. Sindelar. 1973. Reproductive success of squirreltail in medusahead infested ranges. *Journal of Range Management*. **26**:219-221
- Hironaka, M., B.W. Sindelar. 1975. Growth characteristics of squirreltail seedlings in competition with medusahead. *Journal of Range Management*. **28**:283-285.
- Hulbert, L.C. 1955. Ecological studies of *Bromus tectorum* and other annual brome grasses. *Ecological Monographs*. **25**:181-213
- Humphrey, L.D. and E.W. Schupp. 2004. Competition as a barrier to establishment of a native perennial grass (*Elymus elymoides*) in alien annual grass (*Bromus tectorum*) communities. *Journal of Arid Environments*. **58**:405-422.
- Knapp, P.A. 1996. Cheatgrass (*Bromus tectorum* L.) Dominance in the great basin desert. *Global Environmental Change*. **6**:37-52.
- Mack, R. N., 1981. Invasion of *Bromus tectorum* L. into Western North America: An ecological chronicle. *Agro-Ecosystems*. **7**:145-165.
- McGlone, C.M., C.H. Sieg and T.E. Kolb. 2011. Invasion resistance and persistence: Established plants win, even with disturbance and high propagule pressure. *Biological Invasions*. **13**:291-304.
- Pyke, D.A. 1990. Comparative demography of co-occurring introduced and native tussock grasses: Persistence and potential expansion. *Oecologia*. **82**:537-543.
- Salahi, D.O. and B.E. Norton. 1987. Survival of perennial grass seedlings under intensive grazing in semiarid rangelands. *Journal of Applied Ecology*. **24**:145-151.

- Sheley, R.L., M.F. Carpinelli and K.J. Reever-Morghan. 2007. Effects of imazapic on target and nontarget vegetation during revegetation. *Weed Technology*. **21**:1071–1081.
- Tu, M., C. Hurd and J.M. Randall, 2001. Weed control methods handbook. The Nature Conservancy. p 7g.1-7g.7.
- Weber, W.A and R.C. Wittmann. 2001. Colorado flora eastern slope 3<sup>rd</sup> ed. University Press of Colorado, Boulder, Colorado, USA.
- Winslow, S.R. 2002. Propagation protocol for production of *Elymus elymoides* seeds; USDA NRCS - Bridger Plant Materials.

# Yellow toadflax (*Linaria vulgaris*) control: Herbicide pilot study in Hollowell Park

## ABSTRACT

Yellow toadflax is a high priority invasive forb in Rocky Mountain National Park due to its aggressive invasiveness. Chlorosulfuron, an herbicide shown to be effective in controlling toadflax species, is employed by the park for control of yellow toadflax in Hollowell Park. The purpose of this study is to determine the effectiveness of chlorosulfuron in reducing toadflax densities in Hollowell Park. After a single chlorosulfuron treatment, yellow toadflax densities were reduced, but not significantly so. Toadflax density in the untreated control plot increased, suggesting chlorosulfuron may have prevented a similar increase in treatment plots.

## INTRODUCTION

Yellow toadflax (*Linaria vulgaris*), commonly known as butter and eggs or impudent lawyer, is an invasive forb that was introduced to North America for both ornamental and folk medicine purposes and currently occupies more than 40,000 acres of land in Colorado (Beck 2009). Toadflax species are aggressive invaders of disturbed lands and readily reproduce by vegetative means as well as by seed (Beck 2009).

In Rocky Mountain National Park, yellow toadflax is controlled using the herbicide chlorosulfuron (Telar, DuPont) in combination with methylated seed oil (MSO) surfactant. The purpose of this pilot study is to determine the effectiveness of chlorosulfuron for yellow toadflax management; specifically whether chlorosulfuron treatments kill toadflax plants entirely or only damage the crown of the plant.



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## MATERIALS AND METHODS

Hollowell Park, which was the location of a former Civilian Conservation Corps camp, has been invaded by yellow toadflax and was chosen as the site for this study due to the high density of toadflax plants per unit area.

The site is an upland grassy meadow surrounded by coniferous forest and has patches of Canada and musk thistle which were also treated along with the toadflax infestations.

Six treatment plots and one control plot were established in the treatment site. The control plot was located within one meter of a stream which is outside the approved treatment area, as chlorosulfuron cannot be applied at the water's edge. Vegetation density data were collected along 40 m transects at 1 m increments. Density data are a good indicator of plant mortality, which is the focus of this study.

Temporary wood-stake plot markers were placed at the northern end of each transect to ensure accurate plot relocation. The number of yellow toadflax stems rooted within a 0.09 m<sup>2</sup> (1 ft<sup>2</sup>) quadrat were recorded at 40 increments along the transect. The placement of the first increment was determined by randomly choosing a number between 0 and 10, and placing the quadrat at that decimeter mark in the first meter of the transect. All subsequent quadrat placements were one meter farther along the transect. All plots were oriented along a north-south azimuth. Exact GPS locations and azimuths can be found in the appendices.

Chlorosulfuron was combined with aminopyralid and MSO surfactant in a batch mix to allow for the concurrent treatment of yellow toadflax and Canada thistle. Chlorosulfuron was applied at a rate of 2.5 oz/ac with MSO mixed at a rate of 1% of the total solution. Aminopyralid was included in the herbicide mix at a rate of 6 oz/ac. The herbicide mix was applied using backpack sprayers. Yellow toadflax patches were spot-treated, avoiding application on adjacent non-target plants, though no effort was made to avoid non-target plants rooted within a patch of toadflax. Herbicide was applied in mid-August.

## RESULTS

Following herbicide application mean toadflax density decreased 76% from 45 plants/m<sup>2</sup> to 11 plants/m<sup>2</sup> (Figure 1), though this decrease in density was not significant (paired t-test,  $p=0.113$ ).

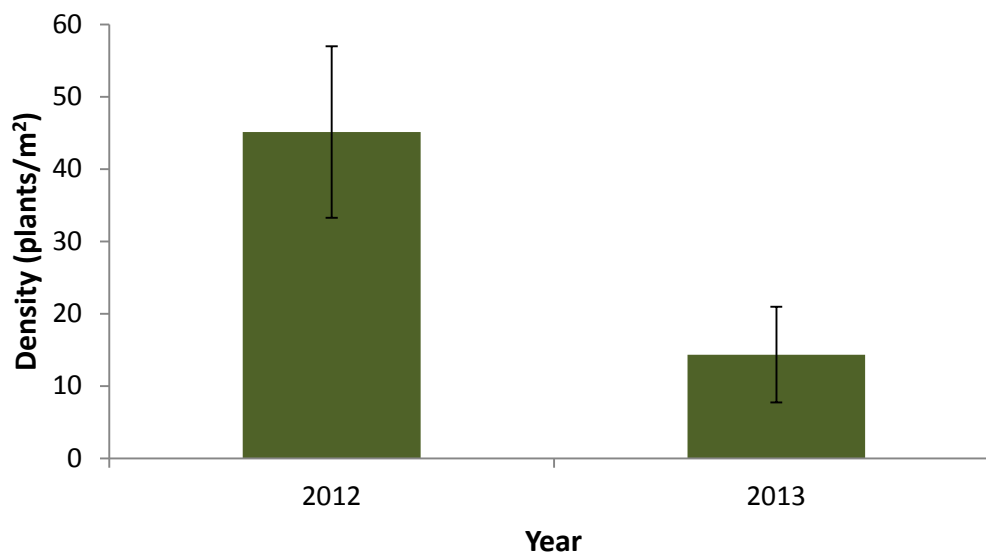


Figure 1. Mean density of toadflax plants in treatment plots before and after herbicide application. Chlorosulfuron was applied in the fall of 2012. (mean  $\pm$  standard error of the mean)

In the control plot, mean toadflax density increased by 31% from 83 plants/m<sup>2</sup> to 120 plants/m<sup>2</sup> between 2012 and 2013 (Figure 2).

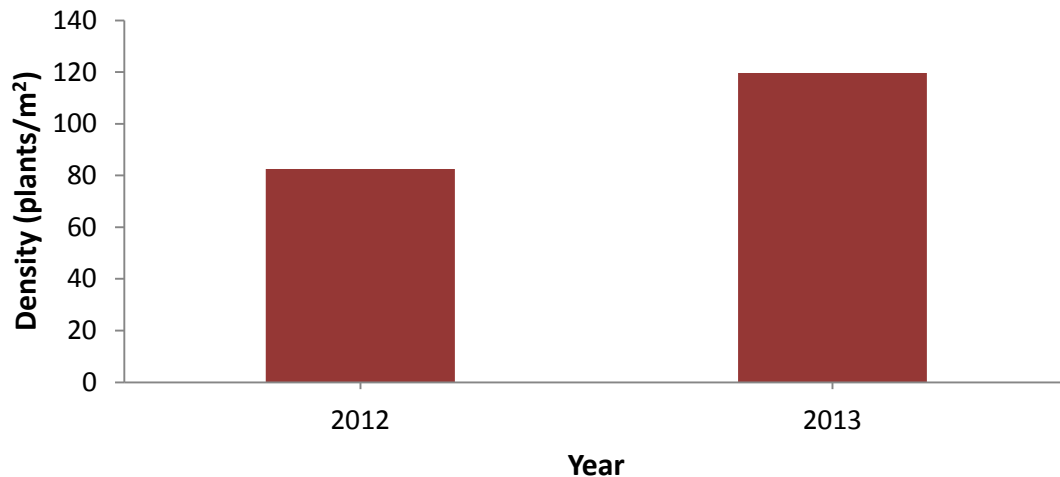


Figure 2. Mean density of toadflax plants in untreated control plots.

## DISCUSSION

Several studies throughout Colorado have shown that yellow toadflax control using chlorsulfuron is highly variable with toadflax reduction ranging from 100% to as low as 35% (Beck 2009). Toadflax populations are often able to recover from a single chlorsulfuron application and yearly applications for three years have been shown to be effective (Beck 2009). Chlorsulfuron has also been shown to control the density and biomass of the related Dalmatian toadflax for as long as three years following application (Jacobs and Cheley 2005).

Although there was not a statistically significant reduction of toadflax density in plots treated with herbicide, there was an increase in toadflax densities in the untreated control plot, suggesting that herbicide treatment may have prevented a similar increase in treatment plots. Continued herbicide application is recommended to further reduce toadflax density in Hollowell Park.

#### LITERATURE CITED

Beck, K.G. 2009. Biology and management of the toadflaxes. Colorado State University Extension.

Jacobs, J.S. and R.L. Sheley. 2005. The effect of season on picloram and chlorsulfuron application on Dalmatian toadflax (*Linaria genistifolia*) on prescribed burns. *Weed Technology*. 19:319-324.



# Bear Lake Road Phase II: Hydroseeding Monitoring

## INTRODUCTION

In the spring of 2012 the second phase of road construction began along Bear Lake Road with the goal of improving deteriorating road conditions, accommodating increased shuttle bus traffic, and improving overall safety of the roadway. Approximately 4.3 miles of road was reconstructed from the Trail Ridge Road intersection to the Glacier Basin Park and Ride. A one mile section of road was entirely rerouted away from Glacier Creek, cutting through old growth montane forest. The area of habitat disturbance resulting from this construction effort was estimated to be 26 ac.

A priority of rehabilitating these impacted sites was to facilitate the rapid reestablishment of native vegetation with the goals of increasing soil stability on exposed steep slopes and discouraging invasion by invasive exotic plants located nearby. Between 2008 and 2012 herbicide was applied in areas of planned disturbance to reduce the abundance of invasive species in those sites that would be hydroseeded.

The purpose of this study is to monitor the success of hydroseeding treatments in establishing robust native plant communities that will stabilize slopes and compete with invading exotic plant species.

## MATERIALS AND METHODS

Hydroseeding was completed on these monitoring sites in the fall of 2012 by WildLands Inc. revegetation contractors. Seed was mixed with bonded fiber matrix (BFM) shredded wood mulch and tackifier, and this BFM and seed mixture was applied at a rate of 3,000 pounds of BFM and 60 pounds of seed per acre. This was applied in several passes of the hydroseeder, with the first pass applying 60 pounds of seed and 500 pounds of BFM per acre and subsequent passes applying an additional 2500 pounds of BFM. This seeding rate resulted in the application 200 pure live seeds (PLS) per square foot of disturbed soil; a significantly higher rate than what is typically used in park restoration rates. A miscalculation in hydroseeding resulted in two slopes with slightly higher and lower seeding rates. These sites, MS1 and MS2, were seeded at 130 seeds/ft<sup>2</sup> and 220 seeds/ft<sup>2</sup>, respectively. This will allow for an interesting comparison of different seeding rates. These seeded sites were also watered throughout the summer of 2013.

Seed for this project was acquired through a variety of sources including seed that was increased from native seed collected within the park by the Upper Colorado Environmental Plant Center in Meeker, Colorado. Seeded species and their respective proportion of the seed mix can be found in Table 1.

Table 1. Seeded species and their proportion and applied rate in the seed mix. Seed numbers are equal to pure live seed.

<i>Scientific Name</i> (Common name)	Percent of mix	Seeds/ft <sup>2</sup>
<i>Antennaria</i> spp. (Pussytoes)	1.00%	2.00
<i>Artemisia frigida</i> (Fringed sagebrush)	6.00%	12.00
<i>Bouteloua gracilis</i> (Blue grama)	6.25%	12.50
<i>Elymus canadensis</i> (Canada wildrye)	22.50%	45.00
<i>Elymus elymoides</i> (Bottlebrush squirreltail)	22.50%	45.00
<i>Koeleria macrantha</i> (Junegrass)	14.00%	28.00
<i>Muhlenbergia montana</i> (Mountain muhly)	14.50%	29.20
<i>Stipa comata</i> (Needle and thread)	1.25%	2.50
<i>Heterotheca villosa</i> (Hairy golden aster)	1.45%	2.90
<i>Oxytropis lambertii</i> (Purple locoweed)	10.75%	21.60
<i>Thermopsis divaricarpa</i> (Foothills golden banner)	0.75%	0.15

Monitoring plots were installed in five hydroseeded sites by Federal Highways Division contractor Russ Hass for frequency sampling. We resampled seedling density in these same plots. At each site a 50 ft baseline running parallel to the roadside was installed and marked with steel pins at both ends. Each individual baseline has six perpendicular transects running upslope. Transect one is located at 0 ft of the 50 ft baseline and each successive transect is located at 10 ft increments along the baseline (i.e. transect 2 at 10 ft on the baseline, transect 3 at 20 ft on the baseline, etc.). Transect length (and by extension the number of quadrats placed on that transect) varied between sites based on the size of the disturbance being monitored. Quadrats were placed along each transect beginning at the baseline with the bottom left corner of the quadrat placed at the intersection of the transect with the baseline (0 ft on the transect). Subsequent quadrats were placed at each 10 ft interval; alternating sides of the transect with each 10 ft interval up to a maximum of 8 quadrats. At the Moraine Park monitoring site quadrats were placed at 6 ft intervals along transects due to the smaller monitoring site.

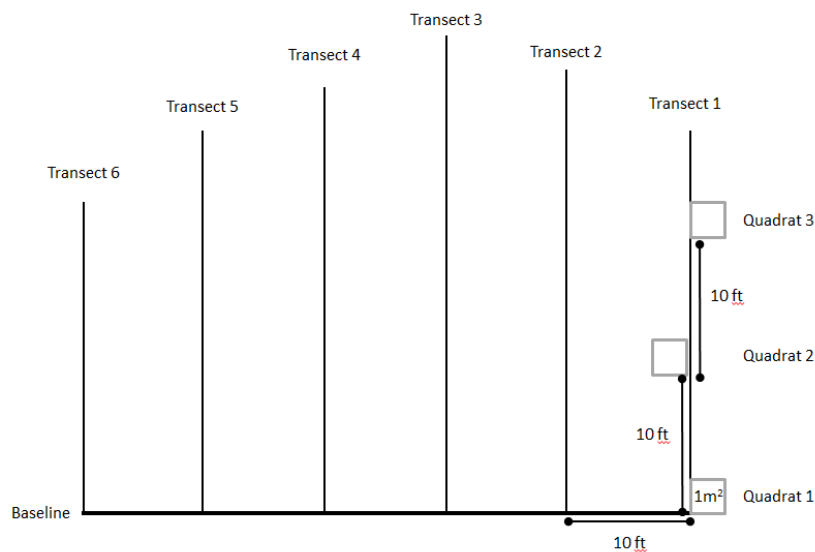


Fig. 1. Monitoring plot design for Bear Lake Road Phase II seeding site monitoring.

Every species identified within a quadrat was recorded and the number of each individual of that species rooted within the quadrat was counted. The first year's data collection densities were summarized across all sites for native seeded, native volunteer, and exotic species. Seeded species were also summarized by individual species and growth form to compare seed mix proportions to those observed in the field.

## RESULTS/DISCUSSION

Overall seedling density of seeded species was approximately 2.5 plants/ft<sup>2</sup>, with native volunteer species and exotic species both below 0.5 plants/ft<sup>2</sup> (Fig. 2). While this seedling density is low compared to the rate seed was applied (200/ft<sup>2</sup>), perennial grass species may require up to two growing seasons to become established following seeding (Winslow 2002). For this restoration project, more than 80% of the seed mix applied to these sites consisted of perennial grass species and our monitoring occurred less than one year after seeding. Continued monitoring of these sites will help to better assess how successful hydroseeding has been in establishing native vegetation on these sites.

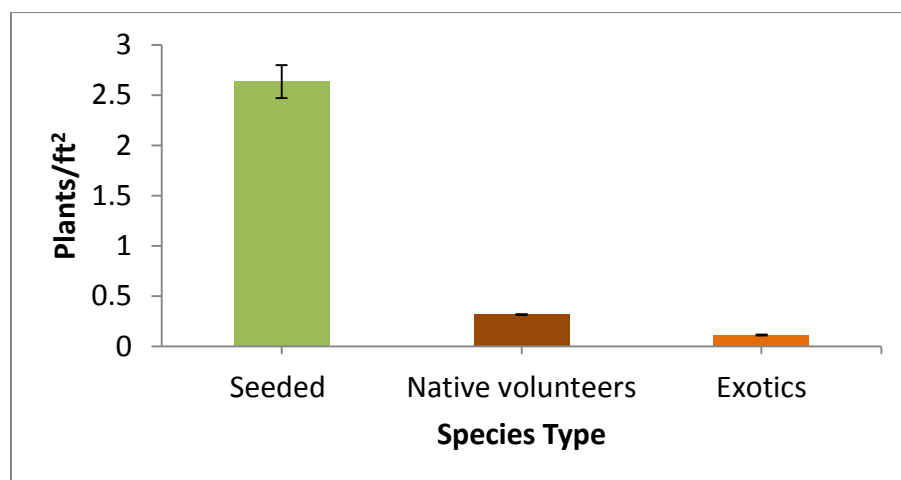


Fig. 2. Seedling density of hydroseeded species, exotic species and native plant volunteer species averaged over all five monitoring sites.

Of the seeded species, *Elymus* species were observed at proportions almost double what would be expected when compared to seeding rates of those species (Fig. 3). Due to the difficulty in differentiating juvenile grasses in the field it is possible that many of the other seeded species tended to get included in the *Elymus* species density measurements. Combining observed seeded species proportions into grass and forb/subshrub categories shows proportions of observed species more closely resembled the seeded proportions (Fig. 4).

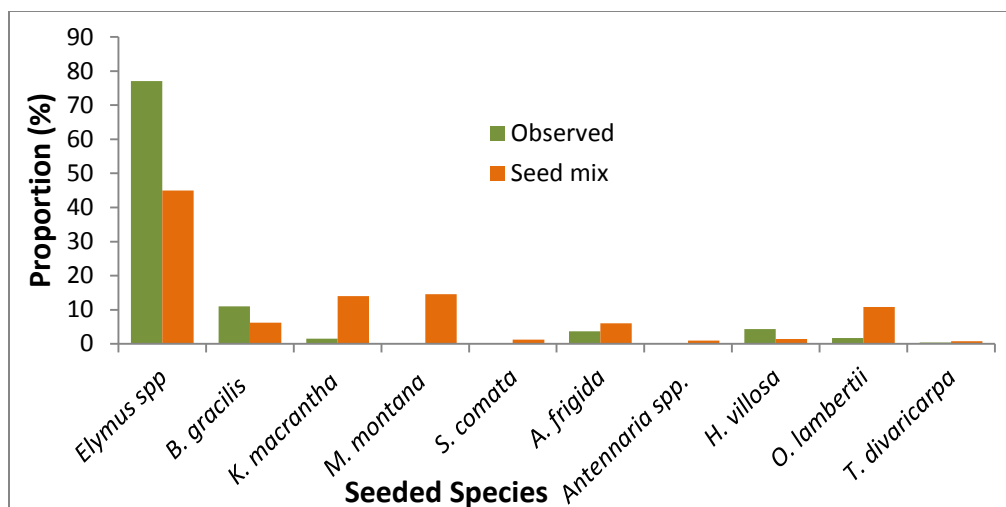


Fig. 3. Seedling proportion observed in the field compared to the proportion for each species in the seed mix used for hydroseeding.

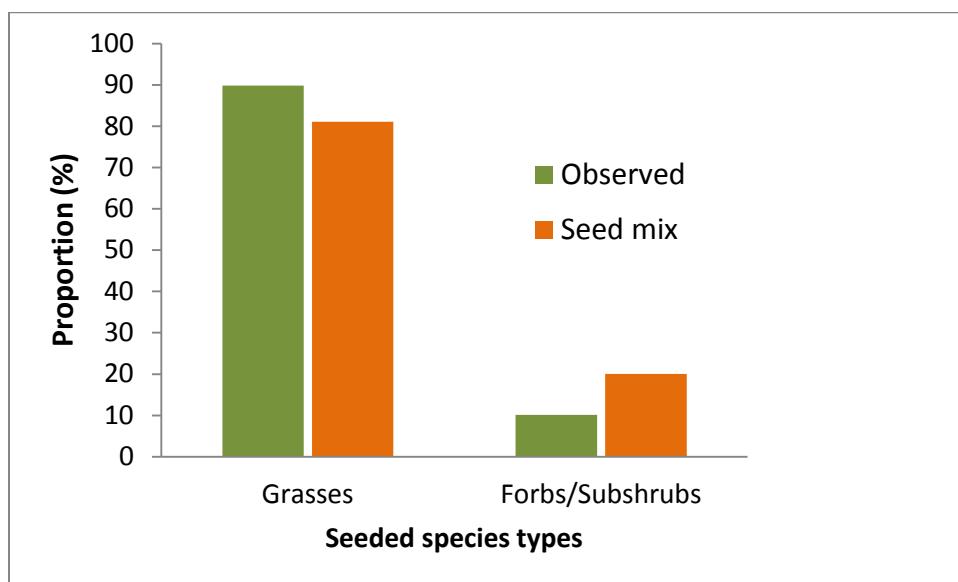


Fig. 4. Seedling proportion observed in the field compared to the proportion for grass and forb/subshrub functional groups.

## CONCLUSIONS

Continued monitoring will provide data for interesting analyses of developing plant communities that incorporate site characteristics such as slope, aspect and elevation, and provide a complete picture of the vegetation dynamics on these sites.

## LITERATURE CITED

Winslow, S.R. 2002. Propagation protocol for production of *Elymus elymoides* seeds; USDA NRCS - Bridger Plant Materials.

## Appendix 1

### **Circular Nested Plot Installation, Data Collection and Analysis**

#### *Installation*

Circular nested vegetation monitoring plots (CNP) have an area of approximately 168.2 m<sup>2</sup> (1810 ft<sup>2</sup>), or 0.01682 ha (1/24 of an acre). The design of these plots consists of a circular plot with three, 7.2 m (24 ft) spokes at 30, 150 and 270 degrees from the center of the plot. A 1 m<sup>2</sup> quadrat is located on each of these spokes 4.9 m (16 ft) from the center of the plot (see Fig. 1).

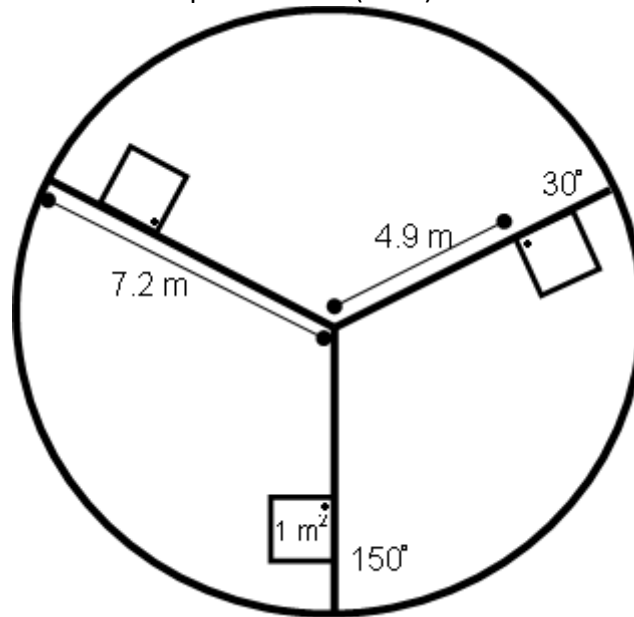


Fig. 1. Diagram of circular nested plot installation. Rebar stakes are located in the center of the CNP as well as 4.9 m from the center stake at 30, 150 and 270 degrees. Square meter quadrats are placed at each spoke so the rebar stake is in the bottom left hand corner of the quadrat when facing away from the center stake. The left side of the quadrat is oriented parallel to the spoke.

CNPs are installed by hammering a one foot length of rebar into the ground at the center point of the CNP. To determine placement of the spokes, a 7.2 m length of rope is secured to the center stake. A knot tied in the rope at 4.9 m from the center stake allows for the consistent location of the stake placement for each spoke. To orient the first spoke at the 30° azimuth, a person stands over the center stake of the CNP and uses a magnetic compass to determine when the rope that is secured to the center stake is approximately aligned with the compass bearing of 30°. When the rope is correctly positioned, a one foot length of rebar is hammered into the ground 4.9 m from the center stake (at the knot). This process is repeated for the 150 and 270 degree azimuths.

For each individual CNP installed for a monitoring project, GPS coordinates and plot elevation are recorded at the center stake using a handheld GPS unit in the NAD 83 CONUS datum setting.

### Data Collection

A 1 m<sup>2</sup> quadrat is oriented such that its left side is parallel to the rope and the stake is positioned in the lower left corner of the quadrat when facing outwards from the center stake of the CNP (see Fig. 1).

Percent cover for all plant species, bare ground, litter, rock and moss/lichen located within each quadrat is estimated using modified Daubenmire cover classes (Daubenmire 1959). Percent cover is estimated as ranges of percent cover as follows: 0, 0-1, 1-3, 3-5, 5-10, 10-25, 25-50, 50-75, 75-95, 95-99 and 100 percent. Each species present in the quadrat is recorded and an ocular estimation of a percent cover range for individual species is then made.

Tree, shrub and forb percent cover are estimated using canopy cover while graminoid (grass, rush and sedge species) cover is estimated using cover at ground level (basal cover). A species is considered to be inside a quadrat if any part of the plant is within a vertical projection of the square meter quadrat (i.e. an overhanging tree or shrub branch is considered inside the quadrat even if the plant is not rooted within the quadrat). Moss and lichen basal cover is only estimated when mosses and/or lichens are growing in soil and not on a rock surface. Standing litter cover is estimated using the same criteria as the living plant tissue would be estimated and dead and down litter cover is estimated basally.

All species within the CNP but not observed within the three quadrats are also recorded. This is done by performing a 20 minute time-constrained walk-through of the entire CNP and recording the species present within the perimeter of the CNP. The walk-through is performed by having one person pull the 7.2 m length of rope taut from the center stake. Another person scans along the rope as the rope-holder walks in a circle keeping the rope taut while walking the perimeter of the CNP. Plant species are recorded and identified as necessary as they are located within the CNP.

If a plant cannot be identified in the field, a sample is collected from outside the CNP and placed in a sealable collection bag for later identification. For forbs and grasses two entire plants should be collected, including the root structure. If a plant is very rare or only located within a CNP, a specimen is not collected and it is recorded as an unknown grass, forb, shrub or tree species with notes taken about its appearance for help identifying it in subsequent monitoring years.

Before collecting quadrat data, the data collection date names of data collectors are recorded for each monitoring plot. At each 1 m<sup>2</sup> quadrat of a CNP a

picture is taken, oriented with the rope parallel to the of the picture with a whiteboard in frame indicating



Fig. 2. Example of photographs taken at each quadrat of a circular nested plot.

and

bottom the plot

name, quadrat azimuth, and date of data collection (Fig. 2)

### *Data Analysis*

Data for each monitoring plot is entered into an Excel spreadsheet for ease of manipulation and entry into statistical analysis and graphing software. The data for each individual plot is then summarized. The mid-point of the cover class is used as the cover value for each species in a quadrat. This is obtained by calculating the mean of the minimum and maximum values of the cover class range. For example, if *Chondrosium gracile* was estimated to have a percent cover of 25-50% in a 30 degree quadrat, the mean percent cover would be summarized as  $(25+50)/2$ , or 37.5% for that quadrat.

After calculating the average percent cover for each species in each quadrat, the average for that species across all three quadrats is calculated. For example, if the average cover of *Chondrosium gracile* was 37.5%, 2 % and 7.5% in the 30, 150 and 270 degree quadrats, respectively, the percent cover of *Chondrosium gracile* for the entire CNP would be  $(37.5+2.0+7.5)/3$ , or 15.67%.

The species in each CNP and their corresponding mean percent cover are then 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 are also grouped according to these criteria. The possible growth habit categories are tree, shrub, graminoid, forb, or moss and lichen. Species are classified as having a particular growth habit based on their designation in the USDA PLANTS profile database and are classified as native or non-native based upon their designation as such in Weber and Wittman (2001). Species are classified as invasive if they are listed on RMNP's list of invasive species or the Colorado Department of Agriculture's schedule of noxious weeds. If there is a particular species that is a focus of a study, its presence and percent cover are also summarized individually.

Data are analyzed using the JMP 7 statistical analysis software package (SAS Institute, Cary, NC, USA). For analysis in JMP, the data are formatted so a single row contained all of the data for one CNP (see past years' analysis ready Excel spreadsheets). This includes plot name, treatment type (e.g. treated or reference), vegetation type (forest, grassland or shrubland), and mean percent cover for each of the species categories (native shrubs, non-native forbs, etc.). The data analysis methods for each project are chosen based on the design of the study and discussed in detail in the studies' respective chapters.

### *Data and Results Presentation*

If a statistically significant ( $\alpha=0.05$ ) difference in cover is detected between subjects, this is graphed using Sigma Plot 10 graphing software (Systat Software Inc., San Jose, CA, USA).

Results of ANOVA analyses are also entered into a table for each main effect and interaction analyzed for a particular study.

## Appendix 2

### Square Nested Plot Installation, Data Collection and Analysis

#### *Installation*

Square nested vegetation monitoring plots (SNP) have an area of  $9 \text{ m}^2$  with sides measuring 3 m in length (Fig. 1). Plots are installed by hammering rebar stakes into the ground at each corner of the SNP and oriented so that the left side of the plot was aligned along the North-South compass bearing. A length of rope is used to create a cross pattern between stakes on opposing corners of the square and this is used to accurately place a  $1 \text{ m}^2$  quadrat directly in the center of the  $9 \text{ m}^2$  plot (Fig. 2). A rebar stake is installed at the southwest corner of the  $1 \text{ m}^2$  quadrat for accurate quadrat placement in future study years.

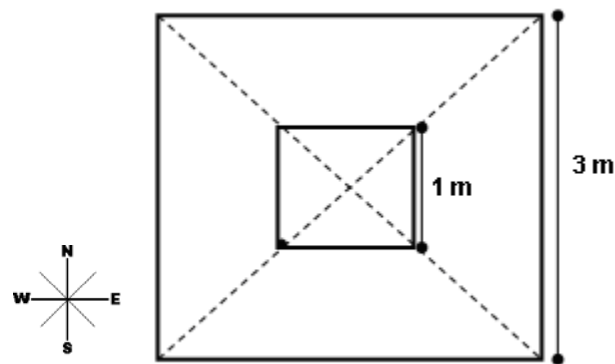


Fig. 1. Square nested plot used to monitor vegetation at the LSR sites. The dashed line illustrates how rope was used to orient the  $1 \text{ m}^2$  quadrat directly in the center of the plot

For each individual SNP installed for a monitoring project, GPS coordinates and plot elevation are recorded at the inner stake using a handheld GPS unit in the NAD 83 CONUS datum setting.

#### *Data Collection*

A long retractable measuring tape is used to indicate the perimeter of the  $9 \text{ m}^2$  plot and  $1 \text{ m}^2$  quadrat is oriented such that the inner stake is located in the southwest corner of the quadrat and all four sides are parallel to the perimeter of the  $9 \text{ m}^2$  plot (see Fig. 1).

Percent cover for all plant species, bare ground, litter, rock and moss/lichen located within the quadrat is estimated using modified Daubenmire cover classes (Daubenmire 1959). Percent cover is estimated as ranges of percent cover as follows: 0, 0-1, 1-3, 3-5, 5-10, 10-25, 25-50, 50-75, 75-95, 95-99 and 100 percent. Each species present in the quadrat is recorded and an ocular estimation of a percent cover range for individual species is made.

Tree, shrub and forb percent cover are estimated using canopy cover while graminoid (grass, rush and sedge species) cover is estimated using cover at ground level (basal cover). A species is considered to be inside a quadrat if any part of the plant is within a vertical projection of the



square meter quadrat (i.e. an overhanging tree or shrub branch is considered inside the quadrat even if the plant is not rooted within the quadrat). Moss and lichen basal cover is only estimated when mosses and/or lichens are growing in soil and not on a rock surface. Standing litter cover is estimated using the same criteria as the living plant tissue would be estimated and dead and down litter cover is estimated basally.

All species within the SNP but not observed within the 1 m<sup>2</sup> quadrat are also recorded. This is done by performing a 20 minute time-constrained survey of the entire SNP and recording the species present within the perimeter of the SNP.

If a plant cannot be identified in the field, a sample is collected from outside the SNP and placed in a sealable collection bag for later identification. For forbs and grasses two entire plants should be collected, including the root structure. If a plant is very rare or only located within a SNP, a specimen is not collected and it is recorded as an unknown grass, forb, shrub or tree species with notes taken about its appearance for help identifying it in subsequent monitoring years.

Before collecting quadrat data, the data collection date and names of data collectors are recorded for each monitoring plot. At each 1 m<sup>2</sup> quadrat of a SNP a picture is taken while facing north, with a whiteboard in frame indicating the plot name, quadrat azimuth, and date of data collection.

#### *Data Analysis*

Data for each monitoring plot is entered into an Excel spreadsheet for ease of manipulation and entry into statistical analysis and graphing software. The data for each individual plot is then summarized. The mid-point of the cover class is used as the cover value for each species in a quadrat. This is obtained by calculating the mean of the minimum and maximum values of the cover class range. For example, if *Chondrosium gracile* was estimated to have a percent cover of 25-50% in a 1m<sup>2</sup> quadrat, the mean percent cover would be summarized as (25+50)/2, or 37.5% for that quadrat.

The species in each SNP and their corresponding mean percent cover are then 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 SNP are also grouped according to these criteria. The possible growth habit categories are tree, shrub, graminoid, forb, or moss and lichen. Species are classified as having a particular growth habit based on their designation in the USDA PLANTS profile database and are classified as native or non-native based upon their designation as such in Weber and Wittman (2001). Species are classified as invasive if they are listed on RMNP's list of invasive species or the Colorado Department of Agriculture's schedule of noxious weeds. If there is a particular species that is a focus of a study, its presence and percent cover are also summarized individually.

Data are analyzed using the JMP 7 statistical analysis software package (SAS Institute, Cary, NC, USA). For analysis in JMP, the data are formatted so a single row contained all of the data for

one SNP (see past years' analysis ready Excel spreadsheets). This includes plot name, treatment type (e.g. treated or reference), vegetation type (forest, grassland or shrubland), and mean percent cover for each of the species categories (native shrubs, non-native forbs, etc.). The data analysis methods for each project are chosen based on the design of the study and discussed in detail in the studies' respective chapters.

#### *Data and Results Presentation*

If a statistically significant ( $\alpha=0.05$ ) difference in cover is detected between subjects, this is graphed using Sigma Plot 10 graphing software (Systat Software Inc., San Jose, CA, USA).

Results of ANOVA analyses are also entered into a table for each main effect and interaction analyzed for a particular study.

## Appendix 3

### Monitoring Plot GPS Coordinates

All coordinates were collected using CONUS NAD83 or NAD 27 datum in region 13T.

#### EFFECTS OF IMAZAPIC APPLICATION ON CHEATGRASS AND NATIVE PLANT SPECIES IN ROCKY MOUNTAIN NATIONAL PARK

Cheatgrass Herbicide Trial		Datum: NAD27
Plot Name	UTME	UTMN
HDR_020C	451370	4468996
HDR_020T	451382	4468966
BLR_052C	448596	4463857
BLR_052T	448591	4463827
BLR_056C	449077	4465821
BLR_056T	449042	4465765
BLR_060C	447197	4463295
BLR_060T	447082	4463325
MPK_064C	448219	4467398
MPK_064T	448114	4467424
MPK_074C	447833	4466433
MPK_074T	447808	4466448

#### EFFECTIVENESS OF COMBINED IMAZAPIC TREATMENT AND NATIVE GRASS SEEDING IN PREVENTING CHEATGRASS (*Bromus tectorum*) REINVASION OF RESTORATION SITES

Seeding Trial		Datum: NAD83
Plot Name	UTME	UTMN
HDR_020HS	*	*
HDR_020LS	451338	4469185
BLR_052HS1	448503	4464028
BLR_052LS1	448484	4464029
BLR_052HS2	448589	4464026
BLR_052HS2	448571	4464026
BLR_060HS	447028	4463538
BLR_060LS	*	*
MPK_064HS	*	*
MPK_064LS	*	*
MPK_074HS	*	*
MPK_074LS	*	*

\*coordinates not recorded

IMPACT OF FIRE ON CHEATGRASS INFESTATIONS IN MONTANE FORESTS OF ROCKY MOUNTAIN NATIONAL PARK

South Lateral Moraine		Datum: NAD83
Plot Name	UTME	UTMN
BSLME_001	450465	4466552
BSLME_002	450450	4466528
BSLME_003	450419	4466533
BSLME_004	450387	4466522
BSLME_005	450332	4466536
BSLME_006	450300	4466537
BSLME_007	450259	4466515
BSLME_008	450233	4466545
BSLME_009	450176	4466525
CSLME_010	449800	4466354
CSLME_011	449753	4466370
CSLME_012	449710	4466363
CSLME_013	449683	4466337
CSLME_014	449584	4466289
CSLME_015	449455	4466268
CSLME_016	449419	4466237
CSLME_017	449420	4466164
CSLME_018	449386	4466141

EFFECTS OF AMINOPYRALID APPLICATION ON CANADA THISTLE (*Cirsium arvense*) AND NATIVE PLANT SPECIES IN ROCKY MOUNTAIN NATIONAL PARK

Canada Thistle Herbicide Trial		Datum: NAD83	
Treatment type	Plot Name	UTME	UTMN
Milestone treatment	UB12T	448368	4469094
Milestone treatment	UB15T	448325	4469123
Milestone treatment	UB20T	448026	4469260
Milestone treatment	UB23T	448246	4469144
Milestone treatment	UB24T	448001	4469268
Control (no herbicide)	UB13C	448363	4469118
Control (no herbicide)	UB14C	448333	4469129
Control (no herbicide)	UB21C	448055	4469253
Control (no herbicide)	UB22C	448283	4469125
Control (no herbicide)	UB25C	447967	4469271

HOLLOWELL PARK TOADFLAX CONTROL STUDY

Canada Thistle Herbicide Trial		Datum: NAD83	
Treatment type	Plot Name	UTME	UTMN
Telar treatment	HP_01T	448657	4465729
Telar treatment	HP_03T	448657	4465651
Telar treatment	HP_04T	448664	4465632
Telar treatment	HP_05T	448613	4465598
Telar treatment	HP_06T	448575	4465471
Telar treatment	HP_07T	448556	4465486
Control (no herbicide)	HP_02C	440615	4465641

## APPENDIX 4. Additional Figures and Tables for Canada thistle Study 2011-2013

ANOVA table for forb *Asteraceae* cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	2.32	0.1661
Within Subjects			
Time	2	3.97	0.0704
Time x Treatment	2	2.36	0.1646

ANOVA table for shrub *Asteraceae* cover. Statistically significant p-values in bold.

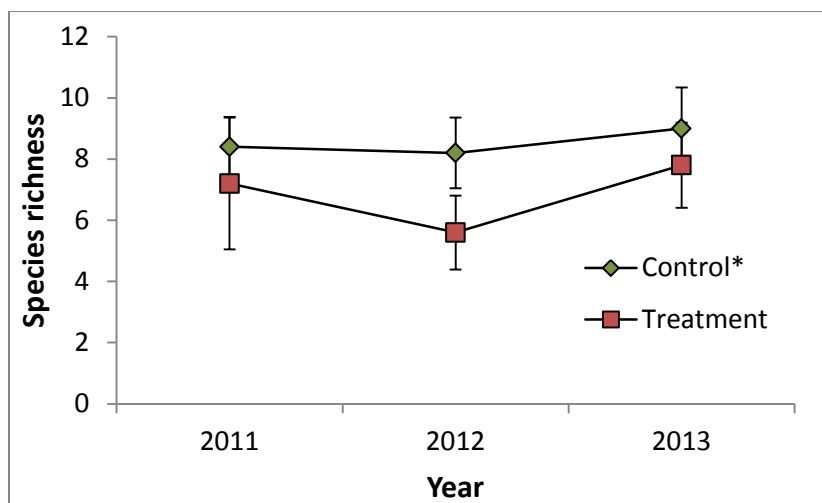
Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	0.33	0.5830
Within Subjects			
Time	2	1.53	0.2815
Time x Treatment	2	1.14	0.3731

ANOVA table for 2011-2013 Aster total cover

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	2.96	0.1237
Within Subjects			
Time	2	3.58	0.0851
Time x Treatment	2	1.08	0.3902

ANOVA table for 2011-2013 Aster total richness. Statistically significant p-values in bold.

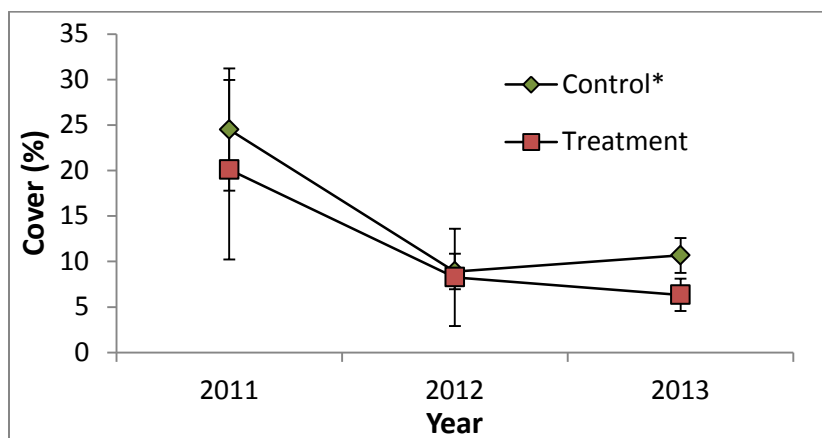
Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	0.80	0.3977
Within Subjects			
Time	2	14.09	<b>0.0035</b>
Time x Treatment	2	3.17	0.1048



Change in total aster richness over time (mean  $\pm$  one standard error of the mean). \*Control plots were treated with herbicide between 2012 and 2013 data collection.

ANOVA table for 2011-2013 total forb cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Treatment	1	0.24	0.6351
Within Subjects			
Time	2	5.53	<b>0.0362</b>
Time x Treatment	2	0.56	0.5948



Change in total forb cover over time (mean  $\pm$  one standard error of the mean). \*Control plots were treated with herbicide between 2012 and 2013 data collection.

Decline in overall forb cover, likely due to drought. Similar to native forbs.

ANOVA table for 2011-2013 total graminoid cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	4.47	0.0675
Within Subjects			
Time	2	11.86	<b>0.0056</b>
Time x Treatment	2	3.39	0.0935

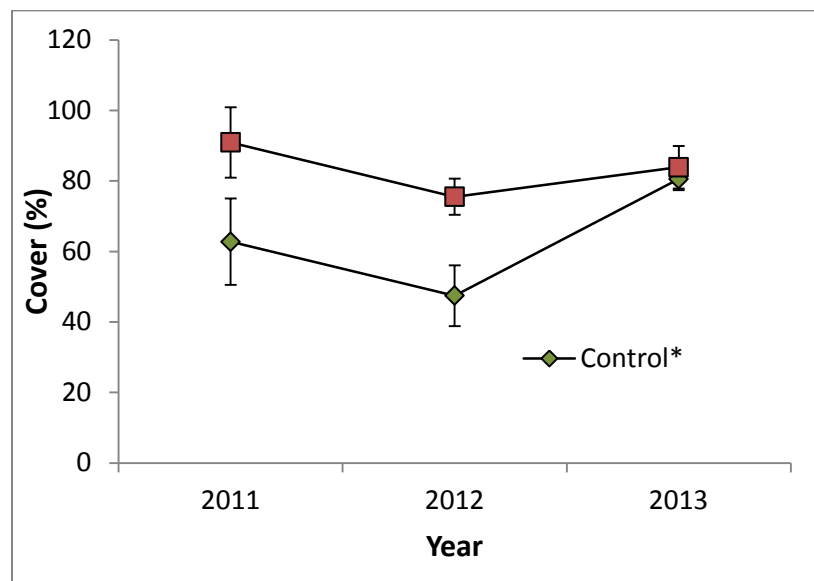


Fig. . Change in total graminoid cover over time (mean  $\pm$  one standard error of the mean).

\*Control plots were treated with herbicide between 2012 and 2013 data collection.

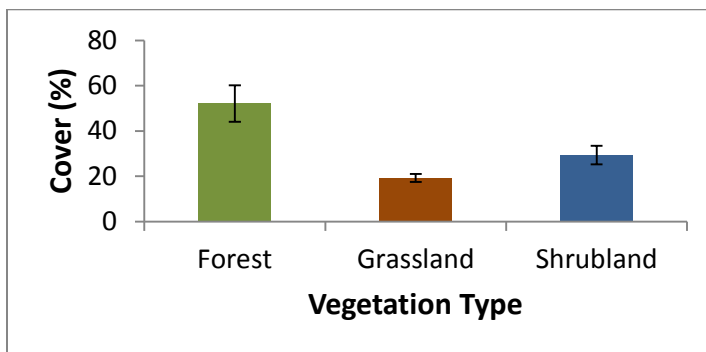
Drop in grass cover in 2012, likely due to drought. Similar to native graminoids.

ANOVA for 2011-2013 moss and lichen cover. Statistically significant p-values in bold.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Treatment	1	0.20	0.6659
Within Subjects			
Time	2	1.78	0.2366
Time x Treatment	2	0.24	0.7964

## APPENDIX 5: Additional Figures and Tables for Nitrogen Study 2013

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	0.20	0.8971
Seeding	1	0.08	0.7797
Veg Type	2	4.97	<b>0.0177</b>
Nitrogen level x Veg Type	6	0.50	0.7995
Veg Type x Seeding	2	0.47	0.6317
Nitrogen level x Seeding	3	0.13	0.9417
Nitrogen level x Veg Type x Seeding	6	0.07	0.9983

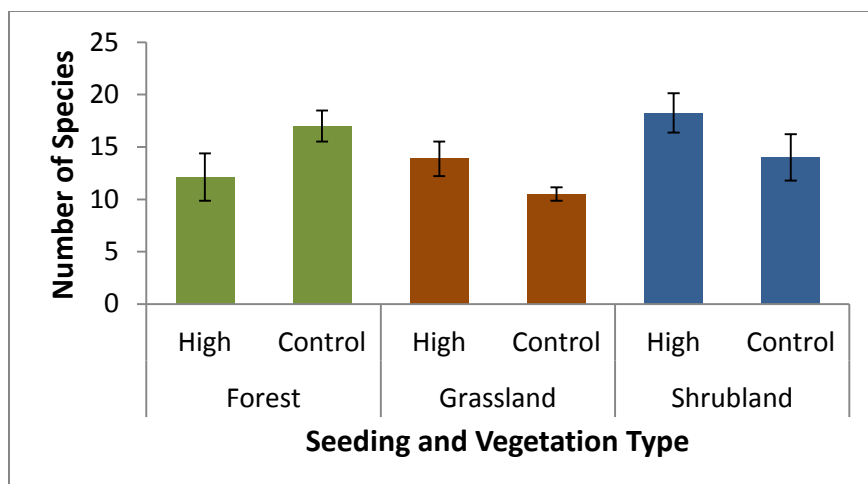


Total native species cover by vegetation type. Forest cover is greatest due to tree canopy cover.

ANOVA for 2013 Total Richness of Natives.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	9.95	<b>0.0003</b>
Seeding	1	0.55	0.4667
Veg Type	2	3.16	0.0642
Nitrogen level x Veg type	6	0.74	0.6231
Veg Type x Seeding	2	6.17	<b>0.0082</b>
Nitrogen level x Seeding	3	0.86	0.4765
Nitrogen level x Veg Type X Seeding	6	0.45	0.8334

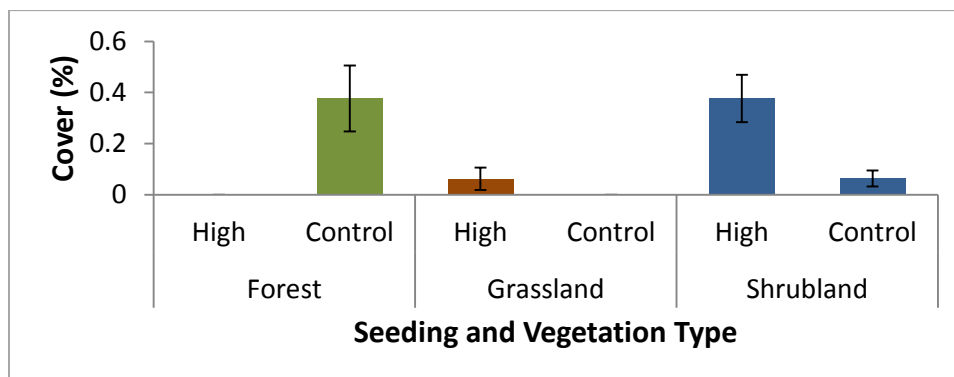




Total native species richness was dominated by forb species. (See native forb results/discussion).

#### ANOVA for 2013 Total Cover of Non-Natives (Non-Invasive)

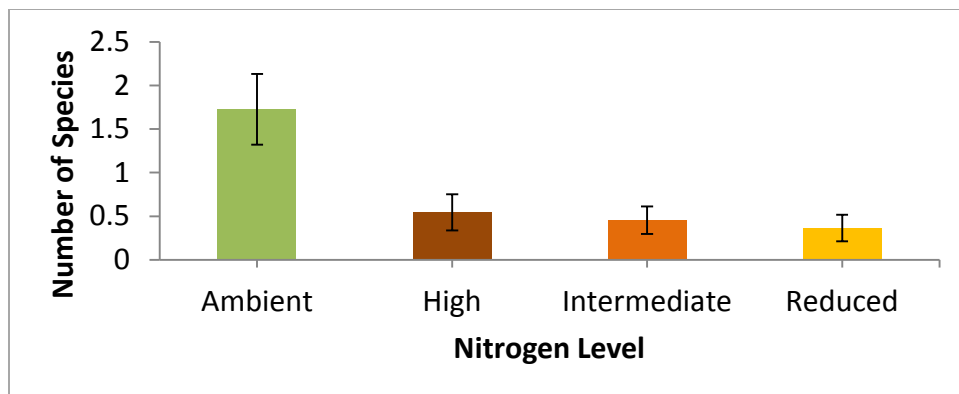
Source of Variation	df	F-statistic	P-value
Nitrogen level	3	0.18	0.9106
Seeding	1	0.00	0.9958
Veg Type	2	1.94	0.1692
Nitrogen level x Veg Type	6	0.22	0.9655
Veg Type x Seeding	2	7.61	<b>0.0035</b>
Nitrogen level x Seeding	3	0.15	0.9299
Nitrogen level x Veg Type x Seeding	6	0.32	0.9174



There were no NNNI graminoids observed in 2013, making these results identical to the NNNI forb results.

#### ANOVA for 2013 Total Richness of Non-Natives (Non-Invasive)

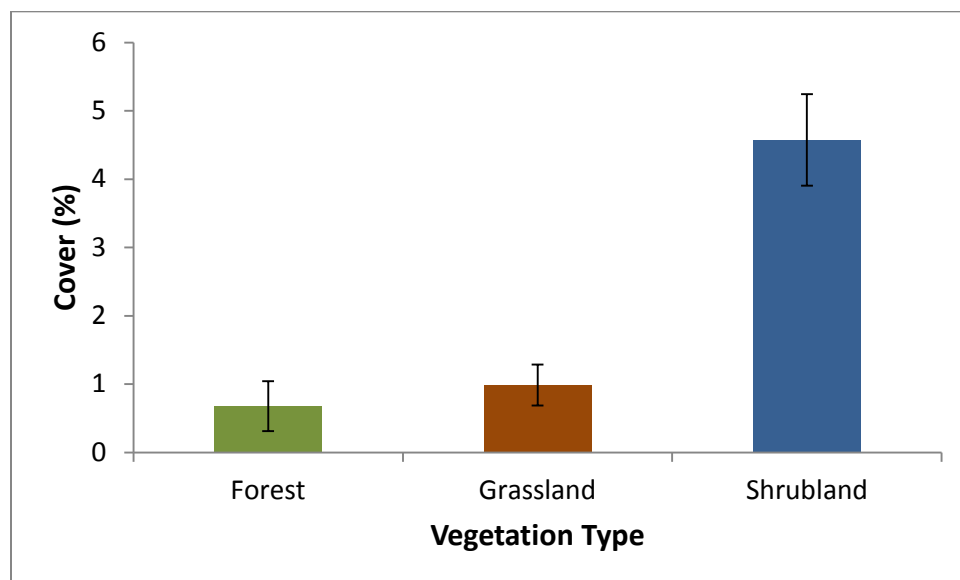
Source of Variation	df	F-statistic	P-value
Nitrogen level	3	6.31	<b>0.0034</b>
Seeding	1	1.06	0.3154
Veg Type	2	0.15	0.8604
Nitrogen level x Veg type	6	0.81	0.5757
Veg Type x Seeding	2	1.97	0.1656
Nitrogen level x Seeding	3	0.83	0.4931
Nitrogen level x Veg Type X Seeding	6	0.30	0.9280



There were no NNNI graminoids observed in 2013, so these results are identical to the NNNI forb results.

#### ANOVA for 2013 Total Cover of Invasive Non-Natives

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	1.32	0.2957
Seeding	1	0.71	0.4085
Veg Type	2	17.44	<b>&lt;.0001</b>
Nitrogen level x Veg Type	6	0.41	0.8608
Veg Type x Seeding	2	1.38	0.2741
Nitrogen level x Seeding	3	0.19	0.9037
Nitrogen level x Veg Type x Seeding	6	0.58	0.7456



Total cover of invasive non-natives by vegetation type. The higher cover in shrublands is explained by cheatgrass cover being included in this group (see cheatgrass results discussion).

#### ANOVA for 2013 Total Richness of Invasive Non-Natives

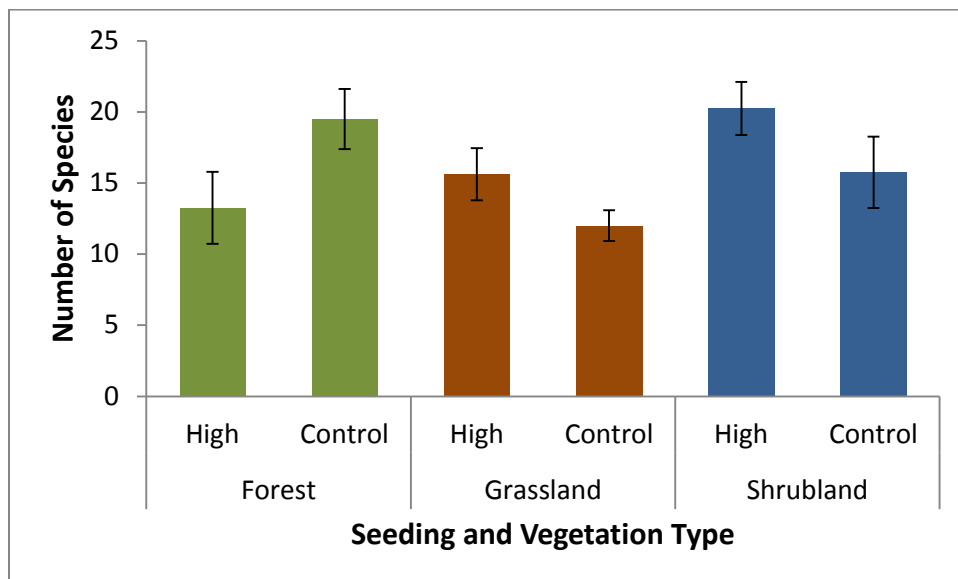
Source of Variation	df	F-statistic	P-value
Nitrogen level	3	0.71	0.5549
Seeding	1	0.00	1.0000
Veg Type	2	0.13	0.8815
Nitrogen level x Veg type	6	1.46	0.2436
Veg Type x Seeding	2	1.14	0.3390
Nitrogen level x Seeding	3	0.71	0.5549
Nitrogen level x Veg Type X Seeding	6	0.28	0.9381

ANOVA for 2013 Total Cover by All Species. Forest has greatest cover, explained by tree canopy cover.

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	0.14	0.9331
Seeding	1	0.10	0.7577
Veg Type	2	9.63	<b>0.0012</b>
Nitrogen level x Veg Type	6	0.40	0.8711
Veg Type x Seeding	2	0.41	0.6666
Nitrogen level x Seeding	3	0.07	0.9778
Nitrogen level x Veg Type x Seeding	6	0.14	0.9882

ANOVA for 2013 Total Richness by All Species (Includes native, invasive, non-native and moss and lichen spp.)

Source of Variation	df	F-statistic	P-value
Nitrogen level	3	12.64	<b>&lt;.0001</b>
Seeding	1	0.22	0.6460
Veg Type	2	3.04	0.0705
Nitrogen level x Veg type	6	0.51	0.7902
Veg Type x Seeding	2	7.35	<b>0.0040</b>
Nitrogen level x Seeding	3	0.79	0.5125
Nitrogen level x Veg Type X Seeding	6	0.55	0.7611



Total cover of all species by seeding treatment and vegetation type.

## Appendix 6: Additional Figures and Tables for Seeding Trial 2011-2013

### ANOVA for total forb cover

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Seeding	2	2.74	0.1237
Veg Type	2	17.63	<b>0.0012</b>
Seeding x Veg Type	4	5.72	<b>0.0179</b>
Within Subjects			
Time	2	7.27	<b>0.0196</b>
Time x Seeding	4	3.61	<b>0.0320</b>
Time x Veg Type	4	2.55	0.0861
Time x Seeding x Veg Type	8	2.89	<b>0.0395</b>

Results nearly identical to native forbs since native forbs are responsible for the large majority of total forb cover.

### ANOVA for total graminoid cover.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Seeding	2	0.14	0.8673
Veg Type	2	2.98	0.1077
Seeding x Veg Type	4	0.98	0.4687
Within Subjects			
Time	2	8.06	<b>0.0153</b>
Time x Seeding	4	0.45	0.7697
Time x Veg Type	4	0.62	0.6572
Time x Seeding x Veg Type	8	1.95	0.1304

Decrease over time due to cheatgrass decline (included with all grasses) combined with native graminoid cover reductions.

### ANOVA for litter cover.

Source of Variation	df	<i>F-statistic</i>	<i>P-value</i>
Between Subjects			
Seeding	2	0.21	0.8159
Veg Type	2	1.85	0.2187
Seeding x Veg Type	4	0.11	0.9752
Within Subjects			
Time	2	16.26	<b>0.0023</b>
Time x Seeding	4	0.22	0.9244
Time x Veg Type	4	3.49	<b>0.0353</b>
Time x Seeding x Veg Type	8	1.26	0.3384

Increased over time in shrubland and forest sites between 2012 and 2013. May be due to drought-induced mortality.

ANOVA for moss and lichen cover.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Seeding	2	0.45	0.6509
Veg Type	2	2.40	0.1522
Seeding x Veg Type	4	0.26	0.8944
Within Subjects			
Time	2	0.41	0.6767
Time x Seeding	4	0.39	0.8155
Time x Veg Type	4	0.98	0.4480
Time x Seeding x Veg Type	8	0.71	0.6790

No changes in moss or lichen cover observed.

ANOVA for total invasive species cover.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Seeding	2	1.20	0.3509
Veg Type	2	4.37	0.0523
Seeding x Veg Type	4	0.61	0.6648
Within Subjects			
Time	2	2.88	0.1221
Time x Seeding	4	0.82	0.5322
Time x Veg Type	4	3.07	0.0522
Time x Seeding x Veg Type	8	2.18	0.0962

Marginal veg type effect because cheatgrass is relatively high in shrubland plots.

ANOVA for total native species cover.

Source of Variation	df	<i>F</i> -statistic	<i>P</i> -value
Between Subjects			
Seeding	2	0.71	0.5199
Veg Type	2	7.09	<b>0.0169</b>
Seeding x Veg Type	4	0.13	0.9684
Within Subjects			
Time	2	2.30	0.1710
Time x Seeding	4	0.16	0.9529
Time x Veg Type	4	0.30	0.8743
Time x Seeding x Veg Type	8	0.50	0.8354

Greatest in forests due to canopy cover, no change over time or in response to seeding.