

Increasing Plant Diversity in *Agropyron cristatum* Rangeland

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Abstract – Introduced species often become invasive and reduce biodiversity, endanger the health of the environment, and reduce resiliency of a grassland. Therefore, efforts to increase diversity of species are important. Burning, seeding, and use of herbicides are methods used to control unwanted plant species, though they are not often used together. We are reporting the use of burning, seeding, and herbicide treatments to establish native species into a rangeland invaded by *Agropyron cristatum* (L.) Gaertn. (Crested Wheatgrass). The study took place in the Grand River Grasslands, South Dakota. A total of 24 plots separated into 8 blocks of 3 plots similar in plant composition and soil type, were allotted to the study. At the beginning, 4 of the blocks were burned then seeding or chemical treatments were applied with one treatment per plot, as follows: seeding with native grass followed by spraying with glyphosate (SEEDCHEM); seeding with native grass only (SEED); and unseeded control (CON). Results showed percentage of introduced grasses significantly declined due to SEEDCHEM with a trend toward increased percentage native grass in both burned and unburned SEEDCHEM treatments. We showed improved nutritional quality of plots with increased percentage of native grasses that increased kilograms of crude protein per hectare. SEEDCHEM appears to be a way to improve grassland diversity and forage quality.

Introduction

Rangelands cover 40% to 50% of Earth's total land area and are an important forage resource for ~ 70% of the global domestic livestock (Brown and Thorpe 2008). Rangelands also provide important ecosystem services beyond forage for livestock, such as food and fiber, biological diversity, wildlife habitat, soil protection, and the ability to sequester greenhouse gases (Sala and Paruelo 1997). The ability of rangelands to provide forage resources for grazing, as well as ecosystem services, is dependent primarily on the health of the range (Brown and Thorpe 2008; Toledo et al. 2014). Healthy range is dependent on attributes such as soil and site stability, hydrologic function, and biotic integrity (Pellant et al. 2020). Plant community composition has a strong influence on rangeland health attributes, especially when unexpected species for a particular ecological site are present and when expected functional and structural plant groups at a site have been altered (Pellant et al. 2020). The more diverse a rangeland is with a greater number of native plant species in different functional and structural groups, the more adaptable it is to variability in precipitation and other weather dynamics (Ernst et al. 2023, Pokorný et al. 2005). It is therefore advantageous to promote rangeland species diversity.

Agropyron cristatum (L.) Gaertn. (Crested Wheatgrass) was established on approximately 10 to 26 million acres (3.2–10.4 million ha) in the western U.S. (Zlatnik 1999) as an effort to preserve soils in abandoned farm sites in the early 1940s. The Natural Resources Conservation Service (NRCS) National Resources Inventory (2018) reports Crested Wheatgrass foliar cover of approximately 24% in sampled areas in the northern Great Plains of the USA (USDA NRCS 2018). Crested Wheatgrass is a cool-season grass that is drought tolerant, palatable to livestock, and easy to establish from seed, resulting in widespread use for both forage and

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conservation purposes. However, Crested Wheatgrass has been reported to decrease plant (Henderson and Naeth 2005) and avian (Sutter and Brigham 1998) diversity compared to native mixed grass prairies, and it lacks the nutritive capacity of native grass when utilized by livestock. While it is not considered invasive in the warm and dry portions of the Great Basin where it is present, Crested Wheatgrass may also be considered invasive in parts of the northern Great Plains, making it a species of concern in many areas (Pellant et al. 2020).

Given that introduced grasses reduce biodiversity, plant succession, and cover, there is great interest in developing strategies for restoring native perennial grasses (Corbin et al. 2004) and improving rangeland productivity and resiliency in Crested Wheatgrass dominated communities.

Fire is part of the natural disturbance regime of Great Plains Grasslands (Anderson, 2006). Documented effects of fire include increases in plant diversity (Anderson 2006), improvement in plant nutrient content (McGranahan et al. 2014), and wildlife habitat (Hovick et al. 2015), as well as reductions of undesirable plant species (Twidwell et al. 2013). These fire effects align with the interest of restoring native perennial grasses and improving rangeland productivity and resiliency in Crested Wheatgrass invaded areas. This study was conducted to document changes occurring in species composition, biomass, and nutritive composition during and after a planned restoration by US Forest Service (USFS) on the Grand River National Grassland district.

We studied two different methods of seeding native grass into burned and unburned Crested Wheatgrass range and measured changes in plant species composition, biodiversity, nutritive value, and forage production the first and third year following planting and compared to unplanted control plots within burned and unburned blocks. A native grass mixture was developed by the FS and seeded into the burned and unburned plots followed by spraying with glyphosate or no spraying. Our study objectives were to evaluate if burning plus seeding a native grass mixture with or without the application of glyphosate will decrease the amount of Crested Wheatgrass while increasing native grass species, and to determine if these treatments could increase the carrying capacity and nutritional quality of forage for livestock.

Materials and Methods

This study was conducted in Perkins County, about 20 km southwest of Lemmon, South Dakota, on the Grand River National Grassland (45° 44' 9.6" N, 102° 21' 39.6" W) operated by the USFS. Vegetation was dominated by Crested Wheatgrass with an understory of *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths (Blue Grama).

We randomly located four blocks on clayey ecological sites at the research location in the spring of 2008 (Fig. 1). Each block contained six plots (18.3 x 27.4m) with three burned on 17 April 2008 three left unburned. Three treatments were randomly applied to the three burned and three unburned plots for each block. The treatments were (1) Seeded native grass mixture followed by spraying with glyphosate (SEEDCHEM), (2) Seeded native grass mixture only (SEED), and (3) Unseeded control (CON).

We seeded the treatments on 12 May 2008 using a no-till grain drill (John Deere 750 model). The seed mixture consisted of *Andropogon gerardii* Vitman (Big Bluestem; 0.54 kg/ha), *Nassella viridula* (Trin.) Barkworth (Green Needlegrass; 2.02 kg/ha), *Schizachyrium scoparium* (Michx.) Nash (Little Bluestem; 0.29 kg/ha.), *Heterostipa comata* (Trin. & Rupr.) Barkworth (Needle and Thread; 0.43 kg/ha), *Calamovilfa longifolia* (Hook.) Scribn. (Prairie Sandreed; 0.22 kg/ha), *Bouteloua curtipendula* (Michx.) Torr. (Sideoats Grama; 0.27 kg/ha),

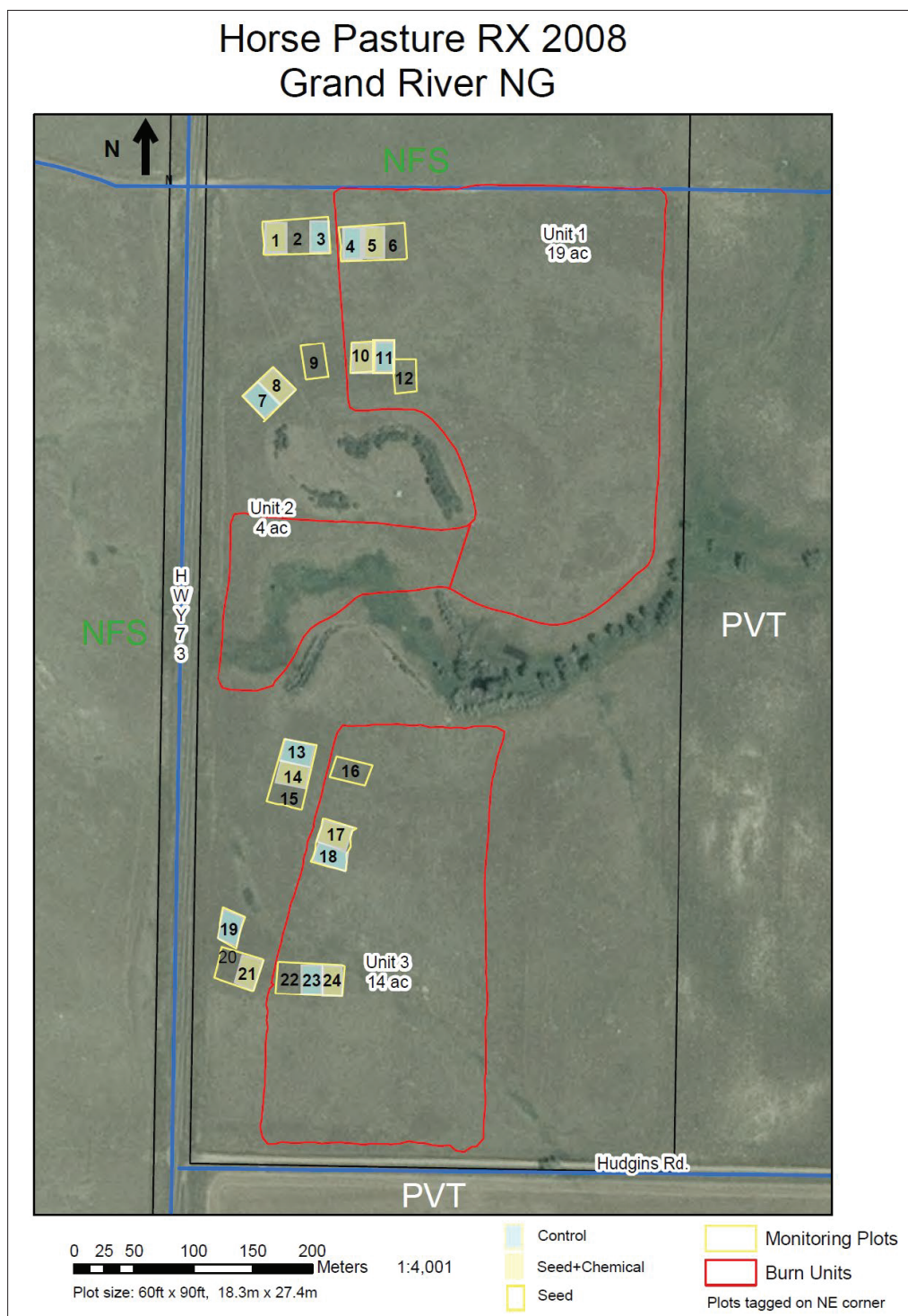


Figure 1. Google Earth map of study section at Grand River Grasslands, near Lemmon, South Dakota, indicating blocks, plots, and burned areas. Green NFS indicates National Forest Service land, white PVT indicates private land.

and *Pascopyrum smithii* (Rydb.) Barkworth & D.R. Dewey (Western Wheatgrass; 0.12 kg/ha). Varieties were unknown. Seeding depth was 1.25 cm with no packing; seeding mixture and rate was determined following FS recommendations. We sprayed glyphosate on plots assigned SEEDCHEM treatment (2.33 L/ha) on 20 May 2008 prior to seedling emergence.

Meteorological data was downloaded from CLIMOD (High Plains Regional Climate Center CLIMOD weather database, collected at the nearest weather station (Lemmon, SD) for the historical and monthly precipitation and temperature for the dates of the study.

We estimated forage productivity using 4 1/8-m²-quadrats randomly placed in each plot. The timing of sampling coincided with peak standing crop (late July–early August). All forage material in each quadrat was cut to ground level, sorted by species, placed in paper bags and dried to a constant weight at 60°C to estimate aboveground biomass in grams per species per quadrat. We then calculated total number of grams present per species to determine the percentage of each present species, then grouped collected species by native grasses (NG), native forbs (NF), introduced grasses (IG), and introduced forbs (IF) for calculation purposes. We observed 56 different species.

We combined dried biomass by species across replicates and then ground the forage samples to pass a 2 mm screen (Thomas Wiley Laboratory Mill Model 4, Arthur H. Thomas and Company, PA, USA). We analyzed biomass samples for the following forage nutritive values following standard wet chemistry forage lab procedures: dry matter (AOAC, 1990; Method 967.03), organic matter (AOAC, 1990; Method 942.05) neutral detergent fiber (NDF; Van Soest et al., 1991), acid detergent fiber (ADF; AOAC, 1990; Method 973.18), and total nitrogen (AOAC, 1990; Method 990.03). Nitrogen was converted to crude protein (CP) using 6.25 N:CP factor. Total CP per plot and digestibility of organic matter using nutritive values for the most common grass species were then calculated.

In vitro determinations of true dry matter disappearance (IVTDM) were performed only on grasses. Assays were performed in ANKOM DaisyII incubators (ANKOM Technology, Fairport, NY). The modified McDougall's buffer described by ANKOM was inoculated with rumen fluid (4:1 ratio) collected from ruminally cannulated cows consuming brome-grass hay. Filter bags (ANKOM F57 filter bags; 25 µm pore size; ANKOM Technology, Fairport, NY) containing 0.5 g of sample were incubated for 48 h. After removal, bags were rinsed in neutral detergent solution using an ANKOM200 fiber analyzer (ANKOM Technology, Fairport, NY) to correct for microbial contamination (Varga et al. 1997).

Data were analyzed as a split-plot with burn as the main plot factor and treatment as the sub-plot factor using PROC GLIMIX (SAS Institute Inc., 2012). We included a repeated statement for year in the biomass analysis. Treatment means were considered significantly different at $p \leq 0.10$

Results

Total annual rainfall measured was 47.7, 50.9, 57.7, and 42.0 cm for 2008, 2009, 2010, and 2011, respectively. Mean annual rainfall near the study site was 130% of the long-term average for the years of the study. The least precipitation occurred in 2011 and was 109% of normal. Mean yearly temperatures were greater than the historical mean for 2008, 2009, and 2011; but less than the mean for 2010. Maximum temperatures during the growing season exceeded the 30-year average all years of the study from May to August, with the maximum temperatures 106% of historical average maximum temperatures in July and August 2011, shortly before and during the time of sample collection of peak biomass.

The SEEDCHEM treatment had a lower percentage (< 0.1%) of IG biomass compared to the CON on both the burned and non-burned plots (Fig. 2). The percent IG biomass

was similar between the burned ($81.3 \pm 2.6\%$) and non-burned ($76.7 \pm 2.9\%$) plots. Native grasses (NG) comprised $12.5 \pm 2.2\%$ and $6.3 \pm 1.2\%$ of relative species composition of burned and non-burned plots of the SEEDCHEM, respectively.

Though forbs were not planted, the effect of seeding treatment on forbs was measured. Introduced forbs (IF) were a relatively minor part of the relative species composition in the seeding year and 2011, however, 2009 had large quantities of sweet clover in all plots, which skewed the results slightly for that year. Because of this artifact, there were no differences ($p > 0.1$) between treatments or burns for IF. Introduced forbs made up $2.5 \pm 2.2\%$ of the relative species composition in the SEEDCHEM treatment compared to $1.4 \pm 0.01\%$ and $2.3 \pm 1.4\%$ in the SEED and CON treatments, respectively. There was no difference ($p > 0.1$) in IF between burned and unburned plots.

Crude protein and digestible organic matter content for native grasses by plot give an overall measure of potential livestock productivity due to treatment (Table 1). Plots that had more crude protein were associated with more plant diversity, though differences were

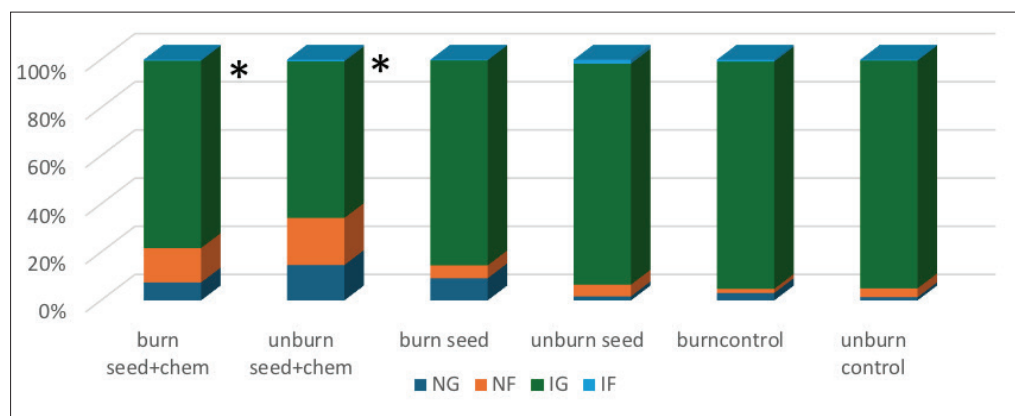


Figure 2. Total biomass by plant group for the third year following treatment. Treatments are burned or unburned and seeded followed by glyphosate treatment, burned or unburned seeded only, and burned or unburned control with no seeding or chemical treatment. Biomass was calculated as grams per species per meter with species grouped by native grass (NG), native forb (NF), introduced grass (IG) or introduced forb (IF). Only the seed+chem treatment was significant for native forb and introduced grass, $p < 0.10$, indicated by the asterisk.

Table 1. Treatment effect on crude protein mass, biomass, and mass of organic matter for two seeding treatments compared to control plots on the Grand River National Grasslands near Lemmon, South Dakota. Means for each nutrient within a year and between treatment types is given in kg/ha (SD), with different lowercase letters indicating significant statistical means ($p < 0.10$).

Nutrients by Year	Control	Seed	Seed and Chemical
2009			
Crude protein	120.2 (29.3)	104.6 (28.6)	104.6 (47.2)
Total biomass	3494.7 (19.3) ^b	2881.5 (7.6) ^b	5001.3 (30.3) ^a
Digestible organic matter	1462.9 (228.3) ^a	1266.6 (497.2) ^b	1476.3 (460.6) ^a
2011			
Crude protein	227.4 (22.1)	188.6 (37.9)	215.1 (66.3)
Total biomass	3834.5 (5.0) ^a	3312.9 (7.5) ^b	4242.9 (10.6) ^a
Digestible organic matter	1228.1 (139.5)	1015.9 (345.9)	1131.3 (157.7)

not significant for all treatments. Protein was higher the second year for all treatments, with CON the highest (227 kg CP/ha; $p < 0.10$).

There were no differences ($p > 0.1$) in biomass production between burned and unburned plots (Table 1). However, biomass was significantly different ($p < 0.1$) in all treatments in 2009, and the SEEDCHEM and CON treatments were greater ($p < 0.1$) than the SEED treatment in 2011.

We found some native grasses assessed in this study were more digestible than Crested Wheatgrass (Fig. 3). Both Big and Little Bluestem were greater ($p < 0.1$) in dry matter digestibility both years than the other grass species ($p < 0.1$). Dry matter digestibility for Blue Grama was not different ($p > 0.1$) by year, and was less digestible ($p < 0.1$) than the Crested Wheatgrass.

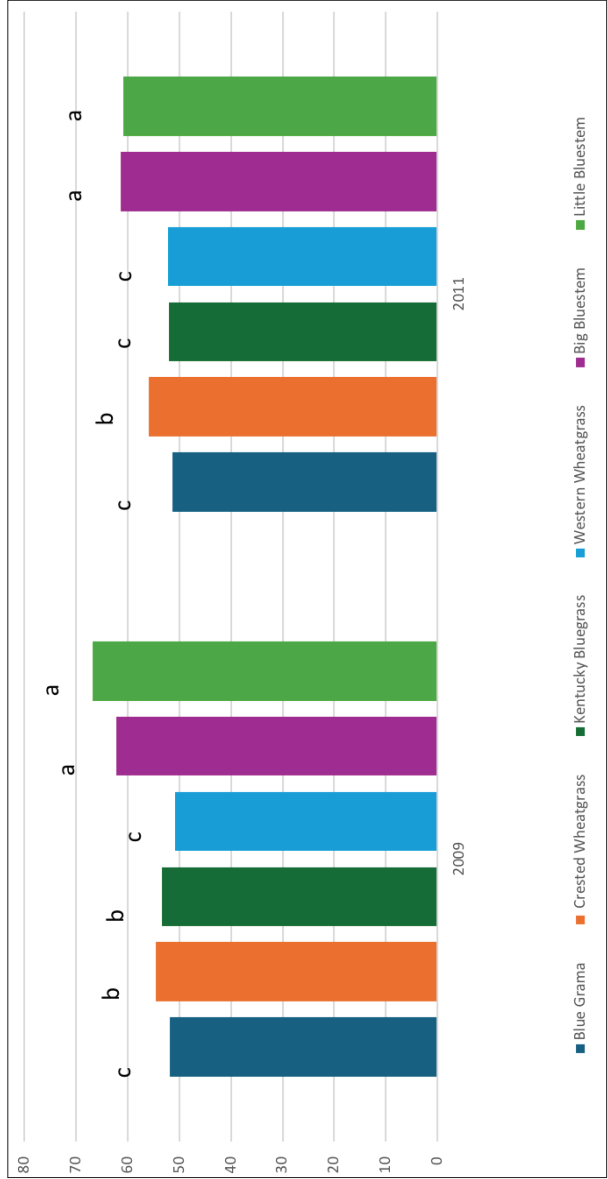


Figure 3. In vitro dry matter digestibility (dry matter basis) by grass species harvested following restoration of Crested Wheatgrass invaded range using three different methods: glyphosate plus seeding with native grass, seeding only, or control on burned and unburned plots. Different lowercase letters next to bars in each year cluster indicate significant differences in digestibility, $p \leq 0.10$.

Discussion

The decline in IG biomass in the SEEDCHEM treatment and increase in NG in both the burn and non-burned plots with the SEEDCHEM treatments suggests that the SEEDCHEM treatment, regardless of being burned or not burned before application, reduced Crested Wheatgrass competition to allow for some native grasses to establish, like what Pellant and Lysne (1998) found. The lack of differences in biomass between SEED and CON treatments can be attributed to the ability of Crested Wheatgrass to produce many seeds and the survivability and success rate of these seeds (Marlette and Anderson 1986; Pyke 1990). Chemical spray at seeding may have provided sufficient control of Crested Wheatgrass enough to allow NG establishment (Archer and Pyke 1991; Wilson and Partel 2003), though additional treatments and possibly adding grazing by cattle to the treatment process may prove more successful than one single treatment and warrant further research. However, grazing alone may not be sufficient, as Crested Wheatgrass can tolerate high levels of grazing pressure (Caldwell et al. 1981; Laycock et al. 1981). The SEEDCHEM treatment could be a desired method for improving diversity in rangelands as the improved biomass carried over for the 3 years of the study.

Since forbs were not planted, forb composition depended completely on the forbs already present and the forb seed bank within the soil. The results suggest that forb abundance could be potentially increased in SEEDCHEM treatments if NF were to be planted. Native forbs can provide important forage to livestock as well as increase native plant diversity of these invaded areas. Further research on the role of forb seeding in northern Great Plains grasslands invaded by Crested Wheatgrass is warranted.

This study is unique from other rangeland restoration studies in that we used a nutritive approach to determine effects of seeding treatments. Forages that are more digestible offer more energy and nutrient absorption for livestock which can increase the carrying capacity of the resource. The greater digestibility of the Big Bluestem and Little Bluestem compared to the Crested Wheatgrass suggests that these native grasses could be focal species used in restoring crested wheatgrass fields or invaded rangelands, though further research is warranted in improving methods of establishment, as these seeded species were only present at small increments in the plots following treatment when compared to the established Crested Wheatgrass.

We noted a year effect on crude protein yield per plot (kg/ha) which was greater in 2011 but concurrent digestibility was lower. It is possible that the high temperatures present during the late development phase led to an increase in protein accumulation in the plants' tissues. Normally this would increase digestibility, however, decreased digestibility indicates that the hot conditions during storage and transport following collection may have negatively affected digestibility, as a possible artifact of heating after collection but before plants could fully dry for processing, causing a Maillard reaction of the protein to the fiber in the plant tissues. Heat damage in forages has been associated primarily with alterations in forage protein digestibility. A Maillard reaction is a heat-induced chemical reaction between protein (amino acids) and sugars. Maillard products generally result in poorly characterized nutrients in forages in terms of ruminant nutrition (Coblentz and Hoffman 2008). Conversely, the increased temperatures present during collection may have indicated stress was occurring in the plants, and individual digestibility measures were reduced due to this heat stress.

Positive increases in crude protein available per acre support seeding methods that increase the native grass proportion of the plot. However, it is important to note that only a few plots had NG proportions greater than 12%. Although these methods show potential

for their use in restoration of grassland, one treatment with glyphosate may not have been adequate. It is possible that further treatments with glyphosate may enhance the composition of the treated plots to further improve native species composition.

Restoring native grasses in Crested Wheatgrass stands is difficult and expensive, which makes finding effective restoration strategies important. We found the most effective strategy to increase plant diversity in this study was seeding followed by an application of glyphosate, regardless of fire use. This option would be attractive to managers in regions where burning is contentious, or difficult to manage. Native species were increased in treatments compared to control plots, though still well below 50% NG composition. Though differences between burned and non-burned plots were not found in this study, fire can provide other ecological benefits which were not measured here. Reestablishing native species presence in invasive-dominated plant communities is especially difficult because the ecological processes necessary to keep plant communities diverse may have been altered in a manner that favors the invasive species. This study showed some methods of restoration that may help shift the plant species composition despite the underlying challenges. The combination of seeding and chemical control seemed to be the best method for improving nutritive contents and diversity of species on Crested Wheatgrass dominated grassland, and burning did not appear to make a difference.

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