Impacts of Cheatgrass on Mammal, Bird, and Butterfly Populations in a Rocky Mountain Foothills Grassland

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2 **Executive Summary** 3 Impacts of Cheatgrass on Mammal, Bird, and Butterfly Populations in a Rocky Mountain Foothills 4 Grassland. Anyll Markevich (Primary Investigator: anyllmarkevich@gmail.com, 303-442-4475), Stephen 5 R. Jones (Grant Recipient: curlewsj@comcast.net, 303-494-2468), Christel Markevich. 12/07/2020. 6 7 8 Understanding the influence of cheatgrass on mammal, bird, and butterfly populations is vital for proper 9 wildlife management, especially as cheatgrass continues to spread across the western United States. Few 10 studies have investigated these relationships. This study aims to give a preliminary indication of the 11 effects of cheatgrass on wildlife populations of wildlife populations within a Rocky Mountain foothills 12 grassland.. 13 We located eight grassland plots, varying in cheatgrass cover, at the base of the Rocky Mountain Front 14 15 Range south of Lyons, Colorado. Remote triggering cameras, point counts, and transect counts were used to quantify numbers of mammals, birds, and butterflies respectively. We used a combination of ANOVA 16 analysis and Linear Correlation Analysis to analyze our data. 17 18 19 We found that mammals were less numerous in areas with high cheatgrass cover. We did not find a statistically significant relationship between birds and cheatgrass, through birds numbers tended to 20 21 decrease with increased cheatgrass cover. Butterflies and butterfly species richness (total number of 22 species) showed statistically significant negative correlation with cheatgrass. 23

Page 2

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Our analysis suggested that mammals and birds are unaffected by cheatgrass up to a certain threshold of cheatgrass cover above which they are repelled. Conversely, butterflies were linearly correlated with cheatgrass cover. The ability of mammals and birds to move across relatively large distances may allow

them to find enough food even in areas interspersed with a certain amount of cheatgrass. Meanwhile the
small home ranges of butterflies and their extreme dependance on local nectar sources may cause
butterfly numbers to be reduced in tandem with the reduced availability of nectar sources in places where
cheatgrass outcompetes the local vegetation.

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Although our study does not prove causal relationships, we believe that the differences in animal populations among plots were in fact driven by cheatgrass infestation. Our belief is supported by the generally weak relationships we observed between animal populations and plot characteristics other than cheatgrass cover.

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Future research should investigate the impact on wildlife of different treatment methods to control cheatgrass. We recommend that such research compare wildlife populations over appropriately long timescales on untreated plots relatively free of cheatgrass, on untreated plots infested by cheatgrass, and on previously infested treated plots. The standard for treatment success should include restoration to conditions similar to those found in untreated, relatively cheatgrass free areas, not just improvement over untreated cheatgrass infested areas.

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44 Additional possible management implications of this study include:

Agencies funding research investigating which cheatgrass removal methods are most beneficial to
 wildlife (including alternative methods such as soil regeneration)

• Biologists accounting for cheatgrass infestation when evaluating the quality of wildlife habitats

Agencies prioritizing native plant restoration in ecologically important areas, such as breeding
grounds

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We studied impacts of cheatgrass (Anisantha spp.) infestation on mammals, birds, and butterflies in a 54 foothills grassland south of Lyons, Colorado. The impacts of cheatgrass on wildlife have been poorly 55 56 studied despite the prevalence of cheatgrass infestation in the United States. We established eight, 75 m 57 radius circular plots in areas of mixed-grass prairie (also containing native shrubs and ponderosa pines) on two Boulder County Parks and Open Space properties. Four of the plots were located in areas with 58 relatively low (3-13%) cheatgrass cover, four plots in areas with relatively high (24-35%) cheatgrass 59 60 cover. We sampled vegetation within each plot using a Point-intercept with Grid Quadrant method. We installed motion-activated wildlife cameras at the center of each plot. The cameras were active over a 3 61 62 month period. We conducted four early-morning point-count bird surveys in each plot between May 29th 63 and July 9th, counting all birds seen or heard perching or foraging within the plot over 8-minute intervals. We counted butterflies within 30 m of 150 m transects bisecting the plots on 6 days between June 6th and 64 August 10th. We found a significant negative relationship between total mammals and cheatgrass cover of 65 plots (p=.02) and an insignificant negative relationship between mammal species richness and cheatgrass 66 cover of plots (p=.15). While bird abundance and bird species richness was greater on low cheatgrass 67 68 plots, these trends were insignificant (p > .10). There was a significant negative relationship between butterfly abundance and cheatgrass (p=.04) as well as a highly significant relationship between butterfly 69 species richness and cheatgrass cover of plots (p=.001). These results suggest that cheatgrass infestation 70 71 in foothills grasslands may reduce populations of mammals, butterflies, and possibly birds. Cheatgrass should be considered when managing wildlife and wildlife habitats. 72

Abstract

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Keywords: Cheatgrass, Foothills Grassland, Mammals, Birds, Butterflies, Rocky Mountains, Invasive
Plants, Ground Nesting Birds, Grass-Dependent Butterflies, Remote Triggering Cameras, Wildlife

Introduction

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This study aims to give a preliminary indication as to whether cheatgrass affects distributions and populations of mammals, birds, and butterflies. We hypothesized that there would be lesser numbers and lesser species richness (total number of species) of native mammals, birds, and butterflies in areas heavily infested with cheatgrass. Testing this hypothesis is critical to good wildlife management and strategic planning of cheatgrass removal, especially in sensitive wildlife habitat. Few studies have investigated the relationships between cheatgrass and large mammals, birds, or butterflies.

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Studies about the effect of cheatgrass on small mammals are abundant and the results are quite consistent: small mammals are negatively impacted by cheatgrass. Freedman et al. (2014) found that the abundance and diversity of small-mammal communities in the Great Basin decreased with increasing abundance of cheatgrass. Similarly, Ostoja and Schupp (2009) found that total rodent abundance was 6.1 times greater in sagebrush-dominated areas relative to cheatgrass-dominated areas in the Great Basin. Although countless studies replicated these findings for small mammals, to our knowledge no such studies have been conducted for larger mammals (ranging from lagomorphs to ungulates).

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The impact of invasive plants on birds has been studied, but not specifically the impact of cheatgrass.
Ortega et al. (2006) found that spotted knapweed reduced chipping sparrows' reproductive success and
site fidelity, likely because of reduced food availability in areas infested by knapweed. Conversely,
Scheiman et al. (2003) found that western meadowlark nesting success was positively associated with
leafy spurge cover in North Dakota. These studies indicate that birds are sensitive to changes in the
vegetative cover of their habitats, and therefore may be sensitive to cheatgrass.

Butterflies present another interesting gap in the scientific literature. Fleishman et al. (2005) concluded
that butterfly numbers within their study area in the Muddy River drainage (Nevada) were not impacted
by non-native plants but were influenced by nectar availability. Cheatgrass has the ability to form
monocultures that outcompete other plants (Young et al. 1987), including potential nectar sources,
indicating that cheatgrass may be detrimental to butterflies. Other research does cite invasive plants as
important stressors in butterfly communities (Keeley, 2017), but none of these studies looked specifically
at relationships between butterflies and cheatgrass.

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To understand the relationships between cheatgrass and numbers of mammals, birds, and butterflies we
selected eight circular plots, each 75 meters in radius, containing varying amounts of cheatgrass cover.
We used a combination of remote-triggering cameras (for mammals), point counts (for birds), and transect
counts (for butterflies) to measure the number and species richness of animals on our plots.

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114 Study Area

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The study area lies at elevations between 1684-1790 m at the base of the Colorado Front Range foothills. 116 117 where the Great Plains abut the Southern Rocky Mountains. Elevation increases gradually from east to 118 west across the study area. Climate within this lower foothills region is characterized by relatively mild 119 but snowy winters and warm, dry summers. The mild winter temperatures result from frequent warm, 120 down-sloping winds, sometimes referred to as Chinooks. Annual precipitation averages 45-50 cm/year, 121 with approximately half of that amount falling during the March-June spring growing season (from US 122 Climate Data, 2020). As a result, maximum greening of native grasses and peak wildflower bloom usually occur in May and June. 123

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In early May, 2020, we established eight, 75 meter radius monitoring plots on the Boulder County Parks
and Open Space owned Pierce and Trevarton properties south of Lyons, Colorado in R 70W, T3N, S 31;
and R 70W, T 2N, S 6 (Figure 1, Table 1). Vegetation on these two properties consists primarily of
foothills mixed-grass prairie, foothills shrub, and ponderosa pine woodland (Baker and Galatowitsch
1985, Colorado Natural Areas Program 1998). Plant names that follow are from Wittmann and Weber
(2011).

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In relatively flat areas with deep soils, the grassland tends to be dominated by green needlegrass (*Nassella viridula*) and western wheatgrass (*Pascopyrum smithii*), with needle-and-thread grass (*Hesperostipa comata*), junegrass (*Koeleria macrantha*), little bluestem (*Schizachyrium scoparium*), and non-natives
such as Canada bluegrass (*Poa canadensis*) and smooth brome (*Bromopsis inermis*) well represented.
Non-native cheatgrass (*Anisantha tectorum*) and crested wheatgrass (*Agropyron cristatum*) tend to thrive
in rocky or upland areas, along with native three-awn (*Aristida purpurea*) and buffalograss (*Buchloe dactyloides*).

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140 Flat-bottomed ravines cutting through the properties support extensive patches of native shrubs, including 141 skunkbrush (*Rhus trilobata*), wild plum (*Prunus americana*), mountain mahogany (*Cercocarpus*) 142 montanus), chokecherry (Padus virginiana) snowberry (Symphoricarpos sp.), wild licorice (Glycyrrhiza lepidota), and hawthorne (Crataegus macracantha). Scattered hackberry trees (Celtis reticulata) and box 143 144 elders (Negundo aceroides) grow in the relatively wet canyon bottoms, along with small patches of native 145 tallgrasses, including switchgrass (Panicum virgatum) and Indiangrass (Sorghastrum avenaceum). Ponderosa pines (Pinus ponderosa) and Rocky Mountain junipers (Sabina scopularum) are scattered 146 147 throughout the study area, becoming most numerous in rocky uplands.

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149	Plot 1 partially contains a one hectare prairie dog colony that extends beyond the plot to the east.
150	Vegetation over this colony consists primarily of cheatgrass and non-native forbs. Within the other seven
151	plots, we observed few obvious signs of disturbance by burrowing animal colonies or by human activities,
152	though a barely discernible two-track road cuts through portions of plots 7 and 8. A heavily-traveled two-
153	lane highway (U.S. 36) passes within 150 m of plots 1 and 5 (Figure 2). Barbed-wire fences border both
154	the Pierce and Trevarton properties, but none pass through our plots.
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156	Methods
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158	Vegetation Sampling
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160	We located the eight circular, 75 m radius plots in areas of the Pierce and Trevarton properties where the
161	primary vegetation type is foothills grassland (Kuchler 1964). When we first established our plot locations
162	we tried to maximize the variability of cheatgrass cover in our plots by choosing 4 plots that appeared to
163	have high cheatgrass cover and 4 plots that appeared to have low cheatgrass cover.
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165	To confirm our visual evaluation of the cheatgrass cover and to allow quantitative analysis we undertook
166	quantitative sampling of plot vegetation. To maximize the uniformity of our sampling (thereby yielding
167	better results), we used a Point Intercept Method with a modified distribution of sampling locations.
168	Standard radial vegetation sampling techniques (Figure 3a) are biased towards vegetation at the center of
169	the plot as they do not account for the non-linear increase of circle area with increased circle radius
170	(caused by the r ² term in $A = \pi r^2$). We used a method that eliminated this bias, providing more
171	representative sampling, while only marginally increasing the time required to sample each plot.
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173 Our method was as follows: we marked the center of each plot with a T-post. During the first two weeks of June we then laid out 8 radial transects 75 meters in length on each plot, originating at the center T-post 174 and extending in each cardinal and inter-cardinal direction. To account for the bias described earlier, 175 sampling locations were pushed outwards away from the center of the plot and closer to the outer edge of 176 the plot (Figure 3b). The exact location of the samples was mathematically determined by splitting each 177 plot into 5 sections of equal area delimited by 5 rings (with the 5th ring being the outer circumference of 178 179 the plot). Each ring's radius was denoted as r_n with r_1 being the radius of the innermost ring. In order to make each section contain an equal area, the radius of each ring was calculated with the following 180 equation: $r_n = \sqrt{n} * (r / \sqrt{n_{rings}})$ where r is the radius of the plot (75 meters) and n_{rings} is the number of rings 181 (5). 182

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The 4 outer sampling locations on each transect were placed halfway between the nearest pair of rings. To further improve the method we rotated the second to innermost sampling location and the second to outermost sampling location by 22.5 degrees around the center post, thereby maximizing the distance between sampling locations (Figure 3c). Instead of laying out an additional 8 transects in order to locate these rotated sampling locations, we triangulated their positions on the 8 main radial transects such that a short sub-transect stretching between each pair of adjacent radial transects could be used to accurately locate the rotated sampling locations (Figure 4).

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To locate the innermost sampling location on each transect, we divided the innermost (circular) ring of radius r_1 into two equal areas split by a ring of radius $r_1/\sqrt{2}$. The innermost sampling locations for the four cardinal transects were placed at the midpoint between r_1 and $r_1/\sqrt{2}$. The innermost sampling locations for the four inter-cardinal transects where placed at $r_1/\sqrt{2}$, as moving them any further inward would excessively crowd them toward the center of the plot. As a result 4 of the 8 innermost samples were 197 located 29 meters from the center T-post and the other 4 were located 17 meters from the center T-post198 (Figure 3d).

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This method greatly increased the uniformity of our sampling on each plot, yielding results much more representative of the actual vegetative cover of our plots while only marginally increasing the time required to sample each plot. The distances for each sampling location from the center T-post can be found in Table 2.

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205 To sample the vegetation at each sampling location we used a Point-Intercept with Grid Quadrant Method 206 similar to the one described in Lutes et al. (2006). We used a 0.7 meter square sampling frame with 2 sets of 5 strings stretched across each side at decimeter intervals, each set of strings positioned directly above 207 208 or below the other set. The frame was systematically placed at each sampling location. A metal pin was 209 lowered at each point where two strings of one set crossed (there were 25 such points), and the pin was carefully aligned to the corresponding strings in the second set of strings below in order to ensure that the 210 211 pin was always at the right location and perpendicular to the frame's plane. We then noted whether the tip 212 of the pin was touching cheatgrass plants, cheatgrass litter, bare ground / rocks, non-cheatgrass litter, or 213 non-cheatgrass plants. The data for each pin were entered into a custom iPad app we had previously 214 developed. The app automatically synthesized and arranged the data by plot, making it exportable as a 215 CSV file. This app allowed us to streamline and accelerate the vegetation sampling process while 216 minimizing errors.

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218 Mammal Sampling

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We mounted a Stealth Cam G45NGX remote-triggering camera 0.9 meters off the ground on each T-post,
secured to a wooden block by zip-ties threaded through sets of holes in the block, such that the camera

could be oriented in each cardinal direction (Figure 5). The cameras were set to maximum sensitivity with
a 15 second recovery time. We chose this short recovery time to ensure we did not miss mammals, while
keeping false positive detections to a manageable level.

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We began observation at 0000 MST on May 14th and ended observation at 2359 MST on August 13th. All cameras were originally pointed north. At intervals ranging from one to two weeks (on May 27th, June 14th, June 24th, July 11th, July 18th, July 24th, and August 7th) we rotated all the cameras clockwise by 90 degrees and replaced the batteries and the SD cards. In this manner all the cameras completed two observational rotations around their posts during the observation period. There was no malfunction or unexpected interruption in the cameras' operation over the course of the study.

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233 We identified and counted all mammals in the images from the remote-triggering cameras without relying on previous or subsequent images for detection or identification of the mammals. Individual mammals 234 were counted regardless of whether they had appeared in other images. Lingering mammals were 235 236 effectively recounted roughly every 15 seconds when they remained in the trigger zone due to the 15 237 second recovery time, thus informally accounting for the duration of animal activity within the trigger 238 zone. All the mammals we detected were categorized as one of the following: mule deer, white-tailed deer, unidentified deer, elk, unidentified ungulate, covote, bobcat, prairie dog, unidentified cottontail, 239 striped skunk, black bear, and unidentified mammal. When performing analysis we excluded mammals 240 241 that were detected on camera rotation days, thereby eliminating data consistency problems resulting from 242 mammals being detected while certain cameras were nonoperational.

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244 Breeding Bird Surveys

246 We counted birds seen or heard from the center points of the eight monitoring plots during 8 minutes on the mornings of 29 May, 16 and 29 June, and 9 July, beginning each survey at sunrise and completing 247 248 sampling of all eight plots by 0730 MST. We varied the order of the plot counts during each replication to reduce temporal biases that might stem from sampling bird populations at varying times of the early 249 250 morning. As we entered each plot we counted any birds flushed from the vegetation. We noted numbers of birds seen or heard perching or foraging within each monitoring plot but did not record numbers of 251 individuals flying over or through the plots without foraging (Ralph et al. 1998). Swallows were counted 252 when they were flying below the tops of the tallest trees and shrubs and their irregular movement patterns 253 254 suggested that they were foraging.

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256 Butterfly Surveys

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We counted butterflies seen along 150 m north / south transects bisecting each monitoring plot during a time interval of 5 minutes on 6 and 20 June; 2, 16, and 27 July; and 7 August. We walked slowly (2 km / hr) along each transect, using binoculars and cameras with telephoto lenses to identify all butterflies seen within 30 m. Counts were carried out between 0745-0945 MST on calm (peak wind velocity \leq 20 km/hr) and clear (mean cloud cover \leq 30%) mornings when the air temperature exceeded 18° C. Plot sampling order was rotated during each replication to reduce temporal biases.

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265 Additional Data Collection

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267 In addition to measuring our primary variables we quantified other properties of our plots, notably slope,

268 distance to highway U.S. 36 (effectively the distance to the nearest human development), shrub / tree

269 cover, and percent bare and rocky ground. We used Google Earth Pro to approximate the first three

variables and data from our vegetation sampling to calculate the percentage of bare and rocky ground ineach plot.

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To measure the slope of the plots we identified the highest and lowest points in each plot and divided the 273 274 difference in altitude of these two points by the distance between them. This gave us an approximation of the slope of each the plot. To measure the distance to highway U.S. 36 we used the "measure" tool in 275 Google Earth Pro to measure the shortest distance between the center T-post of each plot (identified using 276 its GPS coordinates) and the nearest edge of highway U.S. 36. To calculate the shrub and tree cover we 277 278 used a 9 by 9 grid of points cropped into a circle containing 69 points. This was overlaid on the satellite 279 imagery such that it corresponded to the size of a plot, with points spaced by the equivalent of 15 m. For 280 each plot we identified whether each of the 69 points was over a tree, a shrub, or neither.

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282 Data Analysis

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The total cheatgrass cover of each of our plots was calculated by summing up the number of times our vegetation sampling pin touched cheatgrass or cheatgrass liter and dividing the resulting value by the total number of pin samples in each plot (1,000). We used this calculated estimate of cheatgrass cover (essentially the density of cheatgrass plants and cheatgrass litter) in all our analyses.

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We used the software package R to analyze our data with a combination of Linear Correlation analysis
(using Pearson Correlation Coefficients / Tests), as well as ANOVA analysis (using "aov" function in R).
We also verified our assumptions for these tests using the Shapiro-Wilk Normality Test and the FlignerKilleen Test of Homogeneity of Variances (when working with ANOVA analysis). For the data that failed
these tests we used the Kruskal-Wallis Rank Sum Test instead of the usual "aov" function in R.

For the ANOVA analysis we split our plots into two categories: low cheatgrass and high cheatgrass. We defined any plot with less than 0.2 cheatgrass cover as low cheatgrass and any plot with more than 0.2 cheatgrass cover as high cheatgrass. This categorization gave us 4 high cheatgrass and 4 low cheatgrass plots, as we had originally planned when selecting our plot locations. The cheatgrass cover of the low cheatgrass plots varied from 0.031 cheatgrass cover to 0.135 cheatgrass cover, while the cheatgrass cover of our high cheatgrass plots varied from 0.235 cheatgrass cover to 0.35 cheatgrass cover.

We used the R² value of both the Pearson and ANOVA tests to determine which best fit our data. We generally found that our bird and mammal data best fit an ANOVA style model, while our butterfly data clearly showed linear trends. We then consistently used the best fit model for our analysis, except for visualization purposes. When our independent variable was not cheatgrass cover we always used Linear Correlation Analysis.

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Results

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310 Mammals

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312 During the 3 months of camera operation we recorded 18,326 images, 392 of which were identified as 313 containing mammals, for a total of 561 counted mammals (Table 3). We used only 530 mammals in our 314 analyses as the rest were detected on days when we rotated the cameras (see methods section). The most 315 frequently detected mammal species was mule deer (comprising 44% of detections), followed by elk (6% of detections) and coyote (3% of detections). The cameras also detected significant quantities of 316 317 unidentified deer (29% of detections), unidentified ungulates (7% of detections), and unidentified 318 mammals (4% of detections). White-tailed deer, bobcat, cottontail species, and striped skunk represented 319 a combined total of 5% of detections. We detected only one black bear.

Scatterplots suggest a strong negative relationship between total mammals and cheatgrass cover (Figure
6) as well as a weaker negative relationship between mammal species richness (total number of species)
and cheatgrass cover (Figure 7). We then split all mammals into ungulates, carnivores / omnivores, and
lagomorphs / rodents, analyzing each category separately. Ungulates, like total mammals, showed a
relatively strong negative relationship with cheatgrass cover (Figure 8). Carnivores / omnivores were
weakly negatively correlated with cheatgrass cover (Figure 9), while lagomorphs / rodents showed a weak
positive relationship with cheatgrass cover (Figure 10).

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329 Our ANOVA analysis showed greater numbers of mammals on low cheatgrass plots compared to high cheatgrass plots (F(1,6)=9.488, MSE= 932, p=.02, Figure 11a). We found no significant difference in 330 331 mammal species richness between low and high cheatgrass plots, but we did observe a weak negative 332 relationship (F(1,6)=2.778, MSE=1.125, p=.15, Figure 11b). A significant negative relationship was found between ungulates and cheatgrass cover (F(1,6)=8.783, MSE=834, p=.03, Figure 11c), but only 333 334 insignificant relationships were found between carnivores / omnivores and cheatgrass cover (Chi²=0.35, p=.55, df=1, Figure 11d) and between lagomorphs / rodents and cheatgrass cover (Chi²=0.036, p=.85, 335 336 df=1, Figure 11e).

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338 Birds

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During four, 8-minute point-counts, we observed a total of 26 potential breeding species on the 8 plots
(Table 4). Lark sparrows were most abundant (10.25 / count), followed by spotted towhees (5.50 / count)
and western meadowlarks (4.00 / count). Obligate ground-nesters comprised 39.3% of all birds observed.
Shrub-nesters, tree cavity-nesters, cliff-nesters (mostly swallows), lower tree-canopy nesters, and habitat
generalists comprised most of the remaining observed individuals.

Scatter plots suggest a negative relationship between cheatgrass cover and mean birds per plot (Figure
12); and a negative relationship between cheatgrass cover and total bird species per plot (Figure 13).
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Since ground-nesting birds may depend on native grass cover for nesting and foraging (Kelsey 2010, Sheridan et. al. 2020), we expected to find a strong negative relationship between mean ground-nesting birds and mean cheatgrass cover. However, ground-nesting bird results were similar to results for all bird species (Figures 12 and 14). Extent of native grass cover is likely just one of several factors contributing to ground-nesting bird abundance. We observed lark sparrows and western meadowlarks foraging primarily in areas dominated by native grass cover, but we also observed individuals perching frequently on shrubs and small trees, including in areas with cheatgrass (see Discussion section, below).

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Our ANOVA analysis showed an insignificant negative relationship between mean birds per plot and cheatgrass cover (F(1,6)=3.375, MSE=1.333, p=.12, Figure 15a). The negative relationship between mean ground-nesting birds and cheatgrass cover was also insignificant (Chi²=4.0514, p=.12, df=1. Figure 15b). We found that a linear model best fit the correlation between bird species richness (total number of bird species) and cheatgrass cover, showing an insignificant negative trend (r=-0.60, p=.12, Figure 13).

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363 Butterflies

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365 During six, 5-minute transect surveys on each plot, we observed a total of 26 butterfly species on the 8 366 plots (Table 5). See Appendix I for scientific names. Variegated fritillaries (89.67/survey), habitat 367 generalists that occasionally invade the Rocky Mountain region in large numbers in response to 368 environmental stresses in the southern United States (Opler 1999), comprised more than 62% of all 369 butterflies observed. Other numerous species, with host plants in parentheses, included clouded sulphur

Page 17

370	(8.17 / survey; legumes), common checkered-skipper (7.50 / survey; mallows), and orange sulphur (5.50 /
371	survey; legumes). We observed six species that lay their eggs on grasses and that might be particularly
372	sensitive to cheatgrass invasion: common ringlet (0.17 / survey), common wood nymph (3.00 / survey),
373	dark wood nymph (0.33 / survey), green skipper (0.17 / survey), Arogos skipper (0.67 / survey), and
374	taxiles skipper (0.17 / survey).
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376	Scatterplots suggest a strong negative relationship between cheatgrass cover and mean butterflies per plot
377	(Figure 16); and a strong negative relationship between cheatgrass cover and total species per plot (Figure
378	17). The relationship for grass-dependent species appears weaker, due in part to the small number of
379	individuals detected (Figure 18).
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381	Our Linear Correlation Analysis showed a significant negative relationship between mean butterflies and
382	cheatgrass cover (r =-0.73, p =.04, Figure 16) as well as an insignificant negative relationship between
383	mean grass dependent butterflies and cheatgrass cover (r =-0.63, p =.097, Figure 18). A highly significant
384	negative relationship was found between butterfly species richness (total number of butterfly species) and
385	cheatgrass cover (<i>r</i> =-0.92, <i>p</i> =.001, Figure 17).
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387	Discussion
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389	Summary of Results
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391	Results of this single-season study suggest that cheatgrass infestation may negatively impact populations
392	of mammals, birds, and butterflies in foothills grasslands (Table 6). Cheatgrass cover seemed only weakly
393	related to mammal and bird species richness but appeared to have a strong negative impact on butterfly
394	species richness.

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396 We were intrigued that ANOVA analysis was the best-fitting model for mammals and birds, whereas Linear Correlation Analysis worked best for butterflies. We hypothesize that mammals and birds, due to 397 398 their large home ranges, are relatively unaffected by cheatgrass up to a certain threshold level above 399 which mammals and birds avoid areas with cheatgrass. As they forage, areas with sufficient desirable plants may attract them even if the desirable plants are interspersed with some cheatgrass. However, once 400 cheatgrass cover reaches a certain threshold, the foraging in that particular area may become unattractive 401 and the mammals and birds avoid it. Conversely, butterflies have small home ranges and are dependent on 402 403 native grasses and forbs within a small area for reproduction and nectar. Therefore, any reduction in native plant density, even within a relatively small area, may directly drive down butterfly populations. 404 Further research with larger data sets would help to confirm or reject this hypothesis. 405

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407 Additional Factors

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Although our study does not prove causal relationships, we do believe that the differences in animal
populations among plots were driven by cheatgrass infestation. This tentative conclusion is supported by
the generally weak relationships between observed animal populations and plot characteristics other than
cheatgrass cover.

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414 Mean slopes varied among plots, from approximately 0.10 in Plots 1 and 5 to 0.28 in Plot 4. Figure 19 415 shows the relationship between mean slope of each plot and numbers of detected mammals, birds, and 416 butterflies. This figure shows only very weak relationships between slope and the number of mammals 417 (r=-0.59, p=.13) and butterflies (r=-0.56, p=.15). There was no discernible relationship between birds and 418 slope (r=-0.23, p=.59).

420 Shrub and tree cover within the plots varied from nearly 45% in Plot 2, to 4% in plot 5. Shrubs provide resting, hiding, and foraging habitat for deer and elk, the most frequently detected mammals, along with 421 nesting, perching, and foraging habitat for a preponderance of the observed bird species. Whereas 422 butterflies typically derive nectar from blooming wildflowers growing in grassy areas, several locally 423 424 common butterfly species lay their eggs on shrub leaves or perch on shrubs while resting or patrolling breeding territories (Chu and Jones 2020). Figure 20 shows the relationship of shrub and tree cover within 425 individual plots to numbers of detected mammals, birds, and butterflies. This figure indicates a weak 426 positive relationship between shrub and tree cover and numbers of butterflies per plot (r=0.63, p=.09) but 427 428 no discernible relationships between shrub and tree cover and numbers of mammals (r=-0.24, p=.56) or birds (r=0.21, p=.62). 429

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We noticed that cheatgrass appeared to be more abundant in bare or rocky areas. Figure 21 shows the relationship between percent of bare and rocky ground within individual plots to numbers of detected mammals, birds, and butterflies. Here the scatterplots indicate a significant negative relationship between percent of bare and rocky ground and butterflies (r=-0.72, p=.04) but no discernible relationship between percent of bare and rocky ground and numbers of mammals (r=-0.27, p=.51) or birds (r=0.13, p=.77).

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Noting that two of the plots lie within close proximity to a busy highway, we also compared numbers of detected mammals, birds, and butterflies to plot distance from the highway (Figure 22). We found very weak negative relationships between numbers of mammals (r=-0.59, p=.12) and butterflies (r=-0.57, p=.14) and distance to highway U.S. 36. No discernible relationship was found between birds and distance to the highway (r=-0.22, p=.59). This may simply be a result of the plots near the highway being generally lusher than our more remote plots, thus possibly providing more food and shelter for animals. No discernible relationship was found between birds and distance to the highway (r=-0.22, p=.59).

445	We suspect that the fences that border both the Pierce and Trevarton properties may contribute to patterns
446	of mammal distribution and movement, though their effects are difficult to quantify. Barbed wire fences
447	have been shown to restrict movements of large mammals (Gates et al. 2012).
448	
449	A variety of studies have indicated that total volume of foliage can be a strong predictor of abundance of
450	both birds and butterflies (Degraaf et al. 1998, Lee et al. 2015, Mills et al. 1991). Cheatgrass infestation
451	may reduce the total volume of foliage by crowding out native grasses and forbs (Billings 1994,
452	Parkinson et al. 2013). Since cheatgrass is a winter annual that greens up in early spring and becomes
453	senescent by early summer, areas of infestation may become ecological deserts that provide neither
454	breeding habitat nor forage for most birds and butterflies.
455	

456 In contrast, native shrub cover increases the availability of breeding niches while also providing an
457 additional food source for grassland birds and butterflies. Higher density of native grasses, native forbs,
458 and native shrubs may partially explain why our lower-elevation plots supported higher numbers of
459 mammals, birds, and butterflies.

460

461 Future Research

462

Future research might include small mammal trapping and insect netting. As these animals typically have smaller home ranges than large mammals and birds and also depend on native grasses and forbs for food and shelter, we would expect their populations to be negatively impacted by cheatgrass cover. Clipping and weighing of grasses and forbs within nested plots would help to determine the relationship of total volume and weight of native and non-native vegetation to numbers of mammals, birds, and butterflies. Longitudinal studies of plots with comparable amounts of tree and shrub cover would give us a clearer view of the direct impact of cheatgrass cover on wildlife populations.

20

471	Future research should investigate the impact on wildlife of different treatment methods to control
472	cheatgrass. We recommend that such research compare wildlife populations over appropriately long
473	timescales on untreated plots relatively free of cheatgrass, on untreated plots infested by cheatgrass, and
474	on previously infested treated plots. The standard for treatment success should include restoration to
475	conditions similar to those found in untreated, relatively cheatgrass free areas, not just improvement over
476	untreated cheatgrass infested areas. A failure to investigate the effect of cheatgrass treatment methods on
477	wildlife could potentially cause the inadvertent degradation of valuable wildlife habitat. For example,
478	some herbicides are known to negatively impact certain butterfly species (Russell and Schultz 2010).
479	Without studies investigating the effects of herbicides on butterflies, herbicides might be used when
480	alternative methods, such as those suggested by Blumenthal et al. (2010), would be more appropriate.
481	
482	In October 2020 the Calwood fire burned through four of our study plots. A follow-up study,
483	documenting observed fire impacts on cheatgrass cover, native plant cover, and wildlife populations,
484	would offer a rare opportunity to explore the role that fire plays in cheatgrass growth and in the
485	regeneration of native foothills grassland ecosystems.
486	
487	Acknowledgments
488	
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Tables

595 Table 1. Plot information

	1	2	3	4	5	6	7	8
GPS Coordin ates NS	40°9'56.90 "N	40°10'4.2 0''N	40°10'9.1 0"N	40°10'18. 60"N	40°10'35.2 0''N	40°10'33.7 0''N	40°10'47.0 0''N	40°10'54.5 0"N
GPS Coordin ates EW	105°16'12. 50"W	105°16'8. 50"W	105°16'7. 90"W	105°16'6. 50"W	105°15'35. 50"W	105°15'41. 80"W	105°15'34. 30"W	105°15'33. 30"W
Propert y	Pierce	Pierce	Pierce	Trevarton	Trevarton	Trevarton	Trevarton	Trevarton
Altitude (m)	1743	1726	1727	1747	1690	1699	1742	1773
Proximi ty to U.S. 36 (m)	114	185	249	373	130	188	389	484
Slope	0.102	0.108	0.153	0.275	0.102	0.140	0.182	0.229
Cheatgr ass Cover	0.35	0.031	0.061	0.312	0.087	0.135	0.349	0.235
Shrub And Tree Cover	0.116	0.449	0.087	0.188	0.043	0.116	0.174	0.188

606 Table 2. Distances of sampling locations from center post

	Sample Number	Samples on N, E, S, and W Transects	Samples on NE, SE, SW, and NW Transects	Rotated Sample
	1	17 meters	29 meters	No
	2	40 meters	40 meters	Yes
	3	53 meters	53 meters	No
	4	63 meters	63 meters	Yes
	5	71 meters	71 meters	No
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626 Table 3. Total numbers of mammals detected by remote-triggering cameras mounted in the center of

627 monitoring plots

Plot	1	2	3	4	5	6	7	8	Total
Mule Deer	8	27	23	19	65	57	34	2	235
White Tailed Deer	0	2	1	0	0	2	0	0	5
Misc. Deer	6	24	24	6	65	11	16	2	154
Elk	2	7	14	1	4	0	2	3	33
Ungulate	1	9	5	4	9	5	1	5	39
Coyote	6	1	3	0	8	3	0	0	21
Bobcat	0	1	0	1	1	1	0	2	6
Prairie Dog	3	0	0	0	0	0	0	0	3
Cottontail Species	3	0	0	0	0	3	0	0	6
Striped Skunk	0	0	0	2	5	0	0	0	7
Black Bear	0	0	0	0	0	0	0	1	1
Misc. Mammal	0	8	5	0	3	2	0	2	20
Total	29	79	75	33	160	84	53	17	530

642 Table 4. Mean number of birds seen or heard within monitoring plots during four, 8-minute point-counts

643

Species	1	2	3	4	5	6	7	8	Total
Broad-tailed Hummingbird		0.75	0.25	0.75			0.5	0.25	2.50
Mourning Dove		0.5			0.25				0.75
Northern Flicker					0.75				0.75
Western Wood-Pewee								0.25	0.25
Plumbeous Vireo								0.25	0.25
Blue Jay		0.25		0.25					0.50
Black-billed Magpie				0.25		1.50			1.50
Barn Swallow						0.25			0.25
Cliff Swallow			0.25		0.25	0.5	0.25		1.25
Black-capped Chickadee		0.25							0.25
White-breasted Nuthatch								0.25	0.25
Rock Wren				0.25					0.25
American Robin	0.25	0.25		0.25	0.5				1.25
Gray Catbird					0.25				0.25
Brown Thrasher	0.25								0.25
European Starling					0.25				0.25
Lesser Goldfinch			0.25						0.25
American Goldfinch	0.25				0.25	0.25			0.75
Lark Sparrow	1.0	0.75	1.75	0.5	1.75	2.00	1.25	1.25	7.75
Spotted Towhee	0.5	0.5		0.5	0.25	0.5	1.75	1.5	5.50
Yellow-breasted Chat		1.25	0.25		0.25				1.75
Western Meadowlark	0.25	0.5	0.5	0.5	1.25	1.0			4.00
Bullock's Oriole	0.25	0.25							0.50
Brown-headed Cowbird		0.25							0.25
Blue Grosbeak						0.25			0.25
Lazuli Bunting		0.25		1.0		0.25		0.5	2.00
Passerine species							0.5		0.50
Total	2.75	5.75	3.25	4.25	6.00	6.50	4.25	4.25	

Table 5. Mean number of butterflies observed within monitoring plots during six, 5-minute transect

645 surveys

Species	1	2	3	4	5	6	7	8	Total
Two-tailed Swallowtail	0.17						0.33	0.17	0.67
W. Tiger Swallowtail								0.17	0.17
Checkered White	0.17	1.17	0.67	0.17	0.17	0.17	0.67	0.33	3.50
Western White	1.50	0.67	0.17	0.17	1.17	0.67	0.17	0.33	5.50
Cabbage White		1.17	0.17		0.33				1.67
White Species	0.17	0.17	0.33	0.50	1.17				2.33
Clouded Sulphur	1.83	2.67	2.33		0.17	1.00		0.17	8.17
Orange Sulphur	0.33	2.33	1.83		0.83	0.17			5.50
Dainty Sulphur		0.17			0.17	0.17			0.50
Sulphur species		0.67							0.67
Elfin species		0.33							0.33
Melissa Blue			1.33						1.33
Reakirt's Blue								0.17	0.17
Gray Copper					0.17				0.17
Gorgone Checkerspot		0.17							0.17
Red Admiral		0.17							0.17
Variegated Fritillary	11.50	25.83	9.83	9.17	9.83	14.17	4.67	4.67	88.67
Speyeria Fritillary ¹	0.50	3.17	1.00	1.50	0.83	1.00	0.33		8.33
Hackberry Emperor		0.33	0.17	0.17	0.50	0.33	0.17		1.67
Milbert's Tortoiseshell			0.33						0.33
Common Ringlet				0.17					0.17
Common Wood Nymph		0.83	1.00	0.67	0.33	0.17			3.00
Dark Wood Nymph				0.17		0.17			0.33
Silver-spotted Skipper					0.50				0.50
Common Checkered-Skipper	0.17	1.33	3.67		1.00	0.67		0.67	7.50
Green Skipper		0.17							0.17
Arogos Skipper		0.33			0.17	0.17			0.67
Taxiles Skipper							0.17		0.17
Unidentified	0.17			0.17					0.33
Total	16.50	41.67	17.83	12.83	17.33	18.83	6.50	7.33	

¹ Includes both "northwestern" and afrodite fritillary

	Plot	1	2	3	4	5	6	7	8
	Mammals	29	79	75	33	160	84	53	17
	Mammal Species	5	5	4	4	5	6	2	4
	Mean Birds	2.75	5.75	3.25	4.25	6.00	6.50	4.25	4.25
	Bird Species	7	12	6	9	11	9	4	7
	Mean Butterflies	16.50	41.67	17.83	12.83	17.33	18.83	6.50	7.33
	Butterfly Species	8	17	12	9	15	12	7	9
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Figures

- 666 Figure 1. Location of 8 plots within Open Space properties, with green pins indicating low cheatgrass
- 667 plots and red pins indicating high cheatgrass plots (see Methods section)



- 668 Figure 2. Satellite map of 8 plots with green pins indicating low cheatgrass plots and red pins
- 669 indicating high cheatgrass plots (see Methods section)







Figure 4. Positioning of rotated sampling locations (black dots are sampling locations, grey dotsare flagging locations, black lines are radial transects, and grey lines are sub-transects)



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685 Figure 5. Stealth Cam G45NGX remote-triggering camera mounted on T-post using a wooden686 block and zip-ties



Figure 6. Total mammals and cheatgrass cover (showing line of best fit and 95% confidence

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704

interval)

Figure 7. Mammal species richness (total number of species) and cheatgrass cover (showing lineof best fit and 95% confidence interval)





739 Figure 8. Total ungulates and cheatgrass cover (showing line of best fit and 95% confidence

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interval)

Total Carnivores and Omnivores 5-0-0.3 0.2 Cheatgrass Cover 0.1

Figure 9. Total carnivores / omnivores and cheatgrass cover (showing line of best fit and 95%

confidence interval)



Figure 10. Total lagomorphs / rodents and cheatgrass (showing line of best fit and 95%confidence interval)

Figure 11. Bar graphs of mammals and cheatgrass (showing mean and standard deviation)

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e. Total Lagomorphs/Rodents and Cheatgrass Cover





795 Figure 12. Mean birds and cheatgrass cover (showing line of best fit and 95% confidence

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interval)

813 Figure 13. Bird species richness (total number of species) and cheatgrass cover (showing line of814 best fit and 95% confidence interval)





831 Figure 14. Mean ground nesting birds and cheatgrass cover (showing line of best fit and 95%

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confidence interval)



849 Figure 15. Bar graphs of birds and cheatgrass (showing mean and standard deviation)



Figure 16. Mean butterflies and cheatgrass cover (showing line of best fit and 95% confidence

885 Figure 17. Butterfly species richness (total number of species) and cheatgrass cover (showing886 line of best fit and 95% confidence interval)





Figure 18. Mean grass dependent butterflies and cheatgrass cover (showing line of best fit and

904 95% confidence interval)

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Figure 19. Graphs of animals and plot slope (showing line of best fit and 95% confidence

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interval)







957 Figure 21. Graphs of animals and bare ground / rock cover of plots (showing line of best fit and958 95% confidence interval)



Figure 22. Graphs of animals and plot distance to highway U.S. 36 (showing line of best fit and95% confidence interval)

Appendix I. Scientific Names of Mammals, Birds, and Butterflies Mentioned in Text

3	Mammals
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5 6	Common Name	Scientific Name
7	Mule Deer	Odocoileus hemionus
8	White-Tailed Deer	Odocoileus virginianus
9	Deer Species	Odocoileus spp.
10	Elk	Cervus canadensis
11	Ungulate	Ungulata spp.
12	Coyote	Canis latrans
13	Bobcat	Lynx rufus
14	Prairie Dog	Cynomys ludovicianus
15	Cottontail	Leporidae spp.
16	Striped Skunk	Mephitis mephitis
17	Black Bear	Ursus americanus
18	Unidentified Mammal	Mammalia
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20		
21 22	Birds	
23 24	Common Name	Scientific Name
25	Mourning Dove	Zenaida macroura
26	Common Nighthawk	Chordeiles minor
27	Broad-tailed Hummingbird	Selasphorus platycercus
28	Turkey Vulture	Cathartes aura
29	Sharp-shinned Hawk	Accipiter striatus
30	Red-tailed Hawk	Buteo jamaicensis
31	Great Horned Owl	Bubo virginianus
32	Hairy Woodpecker	Dryobates villosus
33	Northern Flicker	Colaptes auratus
34	American Kestrel	Falco sparverius
35	Western Kingbird	Tyrannus verticalis
36	Say's Phoebe	Sayornis saya
37	Western Wood-Pewee	Contopus sordidulus
38	Plumbeous Vireo	Vireo plumbeus
39	Steller's Jay	Cyanocitta stelleri
40	Blue Jay	Cyanocitta cristata
41	Black-billed Magpie	Pica hudsonia
42	American Crow	Corvus brachyrhynchos
43	Tree Swallow	Tachycineta bicolor
44	Barn Swallow	Hirundo rustica

45 Cliff Swallow Petrochelidon pyrrhonota

46	Common Name	Scientific Name
47	Black-capped Chickadee	Poecile atricapilla
48	White-breasted Nuthatch	Sitta carolinensis
49	Rock Wren	Salpinctes obsoletus
50	Townsend's Solitaire	Myadestes townsendi
51	American Robin	Turdus migratorius
52	Gray Catbird	Dumetella carolinensis
53	Brown Thrasher	Toxostoma rufum
54	Lesser Goldfinch	Spinus psaltria
55	American Goldfinch	Spinus psain ia Spinus tristis
56	Lark Sparrow	Chondestes grammacus
57	Chipping Sparrow	Spizella passerina
58	Green-tailed Towhee	Pipilo chlorurus
59	Spotted Towhee	Pipilo maculatus
60	Yellow-breasted Chat	Icteria virens
61	Western Meadowlark	Sturnella neglecta
62	Bullock's Oriole	Icterus bullockii
63	Brown-headed Cowbird	Molothrus ater
64	Common Grackle	Ouiscalus auiscula
65	Virginia's Warbler	Leiothlypis virginiae
66	Yellow Warbler	Setophaga petechia
67	Black-headed Grosbeak	<i>Pheucticus melanocephalus</i>
68	Blue Grosbeak	Passerina caerulea
69	Lazuli Bunting	Passerina amoena
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72	Butterflies	
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74	Common Name	Scientific Name
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76	Western Tiger Swallowtail	Papilio rutulus
77	Two-tailed Swallowtail	Papilio multicaudata
78	Checkered White	Pontia protodice
79	Western White	Pontia occidentalis
80	Cabbage White	Pieris rapae
81	Clouded Sulphur	Colias philodice
82	Orange Sulphur	Colias eurytheme
83	Dainty Sulphur	Nathalis iole
84	Elfin species	Callophrys sp.
85	Gray Copper	Lycaena dione
86	Reakirt's Blue	Echinargus isola
87	Melissa Blue	Plebejus melissa
88	Rocky Mtn. Dotted Blue	Euphilotes ancilla
89	Monarch	Danaus plexippus
90	Variegated Fritillary	Euptoieta claudia
91	Northwestern Fritillary	Speyeria aphrodite

92	Common Name	Scientific Name
93	Aphrodite Fritillary	Speveria hesperis
94	Coronis Fritillary	Speveria coronis
95	Northern Crescent	Phyciodes cocyta
96	Pearl Crescent	Phyciodes pallida
97	Northern Checkerspot	Chlosyne palla
98	Gorgone Checkerspot	Chlosyne gorgone
99	Milbert's Tortoiseshell	Aglais milberti
100	Red Admiral	Vanessa atalanta
101	Painted Lady	Vanessa cardui
102	Hackberry Emperor	Asterocampa celtis
103	Common (Ocher) Ringlet	Coenonympha tullia
104	Common wood Nymph	Cercyonis pegala
105	Silver Spotted Skipper	Cercyonis oeius
100	Common Checkered Skipper	Epargyreus ciarus Burnsius communis
107	Green Skipper	Hesperia viridis
100	Arogos Skipper	Atrytone grogos
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137 Foothills grassland, with green needlegrass in foreground, Pierce Property. 5 June.



140 Western wheatgrass on Plot 5, Trevarton property, 16 June.



143 Prairie dog colony with cheatgrass and non-native forb cover on Pierce Property



Vegetation sampling on Plot 3, 4 June.









155 Plot 3, 24 June.



Plot 4, 24 June. Note extensive cheatgrass cover throughout.



160 161 162 Plot 5, 24 June



4 Plot 6, 24 June. Note shallow ravine with box elder and hackberry trees.



Plot 7, 24 June. Note cheatgrass cover, foreground, and ponderosa pine woodland, background.



70 Plot 8, 24 June. Note ponderosa pine woodland in background.



Hackberry emperor on snowberry bush in ravine near Plot 5.





179 Bobcat, Plot 8.





185 Young mule deer buck prancing through western wheatgrass, Plot 5.



213Appendix III. Github Repository of Cheatgrass Sampling App214

- 215 The code for the iPad app we used to sample cheatgrass can be found here:
- 216 <u>https://github.com/gallus-gallus/Plot-Helper</u>
- 217
- **218** Current functionality my be limited. Questions should be addressed to:
- 219 <u>anyllmarkevich@gmail.com</u>