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# Effects of Mowing and Tebuthiuron on the Nutritional Quality of Wyoming Big Sagebrush

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1 **Effects of Mowing and Tebuthiuron on the Nutritional Quality of Wyoming Big Sagebrush**

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20 **ABSTRACT**

21 Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young) is the  
22 most abundant and widely distributed subspecies of big sagebrush and has been treated through  
23 chemical application, mechanical treatments, and prescribed burning in efforts thought to  
24 improve habitat conditions for species such as greater sage-grouse (*Centrocercus urophasianus*)  
25 and mule deer (*Odocoileus hemionus*). Although the response of structural attributes of  
26 sagebrush communities to treatments is well understood, there is a need to identify how  
27 treatments influence the quality of sagebrush as winter food for wildlife. Our purpose was to  
28 identify how mowing and tebuthiuron treatments influenced dietary quality of Wyoming big  
29 sagebrush in central Wyoming. Two study areas were mowed in January and February 2014 and  
30 tebuthiuron was applied in two study areas in May 2014. We constructed 6 exclosures in each of  
31 these four study areas (24 total), which encompassed 30 m x 30 m areas of treated and untreated  
32 sagebrush within each exclosure. Samples of current annual growth were collected from 18  
33 sagebrush plants from treated and 12 plants from control portions of mowing exclosures during  
34 November 2013–2015 and tebuthiuron exclosures during November 2014–2015. Samples were  
35 analyzed for crude protein and plant secondary metabolites known to influence dietary selection  
36 of sagebrush by sage-grouse and other sagebrush occurring herbivores. Our results suggest  
37 mowing and tebuthiuron treatments may slightly increase crude protein concentrations directly  
38 after treatments without immediate changes in plant secondary metabolites. Slight increases in  
39 dietary quality of sagebrush following treatments coupled with potential trade-offs with loss of  
40 biomass associated with treatments corroborates previous research that treating Wyoming big  
41 sagebrush may have little benefit for sage-grouse and other sagebrush-dependent wildlife. Future  
42 work should evaluate not only how treatments influence sage-grouse habitat use and

43 reproductive success, but how treatments influence other wildlife species in fragile sagebrush  
44 ecosystems.

45 **KEYWORDS:** crude protein, Greater sage-grouse (*Centrocercus urophasianus*), plant  
46 secondary metabolites (PSMs), *Artemisia tridentata wyomingensis*

## 47 **Introduction**

48 Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) is the most widely distributed  
49 subspecies of big sagebrush and provides important cover and foraging resources for many  
50 wildlife species (Beck et al. 2012). Sagebrush not only provides critical vegetative cover for  
51 wildlife, but it is also the primary food source for greater sage-grouse (*Centrocercus*  
52 *urophasianus*; hereafter, ‘sage-grouse’) during late fall, winter, and spring (Connelly et al. 2000;  
53 Wallestad et al. 1975) and pygmy rabbits (*Brachylagus idahoensis*) during winter (Thines et al.  
54 2004). Sagebrush may also comprise greater than 50% of the winter diets of pronghorn  
55 (*Antilocapra americana*) and mule deer (*Odocoileus hemionus*; Austin and Urness 1983; Mason  
56 1952).

57 The loss of sagebrush through both natural and human-mediated disturbances is therefore linked  
58 to the loss of several species (Coates et al. 2016; Connelly et al. 2004). Specifically, sage-grouse  
59 have experienced long-term range-wide declines (Connelly and Braun 1997) and occur in less  
60 than 60% of their pre-settlement habitats (Schroeder et al. 2004). Declining sage-grouse  
61 populations are largely attributed to human mediated loss and fragmentation of sagebrush  
62 habitats. Sage-grouse are a sagebrush obligate species that rely on a variety of sagebrush-  
63 dominated habitats for food and cover throughout the year (Connelly et al. 2004; Crawford et al.  
64 2004). A developing body of research has coupled habitat selection and demographic rates to

65 measure habitat quality and population level consequences for sage-grouse (e.g., Aldridge and  
66 Boyce 2007; Kirol et al. 2015; Smith et al. 2014). Unfortunately, these studies are not often  
67 conducive to long-term monitoring of wildlife following natural or management-directed  
68 changes in habitat quality across landscapes. In addition, the majority of research has focused on  
69 defining sagebrush habitat quality for sage-grouse in terms of height and structural cover, with  
70 myriad studies demonstrating the importance of structure and cover for sage-grouse during  
71 different life stages (e.g. Hagen et al. 2007). However, because sagebrush comprises a substantial  
72 portion of sage-grouse diets, quality of sagebrush habitats should not be defined solely in terms  
73 of structural characteristics.

74 Habitat treatments in big sagebrush communities have been implemented with the intent of  
75 improving sage-grouse habitats by reducing competition between sagebrush overstory and  
76 herbaceous understory to improve important foraging resources for sage-grouse during the  
77 reproductive period (Beck et al. 2012). Treatments often reduce the age structure and density of  
78 sagebrush communities and younger age classes of Wyoming big sagebrush plants contain  
79 slightly higher levels of crude protein (Wambolt 2004). In addition, reduction in sagebrush  
80 density likely alleviates competitive effects between individual plants, allowing greater resource  
81 acquisition of remaining unaltered plants (Casper and Jackson 1997) and possibly greater  
82 nutritional quality. Plant secondary metabolites (PSMs) occur in high concentrations in  
83 sagebrush (Kelsey et al. 1982) and may have negative nutritional and energetic consequences for  
84 herbivores consuming sagebrush (Forbey et al. 2013; Kohl et al. 2015; Stirby et al. 1987).  
85 Mechanisms responsible for tolerating PSMs are relatively unknown for most wild vertebrate  
86 herbivores, but likely include regulated absorption, rapid rates of detoxification, and molecular  
87 insensitivity to cellular toxicity (Sorensen and Dearing 2006; Sorensen et al. 2006).

88 There is considerable evidence that nutritional and chemical quality of the diet is important to  
89 herbivores (Beckerton and Middleton 1982, 1983; Jakubas et al. 1993a,b), including sage-grouse  
90 (Frye et al. 2013; Remington and Braun 1985; Welch et al. 1988). Sage-grouse excrete PSMs  
91 from sagebrush unchanged (Thacker et al. 2012; Kohl et al. 2015), are less sensitive to enzyme  
92 inhibition by sagebrush PSMs (Kohl et al. 2015), and may rely on functional genes within the gut  
93 microbiome (Kohl et al. 2016). Frye et al. (2013) determined that sage-grouse selected black  
94 sagebrush (*A. nova* A. Nelson) with lower PSM concentrations over Wyoming big sagebrush in  
95 winter in southern Idaho. In addition, sage-grouse also selected individual plants within black  
96 sagebrush patches that were higher in nutrient concentrations and lower PSM concentrations than  
97 available plants (Frye et al. 2013). Dietary quality of sagebrush may have a significant impact on  
98 body condition as grouse enter the reproductive period. For example, ruffed grouse (*Bonasa*  
99 *umbellus*) consuming diets with higher crude protein had higher reproductive success (Beckerton  
100 and Middleton 1982) and willow grouse (*Lagopus lagopus*) consuming diets with high  
101 digestibility had higher reproductive success (Brittas 1988). In addition, ruffed grouse  
102 consuming winter diets higher in crude protein and lower chemical defenses had higher  
103 population densities (Beckerton and Middleton 1982, 1983; Jakubas et al. 1993b).

104 Our specific objective was to evaluate how tebuthiuron application and mechanical removal of  
105 sagebrush through mowing influenced the dietary quality of Wyoming big sagebrush. Herbicide  
106 applications, mechanical treatments, and prescribed burning form the major types of treatments  
107 that have been applied in efforts to enhance wildlife habitats in Wyoming big sagebrush (Beck et  
108 al. 2009, 2012; Davies et al. 2009; Hess and Beck 2012). Prescribed burning Wyoming big  
109 sagebrush to enhance habitat for sage-grouse is problematic in most instances because the shrub  
110 structure needed by sage-grouse for nesting, brood-rearing and winter habitat is lost for decades

111 (Beck 1977; Beck et al. 2009; Hess and Beck 2012). In contrast, mechanical and herbicide  
112 treatments may be more suitable to treat sage-grouse habitat than burning because residual  
113 sagebrush remains on treated sites (Olson and Whitson 2002) and shrub skeletons are left behind  
114 that sage-grouse may use for cover (Dahlgren et al. 2006). Only Davies et al. (2009) has  
115 investigated the influence of mowing on crude protein of sagebrush leaves and no studies to our  
116 knowledge have evaluated the influence of herbicide treatment on dietary quality of sagebrush.  
117 Further, increase in crude protein alone does not necessarily indicate an increase in nutritional  
118 quality as PSMs strongly influence selection by sage-grouse (Forbey et al. 2013). We thus  
119 evaluated how mowing and tebuthiuron applications influenced crude protein and PSMs in  
120 leaves of treated and untreated Wyoming big sagebrush plants. We focused on sagebrush  
121 because it is the primary food source for sage-grouse and pygmy rabbits for several consecutive  
122 months in winter (Connelly et al. 2000; Wallestad et al. 1975; Thines et al. 2004) and the  
123 nutritional quality of sagebrush influences patch and plant use by these species in winter (Frye et  
124 al. 2013; Remington and Braun 1985; Ulappa et al. 2014). We used crude protein as a nutrient  
125 variable because it can affect herbivore foraging behavior and reproductive success (Mattson  
126 1980). We chose monoterpenes and polyphenolics (coumarins and total phenolics) because these  
127 classes of compounds exert deleterious effects (e.g., toxicity, increased energy expenditure,  
128 nutrient binding) on herbivores (Dearing et al. 2005) and occur in relatively high concentrations  
129 in sagebrush (Kelsey et al. 1982).

## 130 **Methods**

### 131 *Study Area*

132 Our study area included portions of Fremont and Natrona counties, Wyoming and encompassed  
133 ~3,098 km<sup>2</sup> (735,879 ac; Fig. 1), and was composed of approximately 81% Federal, 6.9% State,  
134 and 12.1% privately administered lands. Average annual 30-year normal precipitation and  
135 temperature were 26 cm and 6.1 °C, respectively (Prism Climate Group 2016). Elevation ranged  
136 from 1642 to 2499 m. The study area was dominated by Wyoming big sagebrush with smaller  
137 amounts of mountain big sagebrush (*A. t. Nutt. ssp. vaseyana* [Rydb.] Beetle), basin big  
138 sagebrush (*A. t. Nutt. ssp. tridentata*), silver sagebrush (*A. cana* Pursh), black sagebrush, and  
139 greasewood (*Sarcobatus vermiculatus* [Hook.] Torr.). Major land uses during the study included  
140 livestock grazing. Treatments consisted of mechanical mowing and aerially broadcasted  
141 tebuthiuron (Spike® 20P, Dow AgroSciences, Indianapolis, IN) to Wyoming big sagebrush in  
142 early brood-rearing habitats during winter and spring 2014. We selected tebuthiuron because it is  
143 a translocated, soil-active herbicide that is partly selective (i.e., selective at low rates or  
144 nonselective at high rates). Thus, at low rates it leaves live sagebrush within the treated  
145 landscape (Olson and Whitson 2002). Treatments followed guidelines of the Wyoming Game  
146 and Fish Department (WGFD) Protocols for Treating Sagebrush to be consistent with Wyoming  
147 Executive Order 2011-5; Greater Sage-Grouse Core Area Protection (WGFD 2011). The only  
148 exception to the WGFD protocols was that instead of grazing rest for 2 growing seasons  
149 following treatments, we installed exclosures to measure post-treatment vegetative response in  
150 the absence of grazing. This was necessitated by the fact that only one allotment in the four  
151 treatment study areas had cross fencing and a rotational grazing system. The remaining treatment  
152 study areas occurred in areas with season-long continuous grazing, making evaluations of un-  
153 grazed post treatment vegetation responses impossible without exclosures. Therefore, we  
154 installed 12, 30 x 60 m exclosures in mowed sites and 12, 30 x 80 m exclosures in tebuthiuron



155 treated sites during May 2014 to serve as controls for livestock grazing. Exclosures constructed  
156 in tebuthiuron-treated areas were larger to account for potential herbicide leaching into the  
157 untreated side. For each treatment type, exclosures were placed such that half of the exclosure  
158 contained treated and the other half contained untreated sagebrush. The general design of these  
159 exclosures was to exclude a 30 x 30 m (0.09 ha) area of untreated sagebrush with an adjoining 30  
160 x 30 m area excluding livestock grazing in treated sagebrush. Treatments occurred in a mosaic  
161 pattern across four general locations (two tebuthiuron and two mowing treatments). During  
162 January and February 2014, 489 ha of sagebrush habitats were mowed to a height of 25.4 cm  
163 across the two mowing treatment areas. Treatments were mowed at this height to be consistent  
164 with previous mowing studies and to minimize soil disturbance (Davies et al. 2009; Pyke et al.  
165 2014). Tebuthiuron application occurred in early May 2014. Contractors applied 0.22 kg/ha  
166 active ingredient to 607 ha across the two study areas, anticipating a 50% kill rate of sagebrush.  
167 Treatments occurred across less than 5% of each study to be consistent with WGFD guidelines  
168 (WGFD 2011).

### 169 *Field Methods*

170 Prior to treatments, we randomly selected 18 Wyoming big sagebrush plants (with at least six  
171 plants less than 25.4 cm) within the treated portion of each mowing exclosure to maximize the  
172 likelihood of at least six plants surviving (assuming less than a 50% kill outcome in treatments)  
173 and 12 plants (with at least six plants less than 25.4 cm) within the untreated portions of each of  
174 the 12 exclosures. We collected 5–8 sprigs from each selected sagebrush plant within each  
175 collection site by clipping the stems with pruning shears and minimizing damage to remaining  
176 leaves and stems. Each plant was marked with a metal plant tag to allow for long term  
177 monitoring of treatment effects on dietary quality of plants. Sagebrush samples were stored in a –

178 20 °C freezer. We were unable to sample at tebuthiuron exclosure locations prior to treatment  
179 because these locations were not yet delineated during the pre-treatment sampling period.

180 During sampling following treatments (November 2014 and 2015), we collected vegetation from  
181 six previously sampled plants that survived treatment, plus an additional six plants in each  
182 treatment that were not sampled during the previous sampling period. Collection and analysis of  
183 new plants allowed us to account for effects of clipping on diet quality. Post-treatment sampling  
184 focused on collecting stems from plants containing new growth during the second winter season.  
185 Because we were unable to sample at tebuthiuron exclosure locations prior to treatment, 2014  
186 sampling at tebuthiuron exclosures was consistent with pre-treatment sampling at mowing  
187 exclosure locations (e.g., 18 plants within the treated portion and 12 plants within the untreated  
188 portions of each of the 12 exclosures).

### 189 *Laboratory Methods*

190 Of the original 18 plants sampled within the treated portions of exclosures, we only analyzed  
191 those plants that survived through the post-treatment sampling periods. In addition, we ensured  
192 that the sizes of plants sampled were similar between treatment and control plots. We found no  
193 differences between new or repeatedly sampled shrubs collected during 2014 in the mowing  
194 exclosures (control and mow) for any of the PSMs analyzed (ANOVA,  $P > 0.05$ ); therefore, new  
195 plants were selected for all analyses for 2015 mowing and tebuthiuron treatments. Six samples  
196 from each exclosure were selected to create composite samples for each independent site,  
197 treatment and exclosure. Composite samples were submerged in liquid nitrogen and sagebrush  
198 leaves were removed from woody stems. We ground composite leaves into a coarse powder  
199 using a mortar, pestle and liquid nitrogen until particles were  $\leq 2$  mm. Samples were then

200 allocated into headspace vials for gas chromatography (50 mg wet weight [ww]) and micro-  
201 centrifuge tubes (100 mg ww) for chemical analysis of coumarins and total phenolics. For crude  
202 protein (% dry matter), a minimum of 1.7 g ww of coarsely ground sagebrush composites were  
203 dried for 48 hours and assessed using combustion method elemental analysis of nitrogen (Dairy  
204 One Forage Laboratories, Ithaca, New York). Monoterpenes of sagebrush were quantified  
205 (AUC/mg dry weight, dw) using headspace gas chromatography (GC) using an Agilent 7694  
206 Headspace Autosampler coupled with an Agilent 6890N gas chromatograph. One ml of  
207 headspace gas was injected into J and W DB-5 capillary columns (30  $\mu\text{m}$  x 250  $\mu\text{m}$  x 0.25  $\mu\text{m}$ ;  
208 Operating conditions: oven temperature at 100°C, loop temperature at 110°C, transfer line  
209 temperature at 120°C, vial equilibrium time of 20 min, a pressurization time of 0.20 min, a loop  
210 fill time of 0.50 min, a loop equilibrium time of 0.20 min, and an injection time of 0.50 min;  
211 Operating conditions for GC: splitless injector at 250°C, flame ionization detector at 300°C,  
212 oven temperature at 40°C for 2 min, then increasing 3°C/min to 60°C, then increasing 5°C/min  
213 to 120°C, then increasing 20°C/min to 300°C, and held at 300°C for 7 min). The make-up gas  
214 was nitrogen and the carrier gas was helium. The inlet pressure was 80 KPa with a flow rate of  
215 1.0 mL/min. Volatile monoterpenes were identified by matching retention times to cocktails of  
216 known monoterpene composition and concentration. Retention times and peak areas were  
217 calculated using HP ChemStation version B.01.00 (Santa Clara, California, USA). Peak areas  
218 were calculated by integrating chromatogram curves. Only compounds with peak areas greater  
219 than 1% of the total area and present in at least 75% of samples were summed to calculate total  
220 monoterpenes used in the analysis. In addition, we included relative concentration of 1,8-cineole  
221 (AUC/mg dw) in analysis because this specific monoterpene is known to influence foraging

222 behavior of herbivores (Bray et al. 1991; Shipley et al. 2012) including sage-grouse (Frye et al.  
223 2013)

224 Coumarins (umol/g) and total phenolics (umol/g) of sagebrush were assessed using colorimetric  
225 assays. Composite leaves were extracted for two separate 3-minute periods in 1.0 ml GC-grade  
226 methanol in a sonicating water bath and filtered through glass wool. For the coumarin assay, 50  
227  $\mu$ l subsamples were pipetted into a 96-well plate in triplicate. Color intensity was measured using  
228 a BioTek Synergy MX multi-mode plate reader (BioTek, Winooski, Vermont, USA) at an  
229 absorbance of 350 nm excitation and 460 nm emission at room temperature. Scopoletin (number  
230 5995-86-8, Acros Organics) diluted in methanol was used as a standard (0 to 80  $\mu$ M). We used  
231 an adapted Folin-Ciocalteu assay to measure total phenolics (Ainsworth and Gillespie 2007).  
232 Samples were diluted with methanol to fit within the standard curve of gallic acid (number 92-6-  
233 15, Acros Organics) diluted in methanol (0 to 580  $\mu$ M). For each sample and standard, 20  $\mu$ l of  
234 the dilution was pipetted in triplicate into 96 well plates. Next, 100  $\mu$ l of 10% Folin-Ciocalteu  
235 reagent was added to each well, mixed and 80  $\mu$ l of 700 mM (7.5%) sodium carbonate was added  
236 and mixed. Plates were allowed to incubate for 2 hours, and were then shaken on the plate reader  
237 for 60 seconds before reading. Color intensity was measured using a BioTek Synergy MX multi-  
238 mode plate reader at an absorbance of 765 nm at room temperature.

### 239 *Statistical Analysis*

240 We assessed monoterpenes, 1,8-cineole, total phenolics, coumarins, and protein for correlations  
241 and found that no variables were correlated above ( $|r| > 0.7$ ). We used linear mixed models  
242 (package nlme; Pinheiro et al. 2016) to test the response of sagebrush dietary quality (crude  
243 protein, total monoterpenes, 1,8-cineole, coumarins, and total phenolics) to mowing and

244 tebuthiuron treatments. Fixed factors included treatment type and year, with exclosures (with  
245 year and type nested within exclosure) treated as a random effect. We performed separate models  
246 for each treatment type (mowing or tebuthiuron) and dietary response to compare differences  
247 between treatments and controls within exclosures. In addition, we assessed differences between  
248 mowing and tebuthiuron treatments during 2014 and 2015, where the response of paired control  
249 plots was subtracted from treatments. We used least square means with Tukey adjustments to  
250 assess *post hoc* differences between treatment and controls or mowing and herbicide treatments  
251 across sampling years when main effects were significant (package lsmeans; Length 2016). We  
252 removed any outliers from analysis and assessed normality of model residuals. We performed all  
253 statistical analyses in R statistical software (R version 3.2.4; R Core Team 2016) and set  
254 statistical significance at  $\alpha = 0.05$ .

## 255 **Results**

### 256 *Mowing Treatments*

257 Comparison of mowing exclosures revealed no differences in crude protein between treatment  
258 and control ( $F_{1,22} = 1.76$ ,  $P = 0.198$ ), year ( $F_{2,44} = 1.65$ ,  $P = 0.205$ ), or treatment by year  
259 interaction ( $F_{2,44} = 2.31$ ,  $P = 0.111$ ; Fig. 2). For total monoterpenes, we found no difference  
260 between treatment and controls at mowing exclosures (Treatment:  $F_{1,22} = 0.13$ ,  $P = 0.722$ ;  
261 Treatment x Year:  $F_{2,44} = 0.42$ ,  $P = 0.662$ ). We detected a difference across years ( $F_{2,44} = 6.26$ ,  $P$   
262  $= 0.004$ ), with lower monoterpene concentrations in 2013 compared to 2014 (*post hoc*,  $P = 0.05$ )  
263 and 2015 (*post hoc*,  $P = 0.003$ ). For 1,8-cineole, we found no difference between treatment and  
264 controls at mowing exclosures or across years (Treatment:  $F_{1,22} = 1.68$ ,  $P = 0.209$ ; Year:  $F_{2,44} =$   
265  $2.20$ ,  $P = 0.124$ ; Treatment x Year:  $F_{2,44} = 0.49$ ,  $P = 0.619$ ). Similarly, we found no differences in

266 coumarin concentrations between treatment and controls at mowing exclosures (Treatment:  $F_{1,22}$   
267 = 0.19,  $P = 0.664$ ; Treatment x Year:  $F_{2,44} = 0.44$ ,  $P = 0.645$ ). However, coumarin concentrations  
268 differed across years ( $F_{2,44} = 20.51$ ,  $P < 0.001$ ), with coumarins lower in 2015 compared to 2013  
269 and 2014 (*post hoc*,  $P < 0.001$ ). For total phenolics, we did not detect differences between  
270 treatment and controls (Treatment:  $F_{1,22} = 0.14$ ,  $P = 0.707$ ; Treatment x Year:  $F_{2,44} = 0.22$ ,  $P =$   
271 0.800), but found differences across years ( $F_{2,44} = 15.1326$ ,  $P < 0.001$ ), with 2015 samples having  
272 lower total phenolic concentrations compared to 2013 and 2014 (*post hoc*,  $P < 0.001$ ).

### 273 *Tebuthiuron Treatments*

274 We found differences in crude protein between herbicide and control treatments ( $F_{1,22} = 9.78$ ,  $P =$   
275 0.005) and year ( $F_{1,22} = 21.90$ ,  $P < 0.001$ ), but did not detect a significant treatment by year  
276 interaction ( $F_{1,22} = 0.18$ ,  $P = 0.677$ ; Fig. 2). We found higher crude protein in tebuthiuron  
277 treatments compared to paired controls during 2014 (*post hoc*,  $P = 0.006$ ) and 2015 (*post hoc*,  $P$   
278 = 0.014), and overall crude protein levels were greater in 2014 compared to 2015 (*post hoc*,  $P <$   
279 0.001). For monoterpenes, we did not detect differences between treatment and controls ( $F_{1,22} =$   
280 0.12,  $P = 0.735$ ) across years ( $F_{1,22} = 3.11$ ,  $P = 0.092$ ) or for the treatment x year interaction ( $F_{1,22}$   
281 = 0.032,  $P = 0.859$ ). For 1,8-cineole, we did not detect differences between treatment and  
282 controls ( $F_{1,22} = 0.00$ ,  $P = 0.985$ ) across years ( $F_{1,22} = 2.47$ ,  $P = 0.131$ ) or for the treatment x year  
283 interaction ( $F_{1,22} = 0.00$ ,  $P = 0.999$ ). We found no differences in coumarin concentrations  
284 between treatment and control in tebuthiuron exclosures (Treatment:  $F_{1,22} = 0.49$ ,  $P = 0.490$ ;  
285 Treatment x Year:  $F_{1,22} = 0.12$ ,  $P = 0.734$ ). However, we did detect differences across years ( $F_{1,22}$   
286 = 7.35,  $P = 0.013$ ), with greater coumarin concentrations in 2014 compared to 2015. Similarly,  
287 we did not detect differences in concentrations of total phenolics between tebuthiuron and

288 control treatments ( $F_{1,22} = 0.79$ ,  $P = 0.384$ ) or the treatment x year interaction ( $F_{1,22} = 2.67$ ,  $P =$   
289  $0.116$ ), but total phenolics were greater in 2015 ( $F_{1,22} = 11.22$ ,  $P = 0.003$ ).

### 290 *Comparison of Mowing and Tebuthiuron Treatments*

291 Comparison between paired differences of treatment and controls indicated a difference in crude  
292 protein between tebuthiuron and mowing treatments ( $F_{1,22} = 11.58$ ,  $P = 0.003$ ; Fig. 3). There  
293 were no differences between year ( $F_{1,22} = 0.19$ ,  $P = 0.663$ ) and the treatment by year interaction  
294 was not significant ( $F_{1,22} = 1.20$ ,  $P = 0.28$ ). Crude protein was greater at tebuthiuron treated  
295 exclosures compared to mowing exclosures during 2014 (*post hoc*,  $P = 0.003$ ), but no differences  
296 were detected during 2015 (*post hoc*,  $P = 0.073$ ; Fig. 3). We found no differences between  
297 treatments, years, or treatment x year interactions for monoterpenes, 1,8-cineole, coumarins, or  
298 total phenolics.

### 299 **Discussion**

300 Relatively little information exists about the effects of management practices on the dietary  
301 quality of shrubs. Although several studies have investigated how management practices  
302 influence diversity and composition of sagebrush communities (Davies et al. 2011a, 2012), only  
303 Davies et al. (2009) have investigated the influence of mowing on dietary quality of sagebrush  
304 and our study is the first to evaluate the influence of herbicide treatments on sagebrush dietary  
305 quality and of any treatment on secondary metabolites. We found that herbicide treatments  
306 resulted in sagebrush plants with greater leaf crude protein content compared to untreated  
307 controls. We did not collect pre-treatment information on herbicide treatments; nonetheless our  
308 results suggest that sagebrush plants treated with herbicide had greater leaf crude protein content  
309 compared to mowing treatments at least during the first year following treatments. However, we

310 did not detect differences in PSMs between treated and untreated plants, or between treatment  
311 types, but our results corroborate others that found annual variation in PSMs (Cedarleaf et al.  
312 1983; Wilt and Miller 1992).

313 Changes in the availability of quality food pose a threat to a variety of terrestrial species. Recent  
314 work from Idaho revealed strong evidence that the nutritional and chemical quality of sagebrush,  
315 not structural cover, explained habitat selection by sage-grouse (Frye et al. 2013) and pygmy  
316 rabbits (Ulappa et al. 2014). This research suggested that wildlife managers should be concerned  
317 with preserving the dietary quality of sagebrush and should identify how management-driven  
318 changes to habitats influence the dietary quality of sagebrush specifically in areas dominated by  
319 Wyoming big sagebrush. Wyoming big sagebrush communities have undergone significant  
320 changes due to invasion of non-native grass species, wildfire, and management practices that  
321 reduce sagebrush (Beck et al. 2012; Davies et al. 2011b). Increases in CO<sub>2</sub>, drought, and  
322 temperatures associated with climate change are likely to reduce the dietary quality of remaining  
323 sagebrush (Bidart-Bouzat and Imeh-Nathaniel 2008; Karban 2011; Robinson et al. 2012). Future  
324 sage-grouse populations may experience both the loss of biomass and reduction in the dietary  
325 quality of existing sagebrush, which could be mitigated or exacerbated by management practices  
326 (Forbey et al. 2013).

327 Sage-grouse are reliant on sagebrush for food during winter (Wallestad et al. 1975) and treated  
328 sagebrush may be used by sage-grouse during this time for food, provided snow cover does not  
329 preclude access to remaining sagebrush canopy. Further, sagebrush is an important dietary  
330 component of female sage-grouse during the pre-laying period prior to new forb growth  
331 (Connelly et al. 2000, Gregg et al. 2006). As such, treatments that increase crude protein or  
332 decrease PSMs in sagebrush may benefit sage-grouse populations. Davies et al. (2009) found



333 slight increases in crude protein levels in treated Wyoming big sagebrush habitats up to 6 years  
334 following mechanical treatments. Similarly, we detected slight increases in crude protein in  
335 tebuthiuron-treated sagebrush without detecting changes in PSMs in tebuthiuron or mowing  
336 treatments. Although the mechanisms for these changes are unknown for sagebrush, increased  
337 protein could be due to new vegetative growth. For example, crude protein increases in grasses  
338 and forbs following fires (Hess and Beck 2014; Powell et al. 2018). Herbicides are known to  
339 alter nutritional quality of plants through changes in plant composition (Soper et al. 1993; Han  
340 and Twidwell 2017) or changes in the soil microbiome (Lekberg et al. 2017), but these responses  
341 are not well described in shrubs. Regardless of mechanism, we agree with Davies et al. (2009)  
342 that minor increases in nutritional quality of treated sagebrush is unlikely to offset the negative  
343 impacts of long term reduction in cover and density for sage-grouse and other wildlife.  
344 Tebuthiuron treatments do leave behind shrub skeletons that sage-grouse may use for cover  
345 (Dahlgren et al. 2006). If sufficient cover remains within herbicide treatments, increased  
346 palatability of sagebrush may improve habitat quality as a result of herbicide treatments.  
347 However, we did not assess how herbicide influences other forage species that may be  
348 particularly important to nesting sage-grouse (Gregg et al. 2008). Beckerton and Middleton  
349 (1982) found that captive female ruffed grouse fed diets with approximately 2% greater crude  
350 protein on a percentage dry matter basis, had greater clutch sizes and mean egg weights, but did  
351 not exhibit increased hatching success. An approximate 10% increase in crude protein in the diet  
352 of captive female ruffed grouse did increase hatching success, but clutch size, mean egg weight,  
353 and hatching success were similar to wild females (Beckerton and Middleton 1982). In addition,  
354 we did not detect differences in PSM concentrations relative to mowing or herbicide treatments,  
355 which may better predict plant and patch selection by sage-grouse compared to crude protein

356 levels alone (Frye et al. 2013). Based on these findings, it is unlikely that marginal increases in  
357 crude protein as a result of herbicide treatments would improve winter habitat use or  
358 reproductive success for sage-grouse.

### 359 **Implications**

360 The sagebrush ecosystem is among the most imperiled ecosystems in the United States (Davies  
361 et al. 2011b) and loss and fragmentation of sagebrush habitats has been identified as a significant  
362 threat for remaining sage-grouse populations (Knick et al. 2003). As such, managers should take  
363 great caution when altering remaining sagebrush habitats. While some evidence suggests that  
364 tebuthiuron treatments may improve breeding habitats for sage-grouse in mountain big sagebrush  
365 communities (Dahlgren et al. 2006, 2015), our results provide evidence that treating Wyoming  
366 big sagebrush communities to benefit sage-grouse may not significantly improve diet quality  
367 relative to detrimental decreases in reduced cover associated with treatments. Specifically,  
368 sagebrush communities recover slowly following disturbances (Baker 2011) and often do not  
369 increase important forb and insect abundance for sage-grouse diets during the breeding season  
370 (Davies et al. 2007, 2012; Fischer et al. 1996; Hess and Beck 2014; Nelle et al. 2000; Rhodes et  
371 al. 2010). Treatments reduce sagebrush cover important for nesting and brood-rearing habitats  
372 (Hagen et al. 2007) and provide only a slight increase in nutritional quality for winter diets  
373 (Davies et al. 2009; this study). In addition, emphasis on improving habitats for sage-grouse does  
374 not reflect the numerous wildlife species that rely on sagebrush communities yearlong. For  
375 example, identifying how to maximize the availability of palatable sagebrush as well as  
376 associated forbs could benefit other herbivores such as pronghorn, pygmy rabbits, mule deer and  
377 elk (*Cervus elaphus*) that rely on sagebrush communities for forage. Further work is needed to  
378 understand the relationship between management practices, changes in cover, diet, and

379 reproductive success of sage-grouse as well as how habitat management targeted at sage-grouse  
380 influences habitat quality for other sagebrush occurring wildlife.

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580

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