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Impacts of a Mixed Ungulate Community on Aspen Forests: From Landscape to Leaf

Aaron C. Rhodes

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

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ABSTRACT

Impacts of a Mixed Ungulate Community on Aspen Forests: From Landscape to Leaf

Aaron C. Rhodes
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Doctor of Philosophy

This dissertation explores the differential effects of an ungulate community (cattle, mule deer, and American elk) on aspen (*Populus tremuloides*) regeneration pre- and post-fire disturbance. It's first chapter examines the differential effects of cattle, mule deer, and American elk on aspen regeneration across variable topography, climate and forest type; aspen dominant to conifer dominant subalpine forests. We found that each ungulate species, if sufficiently numerous, can cause aspen regeneration failure. Also, high elevation, southern aspect and high winter snow pack increase resilience to ungulate herbivory. Chapter two compares the efficacy of four methods of estimating ungulate impact on aspen. We found that direct methods of estimation (meristem removal, and defoliation) are better indicators of ungulate impact than indirect methods (fecal and camera counts). Therefore, we suggest to management that removal of apical meristems be an indicator for relative ungulate use. In chapters 3 and 4 we use differential ungulate exclosure fencing similar and camera traps to model the year to year and monthly use of aspen by ungulates. We model the per unit animal impact on aspen using photo counts. Our results show that ungulates on a per animal basis utilize aspen similarly. However, when adjusted for body size, native ungulates select aspen more often than cattle. Also, we found that while aspen is more palatable in June it is relative use, as measured by removal of apical meristems is significantly higher throughout July and August. We conclude that aspen is relatively more palatable than grass and forb species late season, and becomes and important forage species late season when forbs and grass species nutrient content drops. In chapter five, we quantify the physiological effects of ungulates on aspen regeneration after fire in order to elucidate the physiological mechanisms underlying plant:animal interactions. We show that ungulate herbivory induces high concentrations of defense chemistry (Phenolics), and reduces palatability through lower foliar nonstructural carbohydrates, and that this high investment in defense and lower growth potential related to low palatability causes severe reductions in aspen vertical growth leading to aspen regeneration failure. Together, we examine how biotic and abiotic factors at the landscape level, as well as defense and physiological function at the leaf chemistry level mediate how ungulates influence aspen regeneration and recruitment

Keywords: *Populus tremuloides,* mule deer, American elk, cattle, herbivory, defense chemistry

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TABLE OF CONTENTS

TITLE PAGE i

ABSTRACT ii

ACKNOWLEDGEMENTS iii

TABLE OF CONTENTS iv

LIST OF FIGURES x

LIST OF TABLES xiv

CHAPTER 1 1

ABSTRACT 1

INTRODUCTION 2

MATERIALS AND METHODS 5

Study area 5

Ungulate fecal counts 6

Characterization of Aspen Regeneration 7

Topography 7

Precipitation 8

Statistical Analysis 9

RESULTS 10

Global Model Summary 10

Hypothesis 1: Ungulate effects on aspen regeneration and recruitment 10

Hypothesis 2: Stand Composition Effects 11

Hypothesis 3: Effects of Climate and Topography 12

DISCUSSION 12
Overview 12
Ungulate effects on aspen regeneration and recruitment 13
The role of stand composition in ungulate impacts on aspen 14
The role of topography and climate 15
Ecological implications 17
Conclusion and management implications 18
LITERATURE CITED 20
FIGURES 27
TABLES 31
CHAPTER 2 32
ABSTRACT 32
INTRODUCTION 33
MATERIALS AND METHODS 37
Study area 37
Sampling design 38
Direct methods 38
Indirect methods 39
Fecal Counts 39
Camera Counts 39
Topographical features 40
Statistical Analysis 40
RESULTS 42
Preliminary Topographical Models 42

Top Models for Aspen Height and Density 43
Comparison of Methodologies 43
DISCUSSION 44
Indicators of Height and Density 44
Comparing Indicators of Ungulate Herbivory 46
Management implications 48
LITERATURE CITED 50
FIGURES 57
TABLES 60
CHAPTER 3 63
ABSTRACT 63
INTRODUCTION 64
MATERIALS AND METHODS 67
Study area 67
Study design 68
Characterization of aspen regeneration and recruitment 69
Wildlife cameras 69
Elevation 70
Statistical analysis 70
RESULTS 72
Hypothesis 1: Relative ungulate use patterns across years and site 72
Hypothesis 2: Elevation mediates ungulate:aspen interactions 72
Hypothesis 3: Differential ungulate effects 73
Hypothesis 4: Aspen recruitment thresholds 74

DISCUSSION 75
Changes in relative use across space and time 75
Elevation 75
Differential species impacts 76
Removal of apical meristem thresholds for aspen recruitment 77
Management and ecological implications 78
FIGURES 86
TABLES 91
CHAPTER 4 93
ABSTRACT 93
INTRODUCTION 94
MATERIALS AND METHODS 97
Study area 97
Study design 98
Wildlife cameras 98
Characterization of aspen height and relative ungulate use 99
Elevation 100
Preparation of plant tissue for analysis 100
Foliar defense chemistry 100
Foliar N and starch 101
Statistical analysis 102
RESULTS 103
Ungulate activity patterns across seasons and years 103

Height growth 103
Removal of apical meristems 104
Foliar defense chemistry effects on meristem removal and height 105
DISCUSSION 105
Ungulate Species Visitation Across the Growing Season 105
Ungulate impact on removal of apical meristems and height 107
Foliar chemistry 108
Management and ecological implications 109
FIGURES 116
TABLES 121
CHAPTER 5 123
ABSTRACT 123
INTRODUCTION 124
MATERIALS AND METHODS 127
Study area 127
Study design 128
Leaf collection 129
Ungulate browse intensity 129
Dendrochronology and growth 129
Leaf morphology 130
Photosynthesis measurements 130
Foliar nitrogen 131
Nonstructural carbohydrates 131
Defense chemistry 132

Statistical Analysis 133
RESULTS 134
Experiment one: 1 – 3 years post-fire 134
Experiment two: 26-years post-fire 135
Comparing 3 year and 26 year reponses 136
DISCUSSION 137
Overview 137
Aspen tolerance to ungulate herbivory 137
Resistance to ungulate herbivory 138
Aspen escape from ungulate herbivory 140
Short and long-term herbivory 140
Conclusion 141
LITERATURE CITED 143
FIGURES 149

LIST OF FIGURES

Figure 1–1. Map of the extent of the study sites across central and southern Utah. Paired study sites in adjacent mixed and aspen dominant stands are marked with a single dot. The 8 mountain ranges are marked with numbers: 1) Manti, 2) Fishlake, 3) Monroe Mt., 4) Pavant, 5) Tushars, 6) Markagunt, 7) Aquarius, 8) La Sal.
.....27

Figure 1–2 . Binned means at the Jenk's natural breaks are presented with their associated error bars (± standard error). Weighted linear models that were statistically significant have their r^2, p , and slope (m) values. Non-significant models are denoted with "ns". Height is reported in cm, recruitment is per hectare, aspen suckering is per hectare, and browse percent are presented for American elk, mule deer, and cattle fecal counts.
.....28

Figure 1–3. Regression analysis of a) aspen height and b) aspen recrutiment as a function of binned fecal counts summed for elk, deer and cattle. Error bars represent the standard error around the mean of that bin. c) A frequency histogram indicates the number of sites that have fecal counts ranging from 0 to 71. d) A pie chart of the proportion of sites in low, medium, and high categories of ungulate use. In all four graphics gray scale shading is matched and represents low (light gray), medium (dark gray) and high (black) reductions in aspen height and related to increasing fecal count densities. The three categories were defined based on visual breaks in the data.
.....29

Figure 1–4. Using the zero inflation count model intercept and beta coefficients, this graph presents in panel a) the predicted mean number of aspen recruitment (150 cm $\geq$ x $\geq$ 200 cm) per hectare across the observed total animal fecal counts (Cattle + Deer + Elk) and in panel b) the observed lag winter precipitation (mm). The solid line represents the predicted values at southern aspect and the dashed line represents the predicted mean at northern aspect. Lag winter precipitation was held at the mean 90 mm. Animal fecal counts (Cattle + Deer + Elk), were held at the median = 6.
.....30

Figure 2–1. A map of the study area depicted on a small scale map (1:5,000,000) with partial national forests and fire perimeters. Fires are labeled a, b, and c (Seeley, Box Creek, and Harris Flat, respectively). Inset maps are displayed to the right in a larger scale of 1:500,000. Solid black points mark the study sites in the three inset maps.
.....56

Figure 2–2. Predicted mean height of aspen or mean aspen density is shown across the study period by holding northness at 0 (east or west aspect), and use mean terrain ruggedness. Change in aspen height is presented as a function of percent removal of apical meristems and its interaction with year is presented in panel (a), while percent defoliation and its interaction with year is presented in panels (b) and (c).
.....57

Figure 2–3. Pairwise regression analyses are presented here which the relationships between meristem removal (%), defoliation, ungulate camera counts/day and fecal counts/100 m. The r^2 values represent the full model including year, northness, and terrain ruggedness. The points represent raw values.
.....58
Figure 3–1. Map of the study area – This map shows the larger study area encompassing central and southern Utah. Forest boundaries are in gray and fire perimeters are in black. Three inset maps display black dots at the study sites. At each a four block differential fencing treatment is located. All three inset maps use the same scale.
.....84

Figure 3–2. Browse of apical meristems vary across time and space. This figure presents the mean browse of apical meristems ± SE in fenceless plots at each fire. The comparison uses fenceless plots to assess "natural" levels of ungulate browse.
.....85

Figure 3–3. Plotted results from the analyses involving fences or plotted on the left column for (a) removal of apical meristems, (b) mean aspen height (cm), (c) aspen suckering density (ha^{-1}). The legend for the left column includes: "None" for no ungulates allowed, "D" for deer-only plots, "D + E" for livestock fencing, and "C + D + E" for fenceless plots allowing access to all ungulates. The models involving camera counts are plotted on the right using model predictions for 1 ungulate per camera day and the standard error associated with that ungulate species' impact. Each of the response variable is the same order as the fenced analyses: (d) removal of apical meristems, (e) mean aspen height (cm), and (f) aspen suckering density (ha^{-1}). Only significant models are graphed.
.....86
Figure 3–4. The large panel (a) shows the raw recruitment data (ha^{-1}) plotted with the removal of apical meristems (%). The inset panel (b) shows the modelled recruitment data using 30% removal of apical meristems as the browse threshold. The error bar is one standard error
.....87

Figure 4–1. This map shows the larger study area encompassing central and southern Utah. Forest boundaries are in gray and fire perimeters are in black. Three inset maps display black dots at the study sites. At each a four block differential fencing treatment is located. All three inset maps use the same scale.
.....113

Figure 4–2 . Presented here are the raw visitation rates in ungulates per camera day-1 in separate panels for 2014 and 2014. Ungulate species are color coded and stacked and displayed across each sampled month. The error bar is standard error for the total ungulate community visitation rates.
.....114

Figure 4–3. Presented here are the raw data for mean aspen height (cm) from May through September in separate panels for 2014 and 2015. Legend titles refer to ungulates that were allowed access to the plots. None – no access to these plots. D – deer-only fencing. D + E – livestock fencing that excluded only cattle. C + D + E – fenceless plots. Error bars are one standard error.
.....115

Figure 4–4. Presented here are the raw data for removal of apical meristems (%) from May through September in separate panels for 2014 and 2015. Legend titles refer to ungulates that were allowed access to the plots. None – no access to these plots. D – deer-only fencing. D + E – livestock fencing that excluded only cattle. C + D + E – fenceless plots. Error bars are one standard error.
.....116

Figure 4–5. Presented here are the raw data for foliar chemistry across the growing season of 2015, Error bars are one standard error. Panel a shows phenolic glycosides and condensed tannins, while panel b shows nitrogen, and starch.
.....117

Figure 4–6. Presented here are the predicted effect that foliar chemistry has on rates of removal of apical meristems (%) from May through September in for 2014. Each panel shows the predicted values of meristem removal across the range of observed foliar concentrations of: a) phenolic glycosides, b) condensed tannins, c) nitrogen, and d) starch.
.....118

Figure 4–7. Presented here are the predicted effect that foliar chemistry has on rates aspen height from May through September in for 2014. Each panel shows the predicted values of meristem removal across the range of observed foliar concentrations of: a) phenolic glycosides, b) condensed tannins, c) nitrogen, and d) starch.
.....119

Figure 5–1. A map of our study sites in central and southern Utah. Embedded in the map is a table that presents the date, size, elevation and precipitation (cm) of the fire. National forest boundaries are indicated by solid lines.
.....149

Figure 5–2 . The fence effects for 2015 are presented from experiment one using the model estimates for the mean and one SE around that mean is given as the error bar. NSC refers to nonstructural carbohydrates. Photosynthesis was measured at light-saturated photosynthesis (Asat).
.....150

Figure 5–3. Top panel: a) Mean height is presented in centimeters for both fenced and unfenced plots across years 2013, 2014, and 2015. The error bars are one SE. Bottom panel: b) Mean browse impact is presented in percent of meristems removed for fenced plots only across years 2013, 2014, and 2015. Fenced plots had zero browsing for all three years (data not presented). The error bars represent one SE.
.....151

Figure 5–4. Fence effects are presented for experiment two using the model estimates for the mean and SE around that mean. The error bar is one SE.
.....152

Figure 5–5. Fence effects are presented from experiment 2 using the model estimates for the mean and standard error around that mean. Full model *p* - values are presented in the upper left corner of each panel.
.....153

Figure 5–6. The fence effects for year 3 and 26 post-fire are presented by comparing experiment one and two using the model estimates for the mean and one SE around that mean is given as the error bar. NSC refers to nonstructural carbohydrates.
.....154

LIST OF TABLES

Table 1–1. Fixed effects for each of 4 models for aspen regeneration are presented. Height, density, browse %, and recruitment are in bold with the full model r^2 or rho presented on the same line. Under each model title are the fixed effects that were in the optimal model with their corresponding beta estimates ± standard error, χ^2 and Z statistics for mixed effects models and zero-inflated models respectively, and their corresponding *p* – value.
.....31

Table 2–1. *A priori* model results with topography as a predictor of aspen height. The associated model parameters (K), change in AICc when compared to the top model and the Akaike weights are listed for each model.
.....59

Table 2–2. *A priori* model results with topography as a predictor of aspen density. The associated model parameters (K), change in AICc when compared to the top model and the Akaike weights are listed for each model.
.....59

Table 2–3. *A priori* model results with each of the four methods of estimating ungulate impact as a predictor of aspen height. The associated model parameters (K), change in AICc when compared to the top model and the Akaike weights are listed for each model.
.....60

Table 2–4. *A priori* model results with each of the four methods of estimating ungulate impact as a predictor of aspen height. The associated model parameters (K), change in AICc when compared to the top model and the Akaike weights are listed for each model.
.....60

Table 2–5. Full model goodness of fit are presented for each indicator for both height and density. The marginal coefficient of determination (r_m^2) represents variation explained by fixed effects and the conditional coefficient of determination (r_c^2) represents the variation explained by both the fixed and random effects.
.....61

Table 2–6. Beta estimates for the top models previously selected are presented along with their associated standard error and t-statistic. Aspen height units are centimeters, and aspen density units are the number of suckers $100m^{-1}$. We list the estimate, the standard error associated with it and the t-statistic.
.....61

Table 3–1. This table shows results from analysis of fenced treatments across 2013-2016. F-statistic, p-value, degrees of freedom (df) and marginal model goodness of fit (r_m^2) are presented for each of three response variables. Browse refers removal of apical meristems (%), height is mean height (cm), and density is aspen ha^{-1}. Removal of apical meristem removal had 1 less degree of freedom in the fence category and 3 less in the interaction between fence and year. 88

Table 3–2. This table shows individual effects of ungulates using camera counts to model their individual effect on three response variables. F-statistic, p-value, degrees of freedom (df) and marginal model goodness of fit (r_m^2) are presented for each of three response variables. Browse is removal of apical meristems (%), height is mean height (cm), and density is aspen ha^{-1}. Removal of apical meristem removal had 1 less degree of freedom in the fence category and 3 less in the interaction between fence and year. 89

Table 4–1. Results from analysis of monthly native ungulate visitation rates from May through September in 2014 and 2015. Degrees of freedom, F-statistic, p-value, and full model marginal and conditional goodness of fit (r^2) are presented for mule deer, and American elk. 120

Table 4–2. Results from analysis of monthly mean aspen height and removal of apical meristem rates May through September in 2014 and 2015. Each parameter is listed with its associated degrees of freedom, F-statistic, p-value, and full model marginal and conditional goodness of fit (r^2) are presented for aspen height and removal of apical meristems. 120

Table 4–3. Results from analysis of removal of apical meristems as a function of foliar chemistry on a monthly basses from May through September in 2014. Each parameter is listed with its associated degrees of freedom, F-statistic, p-value, and full model marginal and conditional goodness of fit (r^2) are presented for aspen height and removal of apical meristems. 121

Table 4–4. Results from analysis of aspen height as a function of foliar chemistry on a monthly basses from May through September in 2014. Each parameter is listed with its associated degrees of freedom, F-statistic, p-value, and full model marginal and conditional goodness of fit (r^2) are presented for aspen height and removal of apical meristems. 122

CHAPTER 1

Herbivory Impacts of Elk, Deer and Cattle on Aspen Forest Recruitment Along Gradients of Stand Composition, Topography and Climate

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ABSTRACT

The abundance and composition of large ungulate communities are changing dramatically across Earth's terrestrial ecosystems due to human activities. This has resulted in novel herbivory regimes that create strong top-down effects on biological communities, and can reduce ecosystem resilience. Biotic and abiotic conditions that influence the population structure and behavior of ungulate herbivores are highly variable across space and time, making it difficult to predict ungulate impacts across complex landscapes. Where multiple ungulate species co-exist, it can be challenging to differentiate species impacts on plant communities. We examined the effects of cattle, American elk, and mule deer on aspen regeneration and recruitment along gradients of forest composition and physiographic conditions in 3 National Forests of Utah, USA. Aspen regeneration and recruitment increased with winter precipitation, elevation and higher abundance of overstory aspen. Binned fecal count densities of elk, mule deer, and cattle showed strong inverse correlations with aspen height (elk: $r^2 = 0.83$; deer: $r^2 = 0.92$; cattle: $r^2 = 0.35$) and aspen recruitment (elk: $r^2 = 0.51$; deer: $r^2 = 0.59$). Aspen stem density was negatively correlated with elk ($r^2 = 0.35$) and cattle fecal densities ($r^2 = 0.71$). We found that 60% of the sites we surveyed had low ungulate activity and good aspen regeneration, 32% were intermediate use sites that warrant additional monitoring, and 8% of sites showed high ungulate use that exhibited aspen regeneration failure and lack of recruitment where targeted management is

recommended. We conclude that ungulate impacts in aspen forests should be considered on a site by site basis and should primarily be evaluated based on the total abundance of the ungulate community with precipitation, elevation and stand composition as secondary factors.

INTRODUCTION

Ungulate herbivory strongly shapes plant evolution and plant community assembly (Augustine and McNaughton 1998). Introduction of non-native ungulates, shifts in native ungulate populations, and extirpation of natural predators are changing the extent and intensity of ungulate herbivory across earth's terrestrial ecosystems (Spear and Chown 2009). These changes create novel herbivory regimes that can alter plant community composition and function (Augustine and McNaughton 1998). Ungulate impacts on vegetation are highly variable across landscapes and are modified by both biotic factors (e.g. plant and animal community characteristics) (Veblen and Young 2010) and abiotic conditions (e.g. topography, climate) (Long et al. 2014). This complexity makes it difficult to characterize ungulate impacts on plant community characteristics at broad spatial scales.

The impacts of ungulates on plant communities vary depending on the abundance, composition and behavior of the ungulate community (Koerner et al. 2014.) Differential impacts of ungulate species are mediated by habitat selection and forage preference. Diet preference for plant species is related to differences in body size, physiology and nutritional requirements (Hanley 1982, Koerner et al. 2014). However, in areas where multiple large ungulate species coexist, habitat use overlaps and competition for forage resources can intensify the detrimental effects of ungulate herbivory on palatable plant species (Augustine and McNaughton 1998). Early successional plant species that form the foundation of plant community development tend

to be more palatable (Coley et al. 1985) and are likely susceptible to the damaging effects of changing ungulate communities.

Across the continental forests of the northern hemisphere, aspen spp. (*Populus tremuloides* and *Populus tremula*) are early successional tree species of high ecological value. However, decline of aspen forests across portions of its North American range has uncovered vulnerabilities to stressors including ungulate herbivory that threaten the long-term sustainability of these forest systems (Frey et al. 2004). Aspen is an important food source for several large mammal species including elk, deer and cattle. A growing abundance of these ungulate species in forest landscapes has increased herbivory pressure across aspen's range (Frey et al. 2004, Seager et al. 2013). With the populations structures of multiple ungulate species overlapping and changing it has been difficult to evaluate the impacts of individual ungulate species on forest regeneration and recruitment.

Ungulates select habitat in order to maximize energy and nutrient acquisition efficiency (Hanley 1982), avoid predation (Gervasi et al. 2013) and optimize thermoregulation in adverse climate conditions (Long et al. 2014). Mixed-aspen conifer forests vary in overstory stand composition from aspen dominance, to a mixed condition and finally to conifer dominance in late successional stages. These differences in forest stand characteristics create diverse habitat conditions that may influence patterns of aspen utilization by ungulates (Rogers and Mittanck 2014). Early successional aspen forests are preferred habitat for multiple species of ungulates (Peterson and Peterson 1992, Beck et al. 2006) which may lead to higher use of regenerating aspen.

Climate and physiographic conditions may also modify the effects of ungulate herbivory (Smith et al. 2011a). Variability in precipitation can strongly alter the behavior of ungulates and modify plant community sensitivity to herbivory (Young et al. 2013). Reductions in snowpack

changes the timing and intensity of ungulate herbivory of regenerating aspen forests (Martin and Maron 2012, Mysterud and Austrheim 2014). There is evidence that aspen is susceptible to higher rates of insect herbivory, and disease in drier conditions (Dudley et al. 2015), but less is known about how weather patterns influence ungulate herbivory of aspen.

Topography alters ungulate herbivory patterns by altering habitat use due to variation in terrain ruggedness and forage selectivity related to differences in plant palatability (Augustine and Derner 2014). Higher light availability increases aspen's resilience and tolerance to mammalian herbivory (Lindroth and St. Clair 2013, Wan et al. 2014). Therefore, light gradients due to terrain slope and aspect may alter aspen's susceptibility to ungulate herbivory. Also, areas of steep slopes, or rocky terrain pose higher foraging costs for ungulates, and may influence foraging behavior (Long *et al*. 2014). Extreme relief or ruggedness often provide refuge from ungulate herbivory for preferred forage species (Banta et al. 2005). Despite the well-studied abiotic influences on selection of palatable plants, there are relatively few studies that have examined both the abiotic and biotic factors underlying patterns of ungulate herbivory.

The majority of research examining ungulate impacts on aspen regeneration (aspen < 150 cm) and recruitment (aspen ≥ 150 cm) has occurred in the initial stage of forest succession following disturbance (Seager et al. 2013). However, even in the mid to late stages of aspen forest succession, aspen continue to regenerate and recruit producing multi-aged stands (Kurzel et al. 2007) that increase resilience to ecological stress (Smith et al. 2011b). Poor recruitment of young aspen stems due to ungulate herbivory is thought to increase vulnerability to drought and competitive exclusion by conifers (Frey et al. 2004, St. Clair et al. 2013, Rogers and Mittanck 2014). We know much less about how ungulate herbivory shapes regeneration and recruitment success in mid to late stages of stand succession than in post-disturbance (i.e. fire) conditions.

This is a critical knowledge gap because the vast majority of aspen forests exist in mid to late stages of stand succession.

The objective of this study was to characterize the influence of ungulate herbivory on aspen regeneration and recruitment patterns of intact forests across gradients of physiographic conditions, climate and forest stand composition at a regional scale. We tested three hypotheses: 1) Aspen regeneration and recruitment decrease with ungulate community abundance and the species impacts of elk, deer and cattle varying due to differences in their physiology and anatomy; 2) aspen regeneration and recruitment will be higher in aspen dominated versus mixed aspen-conifer stands because ungulate preference for foraging in aspen stands; 3) climate and physiographic conditions affect aspen recruitment with greater winter precipitation and southern facing slopes increasing regeneration and recruitment success through enhanced physiological performance

MATERIALS AND METHODS

Study Area

This study included 92-paired sites (184 total stands) located across the Manti-La Sal, Fishlake, and Dixie National Forests of Central and Southern Utah, USA (Fig. 1). Sites were selected that had adjacent aspen dominant and mixed aspen-conifer stands greater than 100 m in diameter (identified by satellite imagery), and at least 50 m from roads. Each paired stand consisted of an aspen dominant stand (> 90% aspen) adjacent to a mixed aspen-conifer stand with approximately 50:50 aspen-conifer that provided a contrast in stand composition. The point-quarter method was used to determine overstory tree species composition, and stand density (Pollard 1971). We defined mature overstory trees as those with greater than or equal to 8 cm in diameter at breast height. Aspen dominant stands averaged 93% ± 2 (SD), ranging from

90% to 100% overstory aspen density, and mixed stands averaged 43% ± 2 (SD) ranging from 15% to 60% overstory aspen density. Average tree density in aspen dominant and mixed aspen-conifer stands were 1250 ± 135 and 1540 ± 188 per hectare respectively.

Study sites had slopes ranging from 4% to 52% and elevations ranging from 2600 m to 3200 m, which represents the middle to upper portion of aspen's elevation range in the mountains of the western US (Peterson and Peterson 1992). The adjacency of the paired stands helped limit pairwise variation in aspect, slope, and precipitation. The study sites were grouped into 8 geographic sub-regions based on mountain ranges where they occur: Manti, Fishlake, Monroe Mt., Pavant, Tushar, Markagunt, Aquarius and La Sal (Fig. 1). We used ecoregions separated by mountain range in order to characterize variation in aspen regeneration and ungulate communities across the study region.

Ungulate Fecal Counts

In July 2012, we established one 50 m x 2 m belt transect in each stand, at least 20 m from the stand edge. We randomly selected the direction of each belt transect and marked both end points with 60 cm grade stakes and GPS waypoints. In each transect, we surveyed for fecal counts of American elk (*Cervus elaphus*), mule deer (*Odocoileus hemionus*), and cattle (*Bos taurus*) (Neff 1968). We used the following criteria for counting current year fecal counts: 1) we excluded any feces that were under leaf litter, as this is an indication that they were deposited before the previous fall. 2) Any feces that presented a whitish color, indicative of advanced stages of decay was excluded. 3) Feces was cleared from the plots after being surveyed. We conducted the fecal surveys in July 2012, and July 2013. A pellet group was considered a count when 3 or more pellets were clustered and from the same defecation event based on size and color. Fecal counts were used as a proxy for ungulate use.

Characterization of Aspen Regeneration

In each 50 m x 2 m belt transect mentioned above we surveyed aspen sucker regeneration and recruitment. We measured height, density and recruitment of regenerating aspen. For our study, we defined an aspen sucker as being from 1 cm to 149 cm in height and emerging independently from the ground (unbranched above ground level) within the transect. Aspen stems were considered to be recruiting when they ranged in height from 150 cm to 200 cm tall. Recruitment was defined in this way because that is the height at which aspen usually begins to escape ungulate herbivory (Lindroth and St. Clair 2013).

Browse impact was characterized by examining the percent of apical meristems removed by herbivory from the leading branch and the sub-leading branches in the top 15 cm of an aspen sucker (Jones et al. 2005, Wan et al. 2014). Aspen suckers with multiple subleaders were quite common, and the entire stem was considered browsed if all meristems were removed. Site specific percent browse is reported as the number of browsed stems within each transect versus stems that have apical meristems intact. Meristems were characterized as intact, browsed, or other (damage from frost, pathogen infection, and drought).

Topography

We characterized topography by associating our GPS site waypoints with remotely sensed data from a 30 m x 30 m digital elevation model (USGS DEM, 2013) of Utah. From this DEM we extracted slope, aspect and elevation and related these to our study sites using ArcMap 10.1. Aspect was defined as either north or south aspect where 0° to < 90° and 270° ≥ to 360° was considered north and 90° ≥ South < 270°. We compared north and south because we were interested in the influence of aspect on insolation and microclimate effects.

We used the standard deviation of slope extracted from the 30 m DEM as an index for terrain ruggedness. We chose this index because of its simplicity, and ability to distinguish ruggedness at local and regional scales and its robustness across changes in resolution (Grohmann et al. 2011). We used model selection (comparing AICc values from univariate models) to identify the most appropriate moving window size (number of neighboring pixels used to calculate standard deviation of slope) for each pellet count. We used a 5 x 5 moving window based on comparison of other sizes using multimodel inference. (Grohmann et al. 2011).

Precipitation

We used PRISM (Daly et al. 2009) to extract temperature and precipitation data at the study sites. We considered two seasons of interest; winter given that snow pack is thought to protect aspen at higher elevation from over winter (Jan, Feb, March) herbivory (Peterson and Peterson 1992) and spring (April, May, June) herbivory where precipitation may favor aspen growth. Thirty-year averages ranged from 514 to 994 mm across our sites, providing a fairly wide gradient of precipitation. Annual precipitation averages across the sites were: 2011 = 429 ± 9 mm, 2012 = 359 ± 10 mm, 2013 = 433 ± 10 mm. Average maximum temperature for the winter period was 2 °C in 2012 and 4 °C in 2013, and for summer was 16 °C in both 2012 and 2013. We used multimodel inference to pick the best precipitation and temperature estimator for our data set, and found prior winter precipitation and temperature to fit best. It is important to note that other precipitation and temperature measurements may influence aspen regeneration metrics, but we do not include them here.

Statistical Analysis

To address our first and third hypotheses, we conducted a global analysis of aspen regeneration response to fecal counts as a proxy for ungulate activity, stand composition, precipitation, temperature and topographical variables. All of these variables were included as fixed effects in the mixed-effects linear models with mean aspen height, aspen density, mean aspen browse percent, and recruitment density designated as response variables. For these models (except for recruitment, explained below), we used paired sites (aspen and mixed) nested within ecoregions as the random effects structure and then used a top-down strategy of selection for fixed effects and all two-way interactions using likelihood ratio (maximum likelihood) tests (Zuur et al. 2009). Three way interactions were not tested given the high parameter size and limited sample size. Paired plots were used to test for multicollinearity. We found winter and spring precipitation to be collinear (>0.7, Pearson's r), so only winter precipitation was used in the analysis. R-squared values for linear mixed-effects models were calculated through the MuMIn package by the methods of Nakagawa and Schielzeth (2013).

Recruitment (aspen ≥ 150 cm) was best modeled using a zero-inflated negative binomial distribution for the error structure. We considered time and region to be fixed effects for the zero-inflated models. We removed the least significant fixed effects by using a step-down approach with likelihood ratio tests to find an optimal model. We present Spearman's Rank correlation (rho) of observed versus predicted values for our top model (Potts and Elith 2006).

To test our first and second hypotheses, we pooled ungulate fecal counts into bins and modeled their mean effect on the aspen regeneration across all regions and years. Jenks natural breaks were used to find ten appropriate bins of fecal counts for elk, mule deer, and cattle (Jenks 1967). We calculated the average and standard errors of height, recruitment, density and browse percent within those bins. We then conducted a weighted linear regression for each regeneration

metric and recruitment across those bins. We then summed all ungulate fecal counts into bins and repeated the weighted linear regression to account for the entire ungulate community. All analyses were conducted using the program R (R Core Team 2013), and packages nlme for mixed effects models, MuMIn for comparing multiple models through maximum likelihood, pscl for the zero-inflated model. [Zeileis *et al*. 2008, Barton 2015, Pinheiro *et al*. 2015].

RESULTS

Global Model Summary

The optimal aspen height model included mule deer and cattle fecal counts, stand type and sampling year as fixed effects ($r^2 = 0.14$) (Table 1). Suckering density was best accounted for by elevation, with no other fixed effect included in the optimal model ($r^2 = 0.11$, $p < 0.01$, Table 1). The optimal model for aspen browse percent included elk fecal counts, stand type, and sampling year ($r^2 = 0.08$, $p < 0.01$, Table 1). Antecedent winter precipitation, summed ungulate fecal counts, and aspect provided the best explanation of recruitment of aspen suckers (rho = 0.13, $p = 0.02$) (Table 1). There were no significant two-way interactions between explanatory variables for any of the models.

Hypothesis 1: Ungulate Effects on Aspen Regeneration and Recruitment

Across all study sites 52% of aspen suckers exhibited evidence of being browsed by ungulates. Cattle and mule deer reduced aspen height by 1.1 cm and 0.6 cm per fecal count 100 m^{-2}, respectively (Table 1). Browse impact of aspen increased 0.4% per elk pellet group 100 m^{-2} (Table 1). There was a reduction of 4 aspen recruits ha^{-1} per pellet group of any ungulate species (Table 1).

In our binned regression analysis elk, deer and cattle fecal counts showed strong relationships, with measures of aspen regeneration and recruitment (Fig. 2). For each elk pellet group per 100 m^{-2} there was a 1.2 cm drop in aspen height ($r^2 = 0.83$, $p < 0.01$), 4.7 fewer aspen recruits ha^{-1} ($r^2 = 0.51$, $p = 0.01$), 33 fewer aspen suckers ha^{-1} ($r^2 = 0.35$, $p < 0.01$), and a 0.53% increase in browse impact ($r^2 = 0.60$, $p < 0.01$). For each deer pellet group per 100 m^{-2}, aspen height was reduced by 0.93 cm ($r^2 = 0.92$, $p < 0.01$) and 4.8 fewer aspen recruited ha^{-1} ($r^2 = 0.59$, $p < 0.01$). Each cattle fecal count per 100 m^{-2} was associated with a 0.85 reduction in aspen height ($r^2 = 0.35$, $p = 0.05$), and 103 fewer aspen suckers ha^{-1} ($r^2 = 0.71$, $p < 0.01$).

When fecal counts of all elk, deer and cattle were summed, the average aspen height was reduced by 0.75 cm per fecal count, and average recruitment was reduced by 3 recruits ha^{-1} (Fig. 3). Based on natural breaking points observed in the data, we categorized the number of sites into low, medium, or high use areas, based on reductions in both height and recruitment as a function of ungulate fecal counts (Fig. 3a and b). The analysis indicates that 60% of the 184 study sites were low use areas (≤ 8 fecal counts per 100 m^2), 32% were medium use ($8 < x \leq 26$ fecal counts 100 m^{-2}), and 8% of the sites were high use (> 26 up to 71 fecal counts 100 m^{-2}) (Fig. 3d).

Hypothesis 2: Stand Composition Effects

Aspen suckers in mixed stands experienced 5% greater browse impact than those in aspen stands (Table 1). Aspen suckers in mixed stands were 5.2 cm shorter than aspen suckers in aspen stands (Table1). Stand composition was not an important fixed effect for Aspen sucker density and recruitment did not vary significantly due to overstory stand composition (Table 1).

Hypothesis 3: Effects of Climate and Topography

Southern aspects had 3-fold greater aspen recruitment than northern aspects (Fig. 4a). Antecedent winter precipitation was positively correlated with aspen recruitment (Fig. 4b). Elevation was positively correlated with aspen sucker density such that for each 100 m increase in elevation there was a 405 ± 120 increase in aspen sucker density ha^{-1} ($\chi^2 = 16$, $p < 0.01$) (Table 1). Browse impact was not correlated with topography or climate. Aspen regeneration and recruitment were not significantly related to terrain ruggedness or slope in any of the models.

DISCUSSION

Overview

This study examined the effects of multiple ungulate species on aspen regeneration and recruitment across gradients of forest composition, climate, and topography in forests of central and southern Utah. We found that ungulates have density dependent impacts on aspen regeneration and recruitment. Large variation in aspen regeneration across sites (Table 1) was likely influenced by genetic factors and physiographic conditions (Smith et al. 2011a, Smith et al. 2011b). By statistically binning variation across sites we were able to more clearly identify ungulate effects on aspen regeneration success (Figs 2, 3). Our first hypothesis that regeneration and recruitment decreases with ungulate abundance and that the impacts of elk, deer and cattle differ was partially supported. We found that aspen regeneration and recruitment was inversely correlated with ungulate abundance but there was less evidence that the impacts of elk, deer and cattle differed. In other words, increasing ungulate abundance of any species resulted in fairly similar linear reductions in aspen regeneration and recruitment vigor (Fig. 2). Studies that have considered differential effects of elk, deer and cattle species have also observed significant

impacts by each species (Bork et al. 2013, Walker et al. 2015). Our second hypothesis that aspen regeneration success would be similar between aspen dominant and mixed stands was unsupported given that browse impact was higher and aspen were shorter in mixed stands (Table 1). Our third hypothesis was supported in which elevation, aspect, and winter precipitation each contributed to some of the variation in aspen regeneration and recruitment success (Table 1). Finally, there was strong variation in aspen regeneration and recruitment success across the geographic range of our study.

Ungulate Effects on Aspen Regeneration and Recruitment

The results demonstrate that greater fecal counts of ungulates were negatively correlated with aspen regeneration and recruitment (Fig 2). The data clearly show that high abundance of ungulates can drive complete aspen recruitment failure (Fig. 3b). The data indicate that 8% of the sites had fecal densities at levels that caused aspen regeneration and recruitment failure with 32% of sites showing intermediate impacts where recruitment potential is delayed or at some level of risk (Fig 3d). These areas of high ungulate impact showed impacts that are consistent with other studies where intense ungulate herbivory caused aspen regeneration and recruitment failure in intact stands (Kaye et al. 2005). The majority of our study sites (60%) had aspen that were regenerating well and showed good evidence of recruitment into the overstory (Fig 3d). These sites had lower ungulate activity, as measured by ungulate fecal counts. Our study shows a gradient of impact on aspen regeneration and recruitment that varies strongly according to the density dependence of the ungulate community (Fig. 3). Our regression models show clear linear relationships between binned fecal counts and aspen regeneration responses. However, given that our sampling was not truly random, these proportions may not be representative of the entire

statistical population. These models should allow for a more targeted management approach for controlling the damaging effects of ungulate on aspen forests.

Across North America, there have been many examples where one ungulate species in particular was thought to be driving aspen regeneration failure. For example, aspen loss has been attributed to deer on the Kaibab plateau (Leopold et al. 1947), elk in Yellowstone (Ripple and Beschta 2007), and cattle in Alberta (Kaufmann et al. 2014). Our results suggest that any one of these species independently, or a mixed community of ungulates, when at sufficient abundance can drive aspen regeneration failure (Fig. 3c). Our results indicate that the mean herbivory impacts of elk, deer and cattle on aspen are fairly similar on a per fecal count basis (Fig. 2) suggesting there is little need to separate out species influences to accurately assess the potential impacts of the whole ungulate community. Therefore, characterizing impact of the ungulate community using fecal surveys is both a simple and accurate way of assessing vulnerabilities of aspen forest to ungulate herbivory (Fig. 3b) (Wan et al. 2014).

The Role of Stand Composition in Ungulate Impacts on Aspen

The species composition of forest stands can alter aspen regeneration success (Bartos 2001, St. Clair et al. 2013) and influence ungulate impacts on forest understories (Mason et al. 2010). We found aspen suckers in mixed stands to be browsed 5% more and were 5.2 cm shorter on average than suckers in aspen stands (Table 2). Higher browse of aspen suckers in mixed aspen-conifer stands may be due to reductions in growth rates and defense of aspen due to lower light conditions (Wan et al. 2014). Also, late successional aspen forest communities tend to support less palatable species (Peterson and Peterson 1992), which may increase preference for aspen (Villalba et al. 2014). Also, vegetation type and structure influence ungulate habitat use due to perception of predation risk (Gervasi et al. 2013). While ungulate effects on browse use

and height in mixed stands was only slightly larger, these impacts may be compounded if aspen in mixed stands are continuously utilized year over year. This is consistent with other studies showing that long-term ungulate impacts on aspen regeneration and recruitment grow as conifer abundance increases in forest stands (Kaye et al. 2005, Rogers and Mittanck 2014). High overstory conifer abundance also decrease the vigor of aspen regeneration after disturbance (Smith et al. 2011a), which contributes to long-term loss of aspen stands (Worrall et al. 2013). Our results, suggest that ungulates reduce aspen recruitment potential in both aspen dominant and mixed stands. However, effects are slightly stronger in mixed stands, which may accelerate succession to conifers.

The Role of Topography and Climate

Topography plays a role in the success of aspen regeneration patterns (Smith et al. 2011a) due to its influences on moisture, temperature and animal behavior (Long et al. 2014). In our analysis, higher elevation and southern aspect tended to have positive effects on aspen regeneration density and recruitment, respectively, but had no effect on height or browse percent (Table 1). Southern aspect provides higher insolation in the northern hemisphere, which increases aspen vertical growth rates and stimulates defense chemistry expression against herbivory (Wan et al. 2014). Further, warmer soil temperature on southern aspect can trigger aspen suckering (Peterson and Peterson 1992) and faster growth rates when water is non-limiting. Therefore, increased aspen regeneration and recruitment on southern exposures may be partially driven by higher resistance and tolerance to browse pressure by ungulates (Lindroth and St. Clair 2013).

Elevation contributed to increases in aspen regeneration density in our models (Table 1). Rogers and Mittanck (2014) also found higher regeneration density with increasing elevation.

This positive relationship tracks well with suitable elevation habitat range for aspen. Predictions for aspen habitat suitability suggest aspen at lower elevation are susceptible to changing climate conditions (Worrall et al. 2013). For example, aspen at lower elevation are susceptible to climate related stress including drought (Worrall et al. 2013) and temperature extremes (Martin and Maron 2012, Worrall et al. 2013). Also, deeper snowpack found at higher elevations can reduce overwinter browsing, and improves water relations throughout the growing season (Martin and Maron 2012, Mysterud and Austrheim 2014). Drier conditions characteristic of lower elevations in our study area can also increase susceptibility to disease, and insect herbivory (Dudely et al. 2015), and appears to be true for mammalian herbivory.

Terrain ruggedness can influence aspen regeneration (Rogers and Mittanck 2014), but, our analysis suggests that it was less important than other ecological factors. Refuge for plant communities from ungulates due to terrain ruggedness likely happens at a limited spatial scale, whether on a steep slope (Zegler et al. 2012), or in refuge islands provided by boulders (Banta et al. 2005). Since our analysis was conducted at broad spatial scales and regeneration and recruitment did not vary with terrain ruggedness in our models it supports the idea that the effects of terrain ruggedness on ungulate herbivory occur at smaller spatial scales (Zegler et al. 2012).

Precipitation can have an important role in explaining aspen regeneration success (Dudely et al. 2015), and mediates ungulate impacts on aspen (Martin and Maron 2012). We observed that antecedent winter precipitation increased aspen recruitment success (Table 1, Fig. 4). Winter precipitation likely affects aspen recruitment in two ways. First, drier conditions increase aspen susceptibility to hydraulic failure, disease, and insect herbivory (Worrall et al. 2013, Dudely et al. 2015). Second, greater snowpack reduces ungulate herbivory by decreasing winter browsing (Martin and Maron 2012) and by improving forage quality of other understory

plant species the following summer (Mysterud and Austrheim 2014). Differences in palatability between grasses, forbs, and aspen played an important role in selection for aspen in cafeteria trials (Villalba et al. 2014), suggesting the greater availability of other forage sources due to more favorable weather patterns can decrease ungulate preference for aspen.

Ecological Implications

Shifts in ungulate community abundance and composition across earth's terrestrial ecosystems are altering plant communities. In some aspen forests, there is evidence that ungulate use is decreasing aspen recruitment (Kaye et al. 2005, Rogers and Mittanck 2014) which reduces resilience to environmental stressors of younger cohorts due to low phenotypic diversity (Smith et al. 2011b). Our study suggests that areas of high ungulate use are failing to recruit (Fig 3), and that is driven primarily by ungulate abundance.

Changing climate conditions pose new challenges for managing ungulate herbivory especially for more palatable, early successional species, like aspen (Worrall et al. 2013). Our data show that antecedent winter precipitation has an important impact on the recruitment of aspen (Fig. 4). In areas where winter precipitation regimes are changing, aspen recruitment and resilience may be at risk. Our study provides further evidence that climate will affect the range and distribution of aspen (Worrall et al. 2013, Dudley et al. 2015), through reduction in aspen recruitment. Incorporating an understanding of the influence of physiographic, climate and habitat conditions into management of ungulates can improve plant community resilience to herbivory. Because environmental conditions that interact with ungulate herbivory are shifting due to climate change (Worrall et al. 2013), it is critical that we understand these relationships with greater precision.

While much research has looked at ungulate impact on aspen regeneration after fire or timber harvest (Seager et al. 2013, Kaufmann et al. 2014), few studies have examined how ungulate herbivory affects recruitment in mature aspen stands. We show that aspen regenerating in both aspen dominant and seral aspen-conifer mixed stands are under herbivory pressure (Table 1). High rates of ungulate herbivory may increase rates of succession through selective herbivory of palatable early successional species (Randall and Walters 2011) and reduce stand resilience to disturbance by reducing densities of young cohorts (Smith et al. 2011a). While the effects of heavy ungulate use on post-disturbance sites are generally dramatic and rapid, the majority of aspen forests exist in an intact state, and the ungulate effects on regeneration and recruitment in these stands is less obvious. However, our data demonstrate when ungulate impacts regeneration in aspen dominant and mixed stands they may have important implications for stand resilience to drought and other environmental stressors (Smith et al. 2011b).

Conclusion and Management Implications

Our study explored ungulate use across large environmental and spatial gradients, and found that ungulate herbivory negatively influences aspen regeneration and recruitment. Management of aspen regeneration should include multivariate monitoring that includes both biotic and abiotic factors, and consider temporal and spatial variability in ungulate use on a site by site basis. Care should be taken to avoid broad extrapolation from studies with small ranges of time and space in their experimental design.

Based on our pellet surveys the majority of the region evaluated in our study (60%) experienced low ungulate use with good aspen regeneration. Intermediate ungulate use was observed in 32% of the sites surveyed, which could warrant further monitoring. Only 8% of the study areas experienced severe regeneration and recruitment problems due to high ungulate use

and should be the focus of targeted management (Table 3). Based on our data it appears that fecal counts can be used as a good proxy of ungulate use to identify areas that may be prone to aspen recruitment failure. Fencing, targeted hunts and other techniques (Seager et al. 2013) can be used effectively in high ungulate use stands to stimulate recruitment and the development of multi-cohort aspen stands. Management of ungulates in aspen forests should be considered on a site by site basis and should primarily be evaluated based on the abundance of the total ungulate community.

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FIGURES

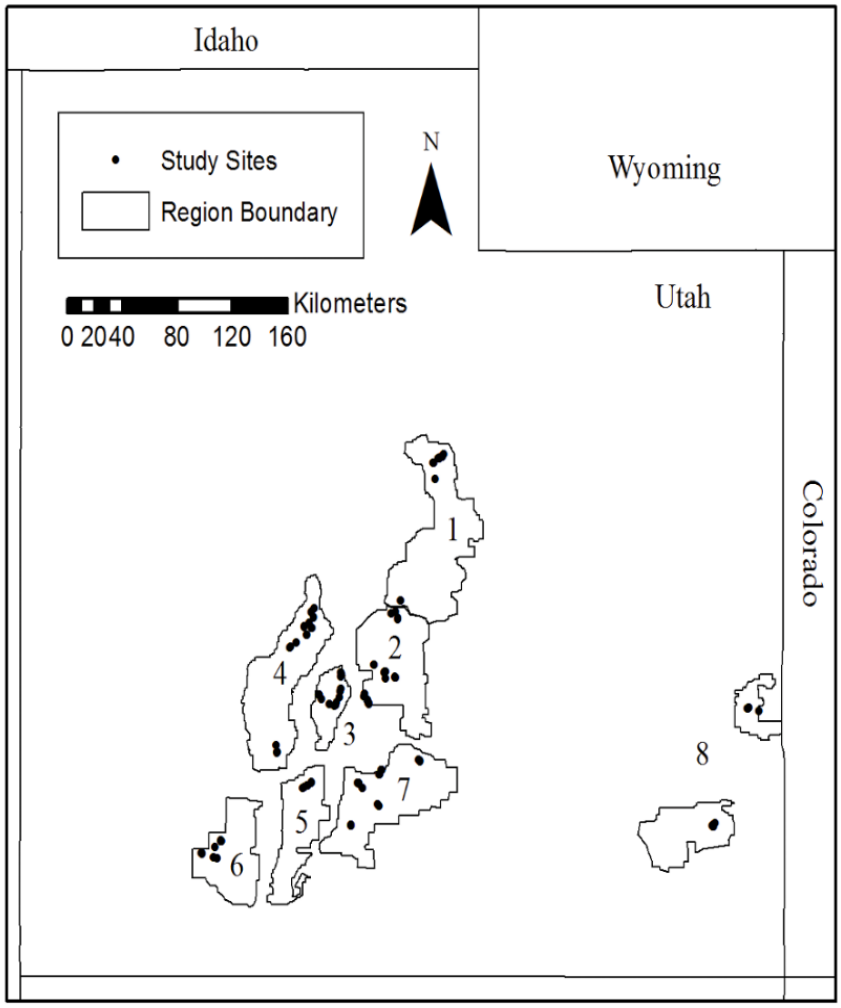


Figure 1–1. Map of the extent of the study sites across central and southern Utah. Paired study sites in adjacent mixed and aspen dominant stands are marked with a single dot. The 8 mountain ranges are marked with numbers: 1) Manti, 2) Fishlake, 3) Monroe Mt., 4) Pavant, 5) Tushars, 6) Markagunt, 7) Aquarius, 8) La Sal.

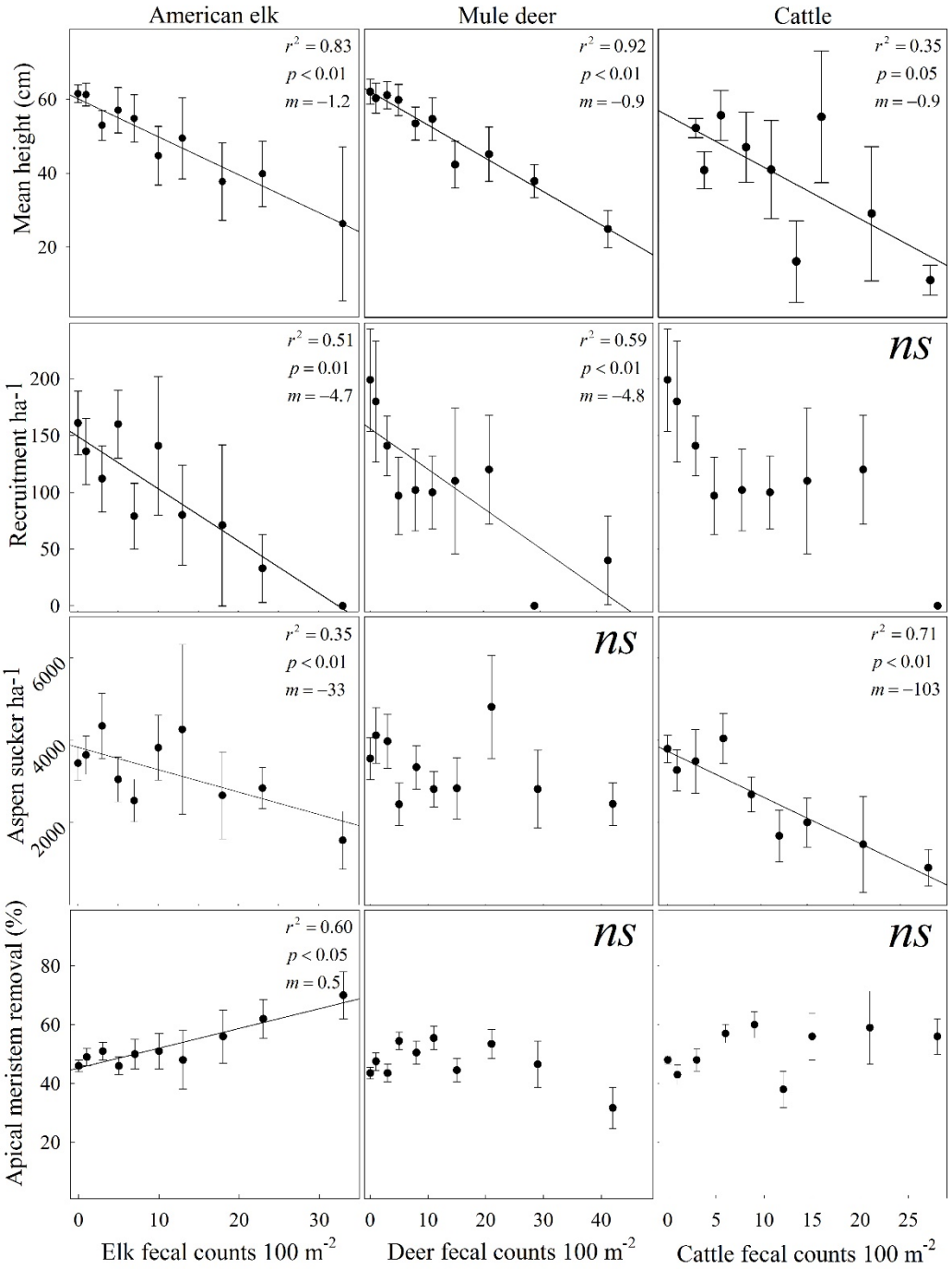


Figure 1–2. Binned means at the Jenk's natural breaks are presented with their associated error bars (± standard error). Weighted linear models that were statistically significant have their r^2, p , and slope (m) values. Non-significant models are denoted with "ns". Height is reported in cm, recruitment is per hectare, aspen suckering is per hectare, and browse percent are presented for American elk, mule deer, and cattle fecal counts.

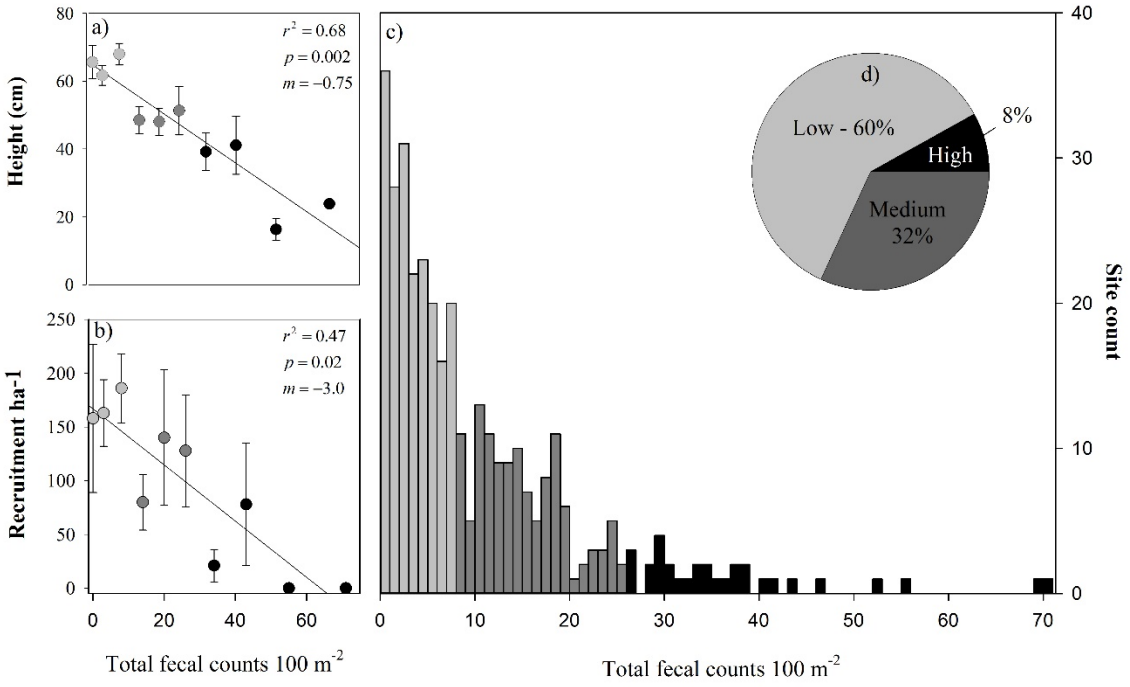


Figure 1–3. Regression analysis of a) aspen height and b) aspen recrutiment as a function of binned fecal counts summed for elk, deer and cattle. Error bars represent the standard error around the mean of that bin. c) A frequency histogram indicates the number of sites that have fecal counts ranging from 0 to 71. d) A pie chart of the proportion of sites in low, medium, and high categories of ungulate use. In all four graphics gray scale shading is matched and represents low (light gray), medium (dark gray) and high (black) reductions in aspen height and related to increasing fecal count densities. The three categories were defined based on visual breaks in the data.

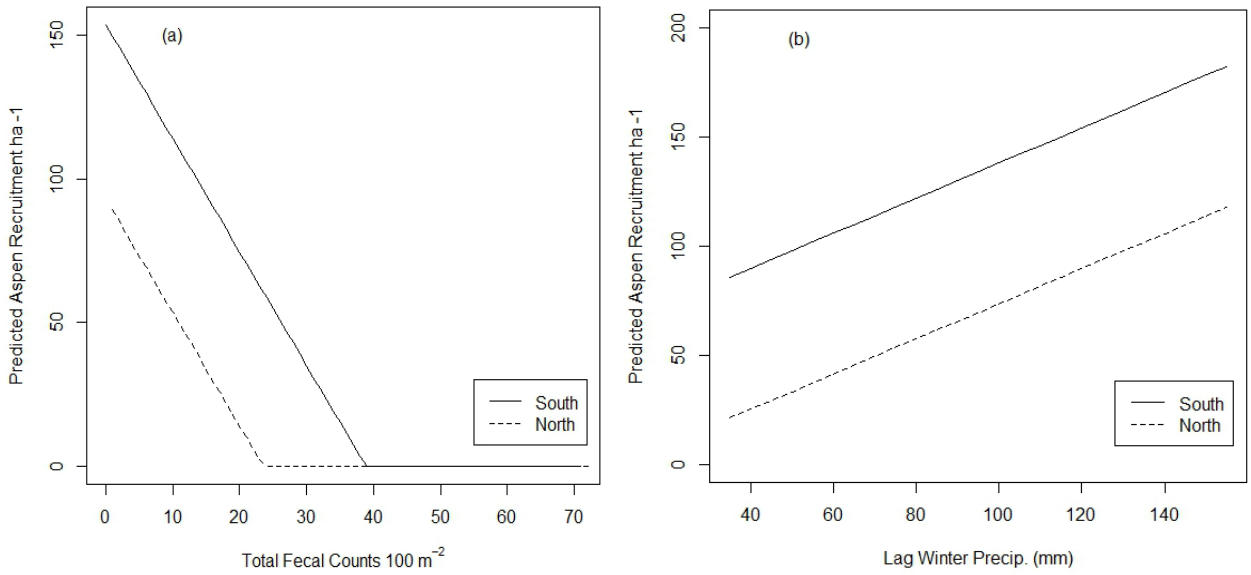


Figure 1–4. Using the zero inflation count model intercept and beta coefficients, this graph presents in panel a) the predicted mean number of aspen recruitment (150 cm ≥ x ≥ 200 cm) per hectare across the observed total animal fecal counts (Cattle + Deer + Elk) and in panel b) the observed lag winter precipitation (mm). The solid line represents the predicted values at southern aspect and the dashed line represents the predicted mean at northern aspect. Lag winter precipitation was held at the mean 90 mm. Animal fecal counts (Cattle + Deer + Elk), were held at the median = 6.

TABLE

Table 1–1. Fixed effects for each of 4 models for aspen regeneration are presented. Height, density, browse %, and recruitment are in bold with the full model r^2 or rho presented on the same line. Under each model title are the fixed effects that were in the optimal model with their corresponding beta estimates ± standard error, χ^2 and Z statistics for mixed effects models and zero-inflated models respectively, and their corresponding *p* – value.

Fixed Effects	**Beta Estimate ± SE**	**Statistic**	***p* - value**	**Full model**
Height		**χ2**		**r2 = 0.14**
Cattle	-1.1 ± 0.30 cm	12	0.005	
Deer	-0.6 ± 0.19 cm	37	<0.0001	
Mixed stand	-5.2 ± 2.0 cm	6	0.02	
Year (2013)	-12.7 ± 2.1 cm	32	< 0.0001	
Density		**χ2**		**r2 = 0.11**
Elevation	405 ± 120 ha-1	16	< 0.0001	
Browse %		**χ2**		**r2 = 0.08**
Elk	0.4% ± 0.2%	3.4	0.04	
Mixed Stand	5% ± 1.4%	9	0.002	
Year (2013)	-6% ± 1.4%	3	< 0.0001	
Recruitment		**Z**		**Rho = 0.13**
Count model μi				
Cattle+Deer+Elk	-4 ± 1 ha-1	-3.9	< 0.001	
Lag Winter Precip.	0.8 ± 0.35 ha-1	2.3	0.02	
South Aspect	65 ± 21 ha-1	3.1	0.002	
Zero Inflation Model πi				
Elevation	-55 ± 2	2.7	0.005	
Lag Winter Precip.	2 ± 0.7	2.8	0.005	
Year (2013)	87 ± 37	2.3	0.02	

CHAPTER 2

Measures of Browse Damage and Indexes of Ungulate Abundance to Quantify Their Impacts on Aspen Forest Regeneration

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ABSTRACT

Ungulate communities are changing at a global scale increasing the need for landscape scale quantification of ungulate impacts on plant communities. To achieve robust monitoring across variable landscapes, managers need quick and reliable indicators for assessing ungulate impacts. Our goal was to evaluate two direct (meristem removal, defoliation) and two indirect methods (fecal counts, camera counts) for evaluating ungulate activity on forest regeneration after fire. We examined the relationships between measures of browse activity (meristem removal, defoliation) of aspen with ungulate community estimates (fecal and camera counts) as a function of variation in physiographic conditions across the study sites. Measurement were made in belt transects at 28 sites across three fires that burned in National Forests in Utah in 2012. Aspen height was best predicted by a model with meristem removal, terrain ruggedness, aspect, and year. Mean aspen height was 5 cm lower for every 10% increase in meristem removal an effect that increased over time. We found moderate correlation between the year and percent defoliation interaction and aspen density. Percent defoliation reduced aspen density in the 2nd and 3rd years but not in the 1st year. Results demonstrated that meristem removal is correlated closely with ocular estimates of defoliation ($r^2 = 0.83$), and quite well with ungulate abundance using fecal counts ($r^2 = 0.65$) and camera counts ($r^2 = 0.63$). Fecal counts and camera counts also

correlated well with each other ($r^2 = 0.74$). Estimates of meristem removal in our study suggest that 75% browse of apical meristems is an important threshold for successful aspen regeneration. Our study highlights the strengths of measurements of meristem removal to predict ungulate effects on forest recruitment potential.

INTRODUCTION

Ungulate herbivory can have strong top-down effects on the abundance and composition of plant communities (Augustine and McNaughton 1998, Carpio et al. 2015, Hobbs 1996, Waller and Alverson 1997). Ungulate communities and populations are changing drastically due to management of ungulate populations and introduction of non-native ungulates (Spear et al. 2009, Waller and Alverson 1997). There is strong evidence that growing ungulate populations limits the regeneration of palatable tree species and reduces plant diversity (Augustine and Frelich 1998, Edenius et al. 2011, Kaufmann et al. 2014, Rooney and Waller 2003). As a result, there is increased interest in understanding how changing ungulate herbivory regimes are affecting forest recruitment at landscape scales (Frerker et al. 2013). However, accurate characterization of ungulate impacts on vegetation at large spatial scales is technically challenging due to the large degree of variation and complexity across landscapes (Frerker et al. 2013, Waller et al. 2009). Therefore, development and comparison of indexes characterizing ungulate impact on ecologically important species is increasingly important under changing herbivory regimes.

Ungulate impacts on ecologically important pioneer plant species are well studied, yet often show conflicting patterns (Bartos et al. 1994, Barnett and Stohlgren 2001, Kaufmann et al. 2010, Randall and Walters 2011, Ripple et al. 2001). Differences in the methodologies used could contribute to the inconsistency in observed results (Waller et al. 2009). Studies that

quantify the impact of ungulates on plant communities generally use two methodological approaches. The first method estimates ungulate population size to infer relative effect on plants across different study areas (Rooney and Waller 2003, Waller et al. 2009). We refer to this first approach as "indirect" because it assumes that the number of ungulates is proportional to their impact on one or more plant species (Morellet et al. 2001). The second approach is to evaluate the ungulate herbivore damage on the focal plant by some measure of damage to plant tissue. We refer to this approach as "direct" given that they use the focal plant species directly in order to evaluate ungulate impact. Selecting optimal methods for estimating ungulate impacts on plant communities is crucial for effective management, yet few studies have explicitly compared the efficacy of multiple methods (Waller et al. 2009).

Indirect methods have been the basis of evaluating ungulate impacts for decades and generally estimate relative abundance through fecal counts, camera counts, or management derived population estimates (Barnett and Stohlgren 2001, Randall and Walters 2011, Rooney and Waller 2003, Seager et al. 2013). Indirect methods have proven useful when compared to other estimates of ungulate habitat use (Loft and Kie 1988), and can inform management decisions (Barnett and Stohlgren 2001, Wan et al. 2014). Fecal counts have been successfully used since the 1940's to estimate relative abundance of ungulates (Bennett et al. 1940) and are often combined with assumptions about defecation rates to extrapolate population size (Neff 1968, Royo et al. 2016). In recent years, camera-traps have been used for estimating animal abundance (Clare et al. 2015, Long et al. 2007), and ungulate behavior and forage patterns in plant communities (Faison et al. 2016a). Use of management derived population estimates have been used to estimate ungulate impact on plants (Seager et al. 2013). However, due to the wide variety of methods (Loft and Kie 1988) and the known inaccuracies in estimating population

sizes (Krebs 1999, Waller et al. 2009) these methods may prove less useful at landscape scales (Frerker et al. 2013).

Indirect methods of quantifying ungulate impacts on vegetation have the advantage of identifying consumers when multiple herbivores are present (Rhodes et al. 2017) but they generally have low model fit and predictability (Rhodes et al. 2017a, Rooney and Waller 2003, Stewart et al. 2009). This is likely due to confounding effects such as: changing forage availability, topography and climate across sites (Frerker et al. 2013, Rhodes et al. 2017a). Linking estimates of ungulate abundance directly to their impact on the plant community is challenging due to the high mobility of ungulates and inaccurate methods of estimating mobile animal populations (Krebs 1998, Waller et al. 2009). For these reasons, there is increasing interest in using direct methods to assess ungulate impacts on focal plant species (Frerker et al. 2013).

Quantifying direct ungulate impacts on plants by measuring browse damage, accurately measures the impact but identifying the ungulate species responsible can be challenging (Frelich and Lorimer 1985, Rooney and Waller 2003, Waller et al. 2009). Direct measurements of ungulate impact on plants include removal of meristems (McInnes et al. 1992, Wan et al. 2014), removal of leaf tissue or indices that use a single plant species as indicators of ungulate impact (Balgooyen and Waller 1995, Frelich and Lorimer 1985, Mason et al. 2010, Rooney and Waller 2003). Ocular estimations of removal of leaf tissue could be analogous to percent cover. Ocular cover estimates, while often considered highly subjective, can be similar to and more efficient than more complex methodologies (Vales and Bunnell 1988). Direct methods sometimes allow differentiation between vastly different herbivores (hares vs. moose, McInnes et al. 1992), but distinguishing between ungulates can be difficult (Faison et al. 2016b).

Aspen (*Populus tremuloides* Michx.) forests are model systems for understanding plant-herbivore interactions because they provide habitat and forage for many herbivore species and their defense chemistry is well characterized (Lindroth and St. Clair 2013). There is concern that heavy browsing of aspen by ungulate in some areas may contribute to aspen decline (Lindroth and St. Clair 2013, Seager et al. 2013). In the Intermountain West, fire is a major driver of aspen regeneration and retention across the landscape (Romme et al. 2005, Smith et al. 2011a). Aspen is particularly susceptible to ungulate herbivory as root suckering occurs after fire (Seager et al. 2013). However, there is considerable controversy over magnitude of the impact of ungulates on aspen recruitment (Kaufmann et al. 2010, Rhodes et al. 2017a, Ripple et al. 2001, Romme et al. 2005). Aspen's vertical escape through height growth is an important defense strategy against ungulate herbivory and sufficient density is needed for stand replacement after fire (Bartos et al. 1994, Rhodes et al. 2017b, Seager et al. 2013). Monitoring of aspen regeneration has shown acute failure of aspen regeneration after fire in some areas (Bartos et al. 1994, Ripple et al. 2001) and stable regeneration despite high ungulate densities (Barnett and Stohlgren 2001, Romme et al. 2005) in other locations. Aspen regeneration success can vary depending on climate, topography and stand composition (Rhodes et al. 2017a, Smith et al. 2011a), which can confound our understanding of the direct effect that ungulates have on aspen regeneration success. Further, quantifying ungulate impacts on plants is inherently complex given the diversity and complexity of ungulate behavior and diet (Beck and Peek 2005, Long et al. 2014).

Exploring the advantages and disadvantages of various methods for quantifying ungulate impacts on aspen regeneration and recruitment is vital to managing for resilience and sustainability of forest ecosystems. Our central research question is: which measures of ungulate impact are best for predicting aspen establishment and recruitment in post-fire environments across large landscapes? The objective of this study was to compare the efficacy of common

methods for estimating ungulate impact on aspen. We measured the height and density of aspen regeneration three years post-fire. We then compared the relative performance of four methodologies (two indirect and two direct) for estimating ungulate impact aspen regeneration. Secondly, we use physiographic features to account for variation in the impact of ungulate species on aspen regeneration due to topography. We predicted that aspect, terrain ruggedness, and elevation would influence aspen vertical growth and density. We hypothesized that aspen height would be better estimated by direct methods given that loss of plant tissue can translate into reduction of height. Finally, we predicted that aspen density would be better estimated by indirect methods of estimating ungulate impact because removal or death of aspen through herbivory is likely related to total ungulate number.

MATERIALS AND METHODS

Study Area

This study was conducted in post-fire environments in mixed aspen-conifer forests across three national forests of central and southern Utah. Our study consisted of 28 sites across three fires that burned in summer 2012 that demonstrated aspen suckering in the fall of 2012 (Fig. 1). Fire sizes ranged from 900 to 19400 hectares. Site elevations ranged from 2500 – 3030 m and slope ranged from 1 - 28 degrees. Thirty year mean annual precipitation ranged from 380 – 800 mm. Dominant tree species in the Seeley and Box Creek fires include; Aspen, Subalpine Fir (*Abies lasiocarpa* (Hooker) Nuttall) and White Fir (*Abies concolo*r (Gordon) Lindley ex Hildebrand). While the dominant conifer component of Harris Flat fire is Ponderosa Pine (*Pinus ponderosa* Douglas ex C.Lawson). Cattle (*Bos taurus*), Mule deer (*Odocoileus hemionus*), and American elk (*Cervus canandensis*) were the most abundant ungulates herbivores at the study sites.

Sampling Design

Eight sites were established in 2012 and the remaining 20 were established in May 2013. The first samples were taken in September 2013. In total, there were 15 sites in the Seeley fire, 8 sites at the Box Creek fire, and 5 sites at the Harris Flat fire. We randomly selected the direction of each belt transect and marked both end points with u-posts and GPS waypoints. In September of 2013, 2014, and 2015 we surveyed aspen sucker regeneration responses and herbivory patterns along these transect lines. We defined an aspen sucker as emerging independently from the ground (unbranched above ground level) within transects. Aspen height and density

Direct Methods

Meristem removal was characterized by examining the percent of apical meristems removed by herbivory from the leading branch and the sub-leading branches in the top 15 cm of an aspen sucker (Jones et al. 2005, Rhodes et al. 2017a, Wan et al. 2014). Aspen suckers with multiple sub-leaders were quite common, and the entire stem was considered browsed if all meristems were removed. Aspen were considered suckers and sampled if they were 1 to 200 cm in height, within the reach of ungulates (Rhodes et al. 2017a). Site-specific percent browse is reported as the number of browsed stems within each transect versus stems that have apical meristems intact. Meristems were characterized as intact, browsed, or other (damage from frost, pathogen infection, and drought).

We treated ocular estimates of defoliation as analogous to ocular estimates of cover. We recorded percent defoliation as observed foliar tissue divided by estimates of potential leaf cover in the absence of herbivory. Ocular estimates of defoliation of aspen were taken from aspen within the 50 m x 2 m belt transects by walking the line and observing leaf cover removal of aspen within the transect. Estimates were given for the total transect and rounded to nearest 5%

in order to increase speed of observations and reduce potential site-to-site, or year-over-year bias. Observations were made by a single observer for the duration of the study to eliminate observer bias. While ocular estimates of cover have the disadvantage of being subjective, training in ocular techniques can standardize estimates (Vales and Bunnell 1988). Ocular estimates of cover can lead to similar results as more time consuming estimates that use complex grids or assessment of multiple subplots (Vales and Bunnell 1988).

Indirect methods

Fecal Counts

In each transect, we surveyed fecal counts of American elk (*Cervus canandensis*), mule deer (*Odocoileus hemionus*), and cattle (*Bos taurus*) (Neff 1968). We used the following criteria for counting first year fecal counts: 1) we excluded any feces that were under leaf litter, as this is an indication that they were deposited before the previous fall. 2) Any feces that presented a whitish color, indicative of advanced stages of decay was excluded. 3) Feces were cleared from the plots after being surveyed. A pellet group was considered a count when 3 or more pellets were clustered and from the same defecation event based on size and color (Neff 1968). Mule deer, American elk and cattle fecal matter were easily distinguishable by their size and shape (Rost and Bailey 1979). Average fecal counts in transects were 16.4 ± 2.1 and ranged from 0 to 57.

Camera Counts

At the end of each transect we placed a trail camera (PC900 HyperFire Professional Covert Camera Trap) (Reconyx, Holmen, WI, USA) to estimate an index of relative use by ungulate species (O'Brien 2011). We used ungulate counts per camera per day (ungulate camera^{-}

^{1} day^{-1}) as an estimate of ungulate presence at each site. Cameras were at one end of each transect facing down the transect line. Cameras were set at medium sensitivity to capture five photos at three-second intervals with a five-minute rest period in between triggers. Each ungulate that appeared at least once in the five-photo set were counted and these counts were summed and divided by the number of active camera days for each plot. Average ungulates per camera day were 1.03 ± 0.17 and ranged from 0 to 5.7.

Topographical features

We considered elevation, aspect, and terrain ruggedness to be important topographic features influencing aspen height and density (Rhodes et al. 2017a). We extracted elevation from a 30 m x 30 m digital elevation model (DEM) of Utah and associated it with the GPS coordinates of our sites. We extracted aspect from the DEM, converted the angles in to radians and took the cosine in order to get a "northness" value, where 1 = North, and -1 = South. We used the standard deviation of slope in a 3 x 3 neighborhood of cells surrounding the pixel of our site to calculate terrain ruggedness (Grohmann et al. 2011). We chose this index because of its simplicity, and ability to distinguish ruggedness at local and regional scales and its robustness across changes in resolution (Grohmann et al. 2011). All extractions of topographical features were completed in ArcMap (ESRI 2016).

Statistical Analysis

We used model selection to determine relative model support for aspen height and density (analyzed separately) using linear regression and variance weighted across years due to heterogeneity of variance. We judged relative model support using Akaike's Information Criterion (AICc) (Akaike 1973, Burnham and Anderson 2002). First, we compared relative

support for preliminary models that included all combinations of topographical features elevation, northness, and terrain ruggedness and with year and fire site against intercept only models for aspen height and aspen density, separately (Tables 1 and 2). After finding an optimal preliminary model, we used model selection to determine relative support for the four methods (direct: meristem removal and defoliation; Indirect: camera counts and fecal counts) of estimating ungulate impact on aspen regeneration after fire. We further broke down total camera and fecal counts by species and compared those models with the rest of the four methods. We then compared the four methods for estimating ungulate effect on the aspen height and density against the best topographic model, and the intercept only model respectively (Tables 3 and 4). In our case, we could not distinguish between ungulate species type by direct measures, and both the indirect measures (fecal and camera counts) showed multicollinearity with fire, making separation of ungulate species problematic. Further, the models that differentiated between ungulate species were less parsimonious than the summed models (Tables 3 and 4). Therefore, we binned all species together for both fecal and camera counts.

As a complement to our model selection, we also compared goodness-of-fit for each model (r^2) (Table 5). Finally, we used linear regression to model each of the four direct and indirect indicators against each other in paired combinations (e.g. removal of apical meristem % vs. defoliation %, or Camera counts vs. fecal counts). For this we used a linear regression and paired each indicator as a response variable in combination with all other indicators as explanatory variables. We also included year and fire site as a fixed effect. We had a total of 6-paired combinations and assess their correlation using the full model. To verify assumptions of normality and homoscedasticity in the error structure for the top model, we visually inspected residual plots. Density counts were square root transformed to meet the assumption of normality,

and in order to use AIC for comparison between aspen density models (Zuur *et al*. 2009). We used the MuMIn (Barton 2015) package of Program R (R Core Team 2015) for all analyses.

RESULTS

Preliminary Topographical Models

Topography appeared in the most parsimonious model for both aspen height and density in the preliminary models. The topography model including both terrain ruggedness and northness had 63% of the Akaike weight and was significantly better than the intercept only model for aspen height (Table 1). The goodness of fit for the top topographical model for height was $r^2 = 0.60$. Terrain ruggedness was positively correlated with aspen height (3.5 cm ± 1.3 cm). The parameter estimate for northness included 0 in its error range and therefore was a poor predictor of height despite being in the top model. Therefore, it was removed from the final model.

Including northness in the model was an improvement over the intercept only model for aspen density, with 75% of the Akaike weight (Table 2). The goodness of fit for the top topographical model for density was $r^2 = 0.26$. The two closest models including terrain ruggedness, or terrain ruggedness and northness had greater than 2 ΔAIC and were, therefore, not included in the subsequent model. The northness parameter estimate included 0 in its error range. Therefore, despite being the most parsimonious model through our AICc methodology, it was a poor predictor of aspen regeneration density. Therefore, it was removed from the final model.

Top Models for Aspen Height and Density

The results of model selection indicated that meristem removal was clearly the top model with 92% of the AIC weight (Table 3). The percent of apical meristems removed showed a strong relationship with height. For each 10% increment in apical meristem removal, the model predicted 5 ± 2.4 cm reduction in height (Table 6). When adjusted for yearly growth patterns, our model indicates that height growth was suppressed at ≈75% apical meristem removal (Fig 2). The maximum and minimum mean heights recorded after three years of growth was 130 and 11 cm respectively and the mean height ± 1 SE at years 1, 2 and 3 were 37 ± 4, 53 ± 5, and 66 ± 7 cm, respectively.

Maximum and minimum aspen density across sites at the beginning of the study was 35,000 and 5000 suckers/ha. By the end of our study the maximum and minimum were 44000 and 500 suckers/ha, respectively. Average aspen density was 17000/ha across the study period. Our top model for predicting aspen density included defoliation (%) with 92% of the AIC weight (Table 4). Neither percent defoliation nor year were significant predictors of density but their interaction was (Table 6). Specifically, defoliation had a slightly positive but statistically insignificant effect on aspen density the first year after fire, but began to reduce aspen density in the 2nd and 3rd year of the study (Fig. 2c).

Comparison of Methodologies

We found that removal of apical meristem and measures of percent defoliation had high goodness of fit for height and moderately good fit for aspen suckering density (Table 5). We found that all four methodologies were significantly correlated, yet the strength of that relationship differed between indirect and direct measures. Mixed modeling showed high correlation between removal of apical meristems and defoliation ($r^2 = 0.83$) and between fecal

counts and camera counts ($r^2 = 0.74$). Correlation between removal of apical meristems and both fecal counts and camera counts were more modest ($r^2 = 0.55$, $r^2 = 0.56$). Correlation was moderate between defoliation and both fecal counts and camera counts ($r^2 = 0.63$, $r^2 = 0.65$). In summary, correlation within direct and indirect methodologies was high and statistically significant, with correlation coefficients between methodologies tending to be lower (Fig 3).

DISCUSSION

Indicators of Height and Density

Despite aspen's high physiological function and growth rates in post burn environments (Wan et al. 2014), aspen regeneration and recruitment success can be reduced by ungulate herbivory (Bartos et al. 1994). Ungulates suppress aspen forest recruitment after fire by suppressing height growth, and reducing suckering density (Romme et al. 2005, Rhodes et al. 2017a, Rhodes et al. 2017b). Our results suggest that meristem removal was the best predictor of aspen suckering height (Table 6). Aspen height growth is a key component to defense against ungulates through vertical escape (Lindroth and St. Clair 2013), and recruitment in the understory is important to long-term aspen stand resilience and health (Kaye et al. 2005, Smith et al. 2011b). These results are consistent with other studies that also found that meristem removal was a useful indicator of ungulate impact on regeneration and recruitment in other forest types (Frerker et al 2013, Royo et al. 2016).

The interaction between percent defoliation and year suggest that changes in density due to ungulate impact are longer term effects (Fig 2c). This is likely because pre-fire stand composition has a stronger influence on initial aspen regeneration density (Smith et al. 2011a) and clonal integration of aspen provide resource storage for aspen which increases resilience

(Rhodes et al. 2016). Stability in initial tree densities after disturbance is consistent with studies in eastern hardwood forests of the US showing that deer browsing reduces vertical growth of hardwood seedlings but not stand density (Royo et al. 2016). Aspen's clonal growth habit through which connected trees share resources may allow browsed suckers to persist for decades without recruiting into the overstory (Rhodes et al. 2017b). Our results indicate that at least three years of monitoring aspen density after fire is necessary to evaluate ungulate impact on aspen forest replacement.

Topography can influence the regeneration success of aspen (Dudley et al. 2015) by modifying ungulate behavior and altering the physiological function of aspen (Long et al. 2014, Rhodes et al. 2017a). Topographical conditions that provide better light or water availability increase aspen resilience to ungulate herbivory (Wan et al. 2014, Rhodes et al. 2017a). Similar to our study, high physiological function at southern aspects contributed to taller and better defended aspen on southern facing slopes (Wan et al. 2014). The high energetic cost of ungulate movement over rugged terrain can reduce habitat selection and vegetation use in these areas (Long et al. 2014). We found that terrain ruggedness increased height of aspen regeneration which suggests that ungulate reduction of aspen height was hindered at rugged sites (Table 6). While elevation is generally correlated with higher aspen regeneration and recruitment (Rhodes et al. 2017a) the limited range in elevation (2500 – 3030 m) in our study likely resulted in little influence of elevation in our study. While only terrain ruggedness estimates were significant, both northness and terrain ruggedness contributed to the most parsimonious models as evidenced by AICc model selection. Our findings support the broader scientific literature suggesting that topographical features should be included when evaluating ungulate impacts on aspen regeneration.

Comparing Indicators of Ungulate Herbivory

We found high correlations within direct and indirect measures of aspen regeneration success with lower but still good correlations between direct and indirect measures (Fig. 3). Ocular measurement of foliar cover are generally considered highly subjective, yet in some cases can provide similar results to more complex or costly methods (Vales and Bunnell 1988). Our results suggest that measuring ocular estimates of defoliation was similar to removal of apical meristems, but with much less investment in time. With a single observer, this quick method tended to correlate well with more time intensive measurements of meristem removal (Fig. 3). Further, direct measures can be extended to estimate local abundances of ungulates and alert managers to high ungulate abundances at the local scale (Fig. 3) (Rhodes et al. 2017a, Royo et al. 2016). This method did not differentiate between missing foliage due to insect herbivory and may not be useful in areas with high insect defoliation.

One of the advantages of estimating ungulate populations or indexing their abundance is to identify which ungulate species is responsible for impacting aspen recruitment. In our case, we could not distinguish between ungulate species type by direct measures, and while indirect measures pellet groups and camera counts accurately identified the species we could not distinguish between species due to multicollinearity between fire location and the mix of species that frequented our plots. However, total fecal counts and camera counts showed good correlations with measures of browsing impact (Fig 3). There is increasing evidence that the abundance of the ungulate community is more important than composition of the ungulate community in assessing impacts on aspen regeneration (Bork et al 2013, Rhodes et al. 2017a, Walker et al. 2015). The data suggest that direct measures of damage to aspen provide good estimates of relative use by the ungulate community (Fig. 3). This is consistent with work in

other forest systems showing good correlations between presence of ungulates and browse impacts (Royo et al. 2016).

Use of browsing indexes are considered a reliable ecological indicator (Freker et al. 2013) and often correspond to fecal counts of ungulates (Royo et al. 2016). There is a growing base of literature that supports the reliability of removal of apical meristems as an ecological indicator for ungulate-aspen interactions (Kaye et al. 2005, Strand et al. 2009, Rhodes et al. 2017a, Wan et al. 2014). Our data suggest that removal of apical meristems is precise with a low standard error around our estimate (± 0.2 cm, Table 6) which may contribute to its use as a monitoring tool and for defining thresholds for ungulate influence on aspen regeneration dynamics. Removal of apical meristems has been used as a monitoring tool for decades (Olmsted 1979, Jones et al 2005) and estimates of thresholds where ungulates begin to influence aspen regeneration are converging at around 30% (Olmsted 1979, Strand et al. 2009, Rhodes et al. 2017b). Our study found that ocular estimates of defoliation may replace or supplement the more work intensive measurement of removal of apical meristems (Table 5). Ocular estimates of simulated defoliation by insects have been shown to have high precision and accuracy with a reduction in bias with experience (Johnson et al. 2016). However, we suggest more work is necessary to develop the ocular defoliation indicator for aspen in the field. This research may compare, among many possibilities: reliability and accuracy across sites and observers.

Use of fecal counts also has a long history of use as an ecological indicator (Neff 1968, Seager et al. 2013). However, the results between studies showing conflicting results that at times are helpful (Rogers and Mittank 2014), but may also offer poor predictive power (Rhodes et al. 2017). Camera traps and pellet counts can achieve high accuracy for estimating ungulate abundance (Marques et al. 2001, Rovero et al. 2009), but neither alone accurately estimates

ungulate impact on their habitat (Morellet et al. 2007). Our data suggest that, from a management perspective, estimating ungulate impact through measurements of damage to aspen tissue may be the most precise, reliable, and feasible.

Management Implications

Ungulate communities are changing on a global scale (Spear et al. 2009) and have the capacity to alter forest regeneration and recruitment (Rooney and Waller 2003). There is an increasing need to monitor ungulate impacts at landscape scales (Frerker et al. 2013). However, the inherent variation in landscape scale topography and ungulate movement confound our ability to link ungulate impact to forest regeneration. In previous studies, ungulates halted aspen regeneration (Bartos et al 1994, Kay 1997) however these studies were conducted in areas known to have high ungulate populations. In regional studies, aspen regeneration failure due to ungulate herbivory has been shown to be much more variable (Rogers and Mittanck 2014, Rhodes et al. 2017a). Therefore, extrapolation of ungulate effects on plant species outside of the study or monitoring area should be used with extreme caution, especially when monitoring lacks spatiotemporal breadth and are known to have high ungulate densities (Suzuki et al. 1999).

In the face of this uncertainty, we present data that identifies critical herbivory thresholds for regenerating trees that is broadly applicable. Meristem removal measurements clearly predicted growth potential in post-fire environments in this study and have also been used successfully to estimate ungulate impacts on recruitment in self-regenerating, stable aspen stands that have not burned (Rhodes et al. 2017a). Impacts of meristem removal on forest recruitment scales well across large landscapes especially when topography is taken into account (Rhodes et al 2017a). We recommend using removal of apical meristems as an indicator of ungulate impact on aspen recruitment and suggest that ≈75% removal of apical meristems (Fig. 2) is an important

threshold for successful aspen regeneration (Jones et al. 2005, Olmsted 1979,). Meristem removal measurements can offer a quick assessment, which warns managers of aspen regeneration problems due to ungulate herbivory. Also, ocular assessment of defoliation could replace surveys of meristem removal when time constraints exist. Both of these direct methods were superior to indirect measurements (fecal counts and camera counts) (Table 3) but indirect measurements still correlated with browse damage and can be valuable in identifying which ungulate species are having the greatest browse impact in a given area.

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FIGURES

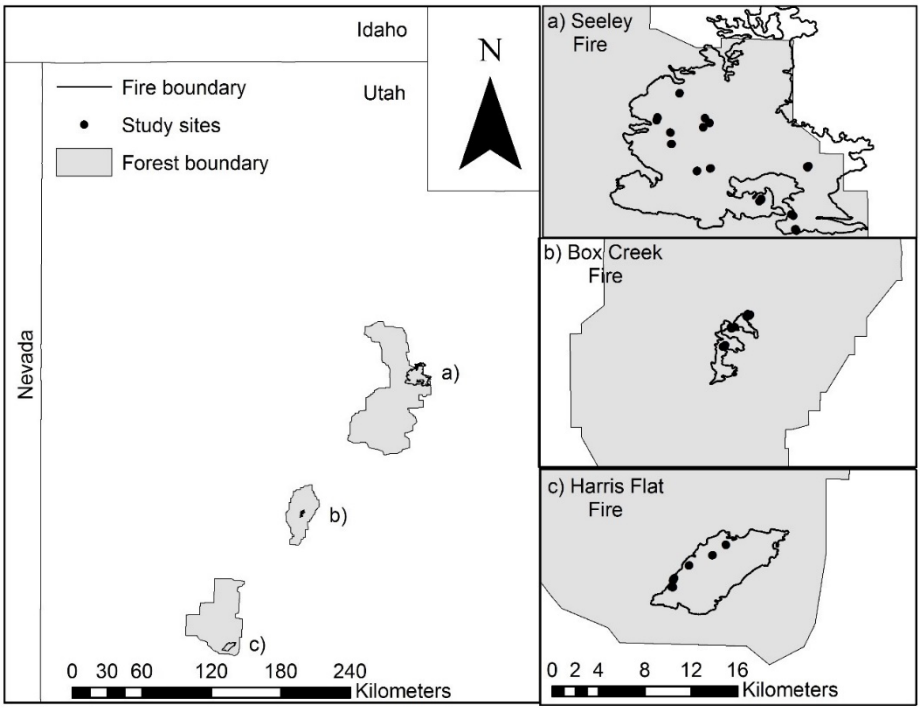


Figure 2–1. A map of the study area depicted on a small scale map (1:5,000,000) with partial national forests and fire perimeters. Fires are labeled a, b, and c (Seeley, Box Creek, and Harris Flat, respectively). Inset maps are displayed to the right in a larger scale of 1:500,000. Solid black points mark the study sites in the three inset maps.

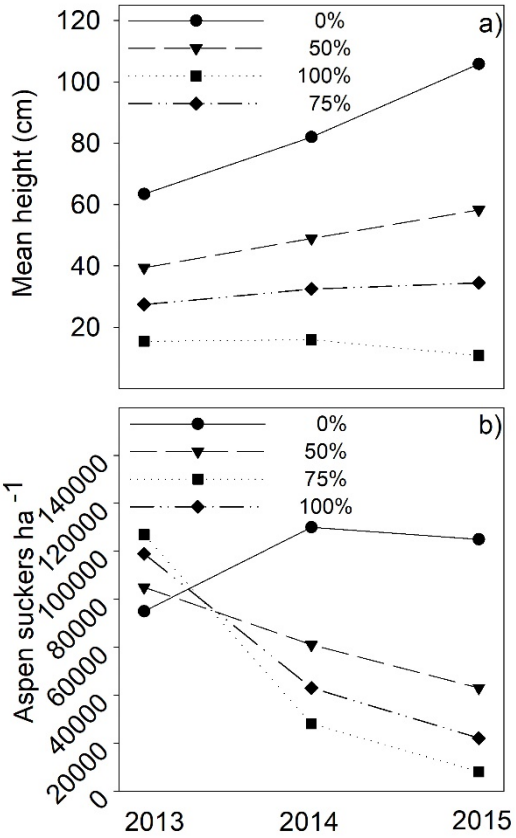


Figure 2–2. Predicted mean height of aspen or mean aspen density is shown across the study period, using the top model from Table 6. For aspen height we account for aspect by holding northness at 0 (east or west aspect). Change in aspen height is presented as a function of percent removal of apical meristems and its interaction with year is presented in panel (a), while the effect of percent defoliation on aspen density and its interaction with year is presented in panels (b).

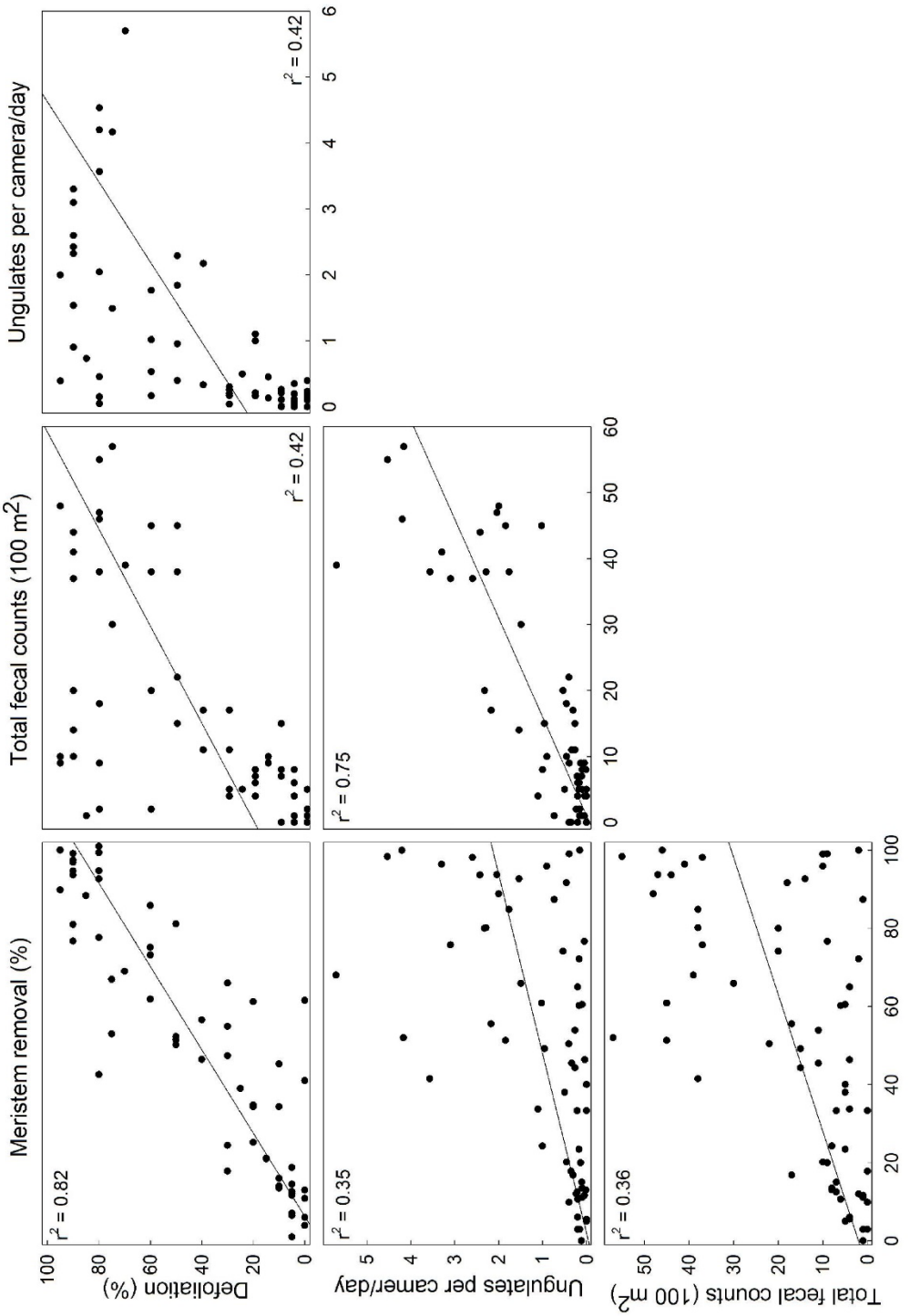


Figure 2–3. Pairwise regression analyses are presented here which the relationships between meristem removal (%), defoliation, ungulate camera counts/day and fecal counts/100 m. The r^2 values represent the full model including year, northness, and terrain ruggedness. The points represent raw values.

TABLES

Table 2–1. *A priori* model results with topography as a predictor of aspen height. The associated model parameters (K), change in AICc when compared to the top model and the Akaike weights are listed for each model.

Aspen Height Topographical Models	**K**	**ΔAICc**	**Weight (%)**
Intercept only	2	53	<0.01
Year + Site	5	4	9
Terrain Ruggedness + Year + Site	6	8	1
Elevation (m.a.s.l.) + Year + Site	6	5	5
Northness + Year + Site	6	7	2
T. Ruggedness + Elevation + Year + Site	7	8	1
Elevation + Northness + Year + Site	7	5	6
***T. Ruggedness + Northness + Year + Site**	**7**	**0**	**63**
T. Ruggedness + Elevation + Northness + Year + Site	8	3	14

Table 2–2. *A priori* model results with topography as a predictor of aspen density. The associated model parameters (K), change in AICc when compared to the top model and the Akaike weights are listed for each model.

Aspen Density Topographical Models	**K**	**ΔAICc**	**Weight (%)**
Intercept only	2	18	<0.01
Year + Site	6	10	<0.01
Terrain Ruggedness + Year + Site	7	7	2
Elevation (m.a.s.l.) + Year + Site	7	12	<0.01
***Northness + Year + Site**	**7**	**0**	**75**
T. Ruggedness + Elevation + Year + Site	8	15	<0.01
Elevation + Northness + Year + Site	8	13	<0.01
T. Ruggedness + Northness + Year + Site	8	3	22
T. Ruggedness + Elevation + Northness + Year + Site	9	15	<0.01

Table 2–3. *A priori* model results with each of the four methods of estimating ungulate impact as a predictor of aspen height. The associated model parameters (K), change in AICc when compared to the top model and the Akaike weights are listed for each model.

Aspen Height Methodology Models	**K**	**ΔAICc**	**Weight (%)**
Intercept only	2	130	<0.01
Topographic Model (TM) + Year + Site	7	65	<0.01
TM + Meristem % removal x Year + Site	11	0	<0.01
TM + Defoliation x Year + Site	11	15	<0.01
TM + Camera Counts* x Year + Site	**11**	**67**	**92**
TM + Camera Counts (cattle, elk, deer) + Year + Site	11	59	<0.01
TM + Fecal Counts x Year + Site	11	16	<0.01
TM + Fecal Counts (cattle, elk, deer) + Year + Site	11	5	7

Table 2–4. *A priori* model results with each of the four methods of estimating ungulate impact as a predictor of aspen height. The associated model parameters (K), change in AICc when compared to the top model and the Akaike weights are listed for each model.

Aspen Density Methodology Models	**K**	**ΔAICc**	**Weight (%)**
Intercept only	2	27	<0.01
Topographic Model (TM) + Year + Site	6	16	<0.01
TM + Meristem % removal x Year + Site	10	9	1
TM + Defoliation* x Year + Site	**10**	**0**	**98**
TM + Camera Counts x Year + Site	10	10	<0.01
TM + Camera Counts (cattle, elk, deer) + Year + Site	10	11	<0.01
TM + Fecal Counts x Year + Site	10	16	<0.01
TM + Fecal Counts (cattle, elk, deer) + Year + Site	10	15	<0.01

Table 2–5. Full model goodness of fit are presented for each indicator for both height and density. Below both total camera and total fecal count models are presented models that differentiate between species for each methodology.

Model	Index	r^2
Aspen Height	Meristem % rem.	0.90
	Defoliation %	0.86
	Camera Counts	0.63
	Cattle, Deer, Elk	0.58
	Fecal Counts	0.61
	Cattle, Deer, Elk	0.59
Aspen Density	Meristem % rem.	0.40
	Defoliation %	0.44
	Camera Counts	0.27
	Cattle, Deer, Elk	0.23
	Fecal Counts	0.28
	Cattle, Deer, Elk	0.11

Table 2–6. Beta estimates for the top models previously selected are presented along with their associated standard error and t-statistic. Aspen height units are centimeters, and aspen density units are the number of suckers $100m^{-1}$. We list the estimate, the standard error associated with it and the t-statistic.

Aspen Metric	Parameter	Estimate	SE	t
Aspen Height	Intercept	61	15	3.9
	Meristem % rem.	-0.5	0.2	-2
	Year 2014	16	16	0.9
	Year 2015	38	16	2.4
	Northness	-5	3.3	-1.5
	Harris Flat	8.7	5.2	1.6
	Seeley	5.1	5.0	1.0
	Meristem % rem:2014	-0.1	0.3	-0.5
	Meristem % rem:2015	-0.4	0.3	-1.6
Aspen Density	Intercept	12	2.9	4.0
	Defoliation %	0.04	0.05	0.8
	Year 2014	2.4	2.5	1.0
	Year 2015	2.4	2.5	1.0
	Harris Flat	2.1	2.2	1.0
	Seeley	1.4	2.1	0.7
	Defol:2014	-0.1	0.05	-2.3
	Defol:2015	-0.2	0.05	-2.9

CHAPTER 3

Differential Effects of Cattle, Mule Deer, and American Elk on Aspen Regeneration and Thresholds for Recruitment After Fire

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ABSTRACT

Human activities are changing the abundance and composition of ungulate herbivore communities globally, which can strongly impact the resilience and function of earth's ecosystems. Impacts from herbivory are compounded in areas where multiple ungulates overlap, which is common in forest ecosystems. The objective of this study was to examine the differential and combined effects of ungulate communities (deer, elk, and cattle) on aspen forest regeneration after fires that occurred in 2012. Ten four-way exclosures with cameras traps were established across three National Forest in Utah to quantify the combined and individual effects of elk, deer and cattle herbivory on aspen height, density and recruitment over time (2013 to 2016). Ungulate activity was nearly 10-fold higher in two National Forests compared to the third, and gradually decreased over time. Meristem removal by ungulates in unfenced plots across sites averaged ~60% which we identified as a critical threshold point for aspen recruitment failure. All three ungulate species had significant and similar effects on aspen regeneration success, but when adjusted for differences in estimated forage intake (animal unit months), differential impacts became apparent (deer > elk > cattle). We estimated that 4 cattle per camera^{-1} day^{-1} and 2.5 deer or elk per camera^{-1} day^{-1} was sufficient to reach the critical recruitment threshold of 60% removal of apical meristems. We conclude that ungulates species differentially influence aspen regeneration and recruitment, and that ungulate browsing impairs aspen recruitment

above 30% meristem removal with recruitment failure occurring above 60% meristem loss.

INTRODUCTION

Native and non-native ungulate populations can influence plant community development and reduce ecosystem stability (Côté et al. 2004, Spear and Chown 2009). The frequency and magnitude of ungulate herbivory can define thresholds for forest regeneration and recruitment (Strand et al. 2009, Wisdom et al. 2006). Ungulate herbivory has particularly strong effects on plant recruitment in post-disturbance environments (Augustine and McNaughton 1998). Ungulate influence on plant communities are often most intense in the early stages of forest succession following disturbance (Wisdom et al. 2006, Augustine and McNaughton 1998). However, quantifying ungulate influence on forest regeneration and identifying forest recruitment thresholds by ungulate species are confounded by overlapping habitat and diet of multiple ungulate species.

What herbivores choose to eat is mediated by complex physiological and environmental cues (Hanley 1982, Hoffman 1989, Long et al. 2014). Interspecific differences in ungulate anatomy drive differences in forage preference (Clauss et al. 2010), providing a framework for understanding ungulate forage selection and their differential impacts on forest recruitment and succession. Ungulate species are generally divided into three classes; “concentrate feeders”, “intermediate feeders”, and “grass and roughage eaters”, hereafter referred to as: browsers, mixed feeders, and grazers (*sensu lato* Hoffman 1989). Body size, mouth anatomy, stomach type (ruminant vs. cecal), and rumino-reticular volume are among the major physiological features that influence diet strategy in ungulates (Hanley 1982). Grazers generally have large body size and/or large rumino-reticular volume that favors selection of lower quality forage like grasses.

Browsers generally have smaller rumino-reticular volume and must select for high-quality forage that passes relatively quickly though the digestive system. Mixed feeders fall between the two previously described types with intermediate anatomical features and diets (Hoffman 1989). Browsers, given their smaller mouth size and need for higher quality forage should select palatable portions of tree species such as meristems and leaf tissue. This conceptual framework predicts differential herbivory impacts on forest regeneration as follows: browser > mixed feeder > grazer (Clauss et al. 2010, Hoffman 1989).

Aspen is a keystone species that provides habitat for hundreds of plant and animal species (Peterson and Peterson 1992). Aspen forests are preferred habitat for deer (*Odocoileus spp.*) and elk, (*Cervus canadensis*) (Beck et al. 2006) and are utilized by cattle (*Bos taurus)* due to the high productivity and forage quality in their understory (DeByle 1985). Aspen forms the foundation of forest successional cycles in many montane and subalpine mixed conifer forests of North America by regenerating through root suckering after fire (Calder and St. Clair 2012, St. Clair et al. 2013). Regenerating aspen suckers, however, are highly palatable and susceptible to ungulate herbivory (Seager et al. 2013) and high rates of ungulate herbivory can result in aspen regeneration and recruitment failure (Rhodes et al. 2017a). Therefore, identifying thresholds at which ungulate herbivory interferes with aspen recruitment is critical to identifying aspen regeneration success and its implication for the resilience of aspen ecosystems.

Quantifying the differential effects of multiple coexisting ungulate species is key to defining thresholds for successful aspen recruitment. Monitoring removal of apical meristems is a good indicator of ungulate effects on aspen regeneration and subsequent recruitment (Rhodes and St Clair 2017). Thresholds for aspen recruitment in self

regenerating aspen forests not triggered by fire have been identified at or near 30% browse removal of apical meristems (Olmsted 1979, Jones et al. 2005, Strand et al. 2009). However, there are no current estimates for browsing thresholds of aspen recruitment in post-fire conditions, and no quantification of interspecific contribution of individual ungulate species on aspen regeneration and recruitment. Aspen tolerance and resistance to ungulate herbivory increases with greater burn severity (Wan et al. 2014a) and fire size (Wan et al. 2014b). Both burn severity and fire size are positively correlated with aspen suckering density and negatively correlated with ungulate influence (Smith et al. 2011, Wan et al. 2014a and b). Therefore, aspen regeneration and recruitment thresholds in response to ungulate herbivory may differ in post-fire conditions.

The effect of ungulate herbivory on forest regeneration is influenced by multiple environmental factors (Dudley et al 2015, Rhodes et al. 2017b) and is highly variable across space and time (Rhodes et al. 2017a, Rhodes et al. 2017b). Topography is an important determinant of ungulate-aspen interactions due to its effects on temperature, precipitation and ungulate foraging behavior (Smith et al. 2011, Rhodes et al. 2017b, Long et al. 2014). Elevation covaries with temperature and precipitation and affects subsequent snowpack persistence and hydrology (Sospedra-Alfonso et al 2015, Morán-Tejeda et al 2013). Snowpack depth and persistence affects ungulate herbivory of aspen by limiting the movement and access of ungulates during the winter and spring periods (Martin and Maron 2012, Brodie et al. 2012, Rhodes et al. 2017b). Moreover, aspen at lower elevations are subjected to warmer and drier conditions (Worrall et al. 2013) which can increase susceptibility to disease and herbivory (Dudley et al. 2015, Strand et al. 2009). Therefore, incorporating a measure of elevation and accounting for gradients of relative ungulate use across space and time provides a better mechanistic

understanding of how ungulate species influence aspen regeneration and forest recruitment.

The central objective of this study was to quantify the individual and combined influence of mule deer, elk, and cattle on aspen regeneration and recruitment in complex, post-fire forest environments. We hypothesized that ungulate use of aspen, as measured by removal of apical meristems, would vary across burned landscapes and years due to natural variability in ungulate visitation over space and time. Second, we hypothesized that aspen regenerating at lower elevations would experience lower height, density, recruitment and due to increased susceptibility to ungulates. Third, we hypothesized that mule deer, elk and cattle would influence aspen height, density, and recruitment differently according to the following theoretical framework: browsers (mule deer) > mixed feeders (elk) > grazers (cattle). Finally, we sought to identify ungulate herbivory thresholds for successful aspen recruitment in post-fire environments.

MATERIALS AND METHODS

Study Area

This study was conducted at three locations in central and southern Utah on the Manti-La Sal, Fishlake and Dixie National Forests that experienced fire in 2012 (Fig. 1). The Seeley, Box Creek, and Harris Flat fires occurred in aspen-conifer mixed forests during peak fire season (June-July 2012). Fire sizes were 19400, 900, and 3400 ha, respectively. Site elevations ranged from 2440 – 3030 m. Thirty-year annual precipitation averages ranged from 25 – 64 cm at these sites (PRISM data, Parameter-elevation Relationships on Independent Slopes Model) (Daly et al. 2009). Dominant tree species in the Seeley and Box Creek fires included; Aspen, Subalpine Fir (*Abies lasiocarpa* (Hooker) Nuttall) and White Fir (*Abies concolo*r (Gordon) Lindley ex

Hildebrand). While the dominant conifer component of the Harris Flat fire was Ponderosa Pine (*Pinus ponderosa* Douglas ex C. Lawson). Cattle, mule deer (*Odocoileus hemionus*), and elk were the most abundant ungulates at the study sites.

Study Design

We selected three sites at Seeley, three at Box Creek, and two at Harris Flat. Sites were selected based on high fire severity (100% mortality of overstory), and pre-fire overstory stands having a significant aspen component (>50% aspen stems pre-fire). At each site, three fence treatments and an unfenced plot were randomly assigned in 30 x 30 m plots. The fence treatments were: 1) mule deer access; 2) native ungulate access (mule deer and elk), 3) full exclusion (cattle elk, and mule deer), and 4) unfenced. Full exclusion fencing was constructed with 3.1 m wooden posts buried 0.8 m into the ground at each corner. Fencing on each of the four sides was fixed on t-posts positioned at 3 m increments. The fencing consisted of 1.8 m of woven wire fencing and topped with a barbless wire set at 2 m. A gate was constructed in one corner of each fenced plot. A fence that allowed only mule deer was also constructed. Deer-only fencing was constructed in similar fashion as the full ungulate exclosure except that a 0.5 m gap was created at the bottom of the fence (VerCauteren et al. 2007). Mule deer were allowed entry through the 0.5 m gap but the fence effectively excluded both elk and cattle. Native ungulate access fencing consisted of 1.8 m wooden corner posts buried 0.6 m into the ground. The fencing was three barbed wires placed at heights of 0.2 m, 0.6 m, and 1.0 m. Unfenced plots were marked at the four corners with 1.8 m u-posts buried 0.6 m into the ground. Fencing may alter ungulate visitation rates when compared to an unfenced plot. Therefore, we complement this design with wildlife camera traps to quantify ungulate visitation in each plot.

Characterization of Aspen Regeneration and Recruitment

Within each 30 x 30 m^2 plot, 25 1 m^2 subplots for surveys of aspen were established in a 5 by 5 grid with a 5 m boundary layer from the fence edge. Aspen density, height, and meristem removal were characterized at the end of August from 2013-2016. We counted aspen suckers as individuals if they had emerged independently from the ground. Removal of apical meristems was characterized by examining the percent of apical meristems removed by herbivory from the leading branch and the sub-leading branches in the top 15 cm of an aspen sucker (Wan et al. 2014a, Jones et al. 2005, Strand et al. 2009, Rhodes et al. 2017a). Removal of apical meristems is reported as the sum of browsed meristems divided by the sum of all stems. In 2016, as we finished this study, we evaluated aspen recruitment by counting aspen stems ≥ 150 cm in height that likely represented successful escape from ungulate herbivory (Seager et al. 2013).

Wildlife Cameras

In each experimental plot, we placed a trail camera to determine ungulate presence by species (PC900 HyperFire Professional Covert Camera Trap) (Reconyx, Holmen, WI, USA). We used ungulate counts per camera per day (ungulate $camera^{-1}$ day^{-1}) as an estimate of relative ungulate use in each of the experimental plots across all sites. Cameras were placed at the corner of each plot facing inward, and were set at medium sensitivity to capture five photos at three second intervals with a five minute rest period in between triggers. Each ungulate that appeared at least once in the five photo set were counted and these counts were summed and divided by the number of active camera days for each plot. Ungulate counts were summed from the month

preceding each aspen survey in order to characterize the relationship between relative ungulate use and aspen regeneration success (May through August period).

Elevation

We associated GPS locations of each study plot with elevation taken from 30 x 30 m digital elevation models (DEM). The DEM we used was a United States Geological Survey product using the UTM Zone 12 projection and NAD83 geodetic network (Universal Transverse Mercator, North American Datum, respectively). We used ArcMap 10.3 (ESRI 2016) to associate our GPS locations with their respective elevation from the DEM.

Statistical Analysis

In order to characterize differences in aspen regeneration across fence types we used linear mixed-effects models with height, density, and percent browse of apical meristems as response variables. We evaluated fence type as a categorical explanatory variable, and ungulate camera counts as a continuous explanatory variable that predicted our response variables in two different sets of models. Photo counts allowed us to evaluate ungulate herbivory as a measure of relative use in a controlled framework provided by the fencing design. We modeled fence, year, and fence by year interaction along with elevation as fixed effects and site location as a random effect. In analysis of photo counts, data from the full exclosure plots was removed given that there was no incidence of browse in these plots resulting in $\mu = 0$ and $\sigma^2 = 0$ for this group, which made modeling problematic.

We present results in two separate groups of models: first, we used fence as a categorical variable and, second, we used camera counts as a continuous variable. Our experimental design allowed for both types of analyses where we have fencing that

controls access, but are able to account for relative ungulate use. Accounting for the site to site variation in ungulate abundance through camera traps is key to improving our knowledge of the influence of ungulates on their environment (Wisdom et al. 2006). In order to quantify the differential influence of ungulate species on aspen regeneration we created a separate model for each ungulate species. We modeled mule deer effects using camera counts from mule deer only plots and subtracted their estimated effect from native ungulate plots to estimate elk effects. We then subtracted mule deer and elk estimated effects from unfenced plots in order to model cattle effects. Here, our modeling assumed that only additive and no interactive effects were present.

In order to evaluate recruitment, we averaged browse across all years for each 30 x 30 m plot and split browsing into high and low categories. High was defined as ≥ 30 % and low was defined as < 30%. This browse limit is consistent with other studies that define removal of terminal buds as a critical threshold for aspen recruitment into the overstory (Olmsted et al. 1979, Jones et al. 2005, Strand et al 2009, Rhodes and St. Clair 2017). For all statistical models mentioned above, we included a heterogeneity of variance identity structure across fence types. We checked assumptions of normality by visually inspecting a histogram of model residuals. We checked assumptions of homogeneity of variance by visually inspecting fitted residuals versus predicted residuals. All analyses were generated using R statistical software (R Core Team 2016) with the MuMIn, (Barton 2015) and nlme (Pinheiro et al. 2015) packages. Conditional and marginal r^2 values were computed for mixed effects analyses (Nakagawa and Schielzeth 2013).

RESULTS

Hypothesis 1: Relative Ungulate Use Patterns Across Years and Site

Removal of apical meristems was highly variable across the three fires and generally decreased over time (Fig. 2). Removal of apical meristems was significantly influenced by fence type, year and elevation, but not the fence by year interactions (Table 1). Removal of apical meristems in unfenced plots was nearly 10 times greater in the Box Creek and Harris Flat fires compared to the Seeley Fire (Fig. 2). Removal of apical meristems trended downward throughout the four year study period across all fencing types. Removal of apical meristems fell from 64% to 51% in unfenced plots from 2014 to 2015. A similar trend was observed in native ungulate and mule deer only plots which fell from 44% to 26% and 33% to 16% respectively. (Fig. 4a). There was 0% removal of apical meristems in full exclosures and no evidence of trespass (no photos or feces) throughout the four year study period.

Hypothesis 2: Elevation Mediates Ungulate: Aspen Interactions

When using fence type as a categorical variable, removal of apical meristems decreased by 3.5% for every 100 meter increase in elevation ($t = 5.9$, $p < 0.01$). Mean height increased 5 cm for every 100 meter increase in elevation ($t = 4.5$, $p < 0.01$). When using camera counts as a continuous variable, elevation was a significant indicator for browse of apical meristems in the cattle model but not the mule deer and elk models (Table 2). Removal of apical meristems was estimated to be 4% lower for every increase in 100 m of elevation in the cattle model. Elevation was a significant indicator for aspen density in the elk and cattle model (Table 2) but not in the mule deer model. Elevation was not significant for height across any ungulate model (Table 2). Elevation was not significant in models for aspen recruitment in 2016.

Hypothesis 3: Differential Ungulate Effects

Our fences differentially excluded ungulate species as designed. We found no evidence of any ungulate entrance into full exclosure plots, and no cattle in native ungulate plots. Mule deer entered, deer-only plots at similar rates as unfenced and native ungulate plots, but there were only one third as many elk in cattle excluded plots compared to unfenced plots (Fig. 3).

Removal of apical meristems varied significantly depending on fence type, an effect that was consistent across all four years (Table 1). There was no evidence of browsing in full exclosure plots. Unfenced, native ungulate plots, and mule deer access plots averaged 64%, 44% and 33% meristem removal over the three-year period an effect that diminished over time (Fig. 4a). Averaged across all fencing types, removal of apical meristems was highest in 2013 and was reduced by 3.5% in 2014, 4.5% in 2015, and 11% in 2016 (Fig. 4a).

Mean aspen height was significantly influenced by all main effects (fence type, year, and elevation) but not the fence type by year interaction (Table 1). Fence type had modest but statistically significant effects on mean aspen height over the four year study period. Over the four-year study period, mean aspen height was reduced by 12.6 cm in mule deer access plots, 16.5 cm in native ungulate access plots, and by 37 cm in unfenced plots when compared to full exclosure plots (Fig. 4b). Mean aspen height averaged across all plots had yearly growth of 25 cm in 2014, 26 cm in 2015, and 16 cm in 2016 (Fig. 4b).

Aspen regeneration density was significantly impacted by fence type and year (Table 1) but elevation and the fence by year interaction were not significant. Aspen regeneration density was highest in 2013, and followed a downward trend across the four year period dropping by as much as 50% by 2016 (Fig. 4c). Aspen densities were

lowest in the full exclusion and mule deer access plots, and were significantly higher in unfenced and native ungulate access plots (Fig. 4c).

When ungulate effects on aspen regeneration were modeled using camera counts: cattle, mule deer and elk each significantly reduced aspen regeneration height, and density, while increasing removal of apical meristems (Fig. . 4). Cattle, elk and mule deer increased removal of apical meristems by 16% ± 9%, 24% ± 4%, and 24% ± 4% for each 1 ungulate camera^{-1} day^{-1}, across the entire 4 year study period (Table 2). Cattle, elk, and mule deer had each reduced mean aspen height by the end of the study. In 2016, full exclusion plots had a predicted height of 151 cm, while 1 ungulate camera^{-1} day^{-1} of each species elk, cattle and mule deer led to a predicted height of 105 cm, 88 cm, and 77 cm, respectively, (Fig. 4e). Our models predicted that, over the entire four year period, elk, and mule deer each reduced aspen regeneration density by 26800 ± 10,400 ha^{-1} and 23,200 ± 6,000 ha^{-1} per ungulate camera^{-1} day^{-1}, respectively while cattle had no significant influence on aspen regeneration density (Table 2).

Hypothesis 4: Aspen Recruitment Thresholds

Aspen in plots subjected to ≥ 30% removal of apical meristems had minimal regeneration success 5 years post-fire while ≥ 57% showed complete recruitment failure (Fig. 5b). In contrast, most plots with meristem removal below 30% were regenerating successfully (Fig. 5a). Aspen recruitment densities in plots with less than 30% meristem removal were 18 fold higher than plots with > 30% meristem removal (15,000 ± 880 aspen recruiting ha^{-1} vs 880 ± 400 aspen recruiting ha^{-1}) ($F = 17$, $p < 0.01$, $r^2 = 0.38$).

DISCUSSION

Changes in Relative Use Across Space and Time

Ungulates have complex behavior and forage patterns across space and time (Long et al. 2014). At regional scales, relative ungulate use can vary greatly across space, making it difficult to extrapolate total and interspecific effects on aspen regeneration (Rhodes et al. 2017b). Our first hypothesis that relative ungulate use would vary across the three fires and across years was well supported. Ungulate relative use, as measured by removal of apical meristems, was nearly 10 times greater in Box Creek and Harris Flat fires when compared to the Seeley Fire and decreased fairly rapidly across years (Fig. 2). Large fire size and high fire severity improve aspen regeneration by increasing tolerance and chemical resistance to ungulate herbivores (Wan et al. 2014a, Wan et al. 2014b). The large size of the Seeley fire (Fig. 1), may have contributed to the reduced use of aspen by ungulates (Fig. 2) and its overall successful regeneration.

Elevation

Elevation mediates the influence of ungulate herbivory in forest systems (Rhodes et al. 2017b). Higher elevation was related to taller aspen and less browse of apical meristems when using fence as a categorical variable. When modeled by camera counts, elevation was associated with reduced browse of apical meristems and increased density. Similarly, a regional study across Utah that found improved aspen regeneration and recruitment at higher elevation in undisturbed stands (Rhodes et al. 2017b). Elevation may be related to differences in snow persistence that will affect the timing of ungulate access to regenerating aspen suckers (Martin and Maron 2012, Brodie et al. 2012, Rhodes et al. 2017b). This suggests that aspen at lower elevation are more

susceptible to ungulate herbivory. If predicted changes in temperature and precipitation occur, aspen at lower elevation may be more affected by ungulate herbivory. (Strand et al. 2009, Worrall et al. 2013).

Differential Species Impacts

At landscape scales, wild and domestic ungulate habitat often overlaps (Wisdom and Thomas 1996) and understanding their differential effects has been difficult to estimate in natural environments (Wisdom et al. 2006). Our second hypothesis that there are fundamental differences in use of aspen between the ungulate species studied was well supported. However, a caveat with our study design is the assumption that there are no significant interactive effects between ungulate species. Competition is stronger between elk and cattle than between cattle and mule deer, likely due to similar diets of mixed feeders with grazer (Coe et. al 2001). Further, cattle can displace elk spatially across gradients of elevation (Stewart et al. 2002) and cattle, elk, and mule deer avoid foraging in the same habitat within 6 hours of visitation but this avoidance is not maintained at longer time scales of 7 days (Stewart et al. 2002). There is evidence that cattle can remove preferred plant species, and change native ungulate habitat use patterns (Madhusudan 2004). However, using cameras to model visitation rates and evaluate them in an additive fashion likely captures the main effects of each ungulate species and has produced similar results to other studies (Bork et al. 2013, Walker et al. 2015).

We found that 1 ungulate per camera^{-1} day^{-1} of any species had similar effects on aspen height and browse of apical meristems (Fig. 4). However, when adjusted to differences in animal diet requirements and comparable AUMs (Cattle 1.0 AUM, elk 0.6 AUM, mule deer 0.2 AUM) (NRCS 2003), differences in ungulate species impacts

were identified (Mule deer > elk > cattle). In other words, the estimated effect of mule deer was greatest, and cattle the least. Therefore, we estimate that while the total forage intake of mule deer is one fifth that of cattle, the total intake of aspen in mule deer diet is higher than cattle based on visitation estimates via camera counts.

This gradient of preference for aspen supports the conceptual framework based on ungulate functional groups (browse > mixed feeder > grazer) (Hoffman 1989). The only difference between our models of ungulate preference was that cattle did not significantly reduce aspen density (Fig. 4). This is consistent with an earlier study in Alberta, Canada which found that cattle have less of an effect on aspen browse, defoliation and mortality rate than browsers or mixed feeders at similar stocking rates (Bork et al. 2013).

Fencing is often used to differentiate individual species effects within ungulate communities (Goheen et al. 2013, Walker et al. 2015, Faison et al. 2016), but fencing cannot always control for natural variability in ungulate visitation and use (Wisdom et al. 2006) (Fig. 2). Therefore, some measure of ungulate use is critical to evaluating their effect (Wisdom et al. 2006). Due to variability in removal of aspen meristems across species in both space and time (Fig. 2), camera counts may be a more accurate estimator for parsing out interspecific impacts of different ungulate species than using fencing treatments alone. When cameras are unavailable, monitoring of removal of apical meristem and pellet counts can help to identify important thresholds for aspen recruitment (Fig. 5) (Rhodes and St. Clair 2017).

Removal of Apical Meristem Thresholds for Aspen Recruitment

Ungulates have the capacity to act as keystone species, exerting disproportionate effects on ecosystem processes (Wisdom et al. 2006, Côté et al. 2004). Our study

suggests that ungulates at sufficiently high rates of use can impair aspen regeneration and recruitment after fire (Fig. 5). We estimated that 2 cattle per camera^{-1} day^{-1} and 1.25 mule deer or elk per camera^{-1} day^{-1} was sufficient to suppress aspen recruitment 30% (Figs. 4b, 5). We identified a conservative threshold at 30% removal of apical meristem (Fig. 5b), that was similar to predicted thresholds of 27% in intact stands (Strand et al. 2009). This is consistent with several other studies that found reduced recruitment at browsing removal of meristems greater than 30% in stands in later stages of forest succession (Jones et al. 2005, Olmsted et al. 1979). However, our results had variability around the 30% threshold (Fig. 5a), with two sites recruiting well (≈20,000 stems ha^{-1}). Therefore, we define >30% as a conservative threshold while ≥ 57% showed complete recruitment failure (Fig. 5). Thresholds in post-burn environments may be higher due to higher growth rates, and higher production of defense chemicals in aspen foliar tissue (Wan et al. 2014a).

Management and Ecological Implications

Cattle, mule deer and elk differ in their preference for aspen. After, adjusting for body size using AUM equivalents (NRCS 2003), we found that differential use of aspen was consistent with the browser-mixed-grazer continuum predicted by ungulate physiology (Hanley and Hanley 1982, Hoffman 1989). Monitoring removal of apical meristems can help managers define ungulate use thresholds for aspen. This study found a conservative threshold at 30% removal of apical meristems and a strict threshold at >57% removal of apical meristems (Fig. 5). We estimated that 2 cattle per camera^{-1} day^{-1} and 1.25 mule deer or elk per camera^{-1} day^{-1} coincided with sufficient relative use to reach 30% browse of apical meristems (Fig. 4d). Managers should also acknowledge site and annual differences in ungulate relative use and can monitor removal of apical

meristems to quantify ungulate impact across space and time (Fig. 2). The influence of ungulates on aspen regeneration after fire are more pronounced in the first two years (Fig. 2) and can lead to long-term reductions in recruitment potential (Rhodes et. al 2017a). Our estimates provide clarity to managers in terms of differential effects between ungulate species, but also cautions that ungulate population size of any species or combination of species at sufficient density can cause aspen regeneration failure (Rhodes et al. 2017c).

Global changes in ungulate communities create novel pressures on plant communities and ecosystem processes (Spear and Chown 2009). Land use change to pasture lands, spread of invasive ungulates, and extinction or extirpation of native ungulates pose threats to ecosystem stability across Earth's rangelands (Bevins et al. 2014, Echeverria et al. 2012, Lopez-Sanchez 2016, Spear and Chown 2009). Human alteration of ungulate population size and communities is closely linked with human population trends (MeyFroidt, et al. 2010, Reid 2012). Therefore, our influence over ungulate communities and subsequent cascading impacts on plant communities is likely to continue and even increase into the future. Therefore, improving our ability to identify individual species impacts on keystone plant species is imperative for mitigating future trends in global change across rangelands (Wisdom et al. 2006). Our data show that theoretical frameworks using both animal species and plant response data can aid in understanding how complex interactions between ungulates and plant communities may unfold in a changing world.

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FIGURES

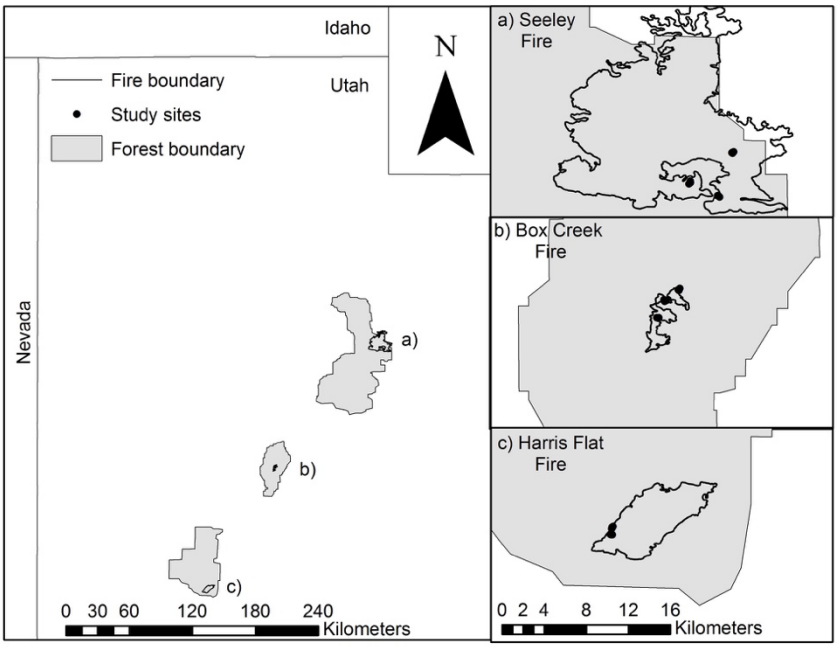


Figure 3–1. Map of the study area – This map shows the larger study area encompassing central and southern Utah. Forest boundaries are in gray and fire perimeters are in black. Three inset maps display black dots at the study sites. At each a four block differential fencing treatment is located. All three inset maps use the same scale.

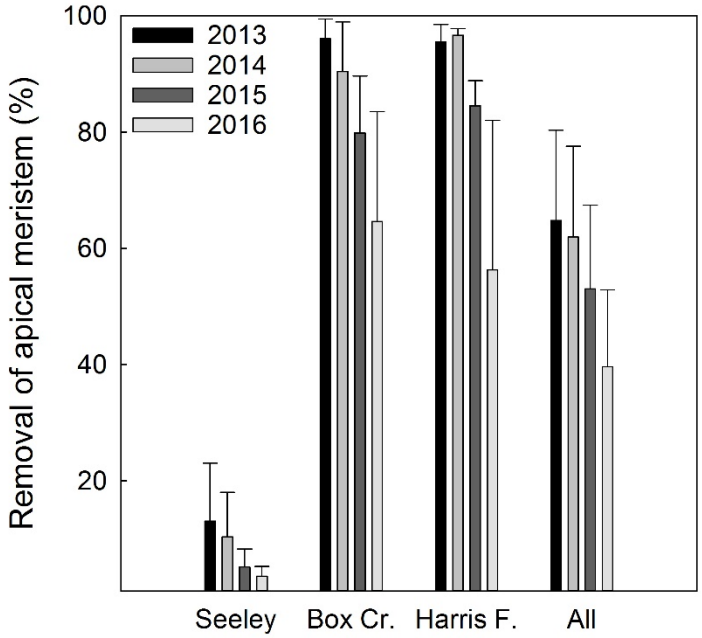


Figure 3–2. Browse of apical meristems vary across time and space. This figure presents the mean browse of apical meristems ± SE in fenceless plots at each fire. The comparison uses fenceless plots to assess "natural" levels of ungulate browse.

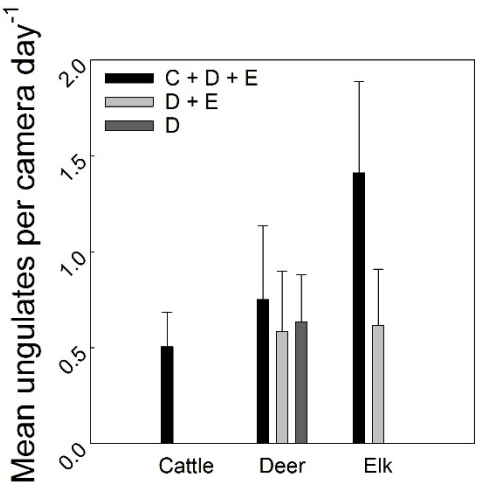


Figure 3–3. Differential exclusion of elk varied between unfenced and native ungulate plots. This Figure presents the mean camera counts of each ungulates species as ungulates per camera day ± SE. Colored bars represent the mean camera count for that species. The legend shows letters for ungulates that are allowed into the plot: "D" for deer-only plots, "D + E" for native ungulate plots, and "C + D + E" for unfenced plots allowing access to all ungulates. The comparison uses unfenced plots to assess "natural" levels of ungulate browse.

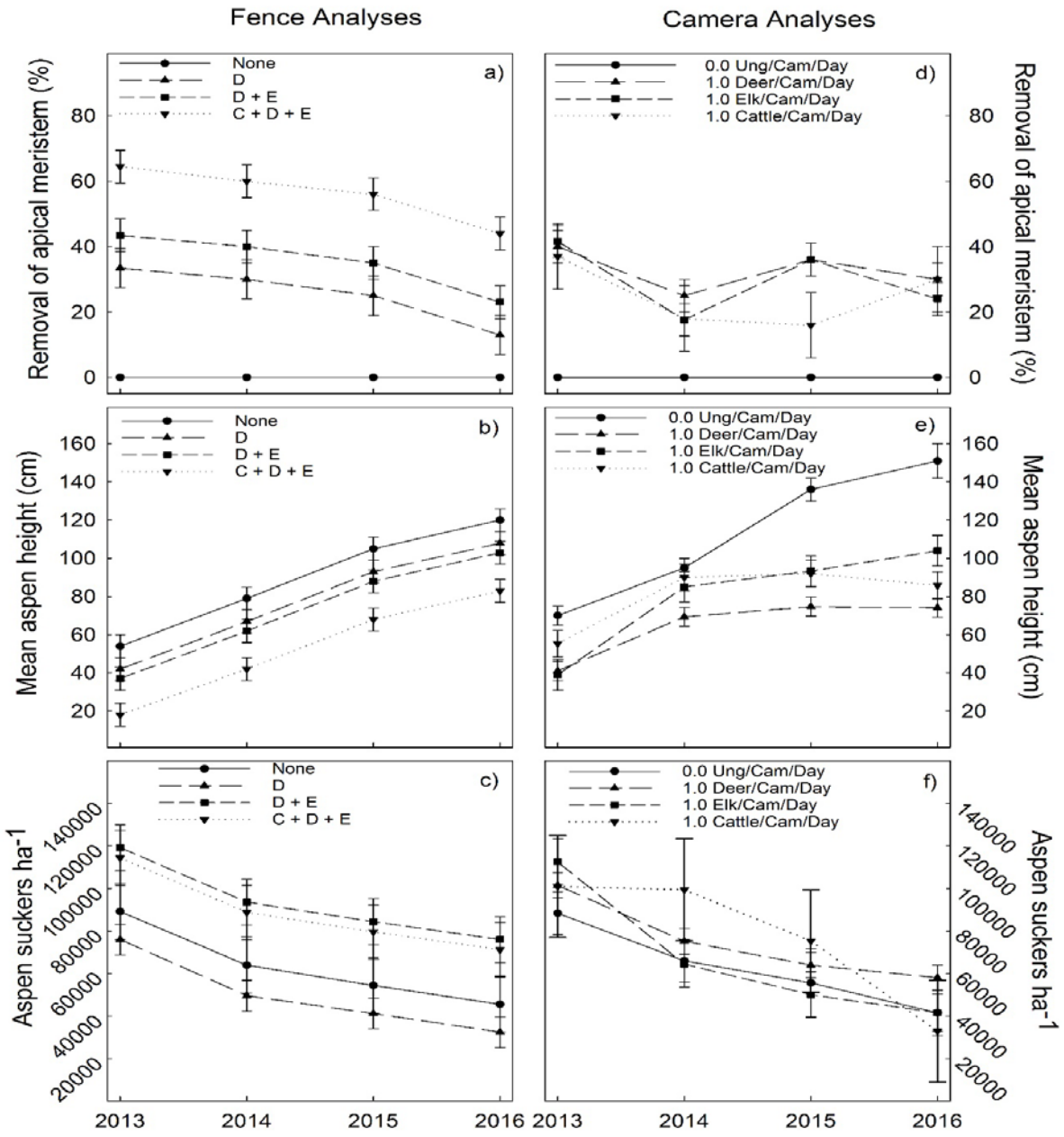


Figure 3–4. Plotted results from the analyses involving fences or plotted on the left column for (a) removal of apical meristems, (b) mean aspen height (cm), (c) aspen suckering density (ha^{-1}). The legend for the left column includes: "None" for no ungulates allowed, "D" for deer-only plots, "D + E" for native ungulate plots, and "C + D + E" for unfenced plots allowing access to all ungulates. The models involving camera counts are plotted on the right using model predictions for 1 ungulate per camera day and the standard error associated with that ungulate species' impact. Each of the response variable is the same order as the fenced analyses: (d) removal of apical meristems, (e) mean aspen height (cm), and (f) aspen suckering density (ha^{-1}).

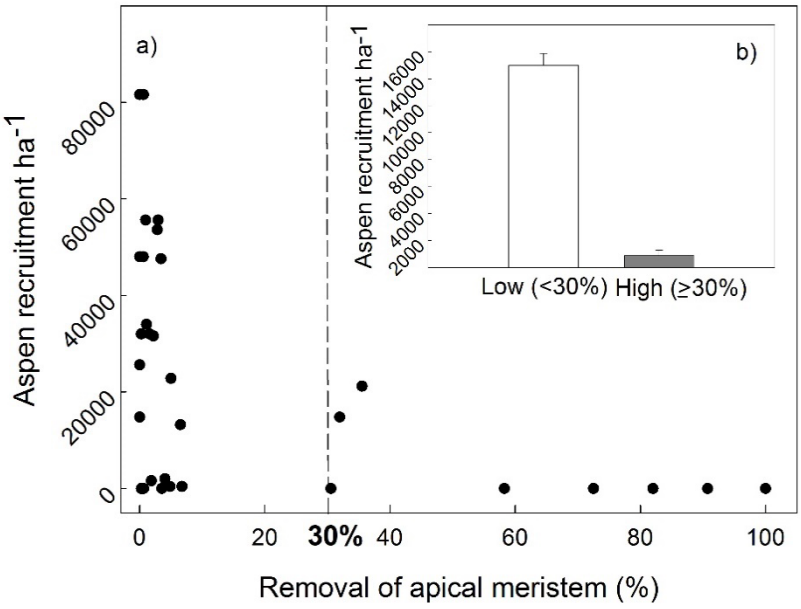


Figure 3–5. The large panel (a) shows the raw recruitment data (ha^{-1}) plotted with the removal of apical meristems (%). The inset panel (b) shows the modelled recruitment data using 30% removal of apical meristems as the browse threshold. The error bar is one standard error.

TABLES

Table 3–1. Results from analysis of fenced treatments from 2013-2016. F-statistic, p-value, degrees of freedom (df) and marginal model goodness of fit (r_m^2) are presented for each of three response variables. Browse refers removal of apical meristems (%), height is mean height (cm), and density is aspen ha^{-1}. Removal of apical meristem removal had 1 less degree of freedom in the fence category and 3 less in the interaction between fence and year.

		Browse		Height		Density	
Parameter	df	F	*p*	F	*p*	F	*p*
Fence	3,81	17	<0.001	10	<0.001	7.3	<0.001
Year	3,81	2.6	0.06	37	<0.001	8.2	<0.001
Fence x Year	6,81	28	0.58	0.6	0.77	0.3	0.58
Elevation	1,81	27	<0.001	19	<0.001	0.3	0.97
Full model r^2			$r_m^2 = 0.12$		$r_m^2 = 0.54$		$r_m^2 = 0.35$

Table 3–2. Effects of each ungulate species using camera counts was modelled through their individual effect on three response variables. F-statistic, p-value, degrees of freedom (df) and marginal model goodness of fit (r_m^2) are presented for each of three response variables. Browse is removal of apical meristems (%), height is mean height (cm), and density is aspen ha^{-1}. Removal of apical meristem removal had 1 less degree of freedom in the fence category and 3 less in the interaction between fence and year.

		Browse		Height		Density	
Model	df	F	*p*	F	*p*	F	*p*
Cattle	1,24	20	<0.001	19	<0.001	3.9	0.06
Year	3,24	1.4	0.26	3.1	0.05	1.4	0.27
Cattle x Year	3,24	0.16	0.92	1.6	0.23	1.2	0.35
Elevation	1,24	11	0.003	2.5	0.13	18	<0.001
Full model r^2			$r_m^2 = 0.67$		$r_m^2 = 0.54$		$r_m^2 = 0.09$
Mule Deer	1,24	119	<0.001	60	<0.001	5.5	0.03
Year	3,24	1.7	0.21	14	<0.001	4.8	0.01
Deer x Year	3,24	1.1	0.02	5.3	0.007	0.11	0.29
Elevation	1,24	6.4	0.36	0.2	0.67	1.1	0.95
Full model r^2			$r_m^2 = 0.81$		$r_m^2 = 0.87$		$r_m^2 = 0.24$
A. Elk	1,24	28	<0.001	8.7	0.007	5.6	0.03
Year	3,24	1.6	0.21	19	<0.001	2.6	0.07
Elk x Year	3,24	2.2	0.12	3.9	0.02	0.18	0.91
Elevation	1,24	0.61	0.44	0.01	0.91	4.2	0.05
Full model r^2			$r_m^2 = 0.56$		$r_m^2 = 0.67$		$r_m^2 = 0.36$

CHAPTER 4

Temporal Patterns of Ungulate Herbivory and Phenology of Aspen Regeneration and Defense

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ABSTRACT

Novel ungulate herbivory regimes are creating strong top-down effects on forest regeneration and recruitment, especially after fire. Defense strategies of tree species against ungulate herbivory include escape through vertical growth and resistance through the production of defense compounds. However, the differential impacts of ungulate species and plant defense responses seasonally are not well understood. Our objectives were: 1) Evaluate the monthly browsing activity of aspen by elk, deer and cattle across two years and 2) examine aspen defense responses to ungulates over a growing season. We used fencing and camera traps to characterize the differential impacts of ungulates on growth, and removal of apical meristems across a growing season. Finally, we examined how growth rates and removal of apical meristems covaried with foliar defense chemicals. Three years after fire, height differences among fence treatments diverged between full ungulate exclusion, deer-only plots, native ungulate plots, and unfenced plots. Aspen heights were: 108 ± 4 cm, 94 ± 4 cm, 89 ± 4 cm, and 65 ± 4 cm, respectively. Fenceless plots had the highest rates of removal of apical meristems by the ends of 2014 and 2015 (61% and 53%, respectively). Native ungulate plots, and deer-only plots both had similar removal of apical meristems in 2014 (37% and 39% respectively). High phenolic glycoside concentrations was correlated with 80% less removal of apical meristems and 4 times taller aspen by September. Therefore, as the season progresses into late summer, phenolic

glycosides represent an important deterrent of ungulate herbivory. In conclusion, our study shows that aspen height growth occurs mainly in June through August and that ungulate use of aspen is highest in July and August. This information allows managers to monitor aspen regeneration after fire during these critical months and mitigate ungulate impact on aspen if regeneration targets are not being met.

INTRODUCTION

Ungulate community composition and abundance are changing at a global scale, which can affect plant communities by altering herbivory regimes (Côté 2004, Spear and Chown 2009). In forest ecosystems, ungulates can have strong top-down effects on successional pattern through their preference for palatable, early successional species (Côté 2004). Meeting nutritional requirements is one of the most important determinants of animal fitness (Parker et al. 2009) and optimizing forage selection is under strong evolutionary pressure (Krebs et al. 1983). Ungulates navigate heterogeneous landscapes where nutritional content and palatability of forage vary across space and time (Bryant et al. 1991). The availability and nutritional content of forage change seasonally, and influence ungulate forage selection and movement. (Augustine and McNaughton 1998, Hobbs 1996, Olsen and Hansen 1977, Zweifel-Schielly et al. 2012). As a result, preference for tree species may change across the growing season.

Interspecific differences in ungulate anatomy drive forage preference (Clauss *et al*. 2010), and influence an ungulate's response to the physical and chemical defense strategies of plants (Robbins et al. 1991). Defense chemistry and plant growth rates (Poorter et al. 2004, Coley et al. 1985) are key determinants of plant susceptibility to ungulate herbivory (Lindroth and St. Clair 2013, Rhodes et al. 2017a). In the early growing season, young plant tissue, is

generally rich in nutrients such as starch and nitrogen, and low in secondary defense compounds that reduce palatability (Augustine and McNaughton 1998, Wan et al. 2014). The seasonal changes in palatability and production of defense chemicals influence temporal patterns of herbivory and alter forage selection by ungulates (Bryant et al. 1991, Boeckler et al. 2011, Zweifel-Schielly et al. 2012). Graminoids, forbs and woody plants experience differential changes in nutritional content across seasons with graminoids and forbs losing nutritional quality toward the end of the growing season more quickly than woody plants (Alldredge et al. 2002). Resorption of foliar nutrients prior to fall senescence reduces leaf nutrients (Lusk et al. 2003, Singh and Tripath 2007), and there is differential success in ungulate feeding guilds in consuming the woody forage in late fall (Hofmann 1989).kl For example, summer feeding trials showed that sheep (*Ovis aries*) preference for grasses and forbs decreased over a season, while proportional use of deciduous woody tissues of quaking aspen increased (Villalba et al. 2014). Seasonal shifts in the palatability and nutrient status of plant tissues are likely a strong determinant of ungulate browsing patterns across time.

Aspen is a keystone species that provides habitat for hundreds of plant and animal species in forests throughout North America (Ellison et al. 2005, Peterson and Peterson 1992). Aspen provide preferred habitat for deer (*Odocileus spp.*) and elk, (*Cervus canadensis*) (Beck et al. 2006) and are utilized by livestock due to the high productivity and quality forage in their understory (DeByle 1985). In aspen habitat, the use of graminoids, forbs, and woody species by ungulate species changes through the growing season (Villalba et al. 2014), and can intensify when multiple ungulate species are present particularly in dry years and in later summer months when competition for forage is high (Beck and Peek 2005). While ungulates utilize aspen habitat throughout the growing season, ungulate selectivity for aspen and aspen habitat increases in late summer (Beck and Peek 2005, Villabla et al. 2014). Aspen plays a foundational role in forest

succession where its post-disturbance regeneration success is critical in determining the structure and composition of mixed deciduous-conifer forests in North America (Calder and St. Clair 2012, St. Clair et al. 2013). Heavy ungulate herbivory is one factor that can lead to aspen regeneration failure and decline (Seager et al. 2013). Understanding timing of ungulate selectivity for aspen may help management mitigate areas where aspen is in decline due to chronic ungulate herbivory pressure (Frey et al. 2004, Seager et al. 2013).

Aspen uses mechanisms of tolerance, resistance, and vertical escape to mitigate the effects of ungulate herbivory (Rhodes et al. 2017a, Lindroth and St. Clair 2013). Two major defense compounds, phenolic glycosides and condensed tannins are found in aspen leaves. Phenolic glycosides reduce herbivory by insects (Donaldson et al. 2006) and mammals (Wooley et al. 2008) while the effects of condensed tannins on ungulate herbivory are less clear (Ayres et al. 1997). Condensed tannins at low doses are thought to improve the digestibility of proteins in ungulates, and may alleviate parasitic infection, but are avoided at high doses (Provenza and Villalba 2010, Bryant et al. 1991). Foliar concentrations of defense chemicals often fluctuate seasonally: Phenolic glycoside concentrations may decline as the season progresses (Lindroth et al. 1987, Boeckler 2011, Holeski et al. 2012) or increase (Wan et al. 2014) depending on environmental conditions. Condensed tannins tend to increase across the growing season (Holeski et al. 2012, Osier et al. 2000). Seasonal variation in foliar chemistry may influence ungulate use of aspen.

The objective of this study was to explore monthly and annual variability in ungulate herbivory in regenerating aspen forests following fire and track corresponding patterns in aspen growth and foliar chemistry. Fence exclosures and cameras were used to control for and identify elk, cattle and deer use and characterize their effects on regenerating aspen. We examined whether defense chemistry production in aspen changed across time and whether it was related to

variation in herbivory of aspen. We tested the following hypotheses: 1) Activity of elk, deer and cattle in aspen habitat will differ across time because of interspecific differences in behavior. 2) Native ungulates will have a greater impact on aspen height and removal of apical meristems than do cattle, given the interspecific differences in diet. 3) Ungulate herbivory of aspen will increase in the late summer season, because of the higher relative palatability of aspen late summer when compared to forbs and grass species. Further, we hypothesize that the detrimental effects of ungulates would increase across years, as aspen are repeatedly browsed. 4) Higher phenolic glycosides and condensed tannins will be correlated with lower browse rates and taller aspen given their reduction in palatability for ungulates.

MATERIALS AND METHODS

Study Area

Our study was conducted at three locations in Central and Southern Utah on the Manti-La Sal, Fishlake and Dixie National Forests that experienced fire in 2012 (Fig 1). The Seeley, Box Creek, and Harris Flat fires occurred in aspen-conifer mixed forests during peak fire season (June-July 2012). Fire sizes were 19400, 900, and 3400 ha, respectively. Site elevations ranged from 2440 – 3030 m. Thirty-year annual precipitation averages ranged from 25 – 64 cm at these sites (PRISM data) (Daly et al. 2009). Dominant tree species in the Seeley and Box Creek fires included; Aspen, Subalpine Fir (*Abies lasiocarpa* (Hooker) Nuttall) and White Fir (*Abies concolo*r (Gordon) Lindley ex Hildebrand). While the dominant conifer component of Harris Flat fire was Ponderosa Pine (*Pinus ponderosa* Douglas ex C.Lawson). Cattle (*Bos taurus*), mule deer (*Odocoileus hemionus*), and elk (*Cervus canadensis*) were the most abundant ungulates herbivores at the study sites.

Study Design

Across the three fires, eight sites were selected: three at Seeley, three at Box Creek, and two at Harris Flat. Sites were selected based on high fire severity (100% mortality of overstory), and pre-fire overstory stands having a significant aspen component (>50% aspen stems pre-fire). At each site, three different fence treatments and a fenceless control were randomly assigned in 30 x 30 m plots. The treatments differentially allowed access to: 1) all animals, 2) mule deer and elk (hereafter referred to as native ungulates), 3) mule deer only, and 4) full exclusion (no access). Full exclusion fencing was constructed with 3.1 m wooden posts buried 0.8 m into the ground at each corner. Fencing on each of the four sides was fixed on t-posts positioned at 3 m increments. The fencing consisted of 1.8 m of woven wire fencing and topped with a barbless wire set at 2 m. A gate was constructed in one corner of each fenced plot for researcher access. Mule deer only fencing was constructed in similar fashion as the full ungulate exclosure except that a 0.5 m gap was created at the bottom of the fence (VerCauteren et al. 2007). Mule deer were allowed entry through the 0.5 m gap but the fence effectively excluded both elk and cattle. Native ungulate access plots (livestock exclusion) consisted of 1.8 m wooden corner posts buried 0.6 m into the ground. The fencing was three barbed wires placed at heights of 0.2 m, 0.6 m, and 1.0 m. The fenceless control plots were marked at the four corners with 1.8 m u-posts buried 0.6 m into the ground. Deer and elk are able to jump into plots while cattle could not.

Within each plot, twenty-five 1 m^2 subplots were established in a 5 by 5 grid with a 5 m boundary from the fence edge. We surveyed our sites at the end of each month (May-September) in 2014 and 2015. During each survey period we characterized aspen height, removal of apical meristems, and collected leaf samples leaf chemistry analysis (2014 only).

Wildlife Cameras

In each experimental plot, we placed a trail camera to determine ungulate presence in all experimental plots by species (PC900 HyperFire Professional Covert Camera Trap) (Reconyx, Holmen, WI, USA). We used ungulate counts per camera per day (ungulate camera^{-1} day^{-1}) as an estimate of ungulate abundance in each of the experimental plots across all sites. Cameras were placed at the corner of each plot facing inward, and were set at medium sensitivity to capture five photos at three second intervals with a five minute rest period in between triggers. Each ungulate that appeared at least once in the five photo set were counted and these counts were summed and divided by the number of active camera days for each plot. Ungulate counts were summed from the 30 days preceding each aspen survey in order to characterize the relationship between ungulate abundance and aspen regeneration success (May – September).

Characterization of Aspen Height and Relative Ungulate Use

Within each 30 m^2 plot, twenty-five 1 m^2 subplots for surveys of aspen were established in a 5 by 5 grid with a 5 m boundary layer from the fence edge. Aspen height and meristem removal were measured every month May through September in 2014 and 2015. We measured relative use of aspen through examining the browse and removal of apical meristems. Removal of apical meristems was characterized by examining the percent of apical meristems removed by herbivory from the leading branch and the sub-leading branches in the top 15 cm of an aspen sucker (Wan et al. 2014, Jones et al. 2005, Strand et al. 2009, Rhodes et al. 2017b). Percent removal of apical meristems is reported as the sum of browsed meristems divided by the sum of all apical meristems measured.

Elevation

We associated GPS locations of each study plot with elevation taken from 30 x 30 m digital elevation models (DEM). The DEM we used was a United States Geological Survey product using the UTM Zone 12N projection and NAD83 geodetic network (Universal Transverse Mercator, North American Datum, respectively). We used ArcMap 10.3 (ESRI 2016) to associate our GPS locations with their respective elevation from the DEM.

Preparation of Plant Tissue for Analysis

Leaf tissue was collected from each site as a pooled sample where 2 fully expanded leaves from the upper portion of the aspen suckers were collected from 8 plants scattered across the experimental plot. Leaf tissue was frozen on site using dry ice and was then stored at -80^{0}C in the lab until analysis. Leaf samples were freeze dried for 48 h in order to preserve the chemical structure of the samples (Lindroth and Koss 1996). Freeze dried samples were ground and homogenized using a Wiley Mini-Mill (Thomas Scientific, Swedesboro, NJ) and dry mass was calculated using an analytical balance (GeneMate GP-600, ISC Brioexpress, Kaysville, UT).

Foliar Defense Chemistry

Phenolic glycosides (salicortin and tremulacin) were extracted from 50 mg of freeze-dried leaf tissue by combining 0.66 ml of methanol in 2 ml screw-cap micro-centrifuge tubes. The samples were then vortexed at high speed for 3 min (vortex cite). The liquid supernatant was removed and placed in the final micro-centrifuge tube. This procedure was repeated 2 more times until 2 ml of sample was extracted. Final concentrations of salicortin and tremulacin were assessed using high-performance liquid chromatography (HPLC, Agilent 1100 Series, Santa Clara, California, USA) with a Luna 2, C18 column (150 x 4.6 mm, 5 μs) at a flow rate of 1 ml

min^{-1}. Compound peaks were visualized under a UV lamp at a wavelength of 280 nm using purified salicortin and tremulacin standards isolated from aspen leaves according to the methods of Lindroth, Kinney, and Platz (1993).

Extractions of condensed tannins from leaf tissue was accomplished by placing 50 mg of freeze-dried leaf tissue in 1 ml of 70% acetone containing 10 mM ascorbic acid in 2 ml screw-cap micro-centrifuge tubes. The samples were then vortexted on high at 4°C for 20 min. The liquid supernatant was extracted and placed in a final micro-centrifuge tube, and the extraction was then repeated until 2ml of sample was achieved. Condensed tannin concentrations were quantified using a spectrophotometer (SpectraMax Plus 384, MDS, Toronto, Canada) using the modified butanol–HCl method (Porter, Hrstich & Chan 1986); purified condensed tannin standard isolated from aspen leaves were used to generate a standard curve (Hagerman and Butler 1980).

Foliar N and Starch

Nitrogen content was determined through the combustion method (Cambell 1991). We prepared 100 mg of dry leaf tissue in tin capsules and analyzed each sample using a nitrogen analyzer (TruSpec, CN Determinator, LECO Corporation, St. Joeseph, MI).

From each leaf sample 20 mg of tissue was suspended in 0.66 ml of 80% ethanol in a 2 ml screw cap microcentrifuge tube. Tubes were placed in a heater vortex at 80 ˚C for 20 minutes. The supernatant was transferred to a separate tube and the extraction was repeated 2 more times. The ethanol extracted tissue samples was then immersed in 1 ml of water in a 2 ml screw cap microcentrifuge tube. The samples were autoclaved for 1 hour at 135 ˚C and the water extract was transferred to another tube and dried overnight in a drying oven at 65 ˚C. Foliar starch concentrations were quantified using the Total Starch Assay Kit (Total Starch Assay Kit,

Megazyme Co., Wicklow, Ireland). The liquid sample was digested in 1 ml of α-amylase. Samples were then boiled for 20 minutes and subsequently cooled for 10 minutes, followed by the addition of 15 µl amyloglucosidase. The samples were mixed in a shaking water bath at 50 ˚C for 45 minutes. Samples were then plated out and 200 µl of GOPOD reaction mix was added to the samples. After 20 minutes at room temperature, absorbance was read at 510 nm on the spectrophotometer. We quantified sample starch concentrations using a starch standard curve from the Megazyme maize starch control at 20, 10, 5, 2.5 and 0 µg / 20 µl concentrations. We ran a positive control sample from aspen leaf tissue with known concentrations of starch to verify that the protocol was working correctly.

Statistical Analysis

In order to understand monthly patterns of aspen height and rates of removal of apical meristems we modeled them both using linear mixed effects models. We tracked these response variables monthly (May through September) throughout 2014 and 2015. We used fence type, month, year and the interaction between fence type and year as fixed effects. In order to understand interspecific use of aspen habitat, we modelled ungulate visitation through ungulate camera^{-1} day^{-1} using the fenceless plots and associated those number to the month end period. We used linear mixed effects models on cattle, deer and elk camera counts (ungulate camera^{-1} day$^{-1)}$ in order to characterize differences in temporal variation in ungulate visitation. . In order to understand ungulate influence on aspen as a function of foliar defense compounds, we modeled removal of apical meristems and aspen height using foliar concentrations of phenolic glycosides, condensed tannins, month and their interactions as fixed effects. For each statistical model we used a heterogeneity of variance identity structure using month nested within fire location, and also used fire location as a random factor.

For all statistical models mentioned above, we included a heterogeneity of variance identity structure across fence types. We checked assumptions of normality by visually inspecting a histogram of model residuals. We checked assumptions of heterogeneity of variance by visually inspecting fitted residuals versus predicted residuals. All analysis were generated using R statistical software (R Core Team 2016).

RESULTS

Ungulate Activity Patterns Across Seasons and Years

Elk visitation varied significantly across months and years, but the month by year interaction term was not significant (Table 1). Elk tended to have a unimodal seasonal pattern of visitation to the fenceless plots with high visitation in July and August (Fig 2). We estimated that July and August had 0.8 and 0.4 more elk per camera day compared to June (Fig. 2). Elk visitation was about 0.23 elk/camera day higher in 2015 compared to 2014 (Table 1). Mule deer visitation was relatively stable across all months and years sampled (Table 1) (Fig. 2). Cattle begin visiting in late July and increased their rates of visitation through August and September (Fig 2). Cattle visitation was controlled by allotment use.

Height Growth

Vertical growth of aspen was significantly influenced by all main effects (fence type, month, year, elevation) and the fence type by year interaction (Table 2). Aspen began the 2014 growing season at similar heights across all fencing types, however differential exclusion of ungulate species began to affect height growth as the months progressed. Throughout 2014, unfenced plots, deer-only, and native ungulate plots had similar height while fenceless plots

diverged, ending the year nearly half as tall as full exclusion fencing (Fig 3). However, by 2015, the height difference among treatments began to diverge with final height of the full exclusion, mule deer-only plots, native ungulate plots, and unfenced plots modelled at heights of: 108 ± 4 cm, 94 ± 4 cm, 89 ± 4 cm, and 65 ± 4 cm respectively (Fig 3). Height growth during the peak vertical growth period was nearly 100% in 2014, and over 50% in 2015 (Fig 3). Height growth showed seasonal patterns across both years with significant height growth May through July (Fig 3). Year over year growth doubled in 2014 and increased by 50% in 2015. Height growth was higher by 3.5 ± 0.1 cm for every 100 m rise in elevation.

Removal of Apical Meristems

Fencing treatment, month and elevation were significant terms significantly influenced removal of apical meristems, but year, and the fencing treatment by year interaction term were not significant (Table 2). Full exclusion plots had no incidence of removal of apical meristem and no evidence of ungulate trespass throughout the study period (Fig 4). Fenceless plots had the highest rates of removal of apical meristems in 2014 and 2015 (61% and 53%, respectively) (Fig 4). Native ungulate plots, and mule-deer only plots both had similar removal of apical meristems in 2014 (37% and 39% respectively). Native ungulate plots and mule-deer plots diverged in 2015 with 38% and 19% meristem removal by September. In 2014, removal of apical meristems in each of the fencing treatments followed a similar trend across the growing season. When averaged across fencing treatments, apical meristems removal increased approximately 20% each month beginning in June until peaking in August and September (Fig 4). Removal of apical meristems in 2015 increased at a lower rate across all fencing types indicating less relative use in 2015 when compared to 2014 (Fig 4). Removal of apical meristems decreased approximately 10% with each 100m increase in elevation.

Foliar Defense Chemistry Effects on Meristem Removal and Height

Foliar concentrations of phenolic glycosides reduced removal of apical meristems in both August and September ($p = 0.01$ and $p < 0.01$, respectively). Our model estimates show that a plot with 5% concentrations of foliar phenolic glycosides was estimated to have 60% meristem removal whereas a plot with 15% phenolic glycosides was estimated to have less than 20% in both August and September (Fig 5a). Foliar concentrations of condensed tannins were not statistically significant for removal of apical meristems (Fig 5c).

Foliar concentrations of phenolic glycosides were associated with increased height in both August and September ($p = 0.01$ and $p < 0.01$, respectively). Our model estimates show that aspen with 15% concentrations of foliar phenolic glycosides were estimated to be 4 times taller than with 5% phenolic glycosides by September 2014 (Fig 5b). Foliar concentrations of condensed tannins were not statistically significant for aspen height (Fig 5d).

DISCUSSION

Ungulate Species Visitation Across the Growing Season

Our three predictions were generally well supported by the results of our experiment. First, visitation to aspen habitat and relative use of aspen differed significantly among ungulate species, as predicted. Mule deer showed a consistent visitation rate over the summer months, while elk had a unimodal pattern of visitation that peaked in July and August (Fig. 2). In September, high cattle activity compensated for reductions in wildlife activity (Fig 2). Second, our prediction that herbivory of aspen by ungulates would increase in the late summer season, and that the detrimental effects of ungulates would increase across years, was well supported (Figs. 3 and 4). Finally, our third prediction that defense chemicals reduce ungulate herbivory

was supported for phenolic glycosides but not condensed tannins, and that this relationship was only significant late in the growing season. We observed that increased aspen height and reduced removal of apical meristems was correlated with high phenolic glycosides concentrations in September (Figs 5a and b)). Our results elucidate the response of aspen to ungulate community herbivory across season and years.

Differential use of habitat and forage by multiple ungulates may have cascading effects on plant communities and forest structure (Stewart et al. 2002, Beck et al. 2006). We found differential patterns of relative use throughout the season and across years by mule deer, elk and cattle. Elk frequented our study plots later in the growing season peaking in July and August (Fig. 2). Intermediate feeders such as elk are highly selective across season and their selectivity is correlated with plant abundance (Zweifel-Schielly et al. 2012). As forbs and grasses begin to senesce and lose both quality and abundance, aspen may become a preferred plant species (Villalba et al. 2014). Mule deer visitation and use of aspen was consistent throughout the growing seasons of both 2014 and 2015 (Fig. 2). Browsers may have disproportionately higher selection for aspen meristems and foliar tissue throughout the growing season and are less dependent on grass and forb species due to their ability to use woody plant material more effectively (Hofmann 1989). Cattle visited our sites beginning in late July, and were observed foraging throughout September (Fig. 2). Visitation by native ungulates dropped significantly in September, but ungulate use of the sites was maintained as cattle relative use increased with a concurrent drop in elk relative use (Fig. 2).

Removal of common food resources by cattle in late summer may cause avoidance of habitat by elk, and could explain the observed reduction in elk visitation in September after cattle begin to use the experimental plots in July (Fig 2) (Stewart et al. 2002, Madhusudan 2004). Native ungulates tend to avoid areas where cattle forage both in the short and long term (Stewart

et al. 2002). Also, cattle compete more strongly with elk given the higher overlap in diet (Stewart et al. 2002). Cattle can consume preferred plant species and reduce selectivity of that habitat by native ungulates (Madhusudan 2004). It is unclear if camera counts of elk were less in September due to changing plant communities, or the increased visitation of cattle.

Ungulate Impact on Removal of Apical Meristems and Height

Vertical escape from ungulate herbivory is critical to aspen forest replacement after fire (Rhodes et al. 2017a, St. Clair and Lindroth 2013). Removal of apical meristems is strongly related to reductions in sucker height of aspen (Rhodes and St. Clair 2018), and lower recruitment into the overstory (Rhodes et al. 2017a). The active height growth period for aspen occurred from May through July, and plateaued in August and September (Fig. 3). This is typical of temporal growth patterns of other *Populus spp* (Yu et al. 2001). Aspen in full wildlife fencing were approaching the critical 150 cm threshold of vertical escape from ungulate herbivory (Fig. 3) and will likely recruit successfully (Rhodes et al. 2017b, Seager et al. 2013). Therefore, focused management of ungulates during the critical growth period may improve the probability of aspen recruitment.

Ungulate herbivory at sufficient levels can block vertical escape of aspen for decades (Rhodes et al. 2017a), and quantifying removal of apical meristems show robust thresholds for aspen escape (Rhodes and St. Clair 2018). Our study showed high rates of relative ungulate use by the ungulate community in the fenceless plots, where removal of apical meristems approached critical thresholds greater than 30% that can impair aspen recruitment success (Lindroth and St. Clair 2013, Rhodes and St. Clair 2018). Monitoring removal of apical meristems during the critical growth period is a good indicator of potential aspen recruitment success (Rhodes et al.

2018). Our results provide insight into both the timing of aspen height growth and ungulate use, which can inform managers on when and where mitigation of ungulate use can be targeted

Foliar Chemistry

Ungulates select for more palatable and nutritious forage in feeding trials (Wooley et al. 2008, Villalba et al. 2014). Phenolic glycosides deters ungulate herbivory in feeding trials (Wooley et al. 2008) and foliar nitrogen is associated with higher protein content and palatability (Poorter et al. 2004). However, the effect of herbivores on foliar leaf traits has been less clear in studies conducted in natural environments (Rhodes et al. 2017a). We found evidence of higher rates of aspen herbivory related to lower concentrations of phenolic glycosides and late in the growing season (Figs. 5a, 5c). While phenolic glycosides influence ungulate browse of aspen in the field (Fig. 5), foliar concentrations of phenolic glycosides had low predictive power (r_m^2 = 0.07) (Table 3). However, even small differences in nutritional quality can have large influences on ungulate performance (White 1983). High phenolic glycosides concentrations were correlated with strong reduction in predicted height (Fig 5b). The differences in phenolic glycosides we observed had important impacts on plant performance and resulting use by ungulates (Fig 5a and b), as seen in other studies (Wooley et al. 2008, Rhodes et al. 2017a). This is consistent with feeding trials showing that increases in phenolic glycosides to reduce ungulate consumption of aspen in feeding trials (Wooley et al. 2008). Our results predict similar trends of higher removal of apical meristems with less concentrations of phenolic glycosides late in the growing season (Fig 5a).

Management and Ecological Implications

Ungulates have strong top-down effects on forest development (Côté et al. 2004) and can cause loss of aspen forests on local scales especially after disturbance (Rhodes et al. 2017a). Monitoring of removal of apical meristems can be used for measuring ungulate impact on aspen regeneration and recruitment and establish appropriate thresholds for mitigation (Rhodes and St. Clair 2018, Rhodes et al. 2018). Using camera counts or removal of apical meristems as a relative use indicator, we identify July and August as periods of high use of aspen by deer, elk and cattle (Fig 4). In areas where there is high relative ungulate use (>30% removal of apical meristems), we suggest targeted management action to protect aspen through temporary fencing or altering ungulate behavior though hazing, changes in hunting seasons, and exclusion of cattle. Removal of apical meristems over 30% has been shown to reduce recruitment potential of aspen both in intact stands and after fire (Rhodes et al. 2018, Strand et al. 2009). Further, we reiterate the influence of foliar defense in mediating ungulate selection of aspen and subsequent effects on aspen forest recruitment following disturbance. Increased concentrations of leaf defense chemicals and high vertical growth rates are essential to aspen recruitment (Seager et al. 2013, Rhodes et al. 2017a). Having a clear understanding of the relationship between foliar palatability and relative use or height growth can allow manager to adjust ungulate numbers in areas where aspen is less palatable.

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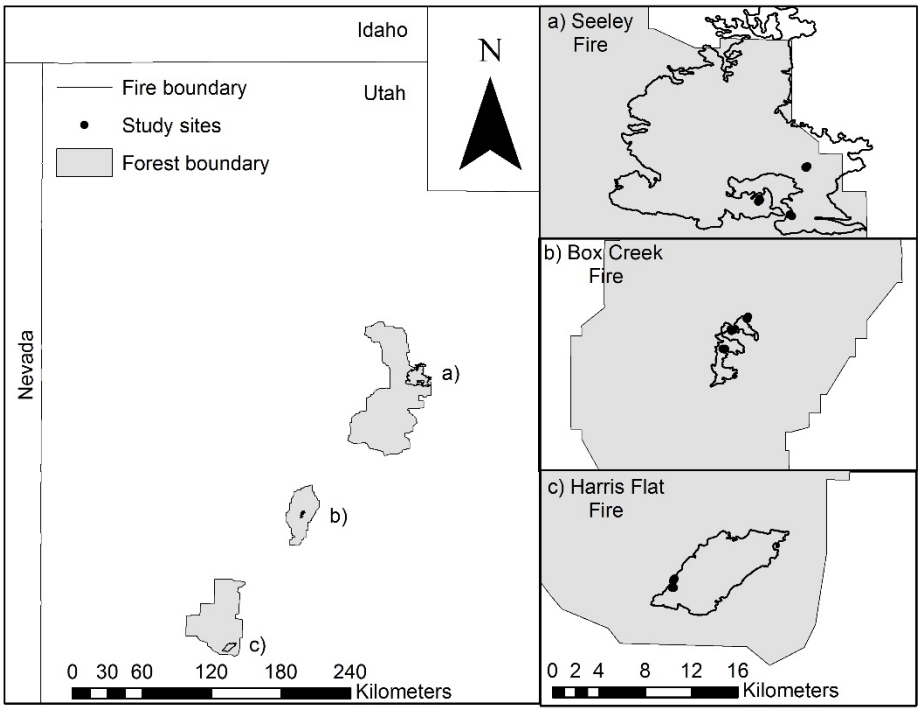


Figure 4–1. This map shows the larger study area encompassing central and southern Utah. Forest boundaries are in gray and fire perimeters are in black. Three inset maps display black dots at the study sites. At each a four block differential fencing treatment is located. All three inset maps use the same scale.

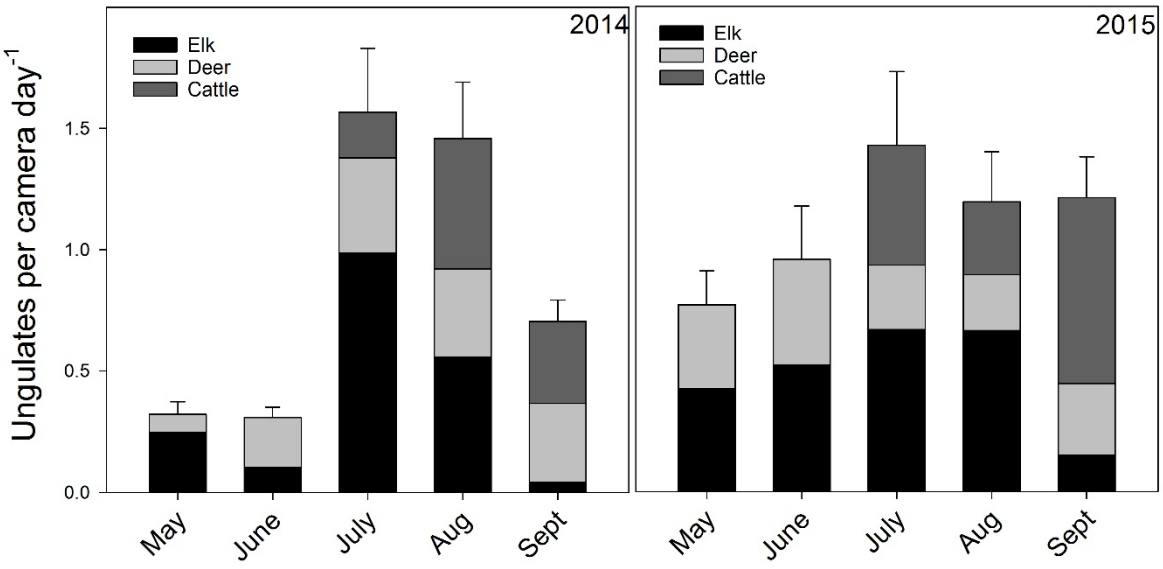


Figure 4–2 . Presented here are the raw visitation rates in ungulates per camera day-1 in separate panels for 2014 and 2014. Ungulate species are color coded and stacked and displayed across each sampled month. The error bar is standard error for the total ungulate community visitation rates.

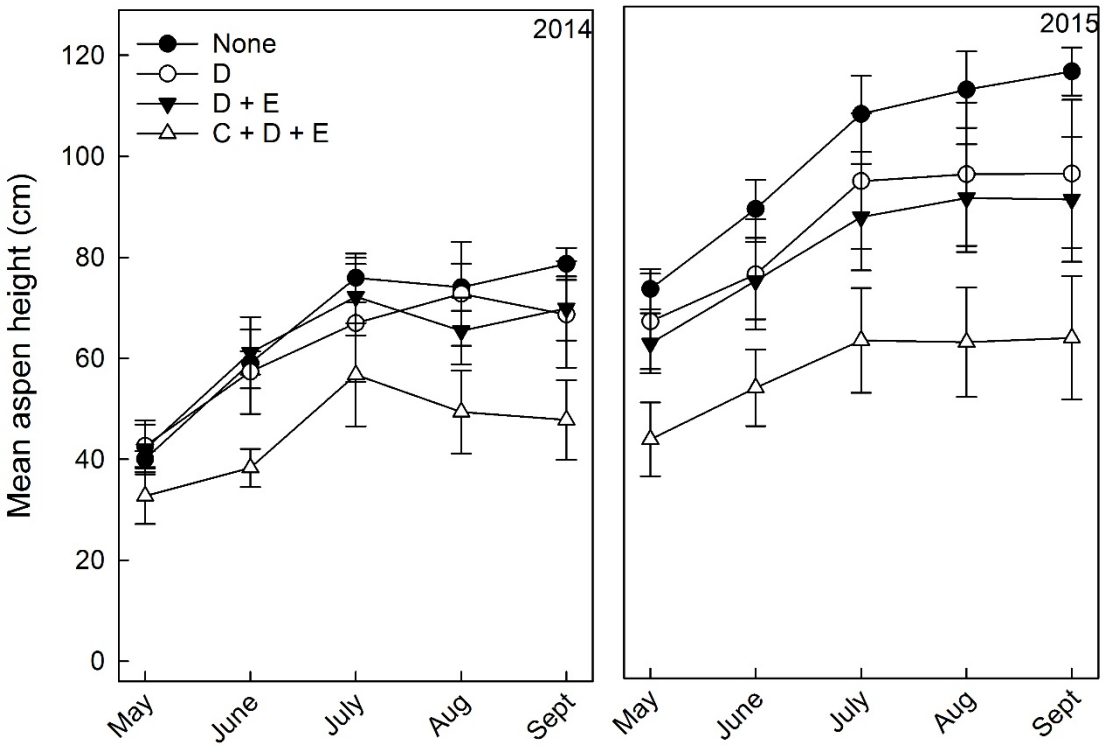


Figure 4–3. Presented here are the raw data for mean aspen height (cm) from May through September in separate panels for 2014 and 2015. Legend titles refer to ungulates that were allowed access to the plots. None – no access to these plots. D – deer-only fencing. D + E – livestock fencing that excluded only cattle. C + D + E – fenceless plots. Error bars are one standard error.

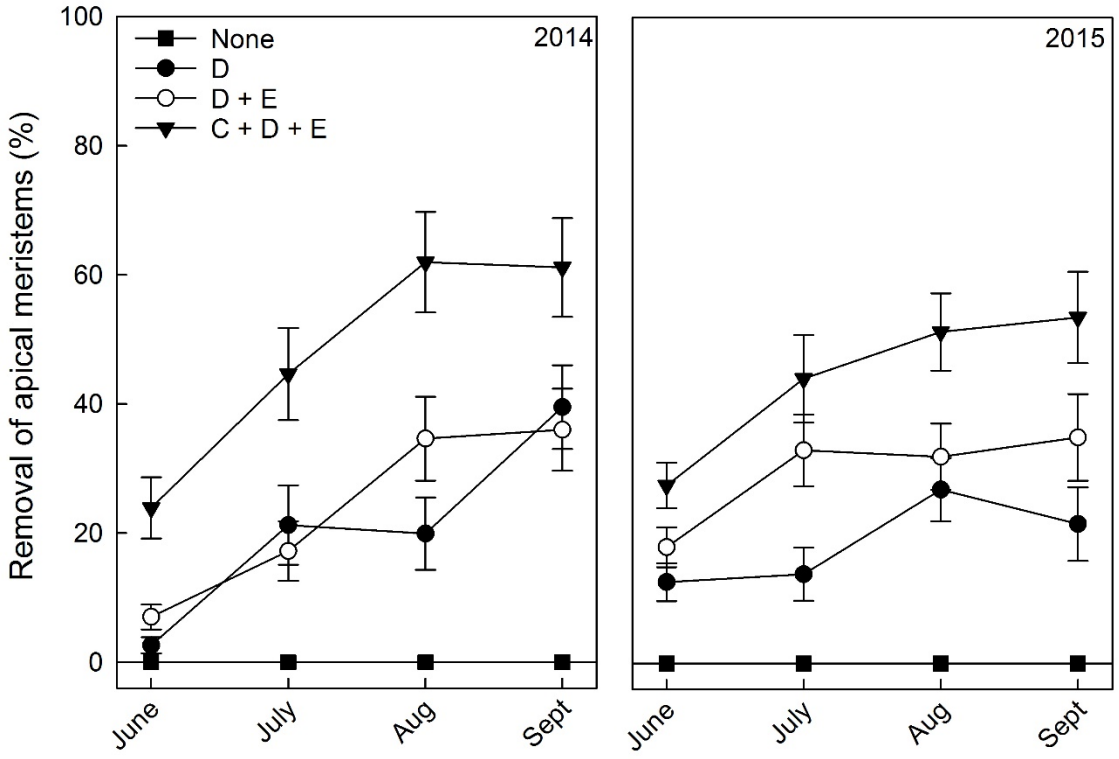


Figure 4–4. Presented here are the raw data for removal of apical meristems (%) from May through September in separate panels for 2014 and 2015. Legend titles refer to ungulates that were allowed access to the plots. None – no access to these plots. D – deer-only fencing. D + E – livestock fencing that excluded only cattle. C + D + E – fenceless plots. Error bars are one standard error.

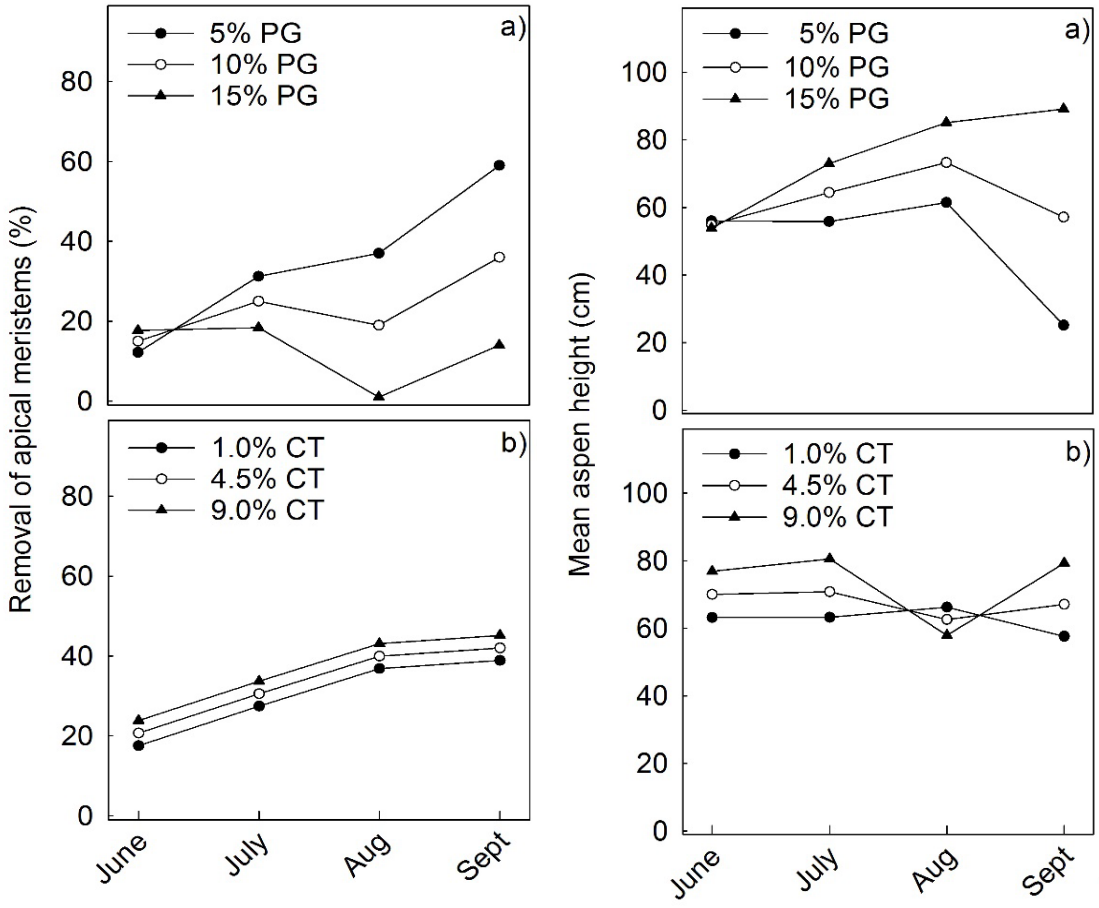


Figure 4–5 Presented here are the predicted effect that foliar chemistry has on rates of removal of apical meristems (%) and height (cm) from May through September in for 2014. Panels a) and b) shows the predicted values of meristem removal across the range of observed foliar concentrations of phenolic glycosides and tannins. Panels c) and d) show predicted values of height across the range of observed foliar concentrations of phenolic glycosides and tannins.

TABLES

Table 4–1. Results from analysis of monthly native ungulate visitation rates from May through September in 2014 and 2015. Degrees of freedom, F-statistic, p-value, and full model marginal and conditional goodness of fit (r^2) are presented for mule deer, and American elk.

	Parameter	df	F	p	$r^2_{m/c}$
M. Deer	Intercept	1,59	2.5	0.11	0.05/0.44
	Month	4,59	0.2	0.92	
	Year	1,59	0.1	0.92	
	Month:Year	4,59	0.9	0.41	
A. Elk	Intercept	1,59	1.6	0.21	0.37/0.51
	Month	4,59	4.1	<0.01	
	Year	1,59	3.8	0.05	
	Month:Year	4,59	0.4	0.84	

Table 4–2. Results from analysis of monthly mean aspen height and removal of apical meristem rates May through September in 2014 and 2015. Each parameter is listed with its associated degrees of freedom, F-statistic, p-value, and full model marginal and conditional goodness of fit (r^2) are presented for aspen height and removal of apical meristems.

	Parameter	df	F	p	$r^2_{m/c}$
Height	Intercept	1, 365	465	<0.01	0.76/0.79
	Fence type	3, 365	28	<0.01	
	Month	4, 365	19	<0.01	
	Year	2, 365	157	<0.01	
	Elevation	1, 365	13	<0.01	
	Fence:Year	6, 365	3.1	<0.01	
Meristem rem.	Intercept	1, 225	10	<0.01	0.34/0.54
	Fence type	2, 225	22	<0.01	
	Month	2, 225	4.4	<0.01	
	Year	3, 225	1.2	0.27	
	Elevation	1, 225	11	<0.01	
	Fence:Year	4, 225	1.7	0.15	

Table 4–3. Results from analysis of height and removal of apical meristems as a function of foliar chemistry on a monthly basses from May through September in 2014. Each parameter is listed with its associated degrees of freedom, F-statistic, p-value, and full model marginal and conditional goodness of fit (r^2) are presented for aspen height and removal of apical meristems.

	Parameter	df	F	p	$r^2_{m/c}$
Height	Intercept	1,113	1101	<0.01	0.37/0.38
	PG (%)	1,113	8.3	<0.01	
	Month	3,113	10	<0.01	
	PG (%):Month	3,113	4.8	<0.01	
Height	Intercept	1,113	137	<0.01	0.08/0.22
	Tannin (%)	1,113	0.1	0.47	
	Month	3,113	3.9	<0.01	
	Tannin (%):Month	3,113	0.4	0.77	
Meristem rem.	Intercept	1,113	6.7	<0.01	0.07/0.25
	PG (%)	1,113	2.3	0.13	
	Month	3,113	2.2	0.09	
	PG (%):Month	3,113	4.1	<0.01	
Meristem rem.	Intercept	1,113	4.4	0.04	0.01/0.26
	Tannin (%)	1,113	0.5	0.47	
	Month	3,113	1.8	0.16	
	Tannin (%):Month	3,113	0.1	0.94	

CHAPTER 5

Ungulate Herbivory Alters Leaf Functional Traits and Recruitment of Regenerating Aspen

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ABSTRACT

Herbivory by ungulates can affect forest regeneration success, but their long-term impacts on tree function and recruitment are less studied. We evaluated strategies of resistance, tolerance, and vertical escape against ungulate herbivory by evaluating leaf traits (photosynthesis, morphology and chemistry) and growth rates of aspen in the presence and absence of ungulate herbivores 1, 2, 3 and 26 years after fires initiated aspen suckering. Over the initial three-year period, approximately 60% of aspen stems in unfenced plots showed evidence of being browsed by ungulates. After three years, aspen in unfenced plots had smaller leaves, were 50% shorter, had 33% lower nonstructural carbohydrate concentrations, and 33% greater concentrations of condensed tannins, when compared to fenced aspen. Aspen exposed to ungulate herbivory over a 26-year period maintained smaller leaves, had lower annual radial growth rates and were still below the critical height threshold of 2 meters required to escape ungulate herbivory for successfully recruitment. In contrast, the average height of aspen protected from ungulates was approaching 6 meters. Over the 26-year period leaves in unfenced plots had 41% lower nonstructural carbohydrate concentrations and greater expression of defense compounds: condensed tannins (63%) and phenolic glycosides (102%) than leaves in fenced plots. Photosynthetic rates were slightly higher 3-years post-fire and 50% higher in unfenced plots 26 years post fire suggesting that changes in leaf anatomy and chemistry due to ungulate

herbivory did not interfere with photosynthesis. Our data suggest that ungulate browsing increases investment in chemical defense, lowers nonstructural carbohydrate concentrations and reduces leaf area, which decreases the recruitment potential of regenerating aspen.

INTRODUCTION

Plants have evolved a wide variety of mechanisms to tolerate, resist or escape herbivory which balance a plant's ability to defend against herbivory with its susceptibility to being eaten (Herms and Mattson 1992, Coley *et al*. 1985). Plants employ three major defensive strategies: tolerance (recovery from herbivory), resistance (herbivore deterrence), and escape, (reducing exposure to herbivores) (Strauss and Agrawal 1999 and Lindroth and St. Clair 2013). Herbivory can strongly influence plant functional traits (Nicotra *et al*. 2011, Carmona *et al*. 2011) including changes in growth patterns, leaf morphology, chemistry and metabolism (Nictotra *et al*. 2011, Carmona *et al*. 2011, Cardenas *et al*. 2014). However, we still lack a complete understanding of how leaf traits are shaped by herbivory and how changes in leaf morphology and chemistry affect tolerance, resistance and escape of plants from herbivores.

Plant morphological, chemical and phenological traits influence herbivore selection of plants (Cardenas *et al*. 2014). Leaf functional traits that determine tolerance, resistance and escape from herbivory are often interrelated. For example, nitrogen content, nonstructural carbohydrates and specific leaf area influence plant susceptibility to herbivory (Porter et al. 2004), yet these traits also affect tolerance and escape through their influence on growth rates (Agrawal 1999, Carmona et al. 2011). Production of secondary defense compounds can be metabolically expensive, and slow growth rates, but effectively reduce rates of herbivory (Donaldson *et al*. 2006, Boeckler *et al*. 2011). High nitrogen content and specific leaf area are correlated with higher rates of photosynthesis and growth (Ackerly and Reich 1999), but also

make leaves more palatable to herbivores (Poorter *et al*. 2004). Also, leaf size and shape alters susceptibility to herbivory (Schudlt *et al*. 2012, Carmona *et al*. 2011) and large leaf size may attract herbivores (Garibaldi *et al*. 2011).

Induction of defense chemistry expression and alterations in leaf morphology and nutrient status can create resistance to herbivory by reducing herbivore preference (Agrawal 2007). However, there is theoretically a trade-off between plant growth and defense against herbivory (Bryant *et al*. 1983, Coley *et al*. 1985) in which highly defended individuals tend to have slower growth rates (Donaldson *et al*. 2006) although there are exceptions when resource availability is high (Wan *et al*. 2014). While short-term plant functional trait and growth responses to herbivory are documented in the literature (Stevens and Lindroth 2005, Donaldson and Lindroth 2007), few studies have documented long-term, chronic impacts of herbivory on leaf traits that impact tolerance, resistance and escape particularly in the context of forest recruitment potential.

Ungulate herbivory can severely reduce plant function (Bryant *et al*. 1983) and hinder the establishment and growth of tree species (Canham *et al*. 1994). As a result, ungulates have been considered "keystone herbivores" due to their impact on forest regeneration and successional processes (Côté *et al*. 2004). Ungulate herbivores reduce woody plant growth through decreases in leaf area and loss of apical meristem tissue (Palacio *et al*. 2008, Millard and Grelet 2010). However, many studies examining the effects of ungulates on tree function use simulated browsing in controlled settings (Strauss and Agrawal 1999, Palacio *et al*. 2013) and examine short-term responses (but see Palacio *et al.* 2008). The artificiality of simulated herbivory draws into question how much inference can be drawn from these studies (Agrawal 1998). Given that ungulate herbivory occurs at broad spatiotemporal scales (Côté *et al*. 2004), research relevant to

management should examine both the short and long-term effects of ungulate herbivory in the context of plant functional responses to ungulate herbivory at the landscape scale.

Aspen (*Populus tremuloides,* Michx.) is an early-succession species that is broadly distributed across North America (Peterson and Peterson 1992). There is increasing concern about loss of aspen across portions of its range, due to changes in fire regimes and ungulate herbivory (Frey *et al*. 2003). Reestablishment of aspen stands after fire through root suckering is crucial to supporting aspen forests in the Intermountain West (Smith *et al*. 2011a). However, aspen is particularly susceptible to ungulate herbivory as root suckering occurs (Seager *et al*. 2013).

Aspen is a model organism for studying the impacts of herbivory in forest systems because it is eaten by hundreds of herbivores across its range and its defense mechanisms are well characterized (Lindroth and St. Clair 2013). Aspen, like all plants, must balance its investment in chemical and physical defense strategies with maintenance costs and growth. To resist herbivory, aspen employs defense chemicals from the shikimic acid pathway: phenolic glycosides, and condensed tannins (Boeckler *et al*. 2011). Phenolic glycosides deter feeding from both insect and mammalian herbivores (Donaldson and Lindroth 2007, Wooley *et al*. 2008, Lindroth and St. Clair 2013). Condensed tannins reduce the nutritional value of plant tissue (Robbins *et al*. 1991). While many studies have focused on how variability in physical and chemical defenses affect herbivore selection and performance, much less is known about how leaf functional traits including leaf morphology and foliar chemistry vary in response to ungulate herbivory under natural field conditions over multiple years.

The objective of this study was to evaluate how herbivory by ungulate communities affect the morphological and chemical traits of leaves and recruitment potential of regenerating aspen over time. We evaluated the effects of ungulates on aspen in the context of defense

strategies of resistance, tolerance, and vertical escape by measuring leaf morphology, photosynthesis, foliar concentrations of nonstructural carbohydrates, nitrogen, defense chemistry and growth rates of aspen in the presence and absence of ungulates 3 and 26 years after fires initiated aspen suckering. We explored the following hypotheses: 1) Aspen demonstrates tolerance to ungulate herbivory by maintaining photosynthesis, foliar starch concentrations and growth rates in response to browsing over time. 2) Aspen exhibits resistance traits to ungulate herbivory through induction of condensed tannins and phenolic glycosides, decreases in leaf N and reductions in leaf size and specific leaf area. 3). Ungulate herbivory impairs vertical escape of aspen 4) Short term and long term herbivory will differ in relation to tolerance, resistance, and escape mechanisms.

MATERIALS AND METHODS

Study Area

Our study was conducted in mixed aspen-conifer forests across three national forests of central and southern Utah. Study sites were located in areas that had experienced fire, and initiated a strong aspen regeneration response. We sampled from eight sites across three fires that burned in 2012 (experiment 1) and three sites from a fire that burned in 1989 (experiment 2) (Fig. 1). Fire sizes ranged from 140 to 19400 hectares. Site elevations ranged from 2795 – 3000 m and slope ranged from 0 - 5 degrees. Thirty year mean annual precipitation ranged from 38 – 80 cm. Dominant tree species in the Seeley, Ferron and Box Creek fires include; Aspen, Subalpine Fir (*Abies lasiocarpa* (Hooker) Nuttall) and White Fir (*Abies concolo*r (Gordon) Lindley ex Hildebrand). The dominant conifer component of Harris Flat fire is Ponderosa Pine (*Pinus ponderosa* Douglas ex C.Lawson). Cattle (*Bos taurus*), Mule deer (*Odocoileus*

hemionus), and American elk (*Cervus elaphus*) were the most abundant ungulates herbivores at the study sites.

Study Design

Two independent exclosure experiments established at different time points (1989 and 2012) were used to test how ungulate herbivory affects aspen leaf traits and sapling growth rates over time. Experiment one was established at 8 locations spread across three independent fires (Seeley, Box Creek, Harris Flat) that occurred in 2012 (Fig. 1). Sites were selected based on high fire severity (100% mortality of overstory trees), and overstory stands having a significant aspen component (> 40% stems/ha). Each study site consisted of a single 30 x 30 m plot surrounded by a wildlife fence paired with a 30 x 30 m unfenced plot separated by at least 50 meters. Replicate fenced and unfenced plot pairs within each fire were separated by at least 4 kilometers. The fences were installed in October 2012. The wildlife fence excluded elk, deer, cattle and sheep but allowed access to all other herbivorous mammals through 14 x 14 cm gaps in the wire. The wildlife fences were constructed with 3.1 m wooden posts buried 0.8 m into the ground at each corner with woven wire 1.8 m in height around the perimeter topped with a barbless wire set at 2 m. Control plots (unfenced) were marked at the 4 corners with 1.8 m u-posts buried 0.6 m into the ground.

Experiment two was nearly identical to experiment 1, but was established more than two decades earlier in the Ferron Fire that occurred in 1989 (Walker *et al*. 2015). Three replicated fence-unfenced plot pairs were constructed following the fire. The plots were slightly larger (0.1 ha) than in experiment 1 (0.09 ha). The three replicated fenced and unfenced plot pairs were separated by at least 3 kilometers.

Leaf Collection

Leaf tissue was collected from 8 aspen saplings (two from each of four equally divided quadrants) in both the fenced and unfenced plots. Leaf measurements and sample collection occurred in early August of 2013, 2014, and 2015 for experiment 1 and early August of 2015 for experiment 2.

Ungulate Browse Intensity

Browse intensity was characterized by examining the percent of apical meristems removed by herbivory from the leading branch and the sub-leading branches in the top 15 cm of an aspen sucker (Jones *et al.* 2005, Wan *et al*. 2014). Aspen suckers with multiple subleaders were quite common, and the entire stem was considered browsed if all meristems were removed. Site-specific percent browse is reported as the number of browsed stems within each transect versus stems that have apical meristems intact. Meristems were characterized as intact, browsed, or other (damage from frost, pathogen infection, and drought).

Dendrochronology and Growth

For experiment one, aspen height was measured on all aspen suckers in twenty-five 1 m^2 subplots in a 5 x 5 grid in each 30 x 30 m plot. Height was measured from the base of the aspen sucker to the tallest aspen stem. Height was averaged across all subplots to get a plot average.

Dendrochronology techniques were used in experiment 2 to determine tree age and radial growth rates of aspen trees over time. At each of the three sites, we cut five trees inside both the fenced and fenceless plots at ground level and collected stem samples at ground level for tree ring analysis. Stem samples were taken back to the lab where they were dried at 60 ˚C for 48 hours. The samples were sanded with 300 grit on a belt sander and then polished by hand with

600 grit sand paper. Then rings were counted and widths were measured using a binocular microscope (VanGuard 1275ZP, Vee Gee Scientific, Kirkland, WA) with a measuring stage (Velmex TA4030H1-S6 CO4060, Velmex Bloomfield, NY).

Leaf Morphology

Two fully expanded leaves without evidence of browsing were collected from the upper portion of aspen suckers from 8 plants at each site. Leaves were frozen on site using dry ice and were then stored in a - 80 ˚C freezer until they could be freeze-dried in the lab. Leaves were freeze-dried for 48 hours in order to preserve the leaf chemistry (Lindroth and Koss 1996). Dry mass was measured using an analytical balance (GeneMate GP-600, ISC Bioexpress, Kaysville, UT). Leaf area was calculated using a leaf area meter (LI-COR 3000 Environmental, Inc., Lincoln NE). Specific leaf area was calculated as square centimeters of leaf area per gram of dry weight leaf tissue. Freeze-dried samples were ground and homogenized using a Wiley Mini-Mill though a #20 screen (Thomas Scientific, Swedesboro, NJ) in preparation for biochemical analysis.

Photosynthesis measurements

Prior to collecting the leaves for lab analysis (see above) measurements of photosynthesis were conducted (16 leaves per plot) during the August 2015 sampling period. Measurements were taken from a single, fully expanded, attached leaf using a LI-COR 6400 gas exchange system (LI-COR, Lincoln, NE). Photosynthetic photon flux density was set to 2000 µmol m^{-2} s^{-1} using the LI-COR 6400-40 LED light source to determine light-saturating rates of photosynthesis (Asat) (Calder et al. 2011). A CO_2 mixer was used to create a baseline concentration of 400 µmol

mol^{-1} of CO_2 in the reference and leaf chambers. Measurements were recorded after reaching a steady state (~ 90 s). Gas exchange measurements were taken between 9:30 and 15:30 hours.

Foliar Nitrogen

The combustion method was used for determination of foliar N concentrations (Campbell 1991) using a nitrogen analyzer (TruSpec, CN Determinator, LECO Corporation, St. Joseph, MI).

Nonstructural Carbohydrates

For each leaf sample, 20 mg of leaf material was suspended in 0.66 ml of 80% ethanol in a 2 ml screw cap microcentrifuge tube. Tubes were then placed in a heater vortex at 80 ˚C for 20 minutes. The supernatant was transferred to a separate tube and the extraction was repeated 2 more times. This ethanol extract was used for determination of glucose and sucrose concentrations. To quantify foliar concentrations of glucose and sucrose we used a Total Starch Assay Kit (Total Starch Assay Kit, Megazyme Co., Wicklow, Ireland). We added 20 µl of the ethanol extract of each sample to three wells for technical replicates. We evaporated the ethanol in a drying oven at 55 ˚C for 20 minutes. We then dissolved the extract in 20 µl H_20. Samples being analyzed for sucrose were treated with 10 µl of invertase and incubated at 37 ˚C for 10 minutes. We then added 200 µl GOPOD reaction mix (Glucose oxidase/peroxidase reagent with O-Dianisidine)) to the standards, and glucose, and sucrose sample wells. We incubated the samples for 20 minutes at ambient temperature and read absorbance at 510 nm using a spectrophotometer. Concentrations were determined using glucose and sucrose standard curves.

For starch analysis, the ethanol extracted tissue samples from the glucose/sucrose analysis were immersed in 1 ml of water in a 2 ml screw cap microcentrifuge tube. The samples

were autoclaved for 1 hour at 135 ˚C and the water extract was transferred to another tube and dried overnight in a drying oven at 65 ˚C. Foliar starch concentrations were also quantified using the Total Starch Assay Kit (Total Starch Assay Kit, Megazyme Co., Wicklow, Ireland). Ethanol extracted leaf tissue was digested in 1 ml of α-amylase. Samples were then boiled for 20 minutes and subsequently cooled for 10 minutes, followed by the addition of 15 µl amyloglucosidase. The samples were mixed in a shaking water bath at 50 ˚C for 45 minutes. Samples were then plated out and 200 µl of GOPOD reaction mix was added to the samples. After 20 minutes at room temperature, absorbance was read at 510 nm on the spectrophotometer. We quantified sample starch concentrations using a starch standard curve from the Megazyme maize starch control at 20, 10, 5, 2.5 and 0 µg / 20 µl concentrations. We ran a positive control sample from aspen leaf tissue with known concentrations of glucose, sucrose and starch to verify that the protocol was working correctly. Glucose, sucrose and starch concentrations were summed together and reported as % nonstructural carbohydrate.

Defense Chemistry

Phenolic glycosides were quantified according to the methods of Lindroth *et al*. 1993. Salicortin and tremulacin were extracted from 40 mg of freeze-dried leaf tissue in 0.66 ml of methanol in 2 ml screw-cap micro-centrifuge tubes. The samples were extracted using a vortex (VorTemp 56, Labnet International Inc., Edison, NJ, USA) for 5 minutes. Following centrifugation the supernatant was removed and placed in a separate 2 ml centrifuge tube. This process was repeated twice with a final sample extract volume of 2 ml. Final concentrations of salicortin and tremulacin were assessed using high-performance liquid chromatography (HPLC, Agilent 1100 Series, Santa Clara, California, USA) with a Luna 2, C18 column (150 x 4.6 mm, 5 μ s) at a flow rate of 1 ml min^{-1}. Compound peaks were visualized under a UV lamp at a

wavelength of 280 nm using purified salicortin and tremulacin standards isolated from aspen leaves (Lindroth *et al.*1993).

Condensed tannins were extracted from 40 mg samples of leaf material by first, suspending the samples in 1 ml of 70% acetone - 10 mM ascorbic acid solution. The samples were then vortexed at 4 ˚C for 20 minutes, and were subsequently centrifuged. Next, the liquid supernatant was transferred to a separate microcentrifuge container and the extraction was repeated a second time. The concentration of condensed tannins was then quantified with a spectrophotometer (SpectraMax Plus 384, MDS, Toronto, Canada), using the modified butanol-HCl method (Porter *et al*. 1985), and quantified using a condensed tannin standard curve. Purified condensed tannin standards were isolated from aspen leaves according to the methods of Hagerman and Butler (1980).

Statistical Analysis

For experiment one, we evaluated leaf chemistry and morphology traits using a mixed effects model with fence type and year as fixed effects nested within each fire. We a used top-down strategy to evaluate fixed effects with likelihood ratio tests using maximum likelihood. Upon finding the optimal mixed effects model, we refit the model using restricted maximum likelihood (REML) (Zuur *et al*. 2009). Fixed effects are presented by their χ^2 and p – value (Table 1). We evaluated aspen height, using fence and year as fixed factors, nested within fire. We accounted for heterogeneity of variance between years by using the identity variance structure. We tested for temporal correlations by examining patterns the auto-correlation function using normalized residuals, and found no general patterns across any analysis. For browse intensity, we only assessed between year differences in unfenced plots. Fenced plots all had zero

browse impact. Therefore, a statistical comparison between the two was unnecessary. We used a generalized linear model with a negative binomial distribution using the identity link function. There were no issues with overdispersion.

For experiment two, we evaluated leaf chemistry and functional traits using linear regression models with fence as the fixed effect. We also used linear regression models for the height, ring width, ring number, and growth rates of trees from unfenced and fenced plots. All response variables were normally distributed.

We compared experiment one and two by using linear mixed effects model with fence-unfenced pairs as a random effect and fence type, year (3 years post disturbance vs. 26 years post disturbance), and their interaction. We used the three pairs from the Seeley fire, and three sites from the Ferron fire to create a balanced design, and reduce regional variance. We report t-statistics for statistically significant terms at $p = 0.05$. For all analyses, we tested model validity through visual inspection of Q-Q plots, and standardized residuals plotted against fitted residuals. All analyses were done in R using the nlme, and MuMIn package (R Core Team 2014, Pinheiro *et al*. 2015, Barton 2015)

RESULTS

Experiment One: 1 – 3 years Post-fire

Aspen leaves in fenced plots were 65% larger than leaves in unfenced plots ($\chi^2 = 16$, $p < 0.001$) (Fig. 2a) but the effect of year and the interaction term were not significant (Table 1). Specific leaf area in fenced plots was 9% lower than in unfenced plots (Fig. 2b) ($\chi^2 = 9.7$, $p = 0.002$). Specific leaf area did not vary across years, and the interactions term was not significant (Table 1).

Nonstructural carbohydrates in leaf tissue from unfenced plots were nearly 33% lower than in fenced plots (Fig. 2c) ($\chi^2 = 26$, $p < 0.001$). Nonstructural carbohydrates did not vary significantly across years and the fence by year interaction term was not significant (Table 1). After 3 years, photosynthesis rates in fenced plots were 11% lower than in unfenced plots (Fig. 2d) ($\chi^2 = 4.7$, $p = 0.03$). Nitrogen did not vary between fenced and unfenced plots, but did vary significantly across years ($\chi^2 = 11$, $p = 0.004$) (Table 1). Nitrogen content in 2014 was approximately 14% lower than in 2013 and 2015.

Phenolic glycoside concentrations were not significantly different in fenced and unfenced plots but did vary significantly across years ($\chi^2 = 21$, $p < 0.001$) (Table 1). Phenolic glycoside concentrations in 2014 were 30% and 35% lower than in 2013 and 2015, respectively. Condensed tannins were 33% higher in unfenced plots than in fenced plots (Fig. 2f). ($\chi^2 = 7.7$, $p < 0.005$), but did not vary significantly across years and the interaction term was not significant (Table 1).

Aspen height varied significantly across fence treatments, year and the fence by year interaction term (F = 46, 30, 5 and $p < 0.001$, $p < 0.001$, $p = 0.01$, respectively). Aspen height in unfenced plots was nearly double that in unfenced plots after 3 years (Fig. 3a). Browse intensity was 0% in fenced plots and approximately 60% in unfenced plots. Browse impact in unfenced plots was consistent from year to year (Fig. 3b) ($\chi^2 = 0.86$).

Experiment Two: 26-years Post-fire

Long-term ungulate herbivory reduced leaf size, but did not affect specific leaf area. Leaves in fenced plots were 39% larger than leaves in unfenced plots (t = - 3.2, $p = 0.005$) (Fig. 4a). Specific leaf area did not vary significantly between fenced and unfenced plots (t = - 0.6, $p =$

0.32) (Fig. 4b). Long-term ungulate herbivory reduced foliar nonstructural carbohydrates, and N content, but photosynthesis was increased. Nonstructural carbohydrates in unfenced plots were 41% lower than in fenced plots ($t = - 9.0$, $p = 0.003$) (Fig. 4c). Foliar nitrogen in fenced plots was 16% higher than in unfenced plots ($t = - 4.6$, $p < 0.001$). Photosynthesis rates in unfenced plots was 50% higher than fenced plots ($t = 4.9$, $p < 0.001$) (Fig. 4d).

Long-term ungulate herbivory caused a strong increase in foliar concentrations of defense chemicals. Phenolic glycoside concentrations of aspen in unfenced plots were approximately two-fold higher than in aspen leaves in fenced plots ($t = 6$, $p = 0.01$) (Fig. 4e). Condensed tannin concentrations of aspen in unfenced plots were 40% higher aspen than in fenced plots ($t = 3.0$, $p = 0.007$) (Fig. 4f).

Long-term ungulate herbivory suppressed aspen recruitment, and reduced the radial growth of aspen stems. Aspen growing in fenced stands were nearly three times taller than aspen growing outside of fences ($t = 5.8$, $p < 0.001$) (Fig. 5a). Annual radial growth in fenced plots was 20% greater than in unfenced plots ($t = 2.6$, $p = 0.02$) (Fig. 5b). Tree age was not significantly different in fenced and unfenced plots ($t = 1.6$, $p = 0.13$) (Fig. 5c).

Comparing 3 year and 26 year Reponses

Ungulate effects on leaf morphology and leaf chemistry were similar in experiments 1 and 2 as indicated by the lack of significance of the fence by year interaction term comparing responses in the 3 and 26-year data sets (Fig. 6a-f). The only exception were phenolic glycosides, which only showed induction in response to herbivory in the longer-term experiment (Fig. 6e).

DISCUSSION

Overview

We evaluated ungulate effects on aspen leaf traits and growth under the conceptual framework of plant resistance, tolerance, and escape from herbivory (Strauss and Agrawal 1998, Lindroth and St. Clair 2013). Our first hypothesis that aspen tolerate herbivory by maintaining photosynthesis, foliar nitrogen and nonstructural carbohydrates showed mixed results. We found increases in photosynthesis, but reduced nonstructural carbohydrates under both short and long-term ungulate herbivory experiments. Foliar nitrogen was maintained throughout the first three years of herbivory and was only reduced by herbivory in the long-term experiment. Our second hypothesis that ungulate herbivory would increase resistance through lower palatability due to increased SLA and smaller leaf size and higher foliar levels of condensed tannins and phenolic glycosides was mostly supported. However, phenolic glycosides were only increased in response to ungulate herbivory over longer periods of exposure (Fig. 4e). Ungulate herbivory caused large reductions in leaf size in both the short and long-term experiments, however our findings for specific leaf area were mixed. Our third hypothesis that ungulate herbivory affects growth and escape was well supported. Ungulate herbivory had strong effects on vertical growth in both the short (3 years) and long-term (26 years). Further, dendrochronology data from the long-term experiment showed consistent reductions in annual ring widths, suggesting that ungulate herbivory was generally strong over the study period. Finally, our forth hypotheses that short and long-term effects on leaf traits across time scales was generally not supported as short and long-term impacts on leaf traits and growth were fairly consistent (Fig. 6).

Aspen Tolerance to Ungulate Herbivory

Increased photosynthesis is an important compensation mechanism after defoliation (Pinkard *et al*. 2011). In our study, ungulate herbivory consistently increased photosynthetic

rates (Figs. 2d, 4d, 6d). While ungulate herbivory had positive effects on leaf level photosynthesis dramatic reductions in leaf size, tree height and canopy area are likely to have drastically reduced photosynthesis on a whole plant basis. These results are consistent with other studies showing that defoliation can increase photosynthesis in remaining leaves (Strauss and Agrawal 1998), but heavy defoliation can result in a net loss of photosynthetic capacity at the plant level (Hoogesteger and Karlsson 1992). Reallocation of carbon stores for growth may then compensate for reduced photosynthetic capacity (Hoogesteger and Karlsson 1992, Palacio *et al*. 2008).

Reallocation of carbon reserves and high relative growth rates (related to SLA) are key mechanisms for compensation after herbivory (Strauss and Agrawal 1998). Our results for specific leaf area were mixed and was only higher within the first three years after ungulate herbivory (Fig. 2b). Specific leaf area was generally lower when subjected to ungulate herbivory over the long-term (Fig. 6b). Higher specific leaf area in response to herbivory can increase relative growth rates (Garnier 1992), but that would only provide partial compensation for the large reductions of leaf tissue observed in this study (Fig. 3b). Reduction of nonstructural carbohydrates in response to defoliation is consistent with other studies (St. Clair *et al*. 2009). Aspen may compensate for tissue loss by metabolizing nonstructural carbohydrates for repair or regrowth of browsed tissue (Pankoke *et al*. 2013) and reallocation of carbon pools may compensate for loss in photosynthetic capacity (Hoogesteger and Karlsson 1992).

Resistance to Ungulate Herbivory

Resistance to ungulate herbivory can be achieved through reduced preference or performance by ungulate herbivory (Strauss and Agrawal 1999). Ungulate herbivory of aspen

increased investment in chemical defense, reductions in foliar concentrations of nonstructural carbohydrates and nitrogen and loss of leaf area. There is increasing evidence that leaf morphology plays an important role in determining susceptibility to herbivory (Schudlt *et al*. 2012, Carmona *et al*. 2011). For example, larger leaf size might attract herbivores (Garibaldi *et al*. 2011) and leaves with higher specific leaf area tend to be more palatable (Poorter *et al*. 2004). Leaf nitrogen content is positively related to palatability (Agrawal 2007), but our study showed that reduction of leaf N in browsed leaves was modest and only occurred under longer browsing exposure (26 years post-fire). Aspen's clonal nature (Rhodes *et al*. 2016) and the high physiological function of aspen in post-burn environments may partially buffer against reductions in foliar N in response to herbivory (Wan *et al*. 2014).

Induction of defense chemistry following defoliation may be a general response that protects second flush leaves from subsequent defoliation (St. Clair *et al*. 2009). Condensed tannins and phenolic glycosides are versatile defense compounds that have been shown to protect against leaf pathogens Holeski *et al*. 2009) and insect (Lindroth and St. Clair 2013) and ungulate herbivory (Min *et al*. 2003, Wooley *et al*. 2008). Given that phenolic glycoside concentrations are greater in younger age cohorts (Smith *et al*. 2011b), our results suggest that ungulate reduction of aspen height could keep aspen in a developmentally juvenile form that maintains higher expression of defense compounds (Bryant *et al*. 1983, Smith *et al*. 2011b). Future research is needed to understand how tree height mediates the developmental controls of phenolic glycoside production to understand whether increases in defense chemistry expression due to browsing are mediated by height reduction.

Aspen Escape From Ungulate Herbivory

While resistance and tolerance are important defense strategies against ungulate herbivory, ultimately vertical escape is necessary for successful aspen recruitment (Seager *et al*. 2013). Our data show that ungulate herbivory can prevent aspen recruitment for decades even with induction of defense chemistry (Fig. 5a). The reduction of annual growth rings throughout the 26-year period suggests that ungulate herbivory has had a constant effect on the growth potential of aspen (Fig. 5b). Studies suggest that browse intensity under 30% are necessary for successful recruitment of aspen suckers (Olmstead 1979, Jones *et al*. 2005), which is well below the 60% we observed over the three-year period of our first experiment. This suggests that browse intensity at our research sites would need to be reduced by at least half for successful recruitment. Further, loss of apical meristems can reduce the recruitment potential in deciduous trees (Palacio *et al*. 2008). Despite strategies of resistance, tolerance and escape, our results suggest that in areas with high ungulate use, these defenses may not be enough.

Short and Long-term Herbivory

Our results show that ungulate impact on leaf chemistry and morphology was similar in the first three years post-fire and 26 years post-fire, but effect sizes increases in magnitude over time (Fig. 6). This suggests that ungulate herbivory can produce short term impacts on leaf function and morphology, and that those effects are magnified by chronic herbivory over time. Further, we found that foliar N was reduced in aspen subjected to long-term herbivory. This is in contrast to studies of simulated browsing which show only short-term effects on C and N pools (Palacio *et al*. 2008), or recovery of growth and nutrient status after simulated defoliation (Hoogesteger and Karlsson 1992). Similar to our study, both simulated browsing and observational studies have shown tree height and growth is drastically reduced under high

browse pressure (Bartos *et al.* 1994, Palacio *et al*. 2008). Removal of apical meristems was high and remained so for the first 3 years of experiment 1 (Fig. 3b). While browsed and defoliated trees can tolerate ungulate herbivory in the short term (Hoogesteger and Karlsson 1992, Palacio *et al*. 2008), our results showed high and consistent levels of herbivory year to year across our sites (Figs. 3b, 5b). The major differences between short and long-term ungulate herbivory was lower foliar nitrogen when exposed to chronic ungulate herbivory and production of phenolic glycosides. These differences may be due to phenotypic differences in development of aspen trees (Smith *et al*. 2011b), and suggest that ungulate herbivory keeps aspen in juvenile stages of development similar to other studies (Bryant *et al*. 1983). We conclude that ungulate herbivory has strong impacts on aspen leaf trait, defense, and growth, which in turn reduced the potential for vertical escape both three years after fire and 26 years after fire.

Conclusion

We conclude that leaf traits, defense chemistry, and growth are strongly influenced by ungulate herbivory and may have cascading effects on leaf function and future susceptibility to herbivory. Aspen under chronic ungulate browse pressure can fail to regenerate (Bartos *et al*. 1994). Our study describes the physiological effects of short and long-term ungulate browsing and how these effects can contribute to aspen recruitment failure. We observed reduced recruitment potential despite aspen's significant tolerance, deterrence and escape defense mechanisms (Lindroth and St. Clair 2013). In our study, increased investment in chemical defenses, and loss of photosynthetic leaf tissue accompanied slow radial and vertical growth rates of aspen (Figs. 3a, 5a, 5b). After 26 years, aspen exposed to ungulate herbivory were still shorter than the critical threshold of 2 meters needed for vertical escape (Fig. 5c). Aspen's ability to survive in this stunted state year after year may be due to aspen's clonal nature which allows

resource storage and resource sharing through the root systems (Rhodes *et al*. 2016). The data indicate that loss of leaf tissue and apical meristems from chronic browsing keeps aspen as a viable food source for ungulates due to a lack of vertical escape and reduces growth potential (Palacio *et al*. 2008, Lindroth and St. Clair 2013). We suggest that in areas that experience high ungulate use mitigation of ungulate herbivory should be employed (see Seager *et al*. 2013 for suggestions) to maintain proper function and recruitment of aspen forests.

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FIGURES

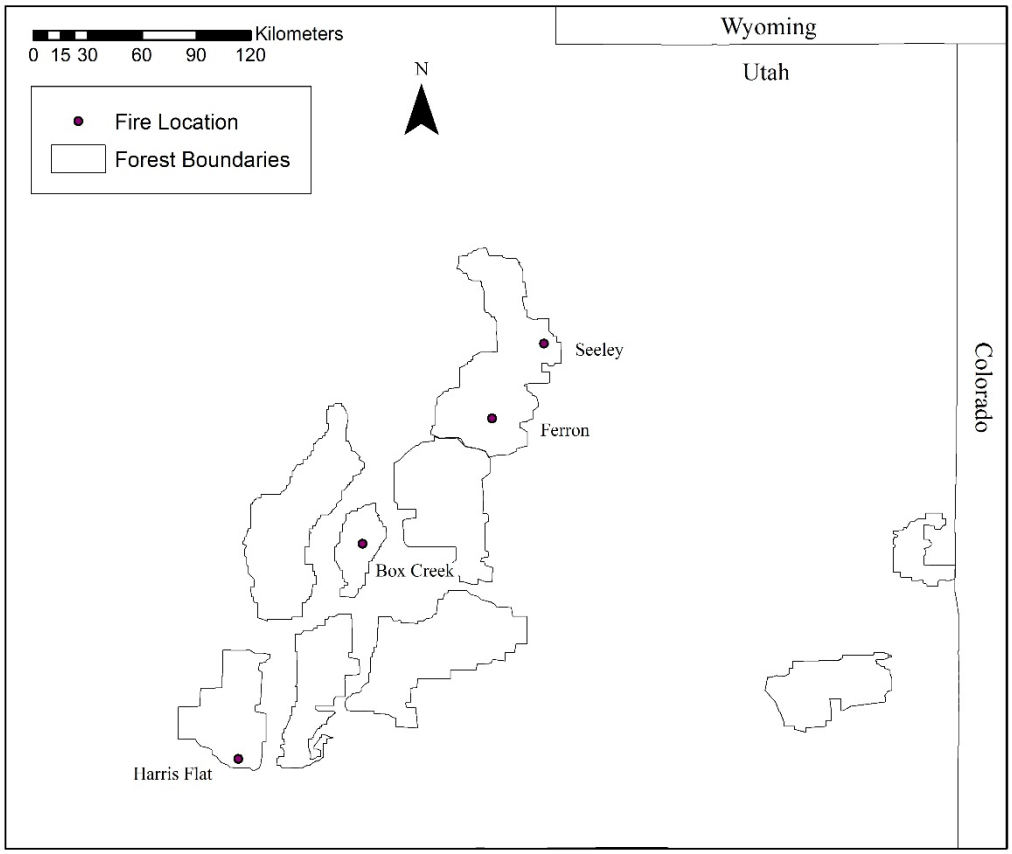


Figure 5–1. A map of our study sites in central and southern Utah. Embedded in the map is a table that presents the date, size, elevation and precipitation (cm) of the fire. National forest boundaries are indicated by solid lines.

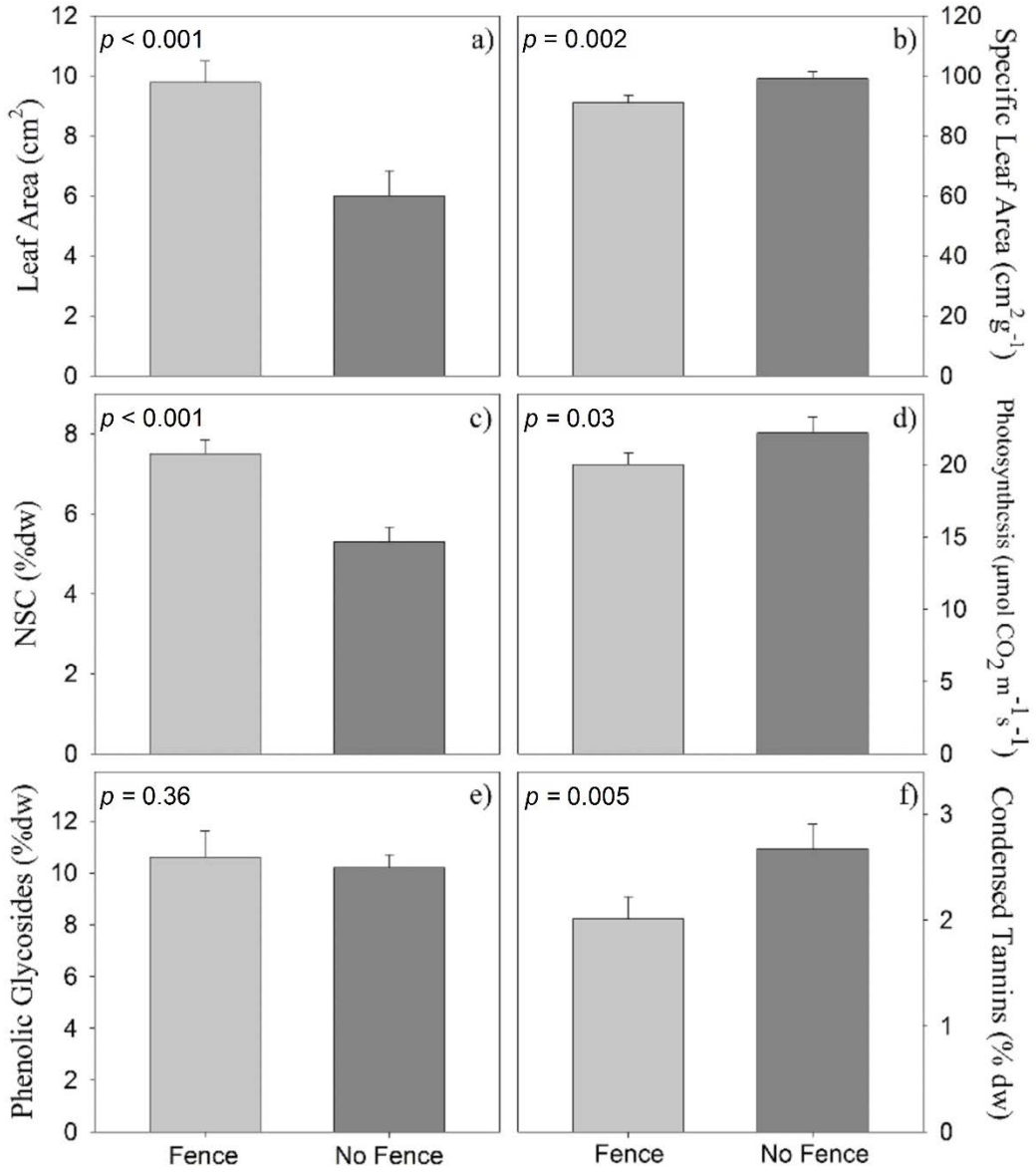


Figure 5–2 . The fence effects for 2015 are presented from experiment one using the model estimates for the mean and one SE around that mean is given as the error bar. NSC refers to nonstructural carbohydrates. Photosynthesis was measured at light-saturated photosynthesis (Asat).

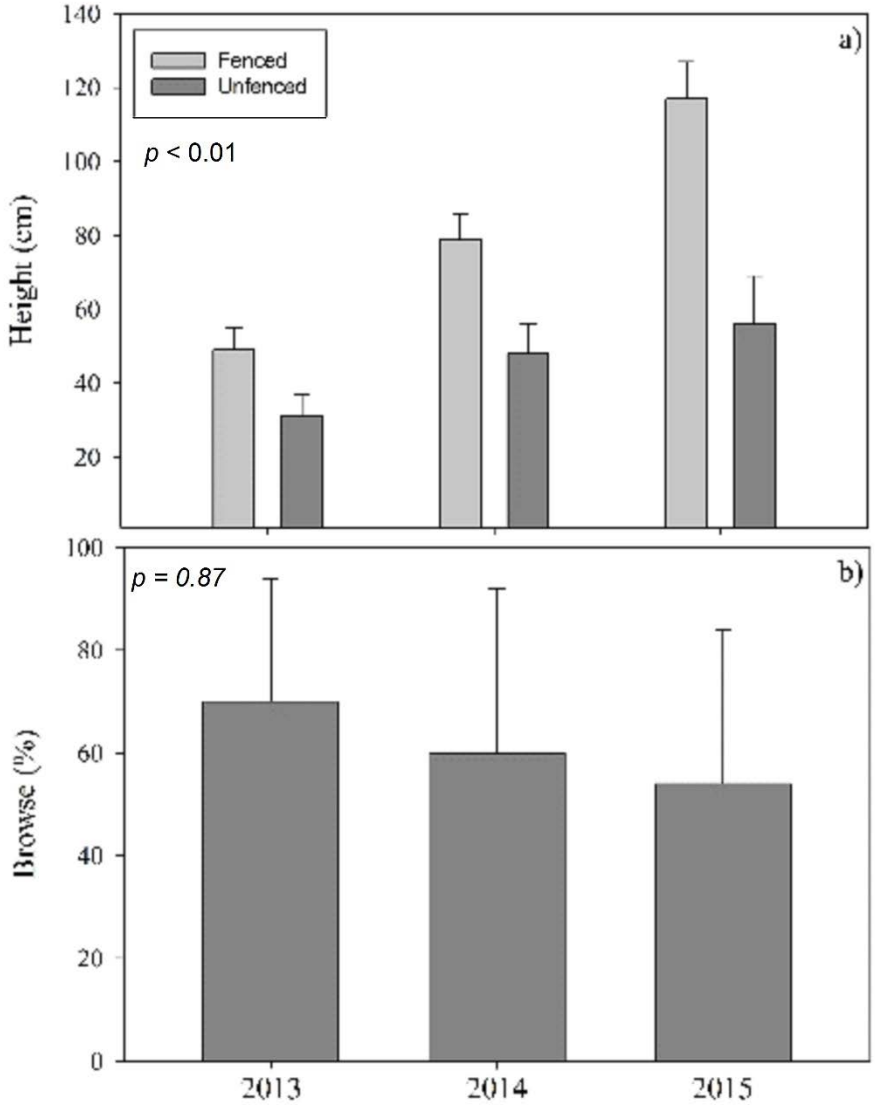


Figure 5–3. Top panel: a) Mean height is presented in centimeters for both fenced and unfenced plots across years 2013, 2014, and 2015. The error bars are one SE. Bottom panel: b) Mean browse impact is presented in percent of meristems removed for fenced plots only across years 2013, 2014, and 2015. Fenced plots had zero browsing for all three years (data not presented). The error bars represent one SE.

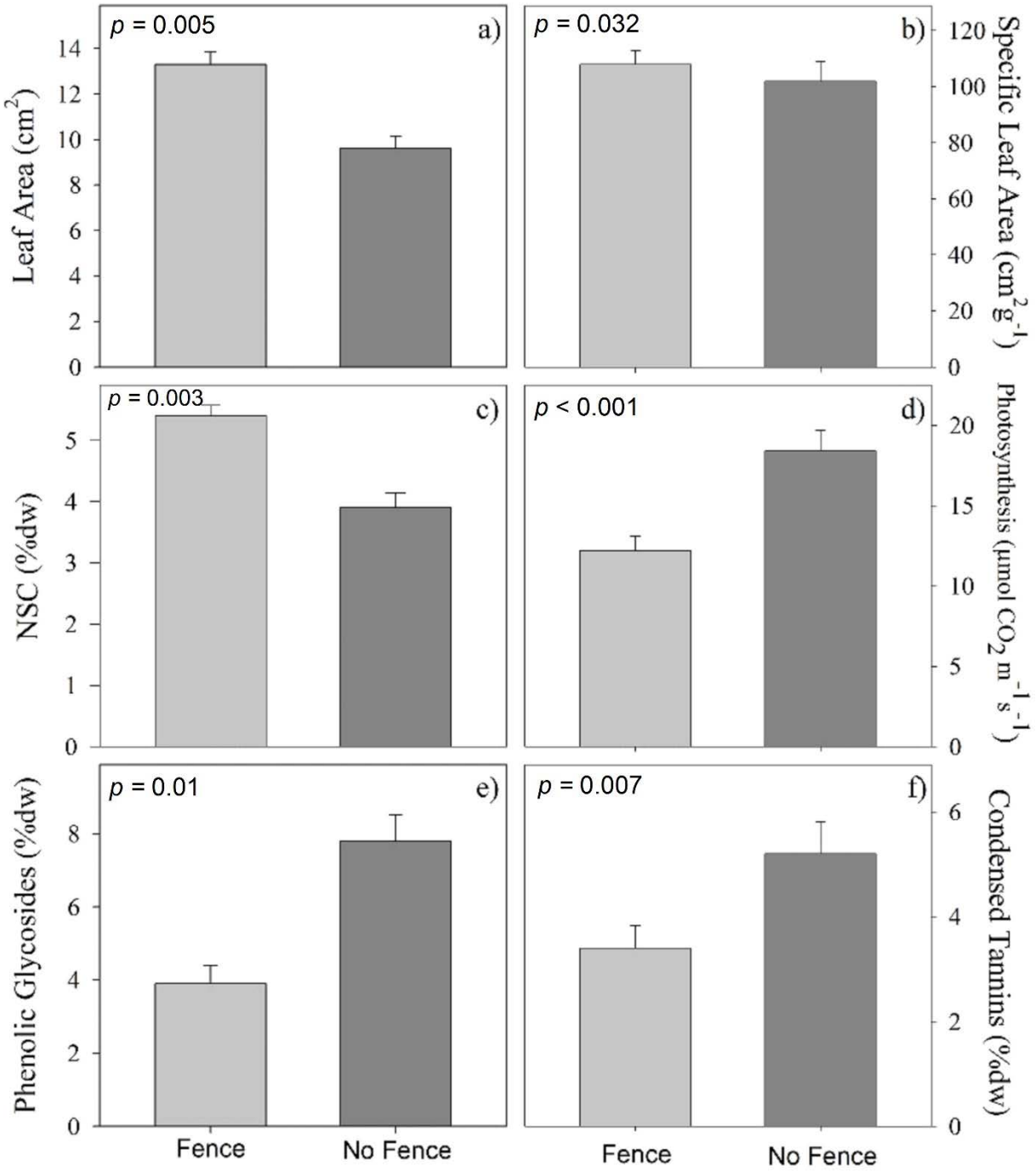


Figure 5–4. Fence effects are presented for experiment two using the model estimates for the mean and SE around that mean. The error bar is one SE.

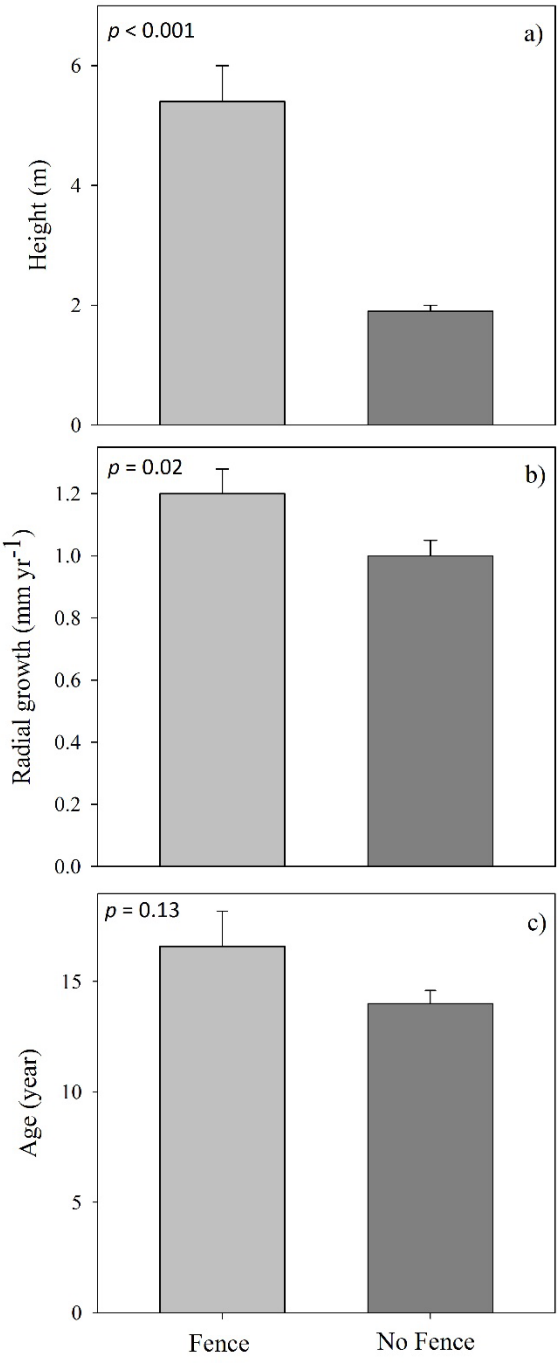


Figure 5–5. Fence effects are presented from experiment 2 using the model estimates for the mean and standard error around that mean. Full model *p* - values are presented in the upper left corner of each panel.

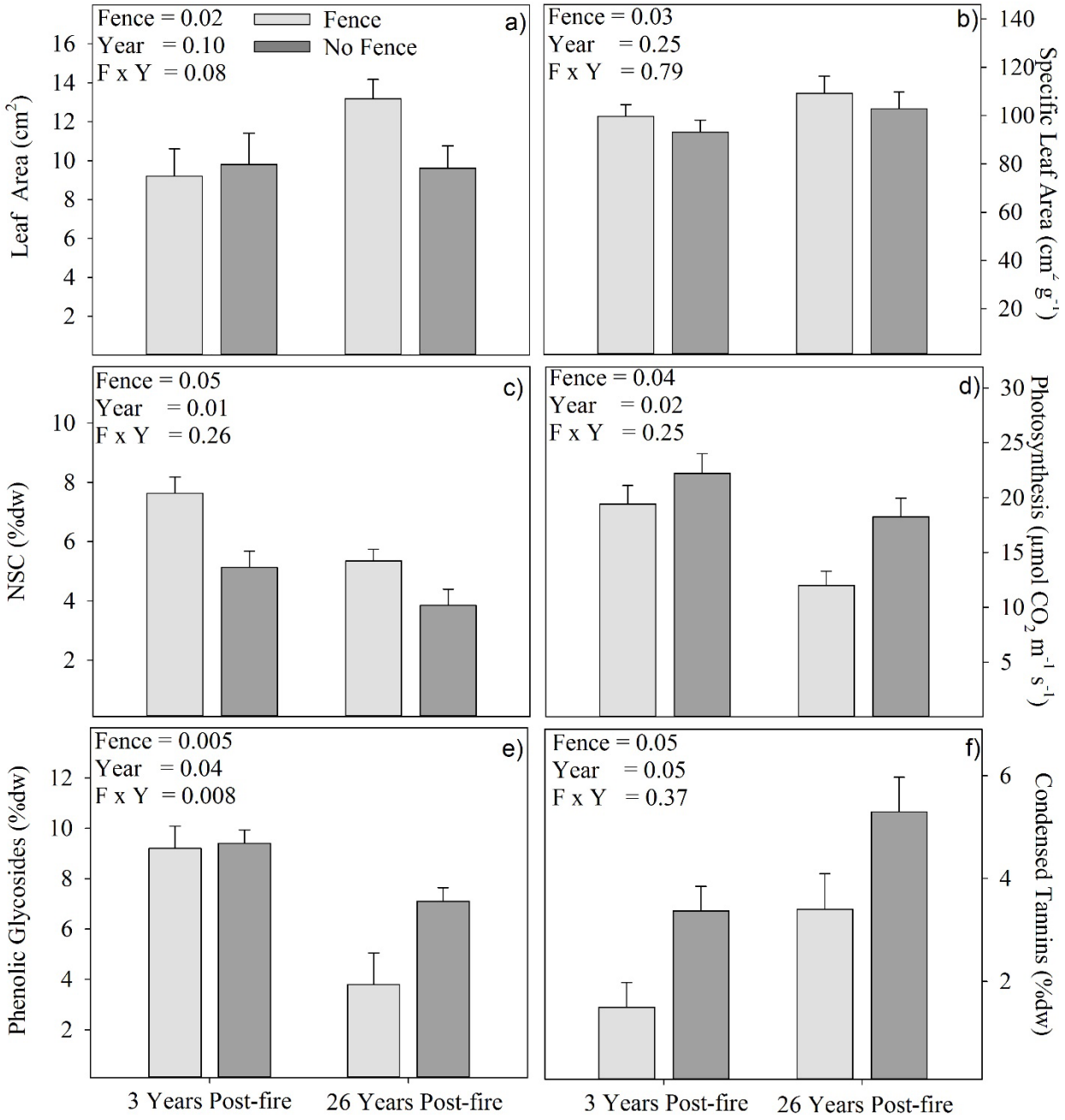


Figure 5–6. The fence effects for year 3 and 26 post-fire are presented by comparing experiment one and two using the model estimates for the mean and one SE around that mean is given as the error bar. NSC refers to nonstructural carbohydrates.

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