Seasonal forage dynamics of three grasses with different origins and photosynthetic pathways in a rural North American cold steppe

J D Scasta

Department of Ecosystem Science and Management, College of Agriculture and Natural Resources, University of Wyoming
jscasta@uwyo.edu

Abstract

Sustainable pastoralism on North American high-elevation cold steppes requires an understanding of forage dynamics relative to potential compositional changes. I sampled forage quality monthly and peak forage mass of a native C4 grass (*Bouteloua gracilis*), a native C3 grass (*Pascopyrum smithii*), and an exotic C3 grass (*Agropyron cristatum*) in 2016, an average precipitation and temperature year, on a 2,195 m elevation steppe grassland in Wyoming USA. Constrained ordination of the forage quality data displayed the first axis by photosynthetic pathways and the second axis by different native/exotic origins (86.3% fit variation explained; permutation test p = 0.003). Forage species, day of year, and their interaction were significant for all forage quality metrics (p < 0.001) indicating that species-specific forage quality characteristics respond to seasonal changes differently. For example, *A. cristatum* had the earliest crude protein peak in April, followed by *P. smithii* from May – July, followed by *B. gracilis* in August. Other examples include lower lignin values for *B. gracilis*, and P and K peaks for *P. smithii*. *A. cristatum* yielded greater forage mass than *P. smithii* or *B. gracilis*. Relative to plant succession and potential compositional change, these three species combined match a broader range of livestock nutritional needs than any single species.

Keywords: *forage ecology, grassland, grazing, herbage, pastoralism, rangeland*

Introduction

Forage-based livestock production and pastoralism continues to be a dominant feature of cold high-elevation steppes globally. These vast and inhospitable ecosystems occur in central Asia, western North America, southwestern South America, South Africa, southern Australia, and a few other areas. These areas are classified as BSk (B – Arid; S – Steppe; k - cold) based on the KÃ¶ppen-Geiger climate classification, are temperate, continental, and characterized by snowfall in the winter and large diurnal temperature gradients (Peel et al 2007). The physiognomy of cold high-elevation steppes makes them unsuitable for farming and crop production due to short growing seasons, cold temperatures, low precipitation, high wind speeds, shallow soils, and difficult terrain (Ormaechea and Peri 2015). However, these features have also conserved native floristic features of cold steppes due to the suitability only for extensive pastoral agriculture (Yabrir et al., 2015). Finally, the cold end of the thermal gradient is a dominant and controlling feature of cold steppes. For example, in a Patagonian steppe, climatic controls of annual net primary productivity (ANPP) were strongly controlled by an interaction of temperature and precipitation, and notably, the lower and cooler end of the thermal

gradient (Jobbágy et al 2002). Thus, photosynthetic pathway of forage species is likely to interact with such thermal extremes that characterize cold steppes.

Sustainability of pastoral livelihoods and livestock production, and the concomitant conservation of flora and fauna, in steppes should include an enhanced understanding of species-specific forage dynamics in the context of potential change (Dong et al 2011, Maamri et al 2015). Moreover, providing livestock with naturally grown forage continues to be the most economical and efficient means of meeting nutritional requirements (Short 2001, Meena et al 2007). From a short-term animal nutrition perspective, the long and cold winters are periods of no forage plant photosynthetic activity and subsequent low forage quality. During these periods livestock may not be able to obtain minimal nutritional requirements (Van Soest 1994). From a long-term forage based production system perspective in steppes, management of grazing relative to forage species, quantity, and quality can induce compositional changes that will have carrying capacity and animal performance consequences (Yousfi et al 2017). In other words, the loss of the most adaptive and productive native species due to mis-management may have negative livestock production consequences (Holechek et al 2004, Derner et al 2008).

Such plant composition and successional changes to native cold steppe plant communities can result from improver grazing management, anthropogenic invasion pathways, or both (Scasta et al 2015). For example, concern about grazing-induced compositional changes relative to both direct and indirect herbivore selectivity effects, and relative to different plant functional traits in Patagonian steppe, have also been demonstrated with intensive grazing leading to greater dominance by low growing grazing-tolerant species (Cingolani et al 2005). Similar plant composition changes have been documented in the northern Great Plains of North America where long-term grazing at heavy stocking rates induced shifts from C3 mid-grass species to C4 short-grass species (Derner et al 2008). While such shifts, relative to grazing are predictable (Augustine et al 2017), the net forage quality and quantity effects are not as well understood. Climate change effects on plant composition, structure, and function further complicate the sustainability of pastoralism on high-elevation steppes (Ganjurjav et al 2016). As one example, a sensitivity analysis of an Inner Mongolia steppe forecasted potential vulnerability of shifting from a grass-dominated state to a shrub-dominated state with increasing temperature, precipitation, and CO_2, or broad declines in herbaceous plant production with decreasing precipitation and increasing temperature and CO_2 (Christensen et al 2004).

Considering that the sustainability of pastoralism on North American high-elevation cold steppes requires an understanding of forage dynamics relative to potential compositional changes, and the need for enhanced quantification of the temporal dynamics of potential changes, I sampled forage quality monthly and peak growing season forage mass (Allen et al 2011) of blue grama - a native C4 grass (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths), western wheatgrass - a native C3 grass (*Pascopyrum smithii* (Rydb.) Á. Löve), and crested wheatgrass - an exotic C3 grass (*Agropyron cristatum* ((L.) Gaertn.) in 2016 on a 2,195 m elevation steppe grassland in Wyoming USA. This information is particularly important for pastoralists in Wyoming USA because Wyoming is the least populous state in the USA with an estimated 69% of the state population classified as rural and > 68% of total state farm receipts derived from rangeland cattle production (USDA ERS 2017). My objectives were to (1) determine how forage quality composition of these three species was explained relative to photosynthetic pathway and native/exotic origins, (2) quantify seasonal trends relative to important forage quality characteristics, (3) analyze annual differences between species for important forage quality characteristics, and (4) elucidate value of a diverse forage assemblage for livestock nutritional demands and potential consequences to shifts to homogenous stands of each of these three forage species.

Materials and Methods

Study Site

The general study area is owned and managed by the University of Wyoming – Laramie Agriculture Experiment Station and occurs in an ecotone between the northern mixed grass prairie and sagebrush steppe of southeastern, WY, USA. The study site consisted of a 5 ha pasture embedded within the larger 400 ha working research ranch. The study site was not grazed by livestock in 2016, the period of study. Study site soils consist entirely of the 165 Forelle-Diamondville association with 3 to 15% slopes and consisted entirely of the R034XY322WY Loamy (10-14SE) ecological site (USDA NRCS 2016). The soils are characterized by a pH of 7.6, cation-exchange capacity of 10.0 milliequivalents per 100 grams, an available water capacity of 0.15 centimeters of water per centimeter of soil, soil bulk density of 1.28 grams per cubic centimeter, 19.3% clay, 57.7% sand, and 22.9% silt in the 0-15 cm layer. The historical "natural" plant community was dominated by *P. smithii* (35%), needle-and-thread (*Hesperostipa comata* (Trin. & Rupr.) Barkworth) (15%), bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Ã•. LÃ¶ve) (10%), big sagebrush (*Artemisia tridentata* Nutt. subsp. *wyomingensis* Beetle & Young) (10%), prairie junegrass (*Koeleria macrantha* (Ledeb.) Schult.) (5%), Indian ricegrass (*Achnatherum hymenoides* (Roem. & Schult.) Barkworth) (5%), and muttongrass (*Poa fendleriana* (Steud.) Vasey) (5%). Total forage dry-weight production on the study site is estimated to be 1,568 kg DM ha^{-1} in favorable years, 1,232 kg DM ha^{-1} in normal years, and 672 kg DM ha^{1} in unfavorable years (USDA NRCS 2016).

Sampling Methods

For the forage quality analyses, I sampled the three dominant grass forage species on our study site, including *B. gracilis*, *P. smithii*, and *A. cristatum*. Entire plants including leaves, stems and seeds were collected by clipping to the ground surface level and immediately placing plant material in a paper bag. The only criteria used for not sampling was to avoid areas clipped the previous month. Forage samples were of a minimum of 50 grams and were randomly collected from the study site on a monthly basis. Sampling began January 13 th 2016 and continued through December 2016. Samples were always collected at the mid-point of each month. Four replicates of each species were collected each month (n = 4; 12 months) for a total of 48 samples per species for the year-long study. Samples were placed in a forced air oven and dried for 48-hours at 60 Â°C (Elgersma et al 2014).

For the forage mass analyses, I sampled the same three dominant grass forage species in late-September 2016. The sampling method was designed to estimate potential maximum forage mass of the three plant species. In other words, the sampling method estimates the potential maximum mass of each species if the plant composition on the study site were comprised of a monoculture of that species (as opposed to relative contributions of each species). This protocol was selected because the plant community dynamics relative to either *A. cristatum* invasion or *B. gracilis* dominance from grazing could shift the plant community and associated forage quality/quantity characteristics different directions with implications for livestock production. These forage mass samples were collected with a 0.25 m^{2} quadrat by clipping all of an individual species within the quadrat to the ground surface level and immediately placing plant material in a paper bag. Sampling was stratified across five randomly selected locations within the study site for five replications (n = 5) per plant species. At a random location, I first determined if all three species occurred. Then I positioned the quadrat on a spot dominated by one of the three species and clipped only the plants of that species. The next two species were then collected within 5 m of the first species in different quadrats positioned in the nearest spot dominated by each of the other two species. Samples were placed in a forced air oven and dried for 48-hours at 60 Â°C (Elgersma et al 2014) and then weighed and converted to kilograms per hectare.

Laboratory Analyses

Monthly samples collected for forage quality were sent to Ward Laboratories, Inc. in Kearney, NE, USA for laboratory analysis and all results were reported on a dry (0.00% moisture and 100.00% dry matter) basis. Ward Laboratories is certified by the National Forage Testing Association (Ward Laboratories Inc 2017). Individual samples were ground at the lab to pass through a 1 mm sieve prior to analyses. Near infrared reflectance (NIR) spectrometry was then conducted using a Foss 2500 at wavelengths between 850 and 2500 nm to quantify presence of key forage quality factors based on universal equations generated from a suite of forage species (Marten et al 1989, Schutte and Lauriault 2015). Specifically, current calibration of the NIR technique are based on 2013 NIRS Level 2 equations (NIRS Forage and Feed Testing Consortium 2017). Key forage quality factors assessed included: crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), NDF 48-hour digestibility (NDFD), *in vitro* true 48-hour digestibility (IVTDMD), total digestible nutrients (TDN), net energy for lactation, net energy for maintenance, net energy for gain, relative feed value (RFV), relative forage quality (RFQ), calcium (Ca), phosphorous (P), potassium (K), magnesium (Mg), ash, fat, lignin, non-fiber carbohydrates, and water-soluble carbohydrates. We had sample quantity issues only for *B. gracilis* in March and because NIRS was not possible, samples were assessed with wet chemistry methods that provide only a subset of forage quality metrics listed above.

Statistical Analyses

In order to account for the complex forage quality data, I first used multivariate statistics by conducting a direct gradient ordination analyses of forage quality response metrics constrained by forage species using redundancy analysis (RDA) which is a constrained form of principal components analysis (PCA), an indirect and unconstrained ordination technique (Gusmeroli et al 2013, Chinea and Arévalo 2014, Hakl et al 2015). The RDA ordination included 'day of year' as a covariate to account for the intra-annual effect on forage quality. Response data was log-transformed, centered, and standardized. All constrained axes were tested for significance at ? 0.05 using a permutation test with 999 iterations and random seed number generator. Ordination analyses were conducted in CANOCO (Lepš and Šmilauer 2003, Ter Braak and Šmilauer 2012).

To further understand forage quality dynamics, I then conducted analyses of variance (ANOVA) separately for each forage quality metric and graphically displayed them with line graphs and bar graphs in four general categories: quality, energy, fiber and digestibility, and mineral nutrient content. For each ANOVA, I used a mixed effects model with Species, Day of Year, and their interaction (Species*Day) as the main fixed effects. I also assigned Day of Year as a repeated effect because each species was repeatedly sampled at a regular and increasing Day of Year interval. To compare means and determine significant differences I assessed the Type II hypothesis test, and evaluated all pairwise comparisons that were considered different at ? 0.05. All forage quality metrics that are percentage data were arcsine square-root transformed to meet the assumptions of normality and distribution for our ANOVA's but all of the raw means and standard errors are presented in the figures. I did not conduct this analysis for the three net energy values (for lactation, for maintenance, for gain) because these are extrapolations based on TDN values and are highly correlated based on our ordination analyses and estimates will be similar to the TDN relationships and analyses. Similarly, to further understand forage mass differences, I also conducted an ANOVA with a mixed effects model with forage species as the fixed effect and sample replication as a random effect to account for soil, water, and topographic influences at the microsite level. To compare means and determine significant differences I assessed the Type II hypothesis test, and evaluated all pairwise comparisons that were considered different at ? 0.05.

Results

Precipitation and temperatures during the study period

Precipitation during our period of study followed the typical seasonal pattern to be expected with the greatest amounts coming in the spring (March, April, May, and June). Late summer and fall also expressed the seasonal decline in precipitation with a drier than normal July and August. It should be noted that these two months were still not the driest on record (Figure 1a). However, precipitation received in August, September, and November offset those lows. Temperature during our period of study also followed the typical seasonal pattern to be expected with the coldest months occurring in the winter (January, February, and December) and the warmest months in the summer (July and August) (Figure 1b).

Figure 1. Box plots displaying the historical monthly (a) precipitation (mm) and (b) mean temperature (Â°C) data distribution from 1895 to
2015 compared to the study period of January 2016 to December 2016 for a cold high-elevation steppe near Laramie, WY, USA.
Time series data were derived from 'parameter-elevation relationships on independent slope model' (PRISM) interpolation.

Forage quality composition

The redundancy analysis (RDA) ordination constrained to the three forage species and day of year revealed that the three forage species have different forage quality composition with unique forage quality parameters for each. The first axis explained 61% of the fitted variation and is largely a function of the differences associated with photosynthetic pathway and growth form – C3 and upright species grouped on the left side and the C4 and low-growing species on the right side of the biplot (Figure 2). Forage quality response variables explaining this difference include *in vitro* digestibility, ash content, and acid detergent fiber (ADF) content that all increase in association with *B. gracilis*, versus lignin, Mg, and non-fiber carbohydrates that all increase in association with *A. cristatum* and *P. smithii*. The second axis explained 25% of the fitted variation, with the first two axis explaining a cumulative of 86.3% of the fitted variation. The second axis is largely a function of species origin differences associated with the exotic and native C3 species, with *A. cristatum* at the top of the biplot and *P. smithii* at the bottom of the biplot, and the associated patterns of these species forage quality composition relative to seasonal changes as demonstrated by the 'Day of year' arrow (Figure 2). Forage quality response variables explaining this difference include lignin and neutral detergent fiber (NDF) that increase in association with *A. cristatum*, versus fat, phosphorous, TDN (and associated net energy content calculations for maintenance, lactation, and gain) and neutral detergent fiber digestibility (NDFD) that all increase in association with *P. smithii*. Finally, the permutation test for significance of all constrained axes was significant (pseudo-F = 3.2; *p* = 0.003) indicating the three forage species had different forage quality composition (Figure 2).

Figure 2. Ordination diagram of forage quality metrics for three grass species based on a redundancy analysis (RDA) constrained by Species
and Day of Year sampling intervals. Forage quality data was collected monthly for 2016 in a cold high-elevation steppe near
Laramie, WY, USA. Significance of constrained axes is based on a Monte Carlo permutation test with 999 iterations.

Crude Protein, RFV, and RFQ

For all metrics of forage quality, forage species, day of year, and the species*day interaction were all significant ($p < 0.0001$) indicating that certain species offer greater quality and respond to seasonal changes differently (Figure 3a-c). Prior to April, crude protein (CP) was below 7% for all species. The first increase in CP was for *A. cristatum* in April to 9.9 ± 1.0% followed by a subsequent decline the rest of the year. In May, *P. smithii* CP increased to the highest level and remained the highest of the three species through June and July with a maximum level of 12.2 ± 0.4%. In August, *B. gracilis* offered the highest CP (9.1 ± 0.3%). Annual mean CP (± standard error) for *B. gracilis*, P . *smithii*, and *A. cristatum* were 4.8 ± 1.6%, 7.1 ± 1.4%, and 5.6 ± 1.2% respectively. Precipitation in November led to an increase in CP for both C3 species in December but not for the C4 species (Figure 1; Figure 3a). Relative forage quality (RFQ) and relative forage value (RFV) followed a similar pattern as expressed by CP (Figure 3b and 3c). *P. smithii* had the highest RFQ of all species in June and July with values exceeding 125. The C4 species *B. gracilis* had low RFQ until July when temperatures warmed sufficiently for substantial photosynthetic activity and it reached an average of 125. *A. cristatum* had the lowest RFQ for 6 months of the year and only exceeded 100 one month (June). RFV followed a similar pattern as RFQ (Figure 3c).

Figure 3. Forage quality data expressed as (a) crude protein, (b) relative feed quality (RFQ), and (c) relative feed value for three grass species
sampled monthly for 2016 in a cold high-elevation steppe near Laramie, WY, USA. Grass species include *Bouteloua gracilis* - a C4
low-growing native, *Pascopyrum smithii* - a C3 upright native, and *Agropyron cristatum* - a C3 upright exotic.

Carbohydrates, Fat, and Energy

For all metrics of energy, forage species, day of year, and the species*day interaction were all significant ($p < 0.0001$) indicating that certain species offer greater energy and respond to seasonal changes differently (Figure 4a-c). Differences in non-fiber carbohydrates was variable with the C3 species (*P. smithii* and *A. cristatum*) generally displaying higher values than the C4 species (*B. gracilis*) (Figure 4a). Differences in water-soluble carbohydrates were not apparent until May when *A. cristatum* offered the greatest content which also occurred in July, August, and September (Figure 4b). Fat content followed a pattern similar to TDN with *B. gracilis* displaying higher values early in the year, *P. smithii* displaying higher values later in the year and the only species to exceed an average of 4% in any given month, and *A. cristatum* consistently displaying the lowest values (Figure 4d). Total digestible nutrients (TDN) were variable until June when *P. smithii* offered the greatest TDN content from then until the end of the year (Figure 4d). *B. gracilis* offered the lowest TDN content four months of the year and was similar to *A. cristatum* seven months of the year.

Figure 4. Forage energy data expressed as (a) non-fiber carbohydrates, (b) water-soluble carbohydrates, (c) fat, and (d) total digestible nutrients (TDN),
for three grass species sampled monthly for 2016 in a cold high-elevation steppe near Laramie, WY, USA. Grass species include *Bouteloua gracilis* - a C4 low-growing native, *Pascopyrum smithii* - a C3 upright native, and *Agropyron cristatum* - a C3 upright exotic.

Fiber and Digestibility

For all metrics of fiber and digestibility, forage species, day of year, and the species*day interaction were all significant ($p < 0.0001$) indicating that certain species have greater fiber content or lower

digestibility and respond to seasonal changes differently (Figure 5a-c). Lignin content was highest in *A. cristatum*, intermediate in *P. smithii*, and lowest in *B. gracilis,* with a greater separation later in the year (Figure 5a). Acid detergent fiber (ADF) tended to be lower in *P. smithii* for at least half of the year than the other two species and *B. gracilis* had the highest ADF in February, April, May, and December (Figure 5b). Neutral detergent fiber (NDF) was variable throughout the year with each forage species having the highest NDF at least one month of the year and A. *cristatum* having the highest NDF in January, June, August, October, November, and December (Figure 5c). The inverse of the lignin and species temporal relationship was reflected similarly in the pattern of NDF 48-hour digestibility (NDFD) and *in vitro* true 48-hour digestibility (IVTDMD) (Figure 5d and 5e). *B. gracilis* had the highest levels of NDFD and IVTDMD, *P. smithii* had intermediate levels of NDFD and IVTDMD, and *A. cristatum* had the lowest levels of NDFD and IVTDMD with the exception of May (Figure 5d and 5d).

Figure 5. Forage fiber and digestibility data expressed as (a) lignin, (b) acid detergent fiber (ADF), (c) neutral detergent fiber (NDF), (d) neutral
detergent fiber digestibility (NDFD), and (e) *in vitro* dry matter digestibility for three grass species sampled monthly for 2016 in a cold
high-elevation steppe near Laramie, WY, USA. Grass species include *Bouteloua gracilis* - a C4 low-growing native,
Pascopyrum smithii - a C3 upright native, and *Agropyron cristatum* - a C3 upright exotic.

Mineral nutrient content

For all metrics of mineral nutrient content, forage species, day of year, and the species*day interaction were all significant ($p < 0.0001$) indicating that certain species have specific mineral content and respond to seasonal changes differently (Figure 6a-d). Ash content was highest in *B. gracilis* (12.0 Â± 2.1%) and lowest in *A. cristatum* (3.0 Â± 1.0%) and *P. smithii* (3.3 Â± 1.1%) (Figure 6a). Calcium (Ca) content was highest in *B. gracilis* (0.41 Â± 0.06%), intermediate in *P. smithii* (0.34 Â± 0.06%), and lowest in *A. cristatum* (0.26 Â± 0.05%) with a similar Ca low in the May sampling period for all three species (Figure 6b). Magnesium (Mg) content was the highest in *P. smithii*, (0.13 Â± 0.01%), intermediate in *A. cristatum* (0.11 Â± 0.17%), and lowest in *B. gracilis* (0.07 Â± 0.02%) (Figure 6c). Phosphorous (P) content was higher in *P. smithii* (0.12 Â± 0.03%) than *B. gracilis* (0.06 Â± 0.02%) or *A. cristatum* (0.07 Â± 0.01%), and the seasonal expression of this difference was apparent in growing season only when *P. smithii* had higher values (Figure 6d). Similarly, potassium (K) content was higher in *P. smithii* (0.46 Â± 0.25%) than *B. gracilis* (0.27 Â± 0.14%) or *A. cristatum* (0.22 Â± 0.13%), and the seasonal expression of this difference was apparent in growing season only when *P. smithii* had higher values (Figure 6e).

Figure 6. Forage mineral nutrient content data expressed as (a) ash, (b) calcium (Ca), (c) magnesium (Mg), (d) phosphorous (P), and (e) potassium (K)
for three grass species sampled monthly for 2016 in a cold high-elevation steppe near Laramie, WY, USA. Grass species include *Bouteloua
gracilis* - a C4 low-growing native, *Pascopyrum smithii* - a C3 upright native, and *Agropyron cristatum* - a C3 upright exotic.

Forage mass potential

B. gracilis forage mass potential did not display inter-species sample differences ($p = 0.08$; Figure 7a) with samples ranging from 188 to 448 kg DM per ha. However, *P. smithii* and *A. cristatum* forage

mass potential both displayed inter-species sample differences (*p* < 0.05 respectively) with some samples higher than others (Figure 7b and 7c). *P. smithii* samples ranged from 480 to 2,064 kg DM per ha and *A. cristatum* samples ranged from 712 to 3,940 kg DM per ha. Comparison of mean species forage mass potential was significant (P < 0.05) with *A. cristatum* yielding greater forage mass (2,012 Â± 560 kg DM per ha) than *P. smithii* (969 Â± 284 kg DM per ha), and both *A. cristatum* and *P. smithii* yielding greater forage mass than *B. gracilis* (325 Â± 48 kg DM per ha) (Figure 7d).

Figure 7. Potential maximum forage mass for three grass species sampled monthly for 2016 in a cold high-elevation
steppe near Laramie, WY, USA. Grass species include *Bouteloua gracilis* - a C4 low-growing native, *Pascopyrum smithii* - a C3 upright native, and *Agropyron cristatum* - a C3 upright exotic.

Discussion

The diverse forage quality and quantity characteristics of the three forage species analyzed in our study indicates that a broader range of livestock nutrition benefits can be provided collectively better than any single species can provide singularly (Provenza et al 2009). The combination of these three species is most beneficial in crude protein by initiating earlier and extending later the optimal crude protein and by only certain species offering adequate levels of certain nutrients during the growing season (i.e., *P. smithii* and P and K). However, these results must be considered within the context of plant succession because the current plant community is different from the historical "natural" plant community. Currently, *A. cristatum* and *B. gracilis* now comprise a substantial proportion of the plant community on the study site. We hypothesize these species have established by different mechanisms. First, *A. cristatum* is present in the region due to its introduction as an alternative forage from Asia and has established at our study site from the movement of hay and vehicles and/or dispersal of seed by other means (Scasta et al., 2015). Second, *B. gracilis* has established from the ecological site immediately to the north (151 Diomandville-Cushool complex with 3 to 15% slopes) where it was a minor component of the plant community and is a consequence of a history of livestock grazing that has also eliminated or reduced perennial cool-season grasses (Derner et al 2008, USDA NRCS 2016). Finally, one additional contemporary difference is the absence of big sagebrush (*Artemisia* species) on the study site, and generally within the Laramie Valley. This is why the area is characterized as a high-elevation steppe rather than a sagebrush steppe.

Our results also demonstrate the compatibility of these three forage species for optimizing livestock nutritional resources in this North American steppe. Implications for successional changes for forage-based livestock productions systems if the plant community approached a monoculture for either of the three forage species assessed also becomes apparent. First, if *P. smithii* was the primary forage species as was historically documented, total forage mass and carrying capacity would be 3x greater than if *B. gracilis* dominated and Â½ of the potential if *A. cristatum* dominated but crude protein, total energy (TDN), forage quality (RFQ and RFV), fat, P, and K would be optimized on an annual basis. However, the early-season crude protein in *A. cristatum* (March) and late-season crude protein in *B. gracilis* (August) would be lost. Second, if *B. gracilis* was the primary forage due to the effects of grazing and loss of the C3 grasses (Cingolani et al 2005, Derner et al 2008), total forage mass and carrying capacity would be 1/3 [rd] to 1/6 [th] lower and early season forage quality below animal nutrient requirements until June due to the cold environment relative to the C4 photosynthetic pathway. However, *B. gracilis* coupled with the other two forage species does provide an August crude protein extension, is the optimal species in terms of lignin content and digestibility (NDFD and *in vitro*), and the optimal source of Ca and ash. Finally, if *A. cristatum* was the primary forage due to continued invasion and selection for this species due to its lower digestibility, total forage mass and carrying

capacity would be 2x to 6x greater but crude protein would be lower, fat would be lowest, and is the worst species in terms of lignin content, digestibility ((NDFD and *in vitro*), and Ca. The presence of *A. cristatum* in native steppe has been documented to produce 2x total forage mass by Hart et al (1983b) which also translated into greater pounds of calf production per unit area. However, *A. cristatum* monocultures are unlikely to meet livestock nutritional needs in the late-summer and fall, especially if moisture is limiting (Angell et al 1990) – an issue that our results also demonstrate.

Because the livelihood of cold steppe pastoralists depends upon the reproduction of ruminant livestock such as sheep and goats and cecal fermenters such as horses, we placed our results in the context of critical nutrients requirements at reproductive stages for each livestock species. First, I consider nutrient requirements for average milking (5 kg milk/day) beef cows of moderate size (450 kg) in the first 3 to 4 months postpartum are 57.5% TDN, 9.9% CP, 0.28% Ca, and 0.23% P (National Research Council 1984, Cheeke 1991). For such cows, the highest TDN values offered by *P. smithii* 6 months of the year are critical, the early boost of CP offered by *A. cristatum* is important for early calving cows and the late boost of CP offered by *B. gracilis* is important for later calving cows, the Ca offered by *B. gracilis* during March and May are critical, and of the three forage species only *P. smithii* meets the minimum P requirement for at least a single month of the year. I can make similar conclusions regarding sheep when the average nutrient requirements for 70 kg ewes in the last 4 weeks of gestation with an expected lambing rate of 130-150% are 59% TDN, 10.7% CP, 0.35% Ca, and 0.23% P and the average nutrient requirements for these same ewes in the first 6-8 weeks of lactation with twin lambs are 65% TDN, 15.0% CP, 0.39% Ca, and 0.29% P (National Research Council 1985, Cheeke 1991). In this scenario, minimum nutrient requirements are even higher than for beef cows, and thus, the forage diversity has similar direct animal production and nutrient requirement implications. Finally, for horses the average nutrient requirements for lactating mares from foaling to 3 months are 13.2% CP, 0.52% Ca, 0.34% P, 0.10% Mg, and 0.42% K (National Research Council 1989, Cheeke 1991). As for beef cows and sheep, different species meet different minimum nutrient requirements at different times and during the summer *P. smithii* is absolutely critical for meeting minimum Mg and K requirements that *B. gracilis* and *A. cristatum* do not meet.

Diverse forage quality and quantity characteristics are critical for sustainable pastoralism (Yousfi et al 2017) on our high elevation steppe study area and are also indicators of species richness and diversity. Moreover, the harsh extremes of the high-elevation, cold, and semi-arid steppe environment of our study suggests that forage species must be well adapted for local climates. Our results however indicate that local adaptation is not important simply at the regional level but also at the temporal level, and specifically at the intra-annual scale. In other words, each of the three species demonstrates a forage quality optima at different times of the year. For *A. cristatum*, this is earlier in the season, for *P. smithii* this is mid-season, and for *B. gracilis* this is late-season as expressed by the respective initiation of photosynthetic activity and crude protein values. The forage quality and quantity features of each forage species also results in tradeoffs that pastoralists will need to be made aware, especially if they are considering conversion to an *A. cristatum* monoculture or are grazing at an intensity that may be leading to successional changes to a *B. gracilis* dominated plant community. Although grazing induced successional changes are a concern for grazing cold high-elevation steppes, these changes occur with a high level of predictability relative to grazing pressure (Augustine et al 2017). Separate from grazing, the biomass allocation patterns of *A. cristatum* in steppe environments are shown to be greater for reproductive shoots and seed production which could lead to spread (Wang 2005). Any conversion to *A. cristatum* monocultures may enhance total forage mass and provide earlier green up and grazing, yet these advantages are offset by the rapid decline in the summer that is more rapid than any other species (Hart et al 1983a). Conceptually and practically, maintaining diverse forage species is critical for sustaining forage-based livestock systems and native wildlife in this high-elevation steppe (Larter and Gates 1991).

Acknowledgements

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