Diet overlap by DNA metabarcoding of mule deer, elk, and cattle in ponderosa pine forest of eastern Oregon

by
Megan Kate Faber

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Environmental Sciences and Fisheries and Wildlife Science
(Honors Scholar)

Presented May 30, 2017
Commencement June 2017

AN ABSTRACT OF THE THESIS OF

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Abstract approved:_____

Becky Kerns

Millions of acres of rangeland in the western U.S. is shared habitat by elk (*Cervus canadensis*), mule deer (*Odocoileus hemionus*), and cattle (*Bos taurus*). Potential competition between these ungulates for the same forage species can be understood from their diet composition. I used DNA metabarcoding methods with *trnL* primers to identify plant DNA fragments from scat samples of mule deer, cattle, and elk that were collected in mid-summer from two pastures in a ponderosa pine forest in the Blue Mountains of Oregon. Diet composition was examined for each ungulate species and between pastures using plant functional groups, families, and individual sequence reads with frequency of occurrence (FOO) and relative read abundance (RRA) metrics. Forbs were found to be major components in all ungulate diets, although more predominate in deer (RRA: 61%) and elk (51%) compared to cattle (37%). Cattle consumed the most graminoids (RRA: 49%), followed by elk (18%) and then mule deer (4%). Mule deer and elk overlap was the highest (80%), followed by elk and cattle (63%), and then mule deer and cattle (51%). Dietary niche overlap was found to be lower for all ungulate interactions in the pasture with higher

productivity and vegetation cover. The importance of forbs to all ungulates suggests that they should be monitored and accounted for in rangeland stocking rates.

Key Words: DNA metabarcoding, mule deer, elk, cattle, diet composition, diet overlap, ecology

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APPROVED:

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I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

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Megan Kate Faber, Author

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Introduction

Mule deer (*Odocoileus hemionus*) and elk (*Cervus canadensis*) share millions of acres of private and public ponderosa pine forest rangeland across the western United States with domestic cattle (*Bos taurus*) (Coe et al., 2004; Vavra, 2005). Forage availability for these species is influenced by a variety of factors such as site productivity, soil type, topography, climate and annual weather patterns, vegetation composition, and the presence and dietary preferences of other ungulates. Monitoring of rangeland health is based on utilization goals of target vegetation, which is assumed to be based on forage value related to ungulate species preference. However, ungulate preference for different species can vary widely based on vegetation composition at fine spatial scales, and actual utilization is difficult to measure in the field. Determining exactly what ungulates are eating is not a trivial task, and better knowledge regarding ungulate diets can help in understanding the interactions between these species and the potential for competition. This can inform range stocking decisions to balance wildlife and cattle numbers with available resources (Holechek et al., 1982). Herbivory is also an agent of change in the form of disturbance in many rangeland systems where resources are limited (Vavra, 2005), so diet composition will also inform how ungulates are influencing and shaping the ecosystem.

Interspecific competition occurs when resources that are used by different species are limited. Diet overlap is often used as a potential indicator of competition (Findholt et al., 2004); however, a demonstration of decrease in species fitness because of sharing limited resources with another species must be observed to be

described as true competition. Other factors that define a niche such as time, space, and habitat type will also play a role in the amount of competition that occurs between species (Torstenson et al., 2006). If diet overlap is high, this can potentially be compensated for with low spatial and temporal overlap to keep overall niche overlap and competition low. Facilitation can also occur between species such that one will improve the forage for another. Elk often prefer areas that cattle have previously grazed, which removes senescent material and allows for new growth (Crane et al., 2016).

Diet selection depends on many factors: different nutrient requirements, forage availability, species anatomy, and presence of competitors (Stewart et al., 2003). Deer are commonly classified as browsers, which consume more woody material than grasses and forbs (Powell and Olson, 1992). This is related to their anatomy of relatively small fore-stomaches (among ruminants) and large salivary glands that make it possible for them to gain nutrients from forbs and woody materials that are heavily defended, nutritious, and are high in cell solubles (Beck and Peek, 2005; Hofmann, 1989). Cattle have complex fore-stomaches, which allows efficient digestion of cellulose-dense grasses and thus are classified as grazers (Hofmann, 1989; Powell and Olson, 1992). Elk anatomy is between these two allowing for both grazing and browsing depending on available resources (Beck and Peek, 2005; Hofmann, 1989). While anatomy may select for certain diets, ungulate diet is found to be widely influenced by the available forage (Ralphs and Pfister, 1992).

Past studies on mule deer, elk, and cattle diets show wide variation in results depending year of data collection, location, season, and diet analysis techniques (Hansen and Reid, 1975). Overall, cattle diets are predominantly grasses, with relatively equal contributions of forbs and browse depending on seasons; elk also consume large amounts of graminoids, although not as much as cattle, and browse is also important in their diets. Mule deer diets are dominated by browse, and they consume very few graminoids (Scasta et al., 2016). Depending on habitat and season, shrubs and forbs can be frequently consumed by all three ungulate species (Beck and Peek, 2005; Holechek et al., 1982; Sandoval et al., 2005).

Diet shifts are seen seasonally with the availability of different vegetation and forage quality. These shifts can result in an increase in diet overlap between species at various times of the year (Hansen and Reid, 1975; Torstenson et al., 2006). While different metrics for niche overlap are used across studies, there is wide agreement that cattle and deer show the least diet overlap (Beck and Peek, 2005; Findholt et al., 2004; Kingery and Mosley, 1996). Highest overlap is often between cattle and elk in spring and late summer when their diets are grass and forb dominated (Beck and Peek, 2005; Findholt et al., 2004; Kingery and Mosley, 1996). Mule deer and elk diets can also have high diet overlap when their diets are both composed of forbs and woody species (Beck and Peek, 2005; Sandoval et al., 2005; Torstenson et al., 2006).

Primary methods for diet analysis are microhistological analysis of feces or rumen contents (Holechek, 1982; Malechek, 1968), direct observation of bites and foraging behavior (Holechek, 1982), spectral methods (Keli et al., 2008), stable isotopes (Stewart et al., 2003) or plant alkane fingerprints (Dove and Mayes, 1996).

Microhistological analysis is the most common method and consists of visually identifying microscopic plant fragments from scat samples. There are limitations to this method: a portion of plant fragments are unidentifiable, differential digestion of different vegetation influences the amounts of remains, and observer skill and bias can influence accuracy. More specifically it is difficult to differentiate grass species and highly digestible forbs and woody material can be underestimated (Sandoval et al., 2005; Scasta et al., 2016).

Advances in molecular techniques make it possible to identify species that occur in scat by sequencing plant DNA using DNA metabarcoding. DNA metabarcoding replicates and sequences short DNA fragments that vary between plant species from residual plant fragments in feces (Niderkorn et al., 2014). Universal plant primers are used to target a variable region of DNA (Taberlet et al., 2007), while family specific primers can be used to gain better species identification (Baamrane et al., 2012; De Barba et al., 2014).

This study is part of a larger experiment in eastern Oregon designed to examine the interaction of fire and grazing impacts on vegetation (Kerns et al. 2011) (Figure 1). Results from Kerns et al. (2011) suggested that cattle grazing exclusion in the study area increased native perennial forb cover, and the cover of some shrubs. The objectives of our study were to 1) use DNA metabarcoding methods to investigate diet composition of cattle, mule deer, and elk, 2) examine dietary niche overlap between ungulate species, and 3) compare differences in diet overlap between two pastures. It was hypothesized that, as seen in past studies, that cattle and elk diets would be a majority of grasses leading to high diet overlap between these two

species. Deer would be very different with high amounts of browse and thus show low diet overlap with cattle and elk. Diets for all ungulates were expected to differ with pasture since species composition was so different, although not as much for the deer and elk which weren't constrained to the pastures.

Methods

Study Site

The study site was located in a ponderosa pine forest of the Blue Mountain ecoregion, Emigrant Creek Ranger District of the Malheur National Forest in eastern Oregon (Figure 1). This part of the state is characterized by old accreted terrains and dry forests of ponderosa pine with historically frequent fire (Franklin and Dyrness, 1973; Kerns et al., 2011, 2006). This study site is part of the larger Season and Interval of Burn study (SIB) (Buscardo et al., 2010; Hatten et al., 2008; Kerns et al., 2011, 2006). Elevation of the stands is between 1600 and 1700 meters and slopes are mostly gentle (3% to 50%). The climate is characterized by short, hot growing seasons and cold winters with the majority of precipitation as snowfall from October through April. Mean annual cumulative precipitation was estimated to be 610 mm per year from 1982 to 2008 (Kerns et al., 2011). Snowmelt normally occurs from May to mid-June and is followed by spring ephemeral annual and biennial plants. Rapid growth and expansion is done by perennial grasses and forbs through June. By August, most of these plants are dormant or senesced.

Three stands from the SIB study were used in this study to complement the SIB experiment on post fire cattle grazing vegetation response. Two of the stands are

within the same Ragged Rock pasture and located in the eastern part of the study area, thus showing similar vegetation patterns (Figure 1). The third stand is in the North Idlewild pasture located 18 km west, showing slightly different vegetation composition and higher productivity (Kerns et al., 2011) (Table 1). Each stand is divided into three treatment units: control (no burn), 5-year spring burn, and 5-year fall burn, ranging from 4-18 ha in size. These burn treatments are part of the landscape mosaic that make up each of the pastures. Each treatment has three plots that were used opportunistically for sampling locations for this study.

Overstory cover is dominated by ponderosa pine (most estimated to be 80-100 years old), while western juniper (*Juniperous occidentalis*) and curl-leaf mountain mahogany (*Cercocarous ledifolius*) also contribute to the open canopy. Ragged Rock understory vegetative cover is dominated by bunch grasses (*Elymus elymoides, Achnatherum occidentale, Poa wheeleri, P. secunda, Bromus carinatus*) (Table 1). These stands are drier and have lower productivity than the North Idlewild pasture. At North Idlewild, total understory vegetation cover is higher and is dominated by sedges (*Carex geyeri*) and forbs (*Arnica cordifolia* and *Kelloggia galioides*) with shrubs (*Berberis repens, Purshia tridentata, Symphoricarpos albus, S. oreophilus, Prunus virginiana* var. *melanocarpa,* and *P. emarginata*) contributing as well. Exotics are slightly less common at North Idlewild than Ragged Rock (Kerns et al., 2011). The main plant association in Ragged Rock is *Pinus ponderosa/Agropyron spicatum*, while at North Idlewild *Pinus ponderosa/Carex geyeri* is the major plant association (Table 1). Vegetation data was collected in the summer of 2015 as part of the SIB study.

These pastures have been grazed by cattle since before the formation of the National Forest Reserves in the 1880s and data are available of stock loads since 1946, which show almost continuous grazing. The area was grazed heavily and for longer seasons from the 1940s through 1990s. Since then, cattle numbers have decreased and the grazing season shortened. In 2016 cattle grazed the area from June to August, which aligns with peak flowering of cool-season grasses (Kerns et al., 2011).

Scat Collection

The experimental treatments and vegetation plot locations were used to spatially distribute our scat collection efforts and provide an unbiased data collection methodology within each pasture. Within each stand (Driveway 26, Driveway 28, Kidd Flat) nine scat samples from each species (cattle, mule deer, and elk) were collected over two days in mid-July 2016 when weather was in the 80s ˚F (27˚C) and dry. This was done by starting at the established vegetation plot centers and searching outward for the freshest, easiest to identify, (and thicker for cattle samples) or what was present in instances of scarce scat. Thus, there was a bias towards larger scats since small or fissured samples had been more exposed and the possibility of contamination was higher.

For cattle scats, the top was lifted off or the pie was broken in half to expose the interior. Latex gloves were worn and tweezers and scoopers were rinsed with 100% ethanol before sampling and not reused. Samples were taken from multiple

locations in the sample and between 1 and 5 mL was placed in a 15 mL centrifuge tube of ethanol (1:4 scat to ethanol).

Mule deer and elk pellets were collected both in 15 mL ethanol tubes and dried in paper bags. For preservation in ethanol (at all Driveway 28 plots), individual pellets were broken and the inside was collected with new tweezers that had been rinsed with 100% ethanol. Since only a small amount could be collected from each, many pellets were used. It was ultimately decided that it would be more efficient and less chance of contamination if this step was completed in the lab. At the other units (Driveway 26 and Kidd Flat) pellets were simply identified and then a handful were placed in paper bags for later prep in the lab. Pellet samples were dry on collection, so samples were simply kept at room temperature in a dry room and in the dark until their processing in October.

Scat Processing

For those samples collected in paper bags and dried (mule deer and elk in Driveway 26 and Kidd Flat), three pellets were randomly selected and cut open with a razor blade to extract 0.5 to 1 mL from the interior of the pellet which was then placed into two 2mL tubes and labeled. For sterilization new razor blades, gloves, and cutting surface (petri dishes) were used for each sample and treated with UV light for one minute prior to use.

For those samples preserved in 100% ethanol (all cattle, and mule deer and elk at Driveway 28), scat was removed from the ethanol by scooping out with a popsicle stick into a petri dish and allowed to dry overnight. Tweezers, washed in bleach,

rinsed in water, and treated for 1 minute of UV light, were used to transfer scat from petri dish to match similar amounts of the dried samples into two 2mL tubes and labeled. One 2mL tube was preserved in the freezer as a backup.

DNA extraction was completed with the Qiamp DNA Stool Mini Kit from Qiagen (Hilden, Germany), with modified protocol with the Levi Lab (at Oregon State University, Department of Fisheries and Wildlife). 1.0 Zirconia/Silica mirobeads (Biospec Products, Bartlesville, OK) and ASL buffer were added to the sample and votexed for 2-5 minutes to break up the sample. This was then centrifuged and the lysate was removed to a new tube. InhibitX tablets were added to each sample and immediately votexed to dissolve and mix the tablet for prevention of plant inhibitors. This was then centrifuged and the lysate was removed to a new tube where Proteinase K and Buffer AL were added and the sample was incubated at 70˚C to further lysate cells and remove contaminating proteins. DNA was then precipitated by adding 100% ethanol. This was run through a QiAmp blood and tissue or DNeasy spin column to collect the DNA. The DNA was washed with AW1 and AW2 buffers, and any residual ethanol was allowed to evaporate. The final wash was done with Buffer AE to release DNA from the membrane and was collected in a 1.5 mL tube and promptly frozen at -20˚C. Each extraction batch consisted of 11 to 13 samples and was accompanied by a blank, that was treated the same followed through sequencing, to test for contamination.

PCR Amplification and Sequencing

Primers *g* and *h* of the *trnL* region of the chloroplast were used for general species identification, and specific primers (ITS1-Poa and ITS2-Ros) in the ITS1 and ITS2 regions of nuclear DNA were used to identify specific grass and rose species (Table 2) (Baamrane et al., 2012; De Barba et al., 2014; Taberlet et al., 2007). These were selected to allow for general taxonomic identification with a more specific focus on grasses and many of the rosaceous shrubs. These primers were selected for their short amplicon length since the study involved degraded, small DNA fragments (Hollingsworth et al., 2011; Valentini et al., 2009). When compared to alignment of known species in Geneious version (10.1) (http://www.geneious.com, Kearse et al., 2012) these three regions showed high conservation at primer binding locations and differentiation in between primers for different species. Nextera adapters were added to these primers, which made them longer by 30 bp.

Multiplex polymerase chain reactions (PCR) were done with all primers using the Qiagen Multiplex PCR kit. A primer mix was created of all six primers at equal 0.2uM concentration and DNA free water. The 20 uL PCR consisted of 1x concentrated Qiagen Master Mix, 1x of primer mix, DNA free water, and 2uL of DNA extract. The PCR profile initial denaturation step of 15 minutes at 95˚C, followed by 35 cycles of 30 seconds at 94˚C, 90 seconds at 58˚C, 90 seconds at 72˚C and a final 10 minutes at 72˚C elongation.

Each PCR reaction was accompanied by a no template control, which had no DNA extract, to check for contamination. There was also a positive control DNA sample of plant tissue that was a mixture of a grass (Poaceae) and curl-leaf mahogany

(Rosaceae) at equal concentrations of DNA (4.0ng/uL) to check for primer success and efficiency. Three PCR replicates were done for each sample.

After the initial amplification, samples were purified using Solid Phase Reversible Immobilisation (SPRI) bead technology from Aline Biosciences. This cleaned up all PCR buffers, polymerase and unamplified genomic DNA. After cleanup, we performed an index PCR using the Nextera XT Index kit from Illumina Inc. (San Diego, CA). This step added an additional forward and reverse tag to allow us to differentiate samples, as well as Illumina adaptors that allow the PCR product to attach to the Illumina flow-cell for sequencing. One more round of PCR cleanup was performed with the SPRI beads and all plates were then sent to the Center for Genome Research and Biocomputing (CGRB) at Oregon State University where PCR products were normalized, pooled and sequenced on the Illumina HiSeq 3000.

Reference Library

Plant specimens of leaves and stems of key forage species that did not have sequences already in GenBank were collected at the time of scat collection. Samples were from elk sedge (*Carex geyeri*), curl leaf mountain mahogany (*Cercocarpus ledifolius*), snowbrush ceanothus (*Ceanothus velutinus*), and wax current (*Ribes cereum*). These samples were placed in brown paper bags, allowed to air dry, and stored in a dark, dry location until processed in November. An unknown grass (most likely, annual bluegrass) was also collected to use in optimizing primers.

Plant DNA extractions were used both for optimizing primers and sequencing key forage species that were not present in GenBank. These extractions were done

with the E.Z.N.A. Plant DNA kit from Omega Bio-tek, Inc. (Norcross, GA) following manufacturer's protocol for dried samples. Initial sample processing was done by finely chopping the dried specimens and grinding with 1.0 Zirconia/Silica mirobeads (Biospec Products, Bartlesville, OK) during the first step of protocol.

The DNA extraction was amplified using primer pair *d* and *g* of the *trnL* region of the chloroplast (Taberlet et al 2007). PCR was performed in a total volume of 20 uL consisting of 1x concentrated AmpliTaq Gold 360 Master Mix (Life Technologies), 0.2uM of each primer, 0.5mg/mL of bovine serum albumin (BSA), DNA free water, and 1uL of DNA template. The PCR profile initial denaturation step of 10 minutes at 95˚C was followed by 40 cycles of 15 seconds at 95˚C, 30 seconds at 54˚C, 60 seconds at 72˚C and a final 7 minutes at 72˚C elongation. Samples were checked for successful amplification with gel electrophoresis and the PCR product was purified following Exosap protocol. They were then Sanger sequenced by the CRGB at OSU.

These sequences were added to the local reference library which was made from selecting sequences of plant species known to occur in the area from the global GenBank library.

Bioinformatics

Sequences for each sample were paired using PEAR v. 0.9.10. Taxonomic assignment was done by first using BLAST against all *trnL, ITS1,* and *ITS2* plant sequences in Genbank, and then again using BLAST against a local database containing *trnL, ITS1,* and *ITS2* sequences from a large subset of plants known to

occur in Northeastern Oregon. When Genbank and our local database produced equal percent match results, we used results from the local database to break ties. Primary analysis was done using the *trnL* sequences (unless otherwise stated) since this is a global plant barcode, while ITS sequences can help in confirming species. Sequencing results from each sample were filtered to only include *trnL* sequences with more than 500 reads. This cutoff was based on the number of reads that were returned for each primer in the positive control sample.

All unique *trnL* sequences were assigned to family and potentially genus from BLAST results. Since general BLAST results were from the global database and many 100% matches were returned for each unique sequence, we were unable to determine exact species of each sequence, however most sequences could be assigned to a family and in some cases genus. From this known information, the sequence was also assigned a functional group and possible species that are known to occur in the area. Functional groups included gramminods (grasses, rushes, and sedges), woody (trees, shrubs, and unknown woody material), forbs, and unknown for families where multiple functional groups were possible, so not to overestimate either forb or woody amounts.

Unique *trnL* sequences that occurred in scats were compared to sequences that were found in no template controls and extraction blanks. Those sequences of high concentrations in no template controls were labeled as contaminants and removed from the dataset. This included sequences for *Solanum lasiocarpum* and *Musa acuminata*, respectively a species of nightshade and banana from Southeast Asia that were obviously not being consumed by these ungulates. There was one sequence that

was found in only one extraction blank, but occurred in many samples. This sequence was labeled as a contaminant and removed for only those samples that were extracted with that blank, this however introduces bias since this sequence could have naturally occurred in these samples as well.

Data Analysis

Diet composition was quantified using two methods: frequency of occurrence (FOO), which measures the number of samples a sequence occurs in, and relative read abundance (RRA) which measures the proportion of the total number of reads in each sample (Kartzinel et al., 2015).

FOO is a simple measure of proportion of samples that the given sequence/family/habit occur in. This can tell us about diet richness and is a less biased metric. Calculations assumed that if a sequence appeared in one to three of the triplicate samples for a given scat sample, it was present in that scat sample. The number of scat samples that the interested element occurred in was then divided by the total number of scat samples of that ungulate species that returned results.

RRA can potentially give us a better understanding of the diet composition as it uses the amount of reads for each element of interest, representing biomass amounts (Kartzinel et al., 2015). This can be a biased measure since the primer may not bind equally to all consumed species (Niderkorn et al., 2014; Yang et al., 2016). RRA was calculated first as the proportion of element of interest reads in a sample divided by the total number of sequence reads in that sample. This was then averaged between the three triplicate samples for each scat sample, and average RRA within each

ungulate species was calculated from these values. Since RRA values were calculated from averages, they did not total to 100%, thus each average value was adjusted to be out of 100%.

Dietary niche breadth was measured using Hulbert's standardized niche breadth (B_A), which is a standardization of Levins' niche breadth metric to be on a 0 to 1 scale, with 0 indicating highly specialized and 1 highly generalized (Hurlbert, 1978):

$$B_{Ajk} = \frac{(1/\sum {p_{ijk}}^2) - 1}{n_{jk} - 1}$$

where p_{ijk} is the proportion of plant sequence/family *i* (RRA) in the diet of ungulate *j* in pasture *k*, and n_{jk} is the total number of plant sequence/family for ungulate *j* in pasture *k*.

Dietary niche overlap was determined between pairs of ungulates in each pasture using Pianka's adaptation of the niche overlap (*Ojk*) metric (Pianka, 1973):

$$O_{jmk} = \frac{\sum_i^n p_{ijk} p_{imk}}{\sqrt{\sum_i^n {p_{ijk}}^2 {p_{imk}}^2}}$$

where p_{ijk} is the proportion of plant sequence/family *i* (RRA) in the diet of ungulate *j* in pasture *k* and p_{imk} is the proportion of prey species *i* in the diet of ungulate m in pasture *k*. An *Ojmk* metric of 0 represents no overlap, while a value of 1 represents complete diet overlap.

Results

We collected 81 scat samples evenly between the three ungulate species. Of these, 77 returned sequences that could be used for diet analysis (cow n=26, elk n=27, deer n=24) (Table 3). These were divided between the two pastures with 18 of each ungulate collected in Ragged Rock and 9 scats from each ungulate in North Idlewild (Table 3). Of the trnL sequences with more than 500 reads, we found 118 unique sequences, 8 of which occurred in our extraction blanks and non-template controls and thus removed from further analysis (Table 5). All sequences were matched from the BLAST library and identified to family (several could only be identified to several possible families) and then assigned functional group based on family, resulting in 33 unique families (Table 5). Those families with multiple functional groups were dependent on genus to be assigned functional group, if genus was unclear then they were classified as unknown, so forbs or shrubs may be under represented, but are not over represented.

Although sequences could not easily be identified to distinct species, if it is assumed that each sequence represents an individual species then RRA, FOO, and richness of sequences represent these statistics of individual plant species. Elk had the highest dietary richness of 75 unique sequences in 26 families, with an average of 15.1 unique sequences per scat (Table 3 and 4). Cattle and deer had lower dietary richness, with 64 and 58 unique sequences in 23 and 22 families, respectively (Table 3). This pattern of overall richness was also observed in the number of unique families and sequences in each scat sample for these three ungulates (Table 4, Figure 2).

Analysis by plant functional group allowed for the characterization of ungulate diets as grazers or browsers. FOO grouped by functional group showed all ungulates were eating forbs (Figure 3). Forbs made up a large percentage of the RRA for all ungulates, but were of decreasing importance for deer (57%), elk (49%), then cattle (36%) (Figure 4, Table 6). Almost all cattle consumed some grass (96%), while only 70% and 29% of elk and deer samples respectively contained a grass (Figure 3). This pattern is also apparent among other graminoids (sedges and rushes) (Figure 3). Similar to what was shown by FOO, graminoids had a high RRA for cattle (49%) and decreased for elk (18%) and deer (4%) (Table 7, Figure 4). Shrubs and trees occurred more often in deer (FOO: 79%, RRA: 50%) and elk (92%, 37%) samples than in cattle (50%, 19%) samples (Figure 3). The RRA for woody plants (shrubs, woody, and trees) was in higher abundance in deer (30%) and elk (25%) diets than for cattle diets (8%).

Diet differences between ungulates were also examined by FOO and RRA by family and individual sequence (Tables 5, 7 and 8, Figures 5 and 6). FOO was similar across ungulates for the predominant forb families (Asteraceae, Onagraceae, and Polygonaceae), while presence of graminoid families (Poaceae and Cyperaceae) were of decreasing importance in elk and deer diets compared to cattle diets as noted above. Woody families (Rhamnaceae, Grossulariaceae, Pinaceae, and Cupressaceae) occurred in more deer and elk samples (Figure 5). RRA by family shows that Asteraceae, Onagraceae, Polygonaceae, Geraniaceae, and Caprifoliaceae are of relatively equal importance between all ungulates (Figure 6, Table 8). Poaceae RRA was very small for deer diets (4%) but, large in cattle diets (39%). Families including

Junacaceae and Cyperaceae make up almost 10% of RRA for cattle, but were not prevalent in elk or deer samples. Rosaceae was eaten the most by elk (21%), followed by deer (14%) and then cattle (12%).

Dietary niche overlap difference between pastures was examined using calculated niche overlap values (*Ojk*). This showed high diet overlap for all ungulates, with deer and elk being the most similar (0.80) and cattle and deer being the least similar (0.51) (Table 9). Diet overlap was higher for all ungulate interactions in the Ragged Rock pasture compared to the North Idlewild pasture increasing between 0.155 and 0.322 (Table 9, Figure 7). The lowest diet overlap is 0.24 between deer and cattle in North Idlewild; the greatest diet overlap is between elk and deer in Ragged Rock (0.78). Calculated niche breadth was relatively similar across species, with deer and elk having slightly larger breadth than cattle and no clear patterns across species between pastures (Table 9, Figure 8).

When RRA was compared between pastures and to known plant cover (Table 1), forage preference could be inferred when vegetation occurred at higher rates in diets than proportionally available (Figures 9 and 10). This showed that while grasses have high cover in Ragged Rock, all ungulates ate lots of forbs which have low cover. In North Idlewild sedges were the primary ground cover (27%), however they only showed up in large amounts in cattle diets (14%) and the cattle were still consuming more grasses (45%). Deer and cattle consumed less forbs (27%, 22%) in North Idlewild even though they had higher cover (18%). Browse species were a large component of deer diets in North Idlewild (53%) where they were more abundant (5.7% cover) compared to Ragged Rock (1.5% cover) (Figure 9).

Forage selection by family (Figure 10) showed that in Ragged Rock all ungulates consumed Asteraceae, Onagraceae, Polygonaceae, and Rosaceae families in much higher amounts than their relative cover. While Poaceae was abundant in Ragged Rock (38% cover), cattle and elk are consuming large amounts of grass (37% and 16% respectively), but did not select for it. The opposite was true in North Idlewild where Poaceae was of low abundance (8%), yet made up relatively more of cattle diets (45%). In North Idlewild, Asteraceae was consumed less than its relative abundance (7%) for all ungulates, especially deer (1%). Deer there consumed large amounts of Rhamnaceae (28%) and other shrubs (Figure 10).

Discussion

Our results show that all ungulate species were consuming large amounts of forbs. While other studies have shown that forbs can be important at specific times of the year for elk and deer (Beck and Peek, 2005; Findholt et al., 2004; Sandoval et al., 2005), none has shown such a high percentage of forbs in cattle diets (Beck and Peek, 2005; Findholt et al., 2004; Holechek et al., 1982; Kingery and Mosley, 1996; Sandoval et al., 2005; Torstenson et al., 2006). Our results are from mid-summer when grasses are not of peak quality, thus ungulates often favor forbs (Beck and Peek, 2005; Torstenson et al., 2006). Forb consumption happens at even higher rates for cattle in the spring in this region of Oregon (Holechek et al. 1982). Past studies may have underestimated the amount of forbs in diets when using microhistogical analysis as forbs can be easily digested leaving no remains to be identified (Sandoval et al., 2005). Major forb families that were consumed by all ungulates include

Asteraceae, Onagraceae, and Polygonaceae. Species within these families are important to monitor as part of available forage for stocking rates.

Grazing exclosures in this SIB study area have showed an increase in forb cover with the removal of cattle grazing and continued mule deer and elk utilization (Kerns et al., 2011). Since cattle are consuming large amounts of forbs (35%) in the summer, this would explain why forb cover would increase with the removal of cattle. Cover for some shrubs also increased, but cattle are consuming these at low rates and most likely are not the only reason for increased cover.

As expected for grazers, cattle consumed the greatest amount of grasses, sedges, and rushes (49%). However, RRA of graminoids were not quite as high compared to past studies that show the majority of cattle and elk to be grasses (Hansen and Reid, 1975; Holechek et al., 1982; Kingery and Mosley, 1996; Powell and Olson, 1992; Torstenson et al., 2006). Elk, as mixed grazers, are often shown to have diets dominated by grasses, not forbs as in our results (Hansen and Reid, 1975; Torstenson et al., 2006). Our results do agree with past studies that deer and elk diets contain browse at higher levels than seen in cattle diets (Beck and Peek, 2005; Findholt et al., 2004; Hansen and Reid, 1975; Kingery and Mosley, 1996; Sandoval et al., 2005). While fifty percent of cattle samples contained browse species, these species are being eaten in small amounts by cattle based on RRA (Figures 3 and 4).

Our results that elk had the highest diet richness among ungulates we sampled differs from many studies, which show deer having the highest richness (Kingery and Mosley, 1996; Torstenson et al., 2006). Overall niche breadth was found to be

slightly greater for deer than elk. Elk have a more pliable diet (Sandoval et al., 2005; Torstenson et al., 2006), which this higher richness would represent.

Low dietary niche overlap between cattle and deer (54%), as shown in our data, is well supported by other studies (Beck and Peek, 2005; Findholt et al., 2004; Kingery and Mosley, 1996; Scasta et al., 2016). Greater overlap was expected between elk and cattle (Hansen and Reid, 1975; Kingery and Mosley, 1996), however our results show the highest overlap between the native ungulates (84%); this diet overlap is supported by other studies although often not at such high levels (Beck and Peek, 2005; Findholt et al., 2004; Kingery and Mosley, 1996; Sandoval et al., 2005). This high overlap could be due to the time of year as the elk in our study are not eating large amounts of grass, but more forbs and browse which is similar to what mule deer are consuming. The food base for deer and elk varies through the season as food quality changes, their highest overlap has been documented in the summer when both are eating forbs (Beck and Peek, 2005). It is likely that although there is high diet overlap, which shows potential for competition, niche overlap is decreased by selecting for foraging grounds where the other is not present so there is less competition (Coe et al., 2004; Vavra, 1996). Elk are known to displace deer from their preferred foraging areas (Coe et al., 2004). We do not have data for our stands on movement patterns or interspecies interactions; however, elk scat was far easier to find than deer scat at most locations, which could indicate that deer are not using the area due to the presence of elk or many other reasons like not liking vegetation cover.

To spatially relate our results, we must consider that average passage time of consumed plants is around two days for these ruminants, with deer digestion

occurring slightly faster than for either cattle and elk (Asano et al., 2005, Spalinger et al. 1993). First physical markers may occur 14 to 19 hours after digestion (Asano et al., 2005), but it is unknown how soon plant DNA is detectable and for how long after consumption. Elk and cattle are known to travel an average of 14 and 11 km, respectively, daily during the summer in the Blue Mountains (Clark et al., 2017). So, the plants detected at scat sampling locations could have been consumed as far away as 30 km for elk and deer that are not constrained by pasture fences. Cattle however are constrained by the pasture and have only foraged within them since their arrival in early July.

Dietary niche overlap was lower for all species interactions in the North Idlewild than in the Ragged Rock pasture. There are many possible reasons for this difference: not having an equal sample size between the pastures (resulting in not identifying as many plant species), difference in forage species available to choose from, different forage species actually being consumed (which could potentially have different primer binding efficiency), or a change in niche breadth.

North Idlewild is more productive, with higher plant cover than Ragged Rock (Table 1). If food is more abundant, it is possible that competition would be lower here as ungulates can differentiate their diets and be more selective in what they eat instead of just having to consume what is available. Niche breadth, however, does not show that diet is more generalized at Ragged Rock for all ungulate species and sample richness does not differ between pastures. Niche breadth does expand for elk in Ragged Rock, however cattle are opposite in that their diet breadth decreases in

Ragged Rock. Deer seem to switch to different food sources between pastures since overall niche breadth is greater while relatively equal in each pasture.

Vegetation cover and forage selection are more helpful in explaining the difference between pastures. At North Idlewild, sedges are in high abundance, but are not selected for by any species. Sedges are only abundant in cattle diets (14%), but still present at a lower amount than expected compared to plant abundance. Cattle at North Idlewild are instead selecting for grasses, even though they were of low abundance. This differs from Ragged Rock where grasses were highly abundant, but were not selected for over forbs. This selection pattern is potentially related to the species composition differences of grasses between the pastures.

All ungulates actively selected for Onaagraceae, Polygonaceae, and Rosaceae families in all locations. Both deer and cattle consumed smaller amounts of forbs, particularly in the family Asteraceae in North Idlewild, even though Asteraceae and forbs are of higher abundance here. Deer instead consumed a large variety of other families, particularly shrubs in the Grossulariaceae, Rhamnaceae, and Rosaceae families. A possible explanation for this would be that mule deer prefer to consume woody species when they are present, but when they are not they will instead select for forbs. This combined with cattle selecting for forbs instead of graminoids in Ragged Rock would account for the increase in diet overlap at the plant species level (even though cattle diet breadth decreases) at Ragged Rock.

Mule deer selecting for forbs when shrubs are in low abundance in Ragged Rock combined with the increase in elk diet breadth could explain the higher overlap here compared to North Idlewild. The higher diet overlap between elk and cattle in

Ragged Rock can be explained with the increase in elk diet breadth and cattle selecting for forbs over grasses. The diet comparison between pastures suggests that when resources are lower (Ragged Rock) different ungulates will respond with different strategies. Mule deer switched the species and functional groups they were consuming, while elk increased their niche breadth and cattle had a slight decrease of niche breadth, but changed in their preference for grasses and forbs.

The separation in space between pastures may lead to different plant species compositions, but also different plant phenology. If forbs were high quality forage in Ragged Rock, but already past their prime in North Idlewild when samples were taken, this could account for why forbs were not selected for in North Idlewild. However this is unlikely as typically plants are seen to senesce later in North Idlewild.

Our analysis is based on the assumption that *trnL* primers we used bind to all plant species present equally. This is key for assuming RRA results represent the amount of biomass consumed of each species (Kartzinel et al., 2015; Niderkorn et al., 2014). Our positive control of equal concentrations of grass and Rosaceae DNA showed relatively equal number of *trnL* sequences, which supports this assumption. However, FOO of specific grass primers (ITS1-Poa) shows that there is grass present in all scat samples for all ungulates, which does not match the FOO calculated from the *trnL* data (Table 7, Figure 5). Possible reasons for this result are that the ITS1-Poa primer has a higher binding efficiency, that low grass concentration to high primer concentration replicates grasses even at trace amounts (for deer samples), contamination from grass ground cover is possible, ITS1-Poa uses nuclear DNA

while *trnL* replicates chloroplast DNA, and ITS1-Poa also has a greater read depth. Our positive control showed ITS1-Poa number of sequences to be three times higher than *trnL* results, supporting high binding efficiency. Comparable results were seen by Kartzinel et al. (2015) and they justified using *trnL* data since it was picking up dominant grasses.

Difference in sample preservation methods could also potentially bias results. When results from the same ungulate species and same pasture, but different preservation methods were compared some difference in unique sequences, but not number of reads was observed. The number of unique sequences was lower for both deer and elk when dried, than when stored in ethanol (Table 10). However, comparison of these species shows that overlap is high between the different sequences observed, and that most sequences observed when dried are also present in ethanol preserved samples. Some difference in sequences is expected as samples were collected at slightly different locations within the pasture. Although the number of reads per sample differed there was no pattern between preservation methods (Table 10).

This project will be continued with results from family specific primers and sequence identification to species. Better identification of what plant species are being consumed should be possible with the specific family primers and a local library database of all species known to occur in the area. Statistical tests will also be informative to test the significance of differences observed. If I were to do this study again, I would include more family specific primers (particularly for Asteraceae (Baamrane et al., 2012)) and make sure that the *trnL* is actually the best universal

plant primer to be using (De Barba et al., 2014; Hollingsworth et al., 2011; Yang et al., 2016). It would be easy and beneficial to double check the identification of scats with a universal mammal barcode primer. Also, it would be ideal to create a local reference library from sequences of local vegetation samples instead of from GenBank. To better understand forage selection, scat should be collected at the same time as vegetation surveys. Enlargement of the project to cover multiple seasons and years would also increase understanding of how variable diets are temporally.

There are multiple management implications of these results that can be applied to rangelands. Since all ungulates are consuming large amounts of forbs, these species are important to include in stocking rate decisions and in utilization measurements. This emphasizes that grasses are not the only vegetation cover that should be considered when monitoring rangeland health or recovery. Foraging selection can be a local phenomenon and potentially differ because of available resources or species composition. To aid in mule deer population increases, shrubs are an important resource to have available on the landscape, more so than graminoids or forbs as they will seek these out when they are available.

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Table 1: Vegetation characteristics of pastures. Data collected in the summer of 2015 as part of the SIB study.

Understory Vegetation *(% cover)*	Ragged Rock	North Idlewild
Total Plant Cover	49.77	58.29
Forbs	7.74	17.83
Grass	38.2	8.14
Rush	0	0
Sedge	2.26	26.65
Shrub	1.36	4.37
Tree	0.21	1.3
Understory Richness	81	72

Table 2: All primers used in this study

Primer name	Taxon	DNA type	DNA region	Forward/ Reverse	Primer Sequence	Reference
g	Plants	Chloroplast	trnL	Forward	GGGCAATCCTGAGCCAA	Taberlet et al (2007)
h				Reverse	CCATTGAGTCTCTGCACCTATC	Taberlet et al (2007)
ITS1-F	Poaceae	Nuclear ribosomal	ITS1	Forward	GATATCCGTTGCCGAGAGTC	Baamrane et al. (2012)
ITS1Poa-R				Reverse	CCGAAGGCGTCAAGGAACAC	Baamrane et al. (2012)
ITS2Ros-F	Rosaceae	Nuclear ribosomal	ITS2	Forward	YCTGCCTGGGCGTCACA	De Barba et al (2014)
ITS2Ros-R				Reverse	CGTKVGYCGCCGAGGAC	De Barba et al (2014)
d	Plants	Chloroplast	trnL	Reverse	GGGGATAGAGGGACTTGAAC	Taberlet et al (2007)

Table 3: Summary statistics of samples collected and results

Pasture	all			Ragged Rock			North Idlewild		
Ungulate	Cattle	Deer	Elk	Cattle	Deer	Elk	Cattle	Deer	Elk
Number of samples collected	27	27	27	18	18	18	9	9	9
Final number of samples	26	24	27	18	17	18	8	7	9
Final number unique trnL sequences	64	58	75	49	50	57	39	27	41
Final number of unique families	23	22	26	19	20	22	16	14	14
Final number of unique ITS1-Poa sequences	56	51	61	42	46	49	35	20	29

Table 4: Average richness of unique sequences and families per scat sample overall and by pasture

Pasture	all			Ragged Rock			North Idlewild		
Ungulate Species	Cattle	Deer	Elk	Cattle	Deer	Elk	Cattle	Deer	Elk
Average unique sequences per sample (st.dev)	10.7 (3.7)	8.9 (5.0)	15.1 (8.3)	11.1 (2.9)	2.3 (5.1)	14.8 (7.8)	9.9 (5.3)	7.9 (4.8)	12.7 (3.3)
Range	4-17	1-18	6-42	6-17	1-18	6-37	4-16	1-14	7-18
Average unique families per sample (st dev)	6 (1.9)	5.4 (2.6)	7.8 (3.3)	6.2 (1.8)	5.47 (2.6)	7.4 (3.1)	5.6 (2.3)	5.1 (2.6)	7.1 (1.3)
Range	3-11	1-9	4-18	3-11	1-9	4-16	3-9	1-8	5-9

Table 5: Unique trnl sequences with assigned family, best match, functional group, frequency of occurrence and relative read abundance. Sequences are indicated if treated as contamination.

Sequence Id #	Taxa	Habit	Contamination	FOO			RRA		
				Cattle	Elk	Deer	Cattle	Elk	Deer
1	Asteraceae	forb	No	1.000	1.000	0.917	0.011	0.014	0.012
2	Asteraceae	forb	No	0.962	0.926	0.833	0.014	0.011	0.009
3	Asteraceae	forb	No	0.846	0.852	0.667	0.010	0.004	0.005
4	Asteraceae	forb	No	1.000	1.000	0.833	0.027	0.020	0.014
5	Asteraceae	forb	No	0.769	0.667	0.667	0.004	0.005	0.010
6	Asteraceae	forb	No	0.654	0.815	0.792	0.005	0.007	0.013
7	Asteraceae	forb	No	0.692	0.667	0.625	0.002	0.002	0.004
8	Asteraceae	forb	No	0.654	0.926	0.792	0.002	0.011	0.011
9	Asteraceae	forb	No	0.538	0.481	0.625	0.002	0.002	0.008
10	Asteraceae	forb	No	0.577	0.333	0.458	0.003	0.001	0.001
11	Asteraceae	forb	No	0.962	0.667	0.542	0.019	0.018	0.012
12	Asteraceae	forb	No	0.346	0.111	0.292	0.015	0.008	0.041
13	Asteraceae	forb	No	0.154	0.074	0.042	0.003	0.002	0.000
14	Asteraceae	forb	No	0.538	0.407	0.500	0.071	0.109	0.078
15	Asteraceae	forb	No	0.038	0.000	0.042	0.000	0.000	0.001
16	Caprifoliaceae, Boraginaceae, Hydrophyllum	forb	No	0.000	0.111	0.042	0.000	0.001	0.001
17	Hydrophylloideae	forb	No	0.038	0.111	0.167	0.000	0.000	0.004
18	Caprifoliaceae	shrub	No	0.077	0.296	0.167	0.001	0.005	0.004
19	Poaceae	grass	No	0.000	0.037	0.000	0.000	0.001	0.000
20	Rosaceae	unk	No	0.692	0.519	0.333	0.056	0.056	0.045

21	Rosaceae	forb	No	0.192	0.222	0.000	0.016	0.022	0.000
22	Rosaceae	forb	No	0.269	0.037	0.000	0.010	0.001	0.000
23	Rosaceae	forb	No	0.000	0.111	0.000	0.000	0.003	0.000
24	Rosaceae	forb	No	0.231	0.370	0.083	0.018	0.017	0.006
25	Fabaceae	forb	No	0.000	0.074	0.083	0.000	0.001	0.002
26	Polemoniaceae	forb	No	0.000	0.037	0.125	0.000	0.000	0.001
27	Poaceae	grass	No	0.038	0.074	0.000	0.004	0.006	0.000
28	Cupressaceae	tree	No	0.000	0.296	0.333	0.000	0.062	0.019
29	Pinaceae	tree	Yes	0.000	0.000	0.000	0.000	0.000	0.000
30	Pinaceae	tree	No	0.115	0.111	0.208	0.006	0.003	0.059
31	Pinaceae	tree	No	0.000	0.037	0.083	0.000	0.000	0.004
32	Pinaceae	tree	No	0.038	0.000	0.000	0.012	0.000	0.000
33	Pinaceae	tree	No	0.000	0.037	0.000	0.000	0.010	0.000
34	Boraginaceae	forb	No	0.038	0.000	0.000	0.001	0.000	0.000
35	Boraginaceae	forb	No	0.077	0.074	0.000	0.001	0.006	0.000
36	Boraginaceae	forb	No	0.000	0.037	0.000	0.000	0.001	0.000
37	Poaceae	grass	No	0.423	0.296	0.083	0.028	0.017	0.009
38	Poaceae	grass	No	0.000	0.037	0.000	0.000	0.000	0.000
39	Poaceae	grass	No	0.077	0.000	0.000	0.001	0.000	0.000
40	Poaceae	grass	No	0.231	0.222	0.083	0.016	0.018	0.004
41	Poaceae	grass	No	0.846	0.593	0.083	0.058	0.057	0.034
42	Poaceae	grass	Yes*	0.500	0.259	0.042	0.127	0.013	0.001
43	Poaceae	grass	No	0.731	0.259	0.167	0.141	0.020	0.005
44	Poaceae	grass	No	0.269	0.259	0.000	0.008	0.029	0.000
45	Poaceae	grass	No	0.038	0.000	0.000	0.000	0.000	0.000

46	Poaceae	grass	No	0.038	0.000	0.000	0.000	0.000	0.000
47	Scrophulariaceae	forb	No	0.038	0.074	0.000	0.001	0.001	0.000
48	Malvaceae	forb	No	0.000	0.037	0.000	0.000	0.001	0.000
49	Onagraceae	forb	No	0.077	0.148	0.250	0.003	0.007	0.019
50	Onagraceae	forb	No	0.000	0.000	0.042	0.000	0.000	0.002
51	Onagraceae	forb	No	0.000	0.037	0.042	0.000	0.000	0.001
52	Onagraceae	forb	No	0.000	0.259	0.167	0.000	0.010	0.003
53	Onagraceae	forb	No	0.000	0.222	0.042	0.000	0.002	0.000
54	Onagraceae	forb	No	0.000	0.037	0.000	0.000	0.000	0.000
55	Onagraceae	forb	No	0.000	0.037	0.000	0.000	0.000	0.000
56	Onagraceae	forb	No	0.538	0.370	0.625	0.064	0.050	0.124
57	Onagraceae	forb	No	0.000	0.074	0.292	0.000	0.002	0.009
58	Onagraceae	forb	No	0.154	0.185	0.333	0.003	0.010	0.027
59	Onagraceae	forb	No	0.000	0.111	0.250	0.000	0.003	0.010
60	Onagraceae	forb	No	0.000	0.000	0.042	0.000	0.000	0.000
61	Apiaceae	forb	No	0.000	0.037	0.042	0.000	0.003	0.011
62	Apocynaceae	forb	No	0.000	0.074	0.000	0.000	0.007	0.000
63	Scrophulariaceae	forb	No	0.000	0.074	0.000	0.000	0.001	0.000
64	Solanaceae	forb	Yes	0.000	0.000	0.000	0.000	0.000	0.000
65	Salicaceae	tree	No	0.000	0.037	0.000	0.000	0.006	0.000
66	Salicaceae	woody	No	0.038	0.074	0.000	0.001	0.022	0.000
67	Apiaceae	forb	No	0.038	0.037	0.042	0.000	0.001	0.001
68	Apiaceae	forb	No	0.000	0.037	0.000	0.000	0.005	0.000
69	Rhamnaceae	shrub	No	0.192	0.333	0.375	0.022	0.027	0.105
70	Fabaceae	forb	No	0.077	0.037	0.000	0.004	0.002	0.000

71	Grossulariaceae	shrub	No	0.038	0.000	0.000	0.001	0.000	0.000
72	Grossulariaceae	shrub	No	0.000	0.000	0.042	0.000	0.000	0.001
73	Grossulariaceae	shrub	No	0.038	0.074	0.125	0.003	0.003	0.002
74	Brassicaceae	forb	No	0.038	0.000	0.042	0.001	0.000	0.042
75	Ranunculaceae	forb	No	0.038	0.000	0.000	0.000	0.000	0.000
76	Ranunculaceae	forb	No	0.000	0.037	0.000	0.000	0.002	0.000
77	Plantaginaceae	forb	No	0.077	0.000	0.000	0.001	0.000	0.000
78	Sapindaceae	woody	No	0.000	0.000	0.042	0.000	0.000	0.001
79	Rosaceae	unk	No	0.077	0.111	0.125	0.001	0.003	0.004
80	Rosaceae	unk	No	0.000	0.000	0.042	0.000	0.000	0.000
81	Rosaceae	shrub	No	0.346	0.741	0.625	0.014	0.093	0.086
82	Rosaceae	shrub	No	0.000	0.037	0.125	0.000	0.002	0.001
83	Rosaceae	shrub	No	0.000	0.037	0.083	0.000	0.000	0.006
84	Lauraceae, Atherospermataceae, Gomortega	tree	No	0.038	0.000	0.000	0.007	0.000	0.000
85	Berberidaceae	shrub	No	0.077	0.074	0.167	0.006	0.003	0.009
86	Berberidaceae	shrub	No	0.000	0.000	0.042	0.000	0.000	0.000
87	Fabaceae, Loasaceae	forb	No	0.000	0.037	0.000	0.000	0.005	0.000
88	Loasaceae	forb	No	0.000	0.037	0.000	0.000	0.003	0.000
89	Loasaceae	forb	No	0.000	0.037	0.000	0.000	0.000	0.000
90	Paeoniaceae	forb	No	0.038	0.148	0.000	0.002	0.004	0.000
91	Fabaceae	forb	Yes	0.000	0.000	0.000	0.000	0.000	0.000
92	Betulaceae	tree	No	0.000	0.000	0.042	0.000	0.000	0.004
93	Lamiaceae	forb	No	0.000	0.000	0.042	0.000	0.000	0.001

94	Scrophulariaceae, Orobanchaceae	forb	No	0.000	0.000	0.042	0.000	0.000	0.000
95	Orobanchaceae	forb	No	0.000	0.000	0.083	0.000	0.000	0.001
96	Solanaceae	forb	Yes	0.000	0.000	0.000	0.000	0.000	0.000
97	Solanaceae	forb	Yes	0.000	0.000	0.000	0.000	0.000	0.000
98	Musaceae	forb	Yes	0.000	0.000	0.000	0.000	0.000	0.000
99	Fabaceae	forb	No	0.115	0.000	0.000	0.002	0.000	0.000
100	Fabaceae	forb	Yes	0.000	0.000	0.000	0.000	0.000	0.000
101	Brassicaceae	forb	No	0.038	0.000	0.000	0.001	0.000	0.000
102	Poaceae	grass	No	0.038	0.000	0.000	0.001	0.000	0.000
103	Geraniaceae	forb	No	0.077	0.259	0.250	0.002	0.008	0.010
104	Polemoniaceae	forb	No	0.000	0.000	0.042	0.000	0.000	0.004
105	Cyperaceae	sedge	No	0.038	0.000	0.000	0.001	0.000	0.000
106	Cyperaceae	sedge	No	0.077	0.000	0.000	0.002	0.000	0.000
107	Cyperaceae	sedge	No	0.038	0.000	0.000	0.001	0.000	0.000
108	Cyperaceae	sedge	No	0.115	0.000	0.000	0.004	0.000	0.000
109	Cyperaceae	sedge	No	0.038	0.000	0.000	0.003	0.000	0.000
110	Cyperaceae	sedge	No	0.269	0.000	0.000	0.016	0.000	0.000
111	Cyperaceae	sedge	No	0.231	0.222	0.083	0.051	0.040	0.016
112	Juncaceae	rush	No	0.346	0.000	0.000	0.015	0.000	0.000
113	Polygonaceae	forb	No	0.077	0.074	0.042	0.002	0.002	0.000
114	Polygonaceae	forb	No	0.500	0.407	0.500	0.070	0.109	0.078
115	Polygonaceae	forb	No	0.038	0.000	0.000	0.000	0.000	0.000
116	Polygonaceae	forb	No	0.000	0.037	0.042	0.000	0.000	0.001
117	Polygonaceae	forb	No	0.000	0.037	0.083	0.000	0.000	0.004

118	Polygonaceae	forb	No	0.000	0.037	0.000	0.000	0.000	0.000

Table 6: Relative read abundance of functional groups overall and by pasture for cattle, elk, and deer

Pasture	All			Ragged Rock			North Idlewild		
Ungulate	Cattle	Elk	Deer	Cattle	Elk	Deer	Cattle	Elk	Deer
Forb	0.364	0.491	0.568	0.428	0.472	0.691	0.219	0.530	0.268
Grass	0.394	0.138	0.036	0.370	0.165	0.021	0.448	0.083	0.073
Rush	0.016	0.000	0.000	0.012	0.000	0.000	0.025	0.000	0.000
Sedge	0.082	0.037	0.005	0.056	0.055	0.007	0.141	0.003	0.000
Shrub	0.054	0.153	0.222	0.056	0.138	0.138	0.051	0.184	0.427
Tree	0.025	0.073	0.077	0.009	0.105	0.067	0.063	0.009	0.103
Unknown	0.064	0.084	0.091	0.068	0.059	0.076	0.055	0.133	0.128
Other Woody	0.001	0.024	0.001	0.001	0.007	0.001	0.000	0.058	0.000

Table 7: Frequency of occurrence of plant families overall and by pasture

	all			Ragged Rock			North Idlewild		
Family	**Cattle**	**Elk**	**Deer**	**Cattle**	**Elk**	**Deer**	**Cattle**	**Elk**	**Deer**
Apiaceae	0.038	0.037	0.083	0.056	0.056	0.059	0.000	0.000	0.143
Apocynaceae	0.000	0.074	0.000	0.000	0.056	0.000	0.000	0.111	0.000
Asteraceae	0.769	0.926	0.833	0.778	0.944	0.941	0.750	0.889	0.571
Berberidaceae	0.077	0.074	0.167	0.056	0.111	0.118	0.125	0.000	0.286
Betulaceae	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.143
Boraginaceae	0.115	0.148	0.000	0.167	0.111	0.000	0.000	0.222	0.000
Brassicaceae	0.077	0.000	0.042	0.056	0.000	0.000	0.125	0.000	0.143
Caprifoliaceae	0.077	0.333	0.167	0.000	0.167	0.235	0.250	0.667	0.000
Caprifoliaceae, Boraginaceae, or Hydrophyllum	0.000	0.074	0.000	0.000	0.000	0.000	0.000	0.222	0.000
Cupressaceae	0.000	0.296	0.333	0.000	0.444	0.471	0.000	0.000	0.000

	all			Ragged Rock			North Idlewild		
Family	**Cattle**	**Elk**	**Deer**	**Cattle**	**Elk**	**Deer**	**Cattle**	**Elk**	**Deer**
Cyperaceae	0.577	0.222	0.083	0.611	0.333	0.118	0.500	0.000	0.000
Fabaceae	0.192	0.111	0.083	0.222	0.056	0.118	0.125	0.222	0.000
Fabaceae or Loasaceae	0.000	0.037	0.000	0.000	0.056	0.000	0.000	0.000	0.000
Geraniaceae	0.077	0.259	0.250	0.000	0.000	0.118	0.250	0.778	0.571
Grossulariaceae	0.077	0.074	0.167	0.056	0.111	0.235	0.125	0.000	0.000
Hydrophylloideae	0.038	0.074	0.083	0.056	0.111	0.059	0.000	0.000	0.143
Juncaceae	0.346	0.000	0.000	0.333	0.000	0.000	0.375	0.000	0.000
Lamiaceae	0.000	0.000	0.042	0.000	0.000	0.059	0.000	0.000	0.000
Lauraceae, Atherospermataceae, or Gomortega	0.038	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
Loasaceae	0.000	0.074	0.000	0.000	0.111	0.000	0.000	0.000	0.000
Malvaceae	0.000	0.037	0.000	0.000	0.056	0.000	0.000	0.000	0.000
Onagraceae	0.538	0.556	0.625	0.667	0.389	0.647	0.250	0.889	0.571
Orobanchaceae	0.000	0.000	0.083	0.000	0.000	0.118	0.000	0.000	0.000
Paeoniaceae	0.038	0.148	0.000	0.000	0.056	0.000	0.125	0.333	0.000
Pinaceae	0.154	0.185	0.208	0.000	0.278	0.176	0.500	0.000	0.286
Plantaginaceae	0.077	0.000	0.000	0.111	0.000	0.000	0.000	0.000	0.000
Poaceae	0.962	0.704	0.292	0.944	0.944	0.294	1.000	0.222	0.286
Polemoniaceae	0.000	0.037	0.167	0.000	0.000	0.176	0.000	0.111	0.143
Polygonaceae	0.538	0.407	0.500	0.667	0.278	0.588	0.250	0.667	0.286
Ranunculaceae	0.038	0.037	0.000	0.056	0.056	0.000	0.000	0.000	0.000
Rhamnaceae	0.192	0.333	0.375	0.222	0.167	0.176	0.125	0.667	0.857
Rosaceae	0.885	0.926	0.667	0.944	0.889	0.647	0.750	1.000	0.714
Salicaceae	0.038	0.111	0.000	0.056	0.056	0.000	0.000	0.222	0.000
Sapindaceae	0.000	0.000	0.042	0.000	0.000	0.059	0.000	0.000	0.000

Scrophulariaceae	0.038	0.148	0.042	0.056	0.111	0.059	0.000	0.222	0.000
Scrophulariaceae or Orobanchaceae	0.000	0.000	0.042	0.000	0.000	0.059	0.000	0.000	0.000

Table 8: Relative read abundance of plant families overall and by pasture for cattle, elk, and deer with percent cover of plants

	all			Ragged Rock				North Idlewild			
Family	**Cattle**	**Elk**	**Deer**	**Cattle**	**Elk**	**Deer**	**Plants**	**Cattle**	**Elk**	**Deer**	**Plants**
Apiaceae	0.000	0.009	0.012	0.000	0.013	0.002	0.04	0.000	0.000	0.036	0.03
Apocynaceae	0.000	0.006	0.000	0.000	0.008	0.000	-	0.000	0.000	0.000	-
Asteraceae	0.151	0.204	0.277	0.181	0.234	0.387	3.33	0.084	0.143	0.012	6.66
Berberidaceae	0.005	0.002	0.010	0.005	0.003	0.001	0.17	0.004	0.000	0.032	2.61
Betulaceae	0.000	0.000	0.004	0.000	0.000	0.002	-	0.000	0.000	0.007	-
Boraginaceae	0.001	0.002	0.000	0.001	0.002	0.000	0.21	0.000	0.003	0.000	0.05
Brassicaceae	0.002	0.000	0.042	0.002	0.000	0.000	0.01	0.002	0.000	0.143	0.01
Caprifoliaceae	0.001	0.006	0.004	0.000	0.003	0.005	0	0.002	0.012	0.000	0.61
Caprifoliaceae, Boraginaceae, Hydrophyllum	0.000	0.001	0.000	0.000	0.000	0.000	-	0.000	0.003	0.000	-
Caryophyllaceae	-	-	-	-	-	-	0.14	-	-	-	0.1
Cupressaceae	0.000	0.056	0.017	0.000	0.081	0.023	0.05	0.000	0.005	0.000	0
Cyperaceae	0.083	0.038	0.005	0.056	0.055	0.007	2.26	0.142	0.003	0.000	26.65
Fabaceae	0.002	0.006	0.000	0.002	0.008	0.001	0	0.001	0.002	0.000	1.92
Fabaceae, Loasaceae	0.000	0.006	0.000	0.000	0.008	0.000	-	0.000	0.000	0.000	-
Geraniaceae	0.002	0.008	0.009	0.000	0.002	0.003	0	0.006	0.021	0.024	0.31
Grossulariaceae	0.005	0.003	0.003	0.003	0.005	0.004	0.35	0.012	0.000	0.000	0.04
Hydrophylloideae	0.001	0.001	0.003	0.001	0.001	0.004	0.09	0.001	0.001	0.001	0.01
Juncaceae	0.016	0.000	0.000	0.012	0.000	0.000	0	0.025	0.000	0.000	0

	all			Ragged Rock				North Idlewild			
Family	**Cattle**	**Elk**	**Deer**	**Cattle**	**Elk**	**Deer**	**Plants**	**Cattle**	**Elk**	**Deer**	**Plants**
Lamiaceae	0.000	0.000	0.001	0.000	0.000	0.001	0	0.000	0.000	0.000	0
Lauraceae, Atherospermataceae, Gomortega	0.006	0.000	0.000	0.009	0.000	0.000	-	0.001	0.000	0.000	-
Liliaceae	-	-	-	-	-	-	0.06	-	-	-	0.02
Loasaceae	0.000	0.007	0.000	0.000	0.010	0.000	0.01	0.000	0.000	0.000	0
Malvaceae	0.000	0.001	0.000	0.000	0.001	0.000	0.01	0.000	0.000	0.000	0.06
Onagraceae	0.079	0.082	0.188	0.090	0.079	0.257	0.68	0.056	0.089	0.021	0.48
Orobanchaceae	0.000	0.000	0.001	0.000	0.000	0.001	0	0.000	0.000	0.000	0
Paeoniaceae	0.002	0.005	0.000	0.000	0.002	0.000	0	0.005	0.012	0.000	0.01
Pinaceae	0.019	0.012	0.059	0.000	0.018	0.043	0.14	0.062	0.001	0.096	1.19
Plantaginaceae	0.001	0.000	0.000	0.001	0.000	0.000	0	0.000	0.000	0.000	0
Poaceae	0.397	0.138	0.036	0.373	0.165	0.021	38.2	0.452	0.084	0.073	8.14
Polemoniaceae	0.000	0.000	0.004	0.000	0.000	0.006	1.23	0.000	0.000	0.001	0.38
Polygonaceae	0.081	0.137	0.077	0.092	0.119	0.096	0	0.055	0.174	0.030	0.07
Portulacaceae	-	-	-	-	-	-	0.69	-	-	-	0
Ranunculaceae	0.000	0.002	0.000	0.000	0.003	0.000	0.01	0.000	0.000	0.000	2.8
Rhamnaceae	0.027	0.029	0.106	0.034	0.020	0.032	0.04	0.011	0.048	0.284	0.48
Rosaceae	0.119	0.207	0.143	0.137	0.144	0.102	0.03	0.079	0.333	0.240	2.2
Rubiaceae	-	-	-	-	-	-	0.3	-	-	-	3.2
Salicaceae	0.001	0.030	0.000	0.001	0.013	0.000	0	0.000	0.063	0.000	0
Saxifragaceae	-	-	-	-	-	-	0.01	-	-	-	0
Sapindaceae	0.000	0.000	0.001	0.000	0.000	0.001	0	0.000	0.000	0.000	0
Scrophulariaceae	0.000	0.001	0.000	0.000	0.001	0.000	0.92	0.000	0.000	0.000	0.25

	Violaceae	-	-	-	-	-	-	0.08	-	-	-	0

Table 9: Diet overlap and breadth calculated from family and sequence RRA

		All		Ragged Rock		North Idlewild	
	Metric	family	sequence	family	sequence	family	sequence
Cattle-Elk	Overlap	0.772	0.628	0.864	0.592	0.431	0.437
Elk-Deer	Overlap	0.837	0.796	0.805	0.767	0.603	0.461
Cattle-Deer	Overlap	0.538	0.506	0.557	0.534	0.312	0.235
Cattle	Breadth	0.158	0.217	0.169	0.206	0.132	0.241
Elk	Breadth	0.235	0.234	0.238	0.281	0.185	0.204
Deer	Breadth	0.234	0.246	0.144	0.207	0.356	0.210

Table 10: Comparison of scat preservation methods (dried vs. ethanol) for deer and elk in the same pasture

	Number of Unique Sequences		Average number of reads per sample	
Collection Method	Deer	Elk	Deer	Elk
Dried (Driveway 26)	28	36	18,923	23,108
Ethanol (Driveway 28)	46	47	23,857	18,132

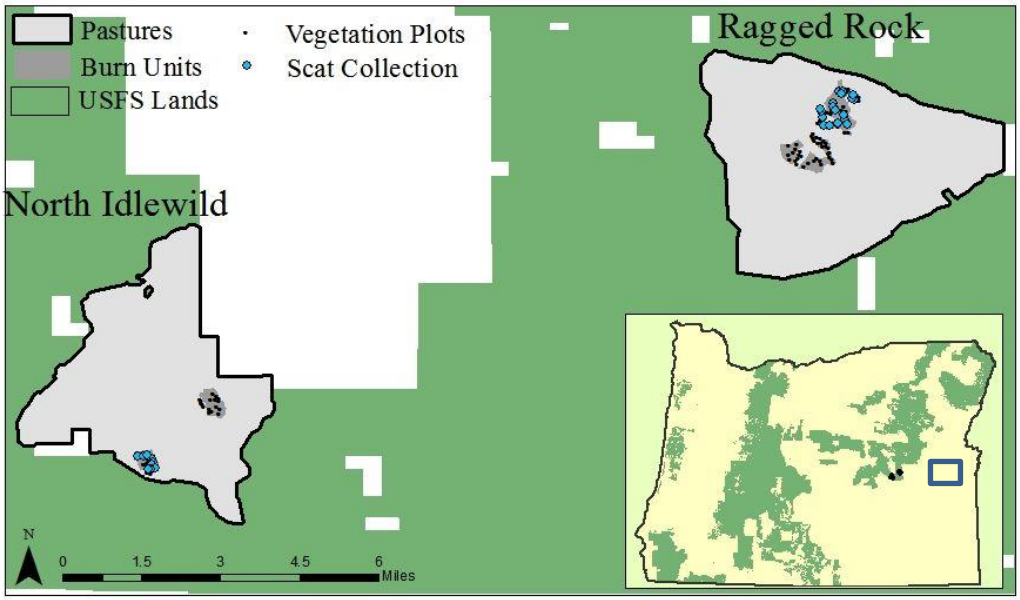

Figure 1: The study area in eastern Oregon, the North Idlewild pasture is located 10 miles west of the Ragged Rock pasture. Scats were collected (blue circles) in SIB treatment units and vegetation plots (black dots) from SIB study were used to characterize pastures.

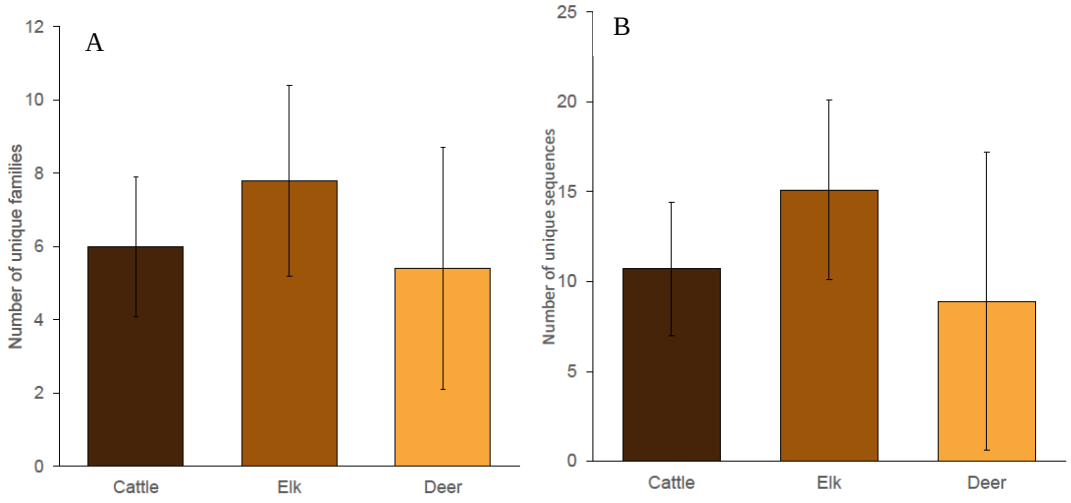

Figure 2: Average number of unique DNA sequences (A) and families (B) found in each scat sample for cattle, elk, and deer.

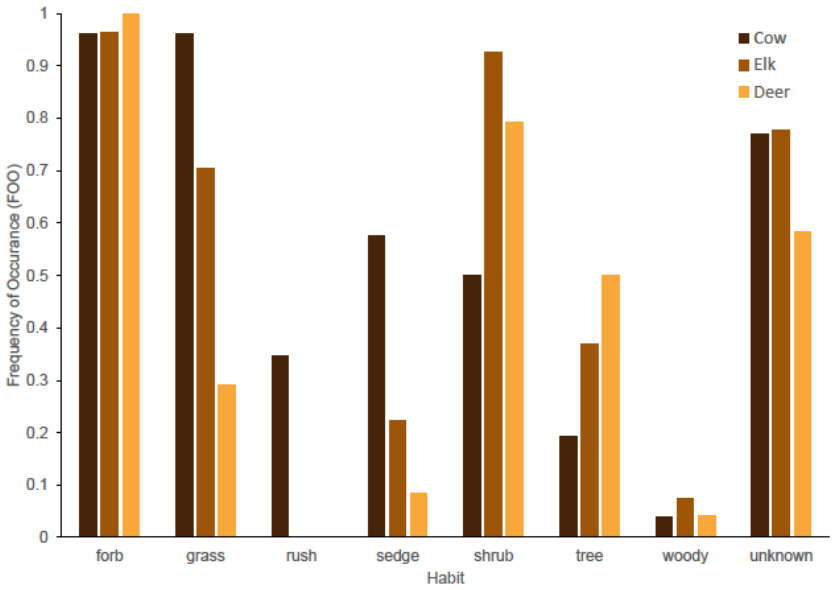

Figure 3: Frequency of occurance (FOO) of functional groups in cattle, elk, and deer scats. Woody includes sequences known to be either of a tree or shrub. Unknown includes sequences from families that could not be identified as forbs or woody.

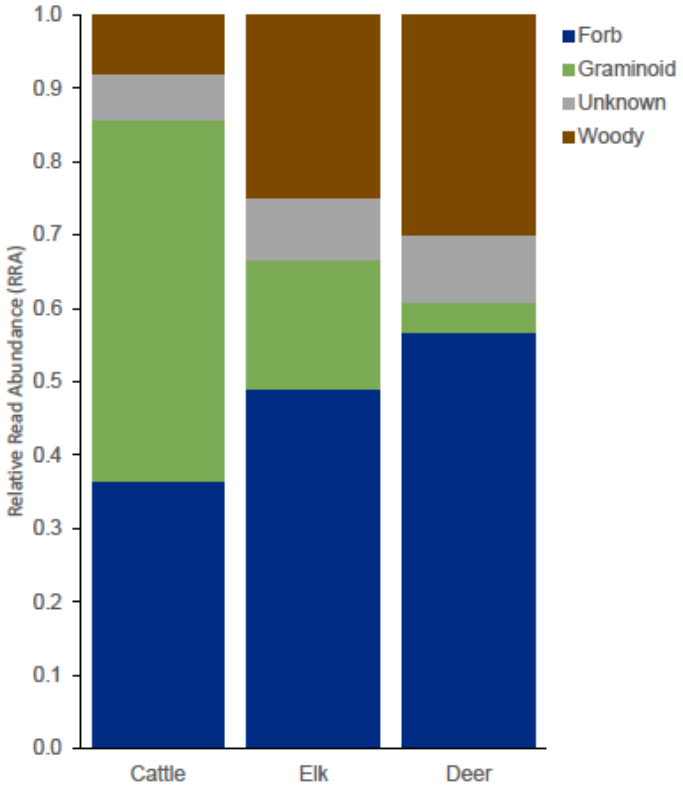

Figure 4: Relative read abundance (RRA) of functional groups compared between cattle, elk, and deer. Unknown includes sequences from families that could not be identified as forbs or woody.

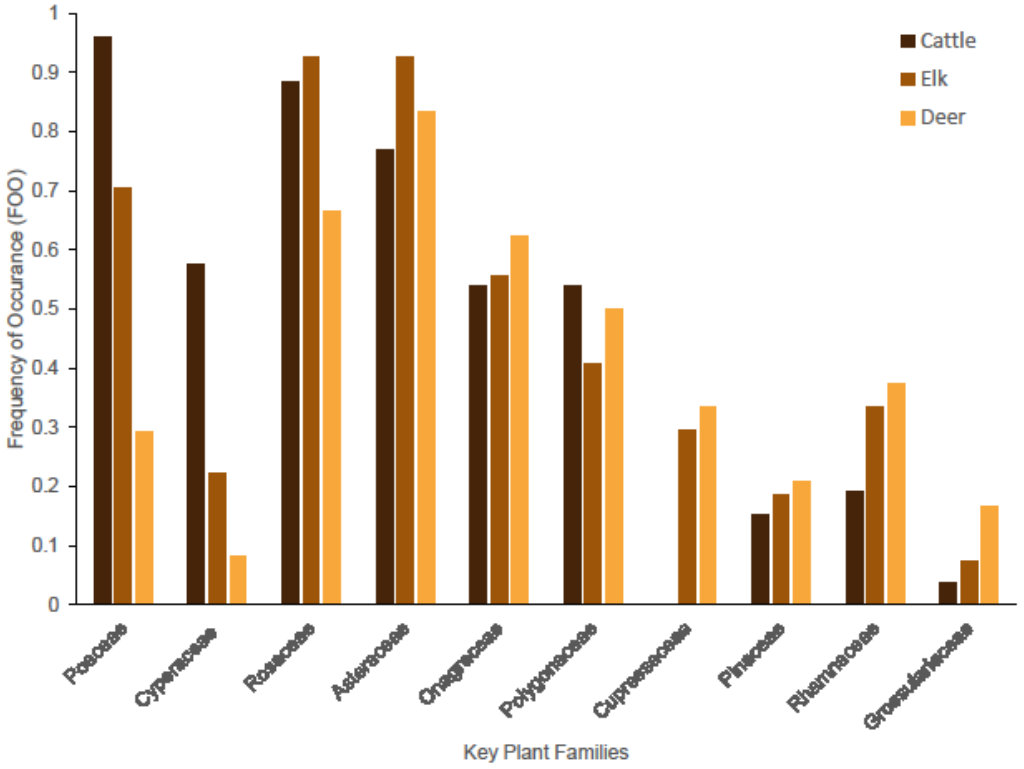

Figure 5: Frequency of occurance (FOO) of key plant families in cattle, elk, and deer scats.

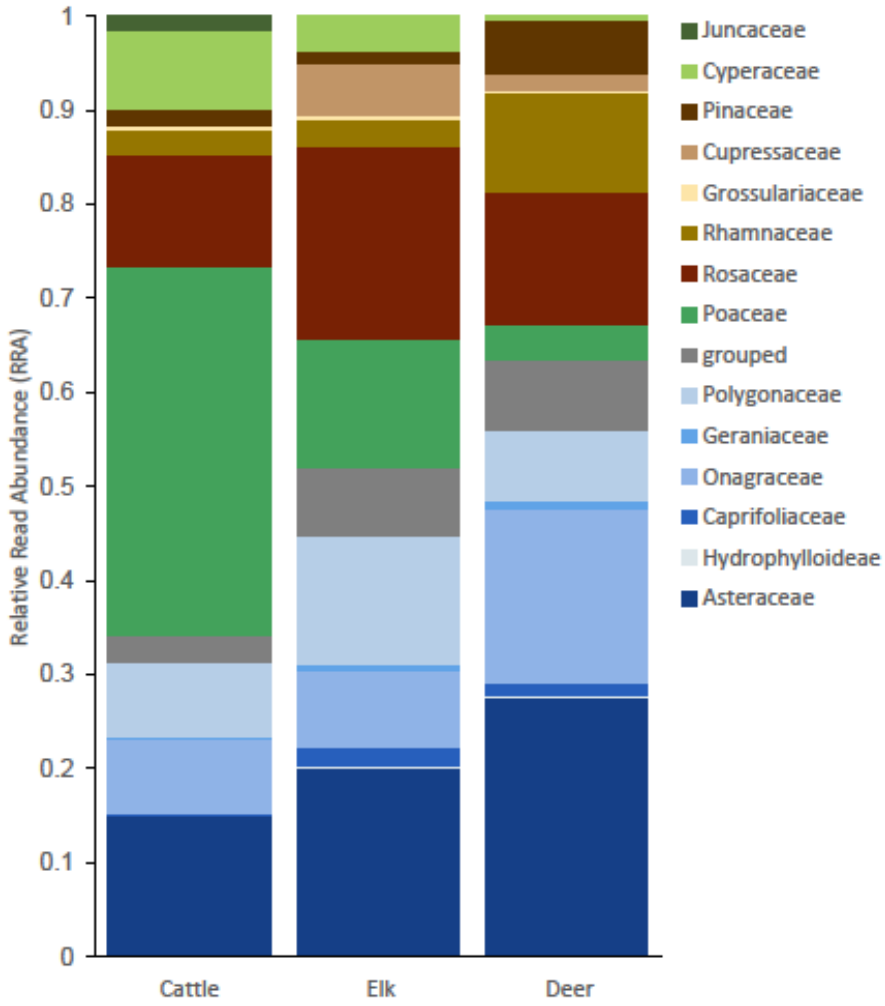

Figure 6: Relative read abundance (RRA) of key plant families compared between cattle, elk, and deer. Grouped families were a low individual occurrence and include Apiaceae, Berberidaceae, Boraginaceae, Brassicaceae, Caryophyllaceae, Ericaceae, Fabaceae, Lamiaceae, Loasaceae, Malvaceae, Orobanchaceae, Paeoniaceae, Polemoniaceae, Ranunculaceae, Salicaceae, and Scrophulariaceae.

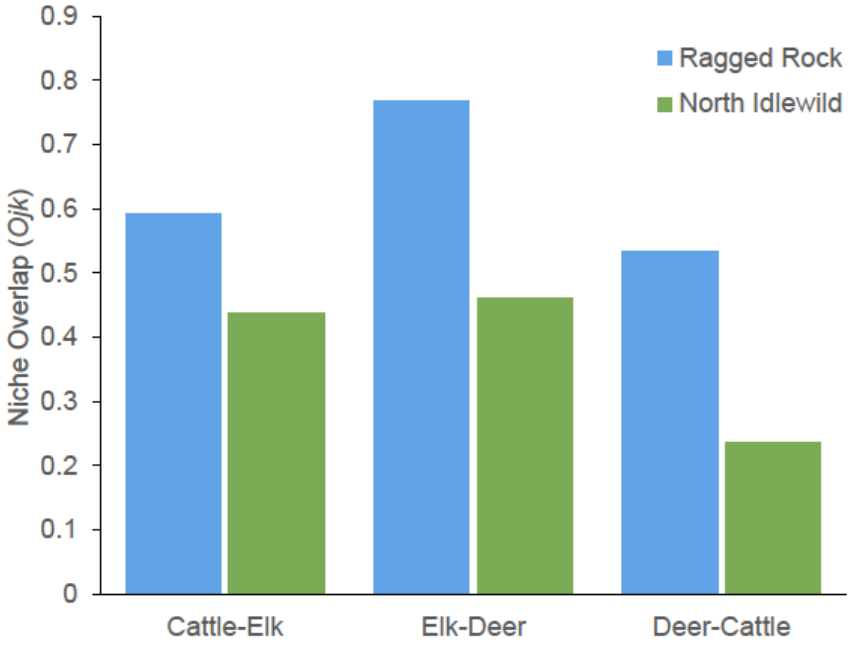

Figure 7: Dietary niche overlap (*Ojk*) for each set of species interactions compared between Ragged Rock and North Idlewild pastures.

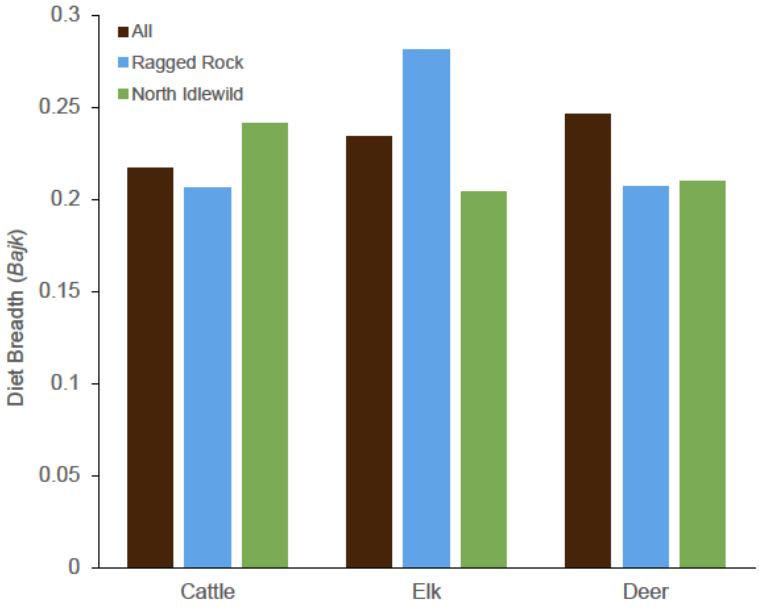

Figure 8: Dietary niche breadth (*Bajk*) for each species compared between Ragged Rock and North Idlewild pastures.

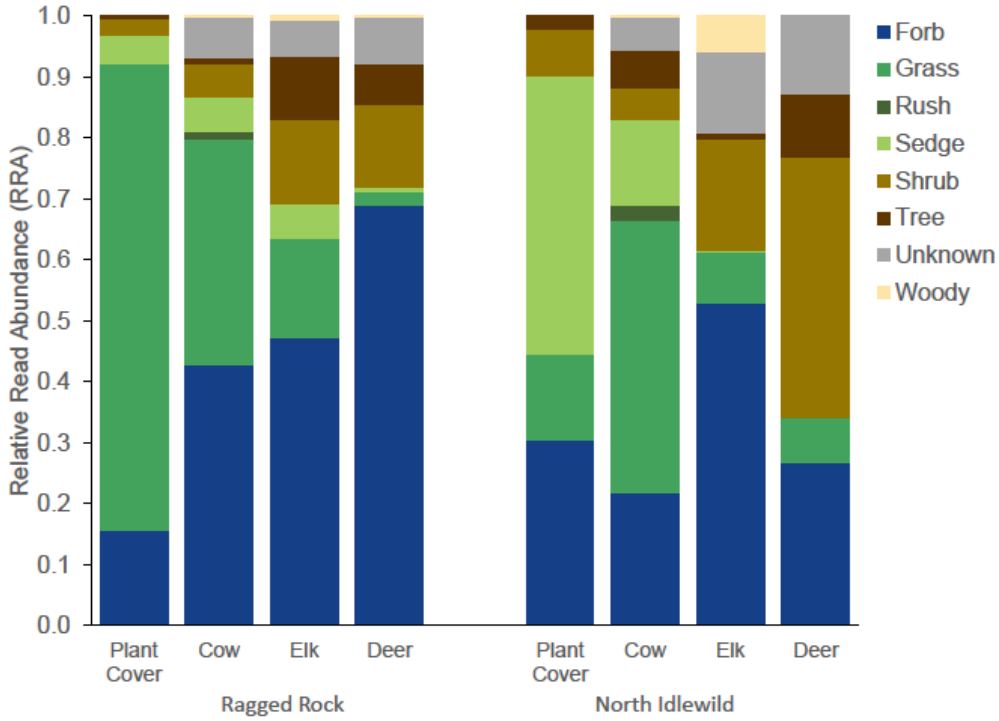

Figure 9: Relative read abundance of functional groups for each ungulate broken apart by pasture and compared to percentage of total plant cover. Total plant cover in Ragged Rock is 50% and in North Idlewild it is 58%.

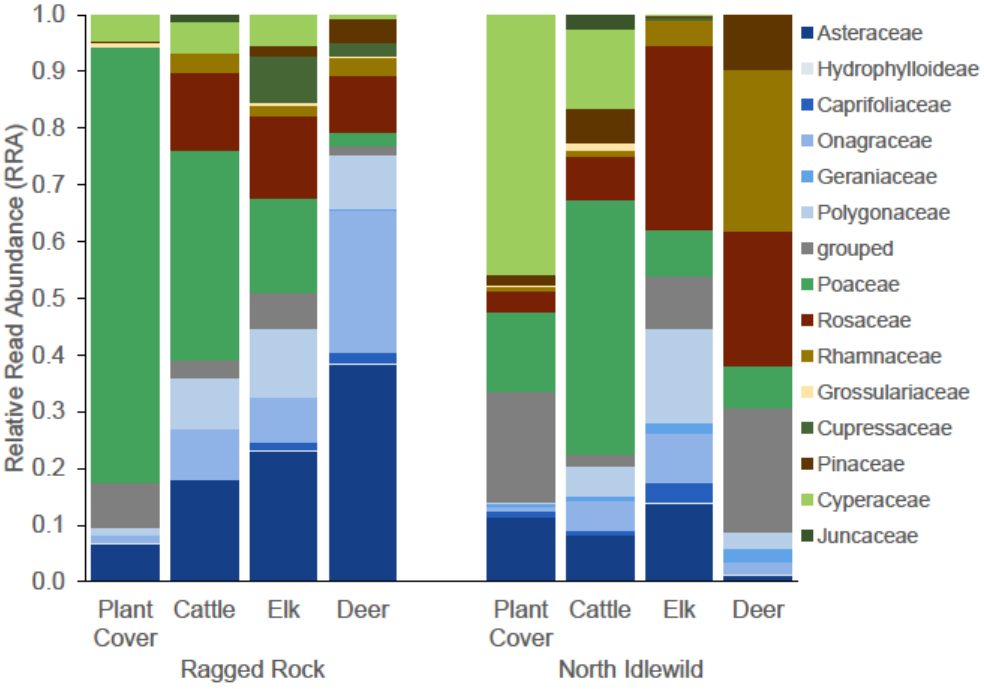

Figure 10: Relative read abundance of plant families for each ungulate broken apart by pasture and compared to percentage of total plant cover. Total plant cover in Ragged Rock is 50% and in North Idlewild it is 58%. Grouped families were at low

individual occurrence in scats and include Apiaceae, Berberidaceae, Boraginaceae, Brassicaceae, Caryophyllaceae, Ericaceae, Fabaceae, Lamiaceae, Liliaceae, Loasaceae, Malvaceae, Orobanchaceae, Paeoniaceae, Polemoniaceae, Portulacaceae, Ranunculaceae, Rubiaceae, Salicaceae, Saxifragaceae, Scrophulariaceae, and Violaceae.