Space, time, and captivity: quantifying the factors influencing the fecal microbiome of an alpine ungulate

Sarah E. Haworth[1,2*]

Kevin S. White[3]

Steeve D. Côté[4]

Aaron B.A. Shafer[1,2]

1. Forensics Program, Trent University, K9J 7B8 Peterborough, Canada
2. Environmental and Life Sciences Graduate Program, Trent University, 7K9J 7B8 Peterborough, Canada
3. Division of Wildlife Conservation, Alaska Department of Fish and Game, Juneau, AK, USA
4. Département de Biologie and Centre for Northern Studies, Université Laval, Québec, Québec G1V 0A6, Canada

*sarahhaworth@trentu.ca

Abstract

The community of microorganisms in the gut is affected by host species, diet, and environment and is linked to normal functioning of the host organism. Although the microbiome fluctuates in response to host demands and environmental changes, there are core groups of microorganisms that remain relatively constant throughout the hosts lifetime. Ruminants are mammals that rely on highly specialized digestive and metabolic modifications, including microbiome adaptations, to persist in extreme environments. Here, we assayed the fecal microbiome of four mountain goat (*Oreamnos americanus*) populations in western North America. We quantified fecal microbiome diversity and composition among groups in the wild and captivity, across populations, and in a single group over time. There were no differences in community evenness or diversity across groups, although we observed a decreasing diversity trend across summer months. Pairwise sample estimates grouped the captive population distinctly from the wild populations, and moderately grouped the southern wild group distinctly from the two northern wild populations. We identified 33 genera modified by captivity, with major differences in key groups associated with cellulose degradation that likely reflect differences in diet. Our findings are consistent with other ruminant studies and provide baseline microbiome data in this enigmatic species, offering valuable insights into the health of wild alpine ungulates.

Keywords: *Oreamnos americanus*, microbiome, ruminant, microorganisms, rumen, goat

Summary: This study characterizes the microbiome of mountain goat (*Oreamnos americanus*) populations across populations and over summer months; we also quantified the effects of captivity to offer more insights into the health of alpine wildlife.

Introduction

The microbiome describes a dynamic community of microorganisms that colonize organisms from birth onwards. Microorganisms in the gut play a key role in host physiological and immunological development (Berg, 1996), with fecal matter containing microbiome DNA that is shed during the digestion and egestion processes (Ingala et al., 2018; Vandeputte et al., 2016). The microbiome can vary according to the host species, age, diet, health, reproductive

status, and external environment, but is directly linked to host health, including metabolism, immunity, and development (Nishida & Ochman, 2017). The fecal microbiome is reflective of a transient response to changes in the host, such as responses to dietary shifts across seasons, but core groups of microorganisms are found in stable relative abundances throughout the life of the host and the relative proportions of these groups can act like a signature of the host's health and environment, with reductions in diversity associated closely with reductions in fitness (Amato et al., 2018; Clayton et al., 2018; Donaldson et al., 2015). The stable relative abundances of the core groups are directly related to the function and the demand of the core groups. The relative ratio of *Firmicutes* to *Bacteroidetes*, for one example, the two most commonly dominant core groups in mammal fecal microbiomes, can be used to discern between carnivorous and herbivorous mammals since each group is responsible for different metabolic demands (Huntington et al., 2019; Kreisinger et al., 2018; Muegge et al., 2011).

Measuring signatures in the fecal microbiome over time, between populations, such as between captive and wild populations of mammals, can help effectively monitor animal health (Bahrndorff et al., 2016; Bik et al., 2016). For example, many captive populations of mammal species suffer from poor health and some conditions have been correlated to fecal microbiome dysbiosis and decreased diversity microbial groups responsible for normal health (Clayton et al., 2018; Li et al., 2018; McKenzie et al., 2017). Actions that target the correction of the microbiome, meaning promoting signatures of core groups that are closer to what is seen in relevant wild and healthy populations, have been suggested as a feasible approach to help alleviate and manage health issues (Li et al., 2018). Moreover, evidence is mounting that supports the link between host phenotypic and genomic variation, and the microbiome (Carthey et al., 2018; Sharpton, 2018); this interaction is particularly relevant when it comes to rapidly changing environments. For example, the host microbial community of coral was critical in facilitating adaptation to warming temperatures (Webster et al., 2017; Ziegler et al. 2017).

Ruminants are herbivorous hooved mammals (ungulates) with specialized anatomical and physiological adaptations to accommodate the cellulolytic fermentation of low-nutrition, high-fiber plant materials (Noel et al., 2017; de Tarso et al., 2016). Numerous extant ruminant populations have been domesticated (e.g. cow, goat), while many of the remaining wild ruminant species are facing population declines that are directly and indirectly driven by human activity,

including agricultural-related activities that have promoted predation, disease and parasitism expansions, environmental change, and have led to significant habitat loss (Smith et al., 2016; Di Marco et al., 2014; Martin, 2001). Non-invasive monitoring of the fecal microbiome of ruminant populations serves as a potential tool that can be used to inform management and conservation decisions aimed at improving the health of captive animals, promoting adaptation to environmental change, and preventing disease and parasitic outbreaks (McKenzie et al. 2017; Amato et al., 2013).

The North American mountain goat (*Oreamnos americanus*) is a sub-alpine ruminant and a symbolic icon of mountain wilderness. Mountain goats are found in fragmented, and occasionally isolated, sub-populations across the Western Cordillera mountain ranges (Shafer et al., 2012; Festa-Bianchet, 2008). Mountain goats show mixed seasonal and sexual habitat selection preferences with relatively small home ranges and limited inter- and intra- population movement, which possibly lends to low genetic variability (Shafer et al., 2012; Ortego et al., 2011; Poole & Heard, 2003). Their longer lifespans, upwards of 12 years, along with seasonally and sexually dependent habitat selection, makes mountain goats a unique study system for attempting to understand how variation in the microbiome supports their highly adapted and unique alpine lifestyle. Furthermore, the longevity of mountain goats, relative to many model and non-model systems, creates opportunities for researchers to conduct prolonged surveillance and infer individual trends over time (e.g. season, year), including non-invasive fecal microbiome studies.

Here we assayed the fecal microbiome of four western North American mountain goat populations, of which one was a captive population (Figure 1). We tested two hypotheses: i) captivity reduces microbial diversity in mammals (McKenzie et al., 2017; Sun et al., 2016; Kohl et al., 2014; Carey et al., 2013); and ii) diversity is negatively correlated to latitude (Dikongué et al., 2017). We further took advantage of temporal sampling in one population to assay changes over three summer months, where we hypothesized a shift in fecal microbiome diversity reflecting the decreased abundance of food resources over the summertime as resources are consumed and depleted (Hicks et al., 2018; Hu et al., 2018; Noel et al., 2017; Festa-Bianchet & Côté, 2008; Hamel & Côté, 2007). Collectively, we predicted that the effect of captivity would drive the strongest differences in the diversity and the composition in mountain goat fecal

104 microbiomes, followed by seasonality and biogeography. This is the first study to establish a
105 fecal microbiome profile for both captive and wild mountain goat populations and contributes
106 valuable baseline knowledge on the diversity of microbiomes for different species (*sensu* McKenzie
107 et al. 2017), while quantifying the influence of three important drivers of diversity and composition:
108 space, time and, captivity.

109 **Methods**

110 **Study species**

111 Mountain goats are part of the Bovidae family and inhabit the mountains of western
112 North America. Mountain goats are an important game species and populations are managed for
113 harvest and non-consumptive uses. Mountain goats can be described as generalist foragers
114 relative to other ruminant species, but it has been shown that they do show preferential selection
115 of forage under some circumstances (Côté and Festa-Bianchet, 2003). During their daily
116 movements, mountain goats spread an abundant amount of compact, dry pellets (feces) multiple
117 times per day; such pellets are well preserved in the alpine environment they occupy.

118 **Study sites**

119 The Calgary Zoo, Alberta, Canada (CZ; 51°N, 114°W) is one of Canada's oldest
120 accredited zoos. The zoo houses a small group of captive mountain goats that are fed year-round
121 an *ad libidum* diet of herbivore pellets and mixed hay (see Table S1 for detailed diet
122 information). This group of mountain goats is accessible for research and veterinary purposes.
123 Caw Ridge, Alberta (CR; 54N, 119W) is an alpine and sub-alpine habitat in western Canada with
124 a regional population of wild mountain goats that have been extensively monitored since 1989
125 (Ortego et al., 2011; Mainguy, Côté, & Coltman, 2009; Festa-Bianchet & Côté, 2008). As of
126 2017, CR was home to about 30 marked mountain goat individuals. This landscape only provides
127 high quantities of quality forage during the summer months (June-August), while the rest of the
128 year it is largely covered in snow or ice and forage availability is limited (Festa-Bianchet &
129 Cote, 2008). Altitudinal migration does not occur in this population. Ship Mountain and Yes Bay
130 (YB; 56N, -132W) each harbor relatively small (n = 40-50 individuals), geographically isolated
131 populations located along the Cleveland Peninsula, southeastern Alaska, United States. Mountain

goats on the Cleveland Peninsula are native and are genetically and morphologically distinct from surrounding mainland populations (Breen et al., submitted). Notably, mountain goats from this region are influenced by a maritime climate and seasonally migrate to low elevations during winter (Fox & Smith, 1988; Smith & Raedeke, 1982). Available diet information for the sampled populations is found in Supplementary Table S1.

Sample collection and DNA extraction

Fresh mountain goat fecal samples (~10 pellets) were collected from CZ (August 2016), SM (July 2016), and YB (July 2016). Larger samples (>10 pellets) were collected from CR during the summer months (June-August 2016). Samples were collected at CR after observing groups of mountain goats defecate, where as those in Alaska were collected by searching areas mountain goats were observed that day. Mountain goats typically defecate in defined, non-overlapping pellet groups and we only selected samples that were clearly from a single individual. Due to the nature of the sampling we were unable to sex or age the animal that defecated. The cool, alpine climate of the sampling environment naturally preserves samples and thus we do not suspect environmental contamination was a major factor. All samples (N=54), except those from CR, were stored immediately in 70% ethanol (EtOH) and at -20°C. CR samples were stored individually in plastic sample bags at -20°C. All surfaces were sterilized with 90% EtOH and 10% bleach solution to prevent environmental contamination. A small portion of a single fecal sample (~1/4 including exterior and interior portions) was digested over night at 56°C in 20 ul proteinase K and 180 ul Buffer ATL from the Qiagen DNeasy Blood & Tissue Kit. The gDNA was extracted from the digest by the Qiagen AllPrep PowerFecal DNA/RNA Kit following manufacturers protocol (Qiagen 80244).

Quality assessment and library preparation

Species identification was confirmed for SM samples as the population is sympatric with Sitka black-tailed deer (*Odocoileus hemionus sitkensis*). The mitochondrial D-Loop was amplified using L15527 and H00438 primers (Wu et al. 2003) and sequenced on an ABI 3730 (Applied Biosystems). The consensus sequences generated in BioEdit (v 7.0.4.1) were screened using NCBI BLAST to identify the species. For all known mountain goat fecal samples DNA concentrations were measured with a Qubit 3.0 Fluorometer per manufacturers protocol

(Invitrogen). Samples were concentrated if the extracted concentration of extracted fecal DNA was below 3 ng/ul. The validated Illumina 16S rRNA Metagenomic Sequencing Library Preparation (#15044223 rev. B) protocol was then followed for library preparation (Rimoldi et al., 2018). The 16S ribosomal ribonucleic acid (16S rRNA) hypervariable region, specifically the V3 and V4 regions, were targeted with four variants of 341F and 805R primers using the primers designed by Herlemann et al., 2011. A unique combination of Nextera XT indexes, index 1 (i7) and index 2 (i5) adapters were assigned to each sample for multiplexing and pooling.

Four replicates of each sample of fecal gDNA were amplified in 25 ul PCR using the 341F and 805R primers. The replicated amplicons for each sample were combined into a single reaction of 100 ul and purified using a QIAquick PCR Purification Kit (Qiagen, 28104) and quantified on the Qubit Fluorometer. Sample indexes were annealed to the amplicons using an 8-cycle PCR reaction to produce fragments approximately 630 bp in length that included ligated adaptors; the target amplicon is approximately 430 bp in length (Illumina 16S rRNA Metagenomic Sequencing Library Preparation; #15044223 rev. B). Aliquots of 100 ng DNA for each sample were pooled together and purified with the QIAquick PCR Purification Kit for a final volume of 50 ul. The final purified library was validated by TapeStation (Agilent, G2991AA) and sequenced in 300 bp pair-end reads on an Illumina MiSeq sequencer at the Genomic Facility of Guelph University (Guelph, Ontario).

Analysis

Bioinformatics and taxonomic evaluation

The quality of the raw sequences was assessed with FastQC (v 0.11.4) and the low-quality cut-off for forward and reverse reads was determined. Forward and reverse reads were then imported into QIIME2 (v 2018.6) for quality control, diversity analysis, and sequence classification. The quality control function within QIIME2, DADA2, was used to truncate forward and reverse reads and perform denoising, and the detection and removal of chimeras. The results of DADA2 with only forward, reverse, and merged reads were analyzed independently; note QIIME2 follows the curated DADA2 R library workflow (https://benjjneb.github.io/dada2/) that requires zero mismatches in overlapping reads for successful merging, since reads are denoised and errors are removed before merging occurs.

Sequencing data were grouped by status (captive or wild), population (CZ, CR, SM, or YB), and collection time (samples from CR collected in June, July, or August) for analytical purposes.

Alpha diversity estimates of community richness included Shannon Index and observed OTUs and estimates of community evenness included Pielou's evenness. A phylogenetic tree was developed in QIIME2 for beta diversity estimates (Supplementary Figure S6). Pairwise sample estimates (beta diversity) included Bray-Curtis, unweighted UniFrac, and weighted UniFrac dissimilarity distance matrices. The taxonomy, to the species level, of all sample reads were assigned using Silva 132 reference taxonomy database (https://docs.qiime2.org/2019.1/data-resources/). The relative proportion of *Firmicutes* to *Bacteroidetes* was calculated for each of the grouped data as variation in the ratio is associated with individual body condition (Donaldson et al., 2006; Ley et al., 2006). For this analysis samples within the groupings were normalized and rarified to the sample with the fewest contigs (6,096 contigs for population and status; and, 7,342 contigs for collection month).

Statistical analysis and visualization

Differences in community richness and evenness by groupings were assessed with the Kruskal-Wallis rank sum test and p-values were adjusted using Benjamini & Hochberg correction (q-values; Storey, 2002). All taxonomic analysis and visualization were computed with the unclassified reads removed. A Wilcoxon rank sum test with continuity correction was used to assess the differences in relative abundance of taxonomic classifications based on origin with all unclassified sequences removed. A Kruskal-Wallis rank sum test was used to assess the differences in relative abundance of taxonomic classifications based on collection month. Differences were treated as significant if the p-value was < 0.01 and a post-hoc (Dunn) test was conducted to determine where the differences occurred between the three collection times.

Statistical PERMANOVA tests were conducted using the ADONIS function from the R package Vegan (v 2.5.2) on Bray Curtis dissimilarity matrices to test for the presence of shifts in the microbiome communities between groups. A detrended correspondence analysis (DCA) was conducted to determine significant communities between groups at each taxonomic level, with only the taxonomic level with the highest resolution (species) reported and visualized. A principle coordinates analysis (PCoA) was also conducted using Bray Curtis dissimilarity

matrices. Data were visualized with the ggplot2 (v 2.3.0.0) package in RStudio (v 3.4.1). Additional materials associated with the analysis are available on Dryad (Accession no. XXXXX) and relevant scripts are available on GitLab (https://gitlab.com/WiDGeT_TrentU).

Results

Samples and quality filtering

A total of 54 fecal samples were selected for this study, but seven samples from SM were excluded because of > 95% identification as black-tailed Sitka deer and five were excluded for quality reasons. A total of ~ 5.37 million paired-end reads were generated from the remaining 42 fecal samples (SRA accession number PRJNA522005). FastQC analysis indicated that both forward and reverse reads lost quality > 250 bp in length (Phred score < 25), so all reads were trimmed to a length of 250 bp and following DADA2 quality filtering, resulted in 896,534 high quality overlapping reads (contigs) kept for taxonomic and diversity analyses. The sequence breakdown for each group can be seen in Supplementary Table S2. Analyses were also conducted on each read set individually, but we only report the results for the merged reads as the patterns were qualitatively similar (just considerably more data). Contigs that were classified to Archaeal lineages (6,013 contigs) were removed from the analysis. The remaining 890,521 contigs were classified into 3,886 unique 16S rRNA ribosomal sequence variants (RSVs) with at least 1 representative sequence, and 3,854 unique RSVs with at least 10 representative sequences.

Assessing diversity and species composition across groups

The three alpha diversity metrics did not show any differences between the captive and wild populations (q-value > 0.89 for all comparisons; Supplementary Table S3; Figure 2); however, there were more unique classifications in wild than in captive mountain goats (Supplementary Table S4). Across the four populations, the alpha diversity metrics did not differ (q-value > 0.57 for all comparisons), but moderate differences between the collection months at CR were observed (q-value = 0.04 for all comparisons; Supplementary Table S3; Figure 2), with a trend toward decreasing diversity as the summer progressed. The significant differences (q-value < 0.01) in the taxonomies observed between June-July, June-August, and July-August at

CR were contributed by 3 classes, 4 families, 3 genera, and 3 species (Supplementary Table S6), and there was no difference in the phyla or orders observed between any months.

The top two phyla based on averaged relative abundance were *Firmicutes* and *Bacteroidetes* (Table 1; Table 2). The important distinguishing taxonomic differences (p-value < 0.01) between captive and wild mountain goats arose from a suite of different groups, ranging from three different phyla to 26 different species (Supplementary Table S5; Figure 3). Notably of the top five groups, they only differed by a *Spirochaetes* identification for captive and by *Proteobacteria* for wild mountain goats (Table 1; Table 2). A similar pattern was observed across populations (Table 1; Table 2) and likewise, *Firmicutes* and *Bacteroidetes* ratios were more-or-less consistent, and only one genus differed across months (Table 2).

Quantifying drivers of microbial diversity

A 4-way PERMANOVA of the Bray Curtis dissimilarity matrix indicated that the captive and wild mountain goats had significant shifts ($p<0.001$) in their microbiome communities (Table 3; Figure 2). The same pattern was observed across populations ($p<0.001$) and over time ($p=0.018$; Table 3). The DCA (at the species level) clearly separated captive from wild as well as the four sampled populations (Figure 4). Similarly, the PCoA at the species level, based on Bray Curtis dissimilarity, weighted UniFrac, and unweighted UniFrac, clearly separated captive from wild as well the four sampled populations (Supplementary Figure S4-S6). No temporal groupings were observed (Figure 4; Supplementary Figures S4-S6), despite the PERMANOVA patterns (Table 3).

Discussion

The structure of the fecal microbiome is influenced by a multitude of biotic and abiotic host-specific factors, including genetics, diet, environment, and health status (Bahrndorff et al., 2016). While unaccounted factors such as age and sex likely contribute to variation in the microbiome (Kook et al., 2018; Bennett et al., 2016; Million et al., 2013), we observed clear effects of captivity (Fig. 3; 4) and trends suggestive of a seasonal effect (Fig. 2; Table 3. This study should therefore inform both basic and applied research of ungulate microbiomes, and potentially inform the management of this enigmatic species by identifying the composition of

275 microbial populations in wild, healthy individuals. Specifically, there is a mounting body of
276 evidence that links the fecal microbiome to the health of individuals and these links may be
277 useful tools in guiding population management in captive and wild populations (Bahrndorff et
278 al., 2016; Pannoni, 2015). Moreover, given the links between host-microbiome and adaptive
279 responses (Webster et al., 2017; Ziegler et al. 2017), it is conceivable that population-level
280 microbiome divergence could, and likely should, be factored into the designation of evolutionary
281 significant units (*sensu* Moritz, 1994) and predictive models as it pertains to adaptive responses.

282 **Comparing diversity metrics**

283 Most mammalian microbiome comparisons to date have shown significant decreases in
284 the alpha diversity (diversity and evenness) in captive ruminant populations; however, there are
285 also studies that show no changes or even increases in diversity in captive populations in other
286 mammals (McKenzie et al., 2017; Kohl et al., 2014). In Bovidae, McKenzie et al. (2017) showed
287 no statistical difference between captive and wild groups, which was also observed in this study
288 on mountain goats. This finding suggests that, for the mountain goats at the Calgary Zoo,
289 captivity has played little effect on overall microbiome diversity, but rather microbiome
290 community structure (Figures 2, 3). Community diversity estimates for mountain goats were also
291 comparable to other unique ruminant populations, such as muskoxen (*Ovibos moschatus*),
292 Bactrian camel (*Camelus bactrianus*), and Norwegian reindeer (*Rangifer tarandus tarandus*), but
293 less comparable to roe deer (*Capreolus capreolu*) and dzo (*Bos grunniens* and *Bos taurus* hybrid;
294 He et al., 2018; Salgado-Flores et al., 2016).

295 There are strong seasonal dynamics in activity, diet, and body condition in wild mountain
296 goats that could lead to disparity between the microbiome community diversity we measured in
297 mountain goats, and other studies from ruminant species. In particular, mountain goats appear to
298 lose and regain a significant amount of body mass (up to 30-40%) between winter and summer
299 (Côté and Festa-Bianchet, 2003) and are exposed to environmental extremes (e.g. 0 to 25°C).
300 Such dynamics may provide important context for understanding seasonal changes in the
301 microbiome. For example, during spring and early summer animals, particularly parturient
302 females, might be oriented to obtaining, absorbing, and utilizing nutrients whereas later in late-
303 summer and fall may be storing nutrients for periods of winter scarcity.

304 The dominance of *Firmicutes* and *Bacteroidetes* phyla is consistent with other ruminant
305 studies (O' Donnell et al., 2017). There were minimal changes in the relative percent *Firmicutes*
306 (from 75% to 72%) and increases in the relative classified *Bacteroidetes* (from 16% to 17%)
307 from wild to captive mountain goats, which is consistent with the general mammalian trends
308 observed in McKenzie et al. (2017). The *Firmicutes* to *Bacteroidetes* ratio (F/B) has been used to
309 give a rough estimate of the function of the microbiome, where a higher F/B ratio in treatment
310 groups compared to control groups in mice and humans has generally been linked with diseases
311 such as obesity and an elevated body mass index (BMI; Koliada et al., 2017). Koliada et al.,
312 (2017) suggested that the association between increase F/B ratios and elevated BMIs arises from
313 *Firmicutes*, a group linked with nutrition absorption and circulation, being more efficiently able
314 to participate in energy utilization than *Bacteroidetes*, a group more associated with nutrition
315 storage. Therefore, increases in the relative abundance of *Firmicutes* can significantly contribute
316 to the hosts' elevated BMI phenotype. The diets of ruminants are typically low in nutrients,
317 especially during winter months, and the elevated F/B ratio in mountain goats detected in our
318 summer samples, relative to some other ruminants, suggests that the metabolism efforts of
319 mountain goat microbiomes are driven to obtain and absorb, rather than store, nutrition; a pattern
320 consistent with expected seasonal energy balance strategies of northern ungulates.
321 *Actinobacteria*, another phylum detected in mountain goats, is associated with body condition
322 (e.g. BMI) and might contribute to the immune system of the host (Koliada et al., 2017; Ventura
323 et al., 2007). An important next step will be to link F/B ratio and relative abundance of
324 *Actinobacteria* to phenotypic attributes like body condition and mass in mountain goats, which is
325 possible in both a captive (CZ) and wild (CR) setting.

326 **Detectable shifts in microbiome community compositions**

327 There were significant shifts in the fecal microbiome community composition between
328 the four different population groups. However, an R^2 value of 0.09 suggests that there are
329 multiple factors, beyond population origin, driving the shifts seen in the fecal microbiome
330 communities. Unmeasured factors like age, sex, and reproductive status would likely explain
331 some of the remaining variation; still, there was considerably less variation explained by captive
332 and wild group designation (R^2=0.03). The spatial differences thus are more prominent in
333 shaping fecal microbial composition than that of captivity, and our model fit is consistent with

other comparisons of captive and wild groups of ruminants (McKenzie et al., 2018). Here we speculate that the northern latitude or geographic isolation of the Alaskan population has contributed to reduced fecal microbiome diversity in terms of species number (Shafer et al., 2012; Table S4), while the shifts at the phyla level are consistent with diet alterations between captive and wild animals with similar patterns seen in Sika deer (Guan et al., 2017).

Interestingly, sample time at CR had the best model fit (R^2=0.15; p=0.018), where the most different genera ($p < 0.001$) between June and July was the *Eubacterium hallii group* (increased in July); similarly, *Clostridiales family XIII AD3011 group* and *Eubacterium hallii groups* increased in July. The *Eubacterium hallii group* is a commonly observed genus in mammal microbiomes and plays a role in glycolysis (Engels et al., 2016); we suggest that the increase of *Eubacterium hallii group* in July relative to June or August might be associated with shifts in forage quality during the peak of summer at CR. Importantly, while the core fecal microbiome appears relatively constant in the mountain goat, at the local-level there are clear shifts in individual bacteria that reflect changes in the microhabitat over time.

Conclusion

We are beginning to understand how the fecal microbiome influences the host and is, in turn, influenced by the host. There is a need to understand how to best apply this knowledge to aid the management and conservation of mammal populations (Li et al., 2018; O' Donnell et al., 2017). The direct connection of the fecal microbiome to both individual and population health makes fecal microbiome assays an important tool for monitoring the health and disease trends of domesticated, captive, and wild populations of mammals (Bahrndorff et al., 2016; Jiménez & Sommer, 2017). This study shows that there are clear differences in fecal microbiome community composition, but not diversity, that can be best explained by a combination of factors, including status, seasonality and population of origin. The baseline microbiome data described here thus has the potential to provide valuable insight into the health of wild mountain goat populations and represents an important frame of reference for the development of future monitoring programs and associated management strategies.

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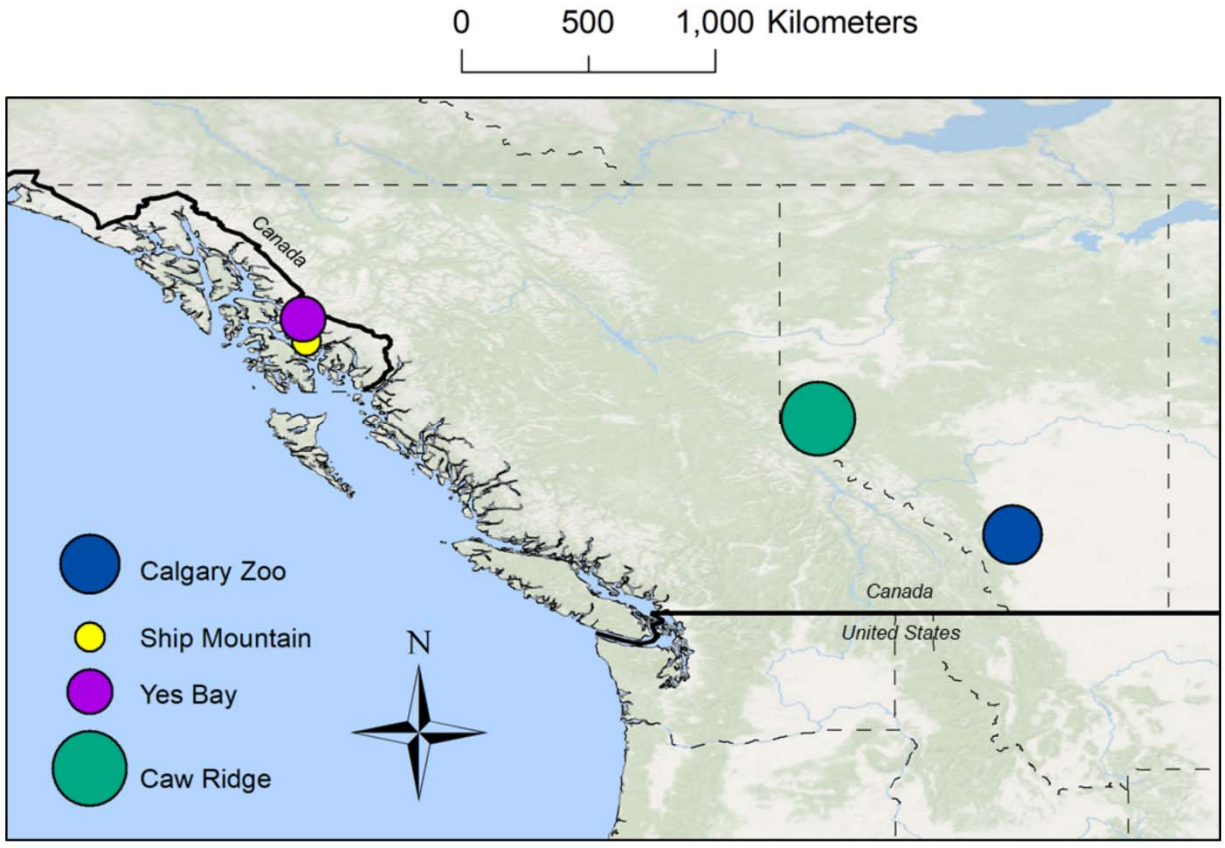

Figure 1. Geographical distribution of study sites in Alaska and Canada. The size of the points reflects the relative number of samples that originate from the study site.

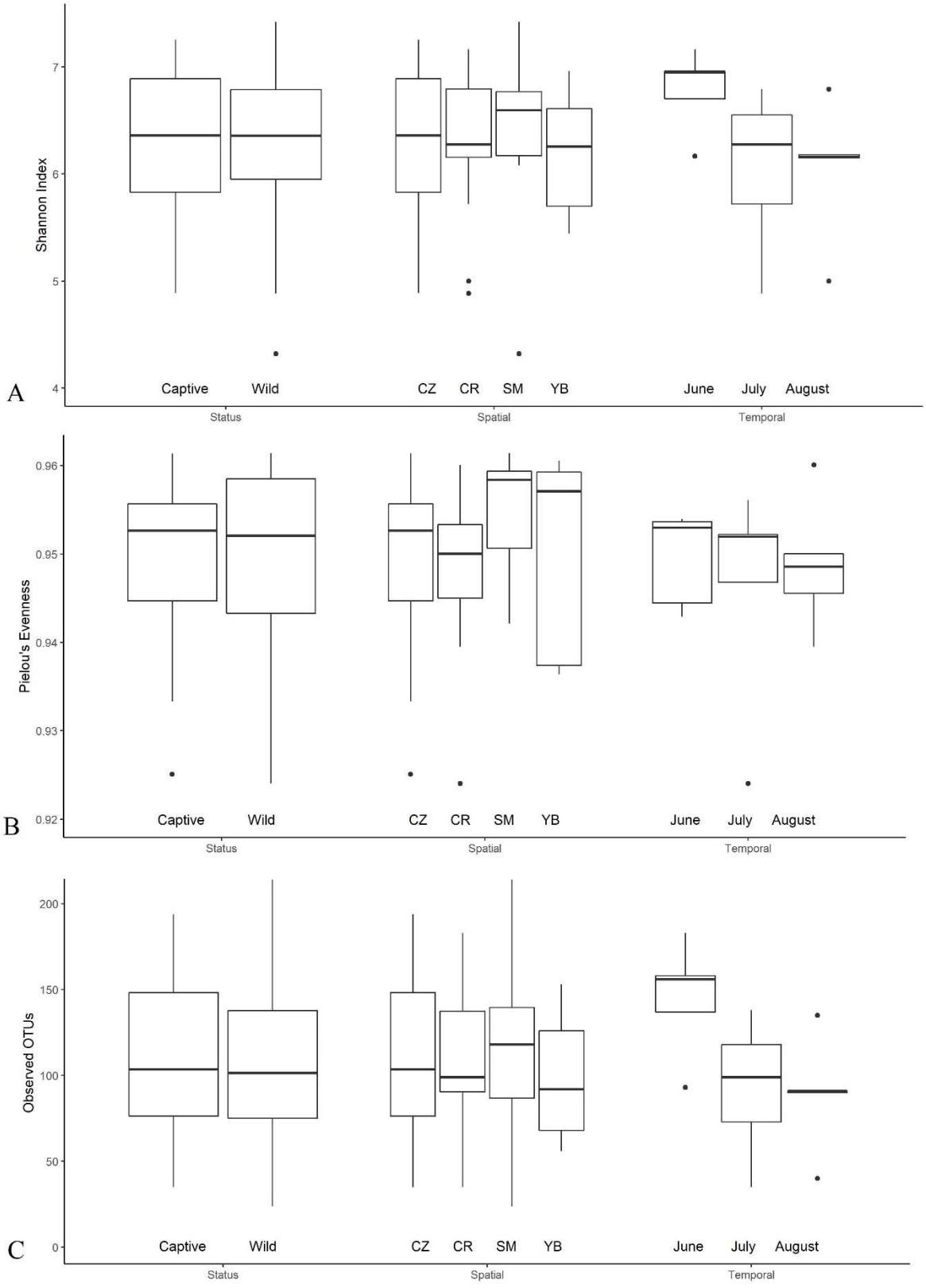

Figure 2. Median (horizontal lines), range (vertical lines) and interquartile range (box) from calculated alpha diversity measured from rarified samples for captive and wild mountain goats, spatial, and temporal comparisons: (A) Shannon Index reflecting community diversity, (B) Pielou's community evenness, and (C) observed number of operational taxonomic units (OTUs).

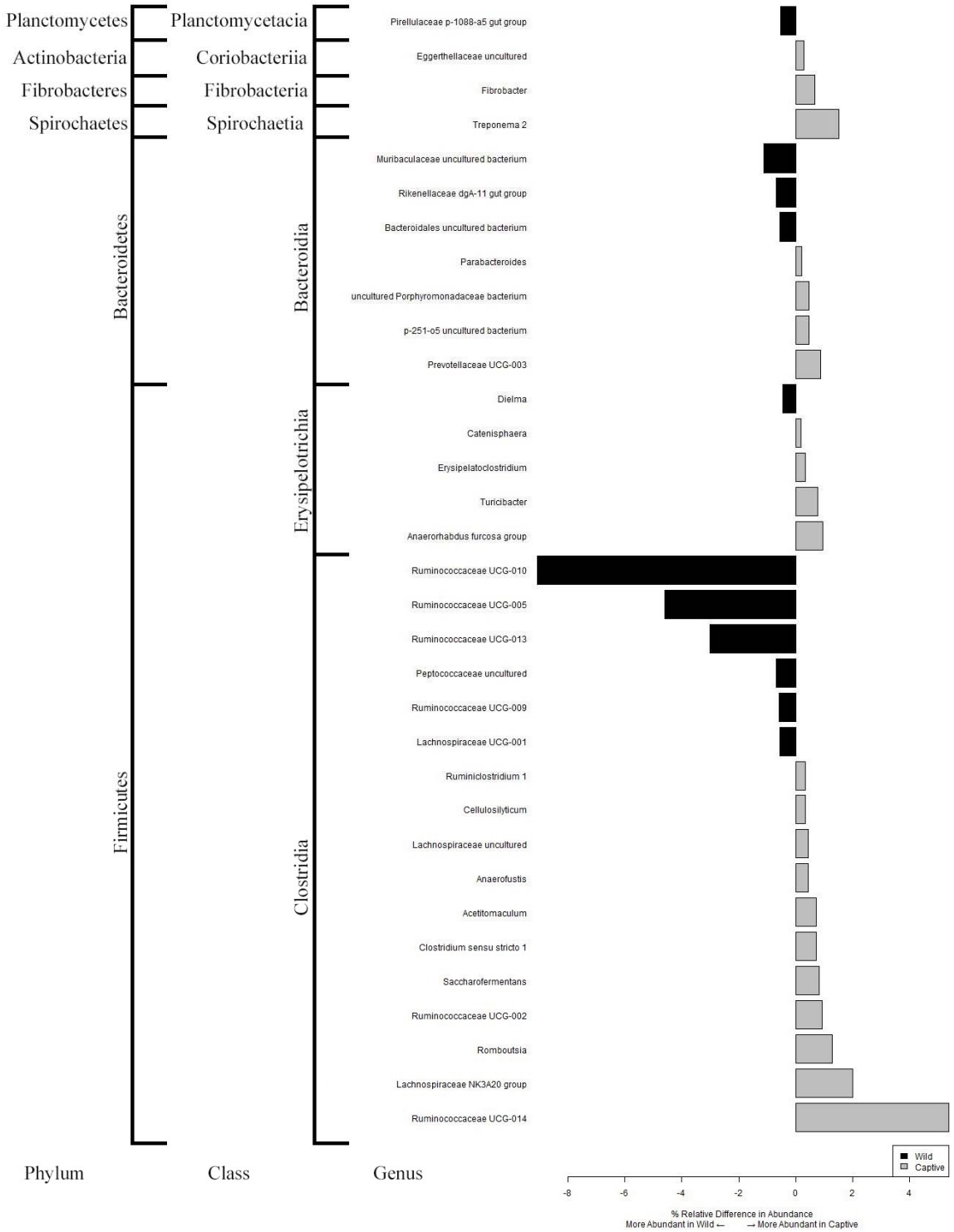

Figure 3. The difference in relative percent abundance of statistically significant genera (p-value < 0.01) that differ between captive and wild populations of mountain goats and their taxonomic breakdown at the phylum and class levels.

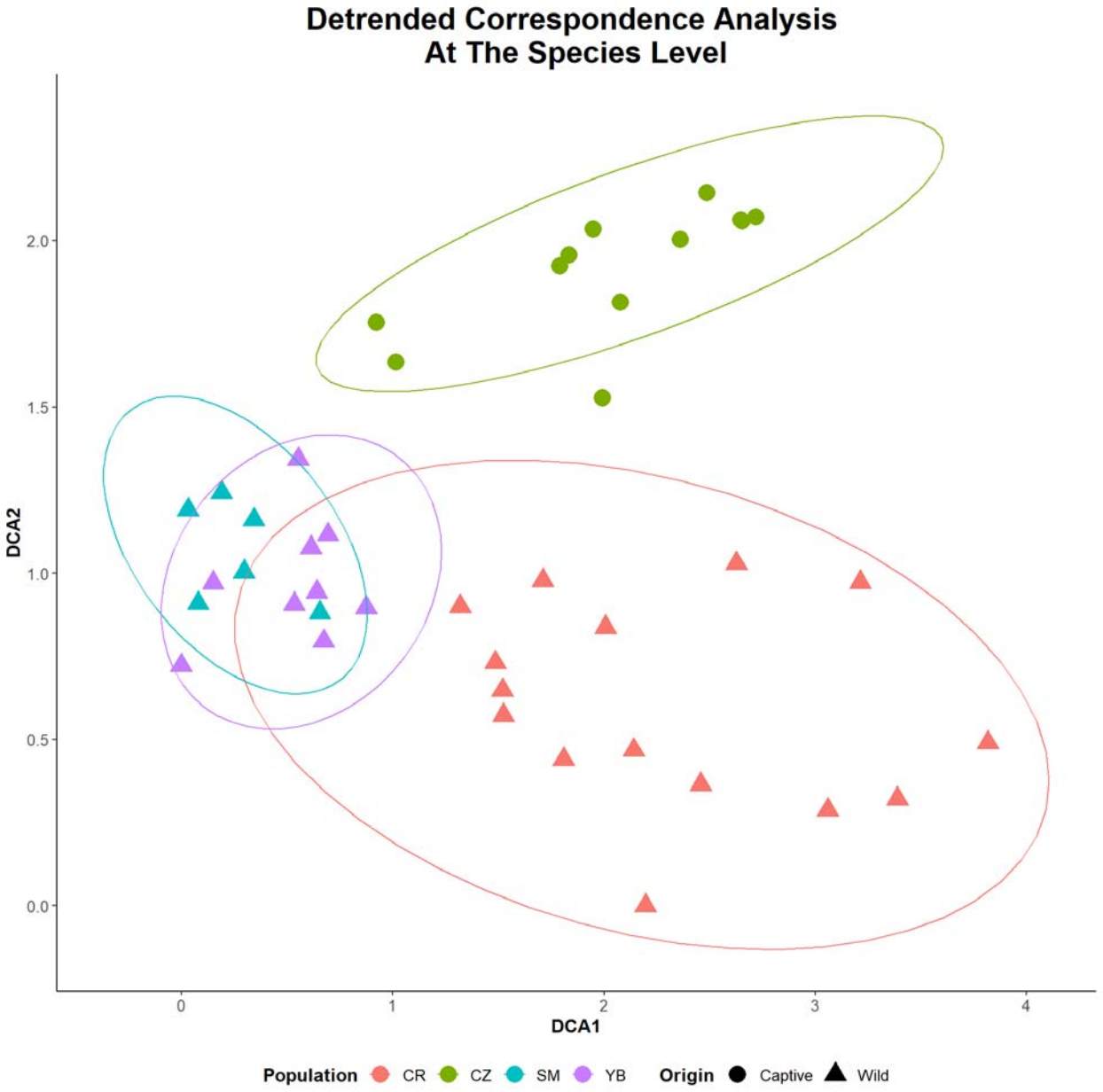

Figure 4. Visualization of the detrended correspondence analysis (DCA) conducted from the species level classification counts by origin and status. The distinction between origin, either Alberta (CR and CZ samples) or Alaska (SM and YB samples), is made clear by DCA1. The distinction between captive (circle samples) and wild (triangle samples) populations are made clear by DCA2.

Table 1. The top five microbiome phyla based on relative abundance for the four mountain goat populations (Calgary Zoo, Alberta = CZ; Caw Ridge, Alberta = CR; Ship Mountain, Alaska = SM; and Yes Bay, Alaska = YB), for captive and wild mountain goats, and for the different collection months at CR. The relative *Firmicutes* to *Bacteroidetes* ratios are also reported. Bolded groups represent a unique classification among groups.

Group						
Status			*F/B Ratio*			
Captive	*Firmicutes* 72%	*Bacteroidetes* 17%	4.35	*Patescibacteria* 4.6%	***Spirochaetes*** 1.6%	*Verrucomicrobia* 1.1%
Wild	*Firmicutes* 75%	*Bacteroidetes* 16%	4.73	*Patescibacteria* 2.5%	*Verrucomicrobia* 1.6%	***Proteobacteria*** 1.3%
Population						
CZ	*Firmicutes* 72%	*Bacteroidetes* 17%	4.35	*Patescibacteria* 4.6%	***Spirochaetes*** 1.6%	*Verrucomicrobia* 1.1%
CR	*Firmicutes* 77%	*Bacteroidetes* 14%	5.63	*Patescibacteria* 3.8%	*Verrucomicrobia* 2.1%	***Actinobacteria*** 1.4%
SM	*Firmicutes* 71%	*Bacteroidetes* 20%	3.54	*Proteobacteria* 2.8%	***Tenericutes*** 1.7%	***Lentisphaerae*** 0.93%
YB	*Firmicutes* 76%	*Bacteroidetes* 17%	4.49	*Proteobacteria* 2.1%	*Patescibacteria* 1.6%	*Verrucomicrobia* 1.5%
Month						
June	*Firmicutes* 74%	*Bacteroidetes* 18%	4.18	*Patescibacteria* 3.5%	*Verrucomicrobia* 1.3%	*Actinobacteria* 0.93%
July	*Firmicutes* 86%	*Bacteroidetes* 5.8%	14.77	*Actinobacteria* 3.2%	*Patescibacteria* 3.1%	*Verrucomicrobia* 0.97%
August	*Firmicutes* 74%	*Bacteroidetes* 15%	4.81	*Patescibacteria* 5.4%	*Verrucomicrobia* 3.2%	***Planctomycetes*** 0.67%

Table 2. The top five microbiome genera, with relative percent classification, for all four mountain goat populations, for captive and wild mountain goats, and for the different collection months at Caw Ridge, Alberta. Bolded groups represent a unique classification among groups.

Group					
Status					
Captive	*Ruminococcaceae UCG-014* 12%	*Eubacterium coprostanoligenes group* 8%	*Ruminococcaceae UCG-005* 6%	***Bacteroides*** 6%	*Christensenellaceae R-7 group* 5.6%
Wild	***Ruminococcaceae UCG-010*** 11%	*Ruminococcaceae UCG-005* 11%	*Eubacterium coprostanoligenes group* 6.5%	*Ruminococcaceae UCG-014* 6.2%	*Christensenellaceae R-7 group* 6.1%
Population					
CZ	*Ruminococcaceae UCG-014* 11.7%	*Eubacterium coprostanoligenes group* 8.1%	*Ruminococcaceae UCG-005* 6.1%	***Bacteroides*** 6.1%	*Christensenellaceae R-7 group* 5.6%
CR	*Ruminococcaceae UCG-005* 11.5%	*Christensenellaceae R-7 group* 9.7%	*Ruminococcaceae UCG-014* 8.8%	*Eubacterium coprostanoligenes group* 6.2%	*Ruminococcaceae UCG-013* 5.1%
SM	*Ruminococcaceae UCG-010* 21.3%	*Ruminococcaceae UCG-005* 9.5%	*Alistipes* 8.8%	*Eubacterium coprostanoligenes group* 5.8%	*Ruminococcaceae UCG-013* 4.4%
YB	*Ruminococcaceae UCG-010* 21.0%	*Ruminococcaceae UCG-005* 10.4%	*Eubacterium coprostanoligenes group* 8.0%	*Ruminococcaceae UCG-013* 7.2%	*Alistipes* 4.8%
Month					
June	*Christensenellaceae R-7 group* 10%	*Ruminococcaceae UCG-014* 10%	*Ruminococcaceae UCG-005* 10%	***Ruminococcaceae UCG-013*** 6%	*Bacteroides* 6%
July	*Christensenellaceae R-7 group* 13%	*Ruminococcaceae UCG-005* 12%	*Ruminococcaceae UCG-014* 9%	***Ruminococcaceae NK4A214 group*** 6%	***Eubacterium brachy group*** 5%
August	*Ruminococcaceae UCG-014* 11%	*Ruminococcaceae UCG-005* 11%	***Eubacterium coprostanoligenes group*** 10%	***Candidatus Saccharimonas*** 7%	*Bacteroides* 6%

Table 3. Results of 4-way, 2-way, and 3-way PERMANOVA comparisons of Bray Curtis dissimilarity matrix for all four mountain goat populations, for captive and wild mountain goats, and for the different collection months at Caw Ridge, Alberta, respectively.

Group	#perm	DF (factor:total)	SS	MS	F.model	p-value	R^2
Status	999	1:41	0.670	0.670	1.395	0.001	0.034
Population	999	3:41	1.801	0.600	1.263	0.001	0.091
Month	999	2:14	0.996	0.498	1.063	0.018	0.151