Gut microbiome composition predicts summer core range size in a generalist and specialist ungulate

J.F. Wolf [1] (https://orcid.org/0000-0003-0773-4456), K.D. Kriss[2], K.M. MacAulay[2] (https://orcid.org/0000-0003-1001-6906), K. Munro[1,3], B.R. Patterson[1,4], and A.B.A. Shafer[1,5] (http://orcid.org/0000-0001-7652-225X)

[1] Trent University, Department of Environmental and Life Sciences, Peterborough, Ontario, K9L 0G2, Canada

[2] Ministry of Forests, Lands, and Natural Resource Operations, and Rural Development, Smithers, British Columbia V0J 2N0, Canada

[3] Ontario Federation of Anglers and Hunters, 4601 Guthrie Drive, Peterborough, Ontario, K9J 8L5, Canada

[4] Ontario Ministry of Natural Resources and Forestry, Wildlife Research and Monitoring Section, Trent University, DNA Building, Peterborough, Ontario, K9L 0G2, Canada

[5] Trent University, Forensic Science Program, Peterborough, Ontario, K9L 0G2, Canada

Corresponding author: jessewolf@trentu.ca

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Abstract

The gut microbiome of animals can vary by age, diet, and habitat, and directly influences individual health. Likewise, variation in an individual's home range can lead to differences in feeding strategies and fitness. Ungulates (hooved mammals) exhibit species-specific microbiomes and habitat use patterns: here, we combined gut microbiome and movement data to assess the relationship between space use and the gut microbiome in a specialist and a generalist ungulate. We captured and GPS radiocollared 24 mountain goats (*Oreamnos americanus*) and 34 white-tailed deer (*Odocoileus virginianus*). During captures, we collected fresh fecal samples and conducted high-throughput sequencing of the 16S rRNA gene. Using the GPS data, we generated Brownian Bridge Movement Models to estimate core (50%) and home range (95%) sizes and calculated the proportion of use for several important habitat types. *Firmicutes* to *Bacteroidetes* ratios were positively correlated with core range area in both species. In mountain goats, we observed a negative relationship between gut diversity and use of both escape terrain and treed habitat. We hypothesize that larger *Firmicutes* to *Bacteroidetes* ratios confer body size or fat advantages that allow for larger core ranges, and that relationships between gut diversity and disproportionate habitat use is stronger in mountain goats due to their restricted niche relative to white-tailed deer. This is the first study to relate core range size to the gut microbiome in wild ungulates and is an important proof of concept that advances the information that can be gleaned from non-invasive sampling.

Introduction

The gastrointestinal tract of animals contains trillions of microbes that influence individual health. Gut bacteria, hereafter termed the gut microbiome, can modify immune responses (Arnolds & Lozupone, 2016), improve and modulate metabolism (De Angelis et al., 2020), and affect behaviour (Shreiner, Kao, & Young, 2015; Zhang, Ju, & Zuo, 2018). While largely stable over time, (Coyte, Schluter, & Foster, 2015; Faith et al., 2013) disturbance of the gut microbiome can lead to disease (Duvallet, Gibbons, Gurry, Irizarry, & Alm, 2017) and impacts metabolic versatility, meaning the ability to survive equally well when presented with a wide range of dietary compositions and habitat (Esposti & Romero, 2017; Tinker & Ottesen, 2016). Gut microbiome diversity has also been shown to impact behaviour; for example, gut microbiome manipulation in mice resulted in higher memory as measured using a passive-avoidance test (Mao et al., 2020). The presence of key gut bacteria species suppressed protein appetite, indicating the ability of the gut microbiome to drive dietary decisions (Leitão-Gonçalves et al., 2017). The mechanistic links are not totally understood, but are thought to follow the microbiota-gut-brain axis, where bacteria have the ability to, for example, generate neurotransmitters that influence cognition (Cryan & Dinan, 2012).

Differences in gut microbiome composition have also been correlated to the landscape; percent urban landcover available to ibises (*Pelecaniformes* spp.) was positively correlated with gut microbiome composition (Murray, et al., 2020). In multiple bird species, microbial community was significantly correlated to habitat type (San Juan et al., 2019). Individuals in farmland habitats also exhibited higher diversity relative to individuals in natural habitats, reflecting the link between gut microbiome composition and ecosystem alteration (Chang, Huang, Lin, Huang, & Liao, 2016). Levels of daily activity and foraging also appear to be influenced by the gut microbiome (Jones et al., 2018; Schretter et al., 2018)

and distinct diet types, such as herbivory and carnivory, are associated with unique microbiome profiles (Ley et al., 2008).

Mammalian herbivores are typified by specific gut microbial taxa as they rely on these bacteria to extract energy and nutrients from food, synthesize vitamins, and detoxify plant defense compounds (Dearing & Kohl, 2017). Ungulates, and ruminants in particular, have specialized anatomical and physiological adaptations to accommodate the cellulolytic fermentation of low-nutrition, high-fiber plant materials (De Tarso, Oliviera, & Bastos Alfonso, 2016). A specialized gut microbiome allows ruminants to digest typically indigestible plant biomass (Kruger Ben Shabat et al., 2016) and as a resultm exploit novel environments. Mountain goats (*Oreamnos americanus*) are large alpine ruminants that are endemic to the mountainous regions of northwestern North America (Festa-Bianchet and Côté 2008). Mountain goats use lower elevation, forested, and warmer aspect habitat during the winter and higher elevation, mountainous terrain in summer (Poole & Heard, 2003; Poole et al., 2009; Taylor et al., 2014; White, 2006). They are considered intermediate browsers and eat a variety of forage, with diets generally dominated by grasses (Festa-Bianchet & Côté, 2003; Hofmann, 1989). In contrast, white-tailed deer (*Odocoileus virginianus*) exploit a variety of habitat and food resources and cover a large geographic range that stretches across most of North America and includes part of Central and South America (Hewitt, 2011). White-tailed deer use woody cover habitats year-round, but can also thrive in urban and rural settings (Grund, McAninch, & Wiggers, 2002; Van Deelen, Campa III, Hamady, & Haulfer, 1998). They maintain distinct seasonal ranges in the northern parts of their range and are considered browsing ruminants as well as both habitat and dietary generalists (Berry, Shipley, Long, & Loggers, 2019).

Our study integrated high-throughput sequencing and GPS telemetry to evaluate the relationship between gut microbiome, core range area, and use of different habitat types of two ungulates living in contrasting environments. We quantified the relationship between key microbiome metrics on core range size and

relative use of different habitat types inferred from GPS tracking of individuals. From an evolutionary perspective, this link between variation in phenotype or behaviour and the gut microbiome assumes selection operates on both the genomes of the constituents (microbiome) and host, otherwise known as the holobiome (Bordenstein & Theis, 2015). We hypothesized that an increase in gut diversity would be linked with larger core ranges, as greater gut diversity would reflect, and possibly drive larger use of space and a more resource-diverse core range, similar to the findings of Ma et al., (2019). High *Firmicutes* to *Bacteroidetes* ratios correspond to larger body size and fat stores; levels of *Firmicutes* increase and promote more efficient calorie absorption and subsequent weight gain (Duvallet et al., 2017; Koliada et al., 2017; Ley, 2010). As such, we hypothesized that larger *Firmicutes* to *Bacteroidetes* ratios would be correlated with larger core ranges, as individuals building up fat stores would generally use more space to forage. This relationship may be impacted by resource distribution, as specialists prefer homogenously distributed resources, while generalists prefer heterogeneously distributed resources, which can impact space use (Marrotte et al., 2020). Consequently, we hypothesized that relationships between proportional habitat use and the gut microbiome would be stronger in specialists as they have a more restricted niche with deviations from this having larger consequences, whereas generalists can make use of a variety of habitat types.

Methods

Animal captures, sample collection and DNA extraction

We captured and radio-collared 24 male and female mountain goats on three adjacent mountain complexes using aerial net-gun capture northeast of Smithers, British Columbia, Canada (Fig. 1). We captured and radio-collared 34 female white-tailed deer (*Odocoileus virginianus*) using baited Clover traps in Marlborough Forest, a deer yard located southwest of Ottawa, Ontario, Canada (Fig. 1). For more information on animal captures, see Wolf, Kriss, MacAulay, & Shafer (2020) and Munro (2020). VERTEX Plus and VERTEX Lite Global Positioning System (GPS) collars (VECTRONIC Aerospace, Germany) were used for mountain goats, while store-on-board (G2110D, Advanced Telemetry Solutions, Isanti, MN) or GSM-upload (Wildcell SG, Lotek Wireless Wildlife Monitoring, Newmarket, ON) GPS collars were used for white-tailed deer. Collars recorded locations every four hours for mountain goats and every five hours for white-tailed deer. During captures we took fecal pellets from each individual and stored them at -20°C; all captures took place during winter. Lab surfaces were sterilized with 90% EtOH and 10% bleach solution and a small portion of a single fecal sample (~1/4 including exterior and interior portions) was digested overnight at 56°C in 20 ul proteinase K and 180 ul Buffer ATL from the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, California, USA). DNA was extracted from the digest with the QIAamp PowerFecal DNA Kit (Qiagen, Valencia, California, USA).

High-throughput sequencing and bioinformatics

The validated Illumina 16S rRNA Metagenomic Sequencing Library Preparation (#15044223 rev. B) protocol was followed for library preparation using slight modifications (Haworth, White, Côté, & Shafer, 2019). The V3 and V4 regions of the 16S ribosomal ribonucleic acid (16S rRNA) hypervariable region were targeted with four variants of 341F and 805R 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 DNA were amplified in 25 µl PCR using the 341F and 805R primers. The replicated amplicons for each sample were combined into a single reaction of 100 µl 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). Samples were purified with the QIAquick PCR Purification Kit and the final purified library was validated on a TapeStation (Agilent, G2991AA) and sequenced in 300 bp pair-end reads on an Illumina MiSeq sequencer at the Genomic Facility at The University of Guelph (Guelph, Ontario).

The quality of the raw sequences was assessed using FastQC v 0.11.9 (Andrews, 2010) and we determined the low-quality cut-off for forward and reverse reads (see Haworth et al., 2019). Forward and reverse reads were imported into QIIME2 v 2019.4 (Boylen et al., 2019) for quality control, sequence classification, and diversity analysis. Merged, forward, and reverse reads were analyzed independently using the quality control function within QIIME2 and DADA2 to perform denoising and to detect and remove chimeras. 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. The taxonomy, to the species level, of all sample reads were assigned using Silva 132 reference taxonomy database. We calculated the relative proportion of *Firmicutes* to *Bacteroidetes* for each of the grouped data. Estimates of diversity included Shannon's Index, observed Operational Taxonomic Units (OTUs) and Pielou's evenness, a measure of diversity that is the ratio of observed diversity to the maximum possible in a sample having the same number of species (Pielou, 1966); these were screened for correlation to one-

another and read-depth. Pielou's evenness and the ratio of *Firmicutes* to *Bacteroidetes* were retained for subsequent analyses. We rarefied sample reads to the sample with the least number of reads.

GPS filtering, core/home range, and proportional habitat use analysis

We used different filtering approaches and seasonal delineations for each species due to the differences in landscapes occupied by mountain goats and white-tailed deer. For the mountain goat data, any N.A. or mortality signals were filtered out as were any GPS points outside of 600 m-2500 m in elevation, as this reflects the maximum and minimum for the study area. Dilution of precision (DOP) values over 10 were plotted against elevation and landscape type to ensure there was no patterns in distribution and that filtering out would not bias downstream analyses. There were no observable patterns and DOP values over 10 were subsequently filtered out. Movement rates between successive GPS points were also calculated, and any movement rates beyond 15 km/hr were also removed from analyses as these were deemed spurious. Seasons were defined as follows: Summer - May 1st to October 31st and Winter - December 1st to April 30th (Cadsand, 2012; Mountain Goat Management Team, 2010; Poole & Heard, 2003; Richard & Côté, 2016). November was excluded from seasonal data as White (2006) noted a large increase in male mountain goat home ranges due to the rut. In white-tailed deer, filtering occurred as above, but with no elevation restrictions as the topography of Marlborough Forest and the surrounding area is effectively flat. As considerable variation in migration dates were observed, movement trajectories for each individual deer were examined to identify the dates of migration movements to and from Marlborough Forest to differentiate between winter and summer ranges. A migration movement was defined as when a deer moved between non-overlapping seasonal ranges and then occupied one seasonal range until the following migration movement (Munro 2020). As movements to and from Marlborough forest were relative to each individual deer's movement, there was no defined date range. No seasonal GPS data were excluded for white-tailed deer as changes in movement patterns and home range size during the rut are minimal in females (Hölzenbein & Schwede, 1989).

We used Brownian Bridge Movement Models (BBMMs) to generate individual core and home ranges using the BBMM package in R (Nielson, Sawyer, & McDonald 2015). A BBMM is a continuous-time stochastic movement model that uses probabilistic and maximum likelihood approaches where observed locations are measured with error to model home ranges (Horne, Garton, Krone, & Lewis, 2007). A minimum of 275 GPS points was required to generate a BBMM and individual isopleths were calculated with a maximum lag time between successive locations of two times the expected fix rate. A location error of 20 m was used as per Sawyer et al., (2009), with a cell size of 25 m^2. We generated 50% and 95% isopleths representing the core and home ranges, respectively. Isopleths were generated separately for each individual during both summer and winter, as ungulates exhibit season-specific habitat use patterns, that vary both by sex and by age-class (Festa-Bianchet & Côté, 2008; Lesage et al., 2000; Mountain Goat Management Team, 2010; Webb, Hewitt, & Hellickson, 2007; White, 2006). We focused on summer isopleths, as deer were baited in winter, which has been shown to bias movement and shift core ranges (Kilpatrick & Stober, 2002). We report only the 50% isopleths, hereafter termed core ranges, to maximize seasonal differences, as they were highly correlated to the 95% isopleths (t_{46}=9.3, r=0.81, p<0.0001) and results were similar between summer 50% and 95% isopleths (Fig. 1, Fig. S1). We generated BBMM isopleths using the R package rgdal (Bivand et al., 2019) and all analyses were conducted in R v.3.6.1. We imported shapefiles into ArcGIS Pro 2.5.0 and core/home range areas were calculated in km^2 for further analyses.

Proportional use of habitats was assessed by calculating the number of GPS points in a given habitat type within an isopleth, relative to the total number of GPS points located in that isopleth. Similar to Johnson's third-order habitat selection, proportional habitat use in this study refers to how specific habitat types were used within a core range (Johnson, 1980). Proportion values in the 50% isopleths were highly correlated to the 95% isopleths (t_{117}=106, r=0.99, p <2.2e-16, Table S1, S2). We selected ecologically relevant features that showed previous evidence for use in both species. Features used in mountain goat models were treed

habitat, Heat Load Index (HLI), and escape terrain (landscape where slope is ≥ 40º; Shafer et al., 2012). These features have shown evidence for disproportionate usage/selection in previous research on mountain goats (Shafer et al., 2012). In the white-tailed deer models, we used forested habitat, treed swamp, and thicket swamp, as each of the three habitat features exhibited >20% core landcover composition in Munro (2020), and thus, were available for usage in the majority of individuals (Massé & Côté, 2013). For the HLI (Mccune & Keon, 2002), the average value of HLI for all GPS points within an isopleth for a given individual was calculated. The Southern Ontario Land Resource Information System (SOLRIS) data set version 2.0 (OMNRF, 2019) was used to determine land cover types for white-tailed deer, while we used the Biogeoclimactic Ecosystem Classification (BEC) dataset (GeoBC, 2019) to determine terrestrial landcover type for mountain goats.

Generalized linear models

We analyzed the associations between core range size, gut microbiome metrics, and age class for both species individually using Generalized Linear Models (GLMs) with the Gaussian family distribution and identity link function. The core range GLMs consisted of core range size as a response variable, a single microbiome metric (*Firmicutes* to *Bacteroidetes* ratio or Pielou's evenness) and age class (adult or subadult) as fixed explanatory effects. The proportional habitat use GLMs consisted of proportion of habitat used as a response variable, a single microbiome metric (*Firmicutes* to *Bacteroidetes* ratio or Pielou's evenness) and age class, as fixed explanatory effects. One exception to this was the HLI GLM, as the response variable was the mean HLI value for GPS points in the isopleth, while the explanatory variables were the same as described above. Individuals 0 - 2 years of age were considered subadults for white-tailed deer, while individuals 0 - 3 years of age were considered subadults for mountain goats (Delgiudice, Fieberg, Riggs, Powell, & Pan, 2006; Festa-Bianchet & Côté, 2008). Effect size and confidence intervals are reported for each model. We conducted k-fold (k=5) linear model cross validation using the Caret package in R (Kuhn et al., 2020) to test for overfitting of our models and quantify the

model's predictive ability. We reported the Scatter Index (SI) and Root Mean Square Error (RMSE): low values in RMSE and SI are indicative of a good model fit and low residual variance.

Results

Bioinformatic filtering and taxonomic analysis

Twenty-three mountain goat and 25 white-tailed deer fecal sample sequences passed QC and a total of ~8.17 million paired-end reads (n_{MG}=5,488,856, n_{WTD}=2,679,668) were generated (SRA accession number PRJNA638162). FastQC analysis indicated that both forward and reverse reads lost quality > 259 bp in length (Phred score <25), so all reads were trimmed to a length of 259 bp. Following DADA2 strict quality filtering, ~1.16 million paired-end reads (n_{MG}=709,457, n_{WTD}=457,541) were kept for taxonomic and diversity analyses. Losing this many reads to quality filtering is typical (see Haworth et al., 2019), as permitted error rates are extremely low in DADA2, resulting in high certainty among retained reads (Callahan et al., 2016). White-tailed deer had higher averages of both Pielou's evenness (mean 0.95, min 0.92, max 0.96, SD 0.012) and *Firmicutes* to *Bacteroidetes* ratio (mean 8.3, min 1.49, max 21.5, SD 6.22) than mountain goats (Pielou's evenness mean 0.92, min 0.84, max 0.95, SD 0.028; *Firmicutes* to *Bacteroidetes* ratio mean 6.90, min 3.51, max 12.10, SD 2.43). Age class and sex averages, in addition to winter data, are shown in Table S3.

Core range and proportional habitat use

Data filtering resulted in 84,932 GPS points for mountain goats (mean per individual 3,679, range 277 - 6,704, SD 761), and 63,900 GPS points for white-tailed deer (mean per individual 2,556, range 831 - 3,558, SD 906). One individual mountain goat was removed due to a small number of GPS points (n = 16). Mountain goats exhibited a slightly larger mean summer core range size of 0.40 km^2 (min 0.01 km^2, max 0.72 km^2, SD 0.17), compared to 0.36 km^2 (min 0.13 km^2, max 0.81 km^2, SD 0.16) for white-tailed deer. Proportional use of habitat values was variable, ranging from 0.05 to 0.91 (means ranged from 0.41 to 0.63) in mountain goats and from 0.00 to 0.84 (means ranged from 0.13-0.34) in white-tailed deer. The mean proportional habitat use values and associated standard deviations are reported in Table S4.

Generalized linear models

Sex was not included in the final model as there were no strong differences in core range sizes between sexes in mountain goats ($t_{17.58}$=0.374, p>0.05) and all white-tailed deer samples were obtained from females. We compared 50% isopleths from summer and winter; summer core ranges were reported here, as winter core ranges produced qualitatively similar results, albeit with weaker signals (Table S5). In both species, a greater *Firmicutes* to *Bacteroidetes* ratio was associated with larger core ranges with both models explaining an equal amount of variance (Nagelkerkes's R^2~0.27; Table 1; Fig. 2). Mountain goat gut diversity increased with core range size, while a decrease in white-tailed deer was associated with larger core ranges; here the mountain goat model explained a relatively large portion of the variance (R^2=0.47; Table 1; Fig. 2). Age-class was a significant predictor in both mountain goat GLMs, but neither of the white-tailed deer models. The use of escape terrain and treed areas were moderately correlated in mountain goats (t_{20}=2.94, p<0.01, r=0.55), and were significant predictors of Pielou's evenness; effect size confidence intervals did not overlap zero in models that measured the relationship between use of escape terrain and treed areas and Pielou's evenness (Table 2). Specifically, a larger Pielou's evenness value was seen in individuals using less treed habitat and less escape terrain (Fig. 3). In HLI GLMs, confidence intervals overlapped zero and exhibited a relative ~2x decrease in R^2 value relative to other mountain goat habitat use models (β= 0.91, R^2=0.12, Table 2). All GLM parameter estimates in the white-tailed deer models had confidence intervals overlapping zero (Table 2). K-fold cross validation of linear models supported retaining age class and microbiome metric as a predictor variable of core range size. RMSE values in models with core range size as the response variable ranged from 0.12 to 0.18, and SI values ranged from 0.30 to 0.51, whereas in proportional habitat use models, RMSE values ranged from 0.11 to 0.33 and SI values from 0.033 to 1.14 (Table S6); this suggests moderate to high support for the models.

Discussion

The link between gut microbiome and host space use and has implications for foraging, activity levels, and ability to use energetically costly habitats. We showed that differences in the gut microbiome between a generalist ungulate and specialist ungulate were linked to patterns of habitat use and core range size. Although the patterns are nuanced, there were some commonalities that collectively suggest the gut microbiome plays a role in determining space use patterns. An increase in *Firmicutes* to *Bacteroidetes* ratios in both species was correlated to an increase in core range sizes (Fig. 2). Increased *Firmicutes* to *Bacteroidetes* ratios are linked to increased Body Mass Index (BMI) and obesity (Duvallet et al., 2017). Both *Firmicutes* and *Bacteroidetes* are involved in energy absorption and carbohydrate metabolism; *Firmicutes* can act as a more effective energy source, leading to more efficient calorie absorption and weight gain, while *Bacteroidetes* are involved with energy production and conversion, as well as amino acid transport and metabolism (Krajmalnik-Brown, Ilhan, Kang, & DiBaise, 2012; Ottman, Smidt, de Vos, & Belzer, 2012; Turnbaugh et al., 2006). In a comparable, study home range size was not correlated with the *Firmicutes* to *Bacteroidetes* ratio in wild rodents (Jameson, Réale, & Kembel, 2020), however, seasonal weight changes are more dynamic in small mammals (Lynch, 1973; Merritt & Zegers, 1991), and we suspect this pattern might be more ubiquitous across temperate ungulates given their specialized gut microbiomes and need to put on fat stores. An individual's gut that is comprised of more *Firmicutes*, bacteria that act as an effective energy source, may be able to increase body fat more efficiently relative to individuals with lower *Firmicutes* to *Bacteroidetes* ratios, with an increase in *Firmicutes* to *Bacteroidetes* ratio helping individuals accumulate fat stores to survive the winter by increasing their core range size.

In large ungulates, increasing levels of body fat is important to survive the winter, when forage is limited relative to the other seasons. In muskoxen (*Ovibos moschatus*), the abundance of *Firmicutes* stayed similar across seasons, while *Bacteroidetes* increased in the summer months, meaning the ratio of

Firmicutes to *Bacteroidetes* is lower in the summer (Bird et al., 2019). Additionally, an increase in microbial diversity in yak (*Bos grunniens*) was noted in spring (Ma et al., 2020). Increased bacterial diversity in spring relative to summer suggests species are capable of modulating bacterial diversity; but note lower ratios of *Firmicutes* to *Bacteroidetes* does not necessarily correspond to higher diversity. The ability to conserve bacteria necessary for adding fat, namely high levels of *Firmicutes* and low levels of *Bacteroidetes*, while exhibiting increases in gut diversity suggests ungulates can prepare for the winter even though the level of gut bacterial diversity is shifting (see Haworth et al. 2019). This concept is of importance to white-tailed deer, as diet turnover is pronounced in Ontario (Brown & Doucet, 1991). Shifting from herbaceous vegetation in the spring to woody browse in the winter may similarly result in increased diversity, while simultaneously conserving bacteria necessary for adding fat.

Both specialists and generalists may shift habitats seasonally, as mountain goats move from alpine summer habitat to subalpine winter areas and white-tailed deer exhibit distinct winter and summer ranges (Grund et al., 2002; Lesage et al., 2000; Poole & Heard, 2003). There was considerably more variation in proportion of habitat used in the generalist white-tailed deer (Table S2), likely a function of their ability to use multiple habitat types and diet sources. Here, proportion habitat use-microbiome models had no relationship, as all proportion habitat use coefficients in white-tailed deer had confidence intervals overlapping zero (Fig. S2, Table 2). Interestingly, the two habitat variables in mountain goats with clear signals were escape terrain and treed areas (Fig. 3), which are related to the defining habitat features of this species: the use of alpine terrain (i.e. above treeline) and steep slopes, (Gross, Kneeland, Reed, & Reich, 2002; Festa-Bianchet & Côté, 2008). Deviations from their specialized and preferred habitat types comes at a cost, as ungulates typically exhibit trade-offs with respect to forage quality and predation risk; avoiding predation can lead to decreased forage quality and abundance (Hamel & Côté, 2007; Hebblewhite & Merrill, 2009). In mountain goats and bighorn sheep (*Ovis canadensis*), energy expenditures increased when travelling uphill or downhill - termed a vertical cost (Dailey & Hobbs, 1989).

A lower *Firmicutes* to *Bacteroidetes* ratio could be linked to the vertical cost associated with spending more time in escape terrain, meaning less-fat or prime conditioned individuals spend more time in escape terrain. Conversely, using more forage-available treed habitat may be correlated with an increase in *Firmicutes* to *Bacteroidetes* ratio, as individuals have increased access to forage, with minimal vertical cost. Collectively, the habitat models had stronger signals in the specialist species compared to the generalist, that we suggest is reflective of their more restricted habitat niche relative to generalists, where microbial deviations generate more prominent shifts in behaviour.

The potential for the gut microbiome to modulate habitat use patterns is particularly important in specialists, as they have a more restricted niche, where deviations from the usage of important habitat types have larger consequences. While there is potential for modulation of proportion of specific habitat usage, the impact of the gut microbiome on the amount of space used may be more explicit. A lower Pielou's evenness value indicates that a given individual has decreased diversity relative to another individual with the same number of gut microbial species. The relationship between Pielou's evenness and core range size differed in direction between species; greater gut diversity in mountain goats was correlated with a larger core range, while a negative relationship was noted in white-tailed deer (Fig. 2). Increased gut diversity might promote individuals moving through and foraging in larger areas, resulting in a positive relationship between diversity and core range size. This is of added importance in a specialist, as the ability to utilize and forage in a more diverse array of habitat types might allow individuals to access and subsist in areas that other conspecifics cannot, thus increasing their competitive ability (Blake & Karr, 1987; McPeek, 1996; Rodewald & Vitz, 2005). Higher relative gut microbiome diversity might allow specialists to forage more like generalists, while lower diversity may limit specialists to their typical niches. The reverse trend in white-tailed deer might reflect the difference in feeding and habitat-use strategies; white-tailed deer are generalists, and thus by definition, all deer can effectively use a variety of habitat and food sources. The negative relationship observed between gut diversity and range size in

white-tailed deer might suggest that deer with higher gut microbiome diversity are able to use more diet sources overall and thus can meet their nutritional requirements within a smaller area relative to individuals with less diverse gut microbiomes. As generalists, deer are not as constrained by specific food and habitat types, and thus, their space use may be less heavily influenced by gut microbiome composition.

We built our models assuming the gut microbiome influences habitat and core range patterns; but this need not be cause and effect, and the relationship is likely reciprocal (Cryan et al., 2019). For example, diet-microbiome covariance has been observed in multiple large-herbivores, and seasonal diet turnover and seasonal microbiome turnover are positively correlated (Kartzinel, Hsing, Musili, Brown, & Pringle, 2019). In this example, our model would assume the gut microbiome impacts diet choice (e.g. Leitão-Gonçalves et al., 2017) and in turn, the correlation between diet turnover and the microbiome contributes to habitat use of a given species. Assessing this relationship clearly needs experimental testing, and we view our study as a proof-of-concept that provides a testable hypothesis. Still, we demonstrated that quantifying the gut microbiome yields information related to space use and linking these two highly complex components of biology aids in our understanding of selection on the hologenome through the interplay between the individual and its microbial genomes and potential traits under selection (e.g. core range size and proportional habitat use). These findings also demonstrate that pellet sampling is useful in determining space and habitat use in managed populations, as it is conceivable with a large enough database and validation, one could predict the distribution and behaviour of animals on the landscape from non-invasively sampled pellets. Ultimately, we utilized a specialist and generalist ungulate to explore the link between the gut microbiome and movement to generate quantitative findings surrounding the impact of the gut microbiome on space use in wild populations. Similar analyses of this kind should clarify the extent to which space use is linked to the gut microbiome in other ungulates and, in turn, aid in assessing the utility of pellets to predict animal distribution and behaviour.

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Data Accessibility

The sequence data generated for the mountain goat and white-tailed deer gut microbiota has been deposited in the Sequence Read Archive (BioProject ID: PRJNA638162; SRA submission: SUB7567698). R scripts used to perform analyses and generate figures are available at https://gitlab.com/WiDGeT_TrentU/graduate_theses/-/tree/master/Wolf_et_al.

Author Contributions

J.F.W. and A.B.A.S. conceived the ideas; K.D.K., K.M.M., K.M., B.R.P., J.F.W., and A.B.A.S. collected materials and data; J.F.W. analysed the data; and J.F.W. wrote the paper with contributions from all co-authors.

Figures

Fig. 1 An example of habitat of both mountain goat (*Oreamnos americanus*) and white-tailed deer (*Odocoileus virginianus*) along with inset sample maps. Colors are reflective of relative elevation and topography with lighter colours indicating higher elevations. Average elevation of mountain goat habitat was 1614.7m, relative to 113.3m for white-tailed deer.

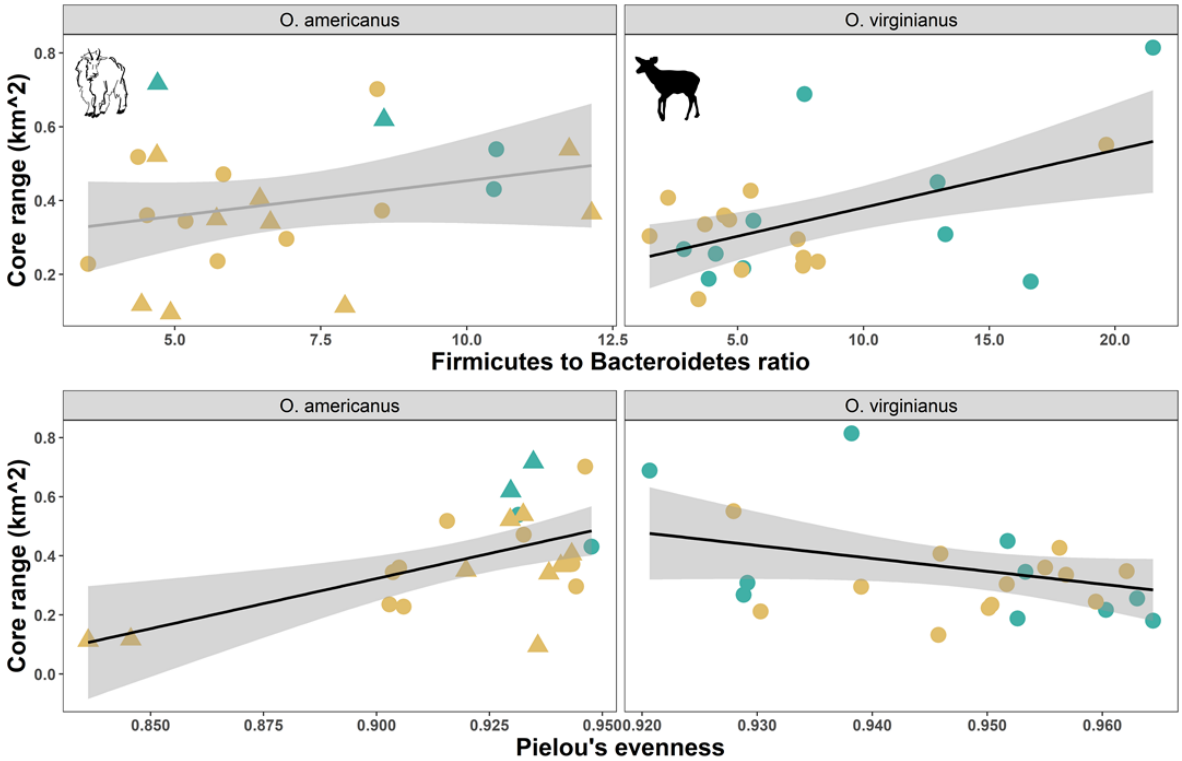

Fig. 2 Microbiome metrics for mountain goats (*Oreamnos americanus*, n=22) northeast of Smithers, British Columbia, and white-tailed deer (*Odocoileus virginianus*, n=25) southwest of Ottawa, Ontario, relative to core summer range size. Females are represented by circles and only mountain goats have mixed-sex data. Adults (≥3 years of age) are represented in yellow, and subadults in blue. Significant Generalized Linear Models (GLMs) are denoted with a black line.

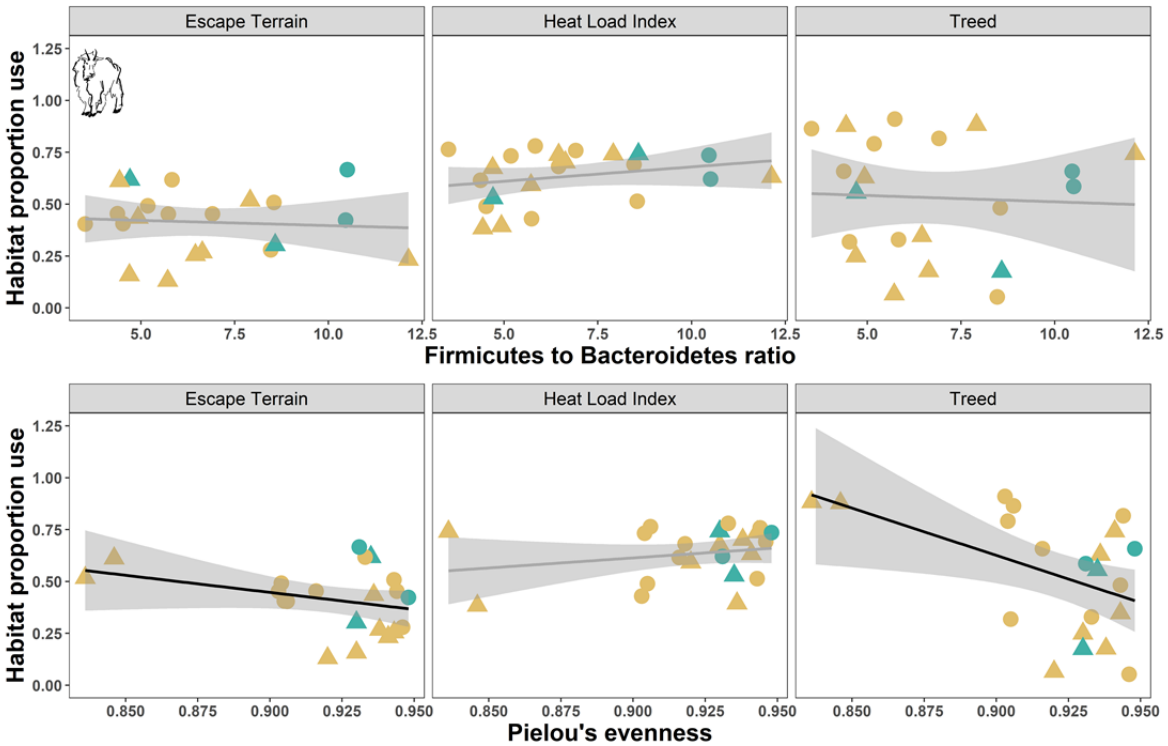

Fig. 3 Microbiome metrics for mountain goats (*Oreamnos americanus*, n=22) northeast of Smithers, British Columbia, relative to habitat selection coefficients of Heat Load Index (HLI), escape terrain, and treed habitat. Females are represented by circles and males are represented by triangles. Adults (≥3 years of age) are represented in yellow, and subadults in blue. Significant Generalized Linear Models (GLMs) are denoted with a black line.

Tables

Table 1. Generalized linear models for core summer range size of mountain goat (*Oreamnos americanus*, n=22) and white-tailed deer (*Odocoileus virginianus*, n=25). The further left column refers to the model where *Firmicutes* to *Bacteroidetes* ratios was a predictor, while the right column refers to the model where Pielou's evenness was a predictor

Mountain goat				
		Core range size		**Core range size**
Predictors	*Estimates*	*CI*	*Estimates*	*CI*
(Intercept)	0.29	0.10 - 0.49	-2.3	-4.1 - -0.42
F:B Ratio	0.010	-0.020 - 0.040	-	-
Pielou's evenness	-	-	2.8	0.85 - 4.8
Age class (Subadult)	0.20	0.020 - 0.38	0.17	0.020 - 0.32
R^2 Nagelkerke		0.27		0.47

White-tailed deer				
		Core range size		**Core range size**
Predictors	*Estimates*	*CI*	*Estimates*	*CI*
(Intercept)	0.22	0.11 - 0.33	5.4	0.62 - 10
F:B Ratio	0.020	0.00 - 0.03	-	-
Pielou's evenness	-	-	-5.3	-10 - -0.32
Age class (Subadult)	0.010	-0.12 - 0.14	0.050	-0.08 - 0.17
R^2 Nagelkerke		0.28		0.21

Table 2. Generalized linear models for habitat proportion use for mountain goats (*Oreamnos americanus*, n=22*)* and white-tailed deer (*Odocoileus virginianus*, n=25) The further left column refers to the model where *Firmicutes* to *Bacteroidetes* ratios was a predictor, while the right column refers to the model where Pielou's evenness was a predictor

Mountain goat

	Escape terrain proportion use		**Escape terrain proportion use**	
Predictors	*Estimates*	*CI*	*Estimates*	*CI*
(Intercept)	0.48	0.28 – 0.69	2.36	0.43 – 4.28
F:B Ratio	-0.01	-0.05 – 0.02	-	-
Age class (Subadult)	0.14	-0.04 – 0.32	0.15	-0.01 – 0.31
Pielou's evenness	-	-	-2.14	-4.24 – -0.05
R^2 Nagelkerke	0.127		0.252	

	Treed proportion use		**Treed proportion use**	
Predictors	*Estimates*	*CI*	*Estimates*	*CI*
(Intercept)	0.56	0.16 – 0.97	4.84	1.23 – 8.45
F:B Ratio	-0.00	-0.06 – 0.06	-	-
Age class (Subadult)	-0.04	-0.40 – 0.32	0.04	-0.25 – 0.34
Pielou's evenness	-	-	-4.69	-8.63 – -0.76
R^2 Nagelkerke	0.0050		0.24	

	Heat Load Index Average		**Heat Load Index Average**	
Predictors	*Estimates*	*CI*	*Estimates*	*CI*
(Intercept)	0.58	0.41 – 0.76	-0.23	-2.02 – 1.55
F:B Ratio	0.01	-0.02 – 0.04	-	-
Age class (Subadult)	-0.07	-0.23 – 0.08	-0.06	-0.22– 0.08
Pielou's evenness	-	-	0.91	-0.99 – 2.90
R^2 Nagelkerke	0.052		0.074	

White-tailed deer

	Forest proportion use		**Forest proportion use**	
Predictors	*Estimates*	*CI*	*Estimates*	*CI*
(Intercept)	0.29	0.11 – 0.47	2.86	-4.64 – 10.36
F:B Ratio	0.00	-0.02 – 0.01	-	-
Age class (Subadult)	0.02	-0.18 – 0.22	0.01	-0.19 – 0.20
Pielou's evenness	-	-	-2.74	-10.63 – 5.16
R^2 Nagelkerke	0.025		0.017	

	Thicket Swamp proportion use		**Thicket Swamp proportion use**	
Predictors	*Estimates*	*CI*	*Estimates*	*CI*
(Intercept)	0.19	0.08 – 0.30	-0.85	-5.37 – 3.67
F:B Ratio	-0.00	-0.01 – 0.01	-	-
Age class (Subadult)	-0.10	-0.23 – 0.02	-0.10	-0.22 – 0.02
Pielou's evenness	-	-	1.10	-3.66 – 5.86
R^2 Nagelkerke	0.12		0.13	

	Treed Swamp proportion use		**Treed Swamp proportion use**	
Predictors	*Estimates*	*CI*	*Estimates*	*CI*
(Intercept)	0.35	0.17 – 0.53	-3.57	-11.02 – 3.89
F:B Ratio	0.00	-0.02 – 0.02	-	-
Age class (Subadult)	0.05	-0.15 – 0.25	0.06	-0.13 – 0.26
Pielou's evenness	-	-	4.14	-3.71 – 11.99
R^2 Nagelkerke	0.012		0.059	