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

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22   **Abstract**

23   Individuals exhibit differences in their microbial composition that have important implications for both

24   population dynamics and ecological processes. The gut microbiome of animals can vary by age,

25   reproductive status, diet, and habitat quality, and directly influences an individual's health and fitness.

26   Likewise, variation in an individual's home range can lead to differences in reproductive behaviour,

27   feeding strategies, and fitness. Ungulates (hooved mammals) exhibit species-specific microbiomes and

28   habitat use patterns that differ by season, sex, and age-class, leading to variation among individuals

29   occupying a similar geographic area. Here, we combined fecal microbiome and movement data to assess

30   the relationship between space use and the gut microbiome in a specialist and a generalist ungulate. We

31   captured and GPS radiocollared 24 mountain goats (*Oreamnos americanus*) and 34 white-tailed deer

32   (*Odocoileus virginianus*). During captures we collected fresh fecal samples and conducted high-throughput

33   sequencing of the fecal microbiome (i.e. 16S rRNA gene) to quantify gut microbial diversity. We

34   generated Brownian Bridge Movement Models from the GPS location data to estimate core (50%) and

35   home range (95%) sizes and calculated the proportion of use for several important habitat types. An

36   increase in *Firmicutes* to *Bacteroides* ratios corresponded to an increase in core range area in both species.

37   In mountain goats we observed a negative relationship between gut diversity and use of both escape terrain

38   and treed habitat, both critical features for this alpine specialist. There were no relationships between

39   habitat use and the gut microbiome in the more generalist white-tailed deer. We hypothesize that larger

40   *Firmicutes* to *Bacteroides* ratios confer body size or fat advantages that allow for larger home ranges, and

41   that relationships between gut diversity and disproportionate use of particular habitats is stronger in

42   mountain goats due to their restricted niche relative to white-tailed deer. This is the first study to relate core

43   range size to gut diversity in wild ungulates and is an important proof of concept that advances the

44   potential type of information that can be gleaned from non-invasive sampling.

45 **Introduction**

46 The gastrointestinal tract of animals contains trillions of microbes that influence each individual's health.

47 Gut bacteria, hereafter termed the gut microbiome, can modify immune responses (Arnolds & Lozupone,

48 2016), improve and modulate metabolism (De Angelis et al., 2020), and affect behaviour (Shreiner, Kao, &

49 Young, 2015; Zhang, Ju, & Zuo, 2018). While largely stable over time (Coyte, Schluter, & Foster, 2015;

50 Faith et al., 2013), disturbance of gut microbiome can lead to disease (Duvallet, Gibbons, Gurry, Irizarry,

51 & Alm, 2017) and impacts metabolic versatility, meaning the ability to survive equally well when

52 presented with a wide range of dietary compositions and habitat (Esposti & Romero, 2017; Tinker &

53 Ottesen, 2016). Gut microbiome diversity has been shown to impact behaviour; for example, gut

54 microbiome manipulation in mice resulted in higher memory as measured using a passive-avoidance test

55 (Mao et al., 2020). Leitão-Gonçalves et al., (2017) showed that the presence of key gut bacteria species

56 suppressed protein appetite, indicating the ability of the gut microbiome to drive dietary decisions. The

57 mechanistic links are not totally understood, but are thought to follow the microbiota-gut-brain axis where

58 bacteria have the ability to, for example, generate neurotransmitters that influence cognition (Cryan &

59 Dinan, 2012).

60

61 Differences in gut microbiome composition have also been correlated to the landscape; percent urban

62 landcover in ibises is positively correlated with gut microbiome composition (*Pelecaniformes spp*; Murray,

63 et al., 2020) and in multiple bird species, microbial community was significantly correlated to habitat type

64 (San Juan et al., 2019). Individuals in farmland habitats also exhibited higher diversity relative to

65 individuals in natural habitats, reflecting the link between gut microbiome composition and ecosystem

66 alteration (Chang, Huang, Lin, Huang, & Liao, 2016). Levels of daily activity and foraging appear to be

67 influenced by the gut microbiome (Jones et al., 2018; Schretter et al., 2018) and distinct diet types, such as

68 herbivory and carnivory, are associated with unique microbiome profiles (Ley et al., 2008). Herbivores in

69 particular exhibit specific gut bacterial compositions, as certain bacteria allow them to extract energy and

70 nutrients from food and detoxify plant defense compounds (Dearing & Kohl, 2017).

71 Mammalian herbivores are typified by specific gut microbial taxa as they rely on these bacteria to extract

72 energy and nutrients from food, synthesize vitamins, and detoxify plant defense compounds (Dearing &

73 Kohl, 2017). Ungulates, and ruminants in particular, have specialized anatomical and physiological

74 adaptations to accommodate the cellulolytic fermentation of low-nutrition, high-fiber plant materials (De

75 Tarso, Oliviera, & Bastos Alfonso, 2016). A specialized gut microbiome allows ruminants to digest

76 typically indigestible plant biomass (Kruger Ben Shabat et al., 2016) and as a result exploit novel

77 environments. Mountain goats (*Oreamnos americanus*) are large alpine ruminants that are endemic to the

78 mountainous regions of northwestern North America (Festa-Bianchet and Côté 2008). Mountain goats use

79 lower elevation, forested, and warmer aspect habitat during the winter and higher elevation, mountainous

80 terrain in summer (Poole & Heard, 2003; Poole et al., 2009; Taylor et al., 2014; White, 2006). They are

81 considered intermediate browser and eat a variety of forage, with diets generally dominated by grasses

82 (Festa-Bianchet & Côté, 2003; Hofmann, 1989). In contrast, white-tailed deer (*Odocoileus virginianus*)

83 exploit a variety of habitat and food resources and cover a large geographic range that stretches across most

84 of North America and includes part of Central and South America (Hewitt, 2011). White-tailed deer use

85 woody cover habitats year-round, but can also thrive in urban and rural settings (Grund, McAninch, &

86 Wiggers, 2002; Van Deelen, Campa III, Hamady, & Haulfer, 1998); they maintain distinct seasonal ranges

87 in the northern parts of their range and are considered browsing ruminants as well as both habitat and

88 dietary generalists (Berry, Shipley, Long, & Loggers, 2019).

89

90 Our study integrated high-throughput sequencing and GPS telemetry to evaluate the relationship between

91 gut microbiome, home range area, and use of different habitat classes of two ungulates living in contrasting

92 environments. We quantified the relationship between key microbiome diversity metrics on home range

93 size and relative use of different habitat classes inferred from GPS tracking of individuals. From an

94 evolutionary perspective this link between variation in phenotype or behaviour and the gut microbiome

95 assumes selection operates on both the genomes of the constituents (microbiome) and host, otherwise

96 known as the holobiome (Bordenstein & Theis, 2015).We hypothesized that an increase in gut diversity

97   would be linked with an increase in area used, as greater gut diversity would reflect, and possibly drive

98   larger use of space and a more resource-diverse home range, similar to the findings of Ma et al., (2019).

99   High *Firmicutes* to *Bacteroides* ratios correspond to larger body size and fat stores; levels of *Firmicutes*

100 increase and promote more efficient calorie absorption and subsequent weight gain (Duvallet et al., 2017;

101 Koliada et al., 2017; Ley, 2010). As such, we hypothesized that larger *Firmicutes* to *Bacteroides* ratios

102 would be correlated with larger home ranges, as individuals building up fat stores for winter would

103 generally use more space to forage. This relationship may be impacted by resource distribution, as

104 specialists prefer homogenously distributed resources, while generalists prefer heterogeneously distributed

105 resources, which can impact space use (Marrotte et al., 2020) Consequently, we hypothesized that

106 relationships between proportional habitat use and the gut microbiome would be stronger in specialists as

107 they have a more restricted niche with deviations from this having larger consequences, whereas

108 generalists can make use of a variety of habitat areas.

109   **Methods**

110   **Animal captures, sample collection and DNA extraction**

111   We captured and radio-collared male and female mountain goats on three adjacent mountain complexes

112   using aerial net-gun capture northeast of Smithers, British Columbia, Canada (Blunt Mountain, Netalzul

113   Mountain, and Goat Mountain) (Fig. 1). We captured and radio-collared 34 female white-tailed deer

114   (*Odocoileus virginianus*) using baited Clover traps southwest of Ottawa, Ontario, Canada (Fig. 1). For

115   more information on animal captures, see Wolf, Kriss, MacAulay, & Shafer (2020) and Munro (2020).

116   VERTEX Plus and VERTEX Lite Global Positioning System (GPS) collars (VECTRONIC Aerospace,

117   Germany) were used for mountain goats, while store-on-board (G2110D, Advanced Telemetry Solutions,

118   Isanti, MN) or GSM-upload (Wildcell SG, Lotek Wireless Wildlife Monitoring, Newmarket, ON) GPS

119   collars were used for white-tailed deer. Collars recorded locations every four hours for mountain goats and

120   five hours for white-tailed deer. During captures we took fecal pellets from each individual and stored

121   them at -20°C; all captures took place during winter. Lab surfaces were sterilized with 90% EtOH and 10%

122   bleach solution and a small portion of a single fecal sample (~1/4 including exterior and interior portions)

123   was digested overnight at 56°C in 20 ul proteinase K and 180 ul Buffer ATL from the Qiagen DNeasy

124   Blood & Tissue Kit (Qiagen, Valencia, California, USA). DNA was extracted from the digest with the

125   QIAamp PowerFecal DNA Kit (Qiagen, Valencia, California, USA).

126

127   **High-throughput sequencing and bioinformatics**

128   The validated Illumina 16S rRNA Metagenomic Sequencing Library Preparation (#15044223 rev. B)

129   protocol was followed for library preparation using slight modifications (Haworth, White, Côté, & Shafer,

130   2019). The V3 and V4 regions of the 16S ribosomal ribonucleic acid (16S rRNA) hypervariable region

131   were targeted with four variants of 341F and 805R primers designed by Herlemann et al., (2011). A unique

132   combination of Nextera XT indexes, index 1 (i7) and index 2 (i5) adapters were assigned to each sample

133   for multiplexing and pooling. Four replicates of each sample of fecal DNA were amplified in 25 µl PCR

134   using the 341F and 805R primers. The replicated amplicons for each sample were combined into a single

135 reaction of 100 µl and purified using a QIAquick PCR Purification Kit (Qiagen, 28104) and quantified on

136 the Qubit Fluorometer. Sample indexes were annealed to the amplicons using an 8-cycle PCR reaction to

137 produce fragments approximately 630 bp in length that included ligated adaptors; the target amplicon is

138 approximately 430 bp in length (Illumina 16S rRNA Metagenomic Sequencing Library Preparation;

139 #15044223 rev. B). Samples were purified with the QIAquick PCR Purification Kit and the final purified

140 library was validated on a TapeStation (Agilent, G2991AA) and sequenced in 300 bp pair-end reads on an

141 Illumina MiSeq sequencer at the Genomic Facility at The University of Guelph (Guelph, Ontario).

142

143 The quality of the raw sequences was assessed using FastQC v 0.11.9 (Andrews, 2010) and we determined

144 the low-quality cut-off for forward and reverse reads (see Haworth et al., 2019). Forward and reverse reads

145 were imported into QIIME2 v 2019.4 (Boylen et al., 2019) for quality control, sequence classification, and

146 diversity analysis. Merged, forward, and reverse reads were analyzed independently using the quality

147 control function within QIIME2 and DADA2 to perform denoising and detect and remove chimeras.

148 QIIME2 follows the curated DADA2 R library workflow (https://benjjneb.github.io/dada2/) that requires

149 zero mismatches in overlapping reads for successful merging, since reads are denoised and errors are

150 removed before merging occurs. The taxonomy, to the species level, of all sample reads were assigned

151 using Silva 132 reference taxonomy database (https://docs.qiime2.org/2019.4/data-resources/). We

152 calculated the relative proportion of *Firmicutes* to *Bacteroidetes* for each of the grouped data. Estimates of

153 diversity included Shannon's Index, observed Operational Taxonomic Units (OTUs) and Pielou's

154 evenness, a measure of diversity that is the ratio of observed diversity to the maximum possible in a sample

155 having the same number of species (Pielou, 1966); these were screened for correlation to one-another and

156 read-depth. Pielou's evenness and the ratio of *Firmicutes* to *Bacteroidetes* were retained for subsequent

157 analyses. We rarefied sample reads to the sample with the least number of reads.

158 **GPS filtering, home range, and proportional habitat use analysis**

159 We used different filtering approaches and seasonal delineations for each species due to the differences in

160 landscapes occupied by mountain goats and white-tailed deer. For the mountain goat data, any N.A. or

161   mortality signals were filtered out as were any GPS points outside of 600 m-2500 m in elevation, as this

162   reflects the maximum and minimum for the study area. Dilution of precision (DOP) values over 10 were

163   plotted against elevation and landscape type, to ensure there was no patterns in distribution, and that

164   filtering would not bias downstream analyses. Movement rates between successive GPS points were also

165   calculated, and any movement rates beyond 15 km/hr were also removed from analyses as these were

166   deemed spurious. Seasons were defined as follows: Summer - May 1st to October 31st and Winter-

167   December 1st to April 30th (Cadsand, 2012; Mountain Goat Management Team, 2010; Poole & Heard,

168   2003; Richard & Côté, 2016). November was excluded from seasonal data as White (2006) noted a large

169   increase in male mountain goat home ranges due to the rut. In white-tailed deer filtering occurred as above,

170   but with no elevation restrictions as the topography of Marlborough Forest is effectively flat. As

171   considerable variation in migration dates was observed, movement trajectories for each individual deer

172   were examined to identify the dates of migration movements to and from Marlborough Forest to

173   differentiate between winter and summer ranges. A migration movement was defined as when a deer

174   moved between non-overlapping seasonal ranges and then occupied one seasonal range until the following

175   migration movement (Munro 2020). As movements to and from Marlborough forest were relative to each

176   individual deer's movement, there was no hard date range. No seasonal GPS data were excluded for white-

177   tailed deer as changes in movement patterns and home range size during the rut are minimal in females

178   (Hölzenbein & Schwede, 1989).

179

180   We used Brownian Bridge Movement Models (BBMMs) to generate individual home ranges using the

181   BBMM package in R (Nielson, Sawyer, & McDonald 2015). A BBMM is a continuous-time stochastic

182   movement model that uses probabilistic and maximum likelihood approaches where observed locations are

183   measured with error to model home ranges (Horne, Garton, Krone, & Lewis, 2007). A minimum of 275

184   GPS points was required to generate a BBMM and individual home ranges were calculated with a

185   maximum lag time between successive locations of two times the expected fix rate. A location error of 20

186   m was used as per Sawyer et al., (2009), with a cell size of 25 m^2. We generated 50% and 95% isopleths

187   representing the core and home ranges. Isopleths were generated separately for each individual during both

188   summer and winter, as ungulates exhibit sex-specific habitat use patterns, that varies both seasonally and

189   by age-class (Festa-Bianchet & Côté, 2008; Lesage et al., 2000; Mountain Goat Management Team, 2010;

190   Webb, Hewitt, & Hellickson, 2007; White, 2006). We focused on summer isopleths, as deer were baited in

191   winter, which has been shown to bias movement and shift core ranges (Kilpatrick & Stober, 2002). We

192   report only the 50% isopleths, hereafter termed core ranges, to maximize seasonal differences, as they were

193   highly correlated to the 95% isopleths (t_{46}=9.3, r=0.81, p<0.0001) and results were similar between

194   summer 50% and 95% isopleths (Fig. S1). We generated BBMM isopleths using the R package rgdal

195   (Bivand et al., 2019) and all analyses were conducted in R v.3.6.1. Shapefiles were imported into ArcGIS

196   Pro 2.5.0 and home range and core areas were calculated in km^2 for further analyses.

197

198   Proportional use of habitats was assessed by calculating the number of GPS points in a given habitat type

199   within an isopleth, relative to the total number of GPS points located in that isopleth. Similar to Johnson's

200   third-order habitat selection, proportional habitat use in this study refers to how specific habitat types are

201   used within a core range (Johnson, 1980). Proportion values in the 50% isopleths were highly correlated to

202   the 95% isopleths (t_{117}=106, r=0.99, p <2.2e-16, Table S1, S2). We selected ecologically relevant features

203   that showed previous evidence for use in both species. Features used in mountain goat models were treed

204   habitat, Heat Load Index (HLI), and escape terrain (landscape where slope is ≥ 40°; Shafer et al., 2012).

205   These features have shown evidence for disproportionate usage/selection in previous research on mountain

206   goats (Shafer et al., 2012). In the white-tailed deer models, we used forested habitat, treed swamp, and

207   thicket swamp, as each of the three habitat features exhibited >20% core landcover composition in Munro

208   (2020), and thus, were available for usage in the majority of individuals (Massé & Côté, 2013). For the

209   HLI (Mccune & Keon, 2002), the average value of HLI for all GPS points within an isopleth for a given

210   individual was calculated. The Southern Ontario Land Resource Information System (SOLRIS) data set

211   version 2.0 (OMNRF, 2019) was used to determine land cover types for white-tailed deer, while we used

212   the Biogeoclimactic Ecosystem Classification (BEC) dataset (GeoBC, 2019) to determine terrestrial

213   landcover type for mountain goats.

Generalized linear models

215   We analyzed the associations between core range size, gut microbiome metrics, and age class for both

216   species individually using Generalized Linear Models (GLMs) with the Gaussian family distribution and

217   identity link function. The core range GLMs consisted of core range size as a response variable, a single

218   microbiome metric (*Firmicutes* to *Bacteroidetes* ratio or Pielou's evenness) and age class (adult or

219   subadult) as fixed explanatory effects. The proportional habitat use GLMs consisted of proportion of

220   habitat used as a response variable, a single microbiome metric (*Firmicutes* to *Bacteroidetes* ratio or

221   Pielou's evenness) and age class, as fixed explanatory effects. One exception to this was the HLI GLM, as

222   the response variable was the mean HLI value for GPS points in the isopleth, while the explanatory

223   variables were the same as described above. Individuals 0 - 2 years of age were considered subadults for

224   white-tailed deer, while individuals 0 - 3 years of age were considered subadults for mountain goats

225   (Delgiudice, Fieberg, Riggs, Powell, & Pan, 2006; Festa-Bianchet & Côté, 2008). Effect size and

226   confidence intervals are reported for each model. We conducted five-fold linear model cross validation

227   using the Caret package in R (Kuhn et al., 2020) to test for overfitting of our models and quantify the

228   model's predictive ability. We reported the Scatter Index (SI) and Root Mean Square Error (RMSE): low

229   values in RMSE and SI are indicative of a good model fit and low residual variance.

230 **Results**

231 **Bioinformatic filtering and taxonomic analysis**

232 Twenty-three mountain goat and twenty-five white-tailed deer fecal sample sequences passed QC and a

233 total of ~8.17 million paired-end reads (n_{MG}=5,488,856, n_{WTD}=2,679,668) were generated (SRA accession

234 number PRJNA638162). FastQC analysis indicated that both forward and reverse reads lost quality > 259

235 bp in length (Phred score <25), so all reads were trimmed to a length of 259 bp. Following DADA2 strict

236 quality filtering, ~1.16 million paired-end reads (n_{MG}=709,457, n_{WTD}=457,541) were kept for taxonomic

237 and diversity analyses. Losing this many reads to quality filtering is typical (see Haworth et al., 2019), as

238 permitted error rates are extremely low in DADA2, resulting in high certainty among retained reads

239 (Callahan et al., 2016). White-tailed deer had higher averages of both Pielou's evenness (mean 0.95, min

240 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)

241 than mountain goats (Pielou's evenness mean 0.92, min 0.84, max 0.95, SD 0.028; *Firmicutes* to

242 *Bacteroidetes* ratio mean 6.90, min 3.51, max 12.10, SD 2.43). Age class and sex averages, in addition to

243 winter data, are shown in Table S3.

244 **Core range and proportional habitat use**

245 Data filtering resulted in 84,932 GPS points for mountain goats (mean per individual 3,679, range 277 -

246 6,704, SD 761), and 63,900 GPS points for white-tailed deer (mean per individual 2,556, range 831 -

247 3,558, SD 906). One individual mountain goat was removed due to a small number of GPS points (n = 16).

248 Mountain goats exhibited slightly larger summer core range size of 0.40 km^2 (min 0.01 km^2, max 0.72 km^2,

249 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

250 use of habitat values was variable, ranging from 0.05 to 0.91 (means ranged from 0.41 to 0.63) in mountain

251 goats and from 0.00 to 0.84 (means ranged from 0.13-0.34) in white-tailed deer. The mean proportional

252 habitat use values are reported in Table S4.

253 **Generalized linear models**

254 Sex was not included in the final model as there were no strong differences in core range sizes between

255 sexes in mountain goats ($t_{17.58}$=0.374, p>0.05) and all white-tailed deer samples were obtained from

256   females. We compared 50% isopleths from summer and winter; summer core ranges were reported here, as

257   winter core ranges produced qualitatively similar results, albeit with weaker signals (Table S6). In both

258   species, a greater *Firmicutes* to *Bacteroidetes* ratio was associated with larger core ranges with both

259   models explaining an equal amount of variance (Nagelkerkes's $R^2 \sim 0.27$; Table 1; Fig. 2). Mountain goat

260   gut diversity increased with core range size, while a decrease in white-tailed deer was associated with

261   larger core ranges; here the mountain goat model explained a relatively large portion of the variance

262   ($R^2=0.47$; Table 1; Fig. 2). Age-class was a significant predictor in both mountain goat GLMs, but neither

263   of the white-tailed deer models. The use of escape terrain and treed areas were moderately correlated in

264   mountain goats ($t_{20}=2.94$, $p<0.01$, $r=0.55$), and were significant predictors of Pielou's evenness; effect size

265   confidence intervals did not overlap zero in models that measured the relationship between use of escape

266   terrain and treed areas and Pielou's evenness (Table 2). Specifically, a larger Pielou's evenness value was

267   seen in individuals using less treed habitat and less escape terrain (Fig. 3). In HLI GLMs, confidence

268   intervals overlapped zero and exhibited a relative ~2x decrease in R^2 value relative to other mountain goat

269   habitat use models ($\beta= 0.91$, $R^2=0.12$, Table 2). All GLM estimates in the white-tailed deer models had

270   confidence intervals overlapping zero. Cross validation of linear models supported retaining age class and

271   microbiome metric as a predictor variable of core range size. RMSE values in models with core range size

272   as the response variable ranged from 0.12 to 0.18, and SI values ranged from 0.30 to 0.51, whereas in

273   proportional habitat use models, RMSE values ranged from 0.11 to 0.33 and SI values from 0.033 to 1.14

274   (Table S5); this suggests moderate to high support for the models.

275

276   **Discussion**

277   The link between gut microbiome and host space use and has implications for foraging, activity levels, and

278   ability to use energetically costly habitats. We showed that differences in the gut microbiome between a

279   generalist ungulate and specialist ungulate were linked to patterns of habitat use and home range size.

280   Although the patterns are nuanced, there were some commonalities that collectively suggest the gut

281   microbiota plays a role in determining space use patterns. An increase in *Firmicutes* to *Bacteroidetes* ratios

282   in both species was correlated to an increase in core range sizes (Fig. 2). Increased *Firmicutes* to

283   *Bacteroidetes* ratios are linked to increased Body Mass Index (BMI) and obesity (Duvallet et al., 2017).

284   Both *Firmicutes* and *Bacteroidetes* are involved in energy resorption and carbohydrate metabolism;

285   *Firmicutes* can act as a more effective energy source, leading to more efficient calorie absorption and

286   weight gain, while *Bacteroidetes* are involved with energy production and conversion as well as amino

287   acid transport and metabolism (Krajmalnik-Brown, Ilhan, Kang, & DiBaise, 2012; Ottman, Smidt, de Vos,

288   & Belzer, 2012; Turnbaugh et al., 2006). In a comparable study home range size was not correlated with

289   the *Firmicutes* to *Bacteroidetes* ratio in wild rodents (Jameson, Réale, & Kembel, 2020), however,

290   seasonal weight changes are more dynamic in small mammals (Lynch, 1973; Merritt & Zegers, 1991), and

291   we suspect this pattern might be more ubiquitous across temperate ungulates given their specialized gut

292   microbiomes and need to put on fat stores. An individual's gut that is comprised of more *Firmicutes*,

293   bacteria that acts as an effective energy source, may be able to increase body fat more efficiently relative to

294   individuals with lower *Firmicutes* to *Bacteroidetes* ratios, with an increase in *Firmicutes* to *Bacteroidetes*

295   ratios helping individuals to accumulate fat stores to survive the winter by increasing their home range

296   size.

297

298   In large ungulates, increasing levels of body fat is important to survive the winter, when forage is limited

299   relative to the other seasons. In muskoxen (*Ovibos moschatus*), the abundance of *Firmicutes* stayed similar

300   across seasons, while *Bacteroidetes* increased in the summer months, meaning the ratio of *Firmicutes* to

301   *Bacteroidetes* is lower in the summer (Bird et al., 2019). Additionally, an increase in microbial diversity in

302   yak (*Bos grunniens*) was noted in spring (Ma et al., 2020). Increased bacterial diversity in spring relative to

303   summer suggests species are capable of modulating bacterial diversity; but note lower ratios of *Firmicutes*

304   to *Bacteroidetes* does not necessarily correspond to higher diversity. The ability to conserve bacteria

305   necessary for adding fat, namely high levels of *Firmicutes* and low levels of *Bacteroidetes,* while

306   exhibiting increases in gut diversity suggests ungulates can prepare for the winter even though the level of

307   gut bacterial diversity is shifting (see Haworth et al. 2019). This concept is of importance to white-tailed

308   deer, as diet turnover between summer and winter is pronounced in Ontario. Shifting from herbaceous

309   vegetation in the spring to woody browse in the winter may similarly result in increased diversity, while

310   simultaneously conserving bacteria necessary for adding fat.

311

312   Both specialists and generalists tend to shift habitats seasonally, as mountain goats move from alpine

313   summer habitat to subalpine winter areas and white-tailed deer exhibit distinct winter and summer ranges

314   (Grund et al., 2002; Lesage et al., 2000; Poole & Heard, 2003). There was considerably more variation in

315   proportion of habitat used in the generalist white-tailed deer (Table S2), likely a function of their ability to

316   use multiple habitat types and diet sources. Here proportion habitat use-microbiome models had no

317   relationship (Fig. 3, Fig. S1), as all proportion habitat use coefficients in white-tailed deer had confidence

318   intervals overlapping zero (Fig. 3, S1, Table 2). Interestingly, the two habitat variables in mountain goats

319   with clear signals were escape terrain and treed areas (Fig. 3), which are related to the defining habitat

320   characteristics of this species: the use of alpine terrain (i.e. no trees) and steep slopes, (Gross, Kneeland,

321   Reed, & Reich, 2002; Festa-Bianchet & Côté, 2008). Deviations from their specialized and preferred

322   habitat type comes at cost, as ungulates typically exhibit trade-offs with respect to forage quality and

323   predation risk; avoiding predation can lead to decreased forage quality and abundance (Hamel & Côté,

324   2007; Hebblewhite & Merrill, 2009). In mountain goats and bighorn sheep (*Ovis canadensis*), energy

325   expenditures increased when travelling uphill or downhill - termed a vertical cost (Dailey & Hobbs, 1989).

326   A lower *Firmicutes* to *Bacteroidetes* ratio could be linked to the vertical cost associated with spending

327   more time in escape terrain, meaning less-fat or prime conditioned individuals spend more time in escape

328   terrain. Conversely, using more forage-available treed habitat may be correlated with an increase in

329   *Firmicutes* to *Bacteroidetes* ratio, as individuals have increased access to forage, with minimal vertical

330   cost. Collectively, the habitat models had stronger signals in the specialist species compared to the

331   generalist, that we suggest is reflective of their more restricted habitat niche relative to generalists, where

332   microbial deviations generate more prominent shifts in behaviour.

333

334   The potential for the gut microbiome to modulate habitat use patterns is especially important in a specialist,

335   as they have a more restricted niche, where deviations from the usage of important habitat types has larger

336   consequences. While there is potential for modulation of proportion of specific habitat usage, the impact of

337   the gut microbiome on the amount of space used may be more explicit. A lower Pielou's evenness value

338   indicates that a given individual has decreased diversity relative to another individual with the same

339   number of gut microbial species. The relationship between Pielou's evenness and core range size differed

340   in direction between species; greater gut diversity in mountain goats was correlated with a larger core

341   range, while a negative relationship was noted in white-tailed deer (Fig. 2). Increased gut diversity might

342   promote individuals moving through and foraging in larger areas, resulting in a positive relationship

343   between diversity and core range size. This is of added importance in a specialist, as the ability to utilize

344   and forage in a more diverse array of habitat types might allow individuals to access and subsist in areas

345   that other conspecifics cannot, thus increasing their competitive ability (Blake & Karr, 1987; McPeek,

346   1996; Rodewald & Vitz, 2005). Higher relative gut microbiome diversity might allow specialists to forage

347   more like generalists, while lower diversity may limit specialists to their typical niches. The reverse trend

348   in white-tailed deer might reflect the difference in feeding and habitat-use strategies; white-tailed deer are

349   generalists, and thus by definition, all deer can effectively use a variety of habitat and food sources. The

350   negative relationship observed between gut diversity and range size in white-tailed deer might suggest that

351   deer with higher gut microbiome diversity are able to use more diet sources overall and thus can meet their

352   nutritional requirements within a smaller area relative to individuals with less diverse gut microbiomes. As

353   generalists, deer are not as constrained by specific food and habitat types, and thus, their space use may be

354   less heavily influenced by gut microbiome composition. It is possible that winter baiting had an impact on

355   the gut microbiome in white-tailed deer, which could have led to weaker relationships.

356

357   We built our models assuming the microbiome predicts habitat and home range patterns; but this need not

358   be cause and effect, and the relationship is likely one of back-and-forth (Cryan et al., 2019). For example,

359   diet-microbiome covariance has been observed in multiple large-herbivores, and seasonal diet turnover and

360   seasonal microbiome turnover are positively correlated (Kartzinel, Hsing, Musili, Brown, & Pringle, 2019).

361   In this example, our model would assume the gut microbiome impacts diet choice (e.g. Leitão-Gonçalves

362   et al., 2017) and in turn, the correlation between diet turnover and the microbiome drives habitat use of a

363   given species. Assessing this relationship clearly needs experimental testing, and we view our study as a

364   proof-of-concept that provides a testable hypothesis. Still, we demonstrated that quantifying the gut

365   microbiome yields information related to space use and linking these two highly complex components of

366   biology aids in our understanding of selection on the hologenome through the interplay between the

367   individual and its microbial genomes and potential traits under selection (e.g. home range size and

368   proportional habitat use). These findings also demonstrate that using pellet sampling is useful in

369   determining space and habitat use in managed populations, as it is conceivable with a large enough

370   database and validation, one could predict the distribution and behaviour of animals on the landscape from

371   non-invasively sampled pellets. Ultimately, we utilized a specialist and generalist ungulate to explore the

372   link between the gut microbiome and movement to generate quantitative findings surrounding the impact

373   of the gut microbiome on space use in wild populations. Similar analyses of this kind should clarify the

374   extent to which space use is linked to the gut microbiome in other ungulates and in turn, aid in assessing

375   the utility of pellets to predict animal distribution and behaviour.

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605 **Data Accessibility**
606 **Author Contributions**
607
608 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
609 materials and data; J.F.W. analysed the data; and J.F.W. wrote the paper with contributions from all co-
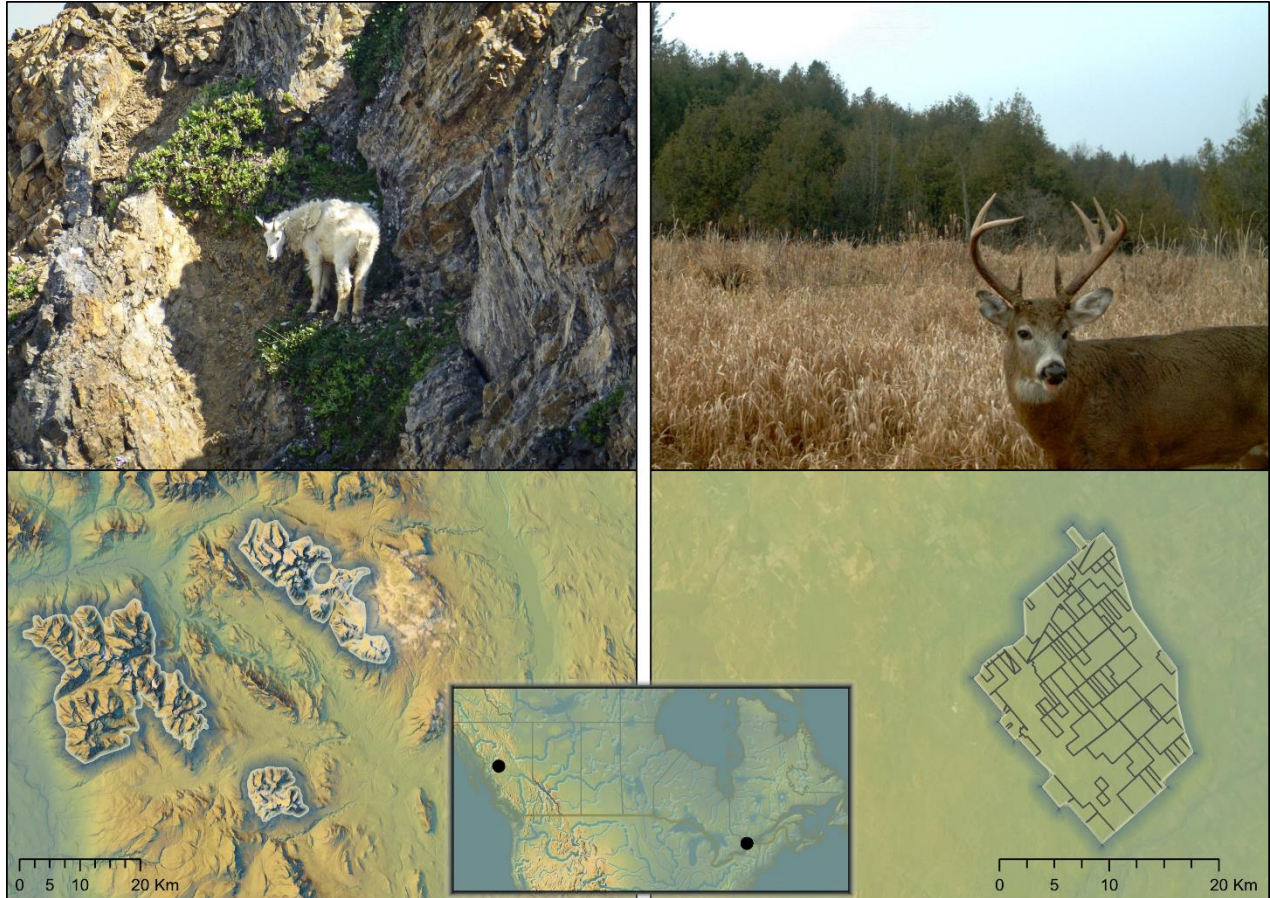610 authors.

611   **Figures**
612

613
614   **Fig. 1** An example of habitat of both mountain goat (*Oreamnos americanus*) and white-tailed deer
615   (*Odocoileus virginianus*) along with inset sample maps. Colors are reflective of relative elevation and
616   topography. Average elevation of mountain goat habitat was 1614.7m, relative to 113.3m for white-tailed
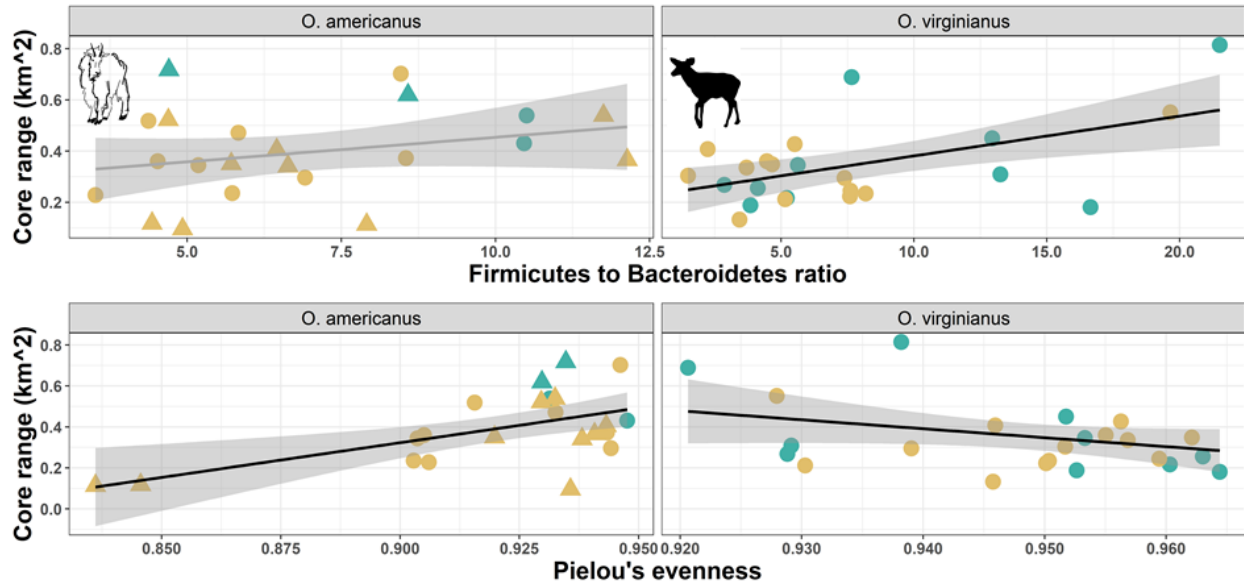617   deer.
618

619
620 **Fig. 2** Microbiome metrics for mountain goats (*Oreamnos americanus*, n=22) northeast of Smithers,
621 British Columbia, and white-tailed deer (*Odocoileus virginianus*, n=25) Southwest of Ottawa, Ontario,
622 relative to core summer range size. Females are represented by circles and only mountain goats have
623 mixed-sex data. Adults (≥3 years of age) are represented in yellow, and subadults in blue. Significant
624 Generalized Linear Models (GLMs) are denoted with a black line.
625

Fig. 3 Microbiome metrics for mountain goats - *Oreamnos americanus* northeast of Smithers, British Columbia, relative to habitat selection coefficients of both Heat Load Index (HLI) and treed habitat (n=21). 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.
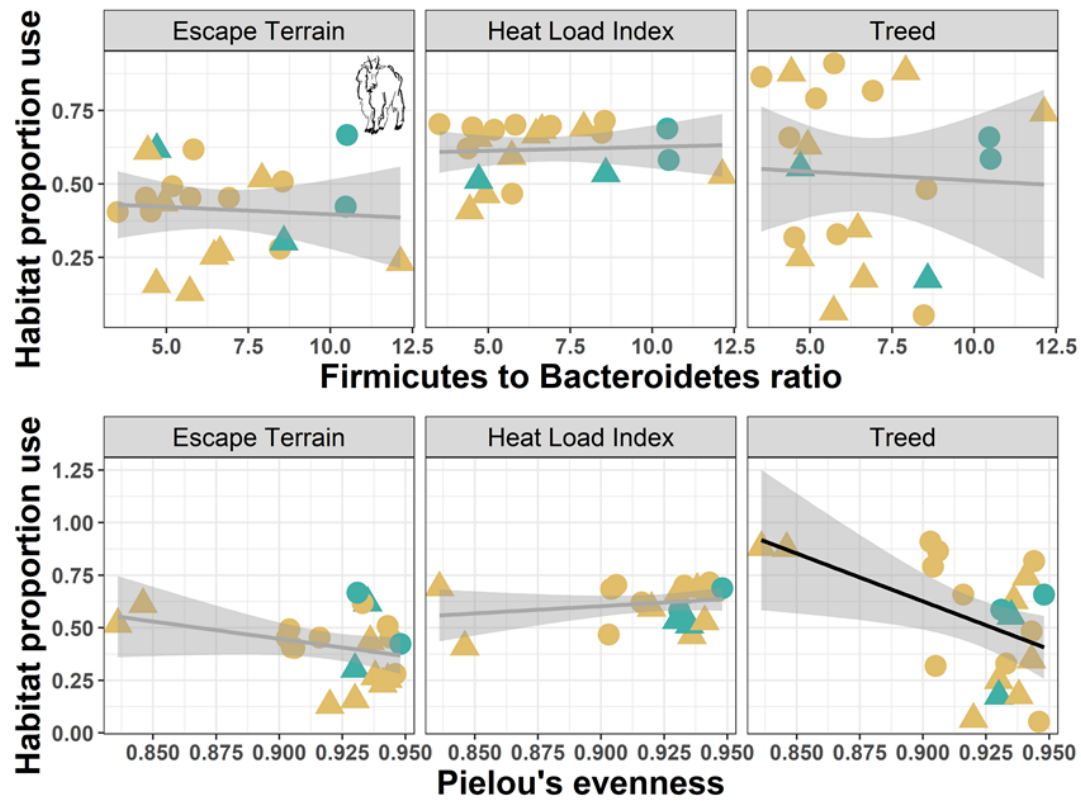
632 **Tables**

633

634 Table 1. Generalized linear models for core summer range size of mountain goat - *Oreamnos americanus*

635 and white-tailed deer - *Odocoileus virginianus* (n_{MG}=22, n_{WTD}=25). The further left column refers to the

636 model where *Firmicutes* to *Bacteroides* ratios was a predictor, while the right column refers to the model

637 where Pielou's evenness was a predictor

638

Mountain goat

Predictors	Estimates	Core range size CI	Estimates	Core range size 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

Predictors	Estimates	Core range size CI	Estimates	Core range size 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

639

640 Table 2. Generalized linear models for habitat proportion use for mountain goats (*Oreamnos americanus)*
641 and white-tailed deer (*Odocoileus virginianus)* (n_{MG}=22, n_{WTD}=25) The further left column refers to the
642 model where *Firmicutes* to *Bacteroides* ratios was a predictor, while the right column refers to the model
643 where Pielou's evenness was a predictor
644

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	

645

	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	

646

White-tailed deer				
	Forest proportion use		**Forest proportion use**	
Predictors	*Estimates*	*CI*	*Estimates*	*CI*

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	

647

	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	

648