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**Spatial variation in diet-microbe associations across populations of a generalist North American carnivore**

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39 **Abstract**

- 40 1. Generalist species, by definition, exhibit variation in niche attributes that promote  
41 survival in changing environments. Increasingly, phenotypes previously associated with a  
42 species, particularly those with wide or expanding ranges, are dissolving and compelling  
43 greater emphasis on population-level characteristics.
- 44 2. In the present study, we assessed spatial variation in diet characteristics, gut microbiome,  
45 and the association between these two ecological traits across populations of coyotes  
46 (*Canis latrans*). We highlight the influence of the carnivore community in shaping these

47 relationships, as the coyote varied from being an apex predator to a subordinate,  
48 mesopredator across sampled populations.

49 3. We implemented a scat survey across three distinct coyote populations in Michigan,  
50 USA. We used carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopic values to reflect consumption  
51 patterns and trophic level, respectively. Corresponding samples were also paired with  
52 16S rRNA sequencing to describe the microbial community and correlate with isotopic  
53 values.

54 4. Though consumption patterns were comparable, we found spatial variation in trophic  
55 level among coyote populations. Specifically,  $\delta^{15}\text{N}$  was highest where coyotes were the  
56 apex predator and lowest where coyotes co-occurred with gray wolves (*Canis lupus*).

57 5. The gut microbial community exhibited marked spatial variation across populations with  
58 the lowest OTU diversity found where coyotes occurred at their lowest trophic level.  
59 *Bacteriodes* and *Fusobacterium* dominated the microbiome and were positively  
60 correlated across all populations. We found no correlation between  $\delta^{13}\text{C}$  and microbial  
61 community attributes. However, positive associations between  $\delta^{15}\text{N}$  and specific  
62 microbial genera increased as coyotes ascended trophic levels.

63 6. Coyotes provide a model for exploring implications of niche plasticity because they are a  
64 highly adaptable, wide-ranging omnivore. As coyotes continue to vary in trophic position  
65 and expand their geographic range, we might expect increased divergence within their  
66 microbial community, changes in physiology, and alterations in behavior.

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## 82 1 | INTRODUCTION

83 Generalist species, by definition, exhibit variation in niche attributes that promote their survival  
84 in changing environments. In North America, coyotes (*Canis latrans*) exist across landscapes as  
85 a widespread, generalist carnivore. They have a high tolerance to human disturbance and few  
86 fixed requirements for survival (Gompper 2002). Furthermore, coyotes and other subordinate  
87 sympatric species have been released from top-down pressures with the extirpation of apex  
88 predators such as gray wolves (*Canis lupus*) and mountain lions (*Puma concolor*) across their  
89 range allowing for their trophic ascension via increased carnivory and nitrogen uptake (Prugh *et*  
90 *al.* 2009; Thornton & Murray 2014). Consequently, the variation in trophic position of coyotes  
91 within their community results in dynamic ecosystem impacts across their range (Crooks &  
92 Soule 1999; Ripple *et al.* 2013). For example, coyotes can affect the abundance of small  
93 mammals (Flagel *et al.* 2017), influence the distribution of zoonotic diseases (Harris & Dunn  
94 2013; Levi *et al.* 2012), and alter the behavior of sympatric carnivores (Flagel *et al.* 2017; Miller  
95 *et al.* 2012; Rich *et al.* 2018).

96 Traits of coyotes are not static. Certain ecosystem characteristics (e.g., climate,  
97 vegetation) induce a myriad of conditions that require flexibility in behavior, physiology,  
98 demography, and ecology. Heterogeneity in habitat use is evident with coyotes occupying a  
99 gradient ranging from heavily urban to forested landscapes (Ellington & Gehrt 2019; Randa &  
100 Yunker 2006). Coyote populations also differ in other behavioral attributes such as sociality and  
101 spatio-temporal activity patterns, which can minimize their risks of intraguild conflict and  
102 enhance resource exploitation (Gese, Morey & Gehrt 2012; Wang, Allen & Wilmers 2015).

103 Because of increasing environmental change and the species' range expansion, traits previously  
104 associated with coyotes as a whole may dissolve and compel greater emphasis on population-  
105 level characteristics. With a wide dietary breadth, consumptive patterns of coyotes vary  
106 seasonally and spatially across populations and habitats (McVey *et al.* 2013; Newsome *et al.*  
107 2015). One method increasingly applied in diet analysis is the use of stable isotopes to assess  
108 trophic structure in food webs, food sources, and niche differentiation (e.g., Brickner *et al.* 2014;  
109 Galetti *et al.* 2016; Layman *et al.* 2007; Manlick *et al.* 2019). The isotopic niche can be  
110 conceptualized as an area in  $\delta$ -space that represents the environmental influences experienced by  
111 a species during the development of various tissues through both consumption and interactions  
112 with habitat (Newsome *et al.* 2007). Consumption patterns and breadth of sampled tissues are  
113 represented with  $\delta^{13}\text{C}$  that ultimately reflects primary production pathways, while  $\delta^{15}\text{N}$  reflects  
114 nitrogen enrichment indicative of increasing trophic position in an individual (Ben-David &  
115 Flaherty 2012; Peterson & Fry 1987). Therefore, stable isotope analyses provide useful insights  
116 to investigate differences in trophic ecology among populations.

117 Diet composition comprising of vertebrates, invertebrates, plant matter, and human foods  
118 by coyotes results in dynamic roles across populations as well as potential variation in their gut  
119 microbial composition throughout their range. For example, if each food item uniquely  
120 contributes microbial colonists and nutrients, a more diverse diet would yield a more diverse gut  
121 microbiome, as recorded in numerous taxa. The emergent pattern results in scaling of microbial  
122 diversity based on guild from herbivores harboring the highest than omnivores followed by  
123 carnivores with the lowest (Ley *et al.* 2008; Youngblut *et al.* 2019). As such, the gut  
124 microbiome can adapt to changes in diet in a manner that is conserved across a wide variety of  
125 mammalian species encompassing carnivores, omnivores, hindgut and foregut fermenting  
126 herbivores, and even humans (David *et al.* 2014; Muegge *et al.* 2011). In addition to the gut  
127 microbiome being governed by diet, community composition of microbes is shaped by  
128 evolutionary history as well as environmental condition such as habitat degradation (Amato *et al.*  
129 2013; Ley *et al.* 2008; Youngblut *et al.* 2019). Closely related species can also exhibit similar  
130 microbial communities (Anderson *et al.* 2012; Brucker & Bordenstein 2012). Other studies  
131 postulate the presence of a 'core' microbiome for a species, describing intrinsic associations for  
132 essential physiological and health functions (Astudillo-Garcia *et al.* 2017; Hamady & Knight  
133 2009; Ley *et al.* 2008). Alternatively, variation in immune competency and infection status can

134 induce trophic differentiation among individuals and populations that influence microbial  
135 attributes (Britton & Andreou 2016). Despite the plethora of comparative studies between  
136 species, fewer studies have assessed differences between wild populations of a species across its  
137 range. Gaining a population-specific understanding of host-microbe associations has  
138 conservation implications for anticipating consequences of environmental change and assessing  
139 vulnerability across a species' range (Kohl *et al.* 2018; Trevelline *et al.* 2019).

140 Here, we assessed the relationship among diet, trophic level, and the gut microbial  
141 community in a spatially explicit manner for coyotes. First, we used carbon and nitrogen isotopic  
142 values to quantify dietary breadth and trophic level across three distinct coyote populations in  
143 Michigan. Then, we inventoried microbial communities for diversity and composition from  
144 associated scat samples. Finally, we evaluated whether dietary breadth and trophic level  
145 correlated with microbial diversity and composition. We expected distinct attributes in both  
146 niche attributes – diet and microbes – among coyote populations. Specifically, we tested the  
147 following predictions in our study: 1) coyote populations will harbor distinct microbial  
148 communities and differ in isotopic signatures from north to south; 2) a more diverse diet and  
149 higher trophic level will correspond to a higher diversity of microbial taxa; and 3) the  
150 relationship between diet and microbes will be maintained despite population-level differences in  
151 the characteristics of each. Because species are nested within complex networks, assessing  
152 population-level variation can have broader implications for understanding drivers of niche  
153 plasticity and evolutionary capacity to assess vulnerabilities to changing environments.

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## 155 **2 | MATERIALS AND METHODS**

### 156 **2.1 | Study areas**

157 We conducted fieldwork for this study in three different areas throughout the state of Michigan,  
158 USA. From north to south (Figure 1): Huron Mountain Club (HMC) in the Upper Peninsula and  
159 two Lower Peninsula sites - University of Michigan Biological Station (UMBS) and Shiawassee  
160 National Wildlife Refuge (SNWR). We selected these areas based on differences in habitat and  
161 climate conditions using latitude as a proxy, levels of anthropogenic disturbance, and differences  
162 in carnivore communities. At our most pristine site, HMC (46.8486° N, 87.7999° W) comprises

163 approximately 5,260 ha of privately-owned mixed secondary and old growth forest. The club is  
164 situated along the southern coast of Lake Superior in Marquette County with ~14  
165 inhabitants/km<sup>2</sup>. UMBS (45.55984° N, 84.71382° W) encompasses approximately 4,000 ha of  
166 mixed deciduous and coniferous forest surrounding Douglas Lake in the northern tip of  
167 Michigan's Lower Peninsula. The station is located east of the town of Pellston in Emmett  
168 County with ~ 27 inhabitants/km<sup>2</sup>. Finally, SNWR (43.3377° N, 84.0273° W), managed by the  
169 U.S. Fish & Wildlife Service, consists of approximately 3,965 ha of marsh, bottomland  
170 hardwood forest, and grasslands. It neighbors the city of Saginaw in Saginaw County with ~ 91  
171 inhabitants/km<sup>2</sup> and fringes both urban development and agriculture.

## 172 **2.2 | Sample collection**

173 We conducted systematic scat surveys from May-August 2016, collecting all carnivore feces  
174 found across our three study areas. Surveys occurred monthly for 7-10 days, utilizing hiking  
175 paths and wildlife trails, and usually included all available habitat types within the study area.  
176 We made concerted efforts during fieldwork to collect mostly "fresh" scat and collected from  
177 interior region to reduce contamination with collection tools cleaned with ethanol between  
178 samples. Opportunistic findings by other researchers, volunteers, and park rangers presumed to  
179 be carnivore were also included. Samples were stored in two of three liquid preservation  
180 solutions: DET, RNAlater or EtOH in 10ml sterile plastic vials as well as dry in sterile bags.  
181 However, nearly 3/4 of samples used for analysis were preserved in EtOH. All samples were  
182 stored at -20°C upon returning from the field until processed. Wet samples were used for  
183 molecular host confirmation and microbial community analysis while dry samples were  
184 processed for stable isotope analysis.

## 185 **2.3 | Molecular confirmation**

186 To make any inferences about niche attributes of the diet or microbiome of coyotes across  
187 populations, we had to first confirm collected scat samples as coyote. We began species  
188 identification by extracting DNA from each potential sample using Qiagen™ QIAamp DNA  
189 stool kits (Qiagen, LaJolla, CA) to yield approximately 200 µl of DNA extract. DNA was then  
190 amplified through PCR using two different primers for mtDNA target regions: a canid-specific  
191 primer amplifying 533 base pairs (Rashleigh, Krebs & van Keulen 2008); and a Carnivora-  
192 specific primer amplifying 126 base pairs (Chaves *et al.* 2012). Samples selected for gene

193 sequencing were submitted to the University of Michigan DNA Sequencing Core for Sanger  
194 sequencing. We analyzed chromatograms for each sequence on the Sequencher and Bioedit  
195 platforms, and high-quality sequences were compared to those of known species submitted to  
196 NCBI Nucleotide BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Samples that matched known  
197 sequences on the NCBI database with 90% or higher query cover and 98% or higher identity  
198 were confirmed as coyote.

## 199 **2.4 | Stable isotope analysis**

200 Confirmed coyote samples were oven dried at 50°C for 48 hours. Dried scat samples were then  
201 gently broken apart over a 0.355 mm fine-mesh sieve (Hogentogler, Columbia, MD) to obtain  
202 only the scat matrix, excluding poorly digested dietary components (e.g., feathers, bones, fur)  
203 that may disproportionately impact isotope values (Reid & Koch 2017). All samples were then  
204 placed into 1.5 ml tubes with 3 – 5 metal beads and homogenized at 6 m/s for 5 minutes. We  
205 then weighed all samples (3 – 4 mg for scat) and created two replicates of each sample, sealed  
206 samples into 5x9 mm tin capsules, and submitted them to University of New Mexico Center for  
207 Stable Isotopes to be analyzed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. Carbon and nitrogen isotope ratios were  
208 measured by Elemental Analyzer Continuous Flow Isotope Ratio Mass Spectrometry using a  
209 Costech ECS 4010 Elemental Analyzer coupled to a ThermoFisher Scientific Delta V Advantage  
210 mass spectrometer via a CONFLO IV interface. Isotope ratios are reported using the standard  
211 delta ( $\delta$ ) notation relative to V-AIR and to Vienna Pee Dee Belemnite (V-PDB). Average  
212 analytical precision was better than 0.1‰ (1s) for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  based on routine analysis  
213 of laboratory standards. The laboratory standards were calibrated against IAEA N1, IAEA N2  
214 and USGS 43 for  $\delta^{15}\text{N}$  and NBS 21, NBS 22 and USGS 24 for  $\delta^{13}\text{C}$ .

## 215 **2.5 | Microbial ecology**

216 We investigated variation in the gut microbial community of coyote by submitting extracted  
217 DNA to the Center for Microbial Systems at University of Michigan. The V4 region of the 16s  
218 rRNA gene was amplified using a Dual indexing sequencing strategy on the Illumina MiSeq  
219 platform. Sequence data were analyzed following a modified standard operating procedure  
220 outlined by Kozich *et al.* (2013). After trimming and aligning sequences, we screened for  
221 chimeras using UCHIME, and then applied a naïve Bayesian classifier to assign each sequence  
222 taxonomic identity against the Ribosomal Database Project (RDP) 16s rRNA gene training set



223 using an 80% pseudo-bootstrap confidence score (<https://rdp.cme.msu.edu/>). Any sequences that  
224 were classified as chloroplasts, mitochondria, and unknown were culled; sequences that  
225 remained were classified as *Archaea*, *Eukaryota*, or *Bacteria*. For quality control, we compared  
226 sequence data to a mock community to measure the error rate and its effect on our analysis.  
227 Afterwards, we organized remaining sequences into groups based on taxonomy and then  
228 assigned them to operational taxonomic units (OTUs) at a 3% dissimilarity level, which has the  
229 advantage of parallelization and reduced memory usage, while shown to be equivalent to not  
230 splitting sequences by taxonomic order (He *et al.* 2015; Kozich *et al.* 2013).

## 231 **2.6 | Statistical Analysis**

232 We used Kruskal-Wallis tests to examine potential differences in both the average stable carbon  
233 and nitrogen values among populations. We then used Dunn's test with the Bonferroni correction  
234 to determine which populations of coyotes were statistically different from one another at  $\alpha =$   
235 0.05 significance level. Finally, we applied a Brown-Forsythe test for both stable carbon and  
236 nitrogen values to explore how the variance of our measurements may differ across populations.  
237 We evaluated variation in microbial diversity using inverse Simpson index on total OTUs across  
238 populations. We used the Bray-Curtis measure of dissimilarity to calculate distances of 1073  
239 sequences per sample with a principal coordinate analysis (PCoA) to compare community  
240 structure. We used permutational multivariate analysis of variance (PERMANOVA) tests to  
241 determine whether the coyote microbiome differed significantly in community structure among  
242 the three populations, pooling sequences for every sample by site for comparison. We also  
243 calculated the average distance for samples to within group median and did not find strong  
244 evidence for group dispersion using a 0.05 significance level ( $F=2.91$ ,  $P\text{-value} = 0.062$ ). We  
245 used a Bonferroni correction to adjust p-values for pairwise population comparisons of  
246 community similarity. We focused our investigation of relative abundance on the most abundant  
247 taxa as OTUs representing at least 25% of reads in at least one sample. We used Pearson's  $R$   
248 correlation to assess the relationship between microbial and dietary attributes across populations.  
249 All statistical analyses were performed in Mothur software (v. 1.39.5) as well as 'vegan', and  
250 'phyloseq' packages in R (v 3.5.1; R Core Team, 2018).

251

## 252 **3 | RESULTS**

253 We collected a total of 357 carnivore scat samples across our study areas and confirmed 58  
254 samples through molecular analyses as coyote for subsequent dietary and microbial analysis  
255 (HMC: n=26; UMBS: n=13; SNWR: n=19).

256 Trophic position was significantly different across populations (Figure 2; Kruskal-Wallis  
257 test;  $\chi^2 = 37.68, p < 0.001$ ). Specifically, coyotes ascended in trophic level moving south with the  
258 highest and most divergent  $\delta^{15}\text{N}$  values at SNWR, as predicted. Though UMBS and HMC  
259 average  $\delta^{15}\text{N}$  values were comparable (Dunn's test:  $z = -2.132, p = 0.049$ ), these populations  
260 both differed significantly from SNWR (to UMBS:  $z = 3.061, p = 0.003$ ; to HMC:  $z = -6.137, p <$   
261  $0.001$ ). Contrary to expectations,  $\delta^{13}\text{C}$  values were comparable across populations ( $\chi^2 = 3.87, p =$   
262  $0.144$ ). The variance of both measured  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values were not significantly different  
263 across coyote populations (Brown-Forsythe test;  $\delta^{15}\text{N}$ :  $t = 0.000, p = 0.999$ ;  $\delta^{13}\text{C}$ :  $t = 1.919, p =$   
264  $0.156$ ).

265 We recorded 507 OTUs with on average 79 unique OTUs (range: 29-232) across our  
266 samples. Specifically, we detected on average 6,642 amplicons within each sample (range:  
267 1,073-16,449). Alpha diversity of OTUs varied significantly among populations and was lowest  
268 at HMC, our northern-most site where coyotes occupied their lowest trophic level, as expected  
269 (Figure 3a,  $\chi^2 = 6.339, p = 0.043$ ). Of the 373 OTUs identified to the genus level, *Bacteroides*  
270 and *Fusobacteria* dominated the microbiota of coyotes and were positively correlated across all  
271 populations (Figure 3b; Figure 4, correlations range: 0.70-0.78). Furthermore, these were the  
272 only two dominant genera that occurred at each site; although, *Bacteroides* differed significantly  
273 across populations and was double to two-thirds more abundant at HMC ( $\chi^2 = 8.472, p = 0.015$ ).

274 We found coyotes from different populations in distinct geographic locations harbored  
275 distinct gut microbial communities based on PCoA clustering, as expected (PERMANOVA:  $R =$   
276  $0.094, p = 0.003$ , Figure 3c). At a finer resolution with pairwise comparisons, HMC clustered  
277 differently from the Lower Peninsula populations (to SNWR:  $R = 0.07, p = 0.027$ ; to UMBS:  $R =$   
278  $0.10, p = 0.015$ ). However, a comparison between the two populations in the Lower Peninsula,  
279 SNWR-UMBS, did not indicate a significant difference in clustering from a PCoA ( $R = 0.04, p$   
280  $= 0.819$ ), contrary to expectations.

281 Clear divergence was evident between consumption patterns and trophic level in relation  
282 to microbial communities across coyote populations. Contrary to our predictions, we did not find  
283 evidence of an association between dietary breadth (i.e.,  $\delta^{13}\text{C}$ ) or C:N ratios and the gut  
284 microbiome of coyotes in any population, as there was no positive or negative correlation  
285 (Figure 4). However, the relative abundance of specific microbial genera was positively  
286 correlated with trophic level, as indexed by  $\delta^{15}\text{N}$ . This association only manifested as coyotes  
287 ascended in trophic position within their respective communities (e.g., Lower Peninsula  
288 populations: UMBS, SNWR). At HMC, where coyotes had significantly lower  $\delta^{15}\text{N}$  values, we  
289 found no correlation to microbial diversity or the relative abundance of specific genera (Figure  
290 4). *Sphingobacterium* showed a strong positive association with  $\delta^{15}\text{N}$  in the Lower Peninsula  
291 populations with the relationship strengthening where coyotes were the apex predator in the  
292 system (Correlations: UMBS- 0.60; SNWR- 0.72). Additionally, we observed a significant  
293 positive correlation between  $\delta^{15}\text{N}$  and *Flavobacterium* (0.59), a genus that only occurred at  
294 SNWR.

295

#### 296 4 | DISCUSSION

297 Understanding spatial variation in niche attributes across a species' range is fundamental to  
298 understanding community structure and mechanisms of coexistence as well as anticipating  
299 vulnerabilities to changing environments (Manlick *et al.* 2019; Mazel *et al.* 2017; Slatyer, Hirst  
300 & Sexton 2013). We evaluated the population-level variation of diet and the gut microbiome of  
301 coyote across a gradient of landscape use and competitor diversity. We found that the  
302 consumption patterns of these populations, reflected in  $\delta^{13}\text{C}$  values, did not differ meaningfully,  
303 while differences were evident in trophic niche (i.e., differences in  $\delta^{15}\text{N}$  values).  $\delta^{15}\text{N}$  increases  
304 approximately 3 – 4‰ per trophic level (Ben-David & Flaherty 2012; Peterson & Fry 1987) with  
305 omnivores exhibiting higher trophic signatures than herbivores (e.g., Steffan *et al.* 2019).  
306 Coyotes ascended to a higher trophic level, having enriched nitrogen values, as larger carnivores  
307 such as gray wolves and mountain lions were extirpated along the north-south gradient of our  
308 study. Consistent with expectation of mesopredator release (Crooks & Soule 1999), we observed  
309 the highest  $\delta^{15}\text{N}$  values at our southern-most site (SNWR), where coyotes serve as apex  
310 predators. Our work presents a novel consideration of how trophic cascades could induce

311 ecological shifts that affect the affiliate microbiome to expand our understanding of  
312 mesopredator release (Estes *et al.* 2011; Ripple *et al.* 2016).

313 Variations in trophic position may be attributed to alterations in resource use or habitat  
314 conditions (e.g., Codron *et al.* 2015; Harris, Garshong & Gray 2018; Resasco *et al.* 2018), which  
315 may vary due to differences in resource availability across the landscape and the ability of a  
316 consumer to access those resources. Additionally, the presence of dominant competitors can  
317 influence the habitat use and feeding behavior of coyotes, subsequently altering nitrogen uptake  
318 (Flagel *et al.* 2017; Merkle, Stahler & Smith 2009). We observed the lowest trophic level and  
319 narrowest dietary breadth at HMC, our northern-most site where coyotes co-occur with gray  
320 wolves. Such sympatry may cause the suppression of subordinate coyotes, forcing individuals to  
321 alter their consumption patterns and switch to alternate food sources. Coyote x wolf hybrids are  
322 known to exist in the Lower Peninsula of Michigan at the UMBS site (Wheeldon, Patterson &  
323 Beyer 2012). These individuals may exert similar top-down pressures on resident coyotes and  
324 potentially alter their trophic interactions.

325 Enrichment along the  $\delta^{13}\text{C}$  axis reflects a shift from  $\text{C}_3$  to  $\text{C}_4$  plant biomass in a  
326 consumer's diet (Ben-David & Flaherty 2012), which can represent a greater reliance on  
327 anthropogenic food sources such as corn and corn-based products (Jahren & Kraft 2008).  
328 Previous works highlight that coyotes in urban areas exhibit wider dietary niche and often exploit  
329 less animal protein (Murray *et al.* 2015; Newsome *et al.* 2015). Consumption patterns of all three  
330 coyote populations indicate a comparable primary production base reliant on  $\text{C}_3$  plant  
331 production, despite our southern-most site (SNWR) having high human disturbance and farming  
332 activity. This may reflect the ability of SNWR coyotes to select preferred prey sources due to the  
333 absence of top-down pressures from larger and more dominant competitor species. The variance  
334 among two of three populations were relatively low, suggesting narrow consumption patterns  
335 that contrast expectations for dietary generalists (Bearhop *et al.* 2004).

336 We detected significant variation in the microbial community among coyote populations,  
337 particularly between the populations in the geographically distinct Lower and Upper Peninsula of  
338 Michigan. Observed differences in trophic position correlated with differences within the gut  
339 microbiome across coyote populations. Similarly, other studies report spatial variation of the  
340 microbiome within wild populations for groups separated by as few as 15 km to greater than

341 1000 km (Gomez *et al.* 2015; Kohl *et al.* 2018). Our findings are particularly notable because  
342 most studies on spatial variation in microbes focus on herbivores, while the omnivorous diet and  
343 trophic variability of coyote represent a greater opportunity for diet-driven microbial plasticity.  
344 The reduction in  $\delta^{15}\text{N}$  values, representing a lower trophic position, do not correlate with  
345 microbial diversity or any other microbial attribute at the HMC site, suggesting a constrained  
346 niche in the presence of large carnivores. We postulate this indicates that a higher trophic  
347 position may promote stronger correlations to microbial attributes (e.g., prevalence, diversity).

348 Fundamentally, coyotes provide a model for exploring niche plasticity and consequences  
349 of environmental change because they are a highly adaptable, wide-ranging omnivore. With the  
350 continued expansion of urbanization and changing climates, exploring this coupling in a  
351 comparative framework to assess implications for consumption and disease dynamics in coyotes  
352 would yield insights into eco-evolutionary processes (Alberti 2015). As coyotes vary in trophic  
353 position across an expanding geographic range, the distinctiveness of their functional traits may  
354 promote increasingly disparate populations and ecological interactions (Bolnick *et al.* 2011). We  
355 might expect increased divergence within their microbial community, changes in physiology,  
356 and alterations in behavior. Furthermore, the observed correlations between diet and the  
357 microbial community may help assign a health status across populations (Trevelline *et al.* 2019).  
358 For example, a high prevalence of Gram-positive Actinobacteria known for its role in producing  
359 biological metabolite could potentially be an immune response to an increase of Proteobacteria  
360 associated with a wide array of pathogens (Ghoul & Mitri 2016). Therefore, identifying  
361 correlations between microbial taxa, driven in part by trophic position and diet, can aid in  
362 assessing vulnerabilities to prioritize conservation efforts and anticipating consequences of  
363 community dynamics induced from anthropogenic pressures at the population level for a species.  
364 Overall, our work elucidating spatial variation in foraging ecology, trophic level, and microbial  
365 affiliates for the coyote further underscores the significance of comparative studies in animal  
366 ecology.

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377 analysis.

#### 378 AUTHOR CONTRIBUTIONS

379 ASC and CCK completed molecular analysis for host confirmations. ASC, CCK, and NCH  
380 wrote the paper. ASC and GIG led sample processing to obtain stable isotopic values, and ASC  
381 completed stable isotope analyses. CCK prepared microbial data. NCH designed and supervised  
382 the study, and completed microbial analysis. All authors contributed to fieldwork for sample  
383 collection and edited the manuscript.

#### 384 DATA ACCESSIBILITY

385 Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.cnp5hqc2h>  
386 (Colborn *et al.* 2020)

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553 **Figure Legends**

554 **FIGURE 1** – Study areas across the state of Michigan, USA. from north – south: Huron Mountain  
555 Club (HMC), University of Michigan Biological Station (UMBS), and Shiawassee National  
556 Wildlife Refuge (SNWR). Animal silhouettes depict the competitor species at each site and \*  
557 indicates known presence of coyote x wolf hybrids.

558 **FIGURE 2** – Mean values and standard error bars of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values from coyote scat  
559 samples across study areas from north to south in Michigan, USA.

560 **FIGURE 3** – Site comparison of microbial community attributes from coyote scat samples across  
561 study areas from north to south in Michigan, USA. a) Boxplot of microbial diversity calculated  
562 from inverse Simpson index ( $\chi^2 = 6.271$ ,  $p = 0.043$ ). b) Community composition of most  
563 abundant genera. c) Principal coordinates analysis of coyote microbiome compositional  
564 differences using Bray-Curtis distances. Populations listed in ascending order in relation to  
565 coyote trophic position within each community (HMC to SNWR)

566 **FIGURE 4**– Correlation matrix between dietary and microbial attributes assessed across coyote  
567 populations from north to south in Michigan, USA. Cells containing “X” indicate non-significant  
568 relationships based on significance level 0.05 from Pearson’s  $R$ .

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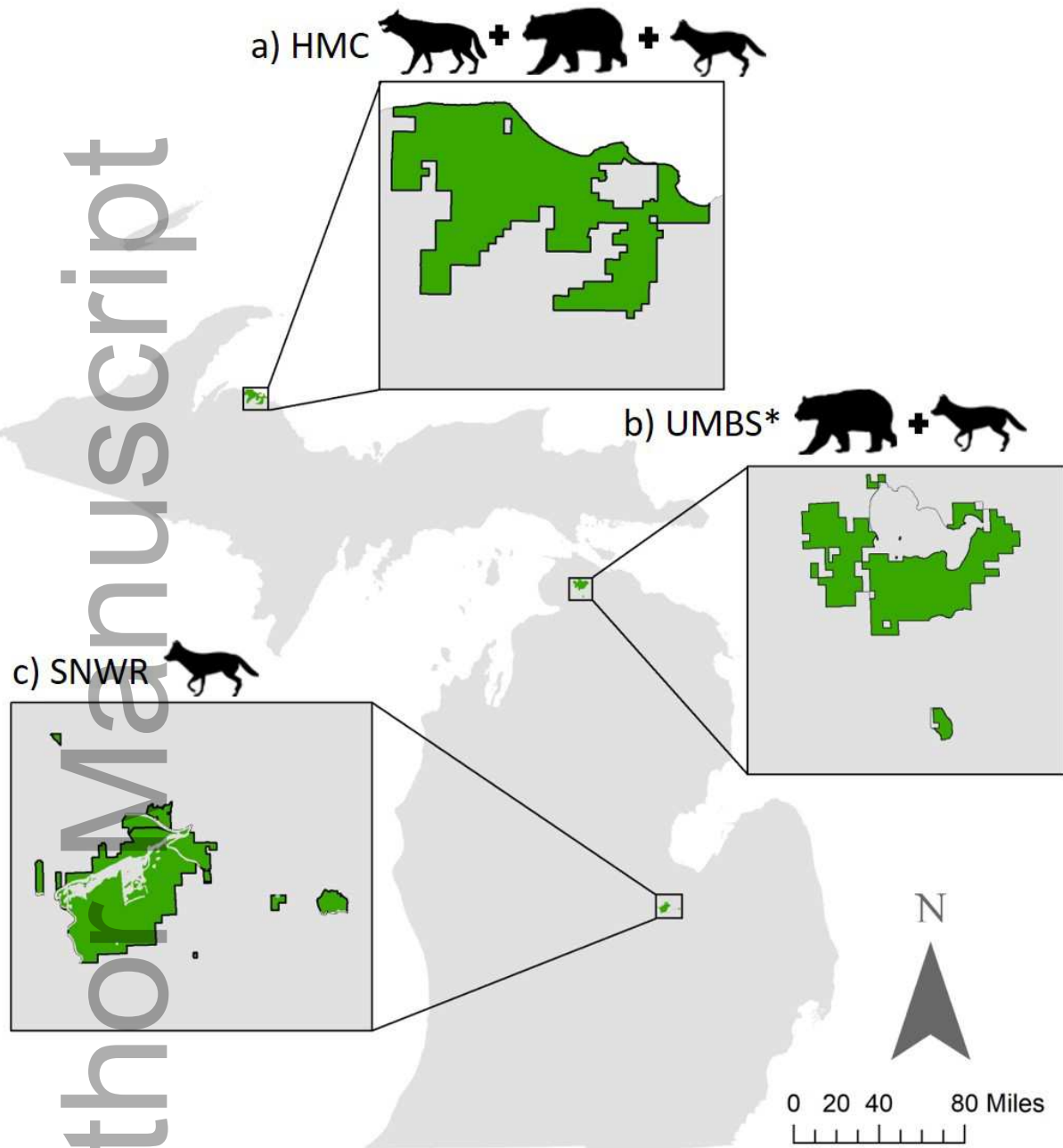
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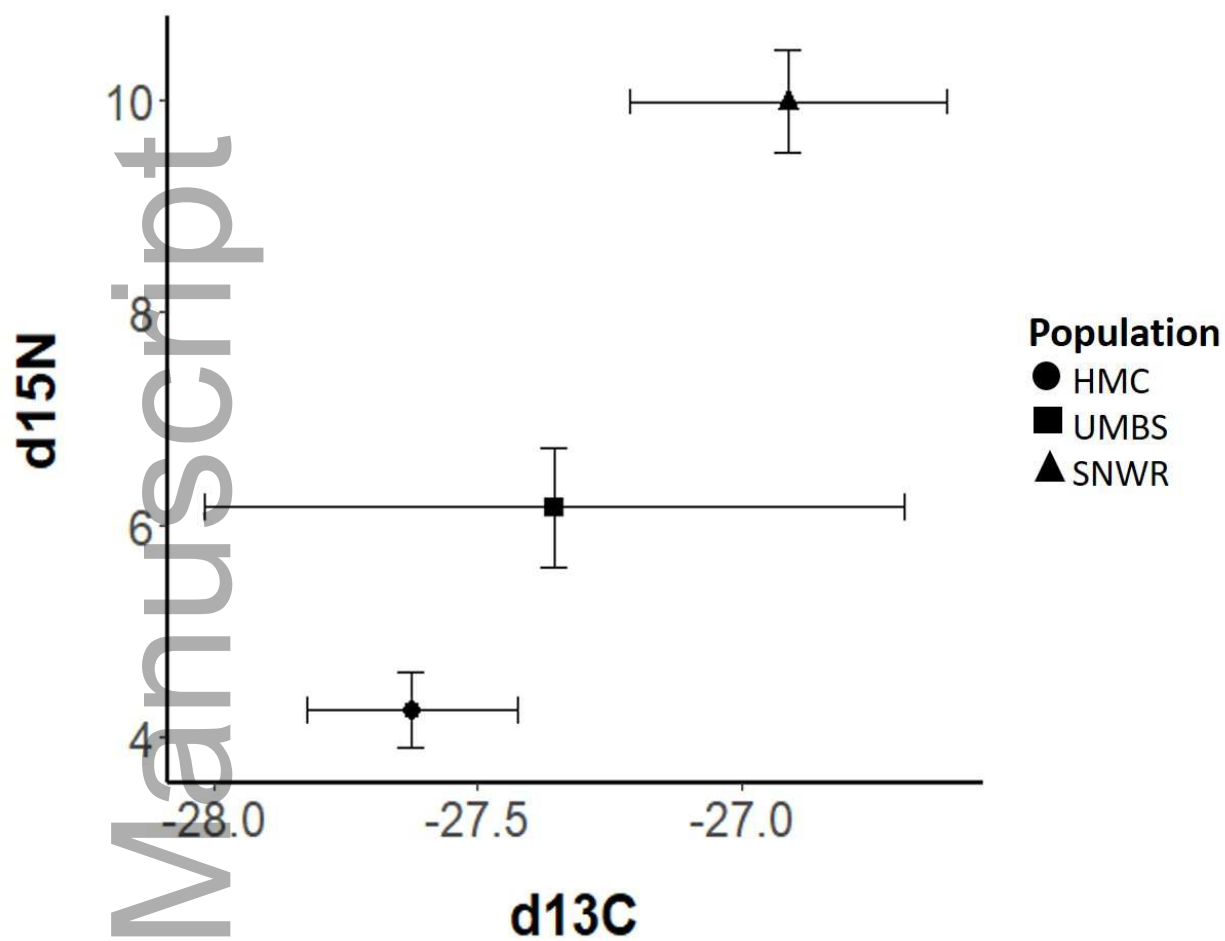
580 **FIGURE 1**

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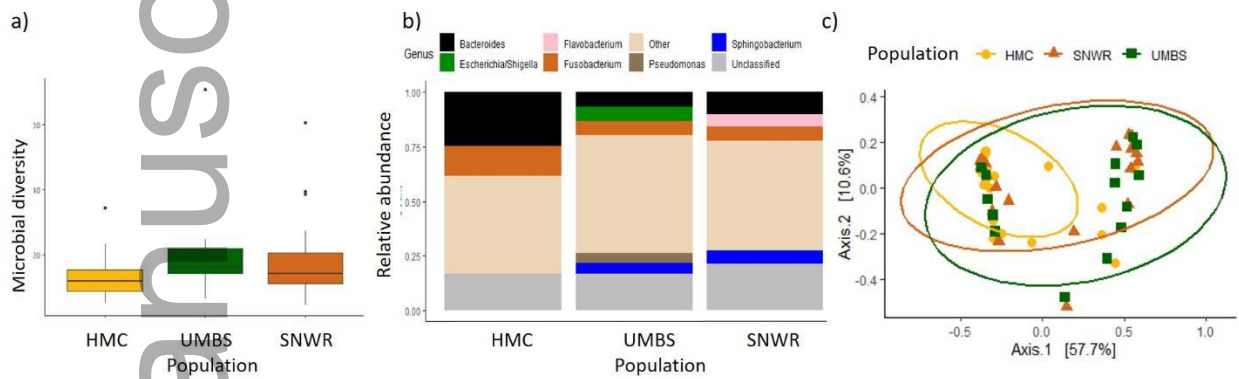
590 **FIGURE 2**



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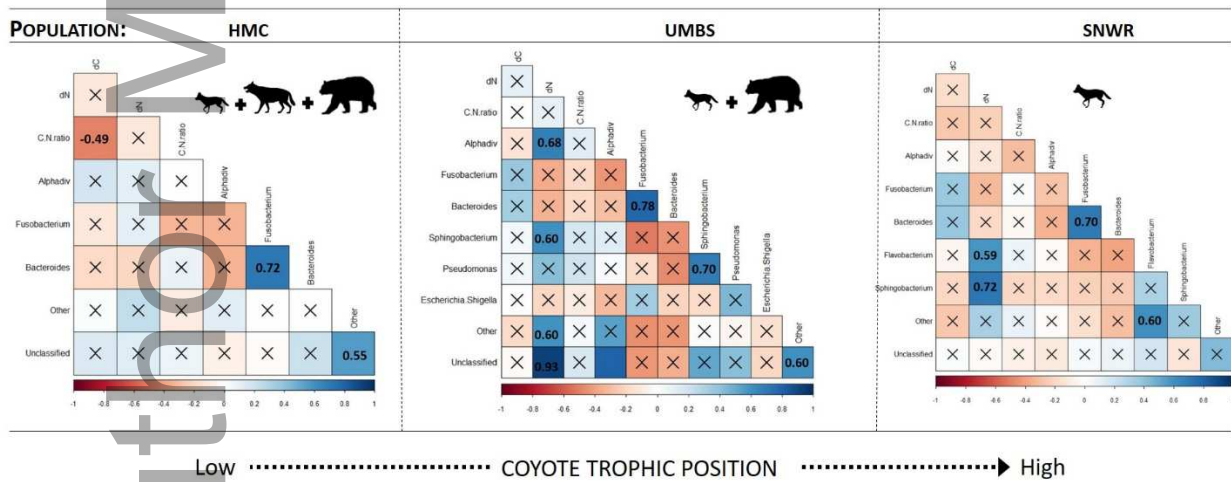
FIGURE 3



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FIGURE 4



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