

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

A. Shawn Colborn | Corbin C. Kuntze | Gabriel I. Gadsden | Nyeema C. Harris 

Applied Wildlife Ecology Lab, Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA

Correspondence

Nyeema C. Harris
Email: nyeema@umich.edu

Present address

Corbin C. Kuntze, Department of Forest & Wildlife Ecology, University of Wisconsin, Madison, WI 53706, USA

Gabriel I. Gadsden, Urban Energy Justice Lab, School For Environment and Sustainability, University of Michigan, 440 Church St, Ann Arbor, MI 48108, USA

Funding information

Huron Mountain Wildlife Foundation; Detroit Zoological Society; University of Michigan Biological Station

Handling Editor: Bethany Hoye

Abstract

1. Generalist species, by definition, exhibit variation in niche attributes that promote survival in changing environments. Increasingly, phenotypes previously associated with a species, particularly those with wide or expanding ranges, are dissolving and compelling greater emphasis on population-level characteristics.
2. In the present study, we assessed spatial variation in diet characteristics, gut microbiome and associations between these two ecological traits across populations of coyotes *Canis latrans*. We highlight the influence of the carnivore community in shaping these relationships, as the coyote varied from being an apex predator to a subordinate, mesopredator across sampled populations.
3. We implemented a scat survey across three distinct coyote populations in Michigan, USA. We used carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopic values to reflect consumption patterns and trophic level, respectively. Corresponding samples were also paired with 16S rRNA sequencing to describe the microbial community and correlate with isotopic values.
4. Although consumption patterns were comparable, we found spatial variation in trophic level among coyote populations. Specifically, $\delta^{15}\text{N}$ was highest where coyotes were the apex predator and lowest where coyotes co-occurred with grey wolves *Canis lupus*.
5. The gut microbial community exhibited marked spatial variation across populations with the lowest operational taxonomic units diversity found where coyotes occurred at their lowest trophic level. *Bacteriodes* and *Fusobacterium* dominated the microbiome and were positively correlated across all populations. We found no correlation between $\delta^{13}\text{C}$ and microbial community attributes. However, positive associations between $\delta^{15}\text{N}$ and specific microbial genera increased as coyotes ascended trophic levels.
6. Coyotes provide a model for exploring implications of niche plasticity because they are a highly adaptable, wide-ranging omnivore. As coyotes continue to vary in trophic position and expand their geographic range, we might expect increased divergence within their microbial community, changes in physiology and alterations in behaviour.

KEYWORDS

bacteria, *Canis latrans*, Michigan, niche, nitrogen, stable isotope, trophic

1 | INTRODUCTION

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

Traits of coyotes are not static. Certain ecosystem characteristics (e.g. climate, vegetation) induce a myriad of conditions that require flexibility in behaviour, physiology, demography and ecology. Heterogeneity in habitat use is evident with coyotes occupying a gradient ranging from heavily urban to forested landscapes (Ellington & Gehrt, 2019; Randa & Yunker, 2006). Coyote populations also differ in other behavioural attributes such as sociality and spatio-temporal activity patterns, which can minimize their risks of intraguild conflict and enhance resource exploitation (Gese, Morey, & Gehrt, 2012; Wang, Allen, & Wilmers, 2015). Because of increasing environmental change and the species' range expansion, traits previously associated with coyotes as a whole may dissolve and compel greater emphasis on population-level characteristics. With a wide dietary breadth, consumptive patterns of coyotes vary seasonally and spatially across populations and habitats (McVey et al., 2013; Newsome, Garbe, Wilson, & Gehrt, 2015). One method increasingly applied in diet analysis is the use of stable isotopes to assess trophic structure in food webs, food sources and niche differentiation (e.g. Brickner, Grenier, Crosier, & Pauli, 2014; Galetti, Rodarte, Neves, Moreira, & Costa-Pereira, 2016; Layman, Arrington, Montaña, & Post, 2007; Manlick, Petersen, Moriarty, & Pauli, 2019). The isotopic niche can be conceptualized as an area in δ -space that represents the environmental influences experienced by a species during the development of various tissues through both consumption and interactions with habitat (Newsome, del Rio, Bearhop, & Phillips, 2007). Consumption patterns and breadth of sampled tissues are represented with $\delta^{13}\text{C}$ that ultimately reflects primary production pathways, while $\delta^{15}\text{N}$ reflects nitrogen enrichment indicative of increasing trophic position in an individual (Ben-David & Flaherty, 2012; Peterson & Fry, 1987). Therefore, stable isotope analyses provide useful insights to investigate differences in trophic ecology among populations.

Diet composition comprising of vertebrates, invertebrates, plant matter and human foods by coyotes results in dynamic roles across populations as well as potential variation in their gut microbial composition throughout their range. For example, if each food item uniquely contributes microbial colonists and nutrients, a more diverse diet would yield a more diverse gut microbiome, as recorded in numerous taxa. The emergent pattern results in scaling of microbial diversity based on guild from herbivores harbouring the highest than omnivores followed by carnivores with the lowest (Ley et al., 2008; Youngblut et al., 2019). As such, the gut microbiome can adapt to changes in diet in a manner that is conserved across a wide variety of mammalian species encompassing carnivores, omnivores, hindgut- and foregut-fermenting herbivores and even humans (David et al., 2014; Muegge et al., 2011). In addition to the gut microbiome being governed by diet, community composition of microbes is shaped by evolutionary history as well as environmental condition such as habitat degradation (Amato et al., 2013; Ley et al., 2008; Youngblut et al., 2019). Closely related species can also exhibit similar microbial communities (Anderson et al., 2012; Brucker & Bordenstein, 2012). Other studies postulate the presence of a 'core' microbiome for a species, describing intrinsic associations for essential physiological and health functions (Astudillo-Garcia et al., 2017; Hamady & Knight, 2009; Ley et al., 2008). Alternatively, variation in immune competency and infection status can induce trophic differentiation among individuals and populations that influence microbial attributes (Britton & Andreou, 2016). Despite the plethora of comparative studies between species, fewer studies have assessed differences between wild populations of a species across its range. Gaining a population-specific understanding of host-microbe associations has conservation implications for anticipating consequences of environmental change and assessing vulnerability across a species' range (Kohl, Varner, Wilkening, & Dearing, 2018; Trevelline, Fontaine, Hartup, & Kohl, 2019).

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

2 | MATERIALS AND METHODS

2.1 | Study areas

We conducted fieldwork for this study in three different areas throughout the state of Michigan, USA. From north to south (Figure 1): Huron Mountain Club (HMC) in the Upper Peninsula and two Lower Peninsula sites—University of Michigan Biological Station (UMBS) and Shiawassee National Wildlife Refuge (SNWR). We selected these areas based on differences in habitat and climate conditions using latitude as a proxy, levels of anthropogenic disturbance and differences in carnivore communities. At our most pristine site, HMC (46.8486°N, 87.7999°W) comprises approximately 5,260 ha of privately owned mixed secondary and old growth forest. The club is situated along the southern coast of Lake Superior in Marquette County with ~14 inhabitants/km². UMBS (45.55984°N, 84.71382°W) encompasses approximately 4,000 ha of mixed deciduous and coniferous forest surrounding Douglas Lake in the northern tip of Michigan's Lower Peninsula. The station is located east of the town of Pellston in Emmett County with ~27 inhabitants/km². Finally, SNWR (43.3377°N, 84.0273°W) managed by the U.S. Fish & Wildlife Service, consists of approximately 3,965 ha of marsh, bottomland hardwood forest and grasslands. It neighbours the city of

Saginaw in Saginaw County with ~91 inhabitants/km², and fringes both urban development and agriculture.

2.2 | Sample collection

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

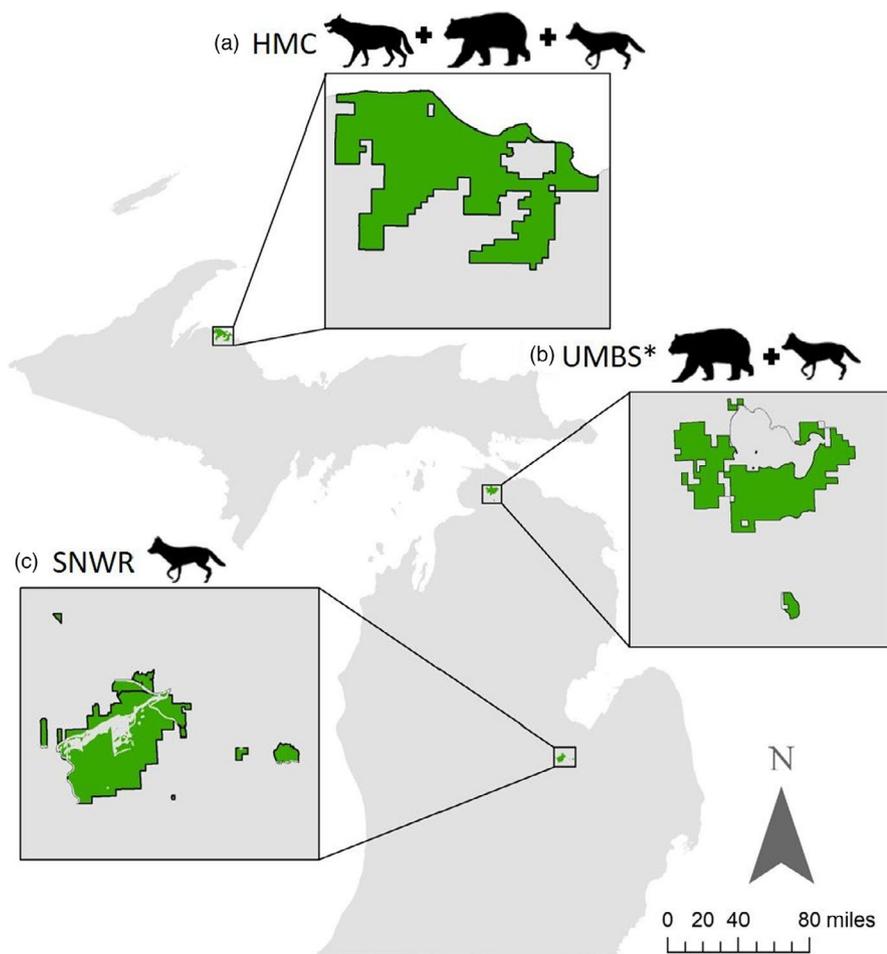


FIGURE 1 Study areas across the state of Michigan, USA: (a) Huron Mountain Club (HMC), (b) University of Michigan Biological Station (UMBS) and (c) Shiawassee National Wildlife Refuge (SNWR). Animal silhouettes depict the competitor species at each site and *indicates known presence of coyote × wolf hybrids

2.3 | Molecular confirmation

To make any inferences about niche attributes of the diet or microbiome of coyotes across populations, we had to first confirm collected scat samples as coyote. We began species identification by extracting DNA from each potential sample using Qiagen™ QIAamp DNA stool kits (Qiagen) to yield approximately 200 µl of DNA extract. DNA was then amplified through PCR using two different primers for mtDNA target regions: a canid-specific primer amplifying 533 base pairs (Rashleigh, Krebs, & van Keulen, 2008); and a Carnivora-specific primer amplifying 126 base pairs (Chaves, Graeff, Lion, Oliveira, & Eizirik, 2012). Samples selected for gene sequencing were submitted to the University of Michigan DNA Sequencing Core for Sanger sequencing. We analysed chromatograms for each sequence on the Sequencher and Bioedit platforms, and high-quality sequences were compared to those of known species submitted to NCBI Nucleotide BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Samples that matched known sequences on the NCBI database with 90% or higher query cover and 98% or higher identity were confirmed as coyote.

2.4 | Stable isotope analysis

Confirmed coyote samples were oven-dried at 50°C for 48 hr. Dried scat samples were then gently broken apart over a 0.355 mm fine-mesh sieve (Hogentogler) to obtain only the scat matrix, excluding poorly digested dietary components (e.g. feathers, bones, fur) that may disproportionately impact isotope values (Reid & Koch, 2017). All samples were then placed into 1.5 ml tubes with 3–5 metal beads and homogenized at 6 m/s for 5 min. We then weighed all samples (3–4 mg for scat) and created two replicates of each sample, sealed samples into 5 × 9 mm tin capsules and submitted them to University of New Mexico Center for Stable Isotopes to be analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Carbon and nitrogen isotope ratios were measured by Elemental Analyser Continuous Flow Isotope Ratio Mass Spectrometry using a Costech ECS 4010 Elemental Analyser coupled to a ThermoFisher Scientific Delta V Advantage mass spectrometer via a CONFLO IV interface. Isotopic ratios are reported using the standard delta (δ) notation relative to V-AIR and to Vienna Pee Dee Belemnite (V-PDB). Average analytical precision was better than 0.1‰ (1s) for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ based on routine analysis of laboratory standards. The laboratory standards were calibrated against IAEA N1, IAEA N2 and USGS 43 for $\delta^{15}\text{N}$ and NBS 21, NBS 22 and USGS 24 for $\delta^{13}\text{C}$.

2.5 | Microbial ecology

We investigated variation in the gut microbial community of coyotes by submitting extracted DNA to the Center for Microbial Systems at University of Michigan. The V4 region of the 16s rRNA gene was amplified using a Dual indexing sequencing strategy on the Illumina

MiSeq platform. Sequence data were analysed following a modified standard operating procedure outlined by Kozich, Westcott, Baxter, Highlander, and Schloss (2013). After trimming and aligning sequences, we screened for chimeras using UCHIME, and then applied a naïve Bayesian classifier to assign each sequence taxonomic identity against the Ribosomal Database Project (RDP) 16s rRNA gene training set using an 80% pseudo-bootstrap confidence score (<https://rdp.cme.msu.edu/>). Any sequences that were classified as chloroplasts, mitochondria and unknown were culled; sequences that remained were classified as *Archaea*, *Eukaryota* or *Bacteria*. For quality control, we compared sequence data to a mock community to measure the error rate and its effect on our analysis. Afterwards, we organized remaining sequences into groups based on taxonomy and then assigned them to operational taxonomic units (OTUs) at a 3% dissimilarity level, which has the advantage of parallelization and reduced memory usage, while shown to be equivalent to not splitting sequences by taxonomic order (He et al., 2015; Kozich et al., 2013).

2.6 | Statistical analysis

We used Kruskal–Wallis tests to examine potential differences in both the average stable carbon and nitrogen values among populations. We then used Dunn's test with the Bonferroni correction to determine which populations of coyotes were statistically different from one another at $\alpha = 0.05$ significance level. Finally, we applied a Brown–Forsythe test for both stable carbon and nitrogen values to explore how the variance of our measurements may differ across populations. We evaluated variation in microbial diversity using inverse Simpson index on total OTUs across populations. We used the Bray–Curtis measure of dissimilarity to calculate distances of 1,073 sequences per sample with a principal coordinate analysis (PCoA) to compare community structure. We used PERMANOVA tests to determine whether the coyote microbiome differed significantly in community structure among the three populations, pooling sequences for every sample by site for comparison. We also calculated the average distance for samples to within group median and did not find strong evidence for group dispersion using a 0.05 significance level ($F = 2.91$, $p = 0.062$). We used a Bonferroni correction to adjust p -values for pairwise population comparisons of community similarity. We focused our investigation of relative abundance on the most abundant taxa as OTUs representing at least 25% of reads in at least one sample. We used Pearson's R correlation to assess the relationship between microbial and dietary attributes across populations. All statistical analyses were performed in Mothur software (v. 1.39.5) as well as VEGAN, and PHYLOSEQ packages in R (v 3.5.1).

3 | RESULTS

We collected a total of 357 carnivore scat samples across our study areas and confirmed 58 samples through molecular analyses as

coyote for subsequent dietary and microbial analyses (HMC: $n = 26$; UMBS: $n = 13$; SNWR: $n = 19$).

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

We recorded 507 OTUs with on average 79 unique OTUs (range: 29–232) across our samples. Specifically, we detected on average

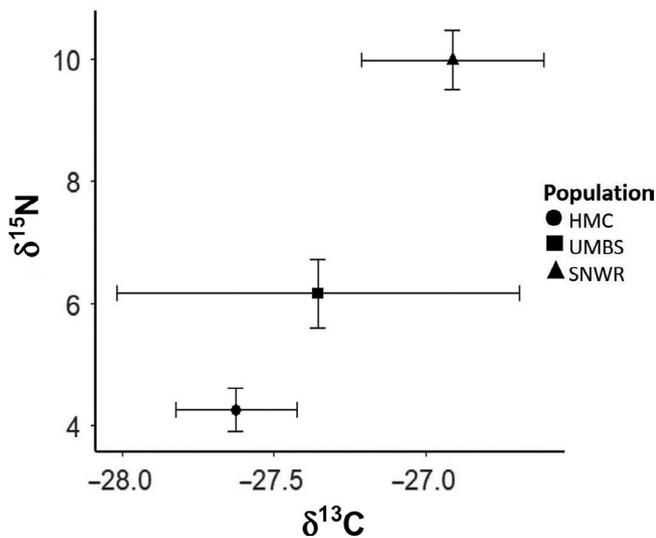


FIGURE 2 Mean values and standard error bars of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values from coyote scat samples across study areas from north to south in Michigan, USA

6,642 amplicons within each sample (range: 1,073–16,449). Alpha diversity of OTUs varied significantly among populations and was lowest at HMC, our northernmost site where coyotes occupied their lowest trophic level, as expected (Figure 3a, $\chi^2 = 6.339$, $p = 0.043$). Of the 373 OTUs identified to the genus level, *Bacteroides* and *Fusobacterium* dominated the microbiota of coyotes and were positively correlated across all populations (Figures 3b and 4, correlations range: 0.70–0.78). Furthermore, these were the only two dominant genera that occurred at each site, although *Bacteroides* differed significantly across populations and was double to two-thirds more abundant at HMC ($\chi^2 = 8.472$, $p = 0.015$).

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

Clear divergence was evident between consumption patterns and trophic level in relation to microbial communities across coyote populations. Contrary to our predictions, we did not find evidence of an association between dietary breadth (i.e. $\delta^{13}\text{C}$) or C:N ratios and the gut microbiome of coyotes in any population, as there were no positive or negative correlations (Figure 4). However, the relative abundance of specific microbial genera was positively correlated with trophic level, as indexed by $\delta^{15}\text{N}$. This association only manifested as coyotes ascended in trophic position within their respective communities (e.g. Lower Peninsula populations: UMBS, SNWR). At HMC, where coyotes had significantly lower $\delta^{15}\text{N}$ values, we found no correlation to microbial diversity or the relative abundance of specific genera (Figure 4). *Spingobacterium* showed a strong positive association with $\delta^{15}\text{N}$ in the Lower Peninsula populations with the relationship strengthening where coyotes were the apex predator in the system (Correlations: UMBS–0.60; SNWR–0.72).

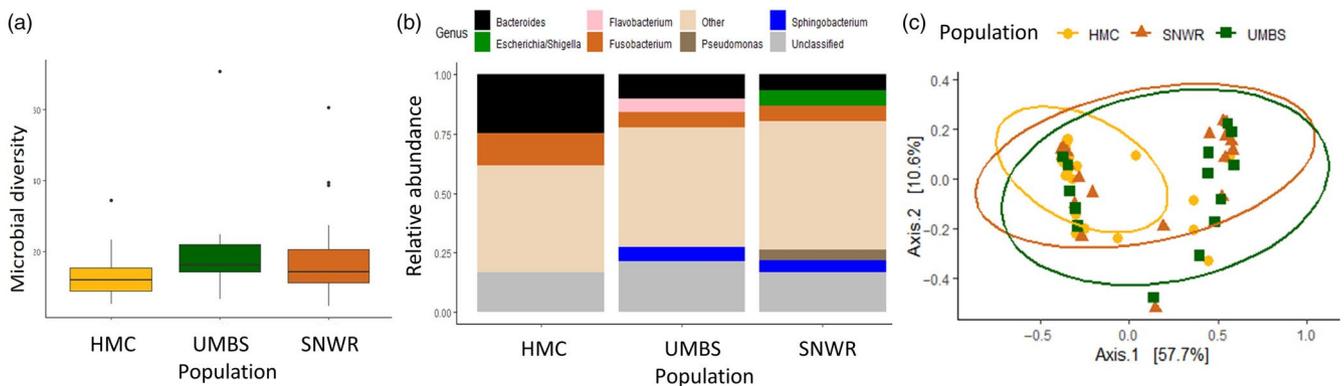


FIGURE 3 Site comparison of microbial community attributes from coyote scat samples across study areas from north to south in Michigan, USA. (a) Boxplot of microbial diversity calculated from inverse Simpson index ($\chi^2 = 6.271$, $p = 0.043$). (b) Community composition of most abundant genera. (c)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

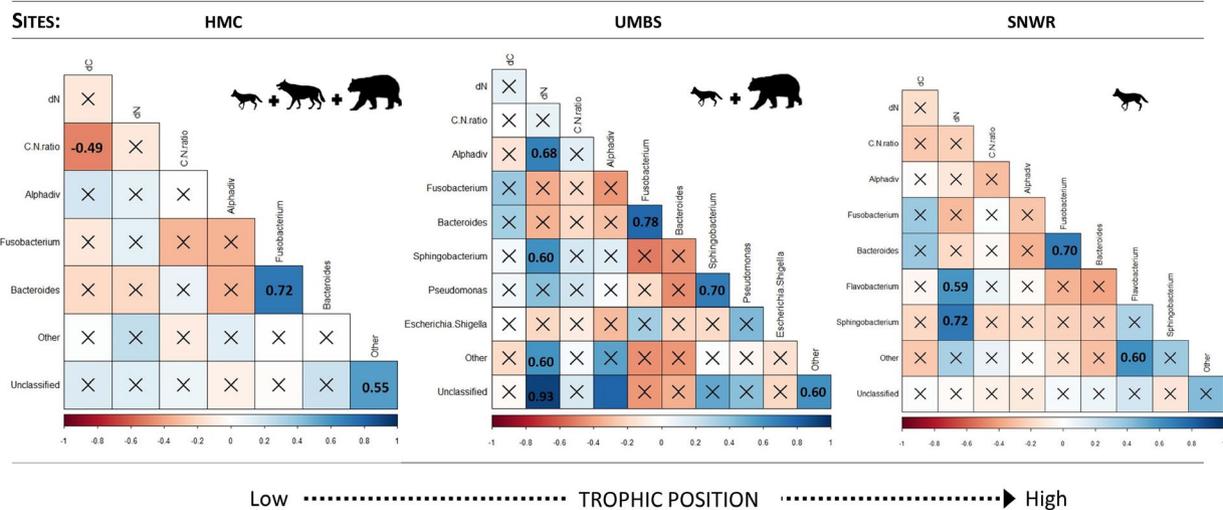


FIGURE 4 Correlation matrix between dietary and microbial attributes assessed across coyote populations from north to south in Michigan, USA. Cells containing 'X' indicate non-significant relationships based on significance level 0.05 from Pearson's R

Additionally, we observed a significant positive correlation between $\delta^{15}\text{N}$ and *Flavobacterium* (0.59), a genus that only occurred at SNWR.

4 | DISCUSSION

Understanding spatial variation in niche attributes across a species' range is fundamental to understanding community structure and mechanisms of coexistence as well as anticipating vulnerabilities to changing environments (Manlick et al., 2019; Mazel et al., 2017; Slatyer, Hirst, & Sexton, 2013). We evaluated the population-level variation of diet and the gut microbiome of coyotes across a gradient of landscape use and competitor diversity. We found that the consumption patterns of these populations, reflected in $\delta^{13}\text{C}$ values, did not differ meaningfully, while differences were evident in trophic niche (i.e. differences in $\delta^{15}\text{N}$ values). $\delta^{15}\text{N}$ increases approximately 3–4‰ per trophic level (Ben-David & Flaherty, 2012; Peterson & Fry, 1987) with omnivores exhibiting higher trophic signatures than herbivores (e.g. Steffan et al., 2019). Coyotes ascended to a higher trophic level, having enriched nitrogen values, as larger carnivores such as grey wolves and mountain lions were extirpated along the north–south gradient of our study. Consistent with expectation of mesopredator release (Crooks & Soule, 1999), we observed the highest $\delta^{15}\text{N}$ values at our southernmost site (SNWR), where coyotes serve as apex predators. Our work presents a novel consideration of how trophic cascades could induce ecological shifts that affect the affiliate microbiome to expand our understanding of mesopredator release (Estes et al., 2011; Ripple et al., 2016).

Variations in trophic position may be attributed to alterations in resource use or habitat conditions (e.g. Codron et al., 2015; Harris, Garshong, & Gray, 2018; Resasco et al., 2018), which may vary due to differences in resource availability across the landscape and the ability of a consumer to access those resources. Additionally, the

presence of dominant competitors can influence the habitat use and feeding behaviour of coyotes, subsequently altering nitrogen uptake (Flagel et al., 2017; Merkle, Stahler, & Smith, 2009). We observed the lowest trophic level and narrowest dietary breadth at HMC, our northern-most site where coyotes co-occur with grey wolves. Such sympatry may cause the suppression of subordinate coyotes, forcing individuals to alter their consumption patterns and switch to alternate food sources. Coyote \times wolf hybrids are known to exist in the Lower Peninsula of Michigan at the UMBS site (Wheeldon, Patterson, & Beyer, 2012). These individuals may exert similar top-down pressures on resident coyotes and potentially alter their trophic interactions.

Enrichment along the $\delta^{13}\text{C}$ axis reflects a shift from C_3 to C_4 plant biomass in a consumer's diet (Ben-David & Flaherty, 2012), which can represent a greater reliance on anthropogenic food sources such as corn and corn-based products (Jahren & Kraft, 2008). Previous works highlight that coyotes in urban areas exhibit wider dietary niche and often exploit less animal protein (Murray et al., 2015; Newsome et al., 2015). Consumption patterns of all three coyote populations indicate a comparable primary production base reliant on C_3 plant production, despite our southern-most site (SNWR) having high human disturbance and farming activity. This may reflect the ability of SNWR coyotes to select preferred prey sources due to the absence of top-down pressures from larger and more dominant competitor species. The variance among two of three populations were relatively low, suggesting narrow consumption patterns that contrast expectations for dietary generalists (Bearhop, Adams, Waldron, Fuller, & Macleod, 2004).

We detected significant variation in the microbial community among coyote populations, particularly between the populations in the geographically distinct Lower and Upper Peninsula of Michigan. Observed differences in trophic position correlated with differences within the gut microbiome across coyote populations. Similarly, other studies report spatial variation of the microbiome

within wild populations for groups separated by as few as 15 km to >1,000 km (Gomez et al., 2015; Kohl et al., 2018). Our findings are particularly notable because most studies on spatial variation in microbes focus on herbivores, while the omnivorous diet and trophic variability of coyotes represent a greater opportunity for diet-driven microbial plasticity. The reduction in $\delta^{15}\text{N}$ values, representing a lower trophic position, do not correlate with microbial diversity or any other microbial attribute at the HMC site, suggesting a constrained niche in the presence of large carnivores. We postulate this indicates that stronger correlations among microbial attributes (e.g. prevalence, diversity) may emerge for individuals at higher trophic levels.

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

ACKNOWLEDGEMENTS

First, we recognize implementing our field research of scat collection was conducted on lands originally belonging to the People of the Three Fires. We thank Huron Mountain Wildlife Foundation, University of Michigan Biological Station and Shiawassee National Wildlife Refuge for their generous support and access to property to conduct fieldwork. We remain grateful to members, past and present, in the Applied Wildlife Ecology (AWE) Lab for assistance with fieldwork and morale. We thank M. Clayson and R. Rivadeneira for assistance with genetic work, and University of New Mexico Center for Stable Isotopes for processing scat samples. This research was supported in part by the University of Michigan Medical School Host Microbiome Initiative, and we thank the Center for Microbial Systems for their assistance in microbiome analysis.

AUTHORS' CONTRIBUTIONS

A.S.C. and C.C.K. completed molecular analysis for host confirmations; A.S.C., C.C.K. and N.C.H. wrote the paper; A.S.C. and G.I.G. led sample processing to obtain stable isotopic values and A.S.C. completed stable isotope analyses; C.C.K. prepared microbial data; N.C.H. designed and supervised the study and completed microbial analysis. All authors contributed to fieldwork for sample collection and edited the manuscript.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.cnp5hqc2h> (Colborn, Kuntze, Gadsden, & Harris, 2020).

ORCID

Nyeema C. Harris  <https://orcid.org/0000-0001-5174-2205>

REFERENCES

- Alberti, M. (2015). Eco-evolutionary dynamics in an urbanizing planet. *Trends in Ecology & Evolution*, 30, 114–126. <https://doi.org/10.1016/j.tree.2014.11.007>
- Amato, K. R., Yeoman, C. J., Kent, A., Righini, N., Carbonero, F., Estrada, A., ... Leigh, S. R. (2013). Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. *The ISME Journal*, 7, 1344–1353. Retrieved from <http://www.nature.com/ismej/journal/vaop/ncurrent/supinfo/ismej201316s1.html>
- Anderson, K. E., Russell, J. A., Moreau, C. S., Kautz, S., Sullam, K. E., Hu, Y. I., ... Wheeler, D. E. (2012). Highly similar microbial communities are shared among related and trophically similar ant species. *Molecular Ecology*, 21, 2282–2296. <https://doi.org/10.1111/j.1365-294X.2011.05464.x>
- Astudillo-Garcia, C., Bell, J. J., Webster, N. S., Glasl, B., Jompa, J., Montoya, J. M., & Taylor, M. W. (2017). Evaluating the core microbiota in complex communities: A systematic investigation. *Environmental Microbiology*, 19, 1450–1462. <https://doi.org/10.1111/1462-2920.13647>
- Bearhop, S., Adams, C. E., Waldron, S., Fuller, R. A., & Macleod, H. (2004). Determining trophic niche width: A novel approach using stable isotope analysis. *Journal of Animal Ecology*, 73, 1007–1012. <https://doi.org/10.1111/j.0021-8790.2004.00861.x>
- Ben-David, M., & Flaherty, E. A. (2012). Stable isotopes in mammalian research: A beginner's guide. *Journal of Mammalogy*, 93, 312–328. <https://doi.org/10.1644/11-MAMM-S-166.1>
- Bolnick, D. I., Amarasekare, P., Araújo, M. S., Bürger, R., Levine, J. M., Novak, M., ... Vasseur, D. A. (2011). Why intraspecific trait variation matters in community ecology. *Trends in Ecology & Evolution*, 26, 183–192. <https://doi.org/10.1016/j.tree.2011.01.009>
- Brickner, K. M., Grenier, M. B., Crosier, A. E., & Pauli, J. N. (2014). Foraging plasticity in a highly specialized carnivore, the endangered black-footed ferret. *Biological Conservation*, 169, 1–5. <https://doi.org/10.1016/j.biocon.2013.10.010>
- Britton, J. R., & Andreou, D. (2016). Parasitism as a driver of trophic niche specialisation. *Trends in Parasitology*, 32, 437–445. <https://doi.org/10.1016/j.pt.2016.02.007>
- Brucker, R. M., & Bordenstein, S. R. (2012). Speciation by symbiosis. *Trends in Ecology & Evolution*, 27, 443–451. <https://doi.org/10.1016/j.tree.2012.03.011>
- Chaves, P. B., Graeff, V. G., Lion, M. B., Oliveira, L. R., & Eizirik, E. (2012). DNA barcoding meets molecular scatology: Short mtDNA sequences for standardized species assignment of carnivore noninvasive samples. *Molecular Ecology Resources*, 12, 18–35. <https://doi.org/10.1111/j.1755-0998.2011.03056.x>

- Codron, J., Duffy, K. J., Avenant, N. L., Sponheimer, M., Leichliter, J., Paine, O., ... Codron, D. (2015). Stable isotope evidence for trophic niche partitioning in a South African savanna rodent community. *Current Zoology*, *61*, 397–411. <https://doi.org/10.1093/czoolo/61.3.397>
- Colborn, A. S., Kuntze, C. C., Gadsden, G. I., & Harris, N. C. (2020). Data from: Spatial variation in diet–microbe associations across populations of a generalist North American carnivore. *Dryad Digital Repository*, <https://doi.org/10.5061/dryad.cnp5hqc2h>
- Crooks, K. R., & Soule, M. E. (1999). Mesopredator release and avifaunal extinctions in a fragmented system. *Nature*, *400*, 563–566. <https://doi.org/10.1038/23028>
- David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., ... Turnbaugh, P. J. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, *505*, 559–563. <https://doi.org/10.1038/nature12820>
- Ellington, E. H., & Gehrt, S. D. (2019). Behavioral responses by an apex predator to urbanization. *Behavioral Ecology*, *30*, 821–829. <https://doi.org/10.1093/beheco/arz019>
- Estes, J. A., Terborgh, J., Brashares, J. S., Power, M. E., Berger, J., Bond, W. J., ... Wardle, D. A. (2011). Trophic downgrading of planet earth. *Science*, *333*, 301–306. <https://doi.org/10.1126/science.1205106>
- Flagel, D. G., Belovsky, G. E., Cramer, M. J., Beyer, J. D. E., & Robertson, K. E. (2017). Fear and loathing in a Great Lakes forest: Cascading effects of competition between wolves and coyotes. *Journal of Mammalogy*, *98*, 77–84. <https://doi.org/10.1093/jmammal/gyw162>
- Galetti, M., Rodarte, R. R., Neves, C. L., Moreira, M., & Costa-Pereira, R. (2016). Trophic niche differentiation in rodents and marsupials revealed by stable isotopes. *PLoS ONE*, *11*, e0152494. <https://doi.org/10.1371/journal.pone.0152494>
- Gese, E. M., Morey, P. S., & Gehrt, S. D. (2012). Influence of the urban matrix on space use of coyotes in the Chicago metropolitan area. *Journal of Ethology*, *30*, 413–425. <https://doi.org/10.1007/s10164-012-0339-8>
- Ghoul, M., & Mitri, S. (2016). The ecology and evolution of microbial competition. *Trends in Microbiology*, *24*, 833–845. <https://doi.org/10.1016/j.tim.2016.06.011>
- Gomez, A., Petrzalkova, K., Yeoman, C. J., Vlckova, K., Mrázek, J., Koppova, I., ... Leigh, S. R. (2015). Gut microbiome composition and metabolomic profiles of wild western lowland gorillas (*Gorilla gorilla gorilla*) reflect host ecology. *Molecular Ecology*, *24*, 2551–2565. <https://doi.org/10.1111/mec.13181>
- Gompper, M. E. (2002). *The ecology of northeast coyotes: Current knowledge and priorities for future research*. Issue 17 of WCS Working Paper. Bronx, NY: Wildlife Conservation Society.
- Hamady, M., & Knight, R. (2009). Microbial community profiling for human microbiome projects: Tools, techniques, and challenges. *Genome Research*, *19*, 1141–1152. <https://doi.org/10.1101/gr.085464.108>
- Harris, N. C., & Dunn, R. R. (2013). Species loss on spatial patterns and composition of zoonotic parasites. *Proceedings of the Royal Society B: Biological Sciences*, *280*, 1847. <https://doi.org/10.1098/rspb.2013.1847>
- Harris, N. C., Garshong, R. A., & Gray, M. (2018). Distinct isotopic signatures reveal effect of ecoregion on small mammals of Ghana. *Journal of Mammalogy*, *99*, 117–123. <https://doi.org/10.1093/jmammal/gyx158>
- He, Y., Caporaso, J. G., Jiang, X.-T., Sheng, H.-F., Huse, S. M., Rideout, J. R., ... Zhou, H.-W. (2015). Stability of operational taxonomic units: An important but neglected property for analyzing microbial diversity. *Microbiome*, *3*, 20. <https://doi.org/10.1186/s40168-015-0081-x>
- Jahren, A. H., & Kraft, R. A. (2008). Carbon and nitrogen stable isotopes in fast food: Signatures of corn and confinement. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 17855–17860. <https://doi.org/10.1073/pnas.0809870105>
- Kohl, K. D., Varner, J., Wilkening, J. L., & Dearing, M. D. (2018). Gut microbial communities of American pikas (*Ochotona princeps*): Evidence for phylosymbiosis and adaptations to novel diets. *Journal of Animal Ecology*, *87*, 323–330. <https://doi.org/10.1111/1365-2656.12692>
- Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq illumina sequencing platform. *Applied and Environmental Microbiology*, *79*, 5112–5120. <https://doi.org/10.1128/aem.01043-13>
- Layman, C. A., Arrington, D. A., Montaña, C. G., & Post, D. M. (2007). Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology*, *88*, 42–48. [https://doi.org/10.1890/0012-9658\(2007\)88\[42:CSIRPF\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2007)88[42:CSIRPF]2.0.CO;2)
- Levi, T., Kilpatrick, A. M., Mangel, M., & Wilmers, C. C. (2012). Deer, predators, and the emergence of Lyme disease. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 10942–10947. <https://doi.org/10.1073/pnas.1204536109>
- Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., ... Gordon, J. I. (2008). Evolution of mammals and their gut microbes. *Science*, *320*, 1647–1651. <https://doi.org/10.1126/science.1155725>
- Manlick, P. J., Petersen, S. M., Moriarty, K. M., & Pauli, J. N. (2019). Stable isotopes reveal limited Eltonian niche conservatism across carnivore populations. *Functional Ecology*, *33*, 335–345. <https://doi.org/10.1111/1365-2435.13266>
- Mazel, F., Wüest, R. O., Gueguen, M., Renaud, J., Ficetola, G. F., Lavergne, S., & Thuiller, W. (2017). The geography of ecological niche evolution in mammals. *Current Biology*, *27*, 1369–1374. <https://doi.org/10.1016/j.cub.2017.03.046>
- McVey, J. M., Cobb, D. T., Powell, R. A., Stoskopf, M. K., Bohling, J. H., Waits, L. P., & Moorman, C. E. (2013). Diets of sympatric red wolves and coyotes in northeastern North Carolina. *Journal of Mammalogy*, *94*, 1141–1148. <https://doi.org/10.1644/13-mamm-a-109.1>
- Merkle, J. A., Stahler, D. R., & Smith, D. W. (2009). Interference competition between gray wolves and coyotes in Yellowstone National Park. *Canadian Journal of Zoology*, *87*, 56–63. <https://doi.org/10.1139/z08-136>
- Miller, B. J., Harlow, H. J., Harlow, T. S., Biggins, D., & Ripple, W. J. (2012). Trophic cascades linking wolves (*Canis lupus*), coyotes (*Canis latrans*), and small mammals. *Canadian Journal of Zoology*, *90*, 70–78.
- Muegge, B. D., Kuczynski, J., Knights, D., Clemente, J. C., Gonzalez, A., Fontana, L., ... Gordon, J. I. (2011). Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science*, *332*, 970–974. <https://doi.org/10.1126/science.1198719>
- Murray, M., Cembrowski, A., Latham, A. D. M., Lukasiak, V. M., Pruss, S., & St Clair, C. C. (2015). Greater consumption of protein-poor anthropogenic food by urban relative to rural coyotes increases diet breadth and potential for human–wildlife conflict. *Ecography*, *38*, 1235–1242. <https://doi.org/10.1111/ecog.01128>
- Newsome, S. D., del Rio, C. M., Bearhop, S., & Phillips, D. L. (2007). A niche for isotopic ecology. *Frontiers in Ecology and the Environment*, *5*, 429–436. <https://doi.org/10.1890/060150.01>
- Newsome, S. D., Garbe, H. M., Wilson, E. C., & Gehrt, S. D. (2015). Individual variation in anthropogenic resource use in an urban carnivore. *Oecologia*, *178*, 115–128. <https://doi.org/10.1007/s00442-014-3205-2>
- Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, *18*, 293–320. <https://doi.org/10.1146/annurev.es.18.110187.001453>
- Prugh, L. R., Stoner, C. J., Epps, C. W., Bean, W. T., Ripple, W. J., Laliberte, A. S., & Brashares, J. S. (2009). The rise of the mesopredator. *BioScience*, *59*, 779–791. <https://doi.org/10.1525/bio.2009.59.9.9>
- Randa, L. A., & Yunker, J. A. (2006). Carnivore occurrence along an urban–rural gradient: A landscape-level analysis. *Journal of Mammalogy*, *87*, 1154–1164.

- Rashleigh, R. M., Krebs, R. A., & van Keulen, H. (2008). Population structure of coyote (*Canis latrans*) in the urban landscape of the Cleveland, Ohio Area. *Ohio Journal of Science*, 108, 54–59.
- Reid, R. E. B., & Koch, P. L. (2017). Isotopic ecology of coyotes from scat and road kill carcasses: A complementary approach to feeding experiments. *PLoS ONE*, 12(4), e0174897. <https://doi.org/10.1371/journal.pone.0174897>
- Resasco, J., Tuff, K. T., Cunningham, S. A., Melbourne, B. A., Hicks, A. L., Newsome, S. D., & Davies, K. F. (2018). Generalist predator's niche shifts reveal ecosystem changes in an experimentally fragmented landscape. *Ecography*, 41, 1209–1219. <https://doi.org/10.1111/ecog.03476>
- Rich, M., Thompson, C., Prange, S., & Popescu, V. D. (2018). Relative importance of habitat characteristics and interspecific relations in determining terrestrial carnivore occurrence. *Frontiers in Ecology and Evolution*, 6, 78. <https://doi.org/10.3389/fevo.2018.00078>
- Ripple, W. J., Estes, J. A., Schmitz, O. J., Constant, V., Kaylor, M. J., Lenz, A., ... Wolf, C. (2016). What is a trophic cascade? *Trends in Ecology & Evolution*, 31, 842–849. <https://doi.org/10.1016/j.tree.2016.08.010>
- Ripple, W. J., Wirsing, A. J., Wilmsers, C. C., & Letnic, M. (2013). Widespread mesopredator effects after wolf extirpation. *Biological Conservation*, 160, 70–79. <https://doi.org/10.1016/j.biocon.2012.12.033>
- Slatyer, R. A., Hirst, M., & Sexton, J. P. (2013). Niche breadth predicts geographical range size: A general ecological pattern. *Ecology Letters*, 16, 1104–1114. <https://doi.org/10.1111/ele.12140>
- Steffan, S. A., Dharampal, P. S., Danforth, B. N., Gaines-Day, H. R., Takizawa, Y., & Chikaraishi, Y. (2019). Omnivory in bees: Elevated trophic positions among all major bee families. *The American Naturalist*, 194, 414–421. <https://doi.org/10.1086/704281>
- Thornton, D. H., & Murray, D. L. (2014). Influence of hybridization on niche shifts in expanding coyote populations. *Diversity and Distributions*, 20, 1355–1364. <https://doi.org/10.1111/ddi.12253>
- Trevelline, B. K., Fontaine, S. S., Hartup, B. K., & Kohl, K. D. (2019). Conservation biology needs a microbial renaissance: A call for the consideration of host-associated microbiota in wildlife management practices. *Proceedings of the Royal Society B: Biological Sciences*, 286, 20182448. <https://doi.org/10.1098/rspb.2018.2448>
- Wang, Y. W., Allen, M. L., & Wilmsers, C. C. (2015). Mesopredator spatial and temporal responses to large predators and human development in the Santa Cruz Mountains of California. *Biological Conservation*, 190, 23–33. <https://doi.org/10.1016/j.biocon.2015.05.007>
- Wheeldon, T., Patterson, B., & Beyer, D. (2012). Coyotes in wolves' clothing. *The American Midland Naturalist*, 167, 416–420. <https://doi.org/10.1674/0003-0031-167.2.416>
- Youngblut, N. D., Reischer, G. H., Walters, W., Schuster, N., Walzer, C., Stalder, G., ... Farnleitner, A. H. (2019). Host diet and evolutionary history explain different aspects of gut microbiome diversity among vertebrate clades. *Nature Communications*, 10, 2200. <https://doi.org/10.1038/s41467-019-10191-3>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Colborn AS, Kuntze CC, Gadsden GI, Harris NC. Spatial variation in diet-microbe associations across populations of a generalist North American carnivore. *J Anim Ecol*. 2020;89:1952–1960. <https://doi.org/10.1111/1365-2656.13266>