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Host phylogeny and functional traits differentiate gut microbiomes in a diverse natural community of small mammals

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Abstract

Differences in the bacterial communities inhabiting mammalian gut microbiomes tend to reflect the phylogenetic relatedness of their hosts, a pattern dubbed phylosymbiosis. Although most research on this pattern has compared the gut microbiomes of host species across biomes, understanding the evolutionary and ecological processes that generate phylosymbiosis requires comparisons across phylogenetic scales and under similar ecological conditions. We analysed the gut microbiomes of 14 sympatric small mammal species in a semi-arid African savanna, hypothesizing that there would be a strong phylosymbiotic pattern associated with differences in their body sizes and diets. Consistent with phylosymbiosis, microbiome dissimilarity increased with phylogenetic distance among hosts, ranging from congeneric sets of mice and hares that did not differ significantly in microbiome composition to species from different taxonomic orders that had almost no gut bacteria in common. While phylosymbiosis was detected among just the 11 species of rodents, it was substantially weaker at this scale than in comparisons involving all 14 species together. In contrast, microbiome diversity and composition were generally more strongly correlated with body size, dietary breadth, and dietary overlap in comparisons restricted to rodents than in those including all lineages. The starkest divides in microbiome composition thus reflected the broad evolutionary divergence of hosts, regardless of body size or diet, while subtler microbiome differences reflected variation in ecologically important traits of closely related hosts. Strong phylosymbiotic patterns arose deep in the phylogeny, and ecological filters that promote functional differentiation of cooccurring host species may disrupt or obscure this pattern near the tips.

KEYWORDS
16S rRNA, community ecology, phylogenetic scale, stable isotopes, symbiosis

1 | INTRODUCTION

In mammals, bacteria of the gut microbiome breakdown complex foods (Hacquard et al., 2015), detoxify plant secondary compounds (Kohl et al., 2014), protect hosts from pathogens (Pickard et al., 2017), and help maintain homeostasis (Cani et al., 2019). Two types of studies on ecoevolutionary trends involving mammals and their gut microbiomes have become common: (i) those focusing on broad phylogenetic patterns (Ley et al., 2008; Muegge et al., 2011; Song et al., 2020; Youngblut et al., 2019) and (ii) those focusing on fine-scale variation within and among closely related populations or species (Grond et al., 2020; Knowles et al., 2019; Moeller et al., 2013). Microbiome variation has been attributed to phylogenetic differences in host characteristics—including behavior

(Moeller et al., 2016), biogeography (Lutz et al., 2019), diet (Muegge et al., 2011), and morphophysiology (Kohl et al., 2018)—although substantial individual level variation occurs as well (David et al., 2014). As a consequence of these many sources of variation, gut microbiomes are thought to often vary in ways that produce a pattern in which the similarity of host-associated microbial communities parallels the phylogeny of host species—a pattern called "phylosymbiosis" (Brooks et al., 2016; Lim & Bordenstein, 2020).

Evidence for phylosymbiosis in mammalian gut microbiomes is so prevalent that the pattern often serves as a null expectation, although reports that phylosymbiosis is weak or absent have recently emerged as noteworthy exceptions to this rule (Mallott & Amato, 2021; Song et al., 2020). These reports highlight a need to elucidate mechanisms that reinforce or diminish phylosymbiotic patterns involving species under investigation. Mechanisms that may reinforce phylosymbiotic patterns in mammals include live birth, parental care, and specific gut anatomies that together create the potential for benefits involving the vertical transmission of bacteria from parents to offspring (Moeller et al., 2018). However, a principal challenge to the interpretation of phylosymbiotic patterns involves substantial variation among lineages in their degree of geographic overlap, functional trait diversity, and diet composition (Ley et al., 2008; Muegge et al., 2011). The degree to which these factors contribute to (or diminish) the emergence of phylosymbiotic patterns probably differs depending on the phylogenetic scale of host species under investigation, the geographic extent of their distributions, and the functional diversity of traits they express (Westoby, 2006). Because microbiome composition hinges partly on phylogeny and partly on host characteristics that may themselves be structured phylogenetically, we need to consider both (i) the extent of microbiome variation that can be explained by host characteristics after accounting for host phylogeny (Groussin et al., 2017) and (ii) the sensitivity of these results to the phylogenetic scale of host species under consideration (Graham et al., 2018).

Whereas prior comparative studies across diverse environments have helped identify host traits that contribute to gut microbiome differences across mammalian lineages, comparatively few have addressed similar questions about hosts that co-occur within ecological communities (Kartzinel et al., 2019; Knowles et al., 2019; Moeller et al., 2013; Perofsky et al., 2019). At finer geographic and phylogenetic scales, ecological niche differences among host species have been shown to explain more microbiome variation than do phylogenetic relationships for bats (31 species, 19 genera) and chipmunks (6 species, 1 genus) studied across extents of ~970 (Lutz et al., 2019) and ~1,560 km (Grond et al., 2020), respectively. These patterns contrast with global findings that have highlighted an overriding influence of host phylogeny on gut microbiomes across the mammalian tree of life (Nishida & Ochman, 2018; Sherrill-Mix et al., 2018). Such observations suggest that the phylogenetic distances among hosts, differences in host functional morphology, or both may contribute to phylosymbiotic patterns, but that the ecological constraints facing closely related hosts in similar ecological communities may erode them.

The paucity of community-level microbiome studies is a missing link for understanding of processes that may generate or disrupt phylosymbiosis. We compared gut microbiomes within a diverse assemblage of co-occurring hosts from a semi-arid Kenyan savanna. We considered the extent to which variation in gut microbiomes could be explained by phylogeny and/or functional traits including body size and diet. This small-mammal community includes species from three taxonomic orders, representing up to 105 million years of evolutionary history, and body sizes that span three orders of magnitude (4–2, 320 g). We tested the hypotheses that: (i) all pairs of species differ significantly in microbiome composition; (ii) interspecific differences in microbiome composition increase with phylogenetic distance; and (iii) interspecific differences in microbiome composition increase with functional trait differences, including body size and diet, after accounting for phylogenetic relatedness. We expected that both phylogeny and functional traits would contribute to variation in gut microbiome composition, but that their relative importance would differ depending on the phylogenetic scale of host lineages included in the analysis.

2 | MATERIALS AND METHODS

2.1 | Study system and sample collection

Our research was conducted at the Mpala Research Centre in Laikipia County, Kenya (0°17′ N, 37°52′E, 1,600-m elevation). A diverse community of small mammals (≲2 kg) occurs at this site, where since 2008, the Ungulate Herbivory Under Rainfall Uncertainty (UHURU) project has sampled at least 17 species (Figure S1; Alston et al., 2022; Goheen et al., 2018, 2013; Kartzinel et al., 2014). Our analysis focuses on three sites distributed across a 20-km transect. Each site contains a series of 1-ha experimental plots, including three pairs of fenced "exclusion plots" that have excluded large mammalian herbivores (>5 kg) and adjacent unfenced "open plots" that are accessible to all species. Compared to open plots, understory vegetation cover is roughly 20% greater and small-mammal diversity is roughly three-fold greater in exclusion plots (Goheen et al., 2013; Kartzinel et al., 2014). Live trapping of small mammals occurs once every 2 months for four consecutive nights using Sherman traps baited with peanut butter and oats ($N = 49$ traps per plot per night per bout). Animals are weighed and identified to species in most cases, although we cannot distinguish congeneric species of *Mus*, *Steatomys*, and *Crocidura* in the field.

Of the three small-mammal orders that occur in these plots, Rodentia (rodents) is the most abundant and species-rich. The rodents in this study range in size from 7 to 97 g (Table 1; Kartzinel et al., 2014) and prior analyses based on both stable isotopes and microhistology support species-level dietary categorizations that range from granivorous to omnivorous (Bergstrom, 2013; Keesing, 2000). The entire intestine tends to be larger in omnivorous than granivorous rodents (Korn, 1992), but omnivores tend to have shorter colons and ceca (Sakaguchi, 2003). Representatives of

TABLE 1 Characteristics of each sampled small mammal species.

Species	*N*	Size (g)	$\delta^{13}C$	$\delta^{15}N$	SEA	Richness	Diversity	Bacteroidetes	Firmicutes
Acomys kempi	5 (3)	30 ± 4				231 ± 78	75 ± 14	0.20	0.64
Aethomys hindei	18 (8)	97 ± 16	−22.9 ± 1.0	8.1 ± 1.4	4.4	291 ± 79	120 ± 3	0.20	0.66
Arvicanthis niloticus	3 (2)	62 ± 10	−15.5 ± 1.8	8.1 ± 1.1	6.3	357 ± 151	129 ± 13	0.25	0.65
Elephantulus rufescens	6 (6)	46 ± 13	−18.1 ± 0.9	10.1 ± 1.0	2.6	411 ± 89	157 ± 38	0.45	0.40
Gerbilliscus robustus	17 (13)	83 ± 21	−16.0 ± 2.5	9.8 ± 1.4	11	345 ± 106	139 ± 45	0.28	0.56
Grammomys dolichurus	7 (7)	34 ± 6	−20.3 ± 2.7	10.2 ± 0.4	5.2	284 ± 12	91 ± 34	0.18	0.71
Lepus sp. A	9 (0)[a]	2320				199 ± 20	83 ± 18	0.26	0.67
Lepus sp. B	8 (0)[a]	2320				231 ± 44	72 ± 17	0.28	0.66
Mus sp. A	3 (3)	7 ± 1				271 ± 113	109 ± 78	0.18	0.76
Mus sp. B	8 (8)	7 ± 2				248 ± 93	61 ± 29	0.27	0.62
Mus sp. C	2 (2)	7 ± 2				284 ± 37	89 ± 27	0.17	0.64
Saccostomus mearnsi	21 (15)	72 ± 22	−23.2 ± 0.8	7.9 ± 1.5	3.4	366 ± 81	152 ± 34	0.17	0.70
Steatomys parvus	4 (4)	14 ± 2				182 ± 18	67 ± 19	0.22	0.68
Taterillus harringtoni	15 (13)	39 ± 9	−12.4 ± 1.5	11.5 ± 1.3	6.1	304 ± 96	113 ± 39	0.36	0.57

Note: We report microbiome data from 126 samples (*N*) and mean body size from the subset of captured individuals in parentheses; range = 2–15 per species; median = 7. Species are listed in the same order as the phylogeny in Figure 1. Isotopic data include mean (±SD) $\delta^{13}C$, $\delta^{15}N$, and standard ellipse area (SEA) for a subset of seven of the most abundant species sampled prior to microbiome analysis. Mean microbiome data include richness, diversity, and the relative read abundance of the two most relatively abundant phyla, Bacteroidetes and Firmicutes.

[a]We did not capture and weigh hares, so these values represent the mean size of *Lepus capensis* and *Lepus saxatilis* in PanTHERIA (Jones et al. 2009, Kartzinel et al. 2019).

other taxonomic orders include two morphologically cryptic species of hares (*Lepus* spp.; Lagomorpha) and the rufous elephant shrew (*Elephantulus rufescens*; Macroscelidea). The hares are the two largest species in the study (~2.3 kg) and are exclusively herbivorous (and coprophagous), producing soft pellets that they reingest to enhance nutrient extraction. These hare species differ in diet composition, but distinguishing them requires evaluation of museum specimens and/or genetics (Kartzinel et al., 2019). The elephant shrew (47 g) is exclusively insectivorous (Bergstrom, 2013), with a colon and caecum that is distinctly shorter compared to herbivores of similar size (Woodall & Mackie, 1987).

To analyse gut microbiomes, we collected 128 fecal samples over a 3-year period (2015–2017) from 14 host species that exhibited a broad variety of diets (Table 1, Table S1). This enabled us to build a community-level data set that included rare and morphologically cryptic host species that were either captured infrequently or were sampled opportunistically (Table S1). The timing and location of sampling for each species varied due to natural variation in the distribution and abundance of small mammal populations within and among plots (Table S1), but this sampling strategy enabled us to obtain remarkable coverage of samples from the vast majority of small mammal species present. A small subset of taxa known to occur at the study sites were not sampled, including *Arvicanthis nairobae*, which is a locally rare congener of *A. niloticus*. Whenever possible, we collected fecal samples directly from the anus of animals during capture. If an animal did not defecate at the time of collection and a fresh fecal pellet was available in the trap, we collected that pellet. To reduce cross-contamination of fecal samples between animals caught on different nights, we removed fecal pellets and food from traps each day. Because hares are too large to capture in Sherman traps, we obtained fresh fecal samples opportunistically from the ground at the southern and central sites. Each fecal sample was stored in an unused plastic bag and placed on ice for transport to the laboratory.

To quantify diet variation within and among species, we measured carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values of hair from a subset of seven species in the same plots over an earlier 4-year study period (2009–2013) involving otherwise similar conditions (e.g., precipitation, vegetation density; Alston et al., 2022). As in other tropical savannas, isotopic differentiation of C_4 (mostly grasses) and C_3 plants enables reliable determination of trophic relationships (Bergstrom, 2013; Kartzinel et al., 2015). We collected 553 hair samples from six rodent species and the elephant shrew (9–211 individuals per species). Hair from the base of the tail was cleaned with a 2:1 chloroform: methanol solution, air-dried, and loaded (~0.5 mg) into tin capsules for analysis. We measured $\delta^{13}C$ and $\delta^{15}N$ with a Costech 4010 elemental analyser coupled to a (i) Thermo Finnigan Delta plus XP isotope ratio mass spectrometer at the University of Wyoming Stable Isotope Facility (Laramie, WY), or (ii) Thermo Fisher Delta V isotope ratio mass spectrometer at the University of New Mexico Centre for Stable Isotopes (Albuquerque, NM). Isotope data are reported as $\delta^{13}C$ or $\delta^{15}N = 1000 \times ([R_{sample} - R_{standard}/R_{standard}] - 1)$, where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio of samples and standards, respectively. Laboratory reference materials are calibrated to the internationally accepted standards Vienna Pee Dee Belemnite limestone (V-PDB) and atmospheric nitrogen (AIR) respectively; units are parts per thousand (‰). Analytical precision was calculated as the mean within-run standard deviation of reference materials, which was ±0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. We characterized diets based on $\delta^{13}C$ as a proxy for the relative use of C_3 versus C_4 plants, $\delta^{15}N$ as a proxy for trophic level, and the standard ellipse area (SEA) of $\delta^{13}C$ and $\delta^{15}N$ as a proxy for dietary variation and overlap among species. We investigated (i) potentially confounding spatiotemporal variation in diets that could obscure correlations with gut microbiomes and (ii) the relationship between host diet composition and microbiome composition after accounting for phylogeny. To assess variation in diet composition that can be attributed to species identity versus ecological changes through space and time, we compared mean $\delta^{13}C$ and $\delta^{15}N$ values according to host species identity, site, and year using ANOVA. Then, to compare dietary overlap between species, we calculated the proportion of SEA overlap for all pairs of species using the R package SIBER (Jackson et al., 2011) in each of two ways: (i) the overlapping proportion of the sum of SEA for both species; (ii) the partial overlap of SEA for each species within the SEA of each of the other species.

2.2 | Microbiome sequencing

To preserve fecal DNA in the field, we transferred fecal samples to lysis tubes containing Zymo Xpedition buffer; we then homogenized the sample by vortexing it in lysis buffer for 30 s and froze it. We used Zymo soil/fecal mini kits to extract DNA from fecal samples and extraction blanks in a laboratory that included separate pre- and post-PCR rooms and equipment and proceeded to sequence microbiomes after visually assessing a lack of amplification in extraction blanks. To analyse microbiomes, we generated amplicons of the V4 hypervariable region of bacterial 16S rRNA using the primers 515f and 806r (Walters et al., 2016). Amplicons were normalized, pooled, quality checked using Qubit and Bioanalyser, and then sequenced in a 2 × 250 bp paired-end Illumina MiSeq run using version 2500 cycle reagents. In addition to amplicons from fecal DNA, we sequenced amplicons of a Zymo microbial community standard (henceforth, mock community) to evaluate accuracy and screen the resulting data for potential contaminants.

2.3 | Host phylogeny construction

We used mitochondrial DNA barcoding to confirm small mammal identity and build a community phylogeny. We obtained fecal samples from eight taxa that were identifiable to species in the field and selected a subset of 2–3 samples per species for host DNA barcoding. We also obtained fecal samples from morphologically cryptic taxa within the genera *Steatomys* ($N = 4$), *Lepus* ($N = 17$), and *Mus* ($N = 13$); we only analysed microbiome data from these samples after

obtaining a diagnostic DNA barcode from the same fecal sample. To obtain these barcodes, we amplified and sequenced two mitochondrial markers: cytochrome *b* and the D-loop of the control region. We used a combination of published and novel PCR primers, and we attempted to identify the phylotypes of cryptic taxa using reference data in GenBank (Appendix S1; Tables S2 and S3). We constructed a phylogeny using concatenated data from both markers and evaluated support with 1,000 bootstraps (Appendix S1). We further used publicly available data from TimeTree (Kumar et al., 2017) to corroborate the overall tree topology and to scale all branch lengths based on genus-level node ages for taxa in the tree (Appendix S1). The resulting community phylogeny comprised 14 small-mammal taxa, including *Steatomys parvus*, two hare phylotypes (provisionally *Lepus* spp. A and B; see also Kartzinel et al., 2019) and three mouse phylotypes (provisionally *Mus* spp. A–C). We treat these phylotypes as equivalent to species in our analysis, pending further taxonomic investigation (Appendix S1; Figure 1, Figure S2–S4). The overall tree topology matched the accepted clade structure (e.g., families, subfamilies, tribes) and bootstrap support for the branching pattern was particularly high near the tips (Figure 1). Comparison of our phylogeny to the genus-level topology of TimeTree revealed ambiguity involving only the short branches of genera within the Arvicanthini tribe, further highlighting the opportunities for taxonomic investigation of small mammals from East Africa (Figure 1).

2.4 | Microbiome analysis

Our strategy to generate data on the relative abundance of bacterial taxa included bioinformatic processing and taxonomic assignments of amplicon sequence variants (ASVs). Briefly, we produced 18,968,931 Illumina sequence reads (median = 145,750 per sample) across all 128 samples and the mock community (Table S1). We removed primer sequences and cut reads to 213 bp using DADA2 (Callahan et al., 2016) in R (R Core Development Team, 2020). We ran the DADA2 sequence-variant algorithm (dadaFs/Rs) on dereplicated sequences with their assigned error rates before merging forward and reverse sequence reads into ASVs and removing chimeras. We assigned taxonomy to all remaining ASVs by comparing them with the SILVA database version 132 (Quast et al., 2013) using the naive Bayesian assignment algorithm in QIIME2 (Bokulich et al., 2018; Thompson et al., 2017). We screened potential contaminants by comparing ASVs from the mock community with reference sequences for the mock community and identified nine ASVs (Table S4) that did not match the mock community for removal from all samples as putative contaminants. These ASVs represented three nontarget fungal taxa, five bacterial taxa and one unknown sequence; only one of these nine ASVs was also present and excluded from analysis as a result (an uncultured Bacteroidetes present in 19 samples; 0%–2% RRA per sample; Table S4). We then removed all putative bacterial sequences not identified to the phylum level as well as nonbacterial ASVs from Eukarya, Archaea, chloroplast, and mitochondria (Table S1 reports read counts at each stage of the pipeline). Before proceeding, we dropped two samples that yielded <5,000 sequences: one from the elephant shrew *Elephantulus rufuscens* and one from the pouched mouse *Saccostomus mearnsi*. To facilitate comparisons of microbiome diversity and composition, we rarefied data representing 7,720,016 sequences across the remaining 126 samples to the lowest read depth (N = 7,017 reads per sample). This final data set contained 9,887 ASVs, including singletons that were retained after rarefying.

To summarize the data for further analysis, we characterized bacterial variation within and between samples. To quantify variation within samples, we used Hill numbers (Hill, 1973) to compare bacterial richness (${}^0D$) and diversity (${}^1D$) in the hilldiv package in R (Alberdi & Gilbert, 2019). We found bacterial richness and diversity to be correlated and thus focus on analyses of diversity. We measured variation bacterial community composition between samples using Bray–Curtis dissimilarity as well as weighted and unweighted UniFrac metrics. UniFrac is a distance metric that includes information on the phylogenetic relatedness of ASVs (Lozupone & Knight, 2005). Unweighted UniFrac gives unique bacterial lineages that contain rare and common ASVs equal weight, whereas weighted UniFrac emphasizes differences in relatively abundant bacterial lineages. To calculate UniFrac, we aligned ASVs in the program MAFFT (Katoh, 2002) and constructed a bacterial phylogeny with FastTree (Price et al., 2009) in QIIME2.

Finally, to characterize functional differences between gut microbiomes of host species, we used Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2; Douglas et al., 2019). We aligned the ASVs from our rarefied data set to a reference tree consisting of marker genes from known bacterial genomes that consisted of 20,000 16S rRNA gene clusters. Based on the relative abundance of each ASV in each sample, the gene family copy numbers were calculated and mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) and KEGG Orthology (KO) databases. Using predicted functional KO pathways, we added the corresponding metabolic pathways using the MetaCyc database (Caspi et al., 2018; Karp & Caspi, 2011). We identified 400 predicted metabolic pathways across 51 MetaCyc categories. Because each ASV can be mapped to one or more pathways and thus generate unequal pathway abundances across samples, we rarefied pathway abundances to the lowest number per sample and the final functional data set comprised 399 predicted pathways across 51 MegaCyc categories (N = 53,512 predicted pathway abundances per sample).

2.5 | Hypothesis testing

We tested our first hypothesis, that species differ significantly in microbiome composition, using both univariate and multivariate measures. To test for statistically significant differences in the diversity of bacterial ASVs within samples from each species, we used Kruskal-Wallis tests. To test for significant differences in microbiome composition among host species, we compared the Bray–Curtis dissimilarity and weighted and unweighted UniFrac metrics

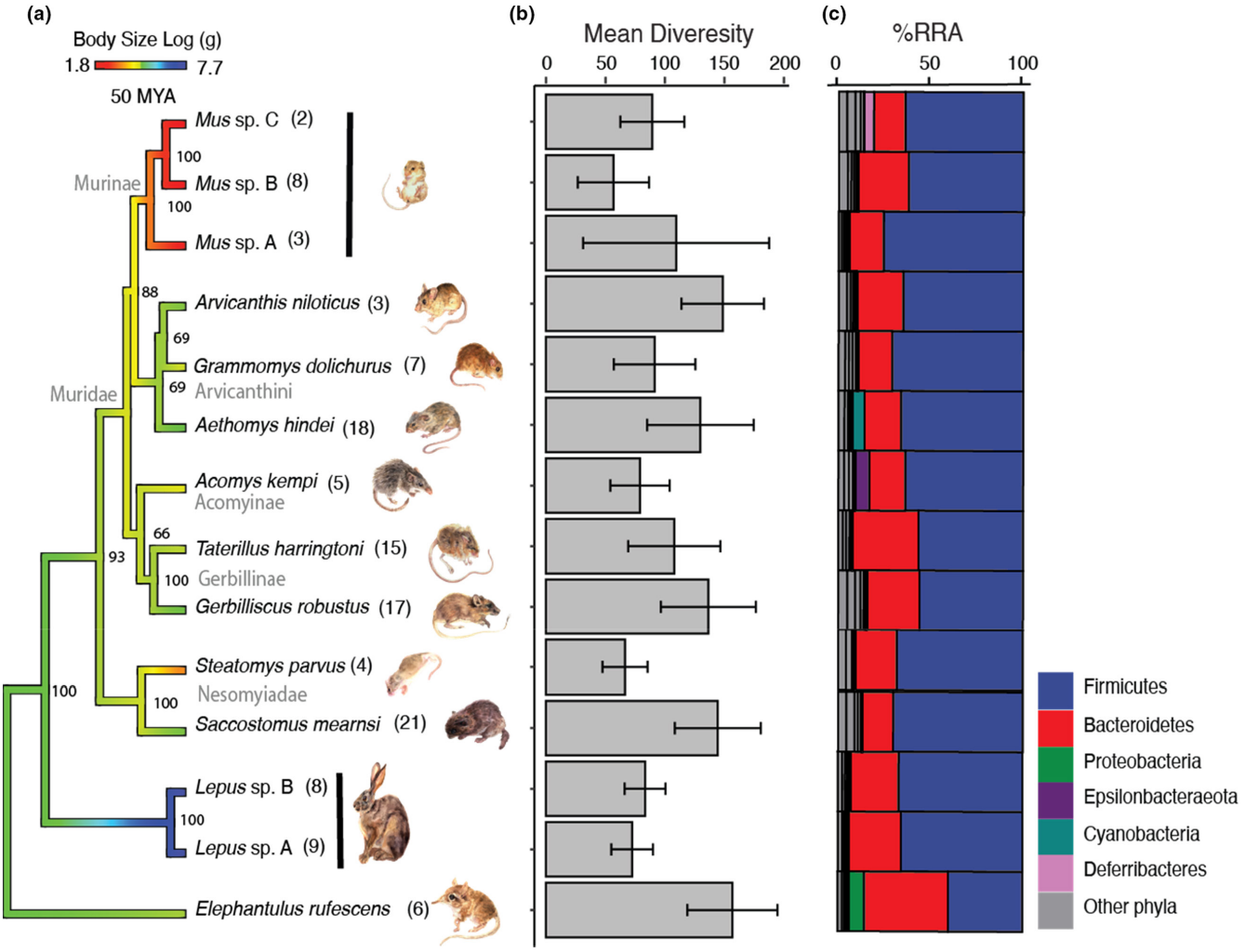

FIGURE 1 Small mammal community phylogeny and gut microbiome compositions. (a) The phylogeny of 14 species includes three taxonomic orders: Rodentia (rodents; subfamily names in grey), Lagomorpha (hares), and Macroscelidea (elephant shrew). The numbers at the nodes represent bootstrap support values, with elephant shrew as the appropriate outgroup. Grey text at the nodes represents relevant family-, subfamily-, and tribe-level clades within the rodents and indicate that the phylogeny represents our best current knowledge of evolutionary relationships within and among these taxa. To visualize body size distributions across the phylogeny, we used maximum likelihood ancestral state reconstruction method implemented by the contMap function in phytools in R. Microbiome sample sizes are shown at the tips in parentheses. (b) Microbiome diversity is shown as the mean number of ASVs per sample ± standard deviation. (c) Stacked bar charts show the relative read abundance (RRA) of six bacterial phyla representing >5% RRA across all samples; the RRA of 19 "other" phyla representing <5% RRA are shown in grey. [Colour figure can be viewed at wileyonlinelibrary.com]

using permutational multivariate analysis of variance (perMANOVA) with 999 permutations in vegan (Anderson & Walsh, 2013; Oksanen et al., 2016). Subsequently, we tested for significant pairwise differences in microbiome composition using pairwise perMANOVA followed by Benjamini-Hochberg corrections for multiple comparisons (Martinez Arbizu, 2017). We performed analysis of multivariate homogeneity in group dispersions to characterize the extent of interindividual variation within host species using ANOVA followed by Tukey's HSD test in vegan. We visualized the microbiome differences among all samples using nonmetric multidimensional scaling (NMDS).

To test our second hypothesis, that differences in microbiome composition increased with phylogenetic distances among species (i.e., phylosymbiosis), we quantified phylogenetic signal in mean microbiome diversity using Pagel's λ based on the geiger (Harmon et al., 2008) and phylosig functions in the phytools package (Revell, 2012). When $\lambda = 1$, the pattern of microbiome diversity is consistent with a Brownian-motion model of evolution and thus the microbiome diversity of closely related species tends to be similar; when $\lambda = 0$, variation in microbiome diversity reflects phylogenetic independence (Freckleton et al., 2002). To test for significant correlations between the pairwise phylogenetic divergence of hosts and their mean pairwise Bray–Curtis dissimilarity and UniFrac distances, we used two approaches that emphasize different characteristics of the data: Robinson-Foulds and Mantel tests. We first used topology-based normalized Robinson-Foulds (nRF) tests in phangorn (Schliep, 2011) to evaluate correspondence between the branching patterns of the host phylogeny and of a UPGMA dendrogram

representing the pairwise Bray–Curtis dissimilarity and UniFrac distances; we visualized topological congruence between the host-phylogeny and microbiome dendrograms using tanglegrams in the dendextend package (Galili, 2015). A nRF score of 0 represents complete congruence while 1 represents complete incongruence. To complement this topology-based approach, we used distance-based Mantel tests to evaluate correlations between the phylogenetic distance separating host species (square-root transformed) and their corresponding microbiome differences, with 999 permutations in vegan. These two tests provide complementary information because Mantel tests measure host evolutionary distances quantitatively whereas Robinson-Foulds tests emphasize the hierarchical clustering of host lineages (Lim & Bordenstein, 2020).

To account for spatiotemporal heterogeneity of sampling and the number of samples used to represent the microbiomes of each host species when testing for phylosymbiosis, we selected representative subsamples of each species in a bootstrapping procedure. In this procedure we randomly selected one representative sample per species, calculated the pairwise differences, and repeated this procedure 999 times. For each of these matrices, we calculated Mantel's *r* and the nRF score. This allowed us to evaluate the distribution of Mantel and Robinson-Foulds test values that would be obtained based on these subsampled data sets, and whether the values based on our full data set differed significantly from this distribution.

To test our third hypothesis, we compared microbiome differences based on host functional traits after accounting for phylogenetic relatedness. We quantified phylogenetic signal in mean body size using Pagel's λ based on mean body size data (log-transformed g) for all species. We tested whether mean body size and the three diet variables (mean $\delta^{13}C$, $\delta^{15}N$, and SEA size (variation within species)) were correlated with mean bacterial diversity using phylogenetic generalized least squares (PGLS) in caper (Orme et al., 2018). We performed PGLS assuming a model of Brownian motion ($\lambda = 1$) and compared results to an ordinary least squares regression ($\lambda = 0$) to avoid errors estimating λ using a relatively small phylogeny. Finally, we used partial Mantel tests to evaluate correlations between mean microbiome dissimilarity based on both Bray-Curtis and UniFrac matrices with (i) mean body size difference (log-transformed) and (ii) isotopic niche separation (1 – proportional SEA), while accounting for phylogenetic relatedness (square-root transformed). We tested for significance using 999 permutations for body size, and 99 permutations for dietary separation due to the smaller number of pairwise comparisons possible. To evaluate the extent to which patterns are consistent across phylogenetic scales, we repeated all statistical tests for a phylogenetically clustered subset of rodent species ($N = 11$) versus the more phylogenetically dispersed suite of 14 host species.

We used information about the functional potential of microbiomes to investigate variation that could be indicative of host dietary differences. As we did for the comparisons of microbiome taxonomy, we calculated Bray–Curtis dissimilarity in the predicted metabolic pathways from the rarified PICRUSt2 analysis to: (i) plot NMDS ordinations, (ii) test for significant differences between host species using perMANOVA, and (iii) perform dispersion analyses. To compare these microbiome-based inferences of diet with isotope-based inferences of diet from the subset of seven species sampled over an earlier time period (2009–2013), we performed a Mantel test for correlation between Bray–Curtis dissimilarity of predicted functional pathways and dietary niche separation (1 – proportional SEA) based on 99 permutations. Finally, we calculated the mean relative abundance of predicted functions pertaining to carbohydrate degradation (CD) and amino acid degradation (AAD). The CD and AAD pathways can be sensitive to the lignocellulosic and protein content of herbivore diets, respectively (Baniel et al., 2021; Gong et al., 2020; Hicks et al., 2018). Thus, we created a ratio of these two values (CD/AAD) such that a high CD/AAD ratio can be interpreted to indicate functional characteristics in a gut microbial community that may be consistent with diets that contain a higher ratio of cellulose and other carbohydrates to protein, and vice versa.

3 | RESULTS

We obtained microbiome data from 126 samples (out of 128) and constructed a robust community phylogeny to test our hypotheses (Table 1; Figure 1). Six of these species were identified with the aid of fecal DNA barcodes, including *Steatomys parvus*, a phylotype matching *Mus minutoides* (*Mus* sp. A), a phylotype matching *Mus* cf. *gerbillus* (*Mus* sp. B), a novel *Mus* phylotype (*Mus* sp. C), and the two unidentified *Lepus* phylotypes (*Lepus* spp. A and B). Of the seven species for which we obtained isotopic data, some exhibited relatively narrow isotopic niches. For example, *Saccostomus mearnsi* and *Aethomys hindei* had low and invariant $\delta^{13}C$ and $\delta^{15}N$ values that were both indicative of primary consumers specializing on C_3 plants, whereas *Gerbilliscus robustus* had a much larger isotopic niche centered around intermediate $\delta^{13}C$ and $\delta^{15}N$ values that reflect a foraging strategy of a generalized omnivore (Figure S5). The strictly insectivorous elephant shrew *Elephantulus rufescens* exhibited a relatively high trophic position, with a similar mean $\delta^{15}N$ value to the rodents *Taterillus harringtoni* and *Grammomys dolichurus*, and with an isotopic niche that was nested entirely within that of *Gerbilliscus robustus* (Figure S5; Table 1). For both $\delta^{13}C$ and $\delta^{15}N$, there were strong and significant differences between host species but little variation through time or space (we found only weak, albeit statistically significant, differences in average $\delta^{15}N$ across sites; Figure S5).

The 9,887 microbial ASVs represented 25 bacterial phyla and included at least 45 classes (2% unclassified), 91 orders (3% unclassified), 177 families (3% unclassified), 385 genera (25% unclassified), and 219 species (58% unclassified). The phyla with the greatest mean RRA across all samples were Firmicutes (63% RRA) and Bacteroides (25%), which accounted for the vast majority of bacteria from all hosts (Figure 1). Firmicutes accounted for ≥56% RRA in all host species except elephant shrew (40%). Bacteroides was the most abundant phylum in elephant shrew (45% RRA) and the second-most abundant phylum in all other species (17% to 36%). Other phyla prominent in at least one host species included Cyanobacteria (mean

3% across species, 7% in *Aethomys hindei*), Proteobacteria (mean 3%, 8% in *Elephantulus rufescens*), and Epsilonbacteraeota (mean 2%, 8% in *Acomys kempi*).

3.1 | Interspecific differences in gut microbiomes

Consistent with our first hypothesis, gut microbiome diversity composition differed significantly among host species. Mean microbiome diversity was greatest for the elephant shrew *E. rufescens*, while rodent microbiomes spanned a broad and intermediate range of diversities with hare microbiomes having comparably low diversity (χ^2 = 59.4, df = 13, P < .001; Table 1). Host identity accounted for 35% of variation in Bray–Curtis dissimilarity, 34% of unweighted UniFrac distances, and 38% weighted UniFrac distances among species based on perMANOVAs; similar results were obtained when we limited the analysis to just the 11 rodent species (Figure 2, Figures S7, S8). Mean pairwise intraspecific unweighted UniFrac distances were lower than pairwise interspecific distances for all comparisons except among *Mus* species, while mean pairwise intraspecific weighted UniFrac were the same or lower than pairwise interspecific distances in all cases (Table S5). Microbiomes were far more similar between species of the same order compared to species of different orders, with the greatest degree of overlap between the two hare species (Tables S5 and S6). Of the 91 possible interspecific comparisons of microbiome composition, most differed significantly in all metrics after correcting for multiple comparisons; only eight Bray-Curtis, eight unweighted UniFrac, and 14 weighted UniFrac comparisons did not differ significantly, most of which involved closely related species (including all contrasts between congeneric pairs of *Lepus* spp. and *Mus* spp.) and similar sized species (including *Mus* spp., *Steatomys parvus*, and *Acomys kempi*; Tables S5 and S6). Unexpectedly, one or all microbiome community metrics did not differ significantly between *Arvicanthis niloticus* and *Mus* spp. (same family) and *Aethomys hindei* and *Gerbilliscus robustus* (similar size). Dispersion analyses revealed significant differences

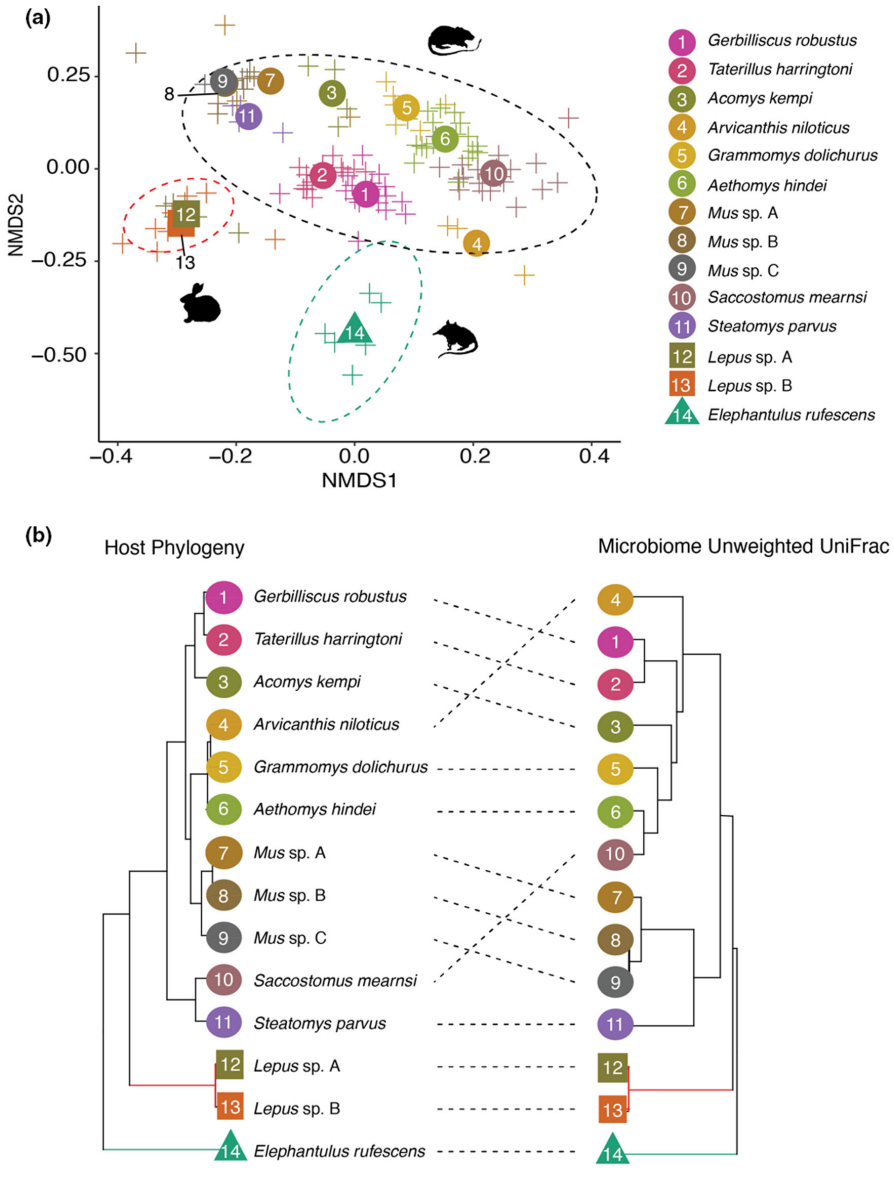

FIGURE 2 Variation in microbiome composition within and among species. (a) We ordinated pairwise unweighted UniFrac distances between 126 samples using NMDS (stress = 0.20). The + marks correspond to each sample and the large shapes indicate the centroids of each species. Species differed significantly in microbiome composition overall (pseudo-$F_{13,112}$ = 4.5, R^2 = .34, $P \leq .001$) and in comparisons involving rodents only (pseudo-$F_{10,92}$ = 3.7, R^2 = .29, $P \leq .001$). Dashed lines represent 95% confidence ellipses around samples from each of the three orders (black = Rodentia; red = Lagomorpha; green = Macroscelidea). (b) A tanglegram connects tips of the small mammal phylogeny (left) to a UPGMA dendrogram representing mean pairwise unweighted UniFrac distances (right). We rotated the trees to minimize crossing of lines. Analogous plots are provided for Bray–Curtis dissimilarity (Figure S6) and weighted UniFrac (Figure S7). Variation in levels of intraspecific microbiome dispersion are reported in Figure S10. [Colour figure can be viewed at wileyonlinelibrary.com]

among species in the degree of intraspecific microbiome variation across metrics (Bray-Curtis: $P < .001$; unweighted UniFrac: $P < .001$; weighted UniFrac: $P = .025$; Figure S9). These overall differences were driven by the subset of species with the highest mean dispersion levels (e.g., *Aethomys hindei* and *Saccostomus mearnsi* for the unweighted UniFrac metric; Figure 2, Figure S9), whereas most Tukey's HSD comparisons were not significant in other dispersion metrics (Table S7).

3.2 | Phylosymbiosis in gut microbiomes

We found mixed support for our second hypothesis that microbiome composition would differ significantly between species in a pattern consistent with phylosymbiosis. On the one hand, the host phylogeny and microbiome dendrogram were topologically congruent and we found significant positive correlations between the phylogenetic distance and microbiome dissimilarity of host species, consistent with expectations under phylosymbiosis. On the other hand, the strength (and significance) of these phylogenetic associations with microbiome composition depended on the metric used to characterize the microbiome (e.g., weighted vs. unweighted UniFrac) and mean microbiome diversity did not exhibit phylogenetic signal for the full community (diversity: $\lambda = 0$, $P = 1$) or for rodents alone ($\lambda = 0$, $P = 1$). The host phylogeny and microbiome dendrogram topologies were most congruent for Bray-Curtis (nRF = 0.58) and unweighted UniFrac (nRF = 0.58), followed by weighted UniFrac (nRF = 0.91). Similarly, phylogenetic distance among host species was significantly correlated with differences in microbiome composition based on Bray-Curtis and unweighted (but not weighted) UniFrac, most strikingly across all species but also within rodents (Figure 3, Figure S10). Hosts from different orders thus contributed most strongly to the phylosymbiotic pattern, which was weaker among closely related rodents (Figure 2, 3, Figures S7, S8, S10).

Our sensitivity analysis showed that (i) differences in microbiome composition inferred from the full data set did not produce Mantel or Robinson-Foulds values that differed significantly from those based on representative subsamples from each host species and (ii) that performing these tests on a subsample from each host species can produce a wide range of results. Results obtained from the full data set fell within the broad ranges of values obtained when selecting a single representative sample per species for both Robinson-Foulds (Bray-Curtis: median nRF value = 0.67, interquartile range = 0.58–0.75, $P = .930$; unweighted UniFrac: median nRF = 0.67, IQR = 0.58–0.75, $P = .860$; weighted UniFrac: nRF = 0.92, IQR = 0.83–0.92, $P = .635$) and Mantel tests (Bray-Curtis: median r statistic = .50, interquartile range = 0.45–0.53, $P = .128$; unweighted UniFrac: median $r = 0.50$, IQR = 0.45–0.54, $P = .161$; weighted UniFrac: median $r = 0.22$, IQR = 0.11–36, $P = .264$). Differences in the degree of microbiome overlap between the pairs of samples used to represent closely related hosts thus produce some imprecision in these parameter estimates, but overall inferences about the presence of patterns that are consistent with phylosymbiosis are robust to this uncertainty in part because hosts from different orders have strongly differentiated gut microbiomes.

3.3 | Functional trait associations with gut microbiomes

Our third hypothesis, that microbiome composition is associated with host functional traits, was largely supported for body size but more moderately supported for diet differentiation. Body size had strong and significant phylogenetic signal among all species in the community, but this signal was diminished when comparisons were limited to the 11 rodent species (all: $\lambda = 1$, $P < .001$; rodents: $\lambda = 1$, $P = .087$; Figure 1). There was a significant positive correlation between body size and mean microbiome diversity among rodents using both OLS and PGLS models (Figure 4); however, when including elephant shrew and hares (the largest species), there was no significant relationship between body size and diversity (Figure 4). Within rodents, body-size differences were correlated significantly with microbiome compositional dissimilarity based on Bray-Curtis and both UniFrac metrics (Figure 3, Figure S10). This correlation remained significant when including elephant shrew and hares for Bray-Curtis and unweighted UniFrac analysis, but not weighted UniFrac (Figure 3, Figure S10).

Isotopic differences were positively and significantly correlated with Bray–Curtis dissimilarity and unweighted UniFrac across all host species and the subset of rodents alike, but dietary differences were not significantly correlated with weighted UniFrac for either set of host species (Figure 3, Figure S10). We also found no significant correlations between microbiome diversity and any of the isotopic dietary metrics ($\delta^{13}C$, $\delta^{15}N$, and SEA; Figure S13), although these analyses had low statistical power because we only had data for a subset of 7 host species.

There were strong and significant differences in the functional composition of microbiomes across all 14 host species as inferred using PICRUSt2 (Figures S11 and S12). Notably, there was a weakly positive and significant correlation between differences in the trophic position of 7 host species inferred using isotopes (1 – proportional SEA overlap) and the functional differences among microbiomes inferred using PICRUSt2 (all: Mantel $r = 0.30$, $P = .05$; rodents only: $r = 0.51$, $P = .03$; Figure S11; Tables S8–S10). As expected, the microbiome with the lowest CD/AAD ratio (functionally indicative of more animal-based diets) was observed in the insectivorous elephant shrew *Elephantulus rufescens* (which had a relatively high trophic position), whereas herbivorous rodents such as *Saccostomus mearnsi* and *Aethomys hindei* had higher CD/AAD ratios indicative of more plant-based diets (Tables S11, S12; Figure S12). Unexpectedly, however, the rodent *Grammomys dolichurus* had the highest CD/AAD ratio despite having a relatively high mean $\delta^{15}N$ and the strictly herbivorous hares had intermediate CD/AAD ratios (Tables S11, S12; Figure S12). The functional composition of microbiomes thus differed significantly between host species in ways that generally corresponded to trophic differences known from isotopic

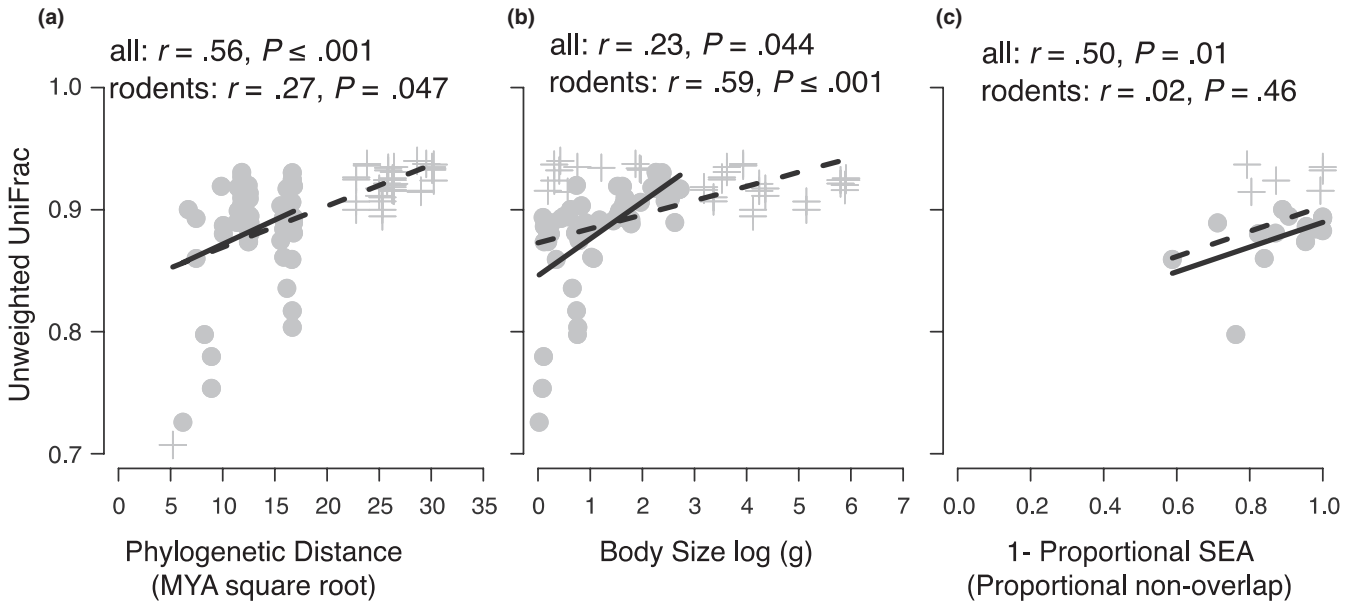

FIGURE 3 Correlations of microbiome unweighted UniFrac distance with phylogeny and functional traits. Predictor variables include (a) host phylogenetic distance (square-root transformed), (b) mean body-size difference, and (c) isotope-based proportional nonoverlap metric as a proxy for dietary differences between pairs of species. Circles represent within-order comparisons and + represent between-order comparisons. Shorter solid trend lines show correlations based on the subset of rodents and longer dashed lines represent comparisons of all species. Correlations involving phylogenetic distance were based on Mantel tests whereas all other correlations were based on partial Mantel tests to account for the phylogenetic relatedness of hosts. Correlations involving phylogeny and body size were evaluated using 999 permutations, but correlations involving diet were limited to 99 permutations due to the relatively small subset of species available for this analysis.

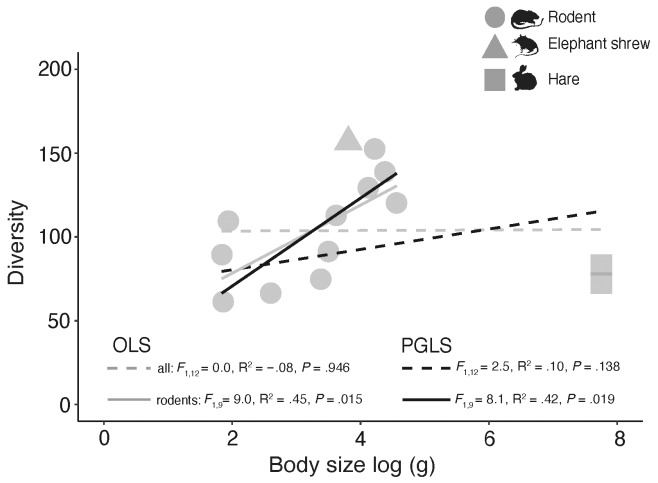

FIGURE 4 Correlations between mean body size and mean microbiome diversity. We compared models based on PGLS (black lines) and OLS (grey lines) for the entire community (longer dashed lines) and the subset of rodents (shorter solid lines).

data collected from these same populations during an earlier study period, although interpreting any predicted functional differences or constituent pathways as indicators of different diet types remains a challenge.

4 | DISCUSSION

Our data show that the strongest phylosymbiotic signal is associated with high-level taxonomic groupings (order), consistent with our hypothesis that microbiome differentiation increases with host phylogenetic distance. At lower taxonomic levels (within rodents), the phylosymbiotic signal is reduced and microbiome differentiation is more clearly associated with differences in body size, which did not in turn exhibit significant phylogenetic signal for the subset of rodents present in this community. The association between microbiome and body size might reflect ecological processes operating in size-structured host communities, including the tendency for larger hosts to contain more diverse and compositionally distinct microbiomes compared to smaller ones (Godon et al., 2016; Sherrill-Mix

et al., 2018). Dietary variation also exerts influence on microbiome composition in ways that may reinforce the phylogenetic separation of disparate herbivorous, omnivorous, and insectivorous lineages or that may conversely reflect the use of similar dietary niches as in the case of some phylogenetically divergent rodent species from this community. The roots of phylosymbiosis are located deep in the phylogeny, but the ecology of local communities may counter or even disrupt this overarching phylosymbiotic signal near the tips of the phylogeny. Our results highlight the importance of scale in the interpretation of phylosymbiotic patterns.

Microbiome differentiation increased with phylogenetic divergence among hosts, consistent with phylosymbiosis, and was most striking among hosts from different orders. For example, the two hares and the elephant shrew shared only 1%–2% of bacterial ASVs with any of the 11 rodent species. Place-based studies that compare microbiome composition across deeply divergent mammalian lineages often report similar results, including comparisons of three orders in Madagascar (Perofsky et al., 2019) and seven orders in Kenya (Kartzinel et al., 2019). The pairs of hosts in our analysis that did not differ significantly in microbiome composition diverged more recently on average (7–33 Ma; median 29 Ma) than pairs that did (11–105 Ma; median 33; Figures 1 and 2; Tables S5, S6). The most closely related host species in our study exhibited a relatively high degree of microbiome overlap and no significant difference in composition (80%–86% shared ASVs by 3 *Mus* spp.; 75% shared by 2 *Lepus* spp.). These two sets of congeners have only subtle morphological differences, and we might have failed to observe this pattern if we had relied solely on field identifications without the aid of host DNA barcoding for identification (Pringle & Hutchinson, 2020). Our results are thus consistent with prior reports that phylosymbiotic patterns are weak or absent among rodents (Grond et al., 2020; Song et al., 2020). Importantly, whereas the presence of some closely related host taxa with undifferentiated microbiomes contributed to a dampening of the phylosymbiotic pattern, the pattern was evident because the phylogenetic scale of host species in this community included taxa with strongly differentiated microbiomes.

The phylogenetic scales at which associations between microbiomes and host lineages become prominent may differ depending on the lineages under consideration, their behaviovrs, and their functional traits. Elucidating mechanisms that contribute to phylosymbiotic patterns at different phylogenetic scales is an emerging priority (Mazel et al., 2018; Moran et al., 2019). Characteristics of mammals that include live birth and parental care can facilitate the vertical transmission of gut bacteria among rodents in the laboratory, and this commonality could be one mechanism behind the emergence and reinforcement of phylosymbiotic patterns (Moeller et al., 2018). The next critical step toward understanding why the strength of phylosymbiotic patterns can vary across mammalian lineages and phylogenetic scales is to consider how divergent characteristics could modify this underlying pattern (Mazel et al., 2018). For example, we observed a strong association between microbiome and body size among rodents, which share a relatively shallow phylogenetic history (Figures 3 and 4). In contrast, associations between microbiome and diet were similar both within the rodent lineage and beyond (Figure 3). Diet as a determinant of microbiome composition was especially clear in comparisons of the rodents *Saccostomus mearnsi* and *Aethomys hindei*, which had broad dietary overlap (Figure S5) and notably similar microbiome compositions given their relatively large phylogenetic distance (Figures 2 and 3). Together, these results reinforce the influence of phylogenetic scale on patterns involving host-microbiome interactions and highlight the challenges associated with generalizing from studies that sample mammalian lineages at broad phylogenetic scales (Godon et al., 2016; Nishida & Ochman, 2018; Reese & Dunn, 2018; Sherrill-Mix et al., 2018).

At the largest phylogenetic scales, gross anatomical differences between host lineages may ensure the differentiation of both diet and microbiome, making it difficult to disentangle the influence of phylogeny and functional traits. For example, whereas previous studies have reported that herbivores generally have greater microbiome diversity than omnivores or carnivores, closer inspections have revealed the dietary effect dissipates when controlling for differences in digestive morphophysiology (e.g., foregut or hindgut fermenters vs. those with simple guts; Nishida & Ochman, 2018; Reese & Dunn, 2018). There is considerable trophic omnivory among rodents in the community that we studied (Bergstrom, 2013), and we found that microbiome diversity in omnivorous rodents tended to be lower than in strictly insectivorous elephant shrews and higher than in strictly herbivorous hares (Figures S5 and S6), suggesting that microbiome diversity may at least sometimes increase with trophic position when controlling for both anatomy (simple guts) and geography. Our comparison of rodents and hares matches the results of another community-level study that found higher microbiome diversity in rodent species compared to a hare species, perhaps reflecting a general pattern whereby rodents with more omnivorous diets and/or higher mass-specific metabolic rates can foster more diverse microbiomes despite having lower average gut sizes than hares (Li et al., 2017). Comparisons of microbiome composition thus depend on both the phylogenetic scale and functional diversity of hosts involved.

The difficulty of sampling all host species from each of our trapping grids prevented spatiotemporal replication of our phylogenetic analyses, but cross-site comparisons of localized phylosymbiotic patterns would be instructive in light of prior work that has shown diets and microbiomes differ among populations (Amato et al., 2013, 2020). Both within and among small-mammal populations, individual-level variation in body size and body condition can occur through time as resource availability, diet quality, and population density vary (Farías et al., 2021; Long et al., 2017). Similarly, microbial communities comprise considerable genetic variation both within and among bacterial taxa that cannot be resolved using 16S rRNA sequencing but that may be resolved by promising new sequencing approaches (e.g., metagenome-assembled genomes, or "MAGs" [Bowers et al., 2018]). Accounting for these sources of variation could reveal patterns in host-microbiome associations beyond what was evident in our study. Prior work has shown that gut microbiome compositions respond to changes in diet on daily

timescales (David et al., 2014), and work to elucidate fine-scale spatiotemporal variation in diet-microbiome linkages could clarify how differences in diet composition might "scale up" to influence phylogenetic patterns (Kartzinel et al., 2019). Notwithstanding these limitations, this small mammal community exhibited sufficient consistency in species-level isotopic niches and microbiome compositions to support this comparative investigation of phylosymbiotic patterns.

Prior studies of phylosymbiosis have emphasized cross-taxon comparisons and many reported relatively low levels of intraspecific replication (e.g., Amato et al., 2019; Grond et al., 2020; Song et al., 2020; Youngblut et al., 2019). By focusing on 14 locally cooccurring small mammal species, we were able to achieve relatively high levels of within-species replication and evaluate the sensitivity of our results to spatiotemporal variation in the samples selected to represent host species. This sensitivity analysis supported our overarching conclusions and highlighted benefits of quantifying intraspecific variation in phylosymbiosis studies. When subsampling microbiome replicates, we obtained Mantel *r* and Robinson-Foulds values that varied widely. Some iterations revealed much larger or smaller values than our complete data set, reinforcing the benefit of accounting for intraspecific variation in comparative phylogenetic studies (Garamszegi & Møller, 2010). Emphasis on sampling phylogenetically distant taxa at the expense of within-species replication could thus complicate efforts to reconcile divergent results in the phylosymbiosis literature (Mallott & Amato, 2021).

The disparate geographic and phylogenetic scales of prior phylosymbiosis investigations have made it hard to generalize. To begin overcoming this challenge, we identified three widely recognized determinants of host-microbiome associations: (i) genetic divergence between host species, (ii) dietary niches of host species, and (iii) functional similarity of host traits. Our initial expectation was that greater divergence in any of these variables would translate into greater microbiome differentiation. Yet, because these variables do not diverge at the same rate, the relative importance of each variable is liable to vary with both the geographic and phylogenetic scales of investigation. Reports of strong phylosymbiotic patterns over broad geographic extents and deep phylogenetic scales may predominate because hosts inevitably differ along multiple functional axes in these comparisons (Ley et al., 2008; Muegge et al., 2011; Song et al., 2020; Youngblut et al., 2019). At intermediate geographic extents and phylogenetic scales, however, studies of closely related hosts may be less likely to identify phylosymbiosis because closely related species can persist in different communities without an ecological requirement to differ in morphology, diet, or other functional axes that shape their microbiome (Grond et al., 2020; Lutz et al., 2019). By focusing within communities, researchers may be better equipped to tease apart the interplay of variables that shape the microbiomes of wild animals (Kartzinel et al., 2019; Knowles et al., 2019; Moeller et al., 2013). At this scale, there are theoretical limits on the cooccurrence of species with similar characteristics (Chesson, 2000), and these limits are borne out in the sized-structured rodent communities of arid ecosystems worldwide (Bowers & Brown, 1982; Kelt et al., 1996). The extent of phylosymbiosis in ecological communities could thus be mediated by a balance between the divergent natural histories of closely related species and the convergent functional characteristics that enable more distant lineages to occupy a similar niche.

Our results show that deep evolutionary splits between mammalian lineages established phylosymbiotic patterns in gut microbiomes and that the strength of these patterns depends on the phylogenetic scales of hosts under investigation. This may be why phylosymbiosis is often absent in investigations of species groups that occur close to the tips (Amato et al., 2019; Kartzinel et al., 2019; Ley et al., 2008; Muegge et al., 2011; Song et al., 2020). Do distantly related hosts share bacterial taxa that enable their convergent use of shared dietary resources? Do diet-microbiome linkages differentially influence host sensitivity to local environmental changes? Do metagenomes facilitate hosts' use of different limiting resources in ways that stabilize coexistence? Whereas prior studies have employed the tools of community ecology to characterize bacterial assemblages, there may be new opportunities to leverage knowledge of microbiomes to study the assembly of functionally diverse host communities in return.

AUTHOR CONTRIBUTION

Bianca R. P. Brown and Tyler R. Kartzinel conceived and designed study; Bianca R. P. Brown, Tyler R. Kartzinel, Jacob R. Goheen, and Leo M. Khasoha conducted fieldwork; Jacob R. Goheen, Robert M. Pringle, and Todd M. Palmer established the study plots; Bianca R. P. Brown and Tyler R. Kartzinel led microbiome laboratory work; Seth D. Newsome led isotope data analysis; Bianca R. P. Brown analysed the data and wrote the initial manuscript; all authors contributed to the final manuscript.

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DATA AVAILABILITY STATEMENT

Illumina 16S rRNA data have been submitted to NCBI's Sequence Read Archive (Accession: PRJNA915597) and the host mtDNA barcodes have been submitted to GenBank (Accession numbers: OQ442724-OQ442745 and OQ124111-OQ124147). Tables

summarizing the taxonomy of 16S rRNA sequences, their total counts in each sample, and their rarefied RRA levels per sample have been posted to Dryad (10.5061/dryad.w0vt4b8tz).

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SUPPORTING INFORMATION

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