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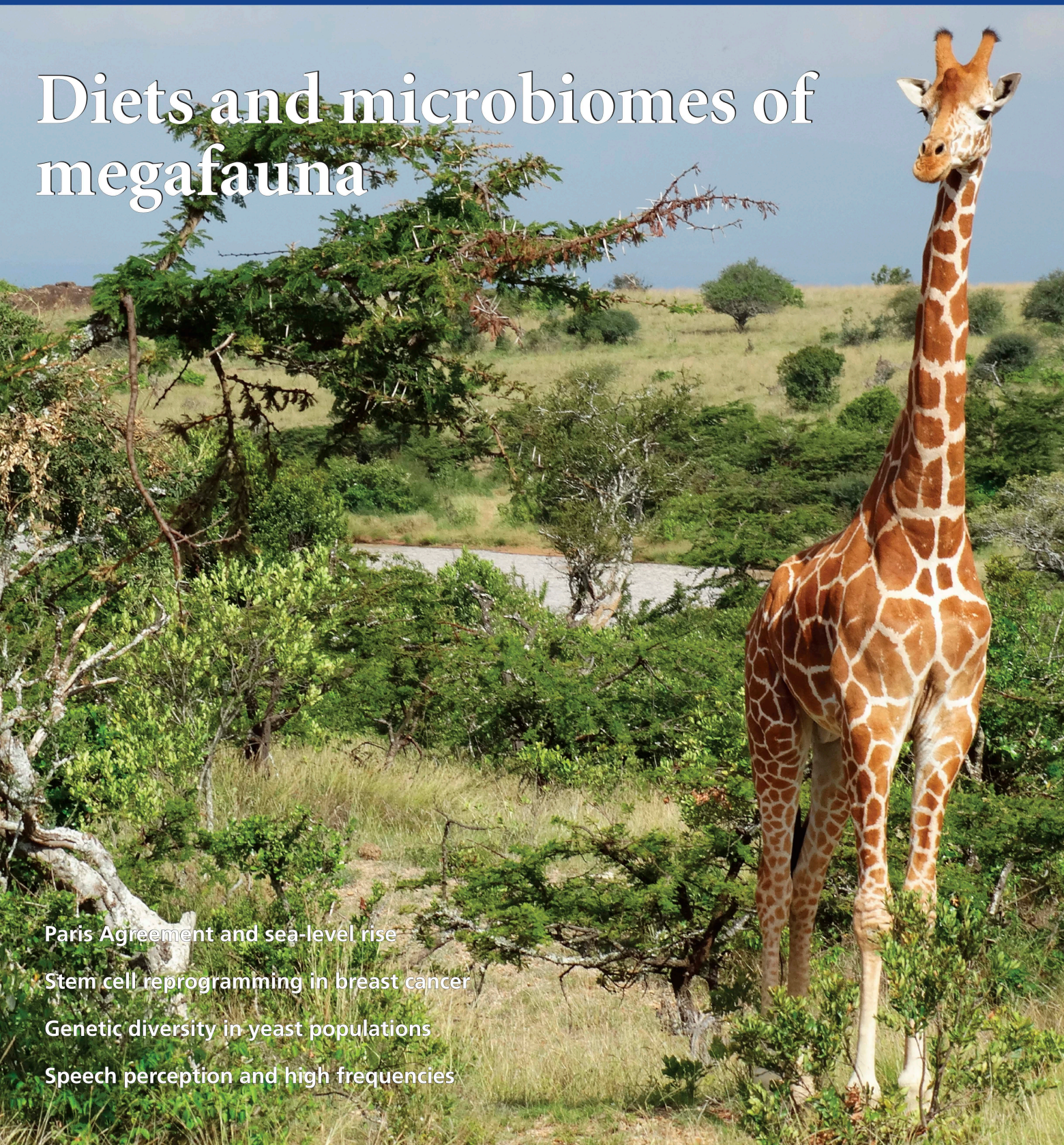
Diets and microbiomes of megafauna



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Covariation of diet and gut microbiome in African megafauna

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A major challenge in biology is to understand how phylogeny, diet, and environment shape the mammalian gut microbiome. Yet most studies of nonhuman microbiomes have relied on relatively coarse dietary categorizations and have focused either on individual wild populations or on captive animals that are sheltered from environmental pressures, which may obscure the effects of dietary and environmental variation on microbiome composition in diverse natural communities. We analyzed plant and bacterial DNA in fecal samples from an assemblage of 33 sympatric large-herbivore species (27 native, 6 domesticated) in a semiarid East African savanna, which enabled high-resolution assessment of seasonal variation in both diet and microbiome composition. Phylogenetic relatedness strongly predicted microbiome composition (r = 0.91) and was weakly but significantly correlated with diet composition (r = 0.20). Dietary diversity did not significantly predict microbiome diversity across species or within any species except kudu; however, diet composition was significantly correlated with microbiome composition both across and within most species. We found a spectrum of seasonal sensitivity at the diet–microbiome nexus: Seasonal changes in diet composition explained 25% of seasonal variation in microbiome composition across species. Species' positions on (and deviations from) this spectrum were not obviously driven by phylogeny, body size, digestive strategy, or diet composition; however, domesticated species tended to exhibit greater diet–microbiome turnover than wildlife. Our results reveal marked differences in the influence of environment on the degree of diet–microbiome covariation in free-ranging African megafauna, and this variation is not well explained by canonical predictors of nutritional ecology.

16S rRNA | DNA metabarcoding | megaherbivores | phylosymbiosis

Links between diet and the gut microbiome are important for health, nutrition, and ecology in mammals. Gut bacteria modify immune responses to maintain health (1), and perturbations in the gut microbiome can cause disease (2). Mammalian herbivores associate with particular gut microbial taxa, in part because they rely on these bacteria to extract energy and nutrients from food, synthesize vitamins, and detoxify plant defense compounds (3). The many services that gut microbes provide to their hosts almost certainly influence not just individual fitness but also the dynamics of the populations, communities, and food webs in which individuals are embedded (4).

The mammalian gut flora has traditionally been studied in the context of 3 major drivers of variation: phylogeny, diet type, and environment. The phylogeny of mammals reflects the evolution of diverse diets and digestive morphophysiologies, as well as coevolution with symbiotic gut bacteria (5). Closely related mammalian species tend to share similar body plans, craniofacial anatomies, and gut architectures. Similarities between species' evolutionary histories and their characteristic gut microbiomes suggest that phylosymbiosis—concordant evolutionary divergence between species and their degree of microbiome dissimilarity (6)—is a globally common phenomenon. Many mammalian lineages have evolved disparate diet types that are associated with different microbiome compositions, such as herbivory and carnivory (5). Moreover, subgroups of species with similar diet–microbiome associations can be distinguished within broad trophic categories; for example, carbon stable isotope ratios that discriminate between grazing and browsing herbivores are associated with differences in gut microbiome compositions (5). At finer grains, studies that experimentally impose dietary treatments (7) or rely on self-reporting of diets by humans (8) have shown that the microbiome is dynamic and responsive to subtle dietary changes within individuals. As a result, microbiome variation among individuals from the same population, among populations of the same species in different environments, and among populations of closely related species is often interpreted in light of dietary variation (3, 9, 10).

Diet–microbiome covariation is predicted to be common, on the grounds that diet composition is a principal determinant of microbial niche diversity in the gut (11). For example, diets containing more high-fiber and plant-based foods should generate a greater diversity of microbial niches than those that do not (5). Likewise, temporal variation in diet could increase the heterogeneity of microbial niches and thereby increase microbiome diversity. However, data deficiencies currently limit even basic comparisons of dietary and microbiome diversity for most mammalian species, which inhibits theoretical development and impedes efforts to explain the causes and consequences of

Significance

Diet and gut microbiome composition are important for health and nutrition in mammals, but how they covary in response to environmental change remains poorly understood—both because diet composition is rarely quantified precisely, and because studies of diet–microbiome linkages in captive animals may not accurately reflect the dynamics of natural communities. By analyzing diet–microbiome linkages in an assemblage of large mammalian herbivores in Kenya, we found that seasonal changes in diet and microbiome composition were strongly correlated within some populations, whereas other populations exhibited little temporal turnover in either diet or microbiome. Identifying mechanisms that generate species-specific variation in the sensitivity of the diet–microbiome nexus to environmental changes could help to explain differential population performance and food-web structure within ecological communities.

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Data deposition: Illumina data and unrarefied sequence count tables are available at Dryad (https://doi.org/10.5061/dryad.c119gm5); mitochondrial DNA sequences are available at GenBank (accession nos. MN262920–MN262991 and MN262700–MN262919).

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microbiome variation (11). Exploring the strength, extent, and generality of diet–microbiome covariation within and between species could help to contextualize the patterns observed across studies and to guide further investigation into the functional significance of variation in gut microbiome composition.

Understanding species-specific differences in diet–microbiome linkages may require studying them in a community context. Many prior studies have investigated species in isolation by drawing samples from geographically isolated populations of different wild species, or from animals kept in controlled settings such as laboratories or zoos (5, 12). The strength of this approach is that it allows researchers to sample species from clades that span the mammalian tree of life and host disparate microbiota; the drawback is that it often leads to comparisons of animals that have evolved and naturally occur within disparate biomes, which might confound the influences of phylogeny, functional morphology, and ecology (13). In addition, animals in controlled settings are rarely able to choose their own diets and are typically not subjected to competition, predation risk, or seasonal variation in the availability of food and water. Studies of populations of the same species from seasonally or geographically heterogeneous environments have often found that dietary changes are associated with microbiome changes (14, 15). These studies provide deeper understanding of intraspecific diet–microbiome covariation in animals that occupy similar habitats and are subjected to natural environmental pressures, but typically include only one or a few species from the entire community. Comparative studies of species sampled at different times and places are inevitably limited in their ability to capture the effects of species interactions—yet we know that animals' interactions with each other, their foods, and their abiotic environments have profound effects on fitness and ecological networks (16–18).

One long-standing obstacle to studying community-wide patterns of diet–microbiome covariation has been the difficulty of precisely identifying foods eaten by free-ranging animals. DNA-based analysis of fecal samples now enables concurrent characterization of both diet and microbiome, creating the opportunity to test for fine-grained variation at the diet–microbiome nexus (19, 20). We performed such analyses on a community of 33 large mammalian herbivore species in a semiarid Kenyan savanna (Fig. 1*A*). The functionally diverse members of this community represent 7 mammalian orders, span sizes from ∼2 to ∼4,000 kg, have various gut anatomies (ruminants, pseudoruminants, hindgut fermenters), include both wild (n = 27) and free-ranging domesticated (n = 6) species (as well as 8 globally threatened and near-threatened species), and occupy a range of herbivore feeding guilds (grazers, browsers, and mixed feeders).

We used these data to evaluate 4 hypotheses about the mammalian gut microbiome that have not previously been tested in a community context. First, we hypothesized that phylogeny is a stronger predictor of microbiome richness and composition (reflecting phylosymbiosis) than it is of dietary richness and composition—in part because the competitive pressures of sympatric coexistence should promote dietary niche differentiation among otherwise similar species but permit resource overlap among otherwise dissimilar species (21, 22). Second, we hypothesized that the individuals and species with the most diverse diets also have the most diverse microbiomes, on the grounds that more diverse diets should be associated with a greater diversity of microbial niches (11). Third, we hypothesized that

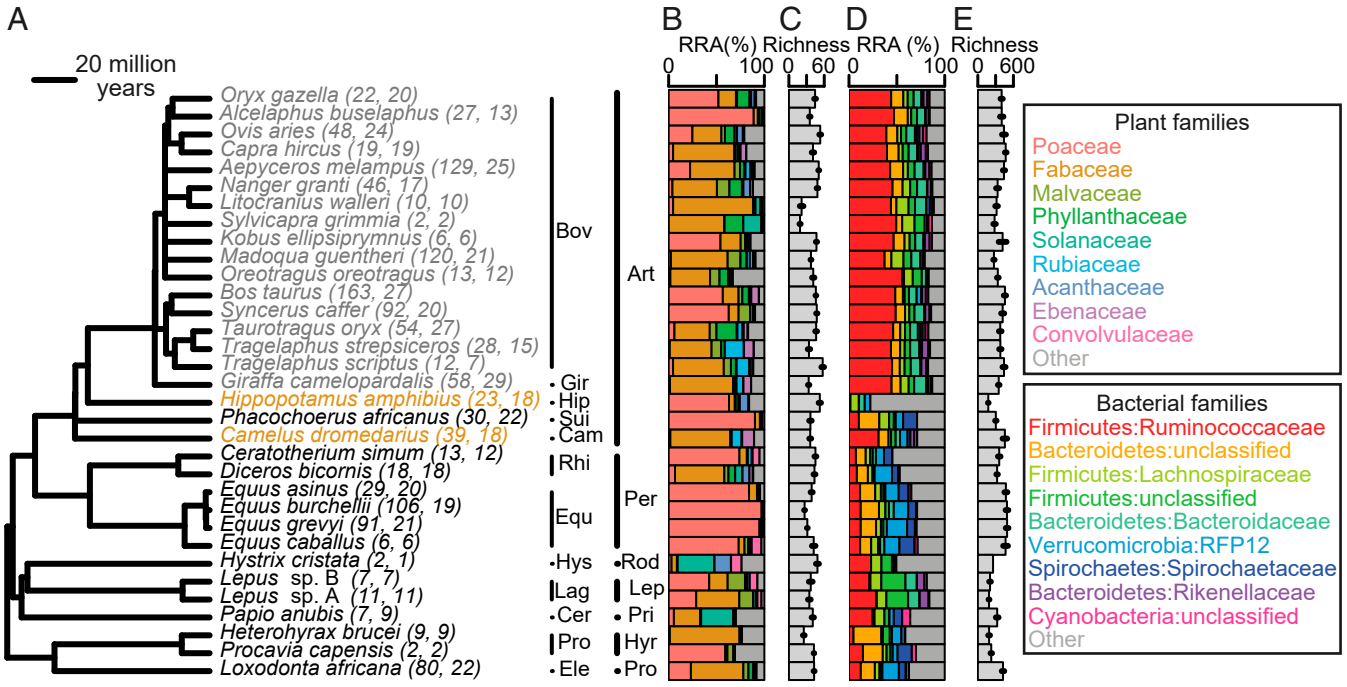

Fig. 1. Phylogenetic variation in diet and gut microbiome composition. (*A*) The phylogeny of 33 sympatric mammalian herbivores in central Kenya, grouped by family and order (identified here by the first 3 letters of families and orders; see also Dataset S1). Species names are in gray for ruminants (toward the top), orange for pseudoruminants (hippo and camel), and black for nonruminants. Sample sizes for each species are listed parenthetically (diet, microbiome). (*B*) The mean RRA of the 9 most eaten plant families and the 45 other plant families, expressed as percentages. There was modest phylogenetic signal in grass (Poaceae) RRA (Pagel's $\lambda = 0.55$, $P = 0.03$), but no phylogenetic signal in the RRA of other abundant plant families (Fabaceae: $\lambda = 0.20$, $P > 0.05$; Malvaceae: $\lambda = 0.62$, $P > 0.05$). (*C*) Mean dietary richness (±1 SE) did not exhibit significant phylogenetic signal ($\lambda < 0.01$, $P \approx 1.0$; diversity yielded similar results: $\lambda < 0.01$, $P \approx 1.0$; Dataset S1). (*D*) Mean RRA of the 9 most prevalent clades of gut bacteria (identified to family when possible and listed by phylum), along with the remaining ≥238 other clades (gray). There was significant phylogenetic signal in mean RRA for 2 of the 3 predominant bacterial families that were identified (Ruminococcaceae: $\lambda \approx 1.0$, $P < 0.001$; Bacteroidaceae: $\lambda = 0.93$, $P < 0.001$; not Lachnospiraceae: $\lambda = 0.98$, $P > 0.05$). (*E*) Mean microbial richness (±SE) exhibited significant phylogenetic signal ($\lambda \approx 1.0$, $P < 0.001$; diversity yielded similar results: $\lambda \approx 1.0$, $P < 0.001$; Dataset S1).

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individuals and species with the most dissimilar diet compositions also have the most dissimilar microbiome compositions, given that variation in the traits, behaviors, and environmental influences that promote differences in diet should also promote differences in microbiome (5, 23). Fourth, we hypothesized that species with the most seasonally variable diets also have seasonally variable gut microbiomes, as expected if microbiome composition is tightly linked to diet composition (14). Based on this hypothesis, we predicted that mixed-feeding herbivores—which frequently switch between grass- and browse-dominated diets in response to seasonal variation in grass availability (higher in wet seasons)—would exhibit the strongest seasonal turnover in their gut microbiomes, for both wild and domesticated species and regardless of gut anatomy.

Results

Diet and Microbiome Composition. We collected fresh fecal samples from 33 mammal species in Laikipia, Kenya, during 5 sampling periods that spanned both wet and dry seasons over 4 y (*SI Appendix*, Texts S1 and S2). We used DNA metabarcoding to characterize diets and microbiomes, sequencing the plant *trn*L-P6 marker and the bacterial 16S-V4 ribosomal RNA marker (refs. 24 and 25 and *SI Appendix*, Text S1). We identified DNA sequences by comparison to a local plant reference database and the GreenGenes bacterial database (refs. 26 and 27 and *SI Appendix*, Texts S3–S5). In total, we analyzed plant DNA from 1,322 fecal samples (median, 23 samples per species; range, 2 to 163) and bacterial DNA from 509 fecal samples (median, 18 samples per species; range, 1 to 29; Dataset S1).

Diets and microbiomes differed widely across herbivore species (Fig. 1). We identified 213 unique food-plant sequences from 54 plant families, out of at least 460 plant species from 66 families that are known to occur at this site (27). These food plants were utilized differently by herbivore species (Dataset S2). Dietary richness averaged 19 to 57 taxa per sample (Fig. 1). The 3 plant families with the highest overall mean relative read abundance (RRA) were Poaceae (grasses; range of mean RRA across species, 0 to 97%; median, 23%), Fabaceae (legumes; range, 2 to 83% RRA; median, 30%), and Malvaceae (mallows; range, 0 to 18% RRA; median, 4%; Fig. 1). The microbiome data included 29,308 bacterial amplicon sequence variants from at least 35 phyla (range, 177 to 494 variants per sample; Dataset S3). The most abundant bacterial families were Ruminococcaceae (range of mean RRA across species, 1 to 55%; median, 37%), Lachnospiraceae (range, 2 to 13% RRA; median, 5%), and Bacteroidaceae (range, 0 to 13% RRA; median, 8%; Fig. 1).

Phylogenetic Signals. Herbivore species differed in the richness and composition of their diets and microbiomes in ways that supported our first hypothesis. Consistent with the conventional categorization of these species into feeding guilds, grass RRA was a key axis of dietary differentiation (Fig. 1 and *SI Appendix*, Fig. S1). There was no statistically significant phylogenetic signal among herbivore species in the mean richness or diversity of plant taxa eaten, and pairs of closest relatives often differed starkly in their relative consumption of plants from different families (Fig. 1). There was a modest but statistically significant phylogenetic signal in grass consumption, but we found no significant phylogenetic signal in the RRA of other major food plant families (Fig. 1). Congeneric zebras, horses, and donkeys (Equidae, *Equus* spp.) all consumed predominantly grass-based diets (range of mean grass RRA, 73 to 97%), whereas other sets of confamilial herbivore taxa diverged sharply in grass RRA: bovids (Bovidae), 0 to 89%; hyraxes (Procaviidae), 59% vs. 1%; rhinos (Rhinocerotidae), 74% vs. 7%; and hares (Leporidae), 42% vs. 29%. In contrast to diet, there was strong phylogenetic signal in the mean richness and diversity of the microbiome. There was also strong phylogenetic signal in the RRA of 2 major bacterial families (Ruminococcaceae and Bacteroidaceae; Fig. 1). Including all plant and bacterial taxa in the analysis, we found significant positive correlations between the phylogenetic distance

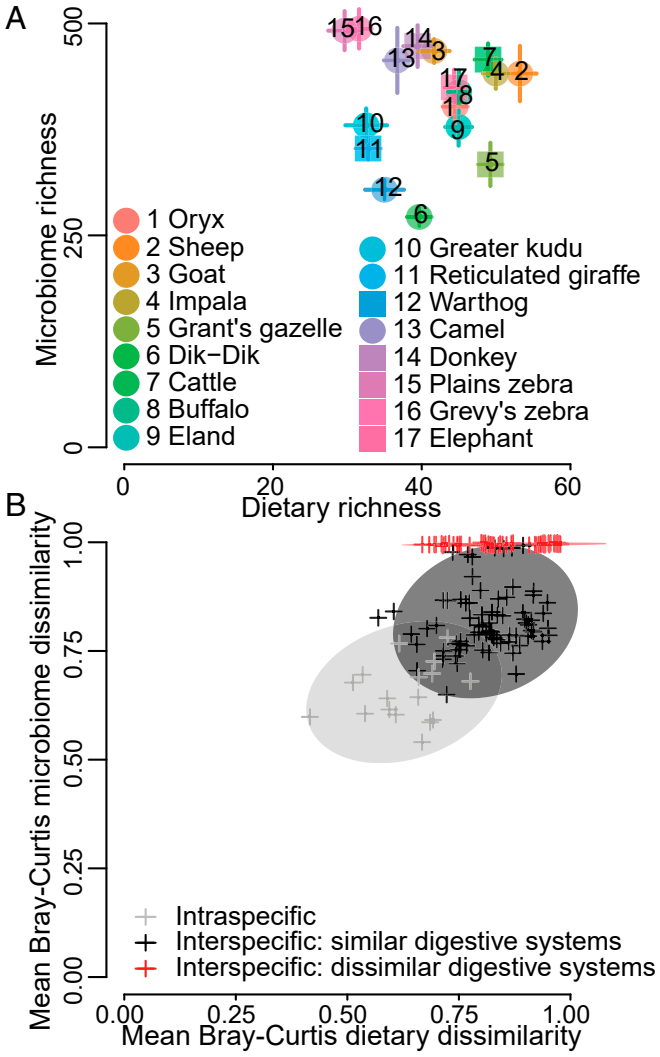

Fig. 2. Dietary richness did not predict microbiome richness across species, but diet composition did predict microbiome composition. (*A*) We found no relationship between mean dietary and microbiome richness (±1 SE) across species (ordinary least squares, OLS: $F_{1,15} < 0.01$, $R^2 < 0.001$, $P = 0.96$; phylogenetic generalized least squares, PGLS: $F_{1,15} < 0.01$, $R^2 < 0.001$, $P = 0.95$). Point colors correspond to the ordering of species from top (oryx) to bottom (elephant) of the phylogeny in Fig. 1 (squares, nonruminants; circles, ruminants/camels). Intraspecific correlations between dietary and microbiome richness within these 17 species are shown in *SI Appendix*, Fig. S3. (*B*) Microbiome dissimilarity within and between pairs of species increased with dietary dissimilarity. Intraspecific comparisons were the most similar (gray crosses), followed by interspecific comparisons between species with similar digestive systems (black crosses). Comparisons between species with dissimilar digestive systems (i.e., ruminants/camels vs. nonruminants, red crosses) had almost entirely distinct microbiomes (all dissimilarities > 0.99), irrespective of dietary overlap (dissimilarities ranging 0.67 to 0.97). Shading represents 95% confidence ellipses. Interspecific comparisons (black and red) revealed a significant increase in microbiome dissimilarity with diet dissimilarity, after accounting for the phylogenetic relatedness of species (partial Mantel: $r = 0.28$, $P = 0.005$). The correlation between intraspecific diet–microbiome dissimilarities across species (gray) was not statistically significant, but the trend was positive (OLS: $F_{1,15} = 0.93$, $R^2 = 0.06$, $P = 0.35$; PGLS: $F_{1,15} = 0.24$, $R^2 = 0.02$, $P = 0.62$). Analagous diet–microbiome comparisons among samples within each species were generally strong and positive (*SI Appendix*, Fig. S4).

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separating herbivore species and the dissimilarity of both their diets and their microbiomes (*SI Appendix*, Fig. S2). The stronger association between phylogeny and microbiome composition (r = 0.91) compared to diet composition (r = 0.20) indicates that shared evolutionary history more strongly constrains closely related species to associate with similar gut bacteria than to share food plants (*SI Appendix*, Fig. S2).

Diet–Microbiome Covariation within and among Species. Microbiome richness was not consistently correlated with dietary richness. Contrary to our second hypothesis that species and individuals with more species-rich diets also have more diverse microbiomes, we found no significant correlation between mean dietary and microbiome richness across species (Fig. 2*A*). Likewise, dietary richness did not strongly predict microbiome richness within species: Only one species (kudu) exhibited a significant correlation (positive) between diet and microbiome richness (*SI Appendix*, Fig. S3 and Table S1).

Consistent with our third hypothesis, there were generally strong correlations between the compositional dissimilarities of diets and microbiomes. Microbiome dissimilarity increased with dietary dissimilarity after accounting for phylogenetic distance between species (Fig. 2*B*). This interspecific correlation in diet–microbiome dissimilarity was heavily influenced by differences between ruminants and nonruminants, which had almost entirely nonoverlapping microbiomes (Bray–Curtis dissimilarity of ∼1)—even when their diets were no more dissimilar than pairs of species with similar digestive systems. Within species, we found a wide range of mean Bray–Curtis dissimilarities among individuals for both diet (0.41 to 0.82) and microbiome (0.54 to 0.78), and these ranges were nearly as broad as the corresponding ranges between species (diet, 0.53 to 0.98; microbiome, 0.65 to 1.0; Dataset S4). Across samples within species, microbiome dissimilarity increased significantly with diet dissimilarity in all but 3 cases (Grevy's zebra, elephant, and dik-dik; *SI Appendix*, Fig. S4 and Table S2).

Seasonal Variation and Covariation in Diet and Microbiome. We evaluated seasonal diet–microbiome linkages within and among species. For this, we used a subset of the data from the 17 best-sampled species, comprising 365 samples that were analyzed for both diet and microbiome (≥15 total samples per species across the 3 largest sampling bouts, and ≥3 samples per species within each bout; range 3 to 17, median 6 samples per species per season). Consistent with our fourth hypothesis, we found a significant positive correlation between the degree of seasonal turnover in diet and microbiome composition across species (Fig. 3*A*). Overall, diet turnover explained 25% of the variance in microbiome turnover; however, several species exhibited considerably higher or lower microbial turnover than predicted by regressions based on diet turnover alone (Fig. 3*A*). In contrast to our prediction that the strongest seasonal turnover would occur in mixed feeders (e.g., elephant, impala), species across the grazer–browser continuum occupied various positions along the seasonal sensitivity spectrum (Fig. 3). The low end of this spectrum included species such as elephant, which exhibited almost as much variation within seasons as between seasons (Fig. 3*D*); other species from multiple feeding guilds exhibited differences between at least the wettest and driest seasons (e.g., Fig. 3 *E* and *F*); and camels (browsers) exhibited uniquely high seasonal turnover in both diet and microbiome (Fig. 3*G*). In general, domesticated species (which occupied disparate positions across the grazer–browser continuum and the phylogeny) exhibited greater turnover in both diet and microbiome than did wild species (Fig. 3 *B* and *C*).

All species differed significantly in diet and microbiome composition across seasons, with the lone exception of the elephant microbiome (*SI Appendix*, Tables S3 and S4). However, microbiome variation among samples mapped significantly onto diet composition (using Procrustes analysis) for only 8 of 17 species, which included ruminants and hindgut fermenters, grazers and browsers, and wild and domesticated species (*SI Appendix*, Fig. S5). These 8 species included all 5 of the domesticated species, but not their closest wild relatives (e.g., cattle but not buffalo; donkey but not zebras). The proportion of variation explained by season was much broader for diet (range, 12 to 73%; median, 27%; *SI Appendix*, Table S3) than for microbiome, which was always low (range, 13 to 28%; median, 20%; *SI*

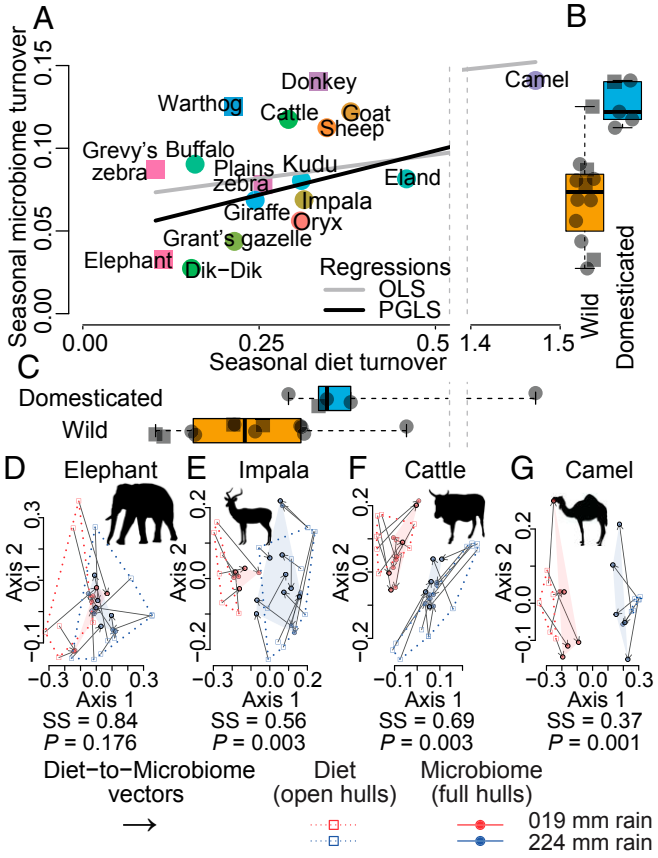

Fig. 3. A spectrum of seasonal sensitivity in diet–microbiome covariation. (*A*) Seasonal turnover in diet composition was positively correlated with seasonal turnover in microbiome composition (squares, nonruminants; circles, ruminants/camels). Trend lines were fit using OLS ($F_{1,15}$ = 5.0, R^2 = 0.25, P = 0.041) and PGLS ($F_{1,15}$ = 5.5, R^2 = 0.27, P = 0.034). Excluding camels, an extreme outlier in diet turnover, would yield similar results, although OLS regression would only be marginally significant (OLS: $F_{1,14}$ = 3.3, R^2 = 0.19, P = 0.090; PGLS: $F_{1,14}$ = 7.5, R^2 = 0.35, P = 0.016). (*B* and *C*) There was greater turnover in domesticated vs. wild species in (*B*) microbiome (phylogenetic ANOVA: $F_{1,15}$ = 19.2, P < 0.001) and (*C*) diet ($F_{1,15}$ = 4.95, P = 0.042). Boxplots show ranges (whiskers), interquartile ranges (boxes), and medians (central lines). (*D*–*G*) Using examples of 4 species at increasing distances from the origin in *A*, we performed Procrustes analyses to visualize how seasonal diet and microbiome compositions mapped onto each other. Procrustes rotates the results of separate principal coordinates analyses of diet composition (open symbols) and microbiome composition (closed symbols) so that results can be compared. For illustration, we show results from the driest and wettest sampling periods, with color-coded minimum convex hulls drawn around the set of points representing diet (open hulls) and microbiome (shaded hulls) compositions in each season. Vectors (arrows) show correspondence between diet and microbiome for each fecal sample; shorter vectors indicate closer compositional correspondence. Below each panel is the Procrustes sum of squares and P value testing whether there is significant dissimilarity in configuration between the diet and microbiome ordinations. Procrustes analyses for all 17 species and all 3 seasons are provided in *SI Appendix*, Fig. S5.

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Appendix, Table S4). The relative abundance of the predominant bacterial families was similar across seasons within species, but the overall richness of microbial taxa differed seasonally for 9 of the 17 species (*SI Appendix*, Table S4 and Fig. S6). Across species, diets were often dominated by relatively few plant taxa in the wet season and were more diverse in the dry season (*SI Appendix*, Fig. S6). For example, camels—the extreme outlier in Fig. 3—specialized on a single shrub species in the wet season (*Acacia brevispica*, mean RRA of 87 to 92% vs. 18% in the dry season) but consumed a more even variety of plants in the dry season (including other *Acacia* species and the shrub *Euclea divinorum*).

Discussion

By focusing on an entire sympatric large-herbivore community and using fine-grained dietary data, we sought to illuminate how host phylogeny, diet, and environmental variation influence diet–microbiome linkages in the presence of natural species interactions and in the absence of confounding geographic variation. We found strong support for hypotheses about diet–microbiome linkages within and across species (Figs. 1 and 2), but less support for hypotheses related to diversity or predictions about the seasonal drivers of diet–microbiome covariation (Figs. 2 and 3). Our results build on previous research into the dietary ecology of African herbivores (21, 28) and the composition of gut microbiomes across the mammalian phylogeny (5, 12), while also revealing marked variation in the degree of diet–microbiome covariation within and among cooccurring species. In particular, we reveal a previously undocumented spectrum in the sensitivity of diet–microbiome covariation to environmental fluctuations (Fig. 3). This seasonal sensitivity spectrum—which could only be detected in light of dietary and microbial data that are highly resolved both temporally and taxonomically—defies explanation by canonical predictors of nutritional ecology (phylogeny, gut morphology, diet type) but does appear to be influenced by species' history of domestication.

Consistent with our first hypothesis, both diet and microbiome were constrained by phylogeny, but the constraint on diet was weaker than that on the microbiome. The weak but significant effect of phylogeny on diet composition accords with competition and niche theory, as ecological constraints on the degree of resource overlap between closely related species should run counter to any phylogenetic constraints on diet composition; in this community, neither constraint has completely obscured the other. Our results affirmed well-known differences in the bacterial lineages that occupy and facilitate digestion in mammals (5), but also revealed unexpected variation among species within these groups. Specifically, and contrary to our second hypothesis, species and individuals with the most diverse diets generally did not have the most diverse microbiomes (Fig. 2). These results are consistent with those previously reported for 2 sympatric equid species (19), but encompass a much larger and more functionally diverse set of species. Furthermore, even though we found that animals with more dissimilar diets also had more dissimilar microbiomes (per our third hypothesis; Fig. 2), and that species with a high degree of seasonal diet turnover also had extensive microbiome turnover (per our fourth hypothesis; Fig. 3), we did not find support for a specific prediction of our fourth hypothesis—namely that mixed-feeding herbivores exhibit the most pronounced temporal turnover in diet and microbiome (22).

Our DNA-based measure of seasonal dietary turnover within each species explained only 25% of the variation in the degree of microbiome turnover (Fig. 3). The unexplained variation is interesting because this community included sets of closely related species and species with similar diets, digestive morphologies (ruminant vs. nonruminant), and body sizes (and hence gut capacities). These phylogenetic and functional characteristics are all known to contribute to microbiome differentiation between species (5, 12), and we therefore expected sets of similar species to respond to seasonal variation in similar ways. The data did not support this expectation. We found, instead, that livestock tended to have stronger diet–microbiome linkages and greater temporal turnover than did wildlife (Fig. 3 and *SI Appendix*, Figs. S4 and S5), even when controlling as much as possible for phylogenetic and functional differences. For example, giraffes and camels occupied similar dietary niches (*SI Appendix*, Fig. S1), but camels had considerably greater microbiome diversity (~30% greater richness) and were an outlier in temporal diet–microbiome turnover (Fig. 3). Similarly, donkeys and zebras are all members of the same genus, all consumed overwhelmingly grass-based diets, and all had similar microbiome diversity (Fig. 1), yet donkeys had much greater diet–microbiome turnover than did either zebra species (Fig. 3). These results contrast with a prior study showing that anthropogenic influences reduced microbiome diversity in a domesticated species relative to a cooccurring wild relative (both equids; ref. 19) and suggest potent anthropogenic effects on both the diets and microbiomes of free-ranging livestock (9).

Obvious potential differences between livestock and wildlife are unlikely to explain our result: These species occupy the same areas within the same landscape, and livestock are herded but choose foods freely without supplemental forage or routine antibiotic treatment (see *Methods*). It is therefore necessary to consider more nuanced hypotheses. Relative to domesticated species, wildlife might have 1) greater variation in traits that underpin plant–herbivore or host–microbiome interactions, 2) more social and demographic heterogeneity, and/or 3) less ability to forage optimally. These nonmutually exclusive possibilities assume different mechanisms underpinning diet–microbiome linkages. The cost-of-domestication hypothesis posits that domestication reduces genetic variation through inbreeding and selective breeding (29), which could homogenize diets and microbiomes within populations (23). The social-network hypothesis posits that physical proximity and demographic similarity constrain foraging opportunities and enhance microorganism transmission, thereby homogenizing diets and microbiomes (15, 30). These homogenizing influences may act strongly on livestock populations, which tend to have biased sex ratios, even age distributions, and relatively dense feeding and sleeping aggregations (31). Finally, optimal foraging theory posits that animals should minimize energetic costs while maximizing food intake (32). There is disagreement about whether domestication is likely to relax selection on "optimal" foraging behavior (33) or to intensify it (34). Theoretically, energetically costly strategies that involve frequent movements between food patches might be necessary for wildlife to reduce the risk of predation, but might be unnecessary for domesticated species that are protected by humans (31, 35); experimentally, at least some domesticated ungulates are genetically more predisposed to energy-saving, infrequent movements than are wildlife (34). Understanding whether these mechanisms contribute to the seasonal sensitivity spectrum could help determine the extent to which such sensitivity represents a cost or a benefit of domestication under different environmental conditions.

Challenges inherent to sampling an entire community of free-ranging animals and caveats inherent to correlative datasets limit our ability to understand sources of variation in the strength of diet–microbiome linkages among species. Limitations inherent to DNA metabarcoding may also obscure variation in animal nutrition, as animals that eat different parts of the same food plant may diverge in nutritional status without diverging in DNA-based dietary profiles. Longer community-level time series of change in both diet and microbiome could be generated using the methods employed here (8, 20), but would be subject to similar limitations. Perhaps more usefully, resources could be manipulated

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experimentally to strengthen inferences about causality in diet–microbiome relationships.

Two complementary bodies of theory suggest that understanding diet–microbiome linkages can improve our understanding of how species interactions shape communities by differentially affecting fitness and population growth. Coexistence theory emphasizes resource competition and niche differentiation within guilds (36), and food-web theory emphasizes trophic regulation of populations (37). Both frameworks can account for species-specific differences in traits such as body size, social structure, and diet selectivity that affect how individuals balance the need for resources against the risk of predation or infection (22, 28). Whereas these characteristics can clearly affect fitness, the health and fitness consequences of microbiome compositions are only well established in biomedical studies on humans and model organisms (1, 3). If diet–microbiome linkages affect demographically vital processes such as health, stamina, and predator evasion, then they could shape the fitness, trophic networks, and coexistence of free-ranging animals.

Methods

We collected samples from adjoining wildlife conservancies with active ranching operations (Mpala and Ol Jogi; Dataset S1 and *SI Appendix*, Text S1 and Fig. S7). Livestock in these conservancies are occasionally treated with antibiotics during disease outbreaks, but no such outbreaks were reported during our study, and, to our knowledge, none of the animals we sampled were treated. Livestock forage and drink during the day following routes that are determined by herders, and are protected from predators at night within corrals (31). We used mammalian mitochondrial DNA markers to confirm the source of a subset of samples, including 2 species of hare that are visually indistinguishable in the field and require further taxonomic investigation (here designated *Lepus* A and B; *SI Appendix*, Text S2). We used Illumina sequencing to generate diet and microbiome data. We identified these sequences, and rarefied the resulting data to enable comparisons of plant and bacterial taxa as percentages of the total rarefied sequences within samples (i.e., RRA; *SI Appendix*, Texts S3–S5). Prior use of this protocol indicated that RRA is likely to be a reliable proxy for consumption of plants at our study site, based on a strong correlation between proportional grass consumption measured using RRA and stable-isotope ratios ($\delta^{13}C$) from feces (38). Although RRA may not always accurately reflect quantitative consumption (39), its use for diet analysis with *trn*L-P6 has been validated using isotopic analyses and feeding trials (40, 41). Together with a published mammalian megaphylogeny (42), we evaluated support for our 4 hypotheses about dietary and microbiome richness, diversity, and dissimilarity (*SI Appendix*, Text S6).

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