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Industrialization restructures the domestic dog gut microbiome while preserving host specificity

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ABSTRACT Industrialization has reshaped human gut microbiomes, but its effects on other human-associated mammals remain poorly understood. Domestic dogs provide an informative comparative system because they have shared human environments and food systems for millennia yet retain distinct host biology. However, most canine microbiome studies have focused on industrialized companion, colony, or controlled-feeding dogs, limiting our understanding of the ecological range of the domestic dog gut microbiome. We analyzed fecal 16S rRNA gene profiles from 261 dogs sampled across Nepal, Thailand, the United Arab Emirates, and the United States, spanning forager, agrarian, pastoralist, urban, and industrialized lifestyles; 257 dogs remained after excluding those with reported recent antibiotic exposure. Lifestyle was the strongest measured correlate of canine gut bacterial composition, and this association persisted in restricted analyses of mature, non-shelter dogs sampled from temperate regions. Industrialized dogs differed from non-industrialized dogs through directional genus-level shifts, prevalence-defined VANISH-like and BloSSUM-like bacterial guilds, coabundance structure, and shifts in predicted functional potential. Non-industrialized dogs were not microbiologically uniform: pastoralist dogs showed non-industrialized gut bacterial community profiles but diverged from a simple forager-to-industrialized continuum. Cross-species comparisons with humans sampled across matched lifestyle categories showed parallel lifestyle-associated restructuring in both hosts, but host species remained the dominant axis of variation, and genus-level responses were largely host specific. These findings expand the ecological baseline for canine gut bacterial communities and identify industrialization-associated lifestyle change as a major axis of gut bacterial community restructuring in a long-term human-associated mammal. More broadly, they show that shared lifestyle transitions can impose parallel ecological pressures across host species without overriding host-specific community assembly.

IMPORTANCE Domestic dogs are one of the closest animal companions of humans, yet we know surprisingly little about how their gut microbiomes vary outside industrialized pet populations. This study shows that industrialization-associated lifestyle change is not only relevant to human microbiomes but also a broader ecological force that reshapes gut microbial communities in animals living alongside us. By comparing dogs across diverse human lifestyles, we show that shared environments can impose parallel pressures on the gut bacterial communities of humans and dogs, while each host maintains a distinct microbial identity. These findings suggest that gut microbial communities are shaped by the interaction between host biology and changing ecological conditions. Expanding microbiome research beyond industrialized settings is therefore essential for understanding what microbial diversity has been retained, altered, or lost during recent lifestyle transitions.

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Industrialization has profoundly altered the ecological conditions under which host-associated microbial communities assemble. In humans, transitions from foraging and agrarian lifestyles to urbanized and industrialized conditions are associated with changes in diet, sanitation, antibiotic exposure, environmental microbial contact, mobility, and built-environment exposure, all of which can restructure the gut microbiome (1–3). Comparative studies across human populations have shown that industrialized populations often differ from non-industrialized populations in gut microbiome composition, prevalence of specific microbial taxa, and inferred functional potential (4–8). These findings have led to the proposal that industrialization is associated with the loss of evolutionarily conserved bacterial lineages common in non-industrialized populations and the expansion of taxa favored in urbanized or industrialized settings (9, 10). However, most evidence for industrialization-associated gut microbiome change comes from humans. Far less is known about whether similar ecological transitions restructure the microbiomes of other mammals that live in close association with humans.

Domestic dogs provide an informative system for addressing this gap. Dogs have lived alongside humans for thousands of years (11–13), occupying human-built and human-modified environments and consuming human-derived foods across much of their evolutionary history (14–16). Their association with humans has tracked major transitions in subsistence, settlement, agriculture, urbanization, and industrialization and has accompanied parallel host adaptations linked to human-associated diets, including genomic changes related to starch digestion (17–21). In contemporary industrialized settings, many dogs consume commercial diets, live in built environments, experience reduced environmental microbial exposure, and receive veterinary antibiotics and other medical interventions (22). In contrast, free-ranging and community dogs in non-industrialized or urbanizing settings often consume household scraps, scavenged foods, livestock-associated resources, or locally available dietary substrates, while experiencing greater environmental exposure and different forms of human management. These contrasts make dogs useful for testing how lifestyle-associated ecological change shapes the gut microbiome of a long-term human-associated mammal.

Despite this potential, most canine microbiome studies have focused on industrialized companion animals, colony dogs, or controlled-feeding cohorts, often involving household pets or research dogs consuming commercial diets in high-income settings (23–32). These studies have provided important insight into canine bacterial communities, but sampling bias limits our understanding of the ecological range of the domestic dog gut microbiome and makes it difficult to determine whether the microbiome of industrialized pets represents a general canine baseline or one endpoint of a broader lifestyle-associated gradient (33, 34). Broader sampling is especially important because dogs occupy diverse ecological roles across human societies, including free-ranging village dogs, agrarian dogs, pastoralist dogs, urban community dogs, and industrialized household pets. These populations differ in diet, environmental contact, livestock exposure, mobility, climate, geography, and human management. Characterizing this diversity is necessary for understanding how canine gut microbial communities vary across natural ecological gradients and for interpreting microbiome variation in industrialized companion animals (34).

A second unresolved question is whether lifestyle-associated microbiome change in dogs resembles changes observed in humans sharing similar ecological transitions. Across mammals and primates, gut microbiomes often retain strong host specificity, including signatures of codiversification (35, 36), host filtering (10, 37), and host-species-specific responses to human-associated environmental change (38). At the same time, coexistence, social transmission, captivity, urbanization, and specialized diets can

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increase microbiome similarity across host species (39, 40). Sympatric chimpanzees and gorillas share more gut bacterial phylotypes than allopatric hosts (40, 41); urban wildlife can acquire human-associated bacterial lineages (38); captive mammals can show partial "humanization" of the gut microbiome (42–45); and specialized diets can drive convergence across distantly related vertebrates (46). Dogs provide a particularly useful test of this balance because they have shared human environments and food systems across generations (17, 18, 47) but remain biologically distinct hosts with their own gastrointestinal physiology, immune filtering, diet processing, and evolutionary history (48–51). Thus, dogs can help distinguish whether shared lifestyle transitions produce taxonomic convergence across host species or instead generate parallel but host-specific microbiome responses.

To address these gaps, we analyze fecal 16S rRNA gene profiles from a globally distributed cohort of domestic dogs sampled across Nepal, Thailand, the United Arab Emirates (UAE), and the United States (USA). The cohort spans five lifestyle categories—forager, agrarian, pastoralist, urban, and industrialized—across broad gradients of geography, altitude, climate, diet, and human association. We use "industrialized" to describe household pets sampled in highly industrialized settings, whereas "urban" refers to free-ranging or community-associated dogs sampled in urbanizing environments. We first test whether lifestyle is a dominant correlate of canine gut bacterial community structure after accounting for major ecological and host covariates. We then identify genus-level taxa, operational VANISH-like and BloSSUM-like prevalence categories, coabundance groups, and predicted functional profiles associated with the lifestyle gradient. Finally, we compare dogs and humans sampled across matched forager, agrarian, and industrialized contexts to test whether shared lifestyle transitions produce cross-host taxonomic convergence or host-specific microbiome responses. Because lifestyle, diet, geography, climate, and human management are inherently coupled in natural ecological systems, we treat lifestyle as an integrated ecological state rather than as a single isolated exposure. We therefore use complementary full-cohort, restricted-cohort, and projection analyses to test whether lifestyle-associated gut bacterial community structure persists after reducing major measured sources of confounding. This framework allows us to expand the ecological baseline for the domestic dog gut microbiome while testing whether industrialization-associated microbiome restructuring extends beyond humans to another long-term human-associated mammal.

RESULTS

A globally distributed canine cohort captures lifestyle transitions across geography and climate

We analyzed fecal 16S rRNA profiles from 261 domestic dogs sampled across four countries and five lifestyle categories spanning broad differences in human association, diet, management, geography, altitude, and climate (Fig. 1A; Table S1). Dogs were sampled from Nepal (n = 151), Thailand (n = 27), the United Arab Emirates (UAE; n = 13), and the United States (USA; n = 70), across altitudes ranging from 10 to 3,840 m. Sampling locations included tropical, arid-desert, temperate, and temperate-cold climate zones, providing a cohort that captured both lifestyle-associated and environmental variation.

Dogs were classified into five lifestyle categories: forager, agrarian, pastoralist, urban, and industrialized. These categories reflected integrated ecological differences in human management, diet, environmental exposure, and the degree of industrialization. Forager, agrarian, pastoralist, and urban dogs were primarily free-ranging or community-associated dogs sampled in Nepal and Thailand, whereas industrialized dogs were household pets sampled in the UAE and the USA. The Nepali cohort spanned four non-industrialized lifestyles across multiple communities and elevations, allowing lifestyle-associated variation to be assessed within a single country as well as across the full globally distributed cohort.

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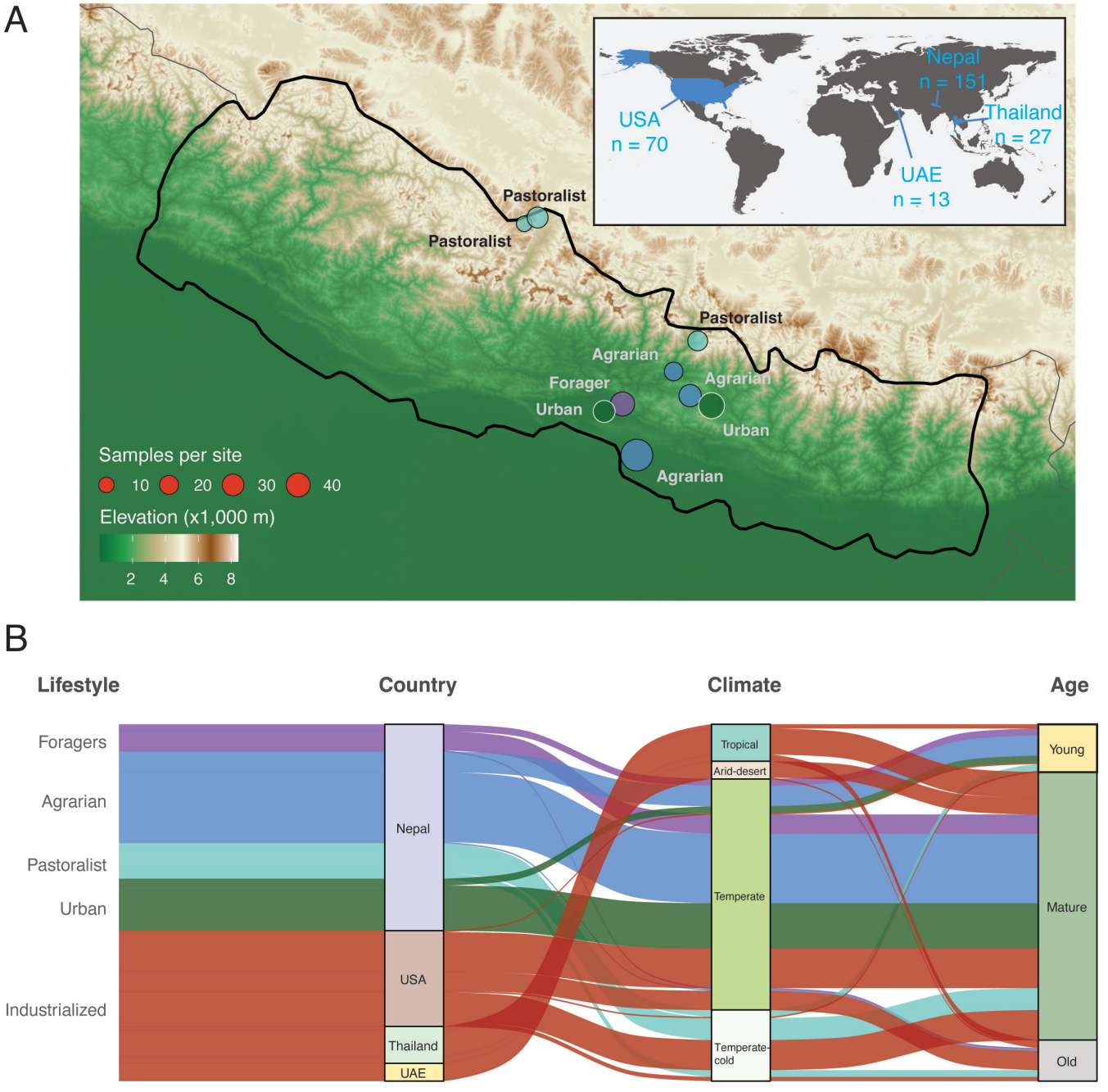


FIG 1 Study design and ecological structure of the globally distributed canine cohort. (A) Sampling locations of dogs across Nepal, Thailand, the UAE, and the United States. The main map shows Nepal sampling locations over elevation relief, with point size indicating sample number per site and labels indicating lifestyle category. The inset map shows the broader geographic distribution of sampled dogs. (B) Alluvial plot showing the relationship among lifestyle category, country, climate category, and age group. Flow colors indicate lifestyle categories. The figure includes all 261 fecal samples passing sequencing and contamination filtering; four dogs with reported recent antibiotic exposure were excluded from downstream microbiome analyses.

Lifestyle categories corresponded to distinct ecological contexts relevant to gut bacterial community composition (Fig. 1B). Forager dogs were sampled from Chepang communities in Korak, where households retain subsistence links to forest-based hunting, fishing, bird trapping, wild plant gathering, and small-scale maize and millet cultivation (52). Forager and agrarian dogs primarily consumed scavenged or household-prepared foods, pastoralist dogs consumed more dairy and animal-derived foods, urban community dogs were sampled in urbanizing settings with greater exposure to

processed human food waste, and industrialized dogs were household pets fed predominantly commercial pet food. Environmental exposure was greatest among free-ranging dogs and lowest among household pets in industrialized settings. Thus, the cohort captures a broad ecological range of domestic dogs, from free-ranging dogs in non-industrialized communities to household pets in industrialized settings, enabling tests of how lifestyle transitions are associated with canine gut bacterial community structure.

Lifestyle is the dominant correlate of canine gut bacterial community structure

After quality control, 16S rRNA gene profiling of 261 dog fecal samples generated a median of 28,617 reads per sample (mean ± SD: 33,578 ± 21,142) and identified 1,472 amplicon sequence variants (ASVs) representing 245 bacterial genera. Four dogs with recent antibiotic exposure showed reduced diversity and were excluded, leaving 257 dogs for downstream analyses. As a within-country proof-of-concept, we first tested whether lifestyle-associated gut bacterial community differences were detectable within Nepal alone, before interpreting the full international gradient. In both weighted UniFrac and Bray-Curtis ordination analyses, Nepali dogs showed lifestyle-associated structure within a single country, with pastoralist dogs departing from the simple forager-to-urban continuum (weighted UniFrac: R^2 = 0.08, P = 0.001; Bray-Curtis: R^2 = 0.09, P = 0.001; Fig. S1). Although the within-Nepal effect size was smaller than the full international contrasts, this analysis shows that lifestyle-associated bacterial community variation was not reducible to broad country-level differences.

Principal coordinate analysis (PCoA) of genus-level weighted UniFrac distances of the 257 dogs showed strong structuring of gut bacterial community composition by lifestyle (Fig. 2A). The first two PCoA axes explained 42.9% of the variation, with PCo1 explaining 29.9% and separating many industrialized dogs from non-industrialized dogs. PCo2 explained an additional 13.0% and captured secondary differentiation among non-industrialized groups, with pastoralist dogs deviating from a simple forager-to-industrialized gradient. Notably, dogs sampled in Thailand clustered closer to dogs from the UAE and the United States than to most non-industrialized Nepali dogs (Fig. S2), consistent with their sampling from an urbanizing food environment in Chiang Mai (53).

We quantified associations between bacterial community structure and measured host or ecological variables using complementary approaches. Marginal PERMANOVA tested associations with the full genus-level distance matrix, whereas EnvFit tested alignment with the leading ordination axes. In PERMANOVA models using weighted UniFrac distances, lifestyle explained the largest share of overall bacterial community variation among measured variables (R^2 = 0.10, P = 0.001), followed by climate category (R^2 = 0.07, P = 0.001). Altitude category explained a smaller fraction of variation (R^2 = 0.02, P = 0.001), as did shelter status (R^2 = 0.01, P = 0.003), whereas age group was not significant (R^2 = 0.007, P = 0.186; Fig. 2B; Table S2). Results were consistent using Bray-Curtis distances (Fig. S3 and Table S2).

EnvFit analyses using the first three ordination axes supported the same overall pattern (Table S2). Lifestyle showed the strongest alignment with ordination structure (R^2=0.43 and 0.49, P = 0.001 for weighted UniFrac and Bray-Curtis, respectively), followed by climate category (R^2 = 0.27 for both distance metrics, P = 0.001) and altitude group (R^2 = 0.22 and 0.26, P = 0.001). Shelter status was not significant in EnvFit analyses (P > 0.05). Although age group explained little variation overall, a small association was detected in EnvFit for both weighted UniFrac and Bray-Curtis ordinations (R^2 = 0.037 and 0.038, P = 0.001). Thus, lifestyle was the strongest measured correlate of canine gut bacterial community composition across both distance-based and ordination-based analyses, with climate contributing additional structure.

Because lifestyle, climate, age, shelter status, and geography are partially coupled in this observational cohort, we next tested whether lifestyle-associated structure persisted after reducing major measured sources of confounding. We repeated beta-diversity analyses in a restricted subset of mature, non-shelter dogs sampled from temperate

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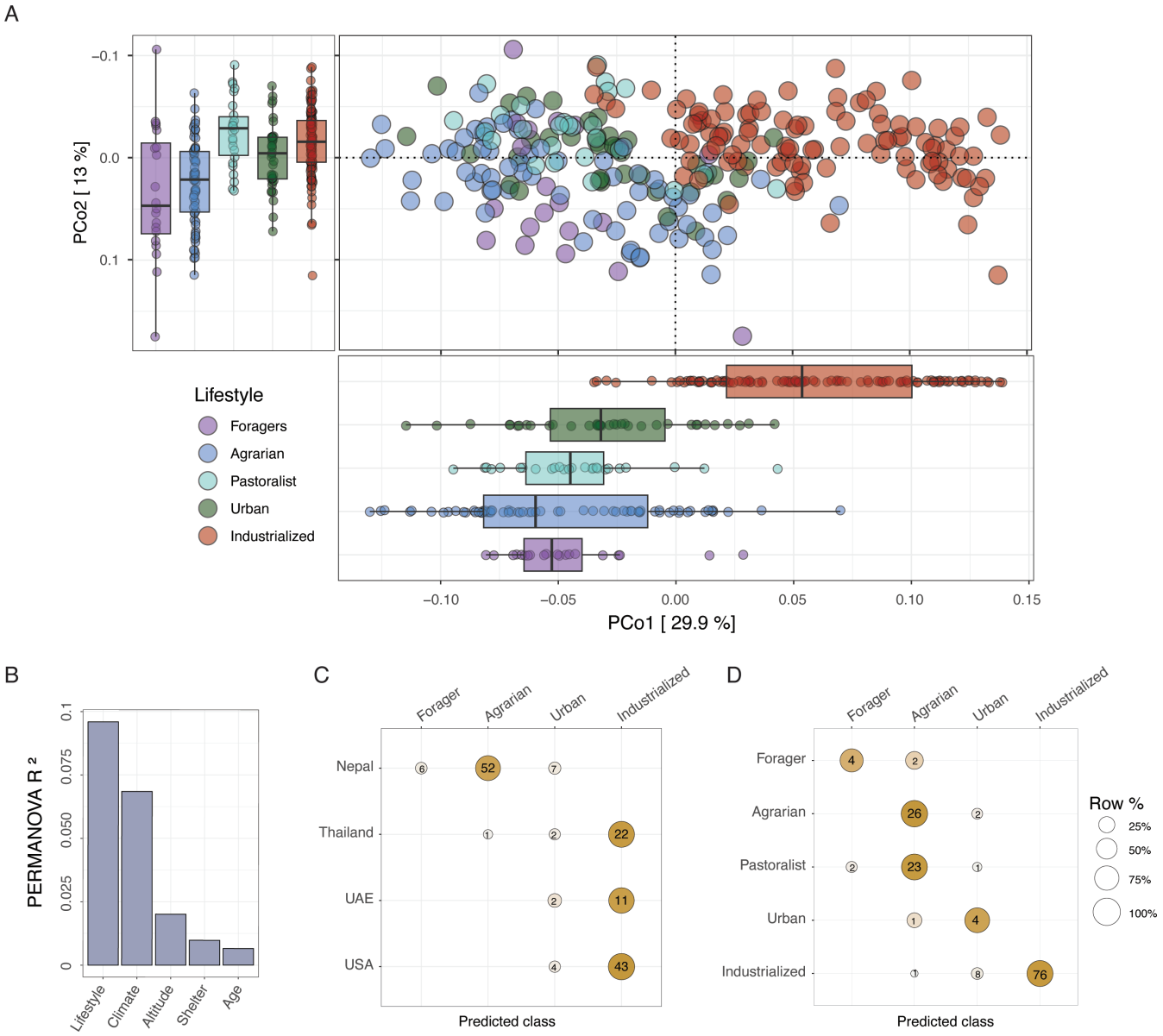


FIG 2 Lifestyle-associated structure in the canine gut bacterial community. (A) Weighted UniFrac PCoA of genus-level bacterial community profiles from 257 dogs. Points represent individual dogs and are colored by lifestyle. Marginal boxplots show sample distribution along PCo1 and PCo2 by lifestyle. (B) Marginal PERMANOVA R^2 values from a multivariable model including lifestyle, climate category, altitude category, shelter status, and age group. (C) Projection of dogs excluded from the restricted random forest training set, summarized by country. (D) Projection of the same excluded dogs summarized by observed lifestyle category. The classifier was trained on mature, non-shelter dogs from temperate regions, excluding Thai and UAE dogs. Balloon size indicates the row-wise percentage of samples assigned to each predicted class, and numbers indicate sample counts. Zero-count cells are omitted.

climate regions, excluding Thai and UAE dogs. This subset retained 107 dogs spanning forager, agrarian, urban, and industrialized lifestyles. Lifestyle remained strongly associated with bacterial community composition in both weighted UniFrac and Bray-Curtis analyses, explaining nearly 20% of variation in each distance metric (weighted UniFrac: R^2 = 0.197, P = 0.001; Bray-Curtis: R^2 = 0.196, P = 0.001; PERMANOVA, Table S2 and Fig. S4). Restricted EnvFit showed similarly strong alignment between lifestyle and ordination structure (weighted UniFrac: R^2 = 0.33, P = 0.001; Bray-Curtis: R^2 = 0.35, P = 0.001; Table S2). These results indicate that lifestyle-associated bacterial community structure was not explained solely by measured differences in climate, age, shelter status, or inclusion of Thai and UAE dogs.

To further test whether the lifestyle signal could be reproduced by arbitrary categorical labeling, we performed a randomized-label sensitivity analysis (Fig. S5). Lifestyle labels were randomly reassigned while preserving the group sizes, and PERMANOVA was repeated 1,000 times for each distance metric in the full cohort of 257 dogs and in the restricted cohort of 107 mature, non-shelter, temperate-climate dogs spanning forager, agrarian, urban, and industrialized lifestyles. In all four analyses, observed lifestyle R^2 values exceeded the randomized-label null distributions: full-cohort weighted UniFrac R^2 = 0.096 vs null 95th percentile = 0.0157; full-cohort Bray-Curtis R^2 = 0.0919 vs 0.0155; restricted-cohort weighted UniFrac R^2 = 0.197 vs 0.0399; and restricted-cohort Bray-Curtis R^2 = 0.196 vs 0.0391. Empirical P values were <0.001 in all cases. Thus, lifestyle-associated bacterial community structure was not reproduced by arbitrary reassignment of lifestyle labels.

We then asked whether lifestyle-associated bacterial community states learned from this restricted data set could classify dogs excluded from the restricted training set. A random forest classifier trained on the restricted mature, non-shelter, temperate-climate subset recovered lifestyle categories with high held-out accuracy (accuracy = 0.864, Cohen's κ = 0.806; Fig. S6A), comparable to the classifier trained on the full data set with 257 dogs (Fig. S6B). When excluded dogs were projected onto this classifier, assignments were biologically coherent by both country and lifestyle (Fig. 2C and D). No excluded Nepali dogs were classified as industrialized; instead, most were assigned to agrarian, forager, or urban classes. In contrast, most Thai, UAE, and US dogs were assigned to the industrialized class, including 22 out of 25 Thai urban community dogs, 11 out of 13 UAE dogs, and 43 out of 47 excluded US dogs. The remaining UAE and US dogs were assigned to the urban class rather than to agrarian or forager classes. These projections supported the placement of Thai urban community dogs near the urbanized/industrialized end of the bacterial community gradient, while retaining their ecological interpretation as urban community dogs rather than industrialized household pets.

The projection analysis also clarified the position of pastoralist dogs. Because pastoralist dogs were not included as a training class in the restricted classifier, they were assigned to the closest available lifestyle category. Most pastoralist dogs were classified as agrarian, with a small number assigned to forager or urban classes and none assigned to industrialized. This indicates that pastoralist dogs carried non-industrialized bacterial community profiles, while also supporting their treatment as a distinct ecological group that does not fall neatly along a simple forager-to-industrialized continuum.

Although altitude aligned with the leading ordination axes in the full data set, its marginal contribution in PERMANOVA was small. This likely reflected the position of high-altitude pastoralist dogs along PCo2, because all high-altitude dogs in this cohort were pastoralist dogs. We therefore interpreted altitude as part of the integrated pastoralist ecological context rather than as an independent predictor in downstream analyses. Downstream models retained lifestyle and climate as primary ecological predictors and age group as a conservative host covariate; shelter status was not retained because it explained little variation overall and applied to a limited subset of dogs.

Finally, we asked whether within-sample diversity followed the same ecological pattern observed for community composition. In generalized linear models including lifestyle and other host and ecological covariates, alpha diversity showed no significant associations with measured predictors after Benjamini-Hochberg correction across all alpha diversity tests, whether measured as observed ASV richness, Shannon diversity, or Faith's phylogenetic diversity (Table S3). Thus, lifestyle-associated variation among dogs was reflected primarily in microbiome structure rather than in overall within-sample diversity.

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Lifestyle is associated with directional restructuring of the canine gut bacterial community

To identify bacterial genera underlying the compositional gradient, we performed genus-level differential abundance analysis using MaAsLin2 (54). In a lifestyle-only model with industrialized dogs as the reference group, 142 out of 245 genera showed significant large-effect differences in at least one non-industrialized lifestyle group relative to industrialized dogs (FDR-adjusted q <0.05 and an absolute MaAsLin2 coefficient >1; Table S4). This broad signal indicated extensive genus-level restructuring across canine lifestyle categories.

To focus on taxa contributing to the main lifestyle gradient, we next identified genera showing monotonic abundance patterns across the forager-agrarian-urban-industrialized axis. Pastoralist dogs were excluded from this monotonic gradient definition because they represented a distinct high-altitude lifestyle rather than a simple intermediate state along the forager-to-industrialized continuum. This analysis identified 48 genera with significant large endpoint contrasts between forager and industrialized dogs (FDR-adjusted q < 0.05 and absolute coefficient > 1; Table S5). These genera separated into two opposing patterns: genera enriched in forager dogs and reduced toward industrialized dogs, and genera showing the reverse pattern (Fig. 3A).

Genera enriched in non-industrialized dogs included several anaerobic gut lineages previously associated with carbohydrate fermentation or short-chain fatty acid production, including *Actinomyces*, *Anaerobutyricum*, *Dorea*, *Sarcina*, *Coprococcus*, *Gemmiger*, *Eubacterium*, and *Oscillibacter* (Fig. 3B) (55–60). Additional genera enriched in non-industrialized dogs, including *Corynebacterium* and *Brucella*, may reflect broader environmental or livestock-associated exposures (61, 62). These patterns are consistent with, but do not directly demonstrate, differences in dietary substrate use and environmental exposure across lifestyles.

Industrialized dogs showed higher relative abundance of a distinct set of genera, including Prevotellaceae-associated taxa such as *Segatella*, Erysipelotrichaceae-associated genera such as *Allobaculum* and *Catenibacterium*, and anaerobic gut lineages including *Blautia*, *Enterocloster*, *Flavonifractor*, *Peptoclostridium*, and the [Ruminococcus] gnavus group (Fig. 3C). Thus, industrialization in dogs was associated not only with reduced abundance of several non-industrialized-associated anaerobes, but also with enrichment of distinct gut bacterial genera.

To determine whether these lifestyle-associated shifts were explained by covarying ecological or host variables, we fit a multivariable model including lifestyle, climate category, and age group. Of the 142 large-effect lifestyle-associated genera identified in the univariate model, 137 remained significant after adjustment (FDR-adjusted q < 0.05 and an absolute MaAsLin2 coefficient > 1; Table S6). Across shared genus-lifestyle contrasts, effect directions were fully concordant between the lifestyle-only and multivariable models, and coefficients were strongly correlated before and after adjustment (r = 0.98; Fig. S7). The median absolute coefficient changed only modestly, from 2.19 to 2.10. These results indicate that lifestyle-associated genus-level shifts were largely preserved after accounting for climate and age.

Climate contributes additional, zone-specific genus-level structure

Because climate was consistently associated with microbiome composition in beta-diversity analyses, we next examined genus-level climate associations in the multivariable model that included lifestyle, climate category, and age group. Eighty-three genera showed significant large-effect associations with climate category using tropical as the reference (FDR-adjusted q < 0.05 and an absolute MaAsLin2 coefficient > 1; Table S6). Lifestyle and climate signals partially overlapped: 66 genera were associated with both variables, whereas 71 lifestyle-associated genera were not climate-associated, indicating that lifestyle captured substantial genus-level variation beyond broad climatic differences (Fig. S8).

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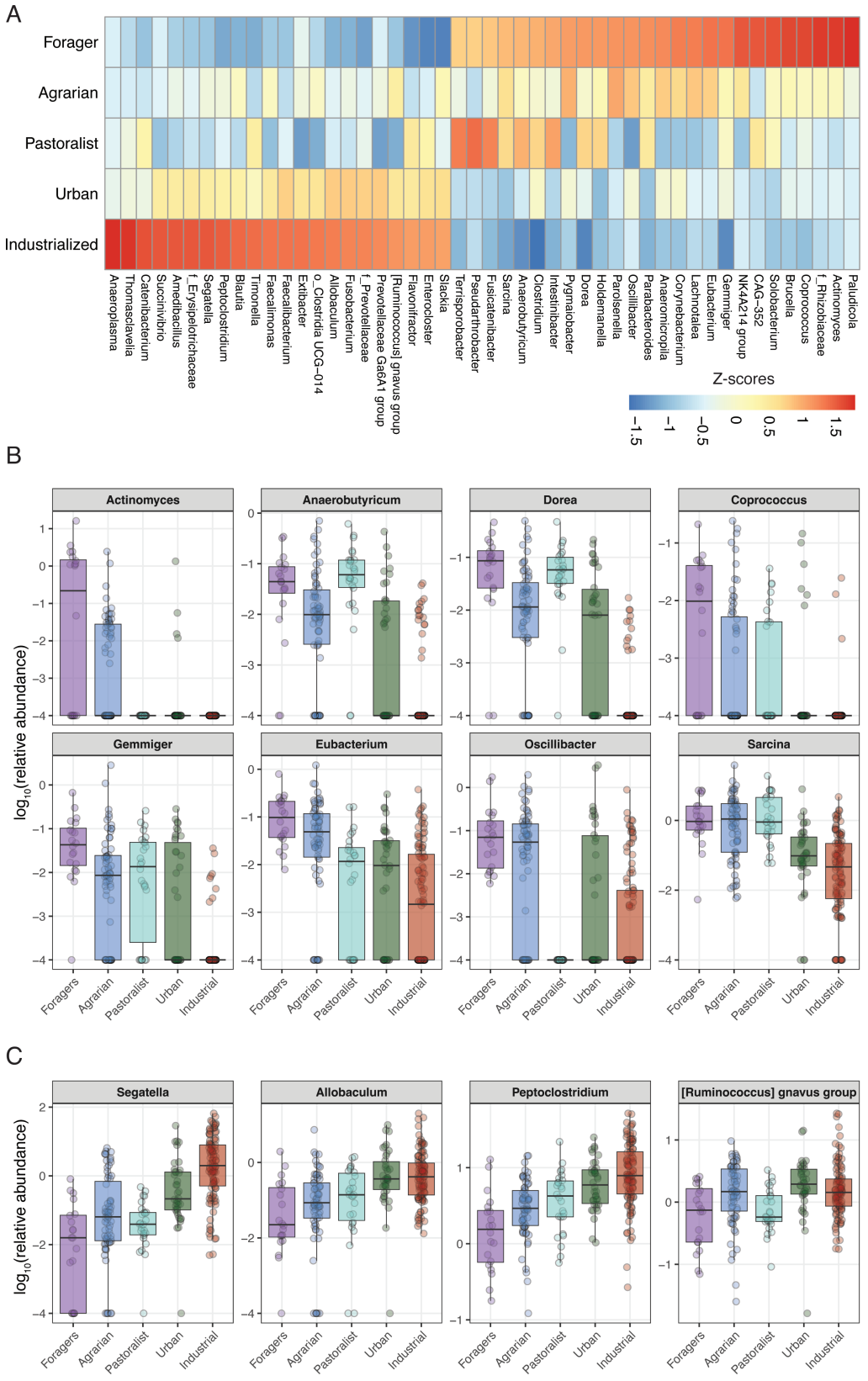


FIG 3 Directional genus-level shifts across the canine lifestyle gradient. (A) Heatmap of genera showing monotonic lifestyle-associated abundance patterns across the forager-agrarian-urban-industrialized axis. Values show row-scaled mean relative abundance within each genus. Red indicates higher relative abundance and blue indicates lower relative abundance

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Fig 3 (Continued)

within each genus. Genera were retained if they showed significant and large endpoint contrasts between forager and industrialized dogs (FDR-adjusted $q < 0.05$ and absolute MaAsLin2 coefficient > 1). (B) Representative genera enriched in non-industrialized dogs and declining toward industrialized dogs. (C) Representative genera enriched in industrialized dogs and reduced in forager or agrarian dogs. Boxplots in panels B and C show log-transformed relative abundance across lifestyle groups, with points representing individual dogs.

Climate-associated genera showed a different pattern from lifestyle-associated genera. Whereas lifestyle-associated genera formed a directional forager-to-industrialized gradient, climate-associated genera formed more discrete climate-enriched blocks (Fig. S9). After excluding genera that were also associated with lifestyle or age, 17 climate-specific genera remained. Most were enriched in tropical environments and included environment-associated genera such as *Sphingobacterium*, *Chryseobacterium*, and *Empedobacter* (Fig. S9), suggesting that warm, humid environments may contribute exposure-related signals not captured by lifestyle alone.

Climate-associated differences were detectable within industrialized dogs sampled across tropical, arid-desert, temperate, and temperate-cold climate categories ($P < 0.05$, PERMANOVA; Fig. S10). This supported climate and geography as additional sources of structured variation, although these effects were weaker than the primary lifestyle-associated gradient. In contrast, age-related genus-level associations were limited: only nine genera showed significant large-effect associations with age after accounting for lifestyle and climate, including a decline in *Bifidobacterium* with age (Fig. S11; Table S6) (63). Thus, climate contributed additional zone-specific bacterial community structure, whereas age had comparatively modest effects in this cohort.

Prevalence-defined VANISH-like and BloSSUM-like genera show structured coabundance patterns across canine lifestyles

We next asked whether lifestyle-associated taxonomic shifts in dogs could be summarized using a prevalence-based framework analogous to the human VANISH/BloSSUM approach. In humans, VANISH taxa are more prevalent in non-industrialized populations, whereas BloSSUM taxa are more prevalent in urbanized or industrialized populations (4, 64). We applied this framework to dogs by comparing forager and agrarian dogs with dogs from industrialized settings. Because these categories were defined within the canine data set, we refer to them as VANISH-like and BloSSUM-like genera and interpret them as operational prevalence groups rather than direct equivalents of the human-defined VANISH and BloSSUM taxa.

This analysis identified 61 genera with large prevalence differences between non-industrialized and industrialized dogs, including 46 VANISH-like genera that were more prevalent in forager/agrarian dogs and 15 BloSSUM-like genera that were more prevalent in industrialized dogs (absolute prevalence difference ≥30%, FDR-adjusted $P < 0.05$, Fisher's exact test; Fig. 4A; Table S7). The non-industrialized-associated, VANISH-like genera included multiple anaerobic gut lineages, including *Coprococcus*, *Dorea*, *Ruminococcus*, *Oscillibacter*, *Anaerobutyricum*, *Anaeromicropila*, *Eubacterium*, *Intestinibacter*, and Christensenellaceae-associated taxa. The industrialized-associated, BloSSUM-like genera included *Flavonifractor*, *Timonella*, *Tyzzerella*, *Anaeroplasma*, *Absiella*, *Empedobacter*, and *Streptococcus*, and several family-level assignments within Erysipelotrichaceae, Muribaculaceae, Enterococcaceae, and Peptostreptococcaceae. Thus, industrialized dogs showed both reduced prevalence of a broad set of non-industrialized-associated anaerobes and increased prevalence of a smaller set of industrialized-associated taxa.

We then tested whether these prevalence-shifting genera were organized into coabundance groups (CAGs) inferred independently from the prevalence classification. FastSpar (65) analyses resolved three CAGs, including two tightly connected modules and a third, more diffuse cluster (absolute $\rho > 0.2$, FDR-adjusted $P < 0.05$; Fig. S12). Because CAGs were inferred from relative abundance patterns across all dog samples

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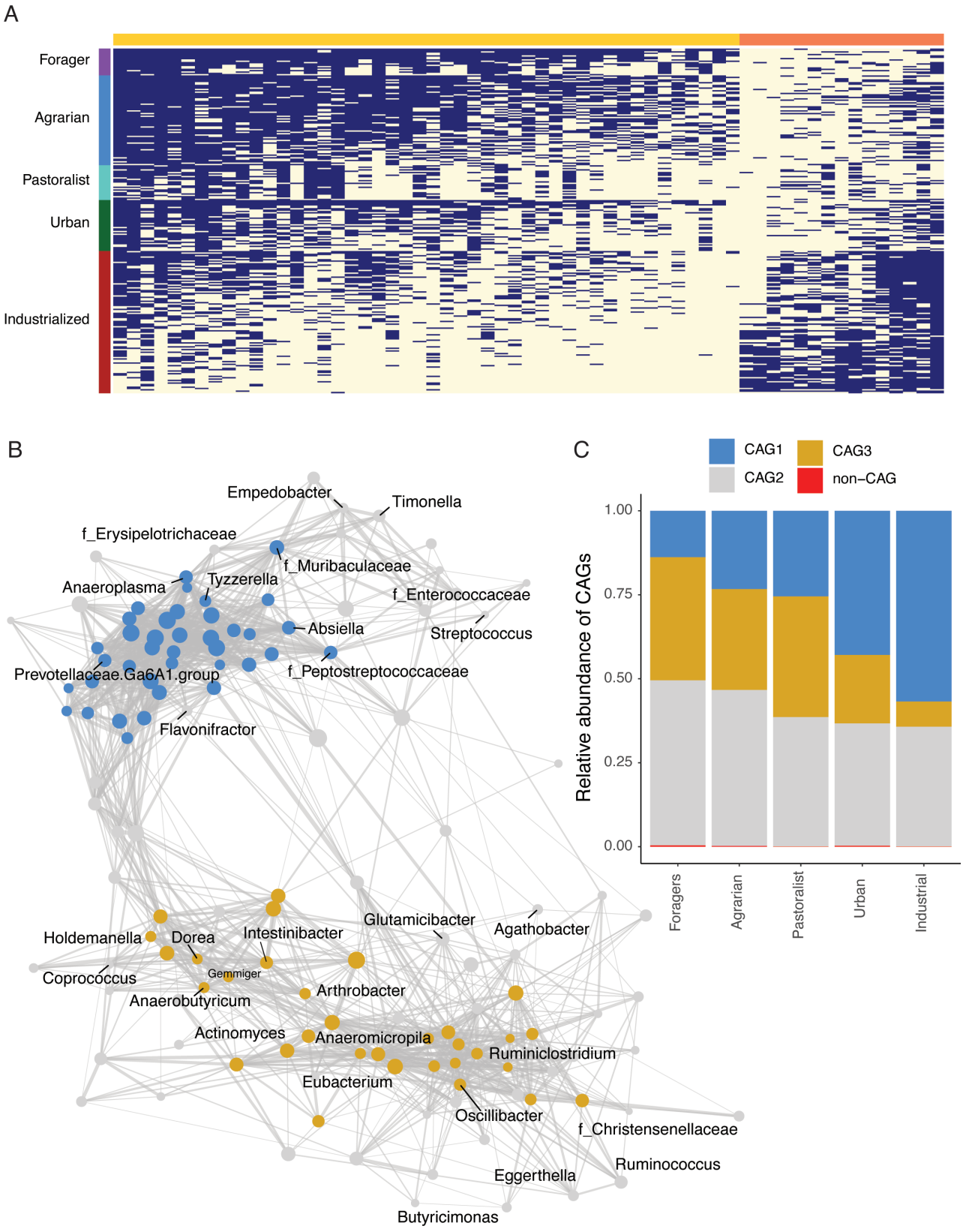


FIG 4 Prevalence-defined bacterial guilds shift across the canine lifestyle gradient. (A) Presence-absence heatmap of VANISH-like and BloSSUM-like genera across dog samples ordered by lifestyle. VANISH-like and BloSSUM-like genera were operationally identified by comparing prevalence between forager/agrarian and industrialized dogs using Fisher's exact tests with FDR correction and requiring an absolute prevalence difference of at least 30%. Rows represent individual

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Fig 4 (Continued)

dogs and columns represent genera; navy indicates presence and pale yellow indicates absence. (B) Genus-level co-occurrence network inferred using FastSpar. Nodes represent genera and edges represent significant positive correlations. Node color indicates CAG membership, node size reflects mean genus abundance, and edge width reflects correlation strength. Key VANISH-like and BloSSUM-like genera are labeled. (C) Mean relative abundance of CAGs across lifestyle categories. Genera assigned to each CAG were summed within samples and converted to relative abundance across CAG and non-CAG taxa.

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without providing lifestyle labels to the algorithm, and because urban and pastoralist dogs were not used to define VANISH-like and BloSSUM-like status, this analysis provided complementary information about the coabundance structure of prevalence-shifting genera. VANISH-like and BloSSUM-like genera were non-randomly distributed across CAGs ($P = 2.8 \times 10^{-7}$, Fisher's exact test; Fig. 4B). BloSSUM-like genera were confined to CAG1 and CAG2, whereas VANISH-like genera were restricted to CAG2 and CAG3. Consistent with this partitioning, CAG-level abundance varied directionally across lifestyles (Fig. 4C). CAG1, which was enriched for BloSSUM-like genera, increased toward industrialized dogs, whereas CAG3, composed primarily of VANISH-like genera, declined across the same gradient. Pastoralist dogs again showed a distinct non-industrialized profile, consistent with their departure from a simple forager-to-industrialized continuum. Together, these results indicate that prevalence-defined bacterial guilds in dogs are non-randomly organized across genus-level coabundance structure and vary directionally across lifestyles, rather than representing only isolated taxonomic shifts.

Predicted functional potential inferred from 16S profiles mirrors the canine lifestyle gradient

We next tested whether lifestyle-associated taxonomic restructuring was accompanied by shifts in predicted functional potential inferred from 16S rRNA gene profiles using PICRUSt2 (66). Because these profiles are predicted from marker-gene data rather than directly measured by shotgun metagenomics, we interpreted them as inferred functional potential.

Using a multivariable model including lifestyle, climate category, and age group, we identified predicted enzyme functions that followed the same monotonic forager-agrarian-urban-industrialized gradient used for genus-level analyses. This analysis identified 341 directional predicted enzyme functions (FDR-adjusted $q < 0.05$ and absolute MaAsLin2 coefficient > 1; Table S8). Most were enriched in forager dogs and declined toward industrialized dogs (307 functions), whereas a smaller set showed the reverse pattern (34 functions). These predicted functions spanned multiple enzyme classes, especially oxidoreductases, transferases, and hydrolases (Fig. S13A).

Predicted MetaCyc pathways showed a similar asymmetry. We identified 85 directional pathways enriched in forager dogs and declining toward industrialized dogs, whereas no pathways showed the reverse monotonic pattern under the same criteria (FDR-adjusted $q < 0.05$ and an absolute MaAsLin2 coefficient > 1; Table S9). These pathways spanned multiple MetaCyc superclass categories, including carbohydrate, lipid, and amino acid degradation and cofactor/vitamin biosynthesis (Fig. S14). Climate-associated predicted pathways were also detected, whereas age-associated functional gradients were comparatively modest (Fig. S13B and Table S10), consistent with the weaker age effects observed in taxonomic analyses. Overall, these results suggest that lifestyle-associated taxonomic restructuring is accompanied by broad shifts in predicted microbial functional potential. Because these profiles were inferred from 16S rRNA gene data, they should be interpreted as hypothesis-generating rather than as direct measurements of metagenomic function or metabolic activity.

Shared lifestyle transitions produce host-specific microbiome responses in dogs and humans

We next asked whether lifestyle-associated microbiome restructuring in dogs paralleled patterns observed in humans sampled across similar lifestyle categories. We compared dogs and humans matched for forager, agrarian, and industrialized contexts using

the human data set from Jha et al. (5). The human data set included 44 individuals: 14 foragers, 20 agrarians, and 10 industrialized individuals. The matched dog data set included 155 dogs: 20 foragers, 67 agrarians, and 68 industrialized dogs. Forager dogs and humans were sampled from the same village, strengthening the ecological comparability of the cross-host comparison.

Because dog and human samples were generated using different 16S rRNA amplicon regions, cross-host analyses were restricted to genus-level profiles. Separate Bray-Curtis PCoA analyses showed lifestyle-associated structure within each host: in both dogs and humans, industrialized samples separated from forager and agrarian samples along the leading ordination axis, whereas forager and agrarian samples were more similar to each other (Fig. S15). Thus, similar lifestyle categories were associated with broad community restructuring in both host species.

However, combined ordination of the 98 shared genera showed that host species remained the dominant axis of variation. PCA of CLR-transformed genus-level profiles separated dogs and humans along PC1, while lifestyle-associated differences occurred within host-specific compositional clusters (Fig. 5A). This pattern was robust to repeated stratified subsampling of dogs to match the number of human samples within each lifestyle category, indicating that host-specific separation was not driven by unequal sample size across hosts or lifestyles (Fig. S16). Thus, shared lifestyle transitions did not produce strong genus-level taxonomic convergence in overall community composition.

We then tested whether the same genera responded to lifestyle transitions in both hosts. Among the 98 shared genera, 17 showed significant directional lifestyle-associated responses in both dogs and humans (FDR-adjusted $q < 0.05$ and absolute MaAsLin2 coefficient > 1; Table S11). Of these, only eight changed in the same direction, whereas nine changed in opposite directions (Fig. 5B). Same-direction overlap was not greater than expected under the shared genus background ($P > 0.05$, one-sided Fisher's exact tests). These results indicate that lifestyle-associated restructuring occurred in both dogs and humans, but the genus-level taxa underlying these shifts were largely host-specific.

To extend this comparison beyond categorical overlap, we next tested whether lifestyle-effect sizes and broad taxonomic features of the shared genera were concordant across hosts. Across all 98 shared genera, dog and human forager-vs-industrialized effect sizes were not positively correlated (Spearman $\rho = -0.13$, $P = 0.202$; Fig. S17), indicating no robust rank-consistent concordance in genus-level lifestyle responses. Sign concordance was also not enriched: 45 genera showed same-sign effects across hosts, whereas 53 showed opposite-sign effects (binomial test, $P = 0.48$). Among the 17 genera with significant directional responses in both hosts, same-direction genera were concentrated within Bacillota (8/8 same-direction genera vs 5/9 opposite-direction genera), but this enrichment was only suggestive and did not reach statistical significance ($P = 0.082$, two-sided Fisher's exact test). Thus, the subset of concordant genera was limited and taxonomically narrow, whereas cross-host lifestyle responses overall showed little evidence of broad genus-level convergence.

Together, the cross-host analyses show that industrialization and associated lifestyle transitions are associated with parallel bacterial community restructuring across human-associated mammals, but these responses are expressed through host-specific bacterial lineages rather than broad cross-host taxonomic convergence.

DISCUSSION

Domestic dogs are long-term human-associated mammals, but most canine gut microbiome studies have focused on industrialized companion animals, colony dogs, or controlled-feeding cohorts (23–32, 47, 67). This has limited our understanding of whether the microbiome of household pets represents a general canine baseline or one endpoint of a broader ecological spectrum. By analyzing dogs across forager, agrarian, pastoralist, urban, and industrialized contexts, we show that lifestyle is the strongest measured correlate of canine gut bacterial community composition. Industrialized dogs were compositionally distinct from non-industrialized dogs and showed directional

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genus-level shifts, structured reorganization of prevalence-defined VANISH-like and BloSSUM-like bacterial guilds, and shifts in inferred functional potential. At the same time, non-industrialized dogs were not microbiologically uniform: pastoralist dogs often departed from a simple forager-to-industrialized continuum, and climate contributed additional zone-specific variation. Restricted analyses further showed that lifestyle-associated structure persisted among mature, non-shelter dogs from temperate climate regions, and classifier-based projections placed Thai urban community dogs near the industrialized end of the bacterial community gradient while assigning pastoralist dogs primarily to non-industrialized classes. These findings expand the ecological baseline for the domestic dog gut microbiome and identify industrialization as a major axis of microbiome restructuring in a long-term human-associated mammal.

A central finding is that lifestyle-associated variation in dogs was reflected in community composition rather than overall within-sample diversity. Alpha diversity showed no significant associations with measured host and ecological variables after multiple-testing correction, whereas beta diversity and genus-level composition differed strongly by lifestyle. Thus, microbiome change associated with industrialization in dogs should not be interpreted simply as a loss of diversity. Instead, our results indicate that lifestyle transitions reorganize community structure by altering relative abundance and prevalence of bacterial lineages. This pattern is consistent with human studies showing that lifestyle-associated compositional differences can occur without uniform reductions in alpha diversity (5, 68).

The genus-level shifts across the canine lifestyle gradient suggest that industrialization is associated with both depletion and enrichment of distinct gut bacterial groups. Non-industrialized dogs showed higher abundance or prevalence of several anaerobic lineages previously associated with carbohydrate fermentation or short-chain fatty acid production, whereas industrialized dogs were enriched for other gut-associated genera, including taxa within Prevotellaceae, Erysipelotrichaceae, Lachnospiraceae, and Peptostreptococcaceae. These patterns likely reflect integrated differences in diet, environmental exposure, human management, and microbial transmission (69, 70). However, because genus-level 16S data cannot resolve strain-level variation or functional heterogeneity within genera, these taxa should be interpreted as markers of lifestyle-associated community restructuring rather than direct evidence of specific metabolic roles.

Applying the VANISH/BloSSUM-like prevalence framework and co-occurrence analyses to dogs provided an additional link to the human industrialization literature (4, 64), but the categories should be interpreted as operational dog-defined prevalence groups. We observed analogous prevalence shifts in dogs: many VANISH-like genera were more prevalent in forager and agrarian dogs, whereas a smaller set of BloSSUM-like genera was more prevalent in industrialized dogs. Because these categories were defined by prevalence differences, we do not interpret their identification as independent evidence that prevalence differs by lifestyle. Instead, the additional information comes from their taxonomic composition, asymmetry, and organization into coabundance structure. These prevalence-shifting taxa were non-randomly distributed across CAGs inferred from abundance correlations across all samples without lifestyle labels, and CAG-level abundance changed directionally across lifestyles. This is conceptually parallel to human industrialization patterns (5, 71), while remaining host- and dataset-specific. The VANISH-like and BloSSUM-like categories therefore should not be assumed to represent the same bacterial lineages or ecological functions across host species. For example, several VANISH genera in humans such as *Segatella* and *Catenibacterium* that are often associated with carbohydrate- or fiber-rich diets in humans (72, 73), were enriched toward industrialized dogs. This may reflect host-specific nutritional ecology, commercial diets formulated with different fiber sources or ratios, or species-level heterogeneity within genus-level assignments.

The pastoralist dogs highlight why non-industrialized canine microbiomes should not be treated as a single "traditional" state. Pastoralist dogs shared free-ranging behavior

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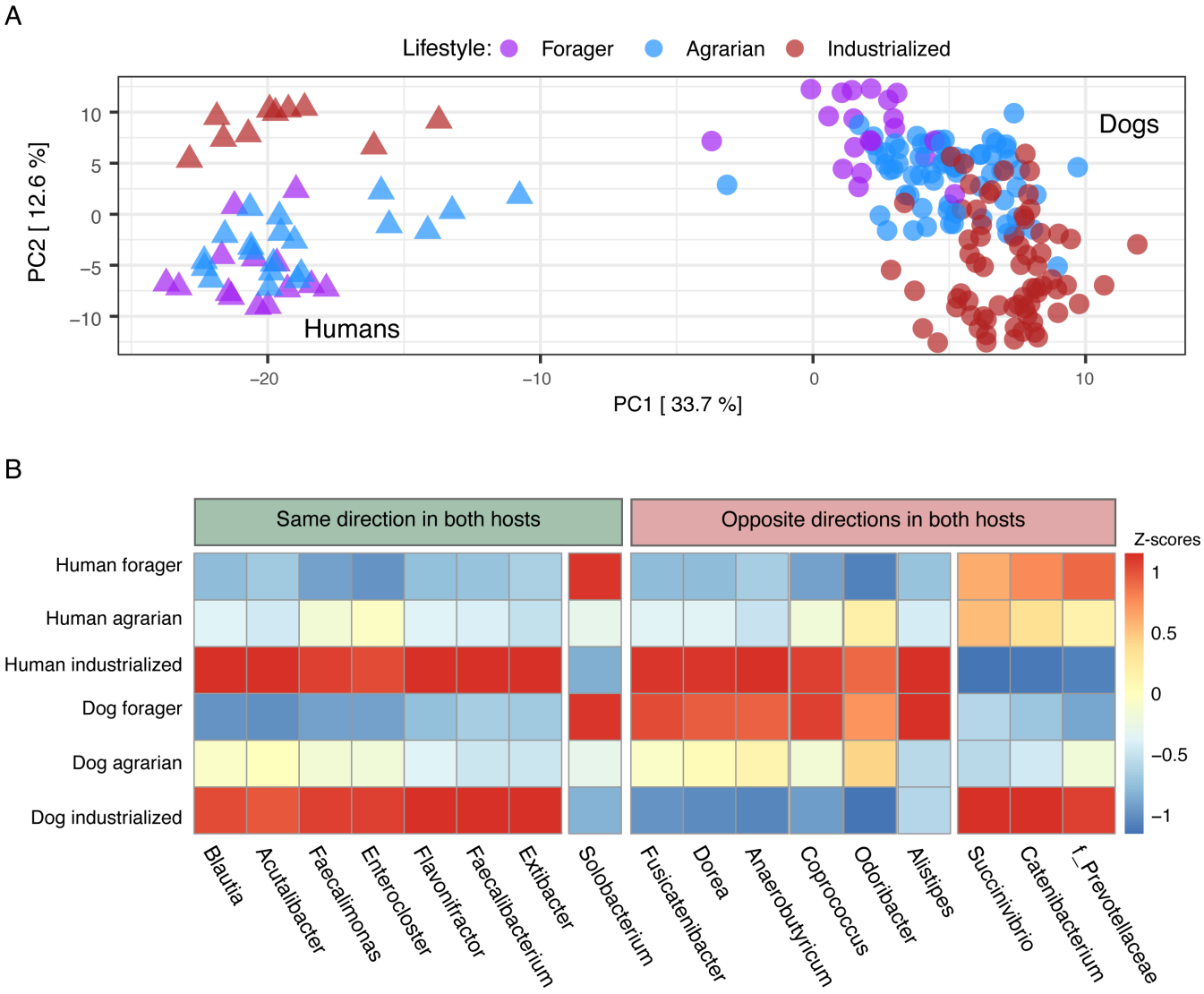


FIG 5 Shared lifestyle transitions produce host-specific microbiome responses in dogs and humans. (A) Principal component analysis (PCA) of centered log-ratio (CLR)-transformed genus-level abundance profiles after restricting the data sets to the 98 genera detected in both dogs and humans. Points represent individual samples, colored by lifestyle category and shaped by host species. Host species is the dominant axis of variation, while lifestyle-associated structure is evident within each host. (B) Heatmap of the 17 genera that showed significant directional lifestyle-associated responses in both dogs and humans, identified independently in each host. Columns show mean relative abundance across lifestyle groups within each host, scaled by row. Genera are grouped according to whether their lifestyle-associated responses occurred in the same direction or in opposite directions in dogs and humans.

and high environmental exposure with other non-industrialized dogs, but they occupied high-altitude settings and had distinct dietary and ecological contexts, including greater access to dairy and animal-derived foods (74). Their separation along the secondary ordination axis suggests that local subsistence ecology can shape gut microbiomes in ways not captured by a simple industrialized vs non-industrialized contrast. Elevation has been associated with gut microbiome variation in both wild mammals and humans, likely through correlated effects of hypoxia, temperature, diet, vegetation, physiology, and geography (75–78). In our data set, however, all high-altitude dogs were pastoralist dogs, so elevation cannot be separated from pastoralist lifestyle, climate, geography, or management. We therefore interpret altitude-associated patterns as part of the broader pastoralist ecological context rather than as evidence for an independent elevation effect. Future studies comparing high-elevation dogs with lower-elevation pastoralist dogs will be needed to test the specific contribution of altitude to the

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dog gut microbiome. The restricted classifier projection supported this interpretation: when forced into available forager, agrarian, urban, or industrialized labels, pastoralist dogs were assigned predominantly to the agrarian class and never to the industrialized class. Thus, pastoralist dogs appear to carry non-industrialized microbiome profiles while representing a distinct subsistence ecology rather than a simple intermediate stage. Together, these findings support treating lifestyle as an integrated ecological state that includes diet, human management, environmental exposure, climate, geography, and local subsistence ecology.

Predicted functional profiles broadly followed the lifestyle gradient, with many inferred enzyme functions and pathways enriched in forager dogs and declining toward industrialized dogs. This pattern is consistent with the observed taxonomic restructuring and suggests that lifestyle-associated community shifts may be accompanied by changes in metabolic potential. However, these profiles were inferred from 16S rRNA gene data rather than measured by shotgun metagenomics or metabolomics and therefore should be interpreted as supportive evidence of predicted functional differences, not as direct evidence of metabolic activity. Future metagenomic and metabolomic studies will be needed to test whether the taxonomic patterns observed here correspond to functional differences in carbohydrate degradation, lipid metabolism, amino acid metabolism, and microbial metabolite production.

The dog-human comparison places these findings in a broader comparative context. Prior studies show that shared ecology can increase microbiome similarity across host species, but that convergence is usually partial and context-dependent. Geographic coexistence, captivity, urbanization, and strong dietary specialization can all increase cross-host similarity (38, 40–46, 79), yet host-specific structure generally persists. Dogs therefore provide a particularly informative natural test of whether shared ecology can overcome host specificity because they have coexisted with humans over long timescales and across shared lifestyle transitions. Our results show that dogs and humans both show lifestyle-associated bacterial community restructuring across forager, agrarian, and industrialized categories. However, combined analyses of shared genus-level taxa showed that host species remained the dominant axis of variation, and lifestyle-associated shifts occurred within host-specific compositional space rather than producing strong cross-host convergence. The genera responding to lifestyle transitions were also largely host-specific, and same-direction overlap was not greater than expected under the shared-genus background. This pattern remained robust after stratified subsampling of dogs to match the number of human samples within each lifestyle category. Additional effect-size and taxonomic analyses further supported this interpretation. Concordant responses were limited to a small subset of shared genera and were largely concentrated within Bacillota, whereas the broader set of shared taxa showed little evidence of coordinated directionality across hosts. Thus, the dog-human comparison does not support a simple model in which industrialization is associated with the same genus-level changes across cohabiting mammals. Instead, it suggests that similar ecological transitions can restructure gut bacterial communities in parallel, while the specific bacterial lineages that respond remain strongly filtered by host biology.

This result clarifies the value of dogs as a comparative microbiome system. Dogs are not simple microbiome proxies for humans, despite their long history of cohabitation, shared environments, and consumption of human-associated foods. Instead, they provide a natural system for separating shared ecological pressure from shared microbial outcome. The absence of strong genus-level dog-human taxonomic convergence indicates that host physiology, immune filtering, gastrointestinal traits, diet processing, transmission history, and evolutionary background can constrain how industrialization-associated pressures are expressed. In this sense, dogs reveal both the reach and the limits of shared human ecology: industrialization can restructure gut microbiomes across human-associated mammals, but it does not erase host-specific community assembly.

Several limitations should be considered. First, this was an observational study across natural ecological gradients, where lifestyle, geography, climate, altitude, and

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human management are inherently coupled. We therefore interpret lifestyle as an integrated ecological exposure rather than as a single causal variable. Restricted analyses showed that lifestyle-associated structure persisted after limiting the data set to mature, non-shelter dogs from temperate climate regions and excluding Thai and UAE dogs, but these analyses cannot fully disentangle all components of lifestyle or exclude unmeasured covariates. Because all high-altitude dogs in this cohort were pastoralist dogs, future studies comparing high-elevation dogs with lower-elevation pastoralist dogs will be needed to isolate the specific contribution of altitude to the dog gut microbiome. Second, 16S rRNA gene sequencing limits taxonomic resolution, and it does not capture strain-level variation, gene content, or directly measured functional activity. Third, dietary, water-source, hygiene, and antibiotic-exposure information were categorical and incomplete for some populations, particularly dogs from Thailand. Raw-food status among industrialized dogs was not systematically available; household-prepared and scavenged foods in non-industrialized settings may also be carbohydrate-rich, and free-ranging dogs may be exposed to antimicrobial residues through wastewater or other environmental sources. Although Thai dogs projected near the industrialized end of the bacterial community gradient, they should be interpreted as urban community dogs with potential processed-food exposure rather than industrialized household pets. Fourth, dog-human comparisons were limited by differences in host biology, sample size, and 16S rRNA gene amplicon region, requiring genus-level harmonization. Stratified subsampling supported the robustness of the host-specificity result, but paired dog-human sampling, shotgun metagenomics, and metabolomics would provide a stronger test of taxonomic, strain-level, and functional sharing.

Conclusions

Our findings expand the ecological baseline for the domestic dog gut microbiome beyond industrialized companion animals and show that industrialization-associated lifestyle transitions are associated with robust, directional restructuring of canine gut bacterial communities. The dog-human comparison further indicates that shared lifestyle transitions can produce parallel compositional restructuring without strong genus-level taxonomic convergence, highlighting domestic dogs as a useful comparative system for studying how human-associated ecological change shapes mammalian gut microbiomes while preserving host-specific community structure.

MATERIALS AND METHODS

Optimization of fecal sample preservation

To select a field-compatible preservation method, we compared three commercial fecal preservation systems with flash-frozen reference samples using fecal samples from seven pet dogs at New York University Abu Dhabi. Microbial profiles differed between flash-frozen and preserved samples, whereas differences among preservation systems were modest (Fig. S18). We selected the Huechenyan fecal collection kit for field sampling because it performed comparably to the other preservation systems and was substantially less expensive.

Sample collection

Dog fecal samples were collected across Nepal, Thailand, the UAE, and the United States to capture variation in lifestyle, climate, geography, altitude, and human association. In Nepal, Huechenyan fecal collection kits were used to collect fecal samples from 151 dogs across nine communities representing four non-industrialized lifestyle categories. Twenty forager dogs were sampled from Chepang communities in Korak at approximately 1,400 m elevation; 67 agrarian dogs were sampled in Chobhar, Parsa, and Nuwakot at 100–1,500 m elevation; 26 pastoralist dogs were sampled from high-altitude regions of Rasuwa, Tsarang, and Lo-Manthang at 2,000–3,840 m elevation; and 38 urban dogs

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were sampled in Chitwan and Banepa at 100–1,500 m elevation. Most Nepali samples were obtained from free-ranging community dogs, except for 15 dogs sampled at the Chobhar rescue shelter. None of the Nepali dogs were reported to consume commercial pet food; instead, they consumed household and scavenged foods.

Outside Nepal, dogs were sampled in Chiang Mai, Thailand (n = 27); Abu Dhabi, UAE (n = 13); and the United States (n = 70), including Louisiana (n = 5), New York (n = 11), Pennsylvania (n = 15), and North Carolina (n = 39). Dogs from Chiang Mai were sampled in urban settings and included free-ranging community dogs brought to a rescue center and sampled on the day of rescue. Their individual diets prior to rescue were not known with confidence, but community dogs in Chiang Mai may forage within an urbanizing food environment where traditional diets coexist with greater access to commercial and processed foods (53). Thai dogs were therefore treated as urban community dogs with potential exposure to processed human food waste, rather than industrialized household pets. Nine dogs from North Carolina were sampled at an animal shelter, whereas the remaining UAE and U.S. dogs were household pets consuming commercial pet food. Individual raw-food status, water source, hygiene exposure, and environmental wastewater exposure were not systematically available for all dogs.

Dogs from Chiang Mai, Abu Dhabi, Louisiana, New York, and North Carolina were sampled using the Huechenyan fecal collection kits. Fecal samples from Pennsylvania pet dogs were collected on ice, transported to the laboratory, and frozen. All international shipments were transported on dry ice, and samples were stored at −80°C until DNA extraction. Samples were processed and sequenced together using a standardized protocol to minimize technical variation.

Dogs were assigned to tropical, arid-desert, temperate, and temperate-cold climate categories based on sampling location using Koppen-Geiger climate classification (80). ET and Df zones were grouped as temperate-cold because both corresponded to cooler, non-arid environments in this data set. Dogs were categorized as young (<0.5 years), mature (0.5–7 years), and older (>7 years), following veterinary life-stage guidelines (81). Metadata including climate category, altitude, age group, and recent antibiotic exposure, when available, are provided in Table S1.

DNA extraction and 16S rRNA gene sequencing

Samples were mechanically lysed using a Bead Ruptor 96 bead mill (Omni International, USA), followed by DNA extraction with the ZymoBIOMICS PowerSoil DNA Extraction Kit (D4303). Extractions were performed in individual tubes, and negative extraction controls were included after every eight samples.

A total of 351 samples, including 309 dog fecal samples and 42 negative extraction controls, yielded sufficient DNA for sequencing. Extracted DNA was shipped on dry ice to Novogene Singapore for library preparation and sequencing. The V3–V4 region of the bacterial 16S rRNA gene was amplified using primers 341F/806R with barcode-tagged forward primers (82). Twenty-nine negative controls and 20 fecal samples failed to amplify. PCR products from the successfully amplified samples, comprising 289 fecal samples and 13 controls, were purified using AMPure XP beads, quantified using the Qubit dsDNA BR Assay kit, pooled, and sequenced on an Illumina platform using paired-end 2 × 250 bp chemistry.

Amplicon sequence processing

Sequencing read quality was assessed using FastQC, and amplicon sequence variants (ASVs) were inferred using DADA2 (83). Reads were filtered and trimmed using maxN = 0, maxEE = 2, truncQ = 2; error rates were learned from the data, ASVs were inferred using pooled processing, paired-end reads were merged, and chimeras were removed. Taxonomy was assigned using the RDP classifier (84) using the SILVA v138 training set (85). ASVs classified as Eukaryota, chloroplast, mitochondria, or unclassified at the phylum level were removed, and ASVs represented by more than 10 reads in more than

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five samples were retained. ASVs were aligned using DECIPHER (86), and a phylogenetic tree was constructed using phangorn (87).

Downstream analyses were conducted in R v4.2.0 using phyloseq (88). Among the 302 sequenced samples, three duplicate fecal samples were removed. Thirteen controls passed sequencing quality filters and were retained for contamination assessment. To identify contamination-like or low-biomass samples, we performed PCoA and partitioning around medoids (PAM) clustering including all negative controls; 15 fecal samples clustered with controls (Fig. S19). Together with the 13 retained controls, these samples were excluded from downstream analyses. Ten additional fecal samples with fewer than 5,000 reads were removed. After filtering, 261 fecal samples remained, comprising 8,763,965 reads and 1,472 ASVs assigned to 245 bacterial genera. Four dogs with reported recent antibiotic exposure were excluded from downstream microbiome analyses, yielding 257 dogs in the final data set.

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Beta diversity analyses

Beta diversity was assessed using weighted UniFrac and Bray-Curtis distances calculated from genus-level abundance data after log(x + 1) transformation as described previously (89). Weighted UniFrac distances were calculated in phyloseq using the phylogenetic tree with normalization enabled. Principal coordinate analysis (PCoA) was performed using phyloseq and visualized with ggplot2 (90).

Associations between gut bacterial community composition and host or ecological metadata were tested using PERMANOVA with adonis2 in the vegan package (91) and 999 permutations. Multivariable models included lifestyle, climate category, altitude category, shelter status, and age group with marginal effects estimated using by = "margin." EnvFit analyses were performed using the first three ordination axes with 999 permutations to assess alignment of metadata variables with major compositional gradients.

To assess robustness to major measured confounders, beta-diversity analyses were repeated in a restricted subset of mature, non-shelter dogs sampled from temperate climate regions, excluding Thai and UAE dogs. This restricted subset was tested using the same weighted UniFrac and Bray-Curtis distance metrics. PERMANOVA models included lifestyle as the predictor variable, and EnvFit analyses assessed alignment of lifestyle with the first three ordination axes.

To evaluate whether lifestyle-associated structure could arise from arbitrary categorical labeling, we performed a randomized-label sensitivity analysis. Lifestyle labels were randomly permuted across samples while preserving the original group sizes, and PERMANOVA was repeated 1,000 times for each randomized-label data set. This analysis was performed for both weighted UniFrac and Bray-Curtis distances in the full cohort and in the restricted mature/non-shelter/temperate cohort. The full-cohort randomization analysis was performed on the genus-level data set after exclusion of dogs with reported recent antibiotic use, using the same PERMANOVA model as the main beta-diversity analysis: lifestyle, climate category, altitude category, shelter status, and age group. The restricted-cohort randomization analysis was performed on mature, non-shelter, temperate-climate dogs from Nepal and the United States, retaining forager, agrarian, urban, and industrialized lifestyle categories. Because age group, shelter status, and climate category were fixed by design in this subset, the restricted-cohort analysis tested lifestyle as the predictor of interest. Empirical P values were calculated as the proportion of randomized-label R^2 values greater than or equal to the observed lifestyle R^2, using a plus-one correction.

Random forest classifiers

Random forest classifiers were trained on log(x + 1)-transformed genus-level abundance profiles using the tidymodels framework (92) with the ranger engine to predict lifestyle category. Models were generated for the full 257-dog dataset and for the restricted mature, non-shelter, temperate-climate subset. The full-dataset model was used only

as a supplemental performance comparison. The restricted classifier was then used for projection of excluded dogs.

Samples were partitioned into training and held-out test sets using an 80/20 partition. Within the training set, fivefold cross-validation and grid search were used to tune mtry and min_n; models were trained with 500 trees. Final models were evaluated on held-out test sets using accuracy, Cohen's kappa, and multiclass ROC AUC calculated from one-vs-all class probabilities with the yardstick package (93).

For the restricted sensitivity and projection analysis, the classifier was trained using only mature, non-shelter dogs sampled from temperate climate regions, excluding Thai and UAE dogs. This restricted training set included 107 dogs spanning forager, agrarian, urban, and industrialized lifestyles. Pastoralist dogs were not included as a training class because they represented a distinct high-altitude ecological context rather than a stage along the forager-to-industrialized gradient. The classifier was trained on log(x + 1)-transformed genus-level abundance profiles using the same modeling framework described above. Model performance was evaluated using an 80/20 train-test split. The trained classifier was then used to project dogs excluded from the restricted training set, including Thai, UAE, additional U.S., excluded Nepali, and pastoralist dogs. Projection results were summarized by country and observed lifestyle category. Balloon plots show row-wise proportions assigned to each predicted lifestyle class, with sample counts shown inside points.

Alpha diversity analyses

Alpha diversity was quantified as observed ASV richness, Shannon diversity, and Faith's phylogenetic diversity. To assess sensitivity to sequencing depth, ASV tables were rarefied from 1,000 to 6,000 reads in 1,000-read increments, with 10 iterations per depth. Rarefaction curves indicated saturation at 6,000 reads; therefore, downstream alpha diversity analyses used estimates calculated at a rarefaction depth of 6,000 reads. Associations between alpha diversity and metadata were tested using generalized linear models with a Gamma distribution and log link, including lifestyle, climate category, shelter status, and age group as predictors. Altitude category was not included because it was collinear with climate category in this data set.

Differential abundance

Genus-level differential abundance was tested using MaAsLin2 (54). Two models were used. First, a lifestyle-only model was fit with industrialized dogs as the reference group to identify genera associated with the primary lifestyle gradient. Second, a multivariable model including lifestyle, climate category, and age group as fixed effects was fit to assess whether lifestyle-associated genera remained detectable after accounting for major ecological and host covariates. In this model, industrialized, tropical, and young dogs were used as reference populations for lifestyle, climate, and age, respectively.

Both models used linear models with cumulative sum scaling normalization, log transformation, and a minimum prevalence threshold of 5%. Genera were considered significant at FDR-adjusted q value < 0.05. Large-effect associations were defined as FDR-adjusted q < 0.05 with absolute MaAsLin2 coefficient > 1, corresponding approximately to a greater than twofold difference in normalized abundance on the log-transformed scale.

To identify directional lifestyle-associated taxa, we applied a monotonic-gradient criterion across the forager-agrarian-urban-industrialized axis. Genera were retained if coefficients followed either a decreasing pattern from forager to agrarian to urban to industrialized dogs or the reverse pattern, and if the forager-vs-industrialized endpoint contrast met the large-effect threshold. Pastoralist dogs were excluded from this monotonic-gradient because they represented a distinct high-altitude lifestyle rather than a simple intermediate stage along the forager-to-industrialized axis.

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Identification of VANISH-like and BloSSUM-like genera

VANISH-like and BloSSUM-like genera were identified as operational, dog-defined prevalence categories using an approach analogous to the human VANISH/BloSSUM framework. For this analysis, forager and agrarian dogs were grouped as the non-industrialized reference set and compared with industrialized dogs. Genus-level abundances were converted to presence/absence, and prevalence differences between groups were tested using Fisher's exact test with Benjamini-Hochberg correction.

Genera were classified as VANISH-like if they were significantly more prevalent in non-industrialized dogs, and as BloSSUM-like if they were significantly more prevalent in industrialized dogs. To focus on robust prevalence shifts, genera were retained if they had FDR-adjusted $q < 0.05$ and an absolute prevalence difference ≥ 0.30.

Coabundance group analyses

Coabundance groups (CAGs) were inferred from genus-level abundance profiles using FastSpar (65) and *P* values were estimated with 1,000 bootstrap replicates. Pairwise associations were filtered to retain genus pairs with FDR-adjusted $P < 0.05$ and absolute correlation coefficient >0.2. Genera involved in at least one significant association were clustered by hierarchical clustering of the filtered correlation matrix, yielding three major CAGs.

For visualization, positive genus-genus associations were plotted as a network plot using igraph (94). To quantify CAG abundance across lifestyles, genera assigned to each CAG were summed within each sample and remaining genera were grouped as a non-CAG fraction. CAG abundances were converted to sample-wise relative abundances and summarized by lifestyle. Enrichment of VANISH-like and BloSSUM-like genera across CAGs was tested using Fisher's exact test, with $P < 0.05$ used as the significance threshold.

Predicted functional profiling and pathway annotation

Predicted functional profiles were generated from 16S rRNA gene data using PICRUSt2 (66) and interpreted as inferred functional potential rather than directly measured microbial gene content or metabolic activity. EC-numbered enzyme features and MetaCyc pathway abundance tables were analyzed separately, retaining features present in at least 10% of samples.

Associations between predicted functional profiles and host/ecological variables were tested using MaAsLin2 with total-sum scaling normalization, log transformation, and linear modeling. Models included lifestyle, climate category, and age group as fixed effects, with industrialized dogs specified as the lifestyle reference group, tropical as the climate reference group, and young dogs as the age reference group. Significant associations were defined as FDR-adjusted $q < 0.05$, and large-effect associations were defined as FDR-adjusted $q < 0.05$ with absolute MaAsLin2 coefficient > 1.

Directional lifestyle-associated predicted functions were identified using the same monotonic-gradient criterion applied to genus-level taxa across the forager-agrarian-urban-industrialized axis. Features were retained if coefficients followed either a decreasing pattern from forager to industrialized dogs or the reverse pattern, and if the forager-vs-industrialized endpoint contrast met the large-effect threshold. Pastoralist dogs were excluded from this monotonic-gradient definition. EC features were assigned to broad enzyme classes using the first digit of the EC identifier, and MetaCyc pathways were annotated using MetaCyc pathway classes (95).

Cross-species comparisons between dogs and humans

Dog data from this study and human data from Jha et al. (5) were restricted to lifestyle categories shared across both data sets: foragers and agrarians from Nepal and industrialized samples from the United States. The human data set included 44 individuals: 14 foragers, 20 agrarians, and 10 industrialized participants. The matched

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dog data set included 155 dogs: 20 foragers, 67 agrarians, and 68 industrialized dogs. Raw human FASTQ files were reprocessed using the same DADA2 workflow and taxonomic assignment strategy applied to the dog data set.

Because human samples were profiled using the V4 region of the 16S rRNA gene and dog samples using the V3–V4 region, cross-host analyses were performed at the genus level rather than at the ASV level. A total of 98 genera were detected in both data sets. Dog and human data sets were first analyzed separately using Bray-Curtis PCoA of genus-level abundance profiles to assess lifestyle-associated structure within each host. Genus-level differential abundance analyses were then performed separately in dogs and humans using MaAsLin2, with industrialized samples specified as the reference group. Directional lifestyle-associated genera were identified using the same monotonic-gradient criterion applied in the dog analysis.

For combined dog-human compositional analyses, data sets were restricted to the 98 shared genera. Zero replacement was performed using the count zero multiplicative method in zCompositions, followed by centered log-ratio transformation. Principal component analysis was then applied to the CLR-transformed genus-level matrix to visualize major axes of variation across hosts and lifestyles.

To test whether lifestyle-associated genera overlapped between host species, the 98 shared genera were used as the background. Same-direction overlap was assessed separately for genera enriched in forager-associated bacterial communities and genera enriched in industrialized bacterial communities using one-sided Fisher's exact tests. We then compared cross-host concordance of forager-vs-industrialized effect sizes across all 98 shared genera. For each genus, we extracted the MaAsLin2 coefficient for the forager-vs-industrialized contrast from the dog and human models, with industrialized samples as the reference group in each host. Concordance of signed effect sizes was tested using Spearman correlation, and sign concordance was assessed using a two-sided binomial test comparing same-sign and opposite-sign effects. Among genera with significant directional responses in both hosts, defined as FDR-adjusted $q < 0.05$ and absolute MaAsLin2 coefficient > 1 in both analyses, same-direction and opposite-direction responses were compared using a two-sided binomial test. Broad taxonomic enrichment among same-direction responders was evaluated using Fisher's exact tests.

Robustness of dog-human PCA patterns to matched subsampling

To assess whether cross-host PCA patterns were affected by unequal sample sizes, we repeated the dog-human comparison after stratified subsampling of dogs to match the number of human samples in each lifestyle category: 14 foragers, 20 agrarians, and 10 industrialized. In each of 100 iterations, dog samples were randomly selected without replacement within each lifestyle category, merged with the full human data set using shared genera, and PCA was repeated using the same genus-level abundance transformation as in the primary analysis. For each iteration, we extracted sample-level PCA coordinates, variance explained, and host-by-lifestyle centroids. Robustness was evaluated from the stability of centroid positions across iterations.

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A.R.J. conceived the study. A. Gautam, D.B., A. Gyawali, P.Y., K.C.M.S., and K.A.-V.H. performed field work and sample collection. A. Gautam and A.R.A. performed experiments. A. Gautam, Kusha Gurung, Kshitiz Gurung, and A.R.J. performed data analyses. A .Gautam, D.B., Kusha Gurung, K.C.M.S., K.A.-V.H., L.S.W., A.K.K., and A.R.J. interpreted the results. A. Gautam, Kusha Gurung, and A.R.J. generated the figures. D.S., K.A.-V.H., L.S.W., A.K.K., and A.R.J. supervised the project. A. Gautam, Kusha Gurung, and A.R.J. wrote the manuscript with input from all authors. All authors approved the final manuscript.

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DATA AVAILABILITY

Summary data necessary to reproduce the analyses presented in this paper are provided in the Supplemental tables. Raw amplicon sequencing data have been deposited in the Sequence Read Archive under accession PRJNA1472931. A phyloseq object containing sample IDs, host-associated metadata, ASV-level count data, taxonomic annotations, and the phylogenetic tree is also included as supplemental data. Additional information needed to reanalyze the data is available from the corresponding author upon reasonable request.

ETHICS APPROVAL

All animal procedures were approved by the New York University Institutional Animal Care and Use Committee (IACUC Protocol #20-0005) and the Nepal Veterinary Council (Reg #535). Fecal samples were collected non-invasively from freshly voided feces or during routine handling with permission from owners, caretakers, or responsible community members where applicable. No animals were subjected to experimental procedures or interventions beyond routine handling for sample collection. Sample collection, transport, storage, and data sharing complied with applicable animal research, biosafety, and material transfer regulations.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental Figures (mSystems00864-26-s0001.docx). Fig. S1 to S19.
Supplemental Tables (mSystems00864-26-s0002.xlsx). Table S1 to S11.

Open Peer Review

PEER REVIEW HISTORY (review-history.pdf). An accounting of the reviewer comments and feedback.

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