Highly specialized plant-based diets drive the decrease of gut microbiota diversity and lignocellulose-degrading function

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Abstract

Dietary specialization represents an extreme adaptive strategy in mammals, yet its effects on the gut microbiome remain poorly understood. Here, we investigated the gut microbiomes of three wild bamboo dietary specialists—the Chinese bamboo rat, giant panda, and Chinese red panda—together with their respective relatives, and extended to additional leaf dietary specialists, including folivorous colobines, folivorous lemurs, and koalas. By integrating 16S rRNA and metagenome data with lignocellulose-degrading enzyme assay experiments, we revealed an unconventional pattern across feeding guilds: highly specialized plant-based diets drive the decrease of gut microbiota diversity and carbohydrate metabolic capacity. Despite shared bamboo diets, bamboo rats showed no gut microbiome convergence with pandas, underscoring gut morphology as a key constraint. Across dietary specialists, polysaccharide metabolism, lignocellulose-degrading gene repertoires, and associated enzyme activities diminished, while pathways for plant secondary metabolite detoxification were enriched. These results emphasize the role of host–microbe compensatory strategies rather than enhanced carbohydrate metabolism in extreme plant dietary adaptation, and call for more natural dietary variability in the captive management of endangered specialists.

Keywords: dietary specialization, gut microbiome, plant-based diet, lignocellulose degradation, adaptive evolution

Introduction

Dietary specialization in mammals represents a long-term evolutionary strategy, wherein species restrict their diets to one specific type or category of food, such as ants (myrmecophagy), bamboos (bamboo specialists), or leaves (folivory) (Chivers & Hladik 1980; Huang et al. 2021; Cheng et al. 2023; Schulz et al. 2025). Such specialization is typically accompanied by pronounced behavioral and physiological adaptations. For example, giant pandas compensate for the low nutrition of bamboo through prolonged feeding; anteaters and pangolins have evolved specialized mouthparts for consuming ants; and folivorous primates possess complex digestive systems that facilitate fiber fermentation (Reiss 1997; Lambert 1998; Delsuc et al. 2014; Nie et al. 2015). These evolutionary adaptations in morphology and ecology provide powerful models for studying host–microbiome interactions under extreme dietary constraints.

Previous studies have extensively explored gut microbiome evolution in dietary specialists and often emphasized convergent microbial adaptations. Unlike myrmecophagous mammals that employ a cooperative host–microbe strategy for polysaccharide metabolism (Cheng et al. 2023), most herbivores lack host-encoded lignocellulolytic enzymes and depend predominantly on microbial fermentation (Ley et al. 2008; Zhu et al. 2011). Giant panda (*Ailuropoda melanoleuca*) and red panda (*Ailurus* spp.), despite belonging to different families, exemplify this phenomenon: both retain carnivore-like digestive tracts but subsist almost exclusively on bamboo. Their gut microbiomes are enriched in *Clostridium_sensu_stricto_1* and genes related to cellulose degradation and cyanide detoxification, indicating microbial convergence compared with omnivorous and carnivorous relatives (Zhu et al. 2018; Huang et al. 2021). In contrast, folivorous primates show weaker cross-lineage microbial convergence, despite harboring microbial genes involved in secondary metabolite metabolism, highlighting the constraining roles of host phylogeny and gut morphology (Amato et al. 2019). An even more extreme case is the koala (*Phascolarctos cinereus*). Its eucalyptus-leaf diet is associated with gut microbiome containing taxa such as *Bacteroides* and Synergistaceae, which have been linked to the detoxification capacity for plant secondary metabolites, enabling it to persist on this highly specialized diet (Shiffman et al. 2017; Bowerman et al. 2026).

The Chinese bamboo rat (*Rhizomys sinensis*), belonging to the Rodentia, is also a

bamboo dietary specialist. Unlike pandas, it possesses a well-developed cecum that supports hindgut fermentation. Previous studies have identified lignocellulose-degrading bacteria (e.g., *Clostridium* and *Bacteroides*) and some carbohydrate-active enzyme families (e.g., GH1, GH3, GH43) in its gut microbiota (Bai et al. 2021; Xiao et al. 2022; Likidkarnchanakornkij et al. 2023). These are capable of breaking down complex plant polysaccharides into oligosaccharides or monosaccharides. However, compared with other rodents that consume different diets, it remains unclear whether the bamboo rat gut microbiome converges with those of giant and red pandas, or whether factors such as gut morphology and host phylogeny, fundamentally alters microbial structure and function. In parallel, giant pandas exhibit lower gut microbial diversity, with cellulose- and hemicellulose-degrading enzyme abundances comparable to those of black bears and other carnivores (Guo et al. 2018, 2020), raising the questions of whether similar reductions occur in other bamboo specialists. More broadly, although considerable progress has been made in characterizing the gut microbiomes of mammals with highly specialized plant-based diets, how these diets shape gut microbiome structure and function remains incompletely understood.

To address these uncertainties, we combined 16S rRNA and metagenomic data with lignocellulose-related enzyme activity assays to characterize the diversity, structure, and function of the gut microbiota in three wild bamboo specialists: Chinese bamboo rat, giant panda, and Chinese red panda (*Ailurus styani*). Furthermore, we performed broad comparative analyses across folivorous colobine monkeys, folivorous lemurs, and koalas, providing a more comprehensive assessment of how dietary specialization influences the assembly of mammalian gut microbial communities.

Materials and Methods

Sample collection

Fresh feces (n=21) were collected from 21 wild Chinese bamboo rats in the Qinling Mountains, Shaanxi Province. And fresh feces were collected from 12 wild giant pandas in the Qinling Mountains and from 9 wild Chinese red pandas in the Qionglai (n=4) and Daxiangling (n=5) Mountains (Table S1). For enzyme activity assays, 28 fresh fecal samples were collected from various animals, including coatis, raccoons, kinkajous, Asian black bears, brown bears, and polar bears from Beijing Zoo, as well

as Brandt's voles and Mongolian gerbils from Inner Mongolia (Table S3). Captive individuals had not received antibiotics or other treatments within three months prior to sampling and were fed standardized diets approximating natural feeding habits. Only apparently healthy individuals were sampled based on field observations. Fecal samples from captive animals were collected immediately after defecation under zoo observation, while field-collected samples were selected to ensure they were as fresh as possible based on appearance and field conditions. To minimize environmental contamination, fecal surfaces in direct contact with the ground were removed. All fecal samples were collected using sterile instruments, placed into sterile sampling tubes, transferred to the laboratory with dry ice containers, and finally kept in −80°C freezer until further analysis.

DNA extraction and sequencing

The total DNA of each sample was extracted according to the protocol of the Invitrogen Easy-DNA kit (K180001, Thermo Fisher Scientific, Inc., USA). DNA concentration and purity were assessed using NanoDrop, and integrity was evaluated by 1% agarose gel electrophoresis. DNA samples were considered qualified when they met the following criteria: concentration ≥2 ng/μL, A260/280 ratio between 1.8–2.0, and no visible degradation on agarose gel electrophoresis. Only qualified samples were used for polymerase chain reaction amplification and library construction. Subsequently, metagenome sequencing of PE150 was performed on the Illumina NovaSeq 6000 platform (Majorbio Bio-Pharm Technology Co., Ltd., China), and 16S rRNA sequencing of PE300 was performed on the Illumina MiSeq platform, targeting the V3–V4 variable region using the following primers 338F: 5′-ACTCCTACGGGAGGCAGCAG-3′ and 806R: 5′-GGACTACHVGGGTWTCTAAT-3′.

Collection of public data

To elucidate the role of dietary specialization in shaping the gut microbiome, we compiled fecal 16S rRNA and metagenomic datasets from three bamboo specialists and their other dietary relatives (Table S1 and S2). Specifically, sequencing data from giant and red pandas were compared with those from other Carnivora species (Ursidae, Procyonidae, and Mustelidae) representing omnivorous and carnivorous counterparts, whereas data from bamboo rats were contrasted with those from other rodents (Spalacidae, Muridae, and Cricetidae) representing herbivorous and omnivorous

relatives.

To further assess the general influence of dietary specialization across distinct feeding guilds, we incorporated publicly available gut microbiome datasets from three additional scenarios: folivorous colobines (four obligate folivores and six non-folivorous monkeys), folivorous lemurs (two obligate folivores and four non-folivorous lemurs), and koalas together with two non-specialized relatives (Tables S5 and S6). Comparative species were selected based not only on phylogenetic relatedness, but also on digestive morphology and dietary breadth. Dietary classifications for each species were assigned according to published studies and the MammalDIET database (Kissling et al. 2014), with corresponding references provided in Table S7. Samples associated with disease conditions and juvenile individuals were excluded based on available metadata.

16S rRNA data analysis

The tags and primers of sequences were removed using Cutadapt (Martin 2011), followed by FLASH (Magoč & Salzberg 2011), to merge the paired-end sequences of 16S rRNA data. Considering that some published datasets targeted the V4 region, which exhibits relatively high reproducibility and consistency across studies (Nagai et al. 2024; Tremblay et al. 2015), sequences from V3–V4 region were trimmed to the corresponding V4 region to enable cross-dataset comparability. This strategy has been shown to reduce biases associated with different variable regions and to yield comparable microbiome profiles (Liu et al. 2020). Specifically, trimming was performed using Cutadapt with the parameter "-g GTGYCAGCMGCCGCGGTAA." Quality control was further performed using Trimmomatic (Bolger et al. 2014) with the parameters "SE -phred33 LEADING:20 TRAILING:20 SLIDINGWINDOW:4:20 MINLEN:50."

Subsequently, all clean data were processed according to the QIIME2 (Bolyen et al. 2019) pipeline. Amplicon sequence variants (ASVs) were generated using DADA2, and taxonomically annotated using the naive Bayes classifier based on the Silva database. The phylogenetic tree was constructed using Mafft based on multiple sequence alignment. Besides, low-abundance ASVs, present in only one sample or with a frequency of <10 (total reads across all samples), as well as eukaryotic, mitochondrial, and chloroplast sequences, were filtered out. For downstream analyses, samples were rarefied to the minimum sequencing depth within each dietary scenario (6,956 and

6,975 reads for bamboo and leaf specialist datasets, respectively) using the phyloseq (McMurdie & Holmes 2013) package. Shannon rarefaction curves reached clear saturation at these depths, indicating sufficient sequencing coverage to capture overall microbial diversity (Fig. S1). Based on the rarefied datasets, α- and β-diversity metrics and the relative abundances of annotated microbial taxa across multiple taxonomic levels were calculated.

Metagenome data analysis

The primers and low-quality sequences of metagenome dataset were removed by fastp (Chen et al. 2018), and host genome sequences were removed by Bowtie2 (Langmead & Salzberg 2012). Clean data was assembled using MEGAHIT (Li et al. 2015) with the parameters "-min-count 2 –k-min 27 –k-max 87 –k-step 10 –min-contig-len 300." Genes were annotated using Prokka (Seemann 2014) with the parameter "--prefix mg." The non-redundant gene set was constructed using CD-HIT (Fu et al. 2012) with the parameters "-aS 0.90 -c 0.95" and further translated into amino acid sequences. These sequences were aligned to the CAZy database using DIAMOND (Buchfink et al. 2015), and aligned to the KEGG database using eggNOG-mapper (Cantalapiedra et al. 2021) with the same parameter "--evalue 1e-5." To enable taxonomic identification, these sequences were also aligned to the NCBI non-redundant database using DIAMOND with the parameters "--query-cover 50 --id 50." These non-redundant genes across all sample data were quantified using Salmon (Patro et al. 2017) with the parameter "--meta." Meanwhile, the abundance of these genes was normalized using transcripts per kilobase of exon model per million mapped reads (TPM).

Lignocellulolytic enzyme activity assay

Cellulose is a polysaccharide composed of glucose, and hemicellulose is a heteropolysaccharide mainly composed of xylan and galactomannan, among which cellulase (EC:3.2.1.4) and β-glucosidase (EC:3.2.1.21) are key enzymes in cellulose degradation pathways, and xylanase (EC:3.2.1.8) and β-mannanase (EC:3.2.1.78) are key enzymes in hemicellulose degradation pathways (Shallom & Shoham 2003; Xu et al. 2019; Houfani et al. 2020). Therefore, these four enzymes were chosen as indicators to compare their activities in the feces of three bamboo dietary specialists and their respective relatives.

Initially, fecal samples were prepared with phosphate-buffered saline (pH 7.4) at a ratio of 1 g/9 ml. Homogenization was performed using the tissue homogenizer (KZ-

III-FP, Servicebio) with ice bath treatment. After centrifugation at 3000rpm/min for 20 minutes, the supernatant was collected. Subsequently, cellulase (anthrone colorimetry), xylanase (3,5-dinitrosalicylic acid method), β-glucosidase (p-nitrophenyl-β-D-glucopyranoside method), and β-mannanase (3,5-dinitrosalicylic acid method) activities were determined using enzyme activity assay kits (Quanzhou Ruixin Biological Technology Co., Ltd., China) according to the instructions. Finally, enzyme activities were quantified by calculating degradation capabilities (the amount of reducing sugar produced per minute per milliliter of supernatant sample). This experiment used a total of 40 fecal samples, including carnivores (polar bears, *n*=2), omnivores (Ursidae, n=4; Procyonidae, n=7; wild Mongolian gerbils, n=5), herbivores (wild Brandt's voles, *n*=5) as well as wild Chinese bamboo rats (CBR1–5, *n*=5), wild giant pandas (GPW1–8, *n*=8), and wild Chinese red pandas (RPW2–5, *n*=4) as bamboo dietary specialists (Table S3). Two replicates were taken from each sample to provide more confident and reliable results.

Statistical analysis

Differences in microbial diversity, gene abundances of lignocellulose-degrading enzyme, chitinase, trehalase and associated GH families, as well as enzyme activity levels, were assessed using the Wilcoxon rank-sum test. Only the $P \leq 0.05$, corrected by the Benjamini-Hochberg method, was statistically significant. Linear discriminant analysis effect size (LEfSe) was used to analyze the differences in microbial abundance, and only a linear discriminant analysis (LDA) score of ≥2 was considered significant. Enrichment of microbial functional pathways was assessed using the Reporter score (Peng et al. 2024) approach, with $|\text{Reporter score}| \geq 1.64$ indicating statistical significance. Bray–Curtis distance and PCoA were obtained using the vegan package, and the significance testing was performed using the *adonis* function. All visual analyses in this study were performed using the ggplot2 package.

To quantify the relative contributions of host and ecological factors to gut microbiome structure and function, MRM model was used. All regression variables were transformed into distance matrices to account for the diverse data types involved. Specifically, host phylogenetic relationships were obtained from TimeTree (https://timetree.org/) and converted into the patristic distance (branch length). Diet (bamboo, herbivore, omnivore, and carnivore), gut morphology (simple and cecum fermentation), and sampling strategy (wild/captive + gut contents/feces), were

represented by the corresponding Gower distances, calculated with the cluster package, which can effectively handle these categorical variables. α-diversity indices (Observed and Shannon) were converted into Euclidean distances to represent microbiome diversity. The relative abundances of functional pathways and ASVs were transformed into Bray–Curtis distances to represent microbiome structure and function, respectively. Given concerns about multicollinearity in the model, Spearman's correlation (ρ) was analyzed using the mantel test to assess the strength of correlations among explanatory variables. Model significance was assessed from performing 1000 permutations with true data using the ecodist (Goslee & Urban 2007) package. Considering within-group heterogeneity, biological replication was conducted by randomly selecting data from each species with the minimum sample size. This process was repeated 100 times to obtain adjusted *P* values.

Results

Comparison of gut microbiome structures among three bamboo dietary specialists

We integrated metagenomic and 16S rRNA datasets, which included wild Chinese bamboo rats (CBR), wild giant pandas (GP), and wild Chinese red pandas (RP), as well as publicly available data from representative Carnivora and Rodentia lineages. Across all comparisons, sampling was broadly balanced between bamboo specialists and their respective relatives: CBR–herbivores–omnivores = 21:22:22 (16S) and 21:16:18 (metagenome); GP–omnivores–carnivores = 28:24:10 (16S) and 26:16:4 (metagenome); RP–omnivores–carnivores = 14:16:7 (16S) and 9:11:5 (metagenome). Detailed sample information was provided in Table S1, Table S2, and Fig. 1A.

Taxonomic profiling based on 16S rRNA data revealed pronounced divergence, rather than convergence, in gut microbiota composition between Chinese bamboo rats and two panda species. The gut microbiomes of Chinese bamboo rats harbored a higher relative abundance in Bacteroidetes (46.2%), alongside Firmicutes (26.2%) and Proteobacteria (12.3%), whereas those of giant and red pandas were dominated by Proteobacteria (48.5% and 78.2%) and Firmicutes (41.3% and 13.9%) (Fig. 1A). At the genus level, Muribaculaceae_unclassified (38.9%), *Clostridium_sensu_stricto_1* (15.4%), and *Pseudomonas* (51.3%) were the dominant taxa in the gut microbiomes of Chinese bamboo rats, giant and red pandas, respectively (Fig. 1B). Notably, *Pseudomonas* (16.0%) was also abundant in giant pandas. These genera displayed significant abundance differences among the gut microbiota of bamboo specialists and

their respective relatives (adjusted P<0.05; Fig. 1B and Fig. S2). In addition, compared with their respective relatives, Chinese bamboo rats showed significantly reduced abundances of multiple genera including *Clostridia_UCG−014*, *Ruminococcus*, *Monoglobus*, *Phascolarctobacterium*, and *Megasphaera*, whereas giant and red pandas exhibited markedly lower abundances of multiple genera such as *Bacteroides*, *Clostridia_UCG−014*, *Fusobacterium*, and *Peptostreptococcus* (Fig. S3).

β-diversity analyses further confirmed the distinct microbial community structures across the three dietary specialists. Principal coordinate analysis (PCoA) based on ASVs abundance and Bray–Curtis distance showed clear separation between the gut microbiota of Chinese bamboo rats and those of giant and red pandas (Fig. 1C). More broadly, gut microbiota differed significantly between Carnivora with simple digestive tracts and rodents with cecum fermentation (R^2=0.08, P=0.001). Spearman's correlation analysis showed that gut morphology was strongly correlated with host phylogeny (ρ>0.5; Table S8), indicating substantial shared variation. Given this collinearity, we performed multiple regression on matrices (MRM) analysis using models that included either gut morphology or phylogeny, along with diet and sampling strategy. In these models, both gut morphology and phylogeny exhibited larger partial regression coefficients than diet and sampling strategy, and were significantly associated with microbiome structure divergence (Fig. 1D and Fig. S4A). Notably, gut morphology showed a stronger statistical association with this variation than host phylogeny (mean coefficient: 0.51 vs 0.45).

Despite these structural differences, all three bamboo dietary specialists exhibited reduced gut microbial diversity. Rodents such as Chinese bamboo rats displayed higher gut microbial diversity than Carnivora species (Fig. 1E), and MRM analysis also indicated that this pattern was strongly influenced by differences in gut morphology (Fig. S5). However, when compared to herbivorous or omnivorous rodents, Chinese bamboo rats showed a significantly lower Shannon index (adjusted P<0.05), while the Observed index was similar. Likewise, both giant and red pandas showed significantly decreased Shannon and Observed indices compared with their closely related Carnivora species (adjusted P<0.05).

Comparison of gut microbiota functional profiles among three bamboo dietary specialists

Metagenomic analyses based on KEGG functional pathways revealed pronounced

functional divergence, rather than convergence, among the three bamboo dietary specialists. Compared with giant and red pandas, the gut microbiomes of Chinese bamboo rats showed significant enrichment in pathways related to carbohydrate metabolism, such as glycosaminoglycan, other glycan degradation, and amino sugar and nucleotide sugar metabolism (Fig. S6). PCoA based on Bray–Curtis distance further demonstrated that rodents with cecum fermentation and Carnivora species with simple digestive tracts formed distinct functional clusters (Fig. 2A, R^2=0.21, P=0.001). Despite sharing a bamboo diet, the functional profiles of Chinese bamboo rats closely resembled those of other rodents rather than giant or red pandas. Given the collinearity between gut morphology and host phylogeny (ρ>0.5; Table S8), we performed MRM analysis using models that included either gut morphology or phylogeny together with diet and sampling strategy. In these models, gut morphology exhibited a larger partial regression coefficient than diet and sampling strategy and was significantly associated with functional divergence (Fig. 2B). Moreover, it consistently showed a stronger statistical association with microbiome variation than host phylogeny (mean coefficient: 0.65 vs 0.56), suggesting that gut morphology captures a substantial proportion of the observed differences (Fig. S4B).

Notably, compared with their other dietary relatives, none of the three bamboo specialists exhibited enhanced functional capacities for carbohydrate metabolism. The gut microbiomes of Chinese bamboo rats lacked significant enrichment in multiple key polysaccharide metabolism pathways, whereas herbivorous and omnivorous rodents exhibited strong enrichment in starch and sucrose metabolism, galactose metabolism, fructose and mannose metabolism, amino sugar and nucleotide sugar metabolism, and other glycan degradation (Fig. 2C). By contrast, the gut microbiomes of Chinese bamboo rats were significantly enriched in pathways involved in degrading plant secondary metabolites (PSMs), including aromatic compounds (e.g., benzoate, aminobenzoate, and styrene), terpenoids (pinene, camphor, and geraniol), and steroid.

Similarly, the gut microbiomes of giant pandas displayed enrichment only in starch and sucrose metabolism compared with those of their carnivorous relatives; however, across most other polysaccharide metabolism pathways, including galactose metabolism, fructose and mannose metabolism, and other glycan degradation, as well as short-chain fatty acid metabolism pathways such as propanoate and butanoate metabolism, they showed substantially lower gene abundances than those of both omnivorous and carnivorous relatives (Fig. 2D). Instead, the gut microbiomes of giant

pandas exhibited significant enrichment in microbial pathways related to plant-derived lipid metabolism, including α-linolenic acid metabolism, biosynthesis of unsaturated fatty acids, and cutin, suberine and wax biosynthesis, in addition to PSMs degradation. Consistent with these patterns, the gut microbiomes of Chinese red pandas also showed significant enrichment in PSMs degradation pathways, while lacking enrichment across many polysaccharide metabolism pathways compared with those of their relatives (Fig. 2E).

CAZyme (carbohydrate-active enzymes) profiles further confirmed the absence of functional enhancement in lignocellulose degradation. Compared with other rodents, especially omnivorous species, the gut microbiomes of Chinese bamboo rats showed significantly lower gene abundance of glycoside hydrolase (GH) families associated with plant fiber breakdown. These included GH113, GH116, GH12, GH120, GH26, GH27, GH3, GH36, GH43, GH45, and GH51 (Fig. 2F). The gut microbiomes of giant pandas exhibited enrichment in only GH116, and those of Chinese red pandas in only GH3, GH43, and GH5, whereas those of their respective relatives displayed broad GH-family enrichment.

Comparison of abilities to degrade lignocellulose among three bamboo dietary specialists

Given that lignocellulose is the primary component of bamboo (Wei et al. 2015; Wu et al. 2017), we analyzed the enzyme-encoding genes related to lignocellulose degradation in the gut microbiomes of three bamboo dietary specialists. Four key enzymes with relatively high abundance were identified, including cellulase (EC:3.2.1.4) and β-glucosidase (EC:3.2.1.21) associated with cellulose degradation, and β-xylosidase (EC:3.2.1.37) and β-mannanase (EC:3.2.1.78) associated with hemicellulose degradation (Fig. 3A and Table S4). Two additional hemicellulose-degrading enzymes, β-galactosidase (EC:3.2.1.22) and arabinofuranosidase (EC:3.2.1.55), were also detected but at lower abundances. The gut microbiomes of Chinese bamboo rats showed significantly higher abundances of lignocellulolytic enzyme-encoding genes than those of giant and red pandas, but these abundances were still markedly lower than those in other dietary rodents (Fig. 3A; adjusted $P<0.05$). In giant pandas, most lignocellulose-degrading enzymes did not display elevated gene abundances compared with their omnivorous and carnivorous relatives; only cellulase was significantly

increased, while β-galactosidase was significantly reduced. A similar pattern was observed in Chinese red pandas: besides a significant increase in β-glucosidase, most lignocellulolytic enzymes were not elevated, and β-mannanase showed a significantly lower abundance than in their relatives.

To obtain direct and accurate evidence for lignocellulose-degrading capacity of the gut microbiota, we collected additional fresh fecal samples for enzyme activity assays, including carnivores (polar bears), omnivores (Ursidae, Procyonidae, and Mongolian gerbils), and herbivores (Brandt's voles) (Table S3). Sample sizes were balanced across each bamboo dietary specialist and its relatives as follows: Chinese bamboo rat–herbivores–omnivores (5:5:5), giant panda–omnivores–carnivores (8:4:2), and Chinese red panda–omnivores (4:7). We then quantified and compared key cellulose- and hemicellulose-degrading enzyme activities in feces, including cellulase, xylanase, β-glucosidase, and β-mannanase, across these species.

Results demonstrated that the gut microbiota of Chinese bamboo rats, giant pandas, and Chinese red pandas displayed similar activities of the four enzymes (Fig. 3B), suggesting comparable bamboo degradation capabilities. However, compared with other dietary rodents, Chinese bamboo rats showed significantly lower activities of cellulase, xylanase, and β-glucosidase (adjusted $P<0.05$), along with lower β-mannanase activity, indicating an overall lower lignocellulose-degrading capacity. Giant pandas also displayed lower or comparable activities of these enzymes compared with their omnivorous and carnivorous relatives; notably, their xylanase activities were significantly lower than those of carnivorous Ursidae species (adjusted $P<0.05$). For Chinese red pandas, both cellulase and xylanase activities were significantly lower than those of omnivorous Procyonidae species (adjusted $P<0.05$), and both β-glucosidase and β-mannanase demonstrated lower activities. Together, these findings indicate that bamboo diet specialization has not enhanced, but rather decreased the lignocellulose-degrading capacity of the gut microbiota in Chinese bamboo rats and two panda species.

Gut microbiome patterns in folivory dietary specialists

To deepen our understanding of the effects of dietary specialization, we further incorporated publicly available gut microbiome datasets from additional hosts with

highly specialized diets (Table S4 and S5). These datasets encompassed three representative scenarios: (1) wild folivorous colobine monkeys (*Piliocolobus badius*, *Colobus guereza*, *Rhinopithecus bieti*, and *Rhinopithecus roxellana*; *n*=28 for 16S; *n*=23 for metagenome) compared with omnivorous Cercopithecidae species (*n*=31; *n*=20); (2) wild folivorous lemurs (*Avahi peyrierasi* and *Indri indri*; *n*=12 for 16S; *n*=4 for metagenome) compared with herbivorous (*n*=11; n=10) or omnivorous (*n*=11 for 16S) lemurs; and (3) wild koala (*n*=5; *n*=5), an obligate eucalyptus feeder, compared with herbivorous (*n*=7; *n*=4) and omnivorous (*n*=8; *n*=7) relatives. Notably, these taxa span different digestive tract morphologies: colobine monkeys possess a ruminant-like foregut, omnivorous Cercopithecidae monkeys rely on colon fermentation, and the remaining species utilize cecum fermentation (Lambert 1998; Hume 2002; Matsuda et al. 2011).

Across all three scenarios, highly specialized leaf-based diets were consistently associated with reduced gut microbial diversity. Both the Observed and Shannon indices were significantly lower in folivorous colobines, folivorous lemurs, and koalas compared with their respective diet-different relatives (Fig. 4A; adjusted $P<0.05$). Functional profiling revealed no evidence of enhanced carbohydrate metabolism in any leaf dietary specialist. In folivorous colobines, most carbohydrate-related pathways, including polysaccharide degradation and short-chain fatty acid metabolism (e.g., starch and sucrose, propanoate, and butanoate metabolism), were markedly reduced in contrast to omnivorous monkeys (Fig. 4B). Instead, the gut microbiomes of these specialists showed significant enrichment in pathways for plant secondary metabolite (PSM) degradation and plant-derived lipids metabolism, including aromatic compounds, terpenoids, and steroids, and α-linolenic acid and linoleic acid. Similar patterns were observed in folivorous lemurs. Compared with herbivorous lemurs, they exhibited substantially reduced abundance of glycan degradation pathways but enrichment of aromatic compound degradation pathways (e.g., nitrotoluene, dioxin, and xylene) (Fig. 4C). The koala mirrored this trend, with no increased polysaccharide or short-chain fatty acid metabolism but significant enrichment in PSMs degradation pathways in contrast to other dietary relatives (Fig. 4D and E).

CAZyme profiles further supported these results. In folivorous colobines, enrichment was limited to GH43, whereas omnivorous monkeys displayed broad enrichment across GH families associated with lignocellulose degradation (Fig. 4F).

Folivorous lemurs also showed limited enrichment (GH5, GH51, GH74), in contrast to the widespread GH family enrichment observed in herbivorous lemurs. Likewise, koalas lacked broad GH family enrichment compared with herbivorous and omnivorous relatives. Consistently, the gene abundances of key lignocellulose-degrading enzymes were generally low across specialists (Fig. 4G). In folivorous colobines, all four major enzymes were reduced, with cellulase (EC 3.2.1.4) and β-mannanase (EC 3.2.1.78) significantly lower than in omnivorous monkeys (adjusted $P<0.05$). Folivorous lemurs exhibited lower or comparable abundances, particularly in β-glucosidase (EC 3.2.1.21). In koalas, three enzymes were significantly reduced (adjusted $P<0.05$), with β-mannanase abundance elevated but not significantly different from herbivorous relatives.

Discussion

In this study, we examined the gut microbiomes of three wild bamboo dietary specialists, namely the Chinese bamboo rat, giant panda, and Chinese red panda, alongside their close relatives with different diets, and further incorporated a case of folivory dietary specialization, including wild folivorous colobines, folivorous lemurs, and koalas. Collectively, these comparisons revealed a pattern that challenges conventional expectations: highly specialized plant-based diets drive the decrease of gut microbial diversity and carbohydrate-degrading function, rather than the increase of carbohydrate metabolic capacity.

Host digestive physiology drives microbiome divergence among bamboo specialists

Despite sharing a bamboo diet, giant and red pandas did not converge with Chinese bamboo rats in gut microbiome structure or function. Instead, bamboo rats exhibited higher microbial diversity, greater Bacteroidetes abundance, and increased abundances of lignocellulose-degrading genes. MRM analysis identified both gut morphology and host phylogeny as key drivers of these differences, with gut morphology exhibiting a stronger association: giant and red pandas retain simple, carnivore-like digestive tracts, whereas bamboo rats rely on cecum fermentation, supporting abundant anaerobic microbes and prolonged food retention time (Kong et al. 2014; Xue et al. 2015; Xiao et al. 2022). Similarly, bamboo lemurs also exhibit higher microbial diversity and distinct community structures compared with giant and red pandas, patterns linked to gut transit

time and host phylogeny (McKenney et al. 2018). The morphological contrast is also reflected in fecal characteristics: bamboo rat feces are smooth and fragile, indicating efficient digestion, while panda feces are coarse and contain abundant undigested residues. These digestive strategies likely generate distinct physiological conditions, including differences in gut pH and immune regulation (Palframan et al. 2002; Belkaid & Hand 2014; Liu et al. 2025), which may further contribute to the microbiome divergence among these bamboo specialists. Notably, although continuous morphological traits (e.g., gut length and retention time) would provide finer resolution, such data are not consistently available across the species included in this study, particularly for wild animals.

Dietary specialization reduces microbial diversity and carbohydrate metabolism

Contrary to the long-standing assumption that fiber-rich diets select for gut microbiota with enhanced carbohydrate metabolism (Muegge et al. 2011; Moeller & Sanders 2020; Milani et al. 2020), none of the three bamboo specialists exhibited enhanced microbial capacities for lignocellulose degradation. This pattern was particularly striking in Chinese bamboo rats, which would theoretically benefit from strong microbial fermentation (Bai et al. 2021). Yet, the microbial diversity, gene abundances for polysaccharide degradation (e.g., starch and sucrose metabolism) and most GH families, were markedly lower than those in other dietary rodents. Enzyme activity assays confirmed significantly reduced activities of cellulase, xylanase, and β-glucosidase. Similar functional constraints were evident in giant and red pandas. Although both species showed enrichment in a few specific fiber-degrading genes compared with their relatives, broader polysaccharide-metabolism pathways, most GH families (e.g., GH113, GH12, GH120, GH36, and GH45), and overall lignocellulose-degrading capacity remained limited, accompanied by pronounced reduction in microbial diversity. These findings indicate that extreme dietary specialization drives the functional limitation rather than the enhancement of carbohydrate-degrading capacity.

This pattern likely reflects strong and persistent dietary selection. Compared with bamboo dietary specialists, their close relatives typically exploit far more diverse food resources (Owen et al. 2000; Watson et al. 2019; Jiangzuo & Flynn 2020; Li et al. 2022): omnivorous rodents mainly consume plant seeds and insects; herbivorous rodents feed on grass, roots, stems, leaves, seeds, and fruits; carnivorous Carnivora species prey on fish, birds, and small mammals; and omnivorous Carnivora species additionally

consume plant material. These foods contain relatively low levels of crude fiber (<22%), whereas bamboo represents an exceptionally fiber-rich (>50% in culms, >25% in leaves, and >15% in shoots) and nutritionally monotonous diet (Langer 2002; Qu et al. 2013; Likidkarnchanakornkij et al. 2023). Such conditions may constrain the gut microbiota of Chinese bamboo rats, giant pandas, and red pandas to a narrow dietary niche breadth (Anders et al. 2022; Gobin et al. 2022), thereby favoring microbes capable of tolerating bamboo-derived secondary compounds or tightly associating with the host intestinal mucosa, such as *Clostridium_sensu_stricto_1* and Muribaculaceae (Wang et al. 2021; Zhu et al. 2024). Meanwhile, specialists progressively lost multiple microbes with alternative metabolic potentials, including carbohydrate-fermenting genera such as *Ruminococcus* (Greene et al. 2018); SCFA-producing genera such as *Phascolarctobacterium* and *Megasphaera* (Wu et al. 2017; Luu et al. 2021); and protein metabolism-related genera such as *Fusobacterium* and *Peptostreptococcus* (Zoelzer et al. 2021). These reductions likely contributed to the simplified microbial composition and contraction of functional gene repertoires (Kraft et al. 2015; Jochum et al. 2020).

Importantly, this pattern extends beyond bamboo dietary specialists to other specialized plant-based diets. Leaf specialists, including folivorous colobines, folivorous lemurs, and the eucalyptus-feeding koala, also exhibited significantly reduced gut microbial diversity and constrained functional potential compared with their herbivorous and omnivorous relatives. Leaves, like bamboo, are fiber-rich and nutritionally monotonous, with recalcitrant lignocellulose comprising ~40–70% of dry mass (Chen 2014; Kiruthika 2024), thereby imposing comparable dietary pressure on the host gut microbiome. Consistent with this, leaf specialists showed reduced abundances of genes involved in polysaccharide degradation and short-chain fatty acid (SCFA) metabolism, which are essential for host nutrition and energy provision (Flint et al. 2008; Clayton et al. 2018), along with no widespread enrichment of lignocellulose-degrading GH families or key enzymes. This trend was particularly evident in folivorous colobines. Despite possessing both foregut and hindgut fermentation systems (Liu et al. 2022), they did not outperform colon-fermenting monkeys in digesting high-fiber diets. This observation aligns with previous reports showing greater enrichment of starch and sucrose metabolism in non-folivorous primates than in folivores (Amato et al. 2019). Notably, because our analyses were

based on fecal samples, they may not fully capture foregut-specific activity. Nevertheless, fecal microbiomes provide integrated signals of gut microbial communities and are widely used for comparative inference across host species. In addition, sample sizes were limited by the availability of wild animal datasets, which may have reduced statistical power. However, the major patterns identified here were consistently supported, and future studies with expanded sampling will help further evaluate the generality of these findings.

Microbial and host-level compensatory adaptations to specialized plant diets

In contrast to reduced carbohydrate metabolism, bamboo and leaf specialists showed enrichment of PSMs (e.g., aromatic compounds, terpenoids, and steroids) degradation. Bamboo and leaves, particularly eucalyptus, contain abundant terpenes, tannins, cyanogenic glycosides, and flavonoids, all of which function as potent chemical defenses (Iason 2005; Zhu et al. 2018; Harenčár et al. 2021). Previous studies suggest that gut microbiota can facilitate the degradation and detoxification of these compounds, supporting host adaptation to plant-based diets (Zhu et al. 2018; Wang et al. 2021; Dearing & Weinstein 2022). We further observed that the gut microbiomes of certain specialists (e.g., giant pandas and folivorous colobines), were enriched in plant-derived lipid (e.g., α-linolenic acid and linoleic acid) metabolism, which may contribute to both energy provision and detoxification (Shiffman et al. 2017; Reszczyńska & Hanaka 2020; Xiao et al. 2022). These patterns suggest that detoxification may represent a key microbial compensatory strategy enabling hosts to tolerate chemically defended, nutritionally monotonous diets. Nevertheless, our findings primarily reflect putative metabolic potential rather than direct functional activity, and further metabolomic evidence is required to validate these pathways.

In addition, across bamboo dietary specialists, gut microbiomes were dominated by taxa such as Muribaculaceae, *Clostridium_sensu_stricto_1*, and *Pseudomonas*, associated with complex carbohydrate degradation, lignocellulose utilization, and SCFA production (Mechichi et al. 2005; McMahon et al. 2007; Xu et al. 2019; Sibai et al. 2020; Smith et al. 2021). Comparable functionally important taxa (e.g., *Clostridium* and Muribaculacaeae) are also observed in folivorous primates and koalas (Amato et al. 2019; Eisenhofer et al. 2023). At the host level, Chinese bamboo rats, koalas, and folivorous primates possess enlarged ceca and colons that enhance fermentation efficiency (Lambert 1998; Grant 2014; Likidkarnchanakornkij et al. 2023), whereas giant and red pandas compensate for their simple digestive tracts through a “high

intake–rapid throughput" strategy and selective feeding on less lignified bamboo tissues (Dierenfeld et al. 1982; Wei et al. 1999; Xue et al. 2015). These microbial and host-level compensatory features enable mammals with highly specialized plant-based diets to achieve dietary adaptation.

Ecological implications and limitations of dietary specialization effects

Notably, the characteristically low microbial diversity in these specialists implies diminished functional redundancy, potentially increasing their gut microbiome vulnerability to dietary perturbations (Costello et al. 2012; Lozupone et al. 2012; Moya & Ferrer 2016). For instance, in captive folivorous primates, dietary diversification with mixed foliage significantly boosts microbial diversity, enriches fiber-metabolizing taxa, and elevates colonic SCFA levels (Greene et al. 2018). This sensitivity may also contribute to health risks in captivity. Captive diets often lack the variability found in natural environments, and gut microbiomes are frequently less diverse than those of wild populations (Greene et al. 2019; Guo et al. 2019; Frankel et al. 2019). These findings have important implications for the management of endangered bamboo specialists (e.g., giant and red pandas) and folivorous species. Diet formulations that better mimic natural dietary diversity may help maintain microbiome stability and support host health.

Dietary classifications in this study represent broad feeding strategies rather than temporally resolved intake (Kissling et al. 2014). As food availability varies across space and time, even specialized species may undergo dietary shifts not captured by such coarse categories (Wu et al. 2017; Kim et al. 2025). Accordingly, our results primarily reflect long-term ecological signatures of dietary specialization, although their resolution may be limited under high dietary variability. Future work incorporating longitudinal sampling and quantitative dietary data will help disentangle the effect of diet composition. Host phylogeny and gut morphology explained substantial variation in gut microbiome, and these effects were minimized by comparing closely related species with similar gut morphological types. Although factors such as season, geography, and captivity-related conditions were not explicitly controlled due to incomplete metadata, their influence is generally weaker than that of diet (Huang et al., 2022; Youngblut et al., 2019). Consistent with this, captive giant pandas (with >90% bamboo in their diet) exhibit lower cellulase and xylanase activities than captive black bears and carnivores (Guo et al. 2018), further supporting the robustness of our conclusions regarding dietary specialization.

In summary, this study provides novel insights into how plant-based dietary specialization shapes gut microbiome structure and function, and expands the understanding of host–microbe co-evolution by showing that adaptive success depends more on host–microbe compensatory strategies than on enhanced carbohydrate metabolic capacity.

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Competing interests

The authors declare no competing interests.

Author contributions

Y.B.H. performed the research design, supervision, and manuscript revision. Y.X.L., M.S.H., L.W., X.Y.Z., H.L., and F.W.W. collected samples and performed experiments. Y.X.L. and Q.W. analyzed data, and Y.X.L. wrote the manuscript. All authors discussed the results, and approved the final manuscript.

Data availability

Raw sequence data generated in this study have been deposited to the GSA database (https://ngdc.cncb.ac.cn/gsa) under the accession number CRA014329. Source data included in this study is presented in a supplementary file.

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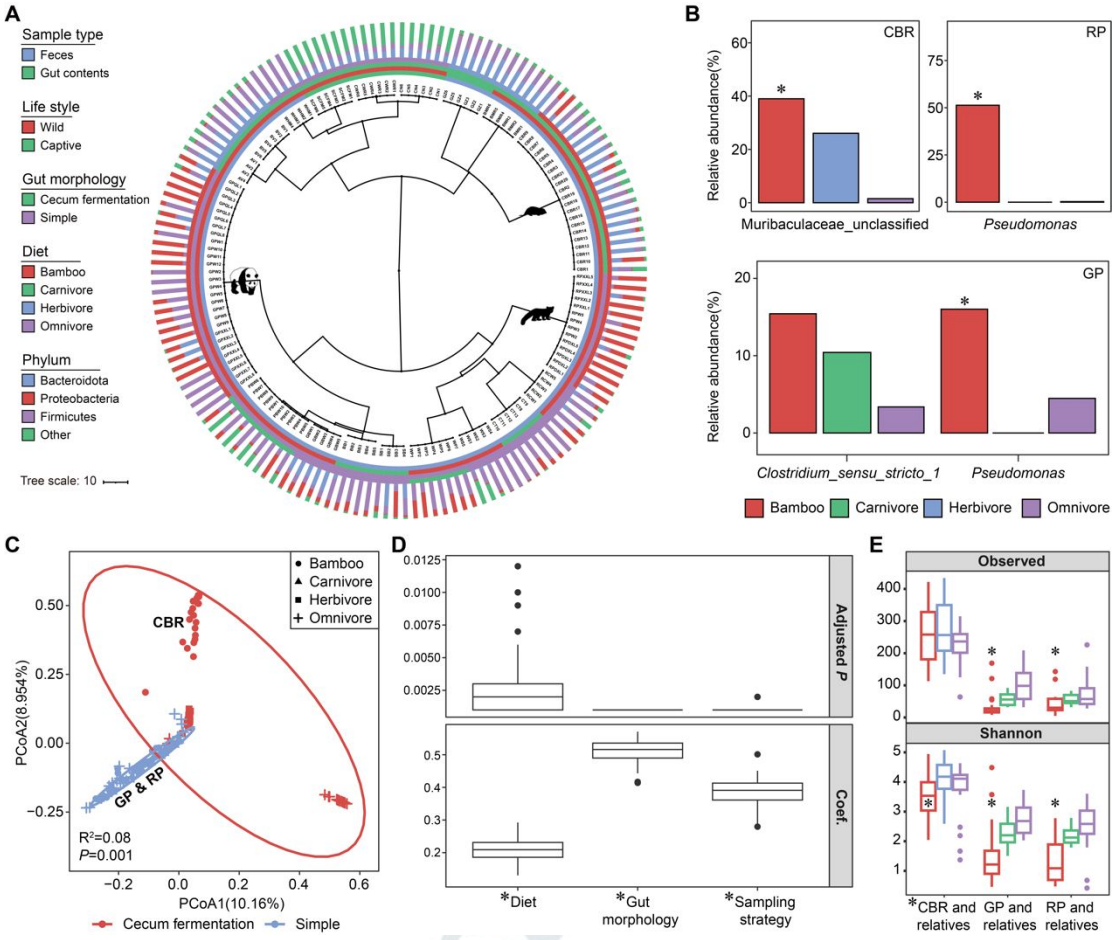

Fig. 1 Comparison of the gut microbiome structure in three bamboo dietary specialists (Chinese bamboo rat [CBR], giant panda [GP], and Chinese red panda [RP]). **A** Phylogeny and sample information. The host phylogenetic tree was retrieved from http:// timetree.org. From the inner to the outer ring, the data mapped onto the tree included sample type, lifestyle, gut morphology, diet, and the relative abundances of microbial phyla in each sample. **B** Differences in the relative abundance of dominant genera. Bars represented the mean relative abundance of each genus within each host group. **C** PCoA plot based on ASVs abundance using Bray–Curtis distance. **D** MRM model result based on ASVs abundance and Bray–Curtis distance. These boxplots showed the adjusted *P* and partial regression coefficients to determine the explanatory degree of diet, gut morphology, and sampling strategy (sample type/lifestyle) on β-diversity differences. **E** Two α-diversity indices of the gut microbiota of CBR, GP, RP, and their diet-different relatives. Four dietary types were displayed: bamboo (red), carnivore (green), herbivore (blue), and omnivore (purple). *Adjusted $P \leq 0.05$. Data represented mean ± standard error of the mean.

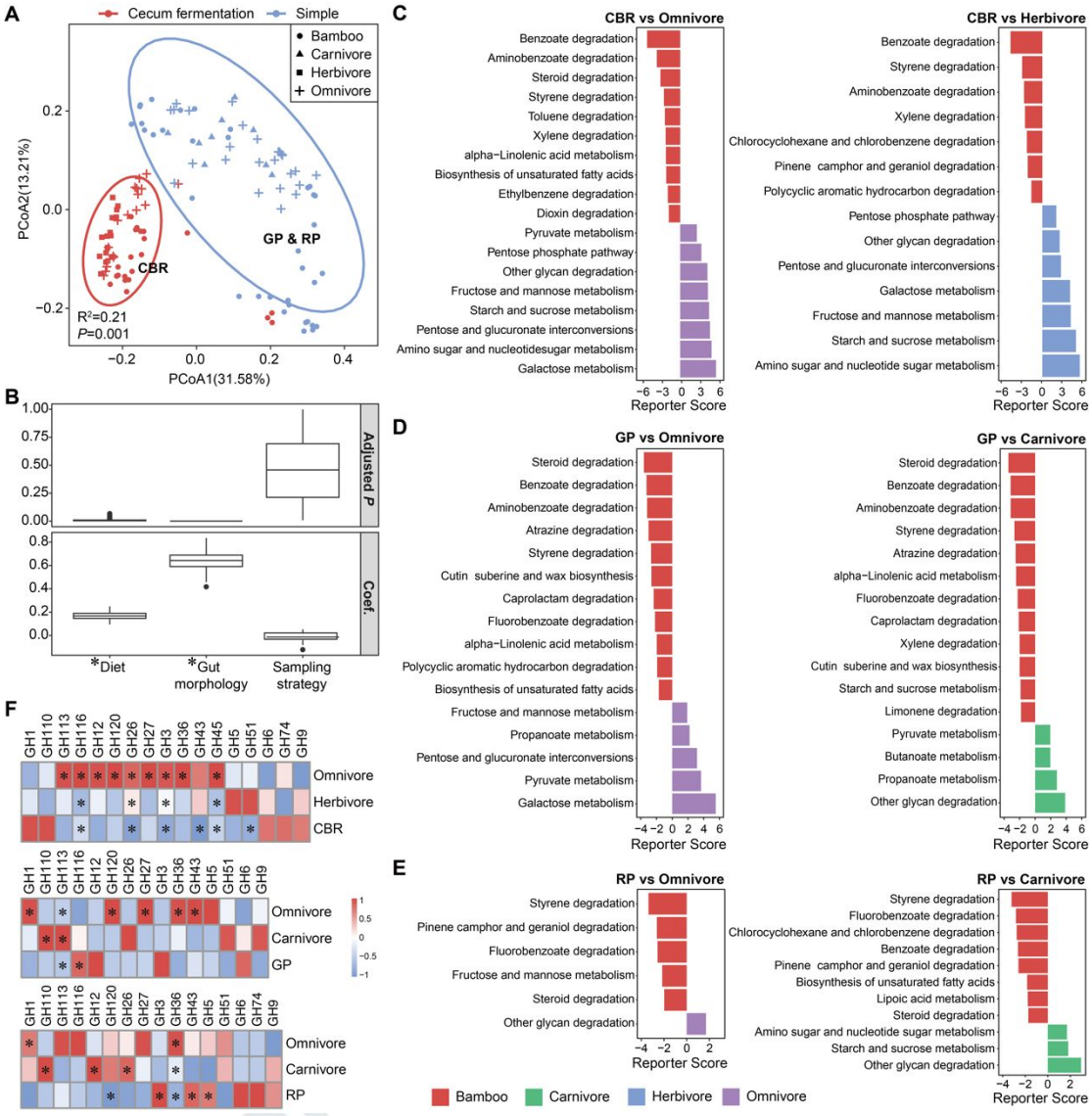

Fig. 2 Gut microbiome functions of the Chinese bamboo rat (CBR), giant panda (GP), and red panda (RP). **A** PCoA plot based on gene-pathway abundance using Bray–Curtis distance. **B** MRM model result based on gene-pathway abundance and Bray–Curtis distance. These boxplots showed the adjusted *P* and partial regression coefficients to determine the explanatory degree of diet, gut morphology, and sampling strategy on the divergences of gut microbiome function. **C–E** Reporter score results of major metabolism pathways in the gut microbiota of CBR, GP, and RP compared with their diet-different relatives. Pathways with reporter scores ≥|1.64| were considered statistically significant at the 0.05 level. Four dietary types were displayed: bamboo (red), carnivore (green), herbivore (blue), and omnivore (purple). **F** Heatmap of GH-family abundances associated with lignocellulose degradation in the gut microbiota of CBR, GP, and RP compared with their diet-different relatives. Red-colored GH families indicated higher abundance. *Adjusted $P \leq 0.05$. Data represented mean ± standard error

of the mean.

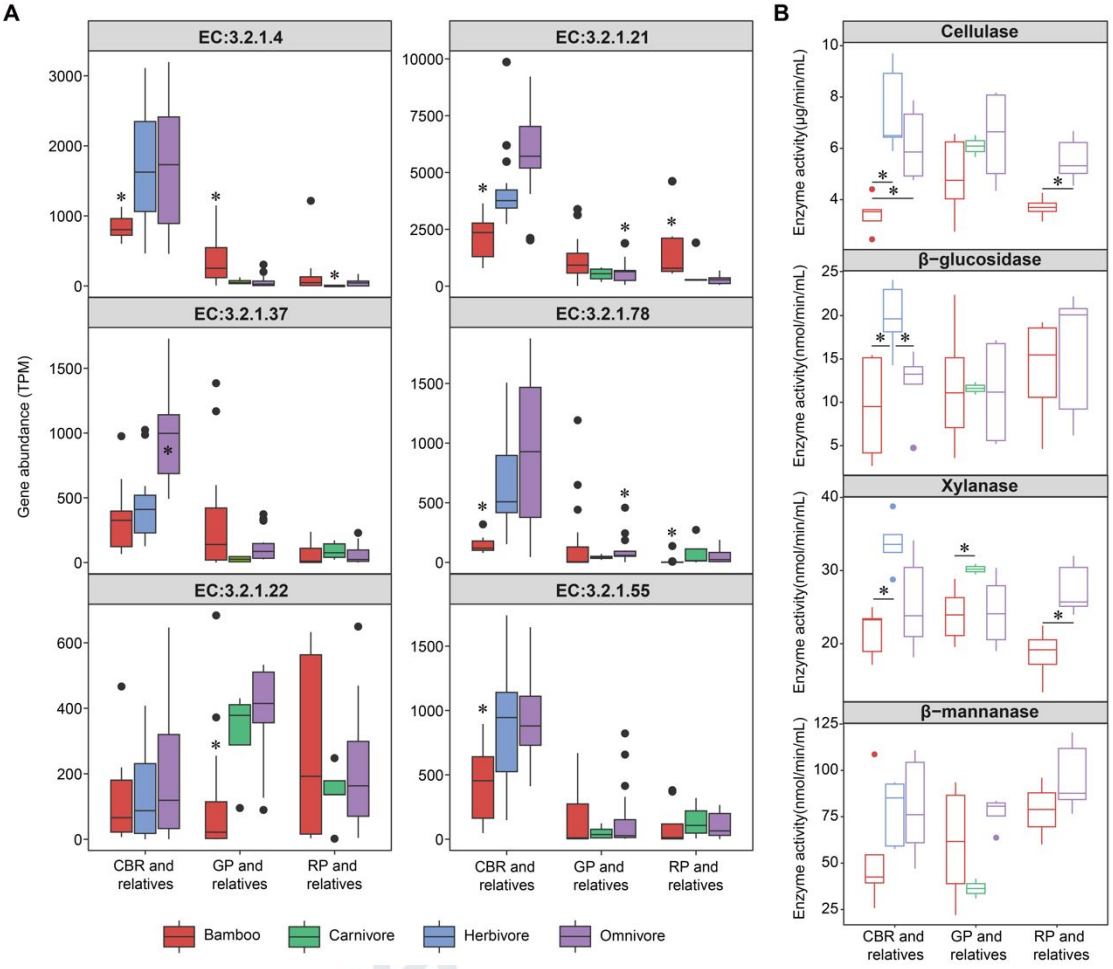

Fig.3 Comparison of abilities to degrade lignocellulose in bamboo dietary specialists (Chinese bamboo rat [CBR], giant panda [GP], and Chinese red panda [RP]) and their diet-different relatives. **A** Different abundances of enzyme-encoding genes associated with lignocellulosic degradation. **B** Activity levels of four key enzymes, including cellulase (EC:3.2.1.4) and β-glucosidase (EC:3.2.1.21) related to cellulose degradation, and xylanase (EC:3.2.1.8) and β-mannanase (EC:3.2.1.78) related to hemicellulose degradation. Four dietary types were displayed: bamboo (red), carnivore (green), herbivore (blue), and omnivore (purple). *Adjusted $P \leq 0.05$. Data represented mean ± standard error of the mean.

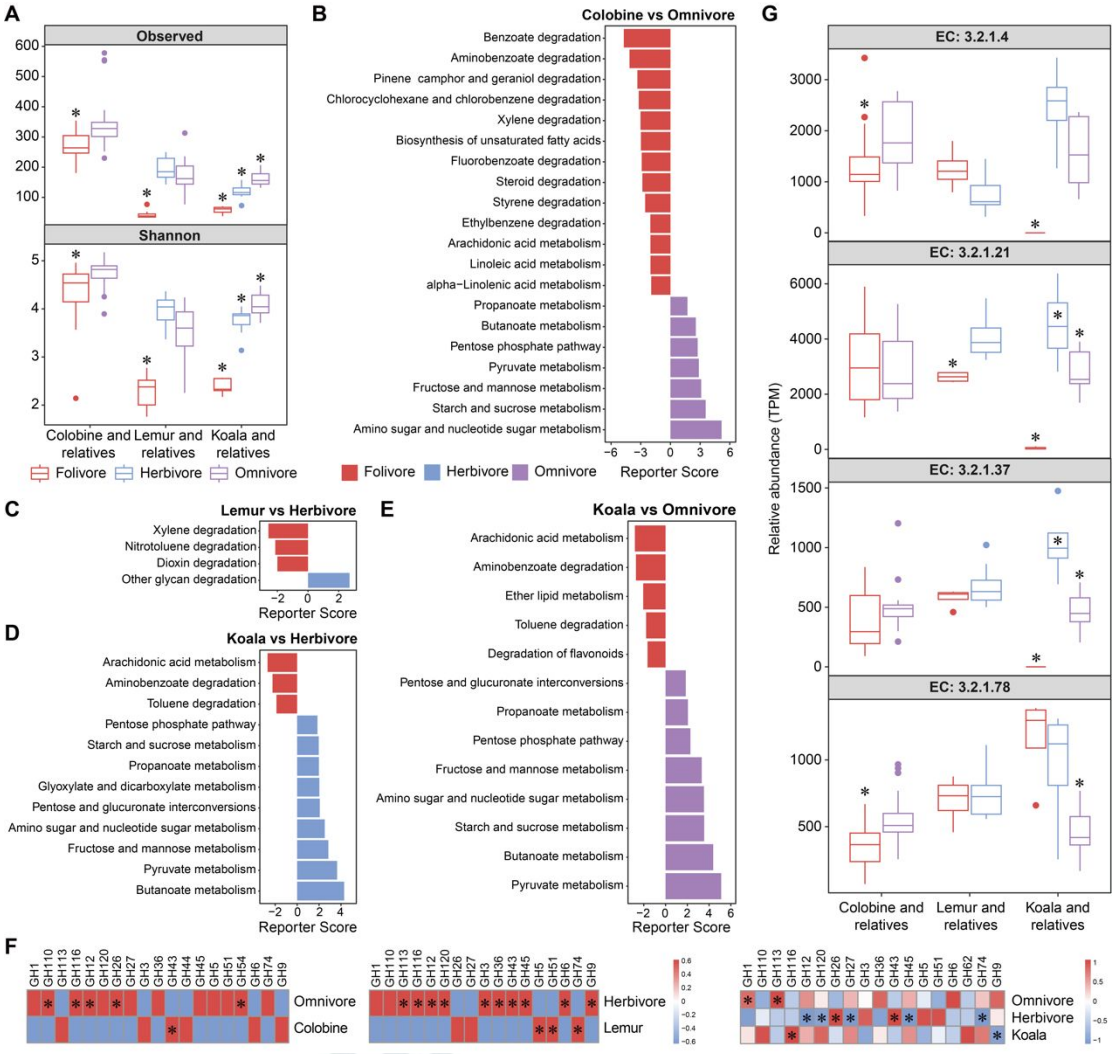

Fig.4 Comparative analysis of gut microbiomes under three folivory scenarios. **A** Boxplots showed gut microbial diversity in folivorous colobines, folivorous lemurs, and koalas compared with their diet-different relatives. Three dietary types were displayed: folivore (red), herbivore (blue), and omnivore (purple). **B**–**E** Reporter score results of major metabolism pathways in the gut microbiota of folivorous colobines, folivorous lemurs, and koalas compared with their respective relatives. Pathways with reporter scores ≥|1.64| were considered statistically significant at the 0.05 level. **F** Heatmaps of relative abundances of GH families associated with lignocellulose degradation in the gut microbiota of folivorous colobines, folivorous lemurs, and koalas compared with their respective relatives. Red-colored GH families indicated higher abundance. **G** Boxplots showed relative abundances of four major lignocellulose-degrading enzymes, including cellulase (EC:3.2.1.4), β-glucosidase (EC:3.2.1.21), β-xylosidase (EC:3.2.1.37), and β-mannanase (EC:3.2.1.78). *Adjusted $P \leq 0.05$. Data represented mean ± standard error of the mean.

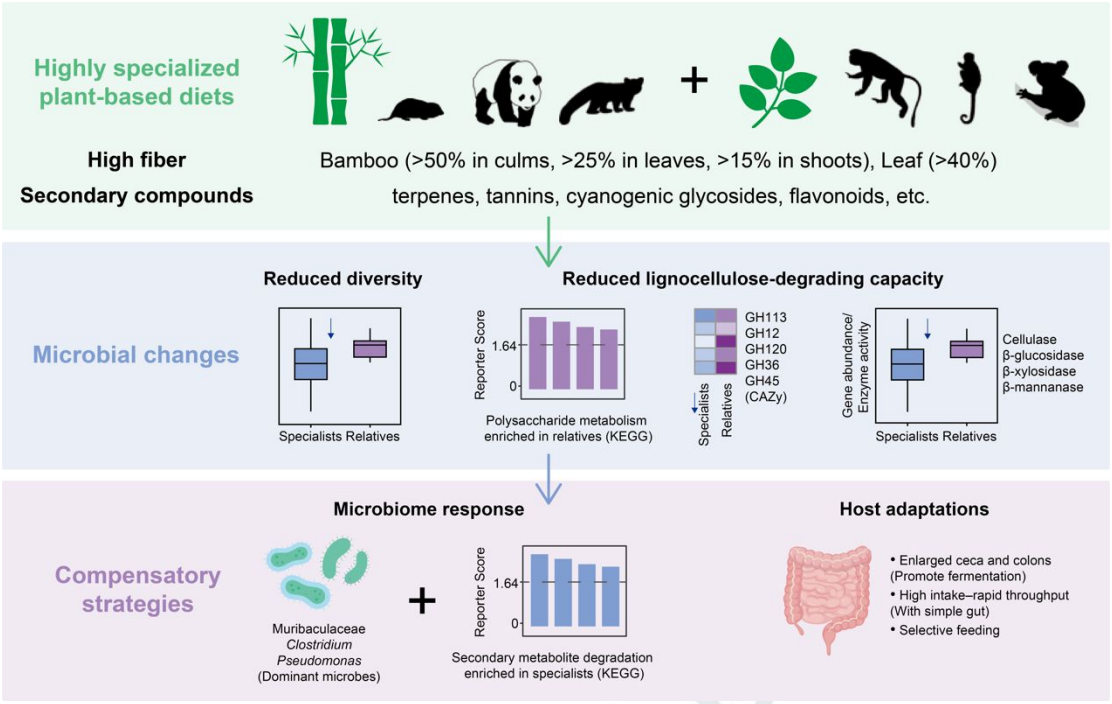

Fig.5 Schematic summary of the main findings of this study.

高度特化植食性驱动肠道菌群多样性及木质纤维素降解功能下降

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关键词：食性特化，肠道微生物组，植食性，木质纤维素降解，适应性进化