

Article

Metagenomic–Metabolomic Integration Reveals Gut Microbiota Dynamics and Metabolic Changes in Super-Geriatric Captive Giant Pandas

Jingheng Wang ^{1,†} , Meiling Cheng ^{2,†}, Feiyun Huang ¹, Lei Chen ¹, Wencai Xu ¹ , Jieyao Cai ¹, Zhoulong Chen ¹, Yanni Zhao ¹  and Xiuyue Zhang ^{1,3,*}

¹ Key Laboratory of Bio-Resource and Eco-Environment of Ministry of Education, College of Life Sciences, Sichuan University, Chengdu 610064, China; wangjingheng@stu.scu.edu.cn (J.W.); fyunhuang@163.com (F.H.); geminist_cl@163.com (L.C.); xwc@stu.scu.edu.cn (W.X.); caijieyao0310@163.com (J.C.); chenzhoulong1010@163.com (Z.C.); zhaoyanni_69@163.com (Y.Z.)

² Key Laboratory of State Forestry and Grassland Administration on the Giant Panda, China Conservation and Research Center for the Giant Panda, Chengdu 610051, China; chml182@126.com

³ The Conservation of Endangered Wildlife Key Laboratory of Sichuan Province, College of Life Sciences, Sichuan University, Chengdu 610064, China

* Correspondence: zhangxiuyue@scu.edu.cn

† These authors contributed equally to this work and share first authorship.

Simple Summary

Advanced age can influence digestive function, metabolism, and immune-related processes in animals. The giant panda is a globally recognized flagship species for conservation, and with continuous improvements in husbandry practices and living conditions, the lifespan of captive individuals has increased. However, information regarding age-associated changes in super-geriatric giant pandas remains limited. In this study, we combined metagenomic and metabolomic analyses to examine fecal samples from captive adult and super-geriatric giant pandas. Our results showed that super-geriatric individuals exhibited a reduction in beneficial microbial taxa and an increased representation of bacteria with reported opportunistic pathogenic potential, together with notable alterations in lipid metabolism. These findings indicate age-associated changes in gut microbial composition and metabolic profiles. This study provides a scientific basis for improving dietary management and health monitoring of super-geriatric giant pandas, contributing to the welfare of captive individuals and supporting conservation efforts for this endangered species.

Abstract

Age-related changes throughout the lifespan are known to influence gut microbiota composition, microbial functional potential, and host-associated metabolic processes. Understanding these age-related variations is important for elucidating their potential physiological implications at different life stages. However, information regarding the gut microbiome and metabolomic characteristics of super-geriatric captive giant pandas (*Ailuropoda melanoleuca*) remains limited. In this study, fecal samples were collected from adult and super-geriatric captive giant pandas and analyzed using metagenomic sequencing combined with untargeted metabolomics. The gut microbiota of super-geriatric individuals exhibited a marked decrease in Bacillota and an enrichment of Pseudomonadota compared with adult individuals. Functional profiling revealed age-associated shifts in microbial metabolic potential, with a transition from biosynthesis-dominated pathways toward pathways related to substrate degradation and energy utilization. Metabolomic analyses further revealed pronounced metabolic alterations in super-geriatric giant pandas, including elevated levels of unsaturated fatty acids and changes in bile acid-related



Academic Editor: Robert Li

Received: 23 December 2025

Revised: 30 January 2026

Accepted: 4 February 2026

Published: 12 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

metabolites. Alterations in gut microbiota composition, particularly the relative enrichment of Pseudomonadota-associated taxa, were associated with inflammation-related metabolic features. Collectively, these findings indicate coordinated changes in gut microbial composition and metabolic profiles during aging. Overall, this study characterizes age-associated alterations in gut microbiota structure and fecal metabolic signatures in super-geriatric captive giant pandas, providing a scientific basis for future studies on microbiota–metabolism interactions and for improving nutritional management and health monitoring strategies in aged individuals of this endangered species.

Keywords: giant panda; aging; gut microbiota; metagenomics; metabolomics

1. Introduction

The giant panda, a member of the order *Carnivora*, originated from an omnivorous ancestor within the bear family (*Ursidae*) but has evolved into an obligate herbivore through long-term adaptation [1–4]. Despite this dietary shift, the species has retained the typical gastrointestinal structure of carnivores. Because giant pandas feed almost exclusively on bamboo and lack genes encoding cellulolytic enzymes in their genome [5], they rely heavily on their gut microbiota to degrade bamboo fibers and facilitate nutrient absorption [6,7]. Previous genomic studies have shown that the panda genome does not contain genes encoding cellulases, making symbiotic gut microbes indispensable for fiber digestion [8]. Among these, certain *Clostridium* species are regarded as major candidates responsible for bamboo fiber degradation [9]. Zhang et al. further demonstrated that the capacity of the giant panda gut microbiota to digest cellulose is limited and that juvenile pandas primarily obtain energy from hemicellulose components of bamboo [6]. This evidence suggests that, over evolutionary time, giant pandas have developed a close symbiotic relationship with specific gut microbial communities to compensate for their enzymatic deficiencies and adapt to a bamboo-based diet [10,11].

The gut microbiota is considered essential for host metabolism and immune regulation [12], and aging is often accompanied by alterations in microbial composition. Previous studies have shown that elderly humans exhibit an increased abundance of Gram-negative bacteria and members of the phylum Proteobacteria, while beneficial genera such as *Bifidobacterium* and *Lactobacillus* are reduced [13,14]. A balanced gut microbiota produces beneficial metabolites such as short-chain fatty acids (SCFAs) and vitamins, maintaining intestinal barrier integrity, whereas dysbiosis has been associated with chronic inflammation and metabolic disorders [14]. Ridlon et al. proposed that gut microbes could modulate host metabolism and immunity by altering bile acid composition and that the “gut microbiota–bile acid axis” could play a critical role in regulating host metabolism and inflammation [15]. Sayin et al. further refined this concept into a well-defined “microbiota–bile acid axis” signaling model [16], suggesting that microbial alterations may mediate aging-related metabolic changes through modulation of bile acids and other metabolites [12].

Metagenomics and metabolomics provide powerful tools to elucidate the gut microbiota composition, functional potential, and interactions with host metabolism [17]. These approaches provide molecular insights into the mechanisms underlying their ecological adaptation and health regulation. In recent years, significant progress has been made in the metagenomic and metabolomic studies of giant pandas, revealing their unique gut microecological adaptations and interactions with bamboo-derived dietary components [18,19]. Previous studies have examined the gut microbial and metabolic characteristics of giant pandas across different life stages, but they mainly focused on juvenile, adult, and elderly

individuals, or they did not integrate and link the two omics datasets [20–22]. In the wild, giant pandas typically have a lifespan of approximately 18–20 years, whereas those in captivity may live up to 25–30 years, with individuals over 20 years of age considered geriatric [23]. Some captive pandas can even survive beyond 30 years. Super-geriatric pandas often exhibit typical aging characteristics such as increased sleepiness and reduced appetite and are prone to age-related conditions affecting vision, dentition, joints, and vital organs including the heart, liver, and kidneys. These physiological and health challenges highlight the growing need for refined health monitoring and management strategies for this vulnerable age group. However, research focusing on the gut microbiome and metabolic characteristics of super-geriatric captive giant pandas, particularly those aged 28 years or older, remains extremely limited. This knowledge gap highlights how little is known about how the gut microecosystem of super-geriatric individuals influences their health and physiological aging. Therefore, conducting in-depth microbiome and metabolomic analyses in this age group is of great importance for developing precise nutritional strategies, disease prevention measures, and targeted health management approaches for aging giant pandas.

In this study, we collected fecal samples from adult and super-geriatric captive giant pandas, with the super-geriatric group comprising mostly very old individuals around 30 years of age. By integrating metagenomic sequencing with untargeted metabolomics, we systematically investigated age-related changes in microbiome composition, functional potential, and metabolite profiles. Our results reveal associations between the gut microecology and host metabolism during aging in captive giant pandas, providing important insights for precision nutrition, disease prevention, and health management.

2. Materials and Methods

2.1. Sample Collection

In total, fecal samples were collected from seven captive giant pandas housed at the Dujiangyan Base of the China Conservation and Research Center for the Giant Panda. The study population included both male and female individuals. Due to the limited sample size, sex was not treated as a grouping factor, and all individuals were analyzed collectively based on age. The sampled individuals comprised adult giant pandas (6–8 years; AD) and super-geriatric giant pandas (28–32 years; SG).

All pandas were maintained under standardized husbandry and feeding protocols and were fed regularly on a daily schedule. No fasting or dietary manipulation was applied prior to sampling. Fresh fecal samples were collected within 10 min after defecation using sterile tools. To minimize potential environmental contamination, the outer layer of feces was removed prior to subsampling. Each individual sample used for metabolomic and metagenomic analyses weighed no less than 1 g. Due to inter-individual variation in defecation time, samples could not be collected at identical time points; however, all samples were collected on the same day following a standardized protocol.

Immediately after collection, fecal samples were snap-frozen in liquid nitrogen and subsequently stored at -80°C until further analysis. One fecal sample was excluded from metabolomic analysis due to contamination and technical issues during library preparation or sequencing. Consequently, six metabolomic datasets and seven metagenomic datasets were included in the final analyses (Supplementary Table S1).

2.2. Metagenomic Analysis

2.2.1. Metagenomic Library Preparation and Sequencing Workflow

Genomic DNA from fecal samples was extracted using the TIANGEN Magnetic Soil and Stool DNA Kit (TIANGEN Biotech, Beijing, China) following the manufacturer's protocol. The extracted DNA was then sheared to an average fragment length of approx-

imately 350 bp using ultrasonication. Subsequent steps, including end repair, A-tailing, and adapter ligation, were performed to meet the library preparation requirements for the Illumina sequencing platform. After library construction, quantitative PCR (qPCR) was used to precisely quantify DNA concentration and assess fragment size distribution. Libraries that met quality control standards were selected and purified. Qualified libraries were pooled according to experimental design ratios and subjected to Illumina paired-end (PE150) high-throughput sequencing on the Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA). Sequencing depth was determined based on the research objectives. The sequencing data are archived in FASTQ format in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) website, accessible under the BioProject identifier PRJNA1368674. All raw reads were quality-controlled using fastp (v0.21.0) [24], applying a filtering threshold of $Q \geq 20$ to remove adapter sequences and ensure sufficient base quality for downstream analyses.

To ensure the accuracy of the metagenomic data and remove host genomic sequences, the reference genome of the giant panda (accession number GCF_002007445.2) was downloaded and used for host sequence filtering. KneadData (v0.12.0) was employed to remove host-derived and potential contaminant sequences during preprocessing of the raw reads.

During sequence alignment, Bowtie2 (v2.5.3) [25] was applied with high sensitivity parameter settings, including—very-sensitive and—dovetail, to improve the detection rate of microbial reads and handle overlapping paired-end sequences. In addition, to maximize the retention of original read structures and information, the parameters—bypass-trim and—bypass-trf were enabled to skip preliminary trimming and tandem repeat filtering steps.

2.2.2. Taxonomic and Functional Annotation

The metagenomic data obtained after host sequence removal were taxonomically annotated using Kraken2 (v2.0.7-beta) [26], with parameters set to --use-names and --report-zero-counts, and the confidence threshold maintained at the default value of 0. Taxonomic classification was performed based on the Kraken database (PlusPF), which includes bacterial, fungal, archaeal, and viral sequences, enabling species-level classification. The resulting output was converted into MPA format using the kreport2mpa.py script. Subsequently, Bracken [27] was employed to re-estimate read abundance and calculate relative abundance across different taxonomic levels, including phylum, genus, and species.

At the species level, alpha diversity was assessed using the Shannon, inverse Simpson, and Pielou's evenness indices, and intergroup differences were evaluated with the Wilcoxon rank-sum test. Beta diversity was calculated based on Bray–Curtis distances and visualized through principal coordinate analysis (PCoA). These analyses were conducted in R using the vegan package (permutations = 999), and differences in community structure were tested using PERMANOVA [28]. The top 15 species with the highest LDA scores were selected as age-related biomarkers through LEfSe v1.1.2 [29–31].

Functional annotation was performed using HUMAnN3, with the database mpa_vOct2_2_CHOCOPhAnSGB_202212, which supports gene family and metabolic pathway abundance prediction [32–34]. Gene family annotation was conducted by mapping against UniRef90 and further linked to KEGG Orthology (KO), Gene Ontology (GO), and level 4 Enzyme Commission (EC) classifications. The resulting tables were organized using the summarizeAbundance.py script [35,36]. Carbohydrate-active enzymes (CAZymes) were annotated using a custom mapping file from CAZy to UniRef90. Annotation results were normalized using the humann_renorm_table function, and stratified abundance tables were generated via humann_split_stratified_table for intergroup comparisons. Significant differences in functional pathway abundances between groups were evaluated using the Mann–Whitney U test, which is appropriate for non-normally distributed data [37].

Based on functional abundance profiles, non-metric multidimensional scaling (NMDS) was performed using Bray–Curtis distances to assess overall dissimilarities among samples, with PERMANOVA used to verify the robustness of the NMDS results. The top 15 most abundant metabolic pathways were selected and visualized as bar plots using the ggplot2 package (v3.4.0) [38].

2.3. Metabolomic Analysis

2.3.1. Sample Preparation and UHPLC-MS/MS Analysis

For each sample, 100 mg of panda feces was ground with liquid nitrogen. Then, 500 µL of 80% methanol solution (Thermo Fisher Scientific, Waltham, MA, USA) was added. The mixture was vortexed and incubated on ice for 5 min, followed by centrifugation at $15,000\times g$, 4°C for 20 min using a centrifuge (Scilogex, Middlesex, CT, USA). An appropriate amount of the supernatant was taken and diluted with LC-MS grade water (Merck KGaA, Darmstadt, Germany) to achieve a final methanol concentration of 53%. The samples were then centrifuged again at $15,000\times g$, 4°C for 20 min. The supernatant was collected and injected into the LC-MS system for analysis. A quality control (QC) sample was prepared by pooling equal aliquots from all individual samples to evaluate system stability and data reproducibility. A blank sample was prepared by replacing the experimental samples with a 53% methanol solution, following the same preparation process as the experimental samples. The blank sample is used to monitor potential contamination and ensure no interference during the analysis.

The UHPLC-MS/MS analysis of giant panda fecal metabolites was performed according to previously reported protocols [39–41] at Novogene Co., Ltd. (Beijing, China), using a Vanquish UHPLC system (Thermo Fisher Scientific, Bremen, Germany) coupled to an Orbitrap Q Exactive™ HF mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Chromatographic separation was achieved on a Hypersil GOLD column (100 × 2.1 mm, 1.9 µm) with a 12 min linear gradient at a flow rate of 0.2 mL/min (Thermo Fisher Scientific, Waltham, MA, USA). For positive polarity mode, mobile phases were A (0.1% formic acid in water) (Thermo Fisher Scientific, Waltham, MA, USA) and B (methanol) (Thermo Fisher Scientific, Waltham, MA, USA); for negative polarity mode, mobile phases were A (5 mM ammonium acetate, pH 9.0) (Thermo Fisher Scientific, Waltham, MA, USA) and B (methanol). The solvent gradient was 0–1.5 min, 2% B; 1.5–3.0 min, linear to 85% B; 3.0–10.0 min, linear to 100% B; 10.0–10.1 min, return to 2% B; 10.1–12.0 min, hold at 2% B. The Q Exactive™ HF was operated in positive and negative ion modes with a spray voltage of 3.5 kV, capillary temperature of 320 °C, sheath gas flow rate of 35 psi, auxiliary gas flow rate of 10 L/min, S-lens RF level of 60, and auxiliary gas heater temperature of 350 °C.

Raw LC-MS data files (.raw) were processed using Compound Discoverer (CD) software version 3.1 (Thermo Fisher Scientific). Feature detection and peak alignment were performed with a retention-time tolerance of 0.2 min and a mass tolerance of 5 ppm. Peak extraction was conducted using a mass tolerance of 5 ppm, a signal intensity tolerance of 30%, a signal-to-noise ratio (S/N) ≥ 3 , and predefined adduct ion settings. Peak areas were used for relative quantification, followed by blank-based background subtraction to remove background-related features and subsequent normalization. Metabolite annotation was performed based on accurate precursor mass, retention-time consistency, isotopic and adduct ion patterns, and MS/MS fragmentation spectra acquired in data-dependent acquisition mode. Experimental MS/MS spectra were matched against mzCloud, mzVault, and an in-house MassList database within Compound Discoverer. According to the Metabolomics Standards Initiative (MSI) guidelines, metabolite identifications in this study should be considered putative annotations (Level 2), as they were based on MS/MS spectral matching rather than confirmation using authentic chemical standards.

2.3.2. Data Analysis and Processing

For multivariate statistical analysis, raw data were processed and normalized using the metabolomics data-processing software metaX (v1.4.16) [42]. Principal component analysis (PCA) was first conducted as an unsupervised method to evaluate overall metabolic variation and detect potential outliers. Partial least squares–discriminant analysis (PLS-DA) was then applied as a supervised method to explore metabolic differences between groups. Variable importance in projection (VIP) values for each metabolite were calculated based solely on the PLS-DA model and were used for subsequent differential metabolite screening. To reduce the risk of model overfitting, PLS-DA was used only as a supportive tool and was presented in the Supplementary Figure S1. Orthogonal partial least squares–discriminant analysis (OPLS-DA) was additionally performed to improve visualization of group separation and to facilitate interpretation of metabolite contribution patterns. Importantly, OPLS-DA results were not used for the identification or screening of differentially expressed metabolites and are presented in the main figures for descriptive and illustrative purposes only. For univariate analysis, Student's *t*-tests were used to assess the statistical significance (*p*-values) of individual metabolites between the two groups, and fold change (FC) values were calculated. Differential metabolites were defined based on a combined criterion of $VIP > 1$ (derived from PLS-DA), $p < 0.05$, and $FC \geq 2$ or $FC \leq 0.5$. KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis of differentially expressed metabolites (DEMs) was performed using MetaboAnalyst (<https://www.metaboanalyst.ca/home.xhtml>, accessed on 30 July 2025). Pathway enrichment significance was evaluated using Fisher's exact test, and pathways with $p < 0.05$ were considered significantly enriched.

To investigate co-variation patterns of metabolites across different age stages, a weighted gene co-expression network analysis (WGCNA) was performed using the R package WGCNA (v1.73) [43,44]. A co-expression network for the metabolite peak intensity matrix was constructed based on this analysis. First, Pearson's correlation coefficients were used to construct a similarity matrix among samples, and the pickSoftThreshold function was applied to determine the soft-thresholding power (β) required for building a scale-free network. Subsequently, the blockwiseModules function was used to construct the network and identify modules. For each module, the module eigengene (ME)—representing the first principal component of metabolite expression data within that module—was calculated using the moduleEigengenes function. Further, Pearson's correlation coefficients were computed between module eigengenes and phenotypic variables (age groups), between individual metabolites and their corresponding module eigengenes (module membership, MM), and between metabolites and phenotypic traits (gene significance, GS). A correlation matrix between metabolites and age groups was then constructed to identify age-associated metabolites, using selection criteria of $|r| > 0.5$ and $p < 0.05$. Finally, hub metabolites were identified based on the combined thresholds of $MM > 0.8$ and $GS > 0.2$ in order to pinpoint key metabolites potentially playing central regulatory roles across different age stages.

2.4. Mantel Test

The Mantel test was applied to evaluate the correlations between gut microbial communities and metabolites. For the metagenomic data, the relative abundances of bacteria, archaea, viruses, and eukaryotes at the genus level were extracted and normalized using total-sum scaling (TSS). Based on these normalized data, Bray–Curtis distance matrices were computed for each microbial group to represent pairwise sample dissimilarities. For the metabolomic data, the top 15 significantly different metabolites identified from untargeted metabolomic profiling were selected. The peak area of each metabolite was standardized using Z-scores, and Euclidean distance matrices were subsequently calculated

to represent sample-wise differences for each metabolite. The Mantel test was performed in R (v4.2.1) using the mantel() function in the vegan package (v2.6-4), with Spearman's rank correlation coefficient as the test statistic. Statistical significance was assessed through 9999 random permutations, and correlations with $p < 0.05$ were considered significant. We focused primarily on associations with large absolute correlation coefficients ($|r| > 0.6$) to highlight the strongest microbe–metabolite relationships.

3. Results

3.1. Changes in Gut Microbiota of Giant Pandas at Two Age Stages

3.1.1. Gut Microbial Composition

Metagenomic sequencing generated taxonomic annotations spanning 43 phyla, 983 genera, and 2916 species across all samples. The microbial community structure showed noticeable differences between the two age groups. At the phylum level, Firmicutes (Bacillota) dominated both groups (Figure 1a, Supplementary Table S2), but its relative abundance was higher in adults (84.10%) and lower in the super-geriatric group (57.06%). In contrast, Proteobacteria (Pseudomonadota) were markedly enriched in the super-geriatric pandas (40.17% vs. 14.98%), while Campylobacterota were also slightly higher in the super-geriatric group (0.97% vs. 0.14%). At the genus level, *Streptococcus* was the dominant genus in both groups (Figure 1b, Supplementary Table S2), though more abundant in adults (78.34% vs. 54.12%), whereas *Escherichia* showed a pronounced increase in the super-geriatric group (35.16% vs. 14.07%). Further analysis identified *Streptococcus alactolyticus* and *Escherichia coli* as the predominant species in both groups (Figure 1c, Supplementary Table S2), with the relative abundance of other taxa being substantially lower.

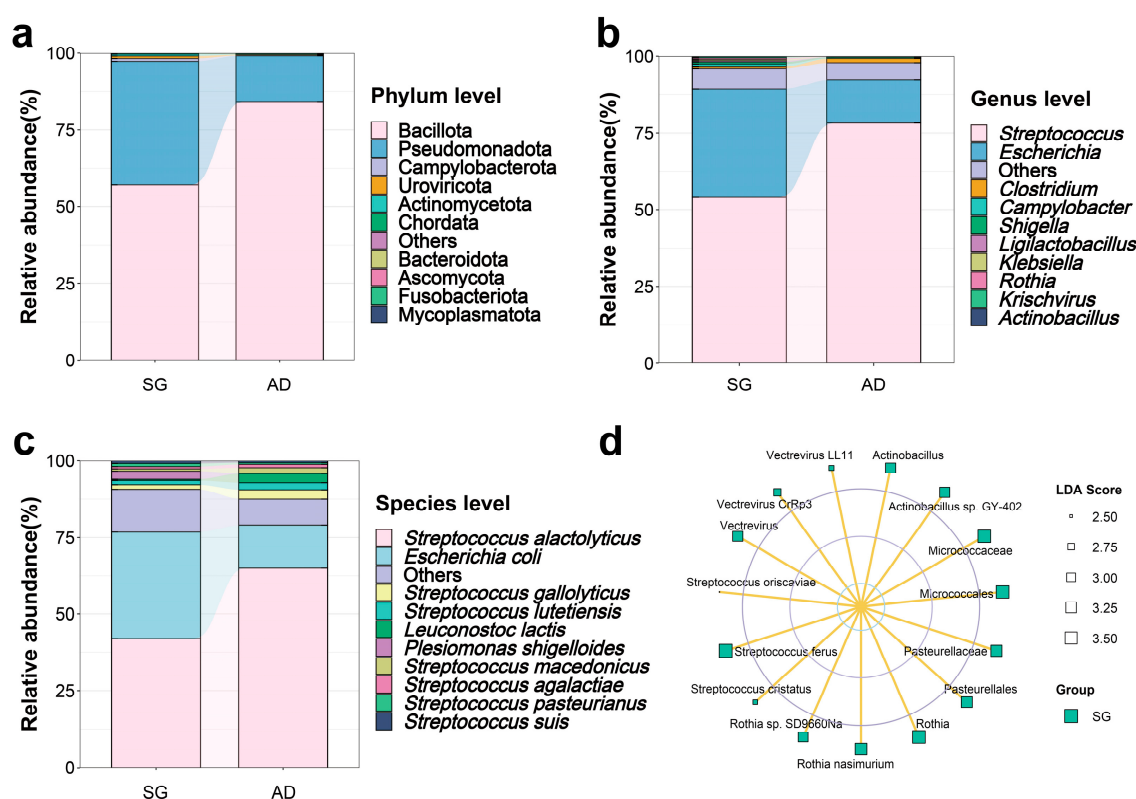


Figure 1. Taxonomic profiling of the gut microbiota in adult and super-geriatric giant pandas across different taxonomic levels: (a) Relative abundance of the top 10 phyla; (b) Relative abundance of the top 10 genera; (c) Relative abundance of the top 10 species; (d) Top 15 age-associated biomarkers identified by LEfSe, ranked by linear discriminant analysis (LDA) score.

Additionally, several beneficial bacterial species, such as *Leuconostoc lactis*, *Enterococcus faecium*, and *Ligilactobacillus ruminis*, were reduced in the super-geriatric group compared to adults. These findings suggest that although the overall gut microbial structure of giant pandas remains similar across age stages, substantial differences exist in the relative abundance of key genera and species, particularly those associated with nutrient metabolism and host health.

We further analyzed the gut microbial diversity between the two groups. Results showed no statistically significant differences in α -diversity. The Shannon index, Simpson index, Pielou's evenness, and species richness indicators (Observed OTUs, Chao1, and ACE) all showed no statistical differences ($p > 0.05$). At the species level, principal coordinate analysis (PCoA) showed no significant separation between adult and super-geriatric samples, and the PERMANOVA test also detected no significant differentiation (Supplementary Figure S2a,b). Further analysis of the LEfSe results (LDA score threshold = 2.0) identified microbial taxa with significantly different abundances between the groups. All significantly enriched biomarkers were derived from the super-geriatric group, whereas no taxa were significantly enriched in the adult group (Figure 1d, Supplementary Table S3). These biomarkers included viruses, bacterial genera, and higher taxonomic ranks, such as Vectorvirus LL11 and CrRp3 virus (both associated with *Actinobacillus*), several *Streptococcus* species, as well as taxa within the orders *Pasteurellales* and *Micrococcaceae*. Among them, *Streptococcus ferus* exhibited the highest LDA score (LDA = 3.68).

3.1.2. Functional Differences in the Gut Microbiota

KEGG Orthology functional annotation revealed clear differences between the two age groups (Figure 2a). In the super-geriatric group, genes related to ribosomal proteins (K02961) and transmembrane transporters, notably the SecY subunit (K02946), were significantly upregulated ($p < 0.05$). In contrast, genes associated with oxidative phosphorylation enzymes, including NADH dehydrogenase, cytochrome c oxidase, and ATP synthase F_0 subunit, as well as pathways related to coenzyme biosynthesis and DNA repair (such as DNA polymerase III δ subunit and MutL protein), were significantly downregulated ($p < 0.05$). Gene Ontology (GO) analysis further supported these functional differences (Figure 2b). The super-geriatric group showed significant enrichment ($p < 0.05$) in categories related to ribosomal structural components (GO:0005840, GO:0003735), cell membrane components (GO:0016021, GO:0005886), nucleic acid binding and translation-related functions (GO:0003677, GO:0003723, GO:0019843, GO:0006412), as well as molecular binding activities, including ATP binding and metal ion binding. In addition, hydrolase activity and ATPase activity were also more abundant in the super-geriatric group.

Despite the lack of significant separation in overall gut microbiota structure between super-geriatric and adult giant pandas (Supplementary Figure S2c), distinct functional differences were observed between the two groups. HUMAnN3 pathway analysis revealed that metabolic pathways associated with the degradation of host-derived compounds, including polysaccharide degradation (PWY-8178, PWY-5154), lipid metabolism (PWY-821), and lipopolysaccharide biosynthesis (P185-PWY), were significantly more abundant in the super-geriatric group than in the adult group ($p < 0.05$) (Figure 2c,d). For example, the relative abundance of the extracellular polysaccharide degradation pathway (PWY-8178) was higher in the super-geriatric group than in the adult group, which was also supported by a higher LDA score in LEfSe analysis (LDA = 3.13, $p < 0.05$). In contrast, several amino acid biosynthesis pathways (e.g., PWY-5910, PWY-7392) exhibited relatively higher abundances in the adult group (Figure 2e). Overall, these results indicate age-associated differences in the functional potential of the gut microbiota between adult and super-geriatric giant pandas.

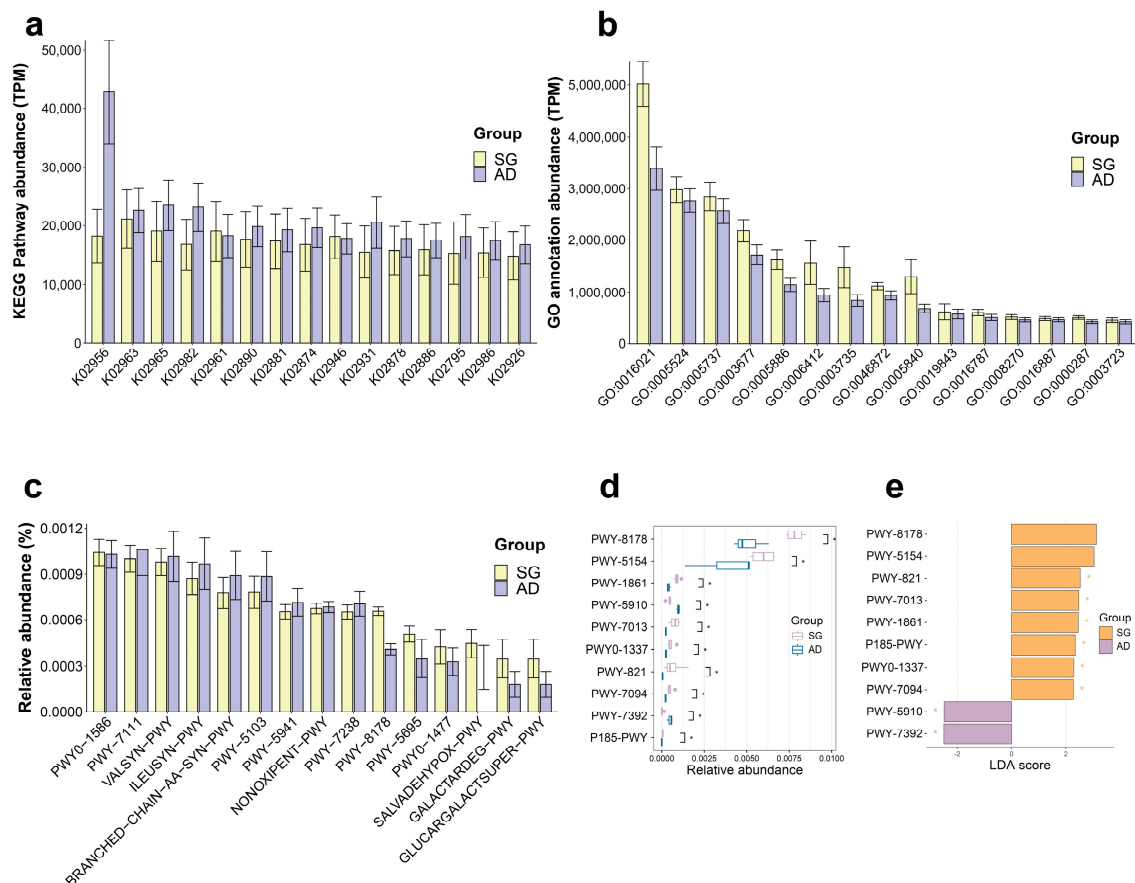


Figure 2. Functional profiling of the gut microbiota in adult and super-geriatric giant pandas: (a) Relative abundance of the top 15 KEGG Orthology (KO) functional categories; (b) Relative abundance of the top 15 Gene Ontology (GO) terms; (c) Relative abundance of the top 15 metabolic pathways annotated by HUMAnN3; (d) Top 15 differential metabolic pathways identified by HUMAnN3; (e) Differential metabolic pathways between adult and super-geriatric groups identified by linear discriminant analysis (LDA). * indicates statistically significant differences ($p < 0.05$).

3.1.3. CAZyme Profiles of Adult and Super-Geriatric Giant Pandas

To further characterize functional features of gut microbiota related to carbohydrate metabolism, we performed a comprehensive analysis of carbohydrate-active enzymes (CAZymes). The annotation results showed that the 15 most abundant CAZyme families were GT83, CBM48, GH3, GH8, GH31, GH73, GH4, GT9, GH24, GT51, GT4, GH23, GH13, GT2, and GH1 (Figure 3a). These enzyme families are involved in the hydrolysis and modification of diverse carbohydrate substrates, including cellulose, hemicellulose, peptidoglycan, and chitin. Group-wise comparisons revealed no statistically significant differences in the overall abundance of CAZyme families between the super-geriatric and adult groups ($p > 0.05$). However, several individual CAZyme families exhibited significantly higher relative abundances in the super-geriatric group, including GH103 (peptidoglycan hydrolase), GH18 (chitinase), and GT4 (glycosyltransferase) ($p < 0.05$; Figure 3b). GT4 family enzymes have been reported to be involved in the biosynthesis of lipopolysaccharide core oligosaccharides.

3.2. Metabolomic Analysis Results

3.2.1. Metabolite Composition and Differences

The gut microbiota plays an important role in shaping the host metabolic landscape through the production and transformation of diverse metabolites. To characterize metabolite compositional differences between super-geriatric and adult giant pandas, untargeted

metabolomic profiling was performed on fecal samples from six individuals using ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS). A total of 877 metabolites were identified, including 623 detected in positive ion mode and 254 in negative ion mode (Supplementary Table S4). Chemical classification analysis showed that these metabolites were mainly composed of lipids and lipid-like molecules (14%), organic acids and derivatives (12%), organic oxygen compounds (5%), and organoheterocyclic compounds (7%) (Figure 4a).

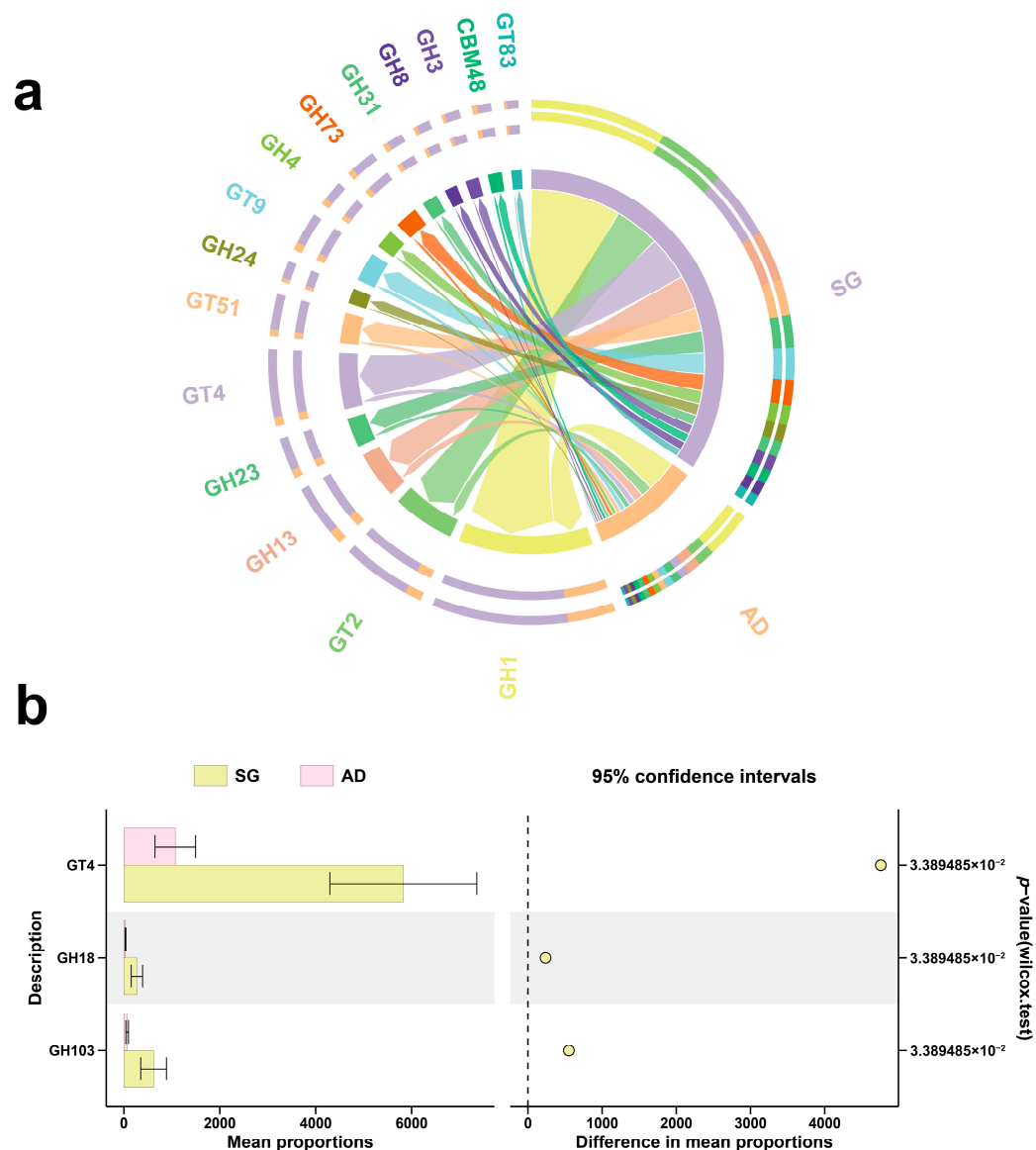


Figure 3. CAZyme profile analysis of the gut microbiota in adult and super-geriatric giant pandas: (a) Distribution of CAZyme categories across all samples; (b) CAZyme families showing significantly different relative abundances between groups, as identified by STAMP analysis.

To explore the metabolic context of the detected metabolites, data from both positive and negative ionization modes were integrated and annotated using the HMDB, KEGG, and LipidMaps databases. Metabolite identification was performed according to the criteria proposed by Sumner et al. [45], and the metabolites reported in this study were classified as Level 2 (putatively annotated compounds) based on accurate mass and MS/MS spectral similarity. Pathway enrichment analysis revealed significant enrichment in pathways related to lipid metabolism, amino acid metabolism, and energy metabolism

(Supplementary Figure S3). In particular, the enrichment of glycerophospholipids and fatty acids indicates prominent alterations in lipid-associated metabolic features, which are commonly involved in membrane composition, signaling processes, and immune-related metabolism. The accumulation of amino acid-related metabolites is associated with alterations in protein and nitrogen metabolism, while enrichment of energy metabolism pathways is indicative of age-associated modulation of metabolic energy utilization under different physiological states.

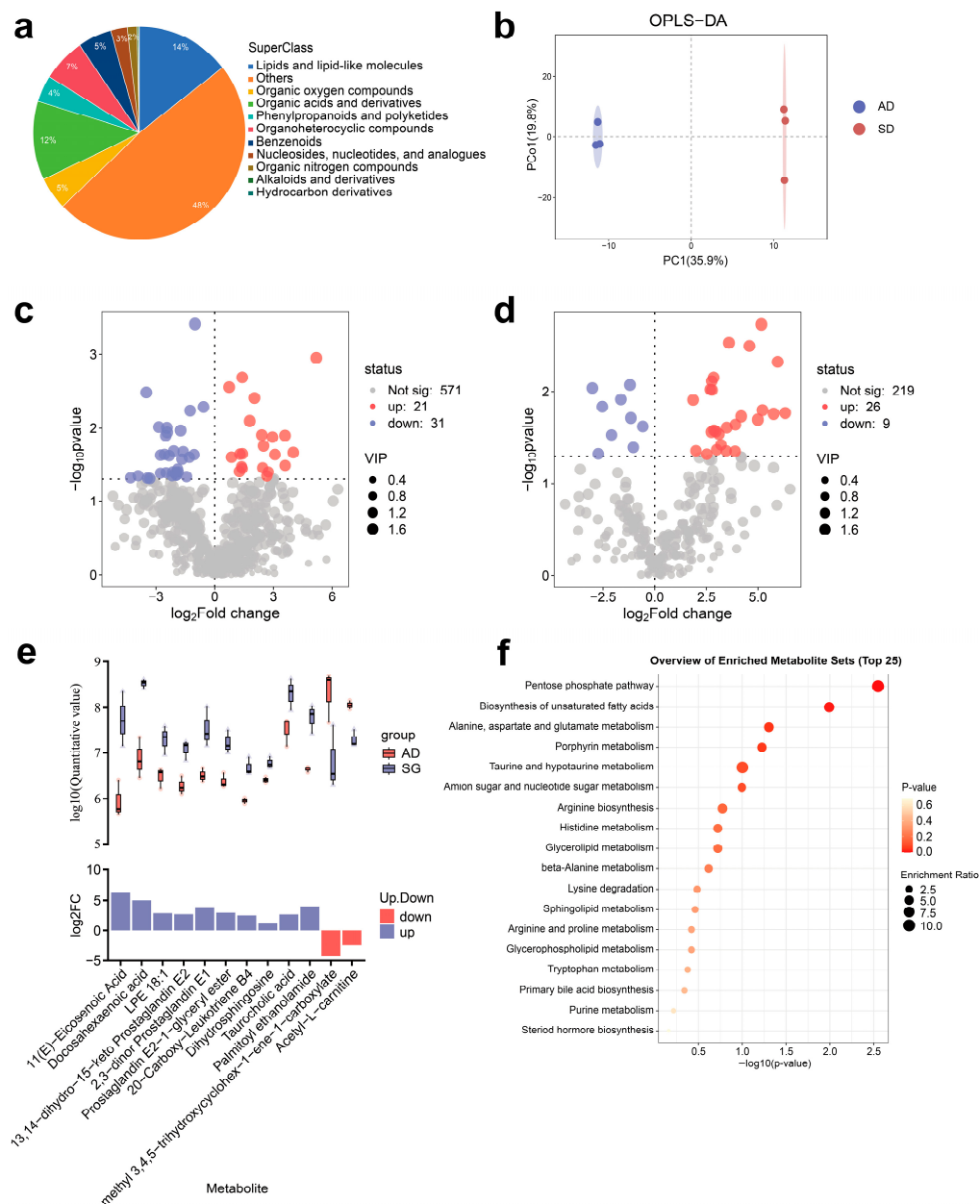


Figure 4. Metabolomic analysis of fecal samples from super-geriatric and adult giant pandas: (a) Classification of putatively annotated metabolites (11 categories; two minor categories with extremely low proportions, ~0.2% and ~0.1%, located to the right of 2% and not easily distinguishable in the un-enlarged figure); (b) OPLS-DA score plot showing separation between the two groups; (c) Volcano plot of significantly different metabolites in positive ion mode; (d) Volcano plot of significantly different metabolites in negative ion mode; (e) Comparison of fold changes among representative differential metabolites; (f) KEGG pathway enrichment analysis of all differential metabolites.

The orthogonal partial least squares discriminant analysis (OPLS-DA) model showed a clear separation of metabolite profiles between adult and super-geriatric giant pandas (Figure 4b), indicating pronounced differences in overall metabolic patterns between the two groups. Based on the predefined selection criteria ($VIP > 1$, $FC \geq 2$ or $FC \leq 0.5$, and $p < 0.05$), a total of 87 significantly different metabolites were identified across both ionization modes (Supplementary Table S5), including 52 metabolites detected in positive ion mode (21 upregulated and 31 downregulated) (Figure 4c) and 35 metabolites detected in negative ion mode (26 upregulated and 9 downregulated) (Figure 4d). Among these differential metabolites, methyl 3,4,5-trihydroxycyclohex-1-ene-1-carboxylate showed a marked decrease in the super-geriatric group ($FC = 0.051$, $p < 0.05$), whereas 11(E)-eicosenoic acid exhibited a pronounced increase ($FC = 79.64$, $p < 0.05$) (Figure 4e). Overall, the metabolite compositional differences between super-geriatric and adult giant pandas were mainly characterized by variations in lipid-related and organic acid-related metabolites.

In super-geriatric giant pandas, notable alterations in lipid- and bile acid-related metabolites were observed. At the lipidomic level, several unsaturated fatty acids and their derivatives exhibited higher abundances (Figure 4e; Supplementary Table S5), including docosahexaenoic acid (DHA) ($\log_2FC = 4.99$, $p < 0.05$) and LPE 18:1 ($\log_2FC = 2.87$, $p < 0.05$). Although arachidonic acid (AA) itself did not show a significant change, multiple downstream AA-derived metabolites were significantly elevated, including 13,14-dihydro-15-keto prostaglandin E2 ($\log_2FC = 2.69$, $p < 0.05$), 2,3-dinor prostaglandin E1 ($\log_2FC = 3.89$, $p < 0.05$), prostaglandin E2-1-glyceryl ester ($\log_2FC = 2.95$, $p < 0.05$), and 20-carboxy-leukotriene B4 ($\log_2FC = 2.49$, $p < 0.05$). Among sphingolipid-related metabolites, only dihydrosphingosine showed a significant increase ($\log_2FC = 1.29$, $p < 0.05$). In addition, taurocholic acid was significantly increased in the super-geriatric group ($\log_2FC = 2.67$, $p < 0.05$; KEGG pathway: primary bile acid biosynthesis). Several other metabolites, including palmitoyl ethanolamide ($\log_2FC = 4.01$, $p < 0.05$) and acetyl-L-carnitine ($\log_2FC = -2.44$, $p < 0.05$), also showed significant differences between groups.

Functional enrichment analysis revealed that the differential metabolites were predominantly and significantly enriched in the pentose phosphate pathway and the biosynthesis of unsaturated fatty acids pathways ($p < 0.05$) (Figure 4f). These pathways are closely associated with energy metabolism regulation and membrane lipid synthesis, suggesting their potential involvement in age-related physiological functional changes.

3.2.2. Identification of Hub Metabolites

To further identify metabolites strongly associated with age, weighted gene co-expression network analysis (WGCNA) was performed. All detected metabolites were clustered into eight co-expression modules (e.g., MEbrown, MEgreen). Among these, the brown module (MEbrown) exhibited the strongest association with age, showing a positive correlation with the adult group ($r = 0.87$, $p = 0.02$) and a negative correlation with the super-geriatric group ($r = -0.87$, $p = 0.02$) (Figure 5). This module contained 189 metabolites, including 52 metabolites that were previously identified as significantly different between groups (Supplementary Table S6).

Based on the combined filtering criteria of module membership ($MM > 0.8$) and gene significance ($GS > 0.2$), a total of 143 hub metabolites were identified within the brown module, of which 40 overlapped with the significantly different metabolites. Among these overlapping metabolites, 28 were significantly decreased and 12 were significantly increased in the super-geriatric group compared with the adult group (Supplementary Tables S5 and S7).

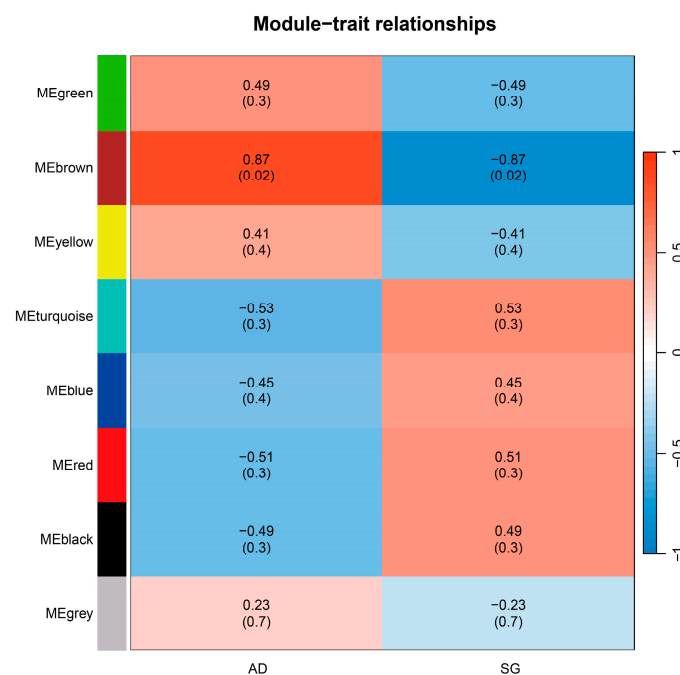


Figure 5. Correlation analysis between fecal metabolites and age-associated modules in giant pandas.

3.3. Correlation Analysis Between Metagenomic and Metabolomic Profiles

Mantel test analysis revealed significant associations between gut microbial community composition patterns and metabolite concentration profiles (Figure 6, Supplementary Table S8). Specifically, variations in bacterial community composition across samples were positively correlated with differences in leucylproline concentration (Mantel $r = 0.68$, $p < 0.05$), indicating an association between this dipeptide and bacterial community structure. The viral community composition showed significant positive correlations with several metabolites, including DL-carnitine (Mantel $r = 0.57$, $p < 0.05$), acetyl-L-carnitine (Mantel $r = 0.51$, $p < 0.05$), N-octyl-2-pyrrolidone (Mantel $r = 0.49$, $p < 0.05$), and 6-hydroxymelatonin (Mantel $r = 0.45$, $p < 0.05$). These metabolites have been reported to exhibit antioxidant or antimicrobial properties in previous studies, suggesting that variation in these metabolites is associated with differences in viral community composition, potentially reflecting broader shifts in host metabolic or immune-related contexts at the ecological level.

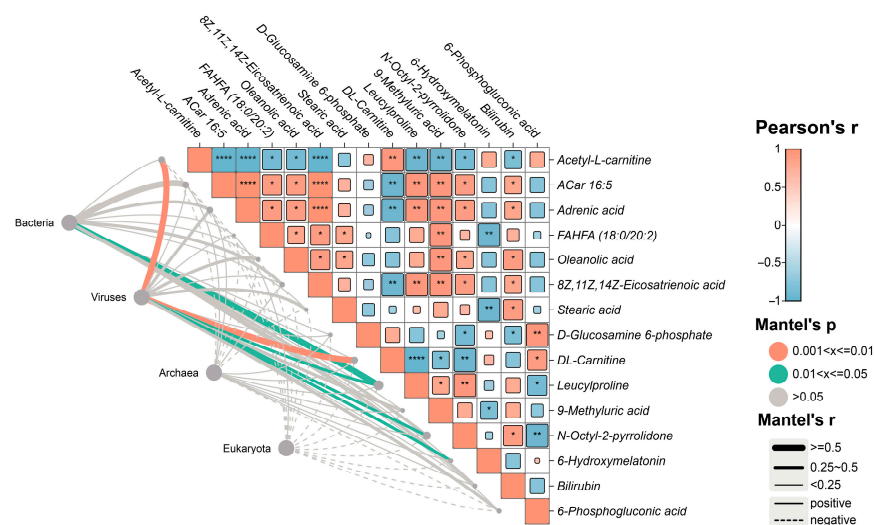


Figure 6. Mantel test-based correlation analysis between metagenomic and metabolomic profiles in giant pandas. Statistical significance is denoted by asterisks: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

In contrast, archaeal and eukaryotic communities exhibited negative correlations with most metabolites; however, none of these associations reached statistical significance. Overall, these distance matrix-based analyses indicate coordinated associations between gut microbial community composition and specific metabolite profiles, highlighting age-related restructuring of the gut microecological and metabolic landscape in giant pandas.

4. Discussion

4.1. Age-Associated Shifts in Gut Microbial Composition and Opportunistic Pathogen Enrichment

The study revealed a distinct enrichment of microbial taxa with reported opportunistic pathogenic potential in the gut microbiota of super-geriatric giant pandas. Among the top 15 significantly enriched biomarkers, all were derived from the super-geriatric group and primarily included bacterial taxa such as *Rothia*, Micrococcaceae, Micrococcales, Pasteurellaceae, *Actinobacillus*, and *Streptococcus ferus*, along with viral sequences related to Vectorvirus LL11 and CrRp3, both associated with *Actinobacillus*. In terms of viral composition, the marked enrichment of Vectorvirus LL11 and CrRp3, together with the significantly higher proportion of Gram-negative bacteria in the aged group, highlights age-associated shifts in the intestinal microbial and viral community structure. Previous studies have shown that Vectorvirus-like viruses are mainly associated with invertebrate vectors and possess broad potential host ranges [46]. Their increased detection in fecal samples from super-geriatric individuals may reflect age-associated changes in the gut microenvironment and virus–bacterium co-occurrence patterns, potentially contributing to shifts in the relative abundance of specific viral populations. Regarding bacterial composition, *Streptococcus cristatus*, an oral commensal bacterium, has been reported to act as an opportunistic pathogen under specific conditions and is associated with diseases such as infective endocarditis [47]. As a member of the core oral microbiota, dysbiosis of *S. cristatus* is closely linked to oral microbiome imbalance and local inflammatory changes [48]. Members of the Pasteurellaceae family, including *Actinobacillus*, are also considered conditional pathogens, and their enrichment may be associated with age-related alterations in host–microbe interactions within the intestinal mucosa of super-geriatric giant pandas [49]. Notably, the higher proportion of Gram-negative bacteria in the super-geriatric group suggests a potential increase in intestinal lipopolysaccharide (LPS)-producing capacity, which may contribute to a pro-inflammatory intestinal milieu [50]. Previous studies have demonstrated that probiotics such as *Leuconostoc lactis*, *Enterococcus faecium*, and *Ligilactobacillus ruminis* can produce short-chain fatty acids (SCFAs), inhibit pathogenic bacteria, and enhance intestinal barrier integrity. Our results showed a reduction in these beneficial microbes in the super-geriatric group, indicating age-associated alterations in gut microbiota structure and potential changes in microbial functional potential. The increase in pathogenic and opportunistic bacteria may reflect a decline in colonization resistance, facilitating the relative expansion of facultative and opportunistic pathogens that would normally be kept in balance [51]. This phenomenon is consistent with previous observations of pathogen enrichment in elderly populations [52]. Overall, these findings should be interpreted as reflecting age-associated shifts in gut microbial and viral community composition and ecological structure, rather than direct evidence of pathogenic activity or causal effects on host inflammatory status.

4.2. Functional Remodeling of the Gut Microbiome and Increased Pro-Inflammatory Potential

The metagenomic functional analysis revealed an age-associated remodeling of gut microbial functional potential in super-geriatric giant pandas. Compared with adults, the super-geriatric group exhibited a notable decline in genes related to energy metabolism and DNA repair, suggesting a reduced microbial functional potential associated with metabolic

activity and stress response [6]. In contrast, genes involved in protein synthesis and cell membrane structure were relatively enriched, possibly reflecting an adaptive shift in microbial functional allocation under altered metabolic conditions [53]. Of particular interest, the GT4 glycosyltransferase [54,55], an enzyme involved in bacterial lipopolysaccharide (LPS) biosynthesis, was significantly increased in the super-geriatric group, consistent with the upregulation of the LPS biosynthetic pathway [56]. This pattern points to an increased representation of Gram-negative bacteria.

In addition to pathway-level functional shifts, age-associated differences were also observed in the profiles of CAZymes. Notably, several CAZyme families involved in cell wall component modification and glycosylation processes, including GT4, showed higher relative abundances in super-geriatric individuals, further suggesting an increased functional potential for LPS-related biosynthesis and structural remodeling of Gram-negative bacteria. Given that LPS is a major structural component of Gram-negative bacterial cell walls, its increased biosynthetic potential may contribute to a pro-inflammatory intestinal milieu. This interpretation is supported by metabolomic findings showing the accumulation of unsaturated fatty acids and alterations in bile acid metabolism, which together may shape an intestinal environment associated with inflammatory processes. Previous studies have reported that aging is accompanied by increased intestinal barrier permeability, which under certain conditions may facilitate the translocation of microbial components and metabolites, thereby contributing to systemic inflammatory responses [57,58]. Age-related alterations in gut microbiota composition have also been proposed to promote the process of “inflammaging” through enhanced LPS production and compromised gut barrier function [57]. In human studies, elevated circulating levels of LPS and LPS-binding protein (LBP) have been recognized as indicators associated with inflammation and metabolic dysregulation [59]. In addition, the reduction in probiotic species such as *Leuconostoc lactis*, *Enterococcus faecium*, and *Ligilactobacillus ruminis* may be associated with decreased short-chain fatty acid (SCFA) production [60–63], which has been linked to impaired intestinal barrier function. Collectively, the loss of beneficial microbes and potential SCFA insufficiency may jointly contribute to the formation of an inflammation-associated intestinal environment in super-geriatric giant pandas [53]. Taken together, these findings highlight the close association between age-related shifts in gut microbial functional profiles and alterations in the metabolite landscape, providing insights into microbiome-associated changes during aging and offering a foundation for future microbiota-targeted strategies in health management. Importantly, these interpretations are based on predicted functional potential derived from metagenomic and metabolomic data, rather than direct measurements of inflammatory activity.

4.3. Age-Related Metabolic Remodeling and Inflammation-Associated Metabolite Profiles

The untargeted fecal metabolomics analysis revealed significant compositional differences in metabolites between super-geriatric and adult giant pandas, which were potentially associated with inflammation- and immune-related metabolic pathways. The super-geriatric group exhibited marked alterations in lipid and organic acid metabolites, characterized by increased levels of unsaturated fatty acids and changes in bile acid-related metabolites. Notably, multiple unsaturated fatty acids and their derivatives were increased in the feces of super-geriatric pandas. Although arachidonic acid (AA) itself was not significantly altered, several downstream AA-derived inflammatory mediators, including prostaglandin- and leukotriene-related metabolites, were markedly elevated. This pattern suggests enhanced AA metabolic activity and increased flux through eicosanoid biosynthetic pathways, rather than accumulation of the precursor itself [64]. In addition, among sphingolipid-related metabolites, only dihydrosphingosine showed a significant

increase, which may reflect age-associated alterations in sphingolipid synthesis or turnover. Alterations in glycerophospholipid composition observed in the super-geriatric group may influence membrane phospholipid remodeling and potentially affect AA availability through phospholipase A₂-related pathways, thereby influencing the production of lipid-derived inflammatory mediators [65–68]. Regarding bile acids, taurocholic acid was significantly increased in the super-geriatric group, suggesting age-related alterations in bile acid metabolism and gut–liver axis–associated signaling. Beyond their role in lipid digestion, bile acids function as important signaling molecules involved in metabolic and immune regulation [69]. Changes in bile acid profiles have been linked to inflammatory and metabolic homeostasis during aging, although the specific mechanisms require further investigation [70]. Collectively, the elevation of multiple eicosanoids together with alterations in unsaturated fatty acids, glycerophospholipids, and bile acid metabolism indicates an age-associated shift toward a metabolically imbalanced state characterized by inflammation-related metabolic features. This pattern is consistent with key characteristics of “inflammaging,” a chronic, low-grade inflammatory state associated with aging. Overall, these findings provide metabolomic evidence supporting an association between age-related metabolic remodeling and inflammation-related pathways in super-geriatric giant pandas, while highlighting the need for further targeted studies to elucidate the underlying mechanisms. It should be noted that, due to the relatively limited sample size and high biological variability, FDR-adjusted *p*-values were not used as the primary criterion for differential metabolite selection, and thus some findings may carry an increased risk of false positives.

4.4. Microbiota–Metabolite Associations Reveal Coordinated Age-Related Microecological Changes

Mantel test analyses revealed significant associations between gut microbial community composition (distance matrices) and fecal metabolite profiles (Figure 6; Supplementary Table S8), indicating coordinated variation between microbial community structure and specific metabolites during aging. Within the bacterial community, leucylproline (Leu-Pro) concentrations were significantly and positively correlated with bacterial community compositional differences (Mantel $r = 0.68$, $p < 0.05$), indicating an association between this dipeptide and bacterial community structure. Previous studies have reported that Leu-Pro, a gut microbiota–derived dipeptide, is involved in inflammatory signaling pathways under pathological conditions [71]. However, such functional evidence originates from experimental systems distinct from the present study. Therefore, based on the correlation-based Mantel analysis, the functional role of Leu-Pro in the gut microbiome of giant pandas cannot be inferred. Instead, the present results indicate that Leu-Pro represents a metabolite ecologically associated with bacterial community variation. For the viral community, the concentrations of acetyl-L-carnitine (ALC), DL-carnitine, N-octyl-2-pyrrolidone, and 6-hydroxymelatonin were significantly and positively correlated with viral community composition. These associations suggest that such metabolites may be linked to variations in viral community structure, potentially through indirect effects mediated by host metabolic state, immune-related processes, or cellular functions. Previous evidence indicates that ALC can modulate intestinal inflammation and contribute to immune homeostasis in mammalian systems [72]. In addition, ALC, DL-carnitine, and 6-hydroxymelatonin have been reported to exhibit antioxidant and antimicrobial properties [73–77], which may be linked to variations in the intestinal microenvironment at an ecological level. In contrast, archaeal and eukaryotic communities exhibited negative correlations with most metabolites; however, these relationships were not statistically significant, possibly reflecting their distinct ecological roles within the gut ecosystem. Overall, these distance matrix–based association analyses reveal coordinated patterns between gut microbial community restructuring and metabolic

profile variation. Importantly, these findings provide ecological-level evidence of association, rather than causal or mechanistic relationships, between gut microbiota composition and host metabolic states during the aging process of giant pandas. It should be noted that the functional pathways discussed in this study are derived from predictive annotations based on metagenomic and metabolomic data and have not been experimentally validated.

4.5. A Gut Microbiota–Metabolite–Inflammation Framework and Implications for Health Management

Changes in gut microbial communities have been reported to influence host immune responses indirectly through microbially derived metabolic products, such as short-chain fatty acids, bile acid derivatives, and microbial lipid mediators [78]. Integrating the findings of this study, we propose a conceptual “gut microbiota–metabolite–inflammation” framework as an ecological and hypothesis-generating model to describe age-associated intestinal changes in super-geriatric giant pandas. With advancing age, alterations in gut microbial composition may occur, characterized by the relative enrichment of bacteria with reported pro-inflammatory potential and a reduction in beneficial microbial taxa. These shifts may be accompanied by the accumulation of inflammation-associated metabolites and/or a decrease in metabolites with protective functions. Such metabolic and microbial imbalances may contribute to the development of a chronic, low-grade inflammatory state, which in turn could further influence gut microbial community structure, potentially forming a self-reinforcing feedback loop. Although causality cannot be inferred from the present data, this framework provides a plausible ecological interpretation of the observed associations among gut microbiota composition, metabolic profiles, and aging. The gut microecological changes identified in this study offer preliminary insights for the health management of aging giant pandas. Given the observed age-associated shift in the gut microbiota toward inflammation-related metabolic features, future studies may explore strategies aimed at modulating gut microbial composition, such as adjustments in bamboo species composition or the cautious supplementation of specific probiotics or prebiotics [79]. However, considering the unique digestive physiology of giant pandas, any dietary or microbial intervention should undergo rigorous safety and efficacy evaluation. In addition, regular monitoring of fecal microbiota composition and metabolite profiles in aged individuals may serve as potential indicators of intestinal health status, enabling the early identification of gut microecological imbalances and timely adjustment of management strategies to support longevity and overall well-being in this endangered species.

5. Conclusions

Through integrated metagenomic and metabolomic analyses, this study identified age-associated differences in gut microbiota composition and metabolic profiles between super-geriatric and adult giant pandas. Compared with adults, super-geriatric individuals exhibited a reduction in several beneficial microbial taxa, an increased representation of Gram-negative bacteria and viral sequences, and an enrichment of predicted functional pathways related to lipopolysaccharide (LPS) biosynthesis, together with alterations in metabolites associated with inflammation-related pathways. Functional analyses further revealed age-related shifts in microbial metabolic potential, including changes in pathways involved in carbohydrate utilization, amino acid metabolism, and secondary metabolite biosynthesis. Collectively, these findings indicate coordinated alterations between gut microbial composition and metabolic profiles during aging. Based on these association-based observations, we outline a conceptual “gut microbiota–metabolite–inflammation” framework as an ecological and hypothesis-generating model to describe age-associated gut microecological changes in super-geriatric giant pandas. Importantly, these results do

not establish causal relationships but provide ecological insights into the links among gut microbiota structure, metabolic remodeling, and aging. These findings offer preliminary implications for the health management of aging giant pandas. Future studies may explore dietary or microbiota-targeted strategies aimed at modulating gut microbial composition while recognizing that any intervention should be carefully evaluated for safety and efficacy given the unique digestive physiology of this species.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ani16040575/s1>. Table S1. The sample information; Table S2. The community structure information; Table S3. The top fifteen LDA scores; Table S4. The metabolomics data overview; Table S5. The differential metabolites between groups; Table S6. The information of significance modules; Table S7. The SDMs in hub metabolites (MM > 0.8 and GS > 0.2); Table S8. The correlation analysis of differential metabolites with domain-specific abundances; Figure S1. Comparison of PLS-DA score scatter plots. (a) Positive ion mode; (b) Negative ion mode; Figure S2. Microbial diversity analysis among different giant panda groups. (a) Species-level α -diversity statistics, where $p < 0.05$ indicates statistical significance; (b) PCoA of species-level microbial community composition; R^2 and Pvalues were calculated using PERMANOVA based on centroids; (c) PCoA generated using HUMAnN functional profiles; Figure S3. Overview of metabolite annotation results across multiple databases. (a) KEGG an-notations in positive and negative ion modes; (b) HMDB annotations in positive and negative ion modes; (c) LipidMaps annotations in positive and negative ion modes.

Author Contributions: Conceptualization, J.W. and X.Z.; methodology, J.W. and F.H.; validation, J.W. and F.H.; formal analysis, J.W. and F.H.; investigation, L.C., W.X. and Z.C.; resources, M.C.; data curation, J.W., M.C., J.C. and Y.Z.; writing—original draft preparation, J.W.; writing—review and editing, X.Z.; visualization, J.W. and F.H.; supervision, J.W., M.C., F.H., L.C., W.X., J.C., Z.C., Y.Z. and X.Z.; project administration, X.Z.; funding acquisition, X.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This work is supported by the Science and Technology Project of Sichuan Province (2025ZNSFSC0252).

Institutional Review Board Statement: All samples in this study consisted of giant panda fecal samples. No live animal experimentation or intervention was involved, the sampling process did not affect animal welfare, and sample collection was conducted at the Dujiangyan Base of the China Conservation and Research Center for the Giant Panda.

Informed Consent Statement: All fecal sampling and associated procedures were conducted by trained staff at the Dujiangyan Base of the China Conservation and Research Center for the Giant Panda in compliance with relevant institutional management regulations.

Data Availability Statement: All data can be found in the main text or the Supplementary Materials. The raw sequence data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) database under BioProject PRJNA1368674.

Acknowledgments: We thank the laboratory members for their assistance and valuable suggestions in this study. We also gratefully acknowledge the Dujiangyan Base of the China Conservation and Research Center for the Giant Panda for providing fecal samples and for their support during sample collection.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Arnason, U.; Gullberg, A.; Janke, A.; Kullberg, M. Mitogenomic analyses of caniform relationships. *Mol. Phylogenet. Evol.* **2007**, *45*, 863–874. [[CrossRef](#)]
2. Krause, J.; Unger, T.; Nocon, A.; Malaspinas, A.S.; Kolokotronis, S.O.; Stiller, M.; Soibelzon, L.; Spriggs, H.; Dear, P.H.; Briggs, A.W.; et al. Mitochondrial genomes reveal an explosive radiation of extinct and extant bears near the Miocene-Pliocene boundary. *BMC Evol. Biol.* **2008**, *8*, 220. [[CrossRef](#)]

3. Xue, Z.; Zhang, W.; Wang, L.; Hou, R.; Zhang, M.; Fei, L.; Zhang, X.; Huang, H.; Bridgewater, L.C.; Jiang, Y.; et al. The bamboo-eating giant panda harbors a carnivore-like gut microbiota, with excessive seasonal variations. *mBio* **2015**, *6*, e00022-00015. [[CrossRef](#)]
4. Wei, M.; Zhu, Y.; Liu, W.; Li, D.; Wei, R.; Deng, L.; Wu, K.; Song, S.; Li, T.; Zeng, W.; et al. Factors influencing bamboo intake of captive giant pandas (*Ailuropoda melanoleuca*). *Sci. Rep.* **2023**, *13*, 6262. [[CrossRef](#)]
5. Wang, H.; Zhong, H.; Hou, R.; Ayala, J.; Liu, G.; Yuan, S.; Yan, Z.; Zhang, W.; Liu, Y.; Cai, K.; et al. A Diet Diverse in Bamboo Parts is Important for Giant Panda (*Ailuropoda melanoleuca*) Metabolism and Health. *Sci. Rep.* **2017**, *7*, 3377. [[CrossRef](#)]
6. Zhang, W.; Liu, W.; Hou, R.; Zhang, L.; Schmitz-Esser, S.; Sun, H.; Xie, J.; Zhang, Y.; Wang, C.; Li, L.; et al. Age-associated microbiome shows the giant panda lives on hemicelluloses, not on cellulose. *ISME J.* **2018**, *12*, 1319–1328. [[CrossRef](#)]
7. Zhan, M.; Wang, L.; Xie, C.; Fu, X.; Zhang, S.; Wang, A.; Zhou, Y.; Xu, C.; Zhang, H. Succession of Gut Microbial Structure in Twin Giant Pandas During the Dietary Change Stage and Its Role in Polysaccharide Metabolism. *Front. Microbiol.* **2020**, *11*, 551038. [[CrossRef](#)] [[PubMed](#)]
8. Zhu, L.; Wu, Q.; Dai, J.; Zhang, S.; Wei, F. Evidence of cellulose metabolism by the giant panda gut microbiome. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 17714–17719. [[CrossRef](#)] [[PubMed](#)]
9. Zhan, M.; Wang, A.; Yao, Y.; Zhou, Y.; Zhang, S.; Fu, X.; Zhou, J.; Pei, E.; Wang, L. An amateur gut microbial configuration formed in giant panda for striving to digest cellulose in bamboo: Systematic evidence from intestinal digestive enzymes, functional genes and microbial structures. *Front. Microbiol.* **2022**, *13*, 926515. [[CrossRef](#)] [[PubMed](#)]
10. Latorre, A.; Dominguez-Santos, R.; Garcia-Ferris, C.; Gil, R. Of Cockroaches and Symbionts: Recent Advances in the Characterization of the Relationship between *Blattella germanica* and Its Dual Symbiotic System. *Life* **2022**, *12*, 290. [[CrossRef](#)]
11. Stepanova, I.; Andreychev, A.; Kulakhmetov, R.; Lobachev, E. Commensals of underground mammals: European mole (*Talpa europaea*, Eulipotyphla, Talpidae) and the greater mole-rat (*Spalax microphthalmus*, Rodentia, Spalacidae). *Biodiversitas J. Biol. Divers.* **2021**, *22*, 4665–4670. [[CrossRef](#)]
12. Ragonnaud, E.; Biragyn, A. Gut microbiota as the key controllers of “healthy” aging of elderly people. *Immun. Ageing* **2021**, *18*, 2. [[CrossRef](#)]
13. Arbolea, S.; Watkins, C.; Stanton, C.; Ross, R.P. Gut Bifidobacteria Populations in Human Health and Aging. *Front. Microbiol.* **2016**, *7*, 1204. [[CrossRef](#)]
14. Gyriki, D.; Nikolaidis, C.G.; Bezirtzoglou, E.; Voidarou, C.; Stavropoulou, E.; Tsigalou, C. The gut microbiota and aging: Interactions, implications, and interventions. *Front. Aging* **2025**, *6*, 1452917. [[CrossRef](#)] [[PubMed](#)]
15. Ridlon, J.M.; Kang, D.-J.; Hylemon, P.B. Bile salt biotransformations by human intestinal bacteria. *J. Lipid Res.* **2006**, *47*, 241–259. [[CrossRef](#)]
16. Sayin, S.I.; Wahlstrom, A.; Felin, J.; Jantti, S.; Marschall, H.-U.; Bamberg, K.; Angelin, B.; Hyotylainen, T.; Oresic, M.; Backhed, F. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab.* **2013**, *17*, 225–235. [[CrossRef](#)]
17. Zhang, X.; Wang, X.; Ayala, J.; Liu, Y.; An, J.; Wang, D.; Cai, Z.; Hou, R.; Zhang, M. Possible Effects of Early Maternal Separation on the Gut Microbiota of Captive Adult Giant Pandas. *Animals* **2022**, *12*, 2587. [[CrossRef](#)]
18. Deng, F.; Wang, C.; Li, D.; Peng, Y.; Deng, L.; Zhao, Y.; Zhang, Z.; Wei, M.; Wu, K.; Zhao, J.; et al. The unique gut microbiome of giant pandas involved in protein metabolism contributes to the host’s dietary adaption to bamboo. *Microbiome* **2023**, *11*, 180. [[CrossRef](#)]
19. Wang, L.; Huang, G.; Hou, R.; Qi, D.; Wu, Q.; Nie, Y.; Zuo, Z.; Ma, R.; Zhou, W.; Ma, Y.; et al. Multi-omics reveals the positive leverage of plant secondary metabolites on the gut microbiota in a non-model mammal. *Microbiome* **2021**, *9*, 192. [[CrossRef](#)] [[PubMed](#)]
20. Yang, S.; Huang, Y.; Li, C.; Jin, L.; Deng, W.; Zhao, S.; Wu, D.; He, Y.; Li, B.; Yu, Z.; et al. The Fecal and Serum Metabolomics of Giant Pandas Based on Untargeted Metabolomics. *Zool. Sci.* **2021**, *38*, 179–186. [[CrossRef](#)]
21. Liu, F.; Li, R.; Zhong, Y.; Liu, X.; Deng, W.; Huang, X.; Price, M.; Li, J. Age-related alterations in metabolome and microbiome provide insights in dietary transition in giant pandas. *mSystems* **2023**, *8*, e0025223. [[CrossRef](#)] [[PubMed](#)]
22. Huang, F.; Jin, X.; Xu, J.; Wu, H.; Gan, M.; Chang, W.; Tan, Q.; Wang, Y.; Li, R.; Li, D.; et al. Gut microbiome-driven longevity mechanisms in giant pandas: Age-associated gut microbial shifts, functional pathways, and probiotic insights. *Curr. Zool.* **2025**, *16*, 1629811. [[CrossRef](#)]
23. Qi, Y.; Su, H.; Hou, R.; Zang, H.; Liu, P.; He, M.; Xu, P.; Zhang, Z.; Chen, P. Giant panda age recognition based on a facial image deep learning system. *Ecol. Evol.* **2022**, *12*, e9507. [[CrossRef](#)]
24. Chen, S. Ultrafast one-pass FASTQ data preprocessing, quality control, and deduplication using fastp. *iMeta* **2023**, *2*, e107. [[CrossRef](#)] [[PubMed](#)]
25. Langmead, B.; Salzberg, S.L. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* **2012**, *9*, 357–359. [[CrossRef](#)]
26. Lu, J.; Rincon, N.; Wood, D.E.; Breitwieser, F.P.; Pockrandt, C.; Langmead, B.; Salzberg, S.L.; Steinegger, M. Metagenome analysis using the Kraken software suite. *Nat. Protoc.* **2022**, *17*, 2815–2839. [[CrossRef](#)]

27. Lu, J.; Breitwieser, F.P.; Thielen, P.; Salzberg, S.L. Bracken: Estimating species abundance in metagenomics data. *PeerJ Comput. Sci.* **2017**, *3*, e104. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Dixon, P. VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* **2003**, *14*, 927–930. [\[CrossRef\]](#)
29. Love, M.I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **2014**, *15*, 550. [\[CrossRef\]](#)
30. Nearing, J.T.; Douglas, G.M.; Hayes, M.G.; MacDonald, J.; Desai, D.K.; Allward, N.; Jones, C.M.A.; Wright, R.J.; Dhanani, A.S.; Comeau, A.M.; et al. Microbiome differential abundance methods produce different results across 38 datasets. *Nat. Commun.* **2022**, *13*, 342. [\[CrossRef\]](#)
31. Wang, Y.; Zhai, J.; Tang, B.; Dong, Y.; Sun, S.; He, S.; Zhao, W.; Lancuo, Z.; Jia, Q.; Wang, W. Metagenomic comparison of gut communities between wild and captive Himalayan griffons. *Front. Vet. Sci.* **2024**, *11*, 1403932. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Suzek, B.E.; Huang, H.; McGarvey, P.; Mazumder, R.; Wu, C.H. UniRef: Comprehensive and non-redundant UniProt reference clusters. *Bioinformatics* **2007**, *23*, 1282–1288. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Beghini, F.; McIver, L.J.; Blanco-Miguez, A.; Dubois, L.; Asnicar, F.; Maharjan, S.; Mailyan, A.; Manghi, P.; Scholz, M.; Thomas, A.M.; et al. Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. *eLife* **2021**, *10*, e65088. [\[CrossRef\]](#)
34. Franzosa, E.A.; McIver, L.J.; Rahnava, G.; Thompson, L.R.; Schirmer, M.; Weingart, G.; Lipson, K.S.; Knight, R.; Caporaso, J.G.; Segata, N.; et al. Species-level functional profiling of metagenomes and metatranscriptomes. *Nat. Methods* **2018**, *15*, 962–968. [\[CrossRef\]](#)
35. Liu, Y.X.; Qin, Y.; Chen, T.; Lu, M.; Qian, X.; Guo, X.; Bai, Y. A practical guide to amplicon and metagenomic analysis of microbiome data. *Protein Cell* **2021**, *12*, 315–330. [\[CrossRef\]](#)
36. Liu, Y.X.; Chen, L.; Ma, T.; Li, X.; Zheng, M.; Zhou, X.; Chen, L.; Qian, X.; Xi, J.; Lu, H.; et al. EasyAmplicon: An easy-to-use, open-source, reproducible, and community-based pipeline for amplicon data analysis in microbiome research. *iMeta* **2023**, *2*, e83. [\[CrossRef\]](#)
37. Parks, D.H.; Tyson, G.W.; Hugenholtz, P.; Beiko, R.G. STAMP: Statistical analysis of taxonomic and functional profiles. *Bioinformatics* **2014**, *30*, 3123–3124. [\[CrossRef\]](#)
38. Wang, D.; Lin, H.; Ma, Q.; Bai, Y.; Qu, J. Manganese oxides in Phragmites rhizosphere accelerates ammonia oxidation in constructed wetlands. *Water Res.* **2021**, *205*, 117688. [\[CrossRef\]](#)
39. Want, E.J.; Masson, P.; Michopoulos, F.; Wilson, I.D.; Theodoridis, G.; Plumb, R.S.; Shockcor, J.; Loftus, N.; Holmes, E.; Nicholson, J.K. Global metabolic profiling of animal and human tissues via UPLC-MS. *Nat. Protoc.* **2013**, *8*, 17–32. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Sellick, C.A.; Hansen, R.; Stephens, G.M.; Goodacre, R.; Dickson, A.J. Metabolite extraction from suspension-cultured mammalian cells for global metabolite profiling. *Nat. Protoc.* **2011**, *6*, 1241–1249. [\[CrossRef\]](#)
41. Yuan, M.; Breitkopf, S.B.; Yang, X.; Asara, J.M. A positive/negative ion-switching, targeted mass spectrometry-based metabolomics platform for bodily fluids, cells, and fresh and fixed tissue. *Nat. Protoc.* **2012**, *7*, 872–881. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Wen, B.; Mei, Z.; Zeng, C.; Liu, S. metaX: A flexible and comprehensive software for processing metabolomics data. *BMC Bioinform.* **2017**, *18*, 183. [\[CrossRef\]](#)
43. Zhang, B.; Horvath, S. A general framework for weighted gene co-expression network analysis. *Stat. Appl. Genet. Mol. Biol.* **2005**, *4*. [\[CrossRef\]](#)
44. Langfelder, P.; Horvath, S. WGCNA: An R package for weighted correlation network analysis. *BMC Bioinform.* **2008**, *9*, 559. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Sumner, L.W.; Amberg, A.; Barrett, D.; Beale, M.H.; Beger, R.; Daykin, C.A.; Fan, T.W.; Fiehn, O.; Goodacre, R.; Griffin, J.L.; et al. Proposed minimum reporting standards for chemical analysis Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics* **2007**, *3*, 211–221. [\[CrossRef\]](#)
46. Shi, M.; Lin, X.-D.; Tian, J.-H.; Chen, L.-J.; Chen, X.; Li, C.-X.; Qin, X.-C.; Li, J.; Cao, J.-P.; Eden, J.-S.; et al. Redefining the invertebrate RNA virosphere. *Nature* **2016**, *540*, 539–543. [\[CrossRef\]](#)
47. Coykendall, A.L. Classification and identification of the viridans streptococci. *Clin. Microbiol. Rev.* **1989**, *2*, 315–328. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Whitmore, S.E.; Lamont, R.J. The pathogenic persona of community-associated oral streptococci. *Mol. Microbiol.* **2011**, *81*, 305–314. [\[CrossRef\]](#)
49. Nørskov-Lauritsen, N. Classification, Identification, and Clinical Significance of Haemophilus and Aggregatibacter Species with Host Specificity for Humans. *Clin. Microbiol. Rev.* **2014**, *27*, 214–240. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Franceschi, C.; Garagnani, P.; Parini, P.; Giuliani, C.; Santoro, A. Inflammaging: A new immune-metabolic viewpoint for age-related diseases. *Nat. Rev. Endocrinol.* **2018**, *14*, 576–590. [\[CrossRef\]](#)
51. O'Toole, P.W.; Jeffery, I.B. Gut microbiota and aging. *Science* **2015**, *350*, 1214–1215. [\[CrossRef\]](#)
52. Ghosh, T.S.; Shanahan, F.; O'Toole, P.W. The gut microbiome as a modulator of healthy ageing. *Nat. Rev. Gastroenterol. Hepatol.* **2022**, *19*, 565–584. [\[CrossRef\]](#)

53. Best, L.; Dost, T.; Esser, D.; Flor, S.; Gamarra, A.M.; Haase, M.; Kadibalban, A.S.; Marinos, G.; Walker, A.; Zimmermann, J.; et al. Metabolic modelling reveals the aging-associated decline of host–microbiome metabolic interactions in mice. *Nat. Microbiol.* **2025**, *10*, 973–991. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Cote, J.; Taylor, E. The Glycosyltransferases of LPS Core: A Review of Four Heptosyltransferase Enzymes in Context. *Int. J. Mol. Sci.* **2017**, *18*, 2256. [\[CrossRef\]](#)
55. Martinez-Fleites, C.; Proctor, M.; Roberts, S.; Bolam, D.N.; Gilbert, H.J.; Davies, G.J. Insights into the Synthesis of Lipopolysaccharide and Antibiotics through the Structures of Two Retaining Glycosyltransferases from Family GT4. *Chem. Biol.* **2006**, *13*, 1143–1152. [\[CrossRef\]](#)
56. Malik, J.A.; Zafar, M.A.; Lamba, T.; Nanda, S.; Khan, M.A.; Agrewala, J.N. The impact of aging-induced gut microbiome dysbiosis on dendritic cells and lung diseases. *Gut Microbes* **2023**, *15*, 2290643. [\[CrossRef\]](#)
57. Ren, J.; Li, H.; Zeng, G.; Pang, B.; Wang, Q.; Wei, J. Gut microbiome-mediated mechanisms in aging-related diseases: Are probiotics ready for prime time? *Front. Pharmacol.* **2023**, *14*, 1178596. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Fransen, F.; van Beek, A.A.; Borghuis, T.; Aidy, S.E.; Hugenholtz, F.; van der Gaast-de Jongh, C.; Savelkoul, H.F.J.; De Jonge, M.I.; Boekschoten, M.V.; Smidt, H.; et al. Aged Gut Microbiota Contributes to Systemical Inflammaging after Transfer to Germ-Free Mice. *Front. Immunol.* **2017**, *8*, 1385. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Boutagy, N.E.; McMillan, R.P.; Frisard, M.I.; Hulver, M.W. Metabolic endotoxemia with obesity: Is it real and is it relevant? *Biochimie* **2016**, *124*, 11–20. [\[CrossRef\]](#)
60. Mishra, S.P.; Jain, S.; Wang, B.; Wang, S.; Miller, B.C.; Lee, J.Y.; Borlongan, C.V.; Jiang, L.; Pollak, J.; Taraphder, S.; et al. Abnormalities in microbiota/butyrate/FFAR3 signaling in aging gut impair brain function. *JCI Insight* **2024**, *9*, e168443. [\[CrossRef\]](#)
61. Koh, A.; De Vadder, F.; Kovatcheva-Datchary, P.; Bäckhed, F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell* **2016**, *165*, 1332–1345. [\[CrossRef\]](#)
62. Louis, P.; Flint, H.J. Formation of propionate and butyrate by the human colonic microbiota. *Environ. Microbiol.* **2016**, *19*, 29–41. [\[CrossRef\]](#)
63. Louis, P.; Flint, H.J. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol. Lett.* **2009**, *294*, 1–8. [\[CrossRef\]](#) [\[PubMed\]](#)
64. Calder, P.C. Polyunsaturated fatty acids, inflammation, and immunity. *Lipids* **2001**, *36*, 1007–1024. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Fabbri, E.; Yang, A.; Simonsick, E.M.; Chia, C.W.; Zoli, M.; Haughey, N.J.; Mielke, M.M.; Ferrucci, L.; Coen, P.M. Circulating ceramides are inversely associated with cardiorespiratory fitness in participants aged 54–96 years from the Baltimore Longitudinal Study of Aging. *Aging Cell* **2016**, *15*, 825–831. [\[CrossRef\]](#)
66. Gaggini, M.; Ndreu, R.; Michelucci, E.; Rocchiccioli, S.; Vassalle, C. Ceramides as Mediators of Oxidative Stress and Inflammation in Cardiometabolic Disease. *Int. J. Mol. Sci.* **2022**, *23*, 2719. [\[CrossRef\]](#)
67. Sun, G.Y.; Shelat, P.B.; Jensen, M.B.; He, Y.; Sun, A.Y.; Simonyi, A. Phospholipases A2 and Inflammatory Responses in the Central Nervous System. *NeuroMolecular Med.* **2009**, *12*, 133–148. [\[CrossRef\]](#)
68. Boilard, E.; Lai, Y.; Larabee, K.; Balestrieri, B.; Ghomashchi, F.; Fujioka, D.; Gobezie, R.; Coblyn, J.S.; Weinblatt, M.E.; Massarotti, E.M.; et al. A novel anti-inflammatory role for secretory phospholipase A2 in immune complex-mediated arthritis. *EMBO Mol. Med.* **2010**, *2*, 172–187. [\[CrossRef\]](#)
69. Baars, A.; Oosting, A.; Knol, J.; Garssen, J.; Van Bergenhenegouwen, J. The Gut Microbiota as a Therapeutic Target in IBD and Metabolic Disease: A Role for the Bile Acid Receptors FXR and TGR5. *Microorganisms* **2015**, *3*, 641–666. [\[CrossRef\]](#) [\[PubMed\]](#)
70. Tang, X.; Zhou, Y.; Xia, L.; Lin, X.; Zhu, Y.; Chen, M.; Wang, J.; Li, Y. Multifaceted Interactions Between Bile Acids, Their Receptors, and MASH: From Molecular Mechanisms to Clinical Therapeutics. *Molecules* **2025**, *30*, 3066. [\[CrossRef\]](#)
71. Yang, L.; He, S.; Wei, A.; Wang, X.; He, L.; Cui, L.; Zhang, S.; Zhong, C.; Zhuo, Y.; Wang, X. Gut microbiota-derived Proline-Leucine dipeptide aggravated sepsis-induced acute lung injury via activating Nod2/NF-kappaB signaling pathway. *Mol. Immunol.* **2025**, *184*, 199–212. [\[CrossRef\]](#)
72. Lin, K.; Zheng, W.; Guo, M.; Zhou, R.; Zhang, M.; Liu, T. The intestinal microbial metabolite acetyl l-carnitine improves gut inflammation and immune homeostasis via CADM2. *Biochim. Et Biophys. Acta (BBA)-Mol. Basis Dis.* **2024**, *1870*, 167089. [\[CrossRef\]](#) [\[PubMed\]](#)
73. Lopez-Maldonado, A.; Pastoriza, S.; Rufian-Henares, J.A. Assessing the antioxidant and metabolic effect of an alpha-lipoic acid and acetyl-L-carnitine nutraceutical. *Curr. Res. Food Sci.* **2021**, *4*, 336–344. [\[CrossRef\]](#) [\[PubMed\]](#)
74. Yi, Y.; Ma, H.; Tan, B.; Wei, H.; Wu, J.; Mao, M.; Wu, J.; Chi, S.; Liu, H.; Yang, Q. Interaction between dietary L-carnitine and lipid levels on growth, lipid metabolism, and antioxidant capacity in juvenile golden pompano (*Trachinotus ovatus*). *Aquac. Rep.* **2025**, *41*, 102714. [\[CrossRef\]](#)
75. Sawicka, A.K.; Renzi, G.; Olek, R.A. The bright and the dark sides of L-carnitine supplementation: A systematic review. *J. Int. Soc. Sports Nutr.* **2020**, *17*, 49. [\[CrossRef\]](#)
76. Alvarez-Diduk, R.; Galano, A.; Tan, D.X.; Reiter, R.J. N-Acetylserotonin and 6-Hydroxymelatonin against Oxidative Stress: Implications for the Overall Protection Exerted by Melatonin. *J. Phys. Chem. B* **2015**, *119*, 8535–8543. [\[CrossRef\]](#) [\[PubMed\]](#)

77. Zimmermann, P.; Kurth, S.; Pugin, B.; Bokulich, N.A. Microbial melatonin metabolism in the human intestine as a therapeutic target for dysbiosis and rhythm disorders. *npj Biofilms Microbiomes* **2024**, *10*, 139. [[CrossRef](#)]
78. Kim, K.-A.; Jeong, J.-J.; Yoo, S.-Y.; Kim, D.-H. Gut microbiota lipopolysaccharide accelerates inflamm-aging in mice. *BMC Microbiol.* **2016**, *16*, 1–9. [[CrossRef](#)]
79. Fusco, W.; Lorenzo, M.B.; Cintoni, M.; Porcari, S.; Rinninella, E.; Kaitsas, F.; Lener, E.; Mele, M.C.; Gasbarrini, A.; Collado, M.C.; et al. Short-Chain Fatty-Acid-Producing Bacteria: Key Components of the Human Gut Microbiota. *Nutrients* **2023**, *15*, 2211. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.