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Exploring fecal microbial activity in zoo felids of varying body mass on a similar diet

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Abstract

Under human care, felids are typically fed similar diets, unlike wild counterparts whose diets vary by body mass and ecology. This study evaluated fecal microbiota and fermentation products in 18 zoo felids from Pairi Daiza Zoo, Belgium, grouped by body mass: under 100 kg ("small") and over 100 kg ("large"), with 9 animals in each group. Fresh feces were collected from the rectum under anesthesia. Microbial composition was assessed via 16S rRNA gene sequencing, while the fecal volatile fatty acids were quantified using gas chromatography. At the phylum level, regardless of body mass, the gut microbiota of zoo felids was predominantly composed of Firmicutes (61.7%), Actinobacteria (16.4%) and Bacteroidetes (12.5%). At the genus level, the most abundant genus was *Clostridium* sensu stricto *1* (15.9%), followed by *Collinsella* (15.7%). Although no significant differences in microbial composition or alpha diversity were found, beta diversity showed body mass influenced overall microbial structure. Smaller felids had significantly higher acetate levels than larger felids ($p < 0.01$). Additionally, acetate proportions were positively correlated with *Clostridium* sensu stricto *13* ($r = 0.6$, $p < 0.01$) and *Peptoniphilus* ($r = 0.5$, $p < 0.05$). These results show particular associations between body mass and the response of the intestinal microbiome to diet, suggesting that a uniform diet may not suit all felids under human care.

Keywords Felids, Fecal microbiota, Fermentation, Body mass, Acetate

Introduction

The body mass of mammalian carnivores is associated with natural hunting and feeding behavior [1]. The Felidae family has the widest range of body mass of all living carnivore families, weighing 1 kg to 300 kg [2]. For example, lions (*Panthera leo*) and tigers (*Panthera tigris*), which weigh over 100 kg, hunt very large mammals; cougars (*Puma concolor*), snow leopards (*Panthera uncia*) and leopards (*Panthera pardus*), with body weights between 15 and 100 kg, hunt smaller prey but a larger number of different species, while black-footed cats (*Felis nigripes*) and bobcats (*Lynx rufus*), which weigh less than 10 kg, prey on small mammals, birds, reptiles, amphibians, and insects. Typically, when relatively small prey is eaten, the entire prey is consumed, including some non-digestible body parts such as fur, skin and bones [3, 4], which has been referred to as 'animal fiber' in nutrition research [5]. The difference in intake of fibrous animal matter may have co-evolved with the digestive physiology of felids [6, 7]. Studies of dog breeds have shown that dog size has significant effects on gastrointestinal physiology, such as the absorption of nutrients and water in the gut, the composition and activity of the gut microbiota, and fecal moisture [8]. Nutrient digestibility and fecal characteristics may also be associated with body mass in felids. Larger exotic felids may be better suited to diets containing small amounts of fermentable fiber, more easily digestible protein sources, or reduced dietary collagen [9]. Whether

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carnivore digestive strategies are associated with particular co-evolved traits is unexplored.

Although exotic felids vary in body mass and diet in the wild, they often are fed similar diets under human care, including commercially-prepared raw meat diets [10]. Usually, feeding recommendations for felids under human care are based on recommendations for domestic cats. However, these recommendations may not be suitable for all species due to size differences [9, 11, 12]. Differences in relative gut capacity may hinder an adequate estimation of energy requirements. Some big felids, such as lions, may not receive satiety signals when their energy needs are met with daily feeding. A major function of satiation is to prevent overconsumption during individual meals, thereby averting deleterious consequences from incomplete digestion [13]. The concomitant meal frequency logically determines the digestive strategy, including features such as gastric emptying and the associated flow and composition of the substrate ending up with the microbiota in the hindgut [1]. As obligate carnivores, felids possess well-developed and active gut microbiota despite their short colon and lack of a functional cecum [14, 15]. Their microbiota is primarily composed of Firmicutes, Fusobacteria, Proteobacteria, and Actinobacteria [16]. Diet-induced changes in the fecal microbiota populations have been documented in wild and human-cared felids [17–20]. Raw meat diets promote proteolytic bacteria (e.g., *Fusobacterium*, *Clostridium*), whereas commercial dry food favors carbohydrate-fermenting taxa like *Prevotella* [21]. Wild felids typically exhibit distinct microbiota compositions due to variations in diet and habitat heterogeneity [18], while captive felids, with more uniform diets and restricted activity, tend to have a more stable but potentially less diverse microbiota [22]. Host genetics, health status, and environmental factors also further shape microbiome structure. However, potential drivers of microbiome differences between felid species—such as body mass—remain unidentified.

In this study, we had the opportunity to sample fresh feces from zoo felids on the same diet but ranging in body mass, which allowed us to look at fecal microbiota and its products as a marker for digestive strategy in relation to species body mass, irrespective of diet.

Materials and methods

Animals and diets

Eighteen felids from Pairi Daiza Zoo (Brugelette, Belgium) participated in this study, including cheetah (*Acinonyx jubatus*) ($n = 1$), puma (*Felis concolor*) ($n = 3$), clouded leopard (*Neofelis nebulosa*) ($n = 2$), lion (*Panthera leo*) ($n = 2$), Javan leopard (*Panthera pardus*) ($n = 1$), white tiger (*Panthera tigris*) ($n = 2$), Siberian tiger (*Panthera tigris*) ($n = 5$), and snow leopard (*Panthera uncia*) ($n = 2$). All felids in this study were housed under relatively similar environmental conditions, including geographical location, ambient temperature, and humidity. Some slight adjustments were made to accommodate species-specific needs. For example, Siberian tigers were housed in enclosures with wooden substrates, while lions and cheetah were housed in grassy enclosures. These variations were primarily designed to mimic the natural habitats of each species while ensuring their well-being. One of the pumas died shortly after the feces collection due to kidney failure (glomerulopathy); no medical or health problems were found for the remaining animals. The basic information of all individuals is shown in Table 1. Visual inspection of the data revealed that felid species did not cover the entire weight range but clustered in these two weight groups. Therefore, they were divided into two groups for further analysis: those below 100 kg ("small") and those (far) above 100 kg ("large").

The animals were fed their regular zoo diet i.e. chunked beef meat with bone top-dressed with a vitamin and mineral premix (Carnicon®; Aveve, Belgium) randomly interspersed with supplemented whole chickens. Feeding quantity depended on the weight of the species (Table 2). Dietary ingredient list and chemical composition are listed in Table 3.

Sample collection

Eighteen felids were anaesthetized with intramuscularly administered ketamin (2.5–5.0 mg/kg, Nimatekl®; Dechra, UK) and medetomidin (0.03–0.05 mg/kg, Dorbene®; Laboratorios Syva, Spain) for the purpose of a routine annual physical health examination at Paira Daiza Zoo (Brugelette, Belgium). This procedure was part of routine health monitoring and did not cause additional discomfort or risk to the animals. Fresh feces were collected from the rectum of each animal during anesthesia and immediately stored at −80 °C for subsequent analysis.

To prepare subsamples for analysis, the fecal samples were placed overnight in a refrigerator (4 °C). For short-chain fatty acid (SCFA) analysis, approximately 1 g of sample was added to pre-weighed 15 ml screw cap tubes (Sarstedt AG & Co. KG, Germany), followed by the addition of 1 ml of 0.1 M phosphoric acid. The contents were vigorously mixed and weighed. For determination of volatile organic compounds, approximately 1 g of sample was added to a pre-weighed 2 ml safe-lock tube (Eppendorf AG, Germany). For microbiota analysis, approximately 2 g of sample was added to 2 ml safety lock tubes (Eppendorf AG, Germany). All samples were immediately stored at −80 °C until analysis.

Table 1 Individual information of the zoo felids in this study

	Name	Latin name	Age (d)	Sex	Average estimated body mass (kg)[‡] [23, 24]
Small felids[*] ($n=9$)	Cheetah1	*Acinonyx jubatus*	3117	M	47
	Puma1	*Puma/Felis concolor*	1285	F	68
	Puma2	*Puma/Felis concolor*	1037	M	68
	Puma3[†]	*Puma/Felis concolor*	-	M	68
	Clouded leopard1	*Neofelis nebulosa*	2583	M	17
	Clouded leopard2	*Neofelis nebulosa*	5476	F	17
	Javan leopard1	*Panthera pardus*	2845	M	54
	Snow leopard1	*Panthera uncia*	1786	F	44
	Snow leopard2	*Panthera uncia*	3618	M	44
Large felids[*] ($n=9$)	Lion1	*Panthera leo*	5411	M	176
	Lion2	*Panthera leo*	5002	F	176
	White tiger1	*Panthera tigris*	3183	F	156
	White tiger2	*Panthera tigris*	3183	M	156
	Siberian tiger1	*Panthera tigris*	1760	M	176
	Siberian tiger2	*Panthera tigris*	1760	M	176
	Siberian tiger3	*Panthera tigris*	1750	M	176
	Siberian tiger4	*Panthera tigris*	1728	M	176
	Siberian tiger5	*Panthera tigris*	1728	M	176

- Data are not available

[*] The animals clustered into two body mass groups: those below 100 kg ("small") and those above 100 kg ("large")

[†] This puma died of kidney failure (glomerulopathy) while in captivity. The sampling time was before death

[‡] Since the exact body mass of the study animals was unavailable from zoo management, we estimated their body mass using values reported in the literature from wild individuals

Table 2 Food composition and feeding amounts for different felid species

	Regular diet	Quantity	Additional Supplement
Cheetah	chunked beef meat	♂:3 kg; ♀:2 kg	vitamin and mineral premix; pieces of chicken (± 500 g); raw egg; ice cube
Puma	chunked beef meat	♂:4 kg; ♀:2 kg	pieces of chicken (± 500 g); ice cube
Clouded leopard	chunked beef meat	♂:3 kg; ♀:2 kg	pieces of chicken (± 500 g); ice cube
Javan leopard	chunked beef meat	♂:3 kg	pieces of chicken (± 500 g); raw egg; ice cube
Snow leopard	chunked beef meat	♂:3 kg; ♀:2 kg	pieces of chicken (± 500 g); ice cube
Lion	chunked beef meat	♂:5–6 kg; ♀:3 kg	vitamin and mineral premix; pieces of chicken (± 500 g); raw egg; ice cube
White tiger	chunked beef meat	♂:5–6 kg; ♀:4 kg	vitamin and mineral premix; pieces of chicken (± 500 g); ice cube
Siberian tiger	chunked beef meat	♂:5–6 kg	None

Table 3 Nutritional composition of beef meat and whole chicken in felid diets, extracted from literature

Item	Beef meat [25]	Whole chicken [26]
DM, %	29.0	24.2
Organic matter (% DM)	93.1	91.1
Crude protein (% DM)	64.5	71.4
Acid-hydrolyzed fat (% DM)	22.2	20
Gross energy, (kcal/g DM)	5.9	5.9

Chemical analysis

The fecal samples were analyzed for dry matter (DM) using lyophilization (CoolSafe™, SCANVAC, Denmark). Briefly, fresh fecal samples were weighed before being frozen at −20 °C and then freeze-dried at −55 °C under a vacuum of 0.1 mbar for 48 h. After lyophilization, the samples were reweighed to determine the DM content. The fecal volatile fatty acids (VFA) analysis, including acetate, propionate, butyrate, isobutyrate, isovalerate and valerate, was performed according to the description of

Gadeyne et al. (2016) [27]. Ten ml of 10% formic acid, containing the internal standard (1 mg 2-ethyl butanoic acid), was added to 2 g fecal sample. After 15 min centrifugation (22,000 g at 4 °C), the supernatant was filtered and an aliquot transferred into a 1.5 ml glass vial. The VFA analysis was conducted using gas chromatography (HP 7890 A, Agilent Technologies, Belgium) equipped with a flame ionization detector and a Supelco Nukol capillary column (30 m× 0.25 mm ×0.25 µm, Sigma-Aldrich, Belgium). The SCFA and branched-chain fatty acids (BCFA) were identified and quantified based on the retention times and peak areas relative to the internal standard.

For the analysis of volatile organic compounds phenol, p-cresol, and indole, the method described by Vossen et al. [28] was used. The method was adapted for the analysis of volatile organic compounds phenol, p-cresol, and indole. The adaptation included 0.5 g of fecal sample was used; there was no internal standard added and the solid phase micro-extraction fiber was exposed to each sample for 40 min at 38.5 °C. Compounds were identified by comparing chromatograms with the National Institute of Standard and Technology Mass Spectral Library (version 2.0, 2005) and by matching retention times with external standards (phenol, x-cresol, indole). Data was obtained by expressing the relative area of specific quantifications ions for phenol, p-cresol and indole (respectively m/z 94, 107 and 117). The detection limit was set at 1×10^4. We report x-cresol as p-cresol.

Microbiota analysis

DNA extraction was conducted with the Qiagen DNeasy® PowerLyzer® PowerSoil® Kit (ref 12,855–100), and the concentration was measured using the Nanodrop ND-1000 spectrophotometer (Nanodrop Technologie). The extracted DNA samples were stored at −80 °C until further analyses. Negative controls were included during DNA extraction and PCR to monitor potential contamination. Blank samples were processed alongside study samples under identical conditions, including dilution where applicable. No normalization of PCR products was performed, meaning that blanks typically did not generate usable reads. Quality control was assessed via gel electrophoresis, and blanks without amplification were not sequenced. Ten µl genomic DNA extract was sent to LGC genomics GmbH (Germany), where the 16S rRNA gene V3-V4 hypervariable region was amplified following the protocol described by Van Landuyt et al. (2020). The PCR mix consisted 1 µl of 10 × diluted DNA extract, 15 pmol of both the forward primer 341 F 5'- NNNNNNNNNTCC TACGGGNGGCWGCAG and reverse primer 785R 5'- NNNNNNNNNNTGACTACHVGGGTATCTAAKCC [29], in a 20 µl volume of MyTaq buffer containing 1.5 units MyTaq DNA polymerase (Bioline) and 2 µl of Bio-Stab II PCR Enhancer (Sigma-Aldrich, USA). Each sample was tagged with a unique 10-nt barcode sequence on both the forward and reverse primers. PCRs were carried out for 30 cycles under the following conditions: 2 min at 96 °C for pre-denaturation; followed by 96 °C for 15 s, 50 °C for 30 s, and 70 °C for 90 s.

DNA bands of amplicons of interest were determined by gel electrophoresis. Approximately 20 ng of amplicon DNA concentration from each sample was pooled with up to 18 samples carrying different barcodes. The amplicon pools were purified with one volume of AMPure XP beads (Agencourt) to remove primer dimer and other small mispriming products, followed by an additional purification step using MinElute columns (Qiagen). Finally, approximate 100 ng of each purified amplicon pool DNA was used to construct Illumina libraries through adaptor ligation using the Ovation Rapid DR Multiplex System 1–96 (NuGEN). The Illumina libraries were pooled and size selected using preparative gel electrophoresis, and sequencing was performed on an Illumina MiSeq using v3 Chemistry (Illumina, USA) with a read length of 2 × 300 bp.

Bioinformatics data processing

The amplicon sequence data were processed using the DADA2 R package following the pipeline tutorial [30]. In the quality control step, primer sequences were removed, and reads were truncated based on quality scores (truncQ = 2). Additional filtering was performed to remove reads containing ambiguous bases or those exceeding the maximum expected error threshold (maxEE = 2.2). After dereplication, unique sequences were denoised using the DADA with the selfConsist sample inference method (pooling = TRUE). The error rates were estimated and visually inspected before proceeding with paired-end read merging, ensuring a minimum overlap of 20 bp and no mismatches (maxMismatch = 0). Chimeric sequences were removed using the consensus method, and the final amplicon sequence variant (ASV) table was generated. Taxonomy was assigned using the Naïve Bayesian Classifier and the DADA2-formatted Silva v138 database [31]. To exclude non-microbial sequences, ASVs classified as chloroplasts or mitochondria were removed prior to downstream analyses. Additionally, singletons (ASVs with a total abundance of 1 across all samples) were filtered out to reduce potential sequencing artifacts.

Statistical analysis

Data analysis and graphical visualization were performed using GraphPad Prism 10.1.2. Model residuals were assessed using residual versus fitted plots and normal Q-Q plots, revealing that they did not conform

to a normal distribution. Consequently, Mann–Whitney tests were employed to evaluate statistically significant differences in SCFA and BCFA concentrations, as well as volatile organic compounds, between body mass groups (small vs. large). Separate Mann–Whitney tests were also conducted to examine microbiota variation by host species. A significance level of $p < 0.05$ was applied. Microbial taxonomic composition and differential abundance analyses were conducted in RStudio v4.1.2 [32], using packages ggplot2 3.3.5 [33] for graph visualizations. Alpha diversity indices (Richness, Shannon, Invsimpson) were calculated on ASV after normalization by scaling with ranked subsampling (SRS) [34] at an average of 32,103 sequences per sample. Shannon index and Invsimpson were used to assess community diversity and evenness, where the Invsimpson is calculated as the reciprocal of the Simpson index (1/D), reflecting the number of equally abundant species in a community. Beta diversity was evaluated by Bray–Curtis dissimilarities and visualized using principal coordinate analysis (PCoA) plots. Permutational multivariate analysis of variance (PERMANOVA) was used to identify difference between the two groups were assessed using, with significance determined at $p < 0.05$. The data analyses were performed using SPSS 29.0 (IBM SPSS Inc., USA), and the Mann–Whitney test was used to assess the proportional taxon abundance and alpha diversity between the two body mass groups. Benjamini–Hochberg standard false discovery rate (FDR-BH) correction was used for multiple testing, with a significance threshold of $p_{adj} < 0.05$.

Advanced correlation clustering heatmap analysis was conducted in RStudio v3.5.1, using package pheatmap 1.0.12 [35]. Spearman correlations were calculated between the relative abundance at the genus level and fermentation products and fecal DM. A correlation greater than 0.5 is considered a highly positive correlation, while a correlation below −0.5 is considered a highly negative correlation, retaining only strong ($|r| > 0.5$) and statistically significant ($p < 0.05$) associations.

Results

Microbial composition and relative abundance

A total of 577,856 16S rRNA gene-based amplicon sequences were obtained, with an average of 32,103 reads (range = 8,859–62,106) per fecal sample. The relative abundance of microbiota at the phylum and genus level among individuals is shown in Fig. 1. At the phylum level, the relative abundance average of the intestinal microbiota of zoo felids was dominated by Firmicutes (61.7% ± 18.4), Actinobacteria (16.4% ± 13.4) and Fusobacteria (12.5% ± 18.1). In addition, Proteobacteria (4.7% ± 5.0) and Bacteroidetes (4.5% ± 8.9) also contributed to the overall composition. At the genus level, 10 genera had an abundance greater than 3% in all sequences. The most abundant genus was *Clostridium* sensu stricto *1* (15.9% ± 16.6), followed by *Collinsella* (15.7% ± 12.9), *Fusobacterium* (12.5% ± 17.6), *Peptoclostridium* (10.7% ± 11.7), and *Blautia* (6.0% ± 7.3). However, considerable individual differences were observed among individuals. Supplementary Fig. 1 shows the relative abundance of bacteria at the family level. To assess the impact of host species, the relative abundance of dominant taxa was compared between felids species. No significant differences were found at either the phylum or genus level (Supplementary Fig. 2).

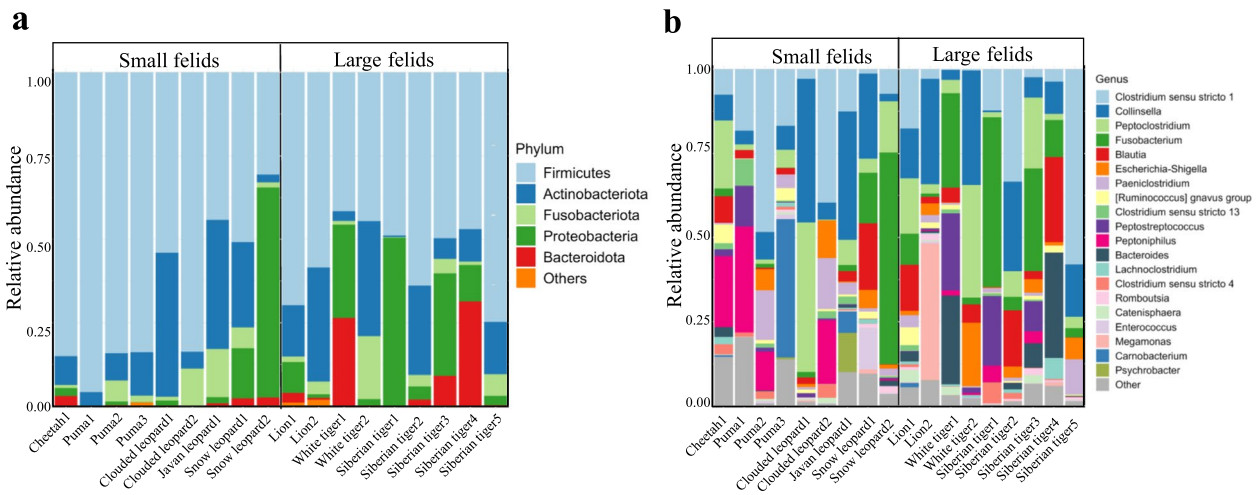

Fig. 1 Microbial composition and relative abundance among individuals: **a**. The top 5 most abundance phyla were selected, and the remaining ones were classified as "Other"; **b**. The top 20 most abundance genus were selected, and the remaining ones were classified as "Other"

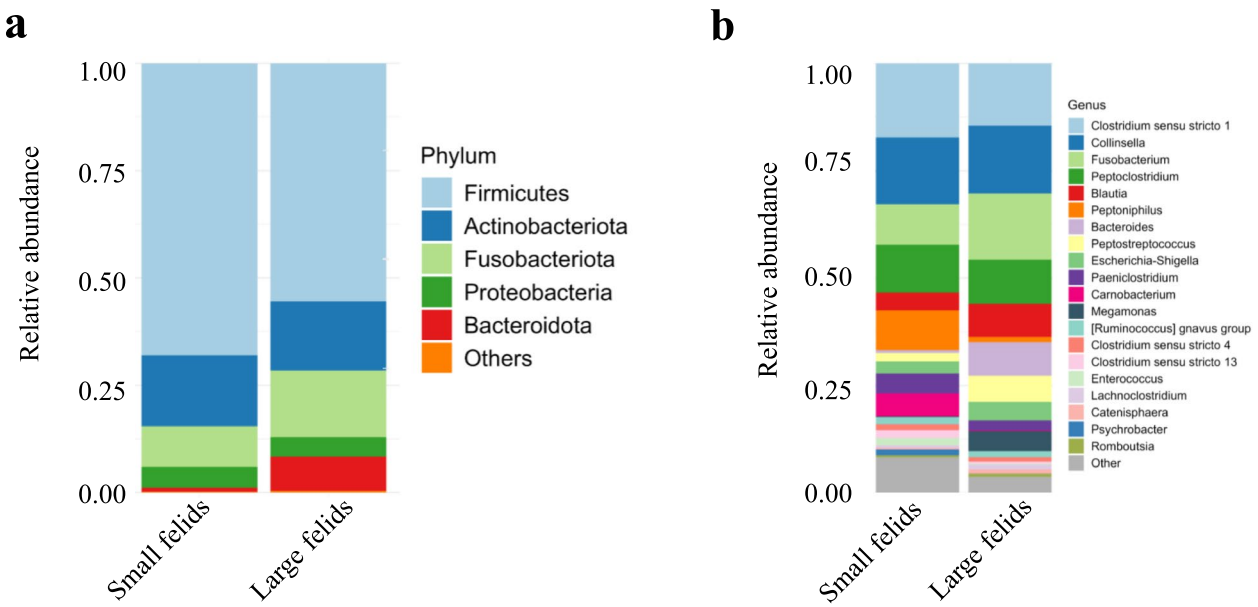

Fig. 2 Impact of body mass on relative abundance of bacteria at the phylum and genus level in different groups. **a** The top 5 most abundance phyla were selected, and the remaining ones were classified as "Other"; **b** The top 20 most abundance genus were selected, and the remaining ones were classified as "Other"

Impact of body mass on microbial parameters

Figure 2 shows the differences in fecal microbiota between the two body mass groups. At the phylum level (Fig. 2a), regardless of small or large felids, the top three bacterial communities were Firmicutes, Actinobacteria, and Fusobacteria. Figure 2b presents the relative abundance at the genus level. In small felids, *Clostridium* sensu stricto *1* was the most dominant genus, accounting for 17.2% of the microbiota, followed by *Collinsella* at 15.6% and *Peptoclostridium* at 11.1%. In large felids, the dominant genus was *Collinsella*, constituting 15.7% of the microbiota, followed by *Fusobacterium* at 15.5% and *Clostridium* sensu stricto *1* at 14.5%. The relative abundance of bacteria at the family level is shown in Supplementary Fig. 3. There was no significant difference in the relative abundance of fecal microbiota between the two groups at the phylum, family and genus level. Alpha diversity indices (Richness, Shannon, Invsimpson) are shown in Fig. 3. After FDR-BH correction, alpha diversity did not differ significantly between body mass groups. Small felids tended to have lower Observed ASV but higher Shannon and Invsimpson indices. Notably, two small felids had the highest Observed ASV values, indicating high variation within groups. Beta diversity, as measured by Bray–Curtis dissimilarities showed a separation between the two body mass groups (PERMANOVA,

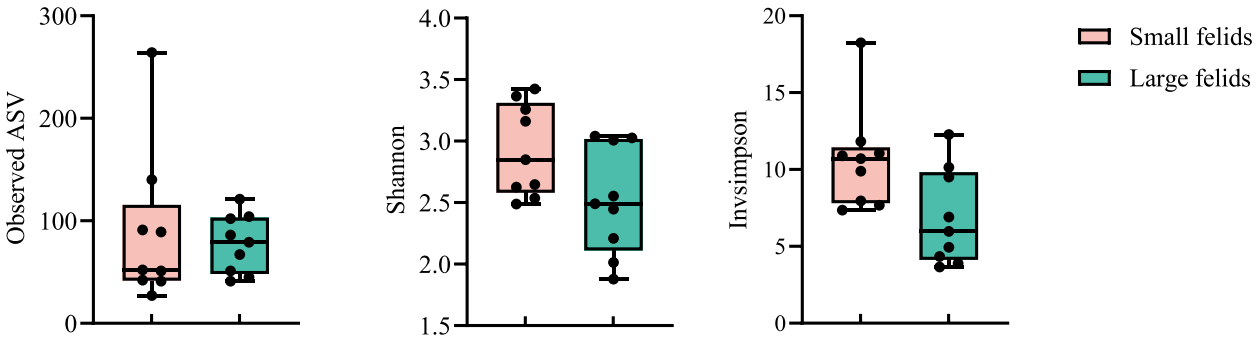

Fig. 3 Alpha diversity measures. Boxplot of microbial richness (Observed ASV), Shannon and Invsimpson of fecal samples from felids in two body mass groups. The boxes denote interquartile ranges (IQR) with the median as a black line and whiskers extending up to the most extreme points within 1·fivefold IQR

$R^2 = 0.139$, $p_{adj} < 0.001$). The fecal microbiota of small felids were more tightly clustered compared to those of large felids (Fig. 4).

Impact of body mass on fecal microbial products production

Figure 5 shows the comparison of the median SCFA, BCFA and volatile organic compounds content between two groups of felids with different body mass. Total SCFA and BCFA was not affected by body mass (Fig. 5a and 5c). Acetate proportion in small felids was significantly higher than in large felids (Mann–Whitney, U = 10, $p = 0.0056$, Fig. 5b). Valerate proportion showed a significant difference between body mass groups (Mann–Whitney, U = 15, $p = 0.0238$, Fig. 5d). However, when analyzed in absolute concentrations, no significant effect of body mass on and BCFA content was observed. Volatile organic compounds vary widely between individuals and were not significantly different between different body mass groups (Fig. 5e). Fecal DM, SCFA, BCFA and volatile organic compounds of different individuals are shown in Supplementary Table 1.

The relationship between fecal microbiota and physiological markers

Figure 6 shows a Clustered Image Map, highlighting the relationship between fecal microbiota at the major genus level and measured physiological parameters. The study identified several significant correlations between fermentation products, fecal DM, and specific bacteria. Acetate exhibited a significant positive correlation with *Clostridium* sensu stricto *13* ($r = 0.6$, $p = 0.0062$) and *Peptoniphilus* ($r = 0.5$, $p = 0.0431$). Fecal DM demonstrated a positive correlation with *Psychrobacter* ($r = 0.6$, $p = 0.0113$). P-cresol was positively correlated with *Catenisphaera* ($r = 0.5$, $p = 0.0412$). Additionally, total BCFA was positively correlated with *Bacteroides* ($r = 0.5$, $p = 0.0545$). Conversely, propionate showed a significant negative correlation with *Clostridium* sensu stricto *13* ($r = -0.5$, $p = 0.0375$), while butyrate was negatively

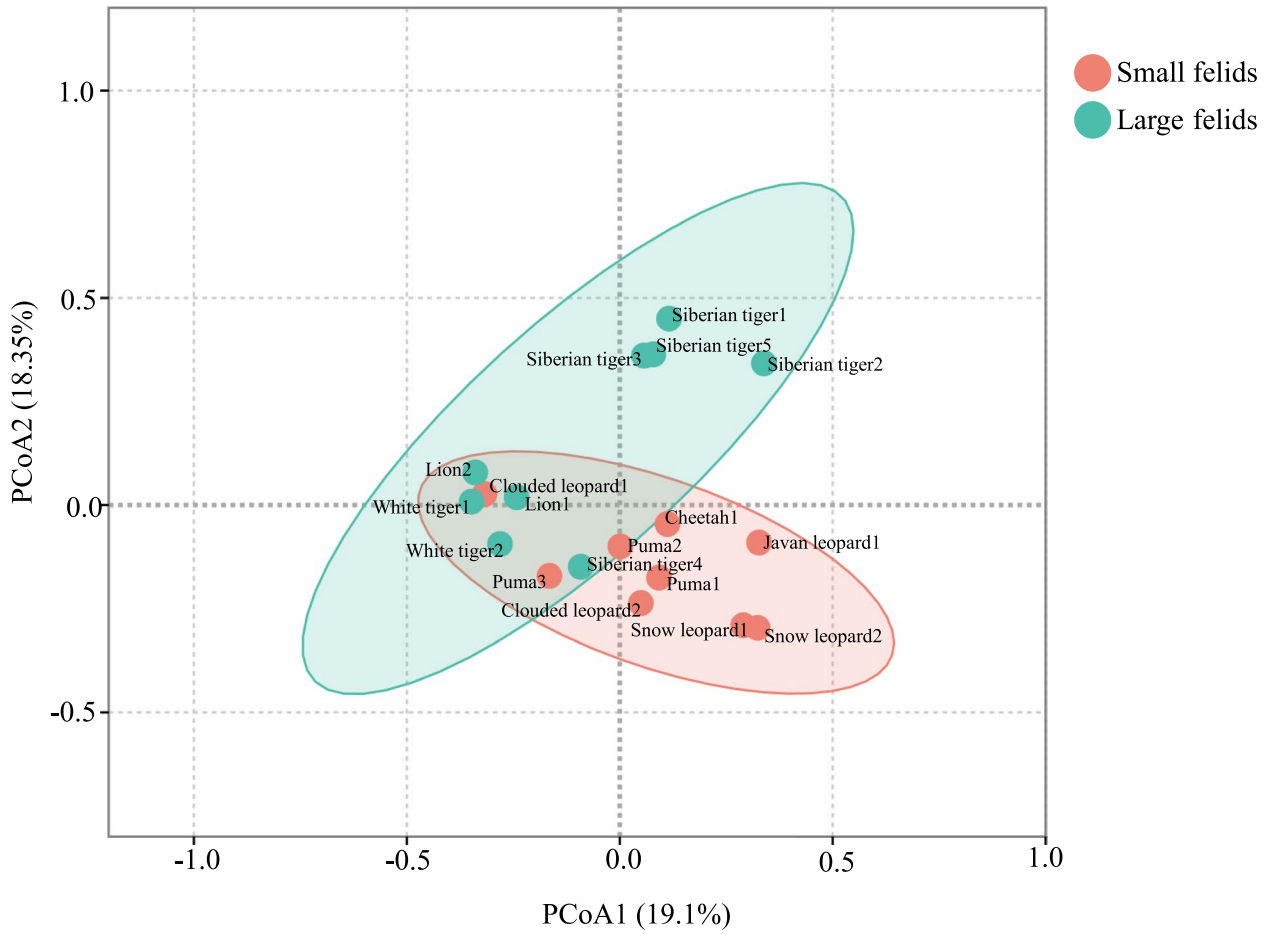

Fig. 4 Principal coordinate analysis (PCoA) plot based on Bray–Curtis dissimilarities of microbial community structure in fecal samples indifferent groups. There are differences in the gut microbiota between the two body mass groups (PERMANOVA, R2 = 0.139, $p_{adj} < 0.001$)

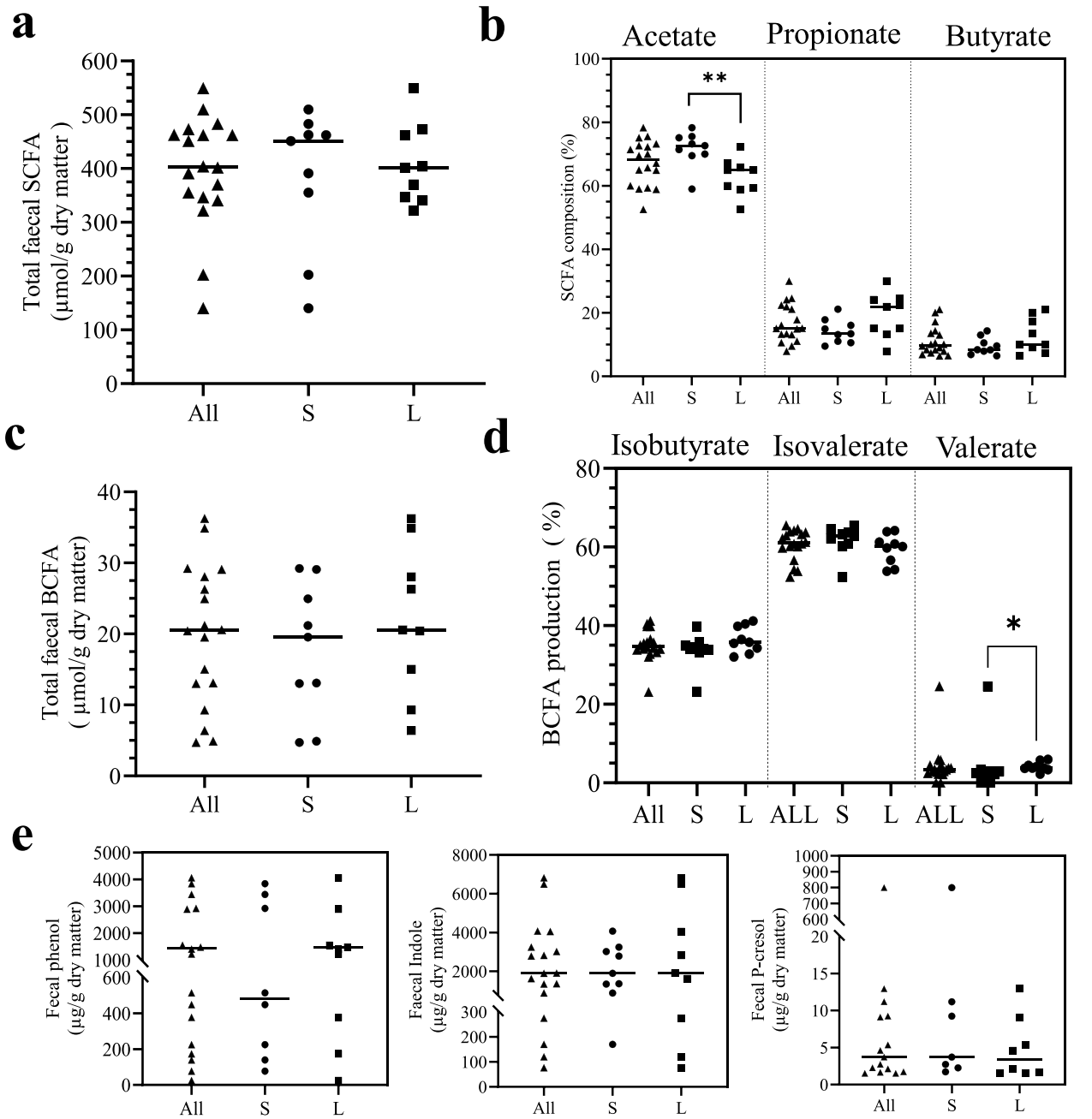

Fig. 5 Impact of body mass on fecal microbial products production (the median as a solid line). a. b. Influence of felid body mass on total fecal major short-chain fatty acids (SCFA, i.e. acetate, propionate and butyrate).c. d. Influence of felid body mass on major branched-chain fatty acids production (BCFA, i.e., isobutyrate, isovalerate and valerate). e. Influence of felid body mass on other microbial metabolites for phenol, indole and p-cresol. Differences between groups with different letters are statistically significant as assessed by Mann–Whitney tests (a double asterisk means that $p < 0.01$)

correlated with *Peptostreptococcus* ($r = -0.5$, $p = 0.0255$). No significant correlations were observed between the remaining fecal microbiota and fermentation. Supplementary Fig. 4 depicts the association between fecal bacteria levels at family level and physiological markers of intestinal function in zoo felids. Indole showed a significant negative correlation with *Streptococcaceae* ($r = -0.6$, $p = 0.0067$).

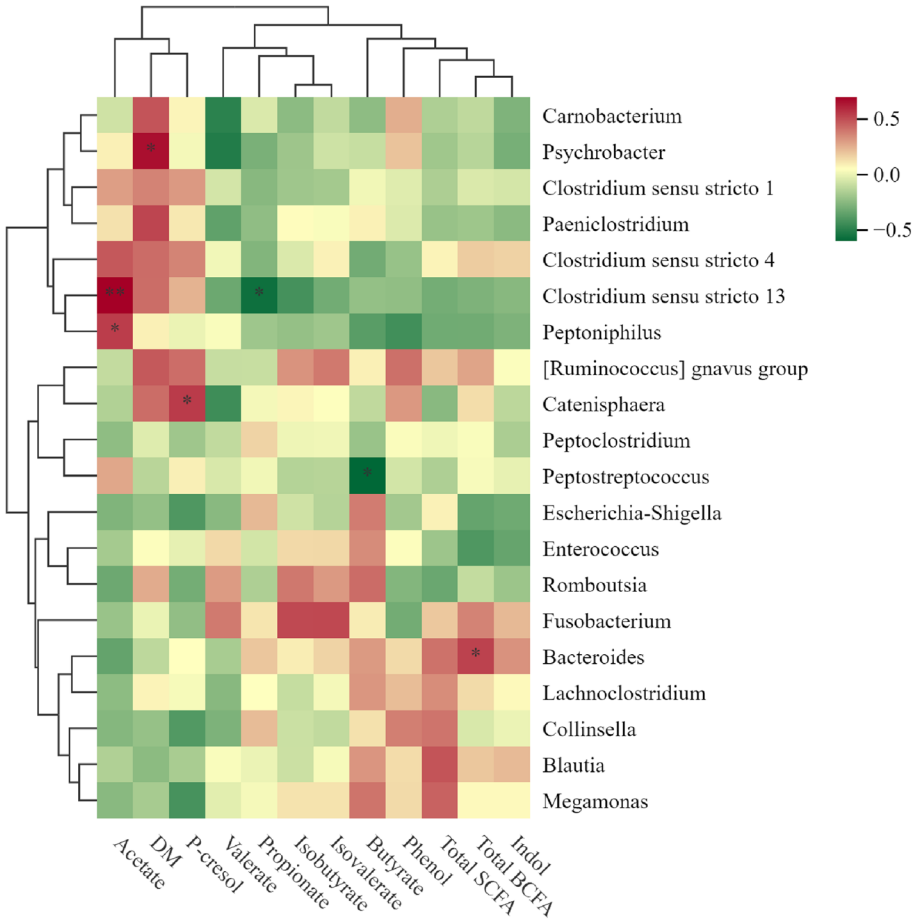

Fig. 6 Correlation heatmap depicts the association between the relative abundance at the genus level and physiological markers of intestinal function in zoo felids. Colors represent correlation strength, with red indicating positive correlations and green indicating negative correlations. The values range from −0.5 to 0.5. An asterisk means that $p < 0.05$; a double asterisk means that $p < 0.01$

Discussion

Our study explored the potential influence of species-specific body mass differences on gut microbiota and fermentation products in felids. While interindividual variation in microbial composition and fermentation patterns were observed, significant differences between body mass groups were most evident in beta diversity and acetate proportion. Geographic location and diet are often key confounding factors that alter gut microbiota [36]. In this study, the influence of these factors was minimized since all felids lived in the same zoo and were provided with similar diets. This allowed us to investigate whether the fecal microbiota composition of felids varied based on species, body mass, or other conditions.

The fecal microbiota of 18 felids at Pairi Daiza Zoo in Belgium was dominated at the phylum level by Firmicutes, Actinobacteria, and Fusobacteria. Similarly, leopards, lions, and tigers fed the same diet in Indian national parks also showed a dominance of Fusobacteria and Firmicutes in their fecal microbiota [16]. High abundances of *Clostridium* sensu stricto *1* and *Collinsella* in both groups were observed for fecal microbiota of felids of different body mass group. Several studies have shown that a natural diet based on whole prey promotes the growth of *Clostridium* in species such as cheetahs, wolves, domestic dogs, and cats [20, 21, 37]. *Collinsella*, in particular, has been reported to influence metabolism by altering intestinal cholesterol absorption, reducing hepatic glycogen production, and increasing triglyceride synthesis [38]. In humans, red meat diets lacking fiber are known to increase the relative abundance of *Collinsella* [39]. It has also been identified as a microbial biomarker for obesity [40]. Although no differences between body mass groups were identified, microbial signatures for high protein consumption were observed, including high abundance of protein-fermenting bacteria such as *Clostridium* and *Fusobacterium*. Conventional wisdom often regards genera like *Lactobacillus* and *Bifidobacterium* as beneficial microorganisms in various mammalian omnivores, including humans, mice, and pigs [41].

However, carnivores rely more on SCFA metabolic pathways driven by protein-associated microbiota rather than carbohydrate-fermenting bacteria [42]. Existing research is primarily based on humans and model animals, which may influence our interpretation of carnivore microbiomes. Given the unique dietary characteristics of carnivores, the criteria for evaluating their gut microbiome health may need reconsideration.

When dietary categories are not considered, microbial diversity generally increases with host body size [43]. This idea stems from the concept that environmental heterogeneity [44], in this case the larger gut, provides more space and diverse conditions for different types of microbes to thrive. However, studies that distinguish herbivores and carnivores by diet found that the positive correlation between body mass and microbial diversity mainly applies to herbivores, while larger carnivores often exhibit a similar or even lower OTU counts compared to smaller ones [45]. Deschamps et al. (2022) noted that fecal microbiota of medium-sized dogs (Shannon index: 4.8) seemed to have higher median diversity compared to small dogs (3.5) and large dogs (2.9). In our study, the fecal microbiota of small felids was more tightly clustered and had a higher proportion of acetate than that of large felids. A hypothesis is that, for felids, body mass's relationship with gut microbiota and fermentation is more influenced by shared ecological factors than by weight per se. De Cuyper et al. (2019) proposed a functional classification of carnivores based on gut capacity and prey size rather than body mass. Small-prey predators tend to consume prey whole, while large-prey predators selectively eat more digestible parts, such as muscles and organs. This difference in feeding strategy affects the intake of indigestible animal fibers like hair, bones, and skin [5], meaning large-prey predators likely ingest less fiber compared to those consuming smaller prey. This contrasts with herbivores, where fiber intake generally increases with body mass as plant fibers are slowly and thoroughly digested by microbes [46]. In natural environments, small-prey predators may exhibit higher gut microbial diversity due to their prey variety and fiber intake. In this study, although felids were categorized into two body mass groups, they were all naturally large-prey predators [1] and were fed the same raw meat diet, which lacked variation in fiber intake. This could help explain why no significant differences in microbial diversity were observed. Future research could investigate whether small-prey predators (which typically consume whole prey in the wild) exhibit higher gut microbiota diversity when fed the same raw meat diet in zoo compared with large-prey predators. Additionally, consuming whole prey may also introduce the prey's microbiome and metabolites, which may influence the carnivore gut microbiome. However, further research is needed because such an effect has only been demonstrated in invertebrate carnivores, e.g. spiders and ladybirds [47, 48]. Further research is needed to confirm this effect on carnivorous species. Considering these factors, it is worth exploring whether felids of different body mass should be provided with diet formulations that better reflect their natural feeding strategies under human care.

In this study, total SCFA, BCFA, and volatile organic compounds in felids were lower compared to other zoo felids fed a beef-based diet [49]. Although most metabolites were not affected by body mass, acetate proportions were significantly higher in small felids. Acetate, propionate, and butyrate are typically present in the colon and feces at an approximate molar ratio of 60:20:20 [50]. In contrast, BCFAs, as byproducts of protein fermentation, generally occur at lower absolute concentrations and may not follow the same distribution patterns as SCFAs. Measuring the health status of carnivores remains a challenge. Fermentation of protein sources is often considered detrimental to intestinal health because many of its by-products, such as ammonia, indoles, and phenolic compounds, are toxic and associated with intestinal disease [51]. Puma No. 3's feces in this study had a very high p-cresol content, and its phenol and indole contents were also at high levels (Supplementary Table 1). Unfortunately, this puma died of renal failure three months after sampling, with pathologies such as glomerulopathy, chronic interstitial kidney disease, fibrosis, and proteinuria. In addition, high concentrations of indole were also detected in the feces of Siberian tiger No. 4, and the protein concentration in its urine was higher than that of other tigers. Although no abnormalities were found on ultrasound, these findings suggest possible impairment of renal function, which could explain why its beta diversity did not cluster with other Siberian tigers even though they lived together. A common primary renal disease in captive cheetahs is glomerulosclerosis, which is rarely found in cheetahs in the natural environment [52–54]. Since the mid-1970 s, sporadic cases of oxalate nephropathy under human care have been reported in pumas, jaguars, leopards, and most commonly cheetahs [55]. In zoos, muscle meat is often the main animal-derived dietary component due to logistical and financial constraints [56]. Meat proteins typically contain high crude protein and are considered highly enzymatically digestible [57]. We still see health issues (i.e. kidney disease) in zoo felids, possibly due to an excess or imbalance of digestible protein [58]. Increased dietary protein results in greater amounts of proteins, peptides, and amino acids reaching the colon for microbial metabolism [59]. Protein fermentation produces potentially toxic metabolites, such as ammonia, phenols, and indoles [41], which may lead to decreased kidney function and an increased risk of

cardiovascular disease [60]. Because animal fiber primarily comes from components like connective tissue (e.g., collagen), bones, hair, or feathers, traditional nutritional analyses, which focus mainly on digestible macronutrients, may overlook the role these indigestible substances play in carnivore diets. Studies in cheetahs have shown that feces have lower concentrations of propionate and butyrate, as well as significantly lower concentrations of serum indoxyl sulphate when fed whole prey compared to supplemented meat [5]. One hypothesis is that the indigestible animal tissue present in whole prey acts as a physical barrier between bacteria and fermentation substrates in the colon, thereby modulating microbial fermentation. Research on domestic cats showed that that whole prey consumption leads to slower digestion, prolonged metabolism, and more variable gastric emptying compared to processed meat diets [21]. Therefore, another theoretical possibility is that animal fiber decreases passage rate, meaning that per time unit, less (and more gradual) highly fermentable matter is presented to the microbiota.

In a study of dogs, *Clostridiaceae* appeared to play a central role in the relationship between the microbiome, macronutrient composition, digestibility, fecal health score, and fecal weight [61]. While certain members of *Clostridium* are considered potential pathogens, the study found that *Clostridiaceae* was positively correlated with higher fecal health scores (i.e., firmer feces) and negatively correlated with fecal output (i.e., reduced feces volume). This suggests that an increase in *Clostridiaceae*, under a meat-based diet, may not necessarily harm the health of dogs and could promote an ideal fecal condition. Canids and felids have different dietary requirements and gut ecology, so we performed similar analyses on felids. There was a significant positive correlation between *Clostridium* sensu stricto and acetate in the feces of zoo felids fed a raw meat diet. Certain *Clostridium* sensu stricto species have been reported to produce acetate in diverse environments, such as plant roots [62], although their functional roles in mammalian guts may differ. Acetate provides energy by participating in the tricarboxylic acid cycle [63, 64]. Earlier we mentioned that small felids had more acetate. One possible explanation is that smaller animals generally have higher energy demands per unit of body weight [65], and an increased availability of acetate could serve as an adaptive strategy to support energy metabolism. Differences in gut transit time or available surface area for fermentation may also influence acetate production. Although acetate has received less attention compared to propionate and butyrate, recent research has highlighted acetate's critical role in maintaining health, particularly in the gastrointestinal tract. For instance, in humans acetate primarily acts through the GPR43 receptor, and in many cases, exerts anti-inflammatory effects by inhibiting NF-κβ activation and HDAC activity, thereby reducing pro-inflammatory cytokine production in various cell types [66]. Our results suggest a potential link between acetate production and gut microbiota in felids, however, we emphasize that correlation does not imply causation. Integrated metagenomic and functional analyses are needed to confirm the metabolic pathways and physiological impacts of microbial fermentation in felids.

Because of its opportunistic nature, this study has some limitations. First, body mass estimates were based on wild felids. Although some zoo-housed individuals may have a higher weight, the distinction between the two body mass groups remained substantial, supporting the validity of our classification. Second, while host species may influence gut microbiota composition, no significant effects were detected, possibly due to the limited sample size. Third, diet remains a potential confounding factor. Siberian tigers were the only individuals not supplemented with additional chicken and four of them showed a single cluster of beta diversity. Lastly, dietary intake varies with body mass and sex, with males typically consuming more than females even within the same species. This factor was not accounted for in our statistical analysis. Future research could explore whether these sex-based differences in dietary intake influence gut microbiota composition. Additionally, incorporating phylogenetic approaches, such as phylosymbiosis analysis, could provide further insights into microbiome differentiation among felid species.

Whether carnivorous mammals, especially felids of different body mass, can have the same diet under human care is a complex question. While body mass can be used as a proxy for estimating feeding volume and frequency, the coevolution of digestive strategies also needs to be considered. In studies of vultures, a strictly carnivorous bird, different digestive strategies were discovered between two sympatric species [67]. This suggests that differential digestive adaptations exist even within taxonomically closely related carnivores. This emphasizes the importance of tailoring diets to the specific digestive capabilities and nutritional needs of different species.

Conclusion

This study represents a comparison of gut microbiota across multiple felid species under similar dietary and housing conditions. Body mass of felids is potentially associated with gut microbiota composition and fermentation products. However, the ecological niche associated with body mass should also be considered. While not yet fully demonstrated, *Clostridium* may play a key role in maintaining gut health in carnivores on a raw meat diet. Our results urge for more attention to fit diets under human care to the specific needs within felids and other carnivorous mammals.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03981-x.

Supplementary Material 1

Supplementary Material 2

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Authors' contributions
Conceptualization, M.S., A.D.C., X.J., and G.P.J.J.; Methodology, M.S., A.D.C., and G.P.J.J.; Investigation, M.S. and A.Q.; Writing – Original Draft, M.S.; Writing – Review & Editing, M.S., A.D.C., X.J., and G.P.J.J.; Resources, M.S., A.D.C., A.Q., and G.P.J.J.; Supervision, A.D.C. and G.P.J.J.

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Data availability
The datasets generated and/or analyzed during the current study are available in the NCBI Sequence Read Archive (SRA), Accession Number: PRJNA1188007.

Declarations

Ethics approval and consent to participate
Anesthesia of the animals for this study was part of routine health monitoring at the zoo and did not cause additional discomfort or risk to the animals. No additional ethical approval required. All animals were cared for in strict compliance with relevant animal welfare regulations, including the European Convention for the Protection of Animals Used for Scientific Purposes (Directive 2010/63/EU), the regulations and policies of Pairi Daiza, and the Royal Decree on the protection of experimental animals—29 th May 2013 (Copy of this document is available from bienetreanimal.wallonie.be/or from Pairi Daiza).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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