Longitudinal Full-Length 16S rRNA Gene Profiling of the Canine Gut Microbiome: Impact of Age, Diet, Birth Mode, and Reproductive Stage

Ph.D. Thesis Booklet

Md Asaduzzaman

Supervisors

Professor Zsolt Boldogkői, Ph.D., D.Sc. and
Dóra Tombácz, Ph.D., D.Sc.

Doctoral School of Multidisciplinary Medical Sciences
Department of Medical Biology
Albert Szent-Györgyi Medical School
University of Szeged

Szeged
- 2026 -

LIST OF PUBLICATIONS

Scientific Paper Included in the Thesis

1. **Asaduzzaman Md**, Oláh Péter, Yaseen Natheer Jameel, Taifi Ahmed, Járay Tamás, Gulyás Gábor, Boldogkői Zsolt, Tombácz Dóra✉

   Longitudinal long-read microbiome profiling in a canine model reveals how age, diet, and birth mode shape gut community dynamics

   MSYSTEMS 11 : 2 Paper: e01279-25 , 17 p. (2026)

   Folyóirat szakterülete: Scopus - Ecology, Evolution, Behavior and Systematics SJR indikátor: D1
   Folyóirat szakterülete: Scopus - Modeling and Simulation SJR indikátor: D1
   IF: 4.6

Publications not Related to the Thesis

1. Ali, N.✉, Sumon, A. H., Fariha, K. A., Asaduzzaman, M., Kathak, R. R., Molla, N. H., Mou, A. D., Barman, Z., Hasan, M., Miah, R., & Islam, F. (2021). Assessment of the relationship of serum liver enzymes activity with general and abdominal obesity in an urban Bangladeshi population. ***Scientific reports***, 11(1), 6640. https://doi.org/10.1038/s41598-021-86216-z

2. Ali, N.✉, Rahman, S., Islam, S., Haque, T., Molla, N. H., Sumon, A. H., Kathak, R. R., Asaduzzaman, M., Islam, F., Mohanto, N. C., Hasnat, M. A., Nurunnabi, S. M., & Ahmed, S.✉ (2019). The relationship between serum uric acid and lipid profile in Bangladeshi adults. ***BMC cardiovascular disorders***, 19(1), 42. https://doi.org/10.1186/s12872-019-1026-2

Record of Scientific Conferences

Since the beginning of my PhD studies in September 2021, I have contributed to a total of 12 conference posters. I served as the first-presenting author on 7 posters and as a contributing author on an additional 5 posters presented at international and national scientific conferences and workshops. All conference records are documented in my ORCID profile https://orcid.org/0000-0003-0434-8517.

ABBREVIATIONS

gDNA: Genomic DNA

DIN: DNA Integrity Number

ONT: Oxford Nanopore Technologies

BCS: Bükki Cserfes

DMD: Duna-menti Dumás

SRT: Serteperti

PPA: Pattogó Parázs

RRT: Rezerta-Réti

LPL: Le Petit Lapin

BARF: Biologically Appropriate Raw Food

HPLC: High-Protein-Low-Carbohydrate

HPMC: High-Protein-Moderate-Carbohydrate

MPHC: Moderate-Protein-High-Carbohydrate

FEDIAF: European Pet Food Industry Federation

ENA: European Nucleotide Archive

NMDS: Non-metric Multidimensional Scaling

CONs: Co-Occurrence Networks

SD: Standard Deviation

bp: Base Pair

1. INTRODUCTION

The gastrointestinal (GI) tract comprises a coordinated system of organs that enables digestion and nutrient uptake while maintaining constant interactions with resident microbial communities. Extending from the mouth to the anus, it comprises multiple anatomically and functionally specialized compartments that collectively process dietary inputs and eliminate waste. In microbiome research, the term “gut” is commonly used to refer to the lower gastrointestinal tract, particularly the intestinal segments that harbor dense and diverse microbial communities comprising trillions of microorganisms, which play important roles in host health and disease[1].

The term *gut microbiota* describes the collection of microorganisms residing in the gastrointestinal tract, including bacteria, archaea, fungi, and protists, whereas the *gut microbiome* additionally incorporates associated viruses, phages, plasmids, and other mobile genetic elements[2,3]. Furthermore, the collective genomes and genes of all members of microbiome are defined as the metagenome[2]. The gut microbiome has been proposed as

a functional ‘new organ’ essential for host survival[4], exerting its effects through the production of diverse metabolites that influence intestinal health and multiple extra-intestinal organs[5]. In dogs, the gut microbiome has also been associated with key functional traits, influencing olfactory performance as well as behavioral and working outcomes, including motivation, aggression, and sociability[6,7].

A well-functioning gut microbiome consists of a stable yet adaptable microbial community that interacts closely with the host, and the maintenance of this equilibrium is strongly linked to both health and disease. The healthy dog gut microbiome is dominated by major bacterial phyla, including *Firmicutes*, *Bacteroidetes*, *Fusobacteria, Proteobacteria, and Actinobacteria*[8,9]. Dysbiosis describes a state in which the normal equilibrium of the gut microbial community is disturbed, involving shifts in microbial composition, diversity, functional potential, and metabolite profiles; in canine, such imbalances have previously been quantitatively assessed using the canine microbiota dysbiosis index (CMDI)[10]. In dogs, dysbiotic

alterations have been associated with diarrheal and irritable bowel–type disorders, obesity, congestive heart failure, chronic kidney disease, gastric dilatation–volvulus, behavioral disorders, infectious diseases, and Crohn's disease[11–16,9,17].

Because of these broad physiological roles, the gut microbiome has become an important subject of investigation in both human and animal models. Domestic dogs (*Canis lupus familiaris*) represent a valuable comparative and translational model in microbiome research, as they share key physiological traits, dietary responses, and environmental exposures with humans[18]. Furthermore, comparative analyses have shown that the canine gut microbiome more closely resembles the human microbiome than those of commonly used laboratory models, such as pigs or mice, particularly with respect to gene content and responses to diet[19]. In our study, we examined fecal samples from the Hungarian Pumi breed.

The canine gut microbiome is influenced by multiple interacting intrinsic factors, including age, physiological state, pathology, and host genetics, as well as extrinsic

factors such as diet, environmental exposure, and medication use, all of which contribute to microbial community development and host health. However, despite increasing research interest, the relative contributions and interactions of these determinants remain incompletely understood in dogs, particularly from longitudinal perspectives spanning early life to adulthood. In addition, the effects of delivery mode, reproductive stage, and dietary variation on microbiome dynamics remain insufficiently characterized, especially at species-level resolution.

Understanding these complex host–microbe interactions requires analytical approaches capable of accurately resolving microbial community composition. Early microbiome studies primarily employed short-read sequencing platforms, such as Illumina, which typically target specific hypervariable regions of the 16S rRNA gene (e.g., V1–V2 or V3–V4). However, partial 16S rRNA gene sequences frequently lack sufficient phylogenetic signal to reliably distinguish closely related species—and, in some cases, ecologically distinct lineages—within the

same genus, thereby limiting taxonomic resolution and biological interpretation[20–22]. Large-scale benchmarking and in silico analyses have demonstrated that short-read sequencing of individual 16S rRNA variable regions is inherently limited in its ability to capture the species and strain-level diversity that can potentially be achieved through sequencing of the complete ~1.5 kb 16S rRNA gene[23].

Within this evolving scientific landscape, longitudinal high-resolution microbiome profiling provides a robust framework for characterizing gut microbiome dynamics over time. In the present thesis, this framework is implemented using full-length 16S rRNA gene sequencing on Oxford Nanopore Technologies (ONT) platform to enable species-level resolution across multiple biological and environmental dimensions. The conceptual and methodological developments outlined above form the foundation of the present thesis, which examines canine gut microbiome dynamics in relation to age, diet, birth mode, and reproductive status.

2. AIMS AND OBJECTIVES

The overall aim of this study was to investigate longitudinal gut microbial community dynamics in purebred Hungarian Pumi dogs using full-length 16S rRNA gene sequencing and to quantify the relative contribution of major host- and environment-related determinants to microbial community assembly.

The specific objectives were as follows:

1) To characterize age-associated maturation of the gut microbiome from birth through adulthood, including diversity dynamics and taxonomic succession.

2) To identify developmental ecological breakpoints, particularly during the weaning transition and associated dietary shifts.

3) To quantify and compare the influence of age, diet, kennel environment, litter, and host sex on microbiome diversity and composition.

4) To define and compare the species-level core microbiome across developmental stages.

5) To assess age-related remodeling of microbial co-occurrence network structure.

6) To determine the impact of delivery mode (cesarean section versus vaginal birth) on early postnatal microbiome composition.

7) To characterize reproductive stage–associated variation in the maternal gut microbiome across pregnancy and lactation.

3. MATERIALS AND METHODS

The primary study population comprised 84 healthy purebred Hungarian Pumi dogs obtained from six dedicated breeding kennels in Hungary, each housing exclusively this breed. The cohort included 55 puppies originating from 12 vaginally delivered litters, their dams (n = 9), and an additional 20 adult male and female dogs serving as adult control animals. Puppies were monitored longitudinally from birth until 81 weeks of age. Neonatal puppies were initially housed indoors in cradles, whereas growing puppies were raised in mixed indoor–garden environments. Adult dogs were maintained in comparable house–garden settings, resulting in broadly similar

environmental conditions across kennels. The mean age of dams was 4.3 ± 1.6 years (mean ± SD), while adult control dogs had a mean age of 5.9 ± 3.6 years (mean ± SD).

A separate subgroup consisting of five puppies (10 samples in total; two samples from each puppy) delivered by cesarean section from a single litter in the Le Petit Lapin kennel was also included. These puppies were born to the same dam that had previously produced vaginally delivered offspring those included in the main cohort. This subgroup was analyzed independently and was not included in the primary cohort.

Puppies were exclusively breastfed until approximately 8 weeks of age, with solid food introduced at around 3.5 weeks alongside continued nursing. All dogs were primarily fed commercial dry diets; however, a subset of adult dogs from one kennel (Duna-menti Dumás) received a raw BARF diet. Diet categorization and nutritional composition followed the 2024 FEDIAF guidelines[24]. No dogs included in this study received antibiotic treatment within six months prior to their first sampling point or during the sampling period.

Stool samples from the primary cohort were collected longitudinally from dams during gestation, lactation, and post-lactation periods; from their offspring from birth through adulthood (up to 81 weeks of age); and from adult dogs included as control animals. Samples from the cesarean-section subgroup, consisting of five puppies, were available only for the 8–10-week age group, yielding a total of 10 samples (two samples per puppy). In total, 463 samples were collected and sequenced, and the sequencing data were deposited in the ENA repository under BioProject accession **PRJEB82125**. Of these, 446 samples from the main cohort and all 10 samples from the cesarean-section subgroup were retained for the final analyses presented in this study.

Genomic DNA (gDNA) was isolated from fecal samples using the ZymoBIOMICS™ 96 MagBead DNA Kit Dx (Zymo Research; Cat. No. D4308-E) in accordance with the manufacturer's guidelines. All extraction procedures were performed under sterile conditions in a Class II biosafety cabinet. Full-length 16S rRNA gene libraries spanning the V1–V9 regions were generated from high-

quality genomic DNA (DIN ≥ 6) using the Oxford Nanopore Technologies Rapid Sequencing Amplicons–16S Barcoding Kit (SQK-16S024), incorporating AMPure XP bead–based cleanup, in accordance with the manufacturer's instructions.

Libraries were pooled in equimolar amounts and sequenced on an Oxford Nanopore MinION device using R9 flow cells (25 flow cells in total). Raw sequencing data were basecalled using Dorado v0.8.0 in super-accurate mode with a quality Q≥10 before downstream processing.

EMU v3.4.5 was used to generate read-count tables per taxon and calculate relative abundances following the workflow described previously[25,26]. Taxon-level read count tables generated by EMU served as input for downstream analyses. Diversity metrics were computed using phyloseq[27] v3.2 in R v4.2 based on unfiltered count data. Taxa were filtered and retained if they exceeded 5% relative abundance and were present in at least five samples. Compositional differential abundance testing across age groups, diet categories, kennels, reproductive states, and delivery modes was conducted using ANCOM-

BC (ancombc R package) on the filtered count tables. The adjusted P-values (*P.adj*) reported in Results correspond to ANCOM-BC outputs.

To assess the effects of age, sex, kennel, kennel-by-litter, and major diet categories, mixed-effects models were fitted using the lme4 package. age was modeled both categorically and with splines to account for diet-age confounding.

A taxonomic tree of the top 350 taxa was illustrated using iTOL[28]. Genus-level hierarchical clustering and heatmaps were generated with ComplexHeatmap[29]. Microbial co-occurrence networks were constructed using SparCC (SpiecEasi) and visualized with igraph and Cytoscape[30]. Comparative statistics (Wilcoxon rank-sum, Student's *t*-test, Mann–Whitney U-test) were performed as indicated in the figure legends, with $P < 0.05$ considered statistically significant unless otherwise stated.

Core microbiome sets were defined at the species level based on prevalence thresholds within each age group. A stringent cutoff (≥90% prevalence) was applied for the

heatmap and Jaccard similarity analyses, whereas a more inclusive threshold (≥80% prevalence) was used for the UpSet plot to capture broader shared community members, following commonly adopted practices in microbiome research[31]. Heatmaps and Jaccard similarity indices were calculated from prevalence tables and overlaps among core microbial sets were visualized using an UpSet plot. All visualizations were conducted in Python (v.3.12.3) using the pandas, numpy, and matplotlib libraries, with the UpSet representation generated through custom plotting code implemented in matplotlib.

4. RESULTS

This study generated a total of 121.30 million high-quality reads (Q ≥ 10). The mean sequencing yield per sample was 266,017.09 ± 127,742.50 reads (mean ± SD). Reads had an average length of 1,521.81 ± 50.07 bp and a mean Q-score of 20.40 ± 1.05. In total, approximately 185.01 GB of raw sequencing data were produced, providing sufficient sequencing depth and read length for reliable species-level taxonomic resolution.

Across the longitudinal cohort dataset, the findings emerged age as the dominant driver of gut community maturation, with a clear transition from early-life microbial profiles toward a more stable, adult-like composition over time. Shannon alpha diversity, increased rapidly during early life, with a marked rise observed during the weaning period (approximately 3.5–6 weeks of age). This increase was followed by a gradual tapering, with diversity values reaching an apparent plateau by around six months of age. This developmental trajectory was consistent across kennels and age strata, suggesting that the maturation pattern is robust to differences in housing environment and kennel-specific management practices. No significant association was detected for host sex on Shannon diversity.

Mixed-effects modeling demonstrated robust associations between specific taxa and age, diet, and kennel environment. SparCC-inferred co-occurrence networks indicated progressive increases in ecological complexity and modular organization with maturation.

A species-level core microbiome comprising 13 taxa was consistently detected across all age groups, although their relative abundances varied systematically with age and were largely dominated by short-chain fatty acid–associated *Clostridia* and the bile acid–transforming species *Peptacetobacter hiranonis*. Similarity and intersection analyses further indicated progressive age-related restructuring of the core microbiome, identifying the weaning period as a major ecological transition followed by increasing stabilization of an adult-like community in later life stages.

A dedicated comparison of delivery mode (cesarean versus vaginal birth) revealed measurable compositional differences in 8–10 week age window, whereas Shannon alpha diversity did not differ significantly. At the taxonomic level, cesarean-delivered puppies exhibited significantly higher relative abundances of *Lactobacillus* (*P.adj* = 0.008) and *Prevotella* (*P.adj* = 0.038), and a lower relative abundance of *Romboutsia* (P.adj = 0.045) compared to vaginally delivered litter . These findings suggest that delivery mode may influence early gut

colonization patterns under otherwise controlled environmental conditions.

The gut microbiota of dams exhibited stage-dependent variation across pregnancy and lactation. Shannon alpha diversity showed a transient reduction during late pregnancy and early lactation, followed by partial recovery after weaning. Despite close temporal proximity, prepartum and postpartum samples displayed distinct compositional profiles. The transition from prepartum to postpartum was characterized by marked turnover among the most abundant genera, including the disappearance of *Blautia* and *Fusobacterium* and the emergence of *Clostridium* and *Streptococcus*. These findings demonstrate dynamic microbiome shifts associated with reproductive status. While the core microbiome composition of dams changed significantly across reproductive stages, these shifts were not associated with specific kennels or diet categories and were not consistently shared across individuals.

Together, these findings highlight age—and the dietary transitions that accompany it—as the principal

determinants of canine gut microbiome assembly, with delivery mode, and reproductive status acting as secondary but biologically meaningful modifiers.

5. DISCUSSION

The present longitudinal, species-resolved profiling of purebred Hungarian Pumi dogs indicates that gut microbiome maturation is predominantly an age-structured process, progressing from an early, variable phase toward a more homogeneous adult community. Alpha diversity increased markedly during early life, particularly around the weaning transition, and plateaued by approximately six months of age, while beta diversity analyses demonstrated gradual age-graded convergence toward an adult-like configuration. The consistency of these trajectories across kennels and litters suggests that age-associated developmental dynamics are largely robust to kennel-level environmental heterogeneity.

Because age and diet are intrinsically intertwined during early development, their independent contributions cannot be fully disentangled; however, adult dietary practices

were largely stable, with BARF feeding confined to a single kennel, thereby limiting diet-related confounding at later stages. The weaning period emerges as a critical developmental window characterized by rapid ecological restructuring, progressive remodeling of microbial association networks, and stabilization of core taxa.

Consistent with the statistical modeling results, age emerged as the dominant determinant of alpha diversity, whereas kennel effects were comparatively modest and host sex showed no significant association. Differences among litters within the same kennel were generally modest. These findings suggest that, under typical husbandry and dietary conditions, intrinsic developmental programming and age-coupled dietary transitions exert a stronger influence than contextual environmental variation on microbiome assembly. From a methodological perspective, these findings emphasize the importance of longitudinal age matching and careful consideration of weaning status in the design and interpretation of canine microbiome studies.

In addition to age-related developmental dynamics, delivery mode was associated with detectable genus-level differences within the 8–10 week age window. Cesarean-delivered puppies exhibited higher relative abundances of *Lactobacillus* (*P*.adj = 0.008) and *Prevotella* (*P*.adj = 0.038) compared with vaginally delivered littermates, despite no significant difference in Shannon diversity between groups. This observation differs from findings in human neonates regarding *Prevotella* and from numerous studies documenting reduced *Lactobacillus* abundance in cesarean-delivered infants[34,35], although it aligns with more recent observations by Pahirah et al., who reported higher *Lactobacillus* abundance in cesarean-born human neonates[36]. These findings suggest that delivery mode–associated genus-level differences are unlikely to be uniform across species or developmental stages and may be influenced by species-specific postnatal ecology, including intensive dam–pup contact and coprophagic behavior, as well as kennel environment, weaning practices, and the later sampling window examined in this study (8–10 weeks). Given that the cesarean cohort comprised a single litter, the results should be interpreted

with caution and validated in larger, multicenter cohorts with detailed documentation of perinatal management.

Beyond early-life colonization factors, stage-linked variation was also observed in dams. Transient reductions in alpha diversity during late pregnancy and early lactation, accompanied by compositional turnover across the pre- to postpartum transition, indicate that reproductive physiology contributes to temporal modulation of the maternal gut microbiome. Although these shifts were not consistently associated with kennel or diet and were not uniformly shared across individuals, they demonstrate that maternal microbial communities are dynamically responsive to physiological state. Such plasticity may represent an additional axis influencing early-life microbial exposure in puppies, potentially interacting with delivery mode and postnatal environmental contact to shape neonatal gut colonization.

Methodologically, full-length 16S rRNA gene sequencing provided species-level resolution within a dense longitudinal framework, enabling fine-scale taxonomic and association analyses while minimizing batch effects.

This work establishes a high-resolution developmental framework for the canine gut microbiome and further reinforces the suitability of dogs as a comparative model for microbiome research.

6. REFERENCES

1. Ley, R. E., Turnbaugh, P. J., Klein, S. & Gordon, J. I. Human gut microbes associated with obesity. *Nature* **444**, 1022–1023 (2006).
2. Marchesi, J. R. & Ravel, J. The vocabulary of microbiome research: a proposal. *Microbiome* **3**, 31, s40168-015-0094–5 (2015).
3. Berg, G. *et al.* Microbiome definition re-visited: old concepts and new challenges. *Microbiome* **8**, 103 (2020).
4. Baquero, F. & Nombela, C. The microbiome as a human organ. *Clinical Microbiology and Infection* **18**, 2–4 (2012).
5. Suchodolski, J. S. Analysis of the gut microbiome in dogs and cats. *Veterinary Clinical Pathol* **50**, 6–17 (2022).
6. Li, Z. *et al.* Analysis and Comparison of Gut Microbiome in Young Detection Dogs. *Front. Microbiol.* **13**, 872230 (2022).
7. Craddock, H. A. *et al.* Phenotypic correlates of the working dog microbiome. *npj Biofilms Microbiomes* **8**, 66 (2022).
8. Kim, H. *et al.* Understanding the diversity and roles of the canine gut microbiome. *J Animal Sci Biotechnol* **16**, 95 (2025).
9. Garrigues, Q., Apper, E., Chastant, S. & Mila, H. Gut microbiota development in the growing dog: A dynamic

process influenced by maternal, environmental and host factors. *Front. Vet. Sci.* **9**, 964649 (2022).
10. AlShawaqfeh, M. *et al.* A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy. *FEMS Microbiology Ecology* **93**, (2017).
11. Kieler, I. N. *et al.* Gut microbiota composition may relate to weight loss rate in obese pet dogs. *Veterinary Medicine & Sci* **3**, 252–262 (2017).
12. Seo, J. *et al.* The gut microbiome in dogs with congestive heart failure: a pilot study. *Sci Rep* **10**, 13777 (2020).
13. Kim, K.-R., Kim, S.-M. & Kim, J.-H. A pilot study of alterations of the gut microbiome in canine chronic kidney disease. *Front. Vet. Sci.* **10**, 1241215 (2023).
14. Hullar, M. A. J., Lampe, J. W., Torok-Storb, B. J. & Harkey, M. A. The canine gut microbiome is associated with higher risk of gastric dilatation-volvulus and high risk genetic variants of the immune system. *PLoS ONE* **13**, e0197686 (2018).
15. Mondo, E. *et al.* Gut microbiome structure and adrenocortical activity in dogs with aggressive and phobic behavioral disorders. *Heliyon* **6**, e03311 (2020).
16. Berry, A. S. F. *et al.* Natural Infection with *Giardia* Is Associated with Altered Community Structure of the Human and Canine Gut Microbiome. *mSphere* **5**, 10.1128/msphere.00670-20 (2020).
17. Maldonado-Contreras, A. *et al.* Dysbiosis in a canine model of human fistulizing Crohn's disease. *Gut Microbes* **12**, 1785246 (2020).
18. Hernandez, J. *et al.* Domestic Environment and Gut Microbiota: Lessons from Pet Dogs. *Microorganisms* **10**, 949 (2022).

19. Coelho, L. P. *et al.* Similarity of the dog and human gut microbiomes in gene content and response to diet. *Microbiome* **6**, 72 (2018).
20. Wagner, J. *et al.* Evaluation of PacBio sequencing for full-length bacterial 16S rRNA gene classification. *BMC Microbiol* **16**, 274 (2016).
21. Ghielmetti, G. *et al.* Advancing animal tuberculosis surveillance using culture-independent long-read whole-genome sequencing. *Front. Microbiol.* **14**, 1307440 (2023).
22. Buetas, E. *et al.* Full-length 16S rRNA gene sequencing by PacBio improves taxonomic resolution in human microbiome samples. *BMC Genomics* **25**, 310 (2024).
23. Johnson, J. S. *et al.* Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nat Commun* **10**, 5029 (2019).
24. FEDIAF (European Pet Food Industry Federation). Nutritional guidelines for complete and complementary pet food for cats and dogs. (2024).
25. Curry, K. D. *et al.* Microbial Community Profiling Protocol with Full-length 16S rRNA Sequences and Emu. *Current Protocols* **4**, e978 (2024).
26. Curry, K. D. *et al.* Emu: species-level microbial community profiling of full-length 16S rRNA Oxford Nanopore sequencing data. *Nat Methods* **19**, 845–853 (2022).
27. McMurdie, P. J. & Holmes, S. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* **8**, e61217 (2013).
28. Letunic, I. & Bork, P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Research* **49**, W293–W296 (2021).

29. Gu, Z., Eils, R. & Schlesner, M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* **32**, 2847–2849 (2016).
30. Su, G., Morris, J. H., Demchak, B. & Bader, G. D. Biological Network Exploration with Cytoscape 3. *CP in Bioinformatics* **47**, (2014).
31. Risely, A. Applying the core microbiome to understand host–microbe systems. *Journal of Animal Ecology* **89**, 1549–1558 (2020).
32. Flores, J. N., Lubin, J.-B. & Silverman, M. A. The case for microbial intervention at weaning. *Gut Microbes* **16**, 2414798 (2024).
33. Chen, L. *et al.* The Maturing Development of Gut Microbiota in Commercial Piglets during the Weaning Transition. *Front. Microbiol.* **8**, 1688 (2017).
34. Biasucci, G., Benenati, B., Morelli, L., Bessi, E. & Boehm, G. Cesarean Delivery May Affect the Early Biodiversity of Intestinal Bacteria1,. *The Journal of Nutrition* **138**, 1796S-1800S (2008).
35. Dominguez-Bello, M. G. *et al.* Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. *Nat Med* **22**, 250–253 (2016).
36. Pahirah, N. *et al.* Comparison of Gut Microbiomes Between Neonates Born by Cesarean Section and Vaginal Delivery: Prospective Observational Study. *BioMed Research International* **2024**, 8302361 (2024).