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Best practices for the experimental design of one health studies on companion animal and owner microbiomes – From data collection to analysis

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ABSTRACT

The relationship between owner and companion animal represents an underestimated opportunity for the studying of One Health relationships between humans, animals, and the environment they share. Microbiome exchanges between owner and pet have been documented for the gut, skin, oral, and nasal microbiomes. These studies give a unique insight into bacterial flows between humans and animals, but come with their specific challenges.

This review discusses the data and sample collection challenges, as well as laboratory, bioinformatic and data analysis challenges specific to One Health studies on companion animal and owner microbiomes. We provide an overview of possible data to be collected and pitfalls to avoid during sample collection and conservation, DNA extraction, and library preparation. We present the main bioinformatics pipelines in sequencing-data microbiome analysis, as well as data analysis specific to pet-owner microbiome comparison. We review and compare three beta-diversity measures (Bray-Curtis dissimilarity, unweighted, and weighted UniFrac distances) for pet-owner distances and the tests to compare them. Finally, we propose a framework with key considerations to bear in mind when designing and carrying out owner-companion animal studies, as well as best practices to implement them.

Although these studies come with additional difficulties compared to species-specific microbiome studies, they offer the opportunity to identify biomarkers, environmental triggers, and impacts of pet-owner interactions across species.

1. Introduction

The One Health paradigm recognises the profound and deep connections between human health, animal health, and the environment. Companion animals represent an understudied portal into exploring shared environmental influences, opening channels for understanding how such influences impact on health [1,2]. Interactions between companion animals and humans take place at multiple scales, from microscopic to macro scales, the population to the microscopic scale. Investigating microbiomes with a One Health lens calls for the exploration of both pathogenic and non-pathogenic interactions [3].

The importance of the micro-organisms in our gut, on our skin and mucosal surfaces - the microbiome – in health and disease are now abundantly clear. It is estimated that environmental influences have at least ten-fold more influence on microbiome variation, and the associated health implications, than host genetics [4]. Pets, mostly companion dogs and cats, are mammals who share our living spaces, are exposed to a subset of the same environmental influences, and, like us, have resident microbiota. Studying their microbiota, therefore, in conjunction with studying pet owner microbiota, represents an important opportunity to understand and dissect common environmental influences on these microbiota. The scale of this opportunity can be envisioned through the lens of 844 million pet dogs and cats worldwide, with 66 % % of households in the USA [5] and 50 % in Europe owning at least one

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pet [6]. Remarkably, the potential to exploit this has been largely neglected to date. However, a few studies have demonstrated exchanges and mutual interactions between the microbiota of humans and companion animals [7–10] or livestock [11].

Human-animal interactions are unique, and various physiological, sociological, psychological, economic factors of both humans and animals come into play [12]. There are a multitude of microbiome-related conditions for which environmental triggers are believed to play a role, on a background of genetic susceptibility, but for which such triggers have not been identified. Examples include inflammatory bowel disease [13], eczema [14], and gluten sensitivity [15]. In some cases, parallel conditions occur in companion animals (atopic eczema and inflammatory bowel disease in dogs) [16]. Studies of companion animals living in shared environments with humans offer the possibility of identifying yet unrecognised common biomarkers, environmental triggers, and pathogenic pathways, across species. Such studies pose significant challenges, as well as opportunities. Examples of challenges of cross-species studies include combining multi-omic datasets across species, methodological differences, and sharing of resources between animal and human health professionals. However, overcoming those challenges may result in added value through new dimensions of and interactions with human health. In this review, we discuss the opportunities, challenges and potential benefits of such studies.

2. Data collection challenges

Investigating pet-owner relationships implies the need to characterise the subjects (age, gender, sex, breed, diseases etc.) as well as their interactions, more specifically the ones that might impact the studied microbiome. The nature, quantity and quality of these interactions may have a significant impact on pet and owner microbiota and should therefore be thoroughly characterised in the data collection process. In order to capture best the complexity of the owner-pet relationships, the focus should not only be on direct pet-owner interactions like petting habits or amount of time they have lived together but also on more "indirect" interactions [12]. Our experience leads us to consider relevant the following questions:

- Does the pet move freely around the house?
- How many hours a day does the pet spend outside?
- Do pet and owner do any kind of training or playing together? If so, what type and how often?
- Are the pet and/or owner in frequent contact with other animals? Could that impact the studied microbiome?
- Are there other cohabiting pets?
- Is the pet allowed on sofas and beds?
- Did the pet have other owner(s) before?
- Do pet and owner live in an urban or rural setting?
- Do pet and owner follow a specific diet? Do they share food?

Specific data to be collected should be considered in accordance with the aims of each project. Moreover, to best identify metadata impacting microbiome sharing between pet and owner, we recommend the inclusion of people that do not cohabit with any pets, as controls.

Power calculations in microbiome studies are often considered difficult, and many microbiome studies do not mention any power calculation or sample size estimation. Being able to estimate sample size is important when considering studies of human microbiome samples, for which data protection is often a consideration. An underpowered study that needs repeating could be considered unethical. However, methods to calculate sample size in microbiome studies do exist, and include the use of pairwise distances and PERMANOVA (micropower R package) [17] or alpha and beta diversity metrics [18] to estimate sample size based on statistical power and effect size. These methods do rely on data for similar projects being available. While animal-owner interaction projects are rare, in the case of the data not being previously available, data from other pet-owner projects or separate cohorts of animal and owner data from similar conditions could be used.

3. Sample collection challenges

Sample collection in microbiome studies faces numerous challenges, such as limiting sampling to one operator to ensure reproducibility, sample storage temperature (especially if participants collect the samples themselves at home), or limiting potential contaminations [19]. Storage temperature has been shown to have an impact on downstream sequencing: better results obtained in faecal samples stored at −20 °C or that used preservation methods such as ethanol or RNALater compared to samples kept at room temperature or + 4 °C [20]. Preservation buffers/tubes have been developed that allow for faecal, oral and skin microbiome samples to be preserved at room temperature for extended periods [21,22]. However, the reliability of microbiota preservation in both pet and human samples would preferably be optimised prior to a study start.

Additionally, having both human and animal participants could require sampling pets and owners in separate locations and/or at different times, such as having to sample owners in a hospital and pets in their home or vice versa. In such cases, sampling sites could have different environmental conditions (humidity, pollutants etc.) and contaminations that should be reduced to a minimum and accounted for with negative controls. Sampling location can also impact stress and anxiety of both owner and pet, highlighting the importance of choosing locations minimising these effects [23]. These precautions are particularly important when working with paucibacterial microbiomes, easily skewed by the presence of contaminants (see 4. Laboratory Challenges). Having to sample pet and owner in separate locations and/or at different times may also increase loss to follow-up due to a more complicated sampling schedule compared to a single sampling time and place. This should be considered in the power calculations. Sample storage and preservation conditions should also be as similar as possible.

4. Laboratory challenges

Due to the potential differences in composition and diversity between human and animal microbiomes, avoiding cross-contamination is crucial. If samples are processed in batches, randomisation of owner and pet samples should be considered so that batch effects can later be identified if present. Adding multiple negative controls such as sampling blank controls, DNA extraction blank controls, and no template amplification controls should also be considered in each batch [19]. Due to the presence of a kitome in most DNA extraction kits, and the different sequencing results that they can produce [24,25], choice of DNA extraction kit should be made accordingly to the studied microbiome and its average biomass. This is particularly important when working with low biomass microbiomes, in which the DNA present in extraction kits and laboratory reagents can come in concentrations similar to that of the samples and can therefore be mistaken for the studied microbiome [19,26].

5. Bioinformatics pipeline challenges and recommendations

Bioinformatics pipelines can be divided into primary and secondary analysis. Primary (upstream) analysis processes data from their raw sequencing output (in the case of Illumina sequencing, BCL or fastq files) to count matrices. In the case of 16S data, primary processing entails a quality check using fastQC or multiQC, trimming of 16S primers using cutadapt, and constructing a table of amplicon counts - operational taxonomic units (OTUs) or amplicon sequence variant (ASVs), using Qiime2, Mothur or DADA2 [27]. The DADA2 package is used to construct an ASV table, remove chimeric reads, and assign taxonomy using a database of choice.

For the above methodology, it is recommended to use reproducible

methods of analysis such as nf-core pipelines (https://nf-co.re/ampliseq/2.9.0/docs/usage). The advantages of those pipelines are their standardisation, allowing for easy comparisons between studies, as well as the fact that the pipeline documents the parameters and software version used, allowing for easy documentation of methodology. The pipelines also contain built-in software containers, thereby solving a software installation problem which can be cumbersome on high performance computing (HPC) systems.

One of the current challenges for 16S data is lack of defined standards for its quality control. There is a lack of publications or tutorials which show an appropriate fastQC output for 16S data, for instance. Parameter adjustments and the desired numbers of chimeric/denoised reads for the DADA2 pipeline are also not well-defined. Finally, an equivalent of the ENCODE guidelines, which have been in place for other types of sequencing data since 2015–2017 [28], does not exist for 16S or metagenomic sequencing. Therefore, detailed and consistent reporting for 16S data analyses is crucial, especially in cases when results are compared to other studies, which could have used other data processing methodologies.

It is notable that for metagenomic data, a skew has been found towards human microbes in most databases, with a need for a database tailored towards animal sequences highlighted previously [29]. It is possible similar database changes could be needed for 16S data. When it comes to taxonomical grouping of amplicon sequencing data, ASVs have been reported to be superior to OTUs, due to the denoising of true biological sequences from potential sequencing error, as opposed to clustering similar sequences into a same OTU, resulting in higher accuracy and sensitivity [30]. ASV results can also be readily compared between studies using the same target region. The manner in which comparison between studies is conducted is another concern. Publications have compared multiple studies by reanalysing raw data using one pipeline [31], however given the large number of 16S studies that have been performed, a method of batch correction of these datasets would be useful. Some batch correction methods have been proposed [32–35], with the most recent being PLSDA batch [36]. The method is already being demonstrated to be beneficial in practice by other groups [36].

An ASV table with its assigned taxonomy can be considered an entry point to secondary analysis, which can be done using packages such as vegan or phyloseq in R and is outlined in the next section.

6. Data analysis challenges

Microbiomes are characterised by their composition and diversity, mainly alpha- and beta-diversity. Alpha-diversity measures the diversity within a sample, characterised by taxa evenness (number of taxa), richness (distribution of taxa within a sample), or both. Richness is commonly estimated by the number of observed species or OTUs/ASVs, or indices such as Faith's Phylogenetic diversity [37] and Chao1 index [38]. Commonly used indices that consider both richness and evenness are Shannon index [39] and Simpson index [40]. It has been argued that, to draw substantial conclusions comparing alpha diversity in groups, one should use models accounting for unobserved species and measurement errors [41].

The diversity between samples is measured by beta diversity. Common metrics used in microbiome analysis include Bray-Curtis dissimilarity [42], unweighted UniFrac distance [43], weighted UniFrac distance [44] and, to a lesser extent, Jaccard index [45]. Jaccard index is a binary dissimilarity metric, that considers only the absence or presence of taxa [45]. It is easy to calculate and interpret but does not consider abundance nor does it account for phylogeny. Bray-Curtis dissimilarity is based on taxa counts in each sample, and considers both the presence/absence of taxa and their relative abundances [42]. The unique fraction metric, or UniFrac, measures the phylogenetic distance between sets of taxa in a phylogenetic tree as the fraction of the branch length of the tree that leads to descendants from either one environment or the other, but not both [43]. Unweighted UniFrac considers the presence or absence of taxa and the branch length fraction that is unique to a community, whereas weighted UniFrac incorporates relative abundance of taxa and weights the branch lengths accordingly [46]. All four metrics vary from 0, if two samples share all the same taxa, to 1, if two samples do not share any taxa and, if accounted for, share no evolutionary history.

Table 1
Comparison of characteristics, strengths, and weaknesses of three distance metrics: Bray-Curtis dissimilarity, unweighted UniFrac distance, and weighted UniFrac distance.

	Accounts for relative abundance of taxa	Accounts for phylogeny*	Strengths	Weaknesses
Bray-Curtis dissimilarity	Yes	No	Easy to calculate (no phylogenetic tree) Used in many studies Relative comparison between groups Sensitive to abundance differences	Does not account for phylogeny Abundant species are weighted more than rare ones
Unweighted UniFrac distance	No (only presence/absence)	Yes	Accounts for phylogenetic relationships among taxa	Requires a rooted phylogenetic tree Binary test of presence/absence of taxa Sensitive to sequencing depth
Weighted UniFrac distance	Yes	Yes	Accounts for phylogenetic relationships among taxa Accounts for rare taxa	Requires a rooted phylogenetic tree

* Requires a phylogenetic tree.

UniFrac incorporates phylogenetic information, that is not reflected in Bray-Curtis dissimilarity, however it requires the construction of a phylogenetic tree, that can be time-consuming. As these metrics have strengths and limitations, their use can depend on the question asked and/or data availability, there is no agreement on which to use for microbiome data analysis. Some studies choose one, whereas others report on beta-diversity with multiple metrics [47–50].

More specifically for pet-owner studies, beta-diversity can be used to estimate the distance, i.e. the "level" of similarity, between pet and owner. As measures all have their strengths and limitations (see Table 1), a comprehensive understanding of similarities in pet-owner pairs can be achieved by ranking pairs according to each measure and using and average rank to identify the least and most similar pairs.

Non-parametric tests, such as Wilcoxon tests, as well as multivariate methods, such as analysis of similarity (ANOSIM) or multivariate analysis of variance with permutation (PERMANOVA), have previously been used for comparisons of alpha and beta diversity between pets and owners [10]. Specifically, ANOSIM describes whether dissimilarity between selected groups is significantly different than the dissimilarity within each group [51]. It is important to note that when looking at pet-owner microbiome interactions, ANOSIM can be used to compare beta diversity between groups within the metadata (for instance, dogs versus owners, between breeds of dogs, or between family units); however, it cannot be used to compare group-to-group distances with one another. Comparisons of specific group-to-group distances (for instance pet-to-owner versus pet-to-non-owner) should be done using non-parametric

Table 2
Proposed framework for experimental design of One Health relationships between companion animal, human, and environment.

	Key considerations/best practices
Study organisation/ Recruitment	Which body sites/microbiome(s) will be sampled? How many times? How many pet-owner pairs are needed? (power calculations) Are non-pet owners (controls) included? What "basic" information is needed on pet and owner? (e.g. gender/sex, age, occupation, pet breed, weight/ height/size, previous owners of the pet, country of birth, country of residence) Define the inclusion/exclusion criteria? (e.g. antibiotic intake in the last 6 months, foreign travel in the last 2 months) Which pet-owner interactions might impact the microbiome(s)? Establish the necessary metadata. Consider including the following categories: - Health data: allergies, systemic diseases, diseases relevant to the studied microbiome, smoking status, drinking alcohol, reproductive status/sterilisation, etc. - Exercise data: amount of daily/weekly exercise, pet and owner exercising together, types of exercise, exercise indoor or outdoor, etc. - Behavioural data: amount of time pet and owner spend together, owner being the pet's primary caregiver, hours of sleep, stress levels, owner working on-site or from home, pet licking owner's face/hands, pet sleeping in owner's bed, pet's behaviour with humans and with other animals, etc. - Household and environmental data: presence of other pets in the household and which pets, type of dwelling (rural/urban), number of household members, living with family or roommates, etc. - Nutritional data: diet type (owner: vegetarian, vegan, omnivore, ketogenic, prescription diet, etc., pet: raw, commercial, prescription, human food, wet or dry food, pet food brand etc.), supplements, probiotics/ prebiotics, type of drinking water (bottled, tap, filtered, etc.), etc. Are pets and owners sampled at the same time and/or in the same place? Determine the biases that may arise from these differences. Try to limit batch effect and cross-contamination, insert multiple negative controls. When working with low-biomass microbiomes: particular attention must be put on the choice of DNA extraction kit.
Laboratory work	How many and which negative controls are included? Choice of DNA extraction method.
Bioinformatics	Use reproducible, well-established packages and pipelines, note package versions and document code. Version control on github is highly recommended. Determine if batch correction is needed. Consider ASV to be the current gold standard for 16S analysis. If possible, use metagenomic data, and complement with 16S.
Data analysis challenges	Rank pet-owner pairs based on multiple beta diversity metrics.

Wilcoxon tests, and data manipulations may be required for such comparisons. PERMANOVA on the other hand is a distance-based method to test the association of microbial composition with covariates of interest [52].

Finally, available genomic databases have a bias towards human-origin bacteria, potentially limiting the consideration of some pet-hosted bacteria [11,29].

7. Proposed framework

Throughout this review, various considerations were described, and recommendations were made for best practices when it comes to studies of pet-owner microbiomes. The most important points are summarised in Table 2. Best practices specific to technical aspects of microbiome studies have been discussed elsewhere [53].

8. Conclusions

Studies of companion animals and their owners, using shared environment to examine as yet unexplored influences on skin, gut and other microbiota are a valuable One Health strategy. In this review, we outline some of the advantages and possibilities of the One Health approach, contrasted with the difficulties inherent in ensuring reproducibility and reliability of data, and provide methodological recommendations for overcoming them. Given the potential for One Health approaches to advance societal goals in respect of predicting emerging infectious diseases, slowing the onward march of anti-microbial resistance, and understanding environmental triggers for inflammatory diseases, to name but a few, the pathway is clear for more research groups to take up this challenge.

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Suzanne B. Clougher: Writing – review & editing, Writing – original draft, Conceptualization. **Dagmara Niedziela:** Writing – review & editing, Writing – original draft, Conceptualization. **Piera Versura:** Writing – review & editing, Validation, Supervision. **Grace Mulcahy:** Writing – review & editing, Writing – original draft, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

References

[1] R. Matheson, C.L. Sexton, C.F. Wise, J. O'Brien, A.J. Keyser, M. Kauffman, M. D. Dunbar, D.A.P. Consortium, J.M. Akey, B. Benton, E. Borenstein, M. G. Castelhano, A.E. Coleman, K.E. Creevy, K. Crowder, M.D. Dunbar, V.R. Fajt, A. L. Fitzpatrick, U. Jeffery, E.C. Jonlin, M. Kaeberlein, E.K. Karlsson, K.F. Kerr, J. M. Levine, J. Ma, R.L. McClelland, D.E.L. Promislow, A. Ruple, S.M. Schwartz, S. Shrager, N. Snyder-Mackler, M.K. Tolbert, S.R. Urfer, B.S. Wilfond, H. M. Stapleton, A. Ruple, Silicone tags as an effective method of monitoring environmental contaminant exposures in a geographically diverse sample of dogs from the dog aging project, Front. Vet. Sci. 11 (2024), https://doi.org/10.3389/fvets.2024.1394061.

[2] C.F. Wise, M. Breen, H.M. Stapleton, Canine on the couch: the new canary in the coal mine for environmental health research, Environ Health (Wash) 2 (2024) 517–529, https://doi.org/10.1021/envhealth.4c00029.

[3] P. Trinh, J.R. Zaneveld, S. Safranek, P.M. Rabinowitz, One health relationships between human, animal, and environmental microbiomes: a mini-review, front, Public Health 6 (2018) 235, https://doi.org/10.3389/fpubh.2018.00235.
[4] D. Rothschild, O. Weissbrod, E. Barkan, A. Kurilshikov, T. Korem, D. Zeevi, P. I. Costea, A. Godneva, I.N. Kalka, N. Bar, S. Shilo, D. Lador, A.V. Vila, N. Zmora, M. Pevsner-Fischer, D. Israeli, N. Kosower, G. Malka, B.C. Wolf, T. Avnit-Sagi, M. Lotan-Pompan, A. Weinberger, Z. Halpern, S. Carmi, J. Fu, C. Wijmenga, A. Zhernakova, E. Elinav, E. Segal, Environment dominates over host genetics in shaping human gut microbiota, Nature 555 (2018) 210–215, https://doi.org/10.1038/nature25973.
[5] M. Megna, Pet ownership statistics 2024, Forbes Advisor (2023). https://www.forbes.com/advisor/pet-insurance/pet-ownership-statistics/. (Accessed 17 September 2024).
[6] FEDIAF European Pet Food, Facts & Figures 2022 - Published with Annual Report 2024, 2024.
[7] L.P. Coelho, J.R. Kultima, P.I. Costea, C. Fournier, Y. Pan, G. Czarnecki-Maulden, M.R. Hayward, S.K. Forslund, T.S.B. Schmidt, P. Descombes, J.R. Jackson, Q. Li, P. Bork, Similarity of the dog and human gut microbiomes in gene content and response to diet, Microbiome 6 (2018) 72, https://doi.org/10.1186/s40168-018-0450-3.
[8] J. Lehtimäki, H. Sinkko, A. Hielm-Björkman, T. Laatikainen, L. Ruokolainen, H. Lohi, Simultaneous allergic traits in dogs and their owners are associated with living environment, lifestyle and microbial exposures, Sci. Rep. 10 (2020) 21954, https://doi.org/10.1038/s41598-020-79055-x.
[9] A.M. Misic, M.F. Davis, A.S. Tyldsley, B.P. Hodkinson, P. Tolomeo, B. Hu, I. Nachamkin, E. Lautenbach, D.O. Morris, E.A. Grice, The shared microbiota of humans and companion animals as evaluated from Staphylococcus carriage sites, Microbiome 3 (2015) 2, https://doi.org/10.1186/s40168-014-0052-7.
[10] S.J. Song, C. Lauber, E.K. Costello, C.A. Lozupone, G. Humphrey, D. Berg-Lyons, J. G. Caporaso, D. Knights, J.C. Clemente, S. Nakielny, J.I. Gordon, N. Fierer, R. Knight, Cohabiting family members share microbiota with one another and with their dogs, eLife 2 (2013) e00458, https://doi.org/10.7554/eLife.00458.
[11] B. Mahmud, R.C. Vargas, K.V. Sukhum, S. Patel, J. Liao, L.R. Hall, A. Kesaraju, T. Le, T. Kitchner, E. Kronholm, K. Koshalek, C.G. Bendixsen, J.J. VanWormer, S. K. Shukla, G. Dantas, Longitudinal dynamics of farmer and livestock nasal and faecal microbiomes and resistomes, Nat. Microbiol. 9 (2024) 1007–1020, https://doi.org/10.1038/s41564-024-01639-4.
[12] K.E. Rodriguez, H. Herzog, N.R. Gee, Variability in human-animal interaction research, Front. Vet. Sci. 7 (2021), https://doi.org/10.3389/fvets.2020.619600.
[13] A.N. Ananthakrishnan, Environmental triggers for inflammatory bowel disease, Curr. Gastroenterol. Rep. 15 (2013) 302, https://doi.org/10.1007/s11894-012-0302-4.
[14] M. Grijincu, M.-R. Buzan, L.-E. Zbîrcea, V. Păunescu, C. Panaitescu, Prenatal factors in the development of allergic diseases, Int. J. Mol. Sci. 25 (2024) 6359, https://doi.org/10.3390/ijms25126359.
[15] A. Levescot, G. Malamut, N. Cerf-Bensussan, Immunopathogenesis and environmental triggers in coeliac disease, Gut 71 (2022) 2337–2349, https://doi.org/10.1136/gutjnl-2021-326257.
[16] A.R. Amaral, L.W. Risolia, M.F. Rentas, P.H. Marchi, C. Balieiro, T.H. A. Vendramini, M.A. Brunetto, Translating human and animal model studies to dogs' and cats' veterinary care: Beta-glucans application for skin disease, Osteoarth. Inflam. Bowel Dis. Manag. Microorgan. 12 (2024) 1071, https://doi.org/10.3390/microorganisms12061071.
[17] B.J. Kelly, R. Gross, K. Bittinger, S. Sherrill-Mix, J.D. Lewis, R.G. Collman, F. D. Bushman, H. Li, Power and sample-size estimation for microbiome studies using pairwise distances and PERMANOVA, Bioinformatics 31 (2015) 2461–2468, https://doi.org/10.1093/bioinformatics/btv183.
[18] C. Casals-Pascual, A. González, Y. Vázquez-Baeza, S.J. Song, L. Jiang, R. Knight, Microbial diversity in clinical microbiome studies: sample size and statistical power considerations, Gastroenterology 158 (2020) 1524–1528, https://doi.org/10.1053/j.gastro.2019.11.305.
[19] R. Eisenhofer, J.J. Minich, C. Marotz, A. Cooper, R. Knight, L.S. Weyrich, Contamination in low microbial biomass microbiome studies: issues and recommendations, Trends Microbiol. 27 (2019) 105–117, https://doi.org/10.1016/j.tim.2018.11.003.
[20] S.J. Song, A. Amir, J.L. Metcalf, K.R. Amato, Z.Z. Xu, G. Humphrey, R. Knight, Preservation methods differ in fecal microbiome stability, affecting suitability for field studies, mSystems 1 (2016), https://doi.org/10.1128/msystems.00021-16.
[21] A.L. Pribyl, D.H. Parks, N.Z. Angel, J.A. Boyd, A.G. Hasson, L. Fang, S. L. MacDonald, B.A. Wills, D.L.A. Wood, L. Krause, G.W. Tyson, P. Hugenholtz, Critical evaluation of faecal microbiome preservation using metagenomic analysis, ISME Commun. 1 (2021) 14, https://doi.org/10.1038/s43705-021-00014-2.
[22] C.-Y. Lin, T.-W.L. Cross, E. Doukhanine, K.S. Swanson, An ambient temperature collection and stabilization strategy for canine microbiota studies, Sci. Rep. 10 (2020) 13383, https://doi.org/10.1038/s41598-020-70232-6.
[23] Q. Tang, G. Jin, G. Wang, T. Liu, X. Liu, B. Wang, H. Cao, Current sampling methods for gut microbiota: a call for more precise devices, Front. Cell. Infect. Microbiol. 10 (2020), https://doi.org/10.3389/fcimb.2020.00151.
[24] A.S. Cheema, L.F. Stinson, C.T. Lai, D.T. Geddes, M.S. Payne, DNA extraction method influences human milk bacterial profiles, J. Appl. Microbiol. 130 (2021) 142–156, https://doi.org/10.1111/jam.14780.
[25] H. Delbeke, I. Casteels, M. Joossens, DNA extraction protocol impacts ocular surface microbiome profile, Front. Microbiol. 14 (2023) 1128917, https://doi.org/10.3389/fmicb.2023.1128917.
[26] A. Glassing, S.E. Dowd, S. Galandiuk, B. Davis, R.J. Chiodini, Inherent bacterial DNA contamination of extraction and sequencing reagents may affect interpretation of microbiota in low bacterial biomass samples, Gut Pathogens 8 (2016) 24, https://doi.org/10.1186/s13099-016-0103-7.
[27] A. Prodan, V. Tremaroli, H. Brolin, A.H. Zwinderman, M. Nieuwdorp, E. Levin, Comparing bioinformatic pipelines for microbial 16S rRNA amplicon sequencing, PLoS One 15 (2020) e0227434, https://doi.org/10.1371/journal.pone.0227434.
[28] J.E. Moore, M.J. Purcaro, H.E. Pratt, C.B. Epstein, N. Shoresh, J. Adrian, T. Kawli, C.A. Davis, A. Dobin, R. Kaul, J. Halow, E.L. Van Nostrand, P. Freese, D.U. Gorkin, Y. Shen, Y. He, M. Mackiewicz, F. Pauli-Behn, B.A. Williams, A. Mortazavi, C. A. Keller, X.-O. Zhang, S.I. Elhajjajy, J. Huey, D.E. Dickel, V. Snetkova, X. Wei, X. Wang, J.C. Rivera-Mulia, J. Rozowsky, J. Zhang, S.B. Chhetri, J. Zhang, A. Victorsen, K.P. White, A. Visel, G.W. Yeo, C.B. Burge, E. Lécuyer, D.M. Gilbert, J. Dekker, J. Rinn, E.M. Mendenhall, J.R. Ecker, M. Kellis, R.J. Klein, W.S. Noble, A. Kundaje, R. Guigó, P.J. Farnham, J.M. Cherry, R.M. Myers, B. Ren, B. R. Graveley, M.B. Gerstein, L.A. Pennacchio, M.P. Snyder, B.E. Bernstein, B. Wold, R.C. Hardison, T.R. Gingeras, J.A. Stamatoyannopoulos, Z. Weng, Expanded encyclopaedias of DNA elements in the human and mouse genomes, Nature 583 (2020) 699–710, https://doi.org/10.1038/s41586-020-2493-4.
[29] R.H. Smith, L. Glendinning, A.W. Walker, M. Watson, Investigating the impact of database choice on the accuracy of metagenomic read classification for the rumen microbiome, Animal Microbiome 4 (2022) 57, https://doi.org/10.1186/s42523-022-00207-7.
[30] M. Chiarello, M. McCauley, S. Villéger, C.R. Jackson, Ranking the biases: the choice of OTUs vs. ASVs in 16S rRNA amplicon data analysis has stronger effects on diversity measures than rarefaction and OTU identity threshold, PLoS One 17 (2022) e0264443, https://doi.org/10.1371/journal.pone.0264443.
[31] N. Bykova, N. Litovka, A. Popenko, S. Musienko, Pet-human gut microbiome host classifier using data from different studies, Microorganisms 8 (2020) 1591, https://doi.org/10.3390/microorganisms8101591.
[32] Z. Dai, S.H. Wong, J. Yu, Y. Wei, Batch effects correction for microbiome data with Dirichlet-multinomial regression, Bioinformatics 35 (2019) 807–814, https://doi.org/10.1093/bioinformatics/bty729.
[33] S.M. Gibbons, C. Duvallet, E.J. Alm, Correcting for batch effects in case-control microbiome studies, PLoS Comput. Biol. 14 (2018) e1006102, https://doi.org/10.1371/journal.pcbi.1006102.
[34] W. Ling, J. Lu, N. Zhao, A. Lulla, A.M. Plantinga, W. Fu, A. Zhang, H. Liu, H. Song, Z. Li, J. Chen, T.W. Randolph, W.L.A. Koay, J.R. White, L.J. Launer, A.A. Fodor, K. A. Meyer, M.C. Wu, Batch effects removal for microbiome data via conditional quantile regression, Nat. Commun. 13 (2022) 5418, https://doi.org/10.1038/s41467-022-33071-9.
[35] Y. Wang, K.-A. LêCao, Managing batch effects in microbiome data, Brief. Bioinform. 21 (2020) 1954–1970, https://doi.org/10.1093/bib/bbz105.
[36] A. Regueira-Iglesias, B. Suárez-Rodríguez, T. Blanco-Pintos, M. Relvas, M. Alonso-Sampedro, C. Balsa-Castro, I. Tomás, The salivary microbiome as a diagnostic biomarker of periodontitis: a 16S multi-batch study before and after the removal of batch effects, Front. Cell. Infect. Microbiol. 14 (2024) 1405699, https://doi.org/10.3389/fcimb.2024.1405699.
[37] D.P. Faith, Conservation evaluation and phylogenetic diversity, Biol. Conserv. 61 (1992) 1–10, https://doi.org/10.1016/0006-3207(92)91201-3.
[38] A. Chao, Nonparametric estimation of the number of classes in a population, Scand. J. Stat. 11 (1984) 265–270.
[39] L.N. Lemos, R.R. Fulthorpe, E.W. Triplett, L.F.W. Roesch, Rethinking microbial diversity analysis in the high throughput sequencing era, J. Microbiol. Methods 86 (2011) 42–51, https://doi.org/10.1016/j.mimet.2011.03.014.
[40] E.H. Simpson, Measurement of diversity, Nature 163 (1949) 688, https://doi.org/10.1038/163688a0.
[41] A.D. Willis, Rarefaction, alpha diversity, and statistics, Front. Microbiol. 10 (2019) 2407, https://doi.org/10.3389/fmicb.2019.02407.
[42] J.R. Bray, J.T. Curtis, An ordination of the upland Forest communities of southern Wisconsin, Ecol. Monogr. 27 (1957) 325–349, https://doi.org/10.2307/1942268.
[43] C. Lozupone, R. Knight, UniFrac: a new phylogenetic method for comparing microbial communities, Appl. Environ. Microbiol. 71 (2005) 8228–8235, https://doi.org/10.1128/AEM.71.12.8228-8235.2005.
[44] C.A. Lozupone, M. Hamady, S.T. Kelley, R. Knight, Quantitative and qualitative β diversity measures Lead to different insights into factors that structure microbial communities, Appl. Environ. Microbiol. 73 (2007) 1576–1585, https://doi.org/10.1128/AEM.01996-06.
[45] P. Jaccard, The distribution of the Flora in the alpine Zone.1, New Phytol. 11 (1912) 37–50, https://doi.org/10.1111/j.1469-8137.1912.tb05611.x.
[46] J. Chen, K. Bittinger, E.S. Charlson, C. Hoffmann, J. Lewis, G.D. Wu, R.G. Collman, F.D. Bushman, H. Li, Associating microbiome composition with environmental covariates using generalized UniFrac distances, Bioinformatics 28 (2012) 2106–2113, https://doi.org/10.1093/bioinformatics/bts342.
[47] C.-C.J. Chang, K. Somohano, C. Zemsky, A.-C. Uhlemann, J. Liebmann, G.A. Cioffi, L.A. Al-Aswad, S.V. Lynch, B.J. Winn, Topical Glaucoma therapy is associated with alterations of the ocular surface microbiome, Invest. Ophthalmol. Vis. Sci. 63 (2022) 32, https://doi.org/10.1167/iovs.63.9.32.
[48] Y. Kang, L. Tian, X. Gu, Y. Chen, X. Ma, S. Lin, Z. Li, Y. Lou, M. Zheng, Characterization of the ocular surface microbiome in keratitis patients after repeated ophthalmic antibiotic exposure, Microbiol Spectr 10 (2022), https://doi.org/10.1128/spectrum.02162-21 e02162–21.
[49] Z. Li, Y. Gong, S. Chen, S. Li, Y. Zhang, H. Zhong, Z. Wang, Y. Chen, Q. Deng, Y. Jiang, L. Li, M. Fu, G. Yi, Comparative portrayal of ocular surface microbe with and without dry eye, J. Microbiol. 57 (2019) 1025–1032, https://doi.org/10.1007/s12275-019-9127-2.
[50] J.W.-K. Yau, J. Hou, S.K.W. Tsui, T.F. Leung, N.S. Cheng, J.C. Yam, K.W. Kam, V. Jhanji, K.L. Hon, Characterization of ocular and nasopharyngeal microbiome in

allergic rhinoconjunctivitis, Pediatr. Allergy Immunol. (2019), https://doi.org/10.1111/pai.13088.
[51] Y. Xia, J. Sun, Hypothesis testing and statistical analysis of microbiome, Gen. Dis. 4 (2017) 138–148, https://doi.org/10.1016/j.gendis.2017.06.001.
[52] Z.-Z. Tang, G. Chen, A.V. Alekseyenko, PERMANOVA-S: association test for microbial community composition that accommodates confounders and multiple distances, Bioinformatics 32 (2016) 2618–2625, https://doi.org/10.1093/bioinformatics/btw311.
[53] J.T. Nearing, A.M. Comeau, M.G.I. Langille, Identifying biases and their potential solutions in human microbiome studies, Microbiome 9 (2021) 113, https://doi.org/10.1186/s40168-021-01059-0.