Environmental Microbiome

RESEARCH Open Access

Investigating the core microbiome concept: *Daphnia* as a case study

Aditi Gurung[1], Martijn Callens[2], François Massol[3], Caroline Souffreau[1], Shinjini Mukherjee[4], Shira Houwenhuyse[5], Robby Stoks[6], Luc De Meester[1,7,8*] and Ellen Decaestecker[9*]

Abstract

Background Host-associated microbiomes play an important role in the ecology and fitness of organisms. Given their significance, it is much debated to what extent these associations are widespread and even obligatory. Such frequent associations are captured by the concept of the core microbiome. The cladoceran *Daphnia* is a pivotal genus in freshwater ecosystems occupying a central position in the food webs of standing waters. With its unique standing in pelagic waters, *Daphnia* serves as a key grazer, regulating algal populations and nutrient cycling, making its microbiome essential to understanding ecosystem function and stability. In recent years, *Daphnia* has become an increasingly popular study system for exploring host–microbiota interactions. There is, however, limited knowledge on the baseline taxa that consistently inhabit this host and potentially contribute to its fitness. Identifying whether such a host-associated "core microbiome" exists for *Daphnia* and, if so, which microbial taxa it comprises is important both for enhancing our ecological understanding of this genus and its ecosystem function and for interpreting future experiments.

Results We compiled a dataset on *Daphnia magna* microbiome based on 12 published studies, comprising gut and whole microbiome samples of both laboratory-cultured and field-grown animals across five countries spanning three continents. To identify core taxa, we employ quantification metrics based on prevalence and a combination of prevalence and relative abundance. Our analysis demonstrates that the *D. magna* microbiome is highly variable, yet, a consistent association with specific taxa, notably *Limnohabitans planktonicus*, is observed especially under laboratory conditions. However, this pattern is tempered by the observation that field-grown animals exhibit a more diverse microbiome with a weaker presence of *L. planktonicus*, challenging its status as a core member.

Conclusions Our analysis suggests that the *D. magna* microbiome is defined by its high variability and few conserved associations, with *L. planktonicus* being the most stable taxon in laboratory settings but not necessarily a core member in natural environments. These findings underscore the need for caution when using laboratory results to interpret natural microbiome compositions and emphasize the need for further research on field-grown animals to better understand the structuring of microbial communities under natural settings.

Keywords Core microbiome, *Daphnia*, *Limnohabitans planktonicus*

*Correspondence:
Luc De Meester
luc.demeester@kuleuven.be
Ellen Decaestecker
ellen.decaestecker@kuleuven.be

Full list of author information is available at the end of the article

© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

Background

All organisms host complex microbial communities, forming symbiotic relationships that often contribute to shaping host health and fitness [1]. These microbiomes have been shown to extend beneficial functions to the host, including nutrient uptake and digestion, production of essential amino acids, host immune system development and direct protection against pathogens, toxins and other environmental stressors [2]. To gain a deeper understanding of these interactions, the concept of the core microbiome was introduced. This concept aims to identify key microbial taxa (and their associated genes) that are characteristic of a specific environment or body site of a host and consistently inhabit this site across all or the vast majority of host individuals [3]. This is in contrast with transient or satellite species, whose frequency in the microbiome is more variable, resulting in a fraction of the microbiome that occurs depending on factors such as diet, host genetic background, physiological status and external environmental conditions [3, 4]. Although the original definition of the core microbiome focused on microbial members that were consistently shared among the vast majority of populations [3], the definition has evolved over the years to encompass different aspects, often identifying key microbes in terms of their spatial distribution, temporal constancy, ecological impact, and overall functional contribution to host well-being (see overview in Table S1, Supporting Information [SI]). These different definitions emphasize two key criteria, namely widespread occurrence and importance for host fitness, which has to some extent complicated the debate on the existence of core microbiomes. This also parallels the dual definition of "symbiosis" defined either by "persistent interactions in close physical association" (original definition) or by "mutualistic interactions in close physical association" (popular definition) which echoes the ongoing debate in microbiome research with regard to how core membership is defined [5]. Specifically, the core microbes defined can vary depending on whether the emphasis is placed on consistency and widespread occurrence across hosts (analogous to persistence in symbiosis) or on their functional contributions to host fitness (analogous to mutualistic interactions in symbiosis). Here, we use the original definition of the core microbiome defined by its consistent presence, rather than by its impact on host fitness. The core microbiome thus identified may contribute to host fitness, but here, we do not use it as a necessary criterion to identify a microbial taxon as a core microbiome member. A better understanding of the core microbiome of a host holds promise for understanding how the microbiome contributes to host biological functioning, helps dissect patterns of microbial colonization, factors shaping host–microbe interactions, and offers critical insights for experimental design allowing the selection of key strains that can serve as reliable indicators of host function and their response to environmental change.

The water flea *Daphnia* is a ubiquitous planktonic crustacean genus found in standing open waters worldwide, with a cosmopolitan distribution spanning all continents [6]. These generalist filter feeders occupy a central position in the food web of freshwater systems as effective grazers of phytoplankton and fish prey [7]. Owing to their convenient features for experimentation and their high responsiveness to both anthropogenic and abiotic (pollution, urbanization, climate warming, salinization [8–11]) as well as natural and biotic stressors (competitors, parasites, predators [12–14];), *Daphnia* species, especially *D. magna* and *D. pulex*, are common model systems in the fields of ecotoxicology, ecology, evolution, and eco-evolutionary dynamics [7, 15]. In recent years, *Daphnia* has also become popular as a model system to study host–microbiome interactions owing to its short life cycle, clonal reproduction, and ability to create germ-free animals that allow manipulation of the (gut) microbiome composition, thus providing a unique opportunity to study the interplay between host genotypes and their microbiomes. *Daphnia* is known to harbor microbial communities both on the surface of its body and in its gut [16], and this microbiome has been reported to affect *Daphnia* reproduction and survival [17, 18]. For example, the microbiome of *Daphnia* has been shown to contribute to the digestion of phytoplankton [19], tolerance against toxic cyanobacteria and heavy metals [1, 20, 21] and to provide the host with essential vitamins and amino acids [22]. However, very little is known about the identity of the microbial taxa that consistently inhabit the host. Some bacterial phyla that have been suggested to be consistently associated with *Daphnia* include Proteobacteria, Actinobacteria and Bacteroidetes [23]. Specific bacterial groups such as *Limnohabitans, Aeromonas*, *Ideonella*, *Pseudomonas*, *Pedobacter*, *Flavobacterium*, *Polaromonas*, Comamonadaceae and unclassified Burkholderiaceae have been regularly reported in association with *Daphnia* [24–26]. The degree to which these taxa consistently occur across individuals isolated from ecologically different habitats and form a stable association with the *Daphnia* microbiome is unknown. Although some earlier work suggested the presence of a core community in *Daphnia* microbiomes [23, 24, 27], a recent study has, however, suggested that *Daphnia* microbiomes may be more dynamic in nature, varying with the conditions of their external environment [28].

In this study, we explore the concept of the core microbiome and explicitly test whether a core microbiome occurs in the model organism *D. magna*. More specifically, we test whether *D. magna* is characterized by a microbiome comprising species shared by most

individuals or whether the microbiome is completely variable among individuals. To test for the existence of a core microbiome, we systematically analyzed an integrated dataset compiled from 12 published studies comprising sequences of the V4 hypervariable region of the 16S rRNA gene of both *D. magna* gut and whole microbiome samples of both laboratory and field origin across five countries.

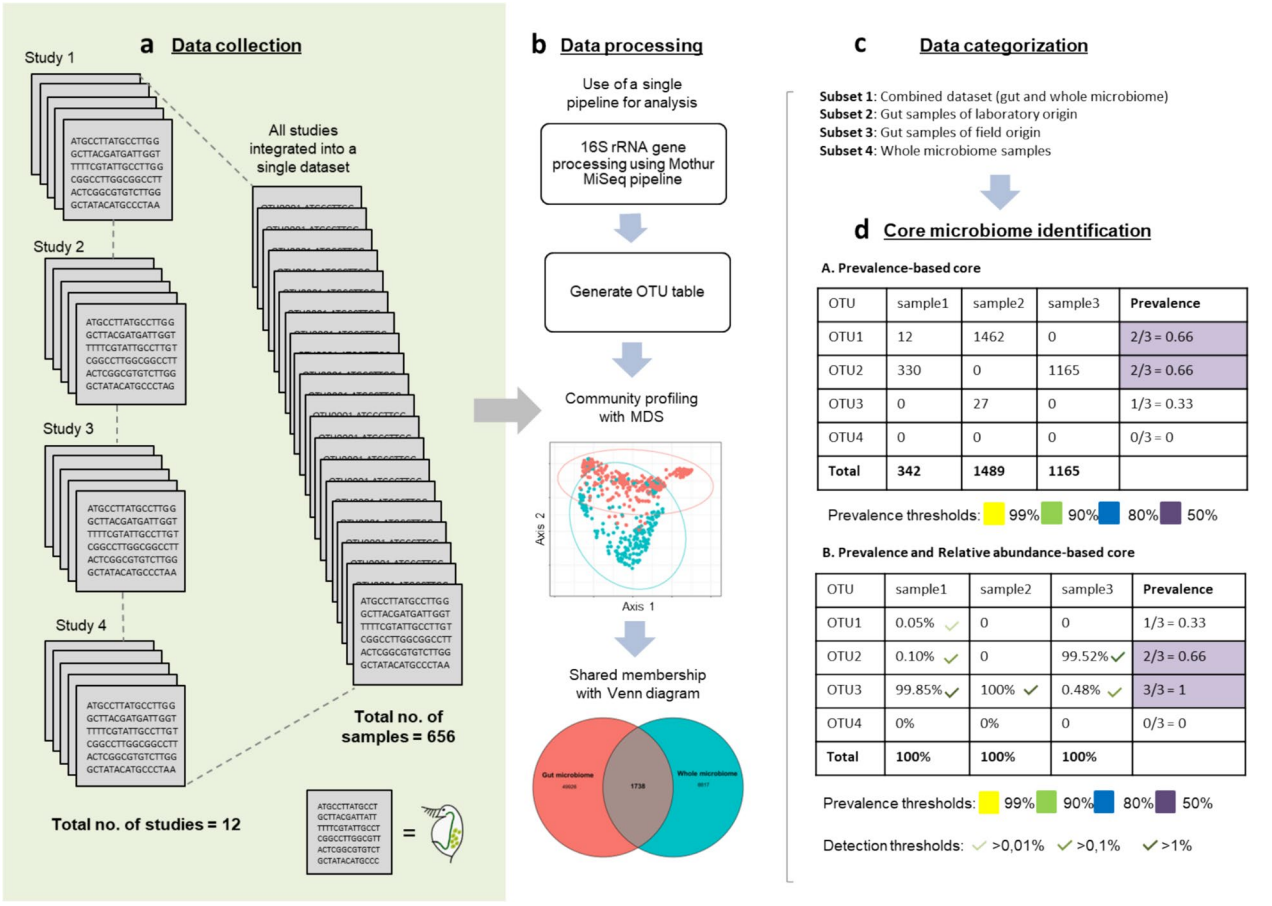
Methods

Search strategy

A total of 145 studies that span a period of 33 years (1989–2022) were identified in a literature search (performed in June 2022) in the NCBI PubMed and Google Scholar databases via the search terms "*Daphnia* microbiome" (57 hits), "*Daphnia* gut microbiome" (18 hits), "*Daphnia* microbiota" (51 hits), and "*Daphnia* gut microbiota" (19 hits). After removing duplicates and reviews and retaining only those papers with relevant 16S rRNA sequence-based data on *Daphnia* microbiomes (both whole-animal and gut samples), either from animals collected in nature or screened as part of experiments, 34 studies were retained (Figure S1, SI). From these studies, pertinent information such as *Daphnia* species and genotype, geographical origin of the *Daphnia* clone, and the sequenced variable region were compiled in a table (Table S2, SI). A second round of pruning (Figure S1, SI) was performed to retain only studies that used the 16S rRNA V4 hypervariable region for metabarcoding, as this region was the most frequently targeted across the dataset and allowed for data standardization via a single pipeline for analysis. As a result, from the 34 studies previously selected, 12 studies that reported on microbial community composition using the V4 region were retained. These 12 studies included a total of 656 samples originating from three continents (five countries; see Table S2, SI), involving the gut as well as whole-body microbiomes of host animals directly collected from their source habitat or grown in the laboratory. Of the 12 datasets, 11 examined the microbiome of laboratory-cultured animals, while one study included both laboratory and field-grown individuals. All field-grown animals in this study were from the same lake (Lake Bysjön, Sweden). All studies focused on the microbiome of *D. magna*. These studies were used in our analysis to identify the extent to which there is a core microbiome in this species. An overview of the analytical steps based on these 12 studies is visualized in Fig. 1.

Data standardization

To account for the variability in the different studies in terms of sequencing methods and taxonomic classification of the microbial taxa, data standardization was conducted. For all the 12 selected studies, raw sequences were obtained in fastq format from the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/home) using their specific BioProject/Accession numbers. These sequences were analyzed in mothur (v.1.48.0) using the MiSeq standard operating procedure [29] to maintain a consistent classification scheme. All sequences were first extracted from the fastq files. Using the *make.contigs* function, reverse reads were converted into their reverse complements and aligned to the forward reads to form contigs. A *screen.seqs* was run to maintain a maximum length of 250 bp (V4 region). Duplicate sequences were merged using the *unique.seqs* command. A customized reference alignment was created to maintain only the V4 hypervariable region. This was accomplished by trimming the SILVA reference database at a starting point of 11,895 bp and an end point of 25,318 bp (determined using the primers 515F and 806R) using the *pcr.seqs* command. Next, with the *align.seqs* command, the sequences were aligned to the customized reference alignment, and a *summary.seqs* was run to determine whether all the sequences were aligned securely. The *screen.seqs* data were rerun with a start at 1968 bp and an end of 11550 bp to ensure that all the sequences overlapped in the same V4 variable region. All overhangs at both ends following alignment were removed with the *filter.seqs* command. A second *unique.seqs* was run to identify duplicates and merge them together. All the sequences were subsequently split by group, allowing differences of up to 2 nucleotides, using the *pre.cluster* command and sorted by abundance. Chimera removal was carried out using the *vsearch* executable (*chimera.vsearch*). The remaining sequences were classified with the Bayesian classifier using the *classify.seqs* commands. Sequences from undesired 16S rRNA gene fragments arising from chloroplasts and mitochondria, Archaea or other unknown groups were removed with *remove.lineage*. The sequences were then clustered into operational taxonomic units (OTUs) using the *cluster.split* command at a cutoff value of 0.03, thereby allowing sequence clustering on the basis of a 97% similarity threshold [29]. This threshold was set to facilitate comparison across studies; we opted for this approach instead of using amplicon sequence variants (ASVs), which resolve sequences to single nucleotide differences and may overestimate diversity and underestimate shared taxa. As a last step, the *make.shared* and *classify.otu* commands were run to make an OTU count table with corresponding taxonomic information. Additionally, to identify the most important OTUs, a species-level identification was carried out. FASTA sequences of these OTUs were extracted using the *otu.rep* function in mothur, specifying the method abundance. The identity of the sequences was then determined with a BLAST search in the rRNA/ITS databases for 16S rRNA gene sequences (Bacteria and Archaea) of NCBI, optimized for



OTU	sample1	sample2	sample3	Prevalence
OTU1	12	1462	0	2/3 = 0.66
OTU2	330	0	1165	2/3 = 0.66
OTU3	0	27	0	1/3 = 0.33
OTU4	0	0	0	0/3 = 0
Total	**342**	**1489**	**1165**	

OTU	sample1	sample2	sample3	Prevalence
OTU1	0.05% ✓	0	0	1/3 = 0.33
OTU2	0.10% ✓	0	99.52% ✓	2/3 = 0.66
OTU3	99.85% ✓	100% ✓	0.48% ✓	3/3 = 1
OTU4	0%	0%	0	0/3 = 0
Total	**100%**	**100%**	**100%**	

Fig. 1 Schematic overview of the analysis starting from the 12 selected studies. **a** Data collection: Raw fastq files from 12 published studies were collected and integrated to form a single dataset comprising a total of 656 *Daphnia* microbiome samples. **b** Data processing: This dataset was processed using the mothur MiSeq SOP pipeline to generate an OTU table which was subsequently used for community profiling and data categorization. **c** Data categorization: Based on the preliminary analysis, the dataset was categorized into the four subsets described. **d** Core microbiome identification: Core species in the *D. magna* microbiome were determined for both combined and separate subsets of gut and whole-animal microbiomes of laboratory and field origin. Metrics used to identify core taxa were based on prevalence only or a combination of prevalence and relative abundance (See Methods for details)

highly similar sequences (megablast). Identification was based on the highest maximum score, > 98% identity and the lowest E value.

Data exploration and grouping of samples for analysis

Because our dataset consisted of samples from different countries, sample types (gut and whole animal samples) and origins (laboratory vs field), a multidimensional scaling (MDS) based on Bray–Curtis distances was performed using the *ordinate* function from the *phyloseq* package in R [30] to explore and visualize whether these different groups differed in community composition (Fig. 2). Significant differences between the communities were determined with a permutational multivariate analysis of variance (PERMANOVA), using the *adonis2* function of the *vegan* package in R [31, 32] specifying *by* = *"margin"* to calculate type III sum of squares. Given the results of this exploratory analysis, in addition to an analysis of the complete dataset, core microbiome species were also identified for different subsets of the data, which resulted in the following hierarchical categorization. Next to the (1) *combined dataset,* comprising samples sourced from both guts and whole-animals of both laboratory and field origin across all five countries, we also analyzed the subset of (2) *gut samples,* comprising all samples sourced from host guts. The latter dataset was further divided into (2a) *gut samples of laboratory origin only* and (2b) *gut samples* of *field origin only*. In the latter dataset, all host individuals were retrieved from the same population. Finally, we separately analyzed (3) *whole-animal samples,* comprising all samples of the full microbiome. This subset consisted of only laboratory-based samples, as the dataset did not contain whole-animal microbiome samples from field animals. To visualize the degree to which taxa were shared among countries, between field and laboratory origins or between whole-body and gut samples, Venn diagrams were generated

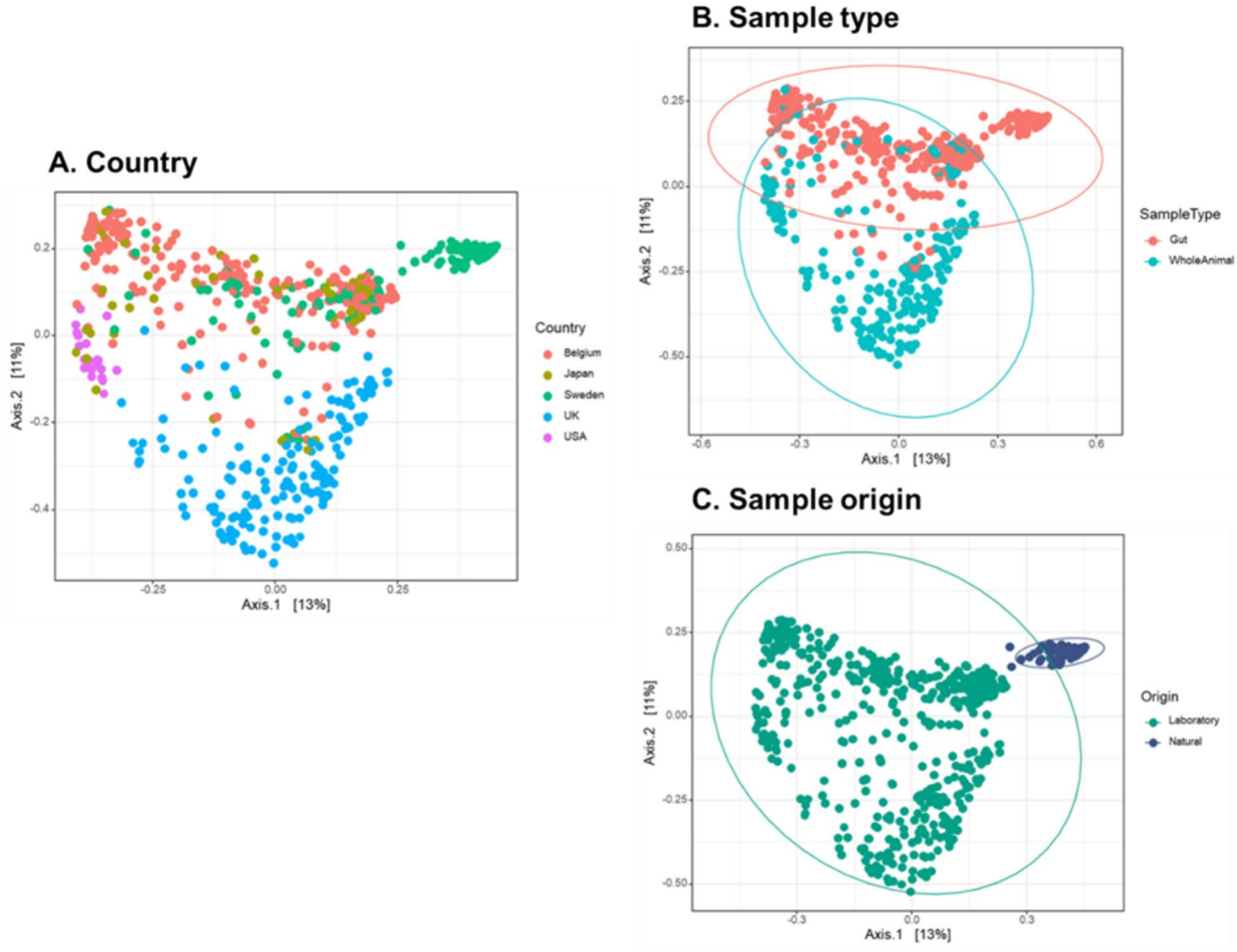


Fig. 2 Multidimensional scaling (MDS) of the microbiome data of all samples based on Bray Curtis dissimilarity index illustrating differences in microbial communities between **A** Countries, **B** Sample type: whole-body versus gut and **C** Sample origin: laboratory-cultured versus hosts grown in the field. Distances between points represent dissimilarities in microbial community composition, with closer points indicating more similar microbiomes as a function of the first two axes in multidimensional space that capture most (in total 24%) of the variation in the dataset. All field-grown animals were retrieved from the same lake

using the *eulerr* package [33] in R (version 4.0.5, R Core team 2021-03-31).

Statistical analyses

All statistical analyses were carried out using the *microbiome* [34], *vegan* [31] and *phyloseq* package [30] in R (version 4.0.5, R Core team 2021-03-31). Raw counts were normalized to relative abundances through total sum scaling (TSS) using the *microbiome* package, to account for variation in library size across samples. To avoid potential noise, samples with less than 1000 reads were discarded. Core/unique members were detected using two quantification metrics, namely a (i) prevalence-based metric and a (ii) metric combining prevalence and relative abundance (as explained below; also Fig. 1). Given that fine-scale data such as operational taxonomic units (OTUs) can often incorporate potential 'noise' in metabarcoding data, the core identification metrics were also analyzed at higher taxonomic ranks (phylum and order levels) via the agglomeration function of the *phyloseq* package *tax_glom*. In this study, we present results from the perspective of a community composition-based analysis and define core members in *Daphnia* from a "common core" standpoint, i.e. shared and recurrent co-occurrences of host and microbiota (Table S1, SI).

Core quantification metrics

Prevalence-defined core taxa

A prevalence-based metric was used to identify microbial taxonomic groups that were widespread and consistently present in all samples or across a preset percentage of *D. magna* samples, irrespective of their relative abundance in the communities. For this metric, a prevalence threshold was set to define the percentage of samples (i.e., host individuals) in which the taxa had to be found in order to be considered a member of the core microbiome. We

used 50%, 80%, 90% and 99% as prevalence thresholds; 99% was used to allow for a by-chance absence of a taxon that is otherwise present in all samples. The *core* function of the *microbiome* package [34] was used for the analysis.

Abundance and prevalence-based core taxa

Prevalence is a good metric to assess whether a given taxon is widespread among hosts, but it poorly reflects its relative dominance within the microbiome of its host. To gauge this, the relative abundances of taxa comprising the host microbiome can be used. However, in our study, relative abundance was not used as a sole criterion for core identification, given that this metric only states whether a given taxon dominates the microbiome when it is present, but not whether it occurs in all or many host individuals. While an approach based on relative abundance could highlight abundant taxa, it would represent the selection of core taxa from a single or few samples that rather reflect condition-specific enrichment and not necessarily consistent dominance across samples. Instead, a combined abundance-prevalence-based metric with a double criterion was used, namely, through a minimum relative abundance threshold and a minimum prevalence threshold. The prevalence threshold was used to include only taxa present in a defined proportion of the samples, whereas the relative abundance detection threshold was used as a criterion to only pick those that met a minimum relative abundance threshold in the host individuals in which this taxon was present. Taxa that were sufficiently widespread with a sufficiently high relative abundance were identified as core members of the microbiome. We used all combinations of the above prevalence thresholds (50%, 80%, 90% and 99%) and three thresholds for relative abundance (10^{-4} or 0.01%; 10^{-3} or 0.1%; 10^{-2} or 1%) as criteria to identify core members of the microbiome. Higher thresholds for relative abundances did not result in the identification of any core member of the microbiome at the selected prevalence threshold range.

Table 1 Results of PERMANOVA, based on Bray Curtis Beta diversity metric assessing the amount of variation in microbiome community composition that can be explained by the categorization variables- country, sample type (gut vs. whole microbiome) and sample origin (laboratory vs. field samples)

Categorization variable	Df	SumofSq	R^2	F	Pr (> F)
Country	4	40.822	0.15972	33.3982	0.001 ***
Sample type	1	2.239	0.00876	7.3266	0.001 ***
Sample origin	1	14.818	0.05798	48.4937	0.001 ***
Residual	647	197.703	0.77354		

Df: degrees of freedom associated with the effect; SumofSq: inertia of the dissimilarity matrix explained by the effect; R^2: squared coefficient of determination (obtained from the inertia explained by the effect and the total inertia of the dissimilarity matrix); F: F-statistic associated with the effect; Pr (> F): *p*-value associated with the sequential permutational F-test, based on permutation of the raw data. ***Asterisks indicate significance values at $p < 0.001$

Results

Available data

Considering all the samples derived from both the gut and whole animals, irrespective of country of origin and whether they were laboratory-cultured or field-sampled, a total of 656 samples were obtained, comprising 423 gut samples and 233 whole animal samples. A total of 25,166,733 sample read counts were available for analysis, which represented 142,150 bacterial operational taxonomic units (OTUs) among 1061 genera belonging to 32 phyla. After removing singletons, i.e., OTUs that had only one read count across all samples combined, and excluding samples with fewer than 10 total reads, 25,083,005 sample read counts remained representing 58,441 OTUs across 654 samples (Table 2).

Hierarchical categorization of datasets

MDS plots revealed distinct clustering patterns in the microbiome community composition across the total dataset (Fig. 2). Samples from the five countries formed five overlapping clusters with notable separation of United Kingdom and Sweden compared to the rest (Fig. 2A). A relatively clear distinction was observed between gut and whole animal samples (Fig. 2B), and a complete separation between samples from laboratory-cultured and field-grown animals (Fig. 2C). PERMANOVA results revealed that country had a significant effect on microbial composition (pseudo-F = 33.398, $p < 0.001$) explaining 15.97% of the observed variance in the microbiome composition across the combined dataset (Table 1). Likewise, sample type i.e., gut versus whole body and sample origin i.e., laboratory versus field also significantly influenced microbial community composition accounting for 0.87% (pseudo-F = 7.326, $p < 0.001$) and 5.79% (pseudo-F = 48.493, $p < 0.001$) of the observed variation in the total dataset, respectively (Table 1). Interaction terms produced undefined results (SumofSq = 0, $R^2 = 0$, pseudo-F = -Inf), indicating insufficient variation or data sparsity in these combinations. As a result, interaction terms were not interpretable and thus the model focused on main effects to explain the variation in the dataset.

To further examine whether methodological differences among studies could contribute to the observed clustering by country, an additional PERMANOVA was performed, where both country and study and their interaction effects were tested. Both variables significantly explained ~ 16% of the observed variation (Country: pseudo-F = 39.305, $p = 0.001$; Study: pseudo-F = 17.94, $p = 0.001$) in microbiome composition (Figure S2, Table S3, SI), suggesting that part of the clustering by country may reflect study specific methodological variation, such as the type of DNA extraction kits used.

Shared taxa among categories

To explore the unique and shared fractions of taxa among the different categories in the combined dataset, a Venn diagram was constructed to visualize shared membership between samples of the different countries, sample types (gut versus whole-body) and sample origin (laboratory-cultured versus field-grown). Overall, a low number of taxa were observed to be shared between categories (Fig. 3A). From a total of 58,441 OTUs present in the total dataset across five countries (Belgium, Sweden, Japan, UK and USA), only 106 OTUs (0.18%) were common to all countries. Similarly, there occurred only 1738 OTUs (2.97%) shared between gut and whole-body microbiome samples (Fig. 3B). A comparison of *D. magna* microbiomes of laboratory and field origin revealed that there was an overlap of 2383 OTUs (4.07%) (Fig. 3C). This reflects a high variability in microbiome composition among hosts derived from different settings, and suggests that a more detailed analysis focusing on specific categories is warranted.

Core microbiome taxa in the combined dataset

No phylum or order met the criterium of occurring in 99% of the samples in this dataset. The phyla Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes occurred in 90% of the samples. At the order level, these were members of Burkholderiales, Chitinophagales, Flavobacteriales and Rhizobiales that occurred in 90% of the samples. The same phyla and orders as mentioned for the prevalence criterium also met the combined criteria of 10^{-4} relative abundance and 90% prevalence. Using the more stringent threshold of 10^{-2} relative abundance at 90% prevalence, only a single phylum, Proteobacteria, and a single order, Burkholderiales, were retained. Table S4 in SI lists all taxa for the various combinations of relative abundance and prevalence thresholds used.

At the OTU level, no taxon was detected at a strict prevalence threshold of 99%. At the 90% prevalence threshold, one particular OTU (OTU1), labeled as *unclassified Comamonadaceae* belonging to the class Betaproteobacteria and family Comamonadaceae, was detected. This OTU was identified as *Limnohabitans planktonicus* in a BLAST search using the rRNA/ITS

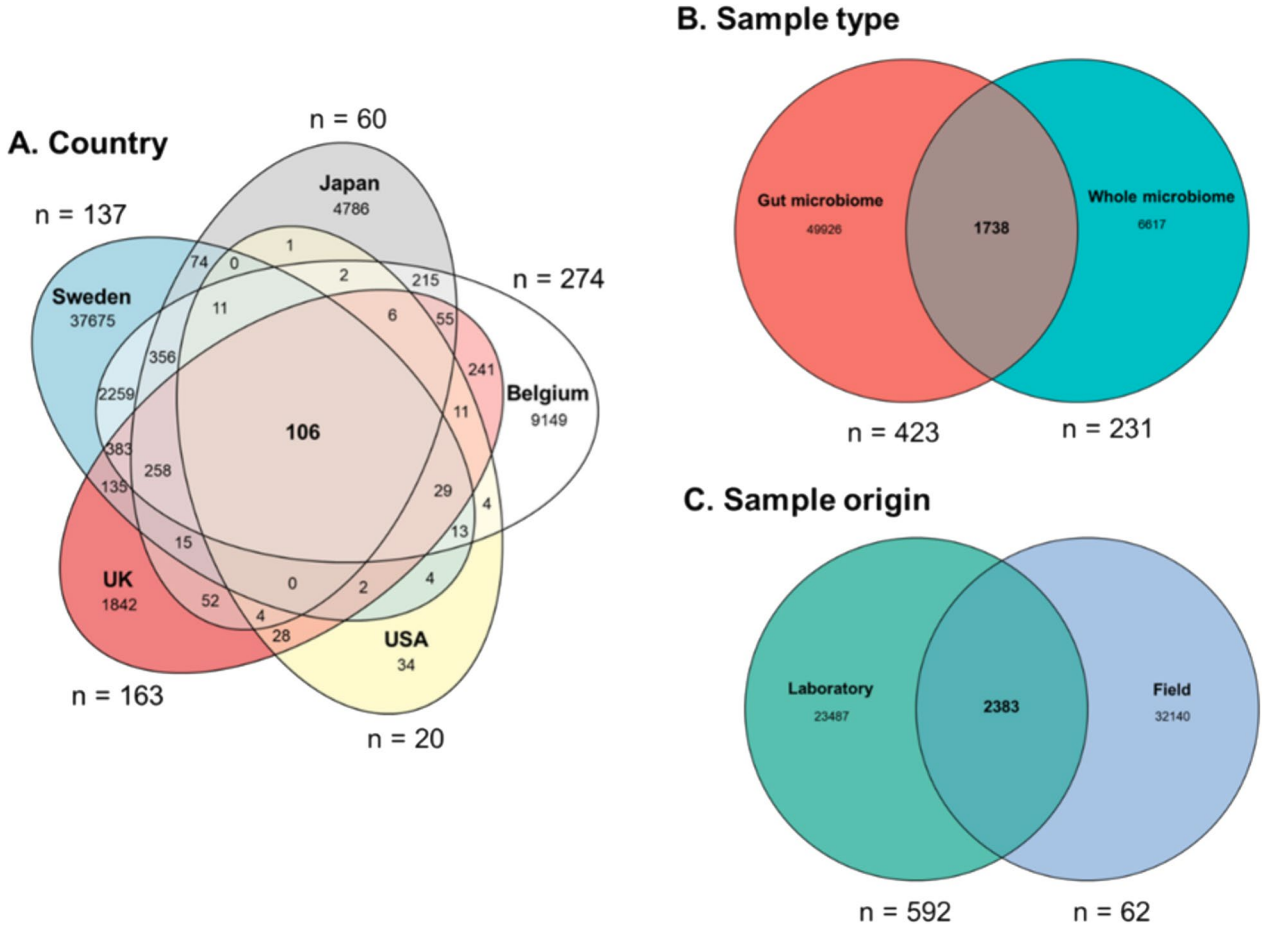


Fig. 3 Venn diagrams representing shared membership between **A** countries, **B** sample type (gut and whole-animal samples), and **C** sample origin (laboratory and field origins)

databases for 16S rRNA sequences in NCBI. At the 80% prevalence threshold, 6 OTUs were identified, including two additional members of the family Comamonadaceae identified as *Variovorax* sp. and *Ideonella paludis, Escherichia/Shigella* sp., *Staphylococcus* sp. and unclassified Chitinophagaceae. Further lowering the prevalence threshold to 50% resulted in the detection of 27 taxa representing eight members of unclassified *Comamonadaceae,* two *Flavobacterium* sp., *Formosimonas* sp., *Acinetobacter* sp., unclassified *Chitinophagaceae, Pseudomonas* sp., unclassified *Rhodobacteriaceae, Escherichia/Shigella* sp., *Shinella* sp., *Polynucleobacter* sp., *Emticicia* sp., *Streptococcus* sp., unclassified *Microbacteriaceae, Staphylococcus* sp., *Bosea* sp., *Hydrogenophaga* sp., *Brevundimonas* sp. *Sphingomonas* sp. and *Bradyrhizobium* sp.. Combining the relative abundance and prevalence thresholds at the level of OTUs, it was observed that only *Limnohabitans planktonicus* (OTU1) met the combined threshold of 10^{-4} relative abundance and 90% prevalence. Other relatively abundant taxa (threshold 10^{-4}) that met the 50% prevalence threshold were unclassified members of the families *Comamonadaceae*, *Chitinophagaceae, and Rhodobacteraceae* and the genera *Formosimonas, Pseudomonas, Escherichia/Shigella, Shinella, Emticicia, Streptococcus* and *Staphylococcus.* At a relative abundance threshold of 10^{-3} and a prevalence threshold of 90% and 80%, only *Limnohabitans planktonicus* was detected. At the highest relative abundance threshold of 10^{-2}, *Limnohabitans planktonicus* was detected in only 50% of the samples.

Core microbiome taxa in the gut microbiome

The results of the core microbiome analysis (prevalence-based and combined criteria of relative abundance and prevalence) on all gut microbiome samples were nearly identical to those of the combined dataset detailed above, and are given in SI Sect. 1. Below, we discuss the separate core microbiome analysis of gut microbiome samples of laboratory-cultured and field-grown animals.

Core microbiome in the gut communities of laboratory origin

This subset consisted of 361 *D. magna* gut samples of laboratory origin, representing 19,030 OTUs belonging to 763 genera and 26 phyla (Table 2). Similar to previous results on the combined dataset, higher-order analysis at the "phylum" and "order" levels revealed that the most prevalent phyla across 90% of the samples were Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes and the most prevalent orders- Burkholderiales, Chitinophagales, Flavobacteriales, Pseudomonadales, Enterobacterales, Rhizobiales and Micrococcales. The same phyla were also selected based on the combined criteria of prevalence and relative abundance at a detection threshold of 10^{-4} across 90% of the samples. The phyla Betaproteobacteria and Bacteroidetes met the more stringent threshold of 90% prevalence and 10^{-3} relative abundance, and only Betaproteobacteria occurred at 90% prevalence and 10^{-2} relative abundance.

At the OTU level, *Limnohabitans planktonicus* (OTU1) exhibited widespread occurrence being identified across almost all samples at 99% prevalence. Other prevalent bacterial taxa identified included *Escherichia/Shigella sp.* (OTU13) and *Ideonella paludis* (OTU8), which were found across 90% of the samples. Taxa identified at 80% prevalence included *Staphylococcus* sp., *Variovorax* sp., *Ideonella paludis*, *Pseudomonas* sp., *Streptococcus* sp., *Polynucleobacter* sp., and unclassified *Microbacteriaceae* and *Chitinophagaceae*. Based on the combined prevalence and relative abundance metrics, *Limnohabitans planktonicus* was identified at 99% prevalence and an abundance threshold of 10^{-4}. For the same detection threshold, *Escherichia/Shigella* sp. was identified at 90% prevalence, whereas *Ideonella paludis* and *Staphylococcus* sp. were detected when the prevalence threshold was lowered to 80%. Using a relative abundance threshold of 10^{-3}, *Limnohabitans planktonicus* was the only taxon identified at both 90% and 80% prevalence. At the same detection threshold, taxa identified at 50% prevalence included *Formosimonas* sp., *Variovorax* sp., *Ideonella paludis, Pseudomonas* sp., *Polynucleobacter* sp., *Staphylococcus* sp. and unclassified *Microbacteriaceae, Chitinophagaceae, Rhodobacteraceae* and *Micrococcales*. At 10^{-2} *Limnohabitans planktonicus* was identified as a core member, and at a 80% prevalence threshold only.

Core microbiome in the gut communities of field origin

This subset was constituted from 62 gut samples originating from animals directly sampled from the same lake and comprised a total of 34,523 OTUs belonging to 493 genera and 22 phyla (Table 2). The most prevalent phyla, present across 99% of the samples in this dataset, included Proteobacteria, Plantomycetes, Verrucomicrobia, Bacteroidetes, Actinobacteria, Firmicutes and unclassified Bacteria. The same high level of prevalence was observed for the orders Burkholderiales, Pirellulales, Rhodobacterales, unclassified Verrucomicrobia, Solirubrobacterales, Rhizobiales, Xanthomonadales, Sphingomonadales, Planctomycetales, Mycobacteriales, unclassifiedBacteria, Acidimicrobiales, unclassified Actinobacteria, Verrucomicrobiales, Bacillales, Rhodospirillales, Micrococcales, Propionibacteriales, unclassified Alphaproteobacteria, unclassified Planctomycetacia, Clostridiales, unclassified Gammaproteobacteria, Legionellales, and unclassified Proteobacteria. With the double criterion combining prevalence and relative abundance, the phyla detected at a threshold of 10^{-4} in 99% of the samples included Planctomycetes, Proteobacteria and Actinobacteria, which

Table 2 Summary information of the sample subsets used in the study and the number of core taxa assigned to each subset based on the core quantification metric employed

Characteristics	Dataset				
	Combined dataset	Gut samples	Gut samples (Lab origin)	Gut samples (Field origin)	Whole animal samples (lab origin)
Total no. of samples	656	423	361	62	233
Total no. of reads	25,166,733	14,743,383	11,148,436	3,594,947	10,423,350
Total no. of singletons	83,709 (58.88%)	64,836 (55.65%)	15,352 (44.65%)	51,753 (59.98%)	20,013 (70.54%)
Total no. of OTUs detected	142,150	116,500	34,382	86,276	28,508
Total phyla	32	29	28	22	26
Total genera	1061	957	820	566	653
After removing singletons and low read samples					
Total no. of samples	654	423	361	62	231
Total no. of reads	25,083,005	14,678,547	11,133,084	3,543,194	10,403,318
Total no. of OTUs	58,441	51,664	19,030	34,523	8,355
Total phyla	32	29	26	22	26
Total genera	962	865	763	493	620
Core Metric	**Core size: No. of taxa assigned to core**				
Prevalence-based					
99%	0	0	1	27	0
90%	1	1	3	86	5
80%	6	6	10	153	13
50%	27	31	34	527	57
Prevalence-relative abundance					
0.0001 (0.01%)					
99%	0	0	1	15	0
90%	1	1	2	32	3
80%	1	2	4	50	6
50%	13	18	25	143	40
0.001 (0.1%)					
99%	0	0	0	4	0
90%	1	0	1	5	1
80%	1	1	1	13	1
50%	6	6	10	30	17
0.01 (1%)					
99%	0	0	0	1	0
90%	0	0	0	2	0
80%	0	0	1	3	1
50%	1	1	1	8	4

belong to the orders Pirellulales, Rhodobacterales, Solirubrobacterales, Mycobacteriales, Rhizobiales, Acidimicrobiales and unclassified Actinobacteria. At a detection threshold of 10^{-3} and 99% prevalence, these included the orders Pirellulales, Rhodobacterales, and Solirubrobacterales. At the most stringent threshold of 10^{-2} at 99% prevalence, only the phylum Planctomycetes, comprising the order Planctomycetacia, was detected.

At the OTU level, the taxa that were widespread were quite diverse, with 27 different taxa detected at 99% prevalence and 153 and 527 taxa detected at prevalence thresholds of 80% and 50%, respectively (Table S4, in the SI). *Limnohabitans planktonicus* was only detected at a prevalence threshold of 80%. The combined relative abundance and prevalence metric revealed 15 dominant taxa at relative abundance thresholds of 10^{-4} and 99% prevalence. These were unclassified *Pirellulales* (OTU 7, OTU 43, 87, 336), unclassified *Rhodobacteraceae* (OTU 11; OTU 71, OTU 75), unclassified *Solirubrobacterales* (OTU 21), *Mycobacterium sp*. (OTU 74), unclassified *Rhizobiales* (OTU 98), unclassified *Pirellulaceae* (OTU 127), *Ilumatobacter* (OTU 138), unclassified *Actinobacter* (OTU 147, OTU 197), and unclassified *Acidimicrobiales* (OTU 182). Likewise, 50 and 143 taxa were detected for the same relative abundance threshold at 80% and 50% prevalence, respectively (see Table S4 in the SI). At a relative abundance threshold of 10^{-3}, 4 taxa were detected at 99% prevalence, namely, unclassified *Pirellulales* (OTU 7, OTU 43), unclassified *Rhodobacteraceae* (OTU 11) and unclassified *Solirubrobacterales* (OTU 21). At a detection

threshold of 10^{-2}, only unclassified *Pirellulales* (OTU 7) remained at 99% prevalence. Unclassified *Solirubrobacterales* (OTU 21) and unclassified *Rhodobacteraceae* (OTU 11) were detected at only 90% prevalence.

Core microbiome in whole-animal samples of laboratory origin

For the whole-animal samples, only samples of laboratory origin were available. This subset comprised a total comprised 8355 OTUs belonging to 620 genera and 26 phyla from 231 whole animal samples (Table 2). When evaluating prevalence at the phylum and order level, the phyla Proteobacteria and Bacteroidetes occurred at 90% prevalence, as did the orders Burkholderiales, Chitinophagales, Flavobacteriales and Cytophagales. Using the double criterion combining prevalence and relative abundance, the phyla Proteobacteria and Bacteroidetes and their respective orders Betaproteobacteria and Chitinophagales met the relative abundance criterium of 10^{-4} and the prevalence criterium of 90%. Only Proteobacteria met the relative abundance threshold of 10^{-3} and 90% prevalence, whereas at the 10^{-2} relative abundance threshold, this phylum was detected only at 80% prevalence.

The OTUs *Formosimonas*, *Limnohabitans planktonicus*, unclassified *Chitinophagaceae*, *Flavobacterium* and *Emticicia* sp. met the 90% prevalence criterion, while *Variovorax* sp., *Brevundimonas* sp., *Bosea*, *Shinella*, *Escherichia/Shigella* sp., *Sphingorhabdus* sp. and unclassified *Comamonadaceae* and *Rhodobacteraceae* met the 80% prevalence criterion. Using the 50% prevalence threshold, 57 taxa were identified (see SI, Table S4). *Limnohabitans planktonicus*, *Formosimonas* sp. and unclassified *Chitinophagaceae* were the three OTUs that met both the 10^{-4} relative abundance and 90% prevalence criteria, while *Flavobacterium* sp. *and Emticicia* sp. met the 10^{-4} relative abundance and 80% prevalence criteria. When the relative abundance threshold was further increased to 10^{-3} and 10^{-2}, *Limnohabitans planktonicus* was detected only at 90% and 80% prevalence threshold, respectively.

Discussion

In this study, we aimed to assess the extent to which the microbiome of the water flea *Daphnia*, and more specifically *D. magna*, harbors a set of highly prevalent and abundant taxa that can be considered a core microbiome, and identify the taxa that constitute that core. To that end, we compiled data on *D. magna* microbiomes across 656 individuals from 12 published datasets (Table S2, SI) targeting the V4 hypervariable region of the16S rRNA gene. Core taxa were quantified based on prevalence thresholds and a combination of prevalence and relative abundance thresholds. Overall, our findings indicate that a large proportion of the microbiome in *D. magna* displays very variable prevalences and relative abundances, with only a low number of taxa (1–5) that are widespread (90% prevalence) and moderately abundant, as also observed in other organism groups [35]. Of these, *Limnohabitans planktonicus* of the order Burkholderiales (phylum Proteobacteria) stands out as the most commonly observed taxon especially in laboratory-cultured samples. A key observation is that the gut microbiome of field-grown animals consistently differs from laboratory-cultured animals and does not feature *L. planktonicus* as the most prevalent and abundant taxon, even though it is still quite widespread. We discuss these results in the following paragraphs and highlight implications of the notable differences between microbiome composition of field-grown and laboratory-cultured animals.

The most prevalent microbiome phyla in *D. magna*, occurring in at least 90% of the gut as well as whole-animal samples, included Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes. These phyla were mainly represented at high prevalence by the orders Burkholderiales, Chitinophagales, Flavobacteriales and Rhizobiales. These phyla were shared among the microbiomes of laboratory-cultured *D. magna*, irrespective of their geographical origin or sample type (i.e., gut or whole animal samples) (Figure S3, SI). The gut microbiome samples of field origin were, however, more diverse than the gut microbiomes of laboratory-cultured animals, and displayed high prevalence of the phyla Planctomycetes, Verrucomicrobia and unclassified Bacteria, with the key orders being Pirellulales, Rhodobacterales, Solirubrobacterales, Rhizobiales, Sphingomonadales, Planctomycetales, Mycobacteriales and Acidimicrobiales. Houwenhuyse et al. [36] similarly reported that, upon bringing field-collected *Daphnia* to the laboratory, the diversity of the microbiome decreases strongly. This reduction in diversity may likely be a consequence of the relatively constant and homogenous culture conditions maintained in the laboratory, with a stable temperature, light:dark regime, pH of the culture medium and a simplified and standardized diet. This phenomenon has also been observed in laboratory-cultured tardigrades, whose microbial communities are predominantly composed of bacterial genotypes originating from food, media, or laboratory reagents [37]. This is in contrast to field environments, where *Daphnia* indiscriminately filter feed on a diverse and assorted range of bacterioplankton communities further broadening the range of microbial taxa available for ingestion and subsequent gut colonization.

Overall, the phylum Proteobacteria constituted the majority (67.85%) of the bacterial sequences in the combined dataset containing both gut and whole animal samples. On average, 38.99% of all detected Proteobacteria sequences in the combined dataset belonged to the family Comamonadaceae. Likewise, this family constituted, on average, 40.52% of the bacterial sequences in the *D.*

magna gut samples and 37.15% of those in the whole microbiome samples. This is in line with other studies that sequenced other parts of 16S rRNA gene hypervariable regions and are therefore not included in the current integrated analysis [16, 23, 24, 38, 39]); in these studies members of Comamonadaceae were observed to be dominant taxa in the microbiome of *Daphnia*. At the OTU level, one single taxon, namely *Limnohabitans planktonicus* (OTU1) belonging to the family Comamonadaceae stands out in terms of prevalence and relative abundance. This pattern in particularly evident in laboratory-cultured animals where this taxon is consistently identified as the most common and abundant taxon in all data-sets of laboratory origin, even though the host individuals were cultured in five independent laboratories in five different countries. In invertebrates, microbiomes are often dominated by only a few species [40–43]. Even species originating from microbially diverse environments, such as soil and marine waters, have been shown to selectively filter environmental microbes and exhibit simple microbiomes that are dominated by one-species associations [44, 45]. A number of vertebrates also have rather species-poor microbiomes [46–48]. For instance, many avian and mammalian microbiomes are dominated by Firmicutes, particularly by the genera *Staphylococcus* and *Streptococcus*, and Proteobacteria [46, 49, 50]. Likewise, gut microbiomes of terrestrial mammals also display a high proportion of *Streptococcus* and Proteobacteria [47, 51].

The genus *Limnohabitans* may be common in the *Daphnia* gut microbiome either due to its potential contribution to host fitness or its dominance in freshwater systems, where it typically comprises 5–30% (up to 50%) of the bacterioplankton communities in non-humic freshwaters [52]. *Limnohabitans* belongs to the R-BT lineage of the class Betaproteobacteria. This genus has been repeatedly reported on body parts such as the external surface, digestive tract, and filtering apparatus of *Daphnia* [22, 24, 27, 38, 53]. Since the natural substrates for the R-BT lineage are autochthonous algae, as witnessed by their enrichment during heavy phytoplankton blooms [52, 54] and on algal extracellular exudates [55], the *Daphnia* gut emerges as an ideal habitat for *Limnohabitans*. The observation that the genus was especially widespread in all laboratory-cultured animals suggests strong selection pressure favoring this taxon. Given that laboratory-cultured *Daphnia* are generally fed with unicellular algae cultured at high densities, it is possible that these algae provide an ideal substrate for *Limnohabitans* to reach high densities in the culture medium. Laboratory culturing, often a preparatory stage to microbiome sampling, has often been shown to change the microbial community structure [36, 56, 57] and increase the abundance of parasitic and commensal microbes [58]. In our study, we observe the high prevalence and dominance of *Escherichia/Shigella* sp., *Ideonella paludis, Staphylococcus* sp., *and Pseudomonas* sp. in the *D. magna* gut of laboratory origin. *Pseudomonas* sp. are often opportunistic bacteria [59] that have been reported in the microbiome of *Daphnia* immediately before death, in what was termed a "sit and wait (SAW) strategy", where these species wait and proliferate during vulnerable stages of the host [60]. An increase in the abundance of *Pseudomonas* sp. has also been reported during antibiotic treatment [26], at which occasion they benefit from lower microbial diversity and a lack of competition. The strong association of *Escherichia sp*. with laboratory-reared *D. magna* reported is in contrast with the findings of Di Cesare et al.,[61], who found *E. coli* only to occur temporarily in the *Daphnia* gut, where the species potentially seek refuge during periods of unfavorable environments [61].

Laboratory studies have shown that the presence of *Limnohabitans* can contribute to host fitness. For instance, inoculation of germ-free animals with *Limnohabitans planktonicus,* strain II D5 and strain DM1 has been shown to restore fecundity in individuals that would otherwise fail to mature or produce short-lived and non-viable juveniles [25]. In another study, an increase in host population size was linked to the dominance of *Limnohabitans sp*. in the *Daphnia* microbiome [17]. *Limnohabitans planktonicus* has also been reported to be an epibiont inhabiting the filter combs of *Daphnia,* where it may play an important role in the transfer of dissolved organic carbon to higher trophic levels in freshwater systems [53]. The functions underlying the beneficial *Limnohabitans–Daphnia* association remain largely elusive, yet recent studies suggest plausible mechanisms. For instance, the metabolic versatility encoded within the *Limnohabitans* genome [22] may facilitate the utilization of a broad spectrum of organic molecules, both within the digestive tract and on the carapace of the host. This may be especially important for non-selective generalist filter feeders such as *Daphnia*, which ingest a diverse array of particulate matter with varying organic matter composition. Moreover, it has been shown that the *Limnohabitans* genome harbors genes for arginine and threonine export, key amino acids crucial for *Daphnia* fitness [62, 63]. Finally, an analysis of annotated genes and functional pathways in *Limnohabitans* also revealed the presence of antibiotic resistance and detoxification genes, secretory and regulatory pumps and osmotic stress response systems, which may support for environmental stress tolerance mechanisms in the host [22]. While these studies suggest multiple pathways through which *Limnohabitans* can contribute to host fitness, our observation that it is much less prevalent in field-grown samples casts doubts on this taxon playing an essential role. Alternatively, such dominance may not always reflect

functional symbiosis but rather niche specialization driven by strong environmental filters typical of laboratory environments, such as stable abiotic parameters and a standardized homogenous diet. In particular, *Limnohabitans* are opportunistic fast-growing generalists [64, 65] that may take advantage of the high algal densities fed to the daphnids maintained in culture, further favoring disproportionate growth of this taxon under laboratory settings. Notably, *Limnohabitans* strains such as 2KL-3 and 2KL-7 have been shown to differ in their effects as compared to the beneficial effect of *Limnohabitans* strain II-D2 and DM1 on *Daphnia* fecundity [25] highlighting the strain-specific nature of this genus [55]. This suggests that *Limnohabitans* exhibits distinct ecological roles with their interactions with the host differing based on substrate utilization patterns and environmental preferences [25, 54, 66]. Additionally, founder effects [67] and priority effects [68, 69] during early establishment of laboratory cultures may shape microbiome assembly in ways that do not necessarily reflect ecological selection or functional relevance. Thus, the observed dominance of *Limnohabitans* may stem from reduced microbial competition in low-diversity laboratory microbiomes, passive introduction with food or water or altered host-microbe dynamics in artificial settings, rather than from an ecologically significant symbiosis.

For the whole-animal microbiome samples of laboratory-cultured animals, core taxa included *Limnohabitans planktonicus, Formosimonas* sp., *Flavobacterium* sp., unclassified *Chitinophagaceae* and *Emticicia* sp.. *Limnohabitans, Flavobacterium* and unclassified *Chitinophagaceae* have been shown to be not only dominant members of the *D. magna* microbiome [70] but also overrepresented on the external surfaces of ephippial cases in dormant resting eggs [71]. The presence of unclassified *Chitinophagaceae* in whole-animal samples likely reflects their capacity to degrade chitin, a principal constituent of the *Daphnia* carapace. The dominance of unclassified *Chitinophagaceae* in the microbiota has also been observed in antibiotic-treated daphnids [27, 72]. In such cases, these microorganisms may exert detrimental effects on host fitness by opportunistically degrading chitin in their host's carapace. Likewise, *Flavobacterium* sp., have also been detected in whole-community analyses of *Daphnia* [17, 24, 72]. The dominance of this group has been observed in *Microcystis aeruginosa*-tolerant genotypes of *Daphnia* [1], suggesting their contribution to the degradation of cyanobacterial toxins. *Flavobacterium* sp. are frequently associated with the lysis and degradation of cyanobacteria in freshwater systems, with their relative abundances and metabolic activity increasing in magnitude during cyanobacterial blooms [73]. In addition, the ability of *Flavobacterium* to digest polysaccharides [74], such as cellulose, hemicellulose and pectins, which are major constituents of the phytoplankton cell wall [16], might contribute to host digestion and nutrient acquisition. The presence of *Emticicia* may be relevant for the fitness of *Daphnia*, as a metagenomics study of key microbiota of *Daphnia* revealed that the genome of *Emticicia* sp. encodes for amino acid biosynthesis and transport, vitamin and mineral transport and carbohydrate breakdown, all of which are essential functions for the optimal survival and growth of *Daphnia.* [22]. *Formosimonas* sp. are a group of free-living bacteria often recovered at high numbers from freshwater systems [75]. Although not detected in other studies, the prevalence of this species in whole animal samples as host symbionts, parasites or neutral associations is unclear.

One of the striking observations of the current study is that the microbiome of field-grown *D. magna* differed strongly from that of laboratory-cultured animals. A key difference is that *L. planktonicus* does not stand out as the most abundant and widespread taxon in the *D. magna* gut microbiome of field-grown hosts. We also observed that the diversity of OTUs that are shared among individuals in the field is much higher than that of laboratory-cultured animals (Fig. 4). However, this latter observation needs to be interpreted with caution, given that all microbiomes of field-grown animals in our dataset were sampled from one single lake (Lake Bysön, Sweden), i.e. were exposed to the same environmental microbial community. This likely also explains why the among-individual differences in microbiome composition seems to be lower for field-grown animals than for laboratory-cultured animals. If we only consider laboratory-cultured animals of genotypes isolated from the same lake as the field-grown animals, then the among-host individual variation in microbiome composition is actually larger than the variation among laboratory-cultured hosts (see Figure S4, SI). The core taxa identified at the highest prevalence (99%) in the gut samples of these field animals comprised various OTUs including unclassified *Pirellulales*, *Rhodobacteraceae*, *Solirubrobacterales*, *Hyphomicrobiaceae*, *Rhizobiales*, *Actinobacteria*, *Acidimicrobiales*, *Isophaeraceae* and the genera *Methylocystis, Roseimaritima, Mycobacterium, Ilumatobacter, Nocardioides* and *Aquihabitans*. While *L. planktonicus* was also detected in these samples, it occurred at a lower prevalence threshold (80%). We hypothesize that given the high diversity of the natural bacterioplankton, the indiscriminate filter feeding mechanism of *Daphnia* results in the uptake of a vast variety of bacterial taxa. Given that microbiomes may strongly affect the fitness and traits of their hosts, the significant differences in microbiome composition of field and laboratory-cultured animals suggest that some caution may be needed when interpreting results of laboratory experiments to infer how *Daphnia* respond in the field [76]. While standardization

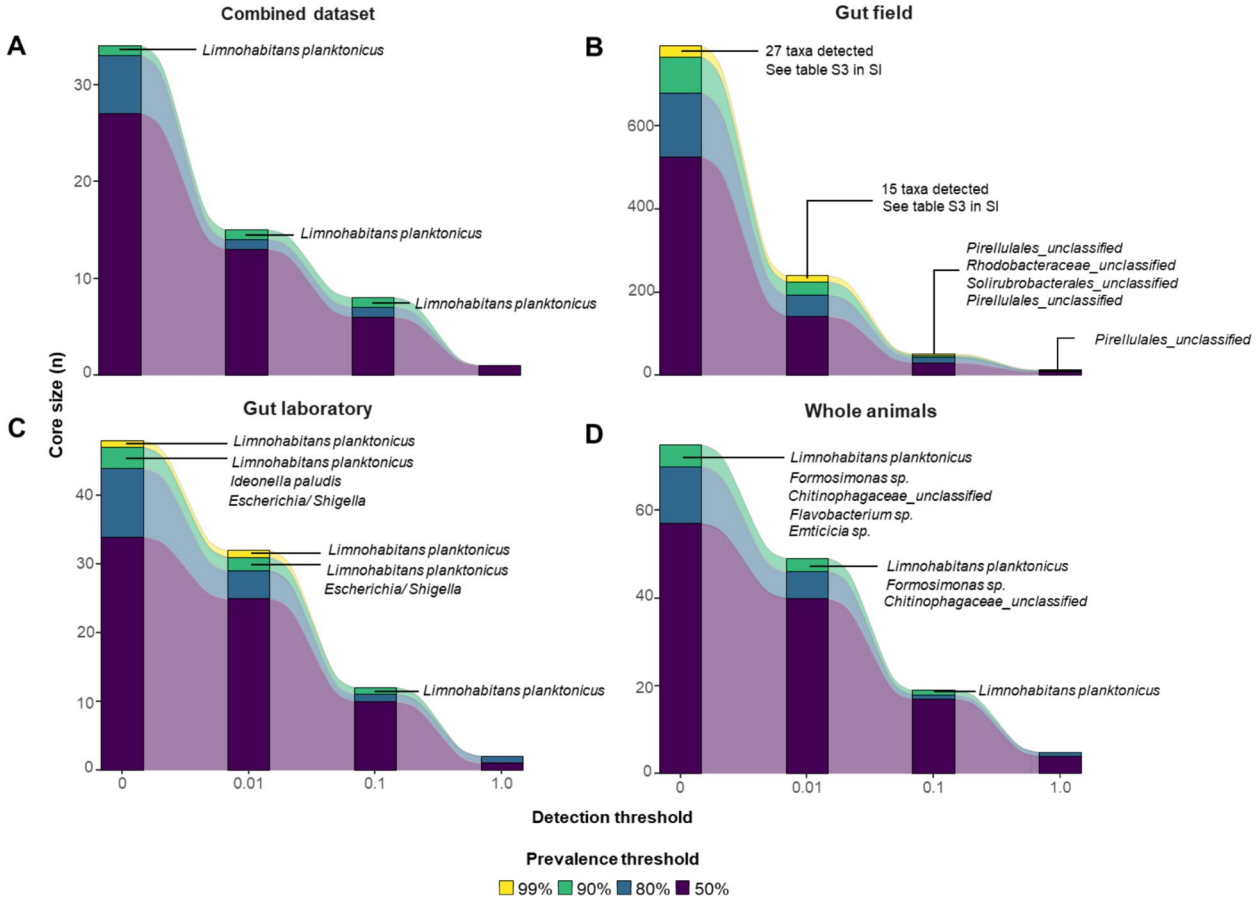


Fig. 4 Core size (no. of OTUs) assigned based on prevalence thresholds (99%, 90%, 80%, 50%) and a combined prevalence and relative abundance detection threshold (> 0.01%, 0.1%, 1%) in the **A** combined dataset, **B** gut samples of field origin, **C** gut samples of laboratory origin and **D** whole animal samples. For the core taxa identified based on prevalence alone, the detection threshold was set to 0. This means that any taxon with a non-zero relative abundance value in the defined proportion of the samples (e.g. 99% prevalence) was included. The core species identified in 99 and 90% of the samples have been labeled (see Table S4 in the SI for details). The alluvial segments or the streamlines between the bars do not represent interpolation but are solely for illustrative purposes to enhance readability and visualization

by growing animals in common garden experiments is often an essential element ensuring experimental control and straightforward interpretation of experimental results, it seems difficult to avoid that it is accompanied by a strong shift in microbiome composition. Our results clearly point towards the need to engage in more studies focusing on the microbiome composition of field-collected animals.

In summary, our findings indicate that the microbiome of *D. magna* is highly variable and can be easily influenced by the external environment. This is in contrast to studies on mice, zebrafish, bees, corals, and other organisms [77–80], where certain bacterial taxa have been detected as constant and stable members of the microbiome, but is in line with recent studies suggesting that core microbiomes may not be omnipresent [35]. Our study shows that in *D. magna,* the proportion of core microbial taxa is quite low. In laboratory-cultured animals, it seems to be limited to one (*L. planktonicus*) or a few taxa that are widespread, although not always in high densities. Our results on field-grown hosts also temper the strong association between *Daphnia* and *L. planktonicus*, since for this dataset we have several other bacterial taxa that seem to be more prevalent than *L. planktonicus.* While *L. planktonicus* is still widespread (prevalence > 80%), its prevalence and relative abundance in the field is lower than in laboratory-cultured animals. The emergent pattern is that the *D. magna* microbiome is highly dynamic and variable with *D. magna* possibly not being universally dependent on specialized microbes and its microbiome likely being strongly determined by what is taken up from the environment.

Our study suffers from some limitations. Our analysis was limited by the availability of only 12 datasets, comprising a total of 656 samples, which may not be sufficient to fully capture the vast diversity of the *Daphnia* microbiome. While we did initially consider integrating datasets sequencing multiple hypervariable regions to

increase the breadth of our analysis, specifically by incorporating V3-V4 and V3-V5 reads and truncating them to extract only the homologous V4 region, this approach proved technically challenging inflating operational taxonomic unit (OTU) counts and generating extremely large distance matrices that further increased computational burden. In addition, we also considered analyzing each hypervariable region separately and merging reads from these different regions into downstream data analysis. However, multiple studies have shown that different targeted hypervariable regions differ in their taxonomic assignment due to primer bias, difference in hypervariable region sequence length and sequence uniqueness across bacterial taxa [81–84]. As a result, taxonomic assignments can vary between regions of the same species, especially at the lower taxonomic levels. This would mean that merging the individual analyses for the different regions would introduce inconsistencies due to the potential differences in taxonomic assignments for the same species (more prominent at the lower taxonomic levels).

We thus focused on the V4 hypervariable region of the 16S rRNA gene, as it was the most commonly sequenced region across studies, while also enabling standardized processing for greater consistency and robust cross-study comparisons. This is in line with literature where most studies investigating the core microbiome focus on analyzing a single hypervariable region of the 16S rRNA gene [85–90] to allow data standardization and reduce biases introduced by non-overlapping regions. However, studies that do combine multiple hypervariable regions, although the input is processed as OTUs or ASVs for analysis, the results are typically collapsed to the genus or sometimes a higher taxonomic level to achieve cross study comparisons [91–95]. While this approach reduces region-specific biases, it compromises ecological resolution given that distinct sequence variants are merged under the same genus. As such, the core microbiome is defined at the genus or family level which may not be accurate as there can arise multiple species within a single genus/family with distinct host functions, metabolic capabilities and ecological roles. In contrast, our synthesis focuses on a single hypervariable region (namely V4 region) of the 16S rRNA gene, thereby allowing OTU-level resolution to be retained for core microbiome analysis. We consider this more biologically relevant as we assume that the ecology of all members within an OTU will be highly similar and that members of an OTU are expected to share most of the adaptations required to persist in association with a particular host. As such, an OTU-based perspective provides a finer scale definition of a core microbiome which is in contrast to that achieved from collapsing OTUs into higher taxonomic levels. However, this choice also represents a limitation potentially underestimating overall microbial diversity that other hypervariable regions or full-length 16S sequencing could better resolve.

Additionally, variation in experimental design, sample collection methods, DNA extraction, library preparation protocols and sequencing platforms across different laboratories and studies could potentially have introduced some heterogeneity and potential biases into the analysis. For instance, in our dataset both country and study explained a comparable portion of variation in microbial community composition, suggesting that technical factors associated with individual studies could partly underlie observed variation.

Given that our study is grounded on 16S rRNA sequencing data, we present results from a community composition-based analysis [96] and focus on identifying a "*common core*" based on consistent taxonomic presence across hosts. This approach follows the original definition of the core microbiome [3] and was selected to maintain clarity and comparability in a rapidly evolving and conceptually fragmented field. This is in contrast to a "*functional core*" which would require complementary data (e.g. metagenomics, transcriptomics, metabolomics) to assess fitness consequences of individual microbial taxa on the host. Future studies integrating multi-omic approaches hold promise for uncovering deeper insights into the functional components of the *Daphnia* microbiome. One further limitation is that all data considered here are snapshots, while the relative abundance of taxa can vary significantly over time [97, 98]. This does not interfere with our effort to identify core taxa given that dynamics in time would average out for the criteria of occurrences and relative abundances. It may rather explain that we observe no taxa that have a very high relative abundance (e.g., > 10%) across many samples. Quite a number of taxa that were observed in our dataset may be transient and not permanent members of the microbiome. Additionally, a crucial factor affecting species richness in microbiome studies is the total number of sequences obtained from each sample. In our study, sequencing depth varied widely among samples. To address such variation, some studies suggest rarefaction [99], whereas others caution that such rarefaction result in important biases [100] and favor approaches based on alpha and beta diversities [101, 102]. In our study, raw counts were normalized to relative abundances using total sum scaling (TSS) to account for differences in library size without discarding samples or having to resort to lower sequencing depth for all studies. This approach was selected such that valid comparisons could be made across samples while allowing the preservation of rare taxa that could potentially contribute to core membership [103]. For instance, deep sequencing efforts have demonstrated that rare taxa constitute

a considerable portion of several microbial communities, often overlooked by shallow sequencing efforts [104, 105]. In addition, accumulating evidence suggests that rare and low abundance taxa in communities, constitute a significant portion of core membership that drive physiologically significant interactions with the host [106].

While these limitations are real, we argue that the main conclusion of our analysis, being that the microbiome of *D. magna* is highly variable, is not affected by them. The key limitation revealed by our study is the surprisingly low number of studies on field-grown animals (only a single field study identified in our analysis), which limits our capacity to draw strong conclusions for field animals and does not allow us to assess the influence of, for instance, geographic origin or the ecology of habitats on microbiome composition. Moving forward, future studies should address this shortcoming and focus more on field-sampled animals. One avenue to increase standardization and thus tractability of such studies is the use of germ-free animals that are exposed to medium directly harvested from natural systems. In addition, metagenomic studies that allow functional inference may shed more light on how widespread microbiome taxa can contribute to fitness of their host.

Conclusions

We conclude from our study that the *D. magna* microbiome is highly variable across host individuals, with most taxa being unique to a small subset of the analyzed host individuals. Yet, amid this large inter-individual variability, a relatively consistent association with a single taxon, namely, *Limnohabitans planktonicus*, which belongs to the family Comamonadaceae, is observed in both the *D. magna* gut and whole animal samples of laboratory origin across samples obtained from five independent laboratories and three continents. This robust association may point towards a symbiotic relationship, with *L. planktonicus* potentially exhibiting features that may contribute to host fitness. In contrast, this association could also be attributed to the fast-growing and generalist nature of this taxon, simply suggesting a neutral or opportunistic association in artificial environments with reduced microbial diversity and competition. Additionally, this putative strong association should be put into perspective due to the observation that the microbiomes of field-grown animals exhibit a relatively weaker presence of *L. planktonicus*, casting doubt on the extent to which one should consider it as a core species of the *D. magna* microbiome. Field-grown animals exhibit a more diverse microbiome in contrast to laboratory-cultured animals which display a reduced diversity when reared under constant and standardized long-term conditions. This suggests that transitioning from natural environments to controlled laboratory settings significantly diminish gut microbiome diversity, resulting in the loss of multiple microbial groups and a reduced core membership, which in turn might pave the path for the dominance and spread of *L. planktonicus*. Ultimately, our research highlights the striking scarcity of studies focused on natural microbiomes in their native environment (with only one field study found in our analysis). This glaring gap emphasizes the urgent need for further investigation into the microbiomes of field animals to truly understand the structuring of microbial communities under natural settings.

List of Abbreviations

OTU	Operational taxonomic unit
ASV	Amplicon sequence variant
ENA	European nucleotide archive
MDS	Multidimensional scaling
PERMANOVA	Permutational multivariate analysis of variance
VSC	Vlaams supercomputer centrum

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40793-025-00834-y.

Supplementary Material 1.

Supplementary Material 2.

Acknowledgements

The research was financially supported by the Research Foundation-Flanders (FWO, ***Fonds voor Wetenschappelijk Onderzoek – Vlaanderen)*** grants, G092619N, G014423N and G061824N, and KU Leuven grant C16/23/003. All analyses were run on the Vlaams Supercomputer Centrum (VSC), funded by the Research Foundation-FWO (Flanders) and the Flemish Government. The authors also thank Maxime Fajgenblat for his valuable assistance in the creation of Fig. 4, providing help with the code as well as insightful suggestions that greatly contributed to the final design. In addition, the authors thank the reviewers and the anonymous commentor on PubPeer for their insightful comments on the preprint version which contributed to the improvement and refinement of the final manuscript. The authors declare no competing interests.

Author contributions

Conceptualization: AG, LDM, ED, RS, MC, FM, SH; Methodology: AG, MC, FM, LDM, ED; Bioinformatic analysis: AG, MC; Writing-original draft: AG, LDM; Writing-review and editing: all authors.

Funding

Onderzoeksraad,KULeuven, Belgium, C16/23/003, Fonds voor Wetenschappelijk Onderzoek,G092619N,G014423N and G061824N.

Data Availability

All raw sequences used in this study are previously published datasets available in the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/home). Please refer to the original publications or Table S2 in SI for additional information on their specific BioProject/Accession numbers. The dataset(s) supporting the conclusions of this article is(are) included within the article (and its additional file(s)).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Laboratory of Freshwater Ecology, Evolution and Conservation, Department of Biology, KU Leuven, 3000 Leuven, Belgium
[2]Animal Science Unit, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), 8400 Oostende, Belgium
[3]Institut d'Ecologie Et Des Sciences de L'Environnement de Paris (UMR7618), Sorbonne Université, Université Paris Cité, Université Paris Est Créteil, CNRS, INRAE, IRD, 75005 Paris, France
[4]Laboratory of Reproductive Genomics, Department of Human Genetics, KU Leuven, 3000 Leuven, Belgium
[5]Department of Biochemistry and Microbiology, Faculty of Sciences, Ghent University, 9000 Ghent, Belgium
[6]Laboratory of Evolutionary Stress Ecology and Ecotoxicology, KU Leuven, 3000 Leuven, Belgium
[7]Department Evolutionary and Integrative Ecology, Research Group Evolving Metacommunities, Leibniz Institute of Freshwater Ecology and Inland Fisheries, 12587 Berlin, Germany
[8]Institute of Biology, Freie Universität Berlin, 14195 Berlin, Germany
[9]Laboratory of Aquatic Biology, Interdisciplinary Research Facility Life Sciences, KULAK, KU Leuven, Campus Kortrijk, 8500 Kortrijk, Belgium

Received: 14 February 2025 / Accepted: 1 December 2025
Published online: 11 December 2025

References

1. Macke E, Callens M, De Meester L, Decaestecker E. Host-genotype dependent gut microbiota drives zooplankton tolerance to toxic cyanobacteria. Nat Commun. 2017;8:1608. https://doi.org/10.1038/s41467-017-01714-x.
2. Stock W, Callens M, Houwenhuyse S, Schols R, Goel N, Coone M, et al. Human impact on symbioses between aquatic organisms and microbes. Aquat Microb Ecol. 2021;87:113–38. https://doi.org/10.3354/ame01973.
3. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature. 2007;449:804–10. https://doi.org/10.1038/nature06244.
4. Parfrey LW, Moreau CS, Russell JA. Introduction: the host-associated microbiome: pattern, process and function. Mol Ecol. 2018;27:1749–65. https://doi.org/10.1111/mec.14706.
5. Wilkinson DM. At cross purposes. Nature. 2001;412:485–485. https://doi.org/10.1038/35087676.
6. Benzie JA. Guides to the Identification of the Microinvertebrates of the Continental Waters of the World: Cladocera: the Genus Daphnia (including Daphniopsis). SPB Acad. Publ.; 2005.
7. Miner BE, De Meester L, Pfrender ME, Lampert W, Hairston NG. Linking genes to communities and ecosystems: *Daphnia* as an ecogenomic model. Proc R Soc Lond B Biol Sci. 2012;279:1873–82. https://doi.org/10.1098/rspb.2011.2404.
8. Almeida RA, Lemmens P, De Meester L, Brans KI. Differential local genetic adaptation to pesticide use in organic and conventional agriculture in an aquatic non-target species. Proc R Soc Lond B Biol Sci. 2021;288:20211903. https://doi.org/10.1098/rspb.2021.1903.
9. Brans KI, Jansen M, Vanoverbeke J, Tüzün N, Stoks R, De Meester L. The heat is on: genetic adaptation to urbanization mediated by thermal tolerance and body size. Glob Change Biol. 2017;23:5218–27. https://doi.org/10.1111/gcb.13784.
10. Geerts AN, Vanoverbeke J, Vanschoenwinkel B, Van Doorslaer W, Feuchtmayr H, Atkinson D, et al. Correction: corrigendum: rapid evolution of thermal tolerance in the water flea Daphnia. Nat Clim Change. 2015;5:956–956. https://doi.org/10.1038/nclimate2810.
11. Wersebe MJ, Weider LJ. Resurrection genomics provides molecular and phenotypic evidence of rapid adaptation to salinization in a keystone aquatic species. Proc Natl Acad Sci U S A. 2023;120:e2217276120. https://doi.org/10.1073/pnas.2217276120.
12. Steiner CF, Nowicki CJ. Eco-evolutionary dynamics in the wild: clonal turnover and stability in *Daphnia* populations. Am Nat. 2019;194:117–23. https://doi.org/10.1086/703484.
13. Decaestecker E, Gaba S, Raeymaekers JAM, Stoks R, Van Kerckhoven L, Ebert D, et al. Host–parasite 'Red Queen' dynamics archived in pond sediment. Nature. 2007;450:870–3. https://doi.org/10.1038/nature06291.
14. Zhang C, De Meester L, Stoks R. Rapid evolution of consumptive and non-consumptive predator effects on prey population densities, bioenergetics and stoichiometry. J Anim Ecol. 2024;93:906–17. https://doi.org/10.1111/1365-2656.14110.
15. Ebert D. Daphnia as a versatile model system in ecology and evolution. EvoDevo. 2022;13:16. https://doi.org/10.1186/s13227-022-00199-0.
16. Qi W, Nong G, Preston JF, Ben-Ami F, Ebert D. Comparative metagenomics of Daphnia symbionts. BMC Genomics. 2009;10:172. https://doi.org/10.1186/1471-2164-10-172.
17. Peerakietkhajorn S, Tsukada K, Kato Y, Matsuura T, Watanabe H. Symbiotic bacteria contribute to increasing the population size of a freshwater crustacean, *Daphnia magna*. Environ Microbiol Rep. 2015;7:364–72. https://doi.org/10.1111/1758-2229.12260.
18. Sison-Mangus MP, Mushegian AA, Ebert D. Water fleas require microbiota for survival, growth and reproduction. ISME J. 2015;9:59–67. https://doi.org/10.1038/ismej.2014.116.
19. Mushegian AA, Ebert D. Presence of microbiota reverses the relative performance of Daphnia on two experimental diets. Zoology. 2017;125:29–31. https://doi.org/10.1016/j.zool.2017.07.007.
20. Sison-Mangus MP, Metzger CMJA, Ebert D. Host genotype-specific microbiota do not influence the susceptibility of *D. magna* to a bacterial pathogen. Sci Rep. 2018;8:9407. https://doi.org/10.1038/s41598-018-27681-x.
21. Fong JC, De Guzman BE, Lamborg CH, Sison-Mangus MP. The mercury-tolerant microbiota of the zooplankton Daphnia aids in host survival and maintains fecundity under mercury stress. Environ Sci Technol. 2019;53:14688–99. https://doi.org/10.1021/acs.est.9b05305.
22. Cooper RO, Cressler CE. Characterization of key bacterial species in the *Daphnia magna* microbiota using shotgun metagenomics. Sci Rep. 2020;10:652. https://doi.org/10.1038/s41598-019-57367-x.
23. Motiei A, Brindefalk B, Ogonowski M, El-Shehawy R, Pastuszek P, Ek K, et al. Disparate effects of antibiotic-induced microbiome change and enhanced fitness in *Daphnia magna*. PLoS ONE. 2020;15:e0214833. https://doi.org/10.1371/journal.pone.0214833.
24. Freese HM, Schink B. Composition and stability of the microbial community inside the digestive tract of the aquatic crustacean *Daphnia magna*. Microb Ecol. 2011;62:882–94. https://doi.org/10.1007/s00248-011-9886-8.
25. Peerakietkhajorn S, Kato Y, Kasalický V, Matsuura T, Watanabe H. *Betaproteobacteria Limnohabitans* strains increase fecundity in the crustacean *Daphnia magna*: symbiotic relationship between major bacterioplankton and zooplankton in freshwater ecosystem. Environ Microbiol. 2016;18:2366–74. https://doi.org/10.1111/1462-2920.12919.
26. Akbar S, Gu L, Sun Y, Zhou Q, Zhang L, Lyu K, et al. Changes in the life history traits of *Daphnia magna* are associated with the gut microbiota composition shaped by diet and antibiotics. Sci Total Environ. 2020;705:135827. https://doi.org/10.1016/j.scitotenv.2019.135827.
27. Callens M, Watanabe H, Kato Y, Miura J, Decaestecker E. Microbiota inoculum composition affects holobiont assembly and host growth in *Daphnia*. Microbiome. 2018;6:56. https://doi.org/10.1186/s40168-018-0444-1.
28. Eckert EM, Anicic N, Fontaneto D. Freshwater zooplankton microbiome composition is highly flexible and strongly influenced by the environment. Mol Ecol. 2021;30:1545–58. https://doi.org/10.1111/mec.15815.
29. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol. 2009;75:7537–41. https://doi.org/10.1128/AEM.01541-09.
30. McMurdie PJ, Holmes S. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE. 2013;8:e61217. https://doi.org/10.1371/journal.pone.0061217.
31. Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, et al. vegan: Community Ecology Package [Internet]. 2024 [cited 2025 Jan 6]. https://cran.r-project.org/web/packages/vegan/index.html. Accessed 6 Jan 2025
32. Anderson MJ. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 2001;26:32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x.
33. Larsson J, Godfrey AJR, Gustafsson P, algorithms) DHE (geometric, code) EH (root solver, Privé F. eulerr: Area-Proportional Euler and Venn Diagrams with Ellipses [Internet]. 2024 [cited 2024 Nov 21]. https://cran.r-project.org/web/packages/eulerr/index.html. Accessed 21 Nov 2024

34. Lahti L, Shetty S. Introduction to the microbiome R package. Preprint at https://microbiome.github.io/tutorials/. 2018;
35. Hammer TJ, Sanders JG, Fierer N. Not all animals need a microbiome. FEMS Microbiol Lett. 2019;366:fnz117. https://doi.org/10.1093/femsle/fnz117.
36. Houwenhuyse S, Callens M, Bulteel L, Decaestecker E. Comparison between the gut bacterial community of laboratory cultured and wild Daphnia. FEMS Microb Ecol. 2023;11:fiad116. https://doi.org/10.1093/femsec/fiad116.
37. Surmacz B, Stec D, Prus-Frankowska M, Buczek M, Michalczyk Ł, Łukasik P. Pinpointing the microbiota of tardigrades: what is really there? Environ Microbiol. 2024;26:e16659. https://doi.org/10.1111/1462-2920.16659.
38. Akbar S, Li X, Ding Z, Liu Q, Huang J, Zhou Q, et al. Disentangling diet- and medium-associated microbes in shaping *Daphnia* gut microbiome. Microb Ecol. 2022;84:911–21. https://doi.org/10.1007/s00248-021-01900-x.
39. Gorokhova E, Motiei A, El-Shehawy R. Understanding biofilm formation in ecotoxicological assays with natural and anthropogenic particulates. Front Microbiol. 2021;12:632947. https://doi.org/10.3389/fmicb.2021.632947.
40. Haynes S, Darby AC, Daniell TJ, Webster G, van Veen FJF, Godfray HCJ, et al. Diversity of bacteria associated with natural aphid populations. Appl Environ Microbiol. 2003;69:7216–23. https://doi.org/10.1128/AEM.69.12.7216-7223.2003.
41. Salem H, Bauer E, Kirsch R, Berasategui A, Cripps M, Weiss B, et al. Drastic genome reduction in an herbivore's pectinolytic symbiont. Cell. 2017;171:1520-1531.e13. https://doi.org/10.1016/j.cell.2017.10.029.
42. Matsuura Y, Moriyama M, Łukasik P, Vanderpool D, Tanahashi M, Meng X-Y, et al. Recurrent symbiont recruitment from fungal parasites in cicadas. Proc Natl Acad Sci USA. 2018;115:E5970–9. https://doi.org/10.1073/pnas.1803245115.
43. Hammer TJ, Janzen DH, Hallwachs W, Jaffe SP, Fierer N. Caterpillars lack a resident gut microbiome. Proc Natl Acad Sci USA. 2017;114:9641–6. https://doi.org/10.1073/pnas.1707186114.
44. Nyholm SV, McFall-Ngai M. The winnowing: establishing the squid–vibrio symbiosis. Nat Rev Microbiol. 2004;2:632–42. https://doi.org/10.1038/nrmicro957.
45. Ohbayashi T, Takeshita K, Kitagawa W, Nikoh N, Koga R, Meng X-Y, et al. Insect's intestinal organ for symbiont sorting. Proc Natl Acad Sci USA. 2015;112:E5179–88. https://doi.org/10.1073/pnas.1511454112.
46. Carrillo-Araujo M, Taş N, Alcántara-Hernández RJ, Gaona O, Schondube JE, Medellín RA, et al. Phyllostomid bat microbiome composition is associated to host phylogeny and feeding strategies. Front Microbiol. 2015;6:447. https://doi.org/10.3389/fmicb.2015.00447.
47. Xue Z, Zhang W, Wang L, Hou R, Zhang M, Fei L, et al. The bamboo-eating giant panda harbors a carnivore-like gut microbiota, with excessive seasonal variations. MBio. 2015;6:10.1128/mbio.00022-15. https://doi.org/10.1128/mbio.00022-15.
48. McKenney EA, Maslanka M, Rodrigo A, Yoder AD. Bamboo specialists from two mammalian orders (Primates, Carnivora) share a high number of low-abundance gut microbes. Microb Ecol. 2018;76:272–84. https://doi.org/10.1007/s00248-017-1114-8.
49. Waite DW, Deines P, Taylor MW. Gut microbiome of the critically endangered New Zealand parrot, the Kakapo (*Strigops habroptilus*). PLoS ONE. 2012;7:e35803. https://doi.org/10.1371/journal.pone.0035803.
50. Hird SM, Sánchez C, Carstens BC, Brumfield RT. Comparative gut microbiota of 59 neotropical bird species. Front Microbiol. 2015;6:1403. https://doi.org/10.3389/fmicb.2015.01403.
51. Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, et al. Evolution of mammals and their gut microbes. Science. 2008;320:1647–51. https://doi.org/10.1126/science.1155725.
52. Šimek K, Kasalický V, Jezbera J, Jezberová J, Hejzlar J, Hahn MW. Broad habitat range of the phylogenetically narrow R-BT065 cluster, representing a core group of the Betaproteobacterial genus *Limnohabitans*. Appl Environ Microbiol. 2010;76:631–9. https://doi.org/10.1128/AEM.02203-09.
53. Eckert EM, Pernthaler J. Bacterial epibionts of *Daphnia*: a potential route for the transfer of dissolved organic carbon in freshwater food webs. ISME J. 2014;8:1808–19. https://doi.org/10.1038/ismej.2014.39.
54. Šimek K, Kasalický V, Zapomělová E, Horňák K. Alga-derived substrates select for distinct Betaproteobacterial lineages and contribute to niche separation in *Limnohabitans* strains. Appl Environ Microbiol. 2011;77:7307–15. https://doi.org/10.1128/AEM.05107-11.
55. Horňák K, Kasalický V, Šimek K, Grossart H-P. Strain-specific consumption and transformation of alga-derived dissolved organic matter by members of the Limnohabitans-C and Polynucleobacter-B clusters of Betaproteobacteria. Environ Microbiol. 2017;19:4519–35. https://doi.org/10.1111/1462-2920.13900.
56. Hammer TJ, McMillan WO, Fierer N. Metamorphosis of a butterfly-associated bacterial community. PLoS ONE. 2014;9:e86995. https://doi.org/10.1371/journal.pone.0086995.
57. Staudacher H, Kaltenpoth M, Breeuwer JAJ, Menken SBJ, Heckel DG, Groot AT. Variability of bacterial communities in the moth *Heliothis virescens* indicates transient association with the host. PLoS ONE. 2016;11:e0154514. https://doi.org/10.1371/journal.pone.0154514.
58. Lighthart B. Some changes in gut bacterial flora of field-grown *Peridroma saucia* (Lepidoptera: Noctuidae) when brought into the laboratory. Appl Environ Microbiol. 1988;54:1896–8. https://doi.org/10.1128/aem.54.7.1896-1898.1988.
59. Preiswerk D, Walser J-C, Ebert D. Temporal dynamics of microbiota before and after host death. ISME J. 2018;12:2076–85. https://doi.org/10.1038/s41396-018-0157-2.
60. Lennon JT, Jones SE. Microbial seed banks: the ecological and evolutionary implications of dormancy. Nat Rev Microbiol. 2011;9:119–30. https://doi.org/10.1038/nrmicro2504.
61. Di Cesare A, Riva F, Colinas N, Borgomaneiro G, Borin S, Cabello-Yeves PJ, et al. Zooplankton as a transitional host for *Escherichia coli* in freshwater. Appl Environ Microbiol. 2022;88:e02522-21. https://doi.org/10.1128/aem.02522-21.
62. Hansen A, Moran N. Aphid genome expression reveals host–symbiont cooperation in the production of amino acids. Proc Natl Acad Sci USA. 2011;108:2849–54. https://doi.org/10.1073/pnas.1013465108.
63. Macdonald SJ, Thomas GH, Douglas AE. Genetic and metabolic determinants of nutritional phenotype in an insect–bacterial symbiosis. Mol Ecol. 2011;20:2073–84. https://doi.org/10.1111/j.1365-294X.2011.05031.x.
64. Horňák K, Zeder M, Blom JF, Posch T, Pernthaler J. Suboptimal light conditions negatively affect the heterotrophy of *Planktothrix rubescens* but are beneficial for accompanying *Limnohabitans* spp. Environ Microbiol. 2012;14:765–78. https://doi.org/10.1111/j.1462-2920.2011.02635.x.
65. Jezberová J, Jezbera J, Znachor P, Nedoma J, Kasalický V, Šimek K. The *Limnohabitans* genus harbors generalistic and opportunistic subtypes: evidence from spatiotemporal succession in a canyon-shaped reservoir. Appl Environ Microbiol. 2017;83:e01530-17. https://doi.org/10.1128/AEM.01530-17.
66. Kasalický V, Jezbera J, Hahn MW, Šimek K. The diversity of the *Limnohabitans* genus, an important group of freshwater bacterioplankton, by characterization of 35 isolated strains. PLoS ONE. 2013;8:e58209. https://doi.org/10.1371/journal.pone.0058209.
67. Santos J, Pascual M, Simões P, Fragata I, Lima M, Kellen B, et al. From nature to the laboratory: the impact of founder effects on adaptation. J Evol Biol. 2012;25:2607–22. https://doi.org/10.1111/jeb.12008.
68. McCafferty J, Mühlbauer M, Gharaibeh RZ, Arthur JC, Perez-Chanona E, Sha W, et al. Stochastic changes over time and not founder effects drive cage effects in microbial community assembly in a mouse model. ISME J. 2013;7:2116–25. https://doi.org/10.1038/ismej.2013.106.
69. Martínez I, Maldonado-Gomez MX, Gomes-Neto JC, Kittana H, Ding H, Schmaltz R, et al. Experimental evaluation of the importance of colonization history in early-life gut microbiota assembly. Elife. 2018;7:e36521. https://doi.org/10.7554/eLife.36521.
70. Pfenning-Butterworth A, Cooper RO, Cressler CE. Daily feeding rhythm linked to microbiome composition in two zooplankton species. PLoS ONE. 2022;17:e0263538. https://doi.org/10.1371/journal.pone.0263538.
71. Mushegian AA, Walser J-C, Sullam KE, Ebert D. The microbiota of diapause: how host–microbe associations are formed after dormancy in an aquatic crustacean. J Anim Ecol. 2018;87:400–13. https://doi.org/10.1111/1365-2656.12709.
72. Cooper RO, Tjards S, Rischling J, Nguyen DT, Cressler CE. Multiple generations of antibiotic exposure and isolation influence host fitness and the microbiome in a model zooplankton species. FEMS Microbiol Ecol. 2017;87:400–13. https://doi.org/10.1111/1365-2656.12709.
73. Williams TJ, Wilkins D, Long E, Evans F, DeMaere MZ, Raftery MJ, et al. The role of planktonic lavobacteria in processing algal organic matter in coastal East Antarctica revealed using metagenomics and metaproteomics. Environ Microbiol. 2013;15:1302–17. https://doi.org/10.1111/1462-2920.12017.
74. Gavriilidou A, Gutleben J, Versluis D, Forgiarini F, van Passel MWJ, Ingham CJ, et al. Comparative genomic analysis of Flavobacteriaceae: insights into carbohydrate metabolism, gliding motility and secondary metabolite biosynthesis. BMC Genomics. 2020;21:569. https://doi.org/10.1186/s12864-020-06971-7.
75. Chen WM, Xie PB, Young CC, Sheu SY. *Formosimonas limnophila* gen. nov., sp. nov., a new member of the family Burkholderiaceae isolated from a freshwater lake. Int J Syst Evol Microbiol. 2017;67:17–24. https://doi.org/10.1099/ijsem.0.001561.

76. Houwenhuyse S, Bulteel L, Vanoverberghe I, Krzynowek A, Goel N, Coone M, et al. Microbiome mediated tolerance to biotic stressors: a case study of the interaction between a toxic cyanobacterium and an oomycete-like infection in Daphnia magna. OSF; https://osf.io/preprints/osf/9n4mg_v1 (2023); https://doi.org/10.31219/osf.io/9n4mg
77. Wang J, Lang T, Shen J, Dai J, Tian L, Wang X. Core gut bacteria analysis of healthy mice. Front Microbiol. 2019;10:887. https://doi.org/10.3389/fmicb.2019.00887.
78. Sharpton TJ, Stagaman K, Sieler MJ, Arnold HK, Davis EW. Phylogenetic integration reveals the Zebrafish core microbiome and its sensitivity to environmental exposures. Toxics. 2021;9:10. https://doi.org/10.3390/toxics9010010.
79. Dew RM, McFrederick QS, Rehan SM. Diverse diets with consistent core microbiome in wild bee pollen provisions. Insects. 2020;11:499. https://doi.org/10.3390/insects11080499.
80. Hernandez-Agreda A, Gates RD, Ainsworth TD. Defining the core microbiome in corals' microbial soup. Trends Microbiol. 2017;25:125–40. https://doi.org/10.1016/j.tim.2016.11.003.
81. Claesson MJ, Wang Q, O'Sullivan O, Greene-Diniz R, Cole JR, Ross RP, et al. Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. Nucleic Acids Res. 2010;38:e200. https://doi.org/10.1093/nar/gkq873.
82. Pinto AJ, Raskin L. PCR biases distort bacterial and archaeal community structure in pyrosequencing datasets. PLoS ONE. 2012;7:e43093. https://doi.org/10.1371/journal.pone.0043093.
83. Tremblay J, Singh K, Fern A, Kirton ES, He S, Woyke T, et al. Primer and platform effects on 16S rRNA tag sequencing. Front Microbiol. Frontiers; 2015;6. https://doi.org/10.3389/fmicb.2015.00771
84. Barb JJ, Oler AJ, Kim H-S, Chalmers N, Wallen GR, Cashion A, et al. Development of an analysis pipeline characterizing multiple hypervariable regions of 16S rRNA using mock samples. PLoS ONE. 2016;11:e0148047. https://doi.org/10.1371/journal.pone.0148047.
85. de Souza YPA, Schloter M, Weisser W, Huang Y, Schulz S. The seeds of *Plantago lanceolata* comprise a stable core microbiome along a plant richness gradient. Environ Microbiome. 2024;19:11. https://doi.org/10.1186/s40793-024-00552-x.
86. Yu J, Jiang C, Yamano R, Koike S, Sakai Y, Mino S, et al. Unveiling the early life core microbiome of the sea cucumber *Apostichopus japonicus* and the unexpected abundance of the growth-promoting Sulfitobacter. anim microbiome. 2023;5:54. https://doi.org/10.1186/s42523-023-00276-2.
87. Wang L, Zhang L, George TS, Feng G. A core microbiome in the hyphosphere of arbuscular mycorrhizal fungi has functional significance in organic phosphorus mineralization. New Phytol. 2023;238:859–73. https://doi.org/10.1111/nph.18642.
88. Simonin M, Dasilva C, Terzi V, Ngonkeu ELM, Diouf D, Kane A, et al. Influence of plant genotype and soil on the wheat rhizosphere microbiome: evidences for a core microbiome across eight African and European soils. FEMS Microbiol Ecol. 2020;96:fiaa067. https://doi.org/10.1093/femsec/fiaa067.
89. Kellogg CA. Microbiomes of stony and soft deep-sea corals share rare core bacteria. Microbiome. 2019;7:90. https://doi.org/10.1186/s40168-019-0697-3.
90. Xia F, Zhou X, Liu Y, Li Y, Bai X, Zhou X. Composition and predictive functional analysis of bacterial communities inhabiting Chinese Cordyceps insight into conserved core microbiome. BMC Microbiol. 2019;19:105. https://doi.org/10.1186/s12866-019-1472-0.
91. Hoffbeck C, Middleton DMRL, Nelson NJ, Taylor MW. 16S rRNA gene-based meta-analysis of the reptile gut microbiota reveals environmental effects, host influences and a limited core microbiota. Mol Ecol. 2023;32:6044–58. https://doi.org/10.1111/mec.17153.
92. Mills S, Dessì P, Pant D, Farràs P, Sloan WT, Collins G, et al. A meta-analysis of acetogenic and methanogenic microbiomes in microbial electrosynthesis. NPJ Biofilms Microbiomes. 2022;8:73. https://doi.org/10.1038/s41522-022-00337-5.
93. Ghosh TS, Shanahan F, O'Toole PW. Toward an improved definition of a healthy microbiome for healthy aging. Nat Aging. 2022;2:1054–69. https://doi.org/10.1038/s43587-022-00306-9.
94. Huang C, Gin C, Fettweis J, Foxman B, Gelaye B, MacIntyre DA, et al. Meta-analysis reveals the vaginal microbiome is a better predictor of earlier than later preterm birth. BMC Biol. 2023;21:199. https://doi.org/10.1186/s12915-023-01702-2.
95. Akinsuyi OS, Roesch LFW, Am Soc Microbiol. Meta-analysis reveals compositional and functional microbial changes associated with osteoporosis. Microbiol Spectr. 2023;11:e00322-23. https://doi.org/10.1128/spectrum.00322-23.
96. Sharon I, Quijada NM, Pasolli E, Fabbrini M, Vitali F, Agamennone V, et al. The core human microbiome: does it exist and how can we find it? A critical review of the concept. Nutrients. 2022;14:2872. https://doi.org/10.3390/nu14142872.
97. Neu AT, Allen EE, Roy K. Defining and quantifying the core microbiome: challenges and prospects. Proc Natl Acad Sci USA. 2021;118:e2104429118. https://doi.org/10.1073/pnas.2104429118.
98. Hegg A, Radersma R, Uller T. A field experiment reveals seasonal variation in the *Daphnia* gut microbiome. Oikos. 2021;130:2191–201. https://doi.org/10.1111/oik.08530.
99. Weiss S, Xu ZZ, Peddada S, Amir A, Bittinger K, Gonzalez A, et al. Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome. 2017;5:27. https://doi.org/10.1186/s40168-017-0237-y.
100. McMurdie PJ, Holmes S. Waste not, want not: why rarefying microbiome data is inadmissible. PLoS Comput Biol. 2014;10:e1003531. https://doi.org/10.1371/journal.pcbi.1003531.
101. Chiu C-H, Chao A. Estimating and comparing microbial diversity in the presence of sequencing errors. PeerJ. 2016;4:e1634. https://doi.org/10.7717/peerj.1634.
102. Martino C, Morton JT, Marotz CA, Thompson LR, Tripathi A, Knight R, et al. A novel sparse compositional technique reveals microbial perturbations. mSystems. 2019;4:10.1128/msystems.00016-19. https://doi.org/10.1128/msystems.00016-19.
103. Ainsworth TD, Krause L, Bridge T, Torda G, Raina J-B, Zakrzewski M, et al. The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. ISME J. 2015;9:2261–74. https://doi.org/10.1038/ismej.2015.39.
104. Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR, et al. Microbial diversity in the deep sea and the underexplored "rare biosphere." Proc Natl Acad Sci U S A. 2006;103:12115–20. https://doi.org/10.1073/pnas.0605127103.
105. Gibbons SM, Caporaso JG, Pirrung M, Field D, Knight R, Gilbert JA. Evidence for a persistent microbial seed bank throughout the global ocean. Proc Natl Acad Sci U S A. 2013;110:4651–5. https://doi.org/10.1073/pnas.1217767110.
106. Chakraborty D, Jousset A, Wei Z, Banerjee S. Rare taxa in the core microbiome. Trends Microbiol. 2025;33:727–37. https://doi.org/10.1016/j.tim.2025.03.002.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.