Titre: Title:	Impact of grazing by multiple Daphnia species on wastewater bacterial communities.
Auteurs: Authors:	Nicolas Tromas, Eyerusalem Goitom, Tiffany Chin, Quoc Tuc Dinh, Sarah Dorner, Ola S. Khawasik, Melania E. Cristescu, & Jean-Baptiste Burnet
Date:	2025
Type:	Article de revue / Article
Référence: Citation:	Tromas, N., Goitom, E., Chin, T., Dinh, Q. T., Dorner, S., Khawasik, O. S., Cristescu, M. E., & Burnet, J.-B. (2025). Impact of grazing by multiple Daphnia species on wastewater bacterial communities. The Science of the total environment, 962, 178364 (11 pages). https://doi.org/10.1016/j.scitotenv.2024.178364

URL de PolyPublie: PolyPublie URL:	https://publications.polymtl.ca/61883/
Version:	Version officielle de l'éditeur / Published version Révisé par les pairs / Refereed
Conditions d'utilisation: Terms of Use:	Creative Commons Attribution-Utilisation non commerciale-Pas d'oeuvre dérivée 4.0 International / Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND)

Titre de la revue: Journal Title:	The Science of the total environment (vol. 962)
Maison d'édition: Publisher:	Elsevier
URL officiel: Official URL:	https://doi.org/10.1016/j.scitotenv.2024.178364
Mention légale: Legal notice:	© 2025 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/bync-nd/4.0/).

Science of the Total Environment 962 (2025) 178364

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Impact of grazing by multiple *Daphnia* species on wastewater bacterial communities

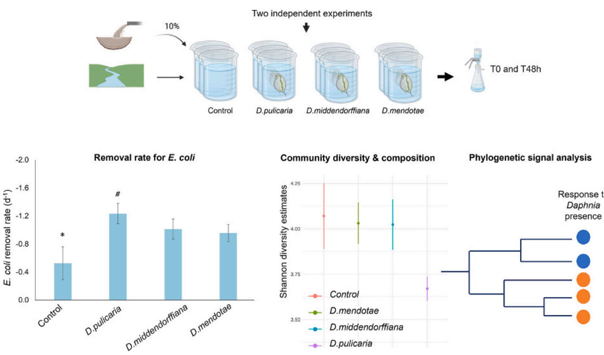
Nicolas Tromas [a,b], Eyerusalem Goitom [c,d], Tiffany Chin [e], Quoc Tuc Dinh [c], Sarah M. Dorner [c], Ola S. Khawasik [e], Melania E. Cristescu [e], Jean-Baptiste Burnet [c,*]

[a] *UMR CARRTEL – INRAE, 74200 Thonon les Bains, France*
[b] *Department of Biological Sciences, Université de Montréal, Montréal, QC H2V 0B3, Canada*
[c] *Department of Civil, Geological, and Mining Engineering, Polytechnique Montréal, Montreal, QC H3C 3A7, Canada*
[d] *Department of Geography, and Environmental Studies, Toronto Metropolitan University Toronto, ON M5B 2K3, Canada*
[e] *Department of Biology, McGill University, Montréal, QC H3A 1B1, Canada*

HIGHLIGHTS

- Study testing impact of three *Daphnia* species on wastewater bacterioplankton
- Exposure to *Daphnia* affects bacterial diversity and community composition.
- *D. pulicaria* displays strongest grazing pressure on fecal bacterial community.
- Microbial response to *Daphnia* is phylogenetically conserved.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Frederic Coulon

Keywords:
Grazing
16S rRNA gene
Fecal pollution
Zooplankton
Metabarcoding
Microcosm

ABSTRACT

Understanding the dynamics of fecal bacterial communities is crucial for managing public health risks and protecting drinking water resources. While extensive research exists on how abiotic factors influence the survival of fecal microbial communities in water, less attention has been paid to the impact of predation by higher organisms, such as the widely distributed grazer *Daphnia*. Nevertheless, *Daphnia* plays a significant role in regulating bacterial communities in natural aquatic ecosystems, and recent studies highlighted its potential as a biofilter in alternative tertiary wastewater treatment systems. In this study, we investigated the influence of three different *Daphnia* species on a wastewater bacterial community, including fecal indicator bacterium *E. coli*. Using a microcosm setup to simulate the discharge of untreated sewage into surface water, we conducted in-depth analysis of bacterial community dynamics through sequencing the 16S rRNA gene. Our results revealed significant changes in microbial diversity and composition following exposure to *Daphnia* grazing, with variations observed among the three *Daphnia* species. *D. pulicaria* exerted the most pronounced impact on microbial diversity, followed by *D. middendorffiana* and *D. mendotae*. A total of 90 taxa exhibited significantly reduced relative abundance in the presence of *Daphnia*, with Firmicutes phylum being the most affected. At genus level,

---

[*] Corresponding author at: Luxembourg Institute of Science and Technology, 41, rue du Brill, L-4422 Belvaux, Luxembourg.
E-mail address: jeanbaptiste.burnet@list.lu (J.-B. Burnet).

https://doi.org/10.1016/j.scitotenv.2024.178364
Received 15 October 2024; Received in revised form 11 December 2024; Accepted 31 December 2024

bacteria typically associated with wastewater (e.g., *Zoogloea* and *Arcobacter*) and gut microbiome constituents (e.g., *Prevotella* and *Akkermansia*) were notably affected by *Daphnia* exposure. The influence of *Daphnia* on bacterial community composition was most pronounced for *D. pulicaria*, while *D. middendorffiana* and *D. mendotae* primarily impacted community structure. Furthermore, we demonstrated that the microbial response to *Daphnia* exposure is phylogenetically conserved, potentially reflecting a grazing resistance or grazer feeding trait. Our findings shed new light on the role of *Daphnia* in controlling bacterial communities in polluted water bodies and underscore its potential as biofilter in wastewater treatment and reuse contexts.

1. Introduction

Grazing by zooplankton is a key mechanism for controlling the bacterioplankton, the phytoplankton and consequently, the entire biotic communities in freshwater resources (Jürgens, 1994; Sanders et al., 1992). Early studies showed that the non-selective filter feeding strategy of many zooplankton species could result in the removal of microorganisms from the water column within a day during zooplankton density peaks (Haney, 1973; Sanders et al., 1994). Among zooplankton communities, *Daphnia* is recognized as an efficient grazer that can alter the structure of the entire food web by affecting the structure of phytoplankton populations (Jürgens, 1994; Porter, 1973; Sarnelle and Knapp, 2005; Sterner, 1989). Grazing could also indirectly disturb the microbial community by altering the availability of nutrients (Sterner, 1986). Species within the genus *Daphnia* are widely distributed and can be found in large permanent lakes as well as small, ephemeral ponds. Through non-selective filtering, *Daphnia* species can retain small (0.1–50 μm) biotic particles such as flagellates, ciliates, and bacteria through sieving (Jürgens, 1994; Peterson et al., 1978). Within that size range, food selectivity can be considered negligible, and *Daphnia* can exert a significant grazing pressure on all microbiological components and thereby affect bacterial community structure and composition (Berga et al., 2015; Degans et al., 2002; Jürgens and Jeppesen, 2000; Langenheder and Jürgens, 2001). Over the last decade, widespread use of high throughput sequencing power has enabled to decipher microbial community structures and dynamics in water and wastewater at unprecedented resolution (Garner et al., 2021) and a few studies have investigated *Daphnia* grazing on bacterial metacommunities using this approach (Berga et al., 2015; Birtel and Matthews, 2016).

Although this large body of studies unfolded the grazing impact of *Daphnia* on natural bacterioplankton communities from freshwater bodies, they did not necessarily address the role of grazing on allochthonous microorganisms such as those introduced into aquatic habitats through fecal pollution. Fecal microorganisms originate from diverse pollution sources such as untreated sewage discharges, agricultural runoff or direct fecal inputs from livestock and wildlife. Understanding the population dynamics of fecal microorganisms is thus of key importance for the control and management of fecal pollution and associated risks for human and ecosystem health (Ferguson et al., 2003). This is especially relevant for fecal indicator bacteria (FIB) as they represent standard microbial parameters monitored within regulatory frameworks for safe drinking and recreational waters (WHO, 2022, 2021). Compared to the role of abiotic factors such as UV or temperature (Blaustein et al., 2013; Espinosa et al., 2020; Hijnen et al., 2006) on the fate of fecal microorganisms in water, grazing by *Daphnia* (and metazooplankton in general) has received much less attention. Early feeding studies reported ingestion of the fecal indicator *Escherichia coli* by *Daphnia* (McMahon and Rigler, 1965). *Daphnia* was also shown to affect the fate of fecal indicators and pathogens under laboratory settings (Burnet et al., 2017; Ismail et al., 2019; Schallenberg et al., 2005). Yet, most studies investigating *Daphnia* grazing on fecal microorganisms have been performed with single (or limited) microbial species instead of mixed communities, often involving high spiking doses that are not representative of the expected removal rates under natural conditions (Burnet et al., 2017; Connelly et al., 2007; Hadas et al., 1983; Ismail et al., 2020; Schallenberg et al., 2005). Also, studies are usually limited to a single grazer species, *Daphnia magna* often being the model organism because of its robustness and ease of manipulation (e.g., Freese and Martin-Creuzburg, 2013; Ismail et al., 2019). With global change expected to increase fecal pollution of freshwater resources (Jalliffier-Verne et al., 2017), there is a pressing need to better understand the role of higher organisms such as *Daphnia* in controlling fecal pollution in natural aquatic systems.

In the present study, we tested three different *Daphnia* species to assess their capacity to impact a wastewater bacterial community including the FIB *E. coli*. Through sequencing of the 16S rRNA gene, we conducted an in-depth investigation of the fate of the bacterial community upon exposure to *Daphnia* using a microcosm setup by mimicking a discharge of untreated sewage into surface water. We hypothesized that *Daphnia* plays a major role in disturbing the microbial community through reducing taxa of fecal origin. On the one hand, *Daphnia* could decrease the species richness through grazing (Berga et al., 2015). In that case, community structure would tend to be composed of more grazing-resistant populations (Jürgens and Gude, 1994). On the other hand, *Daphnia* could also alter the community composition by changing the dissolved organic matter (DOM) - microbes associations (Lampert, 1978; Tanentzap et al., 2019). In such case, *Daphnia* would promote the release of particulate and DOM (De Corte et al., 2023) and potentially increase the bacterial richness (Hu et al., 2022). By manipulating the presence and absence of the different *Daphnia* species, we first tested whether or not *Daphnia* species have similar impact on bacterial population densities (especially on *E. coli*) and structure. We finally dissected the microbial community disturbance by comparing if the microbial response to *Daphnia* presence is a phylogenetically conserved trait, i.e. if closely related species share similar grazer response. The present study provides novel insights on the impact of *Daphnia* grazing on microbial communities in fecally contaminated water and its findings have implications for inactivation models predicting bacterial decay in natural and engineered aquatic systems.

2. Material and methods

2.1. *Daphnia* cultures

Cultures of *Daphnia pulicaria*, *Daphnia mendotae* and *Daphnia middendorffiana* were maintained in the Laboratory for Experimental Ecology and Evolution (LE3) of McGill University. These species were chosen based on 1) their capacity to rapidly yield large populations under the given culture conditions, 2) their acclimation to the sewage-based water matrices used in grazing experiments. A preliminary exposure of several *Daphnia* species (maintained in the same lab under the same conditions) to a diluted wastewater matrix led to the selection of the three *Daphnia* species. *Daphnia* cultures were maintained under standardized conditions in a climate room at (18 °C ± °C with a 12:12 light:dark photoperiod and kept separately in a 10 L-container with ~500 individuals. The artificial FLAMES medium used (Celis-Salgado et al., 2008) mimics a soft water body. The animals were fed twice a week with a mixture of *Ankistrodesmus* sp., *Scenedesmus* sp., and *Pseudokirchneriella* sp.

2.2. Microcosm preparation and experimental set-up

Before the start of the experiment, we conducted a preliminary 48-hour incubation of *Daphnia* species in sewage microcosms at ambient temperature. As our goal was to simulate a fecal contamination event (sewage spiked into river water), a microcosm setup helps reducing the impact of other biotic/abiotic factors present in a real wastewater treatment process. As such, we were not able extend the experiment longer than 48 h as the microbial community is changing very quickly in such setups. We therefore conducted two independent grazing experiments (A and B) in microcosms at ambient temperature for 48 h. For microcosm preparation, surface water (20-L) and a primary effluent (5-L) were collected on Oct 7 and Oct 14, 2017, from a wastewater treatment plant in the Greater Montreal Area, Quebec, Canada. Upon return to the laboratory within 2 h, primary effluent and surface water were immediately mixed in a large container to a final proportion of 1:10 before being dispatched into 2-L glass beaker microcosms containing 1.6 L of the mixed water matrix. Triplicate *Daphnia* microcosms were seeded with 50 parthenogenetic females of similar body sizes of the respective species (*D. pulicaria*, *D. mendotae*, or *D. middendorffiana*) while excluding juveniles. Control microcosms ($n = 4$ and $n = 3$ during experiments 1 and 2, respectively) were added to assess the fate of microbial communities in the absence of *Daphnia*. For both experiments, microcosms were maintained under standard laboratory conditions at 20 °C and 14:10 light:dark photoperiod. At the end of the 48-h incubation period, 10 to 36 *Daphnia* individuals were harvested and preserved in 4 % formaldehyde for body size measurement (Supplementary Fig. 1). During experiment B, an exceptionally high mortality rate was observed (unknown explanation) across the 48-h incubation period for one of the three *D. mendotae* microcosms. The latter could therefore not be used for subsequent analyses.

2.3. Sample processing

Microbial community composition as well as *E. coli* and total suspended solids (TSS) concentrations were assessed in control microcosms at the beginning of the experiment (i.e., after preparing the microcosms, at T_0). Samples were then collected from each microcosm (control and *Daphnia* treatments) after 24 h (T_{24}) and 48 h (T_{48}) for *E. coli* and TSS measurements. At the end of the experiment (T_{48}), samples were also processed for microbial community analysis. Enumeration of *E. coli* was conducted using the membrane filtration method (MI agar) according to USEPA method 1604 (US Environmental Protection Agency, 2002). One hundred milliliters (100 mL) of raw or diluted sample were filtered through a sterile 47-mm, 0.45 μm pore size cellulose ester membrane filter (Millipore) and placed on a 5-mL plate of MI agar (BD Biosciences) containing 5 μg/mL cefsulodine (Sigma Aldrich). Plates were incubated at 35 °C for up to 24 h. Identified *E. coli* colonies were counted, and concentrations were expressed in colony-forming units (CFU) per 100 mL (CFU.100 mL^{-1}). TSS were measured at the start of the experiment (T_0) and at the end of the experiment (T_{48}) in each microcosm by Standard Method 2540D (APHA, 2017).

2.4. Removal rate calculation

The decay rate (k) was calculated using the equation $Ln(C_t/C_0) = -kt$, where C_0 and C_t are the concentrations in culturable *E. coli* (CFU. mL^{-1}) at T_0 and $T_{48,}$ respectively and t is the incubation time (days).

2.5. Molecular analyses

2.5.1. DNA extraction

Samples (100 mL) were filtered through a sterile 47-mm, 0.45 μm pore size cellulose ester membrane filter (Millipore) and stored into a sterile microcentrifuge tube at −20 °C until further processing. DNA extraction was performed using the FastDNA® SPIN kit (MP Biomedicals). Filter samples were homogenized with Lysis matrix A on a FastPrep® instrument at a speed of 6.0 $m*s^{-1}$ for 40 s during 2 successive cycles and were cooled down on ice for 5 min between cycles to avoid overheating. Lysates were centrifuged, and DNA was purified on SPIN Filter columns according to the instructions of the manufacturer and stored at −20 °C.

2.5.2. 16S library preparation and Next Generation Sequencing (NGS)

The V4 region of the 16S rRNA gene was amplified using the universal primers 515F: 5'- GTG CCA GCM GCC GCG GTA A -3' and 786R: 5'-GGA CTA CHV GGG TWT CTA AT -3' (Preheim et al. 2013). Each DNA extract was PCR amplified in triplicate. We follow the Illumina Inc. protocol '16S Metagenomic Sequencing Library Preparation' with small modifications to prepare the sequencing-ready libraries. First step PCR reactions were conducted in 25 μL volumes, which contained 10 mM deoxynucleotides, 5 μM of each primer, and 0.5 U Phusion polymerase (Thermo Scientific Phusion High Fidelity DNA Polymerase). The template DNA was amplified using primers that were appended with overhang adapter nucleotide sequence; MiSeq llumina Forward overhanger: 5' TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG 3′ and MiSeq Illumina Reverse overhanfsuppger: 5' GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G 3′. The thermal cycle profile included an initial 30 s activation step at 98 °C followed by 22 cycles of 20 s denaturation at 98 °C, 35 s annealing at 54 °C and 30 s elongation at 72 °C. Amplification success was verified on 1 % agarose gels. All PCR amplicons were cleaned using AMPure beads following the manufacturer instructions (Beckman Coulter Life Sciences Inc.). In the second step PCR, unique dual indices and Illumina sequencing adapters were attached to each library using the Nextera® Index Kit. The thermal cycle profile for the second PCR included an initial 3-min activation step at 95 °C followed by 8 cycles of 30 s denaturation at 95 °C, 30 s annealing at 55 °C and 30 s elongation at 72 °C. A final cleaning step was carried out for all libraries using the AMPure beads. Libraries were then quantified using the Bioanalyzer system ®Agilent Technologies, normalized and pooled. All libraries were sequenced by Genome Quebec using the pair-end 250 bp Illumina MiSeq technology.

2.5.3. Sequences analysis

All sequences with <30 quality score and/or with <150 bp were excluded using Fastx. We removed primers and adapters using Cutadapt (Martin, 2011) and then used DADA2 (R package version 1.14.1 (Callahan et al., 2016), a workflow for recovering single-nucleotide resolved Amplicon Sequence Variants (ASVs). Forward and reverse read pairs were trimmed and filtered, dereplicated, chimera-checked, and merged using standard parameters (Supplementary Table 1). To reduce non-specific priming, we removed non-target-length sequences, and we assigned the taxonomy against the Silva v132 via the *assignTaxonomy* DADA2 R function. To estimate the accuracy of the DADA2 pipeline, we used BLASTn to evaluate how many sequences from the mock community ATCC® MSA-1002 were recovered. The 20 genomes of the mock community were downloaded from NCBI and their corresponding 16S sequences were extracted using barrnap (version 0.9; https://github.com/tseemann/barrnap). To remove false positive ASV and reduce the impact of samples with low sequencing depth, ASVs observed <10 times and samples with <10,000 sequences were removed (Supplementary Table 2, Supplementary Figs. 2-3).

2.6. Statistical analyses

2.6.1. Removal of E. coli and total suspended solids

For statistical analyses, the calculated *E. coli* and TSS removal rates from both experiments were pooled ($n = 6$). ANOVA 1 with Dunnett's test was used to identify significant ($p < 0.05$) differences between *Daphnia* groups and the control group, and Tukey's HSD test was used to identify significant differences between individual *Daphnia* groups.

2.6.2. *Diversity analysis*

To evaluate the impact of *Daphnia* species presence on bacterial community, we first estimated Shannon diversity with DivNet; which accounts for sampling variation (DivNet R package version 0.3.6) (Willis and Martin, 2022). As Shannon index considers both richness and evenness, we also calculated Faith's phylogenetic diversity (PD) using MetagMisc R package (https://github.com/vmikk/metagMisc, v.0.04). PD is a phylogenetic measure of richness based on the sum of branch lengths. However, since this index is sensitive to sequencing depth, we rarefied the dataset with the phyloseq R function *rarefy_even_depth*. Rarefaction is generally associated with a loss of data, we therefore evaluated Shannon diversity (Microbiome R package, http://microbiome.github.com/microbiome, v.1.20.0) with the rarefied table to compare with DivNet result and observed similar trend.

To compare the microbial composition among samples, we calculate the Bray-Curtis dissimilarity using DivNet. We used the phyloseq R package version 1.42.0 (McMurdie and Holmes, 2013) to calculate the square root of three metrics: Jensen-Shannon divergence (JSD), Weighted, and Unweighted UniFrac (Fuglede and Topsoe, 2004; Lozupone et al., 2007). The UniFrac beta-diversity indexes uses phylogenetic information, however weighted UniFrac also considers species abundance information. As for alpha diversity measures, we used rarefied table for beta-diversity measure sensitive to sequencing depth (McMurdie and Holmes, 2014). We finally performed a principal coordinates analysis (PCoA) (Gower, 1966) to evaluate if the microbial composition from different grazing conditions (different *Daphnia* species) were dissimilar to our controls (i.e., absence of *Daphnia*).

2.6.3. *Differential analysis*

To estimate how *Daphnia* could alter the microbial community (e.g., grazing effect) we used the Multivariable Association Discovery in Population-scale Meta-omics Studies approach (*Maaslin2* R package, version 1.11.0 (Mallick et al., 2021)). We used Maaslin2 R package to identify taxa - at genus level - that significantly decreased in abundance in presence of different *Daphnia* species. “Replicate” variable was included as random effect parameter. Parameters were chosen based on Nearing et al., (2022) where they compared different approaches for differential analysis. *P*-values were adjusted using Benjamini-Hochberg procedure and we only conserved taxa with adjusted *p*-value (q-value) < 0.05.

2.6.4. *Phylogenetic analysis to explore phylogenetic signals*

We first built a phylogenetic tree using ips R package (https://github.com/heibl/ips, v.0.0.11) and RAxML GTRGAMMAIX model) (Stamatakis, 2014) using all ASV sequences previously aligned with the R package Decipher (v.2.2.26) (Wright, 2015). We used the ggtree R package (v3.6.2) (Yu et al., 2017) to visualize the tree with metadata extracted from Maaslin2 analysis at ASV level. In this approach, data from the two independent experiments A and B were pooled and *Daphnia* species were grouped to evaluate; with higher statistical power; if closely related taxa have similar response intensity to *Daphnia* presence. We first calculated the absolute value of the difference between log2FoldChange score for each pair of ASV (with a significant change in abundance based on Maaslin2 result). We then analyzed the relationship between this difference and the phylogenetic distance. If closely related ASV showed similar response to the presence of *Daphnia* (i.e., based on Maaslin2 results), we expected to observe a positive correlation between the phylogenetic distance and the difference of log2FoldChange. To validate this analysis, we also use the phylosignal R package (Keck et al., 2016), https://github.com/fkeck/phylosignal, v.1.3). Phylosignal will use a collection of tools to explore the phylogenetic signal, i.e., if closely related species will display similar trait (here microbial community's response to *Daphnia* presence).

The nature of the microbial response to *Daphnia* could also be evaluated as a categorical variable: positive (the taxa is more abundant in presence of *Daphnia*) or negative (the taxa is less abundant). We therefore analyze this categorical trait using D statistic (Fritz and Purvis, 2010) from the caper R package (https://github.com/cran/caper, v1.0.1). The function *phylo.d* was used with the default parameter and 1000 permutations. This function calculates the *p*-values to test whether D is significantly different from one $P(D > 1)$ and different from 0 $P(D = 0)$. If $D = 1$, it indicates a random distribution, if $D = 0$, the trait is clustered under Brownian motion model.

3. Results

3.1. *Impact of Daphnia on the removal of E. coli and total suspended solids*

Daphnia had a significant impact on the removal of particles from the water including the fecal indicator bacterium (FIB) *E. coli* (Fig. 1). The removal of *E. coli* by *Daphnia* was significant ($p < 0.05$) for *D. pulicaria*, *D. middendorffiana* and *D. mendotae* compared to control microcosms when combining both experiments. When considering experiments separately, the three species exerted a significant ($p < 0.05$) grazing pressure on *E. coli* and particles compared to controls during experiment A, but during experiment B, removal rates of *E. coli* in presence of *D. mendotae* did not exceed those observed in control microcosms.

3.2. *Impact of Daphnia on microbial community diversity and composition*

3.2.1. *Experimental system*

To assess how the different *Daphnia* species might disturb the composition of the microbial community, we first evaluated the DADA2 pipeline using a mock community and recovered 100 % of the ASVs that exactly matched the reference genomes of the expected mock community members. Phyloseq (R package version 1.30.0, (McMurdie and Holmes, 2013)) was then used to analyze the ASVs sequences. A total of 7488 ASVs were obtained from the 8,609,119 sequences processed through DADA2, ranging from 15 to 866,080 reads per sample, with a median of 242,046 reads per sample. Prior to the analysis, four samples with <10,000 sequences were removed from the ASV (Supplementary Fig. 2). Three experimental samples (T481B, T01B, Dmid1A) with <10,000 reads and 3 negative controls were removed. All the analysis were therefore performed on 13 samples for experiment A and 14 samples for experiment B (Supplementary File: metadata_file.csv).

The similarity of the microbial community between the independent experiments A and B was then estimated under initial conditions (T_0) and after 48 h (T_{48}) incubation in control (absence of *Daphnia)* microcosms. Samples from the same experiment tended to cluster at phylum level, whereas microbial community abundances at T_0 differed significantly between both experiments (Supplementary Figs. 4–5). For example, Cyanobacteria and Actinobacteriota phyla were comparatively more abundant in experiment A, while Fusobacteriota and Proteobacteria were more abundant in experiment B (Supplementary Fig. 6). Differences in the initial microbial composition of samples between experiment A and B could arise from short-term changes of raw sewage microbial composition (Guo et al., 2019). Microbial community composition further changed over time (Supplementary Fig. 7). For example, Campylobacterota and Firmicutes phyla were comparatively less abundant after 48 h, whereas Proteobacteria relative abundance increased over time. Global trends over 48 h were similar between both experiments though, with either an increase or a decrease in abundance of some phyla (Supplementary Fig. 7).

3.2.2. *Daphnia impact on microbial diversity*

Microbial diversity changed significantly after 48 h exposure to *Daphnia* grazing, but it differed among species. In the presence of *D. pulicaria*, microbial diversity decreased significantly in all samples at T_{48} (experiments A and B) (regression model, $p < 0.001$, Supplementary Table 3). The same trend was observed in the presence of

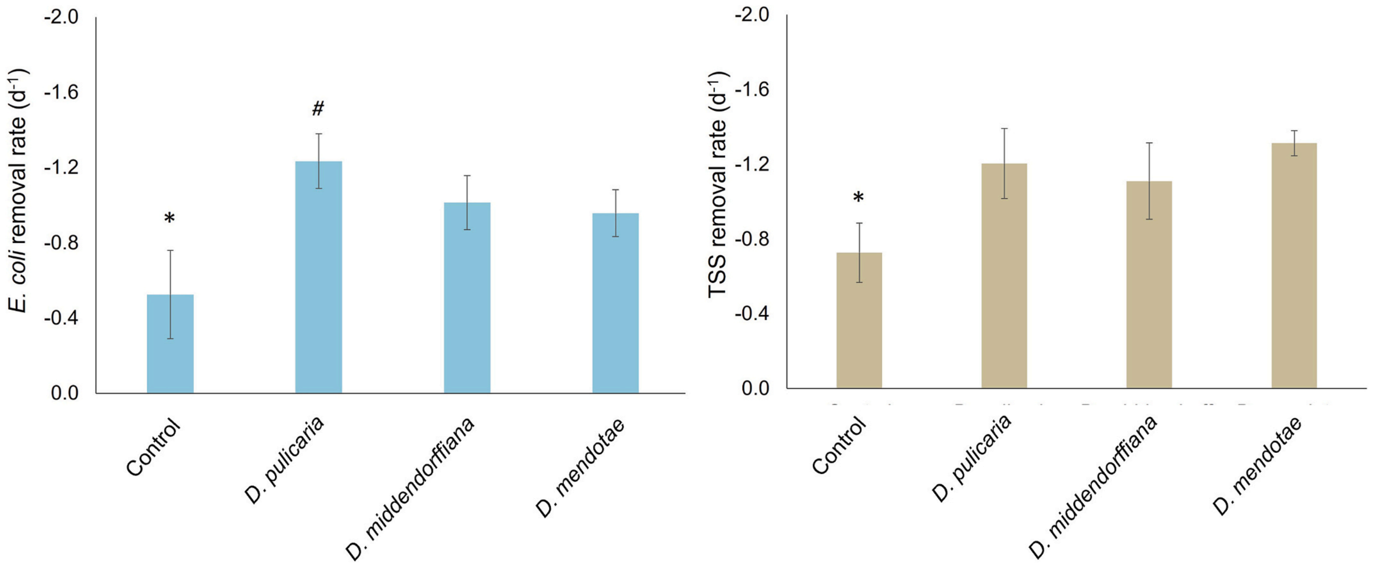


Fig. 1. Impact of three *Daphnia* species on the removal rate (average ± standard deviation, $n = 6$) of a) *E. coli* and b) TSS after 48 h incubation in a mixture of lake water and primary effluent Data from the two independent experiments A and B were pooled for higher statistical power. *significant ($p < 0.05$) differences between control and *Daphnia* groups, $^{\#}$significant differences between individual *Daphnia* groups. TSS, total suspended solids.

D. middendorffiana, but in experiment B only (Fig. 2, Supplementary Table 3), which could be associated with the fact that initial microbial composition differed between both experiments (Supplementary Fig. 4). In contrast, *D. mendotae* did not significantly impact microbial diversity during both experiments compared to controls (Fig. 2). As Shannon diversity combines species richness and evenness, we further calculated the phylogenetic diversity (PD) with rarefied data as this metric is sensitive to sequencing depth. When compared to controls, we observed an increase of PD for *D. mendotae* and *D. middendorffiana* in experiment A, and an increase only for *D. mendotae* in experiment B (Supplementary Fig. 8). *D. pulicaria* showed slightly lower PD values than for the control in both experiments. We therefore concluded that the Shannon diversity decreased was essentially linked to evenness decrease.

To compare the impact of different *Daphnia* species on microbial communities, we performed a pairwise analysis using Bray-Curtis distance (Fig. 3). Microbial communities were more similar in the presence of *Daphnia* than in its absence (controls) in experiment A. This observation was confirmed with other beta-diversity measures considering species abundance (i.e., JSD and weighted UniFrac) (Supplementary Figs. 9–10). In experiment B, *D. pulicaria* and *D. middendorffiana* displayed the most similar community compared to all different pairs. However, Unweighted UniFrac measures, which only considers the presence or absence of taxa, showed that microbial composition was relatively similar between two controls, *D. pulicaria* and *D. middendorffiana* in experiment B (Supplementary Fig. 10). Similarly, *D. pulicaria* microcosms displayed a different microbial community compared to other *Daphnia* microcosms and controls, especially when bacterial species abundance was considered. We can therefore conclude that microbial relative abundance was mainly affected in the presence of *D. pulicaria*. On the other hand, microbial changes observed in presence of *D. mendotae* were mostly driven by changes in community composition.

3.2.3. *Daphnia impact on microbial community composition*

Daphnia species differently impacted the microbial community composition in sewage-impacted freshwater microcosms. *Limnohabitans* was the most abundant genus in both control and *Daphnia* microcosms. In the presence of *D. pulicaria* and *D. middendorffiana*, the abundance of Firmicutes tended to decrease (Fig. 3, Suppl. Table 4). *Flavobacterium* had a higher relative abundance in samples with *D. middendorffiana* and *D. pulicaria* but only in experiment B. For experiment A, *hgcI_clade* was more abundant in presence of *Daphnia* (Suppl. Table 4, Fig. 4).

To evaluate the overall effect of *Daphnia* presence on the microbial community, we performed a differential analysis to compare controls and *Daphnia* treatments. To reduce potential noise due to the low number of replicates in each category, we analyzed microbial community changes at genus level while accounting for inter-replicate variability. For each experiment, we first identified taxa that were significantly more or less prevalent when exposed to *Daphnia* (Supplementary Fig. 11). Taxa abundance varied more in experiment A compared to experiment B (104 and 22 significant changes in abundance, respectively). Fewer taxa were negatively impacted in experiment A, among them the order Burkholderiales which is very most abundant in the *Daphnia* microbiome (Freese and Schink, 2011). Across the two experiments, we found a total of 90 taxa with a significantly lower relative abundance in presence of *Daphnia* (Supplementary Table 5). Firmicutes phylum was the most negatively impacted one (39 taxa with negative log2fold change). At the genus level, several bacteria generally found in wastewater (e.g., *Zoogloea* and *Arcobacter*) and in the intestinal tract such as *Faecalibacterium* and *Lachnospiraceae* (e.g., *UCG-010*) were affected by exposure to *Daphnia*. Taxa associated to the gut microbiome such as *Prevotella* and *Akkermansia* were also impacted. To a lesser extent, 36 taxa were significantly more abundant in presence of *Daphnia*. The Proteobacteria and Actinobacteriota phyla were generally more abundant in *Daphnia* microcosms (17 and 9 taxa with positive log2fold change, respectively). This corroborates previous studies that found these phyla in *Daphnia* microbiomes (Qi et al., 2009). Changes in community composition varied with *Daphnia* species, indicating that the impact on microbial communities depends on the species to which they are exposed (Supplementary Fig. 8). Significant results were found only for *D. middendorffiana* and *D. mendotae* ($Q < 0.05$ with BH *p*-value correction), whereas no significant taxa were impacted by *D. pulicaria* using Maaslin2 approach (Supplementary Table 6). Microbial community was most impacted by *D. middendorffiana,* especially in experiment A, where taxa that belong to Firmicutes were less abundant compared to other phyla (48 % of negatively impacted taxa belonged to Firmicutes). Several taxa belonging to the gut microbiome (e.g., *Intestinibacter*, *Odoribacter*) and to sewage (e.g., *Macromonas*) were also found.

3.2.4. *The nature, but not the intensity of the microbial community response to Daphnia presence is phylogenetically conserved*

Differential abundance analysis was performed for each sample after

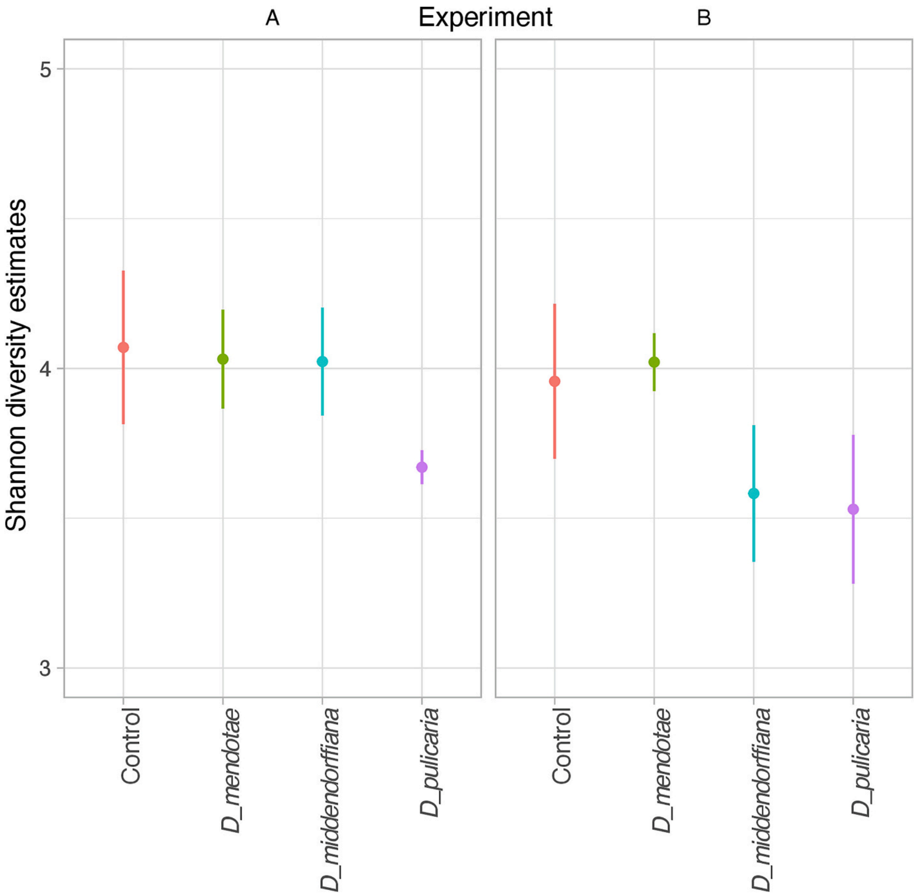


Fig. 2. Shannon diversity estimates in absence (control) and presence of *Daphnia* after 48 h incubation (T_{48}) during experiment A (left) and B (right). Error bars represent a 95 % confidence interval for the true mean Shannon diversity in each group.

exposure to *Daphnia* (at T_{48}) to evaluate the impact of the different *Daphnia* species on the microbial community at ASV level. We then evaluated if the response in terms of abundance changes (i.e., log2-FoldChange) was a phylogenetic trait as already observed in a previous study (Birtel and Matthews, 2016). We first visualized the phylogenetic tree of the ASV that were significantly impacted and their associated traits (Fig. 5).

No significant relationship between phylogenetic distance and the value of the co-response to the presence of *Daphnia* was observed (see Methods, Supplementary Fig. 12). Moreover, the phylosignal analysis confirmed this result using different measures (e.g., Moran's I, Abouheif's Cmean, Pagel's lambda and Blomberg's K, (Keck et al., 2016)) with similar score and *p*-values between *Daphnia* microbial response and a random variable (Supplementary Table 7, Supplementary Fig. 13). This result showed that the intensity of the response (microbial response to the presence of *Daphnia* as a continuous trait) is not due to their phylogenetic relationships. We finally tested if the nature of the response (positive = increase in taxa, negative = decrease in taxa upon exposure to *Daphnia*) could be associated with phylogeny. Calculation of the D metric from the caper R package ($D = 0.140$, $P(D > 1) < 0.05$, $P(D = 0) = 0.154$) showed that the nature of the response was more phylogenetically clustered than expected by chance. Overall, this result showed that bacteria might share similar traits associated with *Daphnia* presence (potentially grazing resistance) and that the nature of the signal but not the intensity is phylogenetically conserved.

3.2.5. *Daphnia pulicaria has an impact on fecal microorganisms*

Based on Shannon diversity analysis, microbial communities exposed to *D. pulicaria* differed most from the controls, implying that the grazing impact on the microbial community was likely stronger than for the two other *Daphnia* species (Figs. 1–3). However, phylogenetic diversity analysis showed similar phylogenetic richness between *D. pulicaria* and control microcosms. Moreover, as mentioned above, no significant changes at genus level were found when using a conservative cut-off for q-value, allowing 5 % of false positive. As *D. pulicaria* displayed the strongest grazing pressure, we evaluated the conservativeness of the differential analysis. Using Maaslin2 default parameter (i.e., Q cut-off to 25 %), a majority (~73 %) of the impacted taxa had a lower abundance and 48 % belonged to Firmicutes in Experiment A. Lower abundances of potential pathogens such as *Klebsiella*, *Raoultella* and *Yersinia* (Supplementary Table 8, Supplementary Fig. 14) were also observed. *D. pulicaria* was also the only species that significantly and negatively impacted the genus *Escherichia* (Supplementary Table 9, $P = 0.03$, $Q = 0.16$). For culture-based *E. coli*, *D. pulicaria* was also associated with comparatively higher removal rates (Fig. 1). As such, among the three tested species, *D. pulicaria* appeared to have the strongest impact of the fecal bacteria community.

4. Discussion

Overall, our results confirmed that *Daphnia* presence could drive the community structure as previously observed (Berga et al., 2015; Birtel and Matthews, 2016; Degans et al., 2002; Jürgens and Jeppesen, 2000) and that *Daphnia* species have different impacts on the microbial communities (Brendelberger, 1991; Peterson et al., 1978). Compared to previous studies, we show here that such trends are also observed for wastewater-associated bacterial communities. We find that alpha diversity is differently impacted by the presence of the three *Daphnia* species, with *D. pulicaria* showing the strongest Shannon diversity decrease (Fig. 2). *D. pulicaria* and *D. middendorffiana* showed similar

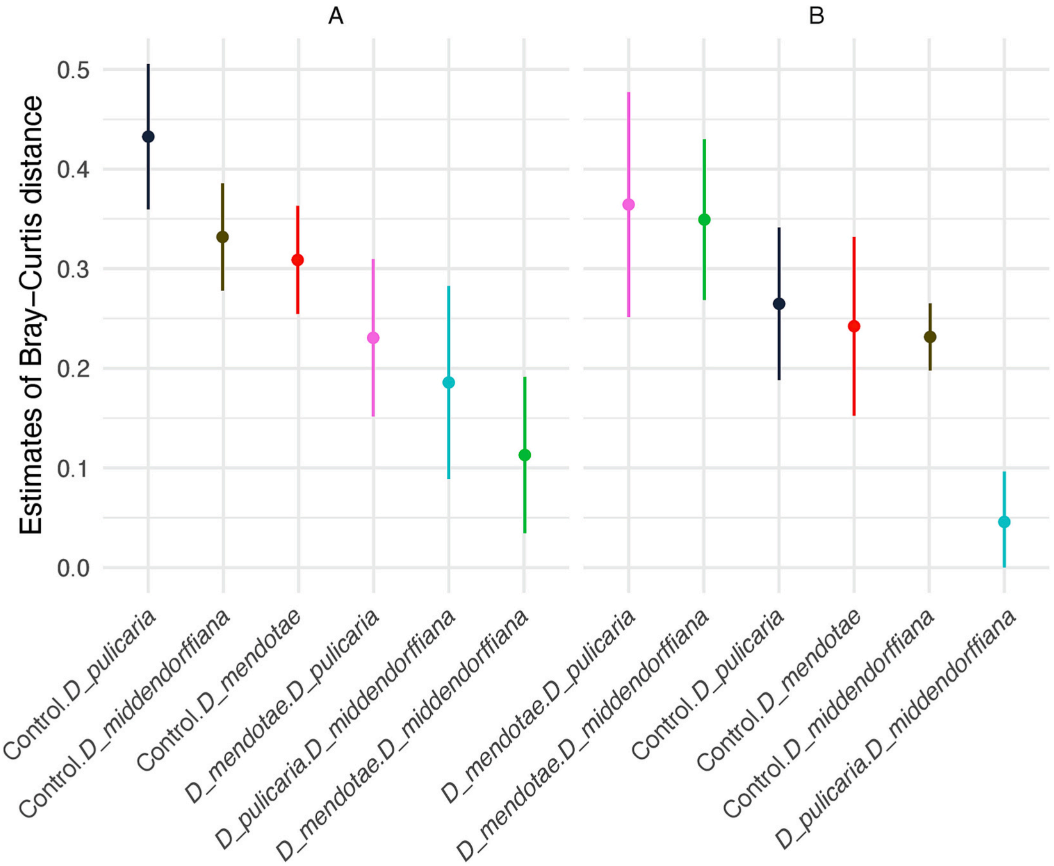


Fig. 3. Pairwise comparison of Bray-Curtis distance. As Bray-Curtis difference distance tends to 0, bacterial community between a pair tends to be similar. Error bars represent a 95 % confidence interval.

impact on microbial diversity and overall similar microbial composition, especially in Experiment B (Fig. 2–3, Supplementary Figs. 8–9)., *Daphnia* presence had a stronger impact on evenness than species richness. It is not clear though if grazing is the main mechanism involved in this observation. *Daphnia* presence could also impact the cross-feeding interactions leading to microbial community disturbance (Attayde and Hansson, 1999; Elser and Urabe, 1999). In addition, the bacterial communities carried by *Daphnia* (Tang et al., 2010) may also interact with wastewater microbiomes in the microcosms and impact their diversity and/or composition. On the other hand, the introduction of such *Daphnia*-associated microbiomes could explain the fact that *D. mendotae* samples displayed a different community composition and a higher phylogenetic richness. The differential analysis confirmed this trend and showed that in presence of *D. mendotae*, a large majority of significant changes were an increase in taxa abundance, especially from the order of *Chitinophagales*. Whether or not this observation could arise from a different composition of *D. mendotae* compared to the other species needs to be tested though. *Daphnia* microbiota is relatively simple and contains an aerobic bacterial community of rather low diversity mainly composed of ß-proteobacteria (e.g. *Limnohabitans* sp.), with also ϒ-proteobacteria (e.g. *Pseudomonas* sp.) and Bacteroidetes (Cooper and Cressler, 2020). Future sequencing of *Daphnia* microbiota could therefore shed additional light on microbial community dynamics when exposed to the grazer.

In the presence of *Daphnia*, *Firmicutes* was the phylum most negatively impacted, with several genera prevalent in wastewater or in the intestinal tract. Most of the changes were observed in experiment A in presence of *D. middendorffiana*. Using a similar approach, we did not find - at genus level - any significant changes in presence of *D. pulicaria*. When we used Masslin2 defaults parameters to explore the potential taxa impacted by *D. pulicaria*, we found a large majority (73 %) of taxa with reduced abundance mainly among *Firmicutes*. Several potential pathogens or emergent pathogens (e.g., *Escherichia, Raoultella, Comamonas*) were also found to have a lower relative abundance. Despite these results that confirmed our experimental observations with *E. coli*, we acknowledge that differential analysis might suffer from the low sample size that would impact the statistical power of such approach.

Our results showed that the intensity of microbial response to *Daphnia* presence was not a phylogenetic signal. However, the nature of the response (i.e. decrease or increase) was significantly associated with their phylogenetic relationships. This result could highlight that grazing resistance or *Daphnia* feeding strategy is a conserved trait in wastewater bacterial community. Seiler et al. (2017) showed that neither the microbial community present in a biofilm nor the plankton phenotype was grazing resistant to its specialized predator, but that the observed community changes mostly reflected the predator feeding trait.

Bacterivory by *Daphnia* is a driver of bacterioplankton abundance and composition in freshwater resources (Degans et al., 2002; Jürgens and Jeppesen, 2000; Langenheder and Jürgens, 2001) but *Daphnia* feeding could also impact the fate of fecal microorganisms including *E. coli* in natural aquatic systems and be valorized in biologically-based tertiary treatment of wastewater (Pous et al., 2021; Serra et al., 2022). Yet virtually nothing is known about the impact of *Daphnia* grazing on the composition and relative abundance of bacterioplankton in freshwater impacted by fecal pollution. The information we provide here therefore expands our knowledge on the interactions between *Daphnia* and bacteria in water, with a focus on wastewater-associated communities, and it has implications for inactivation models predicting bacterial decay in natural and engineered aquatic systems.

Further improvements could be made to the proposed experimental setup to push investigations at a next level. First, the present laboratory-based grazing experiments with *Daphnia* should be expanded to a

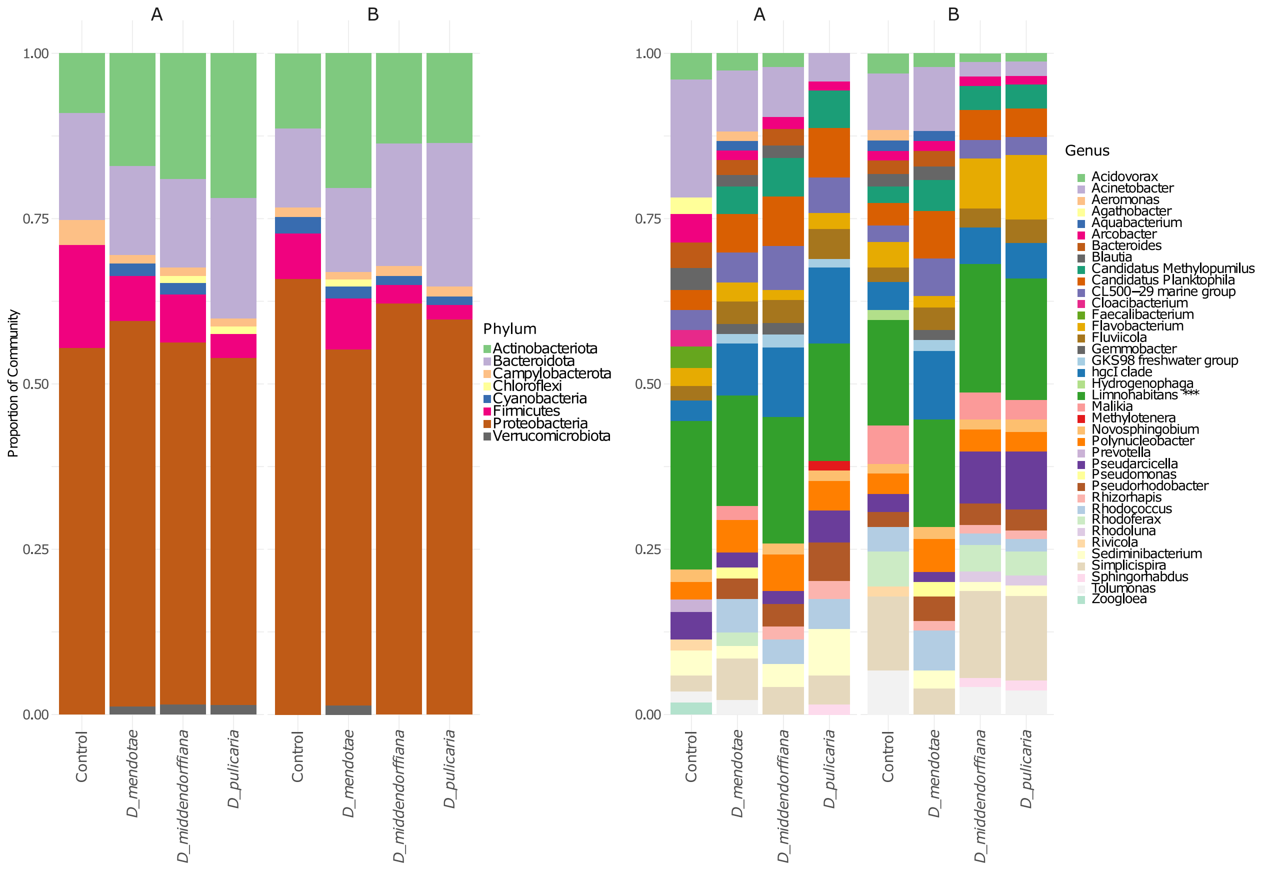


Fig. 4. Microbial composition at Phylum and Genus level of microcosms in absence (control) and presence of different *Daphnia* species after 48 h (T_{48}) of incubation during experiments A and B. Only most abundant taxa (average abundance >0.01) were selected.

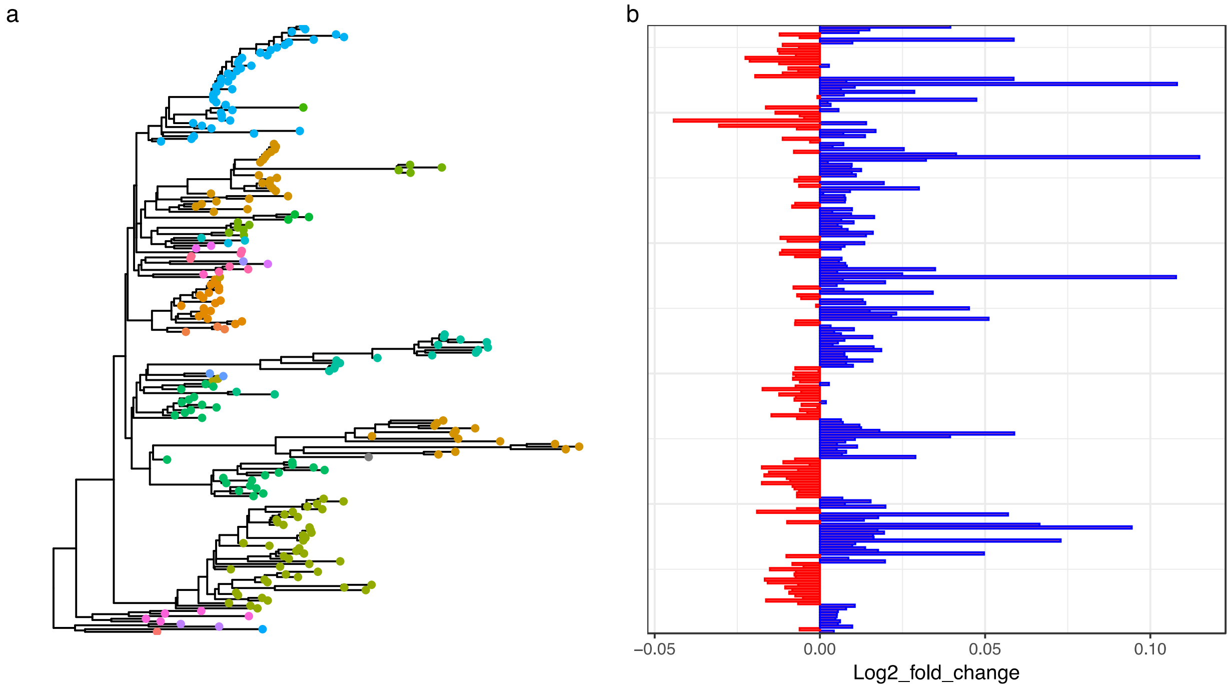


Fig. 5. Annotated phylogenetic tree highlighting microbial community response to *Daphnia* species. (a) Phylogenetic tree of impacted ASV where the different colors represent the taxonomical Class. (b) Barplot of the corresponding log2FoldChange score for each ASV present in the phylogenetic tree.

mesocosm setup to confirm the present findings on biological interactions in more natural conditions. The duration of the experiment could also be increased, and possible trophic cascading effects assessed (Jürgens, 1994). This would help appraise the biological relevance of the removal of *E. coli* and TSS by *Daphnia*. Also, additional *Daphnia* species commonly found in aquatic ecosystems, such as *D. galeta*, *D. pulex* or *D. longispina* could be tested. During preliminary microcosm trials, a pre-selection of the *Daphnia* species that best coped with the sewage mixture was done among 10 species available at the Laboratory for Experimental Ecology and Evolution (McGill University). Several of them were not retained because of their poor survival in the microcosms. Although *D. magna* cultures were not readily available at the time of our study, it would have been interesting to test this model species as well as it may have exceeded the grazing impact of *D. pulicaria* given its large filtration rates (Porter, 1973). In this sense, the Cladoceran *Diaphanosoma brachyurum* (Brendelberger, 1991) could also be an interesting candidate species to be tested. Considering that the mean filter mesh size increases with body length, neonates, and juveniles (which were excluded in our experimental setup) could have generated even higher *E. coli* removal rates than those observed in our study, as they retain small particles more efficiently (Brendelberger and Geller, 1985). Also, despite careful selection of egg-free *Daphnia* individuals of similar body sizes, adults may have varied in age, which in turn could result in different grazing behaviors. Finally, although barcoding provides a wealth of information on the impact of *Daphnia* on bacterial community diversity and relative abundances compared to earlier analytical methods, it is not known if these bacteria are still culturable or if they are injured/stressed and occur in a viable but non-culturable state upon exposure to *Daphnia*. This would be especially interesting for pathogenic species. Early studies suggested indeed that some lake bacteria could survive *Daphnia* gut passage (King et al., 1991). Our study shows that, at least for *E. coli*, both its cultivability and genomic signal were affected by *D. pulicaria*. In future, it could be interesting to further investigate the potential of *Daphnia* to reduce the concentrations of antibiotic-resistant bacteria (ARB) and genes (ARGs) on freshwater ecosystems, as recently reported by (Choi and Kim, 2021).

Aquatic resources pollution by fecal microorganisms is a growing concern worldwide and *Daphnia* can be found in virtually all natural aquatic resources. Yet, metazooplankton grazing on fecal bacteria is still poorly understood. This study therefore brings novelty by addressing the impact of multiple *Daphnia* species on fecal bacteria communities, including the regulatory FIB *E. coli*.

5. Conclusions

In this study, we provide new insights on the interaction between *Daphnia* and bacterial communities with a focus on wastewater-related ones.

- We validated our hypothesis confirming the role of *Daphnia* in removing fecal bacteria from water in a microcosm setup. Microbial diversity and community composition changed significantly after exposure to *Daphnia* grazing, but differences were observed among grazer species.
- Exposure to *D. pulicaria* had the strongest effect on microbial diversity, followed by *D. middendorffiana* and *D. mendotae*. For *D. pulicaria*, this was mainly driven by its impact on microbial relative abundance as it displayed the strongest grazing pressure on the fecal bacterial community including *E. coli* and other wastewater- and gut-related taxa.
- In contrast, *D. mendotae* mostly affected community composition.
- We finally demonstrated that the microbial response to *Daphnia* exposure is phylogenetically conserved and could reflect a grazing resistance or grazer feeding trait. This however needs to be further tested.
- The present findings contribute to our understanding on the value of *Daphnia* as a biofilter for mitigating fecal pollution in aquatic habitats.

Further studies should consider expanding these investigations to other grazers and assess their potential impact on the fate of antibiotic resistant bacteria and fecal pathogens. Also, *Daphnia* populations composed of adults, but also neonates and juveniles could better reflect natural conditions under which the Cladoceran can affect wastewater-related microbial communities.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.178364.

CRediT authorship contribution statement

Nicolas Tromas: Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Eyerusalem Goitom:** Writing – review & editing. **Tiffany Chin:** Resources, Methodology. **Quoc Tuc Dinh:** Investigation. **Sarah M. Dorner:** Writing – review & editing, Supervision, Funding acquisition. **Ola S. Khawasik:** Resources, Methodology. **Melania E. Cristescu:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Jean-Baptiste Burnet:** Writing – original draft, Supervision, Project administration, Methodology, Formal analysis, Conceptualization.

Ethics approval and consent to participate

Not applicable.

Declaration of competing interest

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

Acknowledgements

We would like to thank Jullien Flynn and Tarek Faraj for their valuable help with setting-up the experiments and culturing *Daphnia*. We also thank Michèle Prévost for her scientific inputs to this work. The project was funded by the Canada Research Chair in Source Water Protection to SMD and JBB, ATRAPP project (Genome Canada, grant #10512) to NT and an NSERC Discovery Grant (341423-2012) to MEC.

Data availability

The SRA data of the 16s rRNA sequencing reads have been deposited in the NCBI database with an accession number PRJNA1085780.

References

APHA, 2017. 2540 solids. In: Standard Methods for the Examination of Water and Wastewater, Standard Methods for the Examination of Water and Wastewater. American Public Health Association. https://doi.org/10.2105/SMWW.2882.030.

Attayde, J.L., Hansson, L.A., 1999. Effects of nutrient recycling by zooplankton and fish on phytoplankton communities. Oecologia 121, 47–54. https://doi.org/10.1007/s004420050906.

Berga, M., Östman, Ö., Lindström, E.S., Langenheder, S., 2015. Combined effects of zooplankton grazing and dispersal on the diversity and assembly mechanisms of bacterial metacommunities. Environ. Microbiol. 17, 2275–2287. https://doi.org/10.1111/1462-2920.12688.

Birtel, J., Matthews, B., 2016. Grazers structure the bacterial and algal diversity of aquatic metacommunities. Ecology 97, 3472–3484. https://doi.org/10.1002/ecy.1612.

Blaustein, R.A., Pachepsky, Y., Hill, R.L., Shelton, D.R., Whelan, G., 2013. *Escherichia coli* survival in waters: temperature dependence. Water Res. 47, 569–578. https://doi.org/10.1016/j.watres.2012.10.027.

Brendelberger, H., 1991. Filter mesh size of cladocerans predicts retention efficiency for bacteria. Limnol. Oceanogr. 36 (5), 884–894. https://doi.org/10.4319/lo.1991.36.5.0884.

Brendelberger, H., Geller, W., 1985. Variability of filter structures in eight Daphnia species: mesh sizes and filtering areas. J. Plankton Res. 7. https://doi.org/10.1093/plankt/7.4.473.

Burnet, J.-B., Faraj, T., Cauchie, H.-M., Joaquim-Justo, C., Servais, P., Prévost, M., Dorner, S.M., 2017. How does the cladoceran *Daphnia pulex* affect the fate of *Escherichia coli* in water? PLoS One 12. https://doi.org/10.1371/journal.pone.0171705.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13. https://doi.org/10.1038/nmeth.3869.

Celis-Salgado, M.P., Cairns, A., Kim, N., Yan, N.D., 2008. The FLAMES medium: a new, soft-water culture and bioassay medium for Cladocera. SIL Proc. 1922–2010 (30), 265–271. https://doi.org/10.1080/03680770.2008.11902123.

Choi, J.Y., Kim, S.K., 2021. Changes in antibiotic-resistance genes induced by the grazing effect in three cladoceran species. Microorganisms 9. https://doi.org/10.3390/microorganisms9091959.

Connelly, S.J., Wolyniak, E.A., Dieter, K.L., Williamson, C.E., Jellison, K.L., 2007. Impact of zooplankton grazing on the excystation, viability, and infectivity of the protozoan pathogens *Cryptosporidium parvum* and *Giardia lamblia*. Appl. Environ. Microbiol. 73, 7277–7282. https://doi.org/10.1128/AEM.01206-07.

Cooper, R.O., Cressler, C.E., 2020. Characterization of key bacterial species in the *Daphnia magna* microbiota using shotgun metagenomics. Sci. Rep. 10. https://doi.org/10.1038/s41598-019-57367-x.

De Corte, D., Varela, M.M., Louro, A.M., Bercovici, S.K., Valencia-Vila, J., Sintes, E., Baltar, F., Rodríguez-Ramos, T., Simon, M., Bode, A., Dittmar, T., Niggemann, J., 2023. Zooplankton-derived dissolved organic matter composition and its bioavailability to natural prokaryotic communities. Limnol. Oceanogr. 68. https://doi.org/10.1002/lno.12272.

Degans, H., Zöllner, E., Gucht, K., Meester, L., Jürgens, K., 2002. Rapid Daphnia-mediated changes in microbial community structure: an experimental study. FEMS Microbiol. Ecol. 42, 137–149. https://doi.org/10.1111/j.1574-6941.2002.tb01003.x.

Elser, J.J., Urabe, J., 1999. The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. Ecology 80, 735–751. https://doi.org/10.1890/0012-9658(1999)080[0735:TSOCDN]2.0.CO;2.

Espinosa, M.F., Sancho, A.N., Mendoza, L.M., Mota, C.R., Verbyla, M.E., 2020. Systematic review and meta-analysis of time-temperature pathogen inactivation. Int. J. Hyg. Environ. Health. https://doi.org/10.1016/j.ijheh.2020.113595.

Ferguson, C., Husman, A.M.deR., Altavilla, N., Deere, D., Ashbolt, N., 2003. Fate and transport of surface water pathogens in watersheds. Crit. Rev. Environ. Sci. Technol. 33 (3), 299–361. https://doi.org/10.1080/10643380390814497.

Freese, H.M., Martin-Creuzburg, D., 2013. Food quality of mixed bacteria-algae diets for *Daphnia magna*. Hydrobiologia 715, 63–76. https://doi.org/10.1007/s10750-012-1375-7.

Freese, H.M., Schink, B., 2011. Composition and Stability of the Microbial Community inside the Digestive Tract of the Aquatic Crustacean *Daphnia magna*. Microb. Ecol. 62, 882–894. https://doi.org/10.1007/s00248-011-9886-8.

Fritz, S.A., Purvis, A., 2010. Selectivity in mammalian extinction risk and threat types: a new measure of phylogenetic signal strength in binary traits. Conserv. Biol. 24. https://doi.org/10.1111/j.1523-1739.2010.01455.x.

Fuglede, B., Topsoe, F., 2004. Jensen-shannon divergence and Hilbert space embedding. In: International Symposium on Information Theory, 2004. ISIT 2004. Proceedings, p. 31. https://doi.org/10.1109/ISIT.2004.1365067.

Garner, E., Davis, B.C., Milligan, E., Blair, M.F., Keenum, I., Maile-Moskowitz, A., Pan, J., Gnegy, M., Liguori, K., Gupta, S., Prussin, A.J., Marr, L.C., Heath, L.S., Vikesland, P. J., Zhang, L., Pruden, A., 2021. Next generation sequencing approaches to evaluate water and wastewater quality. Water Res. https://doi.org/10.1016/j.watres.2021.116907.

Gower, J.C., 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53. https://doi.org/10.2307/2333639.

Guo, B., Liu, C., Gibson, C., Frigon, D., 2019. Wastewater microbial community structure and functional traits change over short timescales. Sci. Total Environ. 662, 779–785. https://doi.org/10.1016/j.scitotenv.2019.01.207.

Hadas, O., Kott, Y., Bachrach, U., Cavari1, B., 1983. Ability of daphnia cell-free extract to damage *Escherichia coli* cells. Appl. Environ. Microbiol. 45 (4), 1242–1246. https://doi.org/10.1128/aem.45.4.1242-1246.1983.

Haney, J.F., 1973. An in situ examination of the grazing activities of natural zooplankton communities. Arch. Hydrobiol. 72, 87–132. https://doi.org/10.4319/lo.1971.16.6.0970.

Hijnen, W.A.M., Beerendonk, E.F., Medema, G.J., 2006. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. Water Res. https://doi.org/10.1016/j.watres.2005.10.030.

Hu, A., Choi, M., Tanentzap, A.J., Liu, J., Jang, K.S., Lennon, J.T., Liu, Y., Soininen, J., Lu, X., Zhang, Y., Shen, J., Wang, J., 2022. Ecological networks of dissolved organic matter and microorganisms under global change. Nat. Commun. 13. https://doi.org/10.1038/s41467-022-31251-1.

Ismail, N.S., Blokker, B.M., Feeney, T.R., Kohn, R.H., Liu, J., Nelson, V.E., Ollive, M.C., Price, S.B.L., Underdah, E.J., 2019. Impact of metazooplankton filter feeding on *Escherichia coli* under variable environmental conditions. Appl. Environ. Microbiol. 85. https://doi.org/10.1128/AEM.02006-19.

Ismail, N.S., Olive, M., Fernandez-Cassi, X., Bachmann, V., Kohn, T., 2020. Viral transfer and inactivation through zooplankton trophic interactions. Environ. Sci. Technol. 54. https://doi.org/10.1021/acs.est.0c02545.

Jalliffier-Verne, I., Leconte, R., Huaringa-Alvarez, U., Heniche, M., Madoux-Humery, A. S., Autixier, L., Galarneau, M., Servais, P., Prévost, M., Dorner, S., 2017. Modelling the impacts of global change on concentrations of *Escherichia coli* in an urban river. Adv. Water Resour. 108. https://doi.org/10.1016/j.advwatres.2016.10.001.

Jürgens, K., 1994. Impact of *Daphnia* on planktonic microbial food webs - a review. Mar. Microb. Food Webs 8, 295–324.

Jürgens, K., Gude, H., 1994. The potential importance of grazing-resistant bacteria in planktonic systems. Mar. Ecol. Prog. Ser. 112, 169–188.

Jürgens, K., Jeppesen, E., 2000. The Impact of Metazooplankton on the Structure of the microbial food web in a shallow, hypertrophic lake, 22(6), pp. 1047–1070. https://doi.org/10.1093/plankt/22.6.1047.

Keck, F., Rimet, F., Bouchez, A., Franc, A., 2016. Phylosignal: an R package to measure, test, and explore the phylogenetic signal. Ecol. Evol. 6. https://doi.org/10.1002/ece3.2051.

King, C.H., Sanders, R.W., Shot, E.B., Porter, K.G., 1991. Differential survival of bacteria ingested by zooplankton from a stratified eutrophic lake. Limnol. Oceanogr. 36 (5), 829–844. https://doi.org/10.4319/lo.1991.36.5.0829.

Lampert, W., 1978. Release of dissolved organic carbon by grazing zooplankton. Limnol. Oceanogr. https://doi.org/10.4319/lo.1978.23.4.0831.

Langenheder, S., Jürgens, K., 2001. Regulation of bacterial biomass and community structure by metazoan and protozoan predation. Limnol. Oceanogr. 46 (1), 121–134. https://doi.org/10.4319/lo.2001.46.1.0121.

Lozupone, C.A., Hamady, M., Kelley, S.T., Knight, R., 2007. Quantitative and qualitative β diversity measures lead to different insights into factors that structure microbial communities. Appl. Environ. Microbiol. https://doi.org/10.1128/AEM.01996-06.

Mallick, H., Rahnavard, A., McIver, L.J., Ma, S., Zhang, Y., Nguyen, L.H., Tickle, T.L., Weingart, G., Ren, B., Schwager, E.H., Chatterjee, S., Thompson, K.N., Wilkinson, J. E., Subramanian, A., Lu, Y., Waldron, L., Paulson, J.N., Franzosa, E.A., Bravo, H.C., Huttenhower, C., 2021. Multivariable association discovery in population-scale meta-omics studies. PLoS Comput. Biol. 17. https://doi.org/10.1371/journal.pcbi.1009442.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J. 17.

McMahon, J.W., Rigler, F.H., 1965. Feeding rate of *Daphnia magna* Straus in different foods labeled with radioactive phosphorus. Limnol. Oceanogr. 10. https://doi.org/10.4319/lo.1965.10.1.0105.

McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8. https://doi.org/10.1371/journal.pone.0061217.

McMurdie, P.J., Holmes, S., 2014. Waste not, want not: why rarefying microbiome data is inadmissible. PLoS Comput. Biol. 10. https://doi.org/10.1371/journal.pcbi.1003531.

Peterson, B.J., Hobbie, J.E., Haney, J.F., 1978. Daphnia grazing on natural bacteria. Limnol. Oceanogr. https://doi.org/10.4319/lo.1978.23.5.1039.

Porter, K.G., 1973. Selective grazing and differential digestion of algae by zooplankton. Nature 244. https://doi.org/10.1038/244179a0.

Pous, N., Barcelona, A., Sbardella, L., Hidalgo, M., Colomer, J., Serra, T., Salvadó, V., 2021. Zooplankton-based reactors for tertiary wastewater treatment: a pilot-scale case study. J. Environ. Manag. 278. https://doi.org/10.1016/j.jenvman.2020.111538.

Qi, W., Nong, G., Preston, J.F., Ben-Ami, F., Ebert, D., 2009. Comparative metagenomics of Daphnia symbionts. BMC Genomics 10. https://doi.org/10.1186/1471-2164-10-172.

Sanders, R.W., Caron, D.A., Berninger, U.-G., 1992. Relationships between bacteria and heterotrophic nanoplankton in marine and fresh waters: an inter-ecosystem comparison. Mar. Ecol. Prog. Ser. 1–14.

Sanders, R.W., Leeper, D.A., King, C.H., Porter, K.G., 1994. Grazing by rotifers and crustacean zooplankton on nanoplanktonic protists. Hydrobiologia 288, 167–181. https://doi.org/10.1007/BF00006240.

Sarnelle, O., Knapp, R.A., 2005. Nutrient recycling by fish versus zooplankton grazing as drivers of the trophic cascade in alpine lakes. Limnol. Oceanogr. 50, 2032–2042. https://doi.org/10.4319/lo.2005.50.6.2032.

Schallenberg, M., Bremer, P.J., Henkel, S., Launhardt, A., Burns, C.W., 2005. Survival of *Campylobacter jejuni* in water: Effect of grazing by the freshwater crustacean Daphnia cannata (Cladocera). Appl. Environ. Microbiol. 71, 5085–5088. https://doi.org/10.1128/AEM.71.9.5085-5088.2005.

Seiler, C., van Velzen, E., Neu, T.R., Gaedke, U., Berendonk, T.U., Weitere, M., 2017. Grazing resistance of bacterial biofilms: a matter of predators' feeding trait. FEMS Microbiol. Ecol. 93. https://doi.org/10.1093/femsec/fix112.

Serra, T., Barcelona, A., Pous, N., Salvadó, V., Colomer, J., 2022. Disinfection and particle removal by a nature-based Daphnia filtration system for wastewater treatment. J. Water Process Eng. 50. https://doi.org/10.1016/j.jwpe.2022.103238.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30. https://doi.org/10.1093/bioinformatics/btu033.

Sterner, RW., 1986 Feb 7. Herbivores' direct and indirect effects on algal populations. Science 231 (4738), 605–607. https://doi.org/10.1126/science.231.4738.605.

Sterner, R.W., 1989. The Role of Grazers in Phytoplankton Succession. https://doi.org/10.1007/978-3-642-74890-5_4.

Tanentzap, A.J., Fitch, A., Orland, C., Emilson, E.J.S., Yakimovich, K.M., Osterholz, H., Dittmar, T., 2019. Chemical and microbial diversity covary in fresh water to influence ecosystem functioning. Proc. Natl. Acad. Sci. USA 116. https://doi.org/10.1073/pnas.1904896116.

Tang, K.W., Turk, V., Grossart, H.P., 2010. Linkage between crustacean zooplankton and aquatic bacteria. Aquat. Microb. Ecol. 61. https://doi.org/10.3354/ame01424.

US Environmental Protection Agency, 2002. Method 1604: Total coliforms and Escherichia coli in water by membrane filtration using a simultaneous detection technique (MI medium). USEPA Office of Water, Washington DC. EPA-821-R-02-024. https://doi.org/EPA-821-R-02-024.

WHO, 2021. Guidelines on recreational water quality, vol. 1. Coastal and fresh waters, Geneva.

WHO, 2022. Guidelines for drinking-water quality - fourth edition incorporating the first and second addenda. Geneva.

Willis, A.D., Martin, B.D., 2022. Estimating diversity in networked ecological communities. Biostatistics 23, 207–222. https://doi.org/10.1093/biostatistics/kxaa015.

Wright, E.S., 2015. DECIPHER: harnessing local sequence context to improve protein multiple sequence alignment. BMC Bioinform. 16. https://doi.org/10.1186/s12859-015-0749-z.

Yu, G., Smith, D.K., Zhu, H., Guan, Y., Lam, T.T.Y., 2017. ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol. Evol. 8. https://doi.org/10.1111/2041-210X.12628.