Water Research 253 (2024) 121305

Contents lists available at ScienceDirect

Water Research

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

Microbial communities exhibit distinct diversities and assembly mechanisms in rainwater and tap-water storage systems

Sihang Liu [a,b], Siqing Xia [a,b,*], Xiaodong Zhang [a], Xucheng Cai [a], Jinhao Yang [a], Yuxing Hu [a], Shuang Zhou [c], Hong Wang [a,b,*]

[a] *State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China*
[b] *Shanghai Institute of Pollution Control and Ecological Security, Shanghai 200092, China*
[c] *School of Medicine, Tongji University, Shanghai 200092, China*

ARTICLE INFO

Keywords:
Simulated water storage systems
Rainwater
Tap water
Tank materials
Microbial community
Assembly mechanism

ABSTRACT

Roof-harvested rainwater stored for potable and nonpotable usages represent a clean and sustainable water supply resource. However, the microbial dynamics and mechanisms of community assembly in long-termed operated rainwater storage systems remain elusive. In this study, characteristics of microbial communities in different habitats were systematically compared within rainwater and tap-water simulated storage systems (SWSSs) constructed with different tank materials (PVC, stainless steel and cement). Distinct microbial communities were observed between rainwater and tap-water SWSSs for both water and biofilm samples (ANOSIM, $p < 0.05$), with lower diversity indexes noted in rainwater samples. Notably, a divergent potential pathogen profile was observed between rainwater and tap-water SWSSs, with higher relative abundances of potential pathogens noted in rainwater SWSSs. Moreover, tank materials had a notable impact on microbial communities in rainwater SWSSs (ANOSIM, $p < 0.05$), rather than tap-water SWSSs, illustrating the distinct interplay between water chemistry and engineering factors in shaping the SWSS microbiomes. Deterministic processes contributed predominantly to the microbial community assembly in cement rainwater SWSSs and all tap-water SWSSs, which might be ascribed to the high pH levels in cement rainwater SWSSs and low-nutrient levels in all tap-water SWSSs, respectively. However, microbial communities in the PVC and stainless-steel rainwater SWSSs were mainly driven by stochastic processes. Overall, the results provided insights to the distinct microbial assembly mechanisms and potential health risks in stored roof-harvested rainwater, highlighting the importance of developing tailored microbial management strategies for the storage and utilization of rainwater.

1. Introduction

Growing global population and rapid urbanization have intensified the demand for clean water resources, leading to water scarcity in many regions of the world. The search for alternative and sustainable clean water resources has become increasingly essential (Zhou et al., 2021). Rainwater harvesting and utilization have been widely adopted by millions of people worldwide as a sustainable solution to address the challenges of water scarcity for centuries. In countries such as Australia, Singapore, India and Bangladesh, rainwater harvesting and usage has been successfully achieved at various scales. This includes applications such as toilet flushing, clothes washing, car washing, irrigation, and even using it as potable water (Ahmed et al., 2010; Ghosh et al., 2015). In most domestic rainwater systems, the collected rainwater is stored in tanks before being utilized, similar to the storage of tap water in scenarios of intermittent water supplies (Lundy et al., 2018). However, water storage practices are commonly associated with water quality deterioration issues, including elevated metal concentrations, bacteria regrowth, pathogen proliferation, taste and odor problems (Peter and Routledge, 2018).

Several studies have investigated the water chemistry of fresh and stored rainwater, demonstrating the existence of various contaminants (e.g., organic matters, heavy metals) (Ahmed et al., 2011; Hamilton et al., 2019). However, the understanding of microbial communities and dynamics in long-term operated rainwater storage systems still remain limited (Hamilton et al., 2019). Microbial communities play a critical

---

[*] Corresponding authors at: State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China.

E-mail addresses: siqingxia@tongji.edu.cn (S. Xia), hongwang@tongji.edu.cn (H. Wang).

https://doi.org/10.1016/j.watres.2024.121305
Received 18 December 2023; Received in revised form 8 February 2024; Accepted 10 February 2024

role in determining the overall quality and safety of the supplied rainwater. For instance, previous studies have demonstrated that the microbial communities in rainwater contribute to the removal of organic matters, total nitrogen and phosphorus (Kim and Han, 2011). In other cases, the proliferation of microorganisms in rainwater has led to elevated numbers of human pathogens, posing health threats to rainwater users (Evans et al., 2009).

Existing studies have revealed that storage facility characteristics (i.e., tank materials and status) and environmental factors (i.e., storage temperature, water chemistry, water usage pattern) can strongly affect drinking water microbiology in stored tap water (Schafer and Mihelcic, 2012). For example, cement tanks often exhibit surface corrosion due to high pH levels, resulting in the potential release of particles and other corrosion by-products. These released particles and by-products can create suitable habitats that promote the growth of microorganisms. (Slavik et al., 2020). Chemical leaching often occurs in plastic tanks, which may support the regrowth of microorganisms during long-term storage of water (Manga et al., 2021). Moreover, long detention time and higher temperatures are also favourable to bacterial proliferation, resulting in the shift of microbial communities (Zhang et al., 2014). Compared to tap water, roof-harvested rainwater is typically characterized with higher levels and variations of turbidity and nutrients (e.g., nitrogen and phosphorus) due to the collection of roof-top contaminants (e.g., dirt, animal droppings) (Lim, 2023). In our previous study, we demonstrated that water constituent differences between tap water and rainwater gave rise to complex interactions in storage tanks constructed with different materials, subsequently resulting in distinct regrowth patterns of multiple opportunistic pathogens (Zhang et al., 2021). Nonetheless, it is still unclear how and to what extent microbial communities would evolve in rainwater storage tanks over the long term, particularly when influenced by various tank materials.

Understanding the microbial community assembly mechanisms is critical as it provides insights into whether and how we can manipulate microbial communities to achieve engineering goals (Dini-Andreote et al., 2015). Typically, microbial community assembly can be explained by two complementary theories: niche-based theories (i.e. stochastic processes) and neutral theories (i.e. deterministic processes) (Chen et al., 2019). Neutral theories assume that all species are equivalent toward limited environmental resources (Hubbell, 2001), while niche-based theories suggest that microbial community structures are susceptible to changes of environmental variables (Jongman et al., 1995). Notably, deterministic and stochastic processes can exist simultaneously, with their relative importance varying across space, time, and habitat (Stegen et al., 2013; Wu et al., 2019; Zhou et al., 2014). Numerous studies have deciphered the assembly mechanisms in biological processes in wastewater and drinking water treatment plants, water distribution systems, and biogeochemical cycling in the ocean (Doane et al., 2023; Chen et al., 2022; Lian et al., 2022; Sun and Jing, 2023; Ling et al., 2018). For instance, unravelling the assembly mechanism in wastewater treatment plants serves to evaluate the system maturation and regulate microbial communities in a directional manner (Chen et al., 2022). Therefore, understanding the microbial community assembly mechanisms in rainwater storage tanks would assist in identifying key parameters shaping the microbial community and formulating practical strategies to improve rainwater storage management (Dini-Andreote et al., 2015).

In this study, six lab-scale simulated water storage systems (SWSSs) were constructed with different material coupons (i.e., polyvinyl chloride (PVC), stainless-steel, and cement) and operated in duplicate, which were supplied with either roof-harvested rainwater or dechlorinated tap water. Eighty percent of water changes were implemented at regular intervals throughout the 36-week study to simulate water consumption and refill events. The microbial community characteristics and assembly mechanisms were compared in stored rainwater and tap water during the long-term operation of SWSSs. The results of this study provide new insights into microbial ecology and underlying mechanisms in stored rainwater and tap-water, which are beneficial for developing effective microbial management approaches during water storage practices.

2. Methods and materials

2.1. Simulated water storage system (SWSS) set-up and operation

The details of SWSS set-up had been previously described (Zhang et al., 2021) and are provided in the Supporting Information (SI, Text S1). In brief, simulated water storage systems fed with either roof-top harvested rainwater or dechlorinated tap water were operated in duplicate to mimic household rainwater and tap water storage tanks. Coupons made of three commonly used tank materials (PVC, stainless-steel, and cement) were placed in SWSSs to compare tank material effects on water chemistry and microbiology (Fig. S1). In each SWSS, 6–8 pieces of coupons were hung by strings in 625 ml of water within a 1-L capped glass bottle, ensuring an equal surface-to-volume ratio of 0.44 cm^2/ml. Bottles and coupons were thoroughly cleaned, assembled, and then sterilized at 121 ℃ for 20 min prior to the experimental set-up. Throughout the 36-week operation, all SWSSs were maintained at 25 ℃ in darkness, and weekly water changes of 80 % were performed to simulate water usage events, except during extremely dry periods (i.e., weeks 9, 11, 25, 26, and 29). To replenish the SWSSs, an equivalent volume of recently collected rainwater or tap water was utilized (see detailed information in the SI).

2.2. Sampling, DNA extraction and sequencing

SWSS influents and effluents collected upon water changes were subjected to microbiological analysis and water chemistry analysis (Table S1). For microbiological analysis, 150-ml water samples were filtered through sterile 0.22 μm-pore size mixed cellulose ester membranes (Millipore). Collected membrane filters were fragmented using sterilized tweezers and transferred into 2 mL Lysing Matrix E tubes (MP Biomedicals), which were subsequently subjected to DNA extraction. Meanwhile, 100-mL water samples were collected in conical tubes for water chemistry analysis. For biofilm samples, one coupon was aseptically removed from each SWSS tank at weeks 34, 35, and 36 (12 coupons×3 weeks=36 coupons) and immediately stored in sterile conical tubes containing 30 ml of phosphate buffered saline (PBS) solution supplemented with 0.5 % Tween 80 (wt/vol). Cells were detached from the coupon through sonication in a water bath for 4 min using a frequency of 28 kHz (Guilbaud et al., 2005). The PBS buffer containing detached cells was subjected to membrane filtration and then DNA extraction. Two clean, autoclaved coupons of each material were treated in the same way and used as biofilm negative controls. Genomic DNA on water and biofilm samples, reagent negative controls (blank membranes), and biofilm negative controls were extracted using FastDNA® SPIN Kits (MP Biomedicals) according to the manufacturer's protocol and stored at −80 °C until further processing.

Water samples taken on weeks 3, 16, 20, 24, 28, 32, and 35 and all biofilm samples were selected for Illumina sequencing (Table S1). The primer pair 338F (5′-ACT CCT ACG GGA GGC AGC A-3′)/806R (5′- GGA CTA CCA GGG TAT CTA AT −3′) was used to amplify the V3-V4 region of the bacterial 16S rRNA genes. Paired-end 2 × 250 sequencing of amplicons was performed on a MiSeq platform (Illumina, USA) using previously described methods (Zhang et al., 2020). The raw sequences have been uploaded into the ENA BioProject under accession number PRJEB63249.

2.3. Water quality analysis

The pH, conductivity, total organic carbon (TOC), total nitrogen (TN), ammonia and nitrate concentrations of water samples were analysed using the methods described in the SI (Text S1).

2.4. Bioinformatics and statistics

DADA2 (v1.26) package in R was utilized to trim, quality filter, merge the sequences and generate the amplicon sequence variants (ASVs) (Callahan et al., 2016). The SILVA 138 (nr99) reference database was used to assign taxonomic affiliation of the ASVs with DADA2 default settings. Singletons were removed from the ASV table prior to further analysis. ASV sequences were blasted against the multiple bacterial pathogen detection (MBPD) database to retrieve potential pathogen profiles, with an E-value $< 10^{-10}$ and identity threshold > 99.9 % (Li et al., 2023).

Alpha- and Beta-diversity analyses were conducted using the "phyloseq" (v3.16) package in R (v4.2.3). Principal Coordinate Analyses (PCoA), implemented through the "phylosmith" package (v1.0.6) (Smith, 2019), allowed visualization of microbial community structure variations based on Bray-Curtis distances and differences in water quality parameters using Euclidean distance among different groups. Analysis of similarities (ANOSIM) was used to test the hypothesis that the community differences between groups were similar using the "vegan" package (v2.6–4). Taxa with a relative abundance greater than 1 % were selected to construct the co-occurrence network using the "igraph" package (v1.4.3). Correlations with Spearman-rank coefficient (ρ) > 0.6 and P value < 0.01 were considered valid co-occurrence events (Barberán et al., 2012), which were visualized using the Gephi software version 0.10.1. Redundancy analyses (RDA) and the Mantel test were used to evaluate the associations between water chemistry parameters and microbial communities using the "ade4" package (v1.7–22). Kruskal−Wallis test and Wilcoxon test were used for multiple- and two-group comparisons with the "PMCMRplus" package (v1.9.6), respectively.

2.5. Assembly mechanisms of microbial communities in rainwater and tap-water SWSSs

Modified stochasticity ratio (MST) analysis was conducted using the "NST" package (v3.1.10) to reveal the contribution of stochastic processes in water samples of rainwater and tap-water SWSSs (Ning et al., 2019). Sloan neutral community model (NCM) was also fitted to infer the assembly mechanisms, with the m value representing the immigration rate of new microbial taxa being introduced into a community and the *Rsqr* value explaining the fitness degree to the neutral model (Woodcock et al., 2007). The niche breadths of individual ASVs were determined by the "spaa" package (v0.2.2) based on the Levins' niche breadth index. The average niche breadth from all taxa was calculated as the bulk community niche breadth (*Bcom*). A wider niche breadth indicates that taxa in the microbial community were less influenced by environmental factors (Wu et al., 2018).

3. Results

3.1. Water qualities in rainwater and tap-water SWSSs with different tank materials

Variations in water physiochemical parameters within rainwater and tap-water SWSSs have been previously described (Zhang et al., 2021). In brief, cement tanks exhibited the highest levels of pH and conductivity in both rainwater and tap-water SWSSs due to lime release (Fig. S2 and Table S2). However, a faster decrease in pH and conductivity was observed in cement tap-water SWSSs compared to cement rainwater SWSSs during the early operation stage, resulting in lower pH (~7.2) and conductivity (<40 μS/cm) in cement tap-water SWSS effluents after week 16 (Wilcoxon test, $p < 0.001$). This phenomenon was ascribed to the accelerated release of lime due to the presence of bicarbonate and carbonate ions in cement tap-water SWSSs. This result was further supported by the XRD analysis, which demonstrated that the white precipitates solely present on the glass wall of cement tap-water SWSSs were $CaCO_3$ (Zhang et al., 2021).

Rainwater SWSSs exhibited higher concentrations and greater variations in TOC, TN, ammonia, and nitrate compared to tap-water SWSSs (Wilcoxon test, $p < 0.001$), which could be attributed to the presence of various chemical contaminants in harvested rainwater from polluted air or roofs (Abbasi and Abbasi, 2011). Notably, a marked decrease in ammonia levels and an increase in nitrate concentrations were observed in the effluents of PVC and stainless-steel ("steel" thereafter) rainwater SWSSs compared to their influents (Wilcoxon test, $p < 0.01$). This observation suggested the potential occurrence of nitrification during rainwater storage in PVC and steel SWSSs.

The PCoA analysis of physiochemical data showed a distinct cluster of cement effluent samples in rainwater SWSSs, which was separated from the other rainwater samples (Fig. 1). This result highlights the unique water chemistry in cement rainwater SWSSs. In contrast, less distinction was observed among tap-water SWSSs with different tank materials.

3.2. Microbial communities and potential pathogens in long-term operated rainwater and tap-water SWSSs

A total of 65,032 unique ASVs were retrieved from water and biofilm samples collected in rainwater and tap-water SWSSs. Tap-water SWSSs contained a higher number of ASVs ($n = 49{,}213$) compared to rainwater SWSSs ($n = 19{,}373$) (Fig. S3). Moreover, significantly higher values of Shannon and Chao1 indexes were observed in samples collected from tap-water SWSSs in comparison to samples from rainwater SWSSs (Fig. 2a; Wilcoxon test, $p < 0.001$), illustrating that a more diverse microbial community occurred in tap-water SWSSs.

PCoA plots (Fig. 2b) and ANOSIM analyses revealed that the microbial communities in rainwater SWSS water samples were distinct from those in the tap-water SWSSs (ANOSIM, $R = 0.709$, $p = 0.001$). In the majority of rainwater SWSS water samples, *Proteobacteria* and *Bacteroidetes* were the two most dominant phyla (Fig. S4a), constituting over 70 % of the total sequences. In contrast, the composition of water samples from tap-water SWSSs exhibited a more even distribution (Fig. S4b), with *Proteobacteria, Firmicutes, Bacteroidota* and *Verrucomicrobiota* contributing 35.5 %, 20.4 %, 13.7 % and 12.7 % of the total sequences, respectively. Notably, a variety of faecal-associated bacterial genera, including *Bacteroides* (1.77 ± 5.04 %), *Escherichia* (1.45 ± 2.32 %), and *Lachnoclostridium* (0.87 ± 0.24 %), were exclusively detected in water samples collected from rainwater SWSSs. In terms of biofilm samples, a tighter cluster was observed in samples collected from tap-water SWSSs, in contrast to the scattered distribution of those from rainwater SWSSs (Fig. 1b; ANOSIM, $R = 0.372$, $p = 0.001$). Fig. S5 demonstrated that *Proteobacteria* was the most predominant phylum in both rainwater (42.8 ± 20.1 %) and tap-water (32.5 ± 17.2 %) SWSS biofilm samples.

Fig. 3 demonstrated the relative abundances of bacterial genera containing pathogens in rainwater and tap-water SWSS effluent samples. Previous studies have demonstrated that the genera *Acinetobacter, Clostridium sensu stricto 1, Providencia*, and *Pseudomonas* include pathogen species that specifically infect humans, while the remaining genera contain pathogens that may infect both humans and animals (Yang et al., 2023). Rainwater SWSS effluents (2.91 ± 1.76 %) exhibited a higher relative abundance of retrieved genera than tap-water SWSS effluents (1.76 ± 1.55 %; Wilcoxon test, $p < 0.01$). Hierarchical clustering (Fig. 3a) and ANOSIM analyses ($R = 0.712$, $p < 0.01$) revealed distinct profiles of potential pathogens between rainwater and tap-water SWSS effluents. For instance, *Acinetobacter*, a frequent causal agent of nosocomial infections (Guardabassi et al., 1999), was predominantly found in rainwater SWSSs, with a relative abundance ranging from 0 to 3.4 % in different samples. In tap-water SWSSs, *Rhodococcus* dominated the pathogen profile (0.001 % to 2.4 % of the total sequences), followed by *Bifidobacterium* (0 to 0.8 %) and *Providencia* (0 to 0.15 %). In contrast, no difference was observed in the pathogen profiles of biofilm samples between rainwater and tap-water SWSSs (Fig. S6; ANOSIM, $p > 0.05$).

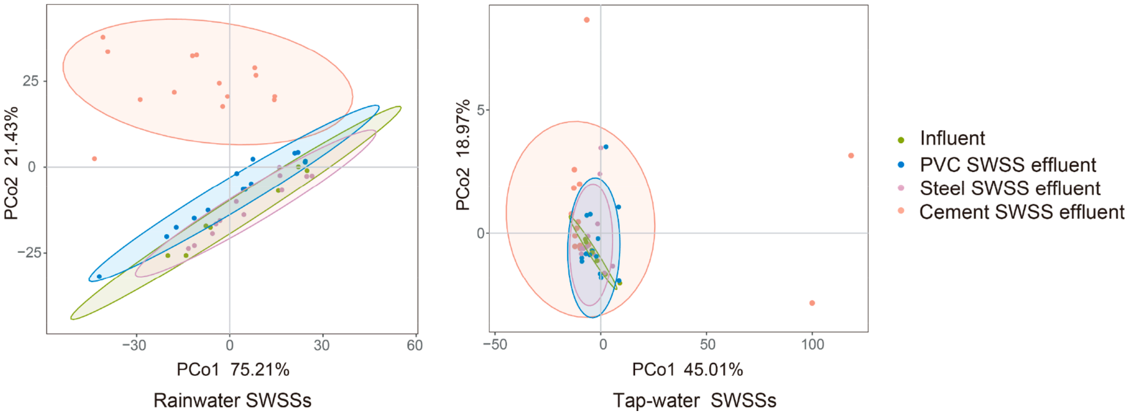

Fig. 1. Principal coordinate analysis (PCoA) of water quality parameters in rainwater (left panel) and tap-water (right panel) SWSS influents and effluents. Analyses were performed based on Euclidean distance matrix derived from water quality parameters including pH, conductivity, TOC, TN, ammonia, and nitrate.

For both rainwater and tap-water SWSS biofilm samples, *Providencia* was the most dominant genus in the pathogen profiles, with the relative abundance up to 30.8 % in individual samples (Fig. 3b). Notably, higher relative abundances of these potential pathogens were found in biofilm samples compared to effluent samples (Fig. S7; Wilcoxon test, $p < 0.001$), highlighting the critical role of biofilm as a potential pathogen reservoir.

3.3. Distinct effects of tank materials on microbial communities between rainwater and tap-water SWSSs

PCoA analysis of microbial communities demonstrated a strong clustering of water samples according to the tank material in rainwater SWSSs (Fig. 4a), and ANOSIM analysis further confirmed this trend ($R = 0.790$, $p < 0.001$). Fig. S8 showed that the precise microbial compositions varied considerably in rainwater SWSSs with different tank material coupons. For example, the dominant genus in cement rainwater SWSSs was *Rubritepida* (26.4 ± 22.1 %), while *Reyranella* (16.5 ± 8.9 %) was the dominant genus in steel rainwater SWSSs. However, no discernible pattern was observed in effluents of tap-water SWSSs with different tank materials (Fig. 4a; ANOSIM, $p > 0.05$). The results illustrated that tank materials exerted distinct impacts on microbial communities in water samples between the rainwater and tap-water SWSSs.

In biofilm samples, distinct microbial community structures were also observed on different tank material coupons in rainwater SWSSs (Fig. 4b; ANOSIM, $R = 0.835$, $p < 0.001$, Table S3). In tap-water SWSSs, the microbial communities in PVC biofilm exhibited a divergence from those found on steel and cement coupons (Fig. 4b; ANOSIM, $R = 0.723$, $p < 0.01$, Table S3). Consistently, Fig. 5 demonstrated that unique microbial compositions in PVC biofilm samples. For example, the genus *Ketobactor* was exclusively found on PVC coupons of tap-water SWSSs, with a relative abundance of 18.7 %.

3.4. Distinct microbial assembly mechanisms and key environmental factors in rainwater and tap-water SWSSs

The modified stochasticity ratio (MST) was calculated to explore relative contributions of deterministic and stochastic processes in rainwater and tap-water SWSSs (Fig. 6a). Cement SWSSs yielded the lowest MST values in both rainwater (0.27 ± 0.20, $p < 0.01$) and tap-water SWSSs (0.4 1± 0.25, $p < 0.01$), respectively, suggesting that microbial assembly in cement tanks was dominated by deterministic processes (i.e., MST<0.5). This conclusion was further supported by the Neutral Community Model (NCM) analysis, which revealed low R^2 values (0.017–0.292) and migration rates (0.019–0.021) in both cement rainwater and tap-water SWSSs (Fig. 6b). In addition, microbial communities in cement tanks also exhibited the narrowest niche width in both rainwater and tap-water SWSSs (Table S4), suggesting that environmental factors exert a more pronounced effect on microbial communities in cement SWSSs compared to SWSSs constructed of other materials.

Higher MST values (0.54 ± 0.24 for PVC, 0.51 ± 0.22 for steel) and R^2 values (NCM, 0.556–0.579, Fig. 6b) were observed in PVC and steel rainwater SWSSs, indicating a prominent role of stochastic processes in community assembly. On the contrary, PVC and steel tap-water SWSSs displayed low MST values (0.41 ± 0.29 for PVC, 0.39 ± 0.24 for steel) and R^2 values (NCM, 0.221–0.252) in community analyses, implying the dominant influence of deterministic processes on tap-water community assembly (Fig. 6b). These results indicated that the interactions between water chemistry (i.e., rainwater versus tap water) and tank materials resulted in different microbial assembly mechanisms.

Redundancy analyses (RDA) and Mantel test were employed to further explore the relationship between microbial community and water physiochemical parameters. As depicted in Fig. 7, only 37.66 % of the variation could be explained by physicochemical factors in rainwater SWSSs. On the contrary, physicochemical factors accounted for 50.20 % of the microbial community variation in tap-water SWSSs. The result echoed the higher contribution of deterministic processes in tap-water SWSSs relative to rainwater SWSSs. In rainwater SWSSs, effluent samples from cement tanks were positively associated with both pH and conductivity (Mantel test, $p < 0.001$). For PVC rainwater SWSSs, microbial communities of effluent samples demonstrated a significant positive association with NO_3^-, and a negative association with NH_4^+ (Mantel test, $p < 0.05$). However, no discernible correlation was identified between the microbial communities and any individual environmental factor in tap-water SWSSs (Mantel test, $p > 0.05$).

3.5. Network analysis revealed more complex microbial interactions in tap-water SWSSs relative to rainwater SWSSs

Tap-water SWSS network was characterized with fewer nodes but a higher average degree, clustering coefficient, and edge number compared to the rainwater SWSS network based on co-occurrence analysis (Fig. 8). The result suggested that more complex microbial interactions might exist in tap-water SWSSs. Moreover, the tap-water SWSS network displayed a higher ratio of negative edges (41.46 %) compared to the rainwater SWSS network (22.99 %). Negative edges may indicate a broad range of co-exclusion interactions within microbial communities, including direct competition, differential niche adaptation and toxin production(Ma et al., 2020). On the contrary, the dominance of positive edges in the rainwater SWSS network (77.01 %) implied that a high portion of microbial taxa may have synergetic relationships or share similar ecological niches in rainwater SWSSs (Ma et al., 2020).

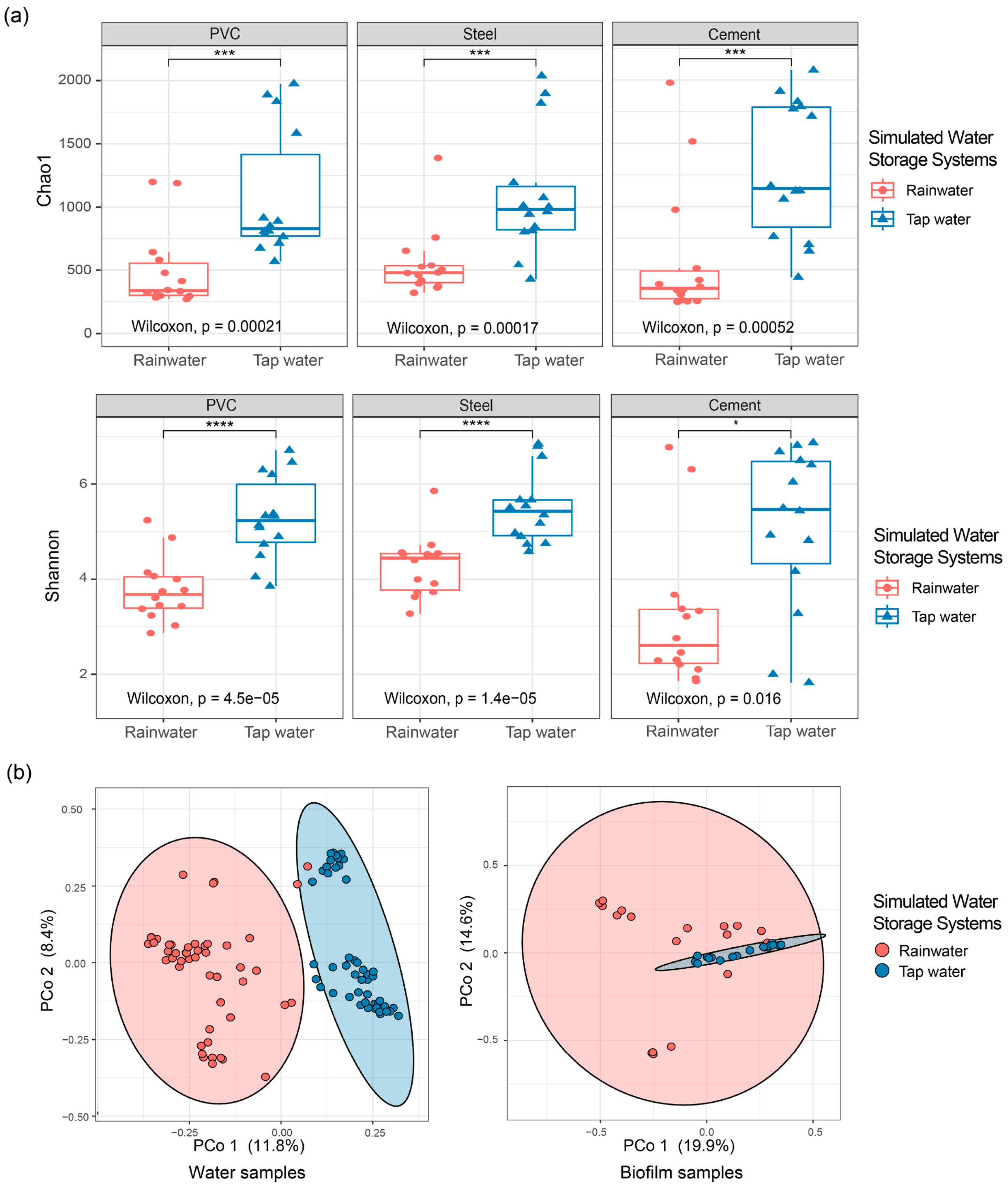

Fig. 2. Microbial community analysis of water and biofilm samples in rainwater and tap-water SWSSs based on Illumina sequencing of 16S rRNA genes. Shannon diversity index (a) and Chao1 index were calculated for SWSS effluent sample s (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). Principal Coordinate Analysis (b) based on the Bray-Curtis dissimilarity matrix were performed on water (influents and effluents) and biofilm samples in SWSSs.

4. Discussion

4.1. Distinct microbial community characteristics between rainwater and tap-water SWSSs

Water quality deterioration frequently occurs during long-term storage (Peter and Routledge, 2018). With the increasing application of rainwater harvesting and usage systems worldwide, numerous studies have investigated the characteristics of water chemistry in stored rainwater. Nevertheless, the comprehension of microbial communities and dynamics within storage systems remain limited, despite their essential role in influencing water quality (Hamilton et al., 2019). In this study, a head-to-head comparison of microbial communities was performed in rainwater versus tap-water SWSSs constructed with different tank materials, providing valuable insights into the microbial ecology and assembly mechanisms in engineered water storage systems.

Rainwater and tap-water SWSS effluents harbored different microbial community compositions, which is not unexpected given the

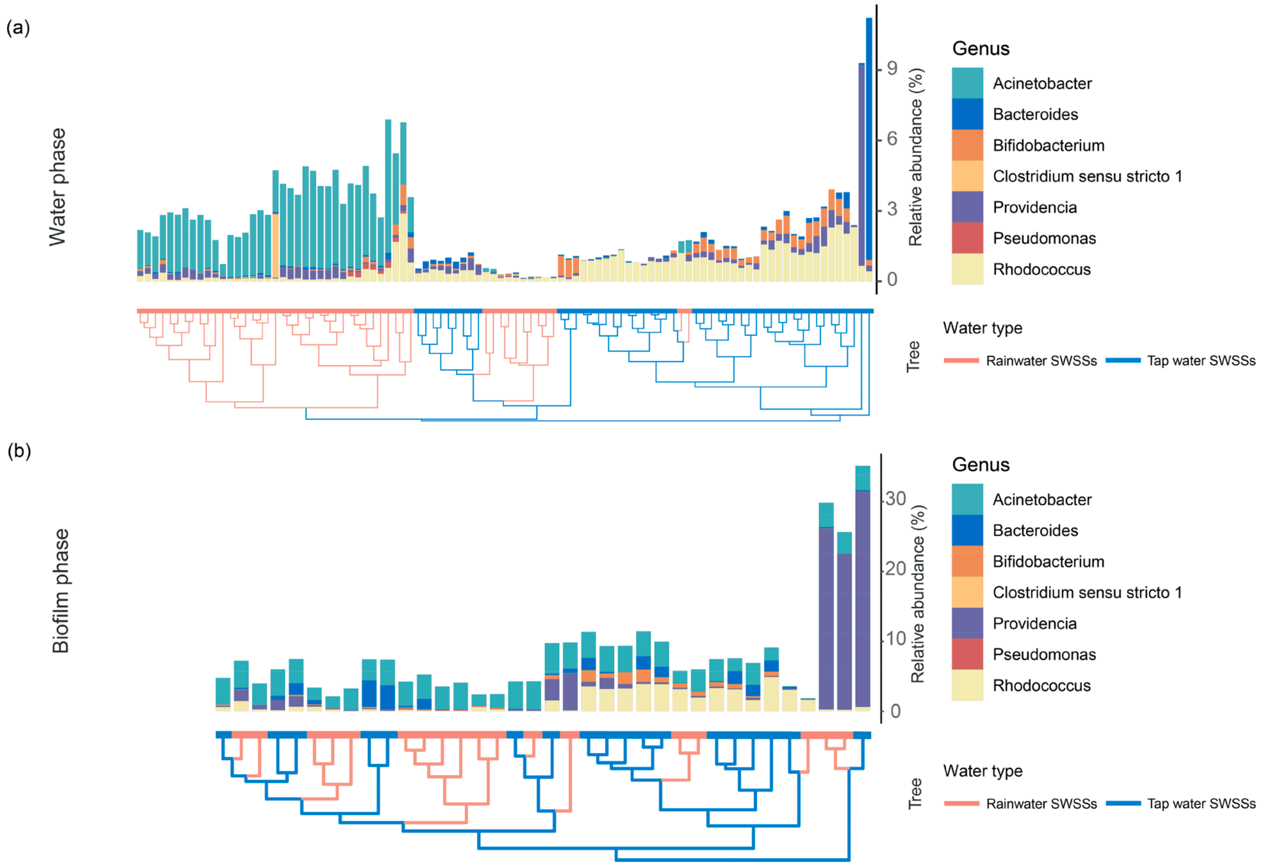

Fig. 3. Relative abundances of genera containing pathogens in (a) water phase and (b) biofilm phase of SWSSs based on MBPD dataset. Hierarchical clustering analysis was performed based on Bray–Curtis dissimilarity matrix.

entirely different sources of influent water. In particular, genera associated with fecal contamination (e.g., *Escherichia*) were solely identified in rainwater SWSSs. These genera may have been introduced to the rainwater SWSSs via roof-top contaminants (e.g., bird droppings) during the harvesting processes (Ahmed et al., 2010; Boretti and Rosa, 2019). Faecal-associated genera are typically absent in tap water due to effective removal during drinking water treatment processes (Nwachcuku and Gerba, 2004). In addition, genera containing pathogens were more enriched in rainwater SWSSs (Fig. 3), which is also likely associated with exogenous contamination during rainwater harvesting. For instance, *Acinetobacter*, a genus commonly found in diverse environments, has been frequently identified among pathogenic genera in roof-harvested rainwater systems (Dobrowsky et al., 2014). The distinct profiles of potential pathogens may imply different microbial risks towards water consumers during the water storage period, which may require different mitigation strategies in these two systems.

It was also noted that rainwater SWSSs harbored a less diverse microbial community relative to tap-water SWSSs (Fig. 1). This might be partially attributed to the higher nutrition level in rainwater SWSSs (Fig. S1), which may favor the growth and enrichment of fast-growing species and subsequently result in a decrease in community diversity (Harpole and Tilman, 2007). The higher number of ASVs observed in tap-water SWSSs may be attributed to the oligotrophic environment, where nutrient-depleted water tends to support a diverse community consisting of sensitive species that thrive within a narrower range of environmental conditions (Burkholder and Glibert, 2013). These findings align with previous studies that have shown an increased microbial diversity in successive ground-water sand filtration units with decreased gradients of organic carbons (Gülay et al., 2016).

4.2. *Varied effects of tank materials on microbial communities in rainwater versus tap-water SWSSs*

Tank materials can significantly alter water chemistry during rainwater storage. For example, elevated pH levels have been observed in cement tanks in the present work and previous studies (Islam et al., 2010; Thomas et al., 2018). Although few studies have elucidated the effect of pH on microbiomes in stored rainwater, the importance of pH on microbial abundances, distribution and diversities has been recognized in various microbial habitats, including soil, lake, and ocean (Jones and Grant, 2000; Luan et al., 2023). Elevated pH is typically associated with reduced richness and evenness of microbial communities, likely due to the enrichment of alkali tolerant and alkaliphilic microorganisms (Luan et al., 2023). We similarly observed the lowest microbial diversity in the cement rainwater SWSSs, illustrating the strong selective pressure of the alkaline environment towards microbiomes. For example, the dominant genus *Rubritepida* in cement rainwater SWSS in our study has been found in alkaline mine wastes with a pH of 9 (Mghazli et al., 2021). Meanwhile, PVC can also change water chemistry by releasing organic carbon or heavy metals (Zhang et al., 2021), which may subsequently encourage bacterial growth or change microbial community structures (Bucheli-Witschel et al., 2012). These factors might account for the distinct microbial community observed in PVC rainwater SWSSs in the present study. Taken together, the distinct microbial community structures in rainwater SWSSs with different materials underscore the pivotal role of tank materials in shaping rainwater microbiomes during long-term storage.

However, negligible influence of tank materials on microbial communities was noted in tap-water SWSS water samples. The result aligned with observations in-building drinking water tanks, revealing similar bacterial communities in water samples collected from storage tanks

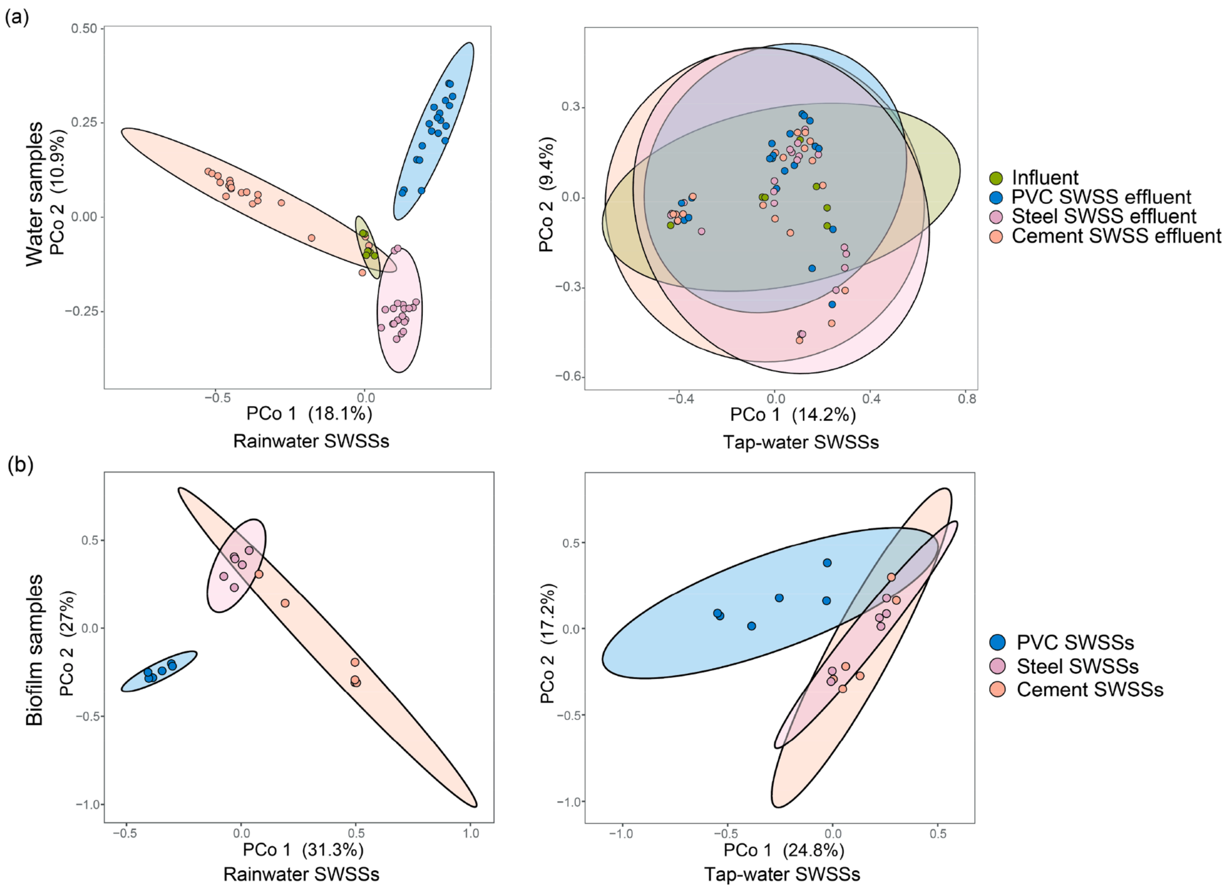

Fig. 4. Principal Coordinate Analysis of microbial communities in rainwater and tap-water SWSSs constructed with different tank materials (PVC, steel and cement). Both water samples (a) and biofilm samples (b) were included in the analysis.

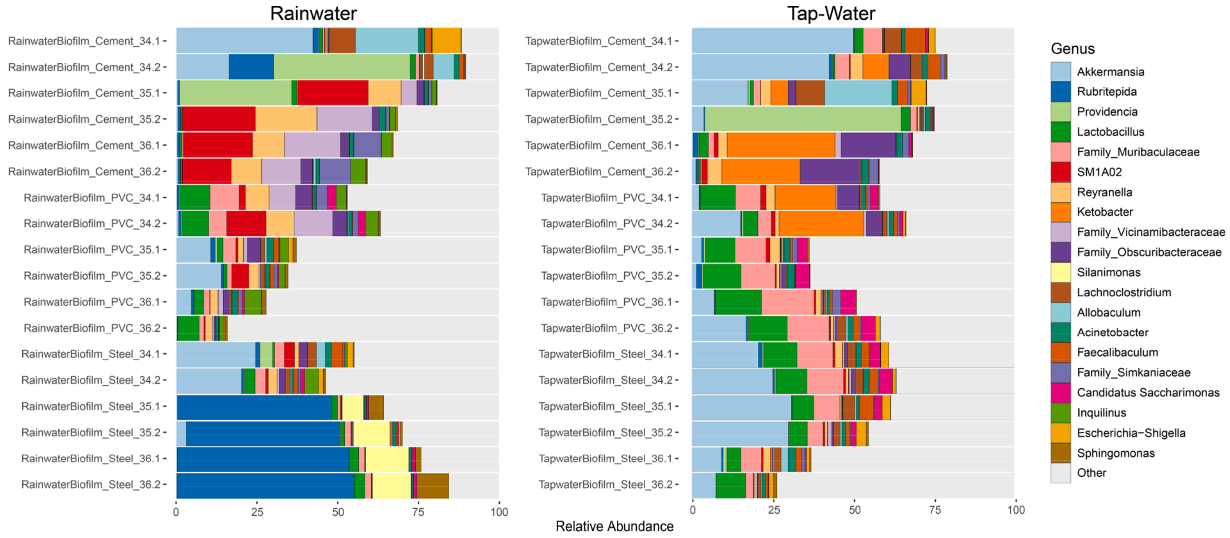

Fig. 5. Distribution of microbial compositions in rainwater SWSSs and tap-water SWSSs biofilm samples at the genus level. Only the most abundant ASVs (top 20) of the biofilm samples were displayed. The numbers 03, 16, 20, 24, 28, 32 and 35 denote the sampling week. The numerical suffixes ′1′ and ′2′ denote the biological replicates of each storage system.

made of PE, stainless steel, and ceramic (Cai et al., 2023). Compared to rainwater SWSSs, the water chemistry varied to a lesser degree in tap-water SWSSs with three different material coupons (Fig. S1). In addition, tap-water microbiomes mainly consist of oligotrophic bacteria, which adopt the k-selection reproductive strategy due to the nutrient-scarce environment (Yin et al., 2022). For instance, the dominant genera in tap-water SWSSs, such as *Hydrogenophaga, Sphingobium*, and *Methyloversatilis*, are slow growers follows the k-selection strategy (Yin et al., 2022). A previous study has shown that high-ratio of k-strategists prevent the regrowth of r-strategists, increasing the stability of microbial communities toward water chemistry change within resource-limited drinking water environment (Favere et al., 2021).

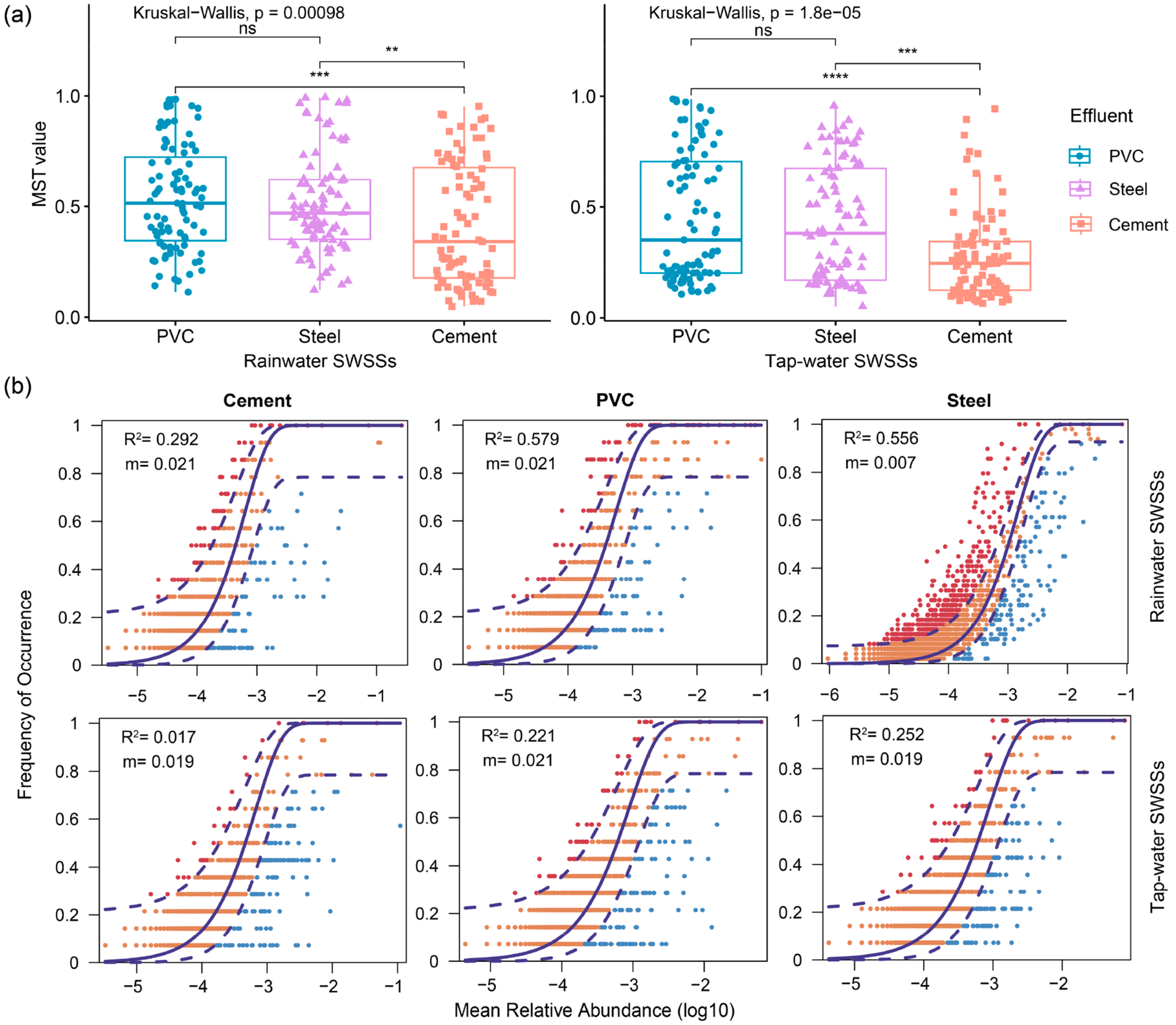

Fig. 6. Microbial community assembly mechanisms in water samples of rainwater and tap-water SWSSs based on (a) modified stochasticity ratio (MST) analysis and (b) neutral community model (NCM) analysis. In (a), MST value > 0.5 indicates that stochastic processes contribute more than deterministic processes in community assembly, and vice versa. In (b), R^2 determines the overall fit to this model, m indicates migration rate. The neutral-model predictions were represented as solid lines, and the dashed lines indicate the 95 % confidence intervals.

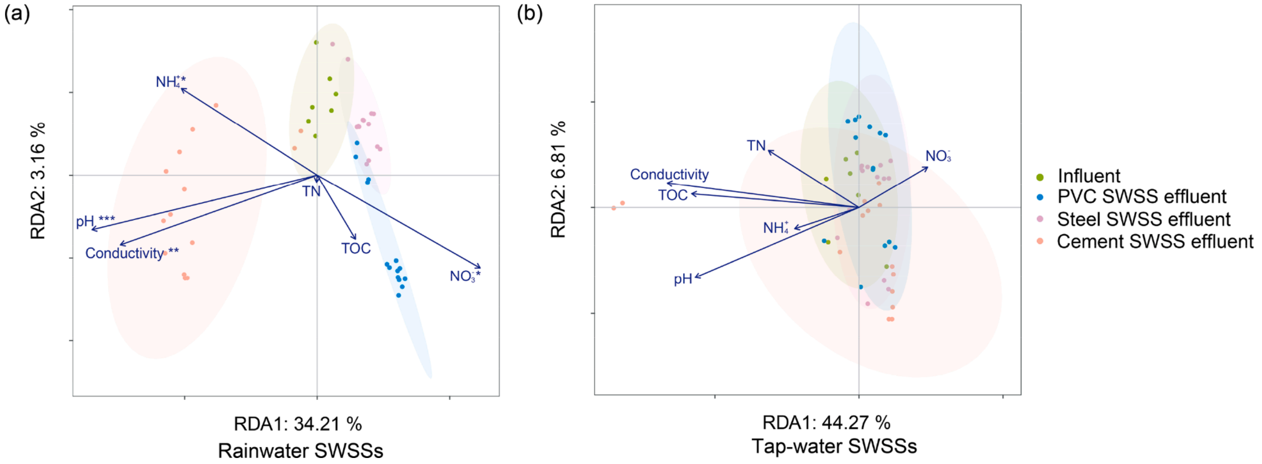

Fig. 7. Redundancy analysis (RDA) exploring the associations between microbial communities and water chemistry parameters in (a) rainwater and (b) tap-water SWSSs. Arrows pointing to sample points represent positive correlations, and arrows pointing to the opposite direction of sample points represent negative correlations. The arrow length corresponds to the variance explained by the environmental variable. *:$p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

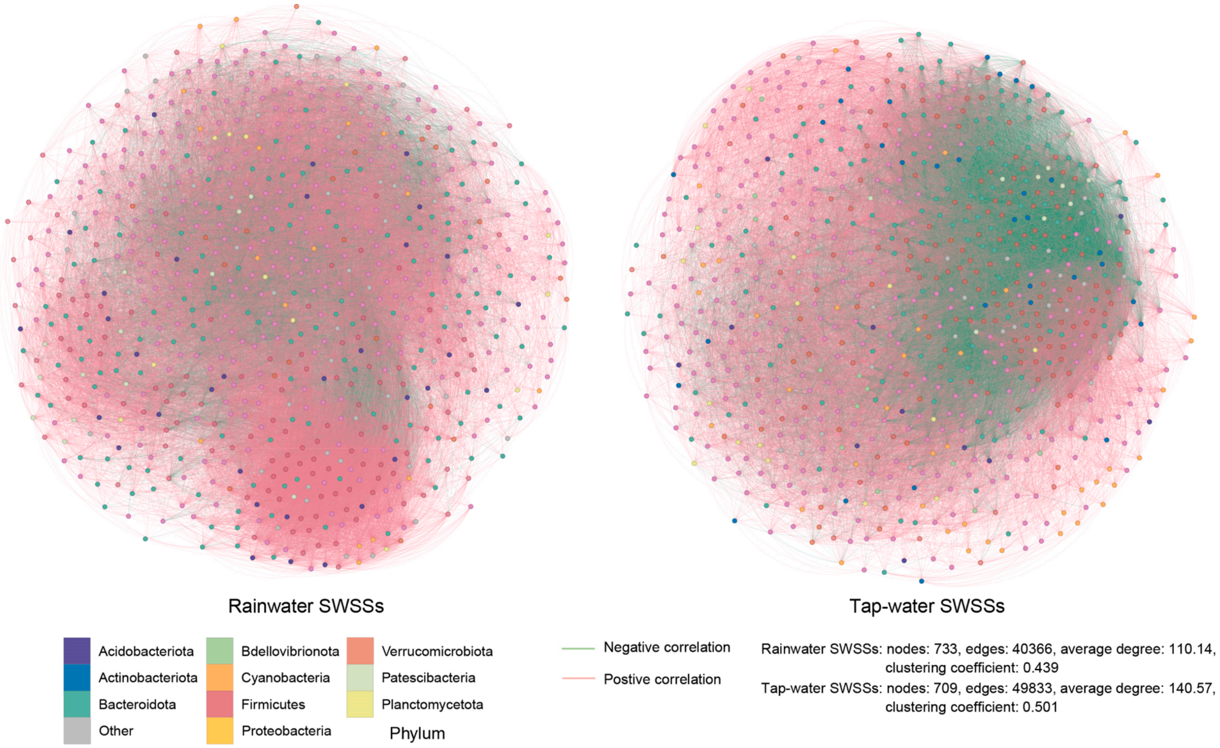

Fig. 8. Co-occurrence network of rainwater and tap-water SWSSs. Each node represents a unique ASV (Amplicon Sequence Variant), and each edge represents a correlation between two ASVs. ASVs of the same color belong to the same phylum, indicating their taxonomic similarity. Negative correlations between ASVs are indicated by green lines, while positive correlations between ASVs are indicated by red lines. The magnitude of red or green lines in the network reflects the relative weight of positive and negative associations, respectively.

These factors might be accountable for the insignificant effect of tank material on tap-water microbiomes. Overall, the distinct effects of tank materials on the microbiomes between rainwater and tap-water SWSS water samples illustrate the complex interplay between water chemistry, tank materials and indigenous water microbiomes, highlighting the importance of developing tailored microbial management approaches for rainwater storage and utilization.

4.3. Distinct community assembly mechanisms in rainwater and tap-water SWSSs

The assembly mechanisms in fresh tap-water and water distribution systems have been previously investigated, revealing varied contributions of stochastic and deterministic processes in different scenarios (Ling et al., 2018; Sun and Jing, 2023). However, few studies have explored the assembly mechanisms of microbial communities in water storage systems. In our study, we demonstrated that deterministic processes played a dominant role in shaping the microbial community in all tap-water SWSSs (Fig. 6). It is suggested that stronger deterministic processes have been commonly observed in extreme environments across both micro- and macro-organism systems, indicating the presence of selective pressures (Stegen et al., 2012). In tap-water SWSSs, the nutrient-limiting condition of drinking water represents an extreme environment, favoring the growth of oligotrophs. In addition, disinfection processes could preferentially select disinfectant-resisting bacteria (e.g., *Mycobacterium, Streptococcus*) in drinking water (Paszko-Kolva et al., 1992; Takayama et al., 2022; Zhang et al., 2021), although chloramine residuals were removed from the tap water prior to the SWSS feeding. For example, our previous study demonstrated notable regrowth of *Mycobacterium* in tap-water SWSSs, in contrast to the sporadic detection of *Mycobacterium* at low concentrations in rainwater SWSSs (Zhang et al., 2021). All of these illustrated the deterministic selections posed by environmental factors in tap-water SWSSs.

For rainwater SWSSs, the dominant role of deterministic assembly was only noted in cement tanks, while stochastic processes predominated the microbial assembly in PVC and steel rainwater SWSSs (Fig. 6). The different assembly mechanisms were likely due to the high discrepancy in environmental chemistry in tanks with different materials. Compared to tap water, roof-harvested rainwater is considered as a nutrient-rich environment, which may favor the proliferation of fast-growing or copiotrophic microorganisms (Dini-Andreote et al., 2015). This nutrient abundance is suspected to reduced competitive pressures in rainwater SWSSs, resulting in the predominance of stochastic processes during microbial assemblies (Chase, 2007). However, the interaction between cement and rainwater in SWSSs leads to unique water chemistry (e.g., high pH and conductivity), which increased the stringency of environmental selection (Dini-Andreote et al., 2014) and resulted in a strong deterministic process (Lauber et al., 2009). This notion is further supported by the Mantel test analysis results, which revealed significant associations between microbial community and pH/conductivity. On the contrary, the water chemistry in PVC and steel rainwater SWSSs had only mild modification compared to their influent rainwater, which might not mask the strong stochastic processes induced by high nutrients (Dini-Andreote et al., 2015). These results further illustrated the important role of tank materials in shaping rainwater microbiomes during long-term storage.

4.4. Biofilm represents a critical ecological niche in rainwater SWSSs

Although species-level identification of pathogens could not be achieved in the present study, higher relative abundances of genera containing pathogens were noted in biofilm samples compared to water samples in both rainwater and tap-water SWSSs (Fig. 3). The result implied that biofilms may provide an ideal habitat for potential pathogens. Enrichment of pathogens in biofilms has been reported in drinking water distribution systems (Li et al., 2023), as biofilm can provide protection to pathogens by creating nutrient-rich microenvironment and physical barrier against disinfectants (Gunn et al., 2016; Kurniawan and Yamamoto, 2019). On the other hand, many pathogens are inherently capable of forming biofilms, as biofilm formation is a fundamental virulence factor (Phillips and Schultz, 2012). For instance, *Acinetobacter*, a common human pathogen identified in our study, harbor highly conserved biofilm-forming genes and are able to form biofilms in various habitats (Colquhoun and Rather, 2020).

In tap-water SWSSs, the impact of tank materials on microbial communities was primarily observed in biofilm samples rather than water samples (Fig. 4b). This result discrepancy can be attributed to the direct contact between microbes and the material coupon surfaces for biofilm samples. The variation in roughness and chemical properties among different materials can influence the microbial adhesion and growth, allowing the establishment of various microbial communities on the surfaces. For instance, plastic surfaces have been found to facilitate the growth of plastic-degrading bacteria by providing carbon and energy sources (Zeenat et al., 2021). In our study, the dominance of *Ketobactor* in the biofilms of PVC tap-water SWSSs is a potential example of this phenomenon, as it is known to be a plastic-degrading genus (Singleton et al., 2023). However, the effect of tank materials might be weakened in the water phase when there is minimal alteration of water chemistry by the tank materials (Section 4.2).

5. Conclusions

This study represents initial efforts to comprehensively compare the microbial community dynamics and profiles of potential pathogens in long-term operated rainwater and tap-water SWSSs with varied tank materials. The experimental design provides a distinctive platform to investigate the assembly mechanisms of microbial communities in different SWSSs and identify key factors contributing to their assembly. The key findings and implications of this study include:

- Distinct microbial communities were observed between rainwater and tap-water SWSSs, with rainwater systems exhibiting higher abundances and unique compositions of potential pathogens. This finding emphasizes the need to implement robust monitoring strategies in rainwater storage systems for microbial risk control.
- The influence of tank materials on microbial communities was more pronounced in rainwater SWSSs, highlighting the crucial role of tank materials in shaping the rainwater microbiomes. It is important to note that experiences in selecting materials for household storage tanks in tap-water systems should not be generalized to rainwater systems, highlighting the need for specific considerations in rainwater system design and material selection.
- Deterministic processes governed the community assembly in tap-water SWSSs and cement rainwater SWSSs, while stochastic processes had a greater impact in PVC and steel rainwater SWSSs. These distinct community assembly processes observed can be attributed to the variations in nutrient levels and the different impact of tank materials in rainwater and tap-water SWSSs. These findings shed light on the intricate interplay between rainwater chemistry and tank materials, underscoring the need for tailored microbial management strategies for the storage and utilization of rainwater.
- Further investigations into the impact of additional environmental and engineering factors, including disinfection methods, will contribute to advancing our understanding of rainwater microbiota and enhance risk mitigation strategies in rainwater systems. Further studies can utilize advanced molecular tools, such as deep sequencing of metagenomes, to identify critical functional genes involved in microbial metabolism within stored rainwater. In particular, these tools can aid in identifying specific virulence genes of pathogens, thereby improving our understanding of the health risks associated with stored rainwater.

CRediT authorship contribution statement

Sihang Liu: Writing – original draft, Visualization, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Siqing Xia:** Supervision, Funding acquisition. **Xiaodong Zhang:** Data curation, Conceptualization. **Xucheng Cai:** Supervision. **Jinhao Yang:** Supervision. **Yuxing Hu:** Supervision. **Shuang Zhou:** Data curation. **Hong Wang:** Writing – review & editing, Supervision, Conceptualization, Funding acquisition.

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.

Data availability

Data will be made available on request.

Acknowledgments

This study was funded by the National Key R&D Program of China (Grant No. 2021YFC3201300), National Natural Science Foundation of China (Grant No. 51878468), and by the Fundamental Research Funds for the Central Universities (2022–4-YB-17). The views presented here do not represent those of the sponsors.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.121305.

References

Abbasi, T., Abbasi, S.A., 2011. Sources of pollution in rooftop rainwater harvesting systems and their control. Crit. Rev. Environ. Sci. Technol. 41, 2097–2167. https://doi.org/10.1080/10643389.2010.497438.

Ahmed, W., Gardner, T., Toze, S., 2011. Microbiological quality of roof-harvested rainwater and health risks: a review. J. Environ. Qual. 40, 13–21. https://doi.org/10.2134/jeq2010.0345.

Ahmed, W., Vieritz, A., Goonetilleke, A., Gardner, T., 2010. Health risk from the use of roof-harvested rainwater in Southeast Queensland, australia, as potable or nonpotable water, determined using quantitative microbial risk assessment. Appl. Environ. Microbiol. 76, 7382–7391. https://doi.org/10.1128/AEM.00944-10.

Barberán, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. ISMe J. 6, 343–351. https://doi.org/10.1038/ismej.2011.119.

Boretti, A., Rosa, L., 2019. Reassessing the projections of the world water development report. npj Clean. Water 2, 15. https://doi.org/10.1038/s41545-019-0039-9.

Bucheli-Witschel, M., Kötzsch, S., Darr, S., Widler, R., Egli, T., 2012. A new method to assess the influence of migration from polymeric materials on the biostability of drinking water. Water Res. 46, 4246–4260. https://doi.org/10.1016/j.watres.2012.05.008.

Burkholder, J.M., Glibert, P.M., Levin, S.A., 2013. Eutrophication and oligotrophication. Encyclopedia of Biodiversity, 2nd Edition. Academic Press, Waltham, pp. 347–371. https://doi.org/10.1016/B978-0-12-384719-5.00047-2.

Cai, X., Hu, Y., Zhou, S., Meng, D., Xia, S., Wang, H., 2023. Unraveling bacterial and eukaryotic communities in secondary water supply systems: dynamics, assembly, and health implications. Water Res., 120597 https://doi.org/10.1016/j.watres.2023.120597.

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, 581–583. https://doi.org/10.1038/nmeth.3869.

Chase, J.M., 2007. Drought mediates the importance of stochastic community assembly. Proc. Natl. Acad. Sci. U. S. A. 104, 17430–17434. https://doi.org/10.1073/pnas.0704350104.

Chen, W., Ren, K., Isabwe, A., Chen, H., Liu, M., Yang, J., 2019. Stochastic processes shape microeukaryotic community assembly in a subtropical river across wet and dry seasons. Microbiome 7, 138. https://doi.org/10.1186/s40168-019-0749-8.

Chen, W., Wei, J., Su, Z., Wu, L., Liu, M., Huang, X., Yao, P., Wen, D., 2022. Deterministic mechanisms drive bacterial communities assembly in industrial wastewater treatment system. Environ. Int. 168, 107486 https://doi.org/10.1016/j.envint.2022.107486.

Colquhoun, J.M., Rather, P.N., 2020. Insights into mechanisms of biofilm formation in acinetobacter baumannii and implications for uropathogenesis. Front. Cell Infect. Microbiol. 10.

Dini-Andreote, F., De Cássia Pereira E Silva, M., Triadó-Margarit, X., Casamayor, E.O., Van Elsas, J.D., Salles, J.F., 2014. Dynamics of bacterial community succession in a salt marsh chronosequence: evidences for temporal niche partitioning. ISMe J. 8, 1989–2001. https://doi.org/10.1038/ismej.2014.54.

Dini-Andreote, F., Stegen, J.C., van Elsas, J.D., Salles, J.F., 2015. Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. Proc. Natl. Acad. Sci. 112, E1326–E1332. https://doi.org/10.1073/pnas.1414261112.

Doane, M.P., Ostrowski, M., Brown, M., Bramucci, A., Bodrossy, L., van de Kamp, J., Bissett, A., Steinberg, P., Doblin, M.A., Seymour, J., 2023. Defining marine bacterioplankton community assembly rules by contrasting the importance of environmental determinants and biotic interactions. Environ. Microbiol. 25, 1084–1098. https://doi.org/10.1111/1462-2920.16341.

Dobrowsky, P.H., De Kwaadsteniet, M., Cloete, T.E., Khan, W., 2014. Distribution of indigenous bacterial pathogens and potential pathogens associated with roof-harvested rainwater. Appl. Environ. Microbiol. 80, 2307–2316. https://doi.org/10.1128/AEM.04130-13.

Evans, C.A., Coombes, P.J., Dunstan, R.H., Harrison, T., 2009. Extensive bacterial diversity indicates the potential operation of a dynamic micro-ecology within domestic rainwater storage systems. Sci. Total Environ. 407, 5206–5215. https://doi.org/10.1016/j.scitotenv.2009.06.009.

Favere, J., Barbosa, R.G., Sleutels, T., Verstraete, W., De Gusseme, B., Boon, N., 2021. Safeguarding the microbial water quality from source to tap. npj Clean. Water 4, 1–6. https://doi.org/10.1038/s41545-021-00118-1.

Ghosh, G.C., Jahan, S., Chakraborty, B., Akter, A., 2015. Potential of household rainwater harvesting for drinking water supply in hazard prone coastal area of Bangladesh. Nat. Environ. Pollut. Technol. 14, 937.

Guardabassi, L., Dalsgaard, A., Olsen, J.E., 1999. Phenotypic characterization and antibiotic resistance of Acinetobacter spp. isolated from aquatic sources. J. Appl. Microbiol. 87, 659–667. https://doi.org/10.1046/j.1365-2672.1999.00905.x.

Guilbaud, M., de Coppet, P., Bourion, F., Rachman, C., Prévost, H., Dousset, X., 2005. Quantitative detection of Listeria monocytogenes in biofilms by real-time PCR. Appl. Environ. Microbiol. 71, 2190–2194. https://doi.org/10.1128/AEM.71.4.2190-2194.2005.

Gülay, A., Musovic, S., Albrechtsen, H.J., Al-Soud, W.A., Sørensen, S.J., Smets, B.F., 2016. Ecological patterns, diversity and core taxa of microbial communities in groundwater-fed rapid gravity filters. ISMe J. 10, 2209–2222. https://doi.org/10.1038/ismej.2016.16.

Gunn, J.S., Bakaletz, L.O., Wozniak, D.J., 2016. What's on the outside matters: the role of the extracellular polymeric substance of gram-negative biofilms in evading host immunity and as a target for therapeutic intervention *. J. Biol. Chem. 291, 12538–12546. https://doi.org/10.1074/jbc.R115.707547.

Hamilton, K., Reyneke, B., Waso, M., Clements, T., Ndlovu, T., Khan, W., DiGiovanni, K., Rakestraw, E., Montalto, F., Haas, C.N., Ahmed, W., 2019. A global review of the microbiological quality and potential health risks associated with roof-harvested rainwater tanks. npj Clean. Water 2, 1–18. https://doi.org/10.1038/s41545-019-0030-5.

Harpole, W.S., Tilman, D., 2007. Grassland species loss resulting from reduced niche dimension. Nature 446, 791–793. https://doi.org/10.1038/nature05684.

Hubbell, S.P., 2001. The Unified Neutral Theory of Biodiversity and Biogeography. Princeton University Press Princeton.

Islam, M.M., Chou, F.N.F., Kabir, M.R., Liaw, C.H., 2010. Rainwater: a potential alternative source for scarce safe drinking and arsenic contaminated water in bangladesh. Water Resour. Manag. 24, 3987–4008. https://doi.org/10.1007/s11269-010-9643-7.

Jones, B.E., Grant, W.D., 2000. Microbial diversity and ecology of alkaline environments. Journey to Diverse Microbial Worlds: Adaptation to Exotic Environments. Springer, pp. 177–190.

Jongman, R.H.G., Ter Braak, C.J., Van Tongeren, O.F., 1995. Data Analysis in Community and Landscape Ecology. Cambridge university press.

Kim, M., Han, M., 2011. Composition and distribution of bacteria in an operating rainwater harvesting tank. Water Sci. Technol. 63, 1524–1530. https://doi.org/10.2166/wst.2011.410.

Kurniawan, A., Yamamoto, T., 2019. Accumulation of NH4+ and NO3− inside biofilms of natural microbial consortia: implication on nutrients seasonal dynamic in aquatic ecosystems. Int. J. Microbiol. 2019, 6473690 https://doi.org/10.1155/2019/6473690.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol. 75, 5111–5120. https://doi.org/10.1128/AEM.00335-09.

Li, L.J., Lin, C., Huang, X.R., An, X.L., Li, W.J., Su, J.Q., Zhu, Y.G., 2023a. Characterizing potential pathogens from intracellular bacterial community of protists in wastewater treatment plants. Environ. Int. 171, 107723 https://doi.org/10.1016/j.envint.2022.107723.

Li, N., Li, X., Fan, X.Y., 2023b. Polypropylene module as a new type of tank for rainwater storage and cleaner production: purification efficacy, bacterial community microecology and potential pathogens characteristics. J. Clean. Prod. 384, 135495 https://doi.org/10.1016/j.jclepro.2022.135495.

Lian, S., Qu, Y., Dai, C., Li, S., Jing, J., Sun, L., Yang, Y., 2022. Succession of function, assembly, and interaction of microbial community in sequencing biofilm batch reactors under selenite stress. Environ. Res. 212, 113605 https://doi.org/10.1016/j.envres.2022.113605.

Lim, H.S., 2023. What happens to nitrogen and phosphorus nutrient contributions from green roofs as they age? A review. Environ. Adv. 12, 100366 https://doi.org/10.1016/j.envadv.2023.100366.

Ling, F., Whitaker, R., LeChevallier, M.W., Liu, W.T., 2018. Drinking water microbiome assembly induced by water stagnation. ISMe J. 12, 1520–1531. https://doi.org/10.1038/s41396-018-0101-5.

Luan, L., Jiang, Y., Dini-Andreote, F., Crowther, T.W., Li, P., Bahram, M., Zheng, J., Xu, Q., Zhang, X.X., Sun, B., 2023. Integrating pH into the metabolic theory of ecology to predict bacterial diversity in soil. Proc. Natl. Acad. Sci. U. S. A. 120, e2207832120 https://doi.org/10.1073/pnas.2207832120.

Lundy, L., Revitt, M., Ellis, B., 2018. An impact assessment for urban stormwater use. Environ. Sci. Pollut. Res. 25, 19259–19270. https://doi.org/10.1007/s11356-017-0547-4.

Ma, B., Wang, Y., Ye, S., Liu, S., Stirling, E., Gilbert, J.A., Faust, K., Knight, R., Jansson, J.K., Cardona, C., Röttjers, L., Xu, J., 2020. Earth microbial co-occurrence network reveals interconnection pattern across microbiomes. Microbiome 8, 82. https://doi.org/10.1186/s40168-020-00857-2.

Manga, M., Ngobi, T.G., Okeny, L., Acheng, P., Namakula, H., Kyaterekera, E., Nansubuga, I., Kibwami, N., 2021. The effect of household storage tanks/vessels and user practices on the quality of water: a systematic review of literature. Environ. Syst. Res. 10, 18. https://doi.org/10.1186/s40068-021-00221-9 (Heidelb).

Mghazli, N., Sbabou, L., Hakkou, R., Ouhammou, A., El Adnani, M., Bruneel, O., 2021. Description of microbial communities of phosphate mine wastes in Morocco, a semi-arid climate, using high-throughput sequencing and functional prediction. Front. Microbiol. 12, 666936. https://doi.org/10.3389/fmicb.2021.666936.

Ning, D., Deng, Y., Tiedje, J.M., Zhou, J., 2019. A general framework for quantitatively assessing ecological stochasticity. Proc. Natl. Acad. Sci. U. S. A. 116, 16892–16898. https://doi.org/10.1073/pnas.1904623116.

Nwachcuku, N., Gerba, C.P., 2004. Emerging waterborne pathogens: can we kill them all? Curr. Opin. Biotechnol. 15, 175–180. https://doi.org/10.1016/j.copbio.2004.04.010.

Paszko-Kolva, C., Shahamat, M., Colwell, R.R., 1992. Long-term survival of Legionella pneumophila serogroup 1 under low-nutrient conditions and associated morphological changes. FEMS Microbiol. Lett. 102, 45–55. https://doi.org/10.1016/0378-1097(92)90112-2.

Peter, A., Routledge, E., 2018. Present-day monitoring underestimates the risk of exposure to pathogenic bacteria from cold water storage tanks. PLoS One 13, e0195635. https://doi.org/10.1371/journal.pone.0195635.

Phillips, P.L., Schultz, G.S., 2012. Molecular Mechanisms of Biofilm Infection: biofilm Virulence Factors. Adv. Wound Care 1, 109–114. https://doi.org/10.1089/wound.2011.0301 (New. Rochelle).

Schafer, C.A., Mihelcic, J.R., 2012. Effect of storage tank material and maintenance on household water quality. J. Am. Water Works Assoc. 104 https://doi.org/10.5942/jawwa.2012.104.0125.

Singleton, S.L., Davis, E.W., Arnold, H.K., Daniels, A.M.Y., Brander, S.M., Parsons, R.J., Sharpton, T.J., Giovannoni, S.J., 2023. Identification of rare microbial colonizers of plastic materials incubated in a coral reef environment. Front. Microbiol. 14, 1259014. https://doi.org/10.3389/fmicb.2023.1259014.

Slavik, I., Oliveira, K.R., Cheung, P.B., Uhl, W., 2020. Water quality aspects related to domestic drinking water storage tanks and consideration in current standards and guidelines throughout the world – a review. J. Water Health 18, 439–463. https://doi.org/10.2166/wh.2020.052.

Smith, S.D., 2019. phylosmith: an R-package for reproducible and efficient microbiome analysis with phyloseq-objects. J. Open Source Softw. 4, 1442. https://doi.org/10.21105/joss.01442.

Stegen, J.C., Lin, X., Fredrickson, J.K., Chen, X., Kennedy, D.W., Murray, C.J., Rockhold, M.L., Konopka, A., 2013. Quantifying community assembly processes and identifying features that impose them. ISMe J. 7, 2069–2079. https://doi.org/10.1038/ismej.2013.93.

Stegen, J.C., Lin, X., Konopka, A.E., Fredrickson, J.K., 2012. Stochastic and deterministic assembly processes in subsurface microbial communities. ISMe J. 6, 1653–1664. https://doi.org/10.1038/ismej.2012.22.

Sun, W., Jing, Z., 2023. Migration of rare and abundant species, assembly mechanisms, and ecological networks of microbiomes in drinking water treatment plants: effects of different treatment processes. J. Hazard. Mater. 457, 131726 https://doi.org/10.1016/j.jhazmat.2023.131726.

Takayama, Y., Kitajima, T., Honda, N., Sakane, N., Yumen, Y., Fukui, M., Nagai, N., 2022. Nutritional status in female patients with nontuberculous mycobacterial lung disease and its association with disease severity. BMC Pulm. Med. 22, 315. https://doi.org/10.1186/s12890-022-02109-5.

Thomas, R.M., Cyrus, S., Abraham, B.M., 2018. Effect of cement on rain water. Int. J. Civ. Eng. Technol. 9, 20–26.

Woodcock, S., Van Der Gast, C.J., Bell, T., Lunn, M., Curtis, T.P., Head, I.M., Sloan, W.T., 2007. Neutral assembly of bacterial communities. FEMS Microbiol. Ecol. 62, 171–180. https://doi.org/10.1111/j.1574-6941.2007.00379.x.

Wu, L., Ning, D., Zhang, B., Zhou, J., et al., 2019. Global diversity and biogeography of bacterial communities in wastewater treatment plants. Nat. Microbiol. 4, 1183–1195. https://doi.org/10.1038/s41564-019-0426-5.

Wu, W., Lu, H.P., Sastri, A., Yeh, Y.C., Gong, G.C., Chou, W.C., Hsieh, C.H., 2018. Contrasting the relative importance of species sorting and dispersal limitation in shaping marine bacterial versus protist communities. ISMe J. 12, 485–494. https://doi.org/10.1038/ismej.2017.183.

Yang, X., Jiang, G., Zhang, Y., Wang, N., Zhang, Y., Wang, X., Zhao, F.J., Xu, Y., Shen, Q., Wei, Z., 2023. MBPD: a multiple bacterial pathogen detection pipeline for one health practices. Imeta 2, e82. https://doi.org/10.1002/imt2.82.

Yin, Q., Sun, Y., Li, B., Feng, Z., Wu, G., 2022. The r/K selection theory and its application in biological wastewater treatment processes. Sci. Total Environ. 824, 153836 https://doi.org/10.1016/j.scitotenv.2022.153836.

Zeenat, Elahi, A., Bukhari, D.A., Shamim, S., Rehman, A., 2021. Plastics degradation by microbes: a sustainable approach. J. King Saud Univ. Sci. 33, 101538 https://doi.org/10.1016/j.jksus.2021.101538.

Zhang, Q.L., Hu, P., Zhao, Y., Feng, G.W., Zhang, Y.Q., Zhu, B.X., Tao, Z., 2014. Anion-controlled assembly of silver-di(aminophenyl)sulfone coordination polymers: syntheses, crystal structures, and solid state luminescence. J. Solid State Chem. 210, 178–187. https://doi.org/10.1016/j.jssc.2013.11.027.

Zhang, X., Xia, S., Ye, Y., Wang, H., 2021. Opportunistic pathogens exhibit distinct growth dynamics in rainwater and tap water storage systems. Water Res. 204, 117581 https://doi.org/10.1016/j.watres.2021.117581.

Zhang, X., Xia, S., Zhao, R., Wang, H., 2020. Effect of temperature on opportunistic pathogen gene markers and microbial communities in long-term stored roof-harvested rainwater. Environ. Res. 181, 108917 https://doi.org/10.1016/j.envres.2019.108917.

Zhou, J., Deng, Y., Zhang, P., Xue, K., Liang, Y., Van Nostrand, J.D., Yang, Y., He, Z., Wu, L., Stahl, D.A., Hazen, T.C., Tiedje, J.M., Arkin, A.P., 2014. Stochasticity, succession, and environmental perturbations in a fluidic ecosystem. Proc. Natl. Acad. Sci. 111, E836–E845. https://doi.org/10.1073/pnas.1324044111.

Zhou, L., Wang, H., Zhang, Z., Zhang, J., Chen, H., Bi, X., Dai, X., Xia, S., Alvarez-Cohen, L., Rittmann, B.E., 2021. Novel perspective for urban water resource management: 5R generation. Front. Environ. Sci. Eng. 15, 16. https://doi.org/10.1007/s11783-020-1308-z.