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Microbiological quality of roof tank water in an urban village in southeastern China

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ABSTRACT

Urban villages are unique residential neighborhoods in urban areas in China. Roof tanks are their main form of water supply, and water quality deterioration might occur in this system because of poor hygienic conditions and maintenance. In this study, water samples were seasonally collected from an urban village to investigate the influence of roof tanks as an additional water storage device on the variation in the microbial community structure and pathogenic gene markers. Water stagnation in the roof tank induced significant decreases in chlorine ($p < 0.05$), residual chlorine was as low as 0.02 mg/L in spring. Propidium monoazide (PMA)-qPCR revealed a one-magnitude higher level of total viable bacterial concentration in roof tank water samples ($2.14 \pm 1.81 \times 10^5$ gene copies/mL) than that in input water samples ($3.57 \pm 2.90 \times 10^4$ gene copies/mL, $p < 0.05$), especially in spring and summer. In addition, pathogenic fungi, *Mycobacterium* spp., and *Legionella* spp. were frequently detected in the roof tanks. Terminal users might be exposed to higher microbial risk induced by high abundance of *Legionella* gene marker. Spearman's rank correlation and redundancy analysis showed that residual chlorine was the driving force that promoted bacterial colonization and shaped the microbial community. It is worth noted that the sediment in the pipe will be agitated when the water supply is restored after the water outages, which can trigger an increase in turbidity and bacterial biomass. Overall, the findings provide practical suggestions for controlling microbiological health risks in roof tanks in urban villages.

Introduction

Over the past few decades, China has experienced rapid urbanization, and it was estimated that approximately 70% of China's population would live in urban areas by 2030 (Deng *et al.*, 2015). Urban villages (called Cheng Zhong Cun in Chinese) refer to a special type of residential community in China. Usually, urban village was originally a real village in an urban-rural fringe area and later became surrounded by constructed urban areas during rapid urbanization. Although they are always characterized with a crowded population, lagged plan-

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ning, and insufficient infrastructure, low rent and living costs are attractive to many migrants. Generally, the water supply systems in urban villages in China are connected with the municipal water supply pipe network. The remarkable feature of this water supply system is the water storage tanks, which are installed on the roofs of self-built apartment buildings in the case of water supply shortages during peak water consumption.

The simple water storage tanks on the roofs of self-built apartment buildings in urban villages are directly exposed to the natural environment. Rainwater and dust can directly enter roof tanks, causing serious exogenous microbial contamination. The warm water in the roof tank due to exposure to sunlight would accelerate the decay of disinfectant residuals (Powell *et al.*, 2000; Miyagi *et al.*, 2017). In addition, the mismatch between the size of roof tanks and the water demand of consumers may contribute to the low water exchange rate and long-term water stagnation (Al-Omari *et al.*, 2008; Al-Bahry *et al.*, 2011; Miyagi *et al.*, 2017). Water stagnation in roof tanks can offer stable environmental conditions, facilitating a series of chemical reactions (Zhang *et al.*, 2021b); and the important one was the decay of chlorine disinfectants. The decay of chlorine disinfectants further facilitated the regrowth of microorganisms, especially opportunistic pathogens, which are often resistant to disinfectants and can thrive under the extreme oligotrophic conditions of drinking water (Falkinham *et al.*, 2015; Zhang *et al.*, 2021a). The existence and regrowth of these microorganisms might cause serious problems in roof tanks in urban villages. Despite the high possibility of microbial contamination, this problem has not been taken into account by the consumers and governments. Compared with the well-managed secondary water supply systems in residential neighborhoods in China (Hu *et al.*, 2021), roof tanks in urban villages might contain greater microbiological risks. To ensure drinking water safety in urban villages, it is necessary to evaluate the microbiological risk in roof tanks in urban villages.

This study aimed to investigate microbial contamination in roof tanks in an urban village from Xiamen, a central city located on the southeast coast of China. The specific objectives were 1) to explore the influence of roof tanks on microbial community structure and pathogenic gene markers, and 2) to identify microbial hazards in roof tanks. These results could help to provide new insight into the microbiological health risks in roof tanks in urban villages, thus providing helpful suggestions for their management.

1. Materials and methods

1.1. *Water sample collection and processing*

An urban village in Xiamen in southeastern China was selected to perform this investigation. Recently, along with rapid economic growth and the significant acceleration of urbanization, a large number of migrant workers poured into Xiamen, and most of them live in urban villages where amounts of apartment buildings were built. Three apartment buildings were selected in this study, and a set of parallel water storage tanks were installed on the roof of each building to supply water to the residents (Fig. 1). The rooftop water storage systems use flotation devices to trigger the drinking water delivery system to refill the water storage tank (stainless steel, and the total volume of each roof tank was 1500 L) when the water line in the roof tank drops to a certain level. In addition, some roof tanks were equipped with a shelter to keep rainwater out, and there was a layer of dust on the outer wall of all selected roof tanks.

The water samples were seasonally collected four times on November 14th, 2019; January 14th, 2020; May 10th, 2020; July 16th, 2020; corresponding to autumn, winter, spring, and summer, respectively, according to the temperature change. For the winter water samples, it is noted that the water outage occurred from 10 pm on January 13th, 2020, to 5 am on January 14th, 2020, and the water samples were collected at 10 am on Jan 14th, 2020, after the water supply was restored. At each sampling time except for the first sampling, water samples of roof Tank1 and Tank2 were collected respectively while only one shared input water sample was collected because of the short distance between Tank1 and Tank2. For the first sampling, both of the input and tank water samples of roof Tank1 and Tank2' were collected respectively because Tank1 and Tank2' was far away from each other. Tap on the first floor of the self-built house was used to collect the input water samples after flushing 5 min at a full flow velocity. For the collection of roof tank water samples, water was drained via a water pipeline that was sterilized by sodium hypochlorite aqueous (NaClO) solution. Twenty liters of each water sample was collected and immediately carried to the laboratory in less than two hours.

For physicochemical analysis of water quality parameters, 500 mL of water was pretreated with a 0.45 μm nitrocellulose membrane (Millipore, USA). Ten mL of water was stabilized with ultrapure nitric acid (0.5% HNO_3, Merck, Germany) for heavy metal analysis. Residual free chlorine was quenched by sodium thiosulfate for the cultivation of bacteria. The microorganisms were collected by filtering various volumes (3 to 6 L) of each water sample through a 0.22 μm nitrocellulose membrane. Propidium monoazide (PMA, Biotium, USA) was then used to treat the collected microorganisms following the procedure of our previous protocol (Hu *et al.*, 2019), which was used for the detection of viable microbes. Finally, the treated membranes were stored at -80°C.

1.2. *Water quality analysis*

Turbidity, pH, dissolved oxygen (DO), and water temperature were measured by portable instruments (Hach model HQ40d and 2100Q, USA). Residual free chlorine was determined using the *N*,*N*-diethyl-p-phenylene diamine (DPD) colorimetric kit (Hach model DR300). Ammonium (NH_4^+-N), nitrite (NO_2^--N), nitrate (NO_3^--N), and total nitrogen (TN) were measured using the UV spectrophotometric method according to the Standard Examination Methods for Drinking Water of China (GB/T 11894-1989 and GB/T 5750-2006). Sulfate ions (SO_4^{2-}) and total organic carbon (TOC) were measured by ion chromatography (ICS-300, USA) and a TOC-V WP analyzer (Shimadzu, Japan), respectively. Concentrations of heavy metals (i.e., Al, Cr, Mn, Fe, Cu, Zn, As, Cd, and Pb) in water samples collected in winter, spring, and summer were detected by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500cx, USA).

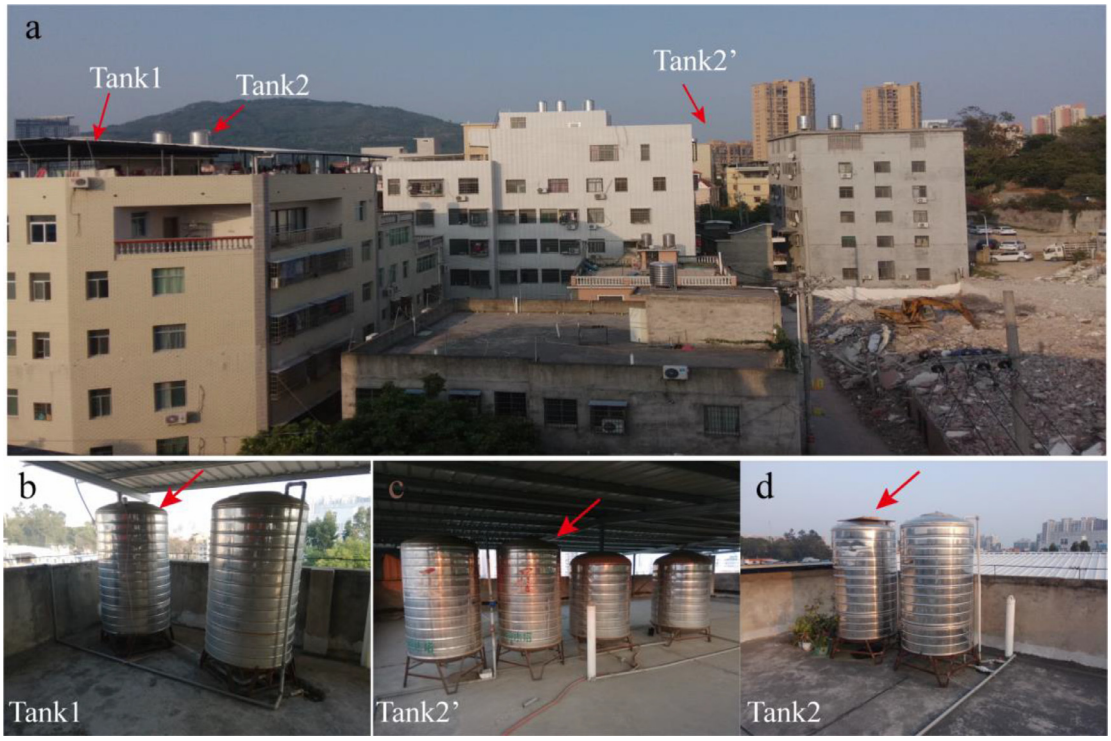

Fig. 1 – (a) The self-built apartment buildings in an urban village in Xiamen. The label in (a) showed the location of the selected roof tanks. (b - d) The roof tanks for collecting water samples.

The total counts of culturable bacteria and fungi were determined by the heterotrophic plate count (HPC) technique. One mL of water sample in triplicate was distributed onto nutrient agar and R2A (Hopebio Co., Qingdao, China) for bacteria. The plates were incubated at 37°C for two days on the nutrient agar plate or at 28°C for five days on the R2A plate. For the enumeration of culturable fungi, 100 mL of the water samples in triplicate were filtered through a 0.22 μm nitrocellulose membrane. The membrane was then attached to dichloran rose bengal chloramphenicol agar (DRBC, Hopebio Co., Qingdao, China) and incubated at 28°C for 5 days.

1.3. *Microbial DNA extraction and PMA-qPCR analysis*

Genomic DNA extraction was conducted using the FastDNA™ Spin Kit for Soil (MP Biomedicals, USA). The DNA concentrations were measured by a NanoDrop ND-1000 microspectrophotometer (Thermo Scientific, USA).

The specific gene abundance of total bacteria, pathogenic fungi, and common waterborne pathogens, including *Acanthamoeba* spp., *Aeromonas hydrophilia, Enterococcus* spp., *Enterococcus faecalis/faecium, Escherichia coli, Hartmannella vermiformis, Legionella* spp., *L. pneumophila, Mycobacterium* spp., *Pseudomonas aeruginosa, Salmonella* spp., *Shigella* spp., and *Staphylococcus aureus*, was quantified using PMA-qPCR assays. Briefly, PMA-qPCR assays were performed on a QuantStudio™ 3 Real-Time PCR instrument (Applied Biosystems, USA) using well-developed methods (Hu *et al.*, 2021). For SYBR Green assays, 20 µL of the reaction mixture was composed of 10 µL Premix (Trans, China), 0.4 µL passive reference dye (50 ×), 0.4 µL each primer (10 µmol/L), and 1 µL of DNA template. For TaqMan assays, 20 µL of the reaction mixture was composed of 10 µL Premix (Vazyme, China), 0.2 µL probe (10 µmol/L), 0.4 µL each primer (10 µmol/L), and 1 µL DNA template. All reactions included the negative and positive controls.

1.4. *Illumina sequencing of bacterial 16S rRNA genes*

The construction procedures of the microbial community library and analysis of the sequencing data were performed based on our recent research (Hu *et al.*, 2021). The V4 region of the bacterial 16S rRNA gene was amplified using the barcode-modified primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). For PCR amplification, each 50 µL reaction mixture included 25 µL Premix (Ex Taq™ Version 2.0; Takara, Dalian, China), 2 µL each primer (10 µmol/L), and 5 µL DNA template. The annealing temperature of the PCR conditions was set at 55°C. Triplicate PCR products of each sample were pooled and purified. After that, equal mass (100 ng) of the purified PCR products was pooled to construct a 16S rRNA library.

All libraries were loaded on the Illumina HiSeq platform (Novogene, Beijing, China) using the PE250 strategy. The Mothur and USEARCH programs were used to identify zero-radius operational taxonomic units (zOTUs) with a similarity threshold of 97% (Edgar, 2010; Kozich *et al.*, 2013). Represonative sequences were taxonomically classified against the EzBioCloud database (Yoon *et al.*, 2017). After the nonbacterial and unassigned sequences were rewmoved, the sequences per sample were rarefied to 12,000. The DNA reads were submitted to the NCBI database with the project accession number PRJNA722664.

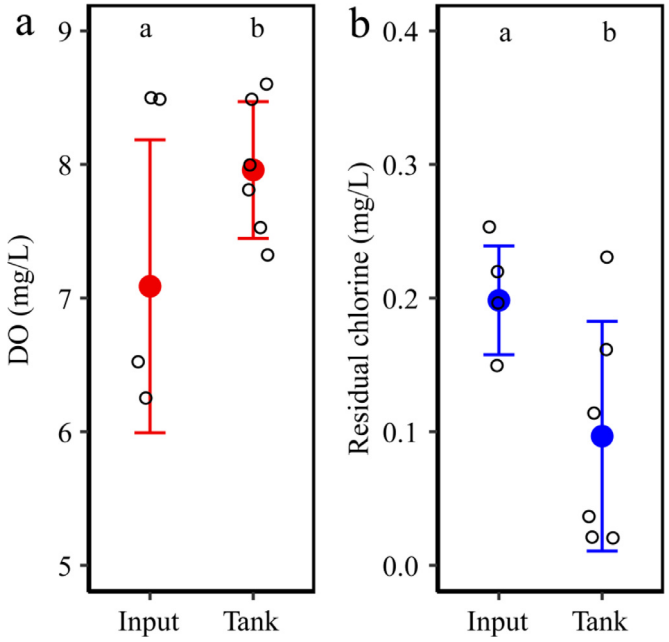

Fig. 2 – The variation of the dissolved oxygen and residual chlorine from input water samples to roof tank water samples collected in autumn, spring, and summer. Groups share no common letters (a and b) are significantly different ($p < 0.05$).

1.5. *Statistical analysis*

Due to the water outage in winter, the variation in water quality parameters from potable water delivery systems to roof tanks was analyzed with the water samples collected on three other seasonal days. A Student paired, two-tailed *t*-test was performed to detect the significant differences of water quality parameters between the input water samples and roof tank water samples with IBM SPSS 22 statistical software. Significant differences were accepted at $p < 0.05$. Nonparametric Spearman rank correlation analysis was employed to evaluate the correlation between water quality indices and biological data. The alpha diversity of the microbial community was calculated using the 'vegan' packages in R software (www.r-project.org/). The Bray-Curtis dissimilarity index was calculated using the 'vegan' package, and nonmetric multidimensional scaling (NMDS) analysis was used to demonstrate the dissimilarity in microbial community structure. The Adonis test was carried out using the 'vegan' package, and a *p*-value less than 0.05 among the groups was considered significant. Redundancy analysis (RDA) was performed with the 'vegan' package to explore the influence of water quality on the microbial community structure at the OTU level.

2. Results

2.1. *Physicochemical analysis of water samples*

The physicochemical properties of the water samples are listed in Appendix A **Tables S1** and **S2**. pH, DO, temperature, residual chlorine, and turbidity ranged from 7.06 to 7.77, 6.25 to 9.27 mg/L, 18.20 to 33.20°C, 0.02 to 0.39 mg/L, and 0.01 to 5.09 NTU, respectively. The concentrations of NH_4^+-N, NO_3^--N, TN, and SO_4^{2-} varied from < 0.02 to 0.08 mg/L, 1.38 to 3.00 mg/L, 1.20 to 2.98 mg/L, and 24.22 to 46.02 mg/L, respectively. The concentrations of NO_2^--N were below the detection limit. The TOC concentration ranged from 0.78 to 1.13 mg/L. The concentrations of all heavy metals were lower than the permittable value of the Standards for Drinking Water Quality of China (GB 5749-2006).

Among the collected water samples, only DO and residual chlorine exhibited the significant changes from the input to roof tank water samples ($p < 0.05$, Fig. 2). The average DO concentration in the input water samples (7.08 ± 1.09 mg/L) was significantly lower than roof tank water samples (7.95 ± 0.51 mg/L, Fig. 2**a**). On the other hand, the average residual chlorine concentration in the input water samples (0.19 ± 0.04 mg/L) was significantly higher than roof tank water samples (0.09 ± 0.08 mg/L, Fig. 2**b**). However, no significant difference was found for other physicochemical water quality parameters (*t*-test, $p > 0.05$).

2.2. *Water biological characteristics*

HPC revealed the total counts of culturable bacteria ranged from 0 to 8.67 CFU/mL by nutrient agar, from 0 to 159 CFU/mL by R2A, while the culturable fungi ranged from 0 to 20.67 CFU/100 mL (Appendix A **Table S1**). PMA-qPCR revealed the consistent presence of total bacteria (1.43×10^4 - 5.79×10^5 gene copies/mL), pathogenic fungi (1.38×10^3 - 3.00×10^5 gene copies/100 mL), *Mycobacterium* spp. (2.48×10^2 - 2.52×10^4 gene copies/100 mL), and *Legionella* spp. (< 10 - 3.54×10^4 gene copies/100 mL) in these collected water samples (Fig. 3). In addition, *Enterococcus* spp. was only detected in spring tank water samples with 52.8 gene copies/100 mL. *Acanthamoeba* spp. and *Escherichia coli* were detected in winter water samples with 4.54 - 8.58×10^2 and 1.20 - 19.0×10^2 gene copies/100 mL, respec-

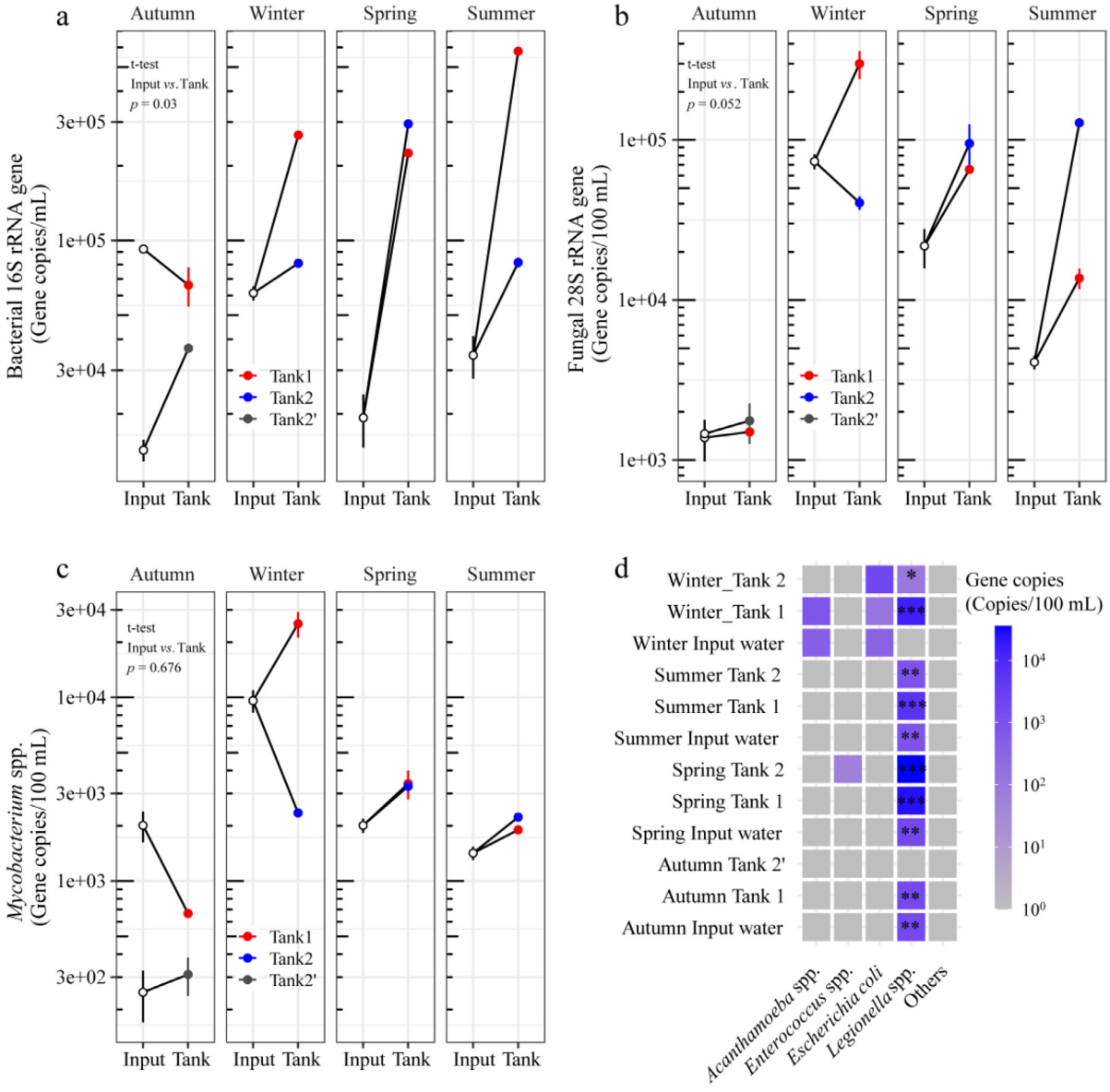

Fig. 3 – Enumeration of (a) bacterial 16S rRNA gene, (b) pathogenic fungi, (c) *Mycobacterium* spp., and (d) *Enterococcus* spp., *Acanthamoeba* spp., *Legionella* spp., *Escherichia coli*, and other pathogens in the collected water samples. A Student paired, two-tailed *t*-test was used to compare the difference in gene abundance between input water samples and roof tank water samples collected in autumn, spring, and summer. $p < 0.05$ presented significant difference. Risk level of *Legionella* was indicated by *, insignificant risk; **, medium risk; or *, high risk.**

tively. The other potential pathogens were under quantification limits.

In general, HPC showed the higher bacterial colony counts with R2A plates in roof tank water samples, but it displayed no significant difference as compared to the input water samples ($p > 0.05$, Appendix A **Table S1**). However, roof tank water samples presented a significantly higher abundance of bacterial 16S rRNA genes ($2.14 \pm 1.81 \times 10^5$ gene copies/mL) than input water samples ($3.57 \pm 2.90 \times 10^4$ gene copies/mL, $p < 0.05$, Fig. 3a). The gene copies of pathogenic fungi and *Mycobacterium* showed no significant difference between input water samples and roof tank water samples (Figs. 3b and 3c), but a sharp increase in the pathogenic fungal abundance in roof tank was noticed in summer (Fig. 3b). In addition, gene markers of *Legionella* exhibited higher abundance in the collected water samples (Fig. 3d).

Due to the detection of high frequency and abundance of *Legionella* in the collected water samples, the risk level of *Legionella* presence in these collected water samples was assessed based on the recommendations of the European Legionnaires' disease Surveillance Network (ELDSNet) (insignificant risk was noted at $< 10^3$ CFU/L, medium risk at $> 10^3$ CFU/L but $< 10^4$ CFU/L, and high risk at $> 10^4$ CFU/L) (European Centre for Disease Prevention and Control, 2017). Usually, there was 3 ribosomal RNA operons in one cell of *Legionella* species (https://rrndb.umms.med.umich.edu/), meaning that the de-

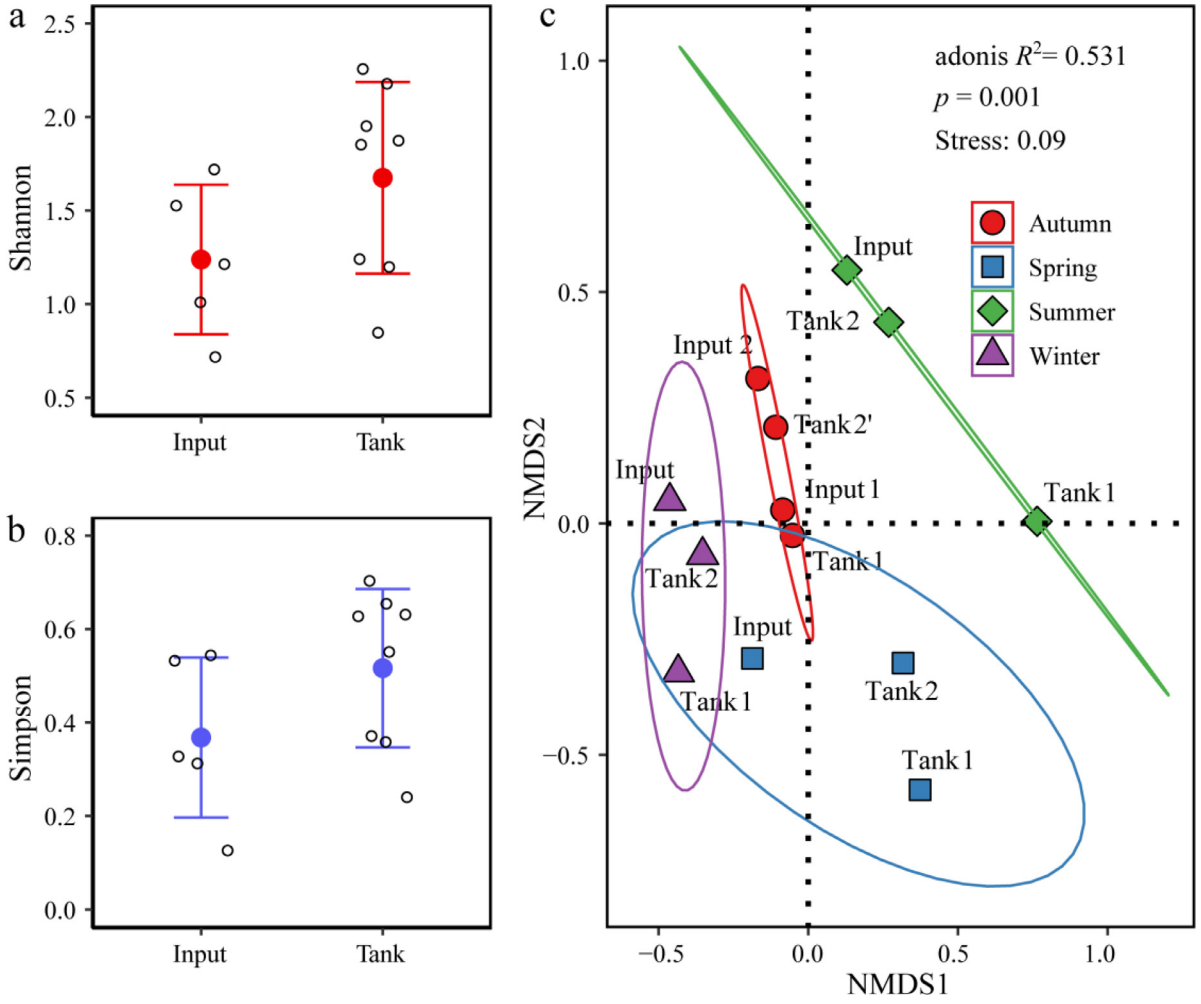

Fig. 4 – The changes in microbial diversity of the collected water sample during the investigation. (a) The Shannon index. (b) The Simpson index. (c) The nonmetric multidimensional scaling (NMDS) ordination.

tected 23S rRNA gene abundance of *Legionella* in this study was three times higher than the cell numbers. The gene copies were then transformed to cell numbers, which was assumed to equal to the CFU counts in this study. It was found that almost all the collected water samples presented at least medium risk of *Legionella* (Fig. 3d).

Notably, a short water outage occurred before the water samples were collected in winter. The turbidity in the Tank1 water sample was increased to a very high level (4.32 NTU, Appendix A **Table S1**). In contrast to the nutrient agar plate, with low bacterial counts (0 - 2.67 CFU/mL), the R2A plate presented the higher counts (6 - 159 CFU/mL, Appendix A **Table S1**). Furthermore, the total bacterial biomass in the input water sample (9.33×10^4 gene copies/mL) with low temperature (18.8°C) after the water outage was strikingly high. It was even higher than that of the water samples (3.47×10^4 gene copies/mL) collected in summer (32.5°C, Fig. 3a). In addition, the detected biomass of pathogenic fungi and *Mycobacterium* in input water samples in winter were also higher than that in other seasons (Figs. 3b and 3c), suggesting that the water outages might lead to the higher microbiological risk.

2.3. *Microbial diversity and composition variation from the drinking water network to the roof tank*

In total, 156,000 high-quality sequences of bacterial 16S rRNA genes were obtained from all water samples. All samples achieved a stable plateau based on library coverage at a distance level of 97% similarity (> 99%, data not shown), suggesting that the microbial community was completely sampled. An obvious increase in the Shannon and Simpson indices was observed from input water samples to roof tank water samples (Figs. 4a and 4b). A striking seasonal separation was seen through different water sampling times in the NMDS plot (Fig. 4c), verified by the ADONIS analysis ($R^2 = 0.531$, $p < 0.05$). In addition, the NMDS plot also revealed a higher variance between the input and roof tank water samples collected in spring and summer (Fig. 4c).

Microbial community composition analysis demonstrated the dramatic shifts along with the seasons, and obvious differences were also observed between input and roof tank water samples in spring and summer (Fig. 5). *Phreatobacter* was the most abundant genus in these water samples, with a rel-

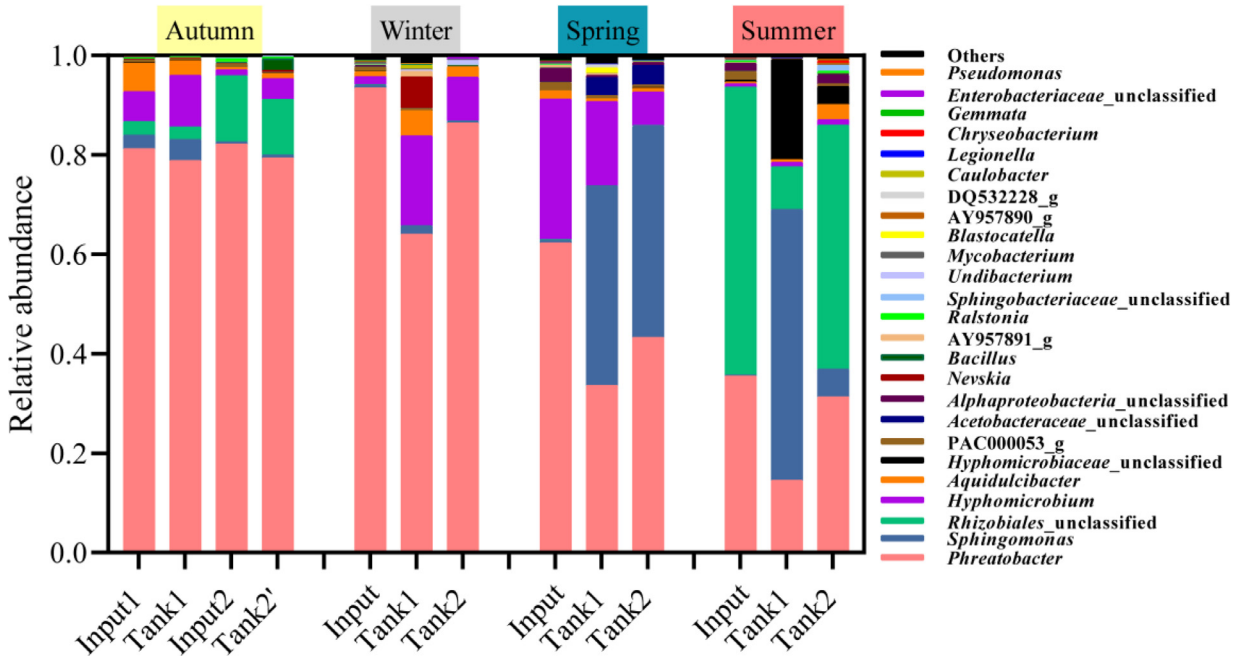

Fig. 5 – Microbial communities present in the collected water samples at the genus level during the investigation.

ative abundance ranging from 14.53% to 93.42%, followed by *Sphingomonas* (0.16% - 54.48%), *Rhizobiales*_unclassified (0.01% - 57.97%), and *Hyphomicrobium* (0.96% - 28.29%). *Sphingomonas* presented a lower relative abundance of 0.48% and 0.16% in input water samples collected in spring and summer but dominated in roof tank water samples with high relative abundances of 40.12% (Tank1-spring), 42.57% (Tank2-spring), and 54.48% (Tank1-summer), respectively. *Hyphomicrobiaceae*_unclassified presented a higher relative abundance in the Tank1 water sample (20.12%) than in the input water samples (0.30%) in summer. *Rhizobiales*_unclassified was dominant in the summer input water sample (49.09%) and Tank2 water sample (57.97%).

2.4. Associations with abiotic and biotic factors

A nonparametric Spearman rank correlation method was employed to assess the relationship between the physicochemical parameters of water quality and the abundance of target microorganisms in the collected water samples. As shown in Fig. 6, the residual chlorine presented negative correlations with all target microorganisms, especially high negative correlations with the bacterial 16S rRNA gene (r = -0.504, p = 0.08) and *Legionella* (r = -0.645, $p < 0.05$). Turbidity was positively correlated with all target microorganisms, presenting high correlations with R2A (r = 0.567, $p < 0.05$), pathogenic fungi (r = 0.698, $p < 0.01$), and *Mycobacterium* (r = 0.720, $p < 0.01$). NO_3^- was also positively correlated with all target microorganisms, presenting high correlations with R2A (r = 0.510), DRBC (r = 0.735, $p < 0.01$), and *Mycobacterium* (r = 0.669, $p < 0.05$). TN displayed positive correlations with DRBC (r = 0.669, $p < 0.05$) and *Mycobacterium* (r = 0.527). SO_4^{2-} displayed a negative correlation with *Legionella* (r = -0.608, $p < 0.05$), and TOC was positively related to pathogenic fungi (r = 0.610, $p < 0.05$). Surprisingly, pH, DO, and temperature displayed no obvious correlations with target microorganisms.

RDA contributed to identify the water quality parameters that influenced the microbial community structure. As illustrated in Fig. 7, RDA1 and RDA2 accounted for 56.98% of the total variation. Residual chlorine was considered the most remarkable explanatory variable, followed by temperature, pH, and DO. However, the microbial community structure was less affected by turbidity.

3. Discussion

3.1. Influence of roof tanks on physicochemical water quality parameters

Water stagnation is a typical feature in roof tanks (Al-Bahry *et al.*, 2011; Miyagi *et al.*, 2017). Previous studies have suggested that water stagnation can affect physicochemical water quality parameters; higher pH, higher heavy metal concentrations, higher TOC concentrations, and lower residual chlorine have been reported in stagnated water in pipelines and roof tanks (Ziadat, 2005; Miyagi *et al.*, 2017; Zhang *et al.*, 2021b). However, only DO and residual chlorine exhibited a significant difference between the water samples collected from the potable water delivery system and roof tanks in this study (Fig. 2). Although the roof tanks were exposed to the outdoor high-temperature environment, no significant difference in the water temperature was found between the input and roof tank water samples ($p > 0.05$, Appendix A **Table S1**).

The concentrations of residual chlorine in roof tank water samples were significantly lower than those in input water samples (Fig. 2**b**). Comparable findings were also reported in previous studies that investigated the water quality in roof

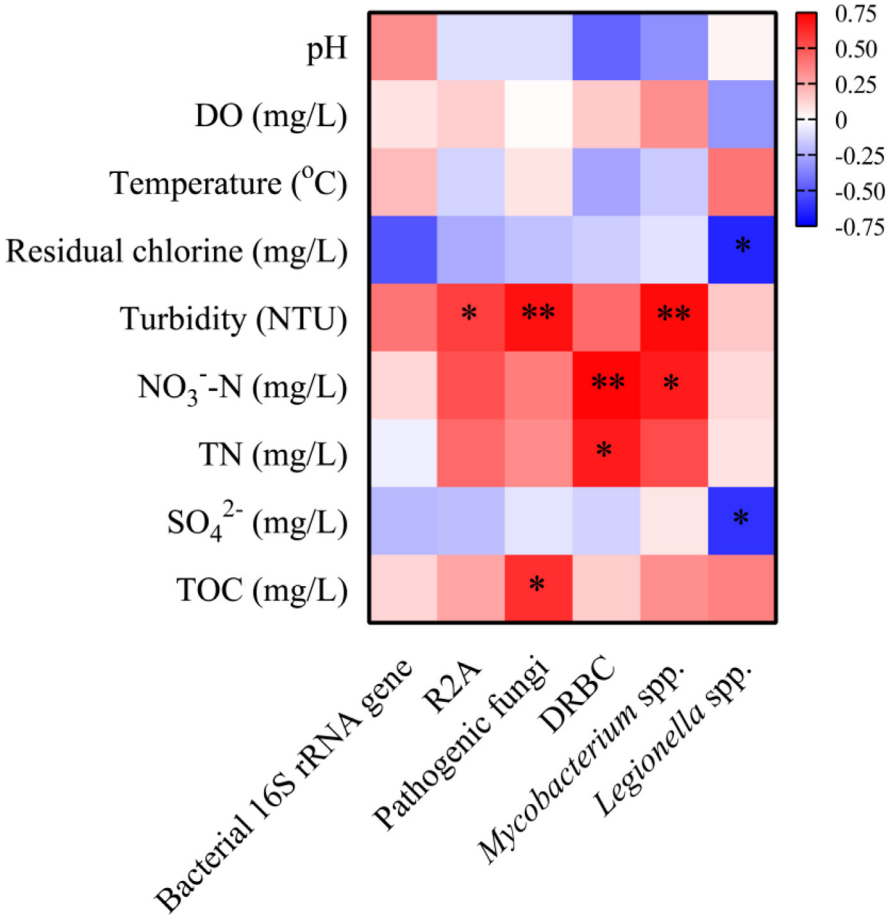

Fig. 6 – Heatmap of the correlations between the abundance of target microorganisms and water quality parameters in the collected water samples. Correlations were performed using the nonparametric Spearman rank correlation approach. *, $p < 0.05$; **, $p < 0.01$.

tanks around the world (Graham and VanDerslice, 2007; Al-Omari *et al.*, 2008; Miyagi *et al.*, 2017), which indicated that chlorine decline is an inevitable problem in roof tanks. The decay of residual chlorine in roof tanks might be due to the reaction with chlorine-consuming substances in stagnated water. This resulted in the chlorine residuals in a few storage tanks being lower than the minimum level (0.05 mg/L) as required by the *Standards for Drinking Water Quality of China* (GB5749-2006). It is noted that residual free chlorine is the most important factor in suppressing the regrowth of microorganisms in drinking water, and therefore, the decline of chlorine disinfectants ultimately induced microbiological risks to the terminal users.

3.2. *Influence of roof tanks on total bacteria and potential pathogens*

Roof tanks offer a good environment for microbial regrowth due to the low chlorine residuals caused by their unique characteristics of low water turnover and long-term water stagnation. Several studies have proven that roof tanks could present the higher microbial biomass (Al-Omari *et al.*, 2008; Al-Bahry *et al.*, 2011; Miyagi *et al.*, 2017), which was in agreement with the detection of high gene abundance of total bacteria in this study (Fig. 3). Compared with the results that there was no difference in the changes of total bacterial 16S rRNA gene abundance from input water samples to tank water samples in secondary water supply systems in residential neighborhoods in Haicang district (Hu *et al.*, 2021), a significant increase of bacterial 16S rRNA gene abundance was observed in this study ($p < 0.05$, Fig. 3**a**). This illustrated the higher microbiological risks in roof tanks in urban villages. However, there was no difference in the bacterial 16S rRNA gene abundance between the tank water samples collected from residential neighborhoods ($6.2 \pm 7.7 \times 10^4$ gene copies/mL, the data were shown in our other study (Hu *et al.*, 2021)) and urban villages ($2.14 \pm 1.81 \times 10^5$ gene copies/mL) in Haicang District ($p > 0.05$).

Diverse waterborne pathogens, such as *Aeromonas, Enterobacteriaceae, Escherichia coli, Pseudomonas, Pasteurella, Salmonella, Serratia*, and *Tatumella*, have been detected in roof tanks (Al-Bahry *et al.*, 2011; Schafer and Mihelcic, 2012; Miyagi *et al.*, 2017). It seems that the high abundance of potential pathogens in roof tanks originated from the drinking water delivery systems in this study, since the biomass of the detected pathogens exhibited no significant difference between the input and roof tank water samples ($p > 0.05$, Fig. 3). In addition, the types of pathogens detected were also less than those of the water samples collected from the water storage

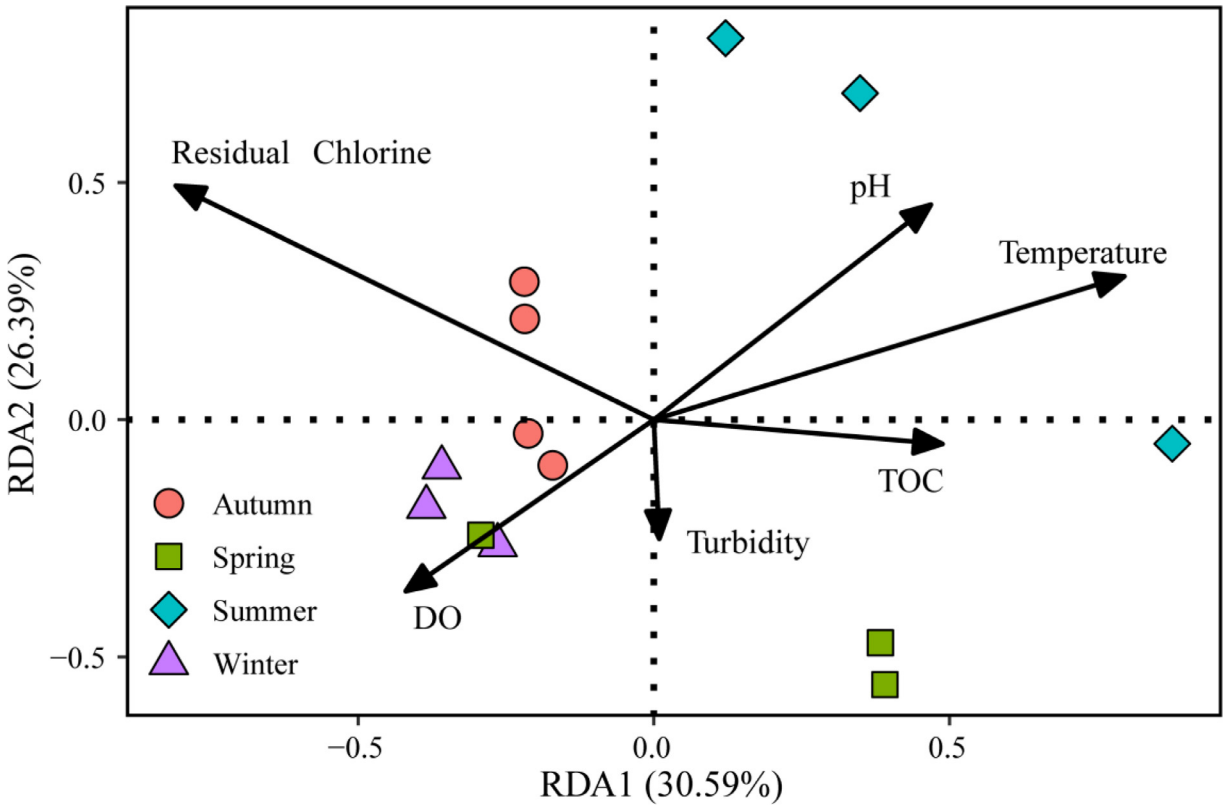

Fig. 7 – Redundancy analysis for the correlation between the microbial community and water quality parameters.

tank in residential neighborhoods in this city (Hu *et al.*, 2021), which might be due to the limited number of samples. Further extensive detection of pathogenic bacteria in roof tanks in urban villages may be needed in future study. Despite this, uncontrolled bacterial regrowth in roof tank water may disturb the biological stability of tap water (Prest *et al.*, 2016). Consumers who used the taps supplied by storage tanks may bear a higher risk of microorganism exposure, since severe proliferation of total bacteria was observed in roof tanks (Fig. 3).

Approximately one-half of the species within *Legionella* are associated with human disease (Fields *et al.*, 2002). Almost all the collected water samples presented positive *Legionella* in this study and might pose a high microbiological risk to terminal users (Fig. 3**d**). Furthermore, diverse *Legionella* species have also been detected in water samples from a Spanish water distribution system (Salinas *et al.*, 2021). In particular, severe *Legionella* contamination has been reported in secondary water supply systems in residential neighborhoods (Li *et al.*, 2018; Hu *et al.*, 2021). It is reported that 70% of Legionnaires' disease outbreaks in the US which were attributed to inadequate residual disinfectant (Garrison *et al.*, 2016). Zhang *et al.* (2021a) has found that the *Legionella* spp. concentrations were strongly correlated with the concentrations of residual chlorine in drinking water distribution systems. In this drinking water system, residual chlorine was negatively correlated with *Legionella* ($r = -0.645$, $p < 0.05$, Fig. 6), and chlorine residuals may not be well maintained with the chlorine concentration low to 0.02 mg/L (Appendix A **Table S1**). These results further highlighted the critical role of the proper level of residual chlorine throughout the drinking water distribution networks, especially the terminal roof tanks.

3.3. *The changes of microbial community structure*

The NMDS plot revealed a clear separation of the seasonal microbial community in this study (Fig. 4**c**), evidenced by the changes in dominant genera among the collected water samples (Fig. 5). *Phreatobacter* was the most represented genus of bacteria in all water samples. The ultrapure water purification system provided the first description of this genus (Toth *et al.*, 2014), and it was a dominant species, referred to group F0723 sp. with 96% gene sequence similarity, in the water distribution system (Stanish *et al.*, 2016; Wang *et al.*, 2018; Perrin *et al.*, 2019). This genus tended to be more abundant in drinking water with reduced chlorine levels (Stanish *et al.*, 2016), as its residual level in the current work was less than 0.39 mg/L (Appendix A **Table S1**). Previous studies demonstrated that chlorination could effectively increase the relative abundance of *Sphingomonas* (Hwang *et al.*, 2012; Jia *et al.*, 2015), as it is a famous chlorine-resistant bacterium (Luo *et al.*, 2021). However, *Sphingomonas* was a dominant bacterial genus only in roof tank water samples in spring and summer (Fig. 5), the chlorine concentration in these water samples was low to 0.02-0.04 mg/L (Appendix A **Table S1**). Similarly, Wang *et al.* (2018) found that the relative abundance of *Sphingomonas* increased with distance from drinking water treatment plants and was accompanied by a decrease of *Phreatobacter*. In addition, *Sphin-*

gomonas might be an unfriendly group in the drinking water distribution system. For example, *Sphingomonas*, isolated from drinking water, presented varying antibiotic resistance profiles, including beta-lactams, ciprofloxacin, and cotrimoxazole (Vaz-Moreira *et al.*, 2011). Wang *et al.* (2018) found that *Rhizobiales*_unclassified was positively correlated with temperature and residual chlorine, which was consistent with our results (Fig. 5 and Appendix A **Table S1**).

3.4. *Key water quality parameter shaping the microbial community*

Diverse factors, such as chlorine residuals, water source, building plumbing age, and pipe material can drive microbial colonization and community shifts in the drinking water distribution system (Li *et al.*, 2018; Goraj *et al.*, 2021). In this study, seasonality, which is strongly linked to water temperature, was the most influential factor that drove shifts in the microbial community (Fig. 4**c**). However, it had almost no influence on microbial biomass (Fig. 6). Residual chlorine was found to be the most important water quality parameter that shaped both the abundance and structure of the microbial community, as indicated by the RDA and Spearman rank correlation analysis (Figs. 6 and 7). This could be ascribed to the enhanced levels of certain dominant or rare species owing to the low concentration of chlorine disinfectant (Zhang *et al.*, 2020), evidenced by the variation in alpha diversity (Figs. 4**a** and 4**b**). However, maintaining an effective disinfectant residual remains a challenge at the distal ends of distribution systems (Zhang and Edwards, 2009; Wang *et al.*, 2014). The concentration of residual chlorine was found to be as low as 0.02 mg/L in spring roof tank water samples in this study (Appendix A **Table S1**), which could not meet the required chlorine dosage (0.05 mg/L) regulated by the Standards for Drinking Water Quality of China (GB 5749-2006). Therefore, residual chlorine might be used as a monitoring indicator to evaluate microbial contamination in roof tanks due to its high correlation with microbial biomass.

Poor hygiene management might be another important cause of microbial contamination in roof tanks in this study. As shown in Fig. 1**a**, a vast majority of roof tanks in this urban village were exposed to the outdoor environment, and a layer of dust was observed on the outer wall of the roof tanks. Although Al-Omari *et al.* (2008) reported no correlation between tank cleaning and chlorine loss, storage tanks with three or more annual cleanings had significantly fewer *Escherichia coli* than those cleaned less frequently (Schafer and Mihelcic, 2012). In addition, the rainwater and particulate matter in the air would enter the roof tanks and deteriorate the water. Therefore, continuous good management is critical for reducing the microbiological risks in drinking water in an urban village.

3.5. *Water quality parameter changes caused by water outages*

Water outages are a public hygiene concern, and they will bring the risks such as increases in turbidity and microbial biomass when the water supply is restored (Kumpel and Nelson, 2013). In this study, the turbidity and biomass of total bacteria, pathogenic fungi, and *Mycobacterium* spp. in input water samples were higher in winter than in other seasons (Fig. 3 and Appendix A **Table S1**) and were also higher than the water samples collected from the storage tanks in residential neighborhoods at the same sampling time (Hu *et al.*, 2021). More importantly, more kinds of pathogenic bacteria were detected in these outage water samples than in other seasonal water samples (Fig. 3**d**), which might be due to the loose sediments in the drinking water network entering the roof tanks. Liu *et al.* (2014) found that loose sediments contributed to approximately 55% - 90% of the microbial biomass in a drinking water distribution system. Higher occurrences of pathogens in winter water samples suggested water outages along with higher microbiological risks, and health risk reduction measures should be strengthened during short-term water outages.

4. Conclusions

This is the first study that revealed the influence of roof tanks on water quality parameters and microbial contamination in Chinese urban villages. The water quality in self-managed roof tanks was expected to deteriorate seriously due to its incomplete infrastructure and lack of management awareness of cleaning roof tanks by managers. Severe decrease in residual chlorine was observed in roof tank water samples, which induced a one magnitude higher level of total viable bacterial gene abundance in roof tank water samples than in input water samples. However, there was no essential difference in the total bacterial 16S rRNA gene abundance between the tank water samples collected from residential neighborhoods and urban villages. Although the sample number was limited, the microbiological risks in roof tanks of the urban village were much less serious than expected and under control. Even so, there is also a need for these households in urban villages to develop an awareness of cleaning the roof tank and maintaining good sanitary by themselves, as well as the need for the government authorities to strengthen the supervision of the physiochemical and microbiological water quality.

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Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2022.01.036.

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