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A comprehensive investigation of the microbial risk of secondary water supply systems in residential neighborhoods in a large city

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ABSTRACT

Secondary water supply systems (SWSSs) are characterized by long water stagnation and low levels of chlorine residuals, which may pose a high microbial risk to terminal users. In this study, the SWSSs of 12 residential neighborhoods in a metropolitan area of 5 million people in southeastern China were seasonally investigated to assess their microbial risks by determining more than 30 physicochemical and biological parameters. Although the microbiological quality of SWSS water met the requirements of the standards for drinking water quality of China, it did deteriorate in various aspects. The heterotrophic plate counts with R2A media were high (> 100 CFU/mL) in some SWSS tank and tap water samples. Propidium monoazide (PMA)-qPCR revealed a one magnitude higher abundance of viable bacteria in the tank and tap water samples (average $10^{3.63\pm1.10}$ and $10^{3.65\pm1.25}$ gene copies/mL, respectively) compared with the input water samples, and *Enterococcus, Acanthamoeba*, and *Hartmannella vermiformis* were only detected in the tanks. In particular, the high detection frequency of *Legionella* in 35% tank and 21% tap water samples suggested it is a supplementary microbial safety indicator in SWSSs. The microbial regrowth potential was more obvious in summer, and Illumina sequencing also demonstrated distinct seasonal changes in the relative abundance of bacterial gene sequences at the genus level. Turbidity and residual chlorine were closely connected with total bacterial biomass, and the latter seemed responsible for microbial community structure alteration. The extremely low chlorine residuals associated with a high abundance of total bacteria (as high as $10^{6.48}$ gene copies/mL) and *Legionella* (as high as $10^{6.71}$ gene copies/100 mL) in the closed valve tanks highlighted the high microbial risk increased by mishandling the operation of SWSSs. This study found that SWSSs possessed a higher microbial risk than the drinking water network, which suggested that the frequency and scope of monitoring the microbial risk of SWSSs in megacities should be strengthened for the purpose of waterborne epidemic disease prevention and control.

1. Introduction

In recent years, increasing numbers of residential neighborhoods (called "Ju Min Xiao Qu" in Chinese) with high-rise buildings have been built in large cities to cope with the scarcity of land resources due to rapid urbanization worldwide. Secondary water supply systems (SWSSs, also meaning onsite water storage systems) are commonly built inside such building infrastructures and provide water to the higher floors to compensate for the deficiency of hydraulic pressure in the municipal pipe network in modern cities (Li et al. 2018a, Lu et al. 2015). As components of water supply infrastructure, SWSSs play an important role in ensuring the health and welfare of residents living on high floors.

Usually, SWSSs are relatively simple, as they are mainly composed of water storage tanks and water pumps, but the mismatch between storage tank size and water demand should be considered since it may result in low water turnover and long water stagnation (Miyagi et al. 2017). Water stagnation is an important driving factor responsible for the deterioration of drinking water quality in storage tanks, such as chlorine

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loss, warm temperature, leaching of metals, and sediment accumulation (Evison and Sunna 2001, Lu et al. 2015, Ziadat 2005). More importantly, these features can trigger serious microbial contamination in SWSSs. High numbers of heterotrophic bacteria, bacterial 16S rRNA genes, fungal counts, and potential pathogens such as *Legionella* and amoebae have been detected in water storage tanks worldwide (Evison and Sunna 2001, Li et al. 2018a, Lu et al. 2015, Novak Babič and Gunde-Cimerman 2021). Customers utilizing the tap water provided by SWSSs, therefore, might be exposed to high microbial risk.

Although microbial contamination in SWSSs has been widely discussed, there are limits in detection methods and research scales. The detection methods used for quantifying microbial contamination are biased, and most researchers tend to use single culture-dependent (Evison and Sunna 2001, Novak Babič and Gunde-Cimerman 2021) or culture-independent (Li et al. 2018a, Lu et al. 2015) methods. Culture-based methods play a paramount role in regular water quality monitoring. However, the microbial community structure in SWSSs is complex (Evison and Sunna 2001, Li et al. 2018a, Lu et al. 2015, Novak Babič and Gunde-Cimerman 2021). Only small fractions of microbial groups can be detected by NA plates, while many can grow on oligotrophic R2A plates, but the majority of microorganisms are in a viable but nonculturable (VBNC) state in drinking water, including most pathogenic bacteria (Guo et al. 2021). These VBNC state pathogens might resuscitate in the intestines and express their virulence (Ramamurthy et al. 2014). Currently, there is still a lack of paradigms combining culture-dependent and culture-independent methods to comprehensively assess microbial risk in SWSSs. In addition, the population size of most cities is continuously expanding; for example, 96 cities in China reached million-inhabitant levels in 2019 according to the *Urban Construction Statistical Yearbook of China* (2020). The water distribution networks of these large cities are huge, interconnected, and complex, which might provide different niches and differently shaped microbiota from those in small-scale distribution networks (Li et al. 2018a). Unfortunately, the seasonal and spatial microbial safety of SWSSs in cities with million-inhabitant population levels remains unknown.

The main purpose of this study was to comprehensively investigate the microbial characteristics and health risk of SWSSs, especially seasonal and spatial influences, in cities with a million-inhabitant-scale population. The specific objectives were 1) to reveal the temporal and spatial microbial community characteristics and potential microbial risks in SWSSs, 2) to determine the relationship between physicochemical water parameters and the microbial community, and 3) to provide qualitative and quantitative support for the scientific management and operation of SWSSs.

2. Materials and methods

2.1. Secondary water supply systems

This study was conducted in residential neighborhoods of Xiamen, Fujian Province, China. Xiamen has accelerated its urbanization in recent years, and its permanent population reached 5.16 million in 2020 according to "*Communiqué of the Seventh National Census of Xiamen City*" (Xiamen Municipal Bureau of Statistics, 2021). Seven water plants jointly serve its six districts with different kinds of water sources, including the Jiulongjiang River, Bantou Reservoir, Shidou Reservoir, and Tingxi Reservoir. A large number of residential neighborhoods with high-rise buildings were constructed, and different types of property companies participate in the management of the residential neighborhoods. Although the local health department has strengthened the water quality monitoring of SWSSs in residential neighborhoods, a systematic microbial risk evaluation has not been carried out. In this study, two representative residential neighborhoods were chosen in each administrative district, and a total of twelve residential neighborhoods were selected (Fig. 1A). Among them, four kinds of SWSS layouts were noted (Fig. 1B). One residential neighborhood in the HuLi district employed an on-site underground tank coupled with a rooftop tank (Fig. 1B-1). Two neighborhoods in the HaiCang district employed one storage tank (Fig. 1B-2). Most of the selected neighborhoods employed double tanks (Fig. 1B-3); however, the water valve of one storage tank in some neighborhoods was closed (Fig. 1B-4). In addition, four residential neighborhoods employed concrete tile water tanks, while the others applied stainless steel water tanks (Fig. 1A).

2.2. Sample collection and processing

Drinking water in SWSSs was seasonally sampled at four time periods: Oct 14th, 2019 - Nov 21st, 2019 (autumn), Dec 27th, 2019 - Jan 14th, 2020 (winter), May 6th, 2020 - June 2nd, 2020 (spring), and July 10th, 2020 - Aug 8th, 2020 (summer). Due to the impact of the COVID-19 epidemic, water samples were only collected from six residential

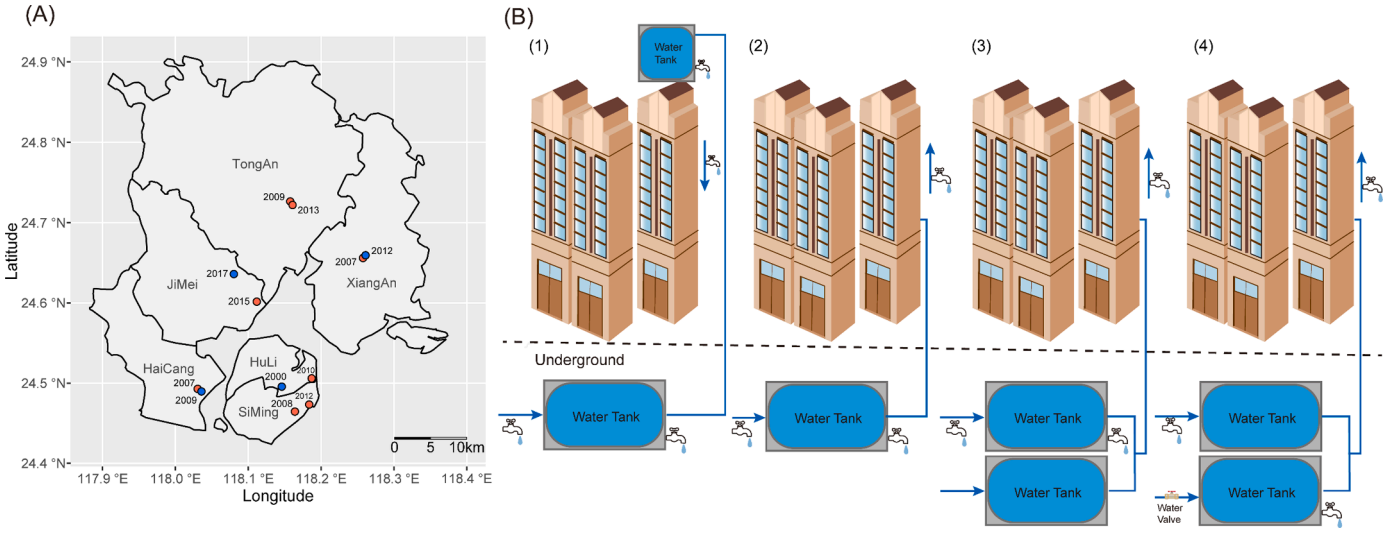

Fig. 1. A) Map showing the location of the sampling sites in Xiamen, in which the residential neighborhoods employing concrete tile water tank are indicated by blue dots, and the others applying stainless steel water tank are indicated by orange dots; The number is the year that the residential neighborhoods were constructed. B) The schematic diagram of the secondary water supply systems selected in this study (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

neighborhoods in winter. A total of 121 water samples were collected during this investigation, and the numbers of water samples in each event were as follows: autumn = 32, winter = 18, spring = 34, summer = 37. In each SWSS, three kinds of water samples (i.e., inputs, tanks, and taps) were collected. For the input water samples, the faucet was chosen on the first floor which was directly attached to the central drinking water distribution systems of the residential building or in the park of the residential neighborhood. For the tank water samples, most of the storage tanks were equipped with siphons that were chosen for sampling, and some tank water samples were collected through the drain pipe of the storage tank. For the tap water samples, floors 7-30 which were connected to the SWSSs of the residential building were randomly selected. At each sampling site, after the tap water and drain pipe water were flushed for 5 min at the maximum flow velocity, the water sample was then collected using a 20 L polyethylene plastic bucket which had been treated overnight with sodium hypochlorite. The collected water samples were then transported to the laboratory within 2 hrs. for later use. With respect to the method of collecting tap water samples after flushing for 5 min, the rationale lies not only in the national guideline for taking tap water samples (The Ministry of Health of the P.R. China, 2006) but also in the real situation as the following, i.e., the daily water consumption per capita in Xiamen was about 163 L (Xiamen Water Resources Bureau, 2019). In general, there are 2-4 persons in a family in Xiamen. The total water consumption by a family per day was about 300-600 L. Although people don't flush water for minutes at one time, the total duration for using water should be much longer. Flushing for 5 min means 150-200 L water passes away, it is about 1/3-1/2 of the daily usage. So taking water samples from this point should be acceptable.

One liter of each water sample was placed in a glass bottle and then stored at 4°C within 24 hrs. for chemical analysis of water quality parameters. Sodium thiosulfate was added to quench the chlorine residuals for bacterial cultivation. Each water sample with the maximum volume was filtered through a 0.22 μm nitrocellulose membrane (Millipore, USA) to collect the planktonic bacteria. All filtered membranes were treated with propidium monoazide (PMA, Biotium, USA) following our previous study (Guo et al. 2021), and the treated membranes were then stored at -80°C for DNA extraction.

2.3. Water quality analysis

The water samples were measured *in situ* for water temperature, dissolved oxygen (DO), pH, and turbidity using portable instruments (Hach, USA). The residual chlorine was measured by the *N,N*-diethyl-*p*-phenylenediamine (DPD) colorimetric method (Hach, USA). A UV spectrophotometric method was used to measure the concentrations of phosphate (PO_4^{3-}), ammonia (NH_4^+-N), nitrite (NO_2^--N), nitrate (NO_3^--N), and total nitrogen (TN) (GB/T 5750-2006 and GB/T 11894-1989). Sulfate (SO_4^{2-}) was measured using an ion chromatograph (ICS-300, USA). Total organic carbon (TOC) was determined using a TOC-V WP analyzer (Shimadzu, Japan). NA and R2A agar (Hopebio Co., China) were used to enumerate the total cultivable bacteria. The membrane filtration method was used to determine the counts of cultivable fungi with dichlorane rose bengal chloramphenicol agar (DRBC, Hopebio Co., China).

2.4. Bacterial DNA extraction and qPCR analysis

Genomic DNA was extracted from bacteria on membranes using a FastDNA™ Spin Kit for Soil (MP Biomedicals, USA) following the manufacturer's protocol.

The bacterial 16S rRNA gene, pathogenic fungi, enteric pathogens (e.g., *Enterococcus* spp., *Enterococcus faecalis/faecium, Escherichia coli, Salmonella* spp., and *Shigella* spp.), and opportunistic pathogens (e.g., *Aeromonas hydrophilia, Acanthamoeba* spp., *Hartmannella vermiformis, Legionella* spp., *Legionella pneumophila, Mycobacterium* spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) were quantified by PMA-qPCR on a QuantStudio™ 3 Real-Time PCR instrument (Applied Biosystems, USA). The qPCR primers, probes, and annealing temperatures are shown in Table S1. Reaction mixtures (20 μL) contained 10 μL of 2 × qPCR master mix: PerfectStartTM II Green qPCR SuperMix (Trans, China) for qPCR without probe and AceQ® Universal U^+ Prob Master Mix V2 (Vazyme, China) for qPCR with 0.2 μL of probe (10 μM), 0.4 μL of passive reference dye (50 ×, for SYBR Green assays), 0.4 μL of primers (10 μM), and 1 μL of template DNA. The quantification limit for all PMA-qPCR assays was 10-100 gene copies/reaction, except for 16S rRNA genes (1000 gene copies/reaction) (Fig. S1).

2.5. Illumina sequencing of bacterial 16S rRNA genes

The bacterial 16S rRNA gene V4 region was amplified with the barcoded primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Song et al. 2019). The PCRs used to generate the sequencing libraries were performed in a 50 μL reaction mixture containing 25 μL of Ex Taq (Takara), 2 μL of each primer (10 μM), and 5 μL of DNA template. Cycling conditions involved an initial denaturing step at 95°C for 5 min, followed by 30 cycles of 30 s at 95°C, 45 s at 55°C, 30 s at 72°C, and a final elongation step of 10 min at 72°C. Each sample was amplified in triplicate. The positive amplicons were pooled, purified, and normalized in equimolar amounts in the final mixture. The 16S rRNA gene library was subjected to Illumina HiSeq sequencing using paired-end 250 bp kits at Novogene Bioinformatics Technology Co. Ltd., Beijing, China.

The raw sequence data were filtered using Trimmomatic (Bolger et al. 2014). Low-quality sequences and chimeras were removed by the UCHIME2 algorithm (Edgar 2016). The high-quality sequences were clustered into zero-radius operational taxonomic units (zOTUs, hereafter referred to as OTUs) at 97% sequence similarity using the Mothur and USEARCH programs (Edgar 2010, Kozich et al. 2013). Taxonomic categories of zOTUs were assigned by the EzBioCloud database (Yoon et al. 2017). After removing the archaeal, algal, and unclassified bacterial sequences, the sampling depth for each sample was flattened to 7,000. Six samples were excluded from further analysis due to the small read library. The DNA reads were submitted to the NCBI database (http://www.ncbi.nlm.nih.gov/) under the access number PRJNA722664.

2.6. Statistical analysis

In this study, water samples collected from the closed valve storage tanks with long stagnation were different from those collected from the open valve storage tanks. Therefore, two types of data were individually used for statistical analysis. In addition, we compared the impact of the storage tank material on the water quality parameters (e.g., residual chlorine, turbidity, and total bacteria) with the water samples collected from the HaiCang district due to their similar SWSS design.

IBM SPSS 22 statistical software was used to perform one-way ANOVA to compare physiochemical and/or biological data between the three groups of water samples. Nonparametric Spearman rank correlation was used to identify the relationship between physicochemical water quality parameters and target microbial biomass (Li et al. 2018a). Redundancy analysis (RDA) was conducted to determine the effects of physicochemical water quality parameters on microbial community structure (Zhang et al. 2021b). Nonmetric multidimensional scaling (NMDS) analysis was applied to assess the similarity in microbial community structure among water samples with Bray-Curtis dissimilarity. The calculated RDA and NMDS analyses used the 'vegan' package in R software (www.r-project.org/).

3. Results

3.1. Water quality characteristics

The variation in water physicochemical parameters is shown in Fig. 2, Fig. S2, and Table S2. The average water temperature ranged from 18.90 ± 0.97°C to 31.63 ± 0.71°C and correlated with seasonal changes (Fig. S2A). A significant increase in DO was observed in the tank and tap water samples (average 7.96 ± 0.63 mg/L and 7.98 ± 0.65 mg/L, respectively) compared with the input water samples (average 7.58 ± 0.85 mg/L, $p < 0.05$, Fig. 2C), especially in summer (Fig. S2C-4). Similarly, the turbidity was significantly higher in the tank water samples (average 0.38 ± 0.33 NTU) than the input water samples (average 0.19 ± 0.10 NTU, $p < 0.05$, Fig. 2D), especially in summer (Fig. S2D-4). Approximately 69% of tank and 48% of tap water samples had increased turbidity of more than 20% relative to input water samples (Table S3), which means that the management of the SWSSs needs to be improved. Furthermore, three tank water samples (SPHLBRT1-1, SUJMA1-1, and SUSMA1-1) were found to be higher than the Chinese drinking water standard (GB 5749-2006) for turbidity (1 NTU, Table S2), with high increased ratio (323% - 950%) of turbidity from input water samples to the three tank water samples was observed (Table S3). In addition, no significant difference in turbidity was observed between the tank and input water samples collected from closed valve tanks ($p > 0.05$, Fig. S4H) and different tank materials ($p > 0.05$, Fig. S5A).

Significant reductions in residual chlorine were observed in the tank and tap water samples (average 0.44 ± 0.20 and 0.42 ± 0.21 mg/L, respectively) compared with the input water samples (average 0.57 ± 0.23 mg/L, $p < 0.05$, Fig. 2E). Approximately 51% of tank and 55% of tap water samples had a chlorine disinfectant decrease of more than 20% relative to input water samples (Table S3), further emphasizing the importance of strengthening the management of SWSSs. High correlation was observed between the change ratio of residual chlorine and the bacterial 16S rRNA gene (Figs. S3C and S3D). Significant decrease in residual chlorine concentration from input water samples to tank and tap water samples was presented in summer ($p < 0.05$, Fig. S2E). Furthermore, a significant loss of chlorine disinfectant was observed in the closed valve tank water samples ($p < 0.05$, Fig. S4I), with a percent

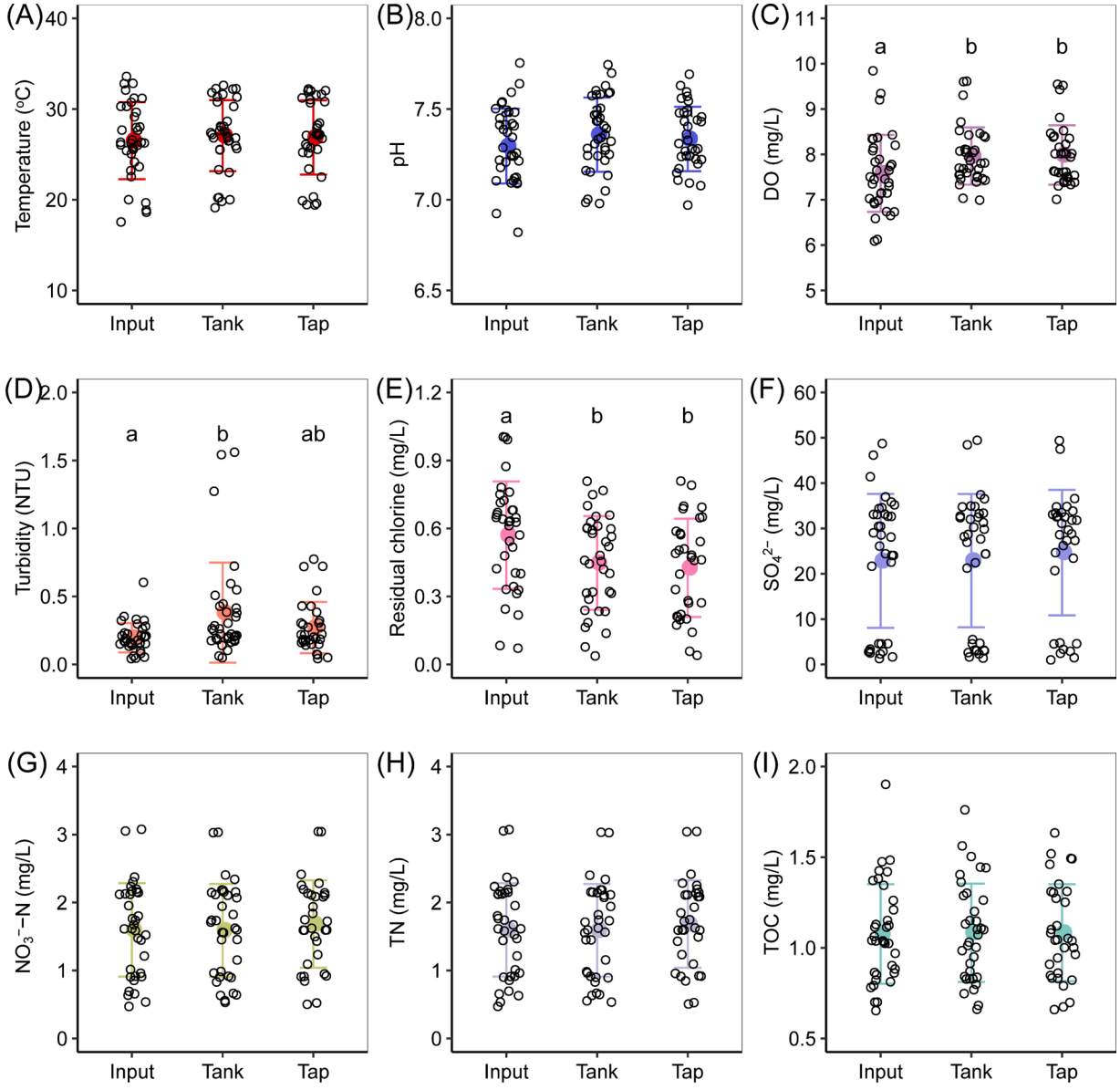

Fig. 2. Boxplot of the levels of A) water temperature, B) pH, C) DO, D) turbidity, E) residual chlorine, F) SO_4^{2-}, G) NO_3^--N, H) TN, and I) TOC in the collected water samples. a and b represent conditions with statistically significant differences ($p < 0.05$).

reduction of free chlorine residual of 45% to 99% (Table S3). Continuous reduction of chlorine disinfectant was observed in the building structure where underground and roof storage tanks were used in combination (Table S2). The residual chlorine concentration of the input water samples varied in different districts, and the HaiCang district was the lowest during the investigation compared with other districts ($p < 0.05$, Fig. 3A). In addition, the tank materials gave little influence on the decrease of chlorine residuals in HaiCang district (Fig. S5B).

In addition, there were no obvious changes in the concentrations of SO_4^{2-}, NO_3^--N, TN, and TOC (Figs. 2F-I). The concentrations of PO_4^{3-}, NH_4^+-N, and NO_2^--N in most water samples were below the detection limits (Table S2). It should be noted that the concentrations of SO_4^{2-}, NO_3^--N, and TN in the districts of HuLi, HaiCang, and SiMing were higher than those in the TongAn and XiangAn districts (Table S2), which could be ascribed to different water sources.

3.2. Bacterial regrowth potential in SWSSs

The bacterial biomass of the water samples quantified by the heterotrophic plate count and PMA-qPCR methods during the investigation are presented in Fig. 4 and Fig. 5, respectively. The results demonstrated that the total cultivable bacteria in the tank and tap water samples were slightly higher than that of input water samples reflected by R2A (Fig. 4A). It is not surprising that few water samples presented positive results with NA (Table S2). However, the abundance of the bacterial 16S rRNA gene was significantly higher in the tank and tap water samples (average $10^{3.63\pm1.10}$ and $10^{3.65\pm1.25}$ gene copies/mL, respectively) than in the input water samples (average $10^{3.08\pm0.91}$ gene copies/mL, $p < 0.05$, Fig. 5A), suggesting significant bacterial regrowth in the SWSSs. In addition, there is no doubt that the potential for bacterial regrowth in summer was higher than that in other seasons (Fig. S6A). A significantly higher abundance of the bacterial 16S rRNA gene was observed in the closed valve tank water samples ($10^{5.32\pm1.16}$ gene copies/mL) than in the input and tap water samples ($10^{2.91\pm1.05}$ and $10^{2.96\pm1.15}$ gene copies/mL, respectively, $p < 0.05$, Fig. S4M). The bacterial biomass of input water samples in HaiCang district was higher than that of the other districts during the investigation (Fig. 3B). However, no difference in the bacterial regrowth potential was observed between the two kinds of tank materials in HaiCang district (Fig. S5C).

3.3. Detection of fungi in SWSSs

Cultivable fungi were detected in most water samples at low levels (< 20 CFU/100 mL, Fig. 4B). However, a high abundance of the pathogenic fungal 28S rRNA gene (average of $10^{3.17\pm0.91}$ – $10^{3.51\pm0.83}$ gene copies/100 mL) was detected (Fig. 5B). No difference in total fungi among the three groups of water samples was observed. In addition, no seasonal variation in fungal biomass was noted (Fig. S6B).

3.4. Occurrence of opportunistic and enteric bacterial pathogens

As shown in Fig. 5, two enteric pathogens and seven opportunistic pathogens were discovered during the investigation. *Mycobacteria* spp. was detected in 74% of the water samples, and a significantly higher abundance of the *Mycobacteria* spp. was detected in the tap water samples ($p < 0.05$, Fig. 5C), especially in summer (Fig. S6C). The detection frequencies of *Legionella* spp. in the tank and tap water samples (35% and 21%, respectively) were higher than that of the input water (9%) with comparable gene copy numbers (Fig. 5D). Specifically, the gene copy numbers of *Legionella* spp. in input, tank, and tap water samples were measured as $10^{0\text{-}3.87}$ gene copies/100 mL, $10^{0\text{-}6.71}$ gene copies/100 mL, and $10^{0\text{-}4.36}$ gene copies/100 mL, respectively. It should be noted that the closed valve tank water samples presented a high abundance of *Legionella* with $10^{2.84\text{-}6.71}$ gene copies/100 mL. *L. pneumophila* with low abundance ($10^{1.99}$ gene copies/100 mL) was only detected in one tank water sample. In addition, the water samples yielded low detection frequencies of *Enterococcus* spp., *Acanthamoeba* spp., *H. vermiformis, Salmonella* spp., *Staphylococcus aureus*, and *Aeromonas hydrophilia* (Fig. 5D). *Enterococcus* ($10^{1.96\text{-}3.43}$ gene copies/100 mL), *Acanthamoeba* ($10^{1.91\text{-}2.38}$ gene copies/100 mL), and *H. vermiformis* ($10^{3.43\text{-}4.10}$ gene copies/100 mL) were only detected in tank water samples. On the other hand, *Salmonella, Staphylococcus aureus*, and *Aeromonas hydrophilia* presented low gene copy numbers ($10^{1.08\text{-}3.38}$ gene copies/100 mL) in tap water samples.

3.5. Microbial community structure in SWSSs

A total of 805,000 high-quality Illumina sequences of the bacterial 16S rRNA gene were obtained from 115 water samples, assigned to 29 phyla and 604 genera. Proteobacteria was the most dominant phylum in the majority of water samples (relative abundance: 28%-99.9%), followed by Bacteroidetes (0-70%), Actinobacteria (0-37%), and Firmicutes (0-13%), according to their overall abundance in pooled water samples (Fig. S7). At the genus level, season-dependent microbial community composition was observed (Fig. 6). *Phreatobacter* was the dominant genus in water samples collected in autumn, winter, and

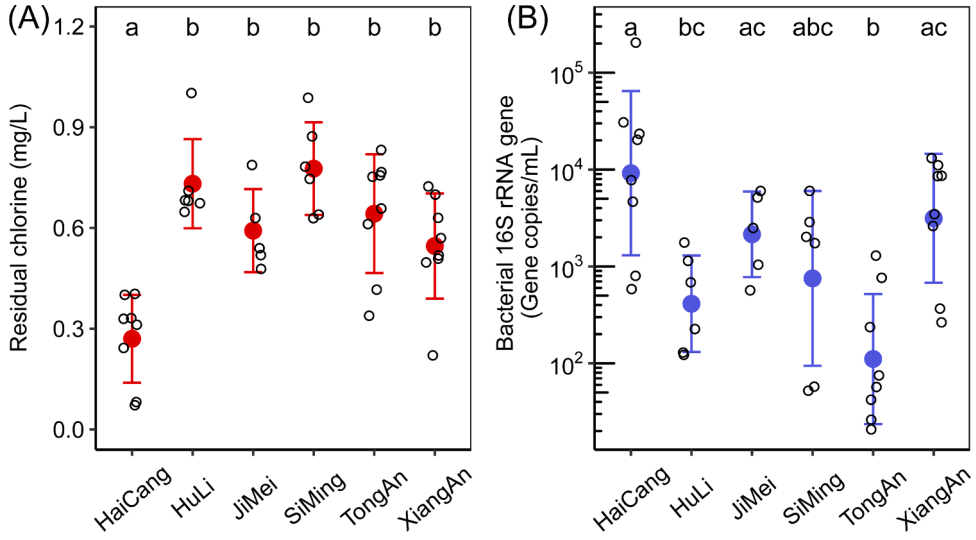

Fig. 3. Boxplot of A) residual chlorine and B) bacterial 16S rRNA gene abundance in the collected input water samples of the six districts. a, b, and c represent conditions with statistically significant differences ($p < 0.05$).

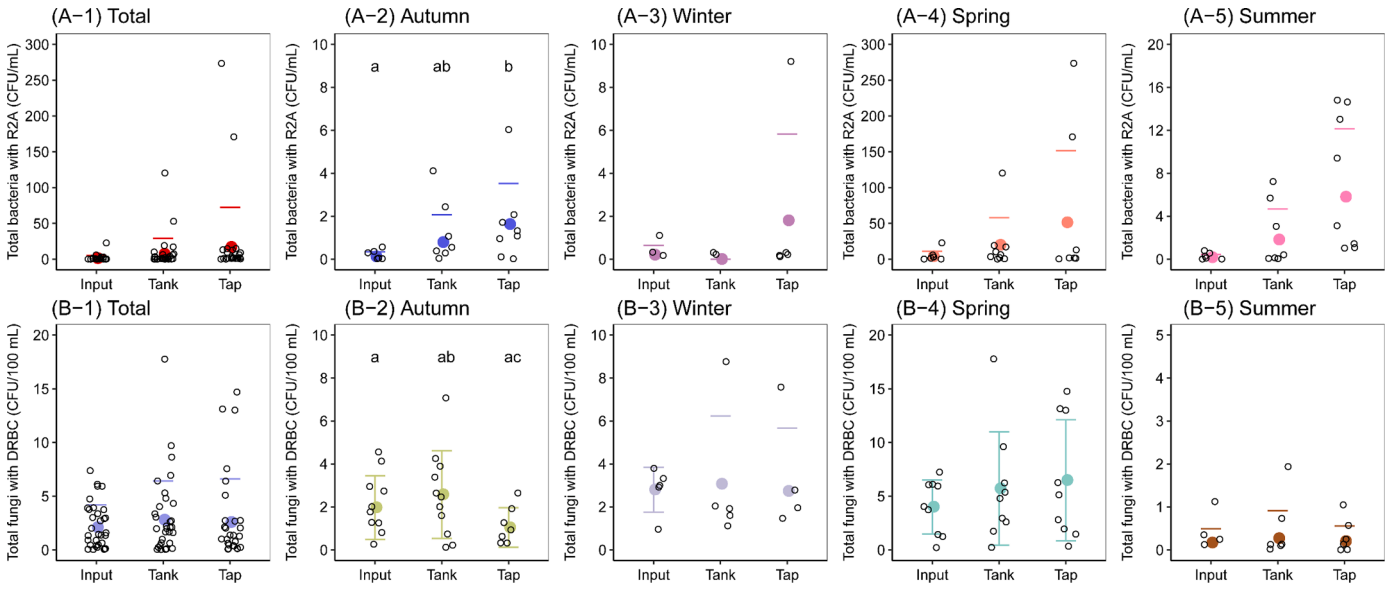

Fig. 4. Boxplot of the total cultural A) bacterial counts and B) fungal counts in the collected total and seasonal water samples. a, b, and c represent conditions with statistically significant differences ($p < 0.05$).

spring. However, *Pseudomonas* and *Rhizobiales*_unclassified became the dominant groups in summer water samples. *Ralstonia* with high relative abundance was concentrated in some water samples collected in autumn.

In general, a clear separation was observed by sampling season for the water samples in NMDS analysis (Adonis: $R^2 = 0.231$, $p < 0.01$, Figs. 7A; Adonis: $R^2 = 0.21$, $p > 0.05$, Fig. 7D). This indicated that the total microbial community structure gradually succeeded as the water temperature rose, and the summer water samples presented a dramatic variation in total microbial composition. The NMDS also revealed tight clustering of water samples by the sampling area (Adonis: $R^2 = 0.159$, $p < 0.01$, Fig. 7B). The type of water sample had little effect on its classification (Adonis: $R^2 = 0.032$, $p > 0.05$, Fig. 7C). However, the closed valve tank water samples presented dramatic community shifts among the three groups of water samples (Adonis: $R^2 = 0.259$, $p < 0.01$, Fig. 7E). This phenomenon suggested that the extremely low chlorine residuals could reshape the unique microbial community composition in SWSSs. In addition, the materials of the storage tank did not affect the microbial variation (Adonis: $R^2 = 0.024$, $p > 0.05$, Fig. 7F).

3.6. Correlation between physicochemical water quality parameters and microbiomes

Correlations between the physicochemical water quality parameters and the abundance of target microorganisms were analyzed by utilizing a nonparametric Spearman rank correlation approach. Residual chlorine displayed negative associations with all target microorganisms (total water samples: r = -0.357 to -0.676, $p < 0.01$) (Table 1). In addition, a high positive correlation was noted between turbidity and total bacteria (r = 0.500, $p < 0.01$) and *Mycobacterium* spp. (r = 0.521, $p < 0.01$) in tank water samples (Table S4).

RDA was performed to analyze the influence of physicochemical water quality parameters on microbial community structure at the OTU level, in which RDA1 and RDA2 explained 25.98% of the total variation (Fig. 8). Temperature was the most significant explanatory variable for the variation in microbial community structure, with a maximum interpretation ratio of 26.92%, followed by SO_4^{2-} (19.08%), DO (12.75%), and residual chlorine (13.15%) (Table 2). pH (3.02%) and turbidity (2.66%) exhibited the lowest influence on the variation in microbial community structure.

4. Discussion

4.1. Occurrence of microbial pathogens in SWSSs

It has been reported that the opportunistic pathogens were ubiquitously existed in SWSSs, and the occurrence pattern of opportunistic pathogens detected in this study was similar to previous studies (Li et al. 2018a, Lu et al. 2015). All studies have reported a high abundance of *Mycobacteria* spp. and *Legionella* spp. and a relatively lower abundance of *Acanthamoeba* and *Aeromonas hydrophilia*. In addition, a high level of pathogenic fungi and relatively low enteric pathogens (i.e., *Enterococcus* spp. and *Salmonella* spp.) were detected in this study (Fig. 5).

The high abundance of fungal 28S rRNA genes was consistent with the results of previous studies investigating fungi in drinking water (van der Wielen and van der Kooij 2013) and concrete tile tank water (Novak Babič and Gunde-Cimerman 2021). This might be due to their high resistance to chlorine (Pereira et al. 2013) and the presence of fungal hyphae with large DNA copies. Novak Babič and Gunde-Cimerman (2021) also found that diverse fungal isolates from a concrete tile tank could produce acids, which threatened the tank material. In addition, the primers used in this study targeted several pathogenic fungal genera (Vollmer et al. 2008). Therefore, further scrutiny of pathogenic fungal species is needed to clarify the exposure risk in local drinking water systems. Attention should also be paid to the disinfection of fungi at SWSSs.

The ubiquitous presence of *Mycobacteria* in SWSSs might be associated with its characteristics of biofilm formation and chlorine resistance (Vaerewijck et al. 2005). A high abundance of *Mycobacteria* has also been found in SWSSs around the world (Li et al. 2018a, Lu et al. 2015). Lin et al. (2019) reported that *M. intracellulare, M. abscessus, M. avium, M. kansasii, M. massiliense*, and *M. fortuitum* were the most frequent clinical species detected in Fujian. High-frequency detection of *Legionella* spp. and relatively low levels of *L. pneumophila* were consistent with the results of previous studies investigating *Legionella* populations in SWSSs (Li et al. 2018a) and drinking water distribution systems (Lu et al. 2016). Furthermore, diverse *Legionella* species were isolated from a drinking water distribution system in Spain (Salinas et al. 2021). *L. pneumophila* is the most important clinical species due to its pathogenicity in Legionnaires' disease (LeChevallier 2019). Despite its relatively low proportion, more attention should be given to *L. pneumophila*. Therefore, the presence of *Mycobacteria* and *Legionella* in local SWSSs

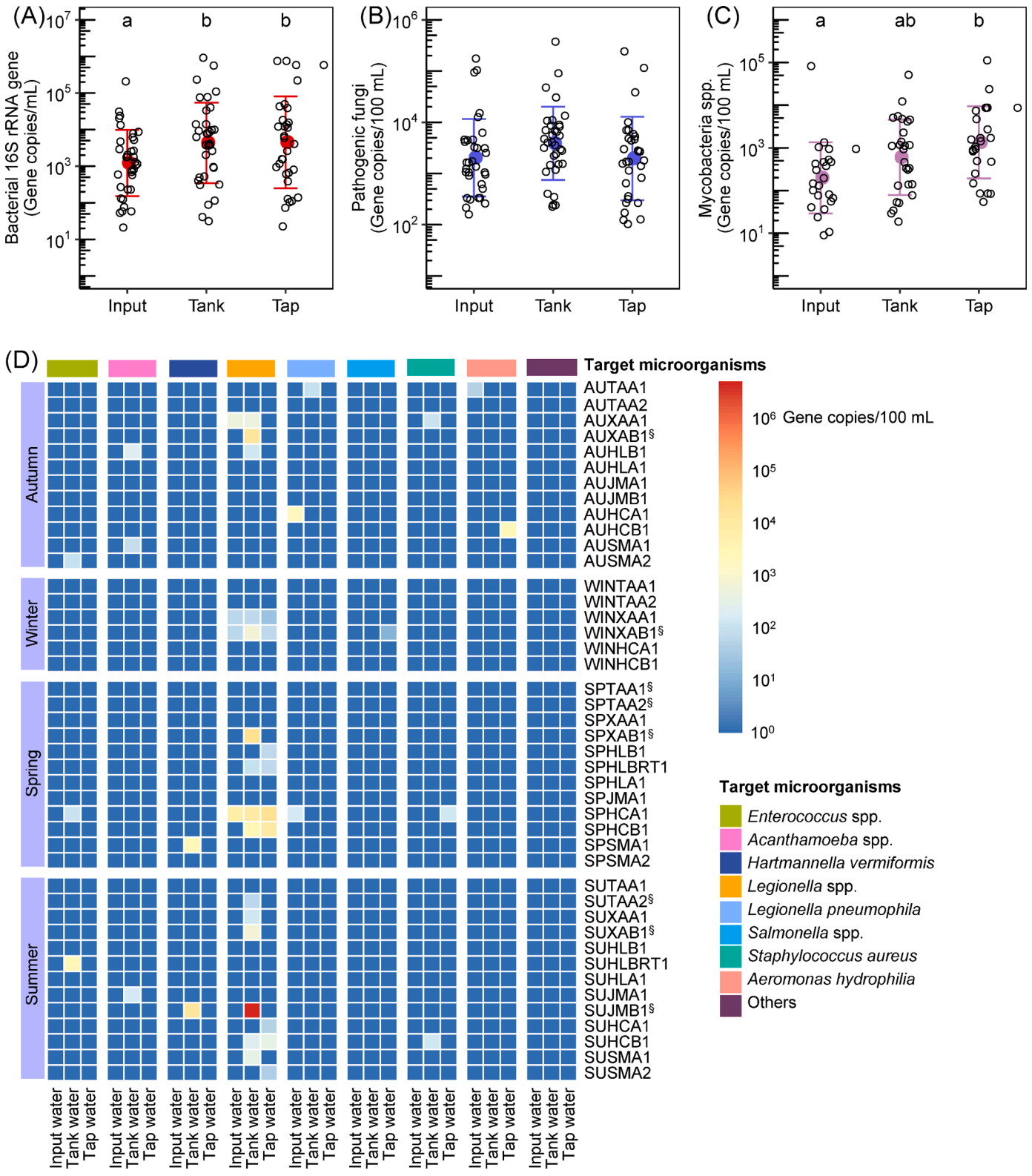

Fig. 5. Absolute abundance of the gene markers of A) bacteria, B) pathogenic fungi, C) *Mycobacteria* spp., and D) other potential pathogens in the collected water samples. Water samples were collected from 6 administrative districts with XiangAn (XA), TongAn (TA), JiMei (JM), HaiCang (HC), HuLi (HL), and SiMing (SM) of Xiamen. Two kinds of water tanks were selected, including stainless steel water tank (A) and concrete tile water tank (B). RT, roof tanks. §, one water valve of the storage tanks was closed. a and b represent conditions with statistically significant differences ($p < 0.05$).

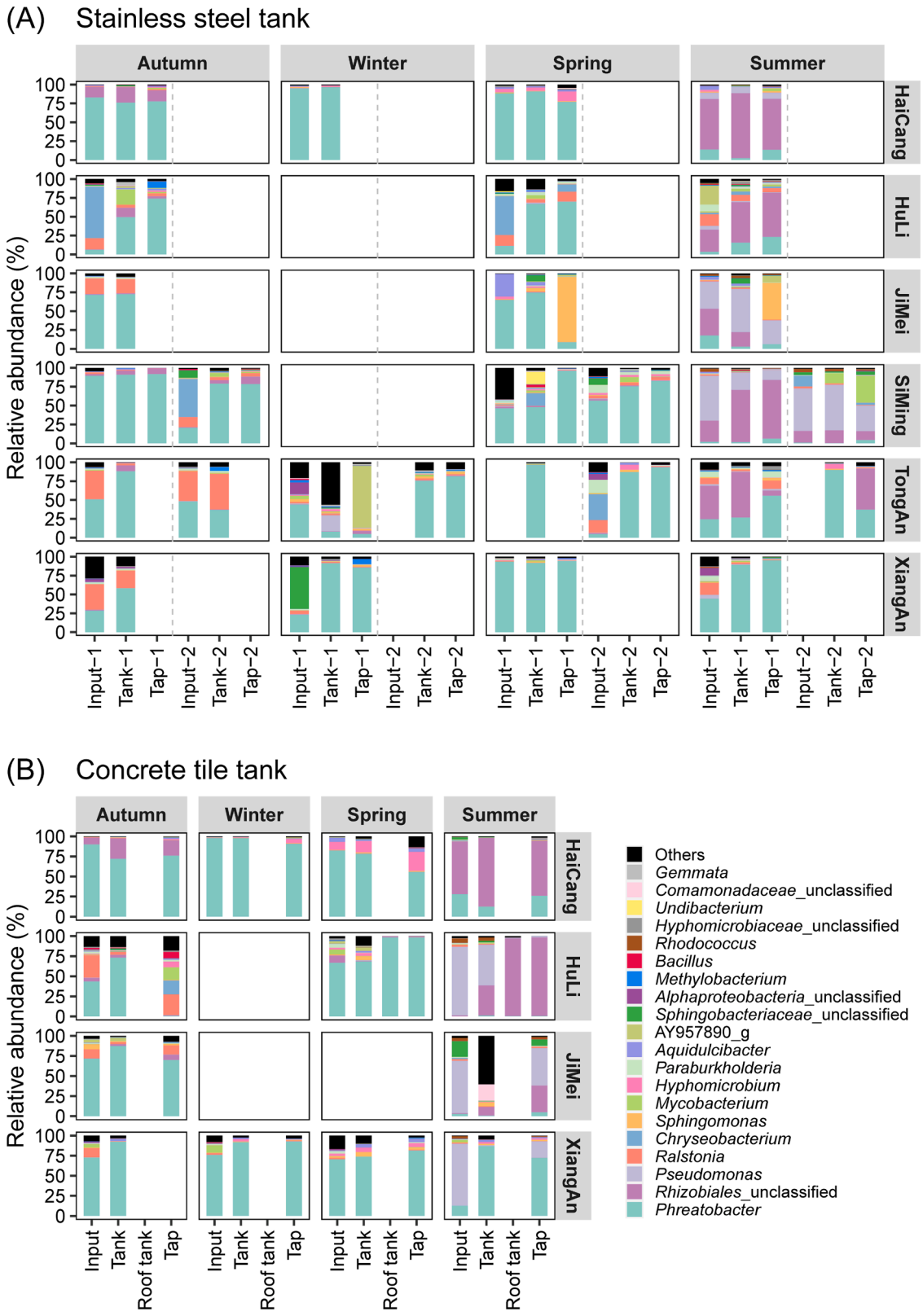

Fig. 6. Relative abundance of bacterial community at genus level observed across the investigation.

can be a potential threat to public health and should be examined thoroughly with complementary techniques, such as selective media, to identify the specific exposure risks of microbial species.

Acanthamoeba is known to produce a rare but serious infection, Acanthamoeba keratitis (Anger and Lally 2008). *H. vermiformis* was one of the most representative genera in drinking water networks (Delafont et al. 2013). It is well known that free-living amoebae, such as *Acanthamoeba* and *H. vermiformis*, can serve as hosts of *Legionella* and *Mycobacteria* (Delafont et al. 2014, Thomas et al. 2014). A high correlation between quantities of *Legionella* and *Acanthamoeba* has been

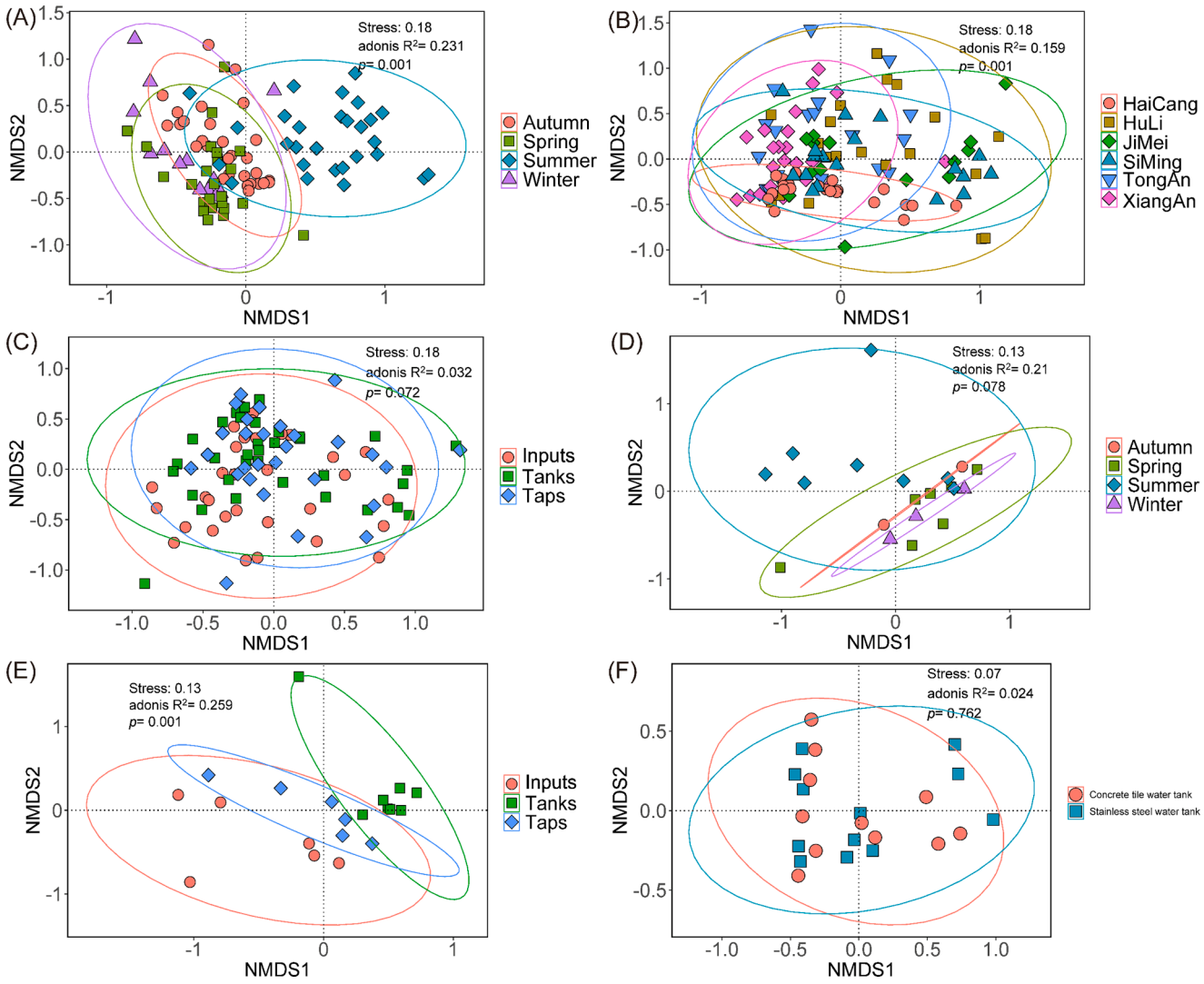

Fig. 7. Non-metric multidimensional scaling analysis (NMDS) of drinking water microbial communities inflected by A) seasonal factors, B) sampling sites, C) water types, D) seasonal factors with closed valve, E) water types with closed valve, and F) storage tanks materials derived from Illumina sequencing.

Table 1
Correlations between target microorganisms and water quality parameters in the collected water samples

Physiochemical parameters	Bacterial 16S rRNA gene	Pathogenic fungi	*Mycobacterium* spp.	*Legionella* spp.
pH	0.279 **	0.23 *	0.327 **	0.102
DO	-0.223 *	0.078	-0.209 *	0.008
Temperature	0.288 **	-0.039	0.310 **	0.044
Residual chlorine	**-0.676** **	-0.357 **	**-0.541** **	-0.442 **
Turbidity	0.365 **	0.356 **	0.320 **	0.218 *
NO_3^-	0.102	-0.284 **	0.091	0.007
Total nitrogen	0.124	-0.234 **	0.122	0.036
SO_4^{2-}	0.159	-0.286 **	0.153	-0.149
TOC	0.151	0.027	0.109	-0.036

Note: Correlations were performed using the nonparametric Spearman rank correlation approach. *, $p < 0.05$; **, $p < 0.01$.
The sample number was 121.

found in SWSS (Lu et al. 2015). However, it seems that there was no positive correlation between these two pathogens in this study (Fig. 5D). It is known that a large volume of water (100 L) is recommended for the detection of protozoans, such as *Cryptosporidium* and *Giardia*, in the *standard examination methods for drinking water* (GB/T 5750-2006).

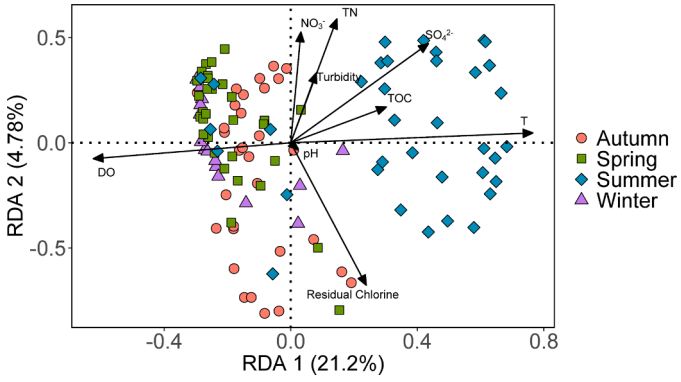

Fig. 8. Redundancy analysis (RDA) constructed to assess the influence of environmental factors on microbial community structure at the OTU level.

Therefore, the low frequency of amoebae species might be partially due to the small sampling volume used in this study.

Table 2
Proportions of total bacterial community structure variation explained by individual environmental factors

	Explained percent (%)	R^2
T	26.92	0.0821
SO_4^{2-}	19.08	0.0582
DO	12.75	0.0389
Residual chlorine	13.15	0.0401
NO_3^-	12.82	0.0391
TN	5.90	0.0180
TOC	3.70	0.0113
pH	3.02	0.0092
Turbidity	2.66	0.0081

4.2. Increased microbial risks in SWSSs

Water storage tanks represent an ideal place for microbial growth given their unique characteristic of low chlorine residuals (Li et al. 2018a, Lu et al. 2015). Higher abundances of the total bacterial 16S rRNA gene and *Mycobacteria* spp. were detected in storage tank and tap water samples than in input water samples in this study (Fig. 5), which was in agreement with the results from Shanghai's residential buildings by Li et al. (2018a). In addition, gene markers of *Acanthamoeba, Enterococcus* spp., *H. vermiformis, Legionella* spp. and *S. aureus* were detected only in tank and tap water samples, rather than input water samples, indicating that deteriorated water gave microbial risks of pathogenic microorganisms in SWSSs. More seriously, a higher abundance and frequency of *Legionella* spp. were detected in the tank and tap water samples than in the input water samples, and similar results were found for bulk water by Li et al. (2018a) and sediments by Lu et al. (2015) in SWSSs, which indicates that SWSSs might be a non-ignorable reservoirs for *Legionella* spp. It is well known that some species (e.g., *L. pneumophila*) of *Legionella* spp. are pathogens with a high capability to cause infection, so high attention must be paid to their extremely high detection. Together, the results indicated that consumers who use the tap waters served by the SWSSs in this study might be exposed to higher microbial risk.

4.3. Dominant bacterial groups in SWSSs

The microbial compositions in this study were similar to those in a previous study in SWSSs, in which Proteobacteria and Bacteroidetes were the dominant microbial groups (Li et al. 2018a), and the relative abundance of the genera detected in this study exhibited distinct seasonal changes at the genus level (Fig. 6). *Phreatobacter* was the main genus in most of the water samples and occupies the dominant position in drinking water across the world (Perrin et al. 2019, Stanish et al. 2016). It is not surprising that a high abundance of *Phreatobacter* was observed since the concentration of residual chlorine in this study ranged from 0.01 to 1.00 mg/L, as Stanish et al. (2016) found that this species was more abundant in low chlorine (< 1 mg/L), which was also confirmed by Perrin et al. (2019). The presented high abundance of *Rhizobiales*_unclassified in the summer might be due to warm water (Wang et al. 2018). Zhang et al. (2021a) found that the average number of OTUs assigned to *Pseudomonas* was significantly higher in stagnate water in indoor pipes, but we could not explain the large percentages of *Pseudomonas* present in summer water samples. In addition, it is still unclear why *Ralstonia* presented high concentrations, and as a suggestion, further studies regarding the genera *Pseudomonas* and *Ralstonia* in SWSSs are recommended due to their high infection levels (Ribas et al. 2000, Ryan and Adley 2014).

4.4. Dominant water quality factors influencing microbial biomass

Turbidity and residual chlorine were water quality parameters that were closely related to microbial biomass in this study. High correlation between turbidity and the total bacterial biomass was only observed in the tank water samples (Table S4), while residual chlorine had a high correlation with the total bacterial biomass overall (Table 1).

In addition, turbidity and residual chlorine were the two important water quality parameters that presented a significant difference between the three groups of water samples in this study (Fig. 2). A significant increase in turbidity in tank water samples might be associated with aging and corrosion of the inner walls (Zhang et al. 2021b) and the local scaling caused by organic acids secreted by bacteria and fungi (Novak Babič and Gunde-Cimerman 2021). Chlorine residuals were the most important factor in inhibiting bacterial regrowth in drinking water, but it is known that maintaining the stability of chlorine residuals in SWSSs is challenging due to water stagnation (Evison and Sunna 2001, Li et al. 2018a, Miyagi et al. 2017).

A high correlation between the change ratio of residual chlorine and bacterial 16S rRNA gene abundance of water samples from inputs to tanks and taps was observed (Fig. S3). Although the change ratio of turbidity presented a low correlation with bacterial 16S rRNA gene abundance, a high correlation between turbidity and the total bacterial biomass was observed in the tank water samples (Table S4). Roos et al. (2017) also reported that acute gastrointestinal illness was strongly correlated with high turbidity. Thus, we set a change ratio of 20% of turbidity and residual chlorine. When the magnitude of the changes exceeds 20%, there might be a greater microbial risk. Unfortunately, at least half of the tank and tap water samples compared with input water samples had changes in turbidity and residual chlorine values by more than 20% in the present study (Table S3). Moreover, the samples with residual chlorine that did not meet the drinking water standard (0.05 mg/L, GB 5749-2006) were mainly collected from storage tanks (Table S2), especially closed valve storage tanks. Therefore, a reliable method to control microbial risk is tuning residual chlorine in the SWSSs. In addition, a supplemental disinfection device, such as UV, can be employed to further reduce the microbial risk in SWSSs (Li et al. 2018b).

4.5. Effect of environmental factors on microbial community structure

In this study, NMDS and RDA illustrated that the season (i.e., water temperature) and sampling area (i.e., the city in which the system is located) were the dominant factors affecting the microbial community structure. The overarching influence of season on microbial communities could likely be ascribed to the water temperature changes over a year (Fig. 7A), as the temperature had the largest interpretation ratio for the variation in microbial community structure (Table 2). In addition, significant microbial regrowth and a reduction in residual chlorine from input water samples to tank and tap water samples were observed in summer (Figs. S2E and S6A), which indicated that the water temperature was one of the most important factors driving the changes in microbiomes. Multiple water sources jointly guarantee the water demand of Xiamen, and Han et al. (2020) found that source water contributed a huge proportion (50% ± 30%) to shaping the tap water microbial community structure. In addition, the interactions between water chemistry, pipe materials, and other factors in the large and complex water distribution system induced significant differences in chlorine residuals among the districts (Fig. 3A) and further shaped the districts' microbiome (Li et al. 2018a, Wang et al. 2015).

Although chlorine disinfectant was not the most important factor that shaped the microbial community structure, a dramatic community shift was observed in the closed valve tank water samples (Fig. 7E), which might be due to the regrowth of some microbial groups under extremely low chlorine conditions. Since a high negative correlation was noted between chlorine disinfectant and bacterial 16S rRNA gene abundance in this study (Table 1), the changes in the microbial community structure and the inactivation of microorganisms caused by chlorine disinfectant cannot be ignored (Martin et al. 2020, Pereira et al. 2013). Therefore, from the perspective of the SWSSs themselves,

residual chlorine was also an important factor inducing the changes in microbial community structure.

4.6. Effect of SWSS operation on the bacterial community

A special secondary water supply mode, double tanks with one tank valve closed, was noted in this study (Fig. 1B4). After closing the water valve of the storage tanks, the bulk water was mainly supplied to the taps through the open valve storage tank, and therefore, the water in the closed valve storage tank might stagnate for a couple of days or even weeks. Serious microbial contamination (e.g., *Legionella* spp.) and significantly low chlorine residuals were observed in this type of tank water sample ($p < 0.05$, Figs. 5D and S4). More importantly, an obvious microbial community structure was present in different water groups in the residential neighborhoods with closed valve tank (Fig. 7E) than in the overall sample sites (Fig. 7C), as evidenced by the Adonis analysis ($p < 0.01$ *vs.* $p > 0.05$). The results illustrated that unreasonable operation of SWSSs could cause serious deterioration of water quality compared with the normal water supply mode, and maintaining the fluidity of the water in the storage tank was important to reduce microbial risk.

Tank materials can strongly influence microbial communities, and Evison and Sunna (2001) found a higher similarity of microbial taxa between water samples from two cast-iron water tanks compared with fiberglass and polyethylene tanks. Among the two kinds of storage tanks located in HaiCang district selected for comparison in this study, no significant differences in residual chlorine, turbidity, microbial biomass, or microbial community structure were observed (Figs. S5 and 7F). This might be due to the bulk water that can be continuously renewed and maintained at the same level of chlorine residuals, and therefore, the microbial regrowth in the selected two storage tanks was not serious and presented only a small difference.

5. Conclusions

This is the first comprehensive investigation of seasonal and spatial microbiomes in SWSSs to reveal the microbial risks over one year in a developed region. The microbial risks were determined by culture-dependent and culture-independent methods, which makes our results more comprehensive, precise, and reliable. We are convinced that the following conclusions have guiding significance for the biosafety control of urban drinking water.

1) On the whole, the water quality in SWSSs could meet the requirements of drinking water standards, including microbial parameters. However, the microbial risk was actually increased based on the detection of specific and non-regulated microorganisms via PMA-qPCR.
2) The detection of diverse pathogens illustrated that the local SWSSs were important reservoirs for viable opportunistic and enteric pathogens. A significant increase in the abundance of the bacterial 16S rRNA gene and Legionella was observed in SWSSs. Together with the detection of Enterococcus, Acanthamoeba, and H. vermiformis primarily in storage tanks, these results highlighted the promoting role of SWSSs in microbial regrowth and colonization of potential pathogens. In particular, the microbial regrowth potential was more obvious in summer, which needs to receive more attention.
3) In view of the very high detected abundance and frequency of Legionella spp. in SWSSs, Legionella spp. was recommended as a supplementary indicator to evaluate the microbial safety of the local SWSSs.
4) Low chlorine residuals were the dominant factor driving microbial regrowth and microbial community shifts in SWSSs. In addition, we should pay attention to the changes in the microbial community caused by regional differences and the increase in water temperature and turbidity.
5) Low chlorine residuals, dramatic changes in microbial community structure, and the high abundance of total bacteria and Legionella in the closed valve tank water samples highlighted that mishandled operation of SWSSs could increase microbial risk.

CRediT authorship contribution statement

Dong Hu: Conceptualization, Methodology, Investigation, Writing – original draft, Visualization. **Huarong Hong:** Conceptualization, Methodology, Investigation. **Biao Rong:** Methodology, Investigation, Supervision. **Yating Wei:** Methodology, Investigation. **Jie Zeng:** Methodology, Writing – review & editing. **Jun Zhu:** Methodology, Visualization. **Lijun Bai:** Methodology. **Feng Guo:** Methodology. **Xin Yu:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that there was no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2021.117690.

References

Anger, C., Lally, J.M., 2008. *Acanthamoeba*: A review of its potential to cause Keratitis, current lens care solution disinfection standards and methodologies, and strategies to reduce patient risk. Eye Contact Lens 34 (5), 247–253.
Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30 (15), 2114–2120.
Delafont, V., Brouke, A., Bouchon, D., Moulin, L., Héchard, Y., 2013. Microbiome of free-living amoebae isolated from drinking water. Water Res. 47 (19), 6958–6965.
Delafont, V., Mougari, F., Cambau, E., Joyeux, M., Bouchon, D., Héchard, Y., Moulin, L., 2014. First evidence of *Amoebae–Mycobacteria* association in drinking water network. Environ. Sci. Technol. 48 (20), 11872–11882.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26 (19), 2460–2461.
Edgar, R.C., 2016. UCHIME2: improved chimera prediction for amplicon sequencing. BioRxiv, 074252.
Evison, L., Sunna, N., 2001. Microbial regrowth in household water storage tanks. J. Am. Water Works Ass. 93 (9), 85–94.
Guo, L., Wan, K., Zhu, J., Ye, C., Chabi, K., Yu, X., 2021. Detection and distribution of vbnc/viable pathogenic bacteria in full-scale drinking water treatment plants. J. Hazard. Mater. 406, 124335.
Han, Z., An, W., Yang, M., Zhang, Y., 2020. Assessing the impact of source water on tap water bacterial communities in 46 drinking water supply systems in China. Water Res. 172, 115469.
Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miSeq illumina sequencing platform. Appl. Environ. Microbiol. 79 (17), 5112–5120.
LeChevallier, M.W., 2019. Occurrence of culturable *Legionella pneumophila* in drinking water distribution systems. AWWA Water Sci. 1 (3), e1139.
Li, H., Li, S., Tang, W., Yang, Y., Zhao, J., Xia, S., Zhang, W., Wang, H., 2018a. Influence of secondary water supply systems on microbial community structure and opportunistic pathogen gene markers. Water Res. 136, 160–168.
Li, W., Li, M., Wen, D., Qiang, Z., 2018b. Development of economical-running strategy for multi-lamp UV disinfection reactors in secondary water supply systems with computational fluid dynamics simulations. Chem. Eng. J. 343, 317–323.
Lin, S., Wei, S., Zhao, Y., Lin, J., Pang, Y., 2019. Epidemiology of human pulmonary infection with nontuberculous *Mycobacteria* in southeast China: A prospective surveillance study. Infect. Drug. Resist. 12, 3515–3521.

Lu, J., Struewing, I., Vereen, E., Kirby, A.E., Levy, K., Moe, C., Ashbolt, N., 2016. Molecular detection of *Legionella* spp. and their associations with *Mycobacterium* spp., *Pseudomonas aeruginosa* and amoeba hosts in a drinking water distribution system. J. Appl. Microbiol. 120 (2), 509–521.

Lu, J., Struewing, I., Yelton, S., Ashbolt, N., 2015. Molecular survey of occurrence and quantity of *Legionella* spp., *Mycobacterium* spp., *Pseudomonas aeruginosa* and amoeba hosts in municipal drinking water storage tank sediments. J. Appl. Microbiol. 119 (1), 278–288.

Martin, R.L., Harrison, K., Proctor, C.R., Martin, A., Williams, K., Pruden, A., Edwards, M. A., 2020. Chlorine disinfection of *Legionella* spp., *L. pneumophila*, and *Acanthamoeba* under warm water premise plumbing conditions. Microorganisms 8 (9), 1452.

Miyagi, K., Sano, K., Hirai, I., 2017. Sanitary evaluation of domestic water supply facilities with storage tanks and detection of *Aeromonas*, enteric and related bacteria in domestic water facilities in Okinawa Prefecture of Japan. Water Res. 119, 171–177.

Novak Babič, M., Gunde-Cimerman, N., 2021. Water-transmitted fungi are involved in degradation of concrete drinking water storage tanks. Microorganisms 9 (1), 160.

Pereira, V.J., Marques, R., Marques, M., Benoliel, M.J., Barreto Crespo, M.T., 2013. Free chlorine inactivation of fungi in drinking water sources. Water Res. 47 (2), 517–523.

Perrin, Y., Bouchon, D., Delafont, V., Moulin, L., Héchard, Y., 2019. Microbiome of drinking water: A full-scale spatio-temporal study to monitor water quality in the Paris distribution system. Water Res. 149, 375–385.

Ramamurthy, T., Ghosh, A., Pazhani, G.P., Shinoda, S., 2014. Current perspectives on viable but non-culturable (VBNC) pathogenic bacteria. Front. Public Health 2 (103), 1–9.

Ribas, F., Perramon, J., Terradillos, A., Frias, J., Lucena, F., 2000. The *Pseudomonas* group as an indicator of potential regrowth in water distribution systems. J. Appl. Microbiol. 88 (4), 704–710.

Roos, A.J.D., Gurian, P.L., Robinson, L.F., Rai, A., Zakeri, I., Kondo, M.C., 2017. Review of epidemiological studies of drinking-water turbidity in relation to acute gastrointestinal illness. Environ. Health. Persp. 125 (8), 086003.

Ryan, M.P., Adley, C.C., 2014. *Ralstonia* spp.: emerging global opportunistic pathogens. Eur. J. Clin. Microbiol. Infect. Dis. 33 (3), 291–304.

Salinas, M.B., Fenoy, S., Magnet, A., Vaccaro, L., Gomes, T.D.S., Hurtado, C., Ollero, D., Valdivieso, E., del Águila, C., Pozuelo, M.J., Izquierdo, F., 2021. Are pathogenic *Legionella* non-pneumophila a common bacteria in water distribution networks? Water Res. 196, 117013.

Song, W., Zheng, M.J., Li, H., Zheng, W., Guo, F., 2019. Profiling population-level diversity and dynamics of *Accumulibacter* via high throughput sequencing of ppk1. Appl. Microbiol. Biotechnol. 103 (23-24), 9711–9722.

Stanish, L.F., Hull, N.M., Robertson, C.E., Harris, J.K., Stevens, M.J., Spear, J.R., Pace, N. R., 2016. Factors influencing bacterial diversity and community composition in municipal drinking waters in the Ohio river basin. USA PLos One. 11 (6), e0157966.

The Ministry of Health of the P.R. China, 2006. Standard Examination Methods for Drinking Water – Collection and Preservation of Water Samples (GB/T5750.2-2006). China Standard Press, Beijing, China (In Chinese).

Thomas, J.M., Thomas, T., Stuetz, R.M., Ashbolt, N.J., 2014. Your garden hose: A potential health risk due to *Legionella* spp. growth facilitated by free-living *Amoebae*. Environ. Sci. Technol. 48 (17), 10456–10464.

Vaerewijck, M.J.M., Huys, G., Palomino, J.C., Swings, J., Portaels, F., 2005. *Mycobacteria* in drinking water distribution systems: ecology and significance for human health. FEMS Microbiol. Rev. 29 (5), 911–934.

van der Wielen, P.W., van der Kooij, D., 2013. Nontuberculous *Mycobacteria*, fungi, and opportunistic pathogens in unchlorinated drinking water in the Netherlands. Appl. Environ. Microbiol. 79 (3), 825–834.

Vollmer, T., Störmer, M., Kleesiek, K., Dreier, J., 2008. Evaluation of novel broad-range real-time PCR assay for rapid detection of human pathogenic fungi in various clinical specimens. J. Clin. Microbiol. 46 (6), 1919–1926.

Wang, F., Li, W., Li, Y., Zhang, J., Chen, J., Zhang, W., Wu, X., 2018. Molecular analysis of bacterial community in the tap water with different water ages of a drinking water distribution system. Front. Environ. Sci. Eng. 12 (3), 6.

Wang, H., Masters, S., Falkinham, J.O., Edwards, M.A., Pruden, A., 2015. Distribution system water quality affects responses of opportunistic pathogen gene markers in household water heaters. Environ. Sci. Technol. 49 (14), 8416–8424.

Xiamen Municipal Bureau of Statistics, 2021. Communiqué of the seventh national census of Xiamen. p. 1, Xiamen, China (In Chinese).

Xiamen Water Resources Bureau, 2019. Water resources bulletin of Xiamen. p. 21, Xiamen, China (In Chinese).

Yoon, S.-H., Ha, S.-M., Kwon, S., Lim, J., Kim, Y., Seo, H., Chun, J., 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int. J. Syst. Evol. Microbiol. 67 (5), 1613–1617.

Zhang, H., Xu, L., Huang, T., Liu, X., Miao, Y., Liu, K., Qian, X., 2021a. Indoor heating triggers bacterial ecological links with tap water stagnation during winter: Novel insights into bacterial abundance, community metabolic activity and interactions. Environ. Pollut. 269, 116094.

Zhang, H., Xu, L., Huang, T., Yan, M., Liu, K., Miao, Y., He, H., Li, S., Sekar, R., 2021b. Combined effects of seasonality and stagnation on tap water quality: Changes in chemical parameters, metabolic activity and co-existence in bacterial community. J. Hazard. Mater. 403, 124018.

Ziadat, A.H., 2005. Impact of storage tanks on drinking water quality in Al-Karak province Jordan. J. Appl. Sci. 5 (4), 634–638.