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Introducing bacterial community turnover times to elucidate temporal and spatial hotspots of biological instability in a large Austrian drinking water distribution network

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ABSTRACT

Ensuring biological stability in drinking water distribution systems (DWDSs) is important to reduce the risk of aesthetic, operational and hygienic impairments of the distributed water. Drinking water after treatment often changes in quality during transport due to interactions with pipe-associated biofilms, temperature increases and disinfectant residual decay leading to potential biological instability. To comprehensively assess the potential for biological instability in a large chlorinated DWDS, a tool-box of bacterial biomass and activity parameters was applied, introducing bacterial community turnover times (BaCTT) as a direct, sensitive and easy-to-interpret quantitative parameter based on the combination of ^{3}H-leucine incorporation with bacterial biomass. Using BaCTT, hotspots and periods of bacterial growth and potential biological instability could be identified in the DWDS that is fed by water with high bacterial growth potential. A de-coupling of biomass from activity parameters was observed, suggesting that bacterial biomass parameters depict seasonally fluctuating raw water quality rather than processes related to biological stability of the finished water in the DWDS. BaCTT, on the other hand, were significantly correlated to water age, disinfectant residual, temperature and a seasonal factor, indicating a higher potential of biological instability at more distant sampling sites and later in the year. As demonstrated, BaCTT is suggested as a novel, sensitive and very useful parameter for assessing the biological instability potential. However, additional studies in other DWDSs are needed to investigate the general applicability of BaCTT depending on water source, applied treatment processes, biofilm growth potential on different pipe materials, or size, age and complexity of the DWDS.

1. Introduction

Drinking water is not sterile but represents an oligotrophic environment naturally harbouring complex autochthonous microbial communities (Abkar et al., 2024; Lan et al., 2024). Typical drinking water contains bacterial cell numbers between 10^3 and 10^6 cells mL^{-1} (Prest et al., 2016). Most of these heterotrophic microorganisms are harmless (Kötzsch et al., 2012), while favourable environmental

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Abbreviations: ATP, adenosine triphosphate; Biostability, biological stability; BTN, bacterial total number (determined by epifluorescence microscopy); CFU, colony forming units; DWDSs, drinking water distribution system(s); HNA, high nucleic acid cell count; HNAP, percentage of HNA cells; HPC, heterotrophic plate count; LI, leucine incorporation; LNA, low nucleic acid cell count; TCC, total cell count (determined by flow cytometry); BaCTT, bacterial community turnover time; TVAC, total viable active count (determined by solid phase cytometry).

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conditions and the presence of easily biodegradable organic substances may permit excessive growth of opportunistic bacteria of environmental origin (Adomat et al., 2020; Wingender and Flemming, 2011). This can increase the potential for biological instability of the drinking water and may lead to aesthetic, operational or hygienic problems posing a risk to system integrity or human health (Prest et al., 2016; van der Kooij and van der Wielen, 2013). Aesthetic problems can be the result of various chemical and microbiological processes leading to taste, odour and colour impairments, e.g. by geosmin production. Although not associated with serious health effects, water containing geosmin is perceived as unsafe by consumers due to its earthy and musty odour (Srinivasan and Sorial, 2011). Operational problems comprise material corrosion caused e.g. by microbial production of corrosive metabolites that can affect the integrity of cast iron mains or pipe lining materials (National Health and Medical Research Council, 2011). While most of Europe's drinking water borne disease outbreaks in the past 30 years were associated with external contamination, some outbreaks were attributed to the growth of hygienically relevant microorganisms in biofilms or stagnating water (Prest et al., 2016; Risebro et al., 2007) in the distribution network after treatment. As there are no further treatment steps between incident and consumer, time and opportunity for remediation is restricted, thus underlining the importance to shed more light on biological stability in drinking water distribution systems (DWDSs).

To achieve and sustain biological stability (hereafter abbreviated as "biostability") in a DWDS, water utilities aim to implement adequate preventive measures to control regrowth through suitable water treatment (van der Kooij and van der Wielen, 2013) or disinfection to supply consumers with microbiologically safe and ideally biologically stable drinking water (WHO, 2017). Large DWDSs, having a vast surface area-to-volume ratio, act as biological and physico-chemical reactors changing water quality during transport (Fish et al., 2017): long residence times in storage tanks, changing temperatures and increasing water age in distal parts paralleled with disinfectant residual decay can positively affect bacterial growth kinetics and may lead to biological instability of the distributed water (Nescerecka et al., 2018).

As a comprehensive biostability assessment of (i) aesthetic (by determining a variety of chemical compounds), (ii) operational (via the determination of corrosive bacterial groups) and (iii) human health impairments (by specifically detecting opportunistic pathogens) would be extremely elaborate, microbiological and chemical indicator parameters are usually measured instead. Within European legislation (Directive (EU) 2020/2184, 2020), only heterotrophic plate counts (HPC) and total organic carbon (TOC) are monitored as indicators of biostability, although no legally defined upper limits for tap water exist.

In their systematic approach for a comprehensive assessment of biological stability, Prest et al., 2016 proposed a complementary combination of direct (full-scale measurements to detect actual changes in the DWDS) and predictive laboratory-scale investigations (determination of growth promoting properties): "Direct" methods that have been used as estimates for biological stability within a DWDS are total bacterial cell counts using epifluorescence microscopy (Servais et al., 1992) or flow cytometry (Hammes et al., 2008); the detection of living and active cells using fluorescent substrates (Reynolds et al., 1997; Riepl et al., 2011), the incorporation of radioactively labelled substrates (Servais et al., 1992) or adenosine triphosphate (ATP)-measurements (Hammes et al., 2010b; Nescerecka et al., 2016). Predictive methods comprise laboratory experiments examining the quality and quantity of organic material as the basic prerequisite for bacterial growth (Prest et al., 2016; van der Wielen et al., 2023). Besides nitrogen and phosphorus, assimilable organic carbon (AOC) or biodegradable dissolved organic carbon (BDOC) are usually limiting growth in drinking waters (Park et al., 2021) and have therefore commonly been linked to bacterial growth potential (Lautenschlager et al., 2013; Liu et al., 2015). Although general thresholds for AOC and BDOC have been recommended to sustain biological stability in specific DWDSs, neither of these time-consuming (time to result up to 14 days) and laborious methods (van der Wielen et al., 2023) has yet been incorporated into legislation.

In this study, our main aim was to test the applicability of bacterial community turnover times (BaCTT) as a new cultivation-independent, quantitative and fast parameter to directly indicate biological instability. BaCTT was calculated from bacterial biomass (based on flow cytometric and microscopic measurements) and biomass production (based on ^{3}H-leucine incorporation). Although individual aspects of BaCTT have been used in previous research, e.g. in ecological contexts (van Driezum et al., 2018), it has never been used for assessing biological stability in drinking water. The added value of the present approach lies in the comprehensive investigation of system-specific properties ("*in-situ* determination") without the need for long incubation periods or the use of standardized but allochthonous bacterial test strains, by only using information derived from the system investigated. Results are obtained within two days and are easily interpretable. BaCTT was applied to identify spatiotemporal patterns of biological instability in a full-scale chlorinated DWDS and to predictively assess the importance of chlorine to maintain biological stability of the drinking water in the DWDS. BaCTT was embedded into a comprehensive set of microbiological and environmental variables to elucidate the main factors driving bacterial dynamics and impacting biostability in the system.

2. Material and methods

2.1. Sampling

The study area was a large chlorinated DWDS in Austria with approximately 3000 km of public pipings, mainly fed by karstic springs. Thin or absent soil covers in karstic spring catchment areas in combination with extended rock cavities prevent effective natural purification of the infiltrating water after precipitation events (Kresic and Bonacci, 2010). The direct hydrological response of the springs leads to a highly dynamic bacterial community depending on discharge (Savio et al., 2018) and to natural fluctuations of raw water quality. From the karstic springs, the raw water travels roughly 200 km to the treatment works with 300,000 m^3 total capacity. A sampling site each was located before (PW01) and after disinfection (PW03) with chlorine gas and chlorine dioxide (final free chlorine concentration $\leq$0.3 mg L^{-1}). In this work, the term "raw water" was used for water before disinfection which is not suitable for human consumption, as opposed to "drinking water". For predictive storage experiments, water from PW01 and PW03 were sampled at five occasions from May to October 2017 in carbon-free 5 L glass bottles. In an additional replicate, residual chlorine was quenched with 5 mL 10 % sterile-filtered sodium-thiosulphate per 5 L (PW03+Thio). Physico-chemical parameters were determined and cooled samples were transported to the lab.

Within the DWDS, seven sampling sites (PW04, PW05, PW06, PW08, PW09, PW11, PW12) were chosen to best reflect the heterogeneity of the network regarding water age, disinfectant residual and temperature. The seven DWDS sites were sampled from taps at ten occasions from May to November 2017. All taps were flame-treated and flushed to constant temperature prior to sampling. Samples were filled into sterilized and carbon-free 500 mL Borosilicate glass bottles and processed within 12 h. Passing through numerous storage containers that also serve as compensation tanks in times of unbalanced water demand and due to a high degree of branching and complexity within the DWDS, an accurate assessment of water age and residence times was impossible, but the local water supply authorities provided a calculation of water ages based on hydraulic simulation models of the DWDS (Table 1). Bacterial biomass and activity parameters for direct assessment of biostability were determined in the lab for each sample (Fig. 1).

2.2. Physico-chemical on-site parameters

Physico-chemical on-site parameters (water temperature, electrical conductivity, pH and Redox potential) were measured *in-situ* at the time

Table 1
List of sampling sites in the DWDS, their respective calculated water age starting from the entry into the DWDS and average measured temperatures ($n = 10$) over the course of 7 months including standard deviations, values in brackets are min-max values.

Sampling site	Calculated water age [days]	Water temperature [°C]
PW04	0.1	8.66 ± 0.47 (7.7–9.4)
PW05	0.5	11.01 ± 0.83 (9.6–12.4)
PW06	0.75	8.3 ± 0.41 (7.8–8.9)
PW08	1.5	15.93 ± 1.79 (12.9–18.2)
PW09	2.3	7.75 ± 0.48 (7.3–9.1)
PW11	3.5	10.6 ± 1.41 (8.7–13.2)
PW12	3.6	18.5 ± 2.8 (13.6–22.5)

of sample collection using a WTW Multi 3430 and a WTW MultiLine P4 meter (WTW GmbH, Weilheim, Germany). Chlorine concentration was determined using a handheld photometer WTW pHotoFlex STD (N. N-diethyl-1.4-phenylendiamin (DPD) method). Turbidity, TOC and DOC concentrations in the raw water source were determined in real-time using a spectro::lyser V2 probe (s::can, Vienna, Austria). Additional turbidity data were available for the sampling sites during the study period, but these did not match the exact sampling dates.

2.3. Heterotrophic plate counts

HPCs for T0-samples were determined with yeast extract agar plates following EN ISO 6222:1999 using pour plate technique. 1 mL sample was used and plates were incubated at 37 ± 2 °C for 2 days and at 22 ± 2

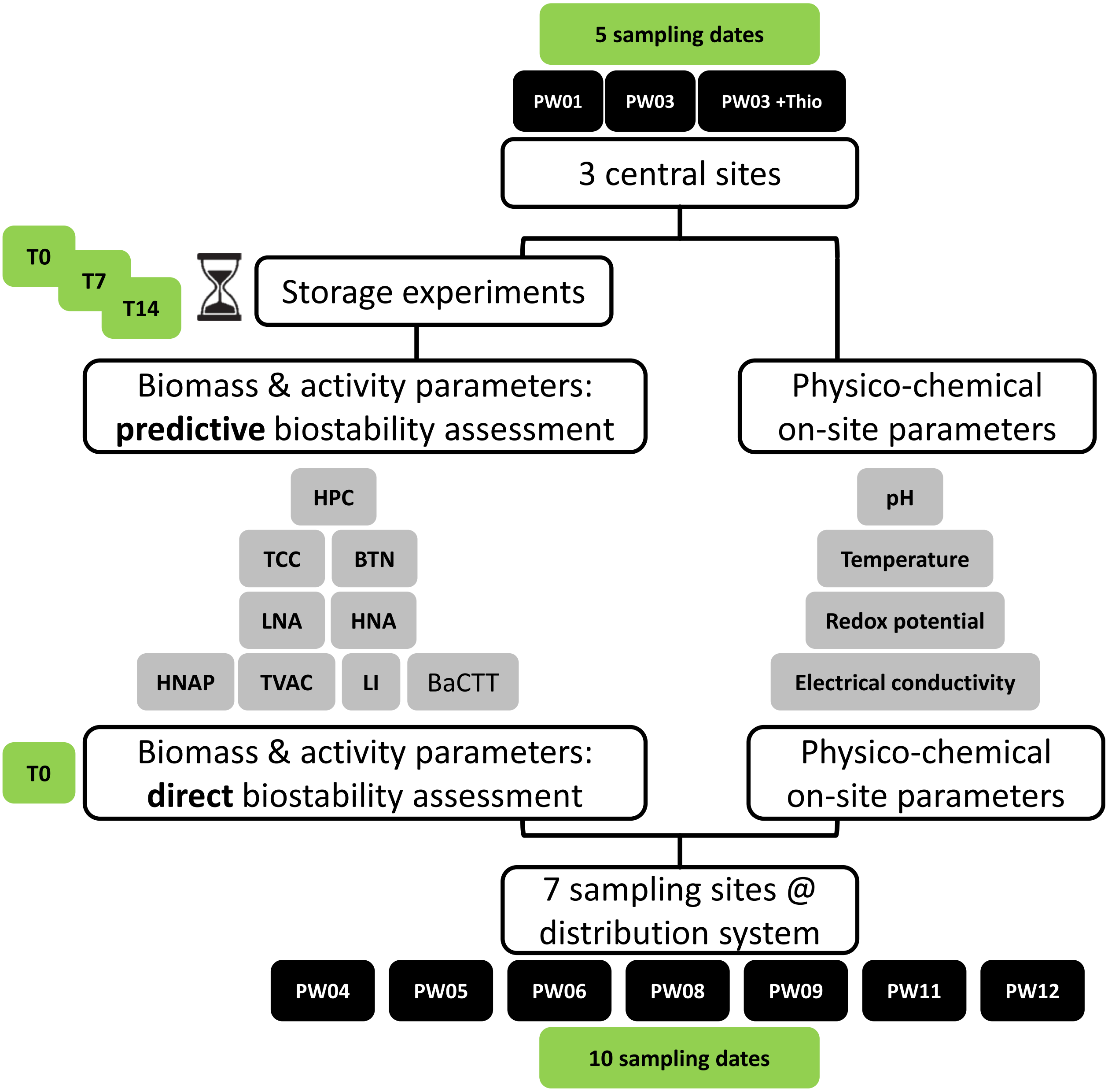


Fig. 1. Sampling scheme of the study. **TCC** = total cell count (determined by flow cytometry); **BTN** = bacterial total number (determined by epifluorescence microscopy); **LNA** = low nucleic acid cell count; **HNA** = high nucleic acid cell count; **LI** = leucine incorporation, **HNAP** = percentage of HNA cells, **TVAC** = total viable active count (determined by solid phase cytometry); **HPC** = heterotrophic plate count; **BaCTT** = bacterial community turnover time; **T0** = analysis without storage; **T7** = analysis after 7 days of storage; **T14** = analysis after 14 days of storage. Physico-chemical on-site parameters were measured directly at the sampling site in the distribution network (PW04–7) and at the central sites before and immediately after disinfection (PW01, PW03, PW03+Thio). Bacterial biomass and activity parameters were analysed in the lab for direct and predictive assessment of biostability.

°C for 3 days.

2.4. Bacterial biomass and activity parameters for direct biostability assessment

2.4.1. Biomass parameters

Two different methods were used for the determination of bacterial cell numbers: all samples were analysed by flow cytometry (total cell counts, TCC), selected samples were also counted by epifluorescence microscopy (bacterial total number, BTN). When comparing the log-transformed data of the two methods, a highly significant correlation ($r = 0.93, p < 0.001, n = 114$, Supplementary Fig. A1) was found. Due to the good comparability to manually counted samples and the high reproducibility of flow cytometry (standard error of <5 %, Hammes and Egli, 2010), only results from flow cytometric measurements are shown in 3.3.1. Microscopy counts were used for visual shape and size determination of the bacterial cells that were subsequently included in bacterial community turnover times (BaCTT) and biomass calculations. TCC were differentiated into cells with high (HNA) and low nucleic acid content (LNA) according to the amount of intracellularly bound DNA dye. TCC were analysed using an Attune® NxT flow cytometer (Thermo Fisher Scientific) equipped with a blue laser (488 nm, 50 mW) after staining with SYBR® Green I (10^{-2} diluted in dimethylsulfoxide; Invitrogen, final working solution 10^{-4}) for 13 min in the dark (Kötzsch et al., 2012). Green fluorescence was collected in the FL1 channel (520 ± 20 nm), and red fluorescence in the FL3 channel (>615 nm). All data were processed with the Attune® NxT software (v2.4) provided by the manufacturer, and electronic gating was used to separate positive signals from noise (Hammes et al., 2008). Cells with high green fluorescence (HNA) clustered in separate populations than LNA cells in FL1 vs. FL3 dot plots.

BTN were determined by filtering 10 mL paraformaldehyde-fixed (final concentration 0.8 %; overnight reaction time at 5 °C ± 3 °C) water sample through 0.22 µm pore-size glass fiber filters (Anodisc 25, Whatman, Germany) and staining the bacteria with SYBR® Gold (Invitrogen, diluted to a final concentration of 1:400 of stock solution) for 13 min in the dark, followed by microscopic counting with an Nikon Eclipse 80i epifluorescence microscope. Cells were classified into large, rod-shaped and coccoid cells with a diameter >1.0 µm and small, coccoid cells with a diameter <1.0 µm (van Driezum et al. 2018).

Bacterial biomass (BBM) values [µg C L^{-1}] were obtained by multiplying TCC [cells mL^{-1}] with an average cellular biomass of carbon per cell. For this work an average cellular carbon content of 15 fg C was calculated from >20.000 visual cell size estimations of 154 samples (Supplementary Table A2). The 154 samples were taken from karstic springs feeding the examined DWDS and showed a high percentage of small coccoid cells.

2.4.2. Activity parameters

Percentage of HNA cells (HNAP) - HNA cells constitute a heterogeneous subgroup and have been described to be responsible for a large fraction of the bulk community activity (Lebaron et al., 2001). As HNA cells have been shown to react sensitively to nutrient inputs and disinfection in drinking water (Besmer et al., 2017; Ramseier et al., 2011), the percentage of HNA cells described as the proportion of highly fluorescent cells measured with flow cytometry can be considered an activity indicator. Ramseier et al. (2011) demonstrated that the membrane permeabilisation rate is smaller for LNA cells than for HNA cells after chlorine and chlorine dioxide disinfection, implying that HNA-cells seem to sustain faster damage than LNA-cells.

Total viable active count (TVAC) - The total number of cells with high intracellular esterase activity was measured via a standardized solid-phase cytometry protocol (TVC bioburden, Biomerieux, France, Riepl et al., 2011; van Driezum et al., 2018): 1 mL sample was filtered through a black, 0.45 µm pore-size polyester CB04 filter and counterstained with 1 mL of CSE/2 (Biomérieux, France). After incubation of $1\,h \pm 5$ min at 37 °C on a ChemSol A4 saturated labelling pad, the filter was transferred to a labelling pad saturated with Chemchrome V6 dye. After incubating for another 30 min at 30 °C the filter was transferred to a membrane holder and immediately enumerated with a solid-phase cytometer (Chemscan RDI; Biomérieux, France) using the Bioburden discrimination settings (Catala et al., 1999). Positive signals detected and identified as viable active cells by the system were validated visually (all signals if $n \leq 100$ or 100 representative signals if $n > 100$). All sample preparation steps were performed under laminar airflow.

Leucine incorporation and bacterial production - Bacterial ^{3}H–leucine incorporation (LI) was measured based on protocols of Kirschner and Velimirov (1999). Briefly, ^{3}H–leucine (4.4 TBq mmol^{-1}, ARC Research Products, USA) was added to triplicate 10 mL samples at a final concentration of 10 nmol L^{-1}. This concentration was proven to be sufficient to saturate leucine uptake and prevent de-novo synthesis of leucine for karstic spring waters (Supplementary Fig. A2). Duplicate control samples were stopped with trichloroacetic acid (TCA, 5 % final conc., Sigma-Aldrich, Germany) directly after the addition of ^{3}H–leucine. Both controls and samples were incubated for 24 h in the dark at respective *in-situ* temperatures. For this period of time, the incorporation of ^{3}H–leucine into the bacterial biomass was linear (Supplementary Fig. A3). After incubation, TCA and 100 µL of 35 % NaCl were added to the samples to enhance protein precipitation for 30 min at room temperature (Kirschner and Velimirov, 1999). After precipitation, the samples were filtered through polycarbonate filters (0.25 mm, 0.2 µm pore-size) and subsequently washed with 5 mL of ice cold 5 % TCA, 80 % ethanol and distilled water for 5 min each for the purification of proteins. Filters were dried overnight in scintillation tubes. After adding 5 mL of scintillation cocktail (Ultima Gold, Perkin Elmer, Austria), radioactive disintegrations per minute were measured in a Perkin Elmer, TriCarb 2300 TR scintillation counter (van Driezum et al., 2018) considering an established standard quench curve.

Bacterial carbon production (BCP) was estimated according to the following equation converting leucine incorporation to biomass production (Simon and Azam, 1989):

$$BCP\ \left[\text{g C L}^{-1}\ \text{h}^{-1}\right] = Leu * M * (\%Leu)^{-1} * \left(\frac{C}{Protein}\right) * ID$$

where leucine incorporation (Leu) is given in mol L^{-1} h^{-1}; M is the molar mass of leucine (131.2 g mol^{-1}); %Leu-is the proportion of leucine in bacterial protein (0.073) that was experimentally determined for aquatic bacteria (Simon and Azam, 1989); C/Protein is the calculated ratio of cellular carbon to protein in bacterial cells (0.86); and ID equals isotope dilution. ID in the present work was 1, because saturation experiments showed that at the applied ^{3}H-leucine concentration of 10 nmol L^{-1} the uptake of radioactive amino-acid was saturated and isotope dilution from intracellular (de-novo synthesis) or extracellular sources was negligible (Supplementary Fig. A2).

Bacterial community turnover time (BaCTT) - The turnover time of the bulk bacterial community [in days] was calculated by dividing the bacterial biomass values (BBM [µg C L^{-1}]) under the assumption of a per-cell carbon-content of 15 fg C by the bacterial carbon production (BCP [µg C L^{-1} day^{-1}]) for each sample (chapter 2.4.1). The values obtained are indicating the number of days in which the total bacterial water community is doubling.

2.5. Predictive biostability assessment (storage experiments)

To assess biostability in a predictive approach (Prest et al., 2016), the growth potential of the natural bacterial communities (corresponds to biomass production potential BPP, van der Wielen et al., 2023) was determined in storage experiments of 14 days ($n = 5$; May–October 2017). Eight replicate 250 mL aliquots of each PW01, PW03 and PW03+Thio were filled air bubble-free into carbon-free, combusted (550 °C, 4 h) and tightly sealed narrow-neck glass bottles with ground glass-stoppers and stored in the dark at an average *in-situ* temperature of 10 °C for 14 days. After 7 days, four of the replicate bottles were sonicated for 2 min in a Branson 5510 Sonicator water bath to detach potential biofilm bacteria from the glass surfaces, homogenized by rigorous shaking and biomass and activity parameters as described above were determined. This procedure was repeated after 14 days for the remaining four glass bottles.

2.6. Statistics

The relationships between the microbiological and abiotic parameters were investigated using Spearman's rank-order correlation. One-way ANOVA was conducted to assess the effects of sampling date and site. For that purpose, results of TCC, HNA, TVAC, BCP and turnover times were log10-transformed to achieve log-normal distribution. Tukey HSD post-hoc test revealed significant differences when variances were homogenous (following Levene's Test). When variances were non-homogenous, the more robust Welch-ANOVA with a Games-Howell post-hoc test was applied. All statistical analyses were performed using Microsoft Excel (version: 14.0.7268.5000) or IBM SPSS (version 25).

3. Results

3.1. Physico-chemical on-site parameters

The sampling sites PW08 (15.9 ± 2.1 °C) and PW12 (18.5 ± 2.9 °C) showed highest average temperatures throughout the sampling period (Fig. 2c) and differed significantly from all other sites (ANOVA, $n = 70$, Tukey HSD, $p < 0.01$). At PW08 and PW12 an increase in the summer months was observed (Fig. 2a, b), whereas the other sampling sites did not show a clear seasonal temperature pattern. At sampling dates in June, July, August and September, average temperatures at all DWDS sites were above the general average (11.5 °C ± 4.1 °C), but did not differ significantly from samples taken in May, October and November (ANOVA, $n = 70$, Tukey HSD, $p > 0.05$).

Redox potential as a measure for the oxidative effect of the residual chlorine in the system was highest at sites of younger water age and decreased with increasing water age: sampling sites PW04 (355 ± 85 mV) and PW05 (370 ± 58 mV) had the highest Redox potential compared to the overall average of 270 ± 87 mV (Fig. 2f). PW04 was significantly different (ANOVA, $n = 70$, Games-Howell, $p < 0.05$) from the two sites with highest water age, PW11 and PW12, while PW05 was

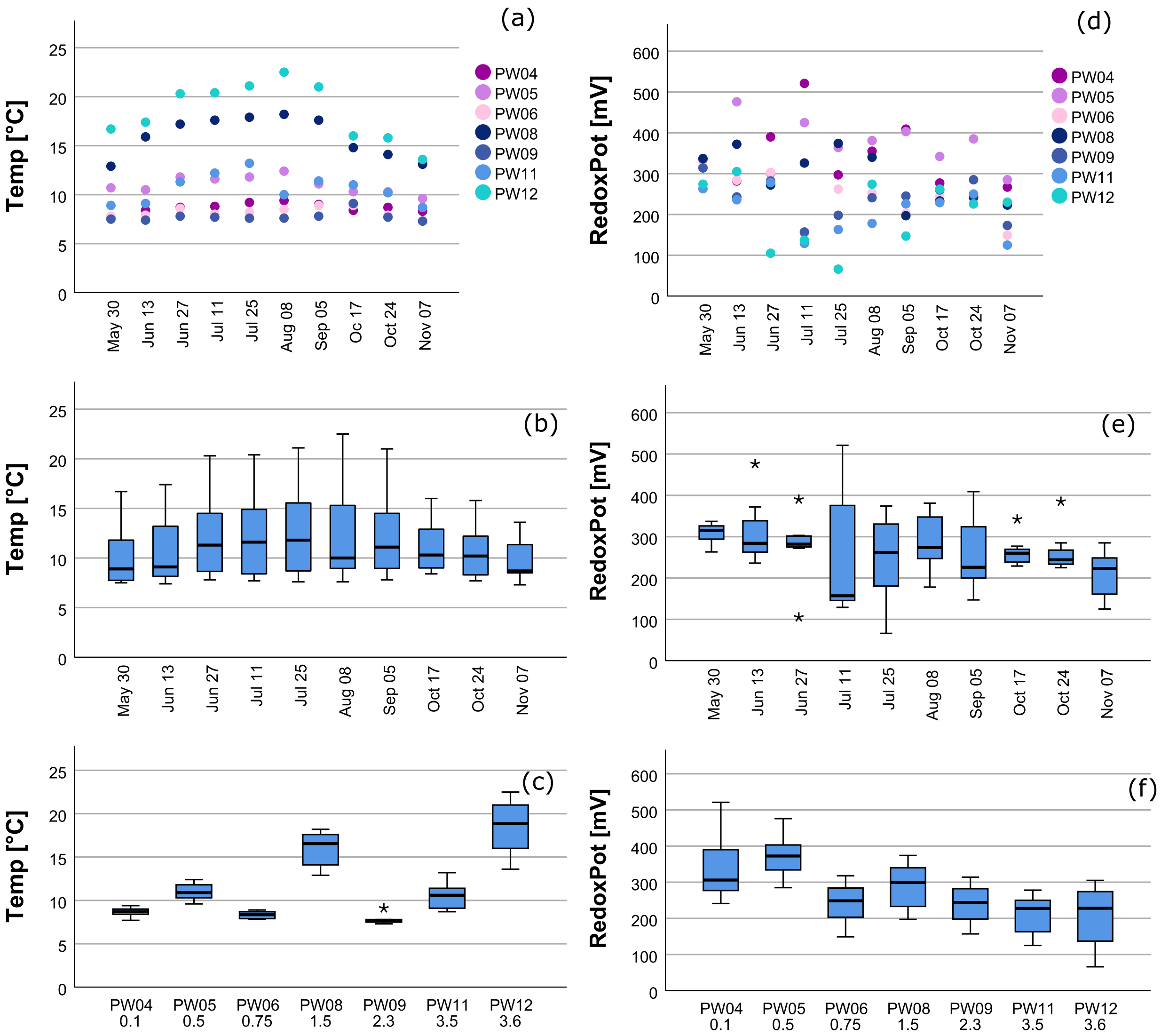


Fig. 2. Water temperatures and redox potential of the DWDS sites over the course of sampling campaign from May to November 2017. (a) and (d) show how temperature and Redox potential, respectively, develop at each sampling site over the course of the sampling period. (b) and (e) show boxplots of temperature and redox potential, respectively, over the course of the sampling period. (c) and (f) show boxplots of the respective parameter sorted according to increasing water age [d] from left to right. Boxes illustrate median values and interquartile ranges, whiskers indicate 5th and 95th percentile; outliers are shown as stars.

significantly different from PW06, PW09, PW11 and PW12 (ANOVA, Games-Howell, $p < 0.001$). No significant seasonal patterns (Fig. 2d, e) were observed (ANOVA, $n = 70$, Games-Howell, $p > 0.05$). Chlorine concentration was excluded from statistical analysis because 44 of 64 measured values were below the detection limit (0.05 mg L^{-1}). Turbidity in the raw water and at the sampling sites was always below 1 NTU, showing no clear patterns (Supplemental Information, Fig. A4, Table A3).

Electrical conductivity ranged from 192 to 471 μS cm^{-1} and showed no significant seasonal trend (ANOVA, $n = 70$, Tukey HSD, $p > 0.05$). The sites PW04, PW05, PW08 and PW12 (average = 339 μS cm^{-1}), differed significantly (ANOVA, $n = 70$, Tukey HSD, $p < 0.05$) from PW06, PW09 and PW11 (average = 242 μS cm^{-1}). No significant differences were found between PW04, PW05, PW08 and PW12 (ANOVA, Tukey HSD, $p > 0.05$). pH values in the DWDS ranged from pH 7.5 to 8.2. PW06, PW09 and PW11 (average = 8.00) were significantly different (ANOVA, Tukey HSD, $p > 0.05$.) from the other sites PW04, PW05, PW08 and PW12 (average = 7.79).

3.2. Heterotrophic plate counts

HPC-measurements lacked clearly interpretable trends. HPC37 values remained low in all samples throughout the investigation period (<5 CFU ml^{-1}). HPC22 showed larger variability and peaked in June at PW05 (140 CFU ml^{-1}), September (90 CFU ml^{-1}) and October (15 CFU ml^{-1}) at PW12, while the rest of the samples remained below 5 CFU ml^{-1} (Supplementary Figs. A5&A6).

3.3. Bacterial biomass and activity parameters for direct biostability assessment

3.3.1. Biomass parameters

TCC showed high fluctuations between sampling dates ranging from 7.4 × 10^{3} cells mL^{-1} to 135×10^{3} cells mL^{-1} during the sampling period. No clear trends related to seasonality or water age were detected. No significant differences were observed between the sampling sites (ANOVA, $n = 70$, Tukey HSD, $p > 0.05$). From May until June, all sampling sites followed a similar declining trend. Thereafter, PW05, PW06, PW09 and PW11 showed remarkable increases in TCC (up to 135×10^{3} cells mL^{-1} in PW06), while PW04, PW08 and PW12 stayed below 50×10^{3} cells mL^{-1} during the summer months, resulting in large interquartile ranges of TCC in July, August and September (Fig. 3a, b). For the last three sampling dates in October and November the trends in TCC were again similar for all sampling sites showing an upward trend

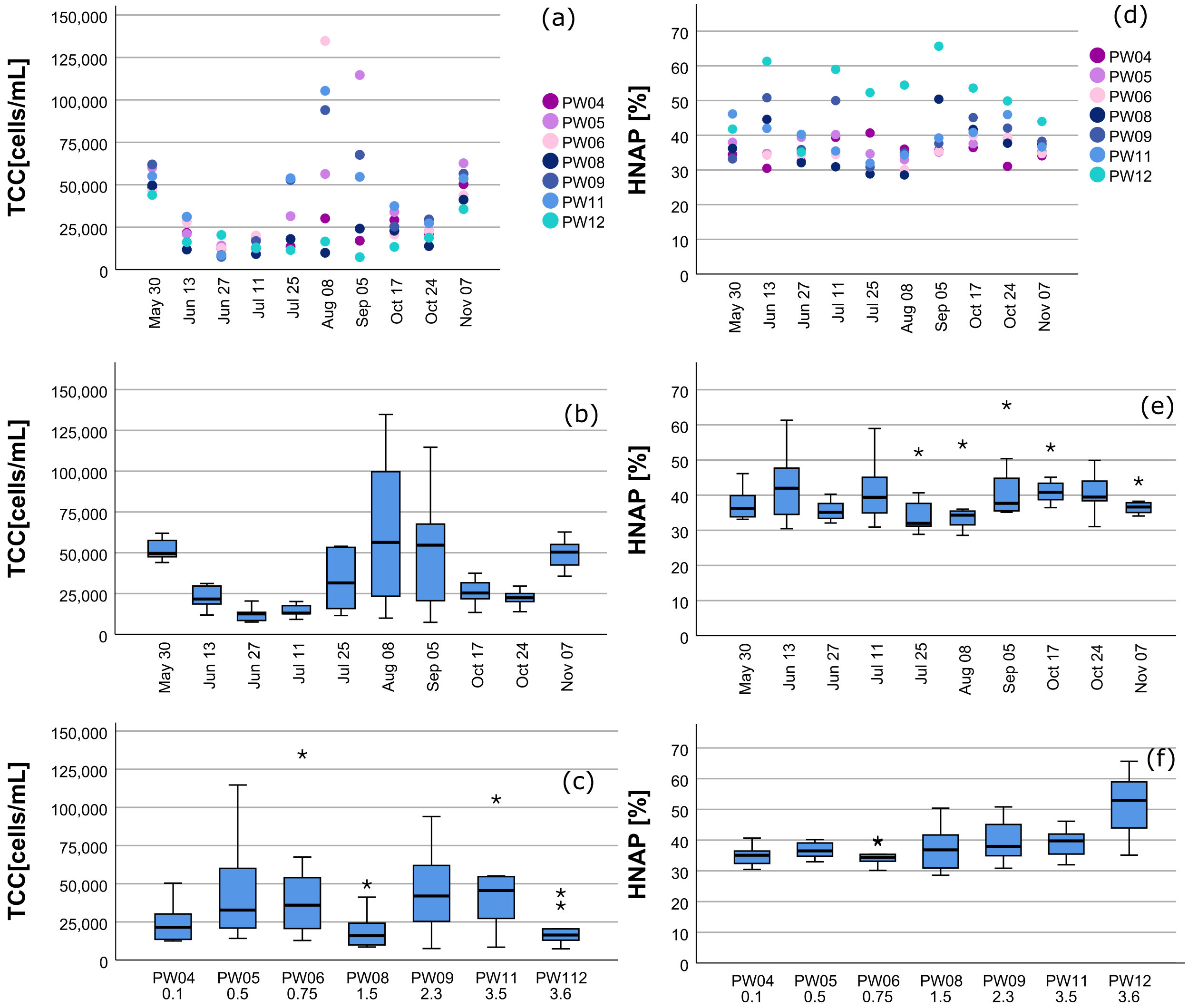


Fig. 3. Total cell counts and percentages of cells with high nucleic acid content (HNAP) of the DWDS sites over the course of sampling campaign from May to November 2017. (a) and (d) show how TCC and HNAP, respectively, develop at each sampling site over the course of the sampling period. (b) and (e) show boxplots of TCC and HNAP, respectively, over the course of the sampling period. (c) and (f) show boxplots of the respective parameter sorted according to increasing water age [d] from left to right. Boxes illustrate median values and interquartile ranges, whiskers indicate 5th and 95th percentile; outliers are shown as stars.

(Fig. 3a, b). HNA values followed the same seasonal patterns (Supplementary Fig. A7).

3.3.2. Activity parameters

Percentage of high nucleic acid cells - HNAP showed different patterns than TCC (Fig. 3d-f) and HNA. HNAP ranged from 28.6 % to 65.6 % during the sampling period; lowest HNAP values were found at PW06 (average = 34.7 %±3.0 %) and highest at PW12 (average = 51.7 %±9.4 %). PW12 was significantly different to all other sampling sites (ANOVA, $n = 70$, Tukey HSD, $p < 0.05$). No other significant differences between the sampling sites were detected.

Total viable active count – The patterns of viable and enzymatically highly active cells were similar to HNAP. With increasing water age, the bacterial communities had more enzyme-active cells. Generally, TVAC contributed only a small share to TCC (between 0.01% - 11.9%) and ranged from 1 cell mL^{-1} to 1,3 × 10^3 cells mL^{-1}. Significant differences between the sampling sites were observed (Fig. 4c). PW04 was significantly different to all other sampling sites (ANOVA, $n = 70$, Tukey HSD, $p < 0.001$). PW12 was significantly different to PW04 and PW05 (ANOVA, Tukey HSD, $p<0.01$), all other sampling sites showed no significant difference to each other (ANOVA, Tukey HSD, $p > 0.05$). No significant differences were observed between the sampling dates (ANOVA, $n = 70$, Tukey HSD, $p > 0.05$, Fig. 4a). Nevertheless, a trend towards higher TVAC values was observed for the spring and autumn samples with bigger interquartile ranges than cells from summer sampling dates (Fig. 4b).

Bacterial carbon production and bacterial community turnover times - Similar to HNAP and TVAC, BCP showed increasing values with higher water age (Fig. 4f). PW04 differed significantly from all other sampling sites apart from PW05 (ANOVA, $n = 70$, Tukey HSD, $p < 0.05$). BCP ranged from 3.05 to 1000 pg C L^{-1} h^{-1}, lowest BCP values were found at PW04 (average = 18.2 ± 27 pg C L^{-1} h^{-1}) and highest at PW12 (average = 293 ± 293 pg C L^{-1} h^{-1}). No seasonal trend was observed for BCP rates at any site, while significantly higher BCP (ANOVA, $n = 45$, Tukey HSD, $p < 0.05$) was detected in September and November compared to the other dates (Fig. 4d, e).

Table 2 shows a heat-map of BaCTT values for all samples, ranging from 13 (PW12, Sep 5) to 6800 days (PW09, May 30). Sampling sites with lower water age (PW04, PW05, PW06) exhibited higher turnover times than sites with higher water age (PW08, PW09, PW11, PW12).

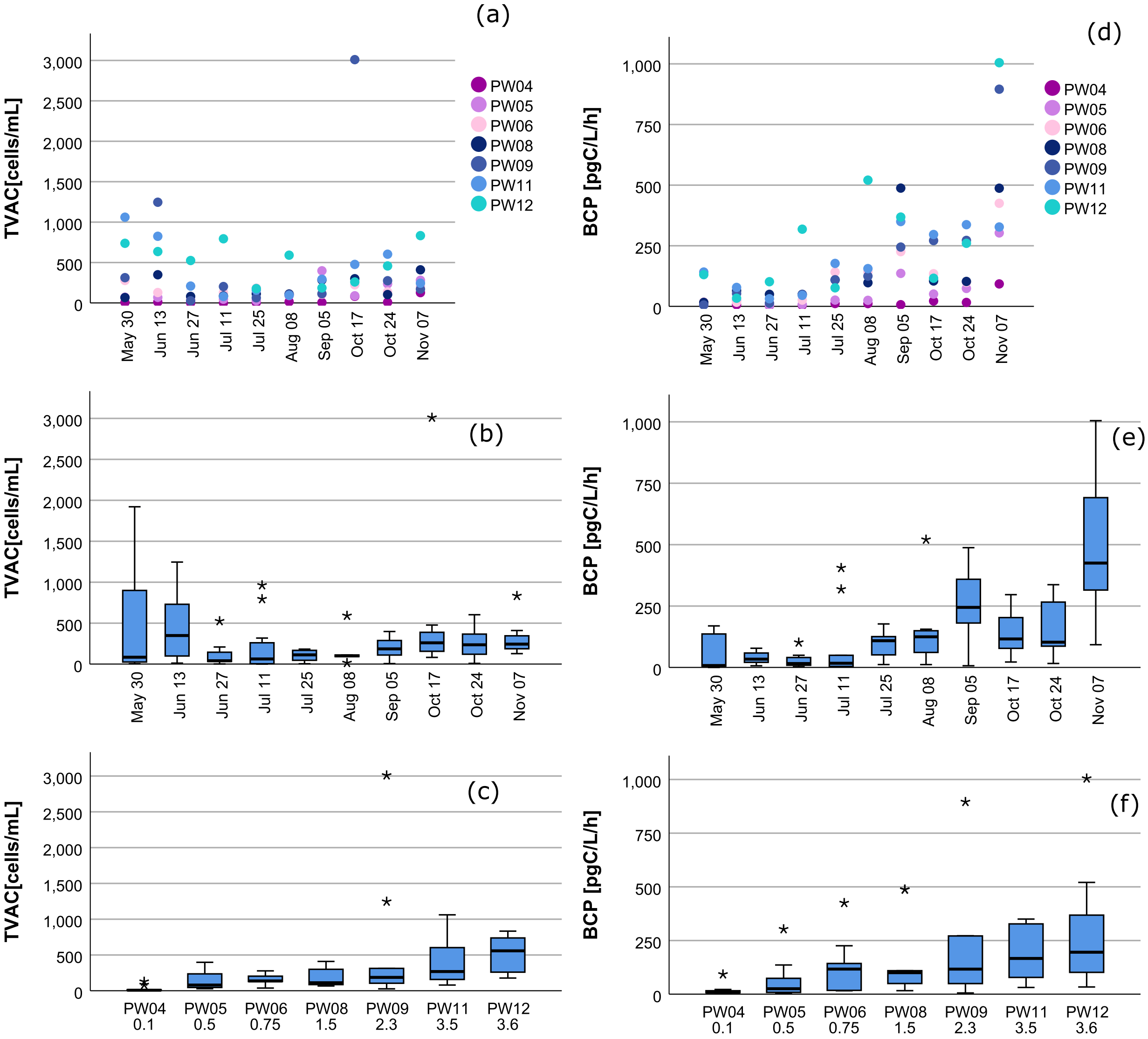
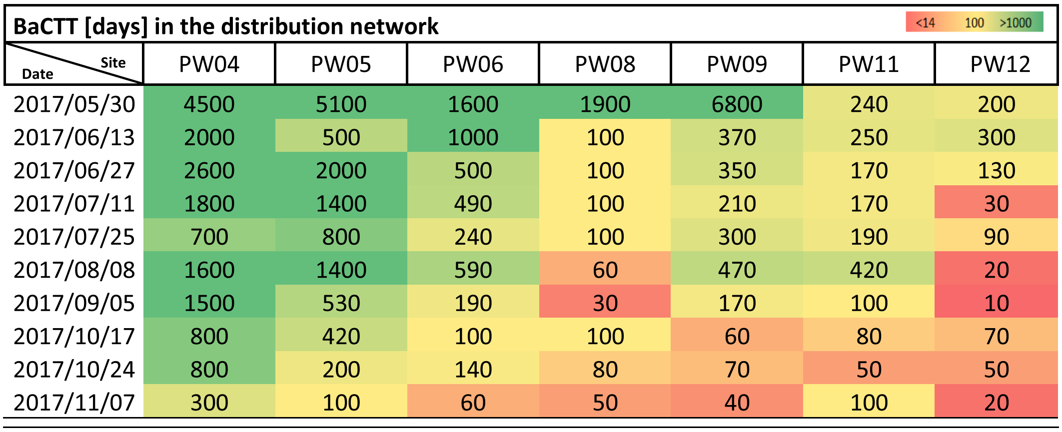


Fig. 4. Total viable active cell counts (TVAC) and bacterial carbon production (BCP) of the DWDS sites over the course of sampling campaign from May to November 2017. (a) and (d) show how TVAC and BCP, respectively, develop at each sampling site over the course of the sampling period. (b) and (e) show boxplots of TVAC and BCP, respectively, over the course of the sampling period. (c) and (f) show boxplots of the respective parameter sorted according toage [d] from left to right. Boxes illustrate median values and interquartile ranges, whiskers indicate 5th and 95th percentile; outliers are shown as stars.

Table 2

Heat-map of bacterial community turnover times (BaCTT, in days) at the drinking water distribution network (DWDS) sites, calculated from bacterial biomass and bacterial carbon production. The legend in the upper right corner describes the colour coding of the table: red meaning short, green meaning long turnover times.



BaCTT [days] in the distribution network

Date \ Site	PW04	PW05	PW06	PW08	PW09	PW11	PW12
2017/05/30	4500	5100	1600	1900	6800	240	200
2017/06/13	2000	500	1000	100	370	250	300
2017/06/27	2600	2000	500	100	350	170	130
2017/07/11	1800	1400	490	100	210	170	30
2017/07/25	700	800	240	100	300	190	90
2017/08/08	1600	1400	590	60	470	420	20
2017/09/05	1500	530	190	30	170	100	10
2017/10/17	800	420	100	100	60	80	70
2017/10/24	800	200	140	80	70	50	50
2017/11/07	300	100	60	50	40	100	20

BaCTT showed a decreasing trend during the investigation period. The highest turnover times were observed in May and were significantly different from values observed in September, October and November (ANOVA, $n = 45$, Tukey HSD, $p < 0.05$).

3.3.3. *Biomass and activity parameters show different correlation patterns*

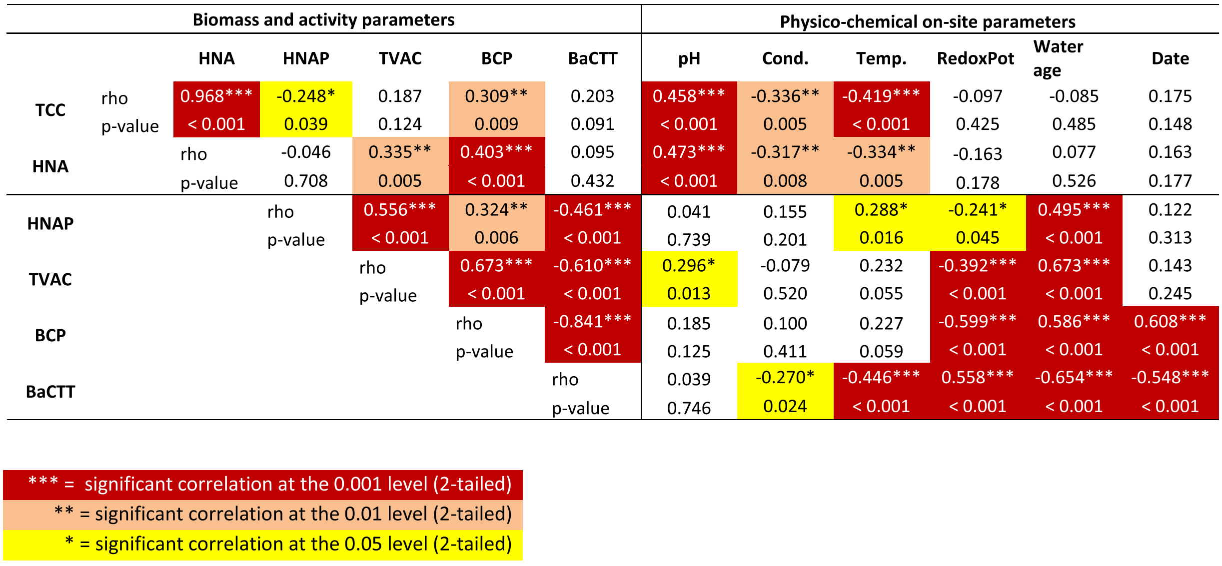
Correlation analysis was performed to assess the key factors controlling biostability in terms of bacterial biomass and activity parameters in the investigated DWDS (Table 3). Biomass parameters (TCC and HNA) were significantly positively correlated with pH and negatively correlated with conductivity and water temperature. No significant correlations were observed with redox potential, water age or sampling date. Both, TCC and HNA were positively correlated with each other and with BCP, while HNA was also positively correlated with TVAC. In contrast, activity parameters (HNAP, TVAC, BCP) displayed completely different correlation patterns. All activity parameters were significantly inter-correlated and showed significant positive correlations with water age and negative correlations with redox potential. In addition, a weak positive correlation was observed with temperature, whereas for TVAC and BCP the correlation coefficients were slightly above the level of significance ($p = 0.05$). A weak positive correlation was also observed for TVAC with pH. BaCTT were positively correlated with Redox potential ($p < 0.001$) and negatively correlated with water age, temperature and sampling date. A weak significant correlation was also observed with conductivity ($p < 0.05$). Interestingly, a significant positive correlation was observed between BCP and sampling date, resulting in a significant negative correlation between BaCTT and sampling date.

3.4. *Predictive biostability assessment (storage experiments)*

In order to predict the biostability of the raw (PW01) and distributed water immediately after disinfection (PW03, PW03+Thio), storage experiments were performed. All measured microbiological parameters in PW01 showed significant increases during 14 days of incubation (ANOVA, $n = 15$, Tukey HSD: TCC ($p < 0.05$), HNA ($p < 0.01$), HNAP ($p < 0.05$), TVAC ($p < 0.01$) and BCP ($p < 0.001$). On average, TCC doubled and HNA tripled during the 14 day incubation period, TVAC increased more than 8-fold and BCP even increased by more than 40-fold (Fig. 5a-e). In contrast, the application of chlorine (both with and without added sodium-thiosulphate) had a pronounced stabilising effect on the

Table 3

Spearman rank correlation coefficient (ρ) matrix between microbiological and physico-chemical on-site parameters measured in the 7 drinking water distribution system (DWDS) sites during 10 sampling events ($n = 70$).



		Biomass and activity parameters					Physico-chemical on-site parameters					
		HNA	HNAP	TVAC	BCP	BaCTT	pH	Cond.	Temp.	RedoxPot	Water age	Date
TCC	rho	0.968***	-0.248*	0.187	0.309**	0.203	0.458***	-0.336**	-0.419***	-0.097	-0.085	0.175
	p-value	< 0.001	0.039	0.124	0.009	0.091	< 0.001	0.005	< 0.001	0.425	0.485	0.148
HNA		rho	-0.046	0.335**	0.403***	0.095	0.473***	-0.317**	-0.334**	-0.163	0.077	0.163
		p-value	0.708	0.005	< 0.001	0.432	< 0.001	0.008	0.005	0.178	0.526	0.177
HNAP			rho	0.556***	0.324**	-0.461***	0.041	0.155	0.288*	-0.241*	0.495***	0.122
			p-value	< 0.001	0.006	< 0.001	0.739	0.201	0.016	0.045	< 0.001	0.313
TVAC				rho	0.673***	-0.610***	0.296*	-0.079	0.232	-0.392***	0.673***	0.143
				p-value	< 0.001	< 0.001	0.013	0.520	0.055	< 0.001	< 0.001	0.245
BCP					rho	-0.841***	0.185	0.100	0.227	-0.599***	0.586***	0.608***
					p-value	< 0.001	0.125	0.411	0.059	< 0.001	< 0.001	< 0.001
BaCTT						rho	0.039	-0.270*	-0.446***	0.558***	-0.654***	-0.548***
						p-value	0.746	0.024	< 0.001	< 0.001	< 0.001	< 0.001

*** = significant correlation at the 0.001 level (2-tailed)
** = significant correlation at the 0.01 level (2-tailed)
* = significant correlation at the 0.05 level (2-tailed)

development of the bacterial communities over the course of 14 days. For all measured parameters, a stabilisation (HNAP, TVAC) or a significant decreasing trend (TCC, HNA, BCP; ANOVA, $n = 14$, Tukey HSD, $p < 0.05$) was observed in the chlorinated samples (PW03). Even when chlorine was neutralized (PW03+Thio), bacterial biomass and activity parameter remained mostly unchanged within 14 days (ANOVA, $n = 15$, Tukey HSD, $p > 0.05$). Table 4 represents a heat map of the calculated BaCTT over the course of 14 days. In the raw water samples (PW01), a significant trend towards short turnover times on the scale of few days to two weeks was observed (ANOVA, Tukey HSD, $p < 0.001$). Chlorinated water (PW03) showed very high turnover times of up to 216.000 days which significantly dropped to a few hundred days during the storage period (ANOVA, Tukey HSD, $p < 0.05$). When the effect of chlorine was quenched by sodium-thiosulfate (PW03+Thio), BaCTT mostly remained at a similar level during the experiment with no significant differences between T0, T7 and T14 (ANOVA, Tukey HSD, $p > 0.05$).

4. Discussion

In this study, the dynamics of the bacterial community in a large chlorinated DWDS were monitored over seven months in terms of biomass, activity and bulk community turnover times in order to assess the potential of biological instability of the distributed water. Samples were taken from central and distal sites along gradients of disinfectant residual, temperature and water age in the course of which a decoupling of biomass from activity parameters was observed. While variations in bacterial biomass parameters reflected changes in raw water and correlated negatively with water temperature, bacterial activity increased significantly with water age and decreasing redox potential and showed a weak positive correlation with water temperature. The application of BaCTT as a new quantitative direct parameter allowed us to identify spatial and temporal hotspots of potential biostability concerns in the investigated DWDS. Additional storage experiments to assess the growth potential of bacterial communities in the raw water feeding the DWDS showed that chlorine addition contributed to a biologically more stable water.

4.1. BaCTT is a highly suitable parameter to assess the potential of biological instability

As proposed by Prest et al. (2016), we applied a multi-parametric approach to assess the potential for biological instability of drinking water in a chlorinated DWDS. By combining a variety of microbial biomass and activity parameters, a more comprehensive and more rapid assessment (van der Wielen et al., 2023) was possible than when exclusively using single parameter-based, cultivation-based or other traditional approaches such as TCC (Prest et al., 2013), HPC (Allen et al., 2004), AOC (van der Kooij et al., 1982) or BDOC (Servais et al., 1987). BaCTT calculated from total bacterial numbers, cell volume estimations and ^{3}H-leucine incorporation into bacterial biomass proved to be a useful quantitative parameter for the direct assessment of the biological instability potential of drinking water. The values obtained are indicating the number of days in which the total bacterial water community is doubling. When values in the range of 10 days are obtained for a DWDS with water residence times of up to two weeks, it is plausible to assume the occurrence of microbiological problems, potentially leading to deterioration of taste and odour, higher risk of corrosion or an increased probability for the growth of opportunistic pathogens (Proctor et al., 2022; van der Kooij, 2000). This parameter could thus represent a sound and easy-to-interpret decision-making tool to rapidly identify potential hotspots of biological instability in a DWDS.

TCC have previously been used to assess biostability in DWDSs (Farhat et al., 2020; Liu et al., 2013). In our studied DWDS, however, TCC turned out to be an inappropriate parameter for biostability assessment, since it reflected raw water characteristics and not bacterial growth dynamics in the system. When using HPC, a limited picture of

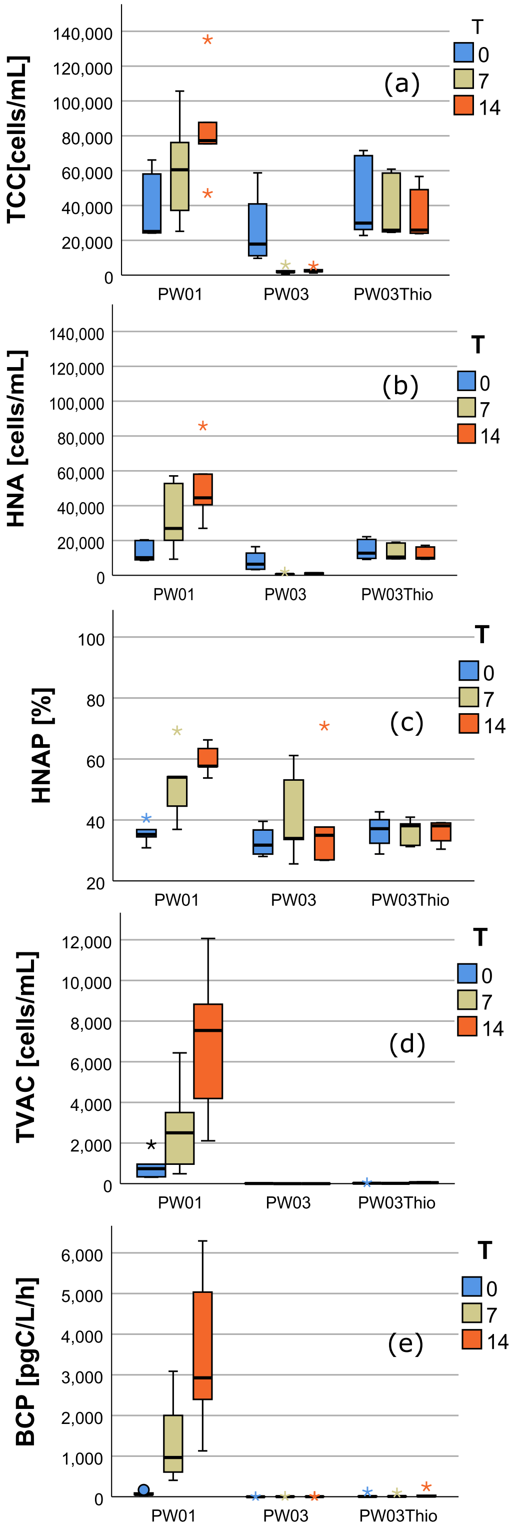
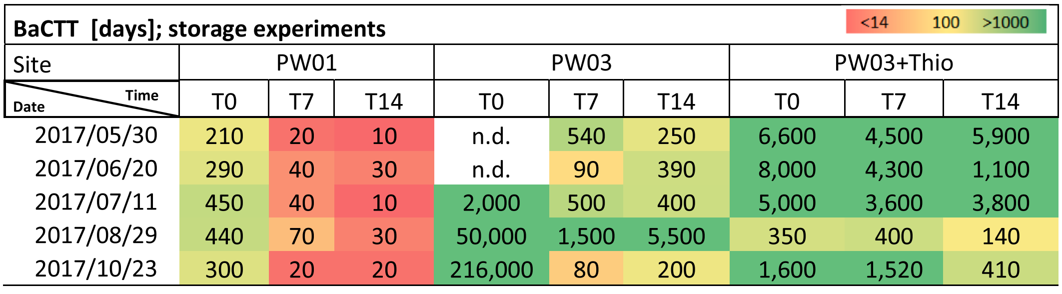


Fig. 5. Storage experiments for predicting biostability of raw water (PW01), water sampled immediately after chlorine disinfection (PW03) and after neutralizing the chlorine with sodium-thiosulphate (PW03+Thio). T0 (on sampling day), T7 (after 7 days of storage), T14 (after 14 days of storage). $n = 5$ for PW01 and PW03+Thio, $n = 4$ for PW03. Biological stability was assessed by microbiological biomass and activity parameters total cell counts (TCC), the count (HNA) and percentage (HNAP) of high nucleic acid cells, total viable active cells (TVAC) and bacterial carbon production (BCP). Boxes illustrate median values and interquartile ranges, whiskers indicate 5th and 95th percentile; outliers are shown as stars.

Table 4

Heat-map of BaCTT (in days) in the raw (PW01) and chlorinated water samples (PW03, PW03+Thio) during the 14 day storage experiments to predict biostability, calculated from bacterial biomass and bacterial carbon production. The legend in the upper right corner describes the colour coding of the table: red meaning short, green meaning long turnover times. T0 = sampling date, T7 = samples stored for 7 days; T14 = samples stored for 14 days. n.d. = not determined.



BaCTT [days]; storage experiments

<14 100 >1000

Site	PW01			PW03			PW03+Thio		
Date / Time	T0	T7	T14	T0	T7	T14	T0	T7	T14
2017/05/30	210	20	10	n.d.	540	250	6,600	4,500	5,900
2017/06/20	290	40	30	n.d.	90	390	8,000	4,300	1,100
2017/07/11	450	40	10	2,000	500	400	5,000	3,600	3,800
2017/08/29	440	70	30	50,000	1,500	5,500	350	400	140
2017/10/23	300	20	20	216,000	80	200	1,600	1,520	410

possible biostability concerns is provided, as only a minor part of the total bacterial community is cultivable (often <1 %, Burtscher et al., 2009) and HPC counts may be too insensitive to allow reliable conclusions. This was also the case in the investigated DWDS, where HPC22 and HPC37 values at the selected sites ranged from 1 to 140 and 1 to 7, respectively, without displaying any interpretable pattern. Using AOC or BDOC as parameters requires incubation of the water for up to three weeks, delaying the availability of results, whilst intervention could be necessary. In the case of AOC, cultures of two selected bacterial species need to be inoculated (*Pseudomonas fluorescens* strain P17 and *Spirillum* sp. strain NOX, van der Kooij et al., 1982, 1984) that might not be representative for the system investigated. Alternatively, inoculating natural bacterial communities (Farhat et al., 2018; Hammes and Egli, 2005) causes a strong disturbance to the water sample and may heavily influence the results.

The approach proposed here uses BaCTT as a quantitative and easy-to-interpret measure for the potential of biological instability. It combines the highly sensitive parameters bacterial biomass and carbon production, while requiring minimal invasive sample handling and delivering results within 48 h. Total bacterial biomass can easily be determined on the basis of TCC, when average values for bacterial cell size and cellular biomass for drinking water bacteria are adopted from the literature. We found bacterial cellular biomass values with an average of 15 fg C per cell, which reflects comparable values from the literature ranging from 10 to 20 fg C per cell for drinking water or oligotrophic environments (Griebler et al., 2002; Loferer-Krößbacher et al., 1998; Servais et al., 2003). TCC are measured either by epifluorescence microscopy (Servais et al., 1992) or flow cytometry in manual or fully automated mode (Nescerecka et al., 2018), for example using the operator-friendly BactoSense (bNovate, Switzerland). Bacterial carbon production, as determined here, was calculated from the incorporated amount of ^{3}H-leucine as a direct and highly sensitive estimate of bacterial activity (Servais et al., 1992b; van Driezum et al., 2018). Incorporation rates of few pg C L^{-1} h^{-1} can be detected. The biggest limitation of this method is that it relies on the use of radioactive substance and thus has to be performed in specialised laboratories that are equipped with a liquid scintillation counter. For detecting low bacterial production values in the oligotrophic drinking water environment, incubation for 24 h is appropriate, but for identifying hotspots of higher activity, shorter incubation times (e.g. 12 h) might be applicable, reducing the time-to-result including sampling handling and analysis from 48 to 24 h. Another drawback of this promising method is that for converting ^{3}H-leucine uptake rates into carbon production rates, a theoretical conversion factor has to be adopted from the literature (Simon and Azam, 1989) that is based on numerous assumptions, such as a constant proportion of leucine in bacterial proteins and a constant proportion of proteins in cellular biomass. However, this conversion factor seems to be very robust over a wide range of environments (Evans et al., 2021; Kallmeyer et al., 2012; Mojica and Brussaard, 2020; Orta-Ponce et al., 2021). Yet, also in other methods determining biostability assumptions on the suitability of test-organisms (AOC) or the effect of sample manipulation (inoculation) have to be accepted.

As a non-radioactive alternative to ^{3}H-leucine, bromo-deoxyuridine could be used as substrate (Pernthaler et al., 2002). However, as this substrate is incorporated into DNA which is much less abundant in bacterial cells than proteins the method is less sensitive. Other indicators of bacterial activity are the percentage of HNA cells (HNAP, Prest et al., 2013), the number of viable and active cells (TVAC, Reynolds et al., 1997) or the amount of ATP (Lautenschlager et al., 2013). All of these parameters are easy to measure automatically via flow cytometry or ATP analysers. However, ATP measurements are likely to be influenced by extracellular ATP (Hammes et al., 2008) and none of the mentioned parameters offers the opportunity to directly calculate bacterial production or turnover rates. For this purpose, long-term incubations (up to 3 weeks) to calculate net production rates from observed cell number changes or ATP content over time, or strong manipulations of the samples (by removing the original bacterial community and adding an inoculum) to obtain relevant gross production estimates would be necessary.

4.2. *Water age, redox potential and seasonal effects are main drivers of biostability in the DWDS*

In the investigated DWDS, an uncoupling of bacterial biomass and activity parameters was observed. While biomass parameters reflected raw water characteristics, bacterial activity parameters were more indicative for the biostability in the system and correlated significantly with community turnover times (negatively), redox potential as a measure of residual chlorine (negatively), water age (positively) and temperature (weakly positively). Moreover, bacterial carbon production rates (positively) and turnover times (negatively) were correlated with date, indicating a seasonally triggered growth-stimulating effect occurring in the DWDS. As stated by Nescerecka et al. (2018) and Wang et al. (2014), changes in growth-promoting parameters in a full-scale network cannot be explained by one single mechanism, but rather by a combination of various processes that occur due to interactions between bulk water, biofilms, disinfection type and residuals, pipe materials and water age.

Higher **water temperatures** are known to favour bacterial growth in DWDSs (Servais et al., 1992a), and especially above 15 °C the risk of regrowth after disinfection increases significantly (Liu et al., 2013). Hence, increased microbial biomass and activities, following higher temperatures in summer (up to 22.5 °C at PW12) were expected in the DWDS. Surprisingly, the biomass parameters were significantly negatively correlated to temperature. Such uncoupling can only be explained by the fact that changes in raw water characteristics (pH, conductivity, cell numbers) over time were higher than effects of temperature on bacterial cell numbers. Indeed, TCC were significantly correlated with pH and conductivity. In contrast to the number of HNA (which followed TCC), the percentage of HNA cells (HNAP) showed the same correlation pattern as the activity parameters. HNAP have been proposed as fingerprint parameter indicating the effect of growth promoting factors on drinking water bacterial community (Lebaron et al., 2001) by linking increasing HNAP with active growth.

Disinfectant residuals significantly inhibit bacterial growth and control biological stability in DWDSs (Gillespie et al., 2014). As chlorine concentrations in the system were too low for reliable quantification, redox potential was used as a measure for the oxidative effect of the residual chlorine in the system. PW04 as the site closest to the treatment had a significantly higher redox-potential than all other sites. The declining trend with distance from the disinfection site (and water age) suggests on the one hand disinfectant residual decay due to longer contact-times with oxidisable material (pipe walls, biofilms, TSS, microorganisms, Van Der Kooij et al., 1999; Lipphaus et al., 2014). On the other hand, increased bacterial activity can contribute to oxygen consumption by respiring microorganisms and hence reduce redox potential. However, available data from the DWDS indicate that oxygen concentrations throughout the distribution network were around 100 % and never lower than 8.6 mg L^{-1} over the entire year implying that the present system is well-oxidised and oxygen depletion does not play a role concerning biological stability.

Water age was reported to be a major factor in water quality deterioration in DWDSs, the main mechanisms being interactions with pipe walls and reactions within the bulk water itself (U.S. EPA, 2002). Along with increasing water age, temperatures of “older” water converge to surrounding temperatures, leading to higher chlorine demand and thus water quality deterioration. Also in the investigated DWDS, water age correlated highly significantly with bacterial activity and decreasing turnover times: at the site with highest water age (PW12) temperatures were significantly higher than at the other sites, suggesting that the combination of decreasing disinfectant residual and increasing temperature most likely triggered the negative effect of water age on biological stability.

Surprisingly, we also observed a significant **seasonal trend** with increasing bacterial activity and decreasing turnover times as a function of date. BaCTT ranged from 200 days at the most distal site PW12 to >4000 days at the most central sites PW04 and PW05 in May and decreased to only 20 days at PW12 and 300 days at PW04 in November. This indicates that in addition to water age, temperature and disinfectant residual also other factors may influence the biological stability in this system. One important factor might be the quality and quantity of organic matter (Supplementary Fig. A8) and inorganic nutrients supporting bacterial growth (Farhat et al., 2018; Hammes et al., 2010a). Indeed, an increase of dissolved organic carbon promoting heterotrophic bacterial production was documented to occur during summer and autumn storm-flow events in the springs feeding the investigated DWDS (Wilhartitz et al., 2009).

4.3. Chlorine effectively prevents potential impairment of raw water biostability

The main finding of the storage experiments was that the addition of chlorine to the raw water samples led to a significant and long-lasting decrease in viable bacterial biomass and activity compared to non-chlorinated samples. In these raw water samples, total cell counts and carbon production increased up to 2- and 40-fold, respectively, during 14 days of incubation at *in-situ* temperature. BaCTT for the chlorinated samples reached up to 5900 days, while dropping to 10–30 days in the non-chlorinated samples, indicating a high bacterial growth- and biological instability potential of the raw water resource over the whole investigation period. Inhibition of bacterial growth by chlorine is an expected and wanted effect of this disinfection medium, ultimately resulting in the destruction and lysis of bacterial cells (Ramseier et al., 2011) after longer exposure times. Cell lysis leads to the release of readily available nutrients promoting niche-occupation by surviving microorganisms (Nescerecka et al., 2016, 2014), most likely by LNA-cells that were proven to be less chlorine-sensitive than HNA-cells (Ramseier et al., 2011). Such an effect was observed in the storage experiments of the chlorinated samples, where BaCTT were lower in the samples without thiosulfate due to the continuous destruction of chlorine sensitive bacterial cells and growth of the chlorine-resistant sub-population, also indicating that the added chlorine has not yet been completely used after 14 days.

5. Conclusions

The uncoupling of bacterial biomass and activity parameters observed in this study strongly underpins the need to use a multi-parametric approach to assess biological stability in DWDSs. Considering only total cell counts as a simple-to-measure indicator for biological stability would have led to different conclusions for this system. The newly proposed direct parameter BaCTT combines both biomass and activity aspects and turned out to be a comprehensive, rapid and easy-to-interpret measure of biostability (as for the potential of biological instability). The major limitation of using BaCTT based on ^{3}H-leucine incorporation is the need for a specialised laboratory and trained personnel. Using BaCTT we could identify hotspots and main drivers of potential biostability concerns (biological instability) in the DWDS that is fed by raw water with a high bacterial growth potential. According to the results of the storage experiments, chlorine addition is important to control bacterial numbers and activity in the drinking water. This was further confirmed by determining redox potential (a measure for the oxidative effect of chlorine residual) as a main factor controlling bacterial regrowth that was strongly influenced by water age, temperature and a seasonal effect. Additional studies in DWDSs of different size, age and complexity using other water sources, disinfection processes, and investigating biofilm growth potential on different pipe materials are needed to investigate the general applicability of BaCTT.

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CRediT authorship contribution statement

Lena Campostrini: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation, Conceptualization. **Philipp Proksch:** Writing – review & editing, Validation, Methodology, Investigation. **Stefan Jakwerth:** Writing – review & editing, Validation, Methodology, Investigation. **Andreas H. Farnleitner:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. **Alexander K.T. Kirschner:** Conceptualization, Data curation, Funding acquisition, Resources, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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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.2024.121188.

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