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Online microbial fingerprinting for quality management of drinking water: Full-scale event detection

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ABSTRACT

Microbial regrowth during drinking water distribution can result in a variety of problems such as a deviating taste and odor, and may even pose a risk to public health. Frequent monitoring is essential to anticipate events of biological instability, and relevant microbial parameters for operational control of biostability of drinking water should be developed. Here, online flow cytometry and derived biological metrics were used to assess the biological stability of a full-scale drinking water tower during normal and disturbed flow regime. Pronounced operational events, such as switching from drinking water source, and seasonal changes, were detected in the total cell counts, and regrowth was observed despite the short hydraulic residence time of 6–8 h. Based on the flow cytometric fingerprints, the Bray-Curtis dissimilarity was calculated and was developed as unambiguous parameter to indicate or warn for changing microbial drinking water quality during operational events. In the studied water tower, drastic microbial water quality changes were reflected in the Bray-Curtis dissimilarity, which demonstrates its use as an indicator to follow-up and detect microbial quality changes in practice. Hence, the Bray-Curtis dissimilarity can be used in an online setup as a straightforward parameter during full-scale operation of drinking water distribution, and combined with the cell concentration, it serves as an early-warning system for biological instability.

1. Introduction

Excessive microbial regrowth may result in a variety of problems during drinking water distribution. Microbial growth and activity can entail aesthetical issues, such as deviating taste and odor, and may eventually form a threat to public health (Skjevrak et al., 2004; Wingender and Flemming, 2011). Biofilms adhered to the pipe walls may cause operational problems, such as loss of pressure, and can accelerate pipe corrosion (Liu et al., 2016). Therefore, it is important to produce drinking water that is biologically stable, which implies *sensu stricto* that the microbial community composition and its abundance remain unchanged during distribution (Lautenschlager et al., 2013; Rittman and Snoeyinck, 1984; WHO, 2011).

"Biostability" is a theoretical concept that is now becoming an operational goal (Prest et al., 2016a). Biostability is most often enforced through the addition and maintenance of various disinfectants in the distribution network to eliminate the presence of viable bacteria (Zhang et al., 2002). However, disinfectants, such as chlorine, only have a temporary effect, and may furthermore result in the availability of dead organic matter that can be used during necrotrophic and heterotrophic growth, resulting in a biologically unstable system (Temmerman et al., 2006). Carcinogenic disinfection by-products are known to be formed when reacting with organic matter (Li and Mitch, 2018). This has encouraged countries, such as Switzerland and The Netherlands, to focus on achieving biostability without maintaining disinfection residuals in the network, but by limiting nutrients and carbon availability (van der Kooij et al., 2013).

To evaluate biostability of drinking water, heterotrophic plate counts (HPC) are commonly used, as this technique is the legal standard (European Communities, 1998, US Congress, 1996, WHO, 2011). However, samples are not taken frequently enough (*e.g.*,

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weekly) and the time-to-result (2–7 days) is too long to anticipate events of biological instability. To facilitate decision-making when detecting events in the abundance or changes in the composition of the microbial community, implementation of online monitoring techniques is necessary.

Though they are only sporadically implemented at full-scale, a variety of techniques for online microbial drinking water quality monitoring are available. Online ATP measurements allow fast (*i.e.*, <10 min) quantification of microbial activity based on luciferin/luciferase enzymatic reactions (de Vera and Wert, 2019; Vang et al., 2014). Though cellular ATP is an indicator for bacterial activity, ATP quantification is prone to interferences of extracellular ATP, and is not always correlated to regrowth (Nescerecka et al., 2016; Vang et al., 2014; Vital et al., 2012). Other enzymatic techniques have been developed for near-real-time selective detection of *Escherichia coli* presence, targeting the specific enzyme β-glucuronidase (Hesari et al., 2016; Koschelnik et al., 2015). Results can be acquired within 1–2 h, with good accuracy. Although this selective method provides information about the hygienic quality of the water, it does not indicate events of general biological instability. Alternatively, recently developed online particle counters and online flow cytometry (FCM) allow the direct quantification of microbial abundance in water using optical technology within 10 min (Besmer et al., 2016; Hammes et al., 2012; Højris et al. 2016, 2018). Flow cytometry is the most established research technique, and when combined with advanced data analysis, it allows assessment of the microbial community characteristics, such as viability and phenotypic diversity (Gillespie et al., 2014; Props et al., 2016). Established ecological biodiversity metrics such as the alpha diversity or the within-sample diversity, and the beta diversity or the community turnover can be calculated from the flow cytometry data based on the phenotypic traits of a microbial community (Props et al., 2016; Whittaker, 1972). Correspondence between the phenotypic and genotypic diversity was shown by Props et al. (2016). The multidimensional flow cytometric data, summarized in the so-called cytometric fingerprint, reacts quickly to changing water quality and conditions (Besmer et al., 2017). The advanced community data analysis is more valuable than the cell counts for decision-making, as it takes into account both changes in the composition and abundance of the microbial community. Yet, as previously stated, it is not often used for microbial monitoring, because translation and interpretation of the results into warnings or concrete actions is currently lacking. This was also one of the major conclusions of an extended review by Safford and Bischel (2019) concerning the implementation of FCM as microbial monitoring tool in drinking water. A key point is to obtain better understanding of the microbial dynamics in full-scale drinking water networks to being able to generate fundamental knowledge about the system and to define the "degree of acceptable change" during events of biological instability (Prest et al., 2016a).

This study aims at demonstrating that advanced data analysis of flow cytometric data can be used for the development of a straightforward parameter to define the microbial water quality in a more holistic way, by not only taking into account the microbial abundance but also the stability of the microbial community composition. Therefore, a water tower with incoming drinking water produced from two different sources was chosen as full-scale model setup during regular and disturbed flow regime. Microbial regrowth in the water tower was evaluated through total cell counts. Also, biological metrics calculated from the flow cytometric fingerprint, such as the Bray-Curtis dissimilarity with a value between 0 and 1 that expresses how different two cytometric fingerprints are, was used for the detection of operational events that resulted in a drastic microbial water quality change (Greenacre and Primicerio, 2014).

2. Materials and methods

2.1. Water tower characteristics

The microbial dynamics of a water tower in Flanders, Belgium were monitored in spring 2018 (April 18th - 27th) and summer 2018 (August 6th - 27th). Before entering the distribution network, the water is treated and disinfected, after which the drinking water is transported to the water tower through a high-pressure piping, and from which it flows to the customers through a low-pressure network (Fig. 1). This water tower was selected specifically because two drinking water feed streams produced from different source waters enter the reservoir. The first feed is produced from surface water (length feeder to tower = 47.58 km, diameter = 0.7–1.0 m) and the second feed stream is produced from groundwater (length feeder to tower = 24.44 km, diameter = 1.0 m). This way, the most important types of drinking water, *i.e.*, produced from either surface water, groundwater or a mixture of both, are included in the monitoring setup. The physicochemical characteristics of the incoming and exiting streams of the water tower are summarized in Table 1. The water tower complex has a total buffering capacity of 6,500 m^3. Based on the average daily intake, 72.48 ± 19.14% of the drinking water entering the tower is produced from surface water, with an average residence time of 6–8 h.

During the monitoring period in April, the water composition was altered in two different ways by closing one of the two feeders. During these events, the incoming water consisted out of drinking water either mostly produced from surface water or completely produced from groundwater (Fig. 1). During the first event on April 24th (07h34 - 23h19), the feed originated for almost 100% from surface water, which is referred to as "event 1". On April 26th (09h06 - 15h29) and also on April 27th (09h13 - 12h30), the feed was 100% produced from groundwater, further referred to as "event

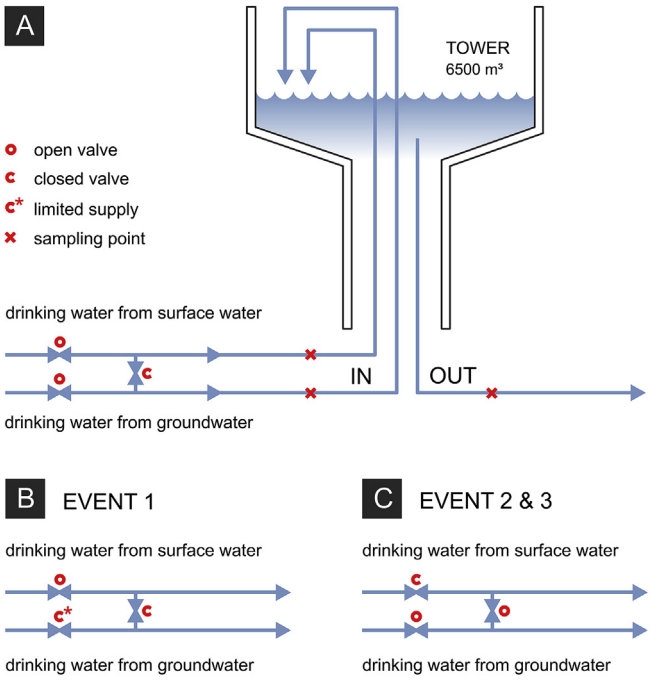

Fig. 1. Layout of the water tower and overview of the sampling points. The configuration of the valves is shown for (A) the normal situation, (B) during event 1, with a change towards a drinking water feed produced from nearly 100% of surface water, and (C) during event 2 and 3, with a change towards a drinking water feed produced from 100% of groundwater.

Table 1
Physicochemical characteristics of the two drinking water feed streams and outgoing stream of the water tower, based on yearly average from 2018 (n = 52). Values are presented as average ± standard deviation. Free chlorine concentrations were below the limit of detection of 30 μg/L. TOC data from the incoming streams in the water tower was not available.

	IN – produced from groundwater	IN – produced from surface water	OUT
Conductivity (μS/cm)	674 ± 44	524 ± 101	551 ± 83
Nitrate (NO_3^- mg/L)	14.15 ± 1.35	10.00 ± 3.17	10.35 ± 2.87
Temperature (°C)	12.9 ± 3.5	13.8 ± 6.2	13.9 ± 5.8
Free chlorine (μg/L)	<30	<30	<30
pH (–)	7.50 ± 0.07	7.89 ± 0.16	7.82 ± 0.12
TOC (mg/L)	–	–	1.58 ± 0.62

2" and "event 3" (Fig. 3). During event 2 and event 3, drinking water produced from groundwater was entering through both feeders (Fig. 1). As seasonal differences are an important factor known to affect the microbial drinking water quality during distribution systems (Nescerecka et al., 2018; Pinto et al., 2014; Prest et al., 2016b), monitoring was repeated in August 2018 without disturbances in the flow, to evaluate the seasonal impact on the biostability of drinking water.

2.2. Flow cytometric measurements

Dynamics of the microbial community of the incoming streams and exiting stream were monitored with online flow cytometry. Samples from all streams were taken automatically every 40 min, were stained and incubated for 20 ± 2 min at 37 °C prior to measurement to allow for stable staining of all cell types. No pretreatment nor dilution was performed. During the monitoring period in April, 933 flow cytometric samples were taken (311 samples per stream), and during the monitoring period in August 2049 samples were taken (683 samples per stream). For automation of these measurements, an onCyt© (onCyt Microbiology AG, Switzerland) robot was coupled to an Accuri™ C6 flow cytometer or an Accuri™ C6 Plus flow cytometer (BD Biosciences, Belgium). The flow cytometers are equipped with a blue (20 mW, 488 nm) and a red laser (12.5 mW, 640 nm), two scatter detectors configured on the blue laser, and four fluorescence detectors with bandpass filters. Three of the bandpass filters are for the blue laser emission (FL1: 533/30 nm, FL2: 585/40 nm, and FL3: 670 LP) and one is for the red laser emission (FL4: 675/25 nm). The lower detection limit of flow cytometry in this type of application is in the order of 10^2 cells/mL and the instrument precision error is below 5%, independent of the cell concentration (Hammes et al. 2008, 2010). The cell concentrations in this study were at least 10 times higher than the lower detection limit, ensuring accurate and precise quantification of cell concentrations in all streams. MilliQ (Merck, Belgium) was used as sheath fluid. A bacteriostatic concentrate solution containing 4.65% EDTA and 0.82% sodium fluoride, (BD Biosciences, Belgium) was added (0.79 vol% final concentration) to prevent bacterial growth in the sheath fluid during the course of the experiments. Staining was performed using a 1000x dilution of SYBR® Green I concentrate (Invitrogen, Belgium) in TRIS buffer with pH 8.2, with a 10 vol% final concentration. Flow cytometry samples were run in fixed volume mode (50 μL) at a flow rate of 66 μL/min. A Cavro® XCalibur Pump (Tecan Trading AG, Switzerland) with 12 channels connects the necessary fluidics, air and waste with the three chambers in the onCyt© robot. The pump regime is adapted to the desired sampling frequency (40 min interval) and size (800 μL total volume) using the onCyt© software.

2.3. Data analysis

Flow cytometric data was analyzed in R (v3.4.4). Flow Cytometry Standard (.fcs) files were imported using the *flowCore* package (v1.44.2) (Hahne et al., 2009). The *FlowAI* package (v1.14.0) was used to check the flow cytometry data quality and to remove events having anomalous values in terms of flow rate stability, signal acquisition and the dynamic range, caused by technical issues (Monaco et al., 2016). Background noise caused by abiotic interferences was removed by manually drawing a gate based on the FL1 and FL3 fluorescence data, as the combination of these two fluorescence parameters results in the most optimal signal and noise separation for drinking water samples (Hammes and Egli 2005, 2010). Further data processing was done using the *Phenoflow* package (v1.1.1) based on the signal height values, with rescaling based on the maximum FL1 fluorescence signal height (FL1-H) after gating of the bacterial population as described by Props et al. (2016). To detect the impact of the disturbed flow regimes on the microbiological quality of water, phenotypic community analysis was performed on the FCM data by the use of advanced fingerprinting (Props et al., 2016; Rogers and Holyst, 2009). In this analysis, the flow cytometry data of every sample is transformed, discretized and concatenated into a one-dimensional vector that serves as basis for further phenotypic community analysis. Fingerprinting was performed using probability binning approach of the package *flowFP* (v1.40.1), constructing a model grid with 5 recursions (2^5 bins) (Rogers and Holyst, 2009). The model was built based on the fluorescence signal height values FL1-H and FL3-H of all samples. From these fingerprints, beta diversity analysis and Bray-Curtis dissimilarity calculations were performed using the *vegan* package (v2.5.4) (Oksanen et al., 2019). Resampling to the lowest sample size (n = 124 cells) was performed prior to beta diversity analysis to account for size-dependent differences.

The Bray-Curtis dissimilarity was chosen above other biological metrics derived from the cytometric fingerprints (*e.g.*, phenotypic typing (Fig. S1) and diversity analysis) to evaluate differences between the microbial community of samples, as, with regard to the industry's needs, this is an easily interpretable, unequivocal parameter quantifying the difference between two cytometric fingerprints. Furthermore, this is a frequently used quantitative dissimilarity index with ecological value (Bray and Curtis, 1957; Legendre and Legendre, 2012). Two identical fingerprints will have a Bray-Curtis dissimilarity of 0, whereas two fingerprints that have no non-empty bins in common, will have a Bray-Curtis dissimilarity of 1 (Greenacre and Primicerio, 2014). A sufficiently large representative set of samples taken during normal operation of the water tower at the beginning of each monitoring period was taken as a baseline (n = 104 in April, n = 106 in August) for dissimilarity comparison. The Bray-Curtis dissimilarity that was assigned to a sample was calculated as the average of the Bray-Curtis dissimilarities between that sample and all of the baseline samples (Fig. 5). As a basis for decisions concerning biological instability, a threshold for event detection was set at the average Bray-Curtis dissimilarity calculated between all baseline samples plus three times the standard deviation of this distribution. This is a rather conservative threshold, that finds its origin in the normal distribution, where it will include 99.87% of the data and thus regard the remaining 0.13%

as deviating events (Howell et al., 1998). This method was carried out to account for inter-sample variability, resulting in a threshold of 0.301 in the case of the monitoring period in April (Fig. 5).

2.4. Statistics

Correlation between variables was calculated using the Pearson correlation coefficient (r_p) for linear correlations. Prior to calculation of correlations, the normality and homoscedasticity of the data was checked and approved (Fig. S2). All statistical analyses were performed in R using the *stats* package (v.3.5.2). The ANOSIM analysis in the *vegan* package (v2.5.4) with 100 permutations was used to determine differences between the cytometric fingerprints of different water types.

2.5. Data availability

The raw flow cytometry data sets have been deposited on FlowRepository and are publicly available under accession ID FR-FCM-Z25U (April monitoring campaign) and accession ID FR-FCM-Z25T (August monitoring campaign).

3. Results

A water tower was monitored in spring using online flow cytometry to simultaneously assess the microbial dynamics in the incoming and exiting streams at high frequency, obtaining the results in real-time by taking a sample of all streams every 40 min (Fig. 1). Incoming drinking water produced from groundwater had a lower average cell density ($3.1 \pm 0.8 \times 10^3$ cells/mL) than incoming drinking water produced from surface water ($1.8 \pm 0.3 \times 10^4$ cells/mL) (Fig. 2). The cell concentrations in the exiting water stream were the highest ($2.7 \pm 0.7 \times 10^4$ cells/mL), and followed the dynamics of the incoming water produced from surface water ($r_p = 0.74$, $p < 0.001$).

The exiting flow showed a recurring diurnal pattern in function of the water demand, with a peak in the morning, and consisted mainly (*i.e.*, 72.48 ± 19.14%) of water produced from surface water (Fig. 3). During the monitoring period in April, the water composition was altered in two different ways by closing of one of the two feeders. During event 1, the water entering the tower originated nearly 100% from surface water, whilst during event 2 and event 3, the water entering the tower was 100% produced from groundwater. During the switch towards 100% of drinking water produced from groundwater, the cell density in the incoming water produced from surface water dropped as well, as groundwater was flowing through both feeders (Fig. 1). During all flow regimes, the total exiting flow rate remained unaffected (Fig. 3).

To evaluate the changes and differences in the microbial community based on the cytometric fingerprints, beta diversity analysis was performed using principal coordinates analysis (PCoA) of the cytometric fingerprints. The analysis reduces the dimension of the cytometric fingerprints and allows visualization of differences between cytometric fingerprints as the proximity of samples. Hence, samples containing microbial communities with similar phenotypic traits are plotted closer to each other. This analysis showed three significantly separated ($R = 0.55$, $p = 0.01$, perm = 100, ANOSIM) communities, associated with both incoming waters and the exiting water (Fig. 4). The difference between the samples of the microbial community of the exiting water (red) and the incoming drinking water produced from surface water (blue) was less pronounced, as the latter comprises for 72.48 ± 19.14% of the incoming feed. During the events, the fingerprint of the exiting water changed. As most of the variance of the data set was captured within the first axis (x-axis), the severity of the change of the cytometric fingerprint of the exiting water during event 2 and 3 (Fig. 4B) that followed this axis, was substantially larger than the shift during event 1 (Fig. 4A). The biggest differences between

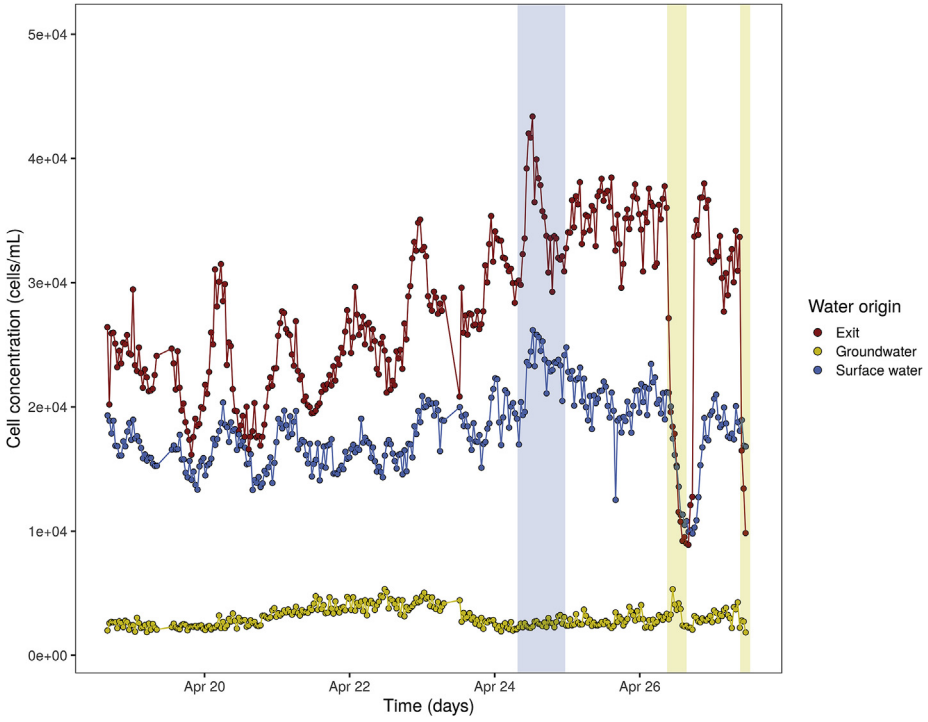

Fig. 2. Flow cytometric total microbial cell concentration of the incoming and exiting water streams of the water tower during the monitoring period in April. Events are highlighted: nearly 100% of the incoming water is produced from surface water (event 1, blue) and 100% of the incoming water is produced from groundwater (event 2 and event 3, yellow). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

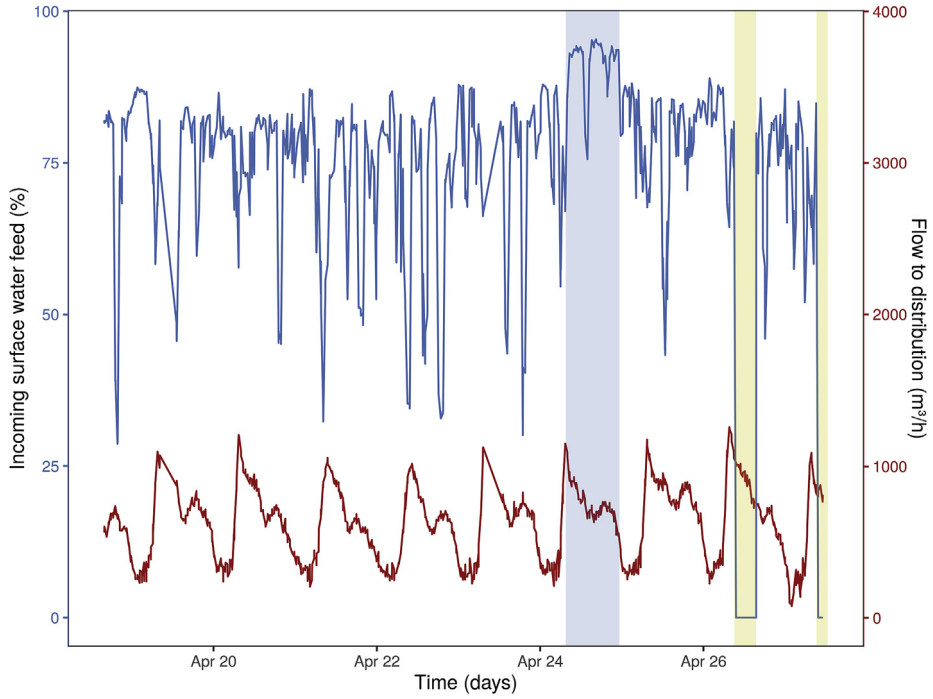

Fig. 3. Percentage of incoming feed produced from surface water (blue) and total exiting flow (red) from the water tower towards the distribution network during the monitoring period in April. Events are highlighted: nearly 100% of the incoming water is produced from surface water (event 1, blue) and 100% of the incoming water is produced from groundwater (event 2 and event 3, yellow). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

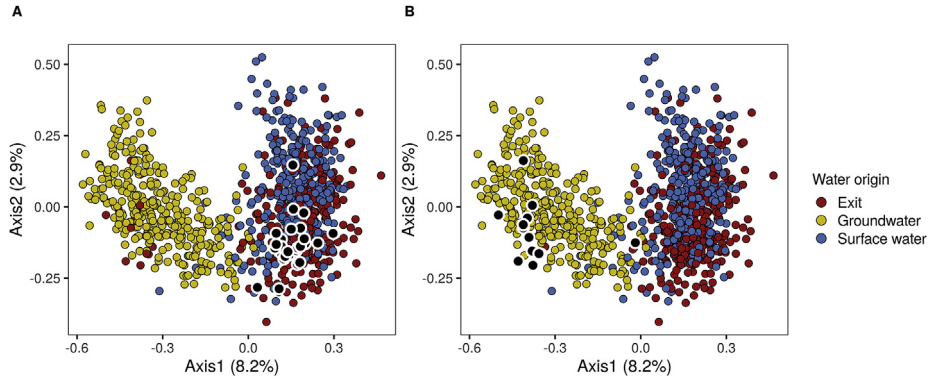

Fig. 4. Beta diversity analysis using principal coordinates analysis (PCoA) of the cytometric fingerprints of all streams based on Bray- Curtis dissimilarities. The effect of the disturbed flow regimes on the exiting water community is highlighted in black: (A) change to feed produced from nearly 100% surface water (event 1) and (B) change to feed produced from 100% groundwater (event 2 and event 3).

exiting samples and their steady state position were observed at the end of events.

As the aim was to provide an unequivocal parameter for online microbial drinking water assessment using advanced flow cytometry data analysis, the proximity of events was calculated using the Bray-Curtis dissimilarity. A sufficiently large representative set of samples taken during normal operation of the water tower at the beginning of the monitoring period was taken as a baseline ($n = 104$) for dissimilarity comparison (Fig. 5). Based on the distribution of the baseline Bray-Curtis dissimilarity, a threshold of 0.301 was set for event detection in the exiting stream. This method indicated a microbial water quality change when switching towards a single feed water produced from groundwater (event 2 and event 3). The feed change towards nearly 100% drinking water produced from surface water (event 1) was not indicated as a microbial water quality change, as all Bray-Curtis dissimilarities compared to the baseline regime were below 0.301 during that event.

Monitoring was repeated in August to evaluate the seasonal influences on the biostability of the drinking water. The regrowth (*i.e.*, the proportional difference between the average cell concentration in the exiting and incoming water) in August was not higher than the regrowth measured in April, although on average, the temperature was higher and free chlorine concentrations were lower (Table 2). The exiting stream contained a higher ($6.1 \pm 2.1 \times 10^4$ cells/mL) average cell density than incoming drinking water produced from surface water ($4.7 \pm 1.8 \times 10^4$ cells/mL) (Fig. 6A). The cell concentrations in the feed produced from groundwater were the lowest ($3.3 \pm 1.9 \times 10^4$ cells/mL). Compared to the measurements in spring, the measured cell concentrations were higher on average in all streams, with a ten times higher microbial load in the incoming water produced from groundwater.

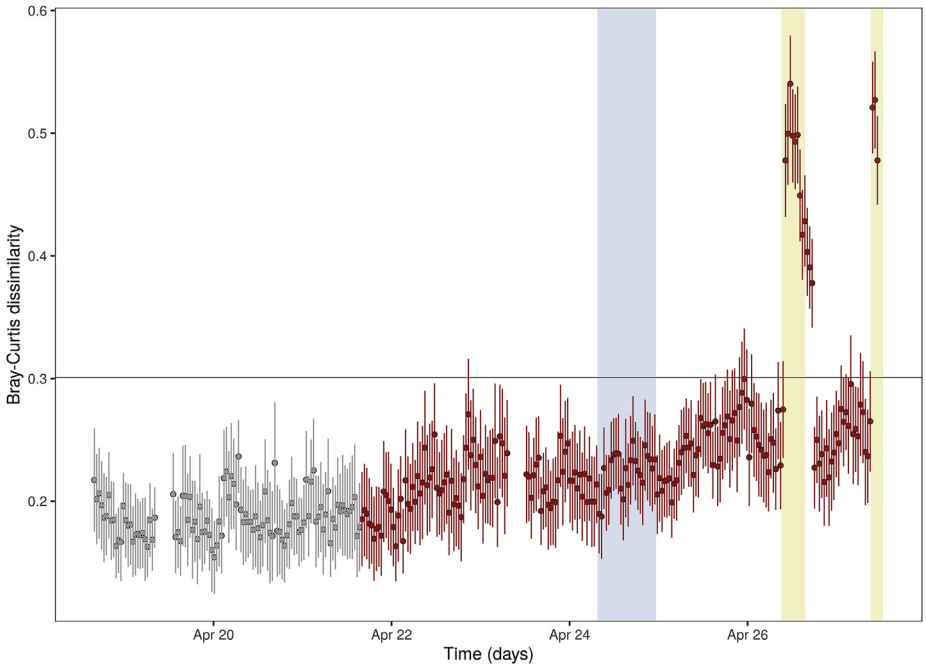

Fig. 5. Bray- Curtis dissimilarity of cytometric fingerprints of the exiting stream from the water tower. Samples in grey (n = 104) are used as baseline as they were taken during a normal feeding regime. Bray- Curtis dissimilarity assigned to a sample is calculated as the average Bray- Curtis distance between that sample and all baseline samples. Based on the distribution of the baseline Bray-Curtis dissimilarity, a threshold of 0.301 was set for event detection. Events with nearly 100% incoming water produced from surface water (event 1) and 100% incoming water produced from groundwater (event 2 and event 3) are respectively highlighted in blue and yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The microbial cell density in the incoming water produced from surface water was 2.5 times higher, and the exiting water had a 2.2 times higher microbial load than in April. The bacterial cell concentration in both incoming waters showed a steadily increase over time, as a result of the loss of residual free chlorine in the system and an exceptionally high water temperature during the extremely hot summer months, as confirmed by the drinking water provider. Based on the distribution of the baseline Bray-Curtis dissimilarity (n = 106), a threshold of 0.391 was set for event detection in the exiting stream. The gradual regrowth attributed to a loss of chlorine residual was not indicated as event in the exiting stream, only at the end of the monitoring period the Bray-Curtis dissimilarity indicated a change in microbial drinking water quality (Fig. 6D). This is in contrast to the incoming streams, where gradual changes were reflected in the Bray-Curtis dissimilarity around August 14–17th (Fig. 6BC). For the incoming streams, the threshold was 0.457 and 0.303 for the stream produced from groundwater and surface water respectively.

4. Discussion

4.1. Continuous full-scale monitoring of microbial drinking water quality using online flow cytometry

Online flow cytometric measurements of the incoming and exiting streams of a water tower showed clear regrowth, despite the short hydraulic residence time of 6–8 h (Fig. 2). The cell concentration of the exiting stream followed the fluctuations of the cell concentration of the incoming water produced from surface water, because this feed stream accounted for 72.48 ± 19.14% of the total incoming feed. These fluctuations can be interpreted as short-term operational variability, and are most likely caused by changes in the drinking water production and/or source water quality (Nescerecka et al., 2018). Mixing of both incoming waters may result in a more balanced nutrient composition, and, hence, facilitate bacterial regrowth in the water tower, because both streams may have different limiting nutrients, which may on the long-term result in higher biological instability. This “mixing effect”, has been previously observed in grab samples of full-scale drinking water distribution systems, where the biological instability (or regrowth potential) was higher in mixed samples than samples produced from single water sources (Nescerecka et al. 2014, 2018; Niquette et al., 2001). However, in this study, regrowth was the highest when feeding the tower only with drinking water produced from surface water. On the contrary, shifting towards a single groundwater source steeply decreased the cell concentration of the exiting stream. These effects stem from the differences in water source, as groundwater generally harbors a 10–100 times lower microbial load than surface water (Besmer and Hammes, 2016). This is often attributed to the lower nutrient concentration, with lower organic carbon concentrations in particular (Hofmann and Griebler, 2018; Prest et al., 2016b). This is also the case for this study in which the total organic carbon concentration (TOC) of the drinking water produced from groundwater was lower (0.74 ± 0.12 mg/L – average of 2018, measured at the production site) than the drinking water produced from surface water (1.77 ± 0.48 mg/L – average of 2018, measured at the production site). Both disturbed flow regimes were reflected in the cell concentration measured using online flow cytometry, showing its value as parameter for monitoring microbial drinking water quality and event detection.

Monitoring was repeated in August to evaluate the long-term biostability of the water, with higher water temperatures and lower free chlorine concentrations (Table 2, Fig. 6A). Compared to the measurements in spring, the measured cell concentrations were higher on average in all streams, with even a ten times higher microbial load in the incoming water produced from groundwater. Furthermore, the bacterial cell concentration of this stream showed a steady increase over time, due to the loss of residual free chlorine

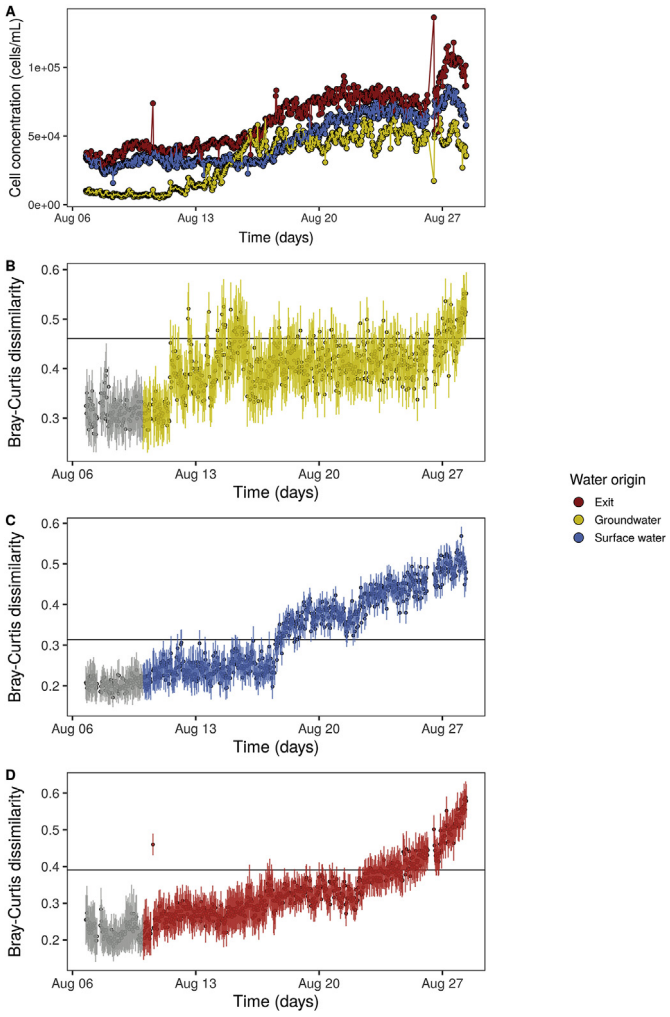

Fig. 6. (A) Flow cytometric total cell concentration of the incoming and exiting water streams of the water tower during the monitoring period in August. Other plots show the Bray-Curtis dissimilarity of cytometric fingerprints of the (B) the incoming stream produced from groundwater, (C) the incoming stream produced from surface water and (D) exiting stream from the water tower. Samples in grey (n = 106) are used as baseline as they were taken during a normal feeding regime. Bray-Curtis dissimilarities of other samples are calculated as the average Bray-Curtis distance between that sample and all baseline samples. A threshold of 0.391 was set for event detection in the exiting stream. For the incoming streams, the threshold was 0.457 and 0.303 for the stream produced from groundwater and surface water respectively.

Table 2
Quantity and properties of the drinking water streams entering and leaving the water tower, compared between spring 2018 (April) and summer 2018 (August). "n.a.": not applicable. Most of the free chlorine concentrations were below the limit of detection of 30 µg/L.

	IN - produced from groundwater		IN – produced from surface water		OUT	
	Spring	Summer	Spring	Summer	Spring	Summer
Flow (m^3/h)	150.2	51.3	502.8	465.3	637.1	485.3
Residence time (h)	n.a.	n.a.	n.a.	n.a.	6	7.86
Temperature (°C)	11.7	19.49	12.45	23.01	12.55	22.25
Free chlorine (µg/L)	<30	<30	<30	<30	40	<30

at the entrance of the system after August 14th, which was confirmed by the drinking water provider. Also, the extremely hot summer months of 2018 resulted in exceptionally high water temperature up to 20 °C for longer periods of time, sustaining bacterial regrowth in the network. Seasonal changes, influencing the drinking water temperature, have been previously observed to affect biostability of drinking water systems (Nescerecka et al., 2018; Pinto et al., 2014; Prest et al., 2016b). Also the loss of residual chlorine caused a clear increase in the microbial abundance in the usually stable incoming stream produced from groundwater. This means that the bacterial community in the water tower is not truly biostable, as bacterial regrowth occurs substantially when the chlorine residual is no longer detected (Lautenschlager et al., 2013). Monitoring of the bacterial regrowth is, thus, valuable to understand biostability in the drinking water network. Besides this, interpretation of the high-dimensional flow cytometric data and quantification of microbial water quality changes in an unambiguous way are needed for full-scale operational use in the drinking water industry (Safford and Bischel, 2019). We therefore propose the development of a parameter derived from flow cytometric fingerprinting for online event detection, as it not only takes into account the microbial abundance, but also changes in the microbial community composition as basis for indicating water quality changes.

4.2. Cytometric fingerprinting for online event detection in a full-scale system

To evaluate the effect of operational changes in flow regime on the microbial community, the cytometric fingerprints of the microbial communities were compared (Fig. 4). The PCoA analysis revealed that samples taken from the feed produced from groundwater harbor a different community than samples taken from both other streams, based on the multidimensional FCM data. This was confirmed in an additional analysis, in which the communities were divided in two different community "types" using k-medoids clustering based on their cytometric fingerprint, as described by Props et al. (2018) (Fig. S1). This algorithm could distinguish two different community types, indicating that the bacterial community in the feed water produced from groundwater was different from the bacterial communities in both other streams. In line with these analyses, the events when groundwater was used as the single drinking water source for the tower could be detected based on the cytometric fingerprints. However, the outcome of these analyses is abstract and lacks nuance, making it difficult to interpret. Consequently, is less suited for operational decision-making in practice concerning microbial water quality changes.

Complementary to the total cell concentration, we propose the additional implementation of the pairwise Bray-Curtis dissimilarity between fingerprints as straightforward metric to detect deviating events that caused a microbial water quality change (Fig. 5, Fig. 6 BCD). Based on the average Bray-Curtis dissimilarity between cytometric fingerprints of samples taken in the baseline regime plus three times the standard deviation, a conservative threshold was determined for event detection. The two independent data sets of April and August showed similar results: an average baseline Bray-Curtis dissimilarity during normal regime of 0.19 ± 0.04 and 0.22 ± 0.05 respectively, resulting in a threshold of 0.301 and 0.391. In this respect, we define an event as two or more subsequent samples having a higher Bray-Curtis dissimilarity relative to the baseline. Single outlying data points (*e.g.*, August 10th, 13h36 in exiting stream, Fig. 6D) are, therefore, not considered as real event of biological instability caused by a water quality change, as the detection of a single event could be caused by imperfect measurements or a short nonstructural change.

When evaluating the monitoring campaign in April, this method indicated a microbial water quality change when switching towards a single feed water produced from groundwater (event 2 and

event 3). The feed change towards nearly 100% drinking water produced from surface water (event 1) was not indicated as a microbial water quality change, as all Bray-Curtis dissimilarities compared to the baseline regime were below 0.301. This is in line with our expectations, as the change from 72% towards a nearly pure feed (almost 100%) originating from surface water is smaller compared to the changing from 28% to 100% feed produced from groundwater. Sudden and large changes were unambiguously detected. However, during the monitoring period in August, gradual regrowth attributed to the temperature increase and the loss of chlorine residual was not indicated as event in the exiting stream. Only at the end of the monitoring period, the Bray-Curtis dissimilarity indicated a change in microbial drinking water quality (Fig. 6D). This is in contrast to the incoming streams, where gradual changes were reflected in the Bray-Curtis dissimilarity around August 14–17th (Fig. 6BC). These changes were also observed in the cell concentrations of these streams. Increase of the drinking water temperature, combined with a loss of chlorine residual will result in regrowth and, thus, biological instability (Gillespie et al., 2014; Hoefel et al., 2005; Nescerecka et al., 2014). The effect of temperature increase and chlorine residual loss was observed in both the Bray-Curtis dissimilarity and the cell concentrations. Overall, we present the Bray-Curtis dissimilarity as a straightforward basis for decision-making concerning the "degree of acceptable change" based on high-dimensional flow cytometric data. Hereby, we define the degree of acceptable change as all Bray-Curtis dissimilarities below the threshold, which is calculated as compared to a representative set of samples taken during the normal flow regime. The combination of the total cell concentration and the Bray-Curtis dissimilarity is a more holistic approach for microbial drinking water quality changes than considering solely the cell concentration, as it also considers the stability of the microbial community composition. The meaning of the Bray-Curtis dissimilarity between cytometric fingerprints can be interpreted in a complementary way to the cell concentration in the water. For example, increasing cell concentrations in combination with a stable Bray-Curtis dissimilarity over time (*e.g.*, event 1), indicate a community that is structurally stable despite the occurrence of (limited) regrowth. Hence, the information of both parameters should be combined for the detection of water quality changes and the development of early-warning systems in the drinking water industry. More specifically, the combination of a "static alarm" based on the total cell concentration to indicate excessive regrowth, and a "dynamic alarm" based on the Bray-Curtis dissimilarity compared to a representative normal baseline situation, forms a holistic basis for a first smart screening for further decision-making concerning the microbial drinking water quality. When placed at strategic points in the drinking water network, such as the water tower discussed in this research, it can be realistically implemented to safeguard the drinking water quality as cost-effective technology providing easily interpretable parameters to the industry.

5. Conclusion

Flow cytometric monitoring of drinking water networks allows the collection of extensive and detailed data compared to *e.g.*, weekly plating. From this full-scale monitoring campaign, it was concluded that even though the microbial water quality complies with the legal standards, the studied full-scale drinking water tower is not always biologically stable. Changes in drinking water source, increase in temperature and lack of residual chlorine drastically impacted both the cell concentrations and Bray-Curtis dissimilarity derived from the flow cytometric fingerprints, compared to the baseline situation. We demonstrated that the analysis of flow cytometric data can be used as basis for the development of straightforward parameters to define the microbial water quality in a holistic way, by combining the microbial abundance (cell concentrations) and the stability of the microbial community composition (Bray-Curtis dissimilarity). This is an example on full-scale of how flow cytometric fingerprinting can be used as sensitive early-warning system for changes in water quality.

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.

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Appendix A. Supplementary data

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