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# Dynamics of bacterial communities before and after distribution in a full-scale drinking water network

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## ABSTRACT

Understanding the biological stability of drinking water distribution systems is imperative in the framework of process control and risk management. The objective of this research was to examine the dynamics of the bacterial community during drinking water distribution at high temporal resolution. Water samples (156 in total) were collected over short time-scales (minutes/hours/days) from the outlet of a treatment plant and a location in its corresponding distribution network. The drinking water is treated by biofiltration and disinfectant residuals are absent during distribution. The community was analyzed by 16S rRNA gene pyrosequencing and flow cytometry as well as conventional, culture-based methods. Despite a random dramatic event (detected with pyrosequencing and flow cytometry but not with plate counts), the bacterial community profile at the two locations did not vary significantly over time. A diverse core microbiome was shared between the two locations (58–65% of the taxa and 86–91% of the sequences) and found to be dependent on the treatment strategy. The bacterial community structure changed during distribution, with greater richness detected in the network and phyla such as *Acidobacteria* and *Gemmatimonadetes* becoming abundant. The rare taxa displayed the highest dynamicity, causing the major change during water distribution. This change did not have hygienic implications and is contingent on the sensitivity of the applied methods. The concept of biological stability therefore needs to be revised. Biostability is generally desired in drinking water guidelines but may be difficult to achieve in large-scale complex distribution systems that are inherently dynamic.

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**Abbreviations**

ANOSIM	analysis of similarity
AOC	assimilable organic carbon
ATP	adenosine triphosphate
DWDS	drinking water distribution system
FCM	flow cytometry
HPC	heterotrophic plate count
MDS	multidimensional scaling
OTU	operational taxonomic unit
TOC	total organic carbon
WTP	water treatment plant

**1. Introduction**

Drinking water distribution systems (DWDSs) serve as a vital network for transporting clean, safe, palatable and ideally biologically stable water. These systems are complex, governed by variable natural and operational conditions that influence the indigenous microbial communities which thrive in the water, biofilm and sediments. Monitoring water quality during distribution and establishing appropriate remedial actions are therefore imperative in the framework of process control and risk management (Smeets et al., 2010). Biological stability is generally defined as the inability of water (and/or pipe material) to promote microbial growth, and many guidelines have been proposed for its evaluation, linked to conventional parameters such as assimilable organic carbon (AOC), biofilm formation rate and heterotrophic plate counts (Liu et al., 2013; van der Kooij, 2000). Since these methods are mostly indicative and often inaccurate, recent studies have recommended more advanced, sensitive tools such as pyrosequencing and flow cytometry that can provide in-depth qualitative and quantitative data on microbial cell concentrations and community structure variations (Lautenschlager et al., 2013; Liu et al., 2013; Prest et al., 2014).

To deliver biologically stable water to end users water utilities normally apply a final oxidative disinfection step (primarily chlorination) and maintain a sufficient disinfectant residual in the network to suppress microbial growth (LeChevallier et al., 1993). Chlorine however has instigated customer complaints concerning taste and odor and has been linked with harmful by-products such as trihalomethanes (Rook, 1976). Chlorination can inhibit certain microorganisms while selecting for opportunistic pathogens that are relatively chlorine-resistant such as *Mycobacterium avium* (Ingerson-Mahar and Reid, 2012). Moreover, disinfectant residuals can react with particles, organics and pipe material releasing AOC that can be consumed by microorganisms, contributing to biological instability (Polanska et al., 2005; Ramseier et al., 2011). As an alternative, high-quality, biologically stable water can be produced by limiting organic carbon and other growth-supporting nutrients during treatment (van der Kooij, 2000) and this is often carried out by benign microbial communities colonizing biological filters.

Previous studies have examined spatial and temporal variations in the microbial water quality, in both chlorinated

and non-chlorinated DWDSs. Distribution effects were mainly determined based on sampling a few locations in the network (Lautenschlager et al., 2013; Liu et al., 2014; McCoy and VanBriesen, 2014) while temporal studies focused primarily on monthly or seasonal variations (Hu et al., 1999; Pinto et al., 2014; Revetta et al., 2010). Most studies reported long-term effects to be more significant than spatial variations, although distribution network samples were rarely compared to the original treatment plant samples. Studies investigating in-depth short-term dynamics are scarce but important for establishing the specific trend characteristic of a particular DWDS, allowing deviations from that trend to be easily recognized and investigated.

In this study, a full-scale, well-maintained drinking water distribution system was sampled intensively over short time-scales (minutes/hours/days) and across two locations (156 samples in total), and analyzed using advanced techniques like 16S rRNA gene pyrosequencing and flow cytometry as well as conventional, culture-based methods like plate counts. The objective of the research was to (i) examine bacterial dynamics at high temporal resolution and (ii) determine the impact of distribution on the water microbiology, in an effort to evaluate the biological stability of systems that apply biofiltration treatment and distribute water without disinfectant residuals.

**2. Materials and methods****2.1. Sampling scheme**

The research was conducted on a drinking water treatment plant in The Netherlands and its corresponding distribution network. The plant treats surface water from the Meuse River by coagulation, flocculation and sedimentation followed by ozonation, rapid dual-medium filtration and granular activated carbon filtration. The filtrate is dosed with chlorine dioxide to a concentration of 0.1 mg ClO<sub>2</sub> L<sup>-1</sup> before being pumped into a storage reservoir located at the plant. The effluent of this reservoir that is distributed contains no detectable disinfectant residual. The drinking water production rate of the plant is 4 × 10<sup>7</sup> m<sup>3</sup> year<sup>-1</sup> (Prest et al., 2014) and it is the sole source of water for this distribution network (no mixing). The pipes in this network are made of cemented steel and (at some locations) PVC. Bulk water samples were collected simultaneously from the outlet of the water treatment plant (WTP) and from one location in the distribution network, both from a continuously running tap to avoid unwanted stagnation influences. The hydraulic residence time was estimated by the local water utility to be 2 days. The research comprised three parts; hour, day and week studies and an overview of these studies is shown in Table 1. In the (i) hour study, samples were taken from the two locations every five minutes for one hour, done in the morning (8–9 am) and afternoon (1–2 pm) of the same day, and resulting in 52 samples. In the (ii) day study, samples were taken every hour for an entire day (48 samples). In the (iii) week study, samples were taken four times a day (at 8 am, 11 am, 2 pm and 4 pm) for one week, resulting in 56 samples. The sampling was conducted in August 2012 when the water temperature was

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