Supplementary Material

Biofilm Detachment Significantly Affects Biological Stability of Drinking Water during Intermittent Water Supply in a Pilot-Scale Water Distribution System

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Running Title

Biofilm detachment in uninterrupted and intermittent water supply systems

Keywords

Intermittent water supply; drinking water microbiome; drinking water biofilms; phenotypic fingerprinting; nitrification; monochloramine

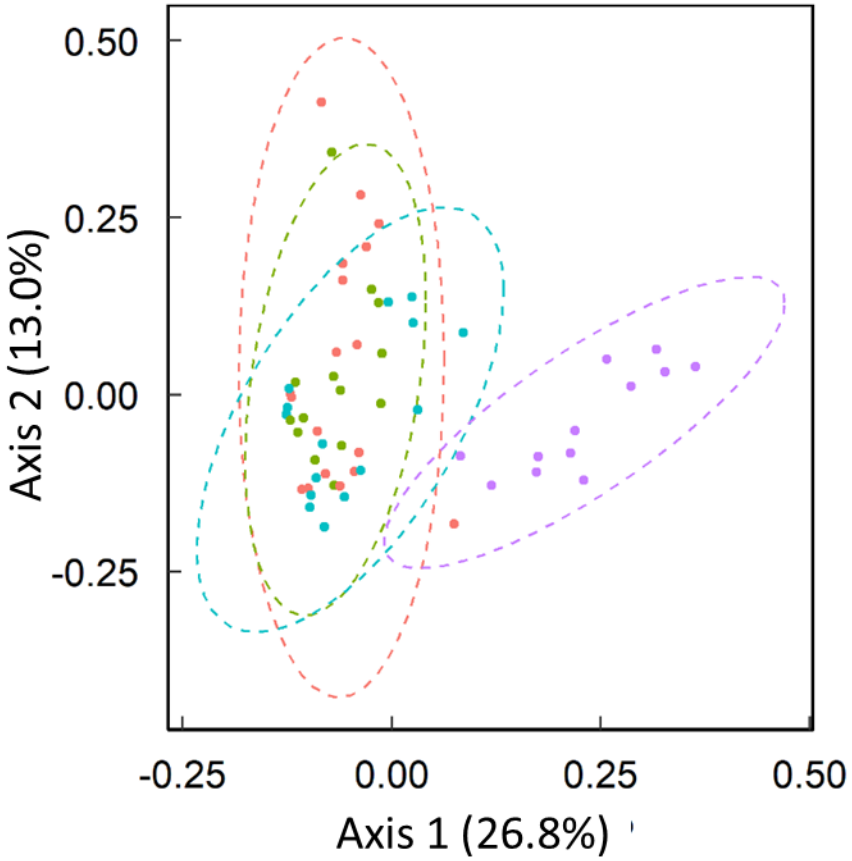
Table S1 Physicochemical parameters measured in bulk water along with the respective instruments and methods

ID	Parameter (unit)	Instrument	Kit used
1	Conductivity (µS/cm)	Myron L Ultrameter II™ 6PFC[E]	-[A]
2	Total Dissolved Solids (mg/L)		-
3	Temperature (°C)		-
4	Monochloramine (mg/L)	Hach DR900 Multiparameter Portable Colorimeter	Cat. No. 10171
5	Ammonia-nitrogen (mg/L)	Hach DR3900 Laboratory Spectrophotometer	TNT830
6	Nitrite-nitrogen (mg/L)		TNT839
7	Nitrate-nitrogen (mg/L)		TNT835
8	Total Nitrogen (mg/L)	Shimadzu TOC-L_{CSH}	-
9	Total Carbon (mg/L)		-
10	Total Inorganic Carbon (mg/L)		-
11	Total Organic Carbon (mg/L)		-
12	Chloride (mg/L)	Shimadzu Prominence HIC-SP	-
13	Sulphate (mg/L)		-
14	Turbidity	HACH DR900 with the program 745FAU method [Turbidity – Absorptometric Method (21-1000 FAU)	

[A] Not applicable

Table S2 Comparison of deviating events identified by Bray-Curtis dissimilarity from metabarcoding and fingerprinting approaches

ID	Repeat	No. of total outflow events	No. of events deviating from inflow based on metabarcoding	No. of events deviating from inflow based on fingerprinting	No. of overlapping deviating events
CWS_{slow} (16)	1st	6	0	0	0
	2nd	6	3	0	0
	3rd	4	0	0	0
CWS_{fast} (41)	1st	15	12	1	1
	2nd	13	12	10	9
	3rd	13	1	0	0
IWS_{fast} (43)	1st	14	7	5	5
	2nd	14	6	2	2
	3rd	15	1	0	0
Sum	-	100	37	18	17



X1	X2	P value (PERMANOVA)	P value (betadisper)
CWS_{slow} inflow	CWS_{fast} inflow	0.494	0.146
CWS_{slow} inflow	IWS_{fast} inflow	0.157	0.934
CWS_{slow} inflow	City water	<0.001	0.095
CWS_{fast} inflow	IWS_{fast} inflow	0.136	0.095
CWS_{fast} inflow	City water	<0.001	0.004
IWS_{fast} inflow	City water	<0.001	0.041

Figure S1 PCoA plot of microbial communities in the inflow for three repeated experiments compared to those in city water (CWS_{slow} n = 324; IWS_{fast} and CWS_{fast}; n = 405) based on 16S rRNA gene metabarcoding. The community diversity of inflow water was stable over time and significantly different from that of city water based on Bray-Curtis dissimilarity indices. Thus, in the subsequent analysis, we grouped the repeats

according to each experiment. Both PERMANOVA and analysis of multivariate homogeneity of group dispersions (variances) via *betadisper* have been carried out with 9,999 permutations.

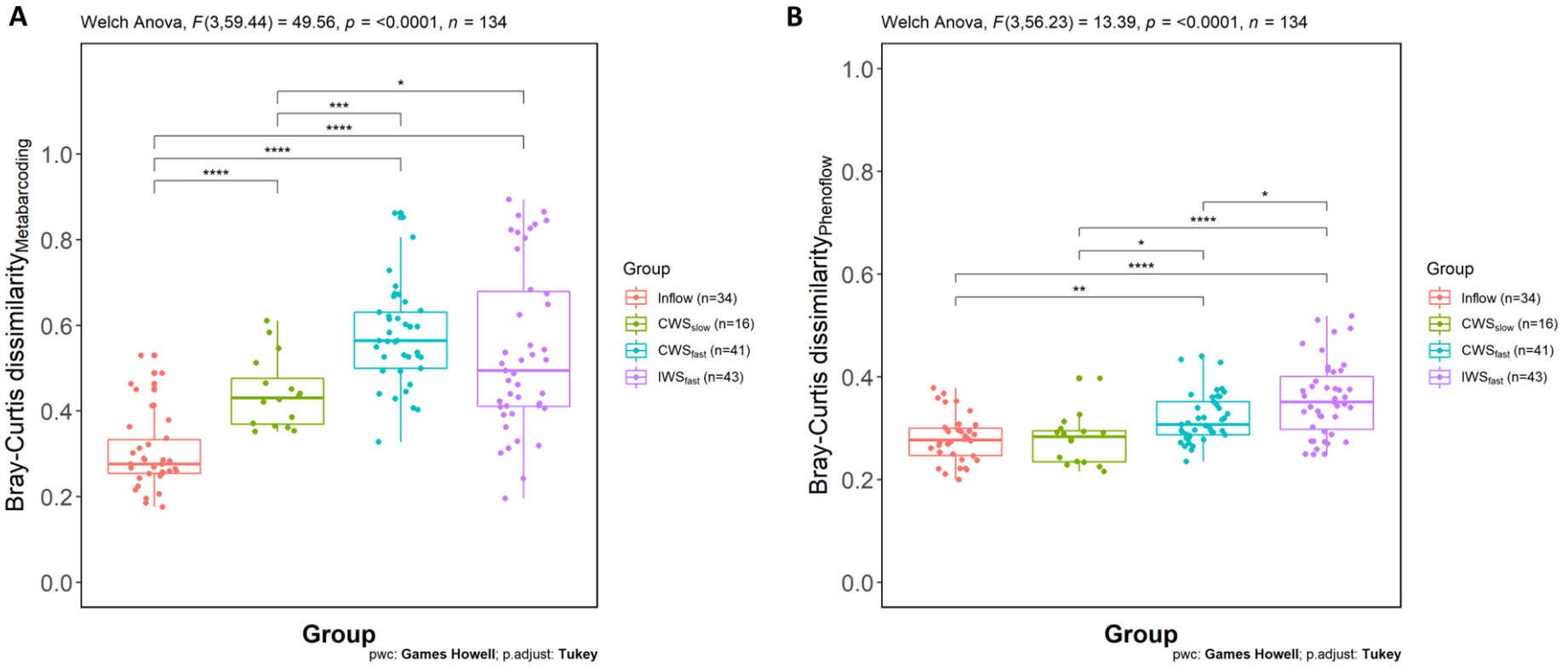


Figure S2. Comparison of average Bray-Curtis dissimilarity indices calculated using (A) 16S rRNA metabarcoding data at the ASV level ($\overline{BC}_{\mathrm{MBC}}$) and (B) flow cytometric fingerprinting data ($\overline{BC}_{\mathrm{PF}}$) from the same samples.

Microbial communities were relatively stable for the inflowing water throughout the experimental period with average Bray-Curtis dissimilarity indices, BC_{MBC} = 0.30 ± 0.09 and BC_{FP} = 0.28 ± 0.05 as shown in Figure S2A and S2B, respectively.

The Bray-Curtis dissimilarity indices in outflow samples from CWS_{slow} based on metabarcoding, BC_{MBC}, were 0.44 ± 0.07, 0.45 ± 0.09, and 0.44 ± 0.12 for Repeats 1, 2 and 3, respectively, and statistically different from those in the inflow and in outflow samples from CWS_{fast} (0.57 ± 0.11) and IWS_{fast} (0.54 ± 0.19) (Figure S2A). A similar trend was observed for phenotypic fingerprinting based on BC_{PF} (Figure S2B).

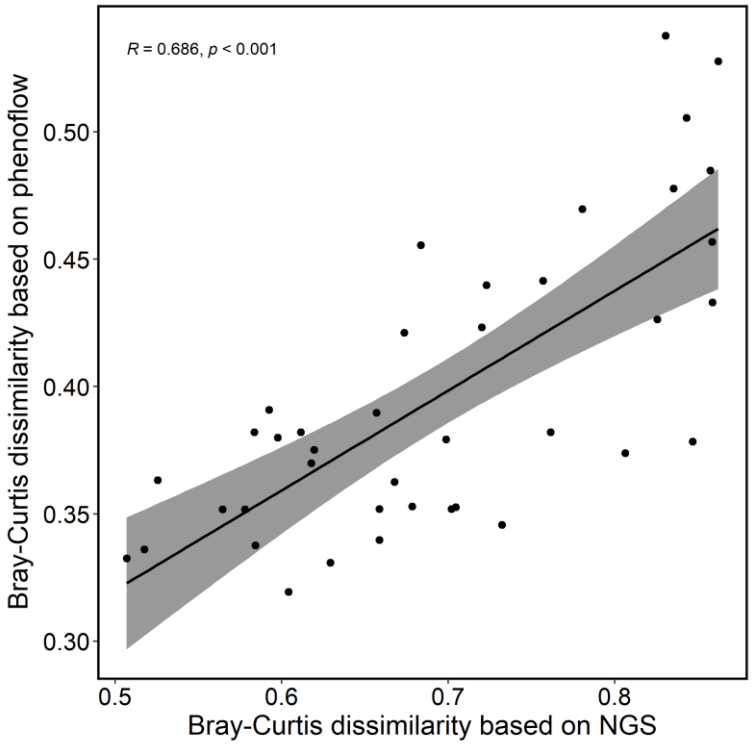


Figure S3 Spearman correlation of Bray-Curtis dissimilarity values during the first 60 seconds from 16S rRNA gene metabarcoding (BC_{MBC}) and Phenoflow FCM-phenotypic fingerprinting data from the same samples. Shaded area indicates 95% confidence interval. Because the deviating events happened mainly during the first 60 seconds, only the Bray-Curtis dissimilarity indices during this period are compared.

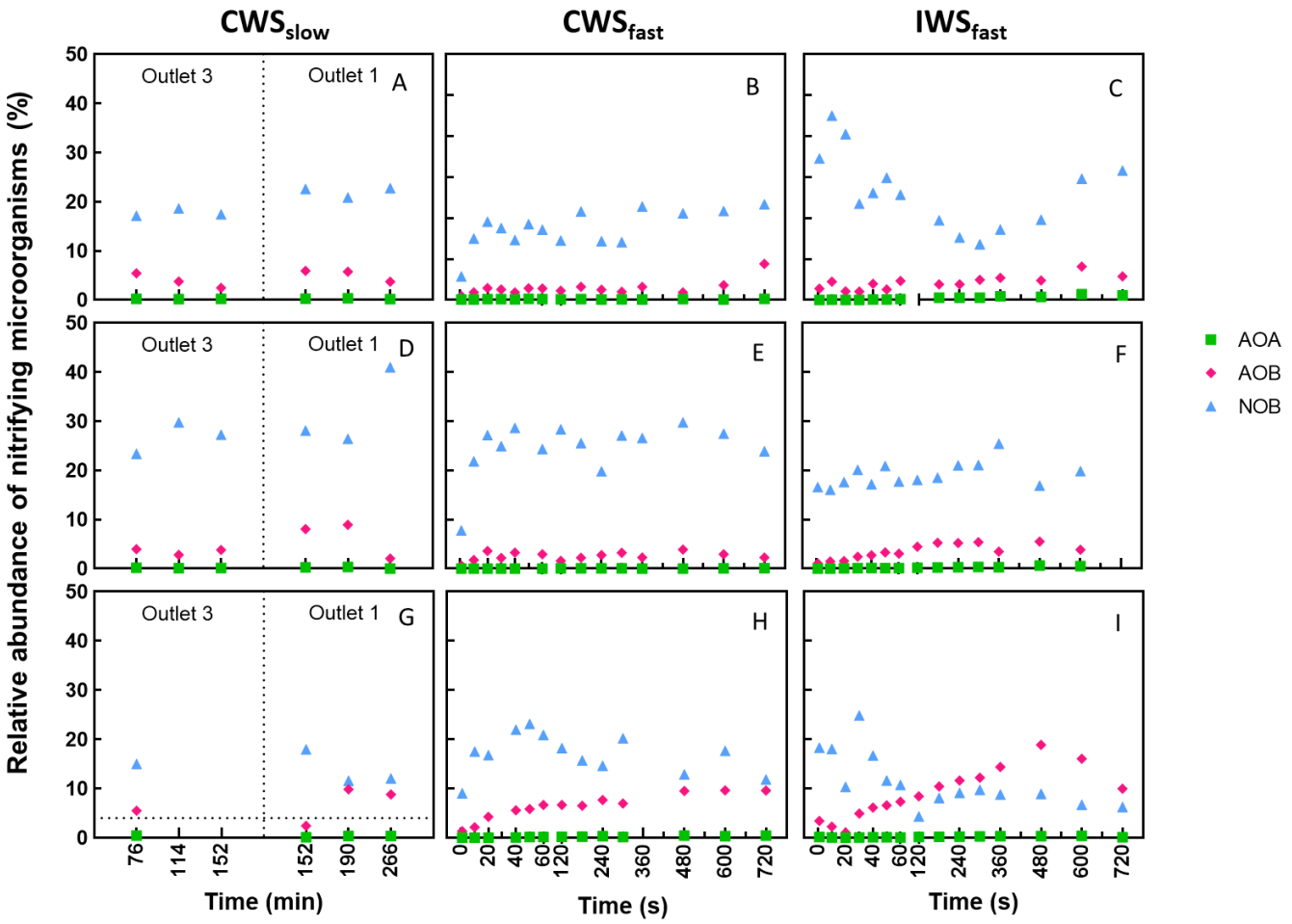


Figure S4 Relative abundance (derived from 16S rRNA metabarcoding) of ammonia oxidizing bacteria (AOB) and archaea (AOA) and nitrite oxidizing bacteria (NOB) in bulk water throughout the experimental period and under all flow conditions.

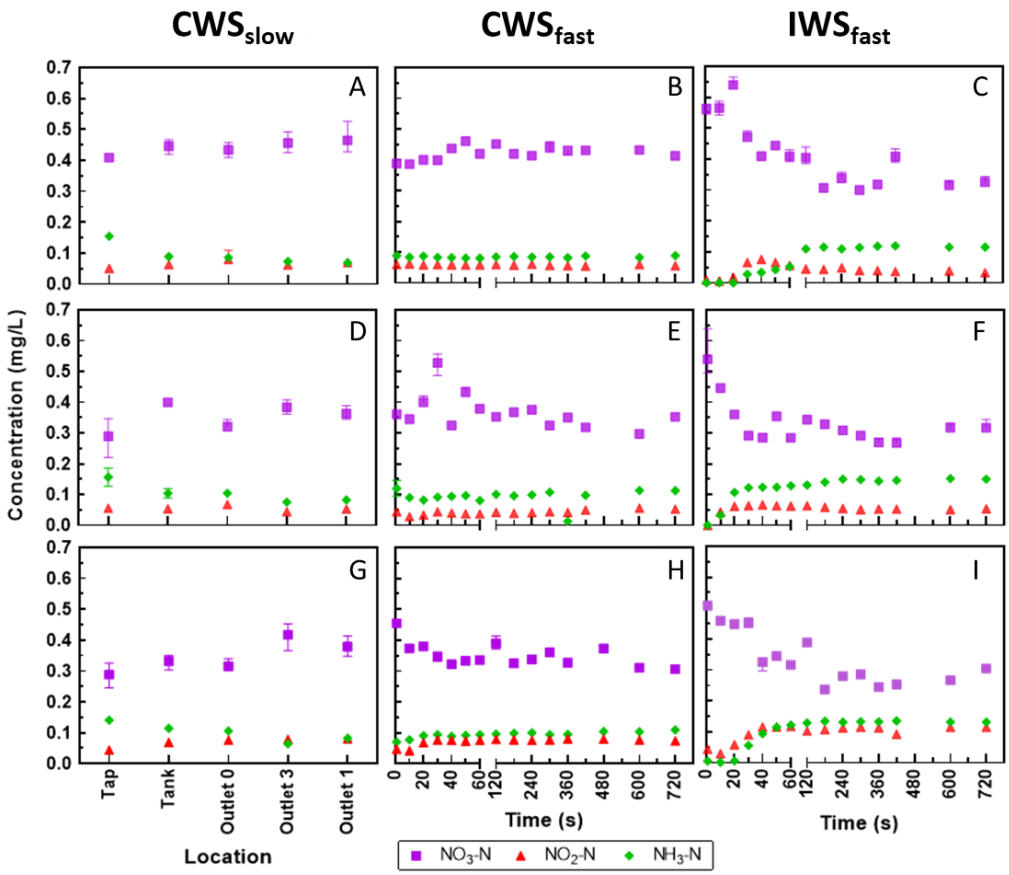


Figure S5 Occurrence of the nitrogen species ammonia (NH_3-N), nitrite (NO_2-N) and nitrate (NO_3-N) in three repeat bulk water samples. Decreasing ammonia concentrations in parallel with an increase of nitrite and nitrate indicate the presence of biofilm communities capable of oxidizing the amine group in the chemical disinfectant monochloramine in the IWS. Error bars indicate standard deviations and measurement was done in biological triplicates (n = 3) for each time point and nitrogen species.