

Supplementary Information - Figures

Bacterial release from pipe biofilm in a full-scale drinking water distribution system

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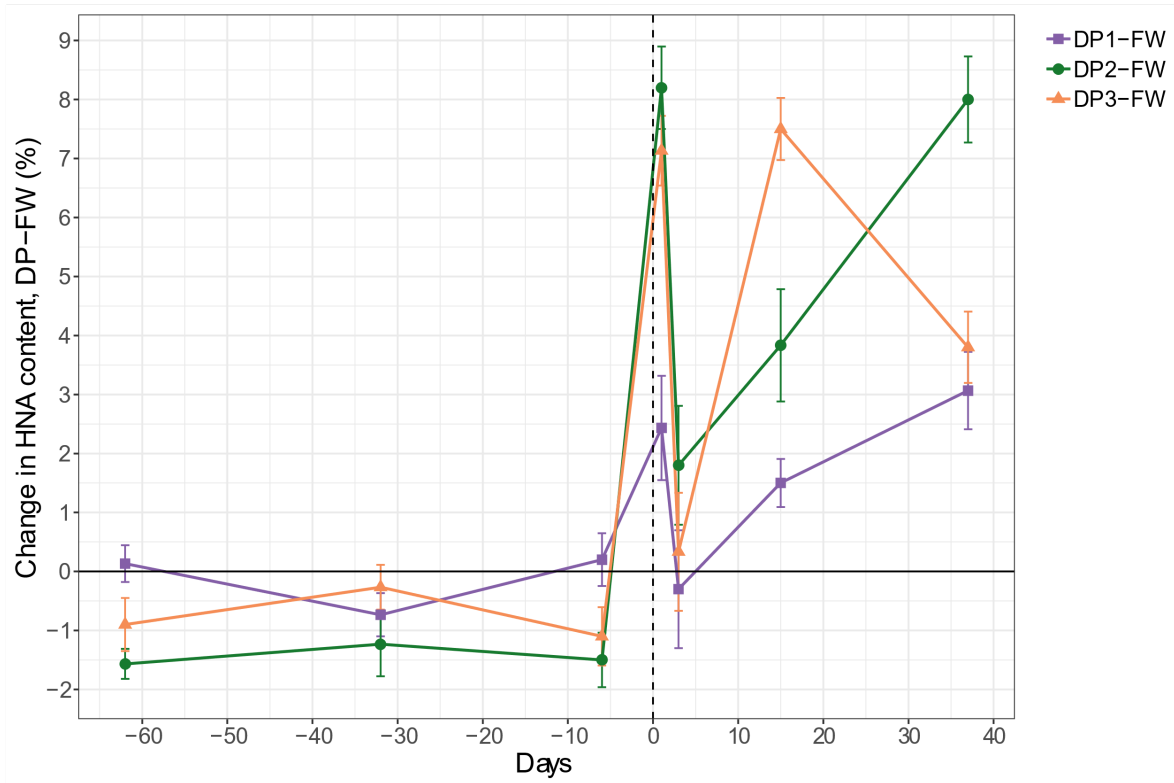
Supplementary Figure 1 – Changes in high nucleic acid (HNA) content bacteria in DWDS

Supplementary Figure 2 – Intact cell concentrations in the DWTP and DWDS

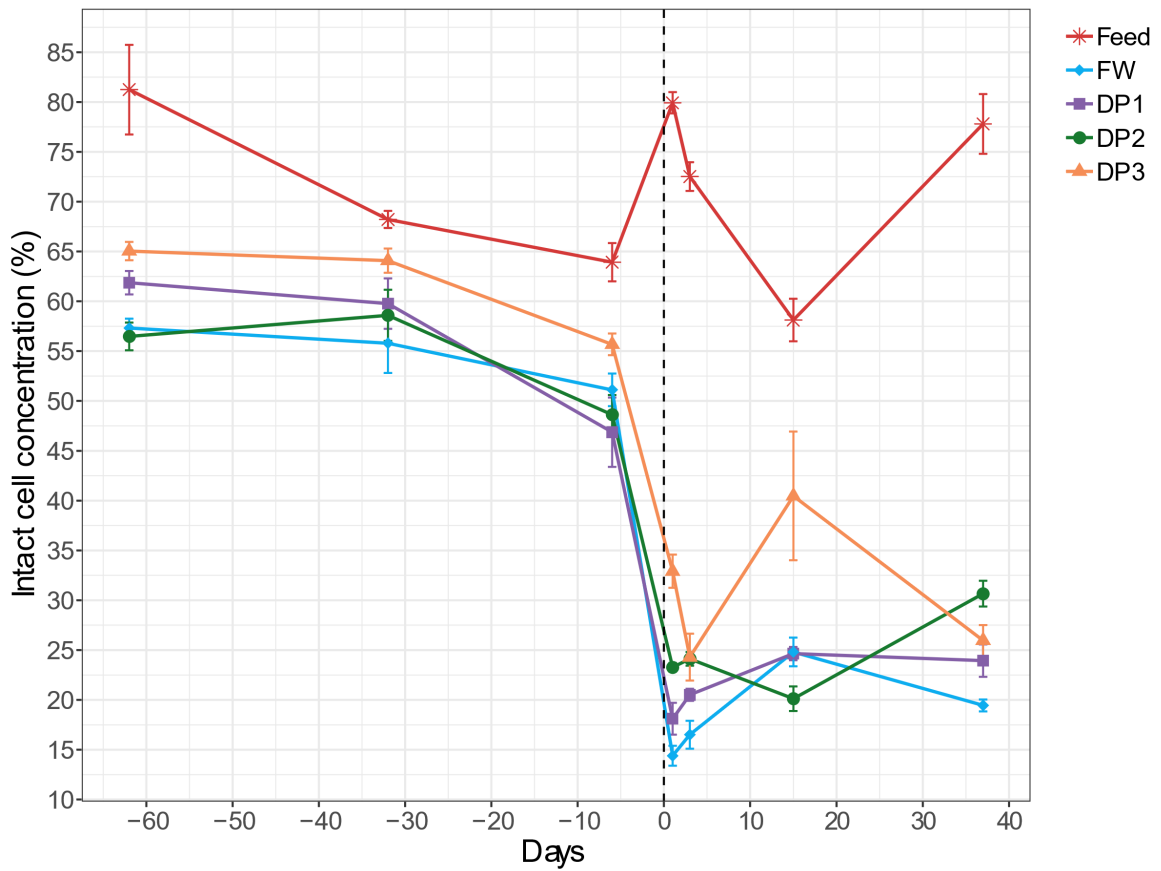
Supplementary Figure 3 – Relative abundance of bacteria in the different samples at the class level

Supplementary Figure 4 – Rarefaction curves

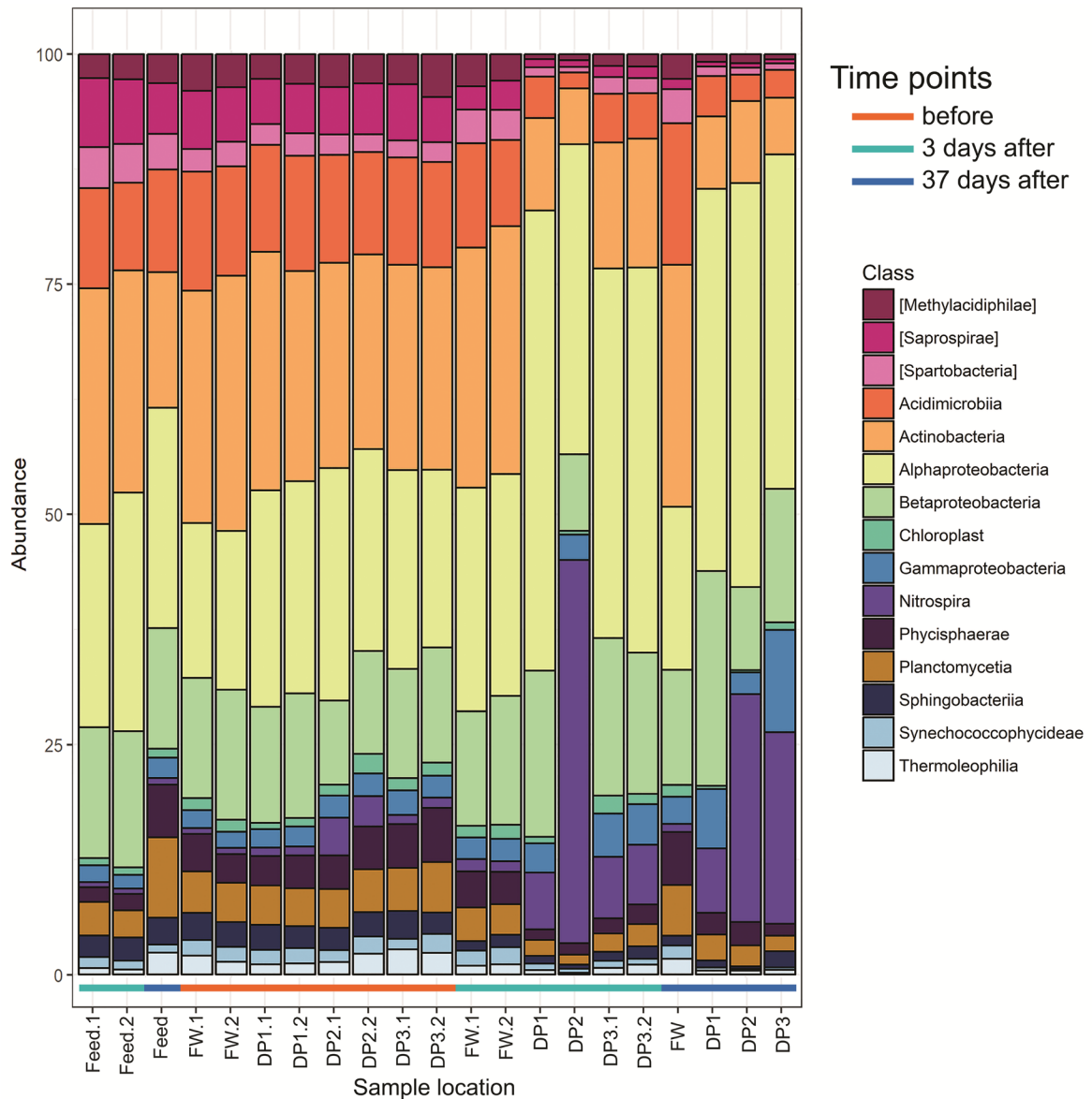
Supplementary Figure 5 – TCC of the distributed water samples after UF installation



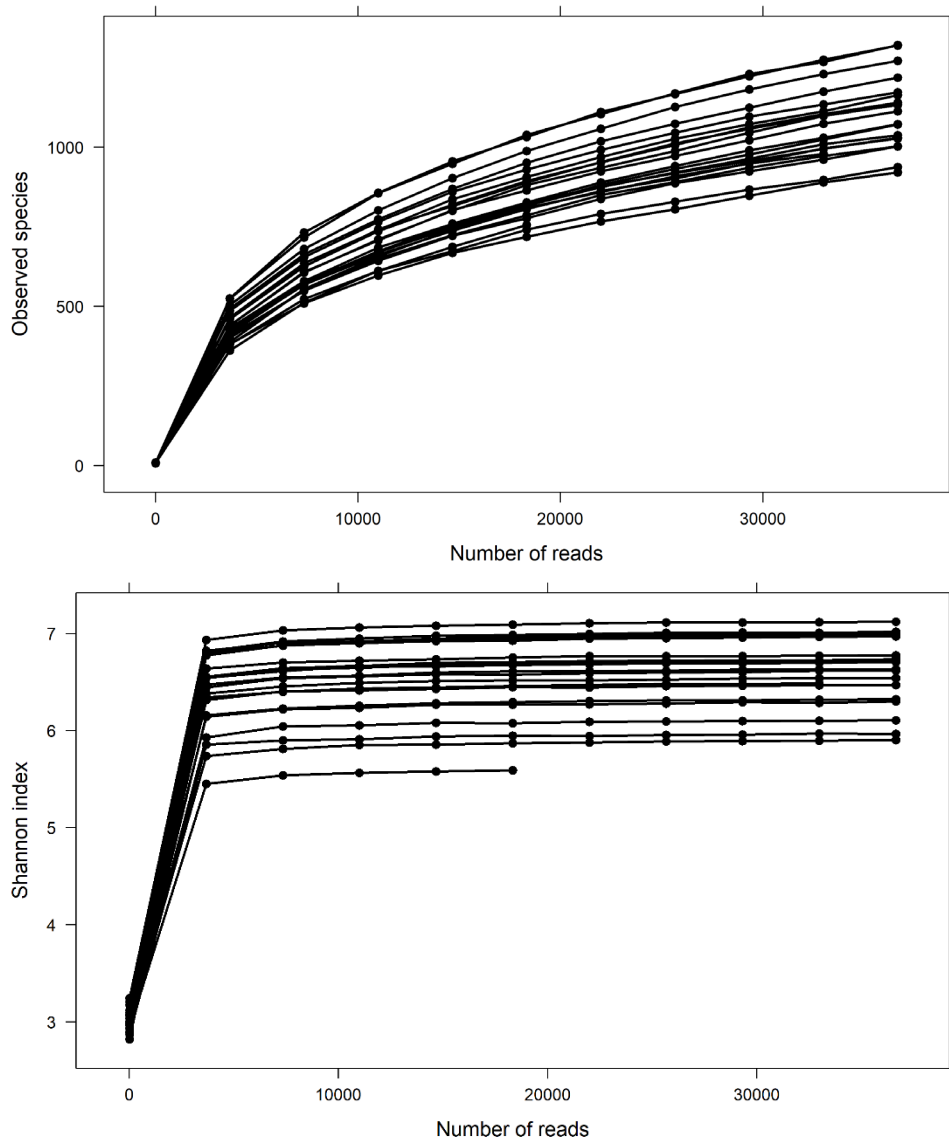
Supplementary Figure 1 – Changes in high nucleic acid (HNA) content bacteria showing an increase in % HNA in distributed water following installation of UF. For each sample, the %HNA in the finished water was subtracted from that measured at DP1 (purple line, squares), DP2 (green line, circles) and DP3 (orange line, triangles) in the distribution system, before and after the installation of UF. Day 0 on the x-axis corresponds to the start of UF (vertical dashed line). Error bars represent the variation in technical triplicates.



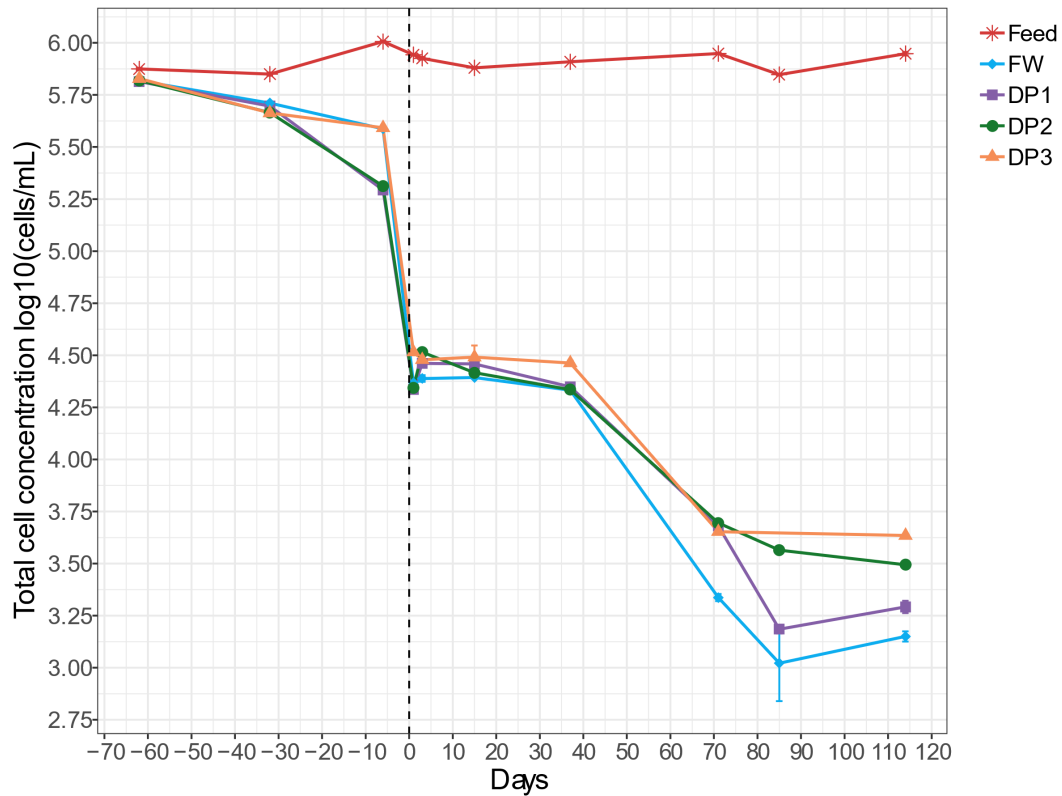
Supplementary Figure 2 – Intact cell concentration measured at the treatment plant for feed water to the UF (red line, stars), finished water (FW, blue line, diamonds), and at DP1 (purple line, squares), DP2 (green line, circles) and DP3 (orange line, triangles) in the distribution system, before and after the installation of UF. The days on the x-axis corresponds to a start of ultrafiltration at day 0 (vertical dashed line). Error bars represent the variation in technical triplicates.



Supplementary Figure 3 – Relative abundance of bacteria in the different samples at the class level. Top 15 most relative abundant classes which contribute to at least 1 % of total reads across all the samples are presented. At each location, one sample was taken before the installation of ultrafiltration (orange line) and two were taken at three and 37 days after the installation (UF1, green line and UF2, blue line). Biological replicates are indicated by a number following the decimal, if included i.e. DP1.1 and DP1.2.



Supplementary Figure 4 – Rarefaction curves based on observed species (number of OTUs) (top) and Shannon index (bottom) for all the 21 samples.



Supplementary Figure 5 – TCC of the distributed water samples before and after UF

installation shows an initial drop between days 0 and 37, when feed water was still used for pH regulation, and a final lower plateau, when only UF-treated water was distributed (day 71-114). TCC is shown for feed water to the UF (red line, stars), finished water (FW, blue line, diamonds), and at DP1 (purple line, squares), DP2 (green line, circles) and DP3 (orange line, triangles). Day 0 on the x-axis corresponds to the start of the UF (vertical dashed line). Error bars represent the variation in technical triplicates.

Supplementary Information - Tables

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Supplementary Table 1 – Output data from DESeq2 combined with the taxonomic annotation of the different OTUs.

Supplementary Table 2 – Conventional chemical and microbial water quality parameters

Supplementary Table 3 – Natural organic matter content

Supplementary Table 1 – Output data from DESeq2 combined with the taxonomic annotation of the different OTUs.

OTU	baseMean	log2FoldChange	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus
807415	433.5328	9.4932781	1.43E-09	5.85E-09	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter
New.ReferenceOTU164	2076.0738	9.3259966	2.71E-06	9.38E-06	Bacteria	Proteobacteria	Alphaproteobacteria	NA	NA	NA
328951	1739.4879	8.9535161	2.00E-12	1.13E-11	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira
264343	975.2421	8.8871578	1.15E-12	6.88E-12	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira
554981	984.9402	8.8368927	1.32E-13	9.17E-13	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA
1143858	306.662	8.7966979	1.52E-10	7.21E-10	Bacteria	Cyanobacteria	4C0d-2	MLE1-12	NA	NA
New.ReferenceOTU371	230.3143	8.7659308	2.21E-18	2.49E-17	NA	NA	NA	NA	NA	NA
New.ReferenceOTU202	341.3616	8.7516688	1.17E-30	2.64E-29	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium
570344	300.7408	8.5713684	6.28E-16	5.03E-15	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NA	NA
New.CleanUp.ReferenceOTU32754	189.374	8.5244266	2.90E-05	9.66E-05	Bacteria	Acidobacteria	Solibacteres	Solibacterales	NA	NA
1062748	598.0293	8.4899176	1.20E-10	6.01E-10	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium
4470200	2036.6014	8.4408388	1.71E-13	1.10E-12	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira
233724	1664.9664	8.2719846	8.54E-21	1.28E-19	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NA	NA
New.ReferenceOTU24	404.3908	8.1076291	2.86E-11	1.51E-10	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira
New.ReferenceOTU155	395.014	8.049085	1.68E-08	6.03E-08	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Coxiellaceae	NA
134302	1951.4365	7.9683312	6.70E-16	5.03E-15	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium
109789	4244.9859	7.9368478	6.56E-10	2.81E-09	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira
568617	388.9368	7.9347294	1.08E-08	4.05E-08	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA
812978	274.1116	7.7282295	2.80E-09	1.09E-08	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA
New.ReferenceOTU480	163.8361	7.3352637	1.53E-03	4.76E-03	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA
564600	503.6496	7.3197144	1.20E-37	3.59E-36	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium
564309	646.4925	6.5569246	1.02E-24	1.83E-23	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	NA
1108960	4063.7182	6.1449385	1.56E-67	7.00E-66	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
1003206	2011.4925	5.9889776	3.98E-83	3.58E-81	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
37092	261.3607	5.5880807	1.76E-10	7.91E-10	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium
692284	1317.3855	5.5199871	2.84E-18	2.84E-17	Bacteria	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae	NA
112204	688.4011	5.3544386	7.60E-18	6.84E-17	Bacteria	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae	NA
New.ReferenceOTU205	1093.1424	5.2954801	1.22E-18	1.57E-17	Bacteria	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae	NA

321513	232.0724	0.9203116	1.32E-03	4.23E-03	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira
New.ReferenceOTU167	114.5191	-0.471999	1.88E-03	5.63E-03	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Limnohabitans

Supplementary Table 2 – Conventional chemical and microbial water quality parameters measured before (day -6) and after (day 3 and day 37) the installation of UF. Samples were taken from the different sampling points in the drinking water treatment plant (feed and finished water) and distribution system (DP1, DP2 and DP3).

Sampling day	-6					3					37				
Sampling point	Feed	Finished water	DP1	DP2	DP3	Feed	Finished water	DP1	DP2	DP3	Feed	Finished water	DP1	DP2	DP3
Alkalinity (mg HCO ₃ /L)	22	56	55	55	54	19	58	61	62	61	20	61	61	61	61
Aluminium (mg/L)	0.014	0.014	0.017	0.016	0.018	0.016	0.029	0.029	0.031	0.029	0.017	0.024	0.023	0.024	0.025
Ammonium (mg/L)	0.005	0.062	0.035	0.033	0.026	0.005	0.049	0.027	0.023	0.03	0.005	0.058	0.027	0.042	0.022
Ammonium-nitrogen (mg/L)	0.005	0.048	0.027	0.026	0.02	0.005	0.038	0.021	0.018	0.023	0.005	0.045	0.021	0.033	0.017
COD-Mn (mg O ₂ /L)	2.3	2.1	2.1	2.1	2.1	1.9	1.2	1.2	1.3	1.2	2.3	1.3	1.3	1.3	1.4
DOC (mg/L)	2.8	2.5	2.4	2.5	2.7	2.8	1	1	1	1	2.5	1	1	1	1
<i>Escherichia coli</i> (CFU/100 mL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Phosphorus (mg/L)	0.0025	0.0025	0.0062	0.0058	0.0025	0.0025	0.0025	0.0025	0.0077	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025
Color 410 nm (mg Pt/L)	13	11	9.5	8.7	12	11	2.5	2.5	2.5	2.5	11	2.5	2.5	2.5	2.5
Iron (mg/L)	0.01	0.01	0.01	0.01	0.01	0.029	0.01	0.01	0.01	0.01	0.021	0.01	0.01	0.01	0.01
Calcium (mg/L)	8.2	19	20	20	19	7.5	21	21	21	21	7.6	21	22	21	22
Coliforms (CFU/100 mL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Conductivity (mS/m)	11	16	16	16	16	11	17	17	17	17	11	17	17	17	17
Copper (mg/L)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.026	0.01	0.01	0.01	0.01	0.026	0.01
Heterotrophic bacteria 7 days (CFU/mL)	17	0.5	0.5	5	6	29	0.5	1	3	22	18	0.5	0.5	3	3
Magnesium (mg/L)	2.3	2.4	2.5	2.4	2.4	2.2	2.2	2.2	2.2	2.1	2.4	2.4	2.4	2.3	2.3
Manganese (mg/L)	0.06	0.059	0.056	0.053	0.05	0.034	0.033	0.033	0.033	0.029	0.014	0.013	0.013	0.012	0.012
Nitrate (mg/L)	1.5				1.5	1.5	1.9	1.9	1.4	1.3	1.5	1.4	1.4	1.5	1.4
Nitrite (mg/L)	0.035	0.0035	0.069	0.046	0.079	0.035	0.0035	0.059	0.03	0.056	0.035	0.0035	0.069	0.036	0.072
Heterotrophic bacteria 3 days (CFU/mL)	13	0.5	0.5	0.5	2	10	0.5	0.5	0.5	0.5	8	0.5	0.5	0.5	0.5
pH	7.5	8	8.2	8.1	8	7.3	8	8.1	8	8	7.3	8.1	8.2	8.1	8
TOC (mg/L)	2.9	2.7	2.7	2.7	2.7	2.8	2	2.1	2.1	2	2.7	1	1	1	1
Hardness (°dH)	1.7	3.1	3.3	3.3	3.2	1.6	3.4	3.5	3.4	3.4	1.6	3.5	3.6	3.5	3.6
Turbidity (FNU)	0.21	0.05	0.29	0.3	0.2	0.22	0.14	0.48	0.19	0.19	0.23	0.05	0.05	0.05	0.11
Temperature (°C)	6.9	6.7	7.4	9	8.2	6.2	6.3	7.2	8.7	7.3	5.7	5.7	6.8	8.2	6.7

Supplementary Table 3 – Natural organic matter content before (day -6) and after (day 3 and day 37) the installation of UF for the different sampling points in the drinking water treatment plant (feed and finished water) and distribution system (DP1, DP2 and DP3).

Sampling day	-6					3					37				
	Feed	Finished water	DP1	DP2	DP3	Feed	Finished water	DP1	DP2	DP3	Feed	Finished water	DP1	DP2	DP3
DOC (Dissolved OC) (ppb-C)	2442	2308	2312	2321	2360	2445	1817	1871	1826	1821	2391	1674	1600	1608	1611
HOC (Hydrophobic OC) (ppb-C)	n.q.	n.q.	71	n.q.	38	n.q.	126	101	88	25	n.q.	81	n.q.	14	14
Inorganic colloids (m⁻¹)	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	0.03
SUVA (L/mg*m)	3.15	2.99	3.1	3.24	3.08	3.29	2.39	2.4	2.55	2.66	3.34	2.44	2.45	2.34	2.52
CDOC (Chromatographic DOC) (ppb-C)	2442	2308	2241	2321	2322	2445	1690	1770	1738	1796	2391	1593	1600	1595	1598
Biopolymers (ppb-C)	65	45	27	38	39	65	24	34	30	30	57	31	35	29	25
Dissolved organic nitrogen (DON) (ppb-N)	7	5	4	5	4	6	n.q.	n.q.	4	5	6	4	4	n.q.	n.q.
N/C ratio (µg/µg)	0.1	0.11	0.14	0.14	0.11	0.09	-	-	0.12	0.15	0.11	0.12	0.1	-	-
% proteins (% of biopolymer)	31	33	41	41	33	27	-	-	36	45	34	35	31	-	-
Humic substances (ppb-C)	1487	1472	1421	1466	1475	1564	957	970	956	983	1562	935	946	942	954
Dissolved organic nitrogen (DON) (ppb-N)	31	32	32	32	32	31	21	22	23	22	32	20	21	19	20
N/C ratio (µg/µg)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Aromaticity (L/mg*m)	3.71	3.5	3.64	3.56	3.56	3.89	3.02	2.99	3.04	2.87	4.11	2.39	2.4	2.42	2.43
Molecular weight (g/mol)	647	566	640	581	601	606	523	525	522	524	685	476	477	482	478
Building blocks (ppb-C)	509	422	478	445	456	448	425	442	433	442	431	340	336	332	340
LMW Neutrals (ppb-C)	509	355	292	352	332	350	276	312	304	330	309	288	283	292	278
LMW Acids (ppb-C)	24	13	24	20	19	18	8	12	15	11	32	0	n.q.	n.q.	0

n.q. = not quantifiable (< 1 ppb; signal to noise ratio)