Supplementary material for:

Biofilms in shower hoses – choice of pipe material influences bacterial growth and communities

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Table S1: Characteristics of Dübendorf tap water. Two types of samples were taken: cold running water to represent the distribution system and hot stagnant water to represent the water that entered the building plumbing simulator (BPS). The first may represent the water entering the system at the end of the 10 minute flushing, while the second represents the water entering at the beginning. Values were averaged for three samples taken on separate days.

	TOC (mg/L)	DOC (mg/L)	Total N (mg-N/L)	Total P (µg/L)	TIC (mg/L)	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)	pH	TCC (cells/mL)
Cold Water	0.9	0.8	1.7	5.9	45.2	68.0	9.1	7.89	1.5×10^5
Hot Stagnant Water	1.0	0.8	1.8	6.9	46.0	68.3	9.2	7.86	1.9×10^5

Abbreviations as follows: TOC – total organic carbon; DOC – dissolved organic carbon; Total N – total Nitrogen; Total P – total Phosphorous; TIC – total inorganic carbon; Ca^{2+} – calcium; Mg^{2+} – Magnesium; TCC – total cell concentration.

Table S2: Primers and Probes used for 16S qPCR and 16S Amplicon PCR. Several similar but clearly distinct reactions were used over the course of this study.

Assay	Name / Alternative	Sequence	Citation
16S qPCR	Bact 349F	AGGCAGCAGTDRGGAAT	[1]
	Bact 806 R	GGACTACYVGGGTATCTAAT	
	Bac516F-FAM	*FAM*-TGCCAGCAGCCGCG GTAATACRDAG-*TAMRA*	
Amplicon PCR for sequencing	Bakt_341F (S-D-Bact-0341-b-S-17) // 341F	CCTACGGGNGGCWGCAG	[2]
	Bakt_805R (S-D-Bact-0785-a-A-21) // 785 R	GACTACHVGGGTATCTAATCC	
	Nextera adapter tail before forward	TCG-TCG-GCA-GCG-TCA-GAT-GTG-TAT-AAG-AGA-CAG-GA	
	Nextera adapter tail before reverse	GTC-TCG-TGG-GCT-CGG-AGA-TGT-GTA-TAA-GAG-ACA-GAG	

Table S3: PCR and qPCR Reaction Conditions. Several similar but clearly distinct reactions were used over the course of this study.

Assay	Holding	Cycling Reps	Cycling	Kit or Mix utilized	Citation
16S qPCR	95 °C 10:00	45 X	95 °C 0:40 53 °C 0:40 72 °C 1:00	Light Cycler 480 Probes Master hot start reaction mix (Roche)	[1]
Amplicon PCR	95 °C 5:00	29 X	95 °C 0:20 51 °C 0:15 72 °C 0:30	Kapa HiFi HotStart ReadyMix (Kapa BioSystems)	
Index PCR	95 °C 3:00	8 X	95 °C 0:30 55 °C 0:35 72 °C 0:35	Kapa HiFi HotStart ReadyMix (Kapa BioSystems)	
Quantification of PCR product	95 °C 5:20	40 X	98 °C 0:20 60 °C 0:45 72 °C 1:00	KAPA Library Quantification Kit (Kapa BioSystems)	

Table S4: Quality Control Steps. Multiple criteria were used to control Illumina sequencing data.

Step	Criteria	Software	Version
Quality Control		FastQC	v0.11.2
Trim bad ends	Trim ambiguous nucleotides Trim quality end:10 Trim quality window: 5 Min length: 200	PRINSEQ-lte	v0.20.4
Merge Reads	Min overlap: 15 Min merge-length : 300 Max error: 5	Usearch	v8.1.1756
Primer Trimming	Overlap: full length Error rate: 0.01 Wild cards allowed	cutadapt	v1.5
Quality Filtering	Amplicon range length: 400-430 Min quality mean: 20 No ambiguous nucleotides Low-complexity filter: dust Low-complexity threshold: 10	PRINSEQ-lte	v0.20.4
Map reads to OTUS	id = 97%	Usearch_ global	
OTU clustering	De-replicate reads Abundance sorting by size = 2 Clustering with read-chimera check (cluster_otus) OTU chimera removal (uchime_ref)	UPARSE	usearch v8.0.1623 _i86linux6 4

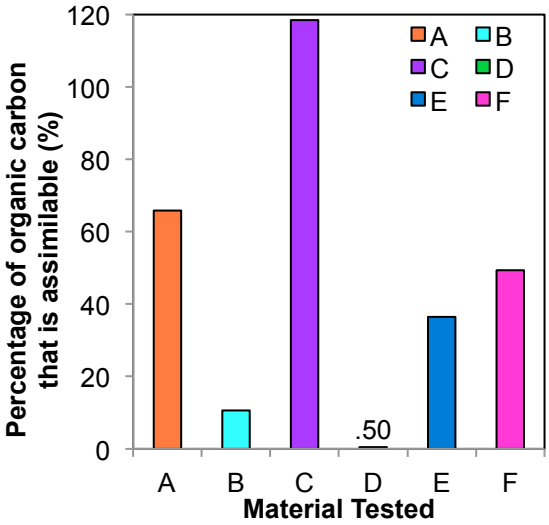

Figure S1: Assimilable Organic Carbon (AOC). The percentage of assimilable carbon in the leachate of 6 pipe materials over three migrations (Day 1, Day 3, and Day 7), using the total of three migrations. This is calculated by comparing the total amount of growth on the leachate to the total amount of organic carbon leached, with the assumption that 1 µg of carbon produces 10^7 cells. Growth supported by the leachate is first converted to equivalent carbon by dividing by 10^7, then equivalent carbon is divided by the total organic carbon (TOC) in the leachate. Data label added for Material D, with an extremely low value. This relationship between TOC and AOC (Figures 2 A and B) is interesting in that it shows that the type of carbon leaching from each substance varies considerably.

Table S5: Nutrient Amendment Study. Warm unchlorinated Dübendorf water was incubated for 72 hours in sterile glass vials with nutrient amendments. The increase in cell concentrations compared to a control with no nutrient additions is reported. This study follows the approach of Prest et al[3]. This shows that the nutrient limitations of the Dübendorf water are complex.

Scenario	Nutrients added				Log increase over unamended water
	Phosphorous (in excess)	Nitrogen (in excess)	Trace elements (in excess)	Carbon (assimilable)	
I	X	X	X		0.05%
II				1 mg/L	16%
III				10 mg/L	18%
IV	X	X	X	1 mg/L	26%

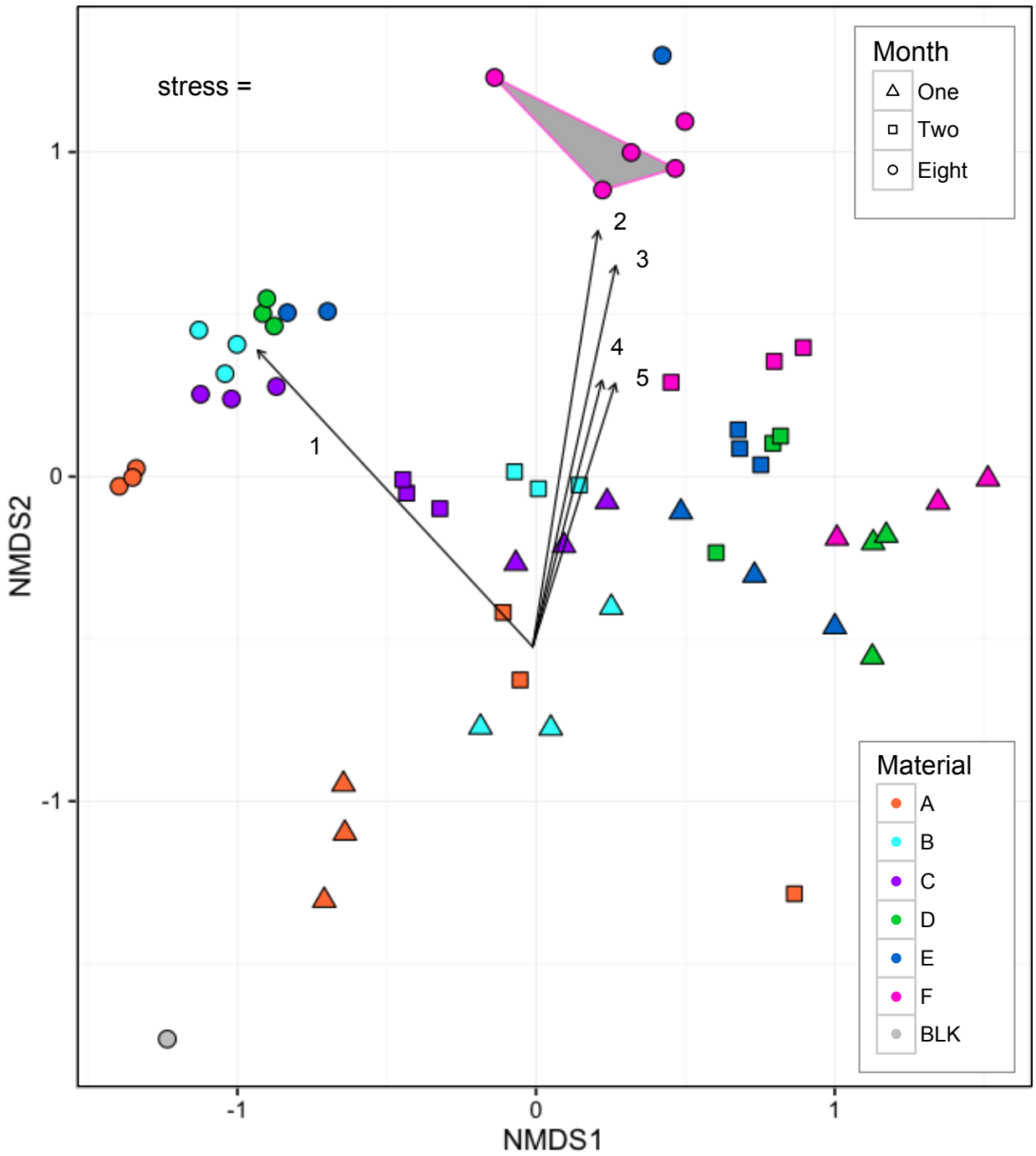

Figure S2: NMDS with five explanatory variables fitted using EnvFit. (1) Month. Measures of biofilm surface concentration: (2) total cells, (3) 16S rRNA gene copies by qPCR, (4) adenosine tri-phosphate, (5) total organic carbon. All vectors are scaled to their R^2 correlation values, and p <0.001 for all measures. This complements Figure 7.

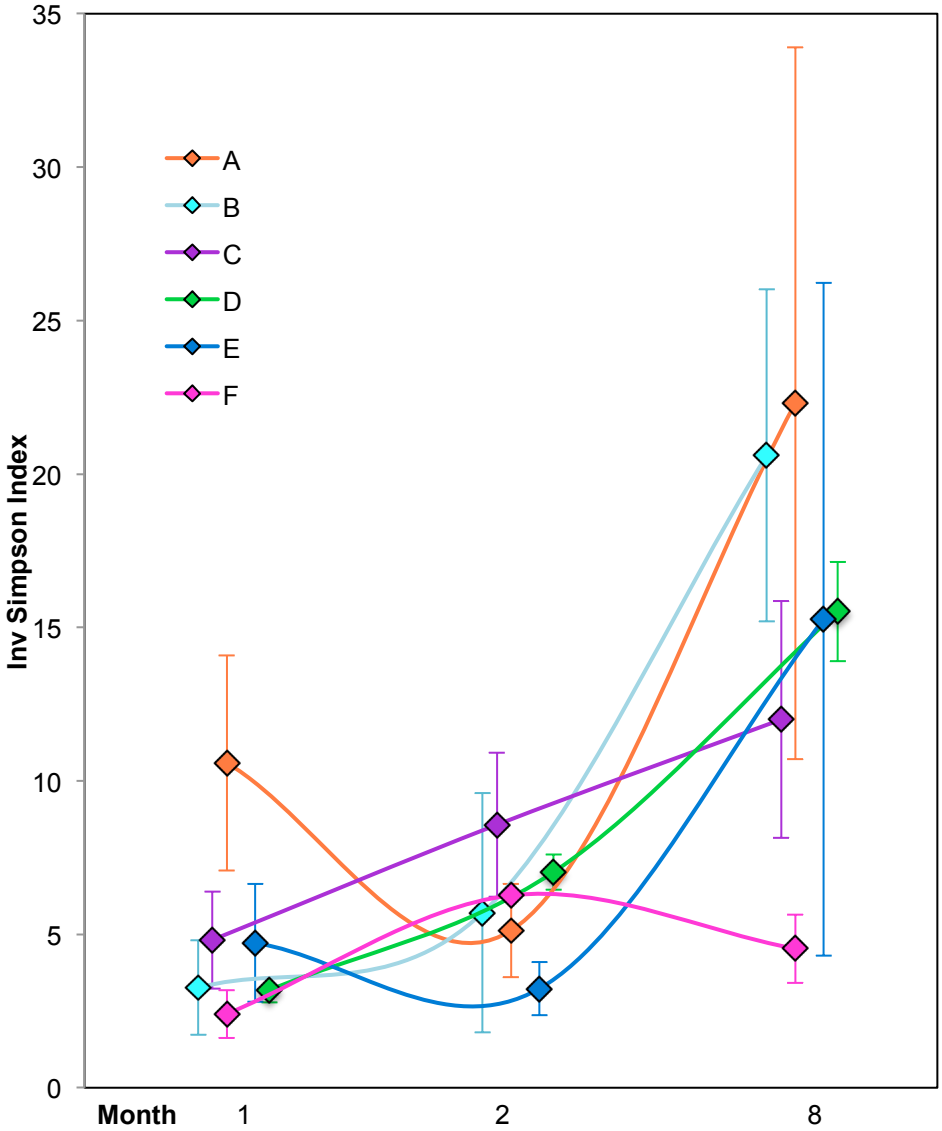

Figure S3: Inverse Simpson Index. The indicator of richness and evenness is higher when communities are more diverse. Samples are grouped by material (color) and month in which the samples were taken. For each point n = 3, and error bars represent standard deviation. Lines connect samples of the same material through time. Samples tend to get more diverse with time.

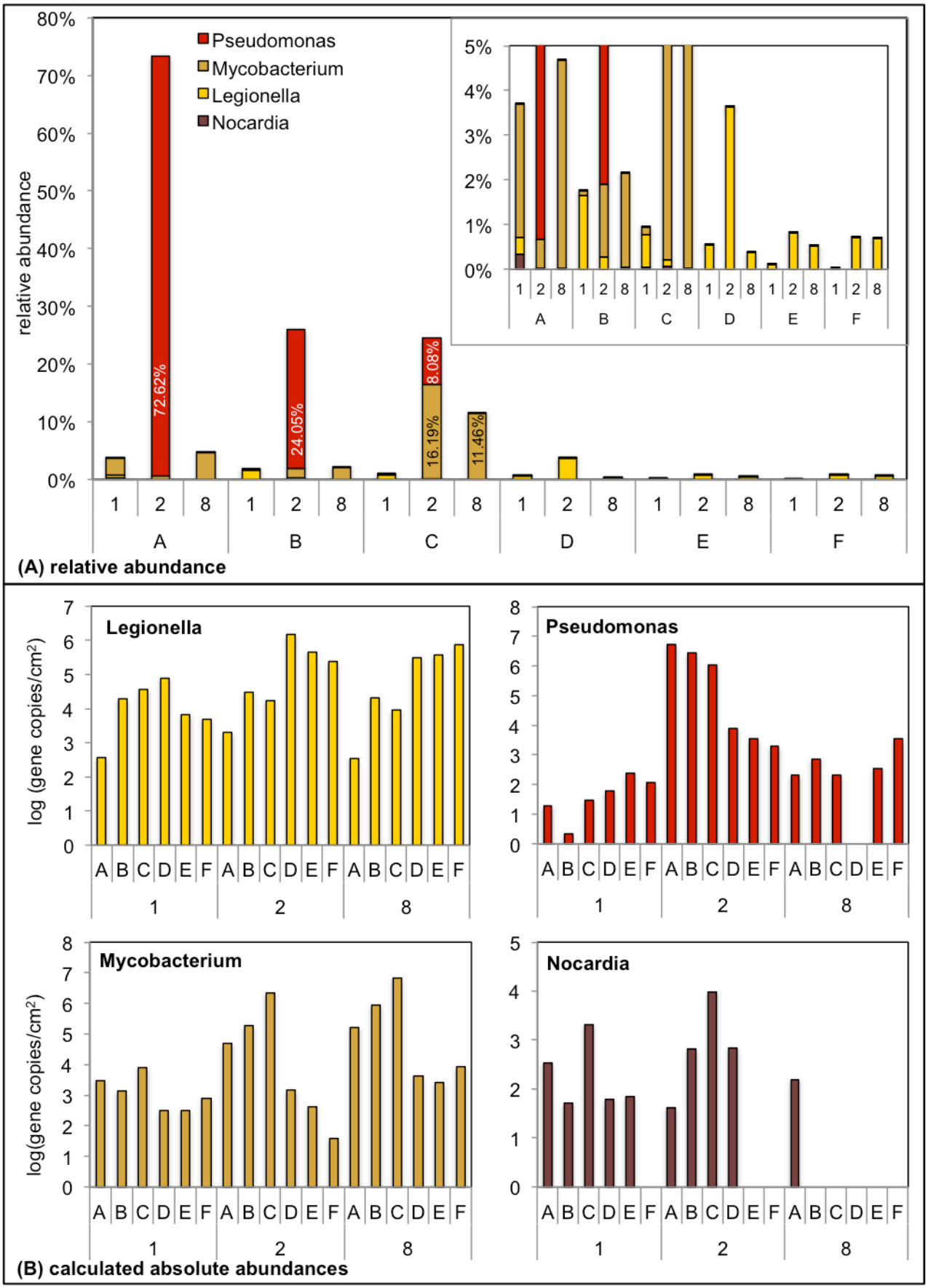

Figure S4: Genera that may contain opportunistic pathogens – (A) relative and (B) absolute abundances. Throughout, each color represents one of four genera that contain opportunistic

pathogens (*Pseudomonas, Mycobacterium, Legionella,* and *Nocardia*). In (A), relative abundances are reported according to month (1, 2, 8) within each material (A-F), with averages reported for each group. An inset in the upper right corner shows higher detail (range 0-5%). All groups with a relative abundance > 5% are labeled with percentage values. In (B), absolute abundances are reported according to material (A-F) within each month (1, 2, 8), with separate graphs for each genus. Absolute abundances are calculated as below using the relative abundance from [S4A] and the absolute abundance from figure [4B]. These values were subsequently log transformed. A similar approach was used by Prest et al. to combine flow cytometry and community abundance data[4].

Formula and example calculation:

[Relative abundance (%)] x [quantification by 16S qPCR] = calculated absolute abundance

for example:

[72.62 %] x [1.1 x 10^7 gene copies/ cm^2] = 7.96 x 10^6 calculated gene copies / cm^2

$\log_{10}(7.96 * 10^6)$ = 6.9 log (calculated gene copies / cm^2)

The genera were chosen due to the possible presence of organisms that cause opportunistic pathogen infections (often with pneumonia symptoms). One representative is given for each below:

- *Pseudomonas – Pseudomonas aeruginosa*
- *Mycobacterium – Mycobacterium avium* complex
- *Legionella – Legionella pneumophila*
- *Nocardia – Nocardia asteroids*

References

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2 A. Klindworth, E. Pruesse, T. Schweer, J. Peplies, C. Quast, M. Horn and F. O. Glöckner, *Nucleic Acids Res.*, 2013, **41**, e1.

3 E. I. Prest, F. Hammes, S. Kötzsch, M. C. M. van Loosdrecht and J. S. Vrouwenvelder, *Water Sci. Technol. Water Supply*, 2016.

4 E. I. Prest, J. El-Chakhtoura, F. Hammes, P. E. Saikaly, M. C. M. van Loosdrecht and J. S. Vrouwenvelder, *Water Res*, 2014, **63**, 179–189.