Towards a probiotic approach for building plumbing – nutrient-based selection during initial biofilm formation on flexible polymeric materials

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Abstract

Upon entering building plumbing systems, drinking water bacteria experience considerable changes in environmental conditions. For example, some flexible polymeric materials leach organic carbon, which increases bacterial growth and reduces diversity. Here we show that the carbon supply by a flexible polymeric material drives nutrient-based selection within establishing biofilm communities. We found that migrating carbon from EPDM coupons resulted in considerable growth for different drinking water communities (0.2 – 3.3 x 10^8 cells/cm^2). All established biofilm communities showed low diversity (29 – 50 taxa/biofilm), with communities dominated by even viewer taxa (e.g., 5 taxa accounting for 94 ± 5 % relative abundance, n = 15). Interestingly, biofilm communities shared some taxa (e.g., *Methylobacterium* spp.) and families (e.g., Comamonadaceae), despite the difference in starting communities. Moreover, selected biofilm communities performed better than their original communities regarding maximum attachment (91 ± 5 vs. 69 ± 23 %, n = 15) and attachment rate (5.0 ± 1.7 x 10^4 vs. 2.4 ± 1.2 x 10^4 cells/cm^2/h, n = 15) when exposed to new EPDM coupons. Our results demonstrate nutrient-based selection during initial biofilm formation on a flexible polymeric material and a resulting benefit to selected communities. We anticipate our findings to help connecting observational microbiological findings with their underlying ecological principles. Regarding initial biofilm formation, attachment dynamics, growth, and selection thereof are important for the management of microbial communities. In fact, managing initial colonization by supplying specific carbon and/or introducing consciously chosen/designed communities potentially paves the way for a probiotic approach for building plumbing materials.

1. Introduction

Uncontrolled microbial growth in building plumbing systems is generally undesirable as it can lead to operational and/or hygienic problems[1,2]. Such growth is caused by changes in environmental conditions, which is what drinking water bacteria experience as soon as they enter a building plumbing system. For example, water temperature increases and fluctuates spatially and temporally, which was shown to alter community composition[3,4]. Also, pipe diameters are considerably smaller (e.g., < 2 cm) compared to main distribution pipes (e.g., ≥ 10 cm), which provides more surface area per water volume[5], and increases the impact of biofilms on the water phase. Regarding operation, flow pattern and rates have been shown to impact biofilm structure and community composition[6,7]. Finally, diverse materials are used for pipes and non-pipe components[8], and some of these support microbial growth by leaching biodegradable substances[9], which is especially critical under long stagnation times of the water[10]. The bottom line is that building plumbing systems often provide more favorable environmental conditions for bacterial growth than the main distribution network and that it is important to understand and control not only their individual but also their combined impact on the drinking water microbiome.

Several previous studies investigated the impact of building plumbing conditions on its microbiome. Overall, microbial community compositions tend to change considerably, e.g., (1) during stagnation[11], (2) while forming biofilms inside flexible shower hoses[12], or (3) due to the combined impact of material, temperature, and stagnation[13]. Considering one of the above in more detail, studies in our research group that were addressing biofilm formation on flexible polymeric materials revealed (1) high bacterial

numbers (i.e., growth) and (2) a considerable loss in species diversity (i.e., selection)[12,14]. Also, Proctor and colleagues[15] observed the development of dissimilar biofilm community compositions when exposing the same drinking water community to different polymeric hose materials. Thereupon, they reasoned for considerable impact of migrating organic carbon on both growth and selection.

In this study, we investigated nutrient-based selection during initial biofilm formation, using a microcosm set up for the simulation of new flexible polymeric material (EPDM) in contact with drinking water. Our hypotheses were: (1) EPDM coupons release biodegradable organic carbon, which increases the potential of bacteria to grow in an otherwise carbon-limited environment. (2) Selection occurs within establishing biofilm communities, irrespective of the initial drinking water community composition. (3) Due to the common carbon supply, biofilm communities will show a certain degree of similarity in their compositions. (4) The selection process will bring advantages for initial biofilm formation, e.g., attachment, growth, etc.. We finally argue that this information provides an opportunity for the development of new, pro-active approaches for the management of biofilms that form on polymeric building plumbing materials.

2. Materials and Methods

2.1 Selection of coupon material and water

The interplay between one flexible polymeric material and five different drinking waters was tested regarding biofilm formation and community selection (Figure 1A). Coupons of ethylene propylene diene monomer (EPDM) rubber (Angst+Pfister AG, Switzerland) with an ethylene fraction of 2 % (w/w) was used as experimental material throughout this study. EPDM is approved for the use in contact with drinking water[16,17], e.g. as rubber seals within building plumbing systems. Two bottled waters and three non-chlorinated tap waters were selected as water matrices, namely: Evian (France; *B1*), Aproz (Switzerland; *B2*), tapped groundwater Dübendorf (Switzerland; *T1*), tap water Dübendorf (Switzerland; *T2*), and tap water Oerlikon (Switzerland; *T3*). The five waters differed in their chemical and biological composition. However, all waters were oligotrophic with low organic carbon and phosphorous concentrations and total bacterial concentrations in the same order of magnitude ($1 – 3 \times 10^5$ cells/mL; Table S1).

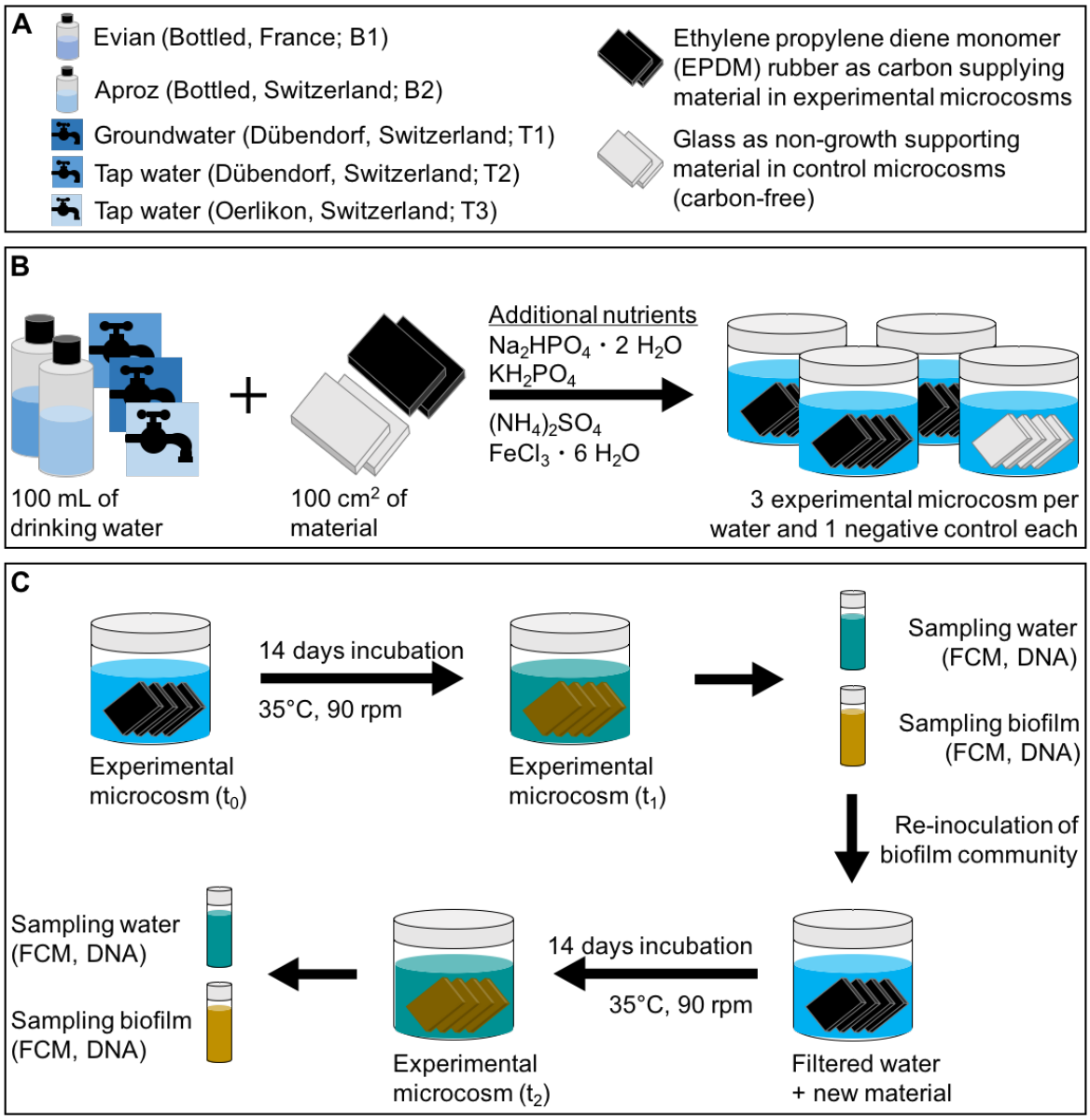

Figure 1 Experimental design. (A) Water and coupon materials. (B) Microcosms contained 100 mL water and 100 cm^2 of coupons. To exclude growth limitation, phosphorous, nitrogen, and iron were added to each microcosm. (C) Microcosms were incubated for 14 days (35 °C, 90 rpm). Biofilms and water were analyzed using flow cytometry for total cell concentrations and 16S rRNA gene sequencing for the community compositions (t_1). New microcosms were set up using filtered water which was spiked with individual biofilm communities. After another 14 days, biofilms and water phases were again sampled and analyzed (t_2).

120

121 **2.2 Microcosm design**

122 Microcosms consisted of 240 mL glass jars (74 x 89 mm) with polypropylene lids
123 including a PTFE lined inlet (Infochroma AG, Zug, Switzerland) (Figure 1). All
124 glassware was cleaned with 1% (0.33 M) hydrochloric acid (HCl, 32%; Fluka, Sigma-
125 Aldrich, Buchs, Switzerland), rinsed with nanopure water, and air dried. The clean
126 glassware was muffled in a furnace (Nabertherm Schweiz AG, Hägendorf,
127 Switzerland) (4.5 h; 500°C). EPDM flat sheets were cut into coupons of 0.2 x 2.6 x 4.3
128 cm (25 cm^2). Prior to use, coupons were cleaned with a 0.1% (v/v) sodium hypochlorite
129 solution (Sigma-Aldrich, Buchs, Switzerland) and rinsed with nanopure water. Glass
130 was used as the control material. Microscope slides (Menzel-Gläser, 1 mm,
131 ThermoScientific) were cut to the same coupon size as the EPDM and cleaned
132 following the same procedure as for the glass jars (above). For each microcosm, four
133 coupons or slides (i.e., 100 cm^2) were stacked (Figure 1), separated by stainless steel
134 springs. The springs and the jar lids were cleaned (60°C, 1 h) in a 100 g/L sodium
135 persulfate solution ($Na_2S_2O_8$, Sigma-Aldrich, Buchs, Switzerland), then rinsed with
136 nanopure water, and air dried. Before use, the bottled drinking water was inverted 3-
137 4 times for uniform mixing, while cold tap water was flushed for 5 min before filling into
138 muffled 1 L SCHOTT Duran® bottles (SCHOTT AG, Mainz, Germany). Each
139 microcosm was subsequently filled with 100 mL water. To ensure unlimited growth
140 conditions, additional nitrogen, phosphorous, and iron were added to the microcosms.
141 The nitrogen/phosphorous buffer contained sodium phosphate dibasic dehydrate
142 ($Na_2HPO_4 \cdot 2H_2O$, 1.28 g/L), potassium phosphate monobasic (KH_2PO_4, 0.3 g/L), and
143 ammonium sulfate ($(NH_4)_2SO_4$, 1.77 g/L) and 3.4 mL of buffer was added to each
144 microcosm. Iron was supplemented in the form of iron (III) chloride hexahydrate ($FeCl_3$

· 6 H_2O, 2.7 g/L), with 50 µL per microcosm. All chemicals were purchased from Sigma-Aldrich (Buchs, Switzerland).

2.3 Migration and growth potential assays

For the assessment of carbon migration from the experimental material (EPDM) and the resulting consequences for bacterial growth, the material BioMig testing method was applied[18]. This method comprises a migration assay and a growth potential assay. In short: for the migration assay, 100 cm^2 of EPDM was incubated (60°C, 24h, without shaking) with 100 mL bottled water (Evian). Over the course of seven days, the EPDM material was transferred into a new microcosm with fresh water every day. After the 1st, 3rd, and 7th day of incubation, the water was sampled and the migrated total organic carbon (TOC) was quantified (see below). In addition, the growth of bacteria in the migration water was assessed. For this, 1 mL of fresh Evian bottled water was inoculated into 20 mL of migration water, with the addition of 690 µL phosphate/nitrogen buffer and 10 µL $FeCl_3$. This test was performed in sterile, muffled 40 mL glass vials with screw caps lined with a PTFE septum (Supelco, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland). Incubation (30°C, 120 rpm, 6 d) was followed by the quantification of the total cell concentration (TCC) using flow cytometry (FCM) (see 2.6.3). For the growth potential assay, 100 cm^2 of new EPDM material was incubated (30°C, 14 d) with 100 mL of fresh bottled water (Evian) and additional nutrients (see 2.2). After 14 d of incubation, the water and biofilm phases were sampled for TCC, allowing for the determination of the bacterial growth potential within the experimental microcosms due to migrating carbon compounds (in direct comparison to a glass control set up without additional carbon)[18].

2.4 Selection experiment

For all five water samples, triplicate microcosms were assembled with the testing material (EPDM) and an additional one containing glass as a control set up (Figure 1, B), as described above. After assembly (t_0), the microcosms were incubated (14 d, 35°C, 90 rpm) (Figure 1, C). After 14 days (t_1), biofilms were removed from the material surface (EPDM and glass; see 2.6.2) and both the biofilm and water phase of each microcosm were sampled for TCC (see 2.6.3) and community composition by 16S rRNA gene sequencing (see 2.6.4). For a second selection step, biofilm samples were re-inoculated into new microcosms. For this, the corresponding drinking water matrix was filtered using sterile bottle top filter units and membrane filters (Whatman® Nucleopore™Track-Etched Membranes, 47 mm, 0.2 µm, Sigma Aldrich). New material was cleaned and stacked, additional nutrients added, and selected biofilm communities were added in a final concentration of 1 x 10^7 cells/microcosm (i.e., 1 x 10^5 cells/mL). After another 14d incubation, biofilms and water phases of all microcosms were again sampled (t_2), following the same procedure. Regarding terminology, in the course of this study, initial drinking water communities are referred to as *original drinking water communities* and the biofilm communities of t_1 and t_2 as *selected biofilm communities*.

2.5 Attachment experiment

Here we compared attachment dynamics of selected biofilm communities with the original drinking water communities. The same microcosm set up was used as described above, with triplicate experimental microcosms (EPDM coupons) and single control microcosms (glass slides). The starting concentration of bacteria in the water phase (TCC, t_0) was adjusted to be the same by diluting the biofilm communities

195 relative to the drinking water TCC. The microcosms were incubated (35°C, 90 rpm)
196 and the TCC in the water phase was measured for all at 30 min intervals over the
197 course of 5 h (t_1 – t_{10}).
198
199 **2.6 Sampling and analysis**
200
201 **2.6.1 Chemical water analysis**
202 Total organic carbon (TOC) was measured using a TOC-V_{CPH} Analyzer (Shimadzu
203 Schweiz GmbH, Reinach, Switzerland). The minimum detection limit of the instrument
204 is 0.1 mg/L. For total phosphorous, samples were chemically digested with potassium
205 peroxodisulfate at 121°C, followed by a reaction to a phosphorous-molybdenum-blue
206 complex and the determination of ortho-phosphate with spectrophotometry. The
207 minimum detection limit of this method was 3.0 µg/L. Total nitrogen concentrations
208 were measured via chemiluminescence using a Shimadzu TOC-L_{CSH} instrument. The
209 minimum detection was 0.5 mg/L.
210
211 **2.6.2 Biofilm removal**
212 All biofilms were removed with an electrical toothbrush (Oral-B®, Advanced Power)
213 and toothbrush heads were replaced after each use to prevent cross-contamination.
214 In short: EPDM or glass coupons were placed into muffled glass petri dishes and
215 covered with filtered (0.2 µm) water. The water volume was always 25 mL per coupon
216 (i.e., a total of 100 mL per microcosm). The coupons were brushed one by one, for
217 approximately 90 sec each (including both coupon sides and the edges). During
218 biofilm removal, 10 mL were saved and after the biofilm removal from all four coupons
219 of a microcosm. This volume was ultimately used to recover biofilm residuals in the

petri dish and on the brush head, by pouring the 10 mL filtered water into the petri dish and brushing without any coupon. The biofilm suspensions of the microcosms were collected in individual, sterile 100 mL SCHOTT Duran® bottles. 10 mL of the biofilm suspension was used for flow cytometry (see 2.6.3). The rest of the biofilm suspension was used for the re-inoculation in the selection experiment (see 2.4), for further steps on community analysis (see 2.6.4), or for the attachment experiments (see 2.5).

2.6.3 Flow cytometry for the quantification of total cell concentrations

FCM was used for the determination of total cell concentrations (TCC) in all biofilm and water samples. For biofilms, a 10 mL subsample of the biofilm suspension (see 2.6.3) was collected and needle-sonicated in a round-bottom glass tube (DURAN®; Faust Laborbedarf AG, Schaffhausen, Switzerland) using the Sonopuls HD 2200 instrument (Bandelin Sonorex, Rangendingen, Germany) and the Sonotrode Sonopuls MS 73 (tip diameter 3 mm, Bandelin). Sonication settings were: 30 sec at 50% power, and 40% amplitude intensity, with the pulse amplitude of the needle being 308 µm. The sonicated biofilm samples were then diluted 10-100x using 0.1 µm filtered Evian water (Millex®-VV, Merck-Millipore), while the water samples were measured undiluted. For the detection of TCC, samples were stained with 10 µL/mL SYBR® Green I (SG, Invitrogen AG, Basel, Switzerland; 100x diluted in 10mM Tris buffer, pH 8). Stained samples were incubated (37°C, 10 min) and measured using a BD Accuri C6® flow cytometer (BD, Belgium) or a CytoFLEX Flow Cytometer (Beckman Coulter International SA, Nyon, Switzerland). Gates and settings were kept the same within experiments. For more detailed information on data analysis and gating strategies see[19].

2.6.4 16S rRNA gene-based community analysis

For sequencing, samples of (1) all original drinking waters (t_0), (2) all selected biofilms (t_1, t_2), and the water phase of the microcosms (t_1, t_2) were concentrated onto 0.2 µm polycarbonate Nucleopore® membrane filters (ø 47 mm, Whatman, Kent, United Kingdom) by vacuum filtration, using sterile bottle top filter units. Filters were immediately frozen in liquid nitrogen and stored at -20°C.

2.6.4.1 DNA extraction

The DNeasy PowerWater® Kit (Qiagen, Hilden, Germany) was used for DNA extraction and performed according to the provided protocol. Extracted DNA was frozen and stored at -20°C until further processing.

2.6.4.2 Library preparation and sequencing

For analyses on bacterial community compositions, the V3-V5 region of the 16S rRNA gene was amplified via polymerase chain reaction (PCR), using the primers Bakt_341F and Bakt_805R[20]. For library preparation, extracted DNA was quantified in duplicates using a Spark® 10M Multimode Microplate Reader (Tecan, Switzerland; Qubit™DNA Broad Range Assay). DNA concentrations were normalized between samples prior to amplification (1 ng DNA / 11 µL). For the PCR, normalized DNA was mixed with 2xKAPA HiFi HotStart Ready Mix (Kapa Biosystems, Roche Holding AG). Primers were added in a final concentration of 0.3 µM (details see Table S2, A). In addition to experimental samples, a negative control (i.e., amplification of sterile water instead of sample-DNA) and a positive control (self-made MOCK community: pure DNA of *Burkholderia xenovorans, Bacillus subtilus, Escherichia coli, Micrococcus luteus, Pseudomonas protegens, Paenibacillus sabinae,* and *Streptomyces*

violaceoruber) were amplified. Additionally, some experimental samples were amplified in replicates. For this PCR, all samples were amplified in duplicates (2 x 25 µL reactions), which were combined prior to clean up. Amplified products were purified using the Agencort AMPure XO System (Beckman Coulter, Inc., Bera, CA, United States). For this, products were attached to magnetic beads, washed with 80% EtOH, and re-suspended in 10 mM Tris, pH 8.0. To enable pooling and re-identification of individual samples, specific sequencing Nextera XT v2 Index Kit adapters (Illumina) were annealed to the amplicons via Index PCR (Table S2, B). Products were again cleaned using the AMPure approach, quantified, and quality was checked using the High Sensitivity D1000 ScreenTape system (Agilent 2200 Tape Station). All samples were normalized to a concordant concentration followed by the pooling of 5 µL per sample. This pool was adapted to a final concentration of 2 mM and the base-pair (bp) length of the product determined with the Tape Station (627 bp).

Sequencing was performed using the MiSeq platform. For this, 0.1M NaOH was added to the pool, centrifuged (300 g, 60 s) and incubated for 5 min (room temperature) prior to the addition of the hybridization buffer HT1. This step was to (1) generate single stranded DNA and to (2) prevent unspecific bindings to the flow cell during sequencing. As a final step, 10% PhiX was added as a sequencing run control (Illumina: Technical Note on PhiX Control). The MiSeq run was a paired-end 600 cycle sequencing run. Data on community composition was generated in collaboration with the Genetic Diversity Center (GDC), ETH Zurich.

2.6.4.3 Processing of sequencing data

Data processing followed a distinct pipeline. First, data quality was controlled using FastQC (Table S2, A). Then, read ends were trimmed and merged (Table S2, B),

which was followed by an *in silico*-PCR and the trimming of the primer sites (Table S2, C). Finally, sequences were filtered (based on quality and size range) (Table S2, D) and amplicon sequence variants (ASV) were generated and taxonomically assigned. The clustering of sequences was performed as presented in a previous study[14]. It is based on an amplicon sequence variant (ASV) approach using UNOISE3, proposed by Edgar and colleagues[21], and includes a correction for sequencing errors and a chimaeral removal. Clustered sequences are called zero-radius operational taxonomic units (ZOTUs). Due to a potential overestimation of the actual number of ZOTUs, an additionally clustering was performed at different identity levels of 99, 98, and 97%. For predictions on taxonomic assignments, the Silva 16S database (v128) and the SINTAX classifier were used (cut-off 0.9). See supplementary information for details on data analysis using R (Version 3.3.0) and RStudio (Version 1.1.477).

3. Results

3.1 Migration and growth potential assays

Applying an established material testing package (BioMig[18]) revealed that a considerable amount of organic carbon migrates from the experimental EPDM coupons and that a substantial fraction of the migrating carbon can be used by drinking water bacteria to grow. The migration assay (60 °C) showed that organic carbon migrated in high concentrations from new EPDM coupons and that it increased the TOC concentration of the water 5-fold (average: 1.1 µg TOC/mL, n = 2) within the first 24 h of migration (Figure 2A). The extent of migration diminished over time. However, even after 7 d of sequential migrations, the TOC concentration of the water with EPDM coupons still increased 3-fold (0.43 ± 0.03 µg TOC/mL, n = 2), equivalent to a rate of 0.3 µg TOC/cm^2/d. These values are typical for flexible materials in contact with drinking water (e.g., in general[14,22], or specifically for EPDM[23,24]). A separate growth potential assay at 30 °C showed that 2.3 ± 0.09 x 10^7 cells/mL (n = 3) were able to grow on migrating carbon from EPDM coupons during 14 days, which is 30x more compared to growth in the absence of EPDM (Figure 2B). Given that the carbon-source for growth was the EPDM coupons, this translated to the growth of 2.3 x 10^7 cells/cm^2 coupon. Of these cells, 57 % (i.e., 1.3 x 10^7 cells/cm^2) were recovered directly from the surface of the EPDM coupons. To summarize, results show that the EPDM coupons favor biofilm formation by (1) providing a surface for colonization and by (2) adding biodegradable organic carbon to the water. Therefore, this material was deemed suitable for the further experiments on biofilm growth and the selection within growing communities (below).

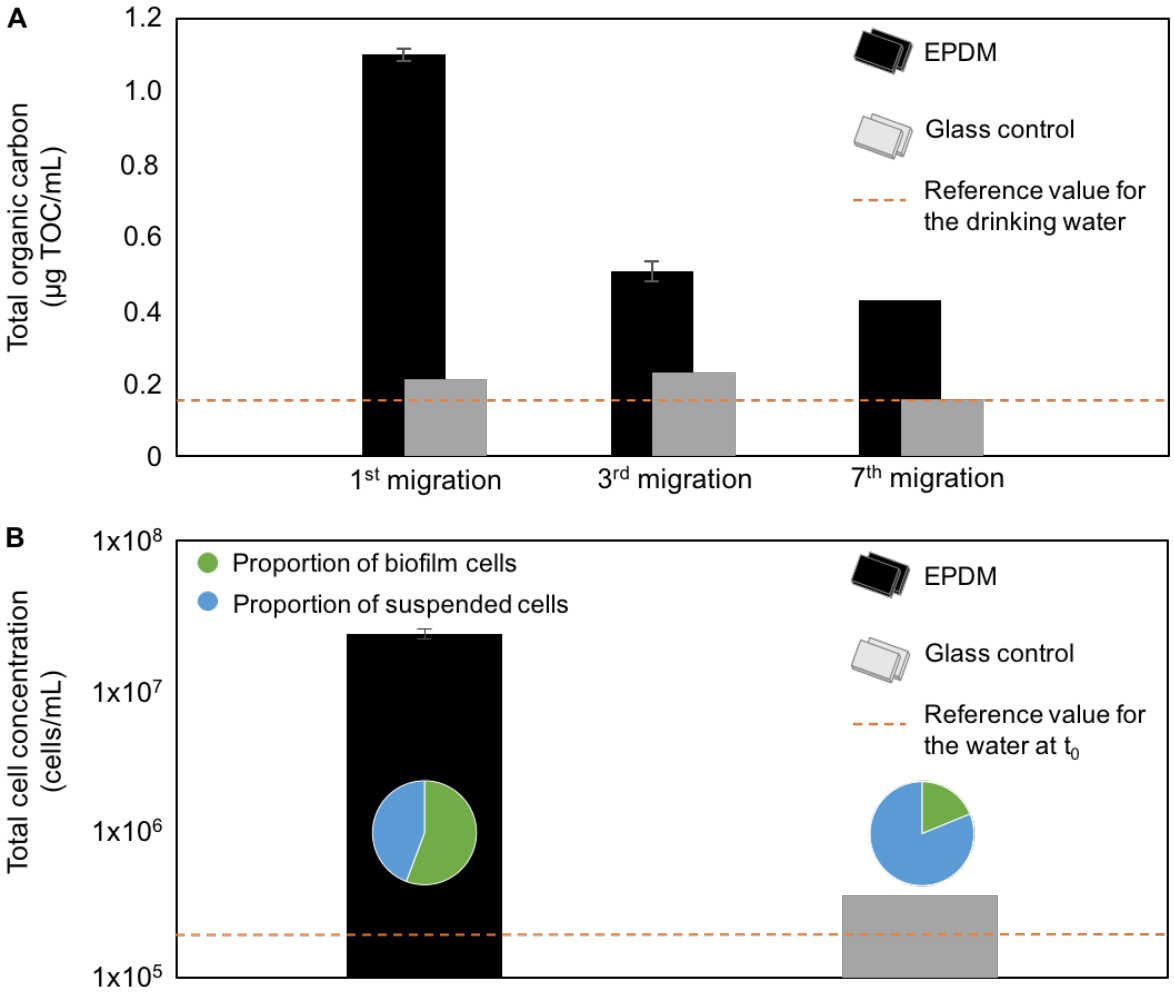

Figure 2 Assessment of carbon migration from EPDM and the resulting potential for bacterial growth. (A) Migration assay for the quantification of total organic carbon (TOC) in microcosms with EPDM or glass coupons. Material was transferred into fresh microcosms every 24 h, with measurements after the 1st, 3rd, and 7th migration. (B) Bacterial growth potential based on EPDM, or glass as control. Total cell concentrations (TCC) are shown per mL for total growth of both suspended and biofilm cells. The conversion of cm^{-2} for biofilm cells to mL^{-1} was based on the water volume to material surface area ratio of 1:1 in the microcosm set up. Proportions of biofilm and suspended cells are indicated via pie charts. Error bars represent the range between duplicate microcosms in (A) and standard deviations for triplicate microcosms in (B).

342 **3.2 Selection experiment**

343 The basic concept and design of the growth potential assay was used to test the
344 growth of five different drinking water communities on identical EPDM coupons (Figure
345 1). All communities showed (1) intensive growth and (2) a considerable loss in taxa
346 diversity (apart from B2), with (3) the development of different biofilm communities
347 involving shared taxa.

348

349 **3.2.1 Considerable growth for different original drinking water communities**

350 Figure 3 shows that after two sequential 14-day cycles of inoculation and incubation
351 (Figure 1), substantial growth was measured for all five waters in the presence of
352 EPDM coupons, ranging within one order of magnitude (0.2 – 3.3 x 10^8 cells/mL).
353 These final concentrations represent both the planktonic and biofilm phases. The
354 proportion of cells recovered directly from the biofilm ranged between 59 – 86 %,
355 equivalent to 0.2 – 2 x 10^8 cells/cm^2. While experimental microcosms had considerable
356 growth, differences were identified. Growth in the absence of EPDM coupons (i.e., in
357 the glass controls) highlighted the impact of the migrating carbon, showing that TCC
358 concentrations were 93 – 99 % lower without the additional carbon source. The
359 proportion of cells in the biofilm was still high with 30 – 93 %, which translates to 1.4
360 ± 0.5 x 10^6 cells/cm^2 (n = 5). These findings confirm our results from Figure 2 on the
361 carbon migration and growth potential based on EPDM coupons. The results show
362 that the growth is high for different drinking water communities and that there was
363 substantial biofilm growth, irrespective of the starting community.

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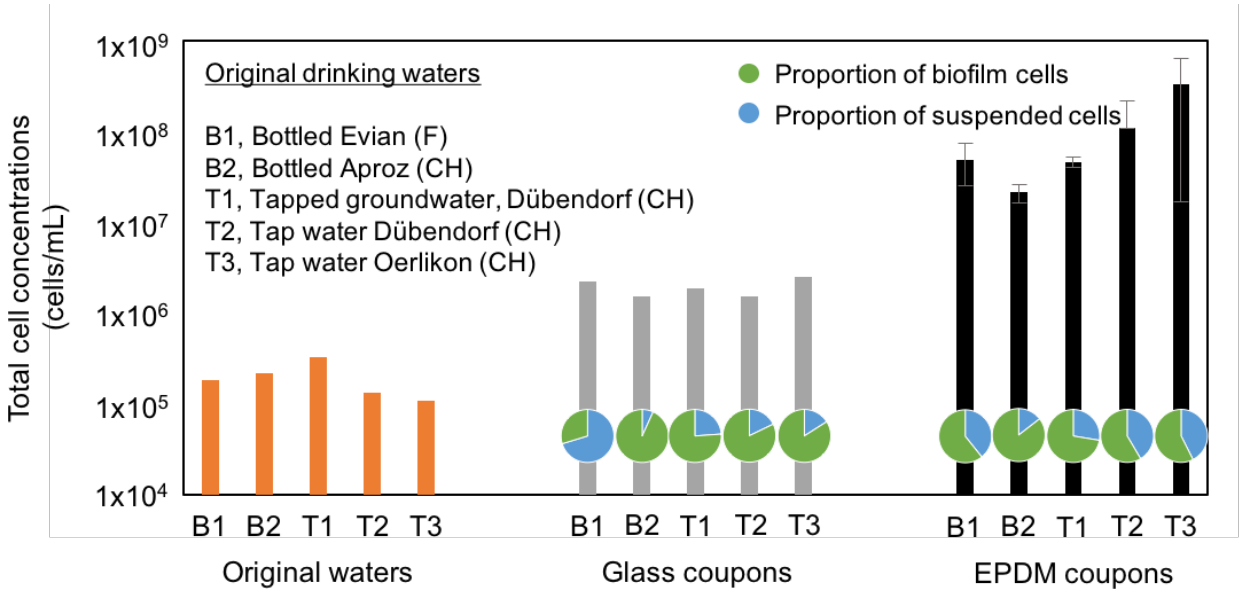

365

366 **Figure 3** Total cell concentrations in the original drinking waters (t_0) and in microcosms
367 at the end of the experiment (t_2), i.e., 2 x 14 d of incubation with an intermediate re-
368 inoculation of biofilms grown at t_1. For growth in microcosms with glass and EPDM
369 coupons, total cell concentrations (TCC) are shown per mL for the total growth of both
370 suspended and biofilm cells. The conversion of cm^{-2} for biofilm cells to mL^{-1} was based
371 on the water volume to material surface area ratio of 1:1 in the microcosm set up.
372 Proportions of biofilm and suspended cells are indicated via pie charts. Error bars
373 represent standard deviations for triplicate microcosms in the EPDM coupon set up.

374

375 **3.2.2 Comparatively low taxa diversity detected in biofilm communities**

376 A comparison of the original drinking water communities with the biofilm communities
377 at the conclusion of the experiment revealed a notable loss in diversity (apart from B2,
378 see below). Richness, i.e., the number of different taxa, decreased and became more
379 comparable between the different waters. Figure 4A shows the richness values for the
380 five original drinking water communities. Interestingly, tapped waters showed
381 considerably more taxa (2'178 ± 131, n = 9) than the bottled waters, with the original
382 water B2 comprising strikingly few taxa (54 ± 0, n = 3) compared to B1 (270 ± 15, n =

3). Overall, biofilm communities comprised comparatively few taxa (29 – 50 taxa), which corresponded to a diversity loss of 46 – 98 % from the original waters. This impressive loss of up to 2'000 individual taxa (tap waters) highlights the relevance of nutrient-based selection within establishing biofilm communities. As a consequence to this loss in diversity (through growth and selection), the similarity between the biofilm communities increased (with respect to diversity), with only 21 % variation in richness between biofilms as opposed to 73 % between the original drinking water communities. Shannon diversity followed a similar trend. Figure 4B shows a comparable dissimilarity between the original tapped water communities (5.9 ± 0.7, n = 9) and bottled water communities (2.3 ± 0.03, n = 3 for B1; 1.1 ± 0.01, n = 3 for B2). The relation between the Shannon Index (H') and its maximum value (H'_{max}) is important for drawing conclusions on diversity, i.e., the closer H' to H'_{max}, the higher the diversity within the community. Here, the relation was 1:1.3 for the tapped waters, 1:2.5 for B1, and 1:3.7 for B2 respectively, indicating a higher diversity in the tapped waters. This difference decreased with biofilm formation, resulting in a comparable degree of diversity. Here, the ratio between H' and H'_{max} is close to 1:3 for all samples. This shows that (1) diversity decreased for (almost) all communities and (2) that biofilm communities are more similar to each other compared to the original drinking water communities. As indicated above, bottled water B2 was the misfit amongst the original water communities with a particularly low initial richness and diversity. Interestingly, this community also grew the least during the selection experiment (Figure 3). This suggests that the initially low diversity did not allow the community to metabolize as many nutrients as for the other more diverse communities. Results on richness and Shannon diversity allowed for the calculation of Evenness. Evenness indices were low for the bottled waters (0.34 ± 0.07, n = 6) and did not change much during the growth

experiment (0.35 ± 0.09, n = 6). For the original tapped waters, Evenness was high (0.77 ± 0.08, n = 9), indicating a rather equal distribution of taxa. For the tapped water biofilms, indices decreased approx. 50 % which resulted in comparable Evenness indices for all samples (0.37 ± 0.1, n = 15).

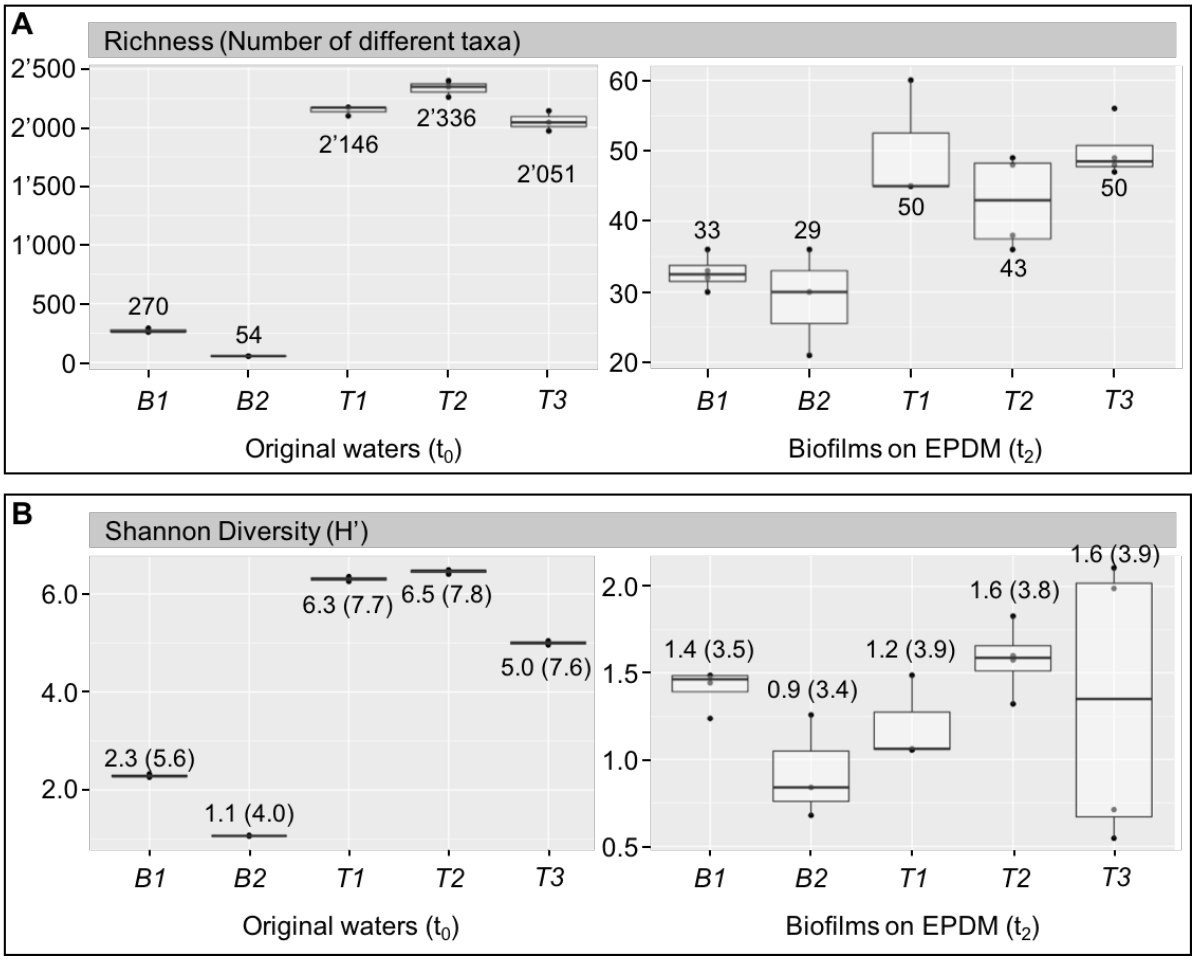

Figure 4 Diversity between microbial communities following growth on EPDM coupons. Alpha-diversity indices richness (A) and Shannon diversity (B) of the original drinking water communities (B1, B2, T1, T2, and T3) and the selected biofilm communities grown on EPDM (t_2) for each experimental water. Results are presented as averaged numbers for richness (A) and averaged Shannon indices with additional information on (H'_{max}). Original drinking waters were sequenced in triplicates and biofilms were sampled from triplicate experimental microcosms (additional sampling

421 points in plot are due to replicate sampling of selected biofilms).

422

423 What is particularly interesting is that, involving already severe loss in taxa diversity
424 and richness (i.e., a strong selection), the low degree of evenness within biofilm
425 communities indicated a dominance of an even smaller number of taxa. This was, in
426 fact, the case with the five most abundant taxa accounting for 95 ± 5 % (n = 15) of the
427 individual biofilm community compositions (Table S4). These results highlight that (1)
428 the bottled waters had low diversities from the start, (2) the number of different taxa
429 decreased during biofilm formation and so did (3) the equality of their distribution. This
430 rendered all biofilm communities more similar, with comparably low diversity and (4)
431 only view taxa dominating the entire biofilm communities.

432

433 **3.2.3 Biofilm growth alters community composition**

434 The decrease in taxa diversity came along with a change in community composition
435 from the original drinking water to the selected biofilm communities. Figure 5 illustrates
436 the dissimilarities between the bacterial communities of the original drinking waters (t_0,
437 triangles) and their corresponding biofilm communities that grew on EPDM coupons
438 (t_2, circles). The distance between original and biofilm communities was large for the
439 tapped waters (e.g., highlighted for T3, Figure 5).

440

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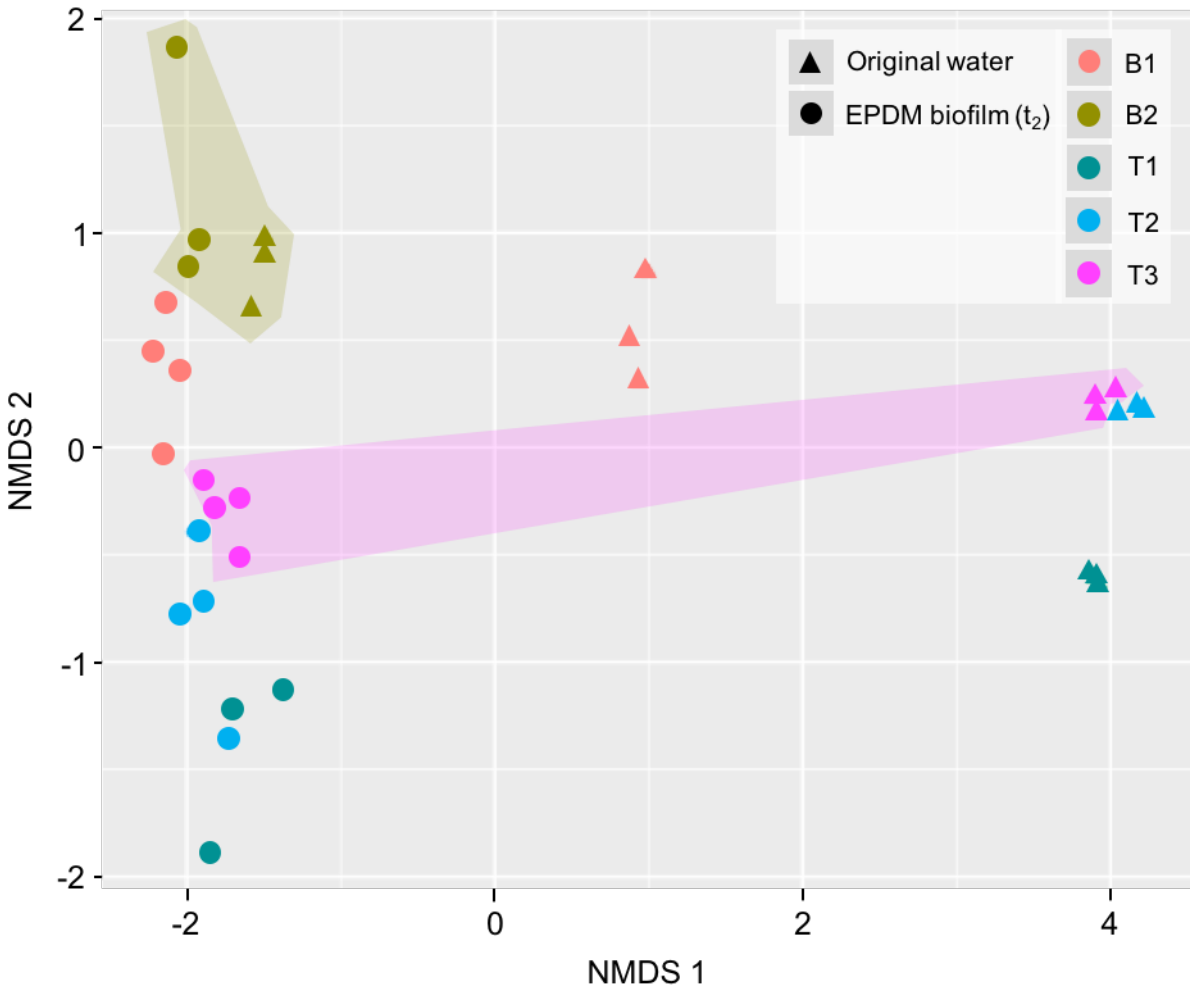

Figure 5 NMDS ordination plot based on Bray-Curtis Dissimilarity. Comparison of five different original drinking water communities (B1, B2, T1, T2, and T3) and their corresponding biofilms that formed on EPDM (t_2). Original drinking waters were sequenced in triplicates and biofilms were sampled from triplicate experimental microcosms (additional sampling points in plot are due to replicate sequencing of selected biofilm samples). Highlighted areas were added manually to emphasize differences between samples.

In accordance with the degree of diversity loss, these community compositions changed considerably more compared to the bottled waters, which was attributed to the higher loss in taxa richness. Here, Bray-Curtis (BC) indices indicated a dissimilarity

of 100 % between original and biofilm communities on taxa level (BC 1.0 ± 0.0, n = 3). In comparison, the dissimilarity was smaller for communities of B2 (highlighted in Figure 5), with BC indicating a dissimilarity of 89 %. The higher degree of similarity for B2 indicated that (1) the community composition changed comparatively little during biofilm formation. This indicates, in combination with the low growth, that diversity in the original B2 did not cover enough metabolic functions to exploit the full growth potential provided by migrating carbon. To emphasize this, 83 % of the most abundant taxa of B2 biofilms (i.e., the 5 most abundant taxa amongst triplicate microcosms) were also detected in the original water community (Table S5), which is high compared to B1 (56 %) or the tapped waters (38 – 50 %). Interestingly, when comparing samples of different origin (i.e., with different starting communities), biofilms were more similar to each other compared to the original drinking water communities. Here, Bray-Curtis dissimilarity between original drinking water communities was 0.94 ± 0.1 (n = 5) and resulted in a BC of 0.83 ± 0.16 (n = 5) between biofilms. In summary, accompanying the reduction in taxa diversity, the community compositions of the original waters changed during biofilm formation and become more similar to each other. What remains unclear is whether the loss in diversity and compositional changes necessarily involved the growth of identical taxa.

3.2.4 Similarities in biofilm communities

Biofilm communities that developed from different (original drinking water) starting communities comprised shared taxa and families. Out of 29 ± 12 taxa/biofilm community (n = 15; including taxa with ≥ 0.01 % relative abundance), only two taxa were present in all biofilms that grew on EPDM coupons (Table S4). These taxa were identified as (1) *Methylobacterium* spp. (0.3 ± 0.1 %, n = 15; ZOTU7) and as (2) a not

479 further identified member of the family Bradyrhizobiaceae (0.5 ± 0.6 %, n = 15;
480 ZOTU23). Taxa that were detected in at least one of the experimental triplicates per
481 set up were (1) a member of Bacilliaceae (0.04 ± 0.05 %, n = 13; ZOTU148), (2)
482 *Aquabacterium* spp. (23 ± 29 %, n = 13; ZOTU1024), and (3) a member of the family
483 Comamonadaceae (10 ± 16 %, n = 14; ZOTU5147). Here, ZOTU5147 was very
484 abundant in the biofilms of B1 (35 ± 14 %, n = 3) and ZOTU1024 in the biofilms of T1
485 (70 ± 9 %, n = 3) and T2 (49 ± 8 %, n =3). Out of 16 ± 6 families/biofilm community (n
486 = 15), four families were present in all EPDM biofilms, namely (1) Bradyrhizobiaceae
487 (0.6 ± 0.6 %, n = 15), (2) Comamonadaceae (43 ± 28 %, n = 15), (3)
488 Methylobacteriaceae (0.3 ± 0.1 %, n =15), and (4) Sphingomonadaceae (3 ± 7 %, n =
489 15) (Table S6). In addition, the families (1) Bacillaceae (0.05 ± 0.05 %, n = 13), (2)
490 Brucellaceae (0.03 ± 0.02 %, n = 13), (3) Burkholderiaceae (22 ± 30 %, n = 13), (4)
491 Caulobacteraceae (4 ± 6 %, n =13), and (5) Xanthomonadaceae (8 ± 13 %, n = 13)
492 were present in at least one triplicate per experimental set up. Of these,
493 Comamonadaceae was highly abundant in the biofilm communities of T1 (71 ± 10 %,
494 n = 3) and T2 (61 ± 14 %, n = 3). For the bottled waters, Burkholderiaceae was
495 dominant in B2 (71 ± 15 %, n = 3) and Xanthomonadaceae showed a high abundance
496 in the biofilm communities of B1, with a relative abundance of up to 18 %. This result
497 highlights that biofilm communities had a certain consistency in their compositions,
498 despite clear differences in their starting communities. The selective pressure during
499 biofilm formation and growth was not only demonstrated by the loss of taxa but also
500 by the dominance of originally rare taxa. Within individual biofilm communities, the five
501 most abundant taxa accounted for 94 ± 5 % (n = 15) (Table S4). From these individual
502 abundant taxa, 53 ± 17 % (n = 5) were detectable in the corresponding original drinking
503 water communities (i.e., the rest was below detection limit of the method). Interestingly,

504 the chance of dominant biofilm taxa also being abundant in the original drinking water
505 community was associated with the degree of initial diversity. For example, a highly
506 abundant taxon in T1 biofilms (70 ± 10 %, n = 3; ZOTU1024) was rarely detected in
507 the original water with a relative abundance of only 0.03 ± 0.01 % (n = 3) (Table S5).
508 Bottled water B2 had the lowest taxa richness and diversity. Here, the most abundant
509 biofilm taxa (71 ± 15 %, n = 3; ZOTU46) was already very abundant in the original
510 water (8 ± 0.4 %, n = 3; ZOTU46). These results show that biofilm formation on carbon
511 supplying EPDM coupons was highly selective and resulted in a considerable loss in
512 taxa richness and diversity. As a result, the composition of biofilm communities differed
513 from their original drinking water communities. Individual biofilms showed, however,
514 similarities regarding dominant organisms, which indicated that, irrespective of starting
515 communities, the environment (i.e., additional carbon supply) was selective for specific
516 taxa and families, which was potentially linked to metabolic functions (see, e.g., [25]).
517

518 **3.3 Attachment experiment**

519 The selected biofilm communities attached more and much faster to new surfaces
520 compared to the original water communities (Figure 6).

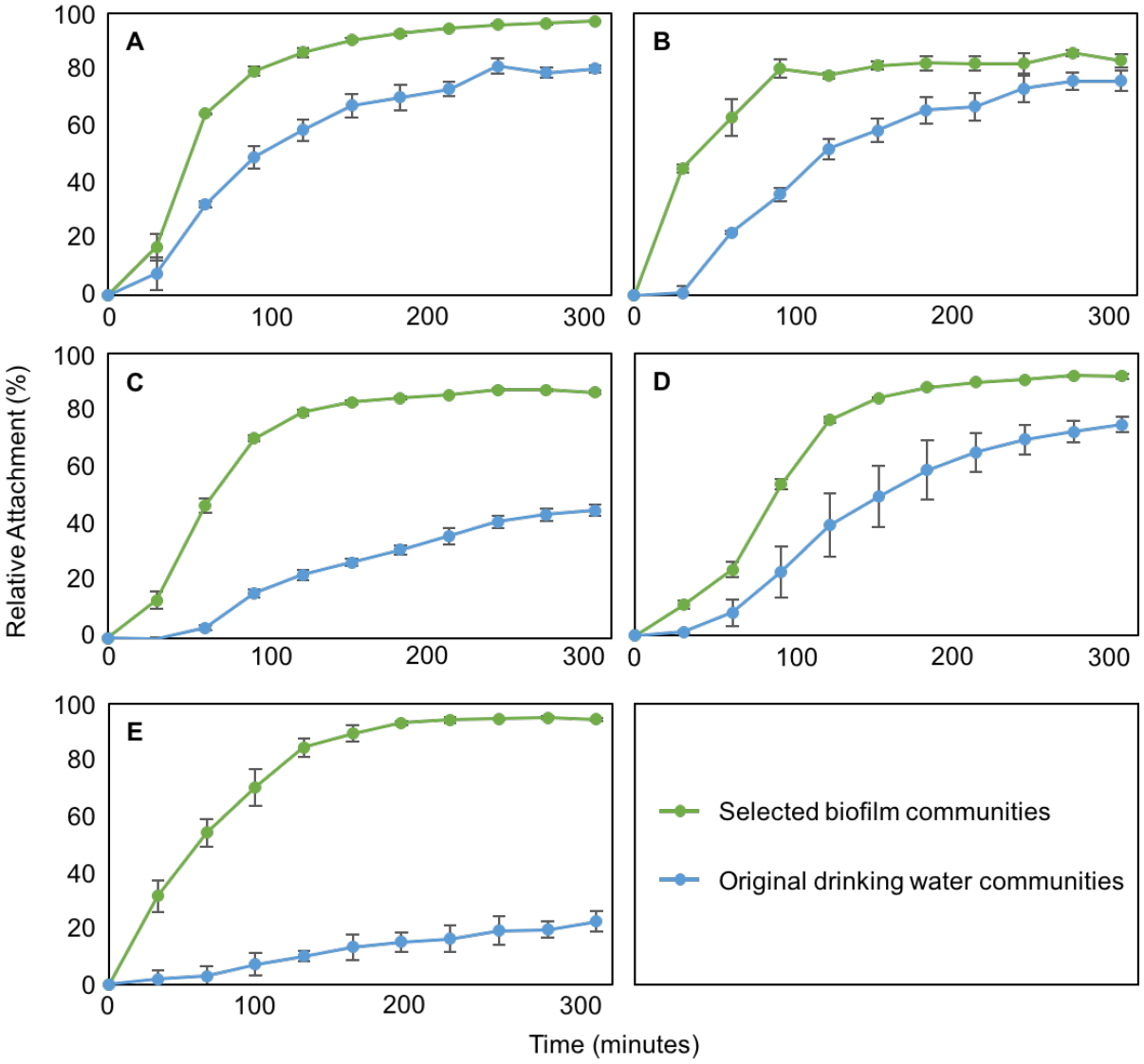

Figure 6 Attachment of selected biofilm and original drinking water communities to EPDM coupons. (A) B1 (bottled Evian; F), (B) B2 (bottled Aproz; CH), (C) T1 (tapped groundwater, Dübendorf; CH) (D) T2 (tap water, Dübendorf; CH) (E) T3 (tap water, Oerlikon; CH). Data points show average values and standard deviations for triplicate experiments.

For the selected communities, 91 ± 5 % (n = 15) of the cells attached within the first 5 hours after exposing freshly suspended cells to new EPDM coupons. This was approx. 30 % more than for the original water communities (69 ± 23 %, n = 15). Between the

original communities, strong differences were measured in maximum attachment. For example, cells from original water B1 attached to 80 ± 1 % (n = 3) within 5 hours as opposed to T1 with only 45 ± 2 % (n = 3). The direct comparison between original and selected communities showed a clear advantage for the selected cells. For example, after 5 hours of incubation one tapped water (T3) showed a relative attachment of 94 ± 1 % (n = 3) for the selected community, but only 22 ± 4 % (n = 3) for the original community (Figure 6E). In absolute numbers, this percentage translates to a maximum attachment of 6.7 ± 0.1 x 10^4 cells/cm^2 (n = 3) for the selected community and 1.4 ± 0.3 x 10^4 cells/cm^2 (n = 3) for the original community (Table S6). In addition to the high maximum attachment, maximum attachment rates were on average 5.0 ± 1.7 x 10^4 cells/cm^2/h (n = 15) in selected communities and 2.4 ± 1.2 x 10^4 cells/cm^2/h (n = 15) in original communities. Regarding T3, maximum attachment rate for selected cells was almost 10-fold higher with 3.9 ± 0.4 x 10^4 cells/cm^2/h (n = 3) as opposed to 4.6 ± 0.9 x 10^3 cells/cm^2/h (n = 3). The comparison of the relative attachment between selected and original communities of all waters showed a 1- to 4-times higher maximum attachment and a 1- to 7-times higher maximum attachment rate for the selected communities. Interestingly, the attachment dynamics were similar with glass coupons as surface (Figure S1). Maximum attachment rates were almost identical between EPDM and glass coupons. Maximum attachment after 5 hours was, however, 6-fold higher on EPDM coupons (Table S7). In summary, a considerably large proportion of planktonic cells attached to the coupons (both EPDM and glass) within a short time. The selected communities attached faster and showed higher absolute values for attachment compared to the original drinking water communities.

4. Discussion

We analyzed biofilm growth on flexible EPDM coupons for five different drinking water communities (Figure 1). The purpose was to study the amount of growth due to biodegradable carbon migrating from the EPDM (Figure 2) and to assess selection within the developing biofilm communities due to this carbon. In the course of biofilm growth (Figure 3), all samples showed a significant loss in species diversity (Figure 4) with, however, the development of different community compositions (Figure 5). The selected communities were in turn more likely to form new biofilms on clean coupons (Figure 6).

4.1 Nutrient-based selection within microbial communities

Community composition in complex biofilms is governed by known ecological principles such as dispersal, selection, drift, and diversification[26]. In the presence of sufficient nutrients, selection within microbial communities is (at least partially) driven by the metabolic potential and growth physiology of individual members. The supply of different/new substrates to an established community allows the growth of different bacterial species based on their metabolic capabilities, resulting in a change in the community composition. For example, Eilers and colleagues[27] (soil communities) and Reintjes and colleagues[28] (marine communities) demonstrated that the addition of particular carbon substrates resulted in bacterial growth, a loss in diversity, and considerable changes in community compositions. Reintjes and colleagues[28] also showed that initially abundant taxa were not abundant in the grown communities anymore. Finally, Wawrik and colleages[29] showed that carbon sources that differ in their complexity select for different bacterial communities, with 70 % dissimilarities

581 between communities grown on different substrates. This happened quickly, e.g., with
582 a developing community growing on acetate being 70 % dissimilar within 18 h of
583 incubation. The establishment of similar communities, relative to the complexity of
584 supplied carbon substrate, indicated that metabolic capabilities (biochemical
585 pathways) are similar amongst growing cells[29]. Our results mirrored many of these
586 findings, in that richness decreased dramatically (Figure 4) and the dominating
587 bacteria after growth on EPDM were completely different to the dominating bacteria in
588 each original water (Figure 5). However, it is noted that even when microorganisms
589 are capable of using the same substrates, growth physiology (i.e., growth rate and
590 yield) allows some species to outcompete others. This so called *competitive exclusion*
591 *principle*[30] was demonstrated by Friedman and colleagues[31] and Christensen and
592 colleagues[32] who correlated the ability to outcompete others to a species' growth rate
593 and yield. Our data (e.g., Figure 5) does not allow separation between selection
594 caused by metabolic capabilities and growth physiology. However, this may be an
595 explanation why some taxa dominated in the microcosms.
596
597 The arguments above explain selection during growth, but may lead to an erroneous
598 conclusion that growth on the EPDM coupons should by default result in similar
599 communities being selected. Here, our data clearly showed that all five original water
600 samples resulted in completely different final communities following growth (Figure 5).
601 This is explained by the fact that many different bacterial species can have the same
602 carbon-degrading functions, i.e., identity does not equal functionality. In this regard,
603 Burke and colleagues[33] showed that communities that are dissimilar in their
604 taxonomical composition (e.g., 15 % similarity) can be very similar regarding their
605 functional composition (with, e.g., 70 % similarity). Recent work by Goldford and

606 colleagues[25] showed that growth on additional carbon sources increased the
607 dissimilarity between developing communities on taxonomic level, however, revealed
608 carbon source specific analogies on family level[25].
609
610 **4.2 Understanding building plumbing microbiomes**
611 One very practical relevance of biofilm growth and selection discussed above is the
612 first colonization of drinking water plumbing systems during the commissioning of new
613 buildings. Here, a wide variety of synthetic plumbing materials[34,35] provides
614 biodegradable organic carbon[36,37] to complex drinking water microbial
615 communities[38,39]. The consequence is biofilm formation and development in the
616 plumbing system, which ultimately affects the microbiological quality of the drinking
617 water[34,40]. Observations in recent years from several pilot-scale and full-scale studies
618 reported considerable changes in drinking water microbial communities after passage
619 through building plumbing systems. For example, Ling and colleagues[11] showed that
620 the community composition of the drinking water changes during stagnation within
621 building plumbing systems compared to the composition within the distribution
622 network. Work from our own group specifically compared tap water, stagnated water,
623 and biofilm communities in shower hoses and showed distinct changes in the
624 microbiome due to the biofilms growing in the hoses[12]. These findings are further
625 supported by data from Ji and colleagues[13] and Dai and colleagues[41] showing that
626 material, temperature, and stagnation time change the microbiology compared to the
627 water community flowing into the rig installations, with, e.g., stagnation resulting in a
628 diversity loss within the drinking water community[41].
629

It is clear that building plumbing systems are per se complex environments, with multiple confounding factors (e.g., temperature, hydraulics, nutrients) affecting bacterial colonization, growth, and microbiome composition. Previous studies suggested that the choice of plumbing material plays a critical role, particularly when the material supplies nutrients for growth[15,24,42]. For example, Rogers and colleagues[43] studied biofilm development on different materials with different extents of growth supporting properties (and their ability to resist invading *Legionella*). Proctor and colleagues[15] studied different shower hose materials and found differences in growth community composition. Along the same lines, we demonstrated in a previous study considerable selection in biofilm communities forming within shower hoses, with differences in the microbiome measurable on small-scale[14]. In the present study, we show an example for EPDM, which is commonly used for drinking water applications[16,24,44,45] (Figures 3, 4, 5). We demonstrated selection, but also showed that selection differed based on the source water (Figure 5). While there is an obvious need and scope for larger observational studies on drinking water microbiomes[46], there is also a clear need for basic laboratory-scale ecological studies that can help to inform on interpretations from complex building plumbing data. Moreover, understanding the basic ecology of building plumbing systems will provide a basis for proactive management of the microbiomes in these systems.

4.3 Managing colonization of building plumbing materials

Better knowledge on growth-dependent selection within biofilm communities can be used to design building plumbing systems where the microbiology is controlled or even specifically tailored to the system. Microbial colonization and growth on building plumbing materials is currently not (properly) controlled. Upon commissioning of a

655 building, all new plumbing material is exposed to complex drinking water communities
656 during the first use. In fact, there is essentially no control over the identity and
657 composition of bacteria that attach and proliferate in the new system, irrespective of
658 the location, source water, disinfectant use, building type, or plumbing materials. To
659 date, there are surprisingly few studies looking at this initial colonization of building
660 plumbing materials, both full- and pilot-scale. A notable exception is the study by
661 Salehi and colleagues[34], where they monitored changes in water chemistry and
662 bacterial growth during the first days/weeks of building occupation. Also, a study by
663 Douterelo and colleagues[47], showed that specific bacteria are dominant during the
664 initial colonization (7 – 28 d) of distribution pipe materials. The fact is, in current
665 practice the owners/operators have effectively no control over the communities that
666 colonize their building plumbing systems.
667
668 Smart use of material properties can control microbial growth (and thus biofilm
669 communities). For example, the use of high quality materials and the avoidance of low
670 quality ones (e.g., flexible hoses) reduces the potential of bacteria to actually grow.
671 For this, standards for material quality requirements have already been implemented
672 in Europe (e.g., [48]) and official tests on carbon migration and corresponding growth
673 potentials have been established (e.g., [17,49]) (e.g., Figure 2). Using such tests to qualify
674 the use of individual materials in new buildings should be a must for the industry. A
675 more expensive but sensible approach is to use materials that do not leach any carbon
676 (e.g., stainless steel plumbing). For example, Van der Kooij and colleagues[50] showed
677 that biofilm growth and the incorporation of *Legionella* spp. was less on stainless steel
678 compared to polymeric PE-X pipes. It is, however, important to take into account that
679 high quality polymeric materials can perform as good as metal piping with regard to

680 microbial growth (see, e.g., [51]). As another possibility, some studies suggested
681 developing and using plumbing materials with anti-microbial properties for minimizing
682 microbial growth and the proliferation of pathogens. For example, Saleh and
683 colleagues[52] showed that a coating containing copper and silver ions resulted in less
684 bacterial attachment and biofilm growth when exposing a *Pseudomonas aeruginosa*
685 isolate (10^5 – 10^6 CFU/mL) to coated glass slides for 2 h, shaking.
686
687 An alternative approach could be to embrace the microbiology of building plumbing
688 systems instead of resisting it. Carbon migration from building plumbing materials can
689 theoretically be used to select and maintain preferred communities. Wang and
690 colleagues[53] provocatively suggested that systems may be redesigned in a
691 pre/probiotic approach to favor certain communities of choice. One way to approach
692 this addresses the concept of niche occupation, which is especially important during
693 the colonization of new surfaces, e.g., during the commissioning of a new building.
694 Niche occupation can result in the exclusion of species due to a more efficient spatial
695 expansion of a competitor or due to better growth physiologies[54]. For the first, Schluter
696 and colleagues[55] emphasized the importance of adhesion during initial attachment for
697 the *evolutionary fate of microbes in biofilms*. For the second, Freilich and colleagues[56]
698 defined the competition for identical nutrient sources as a *win-lose relationship*, which
699 will ultimately allow organisms with better growth yields/rates to outcompete others.
700 This pre/probiotic approach can be taken a step further by introducing, selecting, and
701 maintaining specific antagonistic bacteria that challenge unwanted organisms. For
702 example, several studies showed that a range of aquatic isolates, especially
703 *Pseudomonas* spp., produce *bacteriocin-like substances* that have an antagonistic

704 effect on the establishment of *Legionella* spp. in biofilms[57–59], which can potentially be
705 exploited as probiotic communities against *Legionella pneumophila*.
706
707 Here we propose a combination of the approaches above. We argue for the use of
708 plumbing materials that provide specific substrates and for the targeted colonization
709 of these materials of a benign microbial community. The approach foresees the use
710 of materials that migrate organic carbon in such a quality and quantity that it allows
711 bacteria to grow and to sustain their existence in the developing biofilm. We
712 furthermore propose colonizing these materials with bacteria from a safe source (e.g.,
713 bottled water), pre-selected on the substances migrating from the material (e.g.,
714 Figure 5). This adaption to the nutrients ultimately allows for a rapid colonization (e.g.,
715 Figure 6), growth and long term persistence. A further expansion of the approach could
716 be the use of purposefully designed synthetic communities that specifically include
717 antagonists to specific building plumbing pathogens[57–59]. Combining both niche
718 occupancy capabilities and powerful antagonistic functions within a pre-
719 conditioned/pre-selected community is an unconventional but exciting approach
720 towards the future management of biofilm formation on polymeric materials in contact
721 with drinking water.
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5. Conclusions

- The use of a flexible polymeric plumbing material (here EPDM) increased the biodegradable organic carbon concentration of drinking water, which resulted in substantial growth for bacterial communities of different origin.
- The migrating carbon drove nutrient-based selection within the original drinking water communities, which resulted in (1) a dramatic decrease in taxa richness and diversity, (2) compositional changes in communities, and (3) an increase in similarity amongst growing biofilm communities, i.e., similarities in abundant taxa.
- Selected biofilm bacteria showed better attachment performances to new material surfaces, with more attachment and higher attachment rates.
- This work is a step towards pro-active managing of building plumbing biofilms through nutrient-based selection of specific communities of choice.

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Author contributions

LN: experimental design, experimental work, data analysis, and manuscript writing.

LC: experimental design, experimental work, and data analysis.

FH: experimental design and manuscript writing.

763 **References**

764 1. Gaylarde, C., Ribas Silva, M. & Warscheid, T. Microbial impact on building
765 materials: an overview. *Mater. Struct.* **36,** 342–352 (2003).
766 2. Williams, M. M., Armbruster, C. R. & Arduino, M. J. Plumbing of hospital
767 premises is a reservoir for opportunistically pathogenic microorganisms: a
768 review. *Biofouling* **29,** 147–62 (2013).
769 3. Ji, P., Rhoads, W. J., Edwards, M. A. & Pruden, A. Impact of water heater
770 temperature setting and water use frequency on the building plumbing
771 microbiome. *ISME J.* 1–13 (2017). doi:10.1038/ismej.2017.14
772 4. Henne, K., Kahlisch, L., Ho, M. G. & Brettar, I. Seasonal dynamics of bacterial
773 community structure and composition in cold and hot drinking water derived
774 from surface water reservoirs. *Water Res.* **47,** 5614–5630 (2013).
775 5. Brazos, B. J., O'Conner, J. T. & Abcouwer, S. Kinetics of Chlorine Depletion
776 and Microbial Growth. (1985).
777 6. Kim, J. *et al.* Hydrodynamic effects on bacterial biofilm development in a
778 microfluidic environment. *RSC Publ.* **13,** 1846–1849 (2013).
779 7. Fish, K., Osborn, A. M. & Boxall, J. B. Biofilm structures (EPS and bacterial
780 communities) in drinking water distribution systems are conditioned by
781 hydraulics and influence discolouration. *Sci. Total Environ.* **593–594,** 571–580
782 (2017).
783 8. WHO. *Standards for materials used in plumbing systems*. (2011).
784 9. Holsen, T. M., Park, J. K., Jenkins, D. & Selleck, R. E. Contamination of
785 potable water by permeation of plastic pipe. *Am. Water Work. Assoc.* **83,** 53–
786 56 (1991).
787 10. Zhang, L. & Liu, S. Investigation of organic compounds migration from

polymeric pipes into drinking water under long retention times. *Procedia Eng.* **70,** 1753–1761 (2014).

11. Ling, F., Whitaker, R., LeChevallier, M. W. & Liu, W.-T. Drinking water microbiome assembly induced by water stagnation. *ISME J.* **12,** 1520–1531 (2018).

12. Proctor, C. R., Reimann, M., Vriens, B. & Hammes, F. Biofilms in shower hoses. *Water Res.* **131,** 274–286 (2018).

13. Ji, P., Parks, J., Edwards, M. A. & Pruden, A. Impact of Water Chemistry, Pipe Material and Stagnation on the Building Plumbing Microbiome. *PLoS One* **10,** e0141087 (2015).

14. Neu, L., Proctor, C. R., Walser, J.-C. & Hammes, F. Small-scale heterogeneity in drinking water biofilms. *Front. Microbiol.* **10,** 1–14 (2019).

15. Proctor, C. R. *et al.* Biofilms in shower hoses - choice of pipe material influences bacterial growth and communities. *Environ. Sci. Water Res. Technol.* **2,** 670–682 (2016).

16. Moritz, M. M., Flemming, H. C. & Wingender, J. Integration of Pseudomonas aeruginosa and Legionella pneumophila in drinking water biofilms grown on domestic plumbing materials. *Int. J. Hyg. Environ. Health* **213,** 190–197 (2010).

17. Koetzsch, S. & Egli, T. Kunststoffe in Kontakt mit Trinkwasser. *Aqua & Gas* **3,** 44–52 (2016).

18. Wen, G., Koetzsch, S., Vital, M., Egli, T. & Ma, J. BioMig - A Method to Evaluate the Potential Release of Compounds from and the Formation of Biofilms on Polymeric Materials in Contact with Drinking Water. *Environ. Sci. Technol.* **49,** 11659–11669 (2015).

19. Prest, E. I., Hammes, F., Kötzsch, S., van Loosdrecht, M. C. M. & Vrouwenvelder, J. S. Monitoring microbiological changes in drinking water systems using a fast and reproducible flow cytometric method. *Water Res.* **47,** 7131–7142 (2013).

20. Klindworth, A. *et al.* Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* **41,** 1–11 (2013).

21. Edgar, R. C. Accuracy of microbial community diversity estimated by closed- and open- reference OTUs. *PeerJ* 1–17 (2017). doi:10.7717/peerj.3889

22. Neu, L. *et al.* Ugly ducklings - The dark side of plastic materials in contact with potable water. *npj Biofilms Microbiomes* **4,** (2018).

23. Mao, G., Wang, Y. & Hammes, F. Short-term organic carbon migration from polymeric materials in contact with chlorinated drinking water. *Sci. Total Environ.* **613–614,** 1220–1227 (2018).

24. Park, J. W. *et al.* Evaluation of organic migration and biomass formation on polymeric components in a point-of-use water dispenser. *Water Res.* **165,** (2019).

25. Goldford, J. E. *et al.* Emergent simplicity in microbial community assembly. *Science (80-.).* **361,** 469–474 (2018).

26. Kinnunen, M. *et al.* A conceptual framework for invasion in microbial communities. *ISME J.* **10,** 2773–2775 (2016).

27. Eilers, K. G., Lauber, C. L., Knight, R. & Fierer, N. Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. *Soil Biol. Biochem.* **42,** 896–903 (2010).

28. Reintjes, G., Arnosti, C., Fuchs, B. & Amann, R. Selfish, sharing and

838 scavenging bacteria in the Atlantic Ocean: a biogeographical study of bacterial
839 substrate utilisation. *ISME J.* **13,** 1119–1132 (2019).
840 29. Wawrik, B., Kerkhof, L., Kukor, J. & Zylstra, G. Effect of different carbon
841 sources on community composition of bacterial enrichments from soil. *Appl.*
842 *Environ. Microbiol.* **71,** 6776–6783 (2005).
843 30. Hardin, G. The Competitive Exclusion Principle Published by : American
844 Association for the Advancement of Science Linked references are available
845 on JSTOR for this article : The Competitive Exclusion Principle. *Science*
846 *(80-.).* **131,** 1292–1297 (1960).
847 31. Friedman, J., Higgins, L. M. & Gore, J. Community structure follows simple
848 assembly rules in microbial microcosms. *Nat. Ecol. Evol.* **1,** 1–7 (2017).
849 32. Christensen, B. B., Haagensen, J. A. J., Heydorn, A. & Molin, S. Metabolic
850 commensalism and competition in a two-species microbial consortium. *Appl.*
851 *Environ. Microbiol.* **68,** 2495–2502 (2002).
852 33. Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S. & Thomas, T. Bacterial
853 community assembly based on functional genes rather than species. *Proc.*
854 *Natl. Acad. Sci. U. S. A.* **108,** 14288–14293 (2011).
855 34. Salehi, M. *et al.* Case study: Fixture water use and drinking water quality in a
856 new residential green building. *Chemosphere* **195,** 80–89 (2018).
857 35. Koetzsch, S. & Egli, T. Kunststoffe in Kontakt mit Trinkwasser. *Aqua & Gas* **3,**
858 (2013).
859 36. Schoenen, D. & Schöler, H. Microbial alterations of drinking water by building
860 materials - field observations and laboratory studies. *Am. Water Work. Assoc.*
861 307–317 (1985).
862 37. Connell, M. *et al.* PEX and PP water pipes: Assimilable carbon, chemicals, and

odors. *J. Am. Water Works Assoc.* **108,** E192–E204 (2016).

38. Ma, X., Vikram, A., Casson, L. & Bibby, K. Centralized Drinking Water Treatment Operations Shape Bacterial and Fungal Community Structure. *Environ. Sci. Technol.* **51,** 7648–7657 (2017).

39. Henne, K., Kahlisch, L., Brettar, I. & Höfle, M. G. Analysis of structure and composition of bacterial core communities in mature drinking water biofilms and bulk water of a citywide network in Germany. *Appl Env. Microbiol* **78,** 3530–3538 (2012).

40. Inkinen, J. *et al.* Drinking water quality and formation of biofilms in an office building during its first year of operation , a full scale study. *Water Rese* **9,** 83–91 (2014).

41. Dai, D., Rhoads, W. J., Edwards, M. A. & Pruden, A. Shotgun Metagenomics Reveals Taxonomic and Functional Shifts in Hot water microbiome due to temperature setting and stagnation. *Front. Microbiol.* **9,** 1–17 (2018).

42. Rogers, J., Dowsett, A. B., Dennis, P. J., Lee, J. V. & Keevil, C. W. Influence of plumbing materials on biofilm formation and growth of Legionella pneumophila in potable water systems. *Appl. Environ. Microbiol.* **60,** 1842–1851 (1994).

43. Rogers, J., Dowsett, A. B., Dennis, P. J., Lee, J. V. & Keevil, C. W. Influence of plumbing materials on biofilm formation and growth of Legionella pneumophila in potable water systems. *Appl. Environ. Microbiol.* **60,** 1842–1851 (1994).

44. Kilb, B., Lange, B., Schaule, G., Flemming, H. C. & Wingender, J. Contamination of drinking water by coliforms from biofilms grown on rubber-coated valves. *Int. J. Hyg. Environ. Health* **206,** 563–573 (2003).

45. Meier, T. & Bendinger, B. Survival of pathogens in drinking water plumbing systems: impact factors and sanitation options. *Water Sci. Technol. Water*

Supply **16,** 931–941 (2016).

46. Hull, N. M. *et al.* Drinking Water Microbiome Project: Is it Time? *Trends Microbiol.* **27,** 670–677 (2019).

47. Douterelo, I., Sharpe, R. & Boxall, J. Bacterial community dynamics during the early stages of biofilm formation in a chlorinated experimental drinking water distribution system: Implications for drinking water discolouration. *J. Appl. Microbiol.* **117,** 286–301 (2014).

48. EN16421, D. *Influence of Materials on Water for Human Consumption Enhancement of Microbial Growth (EMG)*. (2014). doi:SS-EN 16421:2014

49. Bundesamt, U. *Leitlinie zur hygienischen Beurteilung von organischen Materialien im Kontakt mit Trinkwasser*. (2016).

50. Van Der Kooij, D., Veenendaal, H. R. & Scheffer, W. J. H. Biofilm formation and multiplication of Legionella in a model warm water system with pipes of copper, stainless steel and cross-linked polyethylene. *Water Res.* **39,** 2789–2798 (2005).

51. Lehtola, M. J. *et al.* Microbiology, chemistry and biofilm development in a pilot drinking water distribution system with copper and plastic pipes. *Water Res.* **38,** 3769–3779 (2004).

52. Saleh, S., Sweileh, B., Taha, S. O., Mahmoud, R. & Taha, M. O. Preparation of polyester-based metal-cross linked polymeric composites as novel materials resistant to bacterial adhesion and biofilm formation. *Molecules* **16,** 933–950 (2011).

53. Wang, H., Edwards, M. A., Falkinham 3rd, J. O. & Pruden, A. Probiotic approach to pathogen control in premise plumbing systems? A review. *Env. Sci Technol* **47,** 10117–10128 (2013).

913 54. Lloyd, D. P. & Allen, R. J. Competition for space during bacterial colonization
914 of a surface. *J. R. Soc. Interface* **12,** (2015).
915 55. Schluter, J., Nadell, C. D., Bassler, B. L. & Foster, K. R. Adhesion as a
916 weapon in microbial competition. *ISME J.* **9,** 139–149 (2015).
917 56. Freilich, S. *et al.* Competitive and cooperative metabolic interactions in
918 bacterial communities. *Nat. Commun.* **2,** (2011).
919 57. Corre, M. H., Delafont, V., Legrand, A., Berjeaud, J. M. & Verdon, J. Exploiting
920 the richness of environmental waterborne bacterial species to find natural
921 legionella pneumophilacompetitors. *Front. Microbiol.* **10,** 1–11 (2019).
922 58. Héchard, Y., Ferraz, S., Bruneteau, E., Steinert, M. & Berjeaud, J. M. Isolation
923 and characterization of a Staphylococcus warneri strain producing an anti-
924 Legionella peptide. *FEMS Microbiol. Lett.* **252,** 19–23 (2005).
925 59. Guerrieri, E. *et al.* Effect of bacterial interference on biofilm development by
926 Legionella pneumophila. *Curr. Microbiol.* **57,** 532–536 (2008).
927