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(Micro)Biological Sediment Formation in a Non-Chlorinated Drinking Water Distribution System

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Abstract: Sediment formation in drinking water distribution systems can lead to brown water at customer taps. Previous studies have shown that sediment formation is closely linked with (micro)biological processes in the distribution system, however the mechanism is not fully understood. Most available studies on discoloration or sediment formation mechanism are based on modeling, pilot-scale experiments, or low frequency data collected during pipe flushing. In this study, long-term sediment development in a large-scale drinking water distribution system was studied at one location over 11 years and at several locations along a known water trajectory during one year. Particulate material was collected at several locations using built-in and mobile filters that were connected to transport and distribution pipes in a semi-continuous manner. The volume of the collected material varied seasonally and the highest volumes were collected in the summer season. The material followed similar variations as temperature, invertebrates biomass and concentration of *Aeromonas*. The results showed that particulate matter of the sediment at downstream distribution locations was not released by the treatment works but instead forms along the distribution network, with increasing particle/floc size, biomass and Fe and Mn content. The large crustacean, *Asellus*, contributed to material production through feces excretion and formation of detritus by degradation of exoskeletons of dead animals. Detailed chemical characterization of the collected material showed the presence of proteins, calcium carbonate and iron precipitates. A similar sediment composition in a reference distribution system where customer complaints about brown water are experienced less frequently suggests that the sediment formation mechanism is the same but that water quality of the treatment effluent impacts the extent of material formation and growth of invertebrates. Overall, the results indicate that sediment formation in the distribution system is the result of complex combinations of (micro)biological and bio-chemical processes, including aggregation of particles with organic and inorganic matter, microbial growth on particles and biofilm, biomineralization, and growth of invertebrates. The determining factors to limit sediment formation, however, could not be identified. Further research is required to focus on the impact of treatment on shaping the distribution system ecosystem.

Keywords: sediments; particles; biological stability; drinking water; invertebrates

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1. Introduction

Water utilities worldwide aim to produce high quality drinking water that is safe and pleasant to drink and to supply it to consumers. Many water utilities, however, experience consumer complaints about discolored water at the tap [1,2]. This also happens in countries, such as the Netherlands, where high quality drinking water is produced through extensive treatment and is supplied through distribution systems with high integrity, low water loss, and minimal risk of external contamination. The drinking water company, PWN, which is located in North Holland, produces drinking water by direct treatment of surface water. In the region where this water is distributed, complaints about discolored water, taste, odor, and on rare occasions, the presence of visible invertebrates at the water meter, represent approximately 85 percent of the total number of water quality complaints (other water quality complaints are related to chemical aspects, such as hardness). The water utility also experiences periods of non-compliance with the Dutch guideline for *Aeromonas* (i.e., <1000 CFU/100 mL), which is monitored in the Netherlands as a biological stability/regrowth indicator [3].

Complaints about discolored water, the presence of invertebrates, and *Aeromonas* growth are closely related to the accumulation of particulate matter, which leads to sediment formation in the distribution system [1,2,4–6]. Previous studies showed that particulate material collected during pipe flushing programs is of biological origin and is closely linked to microbiological processes in the distribution system. Sediments contain organic carbon and nitrogen, as well as high concentrations of bacteria and invertebrates [5–9]. Studies suggest different sediment formation mechanisms, which include biofilm detachment from pipe walls that is caused by hydraulic shear stress [10,11] as well as microbial growth on suspended or deposited particles [6–9].

Most available studies on discoloration or sediment formation mechanisms are based on: (1) modeling [12,13], (2) laboratory or pilot experiments [11], (3) infrequent data that was collected during pipe flushing and mechanical cleaning programs [2,4,5,8,9,14,15], or (4) samples from water tanks located in the distribution system for gravity-pressurized distribution [4,7]. Long-term variations in sediment accumulation, including seasonal changes, have not been studied, and systematic studies of material that accumulates in large-scale distribution systems over time and distance are lacking.

The goal of this study is to provide new insights on the mechanisms of sediment formation in a distribution system in the Netherlands through: (1) the study of long-term temporal variations of material that is accumulated in the distribution system, (2) the study of spatial variations of particulate material from the treatment to downstream distribution locations; and, (3) the analysis and characterization of the collected material. The study was performed in the drinking water distribution system of PWN, in the Netherlands. This water is produced from surface water and is distributed without a residual disinfectant (i.e., no chlorine or chloramine residual). Filter units were installed inside the distribution pipes to keep particles from reaching the consumer's taps. These filters are continuously collecting particulate material which is sampled on a routine basis. Data were collected from this distribution system for 11 years, and this provided a unique opportunity to study seasonal dynamics in sediment build-up in the distribution pipes. These results are compared with a reference distribution system where surface water treatment includes artificial infiltration through sand dunes and where water quality issues (e.g., discolored water, *Aeromonas* non-compliance) occur less frequently.

2. Materials and Methods

2.1. Study Locations

Sediment formation was studied in two separate large-scale distribution systems in the Netherlands. Both water treatment plants treat water from the Ijssel lake, which is a eutrophic surface water. One system (i.e., the main system of this study) has sediment accumulation issues, regular discolored water complaints, and non-compliance to the *Aeromonas* regulation, while the reference system has less frequent complaints or issues.

The study system supplies water that is treated by coagulation, sedimentation, rapid sand filtration, advanced oxidation with H_2O_2/UV, activated carbon filtration, micro-strainers (35 μm) and low dose of chlorine dioxide before the clear water reservoir. The free chorine residual of the effluent of the treatment plant is typically 0.01 to 0.03 mg/L and is not maintained in the distribution system. The treatment facility supplies water to approximately 370,000 people via 170,000 connections through a distribution system of approximately 2700 km of pipes, which are mainly PVC or asbestos cement pipes (an overview of materials used in the distribution system is available in Table S1 in Supplementary Materials). Water is first transported through a large main (900 mm, 20 km) towards an intermediate pumping station. A reservoir in the pumping station is used to level off the consumption peaks (fills during the night and delivers water during the day) and enables constant production at the treatment facility.

Water supplied in the reference system is treated first by coagulation, sedimentation, rapid sand filtration and activated carbon filtration. A split stream of the pre-treated water is further treated by advanced oxidation with H_2O_2/UV, followed by activated carbon, dune infiltration, aeration, rapid sand filtration and UV disinfection. A second split stream is treated by ultrafiltration and reverse osmosis. Both streams are mixed at a ratio of 60/40 for first and second streams, respectively, before distribution. The treatment facility supplies water to 380,000 people via 180,000 connections through a distribution system of 2300 km of pipes, which are mainly PVC and asbestos cement pipes (an overview of materials used in the distribution system is available in Table S1 in Supplementary Materials). An intermediate pumping station with a reservoir is also used to level off consumption peaks.

Information about the water quality at both treatment plants is available in Tables S2 and S3 in Supplementary Materials. A detailed assessment of the biological stability of the study system is also reported by Van der Kooij et al. (2015) [16].

2.2. Long Term Temporal Variations of Sediments at One Location in the Study Distribution System

Collection of particulate material was by polypropylene filter bags of 25 μm pore size (M-Filter, The Netherlands) (Figure 1A). Filters were built into a 63 mm distribution pipe at a location that had an average water age of approximately 54 h (location E1 on Figure 2). There were two, independent filter holders that were made of stainless steel (Twin Filter B.V.) and they were arranged in parallel (Figure 1B). The filter system was originally built near a location where customer complaints about discolored water were regularly recorded, and this filter system was designed to prevent particles from reaching customer's taps. In this system, all water that flowed through the pipe also went through the filters; therefore, all suspended particles or organisms that were larger than 25 μm were collected by the filters. The filters were collected regularly for analysis and replaced with new filters. Information about the volume of water filtered through each filter and the filter replacement frequency is provided in Table 1. In total, 416 data points were generated over 11 years from January 2011 to December 2021.

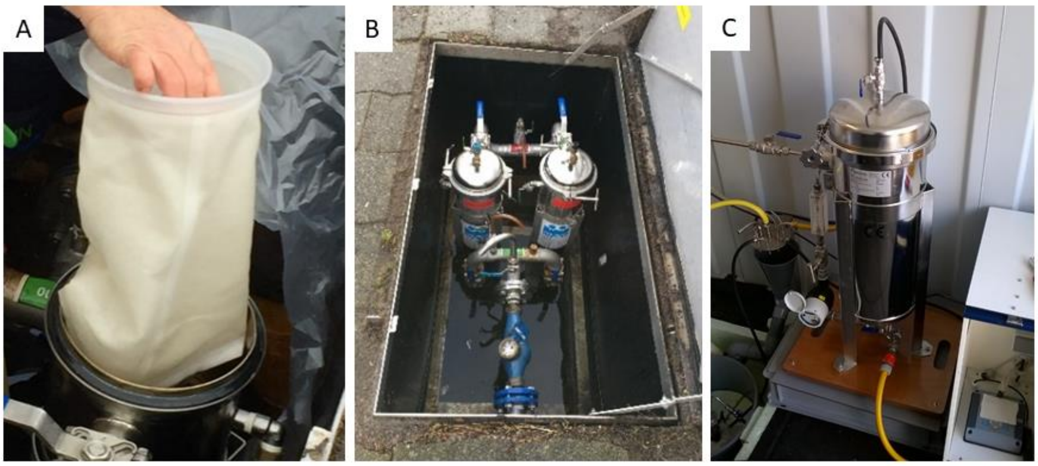

Figure 1. Equipment for the study of particulate material: (**A**) 25 μm filters for particle collection; (**B**) fixed filter holder system built into the distribution system for the temporal study; and (**C**) mobile filter holders for the spatial study.

Table 1. Characteristics of sampling locations and filter installations for the evaluation of spatial variations in the study system (locations A1 to E1) and the reference system (locations A2 to D2) as described in Figure 2. For location E1, data from the 11-year data set was selected to cover the same time period as for locations A1 to D1.

SAMPLE Point	Location	Pipe Diameter (mm)	Flow Range in Pipe (m^3/h)	Flow Velocity in Pipe (m/s)	Type of Installation	Sampling Location in the Pipe	Water Volume Collected Per Week Per Filter	Filters Replacement Frequency	Number of Data Points and Measurement Period
A1	Treatment effluent	900	1980 ± 160	0.88 ± 0.07	Mobile unit	Side	30–40 m^3	T > 12 °C: weekly; T < 12 °C: every two weeks	N = 28 May–December 2017
B1	Transport pipe	900	730 ± 470	0.32 ± 0.20	Mobile unit	Side	60–70 m^3	Every two weeks **	N = 15 May–December 2017
C1	Transport pipe	700	470 ± 190	0.37 ± 0.14	Mobile unit	Measure lance (top, middle, bottom)	30–40 m^3	T > 12 °C: weekly; T < 12 °C: every two weeks	N = 29 May–December 2017
D1	Transport pipe	500	107 ± 44	0.15 ± 0.06	Mobile unit	Measure lance (top, middle, bottom)	30–40 m^3	T > 12 °C: weekly; T < 12 °C: every two weeks	N = 29 May–December 2017
E1	Distribution pipe	150	7 ± 4 *	0.10 ± 0.06	Fixed unit	Full stream	30–40 m^3	T > 12 °C: weekly; T < 12 °C: every two weeks	N = 29 May–December 2017
A2	Treatment effluent	700	1420 ± 570	1.02 ± 0.41	Mobile unit	Side	30–40 m^3	Every two weeks **	N = 18 April–December 2018
C2	Transport pipe	500	190 ± 40	0.26 ± 0.06	Mobile unit	Side	30–40 m^3	Every two weeks **	N = 18 April–December 2018
D2	Transport pipe	300	128 ± 100	0.50 ± 0.40	Mobile unit	Side	30–40 m^3	Every two weeks **	N = 18 April–December 2018

* flow rate at location E is based on flow measurements performed for 5 days in July 2017 only. ** lower replacement frequency due to lower sediment volumes collected at these locations.

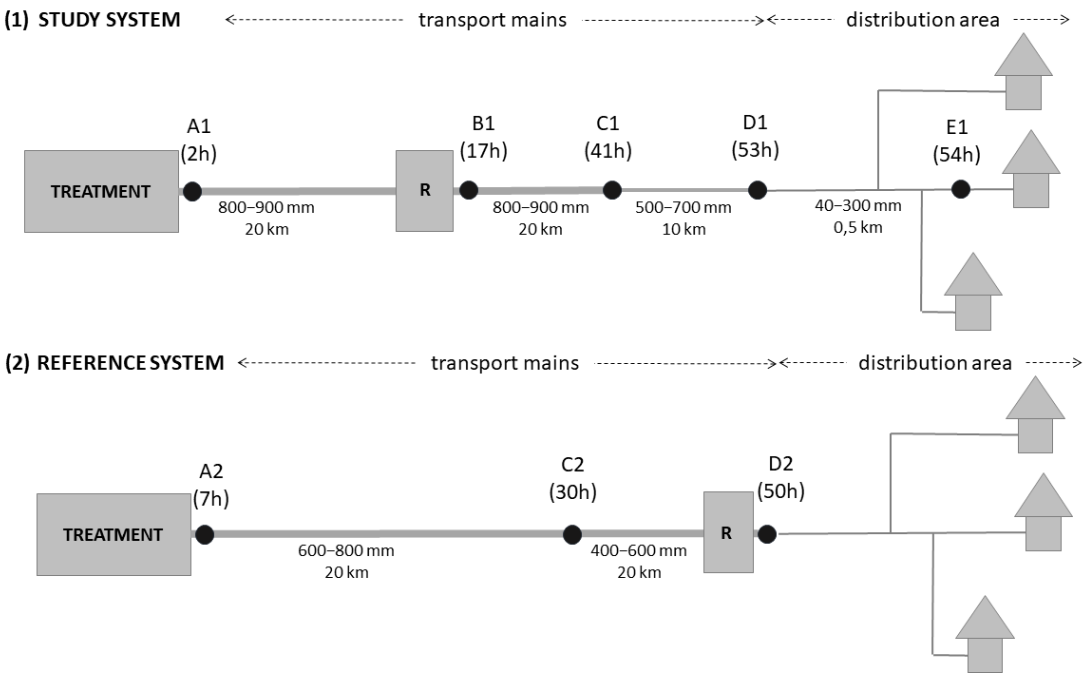

Figure 2. Sampling scheme for the study system (**top**) and the reference system (**bottom**). The sampling locations (A1 to E1 and A2 to D2) are located along known water trajectories from the treatment plants to downstream distribution areas, with intermediate storage reservoirs (R). Distances between locations, pipe diameters and residence times are indicated.

2.3. Spatial Variations of Sediments

The same filter bags (25 µm pore size, M-Filter, The Netherlands) were placed in mobile filter holders (stainless steel, M-Filter, The Netherlands; Figure 1C) at locations along known water trajectories from the treatment plants into distribution in both the study and the reference system (Figure 2). Depending on the location, water was extracted from the pipes in different ways due to site-specific pipe configurations (i.e., the pipe diameter, in a pumping station or underground). Water was from the side of large transport pipes which have high flow velocities and for smaller pipes, the water was from lances which collected water from the bottom, middle, and top levels of the pipe. Information about pipe diameters, sampling methods, volumes of water filtered through each filter, and filter replacement frequency at all locations of the spatial study is provided in Table 1. Samples for the spatial study were collected from May to December 2017 in the study system and from April to December 2018 in the reference system. The number of data points collected per sampling location is provided in Table 1.

2.4. Sediment Analysis

All filters collected for the temporal and spatial studies were sent to Het Waterlaboratorium (Haarlem, The Netherlands) for processing. Filters were flushed with tap water in graduated sedimentation cones and allowed to settle for 24 h. The sediment volume was recorded, and all (if there was a small volume) or part (if there was a large volume) of the particulate material was collected for observation under the stereomicroscope which had 10× magnification. The stereomicroscope allowed for observation and a description of the material that was present in the sample, and this material was listed as detritus, biofilm, feces of invertebrates, sand, rust, calcium precipitates, pipe pieces, etc. The description of the material was always done by the same analyst to avoid human biases. Invertebrates that were >30 µm were also counted and identified at group level. The total biomass was calculated from the number of invertebrates per cubic meter of filtered water using conversion factors of known biomass content per type of invertebrates (Table S4 in Supplementary Materials).

The material was subsequently analyzed for Fe and Mn content from May 2017 for the temporal study and for all filters of the spatial study, except for the filters of locations A1 and B1 which did not have enough volume for accurate Fe and Mn measurements. Note that the results for A1 and B1 are limited in number (i.e., 16 samples for A1 and 12 samples for B1) and were only from 2017. The samples were first dried at 105 °C for 12 h, then burnt at 550 °C ± 25 °C for 2 h to eliminate all organic material. The collected ash was then homogenized in 20 mL tap water and dissolved with 80% HNO3 for further analysis of Fe and Mn content by ICP-MS.

All data for sediment volume, invertebrates concentration and invertebrates biomass were normalized to a cubic meter of filtered water for comparison of results obtained at different times and locations. The filters were in place for one or two weeks and had different volumes of filtered water (Table 1). Sediment volumes and invertebrates biomass are therefore reported throughout the manuscript as mL sediment/m^3 of filtered water or µg biomass/m^3 of filtered water.

2.5. Additional Samples for Detailed Characterization of Sediments

In October and November 2020, extra particulate material was collected from the study system for analysis. Samples were taken from locations A1, C1, D1 and E1 using the same mobile and fixed filter units as for the spatial study. The samples were split into two fractions, one was frozen and the other was dried at 105 °C. Analysis included microscopic observation, FTIR, X-Ray diffractions, and scanning electron microscope coupled with energy dispersive X-ray spectroscopy (SEM-EDX) to provide information on the structure and composition of the sediment.

Microscopic pictures of the frozen samples were taken with a Zeiss AxioPlan 2 imaging microscope (Carl Zeiss, Oberkochen, Germany). Samples were observed with normal light, and all images were obtained with a 400× total magnification. The Fourier transform infra-red (FT-IR) spectrum of the dried sample was recorded on a FT-IR Spectrometer (Perkin Elmer, Shelton, CT, USA) at room temperature, with a wavenumber range from 550 cm^{-1} to 4000 cm^{-1}. The XRD patterns of the dried samples were recorded in a Bragg–Brentano geometry of a Bruker D5005 diffractometer equipped with Huber incident-beam monochromator and Braun PSD detector. Data collection was at room temperature using monochromatic Cu Kα1 radiation (λ = 0.154056 nm) in the 2θ region between 5° and 90°, step size 0.038° 2θ. Samples of approximately 20 mg were deposited on a Si <510> wafer and rotated during measurement. Data evaluation was performed with the Bruker program EVA. An environmental scanning electron microscope (ESEM-FEI Quanta 200F, Houston, TX, US) was used to observe the dried sample. Furthermore, chemical components of the sample were identified by an energy dispersive X-ray spectroscopy (EDX) during the ESEM observation.

3. Results

3.1. Long Term Variations of Sediments at Location E1

Long term variations of sediment volume and characteristics were studied in the study system at location E1, which was in a neighborhood where complaints about discolored water were regularly registered. The volume of particulate material that was collected at location E1 followed similar temporal variations every year (Figure 3). The volumes were low (<0.5 mL/m^3 filtered water) from January to April, but increased rapidly from May to June onwards, (up to 1.5 mL/m^3 on average during the summer months) and decreased slowly over the months September and November. Though this trend was repeatable every year, the maximum values differed from year to year. In some years two peaks were observed, one near the end of June or in early July and one in the end of August or in early September. The seasonal variations in volume of particulate material were congruent to the variations in water temperature, *Aeromonas* concentrations in the water phase, and biomass of invertebrates (>30 µm) in the sediment (Figure 4). The volumes remained low (<0.5 mL/m^3) at temperatures below 12 °C and increased significantly when temperatures increased above 14 °C. A similar trend was observed for the invertebrates biomass and *Aeromonas* concentrations. The legal guideline for *Aeromonas* concentrations (1000 CFU/100 mL) is exceeded every year at the study location. High peaks in sediment volumes and biomass of invertebrates are occasionally observed due to changes in water trajectory and hydraulics caused by pipe maintenance or shut down of the treatment plant. In case of treatment plant shut down, water is supplied by another treatment plant, as the distribution system is an open system.

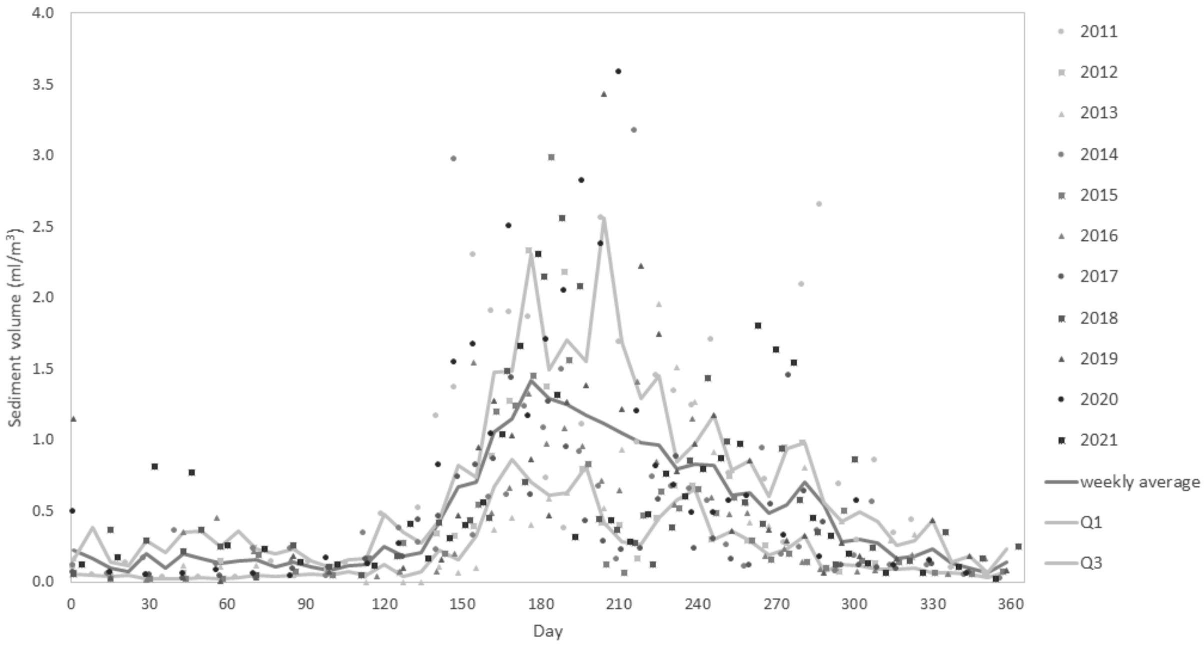

Figure 3. Comparison of the variations over one year for the sediment volume from 2011 to 2021 at location E1 of the study system. Sediment was collected in filters in place for one to two weeks (Table 1), and the volumes were normalized to a cubic meter of filtered water. The grey lines represent the weekly average, lower (Q1 to 25th percentile) and upper (Q3 to 75th percentile) quartiles of all data.

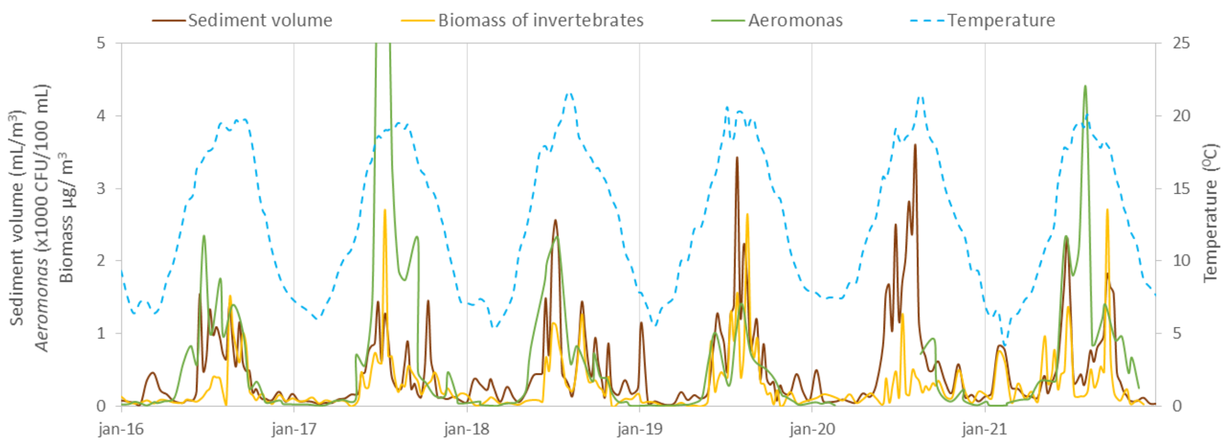

Figure 4. Variations over six years of the sediment volume, water temperature, biomass of invertebrates (>30 µm), and Aeromonas at location E1 of the study system. Sediment was collected in filters in place for one to two weeks (Table 1), and the sediment volumes and biomass of invertebrates were normalized to a cubic meter of filtered water. Aeromonas data in the period March to August 2020 are not available.

The material that was collected contained various invertebrates that were larger than 30 µm (Figure 5), and these included copepods (*Harpaticoida*, *Cyclopoida*, Nauplius larvae), crustaceans (*Asellidae*), water mites (*Halacaridae*), roundworms (nematodes), flat worms (*Turbellaria*), large worms (*Oligochaeta*), water fleas (*Chydoridae*), and larvae of water flies (*Chironomidae*). The most abundant organisms were *Chydoridae* (1.2 ± 2.8 /m^3) and *Harpaticoida* (0.4 ± 0.7/m^3). Dominant genera of *Chydoridae* were *Alona*, with an occasional presence of *Chyrodus* and *Graptolebris*, and all of them were living preferentially in or on sediments. The most dominant genera of Oligochaeta was *Stylaria*. *Harpaticoida* and *Oligochaeta* were found predominantly in or on sediment, which confirms the close link between sediment and abundance of invertebrates in the system. All other groups can live both in sediment or water, depending on the species. The samples have only been analyzed down to the group level, therefore further conclusions about the habitat of the sampled organisms cannot be drawn at this stage. *Asellidae* were present in low quantities compared to other organisms (0.2 ± 0.2 /m^3), however due to their large size (1–10 mm) [4,17] (Table S4 in Supplementary Materials) they contribute the most to the total biomass of invertebrates collected in the sediment (307 ± 396 µg/m^3) (Figure 5B).

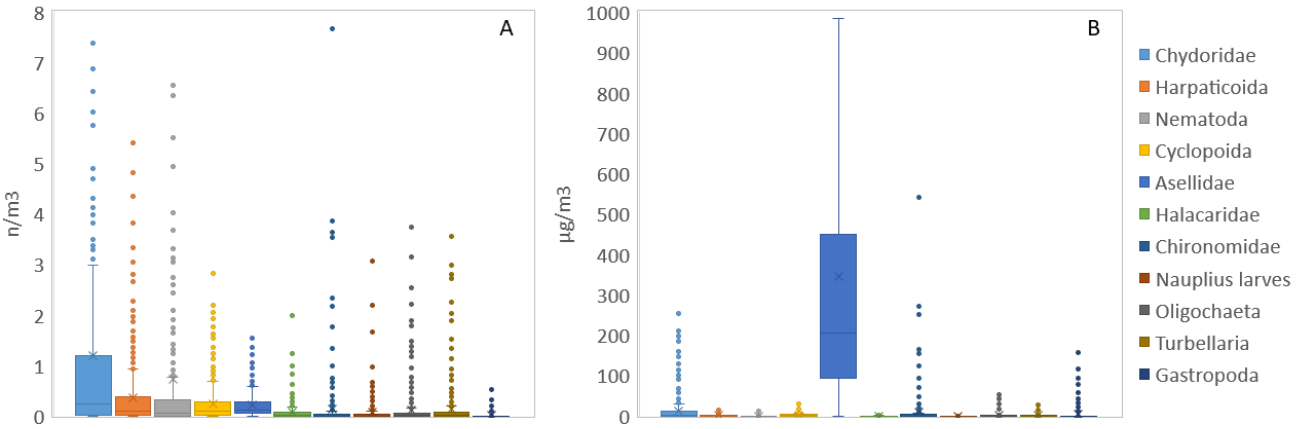

Figure 5. (**A**) abundance and (**B**) biomass of invertebrates collected in sediment of the study system at location E1 over the period January 2011 to December 2021. Invertebrates were collected in filters in place for one to two weeks (Table 1), and the biomass was normalized to a cubic meter of filtered water. Box plots represent the median, lower and upper quartiles of data collected over the entire study period.

3.2. Sediment Characterization at Location E1

Microscopic observation at low magnification (×10) showed that the particulate material collected at the study location E1 contained essentially detritus and feces of *Asellidae* (Figure 6). This result indicates that the material forms through processes related to the life cycles of invertebrates in the system (i.e., the decay of dead organisms and the production of feces) rather than by accumulation of sand or corrosion products. Higher magnification showed that the collected material is made of flocs in some cases bound by filaments, and these are probably filamentous bacteria (Figure 6). FTIR analysis showed peaks at 3309 cm^{-1}, 2957 cm^{-1}, 1653 cm^{-1}, 1423 cm^{-1}, 998 cm^{-1}, and 874 cm^{-1} (which was a strong peak), which indicates the presence of proteins, $CaCO_3$ in a poorly-ordered amorphous form (connected to biomineralization) and sand (Table 2; Supplementary Materials Figure S1). XRD analysis confirmed the presence of $CaCO_3$, as well as SiO_2, NaCl, and iron precipitates (i.e., FeO(OH) and $Fe_2O_3H_2O$) (Table 3; Supplementary Materials Figure S2). The presence of NaCl crystals was confirmed by SEM-EDX, as well as the presence of elements, such as Fe, Mn, Ca, which was in agreement with the XRD analysis (Supplementary Materials, Figures S3–S6).

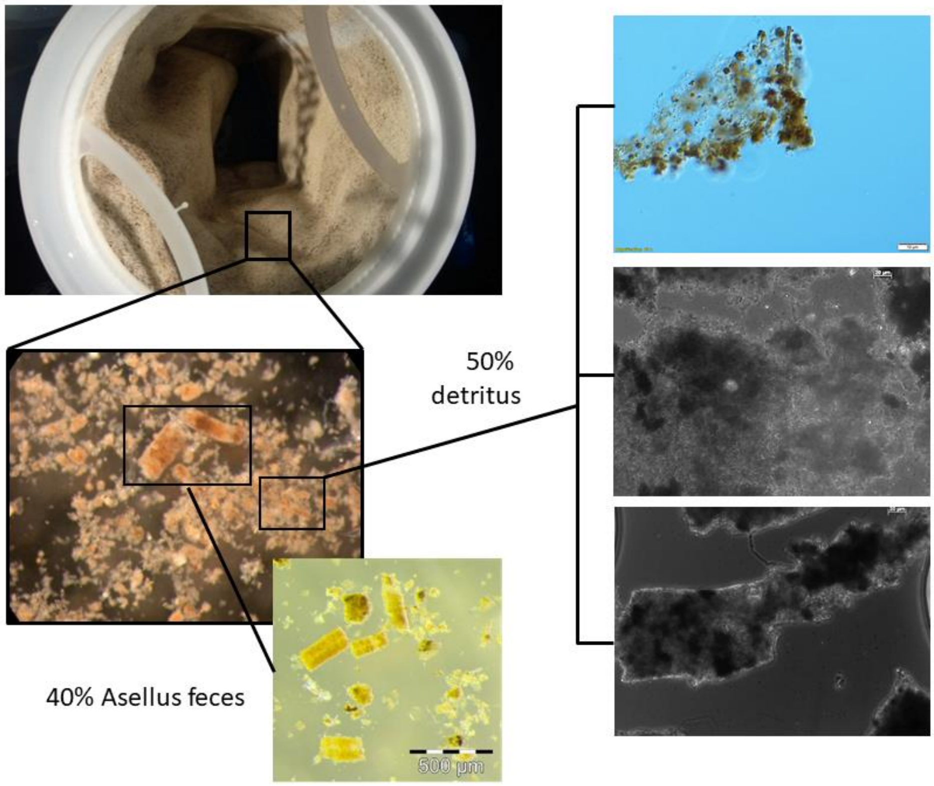

Figure 6. Microscopic images of sediment that was collected at the study location E1 under different magnifications.

Table 2. Summary of wavenumbers in FTIR spectrum of particulate material collected over the distance of the study system at locations A1, C1, D1 and E1. Wavenumbers related to organic functional groups are in indicated in **bold**; wavenumbers that are connected to inorganic functional groups are underlined.

Location	Wavenumber (cm^{-1})	Compounds
A1	**3330**, 2928, **1662**, 1438, **1122**, 874	Proteins, polysaccharides, $CaCO_3$
C1	**3343**, 2923 (strong), **1643**, 1457, 1377, 996, 875, 799, 716	Proteins, $CaCO_3$
D1	2930, **1643**, 1423, 1000, 874	Proteins, $CaCO_3$, sand
E1	**3309**, 2957, **1653**, 1423, 998, 874 (strong)	Proteins, $CaCO_3$, sand

Table 3. Crystalized minerals in particulate material collected over the distance of the study system at locations A1, C1, D1 and E1.

Location	$CaCO_3$ %	SiO_2 %	NaCl %	$FeO(OH) + Fe_2O_3H_2O$ %
A1	17.0	4.48	76.2	2.0 +2.98
C1	75.7	14.8	-	2.3 +14.8
D1	74.6	5.0	-	4.6 + 15.9
E1	78.4	7.2	4.1	3.2 + 7.2

Overall, the analysis of the particulate material that was collected at location E1 indicates that sediment formation is closely related to biological processes, including life cycle of invertebrates, biomineralization, and binding of organisms on sediment. The presence of proteins in the sediment confirms microbial activity on the particles, while the $CaCO_3$ may originate from bio-mineralization processes and/or from degradation of skeletons of invertebrates, such as *Asellidae* or *Cladocera*.

3.3. Spatial Variations

Changes in particulate material from the treatment plant to downstream locations in the distribution system was studied along a known water trajectory to track how the abundance of material and invertebrates develops at location E1. The material build-up along the water trajectory in the study system was also compared with the particulate material development in the reference system where complaints about discolored water are less frequent. The results show that the particulate matter forming the sediment at downstream distribution locations in both systems is not released by the treatment but forms in and along the distribution network. This was shown by a gradual increase in sediment volume and biomass of invertebrates from the treatment plants to downstream network locations (Figure 7). Though this phenomenon was observed in both systems, the volumes of material and biomass collected in the filters were higher in the study system than in the reference system (Figure 7a,b). Volumes of 0.24 ± 0.20 mL/m^3 of filtered water were found at location C1 compared to $0.0.8 \pm 0.0.9$ mL/m^3 at location C2 at comparable flow velocities and volumes of 0.4 ± 0.2 mL/m^3 were found at location D1, compared to 0.2 ± 0.1 mL/m^3 at location D2 at comparable residence times (Figure 2 and Table 1). This result indicates a higher particulate material production between the treatment plant and the distribution location in the study system when compared to similar locations in the reference system. At location E1, sediment volumes were occasionally significantly higher, up to 1.4 mL/m^3. Data from a distribution pipe for the reference system are not available.

The content of Fe and Mn in the collected material also increased along the distribution system in both systems, indicating Fe and Mn accumulation in the sediment (Figure 7). This result correlates with the presence of iron precipitates in the material collected at location E1 as well as at the other locations A1 to D1 (Table 3).

There was a significantly higher sediment formation in the study system than in the reference system (Figure 7), and as a result, a detailed analysis of the sediment is presented only for the study system (analysis of one location in the reference system is provided in Supplementary Materials, Tables S5 and S6 and Figure S7). Microscopic observation revealed increases in not only the sediment volume but also the size of the particles/flocs collected in the filter as water travelled from the treatment to the downstream distribution location D1 (Figure 8). This also confirms measurements of an earlier study where particle counters were placed at the same locations (A1 to E1), and the particle size increased along the path of the water distribution [18].

Chemical analysis of material collected at locations C1 and D1 showed comparable results with analysis of material from E1 location, with the presence of proteins, $CaCO_3$, and iron precipitates (Tables 2 and 3). Material collected at the treatment plant outlet (location A1) also contained polysaccharides and a higher content of NaCl than at downstream locations. The presence of polysaccharides and NaCl at the treatment plant outlet may initiate the formation of aggregates, which can serve as a template for further binding of organic material and minerals, such as Fe_2O_3 and $CaCO_3$, resulting in the formation of larger aggregates [19]. On the other hand, biopolymers can provide a matrix for the attachment and growth of microorganisms.

Besides chemical composition, the composition of the invertebrates present in the collected material was studied in both systems. The composition of invertebrate communities at the treatment plant outlet of the study system and of the reference system were different (Figure 9). At location A1, the biomass of water fleas (*Cladocera*) and of the larvae of water flies (*Chironomidae*) were dominant, while copepods (*Cyclopoida* and *Harpaticoida*) were dominant at location A2. The difference in the invertebrate composition indicates that different ecosystems develop in the treatment steps of both treatment facilities (e.g., activated carbon after direct treatment for the study system and sand filters after dune infiltration for reference system) and flush out into the distribution system. Note that only large organisms (>30 µm) such as copepods, water mites, roundworms, and water fleas were identified; however, it is expected that smaller organisms, such as rotifers, gastrotrichs, and ciliates were also present and in larger abundance. The composition of invertebrate community changed over the distribution system trajectory in both systems, with *Asellidae* not present at early locations but dominating the biomass at downstream locations. In the study system, *Asellidae* were found in all filters collected during the study time; however, in the reference system, *Asellidae* were found in only 5 of 18 sampling dates. Note *Asellidae* numbers are lower than other organisms, but due to their large size, they are dominating the biomass.

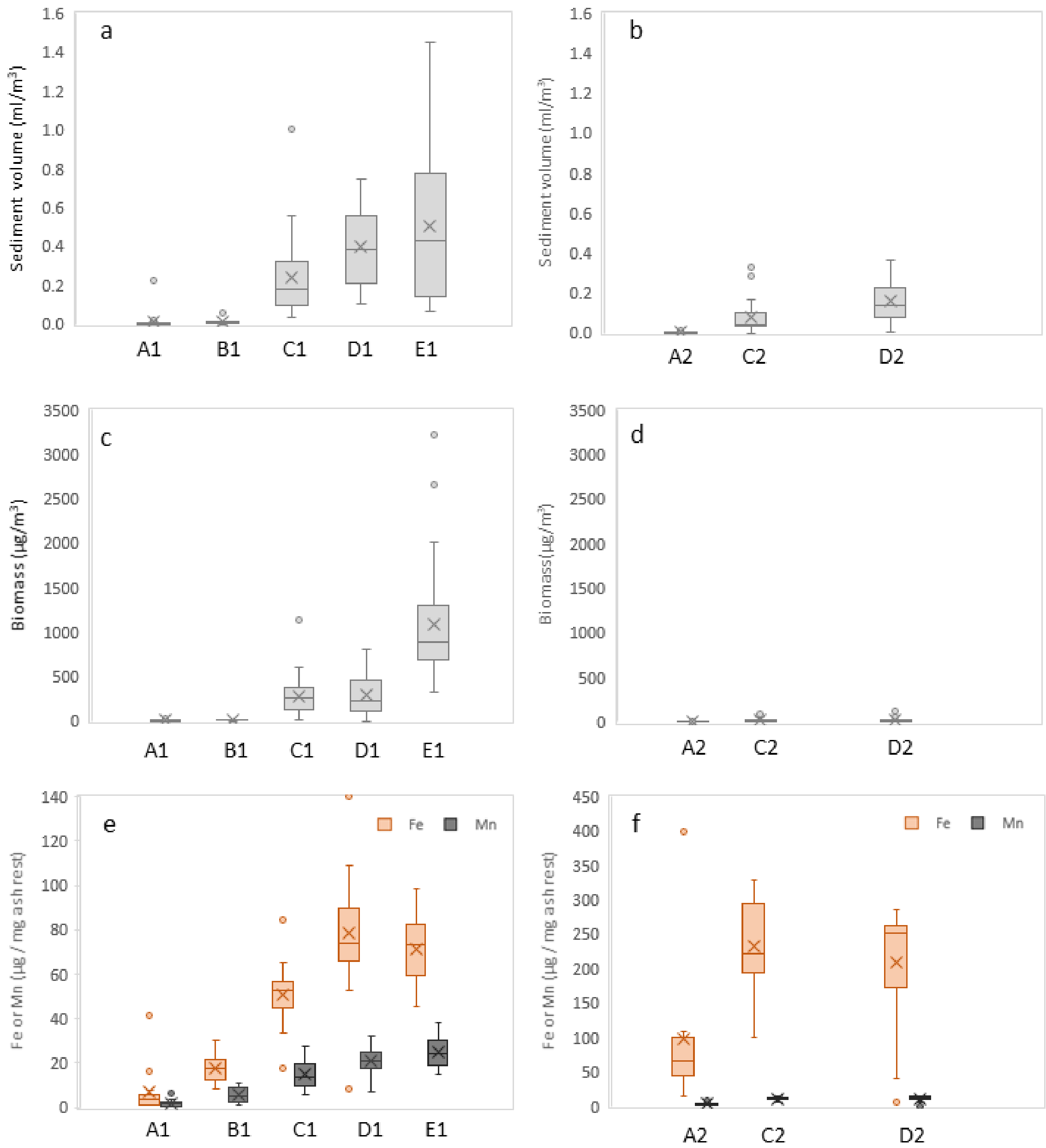

Figure 7. Variations from the treatment plant (A) to downstream distribution locations (D, E) of (**a**,**b**) sediment volume, (**c**,**d**) biomass and (**e**,**f**) Fe and Mn content in particulate material collected in the study system (left graphs, **a**,**c**,**e**) and in the reference system (right graphs, **b**,**d**,**f**) over 8 months. Sediment was collected at all locations in filters in place for one to two weeks (Table 1), and the results were normalized to a cubic meter of filtered water. Box plots represent the median, lower and upper quartiles of data collected over the study period (Table 1).

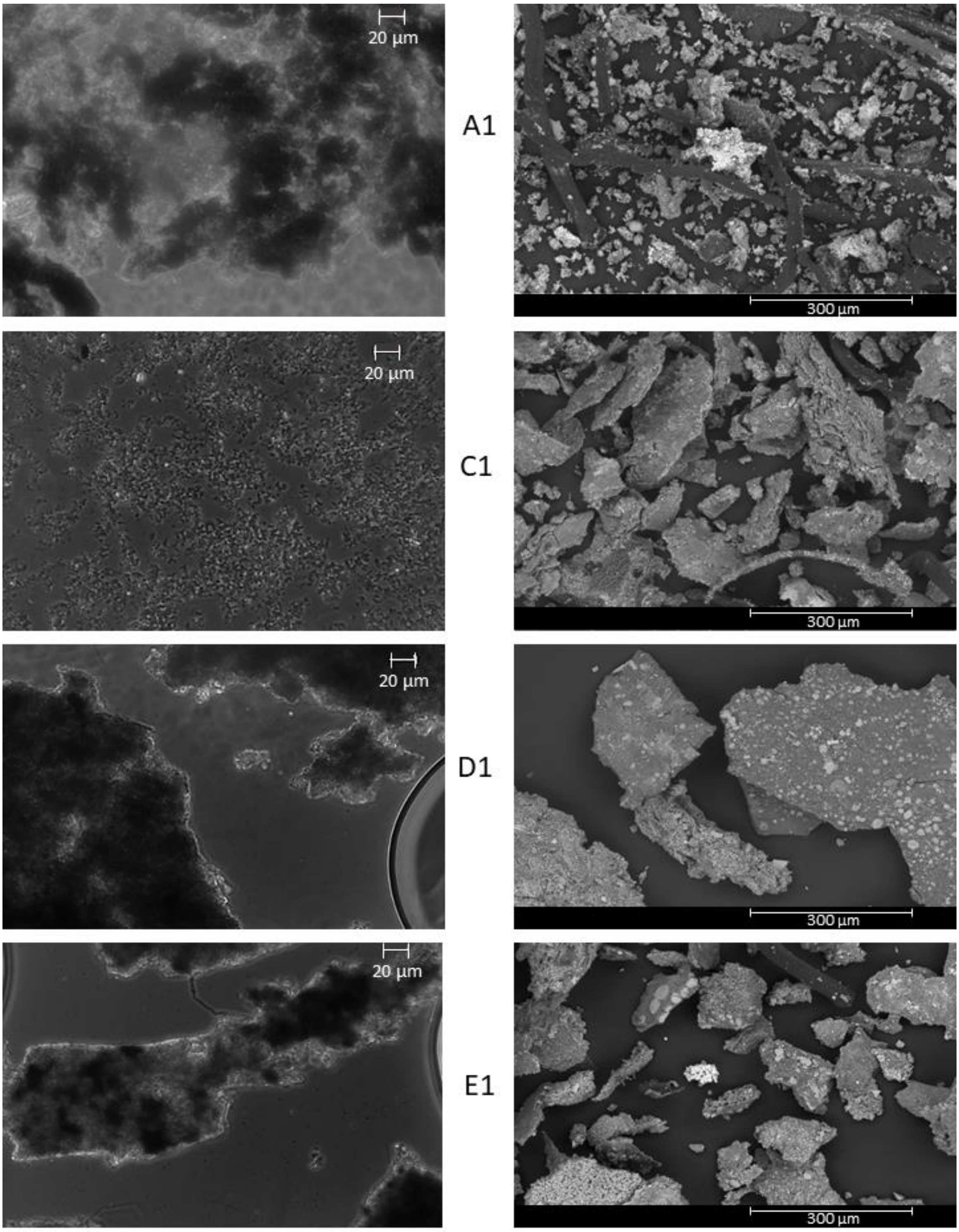

Figure 8. Microscope pictures (**top**) and SEM pictures (**bottom**) of particulate material collected over the water trajectory through the study system at locations A1, C1, D1 and E1.

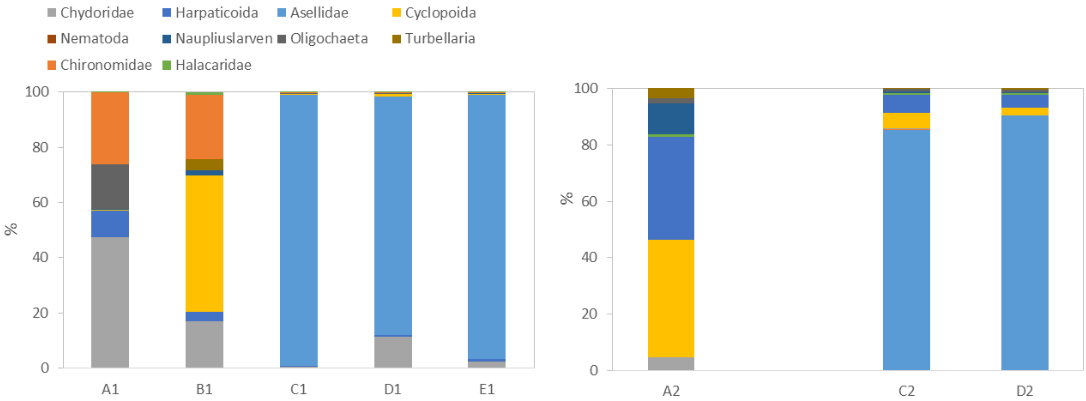

Figure 9. Change over distance in invertebrate composition (based on biomass concentrations) in the study system (**left**) and the reference system (**right**).

4. Discussion

Sediment formation was studied in a large-scale distribution system fed with drinking water produced from surface water and distributing water without a residual disinfectant. The results showed that particulate material collected at downstream distribution locations was not released by the treatment plant, but that it developed while water travelled through the distribution system. The extent of particulate material production was higher in the study system, which has more discolored water complaints than the reference system. Particulate material was formed seasonally and the variations in the volume of the collected material were congruent with seasonal variations in temperature, the number of invertebrates, and the concentration of *Aeromonas*. Analysis of the collected material indicated that sediment formation is closely related to (micro)biological processes and the life cycle of invertebrates.

4.1. Sediment Collection Methodology

The study was performed using a continuous sampling system with 25 µm filters to collect particulate matter from transport and distribution pipes. This approach differs from the traditional methodology for sediment and invertebrate collection from distribution systems that has traditionally been during pipe flushing [2,4–6,8,9,14,15]. The sediment sampling methodology has an impact on the material that is collected. During pipe flushing, an increased velocity results in resuspension of particles that have accumulated for a long time period (several weeks or more). Typical sediment volumes collected during pipe flushing actions range from 5 to 300 mL/m^3 [2,4]. In contrast, the filters used in this study collected particles that were larger than 25 µm, and in suspension under normal hydraulic conditions, including during the regular morning/evening flow velocity peaks which are caused by consumption patterns [18,20,21]. In addition, the filters were replaced regularly (every week or every two weeks). The use of the filters for sediment collection resulted in significantly lower sediment volumes than during pipe flushing actions, with volumes ranging from <0.1 to 5 mL/m^3 of filtered water (Figures 3 and 4). The main advantage of using filters on location is that the method is non-invasive and requires less manual work compared to a flushing event, which is time consuming and cannot be performed on a regular basis. This method also enabled weekly sample collection over a very long period of time (11 years), which yields a unique dataset that provides insights on both spatial and temporal variations of particulate matter in a large-scale distribution system. Having in-line filters for sample collection also allowed for a thorough capture of representative particle sizes: this is relevant as Van der Wielen and Lut [5] showed that the smallest sediment fractions (that are often also the lightest) harbor the highest microbial activity.

Though the sediment was collected using the same type of filters at all locations, it should be noted that the water extraction method from the pipes differed between locations (Table 1). At location E1, all water that flowed through the pipe, flowed through the filter.

In contrast, the water at locations C1 and D1 was extracted through measure lances at three levels of the pipe (top, middle, bottom), thus only a small fraction of the distribution pipe water actually flowed through the filter. The results were, therefore, normalized to a cubic meter of filtered water to account for this difference. Water flowing through the pipes at all other locations (A1, B1, and all locations of the reference system) was extracted from a one single tap from the side of the pipe. At these locations, the flow velocity was high, and it was assumed that most particles remained in suspension. The extraction method from three levels versus the side of the pipe was tested at location B1 and showed indeed no differences in the volume of material collected (data not shown). Note that locations B1 and D2 were located at the effluent of pumping stations and reservoirs where particles can settle. The volume of sediment collected at these locations may, therefore, be lower compared to sediment collected directly in a transport pipe at a location with similar residence time. Observation of sediment at the bottom of the reservoirs during the inspections performed every five years confirmed particle deposition. The sediment at the bottom of the reservoir also contained feces of invertebrates, which indicates that reservoirs can be the place for additional growth of invertebrates that can further colonize the distribution system [22]. The exact impact of reservoirs on water quality should be studied in more detail, by monitoring water quality and particulate matter at the influent and effluent of the reservoirs, as already suggested by Doronina et al. [23].

4.2. A sediment Formation Process Closely Linked to (Micro)Biological Processes

Analysis of the collected particulate material showed that the sediment formation mechanism is closely related to (micro)biological processes. Microscopic pictures and the presence of proteins indicated that there was microbial growth on the particles (Figure 8, Table 2). This result confirms previous studies that showed significant concentrations of heterotrophic plate counts or ATP in material collected through pipe flushing in both chlorinated and non-chlorinated distribution systems [5–9]. Microbial growth on particles can be promoted by organic matter and nutrients contained in the particles. For example, Gauthier et al. [7] showed that deposits that were collected from reservoirs for gravity-pressure distribution in France contained organic matter and nitrogen. The strong seasonal variations observed in sediment volume at location E1 (Figures 3 and 4), congruent with temperature variations, further support the hypothesis that (micro)biological processes play a key role in the sediment formation. Specifically, van der Wielen and Lut [5] and Liu et al. [6] showed that the dominant niche for *Aeromonas* growth is in the sediment. This was also supported here by congruent seasonal variations in sediment volume and *Aeromonas* concentrations in water (Figure 4).

Besides growth on the particles, discoloration events have been associated with biofilm growth on pipe walls [10]. Biofilm growth has been reported in numerous distribution systems in countries where a disinfection residual is maintained in the distribution system as well as in non-chlorinated systems [5,6,9,11,24]. Layers of biofilm grown on pipe walls can detach when varying hydraulic conditions cause high shear stress along the pipe walls [10,15]. The detached biofilm flocs also contribute to particulate material production along the distribution system. In distribution systems that contain cast-iron pipes, microbial growth on the pipe walls can further enhance pipe corrosion through biocorrosion processes, which promotes more production of particles [25]. It has been shown that cast iron pipes promote the most biofilm formation compared to other common pipe materials [26]. Such processes can also occur on appendages such as hydrant and valves that are usually made of cast iron.

Nutrients and bacteria contained in the sediments may be the basis for a trophic web in the distribution pipes. This is supported by seasonal variations in invertebrate numbers, congruent with sediment volume and temperature, and by earlier studies showing large numbers of invertebrates in the distribution systems [2,4,17,27]. The types of organisms found in the studied systems (Figures 5 and 9) were comparable to the organisms found in other distribution systems in The Netherlands and Germany [2,17]. Note that only invertebrates that were greater in size than 30 µm were taken into account in this study, while smaller organisms (e.g., rotifers, gastrotrichs and ciliates) were most likely also present in large abundance [2,27] but not detected/measured to the pore size of the filters and the dense sediment matrix. Among all the organisms found in the distribution system, *Asellidae* were the largest organisms and contributed the most to the total biomass [4,17] (Figure 5; Table S4 in Supplementary Materials). In this study, *Asellidae* were not found at upstream locations but was dominating the biomass at downstream locations where sediment volumes were higher and hydraulic conditions were likely to be more favorable (Figure 9). Earlier studies have shown a link between the presence of *Asellidae* and sediment [2,4]. This can be explained partly by the fact that *Asellidaes* may feed on biofilm and deposited particles but also contributes to material production through feces excretion [2,17] (this study—Figure 5) and decay of exoskeleton of dead individuals. Gunkel et al. [17] showed that the presence of *Asellidae* and its feces induced increased microbial growth, therefore enhancing the microbial processes in the sediment. It should be noted that *Aeromonas* bacteria were found in the midgut glands of *Asellus aquaticus* from different aquatic habitats [28], therefore the presence of *Asellidae* may also increase the concentrations of *Aeromonas* bacteria.

The decay of dead *Asellidae* and other invertebrate exoskeletons can partly explain the presence of $CaCO_3$ in the sediment (Tables 2 and 3); however, the poorly-ordered amorphous form also suggests that biomineralization processes occurred [29]. Biomineralization processes are common in water environments and have been hypothesized to occur in drinking water biofilms [6,30]. Microbial processes might also be involved in the accumulation of Fe and Mn in sediments in distribution systems. An increased Fe and Mn content in the sediment was observed in our study (Figure 7), and was reported in other systems that had [8] and did not have [6] cast iron pipes. The accumulation of Fe and Mn in the sediment and biofilm has been reported earlier [16] and occurred despite low Fe and Mn concentrations in the water at the outlet of the treatment plant and in the distribution system (<10 µg/L). Accumulation of Fe in sediments and biofilms can be the result of particles entrapped in the EPS structure [31], as well as bio-chemical processes. Liu et al. [6] showed evidence of the presence of iron oxidizing and iron reduction bacteria in sediments. Appendages such as hydrant and valves are mostly made of cast iron, even in systems where no cast iron pipes are used and may also contribute to the Fe accumulation through corrosion processes. The role of Fe and Mn in biomass development in drinking water distribution systems seems to be critical and needs to be further investigated.

4.3. Impact of Hydraulic Conditions on Sediment Formation

The increase in material volume collected along the distribution system (Figure 7) may be the result of increasing particle size [18] (Figure 8) combined with different hydraulic conditions. Particles leaving the treatment plant are small (Figure 8) and subject to high flow velocities in large transport pipes (in the range of 0.9 m/s in a pipe of 900 mm diameter in the present study). In such conditions, particles cannot settle but may be entrapped in the biofilm layer on pipe walls [10,32], which also leads to accumulation of inorganics in the biofilm [10]. In the distribution pipes downstream, however, flow rates and velocities are lower (0.1 m/s on average at location E1 of the present study), especially during the night (lowest flow velocities at approximately 0.01 m/s at night at location E1), and the larger particles formed along the water trajectory are more likely able to settle. Particles can also accumulate in the biofilm at the downstream distribution locations and be remobilized under high flow velocities [10]. Earlier studies showed that a minimum flow velocity is required for particle resuspension, usually in the range 0.1 to 0.25 m/s depending on the type of material and conditions [33,34]. This range of flow velocities was achieved daily at location E1 (maximum values at around 0.25 m/s during morning and evening consumption peak), however, the velocity may not be sufficient to resuspend all deposited particles and long-term accumulation can occur. Measurements in large-scale distribution systems showed that particles tend to accumulate at specific locations in the system where local hydraulic conditions are favorable for particle deposition. This phenomenon is also impacted by the characteristics of the particles, such as size, density, and shape, which influence their deposition behavior [13,33,35]. Figure 6 shows that material collected at downstream locations is made of large fluffy particles, however it is difficult to predict their sedimentation behavior. This should be tested for a better prediction of discoloration risks. Overall, it can be concluded that material production occurs along the distribution system but hydraulics in the system are the driving force for transport and deposition of particles in the pipes. The concept of a self-cleaning network has been developed to limit particle accumulation in the pipes [34,36]. The branched distribution system with pipes of small diameters allows for periodic resuspension of particles during everyday peak demand by creating a high flow velocity and shear stress on the pipe walls.

4.4. Sediment Formation Mechanism Hypothesis

Overall, the results indicate that particulate material collected at downstream distribution locations was not released from the treatment plant but developed along the distribution system. Based on the available results and literature, the following formation mechanisms can be hypothesized, as summarized in Figure 10:

- Polysaccharides, NaCl and other inorganic precipitates released by the treatment plant bind to each other to form larger precipitates, which represent a matrix for further binding of small particles, organic and inorganic matter;
- The larger precipitates become an excellent attachment site for bacterial growth, resulting in production of proteins. Biomineralization/bio-chemical processes can lead to the additional formation of, for example, $CaCO_3$ or iron precipitates on the particles;
- The size of aggregates increases along the distribution network as more compounds and microorganisms bind to them;
- Nutrients released by the treatment plant promote biofilm growth on pipe walls. Particles released by the treatment plant or formed along the distribution network can be entrapped in the biofilm. This leads to accumulation of, for example, iron precipitates or other inorganic and organic compounds. Biomineralization may also take place inside the biofilm. In the case of cast-iron pipes or appendages, biofilm growth is known to enhance particle production;
- Hydraulic conditions, grazing and die-off cause detachment of biofilm;
- The formed aggregates or detached biofilm flocs can settle where hydraulic conditions allow (i.e., where flow velocities decrease significantly during the day, typically at night due to decreased water consumption);
- The biofilm on pipe walls and settled particles can be utilized by invertebrates as a food source. The invertebrates contribute to the sediment formation by production of feces and formation of detritus (i.e., the rotting bodies of dead invertebrates). Decay of dead invertebrate (e.g., *Asellidae*) exoskeletons further contributes to the presence of $CaCO_3$ in the sediment aggregates. The presence of *Asellidae* and feces promotes additional microbial growth;
- The microbial growth on the pipe walls and in the aggregates, as well as growth of invertebrates in the sediment, are influenced by temperatures resulting in seasonal processes.

It is not clear at this stage if the dominant process is occurring at the pipe wall, in the water phase or in the sediment; however several studies in literature suggest a combination of different processes, each phase harboring different microorganisms and being the niche of different microbiological mechanisms [5,6,24]. Liu et al. [6] showed with source tracking, based on bacterial community analysis of different phases (water, biofilm, sediment), that resuspension or detachment of sediments (20% contribution) and pipe biofilm (20 to 30% contribution) can cause a significant change in bacterial community in water. Though the current study was performed in a non-chlorinated distribution system, there is sufficient evidence that microbiological processes such as biofilm formation or microbial growth on sediment particles also occur in distribution systems where a disinfection residual is maintained [7,8,11,24]; therefore, the sediment formation mechanism described above is likely to occur in chlorinated distribution systems as well.

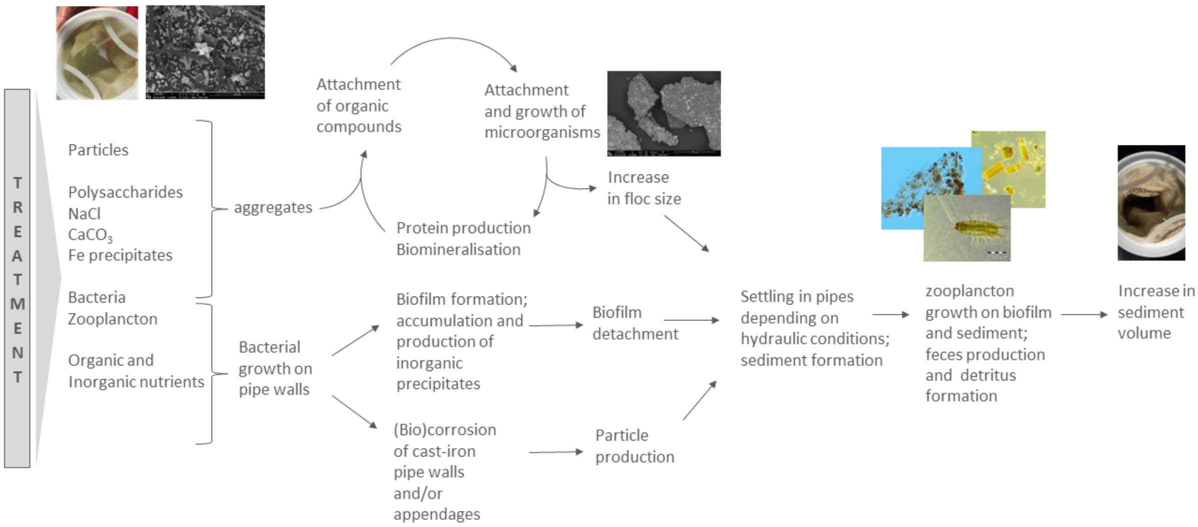

Figure 10. Hypothesis of the sediment formation mechanism.

4.5. Role of Water Treatment

Sediment formation was observed in both distribution systems described in this study. Characteristics of the collected material were similar in both systems (Figure 7; Tables S5 and S6 and Figure S7 in Supplementary Materials), indicating that the general sediment formation mechanism is the same. The extent of particulate matter production and growth of invertebrates was, however, lower in the reference system (Figure 7). Both systems treat water from the same source but with different treatment strategies (one had direct treatment and the reference system has dune infiltration) and distribute water in comparable conditions. The results of this evaluation indicated that the treatment strategy is key in controlling (micro)biological processes in the network. Limiting sediment formation at distribution level requires intensive and curative flushing actions. Reducing residence time in the system or investing in self-cleaning networks could reduce sediment formation: however, this is not feasible on short-to-middle term at a large scale. Actions towards reducing microbial growth and sediment formation in the distribution system should, therefore, focus mainly on treatment strategies.

The two treatment plants described in this study produced different water qualities. Water supplied in the study system has a higher AOC concentration than the reference system and a slightly lower ortho-phosphate concentration (Table S2 in Supplementary Materials). The concentrations of organic and inorganic compounds displayed large seasonal variations in the study system as a result of variations in the treated surface water, while water quality at the reference treatment plant was very stable over the year, as a result of dune infiltration. The different water qualities at the two treatment plants were related to the presence of different invertebrates at the treatment plant outlets, indicating different ecosystems in the last treatment steps (i.e., activated carbon filters at the study system and sand filters at the reference system). Invertebrates can proliferate through treatment and are released by biological filters (e.g., GAC filters, sand filters) into the distribution system [37,38]. Earlier studies have shown that activated carbon filters release large amounts of invertebrates [39]. The differences in both chemical and (micro)biological water quality at the treatment effluents may explain the differences in sediment formation and invertebrate development in the distribution systems; however, the exact components responsible for material production along the distribution system still need to be identified. Studies on biological stability have traditionally focused on the role of biodegradable or assimilable organic carbon concentrations promoting growth in the water phase or biofilm. In recent years, studies have shown that high molecular organic carbon, including biopolymers may play a critical role in microbiological processes in distribution systems [16,40,41]. This study shows that particulate matter and inorganic compounds, including Fe, Mn, and Ca, released by the treatment plant should also be taken into account in future studies.

Improving treatment strategies to limit (micro)biological processes in distribution systems is becoming of increased importance in the context of climate change. The results of this study showed that the sediment formation mechanism is closely related to temperature (Figure 3), with the most sediment formation occurring at temperatures above 12 to 14 °C. Periods of the year where temperatures exceed these values will be longer in the future, which will also impact microbial growth in water and biofilms. In addition, the presence of invertebrates in the system may increase in the future because of increasing water temperatures [27] which will further impact sediment formation. Limiting sediment and its water quality issues currently requires water utilities to implement flushing programs that are labor intensive. For example, the water utility PWN flushes approximately 500 km per year (on average 1 to 2 km of pipes per day). This effort may well have to increase with increasing temperatures if no mitigating strategies at treatment level are identified and implemented.

5. Conclusions

The occurrence of turbid or brown water at the consumers tap causes numerous customer complaints worldwide. These complaints are linked to sediment formation in the distribution system network. To better understand the sediment formation mechanism, spatial and long-term temporal dynamics of sediments were studied in a non-chlorinated drinking water distribution system. Collection and analysis of sediment over 11 years at one location and for one year at several locations along the distribution system led to the following conclusions:

- Sediment is formed seasonally in the distribution system. Sediment formation follows similar variations as temperature, and the presence of invertebrates and *Aeromonas*;
- Particulate material collected at downstream distribution locations was not released by the treatment plant but developed along the distribution system, with increasing particle/floc size, invertebrate biomass and Fe and Mn content with longer distances and residence times;
- The collected material contained proteins, calcium carbonate and iron precipitates;
- The large crustaceans *Asellidae* play a major role in sediment formation through feces excretion, and degradation of exoskeleton of dead animals;
- Sediment formation may be initiated (partly) by aggregation of inorganic precipitates, particles and organic matter released by the treatment. Flocs containing inorganics may also originate from biofilm that is detached from the pipe walls. Particles and inorganic compounds, as well as organic compounds, should, therefore, be closely monitored in future studies;
- Though the sediment formation mechanism seems to be the same in different systems, water quality at the treatment plant outlet impacts the extent of material production along the distribution system and the growth of invertebrates.

Overall, this study provided additional insights into sediment formation mechanism and showed that particulate material production within the drinking water distribution systems is a complex mix of (micro)biological and bio-chemical processes, microbial growth on particles and biofilm, biomineralization and the life cycle of invertebrates. The determining factors to limit sediment formation, however, could not be identified. Further research is required, focusing on how the type of treatment in the water treatment plant impacts the distribution system ecosystem.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15020214/s1, Table S1: Length of different pipe materials in the study system and the reference system, Table S2: Water quality at the outlets of the treatment plants supplying water to the study system (location A1) and the reference system (location A2), Table S3: Microbial changes in the study system and reference system. Comparison of microbial measurements at the treatment effluents (A1 and A2) and of average values of measurements at various distribution locations, Table S4: Biomass per individual for a selection of invertebrates observed in sediment collected from two non-chlorinated distribution systems, Figure S1: FTIR spectra of sediment samples collected in the study system at locations A1, C1, D1 and E1, Figure S2: Examples of XRD spectra, Figures S3–S6: SEM-EDX picture and spectra of sediment collected at locations A1, C1, D1 and E1 in the study system, Table S5: Summary of wavenumbers in FTIR spectrum for samples collected at one location in the reference system by pipe flushing through a 30 µm and a 100 µm filter, Table S6: Crystalized minerals in the samples collected in the reference system, Figure S7: microscope pictures of sediment collected in the reference system through a 30 um and 100 um filter after pipe flushing. Reference [42] is cited in the Supplementary Materials.

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