Report on seasonal bacteria fluctuation in raw water of non-disinfected case study sites in Northern Germany

Deliverable D2.1, WP2

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Abstract

This report presents the findings from task 2.1 of the SafeCREW project, which aimed to monitor seasonal microbial quality changes in source waters of near-natural treatment systems, such as managed aquifer recharge (MAR). Two case study locations, Hamburg and Berlin, were examined to understand microbial dynamics over time. Microbial cell counts in source waters were monitored using flow cytometry (FCM), which enables the analysis of bacteria, protozoa, and viruses. In addition, organic matter in source waters and during near-natural treatment was analyzed using techniques such as Liquid Chromatography-Organic Carbon Detection (LC-OCD), fluorescence spectroscopy, and UV-VIS absorption measurements. These methods provided detailed insights into the type, character and quantity of organic substances, which may influence microbial growth. Notably, biopolymers—organic substances produced during microbial degradation—were identified as indicators of microbial activity and surface water influence. By combining microbiological and organic analyses, a comprehensive monitoring system can be developed that provides extensive information not only on seasonal changes in microbial quality, but also on the underlying causes and influencing factors. This enables targeted and effective control of water treatment processes and helps to ensure high water quality in the face of climate change.

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Abbreviations

CFU	Colony forming units
DCC	Defect cell count
FCM	Flow cytometry
fDOM	fluorescent dissolved organic matter
HNA	High nucleic acid content
ICC	Intact cell count
LNA	Low nucleic acid content
LOD	Limit of Detection
MAR	Managed Aquifer Recharge
NOM	Natural organic matter
TCC	Total cell count
UF	Ultrafiltration
LLOQ	Lower Limit of Quantification
ULOQ	Upper Limit of Quantification

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1. Introduction

This report contains the work conducted in task 2.1 of the SafeCREW project. The aim of this task was to monitor seasonal microbial quality changes in source waters and near-natural treatments such as MAR/bank filtration systems. Two locations of the case study 1 were examined: Hamburg and Berlin. The two case studies are presented in section 2 and 3 separately. Section 4 presents the conclusions for both case studies.

At the case studies seasonal changes in microbial cell counts in source waters have been surveyed using flow cytometry (FCM). FCM allows to obtain information on a large number of particles, including bacteria and protozoa. Monitoring the composition of organic substances in source waters and during near-natural treatment offers a useful and rapid extension of microbiological monitoring. Organic substances serve as a source of nutrients for many microorganisms and may have significant influence on seasonal microbial changes. With the methods applied in task 2.1, such as Liquid Chromatography - Organic Carbon Detection (LC-OCD), fluorescence spectroscopy and UV-VIS absorption measurements, the organic matter was quantified in detail and divided into fractions of different character. These methods provide in-depth insights into the type, character and quantity of organic substances present in the water samples and thus enable targeted monitoring of microbial growth conditions. Organic substances of microbial origin can serve as indicators of increased microbial growth and the increased occurrence of microorganisms. In particular, the fraction of biopolymers produced during microbial degradation processes provides valuable information on bacterial growth. The analysis of the biopolymer fraction can also draw conclusions about the efficiency of a soil passage, in particular whether it is directly hydraulically connected to a surface water body. This size specific organic fraction does not occur naturally in groundwater, which makes its presence a specific indicator of surface water influence.

The Berlin case study plays a critical role in drinking water production through Managed Aquifer Recharge (MAR). Given that natural groundwater recharge is insufficient to meet the Cities water demand, groundwater augmentation is performed using treated surface water. At the waterworks no regular hygienic disinfection is applied, making the aquifer passage the primary hygienic barrier to ensure microbially safe water. In Berlin, an automatic, high-frequency FCM system was deployed at a groundwater augmentation site, and seasonal changes in microbial cell counts were monitored over the course of a year. Complementary large-volume microbial sampling with ultrafiltration (UF) modules was conducted to capture and analyse viruses, bacteria, and protozoa, with microbial recovery performed through backflushing using a surfactant solution. The combined microbiological and organic analyses provided an improved understanding of the microbial quality and its seasonal variations, offering valuable insights for optimizing water treatment processes and ensuring high water quality.

The Hamburg case study site utilizes a unique ditch system for MAR to support groundwater abstraction. Water is extracted through both shallow (up to 35 meters) and deep wells. The shallow wells are hydraulically connected to the ditch system, which is fed by rainwater and, when necessary, deliberately supplemented with river water.

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2. Case study Berlin

The case study CS1B is situated in the north-western part of Berlin (Germany) at the waterworks Berlin-Spandau. The following site description has been adopted and modified from Sprenger (2021). The waterworks Spandau abstracts groundwater in the range of about 25-30 Mm^3/year and recharges 15-20 Mm^3/year through constructed infiltration basins and near-natural lakes and ditches (Figure 1).

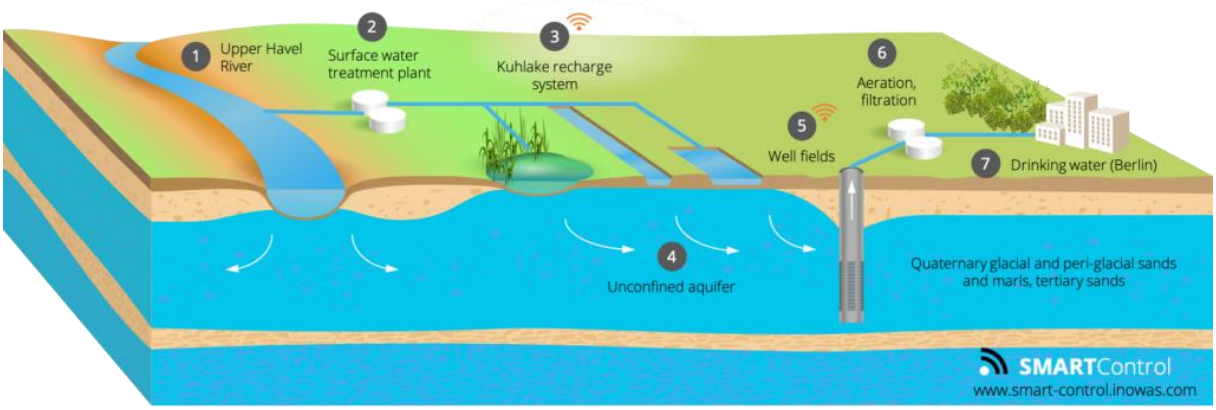

Figure 1: Schematic representation with main components of the MAR facility at Berlin-Spandau waterworks (Sprenger, 2021)

Groundwater augmentation is necessary because natural groundwater recharge does not cover the actual water demand. The source water for groundwater augmentation is abstracted from the Havel River and pre-treated before recharge. Surface water is treated by flocculation and rapid sand filtration before recharge. The technical pre-treatment aims at removing suspended solids and nutrients. The well field Spandau-Nord consists of eight recovery wells with an average abstracted volume of 6.8 M m^3/year. The total depth of the wells is 47-52 meter below ground level, with filter screen lengths between 18 to 19 m. At the Berlin-Spandau waterworks, post-treatment of the recovered groundwater is necessary to ensure that the water meets drinking water quality standards before distribution. The recovered water is aerated to precipitate dissolved iron/manganese and subsequently filtered through rapid sand filters, before being distributed to the water net as drinking water. There is no hygienic disinfection treatment applied on a regular basis. The aquifer passage is therefore the most important hygienic barrier of the treatment scheme.

A hydrogeological cross-sectional view (true to scale) based on water level measurements from 2014-2016 (Sprenger, 2018) and geological explorations (Bruehl and Limberg, 1985) is depicted in Figure 2.

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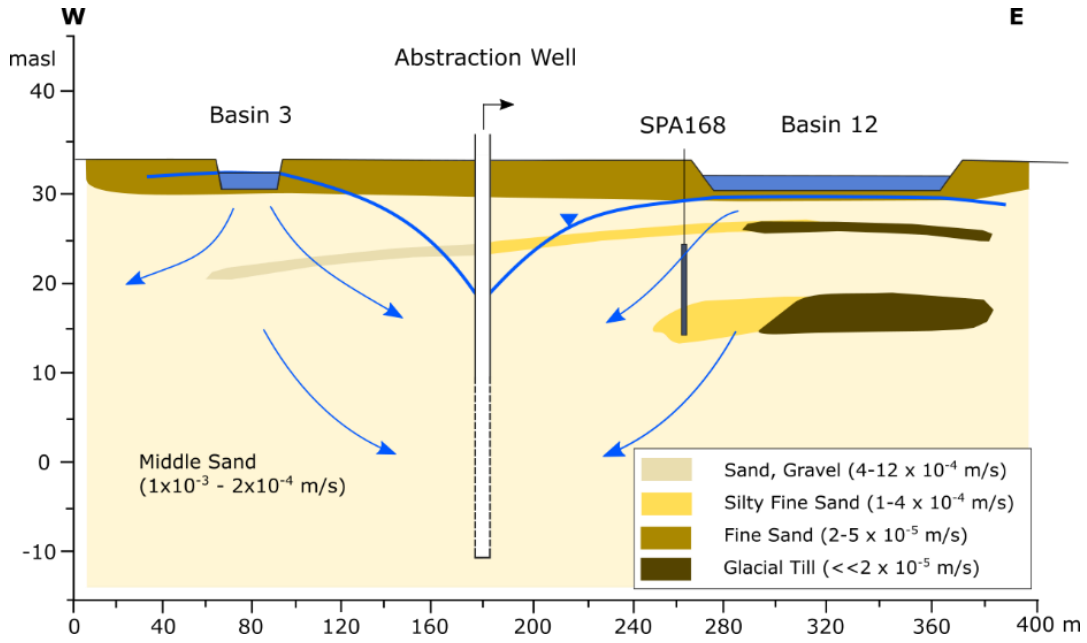

Figure 2: Cross-section of the groundwater recharge site at waterworks Spandau (vertical exaggeration 1:5) (modified from Sprenger (2021))

The geology of the study site consists of quaternary sediments forming an aquifer complex that is characterized by various heterogeneous layers of glacial, peri-glacial and fluvial deposits. This dynamic sedimentary environment results in large small-scale hydraulic differences in the sedimentary sequence, that influences the infiltration and flow pattern to a large extent. The upper layer is composed of fine sands. Below basin 3 this low permeable layer (2-5×10^{-5} m/s) is max 0.5 m, while below basin 1/2 it is around 1 m in thickness (Bruehl and Limberg, 1985). Almost the entire area of infiltration basin 1/2 is underlaid by this low permeable layer, which explains the relative low infiltration rates (Table 1) and the presence of an unsaturated zone of about 0.5 to 1 m thickness even at maximum basin filling level (Figure 2). At basin 3 no unsaturated zone was observed. The glacial till below is composed of extremely unsorted glacial deposit in the form of a ground moraine, deposited as a band that evolves to more coarse grained sediments from east to west (Bruehl and Limberg, 1985). The glacial till is spatially highly variable in thickness and extend and forms due to its very low permeability major flow barriers. However, the hydrogeological observations strongly suggest that the observation well SPA168 is on the groundwater flow path from the infiltration basin to the abstraction well.

Table 1: Geometry and hydraulic parameters of the infiltration basins 1/2, 3 and 4 (Sprenger, 2021)

Basin ID	Area (m^2)	Volume infiltrated 1995-2015 (Mill. m^3/a)	Hydraulic loading (m/d)	Infiltration rate 2015-2016 (m/d)
Basin 1/2	21,900	2.44	0.31	0 – 0.5
Basin 3	8,082	2.42	0.82	0 – 3.3
Basin 4	7,498	2.69	0.98	0 – 3.4

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Field sampling

The field study was carried out from August 2023 to August 2024. During the first 4 months an FCM device was rented and employed on-site. When no FCM was on-site, samples were taken and measured by an lab-based FCM device. This procedure allowed the site to be monitored for a complete year. The first two months the rented FCM device was measuring in parallel at the recharge basin and the observation well and about two months the FCM device was employed at the abstraction well. A BactoSense® was rented because it is the only current flow cytometer on the market that allows for fully automated measurements.

The FCM device was operated in automatic mode at all sampling points, except for inspection visits, where manual measurements were taken additionally. In order to take samples from two different water sources an automated sampling line was installed (Figure 3).

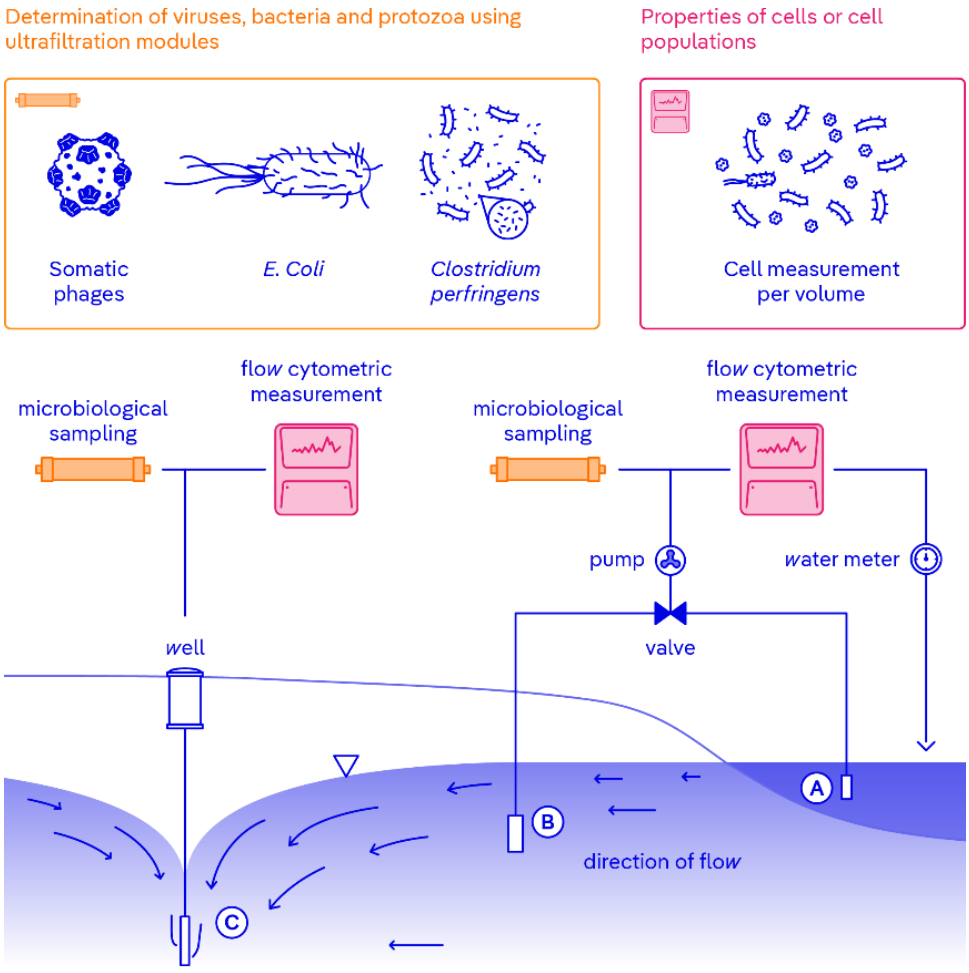

Figure 3: Sampling line with flow cytometry and microbial sampling using ultrafiltration modules.

Two peristaltic pumps were pumping either from the recharge basin (GEMKE, 2020) or the observation well (Solinst model 410). Time controlled magnetic valves were used to control the flow to the FCM device. The flow rate to the FCM device was measured and adjusted if necessary during inspection visits. The magnetic valves were switched every 6 h, resulting in 2 FCM measurements per day for each sampling station. Both pumps were operated 25 min before FCM sampling. The outflow was discharged to the basin. Pumps were operated with santoprene tubes with a diameter of 10 mm and

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13 mm PVC tubes. Prior to the installation of the sampling line the installed equipment including the pipes were disinfected and cleaned with Sodium hypochlorite and purified water. This was done to avoid microbiological contamination of the observation well by the installed equipment. The peristaltic pump and sampling line were installed and stored in a box placed close to the basin and the observation well (Figure 4).

Figure 4: Box with sampling line next to the observation well (left) and intake device at the recharge basin (right).

On November 14th 2023 the FCM equipment was then moved from the recharge basin/observation well to the abstraction well. The FCM device was connected to a sampling bypass at the well head. The outflowing water from the FCM device was collected in a 300 liter reservoir which was lowered into the well chamber and manually pumped dry during regular site visits (Figure 5).

Figure 5: Flow cytometer installed in well chamber (left); flow cytometer maintenance and manual measurements (right)

In addition to the automatic FCM measurements, manual sampling was carried out. A total of 12 sampling campaigns were carried out from August 2023 to August 2024. The recharge basin is in operation all year round. In winter, when temperatures are low, a sheet of ice covers the recharge basin. In summer time layers of algae often cover the water surface (Figure 6).

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September 2023

November 2023

March 2024

May 2024

Figure 6: Recharge basin during the year of monitoring.

It was therefore possible to carry out 12 monthly measurements on site; only one campaign had to be cancelled in January 2024 due to sick leave of staff (Table 2).

Table 2: Overview of measured parameter during field sampling campaigns.

No.	Date of sampling	Monitoring Station	FCM (lab)	Microbial cultivation methods	Hydro-chemistry	NOM, DOC, UV254
1	22.08.2023	Recharge Basin	X	Conventional	Ok	Ok
		Observation well	X	Enriched	Ok	Ok
		Well	X	Enriched	Ok	Ok
2	27.09.2023	Recharge Basin	X	Conventional	Ok	Ok
		Observation well	X	Enriched	Ok	Ok
		Well	X	Enriched	Ok	Ok
3	18.10.2023	Recharge Basin	X	Conventional	X	Ok
		Observation well	Ok	Enriched	X	Ok
		Well	Ok	Enriched	Ok	Ok
4	15.11.2023	Recharge Basin	Ok	Conventional	Ok	Ok

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No.	Date of sampling	Monitoring Station	FCM (lab)	Microbial cultivation methods	Hydro-chemistry	NOM, DOC, UV254
		Observation well	Ok	Enriched	Ok	Ok
		Well	Ok	Enriched	Ok	Ok
5	13.12.2023	Recharge Basin	Ok	Conventional	Ok	Ok
		Observation well	Ok	X	Ok	Ok
		Well	Ok	X	Ok	Ok
6	21.02.2024	Recharge Basin	Ok	Conventional	X	Ok
		Observation well	Ok	Enriched	X	Ok
		Well	Ok	X	X	Ok
7	27.03.2024	Recharge Basin	Ok	Conventional	X	Ok
		Observation well	Ok	Enriched	X	Ok
		Well	Ok	X	X	Ok
8	24.04.2024	Recharge Basin	Ok	Conventional	X	Ok
		Observation well	Ok	Enriched	X	Ok
		Well*	Ok	X	X	Ok
9	22.05.2024	Recharge Basin	Ok	Conventional	X	X
		Observation well	Ok	Enriched	X	X
		Well	Ok	X	X	X
10	19.06.2024	Recharge Basin	Ok	Conventional	X	X
		Observation well	Ok	Enriched	X	X
		Well	Ok	X	X	X
11	24.07.2024	Recharge Basin	Ok	Conventional	X	X
		Observation well	Ok	Enriched	X	X
		Well	Ok	X	X	X
12	14.08.2024	Recharge Basin	Ok	Conventional	X	X
		Observation well	Ok	Enriched	X	X
		Well	Ok	X	X	X

X = sample not available; Ok = sample has been analyzed; *Well off, neighbouring well sampled instead; enriched = sampling with hollow fibre membranes; conventional = 100 ml sampling volume

The observation well was sampled using a Grundfos MP1 submersible motor pump. Before sampling, the stagnant water in the observation well was exchanged at least three-times and during this time the (1) electrical conductivity, (2) temperature, (3) pH, (4) redox potential were measured in a flow-cell (Eijkelkamp) using the Hach HQ2200 multi-meter and associated probes. Samples for NOM characterisation and hydrochemistry were collected in 2x50 ml Falcon tubes, filtered with membranes

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with a 0.45 µm pore size (Chromafil Xtra PA-45/25, Macherey-Nagel, Germany) and stored at around 4 °C.

Microbiological measurements during MAR are challenged that microbes sampled with conventional methods are often found below detection limit in the subsurface. Therefore, an enrichment of indicator organism (*E.coli*, *Enterococci*, *Clostridium perfringens*, somatic phages) was carried out using REXEED 25A hollow fibre membranes with a molecular weight cut-off of 30kDa and a filter surface area of 2.5 m². The method was developed by Korth et al. (2017). The membrane allows the water to flow through, while microbiological organisms, such as bacteria and viruses, are retained. The organisms retained on the membrane are then backwashed in the laboratory to quantify the different types of microbiological indicators. Microbial enrichment was applied in the groundwater (observation well and well), while the recharge basin was sampled by conventional methods (100 ml sample).

Some cultivation based microbial methods (e.g. *Enterococci*, *Clostridium perfringens*) could not be carried out properly due to high turbidity in the observation well. The observation well was therefore regenerated and cleaned in November 3rd 2023 by BWB staff. After cleaning, the turbidity was removed and all measurements could be carried out without restrictions.

Hydraulic residence time (HRT) at the site was determined by temperature and stable isotope measurements (Sprenger, 2018). HRT was found to be 54-61 days (±11 d) in the well and about 10 d in the observation well.

FCM measurements

Sample volumes of 260 µL were taken automatically and mixed with fluorescent stains SYBR®Green and Propidiumiodide. SYBR®Green penetrates all cells and binds to double-stranded DNA (Hammes et al., 2008). Propidiumiodide (PI) binds to DNA, but only penetrates cells with defect cell membrane (Crowley et al., 2016). All cells fluoresce green, while only cells with a damaged membrane are permeable to the red pigment PI. After staining with the two fluorescent dyes, the cells are individually channeled through a glass capillary and detected by a laser light. Every particle encountered by the laser beam causes the light to be scattered, and any fluorescent dye is stimulated to emit light. The resulting scattered light and the fluorescence signal is transmitted to detectors via filters and mirrors and recorded (Kötzsch et al., 2012). After incubation (~10-20 min, 37 °C), samples were analyzed (FL1 channel at 525 nm, FL2 channel at 715 nm) using fixed gates to separate cells and background signals. The detection limit of the FCM device is given with 1×10^2 cells/mL, nominal range of 1×10^3 - 1 x 10^6 cells/mL (Sigrist-Datasheet), and the limit of quantification is 1000 cells per mL (SLMB).

Data from the BactoSens® device were transferred using the data format “flow cytometry standard” (FCS). FCS files were processed with R-studio (R-CoreTeam, 2021) with the R-package FlowCore (Ellis et al., 2019), cell counting and graphs were made with customized R-scripts. Only about 90% of the measured data are stored in the FCS file. Therefore, cell counting conversion of 1000/90 (~11.1) is applied (e-mail communication with Bnovate staff). Measurements are plotted using a 2D density estimation function from the MASS R package to color points by density with ggplot2. This helps to indicate regions with overplotting points (Figure 7, right). FL1 and FL2 are representing the fluorescence channels at wave lengths of 525 nm and 715 nm, respectively (Manickum, 2020). Additionally, fluorescence is measured at 488 nm for the Side Scatter (SSC). The two forward scatter signals (FL1, FL2) are related to particle size, while SSC signals are related to particle complexity and granularity (Manickum, 2020). The green fluorescence (FL1) versus red fluorescence (FL2) scatter plot help to distinguish background from intact and damaged cells.

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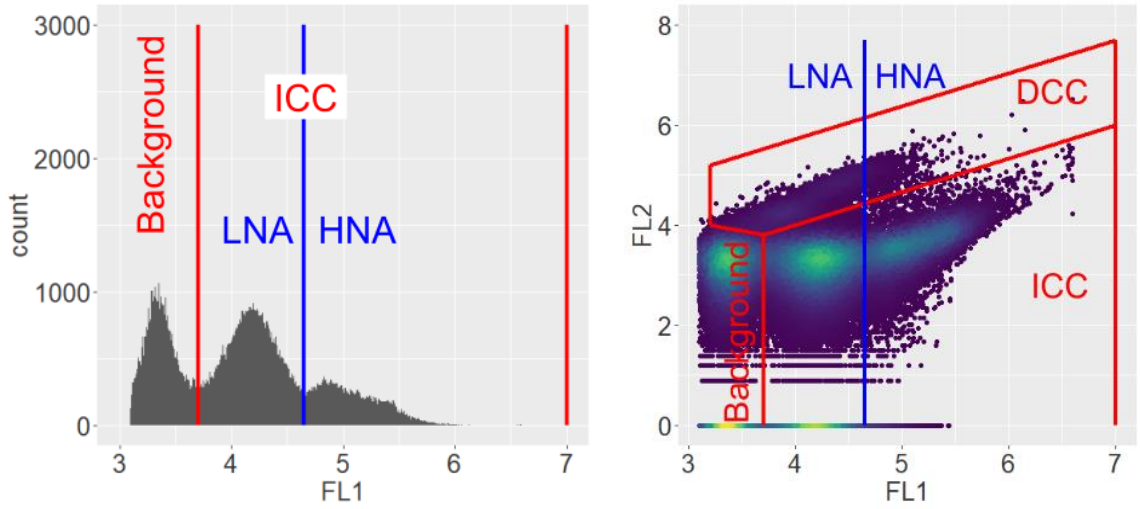

Figure 7: Gating based on single parameter histogram of FL1 (left) and FL1 against FL2 (right) to discriminate LNA/HNA (blue line), intact cell counts (ICC) and defect cell counts (DCC) (red lines)

FCM data is typically analysed through a processing called "gating". Gates are closed polygons in which cell clusters are isolated. The gate boundaries are defined on the basis of one- or two-dimensional representations of FCM parameters. Kötzsch et al. (2012) states that gates need to be corrected only in rare cases, since cell clusters do not change their position with optimally selected settings, but only vary in shape and size within the gates. Fixed gate settings were used for all samples to separate cell counts and background noise (abiotic particles and background signals), to define intact cell counts (ICC) and defect cell counts (DCC) cells, and to distinguish between high (HNA) and low (LNA) nucleic acid content bacteria.

Bulk hydrochemistry and NOM characterisation

Bulk hydrochemistry, NOM, DOC, and UV254 was analysed by the German Environment Agency (UBA). Laboratory analytical procedures are described in Zeeshan et al. (2024) as follows: Iron (Fe) and manganese (Mn) concentrations were determined photometrically according to standard procedures DIN 38,402-A51 and DIN 38,406–2EN. DOC concentrations were quantified with a TOC analyser (Vario TOC cube, Elementar Analysensysteme, Germany). UV absorbance at 254 nm (UV 254) was measured with a spectrophotometer (Lambda 25 UV/Vis Spectrometer, Perkin Elmer, USA). Nitrate (NO_3^-) and ammonium (NH_4^+) were analyzed by ion chromatography (930 Compact IC Flex, Metrohm, Switzerland). Fluorescence was analyzed using a FluoroMax-4 (HORIBA Jobin Yvon, USA) with excitation wavelengths from 220 to 500 nm (5 nm intervals), emission wavelengths from 250 to 600 nm (2 nm intervals) and a scan speed of 4800 nm/min.

Results

The FCM dataset utilized for this monitoring campaign, titled 'CS#1B Monitoring Data of Subsurface Passage in Managed Aquifer Recharge: Raw flow cytometry data (fcs)' is available for access and download from Zenodo at: https://doi.org/10.5281/zenodo.13981204

Bulk hydrochemistry and redox conditions

The bulk hydrochemistry and redox conditions along the flow path is described in the following. The water temperatures measured over one year confirms previous studies (Sprenger (2021); Sprenger et al. (2017)). Seasonal temperature variations are highest in the recharge basin and are gradually attenuated in the groundwater along the flow path. Measured pH show a slight decrease from recharge basin towards the groundwater. Electrical conductivity shows a slight increase from the recharge basin towards the groundwater due to dissolution processes along the flow path (Table 3).

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Table 3: Physico-chemical water quality parameter (min-max)

Sampling station	Temperature (°C)	pH (-)	Electrical conductivity (µS/cm)
Recharge Basin	4.3 - 28.6	7.9 - 8.0	467 - 748
Observation Well	5.7 - 24.5	7.8 - 7.9	415 - 865
Well	11.1 - 17.3	7.8 - 7.9	529 - 853

Based on the Nernst equation, the temperature T [°C] and the probe potential of the electrode Et [mV] the redox voltage relative to the standard probe was calculated according to Wolkersdorfer (2008). The corrected redox voltage [mV] relative to the standard probe and two redox sensitive solutes (Fe, Mn) are shown in Figure 8 as box plots with the median value (horizontal centre line), the 25%- and 75%-quartiles (box = interquartile range), the 1.5 x interquartile range (vertical line) and the extreme values (point).

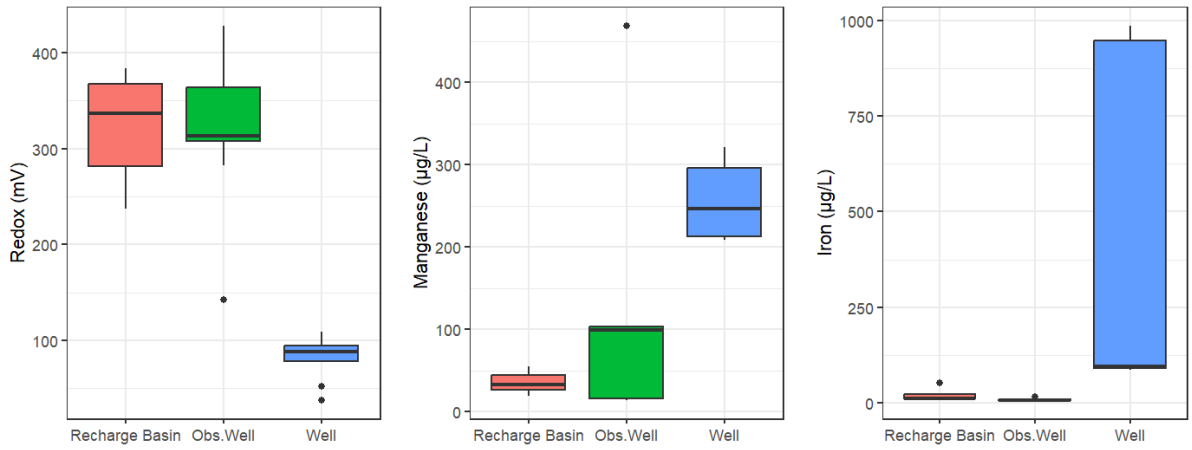

Figure 8: Redox conditions along the flow path

The redox conditions along the flow path are characterised by oxic conditions in the recharge basin and the observation well expressed as high median redox voltage >300 mV and low Mn/Fe concentrations <100 µg/l. Dissolved iron (Fe^{2+}) was measured in previous studies with approx. 1000 µg/L, which is approved by measurements from this study.

Flow cytometry

The results of the microbial monitoring by FCM measurements for the Berlin site are presented in the following. Measurements of total cell counts (TCC) and high nucleic acid (HNA) are shown for the recharge basin, the observation well and the well over one year of sampling (Figure 9).

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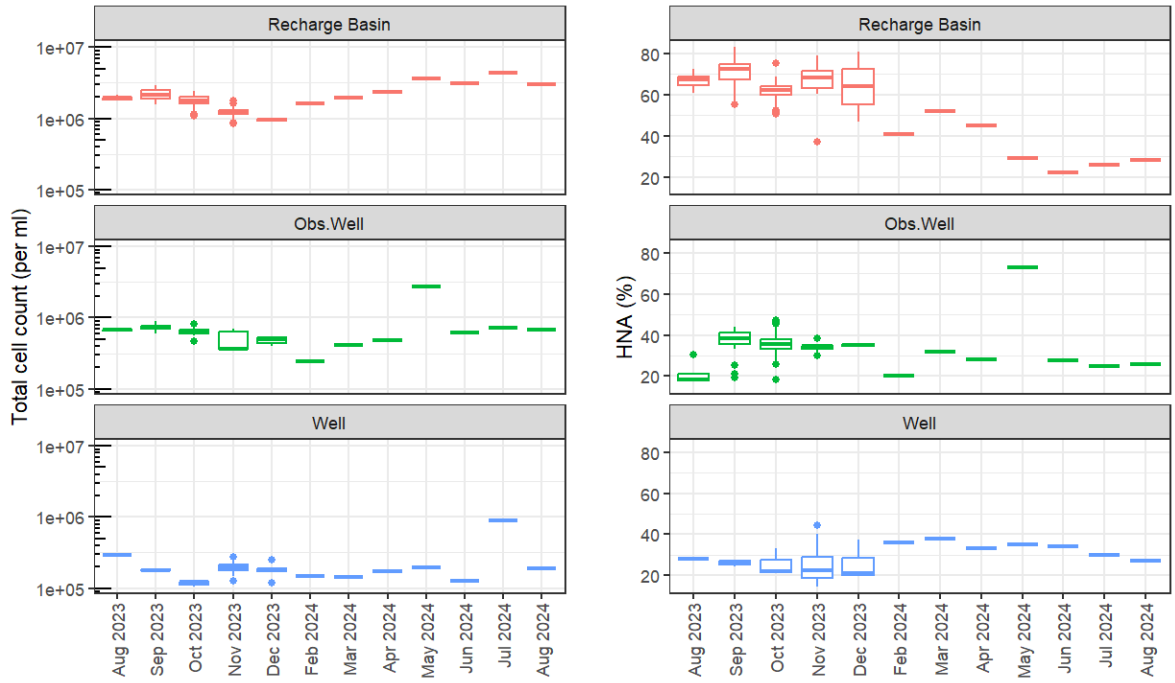

Figure 9: Seasonal monitoring over one year of total cell counts (TCC) and high nucleic acid (HNA) percentage in recharge basin, observation well and well.

In the summer months elevated TCC were measured in the recharge basin. The annual mean of TCC in the recharge basin was 1.88×10^6 cells/ml (sd = 0.56×10^6 cells/ml), while in July 2024 the TCC was more than twice as much (4.37×10^6 cells/ml). It can be concluded, that there is a pronounced seasonality of microbial abundance in the recharge basin. Additionally, a higher percentage of HNA cells were detected in the recharge basin, suggesting increased microbial activity or growth in this area. However, the dominance of HNA cells decreased in the course of the sampling and amounted to approx. 20-40% in the second half of the sampling. The HNA (%) in the recharge basin was not showing seasonality, instead median HNA was found >60% from August to December 2023, while January to August 2024 the HNA was <60% (Figure 9, right).

In the observation well the seasonal fluctuations in TCC were largely attenuated during the aquifer passage. The annual mean of TCC in the observation well was measured with 0.64×10^6 (sd = 0.25×10^6 per ml. The microbial retention expressed as the log reduction of TCC between the recharge basin and the observation well is therefore about one order of magnitude.

In the well the annual TCC variations were found to fall within the range of monthly fluctuations observed in the well, indicating a consistent microbial presence throughout the year. The annual mean of TCC in the well was measured with 0.20×10^6 (sd = 0.10×10^6). There is no influence of operational practice or seasonal environmental influences observed in the well.

Compared to TCC measurements, the DCC and ICC measurements show a similar pattern over time at the three sampling stations.

Ambient air temperature and inflow volume to the recharge basins is shown in Figure 10.

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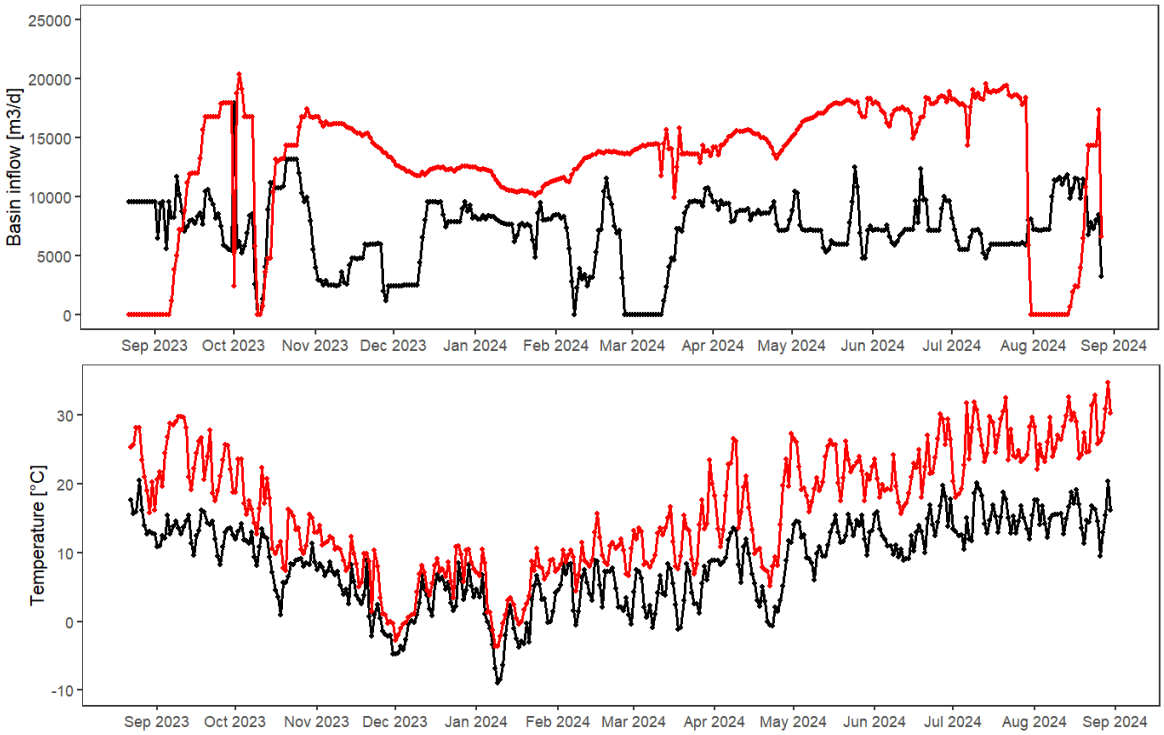

Figure 10: Operational practice (basin inflow volumes, red line = basin 3, black line = basin 12) and environmental conditions (Temperature, red line = max. daily temperature, black line = min. daily temperature)

The FCM measurements in the field led to some difficulties and interruptions. The on-site FCM measurements during the winter months were aborted in some cases. During incubation, the required incubation temperature and duration could not be achieved at outside temperatures of approx. <5°C. The temperature issue was known before, as the device is mainly designed for indoor use. During the routine control inspections, measurements were then taken manually. The failure of these measurements had no impact on the achievement of the task objectives. Even though some measurements could not be taken, meaningful data was still obtained even for the winter period.

The recharge basins were not filled with water at several times during the study period. These interruptions in artificial groundwater recharge may be used to investigate the influence of the basin filtrate on the microbiological composition of the groundwater. At the beginning of November (i.e. 3.11.-20.11.), the recharge basin was only filled with a low amounts of water (Figure 10). After taking into account the HRT of about 10 days, the FCM counting from observation well of the 15.11. were used. For comparison, an FCM measurement from December 6th was used, which was taken about 10 days after a phase of average groundwater enrichment. The example of the influence of operational practice on cell counts is shown in Figure 11.

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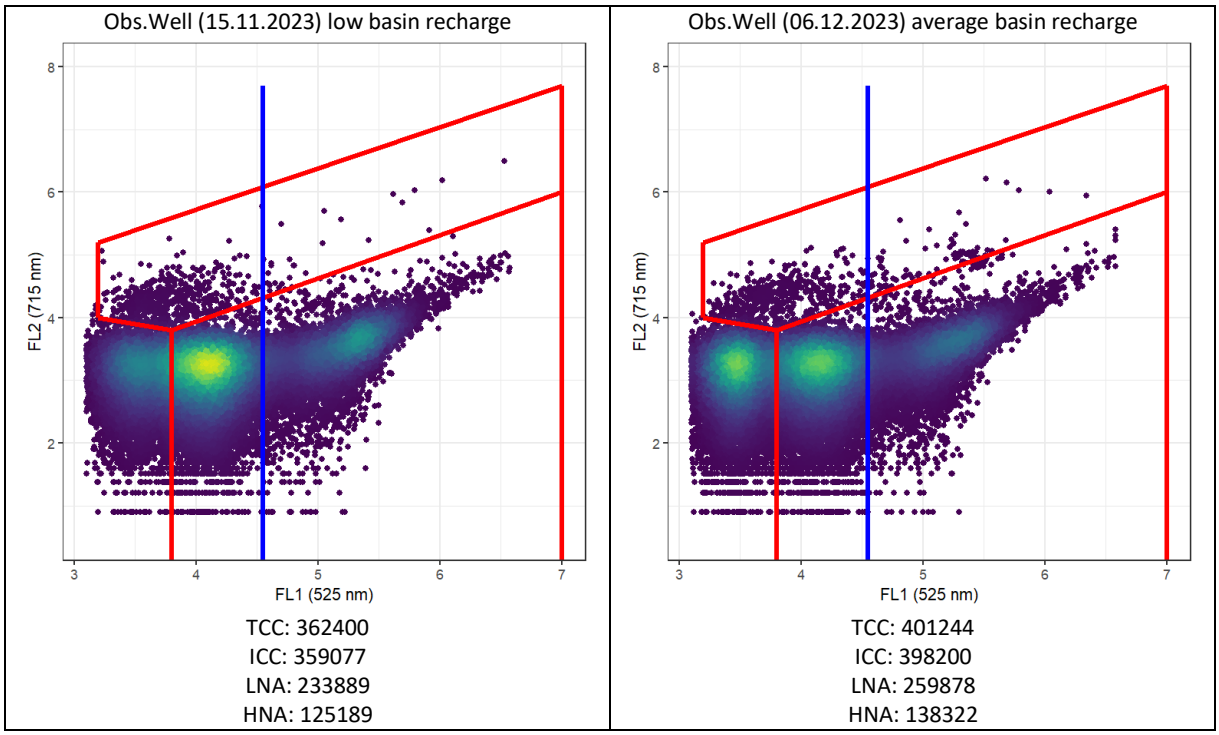

Figure 11: Density plots showing the influence of operational practices (TCC = total cell count; ICC = intact cell count; LNA = low nucleic acid content; HNA = high nucleic acid content)

The increasing influence of the basin recharge on the groundwater in the observation well is indicated by an increase TCC. Considering the fact that HNA cells dominate in the recharge basin, this dominance cannot be observed in the observation well. It can therefore be concluded that the HNA cells are effectively retained during the groundwater passage.

This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101081980.

Natural organic matter

Natural organic matter (NOM) occurs in all water resources and consists of a complex mixture of organic matter (derived from e.g. plant materials) such as fulvic acids, humic acids, proteins and other compounds (Zeeshan et al., 2023). The removal of NOM at the groundwater augmentation site is presented in the following to assess important factors of influence for the Berlin area. The results of the measured DOC (mg/l) and UV254 (1/cm) monitoring along the transect are plotted in Figure 12 as box plots with the median value (horizontal centre line), the 25%- and 75%-quartiles (box = interquartile range), the 1.5 x interquartile range (vertical lines) and the extreme values (points).

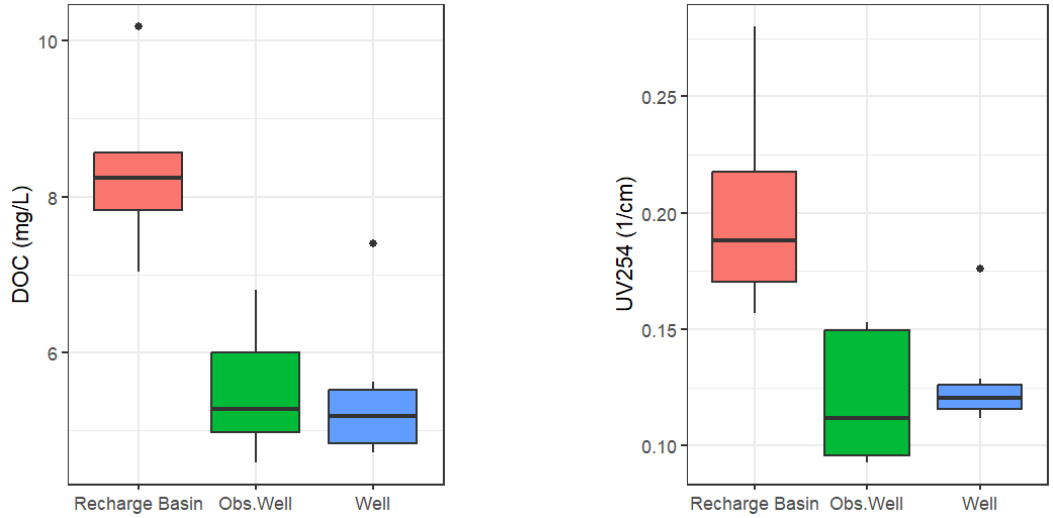

Figure 12: DOC (mg/l) and UV254 (1/cm) measurements

It can be seen that the DOC in the recharge basin is reduced considerably along the transects to a median residual value of 5.3 mg/l in the observation well and 5.2 mg/l in the well. The highest DOC decrease can be observed between the recharge basin and the observation well during the initial phase of the aquifer passage. After this initial rapid decrease, the DOC appears to be stable and supports the theory that the residual DOC under the given field conditions is hardly degradable.

UV254 also shows a rapid decrease during the initial phase of the aquifer passage and appears to be rather stable in the groundwater.

This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101081980.

The excitation emission matrix (EEM) obtained from fluorescence spectroscopy of the recharge basin, observation well and the well collected in November 2023 is presented in Figure 13.

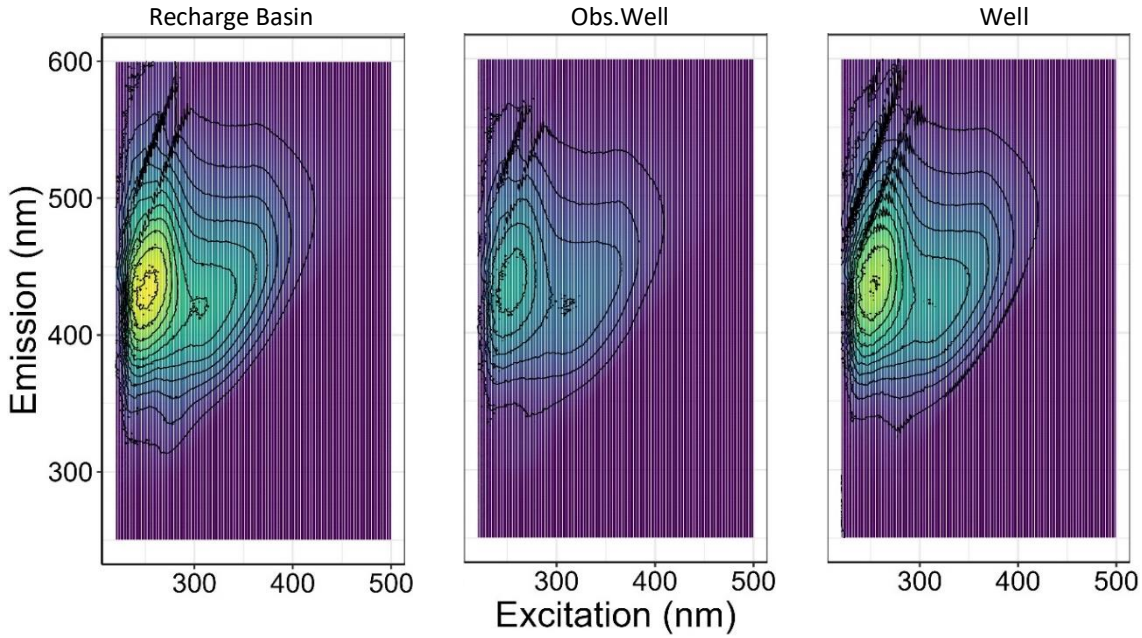

Figure 13: Emission excitation plot of the recharge basin, observation well and well measured in November 2023 with major signal intensities in the region of humic substances.

The plot of the recharge basin shows a concentrated region of fluorescence intensity between 200–300 nm on the excitation axis and 400–500 nm on the emission axis. The highest fluorescence intensity (shown in yellow) suggests the presence of specific fluorophores, which may indicate organic compounds such as humic substances.

In the groundwater EEM intensity represents a water with lower organic matter content and different composition of fluorophores compared to the recharge basin. The reduced intensity suggests less DOC and different types of dissolved organic matter (DOM), possibly indicating fewer humic-like substances and the dominance of non-fluorescent components in the groundwater sample. The increase of peak intensity from the observation well to the well may indicate the presence of organic-rich material in the subsurface, such as sapropel, peat or lignite. Incorporating organic-rich in the subsurface between the observation well and well could gradually leach humic substances, increasing the fluorescence intensity along the flow path. The occurrence of organic-rich sediments in the study area is documented by numerous boreholes.

Cultivation based microbiology and FCM

The relationship between FCM and indicator organisms, i.e. coliform bacteria is investigated in the following. Measured FCM parameter (TCC, ICC, HNA and LNA) and coliform bacteria from the recharge basin are depicted in Figure 14.

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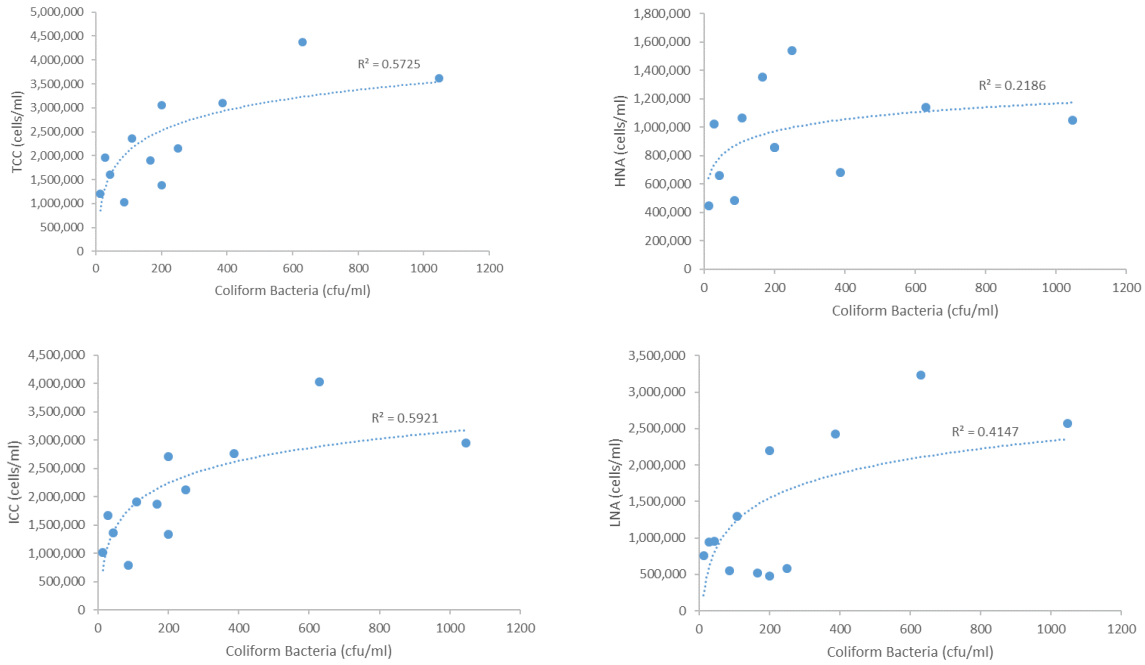

Figure 14: Coliform bacteria and FCM measurements in the recharge basin.

As the concentration of coliform bacteria increases, measured TCC, ICC, LNA and HNA also tends to increase. However, this positive correlation, as indicated by R^2 of the logarithmic trend line, is moderate to weak. The best coefficient is achieved by the ICC measurements, where 59.21% of the variance in ICC can be explained by the coliform bacteria concentration. Overall, Figure 14 suggests that FCM cell count increases with coliform bacteria concentrations, though the relationship is not strongly predictive.

The most important results of the cultivation-based microbiology will be reported in deliverable D4.3. This deliverable will describe a site-specific Quantitative Microbial Risk Assessment (QMRA). It will present the investigated case study to show the benefits of large-volume microbial sampling with ultrafiltration modules. This is to ensure that data and analysis based on it with regard to QMRA are in single report.

This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101081980.

Summary CS1B

The results of the field monitoring program provided new insights into the mechanisms of microbial abundance and bulk organic removal under conditions of groundwater augmentation, dominated by anoxic/anaerobic conditions at the studied field site.

The measurement of total cell counts (TCC) exhibited pronounced seasonality in the recharge basin. High nucleic acid (HNA) cells, indicative of active microbial growth, were dominant in the recharge basin, although their percentage decreased to 20–40% during the latter half of the sampling period. The observation well exhibited attenuated seasonal fluctuations in TCC during aquifer passage, reflecting a log reduction of about one order of magnitude. The well, showed stable microbial concentrations throughout the year unaffected by seasonal or operational changes. DCC (defect cell count) and ICC (intact cell count) measurements exhibited similar patterns across the sampling locations. These findings highlight the effectiveness of aquifer passage in reducing microbial loads and stabilizing microbial activity in groundwater systems, with minimal seasonal influence on the final well water quality.

The results suggest that the aquifer passage effectively reduces DOC levels, particularly during the initial stages of infiltration, a residual fraction of DOC remains relatively constant and is not significantly degraded during the remainder of the aquifer passage. The stability of DOC concentrations after the initial decrease implies that the remaining DOC is likely non-degradable or highly resistant to the prevailing subsurface conditions. This may be due to the presence of humic substances or other complex organic molecules that are not easily broken down by microorganisms or through chemical processes in the aquifer. The EEM shows a typical fluorescence response of dissolved organic matter with a dominant humic-like peak, indicating the presence of organic compounds that contribute to the overall DOC in the sample.

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3. Case study Hamburg

In the CS1H case study site, a managed aquifer recharge (MAR) takes place via a distinctive ditch system for targeted infiltration and thus groundwater recharge. Water is extracted via shallow and deep wells with an annual extraction volume in the millions of cubic meters range. The shallow wells (up to 35 m deep) are hydraulically connected to the ditch system. The ditch system is fed by both rain water and artificially with river water over a large area. Artificial recharge with river water is suspended when there is sufficient precipitation to keep the ditch levels constant. Before being fed into the ditch system, the river water is coarsely sieved to remove larger particles.

The subsoil in this area is of glacial origin and is characterised by high hydraulic conductivity. Climate change increases pressure on the MAR system, highlighting the growing importance of monitoring and management (Escalante et al., 2019; Page et al., 2010). This includes rising temperatures of the source water and furthermore an increase or changing patterns of NOM loads and microbial contamination. Changing precipitation patterns and temperatures can also lead to a shift in the proportion of the demand driven artificially recharged river- and rainwater infiltrating the ditch system. Compared to deep drinking water wells, the hydraulic residence times of the shallow wells are relatively short, which leads to a more relevant influence of the surface water.

For seasonal monitoring of the treatment plants raw water before and after the subsurface passage, a specific ditch was selected that is as displayed in Figure 15 located in close distance of our monitored shallow well (aerial distance ~25 m). This ditch was selected due to its representative characteristics and its influence on the shallow well. The monitored shallow well has a flow rate of approx. 20 m³/h. The selection of the ditch and well enables a systematic investigation of seasonal fluctuations in the drinking water treatment plants raw water quality and microbial load. The proximity of the ditch to the well allows the effects of surface water infiltration and other environmental influences on the groundwater quality to be monitored and analysed.

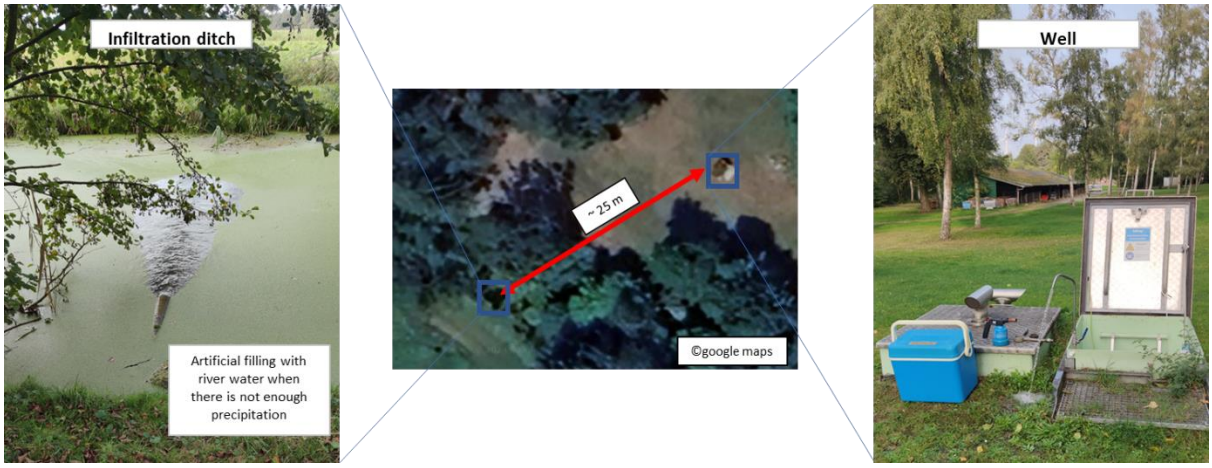

Figure 15: CS1H monitoring site set-up: Infiltration ditch with artificial recharge using river water (left). Hydraulically connected well located approximately 25 meters from the ditch (right).

Sampling

In case study CS1H, monthly sampling was carried out in a similar way to case study CS1B, but without the additional use of a continuous flow cytometer. Sampling began in October 2023 and the monitoring campaign ended in September 2024, analysing microbiological and organic parameters. The infiltration ditch grab samples were collected by using an autoclaved beaker on a telescopic rod (Bürkle, Bad Bellingen, Germany) and the well grab samples were collected by using a flammable sample tap. The

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sample tap was rinsed sufficiently until the water chemistry parameters conductivity, termperature and pH showed constant values. Prior to the microbial sampling, the sampling tap was also flamed. To ensure sample integrity, samples were stored cool and dark until analysis.

Microbiology

Microbial loads were analysed using FCM and cultivation-based methods. For FCM measurements, the CyFlow™ Cube 6 V2 (Sysmex) was used according to the method described by Schuster et al. (2022). Calibration was performed as reported by Ho et al. (2020) using fluorescing 3 µm calibration beads (Sysmex, Germany), mineral water and sterile filtered mineral water (Evian®). SYBR® Green I (SIGMA-ALDRICH®) was used as a stain for total, HNA and LNA cell counts. After the addition of the stain, the samples were incubated for 13 minutes at 37 °C and analysed within one hour in accordance with Ho et al. (2020).

The colony count of culturable microorganisms at 20 °C and 36 °C was determined in accordance with the local public health authorities (German Drinking Water Ordinance, 2023). This was achieved by counting visible colonies under 6- to 8-fold magnification that develop from bacteria present in 1 mL of water, cultivated on nutrient-rich, peptone-containing media (1 % meat extract, 1 % peptone) using the pour plate method. The incubation was conducted at temperatures of 20±2 °C and 36±1 °C for 44±4 hours, resulting in colony forming units (CFU) CFU20 and CFU36. Additionally, E. coli quantification was performed using CHROMagar. For this procedure, 100 mL of the sample was filtered through a membrane, and the filter was cultivated using a similar plate method at 36±1 °C, but with an incubation time of 24±1 hours.

NOM characterisation

The analytical methods - developed in Task 1.2 and reported in Deliverable 1.2 - absorbance, fluorescence spectroscopy and LC-OCD, were used to characterise the NOM composition, in addition to the monitoring on seasonal bacteria fluctuations. Samples were taken in pre-rinsed, muffle furnace-treated TOC-free glassware. An LC-OCD system and ChromCALC software, both developed by DOC Labor Huber (Karlsruhe, Germany), were used for LC analysis and data evaluation, respectively. Absorbance was measured using a Hach Lange DR5000 UV/VIS photometer (Düsseldorf, Germany) and fluorescence using a Horiba Aqualog (Osaka, Japan).

Results

The dataset utilized for this monitoring campaign, titled 'CS#1H Monitoring Data of Subsurface Passage in Managed Aquifer Recharge: Microbial and Organic Composition Analysis' is available for access and download from Zenodo at: https://doi.org/10.5281/zenodo.14008115

Microbial Monitoring

The results of the microbial monitoring and thus seasonal bacteria fluctuation at CS1H are presented in the following. Figure 16 displays the TCC for both the infiltration ditch and the well. It is apparent that the TCC in the infiltration ditch fluctuated greatly from month to month, while the value for the well was subject to less fluctuation. The highest TCC in the ditch was measured at the beginning of the measurement campaign in October 2023 with approximately 5 x 10^6 cells/mL. From November 2023 to January 2024 a drastic decrease to values between 1 to 2 x 10^6 cells/mL was observed. In February 2024, however, the TCC increased by one log level and then showed a decreasing trend until July 2024. A steady increase was observed again from August to September 2024.

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The well, however, showed a relatively constant TCC trend of approximately one log level lower than the ditch. As a result, the subsurface passages' reduction in TCC (%R TCC) was lowest in January 2024 at approximately 68 % and highest in February and September 2024 at approximately 94 %. The lowest TCC in the well was measured in January 2024 with a value of only 8.34 x 10^6 cells/mL. In October 2023 the highest TCC was measured with 2.52 x 10^6 cells/mL. The boxplots of TCC values for ditch and well over the one-year monitoring period, as shown in Figure 16, further illustrate that the TCC in the ditch exhibited significant fluctuations, while the well was less affected by (seasonal) environmental influences.

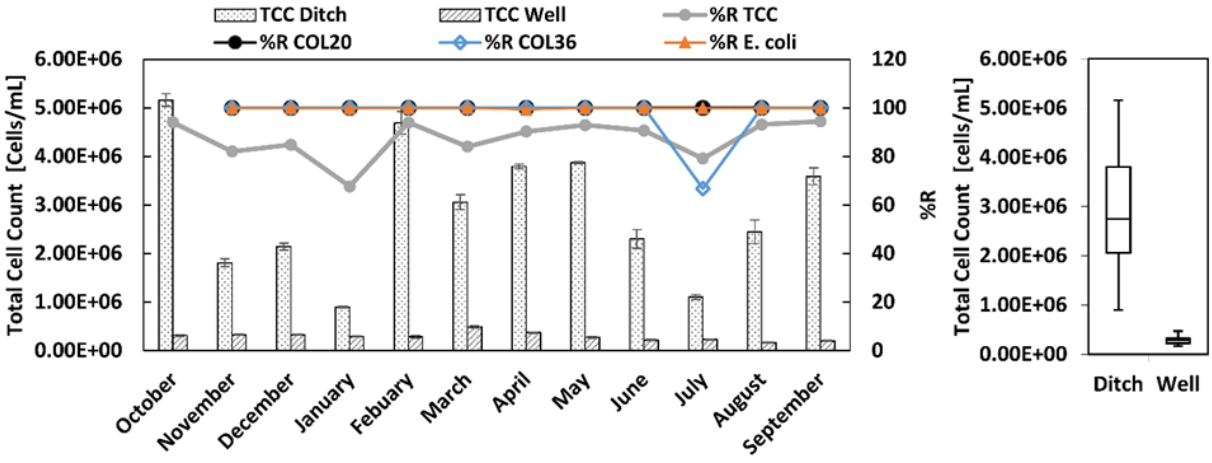

Figure 16: CS1H, Left: One-year seasonal monitoring of TCC in the ditch and the well, along with percentage removal rates (%R) for TCC, Col20, Col36, and E. coli. Right: Boxplots of TCC in the ditch and the well over the entire monitoring period, showing the distribution of total cell counts.

Considering the gating of bacteria in HNA and LNA, there is a different influence on TCC and composition as depicted in Figure 17. In the ditch the fraction of HNA was higher than the LNA fraction for every month. The fraction of HNA bacteria in the ditch was mainly responsible for the observed fluctuating trend in the TCC of the ditch. While in July the composition of the bacteria was about 71 % HNA in the ditch, in April 2024 the gating of the FCM counts revealed up to 99.97 % HNA bacteria. In the well the LNA fraction was predominantly higher during the monitoring period, however, there were months (October, November 2023 and March 2024) in which the fraction of HNA was higher.

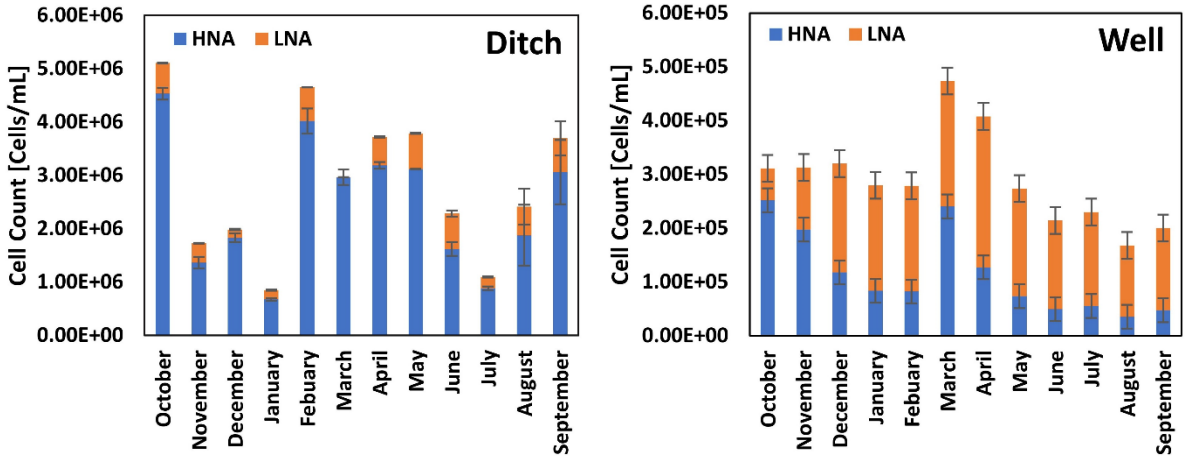

Figure 17: CS1H high nucleic acid (HNA) and low nucleic acid (LNA) cell distribution over the monitoring period for the ditch (left) and the well (right).

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For a comprehensive thorough comparison, additional cultivation methods were utilised alongside FCM. In Figure 18 the counts for the cultivation methods during the CS1H monitoring campaign are shown. The CFU method at 20 °C and 36 °C per mL showed an upper limit of quantification (ULOQ) of 300 CFU/mL.. For the CHROMagar method (*E.coli)*, the ULOQ was 3000 CFU/100 mL. In the ditch, the ULOQ for COL 20 °C was reached from October 2023 to September 2024. For CHROMagar (*E. coli)*, the threshold was met from October 2023 to September 2024, except for December 2024, which recorded only 1300 CFU/100 mL. In contrast, the COL 36 °C values varied significantly, from approximately 50 CFU/mL in October and November to exceeding the ULOQ of 300 CFU/mL in June 2024. An exceptionally low value of 3 CFU/mL was recorded in the ditch in July 2024. Furthermore, no detection for COL 20 °C was observed in the well in any month. For COL 36 °C, only the well July 2024 sample showed a value of 1 CFU/mL, just above the limit of detection (LOD). Consequently, apart from July 2024, reduction rates of 100 % were observed for both COL 20 °C and 36 °C. Additionally, CHROMagar (*E. coli)* yielded values ranging from 0 to 22 CFU/100 mL for the well over the investigation period, leading to log removal rates of up to over 3. However, it is important to contextualise these findings, as the LOQ was exceeded in the ditch in all but one month, suggesting that the actual log removal rates may be significantly higher.

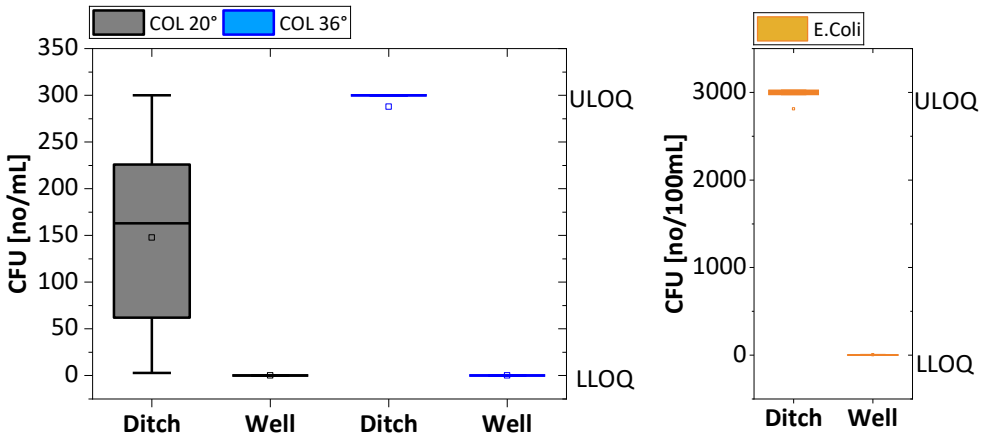

Figure 18: Left: CFU counts per mL for the COL 20°C and 36°C analysis of the infiltration ditch and well throughout the CS1H monitoring period. The Upper Limit of Quantification (ULOQ) is 300, and the Lower Limit of Quantification (LLOQ) is 0. Right: CFU counts per 100 mL for E.coli in the ditch and well during the monitoring period. ULOQ = 3000 and LLOQ = 0. (n = 11 for both graphs)

To further analyze the seasonal bacterial fluctuations, the density plots from the FCM measurements (see Figure 19) were investigated and contextualised. At the beginning of the monitoring campaign in October 2024, the ditch samples showed high cell population and distribution in the density plot and thus the FCM fingerprint of the sample shows an elevated number of HNA cells, indicating favourable environmental growing conditions and a stable cell growth phase. In contrast, in November 2024, there was a significant decrease in TCC and a noticeable decrease in HNA cells, while LNA cells increased. This could indicate stress conditions that affect cell growth. The density plots also show that the TCC continues to decrease in the winter months of December 2023 and January 2024, which is typical for the colder seasons when microbial activity continues to decrease due to lower temperatures and nutrient availability (Liu et al., 2013; Prest et al., 2016). In February an increase in the density of events in the HNA gate could be observed. In contrast, in March the water showed a significantly different fingerprint with almost no events in the left part of the LNA gate and a high density of cells with high nucleic acid content. A strong distribution of LNA to HNA was observed for April and May 2024, and a decrease in the HNA fraction with very high nucleic acid content was observed for June and July 2024.

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The plots for August and September 2024 revealed a prolonged increase in both the HNA and LNA gates.

While the density fingerprint of the FCM measurements for the ditch was subject to large fluctuations and thus the composition of the bacterial population, the picture was different for the well. As aforementioned, the total TCC was more stable for the well. In addition, the shape of the density clouds did not vary to the same extent as for the ditch samples. For the entire monitoring period, a density cloud was observed in the LNA gate and an elongated distribution into the area with a higher nucleic acid content. The shape of the distribution remained relative constant, however slight variations in the density distribution were observed. These variations correspond to the sums of the LNA and HNA gates shown in Figure 17 of the well.

In summary, the investigation of the wells FCM density plots showed less variation in the profile of the bacterial community. On the other hand, the ditch samples had a highly variable population, which can be attributed to two main factors. The lower surface and air temperatures in winter as well as the lower nutrient availability that led to a shift of the populations and a decrease or growth inhibition of the HNA fraction. Furthermore, the strong change in the structure of the density cloud is a further indication that the source water cannot be assumed to be constant. The dependence of the composition of the water in the ditch on precipitation, temperature, and thus on the artificial recharge with the river surface waters, which is not periodic, but rather demand-driven, leads to different FCM fingerprints over the monitoring period. The favourable result, however, is the stability of the FCM fingerprints after the subsurface passage in the well. As shown by the sum parameters for TCC, HNA and LNA as well as the colony-based methods, the population in the well is only slightly influenced by changes in the source water, surface and air temperature, and precipitation patterns, as well as the artificial recharge with river waters.

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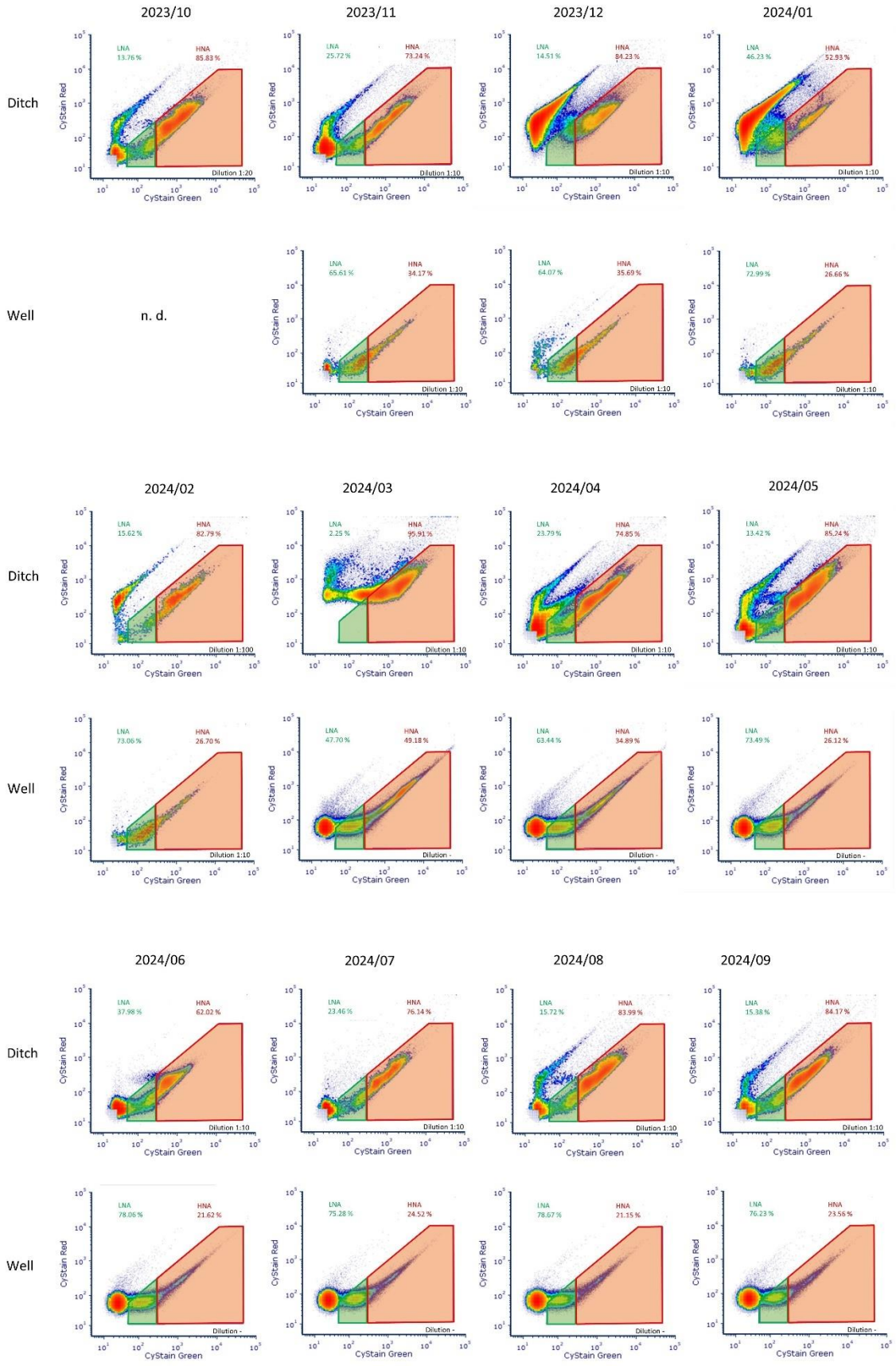

Figure 19: CS1H density plots for seasonal monitoring, displaying the distribution of both LNA (green gate) and HNA (red gate) cell populations and community structure.

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Organic water quality monitoring

The following section presents the quantity and composition of the organic matter during the monitoring period. A similar trend to that observed in microbiological data is evident here as well. As shown in Figure 20 the well exhibits a very consistent concentration of both TOC and DOC, with values around 6.2±0.2 mg/L. In contrast, the infiltration ditch is subject to fluctuations over the monitoring campaign. These variations cannot only be attributed to seasonal changes but rather to additional environmental and operational factors, like precipitation patters and evaporation resulting in artificial recharge with the river waters of different compositions and seasonal loads.

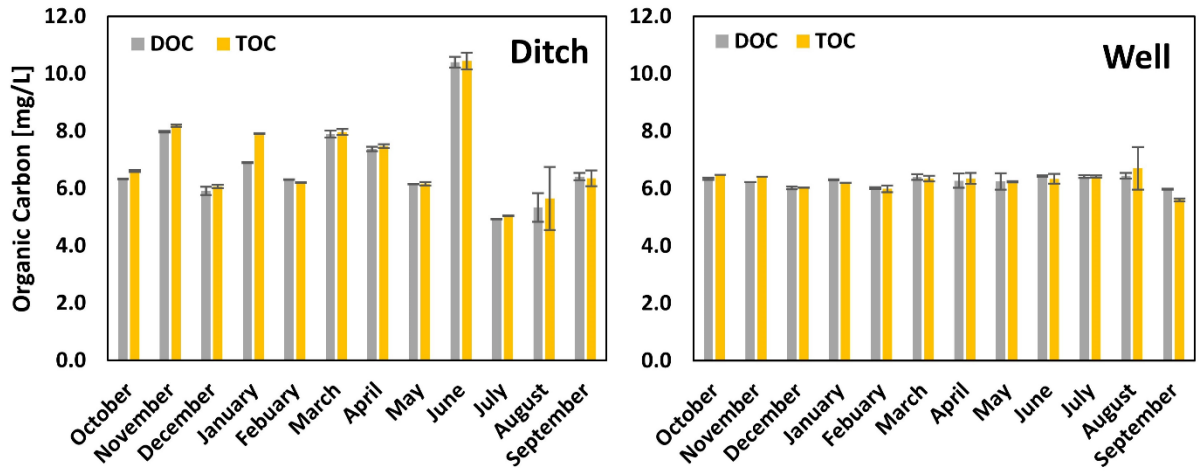

Figure 20: CS1H Total Organic Carbon (TOC) and Dissolved Organic Carbon (DOC) measurements during the monitoring campaign in the ditch (left) and well (right).

Further analysis of the DOC fractions was conducted using LC-OCD. The results are illustrated in Figure 21. In both the well and the infiltration ditch, humic substances dominated the DOC fractions each month, accounting for more than 60 % of the DOC. As mentioned before the DOC concentration in the well was relatively constant. Humic substances consistently represented the largest fraction, followed by Building Blocks and LMW neutrals. Notably, biopolymers were absent in the well.

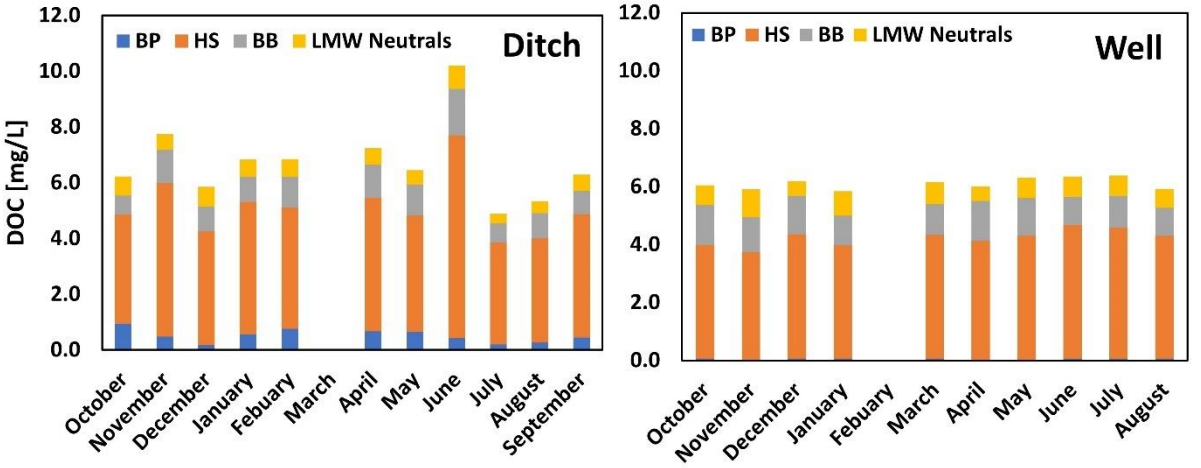

Figure 21: Fractionation of DOC via Liquid Chromatography - Organic Carbon Detection (LC-OCD): Biopolymers (BP), Humic Substances (HS), Building Blocks (BB), and Low Molecular Weight (LMW) Neutrals. LMW Acids signal was assigned to LMW HS due to a strong UV254 signal during their elution time according to Huber et al. (2011).

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In contrast, the infiltration ditch, as shown in Figure 21, exhibited more detailed fluctuations in DOC. Firstly, it was observed that biopolymers were present in the infiltration ditch every month, although to varying extents. The highest concentration of biopolymers was recorded in October, followed by a decreasing trend until December 2023. Subsequently, there was an increase until April 2024, after which the concentration decreased again towards the summer of 2024. The most pronounced quantitative difference was observed in the fraction of humic substances. For instance, in June 2024, the concentration of humic substances was approximately twice as high as in July 2024 or December 2023.

Additionally, Fluorescent Dissolved Organic Matter (fDOM) was analysed. The quantification of relevant peaks in accordance to Coble (2007) is shown in Figure 22. Here, Peak A consistently delivered the strongest signal, while Peak B provided the weakest signal each month. However, the fDOM exhibited a different trend compared to DOC and its fractions. Peaks A, M, C, D, and N showed similar magnitudes in the individual months from October 2023 to September 2024, both in the well and the ditch. The only notable differences between the ditch and well were observed for Peak B and T. The signals of Peak B and T were 10-14 % lower in the well in April and up to 48 % lower in December compared to the ditch. However, they were up to 47 % higher in February in the well than in the ditch.

This can be attributed to seasonal influences. In January, subzero temperatures led to ice formation in the ditch. In contrast to the ditch, the aquifer of the well is better protected from these seasonal changes due to the insulating effect of the surrounding soil. This could explain why the Peaks B and T in the well were approximately twice as high as in the ditch in February. These Peaks are associated with fDOM resulting from fresh microbial degradation processes (Coble, 2007), indicating reduced microbial activity in the ditch due to the lower temperatures.

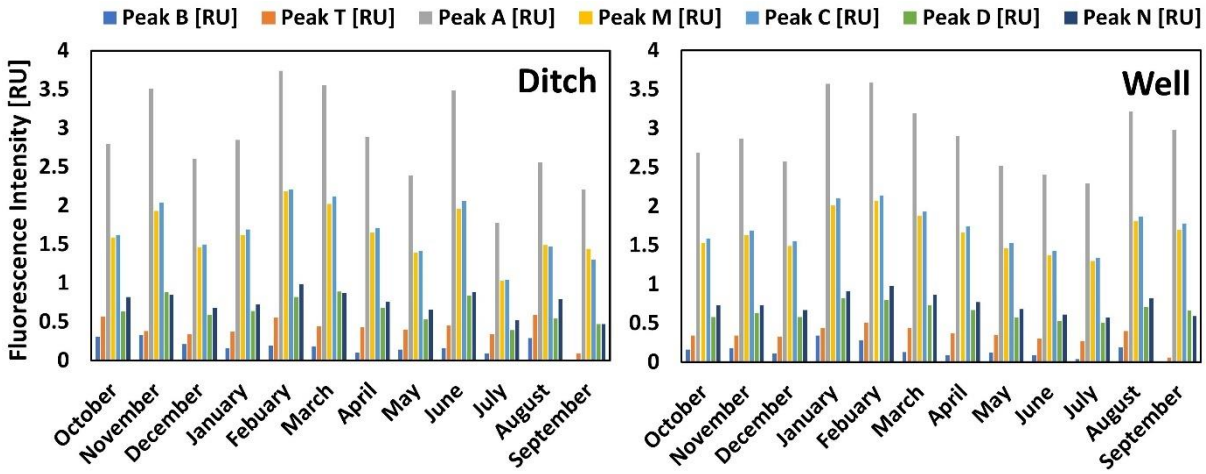

Figure 22 Fluorescence intensity for the fluorescence peaks B, T, A, M, C, D, and N according to (Coble, 2007) for the ditch (left) and well (right) over the entire monitoring period. Peaks B and T are associated with protein-like fluorescent dissolved organic matter (fDOM), while peaks A, M, C, D, and N are linked to humic-like fDOM originating from distinct sources.

Correlation of NOM characterization methods

The analysis of organic matter was used as an extension of the microbial monitoring, as the microbial fluctuations of the raw water in the well might be significantly influenced by the composition of the feed water in the ditch of the managed aquifer recharge system. The analysis of organic matter both in the well and in the infiltration ditch can provide further information, particularly on biopolymers. As described by Huber et al. (2011) groundwater is normally characterised by the fact that no biopolymers

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are present. In lakes, biopolymers originate from algae and have a low N/C ratio. This material is mainly polysaccharides. In groundwater, biopolymers should be absent.

The presence of biopolymers in groundwater can indicate two main factors: A hydraulic contact with surface waters or a high microbial activity in the aquifer. In the first case, this could be caused by leaks or direct infiltration without sufficient soil passage. In contrast, microbial growth and degradation processes occur within the groundwater body in the second case. Monitoring biopolymers therefore offers the advantage of serving as an early warning indicator, allowing conclusions to be drawn regarding whether the subsurface passage provides sufficient removal efficiency and retention of microbial-relevant NOM fractions. During CS1H monitoring campaign no biopolymers were detected in the well, thus underlying the efficiency of the MAR systems subsurface passage.

Furthermore, the concentration of biopolymers in the ditch was correlated with the bacterial loads expressed with the TCC as shown in Figure 23. Excluding the January 2024 data sample taken after the prolonged ice layer on the ditch, a statistically relevant correlation (r^2 = 0.79) for TCC and biopolymers was observed. Therefore, we were able to show that the presence of biopolymers indicates microbial growth since they either act as nutrients or are products of bacterial metabolism.

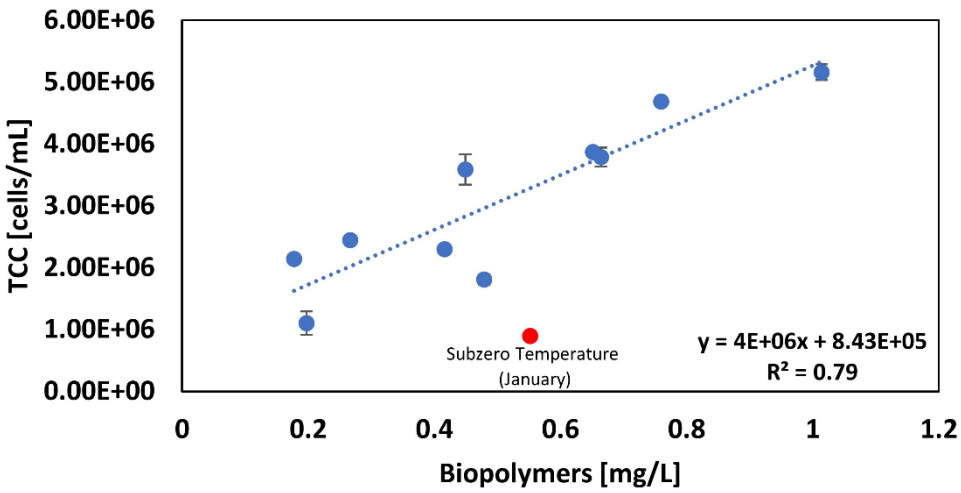

Figure 23: Correlation between total cell count (TCC) levels and biopolymer content in the ditch during the monitoring campaign. The January 2024 sample (marked in red), which was collected after a prolonged ice cover on the ditch, was excluded from the correlation.

A further aim of safeCREWs Task 1.2 was to correlate existing and newly developed methods for monitoring organic matter and to be able to substitute more complex methods, such as LC-OCD measurements, with simpler methods. As an application example within Task 2.1, the peak T of the fluorescence and the biopolymer fraction of the LC-OCD measurement will be assessed. As aforementioned, the concentration of biopolymers correlates with the TCC in the infiltration ditch. Peak T describes organics' autochthonous origin, which is associated with microbial processes and is similar to tryptophan (Coble, 2007). LC-OCD analysis is both time-consuming and costly. However, it has been shown that the concentration and presence of BP in particular can provide useful information and correlations for microbial monitoring. The Peak T of the simpler and cheaper method of fluorescence measurement will be further presented. As depicted in Figure 24 peak T provided a good correlation for both TCC (r^2 = 0.75) and BP concentration (r^2 = 0.75). These correlations emphasise the potential to use not only BP but also microbial-derived fluorescent peaks, such as peak T, as an additional marker for microbial risk assessment. Similar to reporting from Baghoth et al. (2011) who showed that protein like fDOM components correlate well with the BP fraction assessed with LC-OCD

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measurements. Among others Sorensen et al. (2020), demonstrated a strong correlation between TCC and tryptophan-like fDOM. Peak T monitoring can therefore be another link between NOM monitoring and microbial risk assessment.

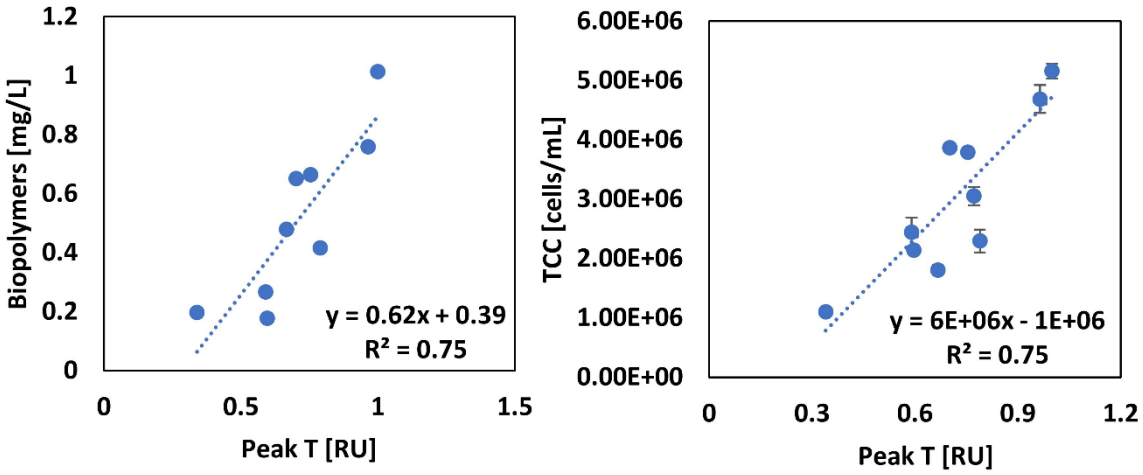

Figure 24: Correlation of fluorescent peak T, associated with protein-like fDOM, with biopolymer content (left) and total cell count (TCC) levels (right) in the infiltration ditch over the CS1H monitoring period.

CS1H Temperature patterns

To gain further insights, the monitoring results were put into the context of temperature trends (see Figure 25). During the seasonal monitoring campaign, a typical temperature pattern was observed: A decrease from summer through autumn to winter, followed by an increase in spring. Notably, there were two periods with sub-zero temperatures: late November to early December 2023, and a longer phase throughout January 2024. The latter period, in particular, led to the formation of an ice layer on the infiltration ditch. Due to the temperatures; in December 2023 and January 2024, a higher LNA bacterial count was observed in the well than in the ditch. This could be explained by the different environmental conditions and dynamics between the ditch and the well. While the ditch was covered by ice, limiting bacterial activity and potentially reducing bacterial loads, the well, which is typically deeper and less affected by surface conditions, has experienced relatively stable temperatures. These stable conditions could have allowed LNA bacteria to persist or even thrive in the well during the colder months, leading to higher counts compared to the partially ice-covered ditch.

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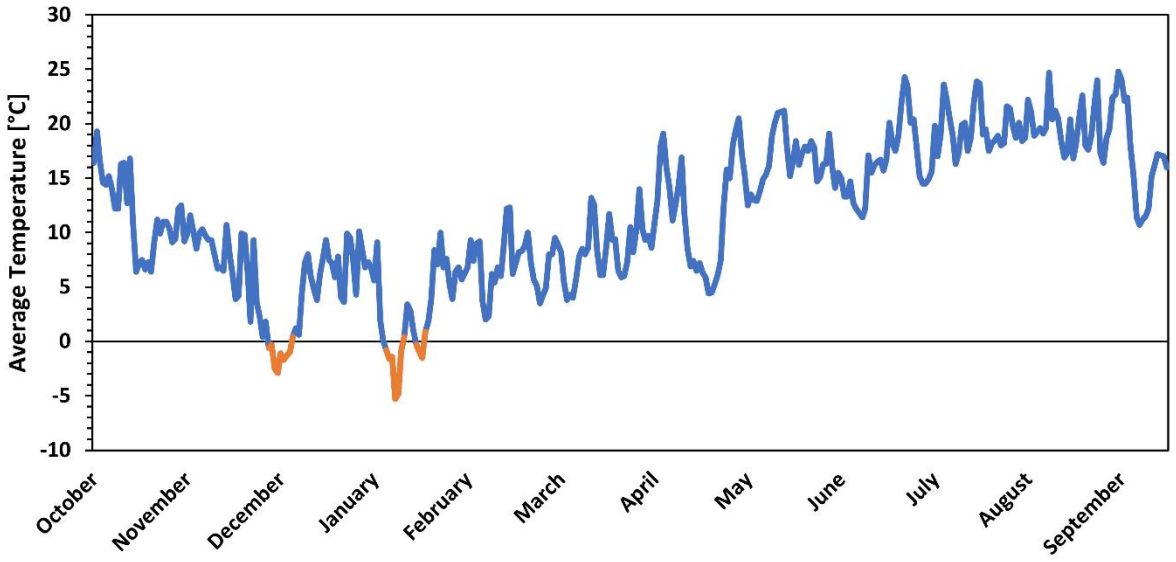

Figure 25: Average daily air temperature near the CS1H site during the monitoring period from October 2023 to September 2024, showing seasonal temperature variations.

Summary CS1H

At the Hamburg case study (CS1H), MAR is conducted via a unique ditch system designed for infiltration, resulting in recharging groundwater for drinking water production. To assess the impact of environmental changes, a specific ditch, located approximately 25 meters from a monitored shallow well, was selected for seasonal monitoring. This proximity enables systematic observation of seasonal fluctuations in raw water quality before and after subsurface passage, with a focus on microbial loads and NOM levels. By examining the interactions between the ditch system and the shallow well, this study aims to evaluate the effects of surface water infiltration and environmental influences on groundwater quality, providing insights into the resilience and performance of the MAR system under climate change conditions.

The monitoring of the seasonal bacterial and NOM loads revealed distinct trends for the infiltration ditch and the well. The results indicated that the changes in the raw water compositions were not only influenced by the different seasons. However, the demand-driven artificial recharge of the ditch with river waters on the one hand and prolonged times without recharge and thus dependence on precipitation, lead to shifts in the ditch's quality in terms of NOM and bacterial loads. In the context of climate change, changes in precipitation patterns are expected, especially prolonged periods without precipitation. Increasing temperatures will lead to increased evaporation and the need for further recharge with river water. Additionally, rising temperatures can lead to higher bacterial loads in the ditches even without artificial recharge and heavy rain events could lead to more surface runoff with higher NOM loads, which may act as nutrients for bacterial growth. Additionally, the higher air and water temperatures will affect the quality of the river water which is used for the artificial recharge. Which finally leads to further challenges CS1Hs' water treatment system which extracts its raw water from the shallow wells connected to the infiltration ditches. However, the generated data shows the effectiveness of the subsurface passage over the prolonged seasonal monitoring campaign of one year. Neither the microbial analyses with FCM and cultivation-based methods were able to find significant deteriorations in the well, even when the ditch showed high general microbial loads as indicated with the FCM or hygienically relevant parameters in accordance to the local health authorities as assessed with cultivation-based methods. The addition of the NOM measurements provided additional insights. Especially the biopolymer fraction as assessed with the LC-OCD can be used as an indicator for the influence or contamination of surface waters to groundwaters. While the NOM composition in the ditch highly fluctuated depending on the seasons as well as on the demand-driven artificial recharge

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with river water, no biopolymers were found in the well over the whole monitoring campaign underlining the effectiveness of the subsurface passage. In addition, a statistically relevant correlation was observed for the concentration of BPs and the TCC in the ditch. Thus, extending microbial measurements with NOM characterisation techniques like LC-OCD can provide additional information and act as an early warning sign for increased nutrients and thus bacterial loads. Furthermore, it can act as an early warning for hindered effectiveness of the subsurface passage. However, LC-OCD analysis is an expensive, time-consuming and complex analysis. Fluorescence may act as an additional method especially the monitoring of fDOM of microbial origin expressed with the fluorescence peak T. We were able to demonstrate that this method can serve as a proxy for the more complex LC-OCD analysis with lower effort, while also reflecting changes in microbial activity through microbial monitoring. It is important to emphasize that both culture-based methods for microbial process monitoring and bulk organic analyses, such as TOC, involve time delay between sampling and analysis result. In contrast, fluorescence spectroscopy and FCM offer potential for real-time analysis, and the generated data demonstrate their potential as monitoring tools.

The microbial composition, analysed in Hamburg through flow cytometry, showed a higher fraction of high-nucleic-acid (HNA) cells in the ditch throughout the year, with the HNA fraction reaching 99.97% in April 2024. The dominance of HNA cells was the primary driver of TCC fluctuations in the adjacent ditch. In the well, low-nucleic-acid (LNA) cells were more prevalent, although periods of higher HNA fractions were observed in specific months. The use of flow cytometry (FCM) and cultivation-based methods showed no significant deterioration in well water quality, and NOM analyses, particularly biopolymer measurements with LC-OCD, confirmed the lack of surface water contamination in the well. A strong correlation between biopolymers and total cell counts (TCC) in the ditch was observed, highlighting the potential of NOM characterization as an early warning for surface water contamination. However, LC-OCD is an expensive and time-consuming, and fluorescence-based methods, such as monitoring fDOM (fluorescent dissolved organic matter), were found to be effective proxies for more complex NOM analyses. Real-time analysis using fluorescence spectroscopy and FCM offers promising tools for monitoring microbial activity and groundwater quality, providing a faster and alternative water quality feedback compared to traditional culture-based methods. These techniques demonstrate potential for improved management of MAR systems under climate change pressures.

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4. Conclusion

The field monitoring program provided valuable insights into the dynamics of microbial abundance, bulk organic parameter removal, and water quality stability during groundwater recharge. Key conclusions combined from the case studies include:

- **Seasonal variations in microbial abundance**: The source water (recharge basin and infiltration ditch) exhibited pronounced fluctuations in total cell counts (TCC). Despite these fluctuations, aquifer passage effectively attenuated these seasonal variations, resulting in a consistent reduction of microbial loads. This indicates that aquifer passage acts as a robust process and buffer, stabilizing microbial concentrations and protecting well water from external seasonal and/or operational influences.

- **DOC removal and residual stability**: The aquifer passage effectively reduced dissolved organic carbon (DOC) levels, particularly during the initial stages of infiltration. However, a residual fraction of DOC remained stable throughout the aquifer passage, suggesting the presence of non-degradable or highly resistant organic compounds. The fluorescence response of dissolved organic matter (DOM) confirmed the presence of these complex organic molecules, which persisted despite microbial degradation efforts.

- **Effectiveness of subsurface passage in microbial and NOM control**: Despite fluctuating microbial loads and NOM levels in the source water, no pathogen indicator contamination was observed in the wells. Biopolymers, often indicative of surface water influence, were absent in the well water throughout the year, demonstrating the effectiveness of subsurface passage in maintaining biostable groundwater quality. A statistically significant correlation between biopolymer concentrations and TCC in the ditch suggests that biopolymers can serve as early indicators of microbial contamination risks.

- **Real-Time Monitoring Potential**: The combination of flow cytometry (FCM) and fluorescence spectroscopy was shown to be effective for real-time monitoring, offering quicker results compared to traditional culture-based methods and bulk organic analyses, such as TOC. These tools present a valuable opportunity for continuous monitoring of microbial activity and organic matter, enabling timely responses to emerging risks.

In summary, the study underscores the critical role of aquifer passage in ensuring groundwater quality, despite external influences, and highlights the value of combining microbial and organic matter monitoring techniques for more comprehensive and timely water quality assessments.

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