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Organic carbon uptake and mineralization in slow sand filters and their relation to process variables

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ABSTRACT

Slow sand filtration (SSF) is a widely used biofiltration method for drinking water treatment, yet quantitative measures of biological activity, specifically in terms of carbon uptake and mineralization, are not well established. This study assessed biological activity in mature SSF systems operated by three Dutch drinking water companies using carbon concentration measurements, isotopic signature analyses, and ^{13}C-labeled glucose tracer experiments. Our results revealed measurable differences in carbon concentrations and isotopic signatures between influent and effluent waters. Specifically, DOC concentrations decreased by up to 0.13 mmol L^{-1}, while DIC concentrations increased by up to 0.84 mmol L^{-1}. Additionally, δ^{13}C-DOC and δ^{13}C-DIC values exhibited shifts of up to +1.9 ‰ and 2.9 ‰, respectively, indicative of carbon uptake and mineralization. Biological activity varied across filters, as reflected in the time required for complete removal of glucose, which ranged from 5 to over 48 h and correlated with assimilable organic carbon (AOC) removal rates. AOC loading appeared to be the primary driver of bioactivity, with the lowest activity found in a filter fed with dune-infiltrated water and the highest in a filter receiving ozonated influent water. These findings highlight the importance of considering source water characteristics and the preceding treatment chain to understand their potential impact on biological activity in SSF.

1. Introduction

Slow sand filtration (SSF) is a well-established method for water treatment, designed to reduce turbidity, pathogens, and organic matter content during the production of drinking water [1,2]. SSF relies on a combination of physical, chemical, and biological processes to ensure the biological stability of the produced water [3]. A key factor in the efficacy of SSF is the biological community within the filter [4,5]. Microorganisms in slow sand filters play a crucial role in consuming and mineralizing organic matter, thereby contributing to the treatment process [6]. Additionally, the microbial community produces extracellular polymeric substances (EPS), which enhance filtration efficiency by promoting particle retention through straining and attachment [7]. Other biological processes that contribute to the filtration process and thereby improve the overall purification efficiency include pathogen predation by protozoa and small invertebrates as well as pathogen inactivation by viruses and phages [8,9].

Despite the critical role of the biological community in SSF, there is a notable absence of quantitative data characterizing the biological activity, particularly in terms of organic carbon uptake and mineralization. This knowledge gap limits our ability to make meaningful comparisons between different SSF systems, hindering the optimization of operational parameters relevant for drinking water production. While previous studies have suggested that factors such as temperature, organic input, and nutrient availability are associated with enhanced filtration outcomes [6,10,11], the specific mechanisms underlying these effects on biological activity remain unclear. More recent research has focused on characterizing the microbial community structure in slow sand filters, which has been found to be shaped by factors including source water, pretreatment, sand size, filter age, seasonality, and filter cleaning

Abbreviations: SSF, Slow sand filtration; Waternet-FS, Waternet-Full-Scale; Waternet-PS, Waternet-Pilot-Scale; DOC, Dissolved organic carbon; DIC, Dissolved inorganic carbon; AOC, Assimilable organic carbon; TSC, Total solid carbon; IRMS, Isotope ratio mass spectrometer; VPDB, Vienna Pee Dee Belemnite.

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[5,12–15]. These studies have shown that microbial communities in slow sand filters are highly dynamic and shaped by both operational and environmental variables. Nevertheless, there remains a critical need to directly link the process variables to microbial functional outcomes (e.g., carbon uptake and removal), which is essential for understanding how the operational factors influence the biofiltration processes in SSF.

In a recent study, we developed and tested three novel approaches to quantitatively assess biological activity in an operational slow sand filter [16]. The first two approaches are based on the comparison of the influent and effluent waters with respect to the concentrations and isotopic compositions of the relevant carbon pools, which provide a general insight into the carbon transformation within the boundaries of the filter. The third approach employs a deliberate introduction of an isotopically labeled tracer (^{13}C-glucose) into the system in a controlled laboratory experiment. Glucose was chosen because it is a simple, readily available, and easily degradable carbon source that is commonly utilized by microbial communities in aquatic environments [17]. The use of glucose as a tracer allows for a targeted study of microbial carbon uptake and mineralization, providing direct insight into the biological processes occurring within an ecosystem. This method has been widely applied in aquatic systems to track the fate of organic matter, with previous studies showing its effectiveness in assessing microbial capacity to assimilate and process organic carbon [18,19]. Our previous research further highlighted the value of this approach in elucidating biological activity within slow sand filters.

The aim of the present study was to employ these novel approaches in the comparison of slow sand filters operated by three Dutch drinking water companies (Waternet, Waterbedrijf Groningen, and Dunea). Our specific interest was to identify how the measures of biological activity determined by the different approaches are influenced by the different conditions and operational parameters characterizing the studied slow sand filters.

2. Materials and methods

2.1. Water production sites

In the Netherlands, the drinking water companies Waternet, Waterbedrijf Groningen, and Dunea utilize SSF during drinking water production. Each company has different water sources, pretreatment methods, and operational conditions. This study specifically examined mature filters at all three water companies alongside a pilot-scale filter at Waternet. Detailed operational characteristics of the investigated filters are listed in Table 1.

Waternet's production site in Weesperkarspel supplies drinking water to Amsterdam. The water originates from seepage water in the Bethune polder and undergoes several treatment steps. Before entering the treatment plant, suspended matter concentrations are first reduced by coagulation and sedimentation, and then water is stored in an uncovered reservoir for about 90 days. Further treatment includes rapid sand filtration, disinfection via ozonation, softening, and activated carbon filtration before reaching SSF. At this site, two types of filters were studied: a full-scale sand filter (Waternet-FS) and a pilot-scale filter (Waternet-PS). The full-scale filter has a surface area of 605 m^2, a sand bed depth of 1.3 m, and started operations 18 years before sampling. The pilot plant filter, which was designed to replicate the operational parameters of the full-scale slow sand filter, began operation in 2020, 3.5 years before sampling. It has a surface area of 2.56 m^2 and a sand bed depth of 1.2 m. Both filters use the same source water, similar sand grain size (0.65–0.68 mm), and slightly different filtration rates (0.30 m h^{-1} for Waternet-PS versus 0.35 m h^{-1} for Waternet-FS).

For the slow sand filter operated by Waterbedrijf Groningen, water is sourced from the Drentsche Aa creek and stored in a mixing basin for 55–60 days. Prior to SSF, the water is treated with coagulation/flocculation, double-layer filtration, activated carbon addition, and UV disinfection. Post-SSF, the water passes through a cascade aerator and additionally undergoes a pH correction step.

The third slow sand filter examined is located at the Scheveningen production site and operated by Dunea. The source water from the Maas river is first pumped into dune lakes (infiltration ponds) for an average residence time of two months. Following dune filtration, the water undergoes softening, activated carbon treatment, cascade aerators, rapid sand filtration, and finally SSF.

2.2. Collection of water samples and sand columns

Water samples from the influent and effluent of the studied slow sand filters were collected on several occasions between 2021 and 2023 (Table 1). In Groningen, the effluent samples could only be collected after the water passed through a cascade aerator and underwent pH correction, i.e., they represent the final drinking water product rather than water immediately after SSF. Separate water sampling specifically targeting assimilable organic carbon (AOC) was carried out for Waternet in February 2023, Groningen in June 2023, and Dunea in March 2021.

Sand columns for the isotope tracer experiments were collected along with the water samples when the filters were scheduled for cleaning, which is typically performed when a decline in hydraulic performance occurs, as evidenced by an increased head loss caused by filter clogging. Sampling occurred a few hours after the filters were drained. Sampling cores (inner diameter of 10 cm, height of 30 cm) were inserted into the sand bed to a depth of 20 cm. After carefully lifting the cores, they were capped to minimize disturbance and placed in a cool box for transport to the laboratory at Utrecht University.

2.3. Stable isotope tracer experiments

Within 3 h after collection, the sand cores were filled with fresh SSF influent water collected on the same day as the sand cores. Each core contained approximately 20 cm of sand and 10 cm of overlying water. The cores were then sealed and a continuous percolation in the downward direction was started. The bottom outlet was connected to the top inlet with tubing to ensure that the percolating water was recycled (Supplementary Fig. S1). In the Waternet-FS and Groningen experiments, highly permeable tubing (Masterflex, BioPharm platinum-cured silicone, L/S) with a CO_2 permeability coefficient of 1.89 $\frac{\text{cm}^3\cdot\text{mm}}{(\text{cm}^2\cdot\text{s}\cdot\text{Pa})\cdot 10^{-10}}$ was used. These experiments showed, however, that this type of tubing allowed significant exchange of CO_2 with the atmosphere on the time

Table 1
Operating characteristics of the investigated slow sand filters at the time of sand columns collection.

Location	Sampling period	Surface size (m^2)	Filtration velocity (cm h^{-1})	Schmutzdecke age (year)	Sand size (d_{50} mm)	Filter cleaning method	Incubation temperature (°C)
Waternet-Full-scale	September 2021	605	35	0.5	0.68	Wet harrowing	18
Waternet-Pilot-scale	June 2023	2.56	30	3	0.65	Scraping	
Groningen	April 2022	1205	18.6	5	0.54	Scraping	12
Dunea	February 2023	1280	21	2.6	0.3–2.25[a]	Scraping	12

[a] Based on d_{10} and d_{90}.

scale of the experiment (see Results). Therefore, subsequent experiments with the columns from Dunea and Waternet-PS used an alternative tubing (Masterflex, PharMed L/S) with about 21-fold lower CO_2 permeability coefficient. The flow rate of 60 mL min^{-1}, which corresponds to a filtration velocity of about 45 cm h^{-1}, was selected to ensure a uniform flow rate for all the locations studied. The flow rate was kept constant by using peristaltic pumps (Masterflex L/S). The cores were kept in the dark in a climate chamber with temperatures adjusted to match the corresponding temperature during sampling (Table 1).

Sixteen hours after filling the sand cores with fresh influent, the isotope tracer experiment was initiated by injecting a ^{13}C-glucose solution (concentration of 18.6 g glucose L^{-1}, ^{13}C atom fraction of 99 %; Cambridge Laboratories) into the overlying water. Each core received 1 mL of the glucose solution, equivalent to 0.6 mmol of glucose-derived ^{13}C per core. To ensure even distribution of the label throughout the columns, the glucose was administered in five injections of 200 μL each, with 10–15 min intervals between injections.

To assess the fate of the glucose-derived ^{13}C tracer, water and sand samples were collected at specific time points and analyzed for carbon concentrations and δ^{13}C in the relevant carbon pools. During the initial hours following the addition of ^{13}C-glucose, overlying water samples were collected frequently from two cores per site for the analyses of glucose, dissolved inorganic carbon (DIC) concentrations, and δ^{13}C-DIC. Once the concentration of dissolved glucose fell below the detection limit (0.002 mmol glucose L^{-1}), duplicate cores per site were sacrificed to collect sand samples for the analysis of glucose-derived ^{13}C incorporation into the solid phase. These sampling time points varied across the studied sites, occurring 6 h, 1 day, and 5 days after the addition of ^{13}C-glucose for the Waternet-FS, Groningen, and Dunea sites, respectively. For Waternet-PS, collecting sand samples after glucose consumption was not feasible; hence, no data on glucose incorporation into the solid phase is available for this location.

When collecting sand samples, the cores were disconnected from the peristaltic pumps and drained from water. The total height of the sand column was noted and the sand was subsequently sampled from three distinct layers: 0–2 cm, 2–5 cm, and 5–10 cm. The top few centimeters of the sand columns were identified as the schmutzdecke based on their distinctly darker appearance compared to the layers below. The thickness of the schmutzdecke was about 3 cm in the sand filter from Groningen and about 2 cm for those at Waternet-FS and Dunea. For Waternet-PS, the color difference was less pronounced, and no distinct schmutzdecke layer was observed. Two sand columns per site sliced directly after core collection (i.e., without filling and percolation with the influent water) were used as controls for the natural isotopic composition of the solid phase carbon.

2.4. Analytical procedures

All water samples were filtered upon collection using 0.2 μm polyethersulfone (PES) filters, except for Waternet-FS, which were filtered using 0.2 μm nylon filters. The δ^{13}C-DOC samples (20 mL) were poisoned to stop biological activity. This was done using 2.5 μL of 50 % H_3PO_4 per mL of water, except for Waternet-FS, which was poisoned with 10 μL of 50 % *w/v* $ZnCl_2$ per mL of water. All filtered samples were stored at 4 °C until analysis.

Glucose concentrations were measured with an enzymatic assay provided by R-Biopharm. The assay is based on the production of NADPH, which was quantified by UV/Vis spectrophotometry at 340 nm [20]. DOC concentrations for samples from Waternet-FS and Groningen were measured using catalytic high-temperature oxidation (Shimadzu TOC-V, model CPH). DOC concentrations for Dunea and Waternet-PS, along with the analysis of δ^{13}C-DOC, were measured at KU Leuven, Belgium, using a high-temperature catalytic carbon analyzer (IO Analytical Aurora 1030W) coupled to a Thermo Scientific Delta XP isotope ratio mass spectrometer (IRMS) via a GasBench and cryo-focusing. Assimilable organic carbon (AOC) was measured at Het Waterlaboratorium, the Netherlands, based on van der Kooij [21].

For the measurement of DIC and δ^{13}C-DIC, 0.7 mL of water samples were injected through a septum into sealed helium-flushed vials containing 0.1 mL 85 % H_3PO_4. The headspace CO_2 was then analyzed using a Thermo Scientific GasBench II coupled with a Delta V Advantage IRMS. Sand samples for the total solid carbon content (TSC, expressed in weight %) and δ^{13}C-TSC were homogenized by manual mixing upon collection, freeze-dried, and subsequently ground with a ball mill grinder. The samples were analyzed with Thermo Scientific EA IsoLink CN coupled with a Delta V Advantage IRMS. All δ^{13}C values are reported relative to the Vienna Pee Dee Belemnite (VPDB) standard.

The porosity of the studied sand columns was estimated by tracer experiments using 550 mg L^{-1} NaCl solution [22], except for the Waternet-FS samples, where it was estimated based on the mass difference between the water-saturated and dry sand samples. The porosities of the sand columns from Waternet-FS, Groningen, Dunea, and Waternet-PS slow sand filters were 0.40, 0.32, 0.38, and 0.44, respectively.

2.5. Data processing

Total carbon content in the examined solute pools (DIC, glucose) was calculated by multiplying the measured concentrations with the total volume of water per core. The water volume comprised both the overlying water and porewater volumes, as derived from the core diameter, heights of the water and sand columns, and sediment porosity.

Total carbon content in the solid phase (TSC) was calculated by combining the measured TSC concentrations in wt% with the total sand volume (determined from the core diameter and sand column height), dry sand density (assumed value of 2.6 g cm^{-3}), and the corresponding sediment porosity. When evaluating the TSC content for the entire 20 cm sand column within the core, the concentrations measured in the 5–10 cm layer were considered representative for the entire section of the column below 5 cm depth.

Transfer of glucose-derived ^{13}C into a particular pool (DIC or TSC) during the isotope tracer experiment was evaluated based on the mass-balance approach described previously by Polerecky et al. [23]. For each pool, the measured δ^{13}C permil-values were first converted to $^{13}C/^{12}C$ isotope ratios according to $R = (1 + \delta^{13}C/1000‰) \cdot R_{VPDB}$, where $R_{VPDB} = 0.0111802$ is the $^{13}C/^{12}C$ isotope ratio of the VPDB standard. Next, the R values were converted to ^{13}C atom fractions according to $x = R/(1 + R)$. The ^{13}C atom fractions determined for the samples collected at time point t (denoted as x_t) and the control (unlabeled) samples (x_0) were then combined to calculate excess ^{13}C atom fractions, $\Delta x = x_t - x_0$, which were finally used to estimate the amount of excess ^{13}C according to $\Delta^{13}C = \Delta x \cdot C$. In the last expression, C is the total carbon content in the pool at time point t, determined from the measured values as described above, and $\Delta^{13}C$ is the amount of ^{13}C originating from the added ^{13}C-glucose that was transferred to the pool between time points 0 and t. Excess ^{13}C-DIC and excess ^{13}C-TSC are thus measures of respiration and incorporation of the added ^{13}C-glucose into the solid phase, respectively.

3. Results

3.1. Carbon concentrations and isotopic signatures

AOC levels decreased after SSF at all locations (Fig. 1A). The filters at Waternet-FS and Groningen exhibited similar absolute AOC removal, whereas the relative removal at Groningen was twice that of Waternet-FS (40 % vs. 20 %). AOC removal at Dunea was lower both in absolute and relative terms, most likely due to the overall lower AOC input. AOC loading rates, calculated as a product of the influent AOC concentrations and filtration velocity, amounted to 0.78 mmol m^{-2} h^{-1} (Waternet-FS), 0.19 mmol m^{-2} h^{-1} (Groningen), and 0.05 mmol m^{-2} h^{-1} (Dunea).

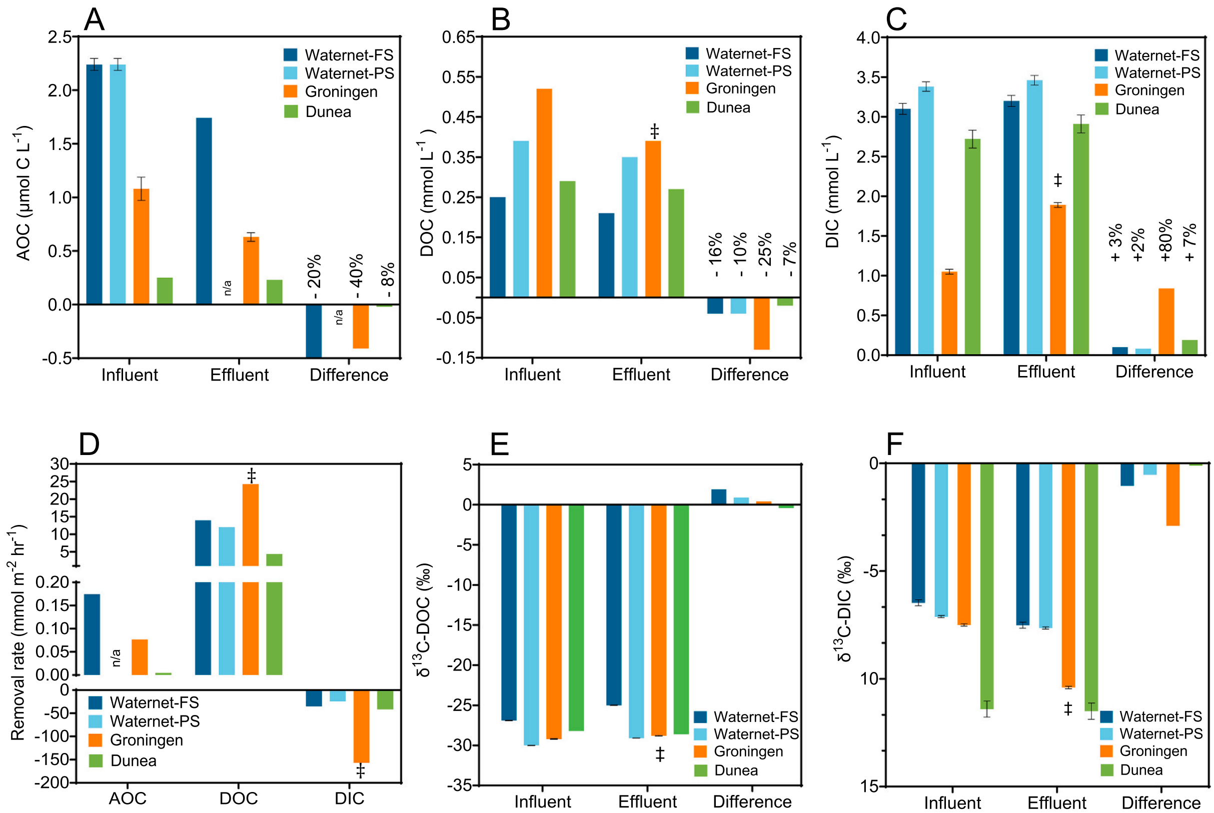

Fig. 1. Carbon concentrations and isotopic signatures in the influent and effluent of the studied slow sand filters, along with their differences. Waternet-FS refers to full-scale and Waternet-PS refers to pilot-scale. n/a indicates missing data. ‡ Groningen samples obtained from SSF effluent that passed cascade aerators + pH correction. (A) Assimilable organic carbon. (B) Dissolved organic carbon. (C) Dissolved inorganic carbon. (D) AOC, DOC, and DIC removal rates, as derived by multiplying the differences in the effluent and influent concentrations with the corresponding in situ flow-through rates to express removal per unit area. (E) Isotopic composition of the DOC pool. (F) Isotopic composition of the DIC pool. Note the various units on the Y-axis. The "difference" is calculated as Effluent – Influent, with bars and percentages showing the absolute and relative values. Data represent a single measurement ($n = 1$) due to practical constraints. Error bars represent the analytical error when available.

DOC concentrations also decreased after SSF at all sites (Fig. 1B). The absolute decrease in DOC was substantially greater than that for AOC, as expected based on the roughly two orders of magnitude higher concentrations of DOC compared to AOC. However, the relative decrease in DOC was smaller than that for AOC. Groningen had the highest DOC influent concentration and achieved the greatest DOC removal, both in absolute and relative terms, followed by Waternet. Similar to AOC, DOC concentration decrease and relative removal were lowest at Dunea.

In contrast to AOC and DOC, DIC concentration in the SSF effluent increased relative to that in the influent, and this increase was again consistently observed at all sites (Fig. 1C). The relative increase was below 10 % at Waternet and Dunea after SSF, but reached up to 80 % in the final drinking water product at Groningen, which underwent aeration and pH correction after SSF. Overall, Waternet had the highest surface area normalized AOC removal rate, while Groningen had the highest rates of DOC removal and DIC increase (Fig. 1D).

SSF altered not only the concentrations of dissolved organic and inorganic carbon but also their isotopic composition. The increase in $\delta^{13}C$ indicated that the DOC in the effluent was more enriched in ^{13}C than in the influent, with the largest change observed at Waternet-FS, followed by Waternet-PS and then Groningen (Fig. 1E). In contrast, the $\delta^{13}C$ of DOC was slightly more negative after SSF at Dunea. The DIC in the effluent was consistently isotopically lighter than in the influent for all locations (Fig. 1F), reflecting the addition of DIC with low $\delta^{13}C$ originating from the mineralization of organic carbon. The largest disparity was observed at Groningen, although it is challenging to determine whether this was due to SSF alone or in part also due to the subsequent cascade aeration and pH correction steps.

For all studied sand filters, TSC concentrations in the sand bed decreased with depth (Fig. 2A). They were similar and highest within the top 2 cm (the schmutzdecke) at Waternet-FS and Groningen, whereas those at Dunea and Waternet-PS were approximately three-quarters and one-quarter of these values, respectively. The contrast in TSC concentrations between the schmutzdecke and deepest sand layer (5–10 cm) varied among the sites, with the highest values at Groningen (0.27 wt%), followed by Dunea (0.22 wt%), Waternet-FS (0.20 wt%), and Waternet-PS (0.04 wt%). However, when considering the relative increase in the TSC concentration in the schmutzdecke compared to the deepest layer, Dunea showed the highest value (4-fold), followed by Groningen (3-fold), and Waternet-FS and Waternet-PS (both around 2-fold). At the Groningen site, the TSC concentration in the intermediate layer (2–5 cm) was around 2-fold higher compared to the deepest layer, likely because the sampled section partially included also the schmutzdecke, which extended down to a depth of 3 cm at this location (assumed based on visual observation). At the other sites, the relative difference in TSC concentrations between the intermediate and lowest layers was less than 1.4-fold.

Vertical gradients in TSC content in the filters can be attributed to preferential DOC retention in the schmutzdecke. If the $\delta^{13}C$ value of the initially present TSC differs from that of the retained DOC, vertical gradients in $\delta^{13}C$-TSC values would be expected. Indeed, at Waternet-FS, Waternet-PS, and Dunea, $\delta^{13}C$ values in the upper two layers fell between the $\delta^{13}C$ values of the input DOC and those of TSC in the deepest layer (Fig. 2B). At Groningen, the $\delta^{13}C$ value of the input DOC was similar to that of the TSC in the deepest layer. At Waternet-FS and Dunea, the TSC in the deepest layer was isotopically heavier than the

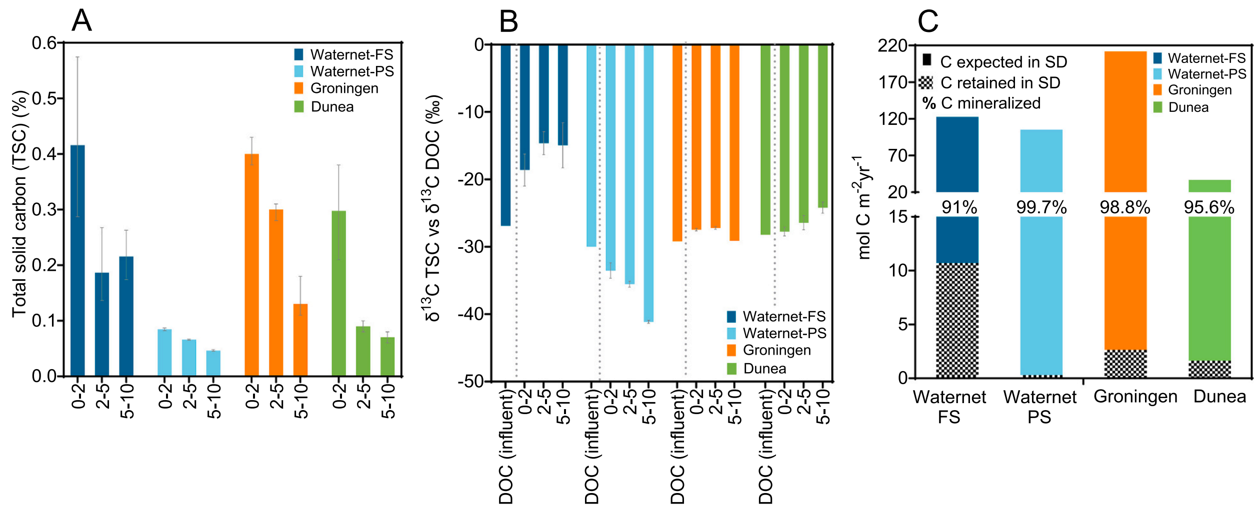

Fig. 2. Carbon contents of the sand collected from different depth intervals and their isotopic signatures. Waternet-FS refers to full-scale and Waternet-PS refers to pilot-scale. Data represents the mean ± range. (A) Total solid carbon (wt%) ($n = 4$), except Waternet-PS ($n = 2$). (B) The isotopic composition of the total solid carbon pool ($n = 2$) along with that in the influent DOC. (C) Expected amount of carbon accumulated in the schmutzdecke (SD) over a year (colored bar), estimated from the DOC removal data shown in Fig. 1D, versus the actual amount found (patterned bar). Note the cut in the Y-axis. The percentages indicate the mineralization of carbon based on the expected C vs actual C found in the schmutzdecke.

DOC in the influent, whereas at Waternet-PS, the $\delta^{13}C$ of the TSC in the deepest layer was lower than that of the DOC in the influent.

The mineralization of DOC was also estimated using a carbon mass balance approach, assuming that DOC retention occurred exclusively in the top 2 cm layer. To estimate the expected annual carbon increase in the schmutzdecke, it was assumed that the initial carbon content of the filter media was similar to that at the 5–10 cm depth before DOC accumulation, and that the DOC input to the filter had remained constant (Fig. 1B) since the last filter cleaning. This value was then compared with the actual TSC content measured in the schmutzdecke at the time of sampling (Fig. 2A). The difference between the expected and actual TSC content in the schmutzdecke was interpreted as resulting from mineralization and subsequent export as DIC. Based on this difference, it was estimated that over 90 % of the DOC removed by the sand filter was mineralized, with the highest mineralization observed at Waternet-PS and the lowest at Waternet-FS (Fig. 2C). It is important to note that, in the absence of data on the initial TSC content of the sand, this calculation relied on a simplifying assumption that carbon accumulation from DOC retention occurred only in the upper 2 cm of the filter, and that the initial carbon content in this layer was similar to that of the underlying 5–10 cm layer. While this assumption was necessary to constrain the mass balance, it does not exclude the possibility that microbial communities throughout the entire filter contributed to DOC degradation.

3.2. Stable isotope tracer experiment

Following the addition of ^{13}C-glucose to the experimental sand columns (approximately 0.6 mmol ^{13}C per core), glucose concentrations decreased approximately linearly over time for all locations (Fig. 3A). However, the time required for glucose to become undetectable in the overlying water, estimated by extrapolating the observed linear trends, varied substantially among the sites. This time was about 5 h for the Waternet-FS columns, longer but similar for the columns from

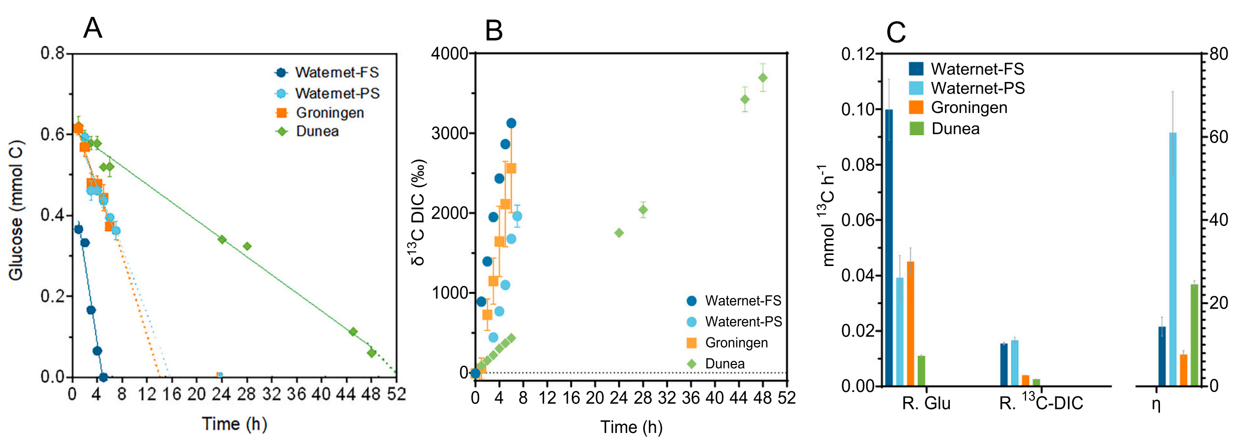

Fig. 3. Dynamics of glucose and the dissolved inorganic carbon pool in the cores during the stable isotope tracer experiments. Waternet-FS refers to full-scale and Waternet-PS refers to pilot-scale. (A) Glucose in the overlying water following glucose addition at time point 0 (Waternet-FS: n = 1, other locations: n = 2). Solid lines represent the linear regression fit. Dotted lines show linear extrapolation of the available data indicating the expected time when glucose became undetectable. (B) Isotopic composition of DIC during the initial hours of the experiments when dissolved glucose was still detectable. Error bars represent the range between duplicate cores. Some error bars are not visible because they are smaller than the symbols. (C) Calculated rates of glucose removal (R.Glu) and excess ^{13}C production in the DIC pool (R. ^{13}C-DIC). η refers to the mineralization efficiency (%) obtained from plotting excess ^{13}C-DIC vs glucose (Supplementary Fig. S3). Error bars represent the standard error of the slopes obtained when calculating the rates based on duplicate cores.

Groningen and Waternet-PS (around 15 h and 16 h, respectively), and reached up to 52 h for the Dunea columns.

Mirroring the decrease in glucose concentrations, δ^{13}C-DIC exhibited a rapid and immediate increase over time for all locations (Fig. 3B). Waternet-FS had the highest initial increase in δ^{13}C-DIC, reaching about 3000 ‰ after 6 h, followed by Groningen, Waternet-PS, and finally Dunea, which only reached around 500 ‰ after 6 h. When accounting for the measured DIC concentrations and calculating the amount of excess ^{13}C in the DIC pool produced by the respiration of the added ^{13}C glucose, the trend among the sites differed. Specifically, because the Groningen site had the lowest DIC concentration (around 1 mmol L^{-1}), its excess ^{13}C-DIC after the first 6 h was similar to that estimated for the Dunea columns and about 5-fold lower compared to Waternet-FS and Waternet-PS (Supplementary Fig. S2).

Based on linear regressions of ^{13}C-glucose and excess ^{13}C-DIC over time when glucose was still detectable in the overlying water, the rates of ^{13}C-glucose consumption and the corresponding excess ^{13}C-DIC production were estimated for each location (details in Supplementary statistical analysis 1). The results indicated that the ^{13}C-glucose uptake rate was highest in sand columns from Waternet-FS, followed by Groningen, Waternet-PS, and Dunea, whereas the rate of excess ^{13}C-DIC production was highest in sand columns from Waternet-FS and Waternet-PS, followed by Groningen and Dunea (Fig. 3C). The fraction of glucose mineralized during the period when glucose was available in the porewater was estimated by plotting excess ^{13}C-DIC versus glucose and calculating the slope of the resulting linear relationship (Supplementary Fig. S3). This yielded mineralization efficiencies of approximately 15 %, 10 %, 25 %, and 60 % for Waternet-FS, Groningen, Dunea, and Waternet-PS, respectively (Fig. 3C).

In addition to excess ^{13}C-DIC production, the complete glucose removal from the water percolating the sand columns led to a substantial ^{13}C enrichment of the TSC pool. This enrichment occurred across all depths of the sand columns, with δ^{13}C-TSC values increasing from about −15 ‰ and −30 ‰ in the control sand columns to between about 30 ‰ and 300 ‰ at the time when glucose was completely removed (compare Fig. 4A and Fig. 2B). Due to the higher carbon concentrations in the schmutzdecke, the concentrations of excess ^{13}C-TSC in this layer exceeded those in the two lower sections of the sand columns (Fig. 4B), except for the columns from Groningen, where the top and middle layers had similar concentrations of excess ^{13}C-TSC. Overall, the concentrations of excess ^{13}C-TSC in the schmutzdecke were highest in the Dunea columns.

The total amount of excess ^{13}C in the TSC pool, summed over the entire sand column, ranged between 0.30 and 0.45 mmol ^{13}C per core, with the highest amount in Dunea and the lowest in Waternet-FS (Fig. 4C). The amount of ^{13}C-TSC in the schmutzdecke at Dunea accounted for almost 45 % of the total ^{13}C in the TSC pool, whereas this fraction was lower at other locations, ranging between 15 and 20 %.

4. Discussion

In this study, we used different approaches to quantify biological activity in slow sand filters. Since each approach yields a different measure of the activity, we first discuss their potential and drawbacks individually. Subsequently, we examine possible reasons behind the observed differences among the studied slow sand filters.

4.1. Approach based on carbon concentrations

One measure of the biological activity in slow sand filters is the ability to remove easily assimilable organic carbon (AOC). Assuming that AOC removal is primarily driven by microbial uptake, differences in AOC concentrations can serve as an indicator of the rates of biological carbon uptake within the filters. This quantity, expressed for example in mol AOC m^{-2} h^{-1}, can be determined by measuring the difference in AOC concentrations between the influent and effluent waters of a slow sand filter and multiplying it with the flow velocity. However, two influencing factors must be considered here. First, abiotic processes may also contribute to AOC removal [24]. Second, the relevance of biotic versus abiotic processes varies among water treatment sites due to differences in the nature and reactivity of AOC [25].

The investigated filters showed differences in both the relative and absolute rates of AOC removal (Fig. 1A and D). The surface-normalized AOC removal rates (mmol C m^{-2} h^{-1}) followed the order: Waternet-FS > Groningen > Dunea, which also correlates with the ranking of the AOC loading rates. In contrast, the relative AOC removal (%) decreased in the order of Groningen > Waternet-FS > Dunea. This suggests that the microbial community at Groningen may be more efficient in AOC removal or that AOC in the influent at Groningen is easier to assimilate. Furthermore, differences in filtration rates might play a role; the shorter residence time (Table 1) in the Waternet-FS filter could have contributed to its lower relative removal efficiency compared to the Groningen filter.

Given that DOC removal exceeds AOC removal by two orders of magnitude (Fig. 1D), it is unlikely that DOC retention in slow sand filters is solely due to biological processes [26]. The significant difference between AOC and DOC removal rates suggests that direct microbial assimilation accounts for only a part of DOC retention. This disparity may stem from the nature of AOC as an operational proxy for

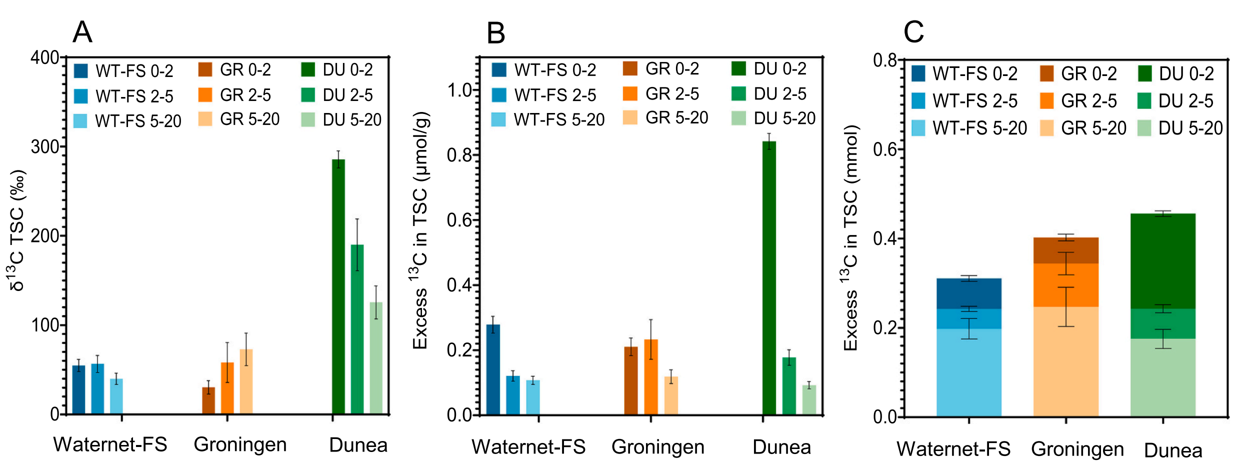

Fig. 4. Dynamics of the total solid carbon pool after complete ^{13}C-glucose uptake (samples were taken on day 0.25 in Waternet-FS, day 1 in Groningen, day 5 in Dunea after glucose addition). WT-FS refers to Waternet-full scale, GR to Groningen, and DU to Dunea. Data represent the mean ± range of samples from two sand cores (n = 2). (A) Isotopic composition, expressed in δ^{13}C notation relative to VPDB. (B) Calculated amounts of excess ^{13}C incorporated into the TSC pool expressed as concentrations per gram for each depth. (C) Total amounts of excess ^{13}C incorporated into the TSC pool per layer in the 20-cm sand column.

degradability, which likely underestimates the fraction of DOC that is truly bioavailable. Instead, abiotic processes, such as adsorption to filter media or straining within the sand bed, are likely responsible for the immobilization of DOC within the filter [10]. Once DOC is immobilized, it can undergo degradation by bacteria through exoenzymatic activity [27] or by small invertebrates [28], thereby entering the biological carbon cycle. However, this process likely operates on much longer turnover timescales than the water residence time within the filter, suggesting that abiotic mechanisms play a dominant role in the short-term retention of DOC.

Assuming that the majority of DOC is removed in the schmutzdecke, our mass balance calculations suggest that, on an annual timescale, most of the DOC removed is mineralized (Fig. 2C). Consequently, we can assume that the DOC removal rate, calculated as the difference in DOC concentrations between the influent and effluent multiplied by the flow rate, corresponds to the rate of organic carbon mineralization. This suggests that a larger fraction of DOC may ultimately be utilized by the biological community, compared to the smaller AOC fraction, which is assimilated by specific bacterial strains in the tests used to measure AOC (e.g., van der Kooij [21]). If DOC removal is then taken as a measure of the biological activity, the ranking of the sand filters would follow this order: Groningen > Waternet-FS ≈ Waternet-PS > Dunea. However, it is important to note that the DOC retention rates derived from our data, and thus the organic matter mineralization rates, could be overestimated. This overestimation results from the assumption that all DOC accumulation and mineralization occurred within the schmutzdecke, neglecting the potential accumulation and removal of organic carbon in the deeper layers [15,16].

We complemented the calculated organic carbon mineralization rates (Fig. 2C) with estimated DIC production rates in the filters (Fig. 1D). After SSF, DIC increased the most in Groningen, followed by Dunea, Waternet-FS, and Waternet-PS (Fig. 1C). The highest increase in DIC at Groningen aligns with the highest mineralization rates deduced from the organic carbon mass balances conducted in full-scale filters, but the increase in DIC concentrations is not solely due to SSF in this location; it is also influenced by aeration and pH adjustment. The larger increase in DIC concentration at Dunea compared to the two Waternet filters contrasts with the calculated organic carbon mineralization rates at these locations as the carbon mineralization rates in Waternet-PS appeared to be higher than in Dunea (Fig. 2C).

Given that DIC concentrations are approximately an order of magnitude higher than DOC concentrations, confidently deducing organic matter mineralization rates in SSF based on changes in DIC is challenging, as the relative increase in DIC is considerably smaller compared to the relative decrease in DOC. Therefore, obtaining reliable, complementary information on organic matter mineralization rates in the investigated filters based on the currently available data is not feasible. To improve the reliability of using DIC as an indicator of organic matter mineralization, more sensitive analytical techniques (e.g., Su et al. [29]) should be employed to capture subtle changes in DIC concentrations.

4.2. Approach based on natural isotopic composition

During organic matter mineralization, the conversion of DOC into DIC is expected to lower the δ^{13}C of DIC because DOC is more depleted in ^{13}C than DIC (Fig. 1E–F). Additionally, kinetic isotope fractionation during mineralization preferentially releases ^{12}C into the DIC pool, resulting in isotopically lighter DIC (lower δ^{13}C-DIC) and isotopically heavier DOC (higher δ^{13}C-DOC) [30–32]. These types of isotope effects have been well utilized to study carbon dynamics in aquatic systems [19,33]. Our data demonstrated that the differences between the influent and effluent with respect to the isotopic composition of the DIC and DOC pools are measurable, opening the possibility to use natural δ^{13}C data to systematically study carbon dynamics in slow sand filters.

δ^{13}C-DOC was consistently higher in the effluent compared to the influent across all sites except for Dunea, where a slight decrease was observed (Fig. 1E). Conversely, δ^{13}C-DIC was lower in the effluent at all locations (Fig. 1F). The most pronounced isotopic shifts were observed at Waternet and Groningen, while at Dunea, the differences were minimal. These findings suggest measurable differences among the studied SSF sites and highlight site-specific biological activity. Furthermore, they support the conclusions based on measured carbon concentrations and mass balances: filters at Groningen and Waternet exhibit higher biological activity in terms of DOC uptake and organic matter mineralization compared to those at Dunea.

A key limitation of this approach is the necessity of a knowledge of the isotopic fractionation factors in order to accurately derive the rates of carbon uptake and mineralization (i.e., to link the rates of DOC uptake and mineralization with the differences in δ^{13}C-DOC and δ^{13}C-DIC). However, the isotopic signature of natural organic matter is heterogeneous [34], which means that the observed differences in the isotopic composition could also be influenced by the preferential degradation of different compounds, each with its own isotopic composition and specific fractionation factor. Therefore, dedicated studies on the effect of dissolved and solid organic carbon mineralization on carbon isotope signatures in slow sand filters would be necessary to utilize the shift in δ^{13}C of dissolved organic and inorganic carbon for making quantitative statements.

Information on the effect of organic carbon adsorption and mineralization on shifts in δ^{13}C values would also enable the use of the isotopic signature of the TSC pool in combination with those of DOC to infer the extent of mineralization of retained DOC over the operational lifetime of the filter. That is, the current δ^{13}C-TSC reflects the δ^{13}C inherited from the initial sand isotopic signature, the δ^{13}C of retained DOC (assuming negligible inorganic carbon precipitation in the filters), and the shift in isotopic signature due to kinetic isotope fractionation effects upon mineralization. The latter is expected to have an even more pronounced effect, according to the Rayleigh distillation model, when only a small fraction of the original organic matter remains [35]. The vertical gradients in δ^{13}C-TSC and measurable differences between the filters, therefore, highlight the potential of δ^{13}C-TSC analyses as a tool for inferring organic carbon retention and mineralization processes in SSF. However, without knowledge of the initial sand isotopic signature and the relevant fractionation factors, the extent of retained DOC mineralization based on δ^{13}C-TSC cannot currently be determined.

4.3. Approach based on an isotope tracer experiment

To address some of the limitations of the approaches discussed in Sections 4.1 and 4.2, we employed isotope tracer experiments for a more direct assessment of organic carbon uptake and mineralization in SSF. For these experiments, we used ^{13}C-glucose, a monomeric carbohydrate that is readily utilized by microbial communities [36]. While we acknowledge that glucose does not represent the full spectrum of organic matter typically found in natural waters, and that its removal rate does not fully reflect the removal dynamics of all bioavailable organic compounds in the SSF influent due to variability in organic matter bioreactivity [37], glucose can nevertheless serve as a useful proxy for assessing the biological community's capacity for organic carbon removal. This approach allows us to assess the potential of the microbial community to process easily degradable carbon and, by tracing the fate of glucose-derived ^{13}C, gain insights into the mechanisms driving the transformation of organic carbon in slow sand filters [16].

In all slow sand filters investigated here, glucose was effectively removed via biological processes. This is evident from the strong correlation between glucose removal (Fig. 3A) and the production of ^{13}C-DIC (Supplementary Fig. S3) in the initial hours following label addition, which confirms the direct coupling between the biological uptake of glucose and its mineralization. The biological community's response to glucose availability varied among the filters. The glucose uptake rate was highest in Waternet-FS, followed by Groningen, Waternet-PS, and Dunea, while the excess ^{13}C-DIC production rates were highest in Waternet-PS, followed by Waternet-FS, Groningen, and Dunea (Fig. 3C).

The contrast between Dunea and the other filters is most apparent from the detection of glucose for up to two days after addition, whereas glucose was quantitatively removed from the overlying water within 24 h for the other locations. These results corroborate the lower biological activity in the filter at Dunea observed with the carbon concentration and natural isotopic composition approaches discussed above. The contrast in mineralization efficiency (Fig. 3C) between the Waternet-PS filter and all other filters indicates a reduced metabolic effectiveness in promoting long-term carbon retention at Waternet-PS. This is evidenced by the greater proportion of glucose being mineralized, rather than assimilated or utilized for other biological processes, following uptake.

The TSC pool, which includes bacteria and other microorganisms within the sand, served as an indicator of label incorporation by the biological community. Isotopic analysis of the TSC provided information about the extent and vertical variability of glucose-derived ^{13}C incorporation into the solid phase (Fig. 4). The schmutzdecke showed the highest excess ^{13}C concentrations across all locations, surpassing the underlying layers (Fig. 4B). This reflects the higher microbial activity in terms of glucose uptake in the schmutzdecke compared to the underlying sand bed. The contrast in excess ^{13}C concentration between the schmutzdecke and the underlying sand column was most pronounced at Dunea. At Dunea, the schmutzdecke accounted for around 45 % of glucose incorporation into the TSC pool within the 20-cm sand column, compared to approximately 15–20 % in Groningen and Waternet. This suggests that the capacity for easily assimilable carbon removal is more concentrated in the schmutzdecke at Dunea than at the other sites. Overall, the underlying sand bed accounted for 55–80 % of glucose incorporation into the TSC pool at all locations, confirming that carbon removal is not limited to the schmutzdecke but also extends to deeper layers in SSF [16], with different filters exhibiting varying levels of activity at different sand depths.

The TSC pool was the largest sink for the added ^{13}C-glucose, incorporating approximately 50 %, 65 %, and 75 % of the label in the Waternet-FS, Groningen, and Dunea experiments, respectively (Fig. 4C). When combining the excess ^{13}C in the TSC and DIC pools (Supplementary Fig. S4), where the latter represents the mineralized label, we were able to account for approximately 65 %, 70 %, and 95 % of the added label in these experiments. The larger missing fraction of the label in Waternet-FS and Groningen experiments could be attributed to the loss of ^{13}C via CO_2 exchange with the atmosphere. The use of highly gas-permeable tubing, combined with continuous system circulation, likely facilitated the escape of ^{13}C-labeled CO_2 from the system. This loss of ^{13}C could have led to an underestimation of the ^{13}C incorporated into the DIC pool, ultimately affecting the carbon mass balance by reducing the apparent amount of carbon mineralized. As a result, this may have compromised our ability to accurately quantify carbon mineralization and close the carbon budget in these experiments. In contrast, the use of less permeable tubing in Dunea's experimental setup minimized this loss, allowing for a nearly closed carbon budget. This highlights the effectiveness of the method while emphasizing the importance of carefully selecting tubing to minimize ^{13}C losses.

4.4. Comparison of filters' biological activities and the potential effects of process variables

The different approaches used to study the carbon uptake and mineralization in SSF indicated distinct activity and unique characteristics of the studied filters. The complexity of the systems and methodological limitations made it challenging to rank the filters with certainty in terms of bioactivity through carbon uptake and mineralization. Nevertheless, as the removal of easily degradable organic carbon is a crucial function of SSF, AOC removal rates, shifts in δ^{13}C-DOC (in the full-scale filters), and glucose uptake rates during the tracer experiments were consistent. Thus, they could collectively provide an assessment of biological activity in view of the removal of easily degradable organic compounds from the water. Based on these metrics, the ranking of bioactivity in the studied filters is as follows: Waternet-FS > Groningen > Waternet-PS > Dunea.

The difference in biological capacity to remove easily degradable organic compounds can be attributed to multiple factors, including source water quality, preceding treatment processes, AOC loading, temperature, filter age, schmutzdecke age, and filter cleaning practices. Given the limited number of filters investigated in this study, it is challenging to confidently identify the individual contribution of these factors to biological organic carbon removal. Nevertheless, their potential roles can be discussed in an attempt to distinguish the most important ones.

The capacity of the biological community in SSF to remove easily degradable organic carbon likely reflects an adaptation to the availability of such carbon sources [38]. Consistent with this, the ranking of full-scale filters in terms of AOC and glucose removal rates follows the trend observed in AOC loading rates (Fig. 5). In particular, the reduced supply of biodegradable organic carbon (or AOC) to the Dunea filters is likely due to the water's approximately two-month residence time during dune infiltration, which occurs prior to its entry into the treatment plant. As the infiltrated water travels through dune sand over an extended period, it undergoes natural filtration processes, including carbon assimilation, that reduce AOC and gradually shape the microbial community toward a composition more similar to that found in the SSF effluent [13]. Studies have reported that drinking water from dune-infiltration systems exhibited greater biological stability compared to water treated directly from surface sources [3,13]. Interestingly, the ranking of filters based on the removal rates of AOC and glucose does not align with the loading or removal rates of DOC. DOC removal is higher for the Groningen filter compared to Waternet, whereas the opposite is true for AOC and glucose removal. This suggests that the turnover of total organic matter may be less important than the availability of easily degradable compounds in determining the efficiency of the biological community in slow sand filters for removing these compounds.

AOC concentrations in SSF influent are influenced not only by the source water composition but also by the preceding treatment processes. For instance, the higher AOC levels at Waternet are likely a result of ozonation, which breaks down larger organic molecules into smaller,

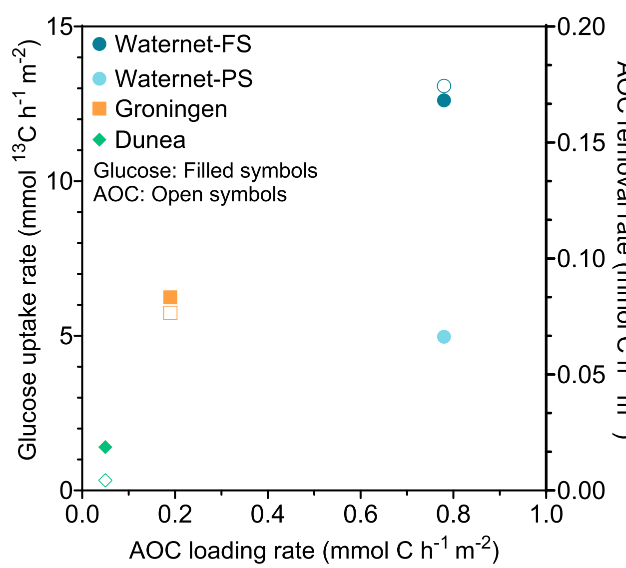

Fig. 5. Correlation between AOC loading and carbon removal rates across the studied slow sand filters. Filled symbols indicate glucose uptake rates (based on Fig. 3C), while open symbols represent AOC removal rates (from Fig. 1D). *Note:* AOC removal rate data for Waternet-PS is unavailable.

more biodegradable compounds, thus enhancing microbial community growth in SSF [39,40]. Research conducted at the same Waternet site [41] found that ozonation led to an increase in AOC by approximately 1120 %. Ozonation can also impact the microbial community structure and metabolic activity in SSF. Rosenqvist et al. [42] reported that ozonation as a pretreatment reduced microbial diversity in slow sand filters and favored communities adapted to metabolize low-molecular-weight compounds generated from ozonated organic matter. This may also explain the improved glucose uptake observed in the slow sand filters at Waternet, where glucose was more readily utilized than at the other sites. In contrast, other pretreatment methods may have different effects on AOC levels; for example, UV treatment, applied at Groningen, has been found to reduce AOC levels in some studies [43], while other studies suggest that UV treatment does not significantly affect AOC concentrations but enhances the low molecular fraction of DOC [44]. Overall, our findings that biological activity in SSF is influenced by AOC loading, which is governed by source water characteristics and preceding treatment steps, are further supported by a recent study by Attiani et al. [13]. In their investigation of slow sand filters from the same water production sites as in this study, they highlighted that the prokaryotic community in slow sand filters is influenced by the interplay of source water characteristics, pretreatment processes, and influent biological stability.

In addition to the higher AOC concentrations, the elevated biological activity observed at Waternet, particularly in terms of glucose uptake, can also be attributed to the higher incubation temperature. In this study, the sand columns collected from Waternet filters were incubated at 18 °C, which was 6 °C higher than the temperatures of the other sites, reflecting the in situ temperature of the filter at the time of column collection (Table 1). This elevated temperature likely contributed to the higher removal rates of easily degradable organic carbon observed in the Waternet experiments. Warmer conditions are known to enhance microbial activity by accelerating metabolic and reproduction rates [45], which in turn facilitates the more efficient breakdown and removal of organic matter [46]. It is important to note that the temperature in all filters fluctuates seasonally, and the temperature differences observed in the experiments are a result of sampling the filters during different seasons. To mitigate potential seasonal effects, future studies should aim to sample filters during the same period and incubate the sand cores at the same temperature. However, this was not possible in the current study due to the timing of column retrieval, which coincided with the scheduled cleaning of the filters.

Other operational parameters, such as filter age, schmutzdecke age, and cleaning procedures, may also contribute to the observed differences in biological activity. The influence of these factors can be assessed by comparing the full-scale and pilot-scale filters at Waternet, as they theoretically share the same source water quality and were incubated at similar temperatures. Specifically, the different responses of the biological communities in the two filters to glucose addition and its mineralization may be attributed to age differences; the Waternet-FS filter had been in operation for 18 years, whereas the pilot-scale filter had been operating for only 3.5 years before sampling. The longer operational history of Waternet-FS, along with the other full-scale filters, may have allowed the biological community to evolve over time, potentially leading to an increased capacity for organic carbon consumption with greater metabolic diversity and efficiency. Supporting this, previous studies have identified filter age as a key factor influencing bacterial community structure, diversity, and functional capacity in SSF [5]. Furthermore, a study conducted at the same Waternet location observed a distinct variation in microbial community composition between two filters differing in age (12 years vs. less than a year old) [13]. However, the study concluded that the variations between different slow sand filters were more strongly influenced by factors such as location, source water, and pretreatment processes, indicating that filter age alone was not the primary driver of community variation across different slow sand filter sites.

Regarding schmutzdecke age, the schmutzdecke at the Waternet-FS filter was cleaned six months prior to columns collection, whereas the schmutzdecke at the Waternet-PS filter had not been cleaned for three years. Despite this, glucose uptake was higher in the cores from Waternet-FS. This difference is likely attributed to the cleaning method used at Waternet-FS: wet harrowing, as opposed to scraping. Wet harrowing involves lowering the water level to just above the schmutzdecke, stirring the sand to dislodge accumulated solids, and flushing the resulting water to waste. This method preserves metabolically active microorganisms [10], in contrast to scraping, which removes the schmutzdecke entirely. In addition, wet harrowing has been shown to enhance biological activity in the deeper sections of the sand bed [6], potentially contributing to the improved performance observed. The importance of the cleaning method was also noted in a study comparing backwashing and scraping, which revealed significant shifts in bacterial communities. Scraping resulted in more pronounced biomass stratification in the deeper sand layers, leading to faster clogging, whereas backwashing promoted a more even distribution of biomass and slower clogging [14]. These findings highlight the impact of cleaning methods on microbial community structure, which can influence the long-term stability and performance of slow sand filters. As recommended by other studies [13], it is essential for water production sites to assess the effects of cleaning procedures on microbial communities prior to implementation. Adopting cleaning methods that preserve biological activity while effectively removing clogging helps increase the efficiency of carbon removal in SSF.

5. Conclusions

Our study demonstrated that stable isotope analysis is a valuable tool for assessing the biological removal and mineralization of organic matter in slow sand filters. Isotopic analyses, including δ^{13}C-DOC and δ^{13}C-DIC measurements in the influent and effluent of SSF, revealed measurable shifts consistent with patterns expected based on the isotope effects of organic carbon uptake and mineralization. However, quantifying the extent of these biological processes based solely on the carbon concentrations and natural isotopic composition remains challenging. Improving measurement precision and identifying relevant isotope fractionation factors are necessary for a more accurate assessment of the biological activity in SSF based on this approach.

Isotope tracer experiments with ^{13}C-labeled glucose provided complementary information about carbon uptake and mineralization rates in SSF. While glucose uptake itself is not a direct indicator of the filters' performance in situ, comparisons between glucose uptake rates and AOC removal rates yielded consistent trends across the locations studied, with higher glucose uptake corresponding to increased AOC removal rates and lower glucose uptake linked to reduced AOC removal rates. Additionally, these experiments provided information about the spatial variability of biological activity within the filters, showing that glucose incorporation occurred along the entire height of the studied sand columns (20 cm) with varying levels of activity observed among the sand layers at each of the sites. These findings demonstrate that isotope tracer experiments can reflect the removal of easily degradable organic compounds, making them a valuable tool for comparing filters and monitoring their biological activity over time. While the use of glucose as a tracer provided valuable insight into the microbial community's capacity to process labile carbon, incorporating additional representative compounds present in SSF influent (e.g., amino acids, fatty acids, or polysaccharides) could further strengthen this approach. Such studies would enable a more comprehensive assessment under conditions that better reflect those in operational full-scale slow sand filters.

Variability in the biological communities' ability to remove easily degradable organic carbon across the investigated filters was primarily driven by AOC loading, which is influenced by both the source water and upstream treatment processes. Pretreatment with ozonation increased AOC loading and led to higher biological activity in filters receiving ozonated influent water. In contrast, source water undergoing dune infiltration resulted in lower AOC loading and reduced biological activity in the corresponding slow sand filter. In this filter, bioactivity was more concentrated in the top two centimeters of the filter, unlike in the other filters, where the contrast between the different depths was less pronounced. This suggests that filters with low AOC loading may rely more on the schmutzdecke layer for carbon removal.

Operational parameters also influenced the biological activity in SSF. Specifically, filter cleaning with wet harrowing resulted in higher levels of biological activity compared to scraping. This suggests that wet harrowing is a more effective method for preserving and enhancing biological activity in slow sand filters, thereby contributing to the overall performance.

To better understand the variability in biological activity across different slow sand filters, future studies should incorporate analysis methods such as phospholipid fatty acids (PLFAs) analysis to track ^{13}C incorporation into microbial biomass to elucidate the abiotic and biotic contribution to ^{13}C retention. This approach would provide detailed information on the spatial and temporal dynamics of microbial communities. It could also clarify how operational factors, including AOC loading and pretreatment methods, directly impact microbial activity and carbon removal efficiency. Furthermore, expanding the dataset by sampling a broader range of filters, especially under more controlled conditions (e.g., during similar seasons), would improve our ability to identify the effects of process variables on microbial activity in slow sand filters.

CRediT authorship contribution statement

Bayan Khojah: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Salima Sadeghi:** Methodology, Investigation, Conceptualization. **Lubos Polerecky:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Jack J. Middelburg:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Dick van Oevelen:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Marcel T.J. van der Meer:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Thilo Behrends:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Thilo Behrends reports financial support was provided by Dutch Research Council. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jwpe.2025.108396.

Data availability

Data will be made available on request.

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