Investigating the community composition and role of meiofauna in carbon cycling in slow sand filters

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Abstract

In the west of the Netherlands, drinking water production includes water infusion into the dunes, followed by multiple filtering and treatment steps such as activated charcoal and rapid sand filters. Slow sand filtration forms the last step and is designed to remove the majority of the remaining organic matter, viruses and bacteria from the influent water. Both physiochemical and biological mechanisms are involved in this. Over time, accumulation of detritus in the top layer (Schmutzdecke) leads to clogging of the filters and requires scraping off which in turn disrupts the bio-active processes in the filter. The microbial community, meiofauna and food web structure that largely make up the bio-active layer had not previously been determined in our sampling locations and have received little to no attention in the scientific community in general. Biological activity is conceived to be important for the performance of slow sand filters. However, it is still enigmatic how the microbial activity is linked to the cleaning function of the SSFs. This thesis explores the meiofaunal community and their potential role in the slow sand filter food web. Samples taken from Waternet (Amsterdam area) contained nematodes (bulk $\delta^{13}C$ = -25.46 ± 0.26 ‰), oligochaetes (bulk $\delta^{13}C$ = -25.48 ± 0.053 ‰), harpacticoid copepods (bulk $\delta^{13}C$ = -25.62 ‰) and mites (bulk $\delta^{13}C$ = -25.91 ‰). The total meiofaunal density in the upper layer was 9 individuals per cm3, a value that is lower than most natural freshwater systems. The comparable $\delta^{13}C$ values of all meiofaunal taxa suggests a similar food source (competition) and/or predation on the meiofauna. Overall, meiofauna may have a limited direct effect on microbial biomass, but may promote microbial production via grazing, enzyme sharing, defecation and other indirect effects.

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waternet dunea DUIN & WATER NWO Netherlands Organisation for Scientific Research

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1. Introduction

The production of drinking water typically involves many steps which vary per production site, depending on the water inlet sources and their quality. In the Netherlands, many water companies use slow sand filters (SSF) in the final purification step within drinking water production after which water is ready for distribution to consumers.

1.1 Slow sand filters

The slow sand beds consist of rectangular compartments (Huisman & Wood, 1974) with a layer of fine sand (0.15-0.3 mm, Visscher, 1990) deposited on top of a graded gravel layer (see figure 1). The overlying space is filled with water and this overhead volume provides pressure needed for the percolation of water through the sand bed over a span of some hours. Valves in the compartments are used to control the flow velocity, overhead water level and drainage. But flow is influenced by many other factors, such as sand grain size, sand bed depth, height of the overhead water and ripening of the filters (e.g., Huisman and Wood, 1974; Bellamy et al., 1985; Langenbach et al., 2010).

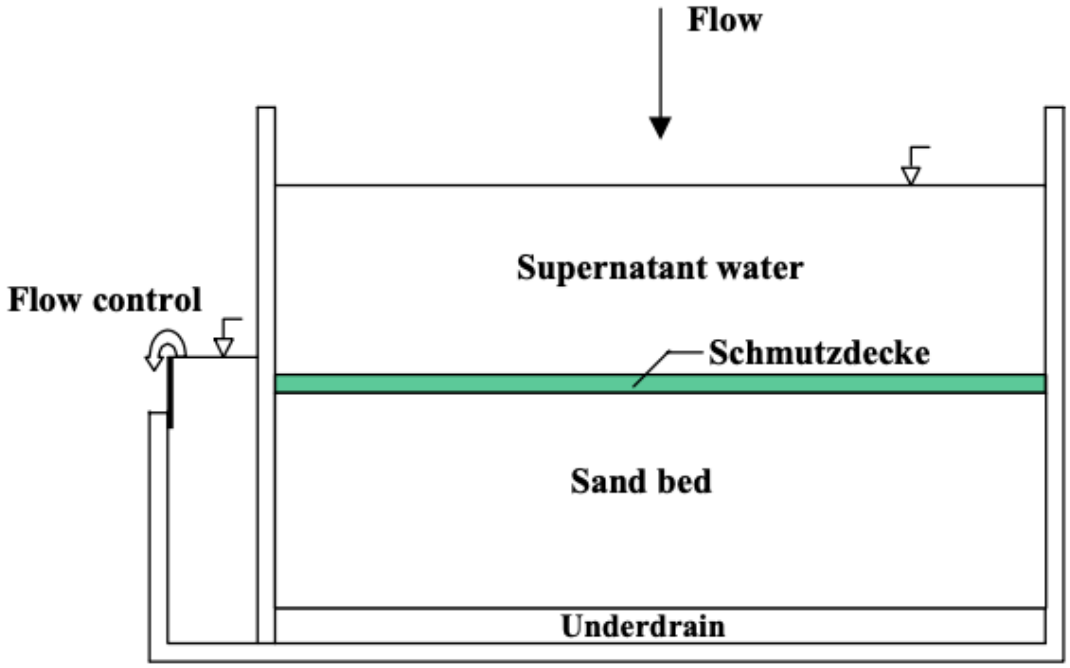


Figure 1. Slow sand filter schematic representation adapted from Huisman & Wood (1974).

As the overlying water passes through the sand bed, colloids, particulate and dissolved organic matter (Collins et al., 1992) and potentially harmful microorganisms, Giardia cysts and parasites are filtered out (Fogel et al., 1993; Verma et al., 2017). Physio-chemical and biological removal mechanisms act concurrently in slow sand filters (Amy et al., 2006). Various researchers have demonstrated the effective removal of pathogenic *E. coli* bacteria, viruses and parasites (e.g. Fogel et al., 1993; Hijnen et al., 2004, Schijven et al., 2014) with particle removal efficiencies in the range 99 % to 99.99 % for biologically mature slow sand filters (Bellamy et al., 1985). There is less consensus on the contribution of the main removal (biological and physiochemical) mechanisms. Starting with physiochemical removal, large particles are retained in the filters when they exceed the size of the pore space (straining) and dissolved compounds may adsorb onto the sand grains (Ives, 1970; Huisman and Wood, 1974). These kind of removal mechanisms may be especially important for removal of particles in the 0.75-10 μm size range (Weber-Shirk & Dick, 1997).

Bioactivity in the form of predation, scavenging, natural death/inactivation and metabolic break down are proposed as the main particle removal mechanisms in SSFs by Haarhoff and Cleasby (1991) and Verma et al. (2017). The number of E. coli bacteria in the filtered water was inversely related to the

number of flagellate and ciliate in a scraped SSF, highlighting the importance of bioactivity in removal (Richards, 1974). Lloyd (1996) on the other hand deemed adsorption onto positively charged surface the primary removal process, but still ascribed an important role to grazing by interstitial fauna, particularly ciliates. The importance of bioactivity was confirmed by Weber-Shirk and Dick (1997) in whose study E. coli and small particles removal decreased after of sodium azide addition to the SSF to inhibit bioactivity.

1.2 Bioactivity

The long retention time of water allows a substantial biological community to build up on top of the sand, the 'schmutzdecke' (Guchi, 2015; Ranjan et al., 2018). This layer is made largely of retained suspended particles, colloids, both organic and inorganic, and a wide range of microorganisms and their secretions (e.g., Ellis et al., 1985; Hendricks, 1991; Ranjan et al., 2018). Over time, the schmutzdecke grows and matures (ripening), increasing particle degradation through bioactivity and particle retention by straining (Bellamy et al., 1985), which fuels further bioactivity (Hendricks, 1991).

Yet, as the schmutzdecke ripens, the filter starts to clog, preventing sufficiently effective percolation of the water (or: increasing hydraulic resistance; Lodge, 1979; Hendricks, 1991). At the point of final headloss, when opening the outlet valve to the maximum does not suffice to reach the desired outflow rate, the schmutzdecke must be scraped followed by a two-month recovery period to allow biological activity to resume (T. Behrends, personal communication, September 2021). But the microbial and meiofaunal communities in SSFs and their functions are largely unknown and by extension, the impact of scraping and the recovery period remain unknown. Since the bioactivity in the SSF seems to contribute to SSF performance in the sense of particle removal efficiency, but is also involved in the eventual SSF headloss and required maintenance, it is important to understand which organisms actually inhabit the SSF, how they relate to one another and what functional roles they play. The remainder of this thesis will look into the meiofaunal community and their (potential) roles in SSFs.

1.3 Meiofauna

Meiofauna are defined as all metazoans passing through a 1 mm but retained on a 42 μm sieve (Higgins & Thiel, 1988), and occurring in all types of sediments, in the sea or in ice, as epiphytes on seagrass or algae, associated with animals and free-living (Vincx, 1996). Majdi et al. (2020) acknowledges 23 taxa as meiofauna, the most common of which in freshwater systems are nematodes, rotifers, oligochaetes, polychaetes, micro-crustaceans, tardigrades, gastrotrichs, chironomids and microturbellarians.

Different size classes and by extension different definitions of meiofauna occur in scientific literature, mostly tied to the sieve mesh size(s) used in research. For instance, Vincx (1996) defines meiofauna as metazoans retained on a sieve of 42 μm, while Majdi et al. (2020) use an upper limit of 1 mm. Silver et al. (2002) talk about invertebrates ranging from 50-500 μm in size while Higgins and Thiel (1988) define meiofauna as metazoans that pass through a 500 μm sieve but are retained on a 40 μm sieve. In some cases, microzooplankton or protists (in particular rotifers and ciliates) are also included in the definition of meiofauna (e.g. Schmid-Arraya & Schmid, 2000).

Meiofauna are often separated into permanent and temporary meiofauna. Permanent meiofauna complete their entire life cycle within the meiofaunal size class and include taxa such as nematodes and copepods (Traunspurger & Majdi, 2017). Temporary meiofauna grow beyond or have smaller larvae than the defined meiofaunal size range (Schmid-Araya et al., 2020). The temporary meiofauna sometimes includes oligochaetes and mites but this depends on the meiofaunal size range employed. A different way of grouping meiofauna uses functional or substrate distinctions between meiofaunal assemblages. Epibenthic meiofauna colonise microbial mats and contain the largest, swimming species or those secreting adhesive substances (Traunsprunger & Majdi, 2017). Interstitial meiofauna colonise the superficial sediment and are typically small, worm-shaped poor swimmers with adhesive organs.

Together, meiofauna consume a wide spectrum of food sources, including detritus, fungi, algae, bacteria, plants, protozoans and other meiofauna (e.g. Schmid-Araya & Schmid 2000; Traunsprunger & Majdi, 2017; Majdi et al. 2020). At the species level, meiofauna often have specialised diets (Carman & Fry, 2002; Moens et al., 2006) and changes in feeding strategies have been observed depending on (seasonal) availability of food sources (Majdi et al., 2012). Despite distinct food preferences many meiofaunal species in freshwater systems are also known to feed opportunistically (Giere, 2009). Meiofauna themselves serve as prey for macrofaunal organisms, e.g. chironomid larvae and flatworms (Ptatscheck et al. 2020).

Benthic meiofaunal communities vary widely in morphology, behavioural patterns, life history traits and functional feeding habits (Majdi, et al., 2020). Meiofaunal assemblages form as a result of a continuous loss, recruitment and colonization via the water column (Palmer, 1988; Giere, 2009). On the cm-m scale, dispersal also depends on meiofaunal migration (Palmer, 1992). Precise populations are influenced by biotic and abiotic factors (Traunsprunger & Majdi, 2017). The large-scale meiobenthic community composition is mostly related to physical and chemical parameters (Higgins & Thiel, 1988), including grain size, water flow temperature and pH (Rundle & Ramsay, 1997). Small-scale distribution at the centimetre scale is mostly governed by organic matter (OM) content (Swan & Palmer, 2000) and bioactivity in the form of predation, bioturbation or competition (Schratzberger & Somerfield, 2020), although oxygenation and interstitial flow are vital too (Traunsprunger & Majdi, 2017). Once meiofauna reach a habitat, a ‘notoriously patchy and unpredictably variable’ spatial distribution tends to form in freshwater systems, in relation to the availability of food sources (aggregations of microorganisms, OM contents, etc.; Silver et al., 2002). Therefore, meiofaunal abundances tend to follow the organic content of the sediments. Similarly, meiofaunal assemblages vary vertically following the availability of specific food sources in different sediment layers (Ingels et al., 2011). The upper few centimetres are richer in oxygen and food sources and usually harbour more meiofauna than the deeper layers, although deeper meiofaunal colonisation does regularly occur (Schmid-Araya, 1997) and can be diurnal or seasonal (Palmer 1990; Stead et al, 2005).

The short population turnover rates, in the order of weeks and months generally, and rapid colonisation of meiofauna enable the community to respond quickly to disturbances (Majdi, et al., 2020). Some meiofauna can enter into dormant stages, making their community extremely resilient too (Traunsprunger & Majdi, 2017). However, recovery can result in community composition changes and by extension, changes in bioactivity. In this context, species richness becomes important. The more diverse the community, the more functional overlap and the less chances of substantial alteration of the ecosystem processes due to the loss of a species (Schratzberger & Ingels, 2018).

The fast growth and turnover rates of meiofauna (high production values with limited standing stock) can make them energetically important compartments to consider in ecosystem energy budgets and (Traunsprunger & Majdi, 2017). But limited quantitative information on meiofaunal biomass and production is available for many freshwater systems and may be vastly different in slow sand filters. More evidence is accumulating on the role of meiofauna as ecosystem structuring and regulating agents (Schmid-Araya et al., 2016). Meiofaunal grazing directly affects the bacterial and detrital stocks and composition. Bioturbation, sediment reworking, bioirrigation, and mucilage secretion modify physical, chemical and biological sedimentary processes both directly and indirectly at various spatial and temporal scales (Giere, 2009; Schratzberger & Ingels, 2018). For instance, in an average sandy sediment, burrowing by meiofauna will completely displace pore water in 1–3 years (Reichelt 1991). Because of meiofaunal bioturbation the transport of solutes with the subsequent stimulation of microbial mineralization can increase up to threefold compared to molecular diffusion (Berg et al. 2001). It remains to be seen if meiofauana are capable of the same ecosystem functions in slow sand filters.

1.4 Aims and outlook

Research into meiofaunal contribution to energy flow and their indirect importance for communities due to habitat engineering and grazing control point to the (indirect) importance of meiofauna in sediments. Despite this, meiofaunal remain a marginal research topic and still less is known about the occurrence and role of meiofauna in other environments such as the SSF. In general, despite the broad-scale, world-wide application of SSFs, remarkably little is known about the organisms present, their food web structure and dynamics, and the processes by which the microbial community contribute to the water purification. This research will make use of the data collected in March 2021 from the slow sand filters of the water company Dunea and continue the data assembling and interpretation of newly collected samples (Waternet samples) with regards to the meiofauna taxa and abundances. Aside from experimental work, the objective of this thesis is to summarise findings on the functional roles and importance of meiofauna in aquatic environments. First, taxa and distribution of meiofauna in the SSFs will be addressed, then the stable isotope (^{13}C) trophic relations will be discussed. Finally, results will be related to the known roles of meiofauna in freshwater communities to explore the potential benefits and challenges that meiofauna bring to the SSF performance.

Ultimately, the results of these experiments should allow for an assessment of the role of meiofauna in the food web of the SSFs under investigation. In turn, this knowledge can help to improve sand bed filter functioning, particularly the optimisation of the length of the recovery period after Schmutzdecke scraping. Water production companies have a special interest in shortened recovery periods and/or lengthened SSF operation periods as these reduce the costs and allow for enhanced drinking water production. Finding a way to optimize sand bed filter performance and duration without scraping off the Schmutzdecke is of interest to all water production companies involved.

2 Material and method

2.1-Sampling sites

2.1.1 Dunea

The water inflow into the Dunea drinking water production plant (Monster, the Netherlands) originates from the 'Afgedamde Maas', a branch of the river Meuse, and the river Lek if required (Dunea, 2021). At Brakel, the water is sieved using microfilters and pumped to Bergambacht where the water is treated with rapid sand filters. This water is pumped into the dunes at Meijendel (Scheveningen). As the water percolates the dunes, it mixes with meteoric water and is purified naturally. After an average residence time of two months, the water is pumped back to the production site where it is softened and pre-treated with activated charcoal and oxidized before entering a rapid sand filter and a slow sand filter. See Appendix A for a detailed overview of the production process.

2.1.2 Waternet

About two thirds of the water used in the drinking water production plant of Waternet (Leiduin, the Netherlands) comes from Lekkanaal (Waternet, 2021). The remaining one third of the water comes from the Bethunepolder with water from the Amsterdam-Rijnkanaal as back-up source. The first step in the production is particle coagulation using iron chloride addition to the water followed by rapid sand filtration and infusion into the dunes in the Amsterdamse Waterleidingduinen. The water is pumped back to Leiduin where it is pre-treated with rapid sand filters, oxidised, softened and filtered with activated charcoal. Finally, the water is filtered in slow sand filters. See Appendix B for an overview of the entire production process.

2.2 Sample collection

In March 2021, fifteen cores were collected from a drained slow sand filter at Dunea, using Plexiglas cores with a diameter of 10 cm and a length of 30 cm. In September 2021, fifteen cores were collected from a drained slow sand filter at Waternet. The cores were pushed into the sand up to 20 cm, excavated from the sand, taken out and transported back to the GEOlab at Utrecht University (Utrecht, the Netherlands). SSF influent water was collected on site as well. Both sampling times, twelve cores were filled with SSF influent water and incubated overnight in the climate chamber at ambient temperatures (10 °C for Dunea, 18 °C for Waternet) before starting the labelling experiment. Out of

the remaining three cores (hereafter: meiofaunal cores) one was frozen (-20°C) and the other two were immediately processed (see meiofaunal extraction below).

2.3 Experimental set-up/labelling experiment

The twelve cores from Waternet were paired (six times two cores, G0 to G5) and set up with a pump that circulated the water through the core in a dark climate chamber (18°C). The overlying water in the twelve cores from Dunea, was stirred instead of pumped through the cores and the climate chamber temperature was lower (10 °C). Only the G0 control cores were used in this research. Waternet's G0 cores were left in the climate chamber for 13 days, after which they were sacrificed and meiofauna were extracted.

2.4 Meiofauna extraction

Two cores from Dunea and two meiofauna cores from Waternet were sliced in three parts (0-2 cm, 2-5 cm and 5-10cm; see Figure 2) and immersed in 8% buffered formaldehyde (pH=7) for at least 24 hours. The duplicate G0 cores of both sites were sliced similarly but were not preserved in formaldehyde. From each section (three depths, four cores, therefore twelve in total per sampling campaign), the meiofauna were extracted using density separation in a procedure modified from Higgins and Thiel (1988). First the formaldehyde was washed off with 32µm-filtered tap water over a 38 µm mesh sieve, then centrifuge tubes were filled up to 20 mL with sample. The colloidal silica polymer Levasil was used as flotation medium to prevent plasmolysis (H.C. Stark, Levasil 200/40%, q = 1.17). Three spoons of kaolin were added to 500 mL of Levasil. The kaolin deposits on top of the heavier sand particles, holding them back during decantation. The Levasil/kaolin mixture was used to fill the tubes up to 50 mL. Samples were thoroughly shaken and centrifuged for 5 minutes at 2800 rpm. The supernatant was washed out over a 38 µm sieve with filtered water and the reminder transferred to Petri dishes using a pipette. The extraction was repeated two more times on the same tube. The Petri dishes were sealed with parafilm and stored at -20 °C awaiting further analysis.

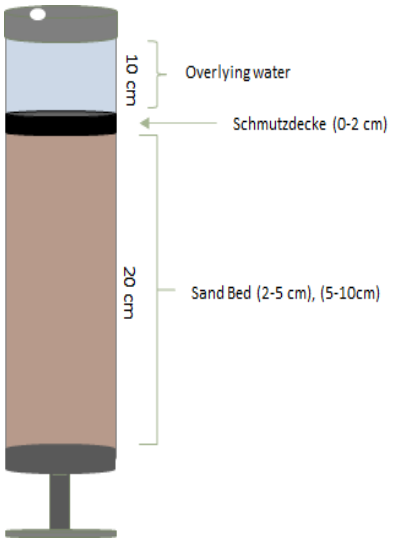


Figure 2. Schematic overview of a SSF core with 10 cm of overlying water, the schmutzdecke at the water-sediment interface and sand in the 20 cm below. The three slices are indicated in the Schmutzdecke (0-2 cm depth) and sand bed (2-5 cm slice and 5-10 cm slice). Figure was obtained from Bayan Khojah (2021).

2.5 Meiofaunal identification and enumeration

A compound microscope (Nikon SMZ800, Nikon Instruments Inc., Tokio, Japan) was used to identify to taxon level and enumerate all mature meiofauna (nematodes, copepods, oligochaetes and mites) at 20x to 40x magnification. Final counts were calculated by extrapolating the counts a part of the layer to the entire layer. While counting, meiofauna were sorted and transferred into small petri dishes containing MilliQ water using a micro-tweezer (Electron Microscopy Sciences, Hatfield, PA, USA) in one petri dish for each taxon of a particular layer in each core (e.g. nematodes in 0-2 cm for Waternet's meiofauna core 1). All samples were stored at -20 °C.

Some meiofauna, especially nematodes, were transferred on glass microscopy slides using a glycerol/filtered water (1:1) solution, followed by evaporation for 2 days at room temperature. Slides were covered with a glass slip and sealed with lacquer. These individuals were photographed using a stereomicroscope (Leica Microsystems GmbH, Wetzlar, Germany). Based on the morphology of their buccal cavities, an attempt was made to classify nematodes into feeding types (deposit feeders, epistrate feeders, suction feeders, chewers; as defined by Traunspurger, 1997).

2.6 Statistical analysis

The similarity between the meiofaunal communities, both horizontally and vertically, were analysed by a one-way analysis of similarity (ANOSIM) with meiofaunal taxa as along the main axis and using the Bray-Curtis dissimilarity index. Horizontal distribution was analysed in terms of the similarity of all layers between the two meiofaunal cores. Vertical distribution was analysed in terms of the similarity of meiofaunal assemblages between different layers of single cores. Meiofaunal assemblages are dissimilar if $p<0.05$. All statistical analyses were performed with PAST software (version 4.09; Øyvind Hammer, Oslo, Norway).

2.7 Bulk isotopic signatures

Meiofaunal taxa (nematodes, oligochaetes, copepods and mites) were transferred into pre-weighted tin cups, using a micro-tweezer. Different tin cups were used for different flow methods. Samples containing about 20 oligochaetes from sorted petri dishes (3 depths, meiofaunal cores 1 & 2 and core G0) were placed in 5x9 mm tin cups (Sercon Ltd, Cheshire, UK). About 25 pre-sorted nematodes were transferred into the cups. One averaged size worm and a small sample of biofilm were also transferred. Due to the limited number of individuals recovered and smaller size (= lower weight), 50 mites pooled from both meiofaunal cores were placed in the smaller tin cup of 4x6 mm (Sercon Ltd, Cheshire, UK) for the more sensitive reduced flow EA-irMS method. A pooled sample of 50 copepods was transferred into small tin cups. Finally, samples of three and five oligochaetes from both meiofaunal cores and G0 (six samples in total) were also placed in tin cups.

All tin cups were dried on a hot plate (40 °C) until their weight stabilised. The final dry weight of the samples was recorded, and the pre-weighed mass of the empty tin cups was subtracted to generate the average weight of each meiofaunal taxa.

Tin cups were placed into an isotope ratio mass spectrometer coupled to a flash elemental analyser (EA-irMS, Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.) to determine bulk $\delta^{13}C$ values and elemental contents (wt % C) of the samples. The delta values represent deviations in the carbon isotope ratio ($R= {}^{13}C/{}^{12}C$) relative to the lab standards Nicotinamide and GQ which have been calibrated against the Vienna Pee Dee Belemnite (VPDB) standard, or in formula: $\delta^{13}C$ (‰) = $(R_{sample}/R_{VPDB} -1) \times 1000$, where R_{VPDB} is 0.0112372. Analytical precision (one standard deviation) was 0.02‰ or $\delta^{13}C$-values calculated from GQ standards with variable weights in the range of the samples and the isotope measurements. The $\delta^{13}C$ values were corrected for ^{17}O interference following Brand et al. (2010), but no corrections were applied for any carbon added through the formaldehyde preservation (see also Majdi et al., 2012). Copepod and mite samples, as well as replicates from oligochaetes were measured with a reduced volume of EA-irMS columns after Carman and Fry (2002) due to sample limitation (see also Schmid-Araya et al., 2016).

2.8 Biomass, secondary production and assimilation

The average dry weight of mature meiofaunal taxa was calculated from the dry weights divided by the number of individuals. The estimated total biomass was calculated by multiplying average dry weights by the total meiofaunal counts. Total carbon (C) values were obtained, by multiplying total biomass by the average %C of each sample as derived from the EA-irMS output. Following Brüchner-Hüttemann (2020) and Majdi et al. (2017), secondary production of every meiofaunal group was calculated using the Plante and Downing (1989) regression formula: $Log_{10} P_y = 0.06 + 0.799 Log_{10} (B) - 0.16 * Log_{10} (M_{max}) + 0.059 T$, where P_y = production (g carbon (C) m^{-2} $year^{-1}$), B = mean biomass (g C m^{-2}), M_{max}- = maximum biomass per taxon (mg C $ind.^{-1}$) and T = mean surface temperature (°C). Yearly values were converted to daily estimated by dividing the Py value by 365 days (see Majdi et al. 2017). The daily production estimates, daily assimilation demands were calculated in mg C, assuming net production efficiencies (NPE = Production⁄Assimilation) of 0.6 for nematodes and 0.4 for other taxa (Smock & Roeding, 1986; Herman & Vranken, 1988).

3. Results

3.1 Meiofauna present

3.1.1 Taxa

In total, four meiofaunal taxa were identified in the SSF sediment cores from Waternet: nematodes (Figure 7a, b, c and d), copepods (Figure 7e), mites (Figure 7c and f) and oligochaetes (Figure 7g and h). Three distinct mouth structures could be recognised among the nematodes, of which the nematode with a stylet (Figure 7a) belongs to the predatory genus Tylenchida (K. Soetaert, 2021, personal communications) that employ a suction feeding strategy. By comparing the pictures (Figure 7) to Figure 3, a large portion of the nematodes in the samples are omnivorous chewers (Figure 3E). The final category of nematode observed (Figure 7d) could be a bacterial feeder or a Tylenchid with a retracted stylet. Many juvenile nematodes were present in the samples (Figure 7c next to the mite) which could not be assigned a feeding strategy based on their mouth structure.

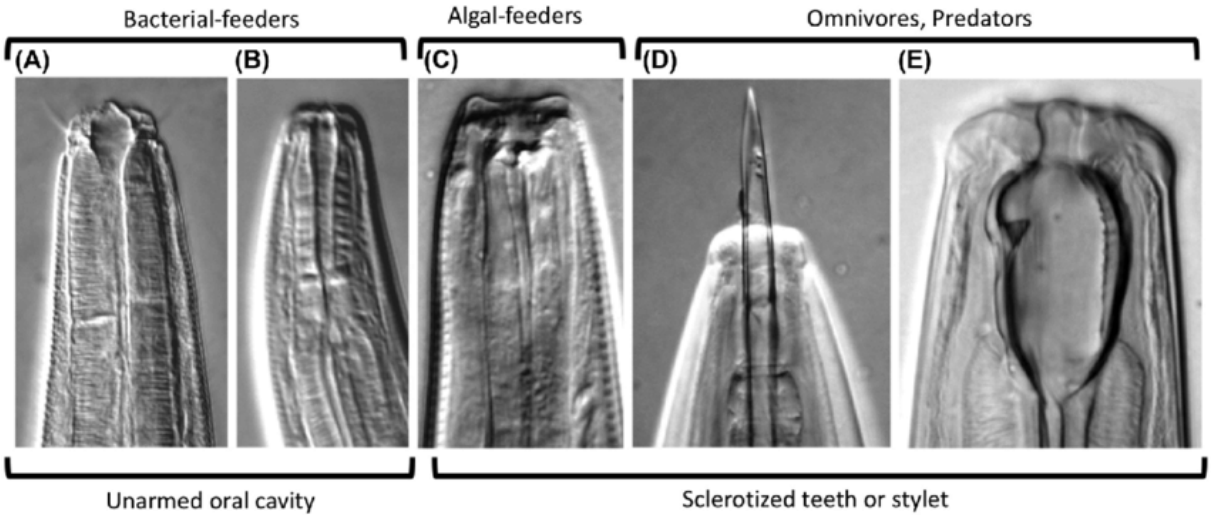


Figure 3. Overview of freshwater nematode-feeding types. (A) and (B) show deposit-feeders with unarmed mouth cavities; (C) Shows an epistrate-feeder with small teeth to crack diatoms; (D) shows a suction feeder with retractable stylet enabling piercing; and (E) shows a chewer with large mouth with teeth. Figure retrieved from Traunspurger (1997).

The copepods in the samples belong to the subgroup harpacticoida, when comparing the sampled copepods (Figure 7e) to Figure 4, especially the picture in figure 4b.

When comparing the oligochaetes (Figure 7g and h to Figure 5, the double, non-forked chaeta (Figure 7h) indicate that, the oligochaetes in the Waternet samples belong to the Enchytraeidae family, the second largest subgroup of oligochaetes (Giere, 2009).

In addition to meiofauna, macrofaunal-sized worms were present in the cores (Figure 6).

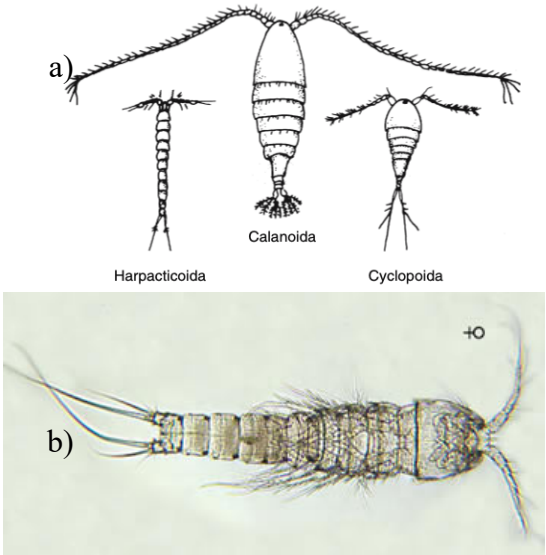


Figure 4.Shoiwing a drawn overview of the three main sub taxa of copepods (4a) and an harpacticoid copepod picture in figure 4b. Retrieved from Giere (2009).

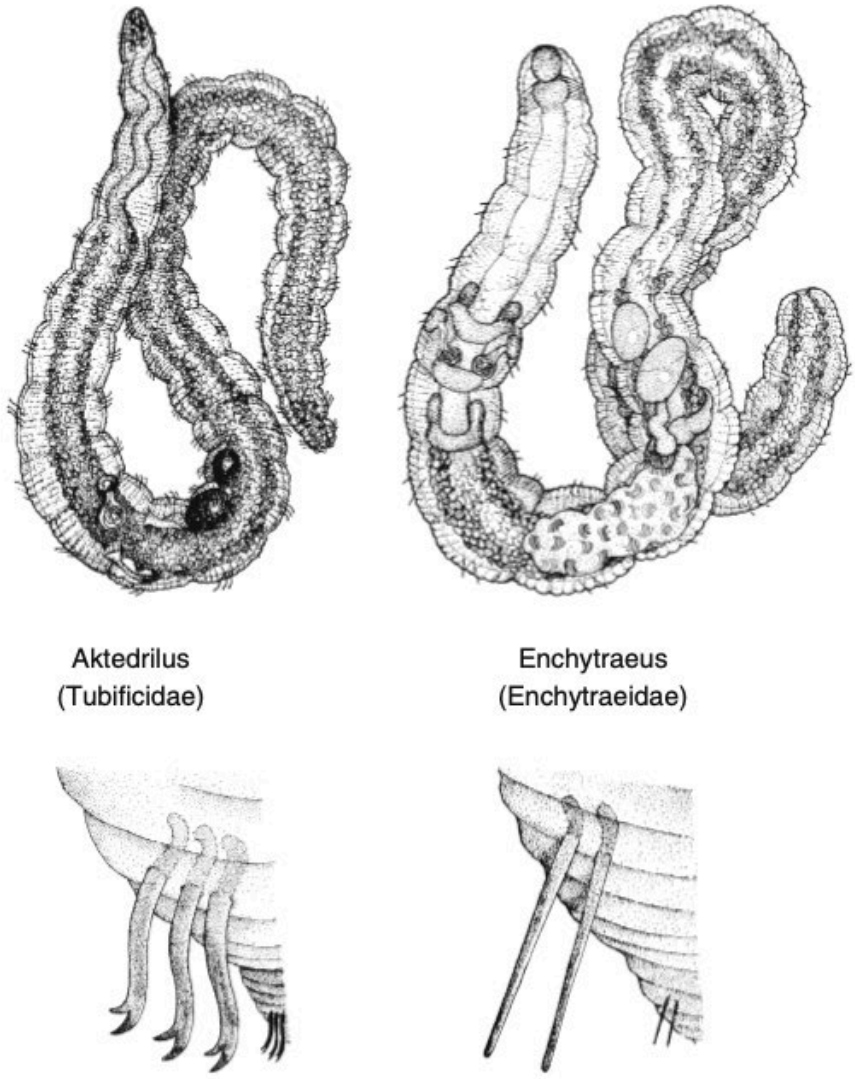


Figure 5. Overview of two main sub taxa of oligochaetes, distinguished based on their chaeta. The left shows the forked chaeta of tubificids while the right shows the straight chaeta of enchytraeids. Retrieved from Giere (2009).

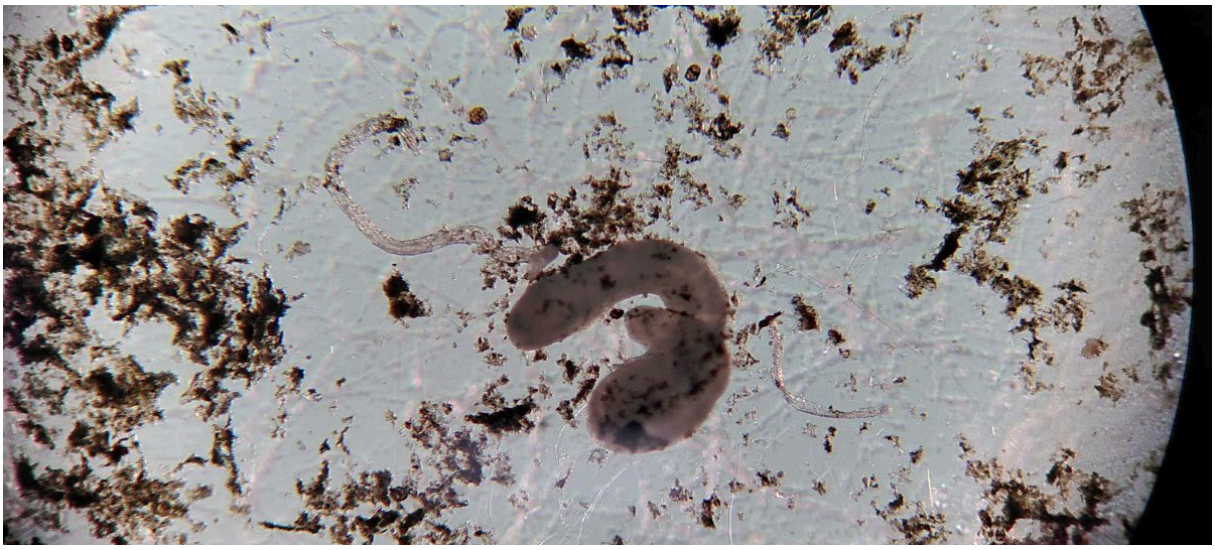


Figure 6. Microscopic picture of a macrofaunal sized worm (dark worm in centre view) and two oligochaetes on either side.

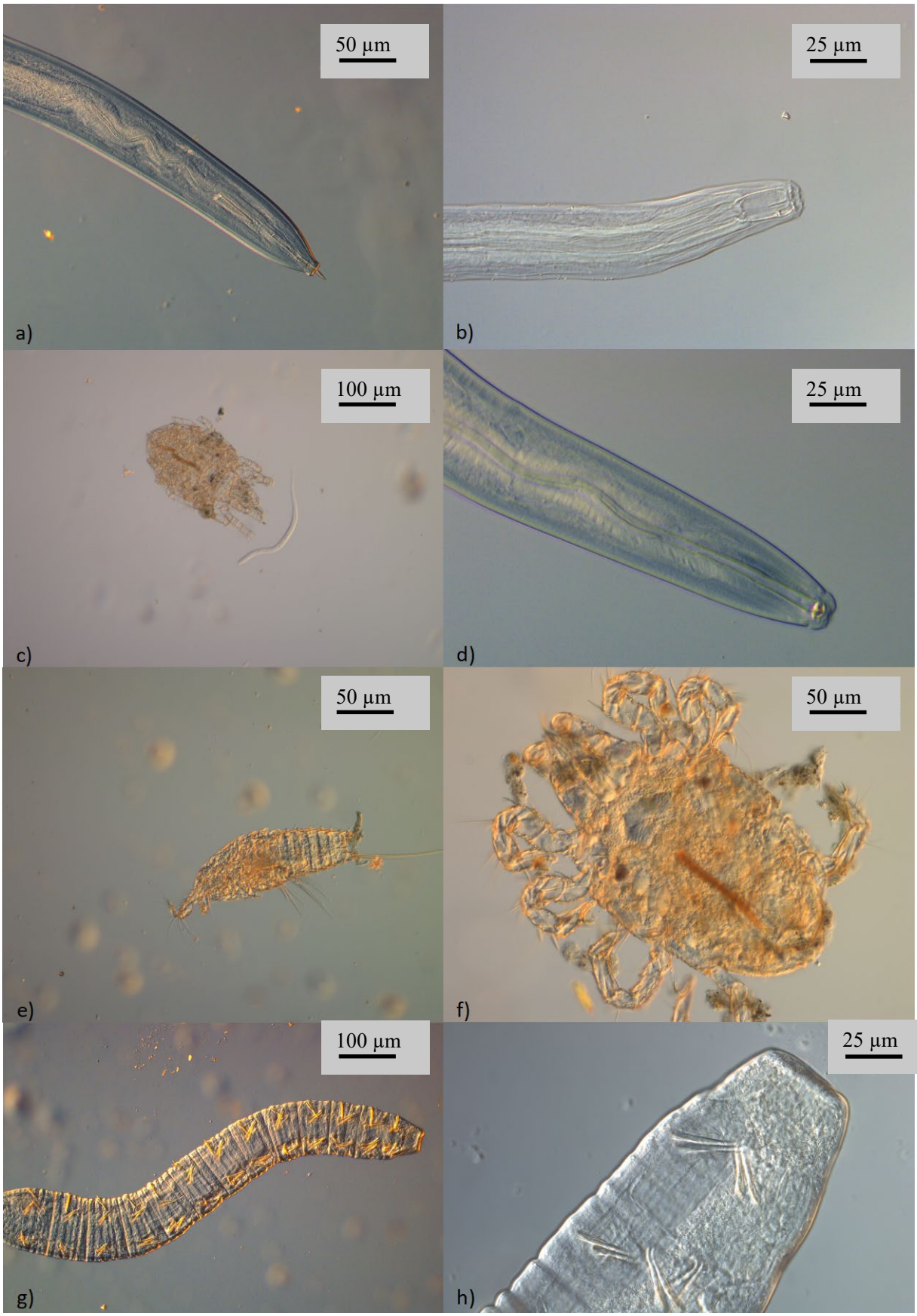


Figure 7. Microscopic images of meiofauna from Waternet's SSF 10-40 times magnified. a), b) and c) al show nematodes with different mouth structures; pictures c) and f) show mites: e) is an image of a copepod and g) and h) show oligochaetes.

3.1.2 Density and distribution

The Schmutzdecke of Waternet's slow sand filters contains the highest meiofaunal count, followed by the 2-5 cm layer and 5-10 cm layer (see Table 1 and Figure 8). The maximum density is nine mature individuals per cm^3, or 685 individuals per 10 cm^2 (over a depth of 10 cm). Oligochaetes numerically outweigh any of the other meiofauna in all layers, except for juvenile nematodes. The general decrease in meiofauna over depth is also seen in samples from Dunea's SSF (see Figure 8; Mishra, S, 2021, unpublished data). Furthermore, densities of copepods and nematodes are similar for the two water companies. Mites occur more frequently in the SSF of Waternet than in Dunea and oligochaetes appear absent in Dunea. Overall, total mature meiofaunal density is higher in Waternet than it is in Dunea, but this is largely due to the oligochaetes, without which densities are comparable.

Table 1

Total meiofaunal counts for two cores taken from Waternet's slow sand filter

	Core 1			Core 2		
	0-2 cm	2-5 cm	5-10 cm	0-2 cm	2-5 cm	5-10 cm
Meiofauna						
Copepod	138	7	0	88	17	10
Mite	133	230	24	131	47	67
Nematode	43	28	32	166	20	35
Nematode (juvenile)	9170	11135	6525	8555	2967	6108
Oligochaete	1328	2117	1303	862	430	1161
Worm	14	0	0	5	7	0

The one-way analysis of similarity showed no statistically significant difference in composition and abundance of mature meiofaunal populations between the two meiofaunal cores ($R = 0.2222$, $p = 0.206$; using total meiofauna estimates). Neither was the meiofaunal composition and abundance in the different layers (the vertical distribution) significantly different at the .05 significance level differ significantly ($R = 0.2222$, $p = 0.2004$; using total meiofauna estimates).

As the possibility that juvenile nematodes were either seriously overestimated (inclusion of silicates similar in size) or underestimated (due to coverage by debris) cannot be excluded, these counts were omitted from the statistical analyses and further calculations.

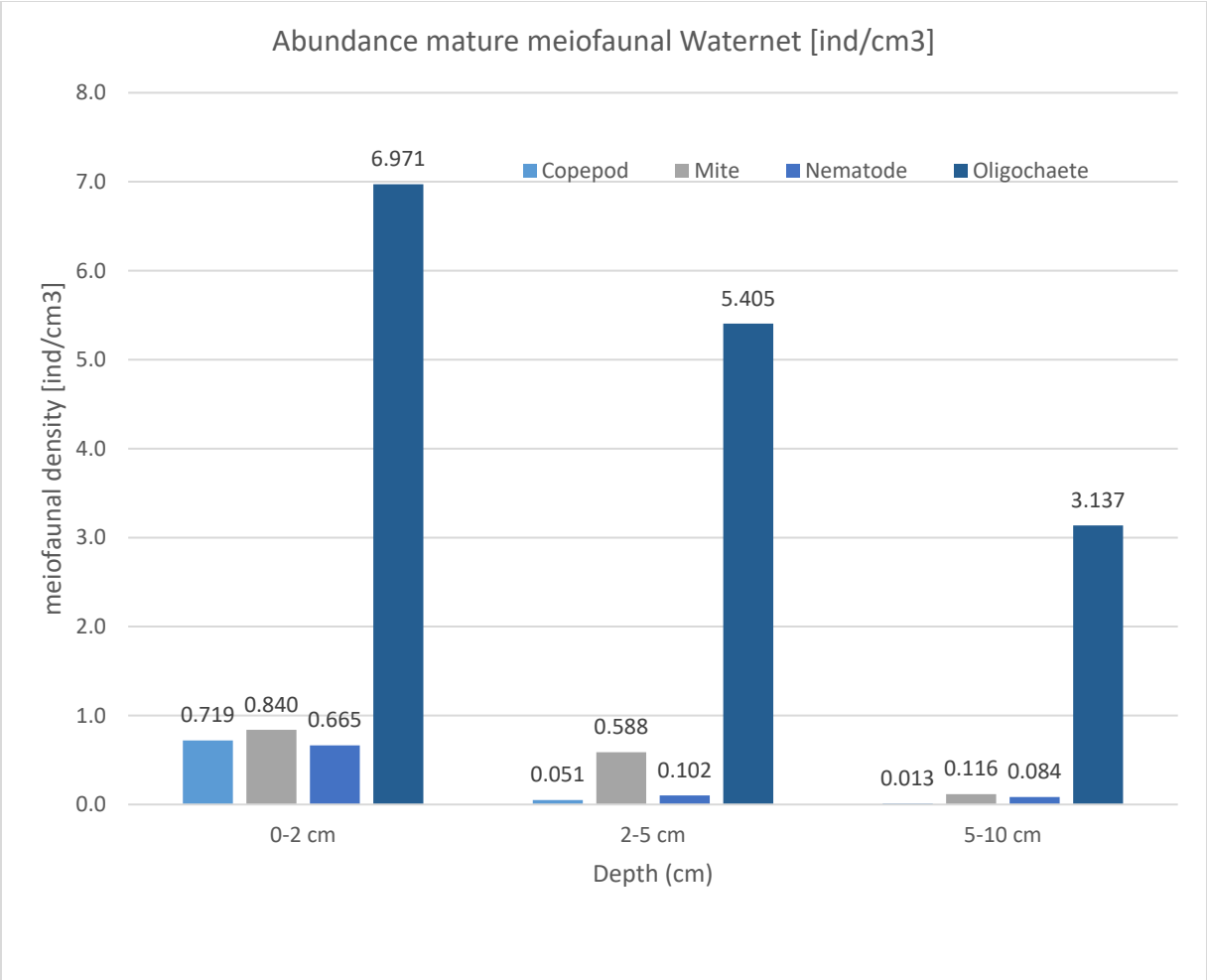
3.2 Bulk isotopic signatures

3.2.1 Bulk ^{13}C signatures

The bulk $\delta^{13}C$ values of all formaldehyde preserved meiofauna (oligochaetes, nematodes, copepods and mites) in Waternet's SSF lie around -25.5 ‰ with the exception of mites which were slightly more depleted ($\delta^{13}C$ = -25.91 ‰; single measurement). Figure 9 shows the bulk $\delta^{13}C$ values: average nematodes bulk $\delta^{13}C$ was -25.46 ± 0.26 ‰, oligochaetes bulk $\delta^{13}C$ was -25.48 ± 0.053 ‰) and (harpacticoid) copepods bulk $\delta^{13}C$ was -25.62 ‰; single measurement). These bulk signatures are in close agreement with the $\delta^{13}C$ values obtained previously for copepods in the schmutzdecke of Dunea, but not the copepods from deeper layers (Mishra, S., 2021, unpublished data).

Due to sample limitation, copepod and mite bulk isotopic signatures in these Waternet samples could not be measured in duplicates, although the signal does represent an average of 50 individuals.

Due to their very small size requiring very large numbers of individuals, combined with the difficulties in picking out and handling juvenile nematodes and time limitation, these juvenile nematodes were excluded from meiofaunal bulk analysis.



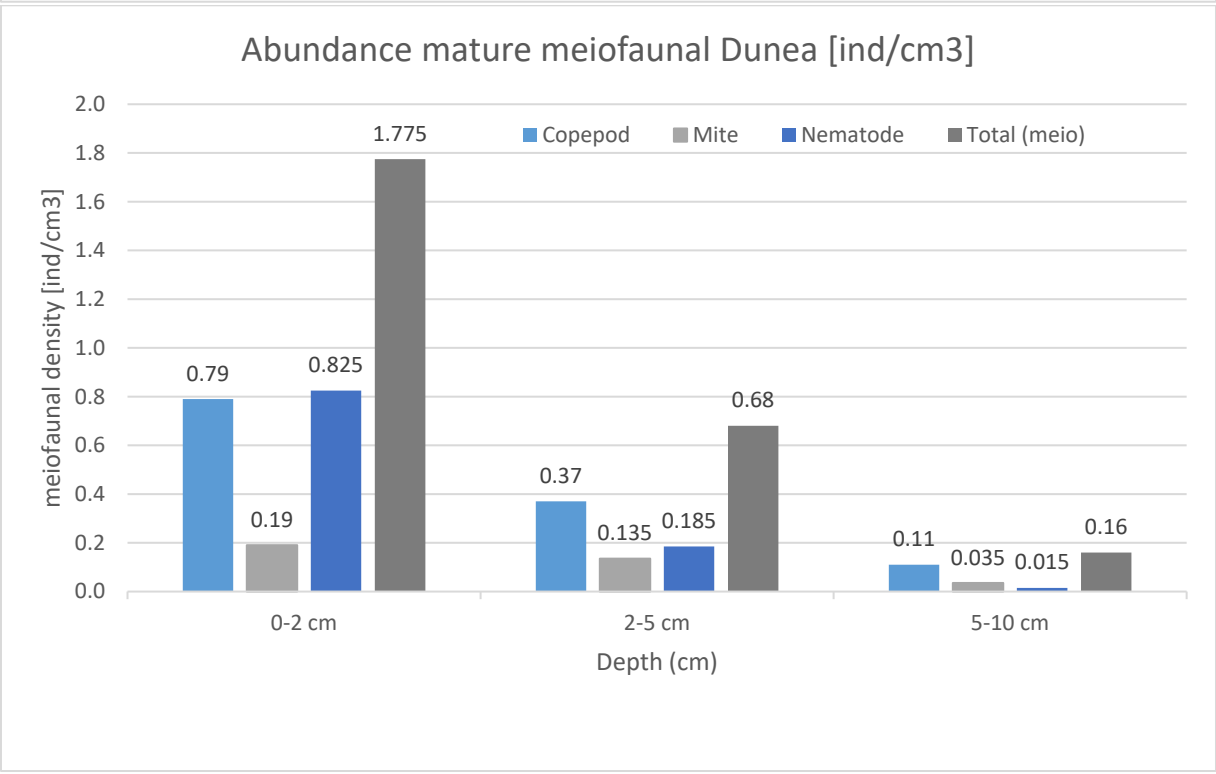


Figure 8. The top figure shows the meiofaunal density per taxon over depth in Waternet's SSF samples while the lower figure shows the densities per taxon and total values for Dunea's SSF. The lower figure (Dunea samples) was adapted from Mishra, S. (2021, unpublished data).

The detritus sample from the Schmutzdecke of core G0 (not preserved in formaldehyde) had a $\delta^{13}C$ value of -26.44 ‰ which is almost 1‰ more depleted than the average preserved meiofaunal bulk signature and 1.5 ‰ more depleted than the unpreserved nematode value. Compared to the trophic shift of +0.5‰ for $\delta^{13}C$ proposed by McCutchan et al. (2003) and even the higher shift of +0.8 ‰ proposed by DeNiro & Epstein (1978), meiofaunal bulk carbon signatures are more enriched than the detritus plus trophic shift would be.

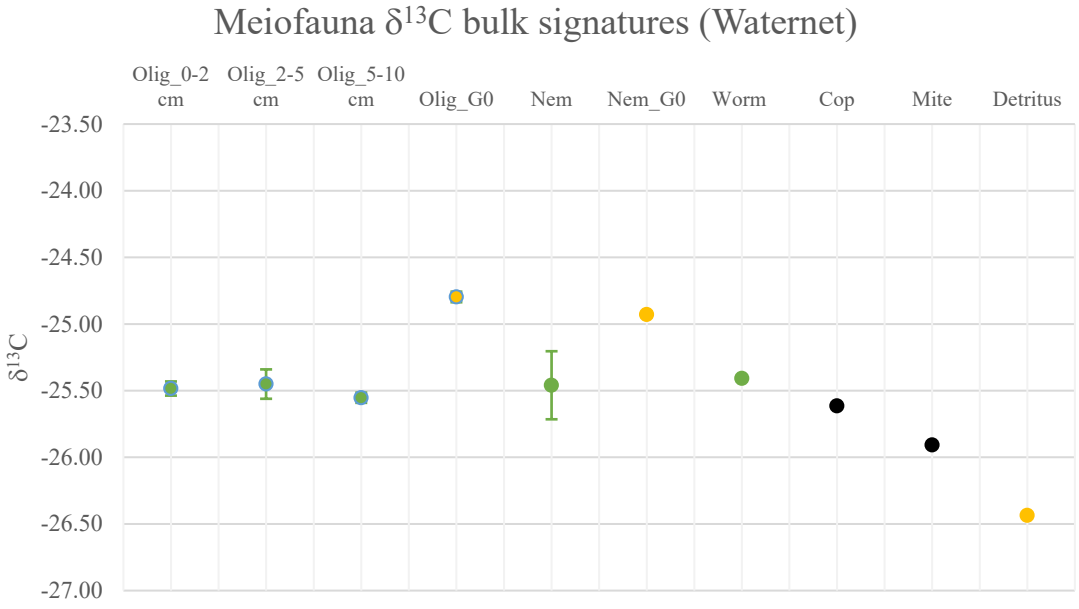


Figure 9. Overview of meiofaunal bulk $\delta^{13}C$ signatures per taxon. For the first six measurements, enough sample was present to measure replicates and standard deviations (SD) could be calculated (error bars represent 1 SD). The green colour represents standard EA-irMS settings performed on samples that were preserve in formaldehyde (meiofaunal core 1 and 2). Yellow dots represent samples originating from core G0 which was not preserved in formaldehyde. Black dots were obtained in a reduced flow EA-irMS setting designed to measure samples at one third of the weight/sample size normally required.

3.2.2 Depth profile

Oligochaete bulk isotopic signatures do not differ consistently over depth (Figure 9). Deeper in the sediment, $\delta^{13}C$ seem to decrease, but the decrease is not consistent with the 2-5 cm layer containing both the most depleted and most enriched samples, and the signatures of the 5-10 cm layer being very close to the more depleted values of the schmutzdecke layer.

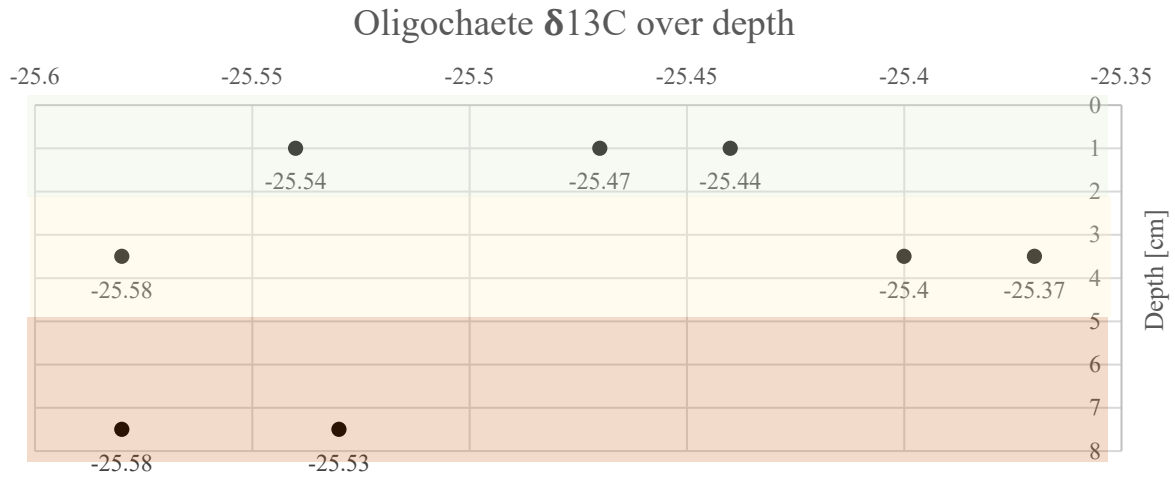


Figure 10. Showing a plot of natural carbon isotope signatures of oligochaetes obtained from different depths. The green colour represents the oligochaete samples from schmutzdecke (0-2 cm), the yellow colour represents the oligochaetes from the 2-5 cm depth layer and the brown colour represent the oligochaetes from a depth of 5-10 cm.

3.2.3 Formaldehyde preservation

A comparison between samples that were fixed in formaldehyde (meiofaunal core 1 and 2) and those that were not (core G0), shows a small offset of 1-4 % (more depleted than G0) or 0.35 to a maximum of 0.81 ‰ higher G0 values in absolute terms. Since the isotopic composition of formaldehyde is unknown, the actual impact of preservation cannot be determined, although it appears to be limited. Since meiofaunal cores are compared to the G0 core, the effect of rehydration of the G0 cores may interfere with an accurate interpretation of the shift resulting from formaldehyde alone.

Table 2

*Comparison of bulk **δ**13C signatures for samples preserved in formaldehyde versus samples that were unfixed.*

Taxon	Sample	Formaldehyde	Unpreserved	Unpreserved/formaldehyde
Oligochaete	Reduced flow (3 ind)	-25.58	-24.53	0.96
Oligochaete	Reduced flow (5 ind)	-25.86	-24.90	0.96
Oligochaete	0-2 cm	-25.47	-24.83	0.97
Oligochaete	0-2 cm	-25.54	-24.77	0.97
Nematode	pooled	-25.28	-24.93*	0.99
Nematode	pooled	-25.64	-24.93*	0.97

Note. Asterisk represents a single measurement (listed twice). All samples were frozen and thawed before isotopic measurement.

3.2.4 Reduced flow method

Due to the limited number and the small size of extracted copepods and mites, these taxa could not be analysed using the conventional EA-irMS settings. Instead, a reduced flow method was used. The sample size of 50 individuals was sufficient to obtain good signals using the reduced flow method at the expense of slightly increased analytical uncertainty. The analytical precision (1 SD) was 0.115 ‰ for Nicotinamide and 0.052 ‰ for the in-house sediment standard 'GQ' in the slow flow compared to 0.073 ‰ for Nicotinamide and 0.072 ‰ for GQ in conventional EA-irMS. A comparison of the slow method and conventional EA-irMS signatures of oligochaetes in meiofaunal cores (5-10 cm depth) and the G0 cores shows that bulk $\delta^{13}C$ signatures for the reduced flow EA-irMS method are very close to the signatures obtained in the conventional method (Table 3), despite containing only about one third of sample weight.

Table 3

*Bulk $\delta^{13}C$ comparison of higher sensitivity (reduced flow) EA-irMS results versus lower sensitivity **δ**13C (normal flow)*

	Reduced flow $\delta^{13}C$		Conventional $\delta^{13}C$	
	No. of ind	$\delta^{13}C$	No. of ind	$\delta^{13}C$
Oligochaete, 5-10 cm	3	-25.58	20	-25.55 ± 0,038
Oligochaete, 5-10 cm	5	-25.86		
Oligochaete G0	3	-24.53*	20	-24.80 ± 0,040
Oligochaete G0	5	-24.90*		

Note. Values with an asterisk indicate samples signals (C weight) that were too low for an accurate measurement. The values obtained with the reduced flow method are single measurements while the conventional method represents an average of multiple measurements of 20 individuals each.

3.3 Biomass, production & assimilation

The mean individual body mass is shown in Table 4, along with the percentage carbon calculated by the elemental analyser. Copepods were the lightest meiofauna with an individual body mass of only 0.66 µg, a value that is more than four times lighter than the oligochaetes. Mites were heavier (1.0 µg

per individual), while nematodes and oligochaetes were heaviest with average individual weights of 1.74 and 3.41 respectively. Weight and carbon contents values obtained for nematodes and oligochaetes in core G0 deviate somewhat from those of the meiofaunal cores and result from multiple differences between sample handling (e.g. preservation in formaldehyde; different number of individuals used; rehydration of core G0; different times of processing; lack of replicates) between the treatment of the meiofaunal and G0 cores.

Table 4
Average mature meio- & macrofaunal weight in Waternet's slow sand filter

	Av weigh t [µg]	% C	Av C weight [µg C]	Biomass [mg/10cm^2]	Total C [mg/10cm^2]	Production [mgC/m^2/d]	Assimilation [mgC/m^2/d]
Meiofauna							
Copepod	0.66*	32.7	0.215	0.0109	0.00357	0.499	1.25
Mite	1.00*	38.0	0.380	0.0402	0.0153	1.47	3.68
Nematode	1.74	41.9	0.729	0.0358	0.0150	1.33	2.21
Nematode G0$^+$	2.56*	46.9	1.20	-	-	-	-
Oligochaete	3.41	45.2	1.54	1.56	0.706	25.0	62.5
Oligochaete G0$^+$	1.13	39.2	0.443	-	-	-	-
Total	1.70	39.4	0.671	1.65	0.740	28.3	69.7
Worm	82*	50.5	41.4	0.136	0.0686	2.38	5.96
Total	-	-	-	1.79	0.809	30.7	75.6

Note. Biomass and Total C refer to 10 cm2 surface extractions (over 10 cm depth). Average weight and carbon are provided in µg whereas total biomass and carbon, production and assimilation are calculated in mg. Values for production and assimilation are calculated in mg per m^2 sediment surface per day.
*Average based on single isotope measurement
+ only partial Schmutzdecke data available

Only the meiofaunal core weights were used to estimate total biomass and total carbon in 10 cm^2 of SSF surface (with a depth of 10 cm). Total meiofaunal biomass in Waternet's SSF is 1.7 mg per 10 cm^2 and increases to 1.8 mg per 10 cm^2 if macrofaunal-sized worms are included. The same Plante and Downing (1989) regression was used to calculate the production and assimilation of the macrofaunal-sized worm. Values for each taxon were summed to estimate total production and assimilation rates in terms of milligram carbon in one m^2 SSF surface (over 10 cm depth) in a day. Total meiofaunal production was 28.3 mg C m^{-2} day^{-1} and total faunal production was 30.7 mg C m^{-2} day^{-1}. Production values represent only the ingested food that is converted to biomass on the sampling day (September 14, 2021). The net production efficiency ratios of Smock and Roeding (1986), and Herman and Vranken (1988) of 0.6 for nematodes, 0.55 for predators and 0.4 for other taxa, were used to convert production estimates into assimilation demands (69.7 mg C m^{-2} day^{-1} for meiofauna and 75.6 mg C m^{-2} day^{-1} in total). Biomass is dominated by oligochaetes and since production and assimilation estimates are calculated from biomass, oligochaetes also dominate the SSFs production and assimilation. Similarly, the worms show a large contribution to production and assimilation due to their relatively big size even if their density in the filter is very low (14 individuals 10 cm^{-2}, see Table 1).

4. Discussion

4.1 SSF meiofauna communities

4.1.1 Taxa

When comparing to samples taken from Dunea, nematodes, copepods and mites seem to be characteristic meiofaunal taxa in sand bed filters in the west of the Netherlands. Waternet's filters are a bit more diverse with oligochaetes, as well as macrofaunal-sized worms present. In streams, increased organic loading allows more oligochaetes and chironomid larvae to thrive in the sediment (Schmid-Araya et al., 2002). And the presence of oligochaetes may therefore indicate a more organic

rich SSF in Waternet compared to Dunea. However, comparisons between meiofaunal communities of Dunea and Waternet are limited by the different observers measuring and different circumstances possibly affecting counts and taxa distributions. This includes different time of year during which was sampled (March versus September), pre-treatment order differences, different water sources and locations.

In freshwater environments, nematodes or copepods typically dominate in abundance and biomass (Giere, 2009). Sediments with a rich detritus/bacteria complex favour nematodes and other taxa linked to the "detritus/bacteria-based food chain" (Castel et al., 1989) rather than the more "microalgae-based" harpacticoids (Montagna et al. 1989). However, neither taxon seems to dominate the SSF in Waternet's or Dunea's samples. This could be due to very stable, oligotrophic conditions of the influent water compared to natural freshwater bodies that do not allow one highly competitive species to dominate.

Many freshwater environments in West-Europe also contain rotifers, annelids, tardigrades, cladocerans, hydrachnid mites, chironomid larvae and oligochaetes (Stead et al., 2003; Reiss & Schmid-Araya, 2008; Majdi et al., 2017). Compared to these freshwater studies, the four taxa recovered form SSFs are limited (see Appendix C for taxa recovered from other sites). Most striking may be the absence of insect larvae (especially chironomids) that seem to inhabit most natural habitats and predate on meiofauna (Ptatscheck et al., 2020). The lack of taxa can result from many factors. First, a taxon may have been missed during microscopic surveillance due to lacking expertise on the part of the investigators, although this explanation alone seems unlikely since multiple investigators must have missed the exact same taxon in multiple cores of the SSFs. Second, initial colonisation of the SSF is much more complex than most natural habitats since many water pre-treatment steps have to be survived (see methods). This point is difficult to assess since the meiofaunal composition along the water source, dune lakes and pre-treatment steps is currently unknown. Third, SSFs may offer only a limited range of microhabitats without plants and with fairly homogenous sand particles, therefore supporting only a limited range of meiofauna. Similarly, the food sources present in SSFs may be limited and homogenously distributed, limiting the possible feeding strategies. In the absence of light and with the limited nutrients in the influent, food sources are derived from externally fixed carbon, or at least to a large extend. Fourth, SSFs are closed off from the outside and as a result, insects are not able to lay down their eggs, live in the benthos as larvae and emerge from the water as mature insects (Giere, 2009). Finally, the use of the 32 µm mesh may have resulted in the loss of the smallest meiofaunal taxa such as rotifers and small nematode species, causing an underestimation of SSF biodiversity (Ptatscheck et al., 2020).

The effect of mesh size on meiofaunal retention was studied by Ptatscheck et al. (2020) who concluded that all tardigrades, microcrustaceans, chironomids and oligochaetes were retained by the largest mesh size investigated (41 µm). Only 9% of the rotifers were retained on the 30-µm meshes and 23% of nematodes were not retained on the 41-µm, while Hummon (1981) showed that nematodes and rotifers were not retained by a 37 µm sieve. Juvenile nematodes were not retained by nets with a mesh size of 35 µm or even by those with 5-µm meshes (Kreuzinger-Janik et al. 2019). A smaller mesh size increases nematode numbers and species diversity (Ptatscheck et al., 2020), but comes at the cost of increasing the amount of detritus retained on the mesh and increasing the time it takes to analyse samples. A smaller mesh size may therefore not be feasible.

4.1.2 Distribution

Both the samples of Waternet and Dunea (Mithra, 2021, unpublished data) showed highest meiofaunal densities in the uppermost layer of the sand filters, yet still contained meiofauna in the lowest layer of 5 to 10 cm. Only two cores per sampling site were analysed and because the layer is homogenised, the precise distribution in the lowest centimetre remains unknown. However, it is likely that meiofauna are present in the lower and may even survive below a depth of 10 cm, since slow sand filters are well-oxygenated systems and meiofauna are found deep in sandy freshwater benthos (Giere, 2009). Majdi et al. (2017) even found higher meiofaunal density and production values the 5-10 cm section compared to the upper layer in a German stream.

In Waternet, samples did not show statistically significant differences in the vertical or horizontal distribution of meiofauna. This evidence is in contrast to the 'notoriously patchy distribution' of meiofauna recorded in the vast majority of aquatic habitats (e.g. Stead et al., 2005). In freshwater systems, community variation occurs mostly on scales of kilometres and centimetres and depends on OM content and substrate heterogeneity (Vincx, 1996). The homogenous conditions of SSFs are therefore in line with a homogenous distribution of meiofauna. The result is also indirectly confirmed by the similarity in $\delta^{13}C$ values between taxa from different cores, indicating the same food source for the same taxa in different locations of the filter (see below).

One potential confounding variable in the enumeration and distribution of SSF meiofauna is the drainage of filters before sampling, exposing meiofauna to air and increasing drying. Different meiofauna respond differently to drought, often with a general downwards migration in for instance coastal with a tidal cycle (Stead et al, 2004; Giere, 2009). Although the cores did not completely fall dry, a potential downward migration can be ruled out by comparing the meiofaunal cores to the meiofaunal community and distribution in the rehydrated control cores (G0).

4.2 The quantitative importance of meiofauna: feeding, biomass and production

4.2.1 Food sources

Following the trophic enrichment factor during food assimilation of +0.5 ‰ for $\delta^{13}C$ (McCutchan et al., 2003), no clear trophic relations between meiofaunal taxa in the SSF of Waternet emerges. All obtained values range around a $\delta^{13}C$ signature of -25.5‰. This result indicates food sources for all taxa are the same or meiofauna predate on one another (=meiofaunal food source) or a combination (omnivory). Detritus (unfixed) has a $\delta^{13}C$ value of -26.4‰ which is at least 1.4‰ more depleted compared to the unfixed nematode and oligochaete samples. The 1.4‰ offset is higher than the trophic shift in ^{13}C resulting from assimilation (+0.5 ‰) and indicates that detritus is likely not the (sole) food source for the meiofauna. Instead, a specific component of the detritus, such as bacteria or algae, may be the food source or a different, more enriched food source contributes at least contributes to meiofaunal diet.

The $\delta^{13}C$ signature combined with the nematode mouth structure (predatory suction and aselective deposit feeding) do not rule out meiofaunal predation on and by nematodes. However, since the trophic shift in $\delta^{13}C$ during assimilation is very low, trophic levels are difficult to establish and $\delta^{15}N$ measurements would be preferred to infer trophic position. Ha et al. (2014) also obtained similar $\delta^{13}C$ signatures for polychaetes and nematodes, but $\delta^{15}N$ values of the three polychaete species were 3 – 4‰ higher than the nematodes, reflecting their higher trophic levels and therefore their predatory relationship (see also Appendix D for the $\delta^{15}N$ over $\delta^{13}C$ plot). Previous work has often demonstrated meiofaunal predatory interactions. For instance, copepods can feed veraciously on nematodes (Ptatscheck, 2021). Most nematodes in the Lambourn stream were omnivores/predators (Schmid-Araya et al., 2016). Some copepods species may be omnivorous or carnivorous depending on the availability of food items and feed on microcrustaceans and oligochaetes (Fryer, 1957). As a rule of thumb, species-rich and detritus-based food webs in systems without primary producers have been linked to a high degree of omnivory (Emmerson & Yearsley, 2004) and in a study of the Lambourn river, most meiofaunal taxa had $\delta^{13}C$ values pointing to a mixed energy source, (Schmid-Araya et al., (2016). This would be in line with the observed aselective deposit feeding mouth structures in the nematodes and the similarity of the isotopic signatures in all taxa ruling out taxa with specialised diets.

The interpretation of stable isotope signatures is not without problems. Many species feed opportunistically and randomly on a wide range of food items (Schratzberger & Ingels, 2018). Stable isotopic signatures represent assimilation over relatively long periods, thereby revealing fluxes of biomass (Majdi et al., 2021). On the one hand, long term, averaged signatures provide a more accurate overall feeding estimate, on the other hand, preferred food sources may be masked by occasional diet shifts (Caramujo et al., 2007; Traunsprunger & Majdi, 2017). Assimilation also depends on digestion, which may be selective in some meiofauna (Majdi et al., 2012). Furthermore, a large numbers of

individuals of a taxon were pooled to obtain enough carbon for the stable isotope measurement. The general food source becomes visible, but at the cost of species-specific variations. On a species level meiofauna have specialised diets but they may change feeding strategy depending on seasonal dietary availability of food sources (Lebreton et al., 2012). All stable isotope samples except the detritus were exposed to freezing-thawing cycles during the enumeration and SI sample preparation. For macrobenthic organisms Dannheim et al. (2007) reported decreases of 1.87‰ in $\delta^{13}C$ because of freezing and thawing. Meiofaunal samples may therefore be almost 2‰ more enriched, becoming even further removed from the detritus carbon signal.

Another issue it the use of formalin which is generally discouraged when determining $\delta^{13}C$ signatures. Formalin may degrade soft-bodied meiofauna, leading to a carbon loss of 8–24% depending on storage time (Traunsprunger & Majdi, 2017). Fixation may enhance weight considerably (Widbom 1984). When comparing the Waternet sample $\delta^{13}C$ signatures of meiofauna that were fixed in formaldehyde to those that were freshly processed, a much smaller shift occurred of only approximately 4% more depletion in nematodes, oligochaetes and copepods. Therefore, formalin fixation does not seem to affect natural signatures much in Waternet's samples. Weight, on the other hand, was increased only in the oligochaete samples when fixed and may not reflect a true difference since the average is based on relatively few, but heavy oligochaetes.

4.2.2 Biomass & production

With an estimated weight of 0.740 mg C 10 cm^{-2} (1.65 mg dwt 10 cm^{-2}), meiofauna in the SSF of Waternet seem to make up a large portion of the biomass compared to the freshwater system estimates (see Table 5 in Appendix C). Overall biomass of 1.65 mg C 10 cm^{-2} is in the same range as the 1-2 g dwt m^{-2} (or 1-2 mg dwt 10 cm^{-2}) reported by Coull and Bell (1979) for shallow littoral bottoms. Nematode mass of 15 µg C 10 cm^{-2} is half of that reported by Blanchard (1990) of 37.69 µg C 10 cm^{-2}. The total meiofaunal SSF biomass estimate is a bit higher than the estimate of Majdi et al. (2012) for the Garonne river, but only for the oligochaetes. Nematodes for instance made up 14.2 mg C m^{-2} of the river sediment (or 0.0142 mg C 10 cm cm^{-2}) compared to an estimated 0.0153 mg C 10 cm cm^{-2} for nematodes in the SSF. SSF biomass was also twice as high as the meiofaunal biomass reported in the Furlbach by Majdi et al. (2017), and seven times the biomass of the Ems. But meiofaunal abundance and species richness often peak in spring and summer and yearly averages are needed for a more accurate comparison of the biomass obtained in September in the SSF to those from literature (e.g. Schmid-Araya et al., 2016; Brüchner-Hüttemann et al., 2020). In contrast to these freshwater systems, SSFs are well-oxygenated and do not contain macrofaunal predators to the same extend as freshwater systems do. In the absence of these two major constraints, meiofaunal biomass can build up to higher values than would be expected from other freshwater habitats.

While meiofaunal biomass appears to be low in most freshwater bodies (0-22%, mostly <1% of total metazoan biomass), meiofaunal contribution to total metazoan production can be high (mostly in the range of 0.8– 10.0 g $C \cdot m^{-2} \cdot yr^{-1}$; Reiss and Schmid-Araya 2010), representing 0.07-52% of total invertebrate production (Boulton et al., 2002). This way, a meiofaunal low standing stock (in terms of biomass) can generate a high secondary production with high P/B ratios. All of this is vastly different in the SSFs. Since the production calculated using the Plante & Downing (1989) regression formula depends on biomass, the production is relatively high in the SSF. The question is whether this a true reflection of secondary production in the SSF since meiofauna do not experience the same predation pressure by macrofauna that is exerted on them in freshwater systems. In the absence of most macrofaunal predators, both grazing pressure and competition with macrofauna may be reduced, allowing more meiofauna to survive to less productive ages. In oligotrophic environments, for instance, meiofaunal biomass is proportionally much larger than it is in oligotrophic environments but production is lower in oligotrophic water (Schmid-Araya et al., 2020). The lowest production/biomass values are reported in the stygobios, with annual ratios close to one (continued below, Giere, 2009).

Another problem when comparing production estimates is the use of a size-frequency in older studies to estimate biomass and production instead of the Plante and Downing regression formula (Schmid-Araya et al., 2020). The use of body size spectra is tricky due to highly dynamic temporal and spatial

fluctuations in biomass and density, and its reliance on distinct cohorts which meiofauna often lack, leading to an underestimated production (Majdi et al. 2017). In general, production estimates across habitats have yielded widely ranging production estimates, probably reflecting differences in populations and habitats, dynamic changes over time and methodical differences (Schratzberger & Ingels, 2018). Estimates appear to be highly habitat specific and may require more methodological consensus before true comparisons are possible (e.g. mesh sieve size, sampling depth).

Production strongly depends on environmental and biotic factors such as temperature and food fluctuations (Plante & Downing 1989) and population traits (growth rates, generation time, etc.) as functions of habitat and system characteristics (Schmid-Araya et al., 2020). Information on specific meiofauna community dynamics and turnover rates is required for a true understanding of production and energy flow within the system (independent of biomass).

The calculated production is lower than the meiofaunal grazing rates due to a substantial fraction of ingested food that is not assimilated (Decho & Castenholz, 1986). Ingestion and assimilation rates can be calculated from production estimates but they are highly species-specific and may differ among ontogenic stages of the same species (Decho & Fleeger, 1988). Since the species in the SSF samples are not known, assimilation calculated for Waternet is only a rough estimate based on taxon-averaged assimilation demands for meiofauna in all aquatic habitats. If a higher resolved food web is available (with trophic positions), the contribution of food sources to the meiofaunal diet can determined and assimilation estimates could be used to investigate the daily ingestion rates of a meiofaunal food sources following the approach of Majdi et al., (2012). With the ingestion rates, a better quantitative assessment of the importance of meiofauna in terms of carbon fluxes can be made.

4.3 The qualitative importance of meiofauna

The ecosystem function of meiofauna is often greatly underestimated in abundance, production estimates and labelling experiments (Majdi et al. 2017) due to methodological and ignoring the interactions that meiofauna engage in (Schratzberger & Ingels, 2018). The full impact of meiofauna on an ecosystem only becomes clear when the complex interactions with the environment, microbes, other meiofauna and macrofauna are taken into consideration.

4.3.1 Abiotic interactions

In the first place, direct ingestion of detritus and microbes may reduce the extend of the schmutzdecke and may slow down the filter maturation. Studies on oligochaete and nematode addition to membrane filtration systems have shown sludge reduction due to direct ingestion of organic matter (Klein et al., 2015). Secondly, reworking, recycling of nutrients, mechanical particle degradation, decomposition, mineralisation, burial, storage of organic matter all occur and are influenced by organisms in the sediment (Schratzberger & Ingels, 2018). Burrowing activities of meiofauna lead to sediment particle reworking and enlarges the water-sediment interface, changing hydrodynamics, nutrient cycling, biogeochemical fluxes and vertical chemical gradients. Sediment permeability and associated solute transport increase due to of meiofaunal burrowing (Derlon et al., 2013). In sand filters, burrowing meiofauna may therefore counteract some of the clogging during filter ripening.

4.3.2 Meiofauna – microbe interactions

Meiofauna interact closely and in complex ways with microbes leading in most cases to stimulation of benthic microbial metabolism (e.g., Majdi et al., 2017). The first way in which microbes are stimulated is via grazing (Schratzberger & Ingels, 2018). Bacterial ingestion rates by meiofauna only reach a few percent of bacterial stock (e.g. Bergtold & Traunspurger 2005) keeping bacteria in the active growing phase (Schratzberger & Ingels, 2018). Meiofauna can also stimulate bacterial population due to their mechanical breakdown of detritus (Tenore, 1977), burrowing activities enhancing oxygenation (Aller & Aller 1992), excretion, secretion of extrapolymeric substances (EPS) and mucous (Hubas et al., 2010) and decaying dead bodies all of which increase nutrients and microbial mineralisation rates in the sediment (De Mesel et al., 2004). Direct meiofaunal uptake of POM and DOM counteract come of this (Meyer-Reil & Faubel, 1980).

Burrowing is the domain of nematodes and oligochaetes (e.g. Svensson et al., 2001). Burrows increase bacteria density and metabolic activity by enlarging the sediment-water interface (Aller, 1978) and intermittent flushing which mobilises nutrients to deeper parts of the sediment (Svensson & Leonardson 1996). Flushing may increase solute transport up to 1.5–3 times the rate of molecular diffusion alone (Aller & Aller 1992). Nitrifying bacteria may profit to a larger extend from the burrowing than others because of the ammonium containing excretions in and around the burrows (Svensson et al., 2001). In the context of the SSFs, burrowing can therefore promote bioactivity and further OM removal without losing the permeability of the schmutzdecke.

Exopolymer secretions (EPS) and mucous are secreted by microorganisms and some meiofauna for protection and adhesion (Giere, 2009). They are made up of mainly polysaccharides and glycoproteins and can be important nutrients for bacteria and meiofauna (Hubas et al., 2010). Since meiofauna have higher C:N ratios compared to bacteria, more nitrogen is excreted in their mucous (Stock et al., 2014), again promoting nitrifying bacteria that can be grazed down by meiofauna ('gardening'; Giere, 2009). When the nitrifying bacteria outcompete other specialised microbes and (aselective) grazing pressure is high, the microbial community can be altered, thereby also changing overall microbial activities. This was the case in the study by Näslund et al. (2010) where PAH-degrading bacteria diminished in favour of nitrifying bacteria when meiofaunal abundances were high.

In addition to its nutrional role, mucous substances compact the sediment and reduce erosion (biostabilisation; Blanchard et al., 2000). Conversely, grazing on EPS or EPS-producing bacteria will destabilise the sediment (Moens et al., 2002). The overall effect of EPS on the sediment is highly variable and depends on the specific habitat. Changes in sediment microtopography in turn affect the sediment-water interface, diffusion and reaction rates, microbial activity and meiofaunal community composition (Schratzberger & Ingels, 2018). The interrelatedness of all causes and effects make it difficult to predict what the practical implications of EPS production could be for SSF functioning. On the one hand, more EPS may promote more microbial mineralisation, increasing biodegradation of OM in the filter and allowing more carbon to be stored in the biomass pool. On the other hand, more EPS could shift the microbial community, with the loss of specialised microbes in favour of nitrifying bacteria, more compaction of the schmutzdecke, speeding up the hydraulic resistance development.

4.3.3 Meiofauna – meiofauna & macrofauna interactions

Inter and intraspecific interactions between meiofauna can be predatory, competitive or facilitating and affect meiofaunal spatial distributions in terms of their density, diversity and species composition (Ólafsson, 2003). A system with as much diversity is desirable because when species diversity increases, the entire ecosystem becomes more resilient since the chance of a species adapting to changes in availability of resources increases and loss of ecosystem processes decreases (Harrison et al., 2014; Schratzberger & Ingels, 2018).

In freshwater systems, fish and macroinvertebrates prey on meiofauna with highly variable effects on meiofauna communities (Silver et al., 2002). Competition over shared food sources forms a more important interaction and significantly reducing meiofaunal counts. The macrofaunal-sized worms in Waternet's SSF may compete with meiofauna over the same food sources (the $\delta^{13}C$ signature is the same), but predation cannot be excluded at this point. Macrofauna may have similar effects on microbes and meiofauna as meiofauna-microbial interactions with intensive reworking of sediments leading to increased nutrient fluxes and mineralisation rates (Braeckman, et al., 2010), tubes and burrows modifying local hydrodynamics and promoting meiofaunal diversity and abundance (Ólafsson, 2003; Giere, 2009), while excretion and secretion promote production. However, overall mineralisation rates in sediments with high meiofaunal abundance do not seem to increase further in the presence of macrofauna (Nascimento et al., 2012).

4.4 Revisiting Dutch SSF meiofauna

4.4.1 Groundwater meiofauna parallel

The SSF habitat is most similar to and preceded (during the water production step of infusion into the dune) by groundwater. Traits of meiofauna in this habitat may therefore be present in the SSF

meiofaunal community and can have consequences for SSF functioning. Homogeneity of sediments and physiochemical conditions are the main features of groundwater systems (Giere, 2009). The habitat has relatively constant, low temperatures, a somewhat lowered pH, a slightly undersaturated oxygen content and oligotrophic conditions. The meiofauna inhabiting these places are called "stygobiotic" in groundwater aquifers and "troglobitic" in caves. Typical meiofauna include crustacean species and copepods (often cyclopoids), ostracods and oligochaetes, while insect larvae that usually dominate riverine and lacustrine systems, are absent (Danielopol et al. 2000). Food sources in groundwater systems are scarce and the food web is simple with few trophic links (Stanford et al. 1994). In addition, meiofaunal distribution is homogeneous with little or no zonation (Giere, 2009). The system depends entirely on heterotrophy and detritus from the surface. The scarcity of predators, limited food sources and reduced competition facilitate the persistence of taxa with low mobility and little competitive strength. Stygobiotic meiofauna move slowly, have slow metabolisms and growth, long generation times and lifetimes, late maturity and low fecundity (a few, large eggs), and hardly show any diurnal rhythms. Yet the stability of the physiochemical environment, absence of predators and reduced competition allow for a high biodiversity combined with the low abundances to persist (Danielopol et al. 2000).

4.4.2 Scraping & recolonization

Schmutzdecke scraping represents a highly disturbing event for all organisms present in the SSF that decimates populations and requires recovery of meiofaunal populations. In freshwater environments, meiofauna dispersing via the water column, such as copepods, recover the most while the more sediment-bound nematodes recolonize slower rapidly although re-entry into the sediment often requires just a few hours or days (De Troch et al. 2005). Complete habitat colonisation by nematodes typically forms a rapid process of a few days in natural water bodies (Ptatscheck & Traunspurger 2014). While overall recolonization of meiofauna takes a few weeks to two months (Peters et al. 2007). Recolonization starts of rapidly but slows down as niches become increasingly occupied, requiring higher specialization for later successful colonization. For nematodes, epigrowth or bacterial feeders establish first (Giere, 2009), followed by deposit-feeders, and later by omnivores and predators (Gwyther & Fairweather, 2002). Since the SSF system reflects the stygobenthic meiofauna, the meiofaunal community may remain more vulnerable and may recover more slowly than would be predicted based on comparisons to other freshwater systems. Mobility, reproduction rates and production are all lower compared to most freshwater systems if the SSF meiofauna reflect stygoobenthic meiofauna. Furthermore, colonisation of meiofauna is influenced by the presence of detritus and substratum complexity (Silver et al., 2002), Since these are likely to be low in scraped filters, meiofaunal recovery is delayed even further.

4.4.3 Potential effects of bioturbation

SSF performance has previously benefited from bioturbation by macrofaunal-sized worms. While mechanical ventilation may be implemented in filters, this could have important implications for the meiofaunal community. Water flow is an important determinant in the settling and persistence of meiofauna in aquatic habitats (Majdi et al., 2017). In streams, faster flow can facilitate deeper penetration of O_2 and OM in the sediment column (Vervier et al., 1992) and deeper meiofaunal colonization. But when flow rates become very high, meiofauna abundances decline (Hakenkamp & Palmer, 2000), while very low flow rates may allow detritus to accumulate and anoxic layers to develop which also lower meiofaunal abundances and affects chemical fluxes. Meiofaunal abundances and diversity are highest at intermediate velocities (about 30 cm/s at the sediment surface; Whitman & Clark, 1984) and changes in flow velocities are tied to changes in meiofaunal communities (Majdi et al., 2017). The smallest organisms are at a higher risk of being washed away from the substrate under high flow conditions (Palmer 1992; Majdi et al., 2012) and many meiofauna are known to react to strong currents by downward migration (Steyaert et al. 2003). But adaptation to local stable conditions occurs (Majdi et al., 2017). The implementation of mechanical bioturbation may initially induce meiofauna to bury into deeper layers to seek refuge against flow erosion, especially if these deeper layers have accumulated more OM (Eisenmann et al., 1999). The more sediment-bound meiofauna (e.g. nematodes) will be less affected by increased flow, than the more epibenthic harpacticoids (McCall & Fleeger, 1995). In general, the mechanical mixing will likely affect the vertical distribution

of meiofauna, creating a surface layer with reduced meiofaunal abundance, as well as change the meiofaunal community composition. Nematodes and oligochaetes may benefit from the increased ventilation while copepods could decline in abundance. Finally, if the density of meiofauna in the schmutzdecke decreases, some of the beneficial interactions with microbes may be lost leading, while less predation and increased ventilation may also stimulate the microbial community. The outcome of the balance is difficult to predict.

4.5 Recommendations

4.5.1 Effect of rehydration

In order to know whether meiofaunal assemblages and their density obtained from the 'meiofaunal cores' in this thesis are influenced by the draining of the SSF prior to extractions, the counts should be compared to the meiofaunal community and distribution over all layers in the rehydrated control cores (G0). Due to time constraints, this was not possible before.

4.5.2 Metagenomics

Provided that reference databases are available, eDNA extraction may dramatically improve and speed up the identification of meiofaunal assemblages from SSF samples, without the need to obtain large cores (see methodology of Majdi et al., 2020). DNA samples can reveal at least which genera are present and in what quantity, but the technique comes with its own disadvantages, including the risks of contamination and false species identification, (b) the potential bias introduced by differential DNA degradation during digestion for quantitative assessment, (c) chimeric reads resulting from meiofaunal gut microbiome, parasites or symbionts (Majdi, et al., 2020). At present Dutch dune meiofauna are being analysed at Naturalis Biodiversity Centre and from an ecological perspective it would be interesting to compare SSF meiofaunal assemblages to those of the source water bodies and the dunes where water is infused before SSF treatment. The outcome of this comparison could contribute to knowledge on habitat preferences, resilience of meiofaunal taxa and dispersal abilities.

4.5.3 Trophic positioning using $\delta^{15}N$ food web

Food web inferences would benefit from $\delta^{15}N$ isotopic. By plotting natural $\delta^{13}C$ versus $\delta^{15}N$ isotopic signatures not only for food sources, but also trophic levels can be identified (Peterson & Fry, 1987). Some example plots involving meiofauna are shown in Appendix D. For oligochaetes, nematodes and worms, $d^{15}N$ could relatively easily be analysed, since the required number of individuals is not high (<40 per sample), but sample collection for mites and copepods might be more tedious. The reduced flow method, allowing less sample material to be analysed, is not possible for $d^{15}N$ signatures. With both signatures, the precise contribution of each food source to a consumer can be estimated using the MixSIR stable isotope mixing model developed by Moore & Semmens (2008).

Opting for the slow flow method with higher subtaxon resolution (e.g. separate nematode feeding groups) instead of collection more samples and measuring the $\delta^{15}N$ isotopic signatures might be more feasible and could also provide interesting insights into feeding habits in the SSF. With limited processing time available, either more samples, of more (sub)taxa with the reduced flow method, or less samples but with trophic position information could be measured.

4.5.4 Temporal resolution

With repeated meiofaunal sampling, more accurate biomass, production and assimilation estimates throughout the year can be determined and compared with the microbial stock population. A future experiment could for instance, obtain (quantitative) eDNA samples every season for the period of a year. Especially meiofaunal stock in the winter, in relation to physiochemical parameters such as water temperature and organic matter contents would help to understand if the meiofaunal community varies across seasons (in terms of diversity and density). On the other hand, large variations are not to be expected since SSF physiochemical conditions are relatively stable over time and sampling risks contamination of drinking water.

4.5.5 Species determination

Determining the species present in SSFs may not only contribute to the knowledge of meiofaunal occurrence in the unique SSF habitat, but could have important implications for SSF functioning. Certain genera and species may be known to have traits that can improve or limit SSF functioning (e.g. burrowing or EPS-excreting species). If the species composition is known, the effects of water production line alterations could also be predicted and used to manipulate the meiofaunal assemblage, such as adjusting mechanical ventilation of the filters and the recovery time after scraping. The recovery time required may also be influenced by the species abundance in the SSF since a higher species diversity increases the resilient of an ecosystem (Harrison et al., 2014; Schratzberger & Ingels, 2018). It is not known whether SSFs abide by this rule, but species richness could be an important parameter to determine the stability and succession of the meiofaunal community linked to bioactivity in the filter with potentially important implications for filter removal efficiency.

5. Conclusion

Waternet's SSFs in the West of the Netherlands is inhabited by nematodes, oligochaetes, copepods and mites as well as macrofaunal-sized worms. Nematodes, copepods and mites were previously also found in SSFs of Dunea, which have a different water source and production line. The nematode, copepod and mites could therefore be characteristics core meiofauna of SSFs in the West of the Netherlands. Compared to most lakes (e.g. Bergtold & Traunspurger, 2005; Stead et al, 2005; Traunspurger et al., 2019) and rivers (e.g. Majdi et al., 2012; 2017), this represents a rather limited range of taxa. Most freshwater systems seem to be dominated by either nematodes or copepods which is not the case in the SSF. The filter environment is closed off from the outside, preventing certain meiofaunal colonisation routes, such as insect eggs deposited in the water. The taxa found, in particular the absence of insect larvae in the sediment, and lack of a dominant taxon in the SSF are in accordance with groundwater meiofauna ('stygobenthic meiofauna'; Giere, 2009).

Another similarity of the SSF habitat with groundwater systems is the relatively stable physiochemical conditions, the dependence on externally produced organic matter and permanently dark conditions. All of these may limit the microniche availability, providing only limited diversity in food sources which are homogenously distributed across the SSFs. These conditions are reflected in the similar carbon isotopic signatures for all meiofauna in Waternet's SSF (indicating similar food sources and/or predation of meiofauna on meiofauna) as well as in the similarity in the communities of different cores in the filter and over depth. The relatively stable physiochemical conditions in the SSF make large (seasonal) variations in the meiofaunal community unlikely. The hypothesized small spatial and temporal variability in the meiofaunal community is in stark contrast with most patchy, highly dynamic assemblages in freshwater systems, except for groundwater and cave systems.

The similarity of the SSF and groundwater habitats may have important implications for meiofaunal traits and ecosystem functioning in the SSF. Groundwater systems do not show predation and competition to the same extend as other freshwater systems (Giere, 2009). The resulting meiofaunal assemblage has a low density but is species rich. Meiofauna typically have low metabolisms, mobility and fecundity rates. If SSF meiofauna are similar to the stygobenthic ones, this would imply that the assemblage takes a relatively long time to recover from scraping events and that the meiofauna are sensitive to changes in physiochemical conditions such as increased water flow (resulting from mechanical ventilation). Meiofaunal production in the SSF may also be lower than the estimates resulting from the Plante & Downing (1989) regression formula simply because metabolic rates are lower and the meiofauna live to relatively old ages in the absence of fierce predation, rather than being kept in the continuously reproducing and active growing phase.

The SSF meiofauna differ from groundwater in a key aspect: meiofaunal biomass is relatively high in Waternet. This is mostly the result of the dominance of oligochaetes that have very high individual weights and probably also relates to the absence of insect larvae and many other macrofaunal predators that would reduce meiofaunal biomass in freshwater systems. As (some of) the highest consumers in the SSF food web, meiofauna may therefore have a role in carbon storage rather than the

carbon flux from microbes to higher trophic levels that is normally associated with meiofauna (Majdi et al., 2020).

Secondary production, the formation of meiofaunal biomass, requires ingestion of organic matter. This can be either grazing on microbes and detritus or via predation on grazing meiofauna. As a result, schmutzdecke growth may be slowed down in the presence of meiofauna and bacterial mineralisation rates may be increased by keeping the bacteria in the active growing phase. More quantitative assessments of meiofaunal grazing require a higher resolved food web in which trophic levels of meiofauna are known. With this information, the percentage contribution of food sources to each taxon's feeding can be determined from which ingestion rates can be calculated. This information provides a quantitative importance of meiofauna in SSFs.

Another aspect that is highlighted in the scientific literature, is the qualitative importance of meiofauna. Meiofauna interact with their abiotic environment, and microbial and macrofaunal organisms in a complex manner (e.g. (Schratzberger & Ingels, 2018). Some meiofauna excrete EPS which may act as biostabiliser of the sediment and provides nutrition to microbes and other meiofauna (Moens et al., 2005), while meiofauna may also graze on EPS or EPS-secreting microorganisms (De Mesel et al., 2004). Other meiofauna (oligochaetes and nematodes) create burrows that increase the porosity of the sediment, affect the local water flow and chemical fluxes (Aller & Aller 1992). The activities of meiofauna may selectively stimulate nitrifying bacteria, while other bacteria are not promoted and may even be hampered by grazing (Svensson et al., 2001). Studies performed in membrane filtration systems have demonstrated that the presence of oligochaetes and nematodes reduces sludge accumulation considerably, as well as increase the sediment porosity (Derlon et al., 2013).

In conclusion, meiofauna may be more sensitive creatures in the SSF compared to most freshwater environments, but in return play a more important role in carbon storage and habitat engineering. Their high biomass, grazing and burrowing activities likely contribute to a slower maturation of the schmutzdecke, keeping the porosity and water percolation higher than would be the case without meiofauna. Higher temporal resolution and taxonomic identification coupled to known species activities and traits is needed to confirm this potential of meiofauna in SSFs.

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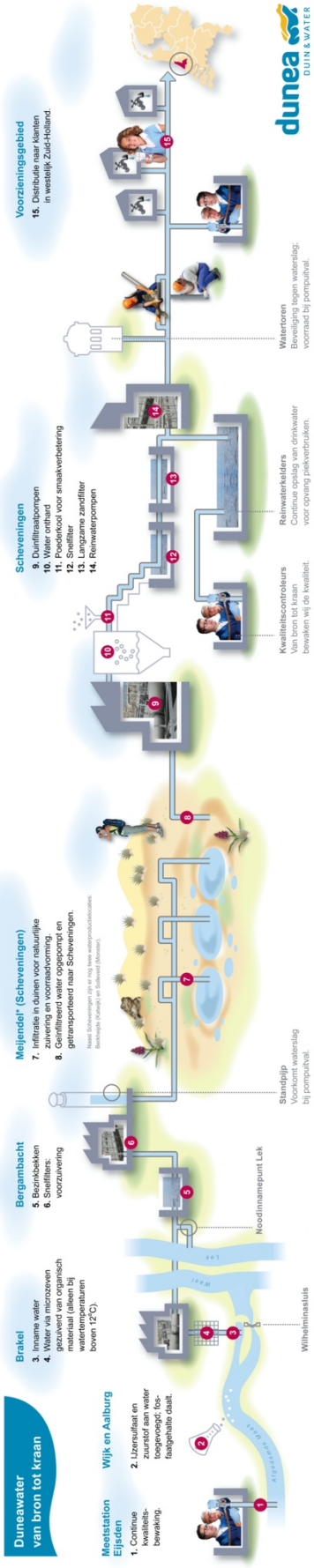
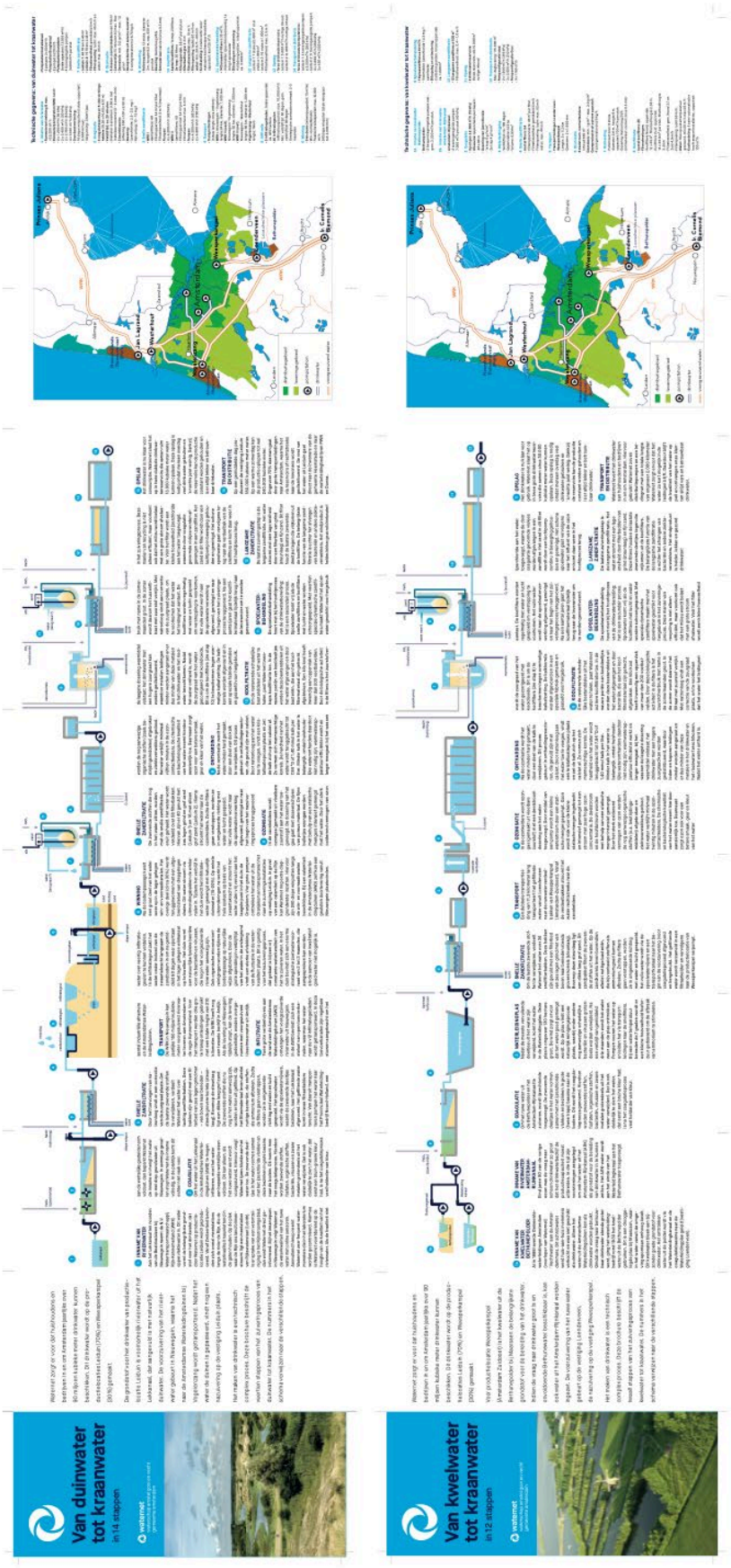
Appendix A. Dunea drinking water production overview



Accessed via: https://www.dunea.nl/over-dunea/pers-en-publicaties

Appendix B. Waternet drinking water production overview



Accessed via:
https://www.waternet.nl/innovatie/schoon-water/drinkwater-van-bron-tot-tap/

Appendix C. Meiofaunal biomass and production in freshwater environments

Table 5
Overview of published biomass, production and P/B in meiofaunal research in different aquatic habitats.

Enumeration	Biomass	Production	P/B	Production method	% total production	Meiofauna	Freshwater system	Location	Reference	Notes
-	-	-	8.4	-	-	Average meiofauna	Average freshwater	Average location	Warwick & Price (1979)	Invalid? (Vranken and Heip 1986; Moens and Vincx 1997b)
-	1–2 g dwt m^{-2}; Peaks of 5 g dwt m^{-2}	-	-	-	<10% of macrofaunal P	Total community	Shallow littoral bottoms	-	Coull and Bell 1979	
-	0.21-0.54 g DM/m^2	-	-	-	-	66% of biomass due to nematodes	Lake	Michigan	Nalepa & Quigley, 1983	
1200 ind. 10 cm^{-2}	-	-	-	-	-	70% nematodes; also turbellarians, gastrotrichs, cladocerans, copepods, rotifers, tardigrades	Lake	Lake Mirror (USA)	Strayer, 1985	
-	-	-	6-50	-	-	Meiofauna	Marine sediment	-	Vranken et al., 1986	
-	Nematodes: 405.53 ± 37.69 µg C 10 cm^{-2} Copepods: 628.12 ± 128.36 µg C 10 cm^{-2}	33.94 µg C 10cm-2 h-1	-	-	-	Nematodes			Blanchard, 1990	
-	-	-	3-6	-	-	-	Stream (gravel)	-	Kowarc, 1990	
-	-	0.1–10 ∝gDW ind^{-1}	-	-	-	-	Stream	-	Poff et al., 1993	
1-10 million per m2	-	-	-	-	-	-	Marine sediment	-	Vinx, 1996	
-	-	3.4 mg C/m^2/day	-	Allometric model	-	deposit feeding meiofauna	-	-	Goedkoop and Johnson (1996)	
Nematodes: 250 ind. 10 cm^{-2}; cyclopoids (20 ind. 10 cm^{-2}	-	-	-	-	-	-	Lakes	Northern	Sarvala 1998	Oligotrophic lakes
millions/m2	-	-	-	-	<5% metazoan P	-	River bed	-	Robertson et al., 2000	
-	-	-	-	-	<5 % metazoan P	-	Lotic	-	Hakenkamp & Morin, 2000	
20–30 ind. per 10 cm^2	-	-	-	-	50% metazoan P	-	River bed (coarse sand)	-	Hakenkamp et al., 2002	Lower B in acidic streams
-	-	-	4.8	-	50-60% of microbenthic P in large lakes; increases in the profundal of smaller lakes	-	European lakes	-	Kurashov 2002	P/B of max 5 for less productive northern lakes

1–12 million nematodes m^{-2}	-	-	8-10	-	Nematodes (80–95% of individuals; 50–90% of biomass)	-	Lake (littoral)	-	Traunspurger 2002; Giere, 2009	
15 - 400 ind. 10 cm^{-2}	-	-	-	-	-	Mostly nematodes, also crustaceans, rotifers, oligochaetes	Lake (eutrophic)	China	Wu et al. 2004	
-	-	-	~1	-	-	-	Deep sea & stygobios	-	Giere, 2009	
50 ind. 10 cm^{-2} harpacticoid copepods; nematodes: 70-189 ind. 10 cm^{-2}	-	-	>10	-	77% nematodes; also rotifers, copepods (harpactocoid)	-	Lake (oligotrophic)	Lake Brunnsee (Germany)	Bergtold and Traunspurger, 2005	
-	-	0.12-1.34 g C/m^2/year (5.2 g dry wt. m^{-2} y^{-1} for permanent meiofauna)	-	-	51% metazoan production	-	Stream	Lone Oak	Stead et al., 2005	40 to 42 µm sieves; acidic, oligotrophic stream
-	-	-	5-58	-	<5% metazoan production	Nematodes	Freshwater	-	Bergtold and Traunspurger 2006	
<5 ind./10 cm^2 cladocerans, copepods; 5 to 217 ind/10 cm^2 nematodes	-	-	-	-	-	harpacticoids, ostracods, cladoceran crustaceans (reservoirs); nematodes, turbellarians, cyclopoid copepods (streams)	River	-	Caramujo et al., 2007	
-	0.06 g C/m^2	0.59-1.15 g C/m^2/year	-	Size-frequency	7-8 of invertebrate P	-	Stream (gravel; chalk)	Lambourn	Tod & Schmid-Araya, 2009	40 µm sieve
-	-	0.8-10 g C m^{-2}-yr^{-1}	-	Size-frequency	~50	-	Stream	-	Reiss & Schmid-Araya, 2010	In Schmid-Araya et al., 2016; 40 µm sieve
>1300 ind./10 cm^2	-	-	-	-	-	Mostly algivorous nematodes (58%); rotifers (26%)	Lake (periphyton)	Lake Erken (Sweden)	Schroeder et al., 2012	48 nematode species
Nem: 369954±32790 ind m^{-2}; chironomid larvae: 14899±1932 ind m^{-2}; oligochaetes 3631 ± 436 ind m^{-2}	Nem: 14.2 mg C m^{-2} Chir: 7.2 mg C m^{-2} Olig: 3.5 mg C m^{-2}	Nem: 2.79 mg C m^{-2} day^{-1} Chir: 1.18 mg C m^{-2} day^{-1} Olig: 0.59 mg C m^{-2} day^{-1}	-	-	37% total faunal production	Nematodes, chironomids, oligochaetes, micro-turbellarians, harpacticoid copepods, rotifers, gastrotrichs, tardigrades, Nauplii gammarids	River (epilithic biofilm)	Garonne	Majdi et al., 2012	40-500 µm size fraction

0.8621653 10^9 ind. m^{-2}	0-5 cm: 0.045 g C/m^2 5-10 cm: 0.061 g C/m^2	Total: 2.58 g C/m^2/year 0-5 cm: 1.27 g C/m^2/year 5-10 cm: 1.31 g C/m^2/year	-	Plante and Downing (1989) regression formula		Tardigrades, rotifers, oligochaetes, gastrotrichs	Stream (sandy)	Ems (NW Germany)	Majdi et al., 2017	30 µm sieve; phosphate-rich, slow-flowing waters; peak density June
>1 10^6 ind. m^{-2} (annual average)	**0-5 cm: 0.203 g C/m^2 5-10 cm: 0.191 g C/m^2**	**Total: 5.46 g C/m^2/year 0-5 cm: 2.85 g C/m^2/year 5-10 cm: 2.61 g C/m^2/year**	**-**	**Plante and Downing (1989)**		**Nematodes, rotifers, micro-turbellarians, harpacticoid copepods, oligochaetes, gastrotrichs, chironomids, tardigrades, Nauplii, gammarids**	**Stream (sandy)**	**Furlbach (NW Germany)**	**Majdi et al., 2017**	**30 µm sieve; nitrate-rich, fast-flowing water; peak abundance February-June**
Nematodes: 710.8 (hard); 254.9 (soft) 10 cm^{-2} Crustaceans: 84.7 (hard); 101.7 (soft) 10 cm^{-2} Rotifers: 234.3 (hard); 166.2 (soft) 10 cm^{-2} Tardigrades 20 (hard); 205.2 (soft) 10 cm^{-2} Oligochaetes 30 (hard); 33.1 (soft) 10 cm^{-2}	Nematodes: 400 (hard); 182.9 (soft) µg (wwt) 10 cm^{-2} Crustaceans: 600 (hard); 432.2 (soft) µg (wwt) 10 cm^{-2} Rotifers: 120 (hard); 5 (soft) µg (wwt) 10 cm^{-2} Tardigrades 40 (hard); 55.4 (soft) µg (wwt) 10 cm^{-2} Oligochaetes 80 (hard); 518.9 (soft) µg (wwt) 10 cm^{-2}	-	-	-	-	Nematodes, crustaceans, rotifers, tardigrades, oligochaetes	Lakes (oligotrophic)	Central and northern Europe	Traunspurger et al., 2019	Literature overview
Nematodes: 1051.6 (hard); 116.3 (soft) 10 cm^{-2} Crustaceans 135.7 (hard); 39.3 (soft) 10 cm^{-2} Rotifers 285 (hard); 41.8 (soft) 10 cm^{-2} Tardigrades 230 (hard); 7.1 (soft) 10 cm^{-2} Oligochaetes 240 (hard); 25.3 (soft) 10 cm^{-2}	**Nematodes: 920 (hard); 539.9 (soft) µg (wwt) 10 cm^{-2} Crustaceans 1480 (hard); 470 (soft) µg (wwt) 10 cm^{-2} Rotifers 400 (hard); 14 (soft) µg (wwt) 10 cm^{-2} Tardigrades 40 (hard) µg (wwt) 10 cm^{-2} Oligochaetes 920 (hard); 750 (soft) µg (wwt) 10 cm^{-2}**	-	-	-	-	**Nematodes, crustaceans, rotifers, tardigrades, oligochaetes**	**Lakes (mesotrophic)**	**Central and northern Europe**	**Traunspurger et al., 2019**	

Nematodes: 950 (hard); 212.5 (soft) 10 cm^{-2} Crustaceans 154 (hard); 25.1 (soft) 10 cm^{-2} Rotifers 304.7 (hard); 186.8 (soft) 10 cm^{-2} Tardigrades 280 (hard); 17.1 (soft) 10 cm^{-2} Oligochaetes 70 (hard); 48.7 (soft) 10 cm^{-2}	Nematodes: 800 (hard); 1040.3 (soft) µg (wwt) 10 cm^{-2} Crustaceans 1680 (hard); 789.3 (soft) µg (wwt) 10 cm^{-2} Rotifers 200 (hard); 25.8 (soft) µg (wwt) 10 cm^{-2} Tardigrades 120 (hard); 1.3 (soft) µg (wwt) 10 cm^{-2} Oligochaetes 800 (hard); 1701 (soft) µg (wwt) 10 cm^{-2}	-	-	-	-	Nematodes, crustaceans, rotifers, tardigrades, oligochaetes	Lake (eutrophic)	Central and northern Europe	Traunspurger et al., 2019	
$6\ 10^5$ (± $2.79\ 10^5$) ind. m^{-2} in May/June; in December: $6.19\ 10^4$ (± $2.39\ 10^4$) ind. m^{-2}	-	**6.1 mg C m^{-2} day^{-1} in May; in August, 1.3 mg C m^{-2} day^{-1}; total annual sec production 2.29 g C m^{-2} $year^{-1}$**	-	**Plante and Downing (1989)**	-	-	-	-	**Brūchner-Hüttemann et al., 2020**	**48% total meiofaunal annual P in the sediment (= 1.10 g C m^{-2} $year^{-1}$)**

Appendix D. Examples of food webs based on $\delta^{13}C$ and $\delta^{15}N$ values

Nitrogen over carbon stable isotope ratio plot showing both trophic level ($\delta^{15}N$) as well as food source ($\delta^{13}C$). If $\delta^{13}C$ (±0.5‰) is the same for different data points, this indicatest he same food source and/or a predatory relationship. An offset of about 3‰ occurs between different trophic levels (McCutchan et al., 2003). If two organism therefore have the same $\delta^{13}C$ signature but one is more enriched in $\delta^{15}N$, the more nitrogen-13 enriched organisms feed on the other organisms.

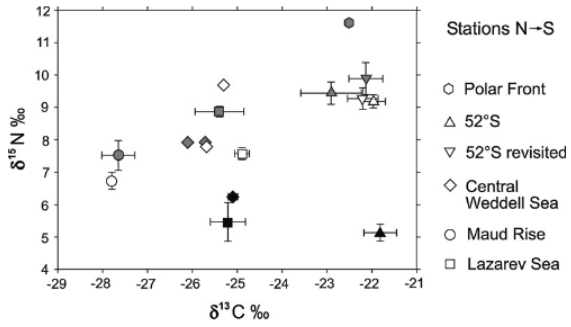


Figure D1. Showing meiofauna from a north-south gradient. Obtained from Veit-Köhler, et al. (2013)

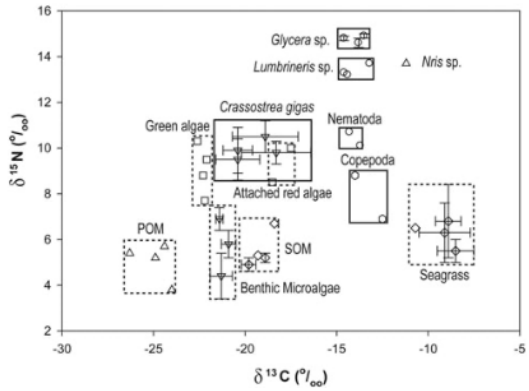


Figure D2. Macrobenthos trophic relations, obtained from Ha et al., 2013

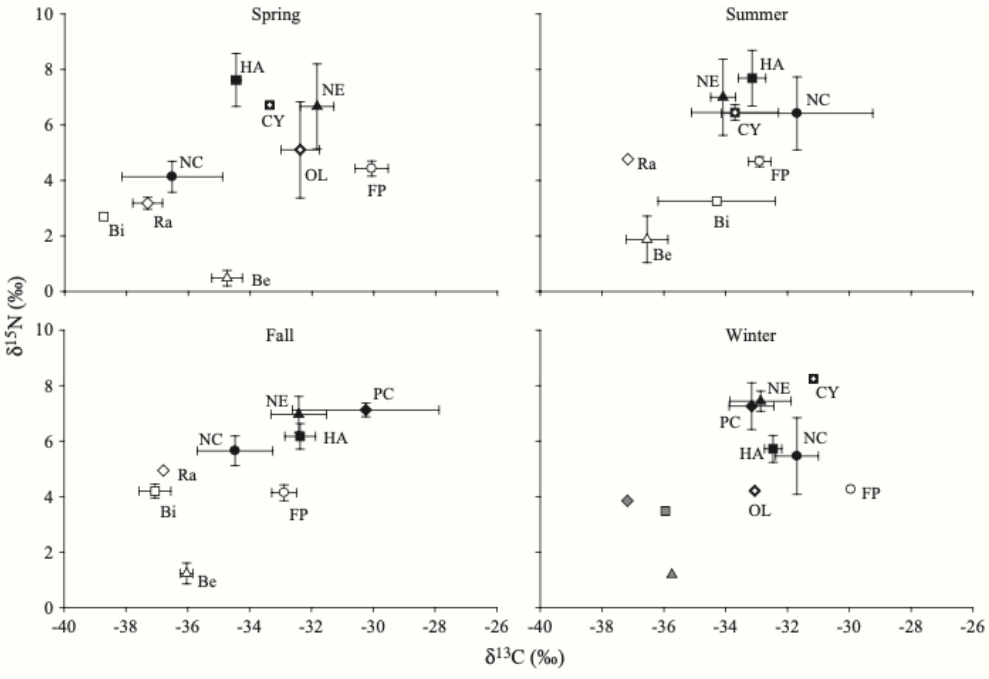


Figure D3. Meiofauna trophic relations in a river over the four seasons. BI = biofilm; OL = oligochaetes; HA = harpacticoid copepods; NE = nematodes. Retrieved from Schmid-Arraya et al., (2016)

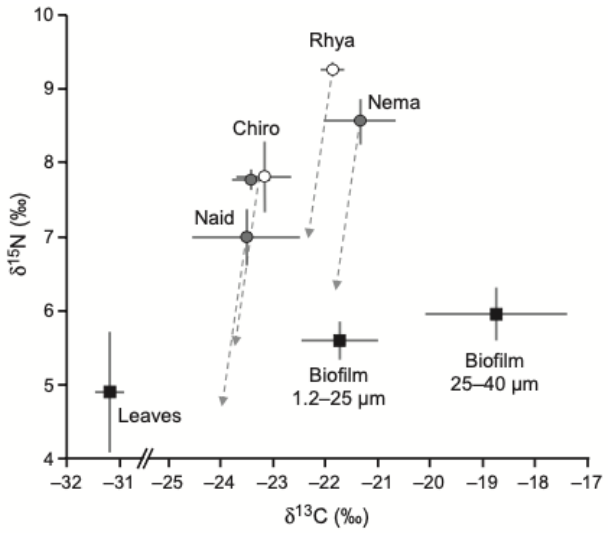


Figure D4. Trophic positions of meiofauna (grey circles) and macrofauna (white circles) from the Garonne river biofilm. The dotted arrows represent the expected trophic enrichment, pointing to the trophic position of the probable main food source. Obtained from Majdi, et al. (2012)

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