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Micro- and nanoplastics in freshwater ecosystems—interaction with and impact upon bacterivorous ciliates

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The ubiquitous occurrence of microplastics and nanoplastics in aquatic environments is of major concern as these priority pollutants are readily ingested by a wide variety of aquatic organisms. Although quantitative data on the interaction of microplastics and even more so on nanoplastics in freshwater environments and their interaction with the aquatic food web are still limited, studies have nevertheless demonstrated that even micro- or nanosized plastic particles can be ingested by various members of the zooplankton functioning as primary consumers. Bacterivorous ciliates are crucial members of the microzooplankton. These fascinating microorganisms are critical components of microbial loops in freshwater environments and are essential links between different trophic levels within the aquatic food web. Ingestion of microscopic plastic particles affects the ciliate cell on a cellular and even on the molecular level. Physical and chemical characteristics such as size, density, and surface properties influence the stability, distribution, retention, transportation, and bioavailability of the microplastic particles for ingestion by ciliates. In turn, the environmental fate of microplastics and nanoplastics can affect their ecotoxicity via surface modifications, such as forming the so-called eco-corona. The consequences of the interaction of ciliates with microplastics and nanoplastics are the potential bioaccumulation of plastic particles through the food web and the possible interference of these emerging pollutants with controlling bacterial and possibly even viral abundance in freshwater environments. Due to the limited data available, studies elucidating the environmental bacterivorous ciliate-micro-/nanoplastics interaction are a priority research topic if we want to holistically assess the environmental fate and ecotoxicity of these pollutants.

KEYWORDS

microplastics, nanoplastics, pollution, ingestion, freshwater, microzooplankton, ciliates, protists

1 Introduction

Pollution by plastics has emerged as one of the most severe environmental threats. The rapid increase in production and consumption of plastic materials has resulted in large amounts of plastic waste being released into the environment; of the 400 million tons of plastic waste that is generated worldwide, only 9% is recycled,

while about 80% accumulates in landfills or remains mismanaged and may end up in the natural environment (Amobonye et al., 2021; OECD, 2022; Haque and Fan, 2023; Lamichhane et al., 2023). It is estimated that up to 14 million tons of plastic waste end up in the ocean annually, making up 50%–80% of all marine debris that accumulates globally, of which 70%–80% are from land-based sources such as rivers, stormwater runoff, wastewater discharges, or transport of land litter by wind (Jambeck et al., 2015; Chaturvedi et al., 2020; Watt et al., 2021).

The presence of microscopic plastic materials in the aquatic environment is a major global concern. Based on the particle size, an important classification criterion as it can affect particle properties and the environmental fate, they could be categorized as microplastics if ranging from 1–1,000 μm and as nanoplastics if ranging from 1–1,000 nm, thus matching scale and prefix, as suggested by Hartmann et al. (2020). However, for the purpose of this review, we adopt the size classification proposed by EFSA (2016) when assessing the presence of micro- and nanoplastics in the food web and Besseling et al. (2019) when addressing the ecological risk of these microscopic pollutants, with an upper size limit of microplastics set at 5 mm and nanoplastics defined as ranging from 1 to 100 nm. Micro- and nanoplastics are considered persistent emerging pollutants due to their environmental and public health impact (Lambert and Wagner, 2018; Lamichhane et al., 2023).

Micro- and nanoplastics are either derived from primary or secondary sources. Primary plastic waste is intentionally manufactured in microscopic sizes and is added to a wide range of products such as household and clothing fibers, drug delivery formulations, cosmetic products, fertilizers, and paints for specific functions (e.g., abrasiveness, thickening, and stability) (Toussaint et al., 2019). Primary microplastics enter the environment through discharge from households, agriculture runoff, and industrial waste, and they account for 15%–30% of all plastics released into the environment (Toussaint et al., 2019; Ter Halle and Ghiglione, 2021; Oliveira et al., 2022). The secondary derivatives are a result of larger plastics that undergo weathering once exposed to the environment due to physical, chemical, and biological agents forming plastic material of microscopic size (Thompson et al., 2009; Gonçalves and Bebianno, 2021; Haque and Fan, 2023). Secondary microplastics greatly contribute to microplastic pollution, accounting for 70%–80% of all plastic released into the environment (Mariano et al., 2021; Kiran et al., 2022).

Microplastics are considered a major threat to aquatic organisms. Their small size range and their varying density allow them to occupy different areas of the water column and sediments, making them bioavailable for interaction with aquatic organisms, potentially enabling detrimental interactions such as ecotoxicity and bioaccumulation along the food web (Lusher, 2015; EFSA, 2016; Rakib et al., 2023). Furthermore, the large surface area to volume ratio of microplastics and their predominantly hydrophobic properties renders them prone to adsorbing organic pollutants, heavy metals, or polymer leaching that may negatively affect the surrounding hydrosphere (Lusher, 2015; Costigan et al., 2022). Microplastics can act as a vector for transporting pollutants to and within the ecosystem via the food chain or by altering the solubility of hydrophobic pollutants when adsorbed onto the plastic particle, which may increase their transport and consequently impact their

distribution and bioavailability (Amelia et al., 2021; Gateuille and Naffrechoux, 2022). In addition, microplastics were identified as possible attachment sites and vehicles for bacterial pathogens, increasing their mobility within aquatic ecosystems (Hou et al., 2021; Pham et al., 2021; Beans, 2023).

The effect of microplastics' interaction with aquatic organisms and the impact on the carbon cycle is well documented for marine environments. Ingestion has been reported for species across various trophic levels, including zooplankton (Cole et al., 2013), copepods (Fibbe et al., 2023), and planktivorous forage fish (Beer et al., 2018). However, not much is currently known about the ecologically important group of bacterivorous ciliates, key members of the microzooplankton. Furthermore, there is still a scarcity of studies on the impact of microplastics in freshwater environments in comparison to studies targeting marine environments (Badea et al., 2023). The aim of this work is, therefore, to undertake a comprehensive review of data available on the effect of microplastics and nanoplastics on crucial members of the freshwater food web, the bacterivorous ciliates, highlight data gaps, and address the factors influencing the bioavailability of microplastics and nanoplastics for ingestion. To understand the environmental fate and the impact of microplastics and nanoplastics in freshwater environments, it is imperative to appreciate their occurrence, distribution, and behavior within this vital ecosystem.

2 Occurrence and distribution of micro- and nanoplastics in freshwater environments

Freshwater environments are vulnerable to small plastic pollution because of the vicinity of waste-generating pathways, i.e., the wastewater treatment plants, landfills, and dumpsites. The contribution of various sources to plastic pollution has been poorly quantified in freshwater environments. The occurrence of microplastics is often correlated with anthropogenic activities; thus, high abundance is generally detected in lakes and rivers in urbanized regions (Schmidt et al., 2020; Dalu et al., 2021; Kunz et al., 2023). Effluents discharged from Wastewater Treatment Plants (WWTPs) are a significant source of microplastics in freshwater environments, as microplastics are not removed quantitatively in the current systems (Wu et al., 2022). Thus, the number of microplastics annually released into rivers via WWTP effluents in Germany was estimated at 7×10^{12} (Schmidt et al., 2020), and a combined daily release of 5×10^8 – 10^9 microplastics via treated effluent for three wastewater treatment plants in the United States (Conley et al., 2019). It is noteworthy that the conventional treatment processes (i.e., stirring, mixing, and pumping) used in the majority of wastewater treatment plants can be a potential source for plastic fragmentation, affecting the size of microplastics and thus releasing substantially larger amounts of much smaller plastic particles into the aquatic environment than assumed (Gangula et al., 2023; Indhur et al., 2023; Monira et al., 2023).

Freshwater environments are considered a major sink for microplastics and are involved in the plastic cycle where larger polymers are transformed, resulting in secondary micro- and nanoplastics. Various studies have reported the occurrence of microplastic in rivers (Horton et al., 2017; Wang et al., 2017;

Hurley et al., 2018) and lakes (Ballent et al., 2016; D'Avignon et al., 2022). Microplastics have also been detected in South African rivers, with a total of 2,406 microplastics per kg detected in the sediment across the Mvudi River in Limpopo, South Africa, which is subjected to various pollution sources, including WWTP discharge (Dalu et al., 2021). Similarly, for the Plankenburg River in the Western Cape province in South Africa, which receives agricultural pollutant inputs as well as inputs through formal and informal residential neighborhoods and industrial activities, up to 9.25 microplastic particles/L were detected in water samples and 2133.33 microplastic particles/kg in sediment samples (Apetogbor et al., 2023). Microplastics were prevalent in the sediment samples of the Vaal River, which supplies potable water to communities in Gauteng, at a concentration of 463.28 ± 284.08 particles/kg sediment dry weight, with small plastic particles of <2 mm accounting for 82% of the total plastic particles (Saad et al., 2022).

The progressive breakdown of plastic debris results in nano-sized plastic particles, changing the plastic properties, reactivity, and impact; thus, nano-sized plastics may have a greater ecological impact than larger polymers such as microplastics. Nanoplastic particles, however, present a technical difficulty in sampling, identifying, and analyzing these smallest plastic particles in the environment (Koelmans et al., 2015; Mariano et al., 2021), even though new analytical techniques have been reported (Mogha and Shin, 2023; Zhang et al., 2023). Nonetheless, nanoplastics have been detected in the North Atlantic Sea, representing a mixture of polystyrene, polyethylene, terephthalate, and polyvinylchloride (Ter Halle et al., 2017) and in surface waters from lakes and streams of Siberia and Sweden at average concentrations of $51 \mu\text{g} \times \text{L}^{-1}$ and $563 \mu\text{g} \times \text{L}^{-1}$, respectively (Trevisan et al., 2022). Although data are lacking, wastewater treatment plants are considered a major source of releasing nanoplastics into the environment. It is assumed that 25% of the nanoparticles that enter the environment are released from wastewater treatment plants (Mohana et al., 2021).

The distinct physicochemical characteristics of micro- and, even more so, nanoplastics have a major influence on their behavior, transportation, and distribution. Microplastics are unevenly distributed within the aquatic environment. The vertical distribution of plastic particles in the aquatic environment is determined by the polymer type, size, shape, density, and chemical composition (Duis and Coors, 2016). Plastic particles with a lower density ($< 1 \text{ g/cm}^3$) than water tend to float on the surface of the water or are suspended within the water column, while particles with a higher density ($> 1 \text{ g/cm}^3$) tend to sink to the bottom; thus, sediments can be a significant sink for microplastics in aquatic environments (Bellasi et al., 2020; Leiser et al., 2021; Viitala et al., 2022). Nanoplastics, conversely, tend to float on the surface or remain suspended in the water column due to the low density and thus may have a shorter retention time in the river systems.

Once they enter the aquatic environment, micro- and nanoplastics are prone to aggregation with either similar particles (homoaggregation) or different types of particles (heteroaggregation). Smaller-sized microplastics are more likely to aggregate than larger-sized microplastics, and such aggregation can change the size, shape, or density of the resulting particle aggregate; this, in turn, has a major influence on the stability

and mobility of such aggregates in waterbodies (Wang et al., 2017; Issac and Kandasubramanian, 2021). Nanoplastics have high colloidal stability due to the negatively charged carbon-containing polymers with many functional groups exposed on the surface, which play a vital role in the formation of aggregates (Kim et al., 2022). Again, aggregation increases their density and may cause them to settle in the sediment. The exposure of *Chlorella pyrenoidosa* to 500 nm polystyrene particles increased the secretion of extracellular polymeric substances and resulted in homo- and heteroaggregation by enabling the attachment of particles to algal cells (Nigam et al., 2022). As extracellular polymeric substances are involved in the auto-flocculation of microalgae, enhanced sedimentation of micro- and nanoplastics would be expected along with that of microalgae floc formation (Li et al., 2023). Several studies have reported on the interaction of nanoplastic particles with minerals in aquatic environments, which affects the environmental stability and transport of nanoplastics (Kim et al., 2022; Zhang et al., 2022). Thus, Nie et al. (2023) reported the heteroaggregation of negatively charged polystyrene nanoplastics with positively charged iron and aluminum hydroxide minerals.

When released into the aquatic environment or taken up by aquatic organisms, micro- and nanoparticles are subjected to biomolecules resulting from the metabolic activities of aquatic organisms that adsorb onto the surface of the particles, forming a biomolecular-coated layer called eco- or bio-corona (Nasser et al., 2020; Ekvall et al., 2021). Such a coating of the micro- and nanoplastics by an eco-corona would affect the hydrodynamic diameter as well as particle surface properties and reactivity (Giri and Mukherjee, 2021), as it provides a barrier between the reactive surface of plastic particles and the aquatic organisms (Natarajan et al., 2020; Xu et al., 2020; Liu et al., 2022). However, the impact on the ecotoxic properties of environmentally aged micro- and nanoparticles sporting a bio-corona might not be straightforward, as both increased and decreased ecotoxic effects have been reported. Giri and Mukherjee (2021) showed that biocoating of 200 nm polystyrene particles representing different surface chemistries with algal EPS reduced the ecotoxicity for cells of *Scenedesmus obliquus*, based on various endpoints. Similarly, Saavedra et al. (2019) demonstrated that exposure of the same particle type to alginate and river humic acid reduced the acute toxicity of such particles for *Daphnia magna*. In contrast, the biocoating of polystyrene nanoparticles with an eco-corona consisting primarily of proteins increased the acute toxicity of the particles for *D. magna* when compared to non-coated particles (Nasser and Lynch, 2016). The increased toxicity could have been due to increased particle size resulting in higher uptake or increased resemblance to food particles due to surface coating. Interestingly, nanoplastics exhibiting an eco-corona persisted longer in the gut, thereby reducing the feeding capacity of *D. magna* for algae. Therefore, more studies considering the potential impact of eco-corona formation on ecotoxic endpoints are needed for crucial members of the aquatic food web. This applies particularly to bacterivorous ciliates that might be negatively affected if a protein-based coating of micro- and nanoplastics occurs in the environment, rendering these particles potentially more attractive due to increased size and a surface coating mimicking natural prey.

3 Effect of microplastics and nanoplastics on aquatic organisms

The presence of plastic particles in the freshwater environment is a major concern for this ecosystem due to their small size, which occupies the same size range as microscopic plankton, making them bioavailable for ingestion by a wide range of organisms. Ingestion of microplastics has been reported in organisms such as Copepods (*Centropages typicus*) (Cole et al., 2013), zooplankton (*D. magna*) (Scherer et al., 2017), freshwater Tubifex worms (*Tubifex tubifex*) (Hurley et al., 2017), freshwater gastropod (*Lymnaea stagnalis*) (Weber et al., 2021), freshwater fish (*Rutilus rutilus*) (Horton et al., 2017), and omnivorous fish (*Puntius proctozysron*) (Kasamesiri and Thaimuangphol, 2020) leading to reduced food intake, delayed growth, tissue damage, oxidative stress, behavioral abnormalities, neurotoxicity, changes in lipid metabolism and mitochondrial bioenergetics, and growth retardation (Botterell et al., 2019; Bhuyan, 2022). However, the sensitivity of different aquatic species—unfortunately not including bacterivorous ciliates—to micro- and nanoplastic exposure differs substantially, as was illustrated by species' sensitivity distributions (Besseling et al., 2019). Upon ingestion, plastic particles can be transferred along the aquatic food web by the interaction of predator and prey representing the same or different trophic levels (Setälä et al., 2014). This was observed in the freshwater diving beetle *Cybister japonicus* after the consumption of zebrafish exposed to microplastics, which affected the diving beetle behavior and predation (Kim et al., 2018). Consequently, the potential for bioaccumulation increases with a decrease in plastic particle size.

Ingestion of nanoplastics has been reported in aquatic organisms such as brine shrimp larvae (*Artemia franciscana*), which affected their feeding, behavior, and physiology (Bergami et al., 2016), marine bivalves (*Mytilus galloprovincialis*), which induced immunomodulation and apoptotic processes (Canesi et al., 2015), and zooplankton (*Daphnia pulex*) causing immobilization, reproduction, and stress defense (Liu et al., 2019). Nanoplastics exhibit higher toxicity than microplastics due to the increased surface area, which enables the particles to be absorbed through tissues and organs, cross biological barriers, and even penetrate membranes (Liu et al., 2021). The buildup of nanoplastics has been detected in various organs of gills, brain, heart, liver, yolk sac, gonads, and digestive organs of vertebrate and invertebrate species, especially at sizes below 100 nm (Trevisan et al., 2022). Thus, Kashiwada (2006) observed polystyrene nanoplastics (39.4 nm diameter) distributed in gills and viscera, but also in testis, liver, and blood after ingestion by the Japanese rice fish (*Oryzias latipes*).

Microplastics and nanoplastics can be readily taken up by aquatic organisms; however, the degree of ingestion is largely influenced by the organismal feeding mode. Suspension or filter feeders are especially prone to ingestion of micro- and nanoplastic particles because they feed mainly on suspended particulate matter, and their feeding mechanism does not differentiate between food and non-food particles of the same size (Gonçalves et al., 2019; Benson et al., 2022). Setälä et al. (2014) reported higher amounts of polystyrene microplastics (2 and 10 μm diameter) in filter feeder

bivalves (*Mytilus trossulus* and *Macoma balthica*) than in deposit feeders (*Monoporeia affinis* and *Marenzelleria* spp.). A study by Scherer et al. (2017) reported an ingestion rate of 6 180 particles h^{-1} for a filter feeder, *D. magna*, 27 times (226 particles h^{-1}) and 52 times (118 particles h^{-1}) more than the ingestion rate for a collector-gatherer, *Chironomus riparius* and surface grazer, *Physella acuta*, respectively. Again, the formation of a bio-corona might affect uptake rates and residence time in the filter feeder exposed to micro- and nanoplastics in the environment, as highlighted above, with potentially adverse toxic effects. Even in non-filter feeding aquatic organisms from higher trophic levels, feeding traits might affect the microplastic uptake, as round goby (*Neogobius melanostomus*, a zoobenthivore) contained more microplastics in the gut than an omnivore (*Pimephales promelas*, fathead Minnow) fish of a similar size (McNeish et al., 2018).

4 Ingestion of microplastics and nanoplastics by ciliated microzooplankton

Studies on the ingestion of microplastics by zooplanktons demonstrate that plastic particles can pose a threat to organisms at the base of trophic levels in aquatic environments (Landry and Décima, 2017). However, studies have reported mainly on the effect of microplastics on mesozooplankton. One group of organisms that is frequently overlooked is the ciliated protozoans, recognized as the main group of microzooplankton.

Ciliated protozoans are filter feeders that can ingest microplastics readily. Microplastic particles $>1 \mu\text{m}$ usually require phagocytosis to enter eukaryotic cells (Liu et al., 2021). Thus, such microplastics enter the ciliate cell by phagocytosis, are internalized, and packaged in food vacuoles (Nilsson et al., 1979). Consequently, microplastic particles (2 μm) were detected in food vacuoles of *Paramecium aurelia* after 10 min of exposure to fluorescent polystyrene beads (Nugroho and Fyda, 2020). Similarly, polystyrene beads (1 and 2 μm) were detected after exposure in the spirotrich ciliates *Blepharisma japonicum*, *Spirostomum teres*, and *Euplotes* sp. (Budzial and Fyda, 2023). Feeding studies have demonstrated that the number of food vacuoles in ciliate cells increases concomitantly with the number of particles ingested (Sherr et al., 1987; Bulannga and Schmidt, 2022). A marine oligotrich ciliate, *Strombidium sulcatum*, ingested polystyrene microplastics with diameters of 0.5, 1.07, 2.14, and 5 μm , which had a negative impact on the abundance and biomass of the ciliate (Geng et al., 2021). The carbon biomass of *Uronema marinum* was reduced after ingestion of polystyrene microplastics (2.14 μm diameter) at high concentrations. At the same time, that effect was not observed at low microplastic concentrations (Zhang et al., 2021). A study by Bulannga and Schmidt (2022) reported that two holotrich ciliates, *Paramecium* sp., and *Tetrahymena* sp., ingested microplastics of 2, 5, and 10 μm diameter at the same rate as biological prey of similar size. Naęcz-Jawecki et al. (2021) reported that secondary micro- and nanoplastics prepared from household materials of PET, PS, PVC, and PhR ($<100 \mu\text{m}$) were ingested at the same degree as primary plastic particles (5 μm diameter) by the ciliate *Spirostomum ambiguum*, though at a lesser

extent compared to nutritional food particles (yeast cells). Earlier groundbreaking studies by Fenchel (1980a; 1980b) reported uptake of latex beads of size 0.09–5.7 μm in fourteen ciliates isolated from freshwater environments. More recently, it was shown that *Paramecium bursaria* exhibited a change in swimming patterns, a reduction in swimming speed, and an increase in oxidative stress when exposed to Carboxy YG microspheres of 1 μm diameter compared to unexposed ciliate cells (Zhang et al., 2022). However, studies comparing the impact of environmentally aged micro- and nanoplastics exhibiting altered hydrodynamic size and surface properties on the feeding pattern of bacterivorous ciliates are unfortunately missing.

Research on nanoplastics is in its infancy. Thus, there is still a lack of quantitative data on nanoplastics in the aquatic environment and their impact on aquatic organisms. However, some studies have described the toxic effect of nanoplastics and/or particles of a similar size range. Compared to microplastics, nanoplastics can be ingested directly or indirectly and can exhibit higher potential risks due to their smaller size and larger surface area, causing toxic effects at cellular and even molecular levels (Gong et al., 2023). Mortimer et al. (2016) detected carbon nanotubes 500 nm in length and 36.5 nm in diameter in the food vacuoles of *Tetrahymena thermophila* after 2 h of exposure in a rich growth medium. The growth of *T. thermophila* was inhibited when exposed to polystyrene nanoplastics of 20.8–24.1 nm diameter due to induced calcium homeostasis, consequently increasing mitochondrial permeability and the generation of reactive oxygen species (Wu et al., 2021). *Paramecium caudatum* ingested starch-coated multi-core magnetite nanoparticles of 200 nm diameter, which acted as a chemoattractant and internalized them within its food vacuoles (Mayne et al., 2019). Ingestion of non-nutritious particles can affect the feeding behavior of ciliates, as observed on a *Paramecium*, which effectively eliminated the cyanobacterium microcystis but with the ingestion rate drastically reduced when the ciliate was exposed to 0.1 mg/L ZnO nanoparticles (Zhang et al., 2022).

Indirect ingestion of nanoplastics can occur through ingesting natural prey with nanoplastics adsorbed on the prey cell. Tang et al. (2022) reported the penetration of nanoplastic particles into bacterial cells across the cell envelope, with nanoparticles accumulating inside the bacterial cells. The same authors observed a large number of 100 nm nanoplastic particles adsorbed and aggregated on the surface of *Acinetobacter johnsonii* AC15, which significantly increased the growth of bacteria, while Dai et al. (2022) observed 80 nm sized positively charged polystyrene embedded into the cell envelope of *Escherichia coli* with no apparent effect on the bacterial cell. Importantly, smaller-sized nanoparticles of ≤ 10 nm can easily penetrate the cell membrane (Frankel et al., 2020; Liu et al., 2023). The translocation through the cell membrane is determined by the surface charge of the particle; positively charged particles display more favorable electrostatic interactions with bacterial membranes than negatively charged particles (Tang et al., 2022). Indirect ingestion of nanoplastics has been reported for filter-feeding invertebrates such as *D. magna*, which ingested *Chlamydomonas reinhardtii* with nanoplastic particles of 51 nm attached to the surface of the algal cell (Chae et al., 2018). Thus, the ingestion of microscopic prey carrying nanoplastics can be reasonably

expected to contribute to nanoplastics uptake and accumulation in bacterivorous ciliates.

5 Impact of micro- and nanoplastics without ingestion

The long retention of micro- and nanoplastics in the environment can have a negative impact on aquatic organisms. Microplastics may constitute a source of exposure to chemical pollutants due to additives contained in the microplastics. The chemical additives are usually of low molecular weight and weakly bound to the polymers, and they leach into the surrounding environment with the progressive weathering of the plastic polymer. The leaching rate is determined by environmental factors, such as temperature, light exposure, salinity, and turbulence (da Costa et al., 2023). Several studies have reported that microplastic leachates reduce microbial abundance and diversity, alter microbial communities, and affect metabolic activities (Li et al., 2020; Yang et al., 2023; Zhang and Liu, 2023). Different types of micro- and nanoplastics would elicit different toxic effects on the microorganisms based on the type of chemical additive on the surface of plastic particles (Brehm et al., 2022). Recycled PET fragments, which are described to contain the most chemical additives, were found to display the strongest ecotoxic effect on a mussel (*Dreissena bugensis*) (Brehm et al., 2022). The emergence success of the freshwater dipteran *C. riparius* and the reproduction in *D. magna* were reduced when exposed to PVC leachates (Scherer et al., 2020; Zimmermann et al., 2020). Even at the base level of the aquatic food web, exposure of *Raphidocelis subcapitata* to polyethylene terephthalate, polyvinyl chloride, and polystyrene microparticles affected the synthesis of biomolecules such as protein, lipid, and carbohydrate (Abinandan et al., 2023), and PVC microplastics shifted the bacterial community of anaerobically digested waste and activated sludge in the direction against hydrolysis-acidification and methanation (Wei et al., 2019). Ciliates are generally sensitive to chemical contaminants, and some ciliate species are considered efficient bioindicator organisms for detecting pollutants in aquatic ecosystems (Bogaerts et al., 2001; Láng and Kóhidai, 2012). Similarly, ciliates would be affected by microplastic leachates, although their response may differ from the responses reported for other non-ciliate plankton members present in aquatic ecosystems.

6 Bioaccumulation of microplastics and nanoplastics in the aquatic food web through ciliates

Ciliated microzooplankton are an integral component of the freshwater planktonic food web and ciliates are described as voracious feeders of bacterio- and phytoplankton, serving as an essential food source for larger zooplankton from higher trophic levels, thereby connecting different trophic levels (Finlay and Esteban, 1998; Montagnes et al., 2010; Setälä et al., 2014). Moreover, ciliated protozoa are an essential source of nutrients for the early stages of fish larvae; thus, there is a direct link between the microbial loop and the fish level within the food web (Montagnes et al.,

2010; Zingel et al., 2019). A trophic link between picoplankton and benthic suspension feeders was reported when the bacterivorous ciliate *Uronema* sp. was grazed by the oyster *Crassostrea gigas* (Le Gall et al., 1997).

Ciliates can be an important route for transferring microplastics present in the water body to organisms in the aquatic food web (Setälä et al., 2014; Carbery et al., 2018; Saeedi, 2023). Transfer of microplastics in the aquatic food web has been demonstrated with Silverlake larvae (*Menidia beryllia*), which ingested a marine tintinnid ciliate, *Favella* sp. containing polyethylene particles (10–20 µm) after 1 h of exposure to plastic particles (Athey et al., 2020). This study further demonstrates that microplastics can be a vector for the transfer of adsorbed pollutants in the food web as ciliates containing microplastics treated with the insecticide DDT were ingested by and detected in the larvae at higher rates than ciliates containing untreated microplastics. Stienbarger et al. (2021) reported that fish larvae of *Centropristis striata* ingested higher numbers of low-density polyethylene microspheres (10–20 µm) through ingestion of the ciliate *Favella* sp. than direct ingestion of the plastic particles from seawater.

7 Factors determining bioavailability of micro- and nanoplastics for ciliates

The toxicity of microplastics and nanoplastics primarily depends on the bioavailability of the particles for ingestion by aquatic organisms, while the bioavailability of micro- and nanoplastics for ciliates is determined by the size, density, and abundance of these priority pollutants in freshwater environments.

7.1 Plastic particle size

Ciliated microzooplankton have a typical cell size range of 20–200 µm and mainly feed on suspended bacterioplankton. They are described as selective feeders that only distinguish food particles based on mechanical properties such as size. Thus, the plastic particle must be within the size spectrum that can be ingested by the ciliate cell as regulated by the oral morphology. Ciliates have a preferred size range of particles they can ingest, which typically is in the bacterial cell size range (Gonzalez et al., 1990). Ingestion of microplastics has mostly been reported for particles matching the size range for ciliate microbial prey (Fenchel, 1980a; Pace and Bailiff, 1987; Sherr et al., 1987). Ciliates of a cell size from 20 to 40 µm show preference for particles up to 5 µm (Bermúdez et al., 2021). The omnivorous freshwater ciliates, *Halteria* cf. *grandinella*, had a high grazing efficiency for planktonic prey size ranges (0.5–5 µm) with the highest grazing rates for 2.78 µm latex beads and no significant ingestion for 0.22 µm latex beads (Jürgens and Šimek, 2000). Some larger-sized ciliates can ingest even larger microplastics up to 10 µm, as reported for *Paramecium* sp., *Euplotes mutabilis*, and *Tintinnopsis lobiancoi*, although they showed high ingestion rates for particles up to 5 µm (Wilks and Sleight, 1998; Setälä et al., 2014; Bulannga and Schmidt, 2022). Furthermore, ciliates can ingest nanosized particles, as demonstrated with *T. thermophila* ingesting particles ranging in

size from 20 nm to 500 nm (Guo et al., 2022); they would, however, require much higher concentrations of nanoplastics to induce food vacuole formation than they would for microplastics. Interestingly, recent studies have shown that viruses, representing the nanoplastics size range and mostly overlooked as potential nutrient sources for ciliates in the aquatic food web, are serving, like bacterial prey matching the lower range of the microplastic size range, as potential prey for ciliates, contributing to growth or at least cell maintenance (Karalyan et al., 2012; DeLong et al., 2022; Bulannga and Schmidt, 2023; Olive et al., 2023). However, when present in aquatic environments, microplastics and nanoplastics may form aggregates of a size that exceeds the particle size range that ciliates can ingest, thereby decreasing the bioavailability of these microscopic plastic particles.

7.2 Plastic particle concentrations

Grazing studies employing ciliates have demonstrated that the ingestion of food particles representing micro and nanoparticle size ranges (e.g., bacterial and viral prey) is concentration-dependent. There is a minimum concentration of prey that can induce particle uptake and food vacuole formation, and the ingestion rate increases with increasing prey concentration until the maximum concentration is reached (Kivi and Setälä, 1995; Pfister and Arndt, 1998). Similarly, the concentrations of microplastics and nanoplastics would have the same effect on the ingestion and food vacuole formation. As observed by Wilks and Sleight (1998), the number of microspheres ingested increased with increasing concentrations in *E. mutabilis*, from ≤ 4 particles \times cell⁻¹ \times h⁻¹ at a concentration from 10² to 10⁴ particles \times mL⁻¹ to 190 particles \times cell⁻¹ \times h⁻¹ at concentrations from 10⁵ to 10⁶ particles \times mL⁻¹ for all particle sizes tested (0.57, 1.90, 3.06, 5.66, and 10 µm diameter). Microplastics in freshwater environments can reach concentrations of up to 10,000 particles/L, which can be adequate for ingestion by ciliates (Kunz et al., 2023). Plastic particles can be ingested simultaneously with biological prey, as observed in *Sterkiella* sp., which contained both the microalgae *Isochrysis galbana* (at an average cell size of 5 µm) and polyethylene microbeads (1–5 µm diameter) (Bermúdez et al., 2021), or *S. ambiguum*, which contained both yeast cells and microplastic particles in its food vacuoles (Należek-Jawecki et al., 2021).

Although the feeding mechanism of ciliates mainly discriminates food particles based on size, some species of ciliates can regulate the uptake of non-nutritious particles by either ingesting the particles at a slower rate or by retaining them within the cell for only a short period of time. Moreover, some species can sense chemical cues of their preferred prey, respond rapidly to the point source, and chemotactically congregate at the location (Müller et al., 1965; Sherr et al., 1988; Gruber et al., 2009). In case of high prey concentrations where the energy requirement of ciliates is satisfied, the toxic effect of microplastics and nanoplastics may be reduced. However, so far, this phenomenon has only been reported in *D. magna*, where the toxic effect of microplastic particles was reduced at high food quantity and was attributed to continuous ingestion of food particles, which increased the rate of egestion (Lyu et al., 2022). It has been observed that once the maximal uptake is reached in ciliates, the rate of food vacuole formation

establishes an equilibrium where a digested food vacuole is egested for every food vacuole formed containing ingested food (Berger and Pollock, 1981; Dolan and Šimek, 1997). However, ciliates may not encounter prey organisms at sufficiently high densities in freshwater environments to reduce the ecotoxicity of micro- and nanoplastics, except for polluted waters, sludge, or sediments rich in organic matter.

7.3 Vertical distribution of plastic particles

Ciliated microzooplankton exist in a vertical gradient within the aquatic environment, and their distribution is influenced by their nutritional and oxygen requirement and prevailing environmental conditions such as pH, temperature, and oxygen availability. Ciliates are categorized into planktonic ciliates that occupy the pelagic zone with the highest abundance in the epilimnion and metalimnion zone or benthic ciliates, which occupy the benthic zone in the freshwater environment and account for 10% invertebrate biomass in this zone (Finlay and Esteban, 1998; Mieczan, 2008; Sonntag et al., 2011; Lei et al., 2014; Zingel et al., 2019). Both ciliate communities, therefore, encounter microplastics and nanoplastics; the pelagic ciliates are more susceptible to nanoplastics and microplastics of low density that float on the surface, suspended in the water column and resuspended particles caused by storms and bioperturbation, while benthic ciliates are more susceptible to microplastics of high density and aggregated nanoplastics settling and accumulating in the sediments.

8 Conclusion and outlook

The presence of microplastics and nanoplastics in freshwater environments has a negative ecological impact. Their size makes these pollutants bioavailable for ingestion by a wide variety of freshwater organisms, including bacterivorous ciliate microzooplankton that occupies a central position within the microbial food loop. This review highlighted the presence and interaction of microscopic-sized plastic particles, ubiquitous in freshwater environments, with ciliated microzooplankton potentially affected negatively once these pollutants are ingested. Physicochemical characteristics such as size, density, and surface properties (e.g., the so-called eco- or bio-corona) influence the vertical distribution, aggregation, and retention of micro- and nanoplastics in freshwater environments and play a key role in determining their interaction with ciliates, ciliate prey, and their environmental fate. At the same time, this review highlighted the need for additional studies to improve our understanding of the interactions between bacterivorous ciliates and micro- and nanoplastics under conditions resembling environmental scenarios. For example, it is presently unclear if environmental aging of micro- and nanoplastics would affect uptake by bacterivorous ciliates and thus interfere with feeding on nutritious prey. Similarly, there are currently no studies available evaluating if environmentally exposed and thus surface coated (e.g., eco-corona) micro- and nanoplastics are more or even less toxic for these filter-feeding microzooplankton members than pristine particles typically employed in laboratory studies. The capability of ciliates to ingest microplastics and

nanoplastics implies that plastic particles that are otherwise too small to be ingested by zooplankton may enter food webs through the ciliates, which serve as food for other members of the aquatic food web and thus enable the transfer of micro- and nanoplastics to higher trophic levels. Bioaccumulation of micro- and nanoplastics might, therefore, occur in the aquatic food web, with possible negative consequences even for humans. Moreover, ingestion of micro- and nanoplastics interferes with protozoans' top-down impact by reducing the ingestion of nutritional food particles such as bacteria and possibly even viruses. The abundance of micro- and, specifically, nanoplastics in aquatic environments is still vastly underreported due to the lack of straightforward, reliable analytical and sampling methods to detect and quantify such particles. Evidently, more studies are needed to understand better the effects that microplastics and, even more so, nanoplastics exhibit on organisms near the base of the freshwater food web, such as bacterivorous ciliates and not yet evaluated other bacterivorous protists such as naked or testate amoebae, which will allow environmental risk assessments of microplastics and nanoplastics not just at the individual but also at the population and the broader ecosystem level.

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