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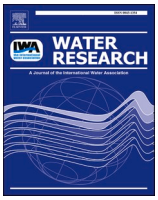

Do weathered microplastics impact the planktonic community? A mesocosm approach in the Baltic Sea

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ABSTRACT

Microplastics (MPs) are ubiquitous pollutants of increasing concern in aquatic systems. However, little is still known about the impacts of weathered MPs on plankton at the community level after long-term exposure. In this study, we investigated the effects of weathered MPs on the structure and dynamics of a Baltic Sea planktonic community during ca. 5 weeks of exposure using a mesocosm approach (2 m^3) mimicking natural conditions. MPs were obtained from micronized commercial materials of polyvinyl chloride, polypropylene, polystyrene, and polyamide (nylon) previously weathered by thermal ageing and sunlight exposure. The planktonic community was exposed to 2 μg L^{-1} and 2 mg L^{-1} of MPs corresponding to measured particle concentrations (10–120 μm) of 680 MPs L^{-1} and 680 MPs mL^{-1}, respectively. The abundance and composition of all size classes and groups of plankton and chlorophyll concentrations were periodically analyzed throughout the experiment. The population dynamics of the studied groups showed some variations between treatments, with negative and positive effects of MPs exhibited depending on the group and exposure time. The abundance of heterotrophic bacteria, pico- and nanophytoplankton, cryptophytes, and ciliates was lower in the treatment with the higher MP concentration than in the control at the last weeks of the exposure. The chlorophyll concentration and the abundances of heterotrophic nanoflagellates, *Astromoeba*, dinoflagellate, diatom, and metazooplankton were not negatively affected by the exposure to MPs and, in some cases, some groups showed even higher abundances in the MP treatments. Despite these tendencies, statistical analyses indicate that in most cases there were no statistically significant differences between treatments over the exposure period, even at very high exposure concentrations. Our results show that weathered MPs of the studied conventional plastic materials have minimal or negligible impact on planktonic communities after long-term exposure to environmentally relevant concentrations.

1. Introduction

Over the last few decades, the accumulation and impacts of plastics in the aquatic environment have become a major global concern (Ostle et al., 2019; MacLeod et al., 2021). The total amount of plastics in aquatic ecosystems is unknown, but it has been estimated that the ocean already contains over 170 trillion floating plastic particles (Eriksen et al., 2023), and up to 12 MT of plastics enter the ocean yearly (Jambeck et al., 2015). With global plastic production increasing (OECD, 2022) and considering the current management of plastic waste (Borrelle et al., 2020), the amount of plastics entering the aquatic systems is projected to triple by 2040 (UNEP, 2021). Research on microplastics (MPs, 1μm- 5 mm) and nanoplastics (NPs <1 μm) has grown rapidly in recent years, showing that these small plastic particles can be found in

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all environmental compartments (Lim, 2021; OECD, 2022).

MPs can cause harmful impacts on marine organisms through multiple mechanisms, including physical effects related to the ingestion, entanglement, or adhesion of MPs (Wright et al., 2013; Enyoh et al., 2020; Bour et al., 2021). Furthermore, there is a potential "vector effect" whereby sorbed pollutants (Koelmans, 2015; Hartmann et al., 2017; Camacho et al., 2019; Fu et al., 2021; Wang and Guo, 2022), additives (Koelmans et al., 2014; Kühn et al., 2020) or pathogenic microbes (Fackelmann et al., 2023) can be transferred from ingested MPs to the tissues. Additionally, exposure to leached plastic additives in water can lead to toxic effects (Oliviero et al., 2019; Gunaalan et al., 2020; Tian et al., 2021; Page et al., 2022; Almeda et al., 2023; Bournaka et al., 2023).

Field studies have demonstrated that ingestion of MPs is very frequent in marine vertebrates (e.g., Duncan et al., 2019; Kühn and van Franeker, 2020), with half of the studied fish found with ingested MPs globally (Wootton et al., 2021). Mortality by marine macroplastic debris is well documented and induced gastrointestinal obstructions have been demonstrated in marine megafauna such as sea turtles and seabirds (Duncan et al., 2019; Roman et al., 2021). Still, few negative effects caused by the ingestion of MPs have been found in wild marine organisms (Porcino et al., 2023). Except for seabirds (Kühn et al., 2020), the role of MPs as vectors of hydrophobic organic pollutants in aquatic organisms seems to be minor compared to other sources (dietary intake and water) under environmentally relevant scenarios (Koelmans et al., 2016, 2022). However, there is increasing evidence that the toxicity of plastic additive leachates is the main driver of the deleterious effect of plastic pollution on aquatic organisms (Capolupo et al., 2020; Gunaalan et al., 2020; Barrick et al., 2021; Beiras et al., 2021; Almeda et al., 2023), and the negative ecological impacts of certain plastic additives have been demonstrated in the environment (Tian et al., 2021).

Marine MPs are very diverse in terms of sources (e.g., textiles, tires, paints) and thus in their polymer and additive composition, making their ecotoxicological characterization challenging. Most of our knowledge of the effects of MPs on marine biota comes from controlled, experimental work in which they are exposed to virgin pristine MPs (beads) of specific pure polymers (Setälä et al., 2014; Kokalj et al., 2018; Botterell et al., 2019; Fulfer and Menden-Deuer, 2021). However, data from studies on the toxicity of MPs from commercial plastic formulations and products that comprise both polymers and functional additives in new and degraded conditions are limited (Botterell et al., 2019; Alimi et al., 2022). Additionally, marine plastic debris undergoes changes caused by weathering processes (e.g., fragmentation, photooxidation, and biofouling) (Gewert et al., 2018; Duan et al., 2021) that modify their physicochemical properties with time (Liu et al., 2020). Changes include the leaching of additives and degradation products that may increase their toxicity to aquatic organisms (Bejgarn et al., 2015; Simon et al., 2021). However, the influence of ageing and weathering processes on the toxicity of MPs is unclear. Some studies have found that biofouling can increase the ingestion of MPs (Vroom et al., 2017) and their sinking rates (Kaiser et al., 2017). Fragmentation of plastic debris into small plastic particles with higher surface areas may accelerate the leaching of additives into the water. The leaching process in small MPs is notably faster, decreasing over time (Teuten et al., 2009; Sarker et al., 2020) and ultimately leading to the release of their hydrophilic additives and consequently reducing their toxicity. Hence, more research is needed to evaluate how ageing and weathering influence the toxicity of plastics to aquatic organisms.

Planktonic organisms are pivotal in aquatic food webs and global biogeochemical cycles. The impacts of MPs on planktonic organisms have primarily been investigated in laboratory microcosm studies, often using a species-specific approach, and focusing on acute exposure scenarios (Botterell et al., 2019). Studies on the acute effects of MPs on planktonic communities have yielded various results, including negative outcomes (Cole et al., 2013, 2015; Fulfer and Menden-Deuer, 2021; Shore et al., 2021), positive responses (Canniff and Hoang, 2018; Chae et al., 2020), and no effects (Rodríguez-Torres et al., 2020; Niu et al., 2021; Traboni et al., 2023). These outcomes seem to be influenced by the choice of species, MPs concentration, type of plastic used, and experimental conditions (Botterell et al., 2019). Additionally, while most acute bioassays focus on single polymer-type exposures, planktonic communities in the environment are exposed to a suite of different plastic polymers. Furthermore, little is still known about the effects of MPs on plankton at the community level under long-term exposure. Community-level experiments are important to evaluate the indirect effects of MPs on other trophic levels. For instance, reduced grazing by zooplankton due to the ingestion of MPs can lead to lower trophic pressure on phytoplankton, stimulating their growth. Dissolved organic matter (DOM) leached by plastics can also stimulate the growth of heterotopic bacteria (Romera-Castillo et al., 2018), which in turn may affect the microbial loop and the rest of the marine food web. More studies with natural plankton communities mimicking real marine environmental conditions are needed to better evaluate the ecological effects of plastic pollution on aquatic systems. Along these lines, mesocosm experiments are useful research tools to help fill the gap between laboratory bioassays at the species level and field studies at the population and ecosystem level (Stewart et al., 2013; Båmstedt and Larsson, 2018).

In this study, our general aim was to assess the effects of MPs on the composition and dynamics of planktonic communities. We hypothesize that exposure to weathered MPs can impact the structure of planktonic communities, particularly at high MP concentrations. Our specific objectives were: 1) to determine the effects of MPs in all plankton components, from bacteria to mesozooplankton, and 2) to evaluate the influence of plastic concentration and exposure time on the effects of weathered MPs on plankton. To accomplish these objectives, we used a mesocosm approach in which a Baltic plankton community was exposed to small size (<120 μm) weathered micronized plastics at two different exposure concentrations for about 5 weeks.

2. Materials and methods

2.1. Plastic materials, weathering, and micronization

Weathered microplastics (MP) were prepared from degraded commercial plastic products frequently identified in marine litter. Products comprised nylon strips (polyamide (PA)), plasticized polyvinyl chloride (PVC) flexible packaging films, polypropylene (PP) plastic sleeves, and polystyrene (PS) cups. These synthetic polymers are commonly found in marine systems (Erni-Cassola et al., 2019) and include both high- and low-density plastics (PP-0.9 g $cm^{-3;}$ plasticized PVC -1.4 g cm^{-3}) (Table 1). All products selected were thin walled (ranging from 0.1 to 0.4 mm) transparent or white. All products had been naturally aged

Table 1
Plastic materials used to produce MPs for this study.

Plastic product description and source	Polymer in product	Pollutants identified using GC–MS after weathering process
Plastic document sleeves https://plant2plast.dk/	Polypropylene (PP)	Propylene monomer Ethanal
PVC packaging film for documents, transparent (21 × 30 cm) National Museum of Denmark's research collection from 1970	Plasticized polyvinyl chloride (PVC)	Hydrogen chloride Bis(2-ethylhexyl) phthalate
PA (nylon) cable strips, transparent (0.5 × 10 cm) www.silvan.dk	Nylon (polyamide) (PA)	None detected
PS cups, transparent (4 cm diameter) https://plant2plast.dk/	Polystyrene (PS)	Styrene monomer

from storage in the dark, indoors in the National Museum of Denmark at 18–25 °C for between 5 and 30 years prior to further treatments. To promote their expected degradation processes with time, all naturally aged products were exposed to accelerated thermal ageing at 70±2 °C in an industrial Memmert convection oven for 30 days, followed by exposure to Danish weather for a period of 60 days. Plastics products were secured to exposure stands facing south in the open courtyard area of the National Museum of Denmark's site in Copenhagen, Denmark. The exposure stands had dimensions of 1.0 × 1.0 m^2 and were adjusted to have 60 ° to the horizontal plane and located 2.5 m above ground (Figure S1). They were constructed from marine grade stainless steel to comply with the standard design for atmospheric corrosion testing (ISO8595, 1999). Accurate light measurements were recorded at 12 hour intervals throughout the 60 day weathering period using HOBO Pendant MX loggers (MX2202 model) attached to the stands and showed an average light intensity of 9633.98 lux. MPs were produced from the weathered plastic products using a Cryomill (Cryogenic Mixer Mill CryoMill, Retsch GmbH, Germany). Weathered plastic items were first cut into small pieces and then ground in the Cryomill at 30 Hz for 2.5 min followed by a recess period of 30 s at 5 Hz for a total of 3 cycles. It was essential to keep the Cryomill colder than -10 °C during the grinding process to prevent the plastics from adhering together. The micronized plastics were sieved through a metal sieve (200 μm or 100 μm steel sieves). The size of the obtained MPs was characterized using a Coulter Counter (Beckman Coulter Multisizer 4e), and the mean sizes of the different polymers are provided in Table S1. Microscope images of the obtained microplastic fragments are shown in Figure S2.

2.2. Chemical analyses of the pollutants in MPs produced from weathered plastics

Gas chromatography-mass spectrometry (GC–MS) of ethyl acetate extracts of MPs was used to study the presence of degradation products and plastic additives qualitatively by the National Museum of Denmark. Small samples of MP (approximately 0.2 g) were placed in a 10 mL headspace vial. GC–MS quality ethyl acetate (2.00 mL) containing 10 μL/250 mL dimethyl azelate (CAS 1732–10–1) as an internal standard was added. This mixture was left to extract for 48 hours and then ca. 40 μL of the liquid extract was transferred to a glass vial and analyzed using a Bruker SCION 456GC-TQMS with a Restek Rtx-5 capillary column (30 m, 0.25 mm ID, 0.25 μm) programmed for at 1 mL min^{-1} helium flow. A sample (1 μL) was injected on the Programmed Temperature Vaporization (PTV) that was programmed to hold 80 °C for 1.00 min, raised to 315 °C at a rate of 200 °C min^{-1} and held at that temperature for 30 min. The split ratio was 15 for the first 0.5 min and then changed to 5. The GC oven temperature was 80 °C for 1.0 min and then raised to 250 °C at a rate of 15 °C min^{-1}, held at that temperature for 4 min, and then increased to 315 °C at 15 °C min^{-1} and held for 15.50 min. The ion source was an electron ionization (EI) at 250 °C and an ionization potential of -70 eV. The mass spectrometer was operated in full scan mode between *m/z* 45 and *m/z* 800. The assignment of peaks was based on searches in the NIST 2.0 mass spectral database (https://chemdata.nist.gov/) and compared with reference compounds when available (CAS 13,674–84–5). Table 1 shows the compounds developed by the weathered plastics. They comprised monomers formed from the oxidation of polymers, including propylene from PP plastic sleeves and styrene from PS cups, additives such as plasticizer bis(2-ethylhexyl) phthalate in PVC, and degradation products such as hydrogen chloride from PVC.

2.3. Description of the mesocosms and experimental setup

The experiment was conducted in October 2021 at the mesocosms facility in the Umeå Marine Sciences Centre, located in the Gulf of Bothnian in the Baltic Sea (N63°34; E19°50). Mesocosms consisted of insulated high-density black polyethylene cylindrical tanks (height 4.86 m, diameter 0.73 m) (Fig. 1). The temperature in the mesocosms was set up at three depths to form a thermocline (from 10.5 °C at the top to 9.5 °C at the bottom) to ensure complete convective mixing of the water every 12 h (Båmstedt and Larsson, 2018) and to mimic thermal conditions in the studied environment. The mesocosms were also equipped with an air bubbling system to create stirring on the surface. Air bubbling was done by lowering a PVC tubing with an outlet diameter of 18 mm to pre-defined depths and regulating the air pressure to give a bubbling frequency of 1–2 bubbles per second from 10 cm depth. Each mesocosm had its light source (Valoya R-258) which was set to an intensity of ~170 μE m^{-2} s^{-1} and 12:12 h light: dark cycle to mimic the environmental light conditions in this period.

Nine mesocosms were filled simultaneously with 2 m^3 of surface seawater (salinity ~4.6 PSU) from an inlet 800 m offshore of the facility at 2 m depth. A 7 × 7 mm mesh was placed at the inlet to avoid the entry of fish into the mesocosms. After filling, the mesocosms were left for 48 h before starting the experiment.

The mesocosms were prepared in triplicates with the following treatments: seawater without MPs ("Control" =CTRL), an

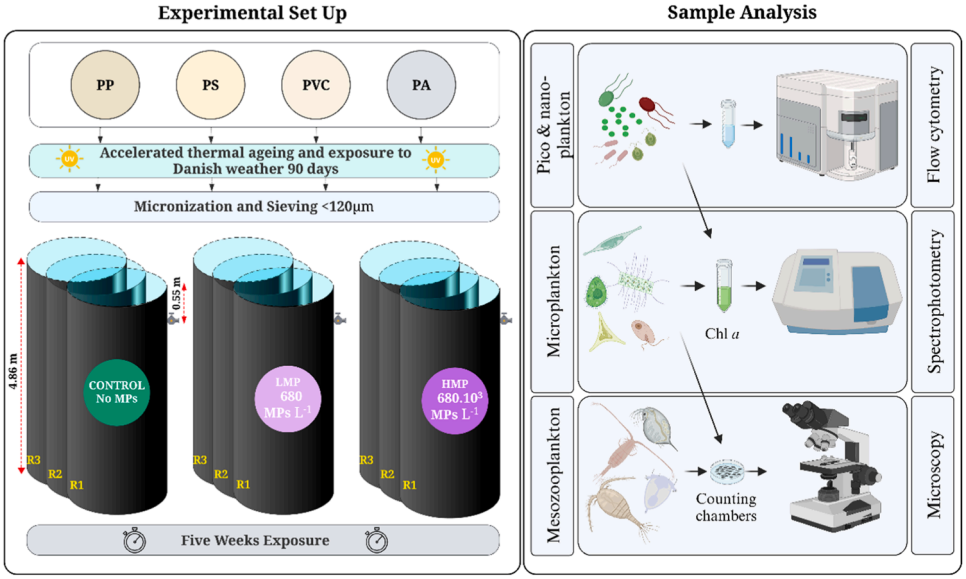

Fig. 1. Experimental setup and sample analysis methods during the experiment.

environmentally relevant exposure concentration of MPs (680 MPs L^{-1}), ("Low MPs concentration" =LMP), and a very high exposure concentration of MPs (680 × 10^3 MPs L^{-1}) ("High MPs concentration" = HMP) (Fig. 1). Our "low exposure level" is within the highest range of concentrations that has been previously reported. Commonly, microplastic concentrations in marine surface waters are typically < 1 MPs L^{-1} (e.g., Rist et al., 2020; Gunaalan et al 2023; Campillo et al. 2023) but dozens to thousands of MPs per litter have also been reported in water samples from several aquatic compartments/sites (Song et al., 2014, Vollertsen and Hansen, 2017; Badylak et al. 2021, Osorio et al., 2021, Uogintė et al., 2022; see section 3.7 for details). Our high exposure level of MPs was chosen because it is a concentration in the order of magnitude at which effects of MPs have been reported in planktonic organisms in laboratory studies (e.g., Cole et al., 2014). To obtain the desired exposure concentrations based on the coulter counter estimations (S.I. Table 1), 0.001 g of each polymer for the low MP treatment (LMP) and 1 g for the high MP treatment were used. Thus, the exposure levels in terms of plastic mass were 2 μg L^{-1} and 2 mg L^{-1} for the respective experimental treatments. Before being added to the mesocosms, the MPs from the different materials were weighed and pooled in 2 L glass bottles with 0.1% Tween 80 to prevent aggregation. The same amount of 0.1% tween solution (2 L) was added to the control mesocosms. The final nominal concentration of Tween 80 in the mesocosm was 0.0001 %, which is nontoxic to plankton (Rodríguez-Torres et al., 2020).

The physicochemical parameters were regularly monitored. A Seaguard CTD SW (product no. 4320, serial no. 89) was used to measure temperature, salinity, oxygen, and fluorescence (as a proxy of total chlorophyll). Light levels were quantified using a spherical underwater quantum sensor (LI-COR LI-1400, LI-193). Nutrient analysis, including nitrate (NO_3^--N), phosphate (PO_4^{3-}-P), ammonia (NH_4^+-N) and silicate (SiO_2-Si) was performed as described in Grasshoff et al. (1983) using a continuous segmented flow analyzer (QuAAtro SEAL Analytical). There were not significant differences ($p > 0.05$) on nutrient concentration among treatments along the experiments (Fig. S3). All the other abiotic parameters were stable ($p > 0.05$) in all the treatments (Supporting information, Table S2, Fig. S4). In instances where a decline in nutrient concentrations was observed (e.g., nitrate and silica), adjustments were made to restore them to their initial levels to prevent nutrient depletion

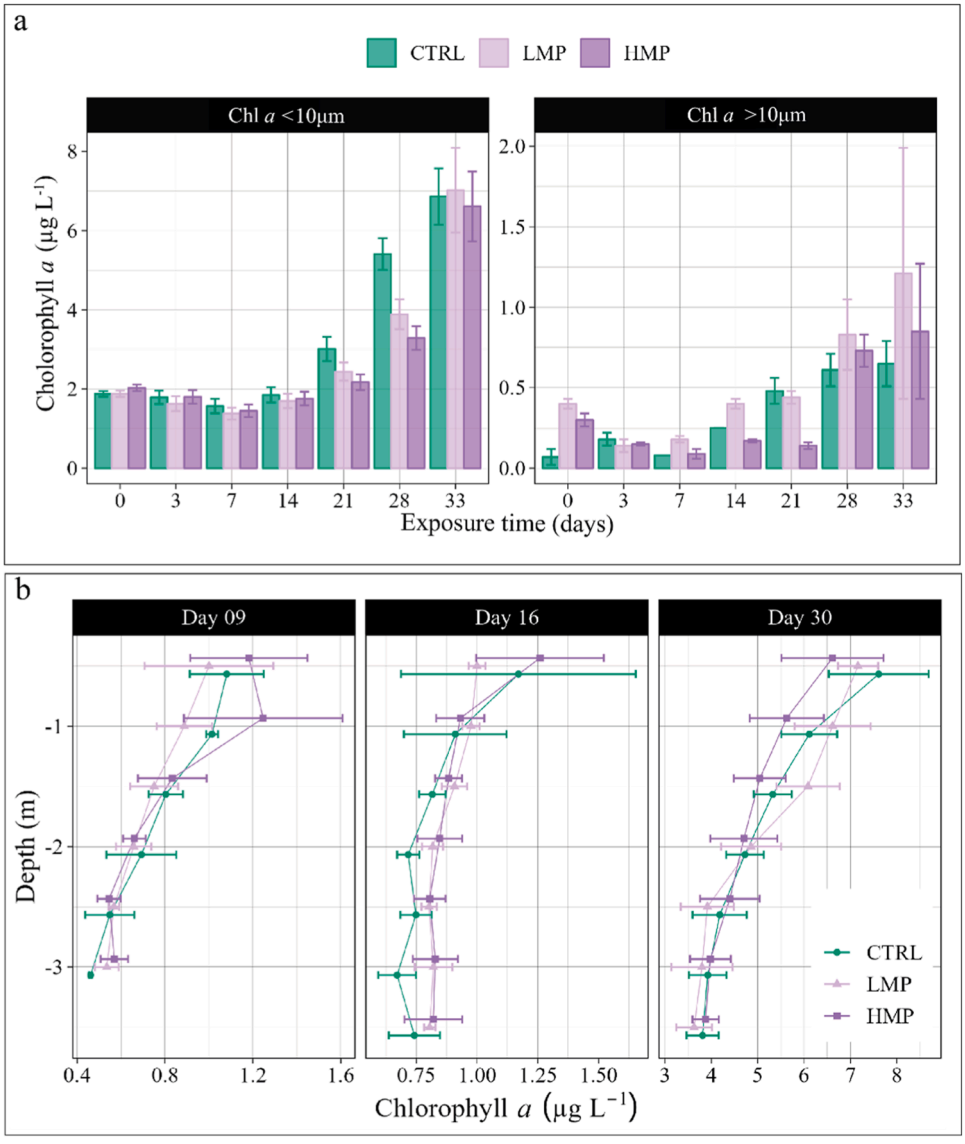

Fig. 2. Top Panel (a): The concentration of chlorophyll a in the mesocosm (up to 0.55 m) for each treatment throughout the experiment. The treatments were Control (no MPs), LMP (environmentally relevant MP concentration, 680 MPs L^{-1}) and HMP (high MP concentration, 680 × 10^3 MPs L^{-1}). Error bars represent the standard deviation of the mean (n=3). Bottom Panel (b): Vertical distribution of total chlorophyll a (estimated from the CTD fluorescence) in the different treatments after 9, 16, and 30 days of exposure.

and potential collapse of primary production.

2.4. Sampling procedures and sample analysis

Seawater samples (20 L) for analyses of the plankton community and chlorophyll (Chl) concentration were taken from the mesocosms at 55 cm depth. This depth was chosen for sampling due to the high concentration of Chl. *a* found when measuring the distribution in the water column (Fig. 2). The seawater samples were obtained from an outlet valve and transferred to carboys. The sampling was done periodically, twice in the first week, and weekly after the first week. To maintain the stable conditions of light and aeration, we kept the water levels in the mesocosms constant by refilling them with 0.2 μm filtered seawater after each sampling. The dilution of mesocosm tanks was minor since only 1% of the total water volume was replaced during each sampling. Subsamples from the collected mesocosm seawater were taken for analyses of chlorophyll *a* (Chl *a*), pico- and nano-plankton, microplankton and mesozooplankton (Fig. 1).

2.4.1. Chlorophyll measurements

To determine the total Chl *a*, 250 mL of collected seawater samples were filtered through a GF/F filter (0.8 um) using a vacuum filtration system. For the Chl $a > 10$ μm measurements, 500 mL water samples were initially filtered using a 10 μm sieve and subsequently concentrated in a GF/F filter. The filters were placed individually in Falcon tubes with 10 mL of 96 % ethanol for the Chl extraction. The tubes were shaken for 5 min at 150 rpm (Edmund Buhler SM-30 Universal Shaker) and left in darkness for 24 h. The tubes were then centrifuged for 10 min at 3500 rpm (Beckman Coulter Avanti J-26 XP) and the Chl *a* concentration was measured using a spectrofluorometer (LS 30 Perkin Elmer) calibrated against a 96 % ethanol sample, ex λ 433 nm, em λ 673 nm.

2.4.2. Pico and nano-plankton analysis

For the determination of pico- and nano-sized plankton abundances, subsamples of 5 mL were taken with an automatic pipette, fixed with glutaraldehyde (0.5 % final concentration), and stored at -80 °C and until analysis. The samples were thawed and pico- and nano-phytoplankton were analysed on an Attune® Acoustic Focusing Flow Cytometer (Applied Biosystems by Life Technologies). The phytoplankton were grouped based on their pigmentation on biplots of green vs. red fluorescence. Before counting bacteria and heterotrophic nanoflagellates (HNF) the DNA of the cells was stained SYBR-green-I and groups were discriminated on biplots of side scatter vs. green fluorescence and red vs. green fluorescence, respectively. We only count free-living pelagic bacteria, not those that are particle-associated. Actively dividing bacterial cells contain more DNA, therefore the ratio of High Nucleic Acid (HDNA) bacteria to Low Nucleic Acid (LDNA) bacteria is here used as an indicator of the relative activity of the bacterial community.

2.4.3. Phytoplankton and zooplankton analysis

For determination of the abundance and composition pf microplankton, 250 mL subsamples were fixed with Lugol's solution (1 %) allowed to settle for 24 h in Utermöhl chambers. The whole chamber was counted under an inverted microscope at magnification x20. Since we used Lugol's solution for preserving the microplankton samples, we did not distinguish between autotrophic and heterotrophic dinoflagellates, and all dinoflagellates were placed in the category of microphytoplankton. To estimate the abundance and composition of mesozooplankton, 18 L seawater subsamples were concentrated by filtering onto a 100 μm mesh sieve. The samples were fixed with 1 % Lugol's solution and counted under a stereomicroscope.

2.5. Statistical analysis

We carried out two distinct statistical analyses in this study. Firstly, we conducted a two-way analysis of covariance (ANCOVA) to determine main and interaction significant effects of the "treatment" and "time" on the Chl concentration and the abundance of the different planktonic groups. The assumptions of ANCOVA analyses were evaluated and fulfilled and the analyses were conducted using the raw data. Secondly, we employed Generalized Additive Mixed Models (GAMMs) to investigate effects of "treatment" on abundance of each plankton group, as well as the whole size classes. This analysis was based on the following model formulation:

$$\log\left(N+1\right)_{g,t,d} = a + MP_t * G_g + s(Day_d) + e \quad (1)$$

where the log-transformed abundance of a given organism group (*g*) under treatment (*t*) and day (*d*) is a function of the overall intercept (a), as well as the treatment level (MP) and group (G) in question. In this case, prior to the analysis, the abundances were corrected for any potential bias due to differences in the initial densities between the control and treatment at day 0 (Fig. S5). In order to reflect differences in the amount of MP we specified treatment as an ordered factor (i.e., with levels: Control<LMP<HMP) and included an interaction term (*) between treatment and group to reflect potential group-specific responses. Furthermore, we included day as a random effect to account for temporal fluctuations in group abundances throughout the duration of the project where a non-linear smoothing function (s) was applied to represent any temporal variability. In addition, we also accounted for potential temporal autocorrelation by including an AR (1) correlation structure in the model residuals (*e*). Finally, we fitted one model for each size-class and compared the responses to MP across size-class and organism group by predicting the abundances for each treatment and day using the set of fitted models. A significance level of 0.05 was considered in all the tests. All statistical analyses and visualizations were performed in the software R (version 4.0.2) using the package "mgcv" (Wood, 2017), "ggplot2" (Wickham, 2016).

3. Results and discussion

3.1. Effects of MPs on chlorophyll concentrations

The initial concentration of total Chl *a* was approximately 2 μgL^{-1} at the beginning of the experiments. This concentration remained stable during the first two weeks of the study but experienced a notable increase in subsequent weeks, reaching up to 7 μgL^{-1} (Fig. 2a). These Chl concentrations are similar to those commonly found in the Gulf of Bothnia in the Baltic Sea (e.g, Fleming-Lehtinen et al., 2008). The Chl > 10 μm represented a minor proportion (4–12%) of the total Chl, indicating that smaller size- fractions such as pico- and nano phytoplankton majorly contribute to total phytoplankton biomass (Fig. 2a). Notably, no significant effects were detected in either Chl. $a > 10$ μm or Chl. $a < 10$ μm among the treatments (Fig. 2a). Furthermore, the vertical distribution of total Chl *a*, as estimated from CTD fluorescence measurements, did not exhibit any differences among the treatments (Fig. 2b). Other studies have found that exposure to micro-nano plastics influences the composition of photosynthetic pigments and the overall photosynthetic efficiency of microalgae (Wu et al., 2019; Zhao et al., 2019). For example, pristine polystyrene (size 5 μm) at high concentrations (100 mg L^{-1}) leads to reduction in the content of Chl *a, c* and carotenoids in the diatom *Phaeodactylum tricornutum* (Chen et al., 2021). Conversely, amino modified polystyrene exhibited negligible effects on the photosynthesis of *Chaetoceros eogracile* (Seoane et al., 2019) and regular polystyrene did not significantly affect the photosynthesis of *Dunaliella tertiolecta* (Sjollema et al., 2016). However, it is important to highlight that the inhibitory effects of MPs on photosynthetic efficiency have been reported to be less pronounced when photosynthetic microorganisms are given time to acclimate (Li et al., 2021). This increased tolerance to MPs was also observed in *Phaeodactylum tricornutum* exposed to aged polystyrene (Chen et al., 2021). Our findings are in line with these minor

effects of MPs on primary producers; this can be attributed to the potential decrease in toxicity of weathered plastics after losing their soluble additives and the absence of negative changes of MPs on grazers abundance as discussed in the next sections.

3.2. Effects of MPs on pico and nano-plankton

The abundance and composition of picoplankton (< 2 μm) varied throughout the duration of experiment (Fig. 3; Fig. S6). The concentration ranges of heterotrophic bacteria (1–1.5 × 10^6 cells mL^{-1}), picoeukaryotes (1–4 × 10^4 cells mL^{-1}), nanophytoplankton (200–2000 cells mL^{-1}) and heterotrophic nanoflagellates (1500–5000 cells mL^{-1}) found in our study are commonly observed in Baltic waters (Dedman et al., 2022). Overall, the ANCOVA showed no statistically significant differences among treatments in the abundance and composition of any of the pico-nano plankton groups nor in bacterial activity (Fig. 3; Table S3). However, small changes were observed, where the bacterial activity increased during the first few weeks in the presence of MPs (Fig. 3a), as reflected by higher bacterial abundance in the treatments with MPs during the first half of the experiment (Fig. 3a). Though, by the end of the experiment (days 20–32), the bacterial abundance was up to 16 % lower in the high MPs treatment compared to the control (Fig. 3a). The high bacteria activity and concentration observed in the MP treatments compared to the control during the first weeks can be related to the release of dissolved organic carbon (DOC) from MPs. It has been demonstrated that DOC leaching from plastics stimulates the activity of marine planktonic bacteria (Romera-Castillo et al., 2018). Leaching of DOC from MPs decreases drastically with time and the DOC is rapidly used by the bacteria (Romera-Castillo et al., 2018), which explains why this stimulation was only observed during the first weeks of the experiment. Low nutrient levels can promote the attachment of free planktonic bacteria to the surface of MPs to form biofilms (Stanley and Lazazzera, 2004; Kesy et al., 2019). Therefore, the decrease in the abundance of free-living bacteria observed in our study can be related to the decrease in nutrients observed in the last weeks of the experiment (Fig. 3; Fig. S3).

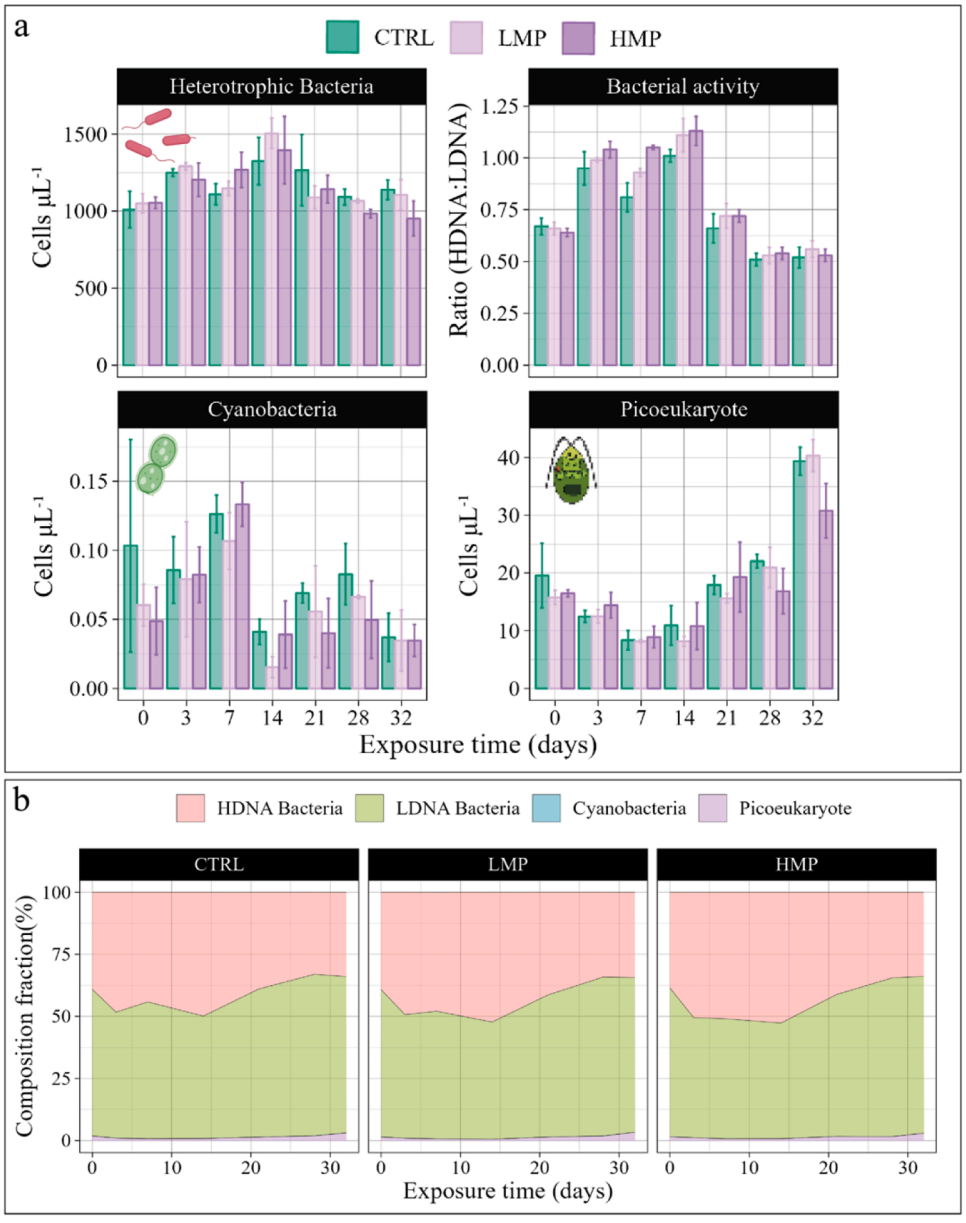

Fig. 3. Top Panel (a): Composition and abundance of picoplankton throughout the experiment, where the top-right panel shows the ratio of bacterial activity. Bottom Panel (b) Compositional (%) changes of the abundance of picoplankton throughout the exposure time.

The concentration of the smallest phytoplankton (picophytoplankton <2 μm) increased in all treatments after day 14 (Fig. 3b; Fig. S6). The pico-phytoplankton community was mainly composed of eukaryotes and only a few cells of the cyanobacteria *Synechococcus* were detected (<200 cells mL^{-1}). The picoeukaryotes remained statistically unaffected by MPs (ANCOVA; GAMM, Table S3 & S4), but the abundance in HMP treatment were 22 % lower compared to control at the end of the experiment (T32; Fig. 3). The large surface-to-volume ratios of small phytoplankton can make picophytoplankton more susceptible to plastic leachates than larger phytoplankton. Other studies have found that plastic leachates for new and weathered plastics negatively affect the cyanobacteria *Prochlorococcus* in laboratory bioassays, but the effects were observed at solid-to-liquid ratios several orders of magnitude higher than the ones used here (Tetu et al., 2019; Sarker et al., 2020). Contrary to these findings, our GAMM model indicates a positive linear response for cyanobacteria, as indicated by the significant group-specific interaction term with MP treatment (Table S4). However, cyanobacteria only contributed marginally to the total picophytoplankton, and the overall effect of MPs on picoplankton remained minor and non- statistically significant (ANCOVA; GAMM model, Table S3, Table S4). This supports the general conclusions from Galgani et al., (2019) demonstrating, minor changes in the microbial communities after ca. 2 weeks of exposure to PS beads (430 MPs L^{-1}) in mesocosm experiments.

The concentration and composition of nanoplankton (2–20 μm) also varied during the experiment (Figure 4; Figure S6). The abundance of heterotrophic nanoflagellates (HNF) along the experiment varied greatly among treatments (Fig. 4), with a small significant decreasing effect of MPs according to the GAMM model, but non-significant according to the ANCOVA (Table S3). The HNF had a higher predicted abundance compared to control, especially in the LMP treatment according to the GAMM model (Figure S7). The lower abundance of small phytoplankton (picoeukaryotes, cyanobacteria, and autotrophic nanoflagellates) in the MP treatments compared to control from day 21 and onwards was also apparent in the Chl a concentration values, except for the last sampling day (T32) (Fig. 2a, 3a, 4a). The nanophytoplankton showed a 53 % lower abundance in the HMP treatment compared to the control during the last weeks of the experiment (T28), but this effect was reduced to 12 % by the end of the experiment (T32) (Fig. 4). The GAMM model only indicated statistically negative effects of MPs on nano-phytoplankton (Table S4), while ANCOVA did not show any significant differences between treatments for pico and nano-plankton (Table S3).

3.3. *Effects of MPs on microplankton*

The microphytoplankton community was mainly composed of cryptophytes, diatoms, and dinoflagellates whereas protozooplankton was dominated by ciliates and sarcodines (*Astramoeba* sp) (Fig. 5; Fig. S8). The abundance of the microplankton groups was overall not significantly affected by the exposure to MPs (ANCOVA; GAMM, Table S3, S4 & S5). However, we observed a 47 % higher concentration of diatoms in the MP treatments than in the control on the last sampling day and a lower concentration of cryptophyte cells in the high concentration MP treatment in the last phase of the experiment (43 % at T32) (Fig. 5). Dedman et al., 2022 suggested that larger phytoplankton are

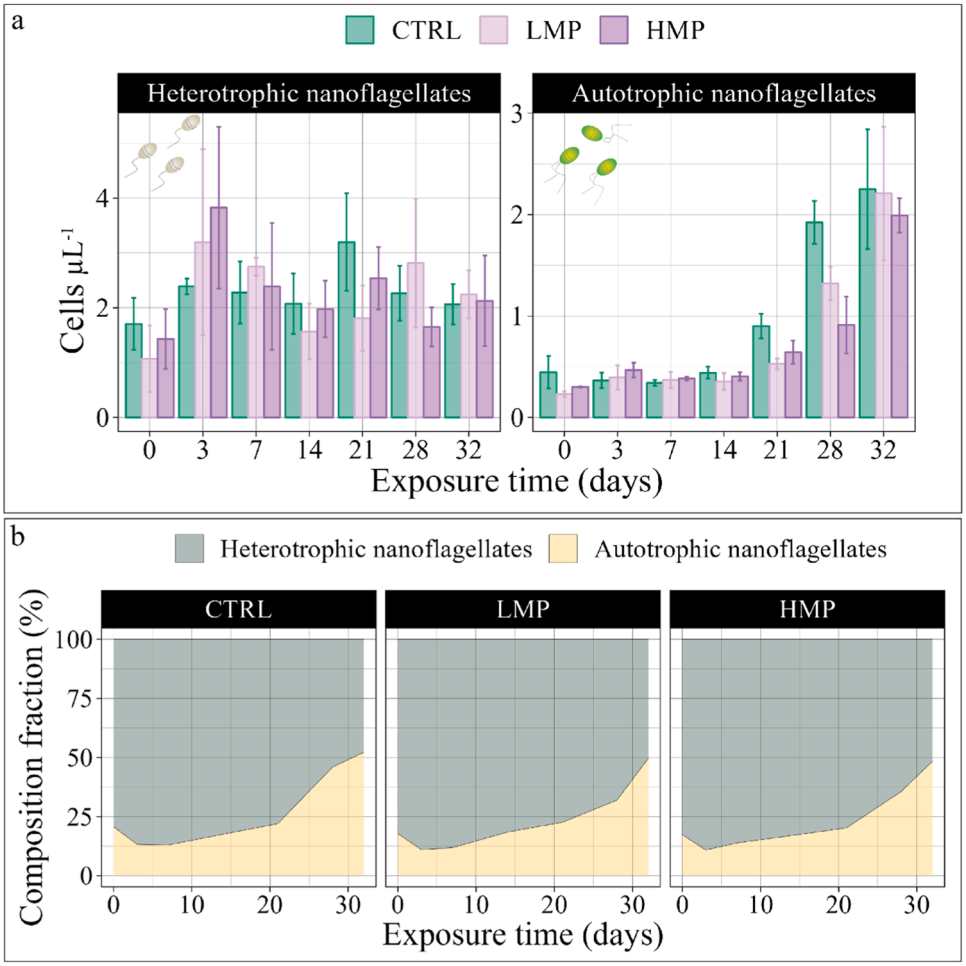

Fig. 4. Top Panel (a): Composition and abundance of heterotrophic and autotrophic nano-flagellates throughout the experiment. Bottom Panel (b): Compositional (%) changes of the abundance of nano-plankton throughout the exposure time.

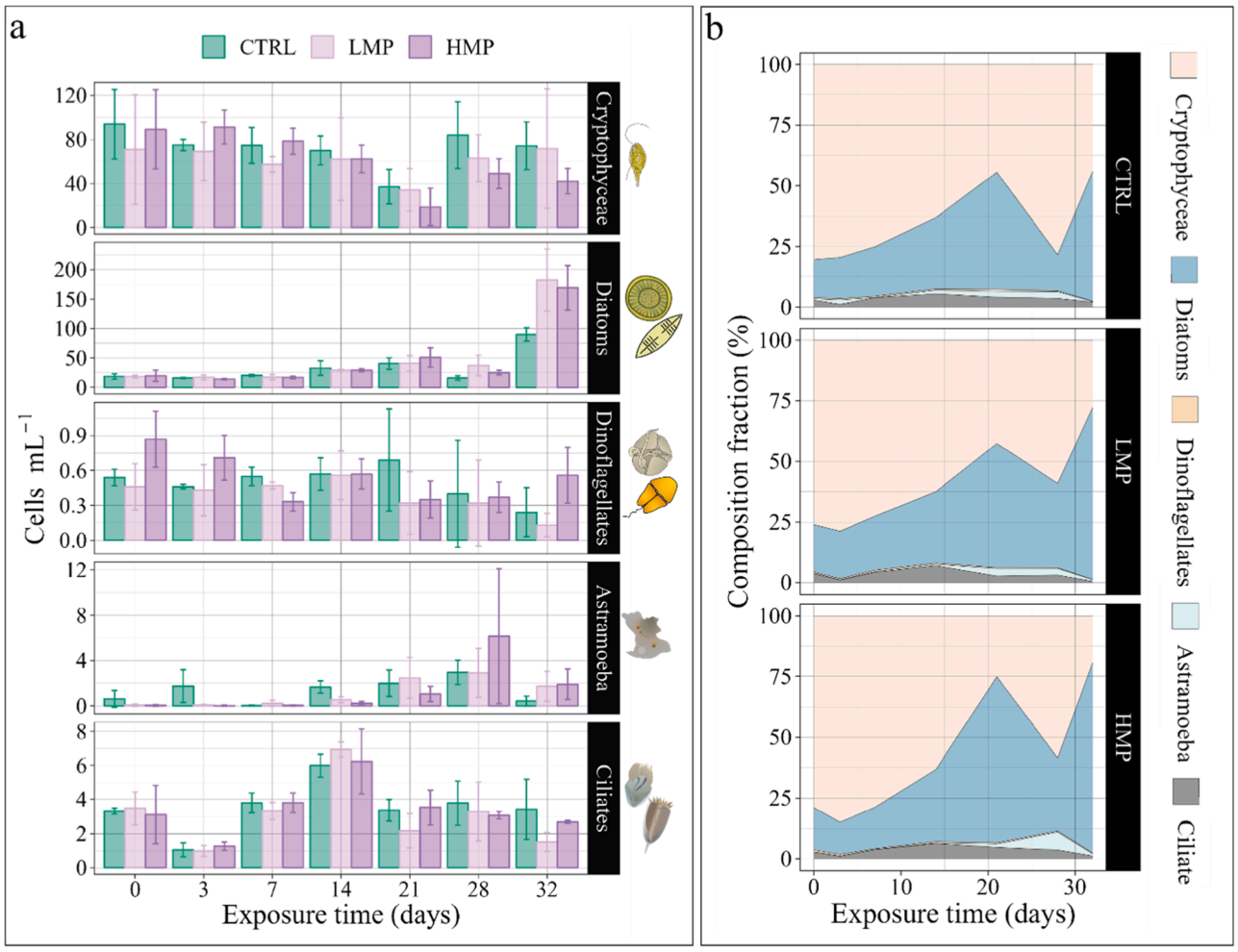

Fig. 5. Left Panel (a): Abundance and succession of micro-plankton throughout the exposure period. Right Panel (b): Micro-plankton compositional changes the abundance during the exposure time.

more susceptible to negative effects of MPs due to aggregation and co-sedimentation of cells with plastic particles. However, we did not observe any statistically significant effects of MP either diatoms or cryptophytes (Fig. 5; Table S3 & S4).

The effects of small MPs (1–200 μm) on autotrophic microplankton have been mostly investigated in bottle incubations (Mendonça et al., 2023; Prata et al., 2019) and, in some cases in mesocosms, mainly in freshwater (Yıldız et al., 2022; Marchant et al., 2023). Laboratory experiments in microcosms show that MPs only negatively affect micro-phytoplankton at very high concentrations of MPs (0.1 g L^{-1}) compared to environmental concentrations (Gambardella et al., 2019; Chae et al., 2020). In some cases, stimulation of growth has been found in some microplankton species after exposure to high concentrations of plastics (0.1 g L^{-1}) (Canniff and Hoang, 2018; Chae et al., 2020). The effects of plastics on photosynthetic organisms not only depend on the concentration of plastics but also on the size of the particles. Studies indicate that NPs can be absorbed through the cell wall of autotrophic microplankton, which is not possible for larger particles (Bhattacharya et al., 2010; Zhang et al., 2017). In our study, the MPs used were sieved to be < 120 μm and, although only the fraction between 4 – 120 μm was measured, it is expected that smaller MP particles, including NPs, were also added to the mesocosms. Despite potential exposure to NPs, our study did not find any negative effects on microplankton. Similar findings have been reported in mesocosm experiments involving freshwater communities exposed to MPs, where a limited impact on plankton was observed (Yıldız et al., 2022; Marchant et al., 2023).

The effects of MPs on planktonic protozoans are less known than for metazooplankton. Ingestion of MPs in the laboratory has been observed in ciliates (Setälä et al., 2014; Zhang et al., 2021b) and dinoflagellates (Fulfer and Menden-Deuer, 2021). A study on the marine ciliate *Uronema marinum* showed that the abundance, volume, and biomass of this species decreased after grazing polystyrene beads (~10^6 MPs mL^{-1}) (Zhang et al., 2021a). Similarly, Fulfer and Menden-Deuer, (2021) found a reduction in the growth of dinoflagellates at MP concentrations of ~10^5 MPs L^{-1}and Geng et al. (2021) reported detrimental effects on the protozoan growth after exposure to MPs (2 × 10^5–1 × 10^6 MPs mL^{-1}). These studies overlap with our high MPs concentration and suggest that protozoans are quite sensitive to the MPs. However, we did not observe any statistically significant changes in the abundance of Astramoeba while ciliates showed a small increasing effect of MPs (GAMM, Table S4) possibly caused by internal trophic interactions. In line with our results, Nałęcz-Jawecki et al., 2021 did not find any negative effects of MPs (10^3–10^6 MPs mL^{-1}) on ciliates. Planktonic protozoans play a crucial role as major phytoplankton grazers and are key players in the microbial loop (Setälä et al., 2014; Sherr and Sherr, 2002). However, trophic cascading effects due to MPs were not observed in our study due to the absence of overall negative effects of MP on the planktonic protozoans.

3.4. *Effects of MPs on mesozooplankton*

The two most abundant metazooplankton groups were copepods, including their different developmental stages (nauplii and copepodites)

and rotifers (Fig. 6). The planktonic copepod *Eurytemora affinis* accounted for 97 % of the total adult copepods found initially in the community (Fig. 6). *Synchaeta* sp., *Keratella cochlearis, K. quadrata* were the main species of rotifers, with *Synchaeta* sp being the dominant species. Other metazooplankton species, including *Bosmina coregoni maritima, Acartia bifilosa,* and barnacle nauplii were found at low concentrations. The development in the copepod population is illustrated by the dynamics in the composition of their life stages (Fig. 6), where a notable abundance of nauplii at day 7 was followed by a high abundance of copepodites at day 14, which turn was followed by a high abundance of adult copepods towards the end of the experiment. *Synchaeta* sp. was initially the numerically dominant metazoan species, accounting for 98.1 % ± 1.1 % of the community, but their abundance was drastically reduced after 3 days in all the treatments (Fig. 6). The used MPs are in the size spectra of the prey ingested by metazooplankton (Heinle and Flemer, 1975; Hansen et al., 1994), However, we found no significant differences between the treatments in any of the meso-zooplankton taxa, life stages or the total metazoan community (Fig. 6; ANCOVA;GAMM, Table S3, S4, S5). These results indicate that the potential ingestion of MPs did not cause any impacts on meso-zooplankton at the population level under the studied exposure levels. Bottle incubation studies have shown that ingestion of virgin MPs can cause sub-lethal negative effects on copepods (Cole et al., 2015, 2013; Shore et al., 2021) or non-effects (Vroom et al., 2017; Rodríguez-Torres et al., 2020). Some copepod species are efficiently rejecting MPs (Xu et al., 2022) or show a low ingestion of MPs due to their foraging behavior (Rodríguez Torres et al., 2023), which decreases the risk of MPs ingestion and their effects. Yet, leachates of microplastics from other plastic materials can be acutely toxic to copepods and other metazooplankton (Bejgarn et al., 2015; Almeda et al., 2023; Bournaka et al., 2023) but commonly at higher concentrations than used here (> 200 mg L^{-1}).

Taken together, we found no uniform and overall relationship between plankton abundance and MPs exposure in any of the size classes/taxonomic groups. However, we found a few taxa-specific responses among the smallest plankton (i.e., cyanobacteria and nano-plankton) according to the GAMM model (Table S4). Whether the derived relationships arise from direct effects related to the presence and exposure to MP, and/or through indirect biotic effects, channeled through competition or trophic interactions is difficult to disentangle. Overall, the effects of the studied MPs on the abundance and composition of the planktonic community were minor and, in most cases, non-significant compared to control according to both statistical analyses.

3.5. *Influence of thermal ageing, weathering, and leaching on the toxicity of microplastics*

In the environment, plastics are fragmented and degraded by the action of abiotic factors and mechanical abrasion (Andrady, 2011; Dimassi et al., 2023; Luo et al. 2022). Thermo-oxidative degradation and photodegradation are two key processes in the ageing and weathering of plastic debris (Zhang et al., 2021a; Luo et al., 2022). The MPs used in this study come from plastics aged by accelerated thermal degradation and weathered by outdoor exposure to Danish weather for 60 days.

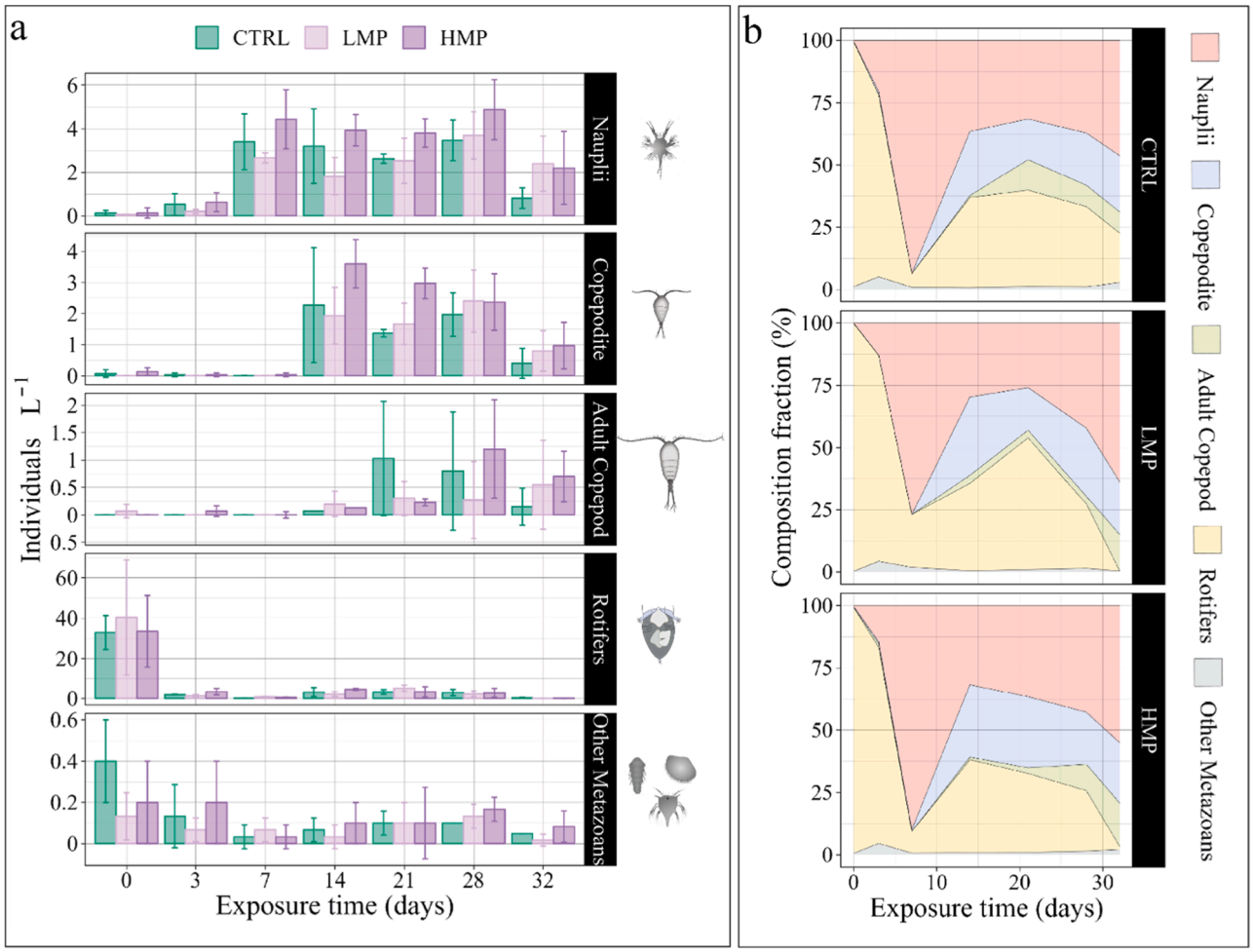

Fig. 6. Left Panel (a): Abundance and succession of meso-zooplankton throughout the exposure time. Right Panel (b): Mesozooplankton compositional the abundance changes during the exposure period.

These processes affect the physical and chemical structure of plastics and accelerate the release of oligomers and additives (Paluselli et al., 2019; Yan et al., 2021; He et al., 2023). Therefore, aging and weathering may increase their ecological risks since the toxicity of MPs correlates with the content of leached additives (Capolupo et al., 2020; Beiras et al., 2021). The influence of aging and weathering processes on the toxicity of MPs are complex, however, and studies have shown contrasting results (Baudrimont et al., 2020; Simon et al., 2021). Additionally, toxicity of plastic can be higher during the initial stages of aging, decreasing with aging time (Sarker et al., 2020; Pflugmacher et al., 2021). In our study, despite using micronized aged/weathered materials, we did not find a significant toxic effect of MPs on the plankton community. It is important to note that the composition of plastic additives can greatly differ based on the manufacture and intended application of the plastic (Hahladakis et al., 2018; Andrady and Rajapakse, 2019). For instance, PVC typically contains around 80 % plasticizers, which also includes heat stabilizers such as the metals Pb, Sn, Ba, Cd, and Zn. In the case of PS, colorants are often added to achieve bright and transparent colors, while PP incorporates antioxidants that can delay the degradation when exposed to UV light (Hahladakis et al., 2018). Additionally, both nylon and PS have the potential to depolymerize into oligomers and monomers. At the used exposure levels, the studied conventional plastics did not influence the plankton community significantly, suggesting that the materials used in our study have a low number and concentration of potential toxic additives.

3.6. Ecological implications

The concentrations of MPs used in our study are higher than those commonly found in marine surface waters (< 1 MP L^{-1}, (Rist et al., 2020; Botterell et al., 2022; Gunaalan et al., 2023; Campillo et al., 2023). Our lower exposure concentration (LMP) is considered environmentally relevant since dozens to thousands of MPs per litter has been reported in water from several aquatic compartments/sites, such as the marine surface water microlayer (up to ca. 200 MPs L^{-1}, Song et al., 2014,), wastewater effluents (e.g., up to 5800 MPs L^{-1}, Vollertsen and Hansen, 2017; Uogintė et al., 2022), river mouth surface water (up to 57 MPs L^{-1} Osorio et al., 2021) and marine lagoons (up to 76,000 MPs L^{-1}, Badylak et al., 2021). Our higher MP exposure level (HMP) is a very extreme scenario compared to surface water concentrations of MPs but it aligns with exposure concentrations commonly used in microcosms experiments (Setälä et al., 2014; Cole et al., 2015; Canniff and Hoang, 2018; Fulfer and Menden-Deuer, 2021; Shore et al., 2021), where negative effects on plankton have been observed. With the focus on additives, the toxicity of plastic leachates is commonly based in terms of mass and solid to liquid ratio (Beiras et al., 2019; Almeda et al., 2023). The mass of conventional MPs in the marine environment is variable; it ranges from micrograms to a few milligrams per cubic meter in surface waters (Rist et al., 2020; Dibke et al., 2021; Gunaalan et al., 2023; Ikenoue et al., 2023). The mass of plastics in sediments is typically higher than in the water column, with values up to 34.5 mg kg^{-1} in surface sediment (Kim et al., 2023). Therefore, similar to the exposure level in terms of particles, the mass of plastic used in the LMP treatment in this study was in the upper range of concentration reported for surface waters. However, we did not find statistically significant impacts of the studied weathered MPs on the brackish planktonic community even at a high MP concentration. Our study suggests that the MPs from weathered conventional plastics have a negligible impact on primary and secondary producers in the plankton food web at environmentally realistic concentrations.

4. Conclusions

The present study shows that conventional aged and weathered micronized (<120 μm) plastics do not affect the structure and dynamics of a planktonic community in the Baltic Sea after long-term exposure to MPs. It is important to note that the results obtained here cannot be generally extrapolated to all kinds of plastics. For instance, other types of plastics like crumb rubber, tire wear particles, and cigarette butts exhibit much higher additive-related toxicity compared to the plastics used in this study, some of which are common in human consumption product like PS cup lids. To gain a more comprehensive understanding of the impacts of plastic pollution on plankton communities, further investigations with MPs derived from other plastic materials, especially those with high levels of functional additives or potentially harmful leachates are needed.

CRediT authorship contribution statement

Linea Gry Ebbesen: Formal analysis, Investigation, Writing – original draft. **Markus Varlund Strange:** Formal analysis, Investigation, Writing – original draft. **Kuddithamby Gunaalan:** Data curation, Formal analysis, Investigation, Writing – original draft. **Maria Lund Paulsen:** Formal analysis, Investigation, Writing – review & editing. **Alicia Herrera:** Investigation, Writing – review & editing. **Torkel Gissel Nielsen:** Investigation, Supervision, Writing – review & editing, Conceptualization. **Yvonne Shashoua:** Formal analysis, Investigation, Writing – review & editing. **Martin Lindegren:** Formal analysis, Investigation, Writing – review & editing. **Rodrigo Almeda:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

RODRIGO ALMEDA reports financial support was provided by European Commission. RODRIGO ALMEDA reports financial support was provided by JPI Oceans. TORKEL NIELSEN reports financial support was provided by Velux Foundation. RODRIGO ALMEDA reports financial support was provided by The Canarian Science and Technology Park Foundation of the University of Las Palmas de Gran Canaria. RODRIGO ALMEDA reports financial support was provided by Spanish Ministry of Science and Innovation. RODRIGO ALMEDA reports financial support was provided by Spanish National Agency of Research. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.121500.

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