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Seafloor sediments as microplastic sinks in the northern Baltic Sea – Negligible upward transport of buried microplastics by bioturbation☆

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ABSTRACT

Microplastics (MPs) are ubiquitous in the marine environment. High concentrations of MPs are found from seafloor sediments, which have been proposed to act as their final sinks. Because bioturbation is an important process affecting the burial of MPs, a mesocosm experiment was established to study whether sediment infauna may also promote MP return to the sediment surface. Thin layers of frozen sediment containing an environmentally realistic concentration (<1300 MPs per kg of dry sediment) of MP fragments in two size classes (>500 μm and 100–300 μm) were added to depths of 2 cm and 5 cm in the experimental cylinders filled with sediment. The displacement of these MPs, made of acrylonitrile butadiene styrene (ABS), by a community of common benthic invertebrates in the northern Baltic Sea (clam *Limecola balthica*, polychaete *Marenzelleria* spp., gammarid *Monoporeia affinis*) was studied in a 10-week experiment. After the experiment, the MPs were extracted from each sediment layer and the animals were examined for MP ingestion. The results indicated that the transportation of MPs to the sediment surface by bioturbation was negligible. Thus, in the Baltic Sea, the seafloor may act as a sink for once sedimented MPs, reducing simultaneously the MP exposure of the macrofauna feeding on the sediment surface.

1. Introduction

Seafloors are proposed to act as final sinks to the plastic debris distributed globally in our oceans (Cózar et al., 2014; Eriksen et al., 2014; Woodall et al., 2014). Plastic polymers denser than seawater will sink and end up to the sediments (Andrady, 2011), and also buoyant plastic materials can eventually be deposited to the seafloor when their density increases e.g. as a result of biofilm formation (Lobelle and Cunliffe, 2011). The smallest fragments of plastics, microplastics (<5 mm, hereafter referred to as MPs) (GESAMP, 2016), can also sink when incorporated to descending phytoplankton aggregates (Long et al., 2015) or when ingested and subsequently egested in fecal pellets of animals (Cole et al., 2013).

The MPs prevalent in marine sediments vary with size, shape, polymer type, and origin. Most of them are fragments from larger plastic items (Shim et al., 2018) and hence classified as secondary MPs (GESAMP, 2016), but in some areas the dominant fraction consists of primary MPs that are intentionally manufactured to small size (Norén, 2007). MPs are spread from shallow coastal areas (Thompson et al., 2004; Claessens et al., 2011; Vianello et al., 2013) to deep sea floors (Van Cauwenberghe et al., 2013; Woodall et al., 2014; Fischer et al., 2015; Bergmann et al., 2017), where their concentration can reach up to 4356 particles kg^{-1} sediment (dry weight) (Bergmann et al., 2017). Because of their small size, MPs are available for ingestion, capable of causing physical and chemical harm to the animals (von Moos et al., 2012; Rochman et al., 2013; Wright et al., 2013; Ogonowski et al., 2016), and subject to trophic transfer (Setälä et al., 2014). Globally, marine sediments cover most of the seafloor, and benthic animals living in these habitats form the largest faunal assemblage on the planet in areal coverage (Snelgrove, 1998). As evidence on MPs occurring in benthic food webs is already present (Van Cauwenberghe et al., 2015; Taylor et al., 2016), their accumulation to seafloor raises concerns about their current and future effects in these habitats.

Animals living in and on the seafloor sediments are continuously modifying the physical, chemical and biological properties of their habitat in a process called bioturbation (Rhoads, 1974; Aller,

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1982; Lohrer et al., 2004). Bioturbation includes all animal activities, such as burrowing, feeding, defecation and ventilation, that directly or indirectly affect the sediment structure by transporting solutes or particles in the sediment-water interface (Kristensen et al., 2012). Bioturbation plays an important role for example in global carbon and nutrient cycling, as well as in the metabolism, dispersion and burial of marine pollutants (Rhoads, 1974; Snelgrove, 1998). In addition to natural particles, such as sand grains, bioturbation also affects the distribution of MPs in sediments. Uniform plastic spheres of different sizes have been used to assess sediment mixing rates by benthic fauna by placing them to sediment surface both in laboratory and *in situ*, and consequently these particles have been quantified deeper within the sediment (Wheatcroft, 1991; Kearns et al., 1996; Ciutat et al., 2005; Viitasalo-Frösén et al., 2009). Besides these primary MPs, bioturbation has also recently been shown to have an important role in burying secondary MPs in a similar manner in the Baltic Sea sediments (Näkki et al., 2017; Gebhardt and Forster, 2018).

Although studies on MP abundance and bioturbation-induced burial in the sediments support the hypothesis of seafloors being the ultimate sink for MPs, little is known on the stability of these MP reservoirs. In addition to contributing to burying MPs in sediments, animals can move particles to other directions as well (Wheatcroft et al., 1990). Bioturbation might therefore also act as a countering process that promotes the return of buried MPs to the sediment-water interface, and facilitates their subsequent resuspension to the water column. As a result, the MP load on the seafloor would concentrate to the biologically active near-surface sediments, which might cause adverse effects to benthic fauna. As deposit-feeding is the dominant feeding strategy in soft, muddy sediments (Shull, 2009), the higher MP concentrations in surficial sediments might lead to increased risk for benthic communities through higher exposure and ingestion probability. Furthermore, MPs incorporated in loose surface sediments might be subject to resuspension by wind-driven waves, tidal streams and upwelling events (Rhoads, 1974) affecting the animals not only on the seafloor, but in the demersal habitat above.

More than half of the Baltic Sea seafloor comprises soft, fine-grained muddy and sandy sediments (Kaskela and Kotilainen, 2017), but knowledge on MP concentrations and their fate in these areas is scarce. This study aimed to investigate whether bioturbation by a typical northern Baltic Sea invertebrate community can re-distribute buried MPs back to the sediment surface. The invertebrate communities in these areas are dominated by the clam *Limecola balthica*, polychaete *Marenzelleria* spp. and amphipod *Monoporeia affinis* (Josefson et al., 2012; Rousi et al., 2013; Gogina et al., 2016), which were therefore selected as the study species. They are all deposit-feeders that typically reside in different depths within sediments, and their capability for downward particle transport has been described previously (Viitasalo-Frösén et al., 2009; Näkki et al., 2017). We constructed a mesocosm experiment using secondary MPs in two different size fractions placed at two different depths within sediment to study the influence of burial depth and MP size in their transport. In this experiment, we studied whether 1) the benthic invertebrate community is able to transport buried MPs to the sediment surface, 2) the transport is affected by the burial depth or size of MPs.

2. Materials and methods

2.1. Experimental design

Sediment and animals were collected onboard R/V Saduria on the SW coast of Finland, northern Baltic Sea, in the vicinity of Tvärminne Zoological Station in April 2017. Clams (*L. balthica*) were caught with a bottom trawl (start 59°51′269 N 23°16′135 E, end 59°51′294 N 23°16′421 E, depth 34–36 m), and the sediment samples containing *Marenzelleria* spp. and *M. affinis* were taken with van Veen grab sampler in three different locations containing muddy sediments (59°51′131 N 23°14′653E, depth 7 m; 59°51′185 N 23°15′185 E, depth 21 m; 59°51′319 N 23°15′822 E, depth 16 m). Three sibling species of *Marenzelleria* spp. are found from the Baltic Sea: *Marenzelleria viridis*, *M. neglecta* and *M. arctia* (Bastrop and Blank, 2006). As they can only be reliably identified by their genotype (Bastrop and Blank, 2006), we did not differentiate them in this study. To remove all the resident macrofauna, the sediment was sieved through 1 mm and 0.5 mm sieves. *Marenzelleria* spp. and *M. affinis* found on the sieves and *L. balthica* from the trawl were transferred to temperature-controlled room at 4 °C. The animals were placed in acclimation aquaria for 4 days in a constant water flow of ambient seawater (temperature 4 °C, oxygen 13 mg L^{-1}, salinity 5–6).

All sediment that had passed through the 1 mm sieve was left to settle for 2 nights in seawater, after which the cleared water was carefully removed. Sediment from three locations were mixed together to obtain sufficiently homogenous sediment and 4 cm thick layer of sediment was placed into each of 16 transparent experimental cylinders (height 20 cm, Ø 14 cm) equipped with a removable base. These experimental cylinders were covered with steel mesh lids (mesh size 500 μm), and placed in the same temperature-controlled room with the animals. A hose with drilled small holes was placed horizontally on top of each set of 4 cylinders to provide a gentle dripping of ambient seawater to the cylinders.

Secondary MPs were produced by grinding four colors (pink, green, yellow, red) of children's toy bricks made of acrylonitrile butadiene styrene (ABS). ABS was chosen because (i) its density, 1.03–1.09 g cm^{-3} (Wypych, 2016), enables the material to sink in the brackish Baltic Sea water, (ii) it was available in different colors, and (iii) easy to grind to different size fractions using a kitchen grater. The generated plastic fragments were suspended in Milli-Q water and sieved into two different fractions: >500 μm (pink MPs, green MPs) and 100–300 μm (yellow MPs, red MPs). A total of 32 Eppendorf tubes were pre-weighted, and each of the 16 tubes received 150 fragments of pink MPs (>500 μm) and another 16 tubes 150 fragments of green MPs (>500 μm), which were hand-picked under a microscope (Leica CLS 150 EXE, VWR CL-150, magnification 0.63–5.0 ×). These tubes were then dried in 50 °C and weighed (Precisa EP 225SM-DR) to determine the mass of MPs. The fragments in the smaller size fraction (yellow and red MPs, 100–300 μm) were suspended separately in Milli-Q water and their concentration was adjusted to approximately 300 MP mL^{-1}. 0.5 mL were then taken from both of the solutions and added into 32 separate Eppendorf tubes. Similar subsamples from both solutions were also added into five pre-weighed Eppendorf tubes, dried in 50 °C, weighed (Precisa EP 225SM-DR) and counted (Leica CLS 150 EXE, VWR CL-150, magnification 0.63–5.0 ×) to obtain an estimate of their numbers (average ± SD concentrations in 0.5 mL^{-1}: red MPs = 161 ± 24.0; yellow MPs = 163 ± 20.7).

To spike the MPs to specific depths in the sediment, frozen sediment layers with MPs were prepared (Gilbert et al., 2007) to be added to the cylinders together with fresh, original sediment from the same homogenous sediment pool. Sediment layers with a thickness of 1 cm were made in similar cylinders that were used in the experiment; the bottom of the cylinder was lined with non-adhesive baking paper, 1 cm of sediment was added and MPs gently mixed into the sediment. Baking paper, a disc made of cardboard and another sheet of baking paper was then placed on top to prevent mixing with the next sediment layer. Two types of frozen layers containing MPs were made; the first type contained 150 green (>500 μm) MPs and on average 161 red MPs

(100–300 μm), and the second type 150 pink (>500 μm) and on average 163 yellow (100–300 μm) MPs. All layers containing MPs were frozen overnight at −20 °C.

One frozen sediment layer containing green (>500 μm) and red (100–300 μm) MPs was placed on top of the earlier added sediment in every experimental cylinder. Several white glass beads (Ø 3 mm) were placed on the edges of the frozen layers to mark the border between the neighboring layer and to monitor the compaction of sediment in the transparent cylinder (Fig. 1). Their location was marked to the outside wall of the cylinders as they were expected to sink when the frozen sediment layer melted and the sediment compacted; the distance between the mark and the bead would at the end of the experiment show how much the frozen, spiked sediment layer had subsided. After placing the beads, homogenous sediment was added to create approximately 2 cm thick layer, and a frozen layer containing pink (>500 μm) and yellow (100–300 μm) MPs was placed on top of that with glass beads. Finally, approximately 2 cm of sediment was added to cover the frozen layer. After adding the MP-containing frozen sediment layers, the total number of MPs in each experimental cylinder was on average 624 MPs, which consisted of 150 pink, 150 green, 161 red, and 163 yellow particles. Together they weighed approximately 18.3 mg, and formed a concentration of 1219 MPs per kg (dry weight) and 35.7 mg per kg (dry weight) in each of the experimental cylinders. After settling and compacting, the thickness of the sediment was 9.5 cm, and the MPs incorporated in the frozen sediment layers ended up in the targeted depths of approximately 2 cm (pink >500 μm, yellow 100–300 μm) and 5 cm (green >500 μm, red 100–300 μm). Oxygen content, temperature and salinity of the ambient seawater dripping to the experimental units were 13.42 mg L^{-1}, 4.3 °C, and 5–6, respectively, and 12:12 light-dark cycle was established to the room.

Grain size analysis was performed from the same pool of sediment that was added to the experimental cylinders. Approximately 400 mL of wet sediment was covered with 6% H_2O_2 for 48 h and stirred twice a day to digest the organic material. Sediment was sieved with the aid of water through a stack of sieves (mesh sizes 500, 250 and 63 μm), and each fraction was placed into separate pre-weighed container and dried at 60 °C. Water and the smallest size fraction that passed the <63 μm sieve were left in a bucket to settle for three days. The water was carefully removed without disturbing the sediment at the bottom and the sediment was then washed into a pre-weighed container and dried at 60 °C before weighting (Sartorius LG620 Masterpro). Using the classification of Blott and Pye (2001), the sediment used in this experiment can be classified as very fine-grained: the dominant fraction (73.6%) from the dry weight consisted of silt and clay (<63 μm), followed by fine and very fine sand (63–250 μm; 21.3%). The proportion of medium sand (250–500 μm) was 3.8% and coarse sand (>500 μm) 1.3%. The dry weight of the sediment in one experimental cylinder was 512 g.

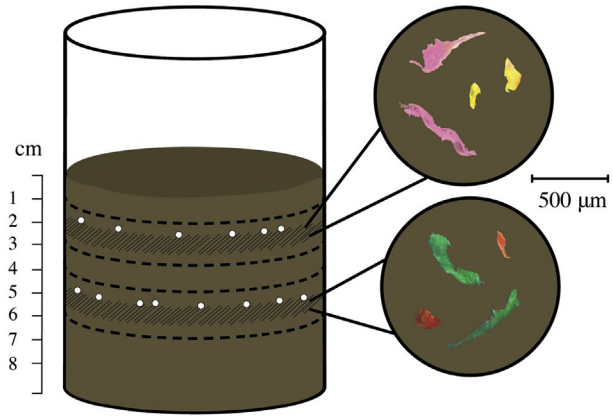

Fig. 1. Experimental cylinder showing how the sediment layers were cut at the end of the experiment. The shaded areas represent the location of frozen sediment layers containing MPs, and the glass beads marking their location are shown white.

2.2. Running the experiment

On the next day the experiment was started by adding the acclimatized animals to the experimental cylinders. 14 *L. balthica* (size range 15–21 mm, average 18.1 mm), 7 *Marenzelleria* spp. (size range 10–70 mm, average 34.1 mm) and 6 *M. affinis* (size approx. 6–7 mm) were placed in each of the animal cylinders (n = 8) in natural densities: *L. balthica* 908 ind. m^{-2}; *Marenzelleria* spp. 454 ind. m^{-2}; *M. affinis* 390 ind. m^{-2} (Rousi et al., 2013). The rest of the cylinders (n = 8) were controls containing only MPs. During the experiment, ambient, unfiltered seawater was constantly dripping from the hose to provide oxygen and food to the cylinders. Two days after the beginning of the experiment 5 dead, unburrowed clams found on the top of the sediment were replaced with living ones and live *Nannochloropsis* algal concentrate (PhytoMaxx, NYOS® Aquatics, Germany) was added to the cylinders in a final concentration of approx. 200 000 cells mL^{-1} as supplementary food for the animals. The temperature and oxygen levels were measured weekly from the water phase of the cylinders and *Nannochloropsis* was added as described once per week throughout the study period. Dead amphipods were picked up from the sediment surface and stored in a freezer (−20 °C).

Because the experiment was carried out in spring and the water to the cylinders came directly from the sea, the temperature followed the environmental conditions and was in a slow constant rise as the spring proceeded. Nevertheless, the conditions stayed relatively stable; during the experiment the water had a mean temperature and SD of 7.3 ± 3.0 °C and mean oxygen content and SD of 10.8 ± 2.0 mg L^{-1} in the animal cylinders, and 7.3 ± 3.0 °C and 11.2 ± 1.3 mg L^{-1} in the control cylinders. The animals appeared to be in good health, judged by the burrow openings, new feces, and frequent sightings of *Monoporeia affinis* and siphons of *Limecola balthica* on the sediment surface.

The experiment was ended after 72 days. The mean depth of the light-colored oxygenated layer in the top of the sediment was determined by measuring it from four randomized spots around each cylinder. The light brown oxygenated layer penetrated deeper into the sediment in the cylinders containing animals (1.97 ± 0.25 cm) compared to cylinders without animals (0.49 ± 0.20 cm) (Mann-Whitney *U* test: $U = 0$, $p = 0.000$), indicating improved oxygen conditions in the animal-containing, bioturbated sediments. Below the light-colored layer, the sediment color was dark, greyish brown, but the burrows of *Marenzelleria* spp. were extending throughout the core in animal cylinders. The sediment from the cylinders was sliced horizontally into five layers with a HAPS corer sample ejection aggregate with a cutting plate on top (see Näkki et al., 2017 for more information). The depth of spiked sediment layers was determined by the location of glass beads. To ensure that these spiked sediment layers would not contaminate the neighboring layers when cutting, we left approximately 0.5 cm of extra space under and above them. From top to bottom, the thicknesses of cut sediment layers were 1 cm, 2 cm, 1 cm, 2 cm and 3 cm (Fig. 1). All sediment layers were sealed in ziplock bags and frozen in −20 °C.

2.3. Sample processing

The frozen sediment layers were thawed in room temperature

on wide trays. Animals were hand-picked to separate tubes and frozen again in −20 °C until further processed to study MP ingestion. Saturated NaCl was used for processing the sediment samples according to Näkki et al. (2017) with minor modifications; supernatant was only decanted and the walls of the funnel were rinsed both with tap water and water mixed with a drop of washing up liquid (Fairy, Procter & Gamble) to reduce the amount of MPs sticking to the walls. MPs on the filters were counted under a Leica MZ 7.5 microscope (Leica CLS 150 EXE, VWR CL-150, magnification 0.63–5.0 ×).

For the enumeration of potentially ingested MPs, frozen animals were thawed in room temperature, rinsed with tap water and placed individually in small glass vials. Animals were treated with enzymatic digestion protocol according to Railo et al. (2018): a solution containing 50% of SDS (5 g/L, Sigma-Aldrich), 25% of Biozym F and 25% of Biozym SE (both from Spinnard, Bad Segeberg, Germany) was added to the vials and incubated 48 h at 37.5 °C. The disintegrated samples were filtered onto 100 μm mesh size filter and examined under stereomicroscope. As amphipods did not fully disintegrate due to the chitin in their carapace, they were manually torn on a filter with tweezers after enzymatic digestion.

2.4. Statistical methods

All statistical tests were performed with SPSS (version 23). We tested the proportions of different colors of MPs (indicating the particle size and original burial depth) related to their total number retrieved from each cylinder; this approach was chosen to be able to make comparisons between particles that had different recovery rates. The data did not fulfill the assumption of normality with or without transformations; thus, we had to apply a nonparametric Mann-Whitney *U* test for independent samples for statistical testing. The results are given in average with standard deviation.

3. Results

3.1. MP recovery from the sediment

Even though the density of saturated NaCl (1.2 g cm^{-3} (Hidalgo-Ruz et al., 2012)) enables the flotation of ABS, the overall recovery rate of MPs was 63%. 62 ± 6.1% of MPs were recovered from control cylinders, and 65 ± 4.5% from animal cylinders (Fig. 2); this difference was not significant (Mann-Whitney *U* test: $U = 24$, $p = 0.442$). The recovery of larger particles (>500 μm) was higher compared to the particles in the smaller size fraction (100–300 μm) (Mann-Whitney *U* test: $U = 6$, $p = 0.000$); on average 83–89% of green and pink particles were retrieved compared to the 32–56% of red and yellow particles.

The extraction efficiency in the >500 μm size fraction between green and pink particles placed in different depths did not differ (Mann-Whitney *U* test: $U = 87$, $p = 0.128$), whereas in smaller size fraction more red particles were recovered compared to yellow particles (Mann-Whitney *U* test: $U = 30$, $p = 0.000$). This difference was most likely due to the pale color of yellow particles, which made it harder to detect them from the filters.

3.2. Fragmentation of MPs

During counting particles from the filters, we noticed small fragments derived from the pink and green particles (originally >500 μm). Fragmentation of green and pink MPs (>500 μm) into smaller particles was observed in all experimental cylinders, and was presumably caused by the stirring phase during the NaCl extraction. To obtain an estimate of their fragmentation, they were all counted separately as small green and pink particles. Green MPs

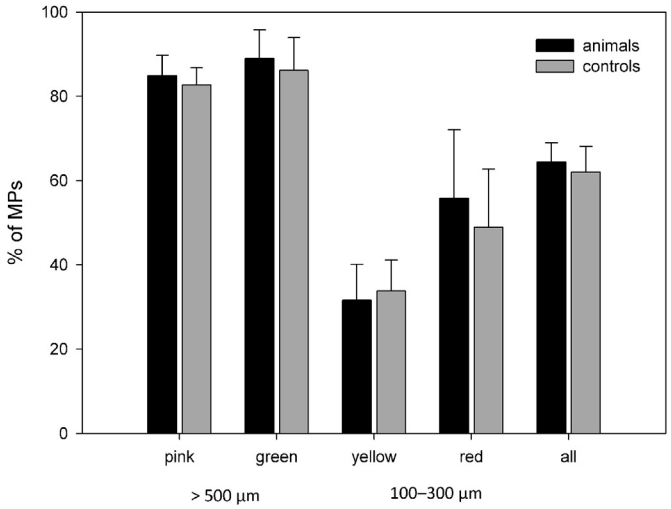

Fig. 2. Extraction efficiencies (average + standard deviation) of different sizes of MPs used in the experiment in animal (n = 8) and control (n = 8) cylinders.

fragmented more than pink MPs (Mann-Whitney *U* test: $U = 68$, $p = 0.023$): on average 7.6 ± 4.6 small green fragments and 4.3 ± 2.1 small pink fragments were found from each cylinder.

3.3. Distribution of animals

Out of 48 *M. affinis* individuals, 5 were retrieved from the frozen sediment layers, and from these, four were found from the uppermost sediment layer (depth 0–1 cm) and one from the second layer (depth 1–3 cm) (Fig. 3). Twenty individuals were found dead on the sediment surface at different times, mainly towards the end of the experiment, and the rest were not found. All clams (112 ind.) were found, and were distributed across the whole sediment column. 48 out of 56 *Marenzelleria* spp. individuals were retrieved; they tended to occupy the deeper parts of the sediment column.

3.4. MPs in the topmost sediment layer

When all colors of spiked MPs were pooled together, significantly more MPs were found from the surface layer of animal cylinders 1.0 ± 1.0% compared to control cylinders 0.2 ± 0.5% (Mann-Whitney *U* test: $U = 11$, $p = 0.028$). The most common particles found from the top layer in both types of cylinders were yellow (100–300 μm) and pink (>500 μm) MPs, which were originally placed at a short distance away from the surface (2 cm depth). On average, only 2.1 ± 2.5% of the particles placed to the second layer were found from the surface layer in animal cylinders, whereas in control cylinders 0.5 ± 1.1% of the particles ended up to the surface layer. In contrast, green (>500 μm) MPs placed at 5 cm depth into the sediment were completely absent from the surface layer in both animal and control cylinders and only one red (100–300 μm) MP from the same original layer was found from the surface of animal cylinders (Fig. 4). A higher percentage of yellow, pink and red particles were found from the surface layer of animal cylinders compared to control cylinders. However, the difference was not significant (Mann-Whitney *U* test: yellow MPs, $U = 13$, $p = 0.050$; pink MPs, $U = 20$, $p = 0.234$; red MPs, $U = 28$, $p = 0.721$). Furthermore, our results also show that animals transported pink and yellow MPs similarly regardless of their size, since we found no significant differences between their upward transport in animal cylinders (Mann-Whitney *U* test: $U = 41$, $p = 0.382$).

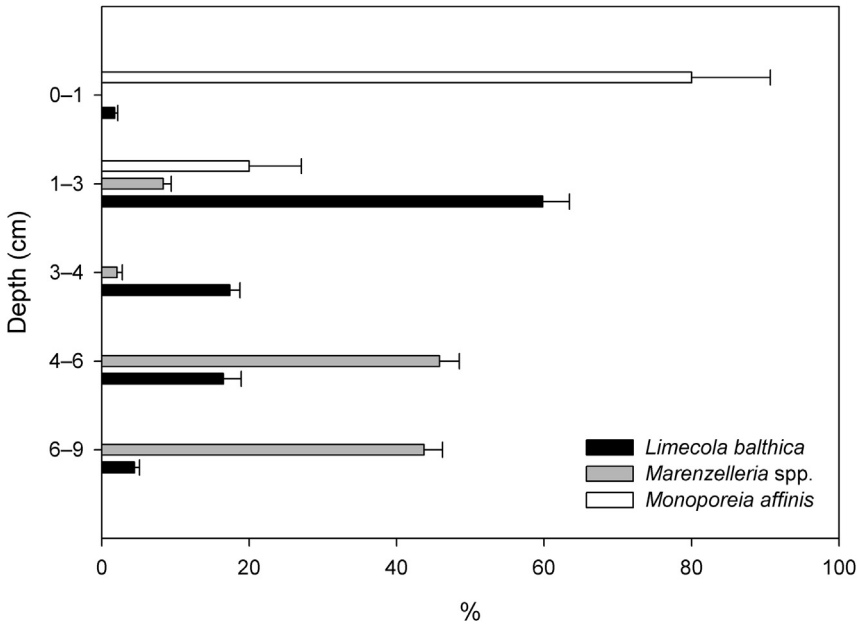

Fig. 3. Distribution of animals (average + standard deviation) at the end of the experiment in five layers of sediment (*Limecola balthica* n = 112; *Marenzelleria* spp. n = 48; *Monoporeia affinis* n = 5).

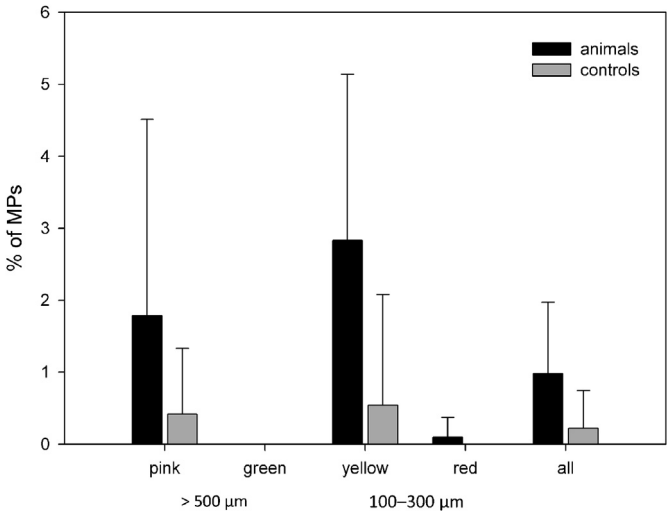

Fig. 4. Proportion of different MPs (average + standard deviation) recovered from the uppermost sediment layer (0–1 cm) in animal (n = 8) and control (n = 8) cylinders. Pink and yellow MPs were originally placed to the depth of 2 cm, and green and red MPs in the depth of 5 cm.

3.5. MP ingestion

We found only one yellow particle (100–300 µm) from one *L. balthica* individual. This individual was found from the second sediment layer (1–3 cm), where the yellow MPs were originally placed at 2 cm depth. The two other species in this experiment, *M. affinis* (including individuals that died during the experiment) and *Marenzelleria* spp., did not contain any of the spiked MPs.

4. Discussion

4.1. Upward transport of MPs by bioturbation

Our results demonstrate that the benthic macrofauna in the northern Baltic Sea may be able to redistribute buried MPs towards the sediment surface in small quantities. During our 10-week long experiment, approximately 2% of MPs placed to the depth of 2 cm ended up to the surface layer (0–1 cm) in the presence of animals. Small numbers of MPs were also found from the top layer in the control cylinders, and such slight mixing of particles and dissolved contaminants in control cores seem to be common in bioturbation studies (e.g. Quintana et al., 2007; Josefsson et al., 2011). It is suggested to stem from the activities of meiofauna and juvenile macrofauna (Quintana et al., 2007; Viitasalo-Frösén et al., 2009; Renz and Forster, 2013), compaction of the sediment and the subsequent movement of pore water (Josefsson et al., 2011), or to be a mechanical artifact from slicing the sediment layers (Renz and Forster, 2013). Although we had a small number of replicates and high variability in the redistributed MPs in cylinders, our observation of the upward transport of MPs by bioturbation is supported by earlier laboratory studies where polystyrene beads (Ø 100 µm) were transported upwards by oligochaete worms (Naididae) (Kearns et al., 1996), and luminophores (i.e. natural sediment grains covered with fluorescent paint) were transported upwards by the tellinid bivalve *Abra nitida* and the polychaete *Scalibregma inflatum* (Gilbert et al., 2007).

As most infaunal species typically inhabit the oxygenated sediment layer just below the sediment-water interface (Pearson, 2001), bioturbation is concentrated in the surficial sediments, varying in depth depending on the species composition. In the Baltic Sea, the depth of bioturbated layer is typically less than 5 cm (Hedman, 2008), which is in line with our results: green and red MPs from 5 cm depth rarely ended up to the surface, whereas particles placed in the depth of 2 cm were more often transported upwards in the animal cylinders. This pattern suggests that the deeper in the sediment the MPs are buried, the less probable is their return to the surface.

An unexpected drawback in the experiment was the fragmentation of MPs. As the fragmentation of red and yellow particles (100–300 µm) could not be monitored because of the 100 µm mesh size of filters, it is impossible to say whether they fragmented and whether this fragmentation caused over- or underestimation of

particles, especially when there could have been differences in fragmentation depending on the color. We observed differences in the numbers of fragments detached from green and pink MPs, but fortunately these fragments did not skew the counts, as they were considerably smaller (approx. 100–300 μm) than the original fragments (>500 μm). The variation in fragmentation may arise from different batches, coloring agents, or the age of the material. Nevertheless, when processing environmental samples, it must be considered that fragmentation of already weathered, fragile particles during the extraction process can lead to overestimation of MP concentrations and distortion of their original size distribution.

The recovery of MPs was affected by the size and color of the particles. The yellow MPs (100–300 μm) had the lowest recovery rate (<40%) partly due to their weakest visibility, whereas >80% of the bigger pink and green particles (>500 μm) were recovered. However, the recovery rates are comparable to other studies conducted with similar methods; recoveries ranging from 34.3 to 97.5% has been achieved, recovery being higher for larger particles and decreasing with size (Claessens et al., 2011; Näkki et al., 2017). The low recovery rate may have led to underestimation of particle loads in the surface layer, but assuming that the extraction efficiency has been similar in all layers, examining the proportions of particles found in each layer enabled the comparisons between different-sized particles and presumably reflected their true distributions.

4.2. The effect of species composition

The distribution patterns of animals at the end of the experiment corresponded well to their typical preferred depths; *M. affinis* is found on the sediment surface, whereas *L. balthica* and *Marenzelleria* spp. are usually distributed deeper within the sediment (Karlson et al., 2005; Viitasalo-Frösén et al., 2009; Näkki et al., 2017). The distribution of animals combined with the species' characteristics helps to explain why only minor upward transport of MPs was observed. For example, the near-surface layers (0–3 cm) were occupied by *M. affinis*, which displaces particles in the sediment-water interface and causes their resuspension to the overlying water (Hedman et al., 2008). Even though the mortality of amphipods was high, the deaths mostly occurred in the latter half of the experiment. Hence, it can be assumed that they could have affected the MP distributions during the first 5 weeks, after which their bioturbation activity gradually declined. We suspect that the loss of amphipods during the experiment was at least partially due to the phase of their life cycle: in shallow waters *M. affinis* typically have a life cycle of 1 year, and females often die after releasing their brood in spring (Segerstråle, 1950; Cederwall, 1978). This hypothesis is supported by the timing of our experiment, as well as finding numerous juvenile individuals during MP extraction.

Regardless of the loss, the impact of *M. affinis* has probably been minor, as an earlier study found no evidence that this amphipod would transport zooplankton resting eggs upwards in sediments (Albertsson and Leonardsson, 2000). In contrast, the bioturbation activities of *M. affinis* have rather been associated to particle burial (Viitasalo, 2007). Similarly, *Marenzelleria* spp., are not usually considered efficient particle bioturbators (Hedman et al., 2008; Viitasalo-Frösén et al., 2009; Renz and Forster, 2013), even though they are known to move particles in various directions when feeding and maintaining burrows (Quintana et al., 2007), and burrowing deeper than most macrofauna in the northern Baltic Sea (Karlson et al., 2005). Their weak particle-reworking capabilities coupled with their moderate density might explain the absence of MPs that were placed at the 5 cm depth from the surface. Both the *Marenzelleria* spp. and *M. affinis* were, however, introduced to the cylinders because they are important components of the benthic macrofauna in many areas, and we wanted to examine the effect of the whole community on the MP distribution.

L. balthica is known to be relatively efficient downward particle-displacer in the Baltic Sea compared to *M. affinis* and *Marenzelleria* spp. (Viitasalo, 2007; Viitasalo-Frösén et al., 2009), but their capability to promote upward transport of particles is unknown. The distribution of *M. affinis* and *L. balthica* in the surface layers where MPs were transported upwards suggest that the activities of either *M. affinis* or *L. balthica*, or both of these species together, may have contributed to the observed minor upward transport of MPs in this experiment. Based on the patterns of animal and MP distributions in our experimental cylinders, it seems that MPs at different depths are subject to varying bioturbation pressures, which arise from the preferred residence depth of species as well as their specific characteristics, such as the feeding range and mode. These are likely to further affect the potential redistribution of MPs within the sediment.

4.3. Ingestion of MPs

Despite the fact that yellow and red MPs (100–300 μm) were within the feeding range of *L. balthica* (Gilbert, 1977; Viitasalo, 2007), only one individual had ingested one yellow particle. This is likely due to the fact that *L. balthica* is a surface deposit feeder, feeding either by siphoning the material from the sediment surface or filtering the overlaying water (Lin and Hines, 1994), and is therefore not exposed to buried MPs while feeding. In our previous study with similar MP size, 25% of the examined *L. balthica* individuals ingested MPs that were allowed to settle on the sediment surface (Näkki et al., 2017). Although the concentration of MPs was slightly higher in the previous study (1790 MP kg^{-1} dry sediment), it is probable that the difference between these results is simply explained by the location of the MPs in the sediment.

M. affinis is also a surface deposit feeder (Lopez and Elmgren, 1989), but the available MPs were outside its ingestible size range of <60 μm (Ankar, 1977). Similarly, *Marenzelleria* spp. are primarily surface deposit feeders (Hedman, 2008). The smaller size fraction of MPs (100–300 μm) was partly in the feeding range of *Marenzelleria* spp. (Bock and Miller, 1999), and they have previously been found to ingest MPs when exposed to 10 μm polystyrene beads in experimental conditions (Setälä et al., 2016). It is probable that the low concentration of ingestible MPs in the sediment combined with the particle-selection performed by the polychaetes lowered the possibility of MP ingestion. As they are known to select small particles over larger ones (Bock and Miller, 1999), and most of the sediment consisted of silt and clay and was thus smaller than the MPs, the polychaetes may have selected sediment particles instead of MPs.

It has been proposed that deposit-feeding is the most important mechanisms of particle mixing in marine sediments (Jumars and Wheatcroft, 1989), affecting especially the displacement of small particles that are more probably ingested than larger ones (Wheatcroft, 1992). However, as the ingestion of MPs in our study was negligible, and the size of MPs did not affect their upward transport in animal cylinders, we can conclude that the means of transport in this experiment has probably been through animal movement rather than feeding, which further explains why only minor transport of MPs was observed.

4.4. Implications

Our previous study conducted with a similar experimental setup found out that 8% of secondary MPs placed on the sediment surface were transported downwards in just 3 weeks (Näkki et al., 2017), whereas the results from the present 10-week study suggest a lower rate of upward transport. Thus, based on these two studies, in

the northern Baltic Sea the net transport of MPs could be downwards, further supporting the general idea of seafloors acting as MP sinks (Woodall et al., 2014). It is presented that bioturbation is likely to result in heterogeneous mixing of the sediment; for example, unidirectional downward transport can happen when particles on the sediment surface fall to the collapsing burrows of benthic animals (Kristensen et al., 2012). Differences in particle-mixing can also arise for example from particle sorting during feeding and fecal deposition sites, which are species-specific (Snelgrove, 1998; Kristensen et al., 2012). Because of this species-specificity of bioturbation (Viitasalo-Frösén et al., 2009; Janas et al., 2017) our results only apply to the study area, where the most abundant benthic macrofauna are small-sized, mainly shallow-burrowing surface deposit feeders, and mostly classified as biodiffusors, which transport particles in a random manner over short distances (Michaud et al., 2005; Hedman et al., 2008; Renz and Forster, 2013). In contrast, upward conveyors, such as the lugworm (*Arenicola marina*), ingest subsurface sediment and defecate on the sediment surface (Rhoads, 1974; Riisgård and Banta, 1998). This species has recently been shown to efficiently bury MPs deposited on the sediment surface (Gebhardt and Forster, 2018), but it is still unknown in what extent its feeding mode would also redistribute MPs buried down to its feeding layer back to the sediment surface. Being aware of the characteristics of local fauna is crucial when trying to understand the dynamics of MPs on the seafloors.

It has been suggested that MPs tend to accumulate in areas with weak water flow alongside with fine-grained sediments, associated contaminants and organic carbon (Strand et al., 2013; Vianello et al., 2013; Maes et al., 2017; Willis et al., 2017). These muddy sediments provide ideal habitats for a variety of organisms, of which majority are deposit feeders (Shull, 2009). It has been estimated that the surface sediments of fine-grained seafloors are passed through the benthic fauna at least once or in some cases even multiple times a year (Rhoads, 1974). Species occupying muddy environments may be likely exposed to high MP concentrations, as also suggested earlier by Maes et al. (2017). High concentrations of MPs have already been detected in the sediments around the world: 672–2157 MP kg^{-1} sediment (d.w.) in the Lagoon of Venice (Vianello et al., 2013), 3146 MP kg^{-1} (d.w.) in the southern North Sea (Maes et al., 2017), and 4356 particles kg^{-1} (d.w.) in deep sea sediments at Fram Strait (Bergmann et al., 2017). The concentration used in our experiment (1219 MP kg^{-1} d.w.) corresponds or even falls below to these values, but exceeds the concentration so far observed in the Baltic Sea, where on average 34 particles kg^{-1} sediment (d.w.) were found from the Gdansk Bay (Zobkov and Esiukova, 2017). With the concentration and MP size distribution used in our experiment, the probability for ingestion to the Baltic Sea communities seems to be low. However, alongside concentration and particle size, our results demonstrate that when assessing the risk of ingestion and transport of MPs by benthic fauna, the vertical distribution of MPs with respect to the existing species are also important. According to the present knowledge, most of the MPs are found near the sediment surface, and their numbers are decreasing with the increasing sediment depth (Martin et al., 2017; Willis et al., 2017); for example, in the western continental shelf of Ireland, 97% of the MPs (>250 μm) were found from the topmost 2.5 cm of the sediment (Martin et al., 2017). As the quantities of MPs in the ocean are inevitably increasing (Law and Thompson, 2014), benthic habitats are subject to constantly increasing plastic contamination. To be able to assess the ultimate fate and impacts of MPs in the marine environment, more information is certainly needed on the vertical distribution of MPs in natural sediments, their availability to benthic fauna, and the influence of bioturbation in their distribution.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.02.099.

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