Science of the Total Environment 856 (2023) 159106

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Multi stress system: Microplastics in freshwater and their effects on host microbiota

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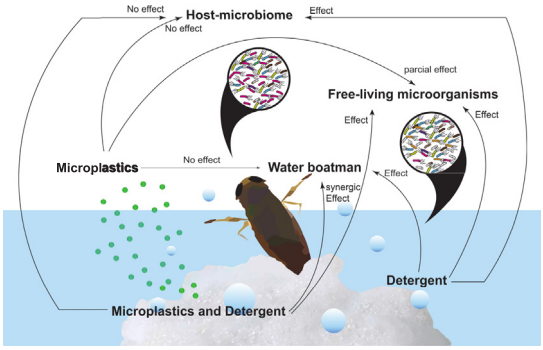
HIGHLIGHTS

- The detergent and microplastics together had negative effects on survival.
- Microplastics acute the toxicity of detergent, causing mortality in fewer hours.
- The microbiome in the host showed to be more stable than the free-living microorganisms.
- The combined exposure of detergent and microplastics showed no effect on the host-microbiome.
- The microbiome is affected by the microcosm method compared to what is found in nature.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Thomas Kevin V

Keywords:
Host microbiome
Water boatmen
Environmental stress
Water pollutants
Sodium dodecyl sulfate
Ecotoxicology

ABSTRACT

Microplastics are persistent and complex contaminants that have recently been found in freshwater systems, raising concerns about their presence in aquatic organisms. Plastics tend to be seen as an inert material; however, it is not well known if exposure to plastics for a prolonged time, in combination with organic chemicals, causes organism mortality. Ingestion of microplastics in combination with another pollutant may affect a host organism's fitness by altering the host microbiome. In this study, we investigated how microplastics interact with other pollutants in this multi-stress system, and whether they have a synergistic impact on the mortality of an aquatic organism and its microbiome. We used wild water boatmen Hemiptera (Corixidae) found at lake Erken located in east-central Sweden in a fully factorial two-way microcosm experiment designed with polystyrene microspheres and a commonly used detergent. The microplastic-detergent interaction is manifested as a significant increase in mortality compared to the other treatments at 48 h of exposure. The diversity of the microbial communities in the water was significantly affected by the combined treatment of microplastics and the detergent while the microbial communities in the host were affected by the treatments with microplastics and the detergent alone. Changes in relative abundance in Gammaproteobacteria (family Enterobacteriaceae), were observed in the perturbed treatments mostly associated with the presence of the detergent. This confirms that microplastics can interact with detergents having toxic effects on wild water boatmen. Furthermore, microplastics may impact wild organisms via changes in their microbial communities.

1. Introduction

Anthropogenic pollutants have a major impact on aquatic environments (Schmeller et al., 2018; Sibley and Hanson, 2011). They are released in freshwater bodies every day and contribute to the occurrence of chemical micropollutants and microplastic (Schmeller et al., 2018; Zettler et al.,

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http://dx.doi.org/10.1016/j.scitotenv.2022.159106
Received 7 July 2022; Received in revised form 24 September 2022; Accepted 24 September 2022
Available online 30 September 2022

2013). We have known for a long time that pollutants affect mortality of aquatic organisms (Chandini, 1989; Czyzewska, 1975; Kwok et al., 2007; Sobrino-Figueroa, 2018; Vargas and Dussán, 2016). For example, Sobrino-Figueroa (2018) found that exposure to commercial detergents increased mortality rates of various organisms including plants, invertebrates, and fish. Pollutants originating from anthropogenic activities are especially detrimental to invertebrates in freshwater systems (Häder et al., 2020). Thus, embryos and larval stages of aquatic invertebrates have commonly been used in water ecotoxicology research (Burgess et al., 1995; Byrne, 2012). Early experimental studies generally focused on one pollutant at a time; however, natural environments are likely to involve multiple pollutants. Thus, recent studies have looked at combined effects of pollutants on mortality, fitness, and life history traits (Gomez Isaza et al., 2020; Kwok et al., 2007; Varg et al., 2021). The emerging pollutant combined with the proliferation of chemicals from human activities represent a new challenge for water ecotoxicology. Hence, it is essential to study the synergistic effects of multiple pollutants on the survival of aquatic organisms (Byrne, 2012; Häder et al., 2020).

Besides larger organisms, pollutants have also been shown to affect micro-organisms, such as bacteria (Babich et al., 1980; Breton et al., 2013; Merrifield et al., 2013). For example, cadmium has been shown to inhibit the activity of free-living bacteria (Shi and Ma, 2017). Multiple stressors might be synergistic and acute the effects of the pollutants in the bacterial communities in aquatic ecosystems affecting for example organic matter (re)cycling, microbial trophic interactions and nutrient availability (Cabrerizo et al., 2019; Sadeq et al., 2021). Furthermore, host-associated bacteria, such as gut microbiota have been shown to be affected by pollutants and its synergistic interactions (Breton et al., 2013; Varg et al., 2022b; Wu et al., 2014; Yuan et al., 2019). Host microbiota are important to their hosts for biochemical and physiological processes and can have important effects on the host's health, immune regulation, growth, development and even on behavior through the gut-brain axis (Dehler et al., 2017; Nicholson et al., 2012; Roger et al., 2016; Stilling et al., 2014). Thus, disturbing the host microbiota with pollutants may negatively impact the host. The composition of host microbiota is not only influenced by environmental factors but also by the host (e.g., host genetics and diet; Ley et al., 2008, Medina and Sachs, 2010, Dehler et al., 2017). It has been hypothesized that hosts are under strong natural selection to control their microbiomes (Berg et al., 2020; Foster et al., 2017). Therefore, external disturbances may have less of an effect on microbiomes compared to host factors.

Detergents as pollutants in aquatic environments have been extensively studied. Toxicity studies of detergents in aquatic organisms have been carried out since 1950 (Czyzewska, 1975; Gerasimidis et al., 2019). It is well known that exposure to these products can decrease activity in living organisms and that prolonged exposure usually ends up in damaging biological functions, paralysis and even death of the organism (Czyzewska, 1975; Gerasimidis et al., 2019; Sobrino-Figueroa, 2018). Besides larger aquatic organisms, detergents have also been shown to affect the abundance and diversity of micro-organisms (Baker et al., 1941; Gerasimidis et al., 2019; Warne and Schifko, 1999) Antimicrobial activities of detergents can take many forms, including the limiting of metabolic activities and growth rate, destruction of bacterial cell membranes, inhibition of cellular metabolism, as well as bactericidal and bacteriostatic effects (Baker et al., 1941; Brandt et al., 2001; Klebensberger et al., 2007; Mousavi and Khodadoost, 2019). While we have good knowledge of the effects of detergents on aquatic macro-organisms as well as free-living micro-organisms, we know relatively little about the effect of detergents on host-associated micro-organisms, (e.g., the gut microbiota of aquatic macro-organisms).

Plastic pollutants have been identified recently as a major problem in aquatic environments (Beer et al., 2018; Eriksen et al., 2014; Horton et al., 2017; Menéndez-Pedriza and Jaumot, 2020) around the world (Eerkes-medrano et al., 2015; Wagner and Lambert, 2018). Especially worrisome are small plastic fragments, beads, granules, and fibers below 1 mm in diameter that are referred to as microplastics (Cole et al., 2015; hereafter "MPs"). Studies have shown that the exposure to MPs could induce mortality in aquatic invertebrates such as *Hyalella azteca* and *Daphnia magna* (Au et al., 2015; Renzi et al., 2019). MPs can also affect the microbiomes of aquatic organisms by ingestion; MPs could provide a long-lasting, novel substrate for microorganism to attach and grow (Amaral-Zettler et al., 2020; Varg et al., 2022a). Therefore, this new carbon source may influence the microbial community.

Plastics may interact with detergents, as some plastics can also be used as a basis for surfactants that are used in the manufacture of industrial and domestic detergents and pesticides (Sibley and Hanson, 2011). Both MPs and detergents may affect free-living as well as host-associated microbes (Mousavi and Khodadoost, 2019; Varg et al., 2022b). Furthermore, plastics as a floating substrate facilitate transportation of pollutants. However, interactions of MPs with other pollutants are not well known in water but may explain the pathophysiology of many reported biological activities in diverse organisms and their microbiomes (Pietroiusti et al., 2015; Wang et al., 2018).

In this study we investigated the effects of MPs and a detergent on water boatmen from the family Corixidae (Hemiptera). We evaluated mortality over time as well as community composition of free-living and host-associated microbiota, both in the presence and absence of MPs and sodium dodecyl sulfate (SDS; a detergent), in a two-way factorial design. It has been shown that plastics can enhance the growth of bacteria that are able to colonize plastic surfaces and use MPs (Horton et al., 2018; Zettler et al., 2013). Therefore, we hypothesized that presence of MPs would result in changes in the microbial community both in the water and in the host. We also hypothesized that because of the toxicity of detergents and their effects on host microbiomes (Czyzewska, 1975; Gerasimidis et al., 2019), water boatman will have higher mortality in treatments with SDS. Similarly, it has been reported that chemical pollutants could be adsorbed on MP surfaces, thus increasing the effect of the pollutant (Felten et al., 2020; Skjolding et al., 2016). For this reason, we hypothesized that water boatman mortality in the treatments with a combination of SDS and MPs would be highest and microbial diversity lowest.

2. Methods

2.1. Lake water sampling and wild water boatmen collection

The experiments and the water collection from lake Erken, Nortällje, Sweden (59.510°N 18.360°E) were carried out in two occasions; summer 2016 and summer 2017. Near shore water was sampled close to the limnological field station, at a depth of 30 cm. The water was filtered with a 165 μm mesh to remove phytoplankton, zooplankton and other big particles. The filtered water was immediately used in the microcosm experiment (described below) and five aliquots of 50 ml were also immediately frozen and stored at −20 °C for further microbiome analysis of the lake water. Nymphs from an aquatic bug, water boatmen (Hemiptera: Corixidae) were collected from the same near-shore location with a fish net and stored in a flask with lake water. The samples were transported to Erken laboratory 5 min walking from the shore of the lake and stored for 1 h while the treatments were prepared. 9 Water boatmen directly from the lake were immediately frozen and stored at −20 °C for further microbiome analysis.

2.2. Microcosms

The microcosm experiment was carried out in 1 l flask filled with 500 ml of filtered lake water (see above). We tested the effects of microplastics (MPs) and Sodium dodecyl sulfate (SDS) in a two-way factorial design, replicated 6 times (Fig. 1). In the MPs treatments, we used 1 μm Fluoresbrite® Yellow Green Microspheres (CAS# 0009003536, Polysciences, Inc.) at a concentration of 4000 particles/ml (2.2 × 10–9 g/ml). The MPs concentration was chosen based on previous reports, as well as based on the concentration found in contaminated water environments that is similar to or lower than the one used here (Klein et al., 2015; Lei et al., 2018; Varg et al., 2021; Wagner and Lambert, 2018). According to

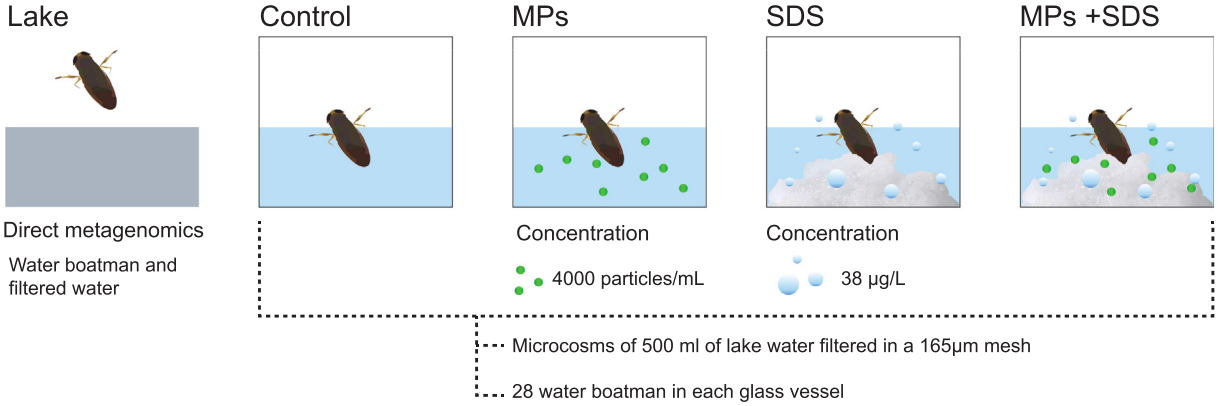

Fig. 1. Graphical representation of the experimental design. Heteroptera water boatmen from the family Corixidae were exposed to 1 µm polystyrene-base latex MPs, sodium dodecyl sulfate (SDS), and the combination of both MPs and SDS. Water boatmen in the control were not exposed to MPs or SDS. The lake treatment is the direct metagenomics of the water boatman and the 165 µm filtered lake water (i.e. No microcosm was made for the lake treatment), 6 replicates per treatment were used.

the manufacturers the microplastics was packaged as 2.5 % aqueous suspension, and no surfactants were listed as ingredients To remove the leached dye in the solution, MPs were centrifuged at 6000 rpm and resuspended in freshwater. The concentration of SDS Sigma-Aldrich® (CAS#$ 51-21-3) in the SDS treatments were 38 µg/l, similar to previous EC^{95} toxicity studies (Sobrino-Figueroa, 2018; Warne and Schifko, 1999). After adding the SDS to their treatments, they were stirred with a magnetic stirrer until the surfactants were dissolved and avoiding the formation of bubbles. The microcosm experiment was carried out in the lake with half of the flask submerged in the water to achieve lake temperature and same lake environmental conditions. Furthermore, the microcosms were open allowing bacterial dispersal.

The experiment started after adding 28 water boatmen to each replicate. Mortality was assessed after 24, 48, 72 and 168 h by observing the water boatmen movement; when there were uncertainties in mortality, a sterile plastics stick was used to poke the water boatmen to elicit a response. After 48 h, three haphazardly picked water boatmen and 50 ml of water were extracted from each replicate. Therefore, 25 water boatmen were considered as the total number of water boatmen in each microcosm. The three water boatmen from each replicate were pooled and stored at −20 °C for further DNA extraction. The 50 ml water samples were filtered through a 2 µm filter and the content of the filter was stored at −20 °C for further DNA extraction. pH was measured throughout the experiment (Fig. S1), and Dissolved Organic Carbon (DOC) was measured at the beginning and at the end of the experiment (Fig. S2).

2.3. Microbiome and library preparation

Total DNA from the water samples as well as from the water boatmen was extracted using the DNeasy PowerSoil kit from Qiagen No./ID: 12888-100. 16S primers 515F and 806R were used to amplify the hypervariable region V4 of the 16S rRNA gene through PCR using Phusion High-Fidelity DNA Polymerase (ThermoFisher scientific, F-530XL) all by triplicate. To attach the specific illumine barcodes to the previously amplified 16S region a Second PCR was performed (all by triplicate) following the PCR protocols of the earth microbiome project (Caporaso et al., 2012; Zha et al., 2018). In between each PCR step, the result of the PCR was purified using magnetic beads Agencourt AMPure XP from Beckman coulter life sciences. The samples were pooled with an equal concentration of DNA. An Electrophoresis of the DNA pool was run to perform a final purification step using the QIAquick Gel Extraction Kit from Qiagen, No./ID: 28104. The purified gel band was between 400 and 500 bp. The resulted amplicons were sequenced on an illumina Miseq at the Science for Life Laboratory (SciLifeLab) NGI platform hosted by Uppsala University.

2.4. Statistical analyses

To test if there were differences between summer 2016 and 2017 a Linear mixed models (LMM) were carried out using the water beetle mortality as response variable and summer as fixed factors, and vessel was used as random effect. The model were performed using the lm4 R package (Bates et al., 2015).

2.4.1. Mortality

The effect of MPs and SDS individually and its combined effect on water boatman mortality was analyzed in four different models, one for each observed time (24, 48, 72 and 168 h). In all cases a poisson generalized linear model was used. Mortality was used as response variable and it was measured as percentage. MPs and SDS were used as fixed factors. To further investigate the effects of exposure between treatments, post-hoc Tukey tests were used using the emmeans R package (Lenth et al., 2020).

2.4.2. Microbiome

The amplicon sequence variant (ASV) table of the sequenced water boatmen and filters was created using Demultiplexed data from the SciLifeLab and following the DADA2 pipeline 1.8 (Callahan et al., 2016). Taxonomy was assigned using SILVA database (Quast et al., 2013), taxa diversity was measured, and the top 6 abundant phyla were identified using the R package lattice and MASS (Venables and Ripley, 2002; Sarkar, 2008). Chao, Shannon and Phylogenetic diversity index were estimated as alpha diversity using the R packages fossil, vegan, ape and picante respectively (Kembel et al., 2010; Vavrek, 2011; Oksanen et al., 2019; Paradis and Schliep, 2019). Linear models (LM) were carried out to observe the effect of the exposure of MPs and SDS in the response variables diversity indexes followed by a Variance Analysis (ANOVA). To test beta diversity Permutational Multivariate Analysis of Variance (PERMANOVA) were performed. ASV Bray-Curtis distance matrix was used as response variable and MP and SDS as fixed factors. Moreover, a Principal Coordinates Analysis (PCoA) was performed to visualize how microbial communities change between treatments using vegan R package.

To test the effects of the factors MPs and SDS on the relative abundance of the main 6 phyla a Multivariate Analysis of Variance MANOVA were performed, in the microbiomes of water and water boatmen. To observe the univariate effects in each main phyla, separate Generalized linear model (GLM) with a quasi-Poisson family were performed using the relative abundance of each phyla as response variable and MPs and SDS as fixed factors. All statistical analyses were executed in R statistical Computing Language 3.6.2 (R Core Team, 2020). The phylogenetic tree, and the taxonomy plots were created using Qiime 1.9.9 (Caporaso et al., 2012).

3. Results

We carried out the experiment in two seasons; summer 2016 and summer 2017. There was no difference in mortality between seasons (Season: $\chi^2 = 0.1022$, $p = 0.7492$), therefore we pooled the collected data between years and analyzed it as the whole data set.

3.1. Mortality of water boatmen

Mortality at 24 h was highest in the MP treatments (with a mean mortality of 13.3 % for MPs and 19.3 % for the combination of MPs and SDS) compared to treatments without MPs (Fig. 2a). There were significant effects on the mortality by the MPs and the SDS individually but no interactive effect (MPs: $\chi^2_{1,23} = 121.368$, $p < 0.001$; SDS: $\chi^2_{1,23} = 4.428$, $p = 0.035$; MPs:SDS: $\chi^2_{1,23} = 2.667$, $p = 0.10242$).

At 48 h the trend is the same for the treatments with MPs that shown the highest mortality, the combined treatment of MPs and SDS shown a mean mortality of 47.3 % and the MPs alone shown a mean mortality of 20 % (Fig. 2b). The model showed significant effects on the mortality by the MPs and the SDS alone and a marginal non-significant effect on the two-way interaction (MPs: $\chi^2_{1,23} = 296.950$, $p < 0.001$; SDS: $\chi^2_{1,23} = 65.902$, $p < 0.001$; MPs:SDS: $\chi^2_{1,23} = 3.780$, $p = 0.052$).

A drastic change is observable at 72 h where the exposure of SDS alone reaches a mean mortality of 86.7 %, similarly high compared to the combined MPs and SDS treatment that reach 100 % mortality. The mortality in the MP treatment was nearly constant 48 h to 72 h with a mean mortality of 24 % (Fig. 2c). The two-way interaction had significant effects on the mortality of the water boatmen (MPs: $\chi^2_{1,23} = 20.37$, $p < 0.001$; SDS: $\chi^2_{1,23} = 697.56$, $p < 0.001$; MPs:SDS: $\chi^2_{1,23} = 20.99$, $p < 0.001$).

At 168 h the treatment with SDS alone reach 100 % mortality whereas the MPs alone had a mean mortality of 35.3 % (Fig. 2d). The combined exposure of MPs and SDS had significant effects on the mortality of the water boatmen (MPs: $\chi^2_{1,23} = 6.57$, $p = 0.010$; SDS: $\chi^2_{1,23} = 535.74$, $p < 0.001$; MPs:SDS: $\chi^2_{1,23} = 24.81$, $p < 0.001$).

3.2. Main bacterial phyla

The 6 main phyla that were observed in the water boatmen samples were Proteobacteria (94.888 %), Tenericutes (3.978 %), and in lower relative abundance Bacteroidetes (0.608 %), Cyanobacteria (0.288 %),

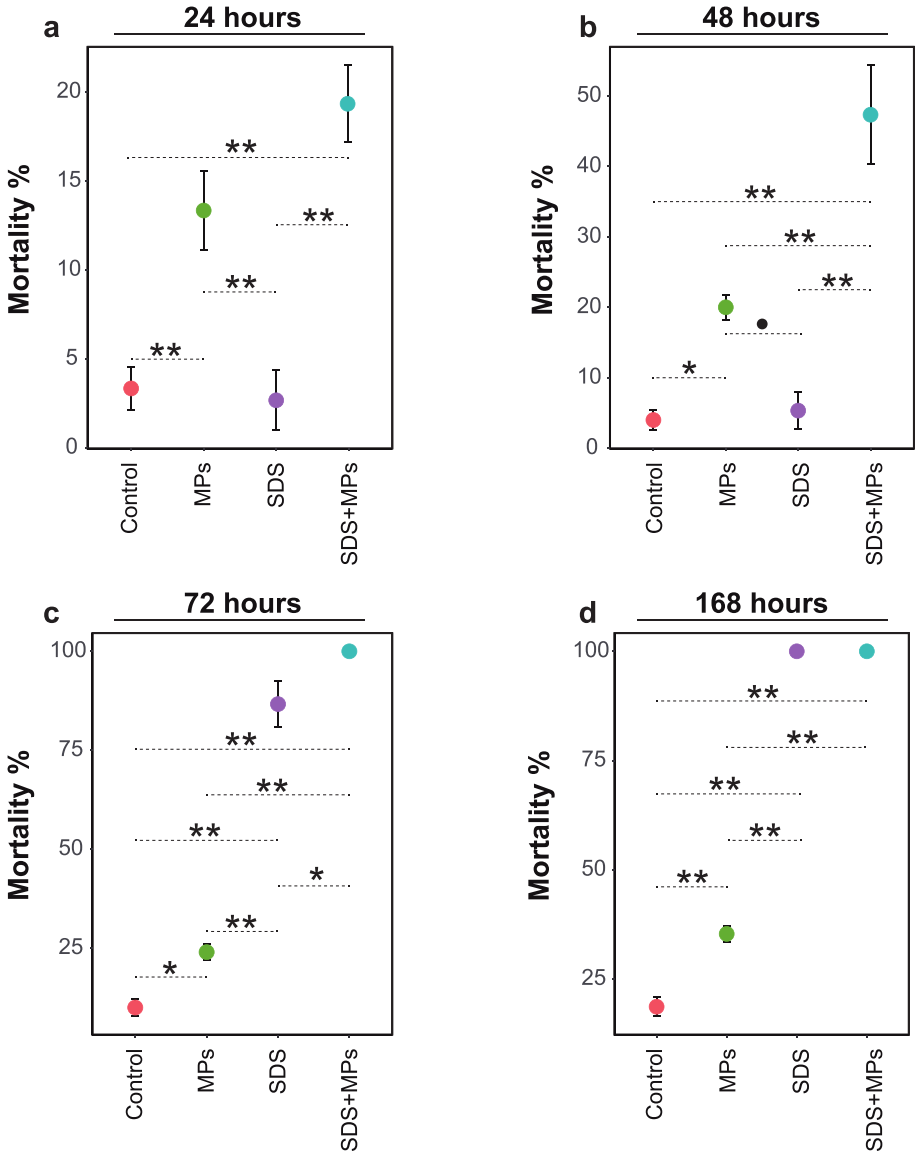

Fig. 2. Water boatmen mortality exposed to MPs, SDS and the combination of MPs and SDS at (a) 24 h, (b) 48 h, (c) 72 h, and (d)168 h. The control treatment was not exposed to either MP or SDS. Significant differences between treatments were tested using post-hoc Tukey tests and are indicated with asterisks (***: p-value < 0.001; **: 0.001 < p-value < 0.01; *: p-value < 0.05; •: 0.05 < p-value < 0.09).

Gracilibacteria (0.098 %) and Actinobacteria (0.086 %) (Fig. 3). The MANOVA showed that there was only a marginal non-significant effect of the MPs on the water boatmen phyla composition (Table 1). In the univariate analyses of the main phyla, the exposure to MPs significantly increased the relative abundance of Cyanobacteria and significantly decreased the abundance of Actinobacteria (Table 1). A marginal non-significant effect on Bacteroidetes was also observed. SDS showed significant negative effects on the relative abundance of Bacteroidetes, Cyanobacteria, Gracilibacteria and Actinobacteria (Table 1). The post hoc test on the water boatmen showed significant differences between treatments in the relative abundance of Bacteroidetes and Actinobacteria (Table 2).

In the water, Actinobacteria (61.839 %) and Proteobacteria (32.825 %) are the main observable phyla followed by Choroflexi (3.516 %), and Bacteroidetes (1.228 %). Verrucomicrobia (0.440 %) and Acidobacteria (0.073 %) can be found but with low relative abundance (Fig. 3). The MANOVA showed that there is a significant effect on the composition of the main phyla to the exposure of SDS (Table 1). Individually, there were significant negative effects of the SDS exposure on the relative abundances of Bacteroidetes, Verrucomicrobia and Acidobacteria, and a significant positive effect on the relative abundance of Choroflexi (Table 1). The exposure to MPs only had a positive effect on Verrucomicrobia (Table 1). Actinobacteria the most abundant phyla in the water showed a marginal but non-significant effect on its relative abundance by the MPs exposure (Table 1). There was however no interaction between MP and SDS influencing the relative abundance of any phyla, neither in the water boatmen nor in the water (Table 1). The post hoc test showed significant differences in the relative abundance of Bacteroidetes and Choroflexi, Verrucomicrobia (Table 2).

When looking at the taxonomic level class, Alphaproteobacteria and Gammaproteobacteria dominated the microbiome of the water boatmen (Fig. S2a). The relative abundance of Gammaproteobacteria in water boatmen directly from the lake (16.5 %) is lower than in the water boatmen from the experiment (control 25.7 %, MPs 28.7 %, SDS 37.8 % and the combined SDS and MPs 38.7 %) (Fig. S3a). In the water collected from the lake the class Actinobacteria (56 %) showed the highest relative abundance followed by Alphaproteobacteria (21 %) and Betaproteobacteria (9.5 %). The control treatments and the MPs treatment show similar relative abundance of Actinobacteria, but Betaproteobacteria become the second most abundant class followed by Alphaproteobacteria. On the other hand, it is observable that Alphaproteobacteria disappeared and Gammaproteobacteria increased in the treatments with SDS (Fig. S3b).

3.3. Alpha diversity

The alpha-diversity indexes show that the bacterial diversity in water is higher than the bacterial diversity in the water boatmen for all the diversity-indexes (Fig. 4). In the water boatmen, the Microbial diversity measured as Shannon index, increased in the SDS treatments (Table 3). There was also a marginal non-significant effect of SDS as well as MP on the phylogenetic diversity (Table 3). The pairwise post hoc test showed no significant differences between treatments, but there is a marginal non-significant difference between Control and the combined MPs and SDS treatment.

In the water, SDS had significant effects on all the diversity indexes (Table 3). The MP treatment on the other hand only had an effect on the Shannon index. The combined exposure of MPs and SDS decreased the microbial diversity and showed significant two-way interaction on the Chao and Shannon indexes (Table 3, Fig. 4). The post hoc test showed significant differences between treatments in all the diversity indexes. (Table 4). For the Chao diversity index the control and the MPs differed significantly from SDS and the combined SDS and MPs, and the SDS alone is significantly different from the combined SDS and MPs treatment. For the Shannon index the control, the MPs and the SDS differed significantly from the combined SDS and MPs treatment. Phylogenetic diversity index showed that the control differed significantly from all the treatments and SDS is significantly different from the MPs and the combined SDS and MPs treatment (Table 4).

3.4. Beta diversity

The water boatmen microbiome is clustered in two distinct groups, i.e. treatments with SDS and treatments without SDS, while the water microbiome clustered by treatment (Fig. 5). PERMANOVA results showed that the beta diversity of water boatmen was affected by the presence of

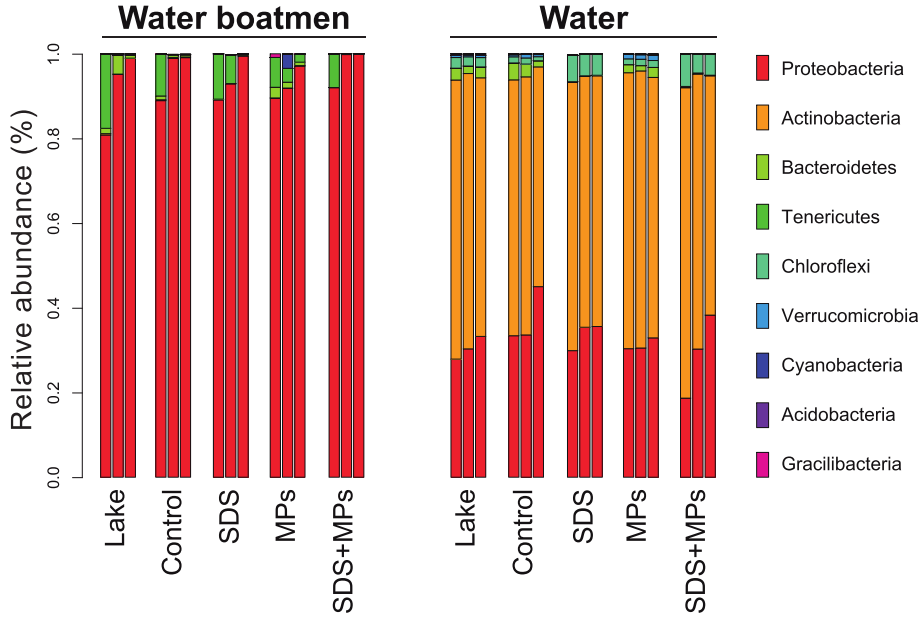

Fig. 3. Relative abundance in order of importance of the 6 main phyla in the water boatmen: Proteobacteria, Tenericutes, Bacteroidetes, Cyanobacteria, Gracibacteria, and Actinobacteria and in the water: Actinobacteria, Proteobacteria, Bacteroidetes, Chloroflexi, Verrucomicrobia, and Acidobacteria. For the exposure to MPs and/or SDS. The control replicates were not exposed to either MPs or SDS. The lake factor is the microbial diversity of water boatmen and water directly taken from the lake, no microcosm procedure or MPs and/or SDS exposure was executed to this factor.

Table 1
Results of the model testing effects of exposure to MPs, and exposure to SDS, and the two-way interaction on the relative abundance of the six main microbiota phyla in the water boatmen and in the water (MANOVA). As well as the model testing effects of the metioned factors on the relative abundance of the individual six main microbiota phyla. Significant and marginally non-significant p-values are highlighted in bold.

	Water boatmen				Water		
	SDS	MPs	MPs:SDS		SDS	MPs	MPs:SDS
Manova				Manova			
Pillai	0.895	0.932	0.888		0.981	0.88	0.840
Appox *F*	4.264	6.844	3.985		26.318	3.660	2.6285
p-Value	0.131	**0.071**	0.142		**0.011**	0.157	0.229
Proteobacteria				Actinobacteria			
χ^2	0.22	0.011	1.211		0.405	3.16	0.135
p-Value	0.639	0.915	0.271		0.525	**0.075**	0.713
Tenericutes				Proteobacteria			
χ^2	0.034	0.191	0.450		0.647	2.115	0.021
p-Value	0.853	0.662	0.502		0.421	0.146	0.886
Bacteroidetes				Chloroflexi			
χ^2	26.29	3.755	2.265		102.027	0.300	0.127
p-Value	**<0.001**	**0.053**	0.132		**<0.001**	0.584	0.721
Cyanobacteria				Bacteroidetes			
χ^2	5.463	5.041	0.101		74.443	2.497	1.140
p-Value	**0.019**	**0.025**	0.75		**<0.001**	0.114	0.286
Gracilibacteria				Verrucomicrobia			
χ^2	7.655	2.575	0.382		355.77	23.41	<0.001
p-Value	**0.006**	0.109	0.536		**<0.001**	**<0.001**	0.999
Actinobacteria				Acidobacteria			
χ^2	25.82	11.498	0.079		143.27	0.859	<0.001
p-Value	**<0.001**	**<0.001**	0.778		**<0.001**	0.354	1

SDS (MPs: $F_{1,8} = 0.625$, $p = 0.617$; SDS: $F_{1,8} = 13.160$, $p = 0.002$; MPs: SDS: $F_{1,8} = 0.660$, $p = 0.596$). The beta diversity of the water microbiome on the other hand was significantly affected by the SDS and showed marginal non-significant effects by the presence of MP (MPs: $F_{1,8} = 2.346$, $p = 0.086$; SDS: $F_{1,8} = 23.706$, $p = 0.001$; MPs:SDS: $F_{1,8} = 1.274$, $p < 0.241$).

4. Discussion

Detergents are recognized as pollutants with multiple effects on the fitness of living organisms. Moreover, detergents can occur together with microplastics in nature, and they are even manufactured together with detergents, resulting in them being released together into natural environments. We have shown here that the interaction of these two pollutants leads to the toxicity of SDS being enhanced by the presence of MPs.

4.1. Mortality

We expected to find a higher mortality in the treatments with SDS since detergents are found to have toxic effects (Czyzewska, 1975), but also because the detergent's bactericidal activity can induce dysbiosis in the host microbiome (Gerasimidis et al., 2019). It is important to highlight that the concentration of SDS used here is similar or lower than the ones used in other experiments assessing negative effects of SDS and other surfactants (Fei et al., 2020; Harada et al., 2007). Initially, it was not possible to observe an apparent SDS-exposure effect on the mortality of water boatmen. This changed after the third day of exposure, suggesting that prolonged exposure is needed to observe the mortality effects of SDS. Such a delayed response in mortality from SDS has also been shown by Harada et al. (2007), who tested the effects of SDS in the survival rate of the nematode *Caenorhabditis elegans*.

In this study, the exposure to MPs alone resulted in low mortality. But, there are several studies with different results, including studies that found that MPs induce negative effects in aquatic invertebrates such as *Daphnia magna* (Besseling et al., 2014) and *Hyalella azteca* (Au et al., 2015), as well as no biological effects in *Crassostrea gigas* (Revel et al., 2020). Nevertheless, the results of those studies showed that the effect depends on the material, shape, and concentration of MPs (Aljaibachi and Callaghan, 2018; Au et al., 2015; Besseling et al., 2014; Renzi et al., 2019; Revel et al., 2020).

Interestingly, the mortality of water boatmen was higher in the combination of MPs and SDS at 24 and 48 h. This suggests that the interaction of the SDS and MPs is reducing the time that it takes the SDS to become toxic to the water boatmen (Fig. 2). This might be explained by a possible synergic effect of MPs and SDS but also by the way SDS can interact with the MPs (Horton et al., 2017; Wagner and Lambert, 2018). In this perspective, the MPs could transport other pollutants (e.g., via adsorption), making it impossible for the water boatmen to dispose of this associated pollutant through their gut tract. MPs could also be translocated together with the associated pollutant into different tissue (Pietroiusti et al., 2015;

Table 2
Test post-hoc checking differences between treatments Control, MPs, SDS, and SDS + MPs on the relative abundance of the individual six main microbiota phyla for both water boatmen and water. Only significant and marginally non-significant p-values are shown in highlighted in bold.

Variable water boatmen	Contrast	p-Value	Variable water	Contrast	p-Value
Bacteroidetes	MPs − SDS	**0.007**	Chloroflexi	Control − SDS	**0.01**
				Control − MPs + SDS	**<0.001**
	MPs − MPs + SDSs	**0.022**		MPs − SDS	**<0.001**
				MPs − MPs + SDS	**<0.001**
Actinobacteria	Control − MPs	**0.025**	Bacteroidetes	Control − SDS	**<0.001**
	Control − SDS	**0.001**		Control − MPs + SDSs	**<0.001**
	Control − MPs + SDS	**<0.001**		MPs − SDS	**0.003**
	MPs − MPs + SDS	**0.0633**		MPs − MPs + SDS	**0.001**
			Verrucomicrobia	Control − MPs	**<0.001**

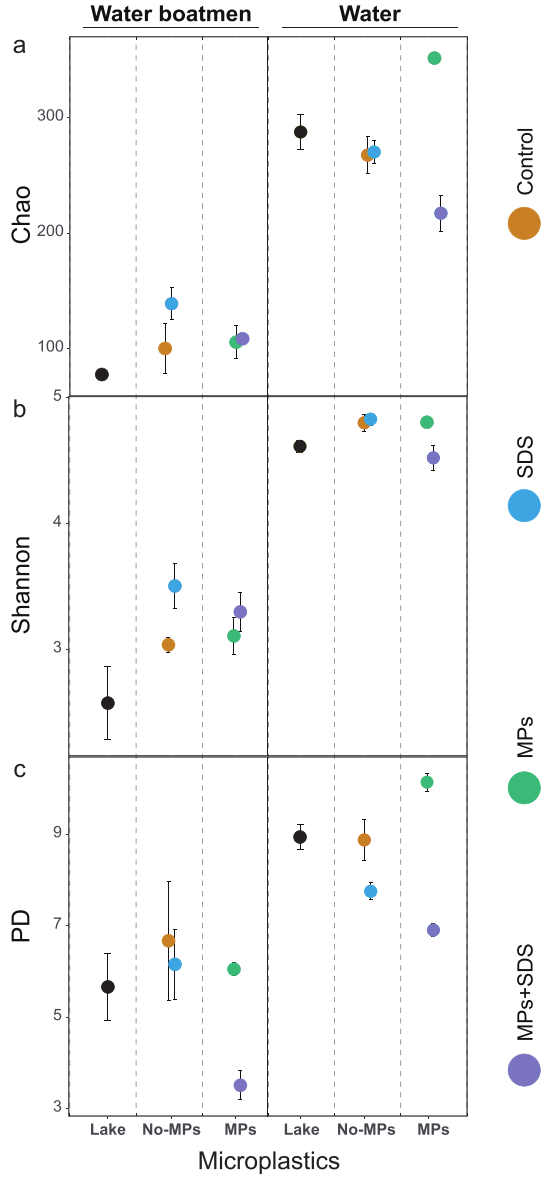

Fig. 4. Microbial diversity indexes, In the left the water boatmen and in the right the water microbial diversity, (a) Chao index, (b) Shannon index, and (c) Phylogenetic diversity index for the exposure to MPs and/or SDS. The control replicates were not exposed to either MPs or SDS. The lake factor is the microbial diversity of water boatmen and water directly taken from the lake, no microcosm procedure or MPs and/or SDS exposure was executed to this factor.

Wagner and Lambert, 2018). In our case, this entrapment might be the reason of the increased mortality in the treatments with MPs and SDS.

4.2. Effect on the water boatmen microbiome

MPs affected individual bacterial phyla such as Cyanobacteria but not the alpha nor the beta diversity of the microbiome. Previous and similar studies have shown that aquatic biota are negatively impacted by the exposure of MPs (Eerkes-medrano et al., 2015; Felten et al., 2020; Redondo-Hasselerharm et al., 2018; Rist et al., 2017; Silva et al., 2019). In a meta-analysis by Foley et al. (2018), MPs lead to negative effects on the consumption of aquatic organisms with less strong evidence of the effects on growth, reproduction, or survival of aquatic organisms. Here we showed that MPs can have a more indirect effect impacting single microbial taxa. However, more studies are necessary to improve our understanding of how MPs can indirectly affect free-living and host-associated microbes (Amaral-Zettler et al., 2020; Foley et al., 2018).

Table 3
Results of the model testing effects of exposure to MPs, exposure to SDS, and the two-way interaction on the diversity indexes (Chao, Shannon and Phylogenetic) for both water boatmen and water. Significant and marginally non-significant p-values are highlighted in bold.

Water boatmen	Water					
	SDS	MPs	MPs:SDS	SDS	MPs	MPs:SDS
Chao						
$F_{1,8}$	1.996	0.708	1.464	28.524	1.5812	30.8814
p-Value	0.195	0.424	0.261	**<0.001**	0.244	**<0.001**
Shannon						
$F_{1,8}$	5.405	0.229	0.948	3.934	5.621	5.938
p-Value	**0.048**	0.645	0.359	**0.083**	**0.045**	**0.041**
Phylogenetic						
$F_{1,8}$	3.879	4.444	1.704	65.539	0.599	15.381
p-Value	**0.084**	**0.068**	0.228	**<0.001**	0.461	**0.004**

Toxicity of detergents in aquatic organisms has been studied since the 1960s (Sobrino-Figueroa, 2018). It is also well known that surfactants such as SDS show bactericidal activity by damaging membranes and proteins of bacteria (Klebensberger et al., 2007). However, knowledge related to how detergent could affect host-microbiome of aquatic invertebrates are scarce. Here we show that SDS is the main driver of microbial changes in beta diversity as well as in the increase of alpha diversity in water boatmen. Even though detergents should decrease the bacterial diversity, it has been found that bacteria with efflux pumps and Clp-proteases mechanisms can survive in the presence of detergents and grow in low SDS concentrations (Klebensberger et al., 2007; Poole, 2004; Rajagopal et al., 2002). Moreover, our results showed that the increase in relative abundance of Gammaproteobacteria in the water boatmen was mainly driven by SDS exposure (Fig. S3a). This suggests that SDS might cause dysbiosis, and have an effect on the presence of this bacterial class (Gerasimidis et al., 2019). For example, Gerasimidis et al. (2019) found that the addition of detergents increase the relative abundance of *Escherichia/Shigella* and *Klebsiella* all members of Gammaproteobacteria (Gerasimidis et al., 2019).

We hypothesized that the SDS can interact with MPs thus increasing the effect of the pollutant in the microbiome. However, when the SDS and MPs were combined there was no interaction effect on the host microbiome. The post hoc test showed only marginal not significant differences between the

Table 4
Test post-hoc checking differences between treatments Control, MPs, SDS, and SDS + MPs on the diversity indexes (Chao, Shannon and Phylogenetic) for both water boatmen and water. Only significant and marginally non-significant p-values are shown in highlighted in bold.

Variable water boatmen	Contrast	p-Value
Phylogenetic	Control − MPs + SDS	**0.078**
Variable water	**Contrast**	**p-Value**
Chao	Control − SDS	**0.006**
	Control − MPs + SDS	**0.078**
	MPs − SDS	**0.0070**
	MPs − MPs + SDS	**0.0628**
	SDS − MPs + SDS	**<0.001**
Shannon	Control − MPs + SDS	**0.059**
	MPs − MPs + SDS	**0.038**
	SDS − MPs + SDS	**0.0557**
Phylogenetic	Control − MPs	**0.071**
	Control − SDS	**0.042**
	Control − MPs + SDS	**0.004**
	MPs − SDS	**0.001**
	SDS − MPs + SDS	**<0.001**

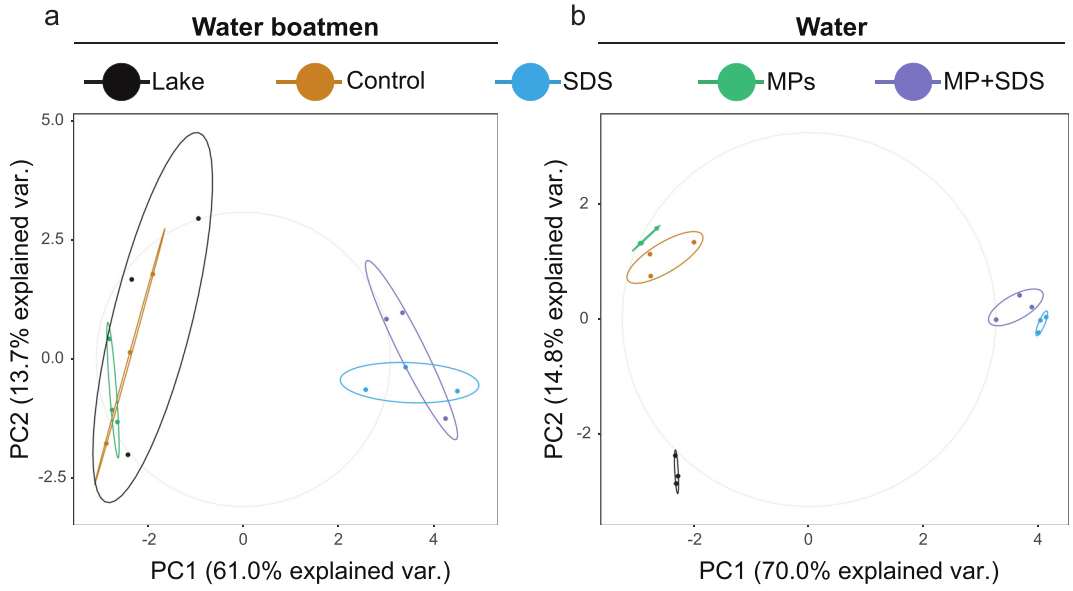

Fig. 5. Principal Coordinates Analysis showing the microbial composition clusters of each treatment. The left shows the beta diversity of the water boatmen and to the right shows the beta diversity of the water. The microbial composition is also coded following the exposure treatment to MPs (Mp), Sodium dodecyl sulfate (SDS), a combination of both, and without either of them (Control). The lake factor is the microbial diversity of water boatmen and water directly taken from the lake, no microcosm procedure or MPs and/or SDS exposure was executed to this factor.

control and the combined treatment of MPs and SDS in phylogenetic diversity, being lower in the combined treatment of MPS and SDS. We argue that the interaction between MPs and an additive pollutant could highly affect the microbiome but with higher complexity than the direct effects of the pollutant on the host. The interaction between MPs and a toxic pollutant could depend on many factors such as the concentration of the pollutant, the size of the MPs, and how the microbial communities can use the MPs with the additional pollutant as a surface to attach themselves. In our case, it can be that in the combined treatment of MPs and SDS, the SDS might be adsorbed by the MPs allowing new microbial taxa to grow on the microplastics (Amaral-Zettler et al., 2020). However, we observed that the changes in the microbiome in response to MPs and additional pollutant is low. This might be because of the low concentration of the SDS (i.e. low bactericidal effects) and also because the microbiome is under strong influence by the host where the host is modulating the gut environment to select certain microorganisms (Berg et al., 2020; Foster et al., 2017).

4.3. Effect on water microbiome

Our treatments affected the water microbiome stronger than it affected the microbiome from the water boatmen. This effect has been shown many times in previous studies and is attributed to the intrinsic selection pressure excised by the host on the outside microbiome (Foster et al., 2017; Hanning and Diaz-Sanchez, 2015). In this way, the host can be seen as a different environment with its own ecological community of microbes (Douglas and Lindsey, 2016). The changes we found in the diversity indexes in the water microbiome might be due to MPs and SDS exerting a selection pressure decreasing the microbial diversity, and selecting the microorganisms with SDS resistance or the ability to use these pollutants as a carbon source. (Liu et al., 2020; Rajagopal et al., 2002). Furthermore, microorganisms could use plastics as a sole carbon source suggesting that the plastics particles can change the ecology of microbes (reviewed by Amaral-Zettler et al., 2020). Interestingly, the relative abundance of some taxa in the water directly collected from lake Erken was different compared to the control treatment (Figs. 5b and S1b). This might be probably because the microcosm itself is working as a closed environment with less microbial dispersion and less flux of resources and organic waste compared to the intrinsic flux of the lake.

In the water, we found that MPs increased microbial alpha diversity. This could be a result of MPs serving as a substrate for bacteria to proliferate on, thus increasing the possibility of new bacterial colonies becoming established (Amaral-Zettler et al., 2020; Zettler et al., 2013). In contrast, the exposure to SDS had a major effect on the main taxa and on the total relative abundance of microbes in the water. This effect could be attributed to the bacterial toxicity of SDS (Klebensberger et al., 2007). The combined exposure of MPs and SDS had a major negative effect on microbial diversity indices. This is in agreement with the hypothesis of SDS interacting with MPs thus amplifying the effect of SDS, as has been reported in previous studies (Felten et al., 2020; Skjolding et al., 2016; Wang et al., 2018). On the other hand, it is important to highlight that these effects are not necessarily obvious in water boatmen because of selection forces exert by the host to host control its microbiome. This is the main reason why communities found in external aquatic environment are different from communities found in the gut tract. It has been shown that the host exerts a robust response to perturbation allowing the host to keep key microbial species (Dethlefsen and Relman, 2011; Faith et al., 2013; Foster et al., 2017). Nevertheless, in the water, there are still microbe-microbe interactions that could provide stability to the microbial communities because of the intrinsic microbial cell competition determining whether a strain can persist (Buffie et al., 2015; Coyte et al., 2015; Nadell et al., 2016). However, under pollution stress bacteria with pollutant resistant mechanism might persist.

5. Conclusion

Our study highlights the importance of considering the interaction of MPs with pollutants when assessing the effects of activities of both industry and individuals on natural environments. The interactions in the environment are complex resulting in multi-stress systems that are poorly studied. The effect of multiple pollutants on host-microbiomes represents a new frontier in ecotoxicology research. This study explores the connections between the microbiome, environmental pollution, and host fitness. Additionally, we evaluated the reproducibility of the experiment over two years. Considering the insights gained from this study, we recommend that future research focus on performing metagenomic and metatranscriptomic analyses together in wild organisms at different years to evaluate the changes in gene expression and microbial communities in water and in the host-

microbiome as well as the reproducibility of the experiment. We want to acknowledge that the microcosm technique has its limitations in maintaining the lake microbiome. As we have shown here, the microcosm can be a source of stress that can affect the microbial communities and change the wild microbiome in the collected water.

CRediT authorship contribution statement

J.E.V. conceived the project and designed the experiment. J.E.V. performed the experiment as well as the DNA extraction and DNA library preparation. J.E.V. performed the microbiome analyses and the statistical analyses with advise from R.S.. J.E.V. wrote the paper, R.S. give comments to the paper. All the authors approved to the final version of the manuscript.

Data availability

Data is available on request from Javier Edo Varg at jedovarg@gmail.com. Data have been uploaded to the European nucleotide archive (ENA) and it will be available after publication. With accession number: PRJEB45596 and the study unique name: ena-STUDY-UPPSALA UNIVERISTY-09-06-2021-23:36:54:808-767.

Declaration of competing interest

We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript and agree with submission to Science of the Total Environment. We declare no conflict of interest associated with this publication

Acknowledgements

Financial support for this study came from Linnéska stipendiestilftelsen and the Department of Ecology and Genetics at Uppsala University. We want to thank Nemira Žilinskaitė, Malin Bohman Gineta Šukytė, Gabrielė Ivaščenkaitė and Tania Safa for their invaluable contribution collecting the water samples and performing microcosms at Erken research school in the summer of 2016. We also thank to Erken research school for providing their installations and some of the equipment to perform this experiment. Sequencing was performed by the SNP&SEQ Technology Platform in Uppsala. The facility is part of the National Genomics Infrastructure (NGI) Sweden and Science for Life Laboratory. The SNP&SEQ Platform is also supported by the Swedish Research Council and the Knut and Alice Wallenberg Foundation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.159106.

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