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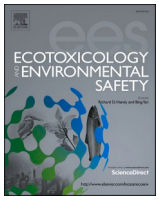

Microplastics in freshwaters: Comparing effects of particle properties and an invertebrate consumer on microbial communities and ecosystem functions

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ABSTRACT

The effects of microplastic (MP) accumulation in freshwaters on organisms and ecosystem functions are poorly understood, as are the roles of MP particle properties in regulating these effects. In freshwater microcosms, we quantified variation in microbial communities and ecosystem functions and compared effects of MP concentration (0, 1000, 50000 particles/$kg_{sediment}$), shape (sphere, fragment, fibre), and polymer (polyethylene, polyethylene terephthalate, polypropylene, polystyrene) with those of a model invertebrate consumer (*Chironomus riparius*). We detected multiple effects of specific MP properties, especially associated with MP fragments and fibres, and the polymer polypropylene. These effects included increases in microbial abundance, consumer biomass and ecosystem respiration, as well as decreases in microbial enzyme activity and water chlorophyll-a. MP presence was also associated with increased relative abundance of microbial taxa reported to degrade plastics. However, consumer presence mostly had stronger effects (effect sizes ranging from ± 11 −313 %) than MP exposure (effect sizes ranging from ± 1–89 %) on microbial communities and ecosystem functions. Furthermore, several MP effects were only detected when chironomid consumers were absent. Overall, our findings suggest that MP effects on microbes and ecosystem functions are often relatively small and variable, depending on particle properties and consumer presence. Nevertheless, the number of MP effects detected highlights the need for further investigations of interactions between MPs and other environmental drivers, to more thoroughly assess the risks of MP pollution for freshwater ecosystems.

1. Introduction

Microplastics (MPs), ranging in size from 1 to 5000 μm in diameter, are well-recognized as global pollutants (Hale et al., 2020). However, the significance of MPs for the biodiversity, community composition, and functioning of ecosystems remains unclear. This uncertainty reflects the focus of much MP research on quantifying the impacts of a narrow range of MP types, especially polystyrene (PS) or polyethylene (PE) microspheres, on single test species (Rozman and Kalčíková, 2022). Microspheres represent ≤ 10 % of MPs in the environment, which are instead dominated by microfibers (≥ 45 %) and fragments (≥ 29 %) comprising a wider polymer range, including PE, PS, polyethylene terephthalate (PET) and polypropylene (PP) (Burns and Boxall, 2018). Our limited knowledge of how the wider array of MP types occurring in the environment impacts communities and ecosystems hinders priority setting in environmental monitoring, policy and management actions targeting plastic pollution.

In freshwater habitats, MPs are likely to integrate with stocks of fine particulate organic matter (FPOM; ø = 0.45–1000 μm). Both MP and FPOM have overlapping particle size ranges and densities, which leads to both types of particles accumulating in similar depositional habitats (Hoellein et al., 2019). FPOM particles are comprised of faecal matter, microbial cells and other organic fragments or micro-aggregates (Shepard and Minshall, 1984) and are an important food resource for

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various macro-organisms, including particle-feeding Chironomidae (Diptera) larvae (Vannote et al., 1980). The development of microbial biofilms improves the palatability of naturally occurring FPOM for particle-feeders, which often gain the bulk of their nutrition from microbes growing on FPOM rather than from the particles themselves (Cummins and Klug, 1979). FPOM is additionally used by many chironomid species in construction of dwelling tubes (Brennan et al., 1978). Chironomid tube construction and FPOM consumption modify benthic habitats and contribute to nutrient cycling (Hölker et al., 2015). The entry of MPs into FPOM pools might directly affect both chironomids and the ecosystem functions they regulate, through negative physiological effects of MP consumption, or indirectly, by altering the structure and function of microbial communities associated with FPOM.

The fate and impact of MPs in freshwater environments, including interactions with microbial organisms and consumers, are regulated by multiple physico-chemical particle properties, including density, surface area and propensity to support biofilm growth. These properties vary with particle polymer and shape. For example, denser polymers (e.g. PET and PP) are more likely to sink rapidly and enter benthic FPOM stocks (McKie et al., 2023). Irregular particle shapes with higher surface area (e.g. fragments) allow for increased biofilm formation rates (Hossain et al., 2019). In turn, this increases MP consumption by particle-feeders (Vroom et al., 2017), despite the negligible nutritional value of MPs *per se*, with potential negative physiological consequences for consumers (Silva et al., 2019; Scherer et al., 2020). Increased biofilm biomass may also alter ecosystem functions, such as community respiration (Haggerty et al., 2014). Further cascading effects of biofilm formation might be mediated through changes in microbial community composition. For example, biofilms with a higher proportion of cyanobacteria are correlated with increased lipid content in chironomid consumers (Wei, Liao and Wang, 2016), and microbial community composition can also affect biofilm respiration rates (Hölker et al., 2014). Additionally, MPs are more likely than FPOM to be colonised by plastic-degrading taxa, such as diverse representatives from the phyla Proteobacteria and Actinobacteria (Gambarini et al., 2021), but the extent and ecological consequences of divergence in microbial communities on MPs compared with naturally-occurring substrates are poorly known.

In food webs, microorganisms provide essential metabolic functions, from primary production to decomposition, and regulate flows of nutrients and energy to higher trophic levels (Moënne-Loccoz et al., 2015), and virtually all detritus is replete with microbes (Steffan and Dharampal, 2019). Accordingly, any understanding of indirect impacts of MPs on food webs needs to incorporate knowledge of how MP exposure alters microbial communities and ecosystem functions. To address this, we conducted a laboratory microcosm experiment to investigate the effects of MP particle shape (Fragment, Fibre, Sphere), polymer (PE, PET, PP, PS) and concentration (0, 1000, 50000 p/kg) on microbial communities and coupled ecosystem functions. We further manipulated the presence of the chironomid *C. riparius* as a model particle-feeder, known to ingest MPs even when alternative organic particles are readily available (Scherer et al., 2017). The term *ecosystem functions* here refers to both the direct quantification of ecosystem processes (e.g. rates of ecosystem respiration) and the outcomes of ecosystem processes (e.g. chlorophyll concentration), since both types of variables provide insight into ecosystem functioning. We hypothesised that (H1) microbial community composition differs between microcosms with and without microplastics, and (H2) that MP fragments are mostly associated with increases in microbial abundance and enzymatic activity, consumer biomass and lipid content, and ecosystem functions (chlorophyll a concentration and respiration), reflecting their greater surface area for biofilm formation, relative to spheres. Finally, we hypothesised that (H3) MPs composed of denser polymers (PS and PET) with irregular shapes (fragments and fibres) have stronger effects on benthic microbial communities and chironomid consumers, due to more rapid deposition rates, compared with buoyant polymers.

2. Methods

2.1. Overview of study design

We conducted a microcosm experiment in a controlled climate cabinet (Aralab FitoClima 1200) set at 20 ± 1 °C, 80 % humidity (to reduce evaporation from microcosms) and 35 μmol s^{-1} m^{-2} light intensity on a 16:8 day-night cycle, at the Uppsala campus of the Swedish University of Agricultural Sciences. Four variables were manipulated: Chironomid presence (two levels: absence or presence with 10 individuals per microcosm, corresponding to 1410 individuals m^{-2}) and MP concentration (three levels: absent, low (1000 p/kg) and high (50,000 p/kg)). Two further factors were varied in microcosms with MPs present: MP polymer (four levels: PE, PET, PP and PS) and MP shape (three levels: fragment, fibre and sphere). Some polymer-shape combinations are not readily available (i.e. rarely or never manufactured in the targeted size range) and are thus excluded from our experimental manipulations (see Supplementary information, Fig S1, for details). Due to the total number of microcosms (240) in the experiment, treatment replicates were evenly distributed across random blocks both spatially (six cabinet shelves) and temporally (June and July; Supplementary information, Fig S1). We quantified effects of MP treatments on microbial community composition (from both benthic and water column habitats), diversity, abundance and enzymatic activity. We also quantified chironomid growth and body condition (lipid content) and ecosystem functions (respiration and water chlorophyll-a concentration).

2.2. MP preparation

PS and PE microspheres (Fig. 1a-b) were purchased from Cospheric (PSMS-1.07 38–48 μm and CPMS-0.96 10–63 μm). PET microfibers (Fig. 1c) were produced at the Norwegian Institute of Water Research (NIVA, Oslo) by collecting loose fibres from washing machine effluents loaded with microfiber blankets (“Skogsklocka”; IKEA). PP microfibres were produced by cutting a surgical mask (Medical Face Mask Type IIR, Zhende medical) with a sharp pair of stainless steel scissors. MP fragments (e.g. Fig. 1d) of all polymers (PE, PET, PP, PS) were produced by freezing pre-manufactured pellets in liquid nitrogen before grinding using stainless steel equipment on a ball mill (MM400, Retsch), to prevent MP particles melting during the grinding process. All MP fragments and spheres were filtered through nested stainless-steel sieves to retain the size fraction 25 – 63 μm. Due to their elongated shape, few MP fibres passed through the 63 μm sieve. Instead, we filtered fibres using a 25 μm sieve only, retaining all fibres that do not pass through the sieve. We washed MP particles three times with 95 % ethanol and ultrapure water to remove adhering chemicals and particles. Densities of the polymers used were: (high density) PE 0.95 g cm^{-3}, PET 1.34 g cm^{-3}, PP 0.91 g cm^{-3} and PS 1.04 g cm^{-3}.

We took images of MP particles under a stereomicroscope, and quantified aspects of their morphology after Zhao et al. (2018). The 2D diameter (and length for fibres) of all particles, and perimeter and circularity of the more rounded fragment and sphere particles were measured using imageJ (version 1.53a; Schneider et al., 2012). Fibres were clearly distinct from the other two particle types, and were especially characterised by their elongated shapes, with the median length being 499.3 μm and the median width being 28.4 μm. Fragments had comparable median particle diameter size ranges with spheres (40.5 vs 48.1 μm). To further distinguish the morphological characteristics of fragments and spheres, we calculated 2D particle circularity (corresponding to shape factor in Zhao et al., 2018) as an index of 3D surface area. We did not quantify this metric for fibres since the dimensions of these particles were markedly different from the other particles, making comparisons less meaningful. The circularity of particles were calculated using the following formulas:

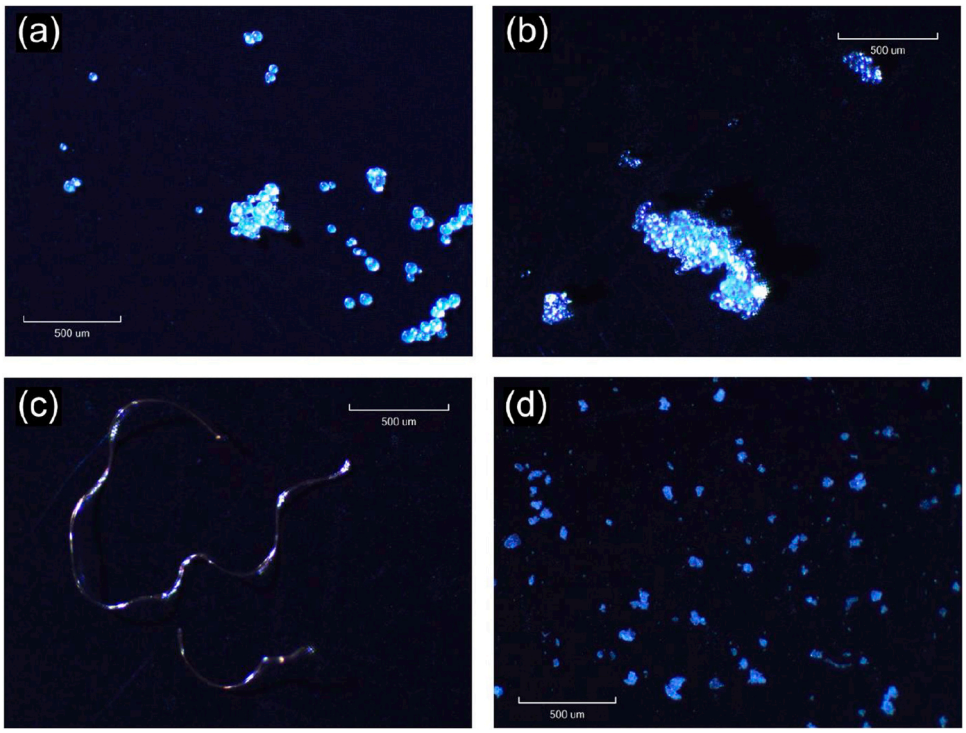

Fig. 1. Microscope images of (a) polyethylene spheres, (b) polypropylene spheres, (c) polyethylene terephthalate fibres and (d) polystyrene fragments used in the microplastic treatments of this study. All scale bars are 500 μm.

$$Circularity = \frac{4\pi Area}{Perimeter^2}$$

Circularity is a measure between 0 and 1 which describes how closely a 2D shape resembles a perfect circle (Cox, 1927). Decreasing circularity is indicative of increasing particle surface area. This 2D metric illustrates the increased average 3D surface area available on MP fragments (circularity= 0.66 ± 0.06) relative to MP spheres (circularity= 0.81 ± 0.08) used in our study. Circularity was not a meaningful measurement for fibres, which were elongated rather than round, but for comparison, the median 2D surface area (calculated from length x diameter) of fibres was 14,200 μm^2, while that for fragments and spheres were 1010 and 1750 μm^2 respectively. Further details on MP particle characterisation are in supplementary information (Section II).

To guide the definition of our MP concentration treatments, we conducted a compilation of published global freshwater sediment concentrations of MPs (Supplementary information, Fig S2). We found that the mean concentration of MPs was 1069.3 p/kg with 99.3 % of all recorded observations below 50,000 p/kg. Thus, we applied MPs: at 1000 $p/kg_{sediment}$ ("low"), the approximate global mean, and at 50,000 $p/kg_{sediment}$ ("high"), which is significantly higher than 95 % of recorded concentrations.

2.3. Microcosms: set-up and conditioning

The microcosms consisted of glass crystallisation dishes (300 mL), each containing 80 g of autoclaved sand (Fontainebleau Sand, VWR) and 0.11 g of a laboratory-prepared FPOM mixture (consisting of a ratio of 1:1:2 of ground alder leaves, Tetra Phyll® fish food flakes and birch leaves). This FPOM mixture is readily consumed by chironomid larvae, and supports rapid larval growth and development to pupation after two weeks when incubated at 20 °C (Kong et al., 2023). MPs were added to treatment replicates through stock suspensions that were made by suspending MPs in 100 mL 95 % ethanol (filtered through 10 μm filter paper to remove possible MP contamination). The mass of MPs added into the stock suspension was calculated by estimating the individual mass of MP particles (see Supplementary information, Section II, for details). Two separate MP stocks were made for each MP type for the low and high concentration treatments, to ensure comparable volumes of ethanol were added to each treatment replicate. MP stock suspensions was sonicated for 1 minute in an ultrasonic bath (VTUSCT5, Velleman Group) before application to improve the uniformity of particle distribution. The ethanol in the MP suspension was left to evaporate in the dry microcosm for 24 hrs before the addition of 150 mL stream water, which we anticipated would be sufficient to exclude any effects associated with the suspension media. To confirm this, we established "ethanol controls", identical to the control microcosms used in the experiment, but with addition of the suspension media only (95 % ethanol). There were no significant differences between these ethanol controls and those used in the experiment for any of our response variables (all $p > 0.070$).

Water was collected from a stream near Uppsala (Hågaån, 59.80497 N, 17.60476E) on 28th May and 25th June 2021 for use in the experiment. We filled 5 L containers just below the water surface for immediate transport to the laboratory. The water collection site was situated ca. 50 m upstream of a road bridge to minimise contamination from road and tire wear particles, but the catchment area includes multiple potential sources of MP contamination (predominantly agricultural fields, but also urban areas). Hence, we filtered the water through a 25 μm sieve to remove MPs falling within the size range of our experimental MP manipulations. Microcosms were left to condition for eight days after stream water addition to allow microbial biofilm growth on sediment particles. After eight days, ten first instar chironomids (<24 h old) were added to the respective microcosms using sterile plastic pipettes, which had been pre-soaked in ultrapure water to remove any potential adhering chemicals (see Supplementary information, Section II for details). Chironomid larvae were hatched from egg masses obtained from an in-house culture, at 20 ± 1 °C in the same controlled climate cabinet as the experimental microcosms. The experiment was maintained for another 13 days following chironomid addition (21 days total).

2.4. Microbial community measurements

2.4.1. Microbial community structure and diversity

At the end of the experiment (day 21), we sampled microbial communities from both the water column and the benthic FPOM layer that was deposited on the inorganic sand substrate in our microcosms, with approximately 100 mL of water and 1.5 mL of organic sediment collected per microcosm. Cells from water samples were collected by vacuum filtration through ⌀ 47 mm 0.2 μm Supor® membrane filters (Cytiva). Water sample filters and sediment samples were stored at −80 °C until further processing and analysis. Samples from the water initially added into the microcosms were also collected, filtered and stored at the start of the experiment. Similar analyses were not conducted for the initial FPOM mixture, but we do not expect major differences between replicates as the material added was identical in all microcosms and had been stored dry for ≥ 1 year prior to the experiment.

DNA extraction was conducted using DNeasy PowerSoil kits (Qiagen) and DNA concentration quantified with a Qubit fluorometer (Invitrogen). Sample preparation for 16S rRNA amplicon sequencing was performed following the protocol from (Sinclair et al., 2015). Microbial 16S rRNA genes were amplified with bacterial primers 341 F (CCTACGGGNGGCWGCAG) and 805RN (GACTACNVGGGTATCTAATCC) (Herlemann et al., 2011). Amplicon sequencing was conducted using the Illumina Miseq platform with Reagent kits v3 and 2 × 300 cycles. The obtained raw data were analysed using the open-source bioinformatics pipeline DADA2 (version 1.16) (Callahan et al., 2015) in R (4.0.2 version). Forward and reverse primers were trimmed and the sequences were cut to lengths of 300 and 222 bp, respectively, with the quality criterion of maxEE = (3, 2) and truncQ = 2, as calculated *in silico* by FIGARO (Weinstein et al., 2019). Taxonomic profiles were assigned based on amplicon sequence variants (ASVs) using the rRNA database SILVA, release 138.1 (Quast et al., 2013). Samples with sequencing depth that were too low (less than 1000 reads) were excluded from analysis (10 out of 484 samples). The sequencing raw data can be accessed via the National Centre for Biotechnology Information (NCBI) database (BioSample accession SAMN37759159 under BioProject PRJNA1026802). Microbial community diversity was evaluated using the Hill diversity index q^0 and q^1 (Lucas et al., 2017).

2.4.2. Microbial abundance

Water samples were collected from dishes on the first day after the conditioning period (Day 8 of the experimental timeline) and at the end of the experiment (Day 21). Samples were fixed with 37 % formaldehyde buffered with sodium tetraborate to a final concentration of 2 % and stored at 4°C in the dark. During analysis, samples were stained with SYBR Green®, incubated for 5 mins at room temperature and filtered through a 20 μm cell strainer to remove any MPs. The abundance of microbial cells was analysed by analytical flow cytometry (CytoFLEX, Beckman Coulter) by loading 100 μL of sample on 96 well plates and using a flow rate of 10 μL/min. Negative controls of stained milliQ water and 0.22 μm filtered samples were used to identify and exclude background particles.

2.4.3. Enzyme activity

Two enzymes were targeted in this study: β-glucosidase as an important enzyme in cellulose degradation, and chitinase that degrades the chitin exoskeleton molts (exuviae) of chironomid larvae. Activities of β-glucosidase and chitinase were measured using methylumbelliferone (MUF)-linked substrates according to Renes et al. (2020). Substrates for these enzymes (cellulose and chitin) represent the two most abundant biopolymers on earth and are hence of central biogeochemical importance. The substrate for β-glucosidase was 4-Methylumbelliferyl--beta-D-glucopyranoside (M3633, Merck) and for chitinase 4-Methylumbelliferyl-N-acetyl-β-D-glucosaminide (M2133, Merck). Enzymatic assays were carried out on water samples collected on the penultimate day (day 20) of the experiment. Samples, blanks and MUF standards were incubated with MUF-linked substrates at 20 °C (β-glucosidase for 2 hrs and chitinase for 1 hr) under saturating conditions (0.4 mM β-glucosidase and 5 μM chitinase). The method was optimized for substrate concentration prior to sample processing by determining the saturation point of a range of concentrations for both enzymes and the appropriate incubation time to obtain a clear signal (Details can be found in the supplementary information section IV). After incubation, 1 M glycine buffer was added to halt the reactions and enhance the fluorescent signal that was measured at λex/em = 355/460 nm (Hidex Sense microplate reader).

2.5. Chironomid measurements

At the end of the experiment, chironomids were retrieved from each microcosm and survivorship was recorded. Retrieved larvae were kept in tubes filled with deionised water and left overnight to allow them to empty their guts. Chironomids were then frozen at −20 °C before determination of dry biomass, with lipid content then quantified using the method described in Reznick (1983). Chironomid samples were oven dried at 60 °C for 48 hours and then weighed to the closest 0.001 mg. Subsequently, chironomid samples were submerged in 1.5 mL of diethyl ether for 72 hours to extract lipids, and then oven dried at 60 °C for 48 hours. Once dry, chironomid samples were re-weighed to estimate their lipid-free dry mass. Lipid content was calculated by subtracting lipid-free dry mass from dry biomass.

2.6. Ecosystem process measurements

2.6.1. Chlorophyll-a concentration

Chlorophyll-a concentration in water has been shown to correlate with phytoplankton photosynthetic rates (Huot et al., 2007), and was quantified in our experiment as a proxy for primary productivity. Water samples were collected at the end of the experiment (day 21) and filtered through ⌀ 25 mm 0.2 μm Whatman® membrane filters, wrapped in aluminium foil and stored at −20 °C. Chlorophyll from cells that were immobilized on filters was extracted using 99.5 % ethanol for 22 hrs at 4 °C in the dark. Subsequently, filters were removed and the extracts centrifuged at 1000 x g for 5 minutes to pelletize any remaining particles. 200 μL of extracted samples and a Chlorophyll-a standard series (Sigma-Aldrich, from spinach) were pipetted into black solid-bottom 96-well plates. Chlorophyll-a concentration was measured using $\lambda_{ex/em}$ = 444/680 nm in a microplate reader (Hidex Sense).

2.6.2. Community respiration

Prior to collection of microbial and chironomid samples at the end of the experiment, community respiration was quantified as detailed in Kong et al. (2023). Microcosms were completely filled with oxygen-saturated autoclaved stream water and the initial dissolved oxygen (DO) concentration measured once using calibrated optical sensors (*Firesting* O_2 Meter, PyroScience, Aachen, Germany). Microcosms were then sealed with parafilm and incubated in the dark for 3 hours at 20 °C, after which the final DO concentrations were measured. Community respiration was calculated as the difference between the initial and final DO concentration, and reported as mg O_2 consumed l^{-1} hr^{-1}.

2.7. Data analysis

2.7.1. Univariate responses

We used nested linear mixed models (LMMs) to test the effects of consumer presence, MP particle properties (concentration, polymer and shape) and experiment day (for microbial abundance only) on our univariate response variables. These include all microbial parameters (diversity, abundance, enzyme activity), chironomid responses (survival, growth and lipid content) and functional variables (respiration, primary productivity). Experimental blocks, which are cabinet shelves nested

with month (i.e. spatial blocks nested within temporal blocks), were set as random effects to account for any potential environmental gradients within the temperature cabinets. The model also includes a nesting structure for fixed effects, since all polymer-shape combinations were not available, with all MP particle characteristics nested within MP presence (Supplementary information, Fig S1).

The nested design of the statistical tests prevented us from directly comparing all interactions between MP shape, polymer and concentration against the control. To address this, we calculated log response ratios (LRRs) for all univariate responses by blocks, i.e. treatment values were divided by the control within the same experimental block. Treatment effects were determined to be significantly different from the control when 95 % confidence intervals do not overlap zero. Consequently, we denoted treatments as significant with (+) when the 95 % confidence interval of LRRs are above zero, and (-) when it is below zero on the figures presented here. We additionally report effect sizes as the percentage difference in treatment effects from the control, based on means pooled at the block scale.

2.7.2. Microbial community data

The complex structure of both fixed and random factors in our experimental design prevents us from conducting analysis of our microbial community data testing all nested factors simultaneously, using e.g. Permutational Multivariate Analyses of Variance (PERMANOVA; Truchy et al., 2020). Instead, we first visualized dissimilarity among microbial communities using Non-metric Multi-Dimensional Scaling (NMDS), based on Bray-Curtis distance of ASV read counts, normalized to 100 % for each sample, to identify the dominant factors structuring microbial communities, and supported these observations with a simplified PERMANOVA fitted with sampling habitat (water column or sediment), sample month and chironomid presence as factors. We then used this information to split the data set into subsets for further analyses of MP effects using Linear Discriminant Analysis (LDA) to highlight any differences in microbial relative abundance between given subsets. The LDA involved two rounds of testing. The first round employed the Kruskal–Wallis' test to determine if there were statistically significant differences between any two samples, while the second round utilized the Wilcoxon test to assess differences between multiple sets of pairs, with a significance threshold set $p \leq 0.05$.

All data analysis was carried out in R v4.3.1 (R Core Team, 2023). Multivariate PERMANOVAs and NMDS analyses were conducted using the R package *vegan* (v2.6–4; Oksanen et al., 2018). R packages *Phyloseq* (v1.44.0; McMurdie and Holmes, 2013) and *MicrobiotaProcess* (v1.12.3; Xu et al., 2023) were used to perform ANOVAs and LDAs for analyzing the difference in relative abundance of microbial community on ASV level across samples. Hill diversity indices, which measure microbial community diversity, were computed for each sample using *hillR* (v0.5.1; Chao et al., 2014). In this process, ASVs were rarefied to correspond with the minimum read count observed across the samples (1152 ASVs). For the LMMs for univariate responses, *lme4* (v1.1.34; Bates et al., 2015) was used to construct the models and *lmerTest* (v3.1.3; Kuznetsova et al., 2017) to calculate *p*-values for the ANOVAs of the models.

3. Results

3.1. Microbial communities

Microbial communities were structured especially by habitats (sediment vs water column) and temporal blocks (June vs July) with chironomid presence being also important for the sediment samples (PERMANOVA, $p < 0.050$). We thus split our subsequent LDA analyses according to these effects (see Methods). To highlight any overall effect of MP treatment on the microbial community across different taxonomical levels, we also investigated read counts of ASVs by comparing MP-treated replicates (pooled across all MP types) against their paired controls (Supplementary Information, Figure S3). Here, we report on taxa that had higher relative abundance when exposed to MPs, data for all taxa are available in supplementary information (section V). At the genus level, MP presence was associated with increased abundance of the Phyla Bacteroidota (synonym Bacteroidetes), Pseudomonadota, Verrucomicrobiota, and Proteobacteria, with members assigned as *Flavobacterium, Rhizobiales Incertae Sedis, Pseudomonas, Niveispirillum, Pseudorhodobacter, Lueolibacter, Phodospirillales, Cellvibrio*.

3.2. Microbial community diversity, abundance, and enzymatic activity

We report all effect sizes (as percent difference from controls) for our univariate microbial, consumer and functional response variables in Table 1. In the water column, chironomid presence reduced microbial taxa richness ($F_{1,\ 186}=12.2$, $p < 0.001$) and Hills-Shannon diversity ($F_{1,\ 186}=5.00$, $p < 0.050$) relative to controls (Table 1). MP concentration had a small effect on water column microbial taxa richness ($F_{1,\ 186}=5.27$, $p < 0.050$), which decreased relative to the controls when exposed to the low MP concentration (Fig. 2). In the sediment, chironomid presence had a effect on microbial diversity ($F_{1,\ 189}=22.6$, $p < 0.001$), which was reduced relative to controls (Table 1).

After the microbial conditioning period (Day 8), microbial abundance (Fig. 3a) was lower under the PP treatment relative to controls ($F_{3,\ 220}=3.36$, $p < 0.050$; Table 1). Final microbial abundance (Day 21) was greater in the presence of chironomid larvae relative to chironomid absence ($F_{1,\ 217}=18.3$, $p < 0.001$; Table 1). Additionally, there was a significant interaction between chironomid presence and MP concentration for the final microbial abundance ($F_{1,\ 194}=6.75$, $p < 0.050$). In the presence of chironomids, the high MP concentration led to a large increase in microbial abundance relative to the controls (Fig. 3b). In contrast, exposure to the high MP concentration in the absence of chironomids led to a small decrease in microbial abundance relative to the controls (Fig. 3b).

Chironomid presence had a large effect on enzyme activity, increasing chitinase activity ($F_{1,\ 196}=119.53$, $p < 0.001$) and β-glucosidase activity ($F_{1,\ 193}=191.18$, $p < 0.001$; Table 1) relative to controls (Table 1). There was a small effect of MP shape on β-glucosidase activity ($F_{2,\ 192}=3.20$, $p < 0.050$), with activity rates lower for fibres relative to controls (Table 1).

3.3. Chironomid survivorship, growth, and lipid content

Chironomid survivorship averaged 85.7 % overall, and was not affected by any MP treatment ($p > 0.05$). The biomass of individual chironomids was significantly affected by MP shape ($F_{2,\ 102}=5.04$, $p < 0.010$), with higher biomass recorded when exposed to MP fragments relative to controls (Fig. 4a). Larval lipid content was significantly affected by MP concentration ($F_{1,\ 83}=6.62$, $p < 0.050$), with mean lipid content greater under the high MP concentration relative to controls (Fig. 4b).

3.4. Chlorophyll-a concentration and ecosystem respiration

Chlorophyll-a concentrations were higher in microcosms with chironomids than those without ($F_{1,\ 195}=81.17$, $p < 0.001$; Table 1). There was also a significant three-way interaction between chironomid presence, MP polymer and MP concentration ($F_{3,\ 195}=3.72$, $p < 0.050$). Chlorophyll-a concentrations were decreased in the absence of chironomids and at high concentrations of PP relative to controls (Fig. 5a).

Overall ecosystem respiration was greater in microcosms with chironomids compared to those without ($F_{1,\ 206}=34.8$, $p < 0.001$; Table 1). In the absence of chironomids, MP presence was associated with an increase in respiration relative to controls ($F_{1,\ 102}=5.13$, $p < 0.050$; Table 1). Furthermore, there was a significant effect of MP polymer ($F_{3,\ 102}=2.87$, $p < 0.050$), with PP MPs increasing microbial respiration relative to controls (Table 1). This effect appears to be primarily driven

Table 1
Table comparing the mean values (± SE) of microbial, consumer, and ecosystem-level responses between control and chironomid presence or microplastic treatment groups, including only significant effects ($p < 0.050$) as tested by linear mixed models.

Measure	Mean ± SE		Change %	Mean ± SE		Change%	MP Notes
	Control	*Chironomid*		*Control*	*MP*		
Microbial Diversity							
Water	37 ± 4	32 ± 4	−11.3				
Sediment	126 ± 15	102 ± 6	−19.0				
Microbial Richness							
Water	104 ± 13	87 ± 12	−16.8	99 ± 12	89 ± 12	−9.3	Low
Microbial Abundance ($x10^6$ cells/mL)							
Day 8				8.4 ± 1.2	7.5 ± 0.7	−10.8	PP
Day 21 +chiro presence	4.6 ± 0.4	7.6 ± 0.6	+ 65.1	6.1 ± 0.9	8.7 ± 1.1	+ 88.8 *	High
Day 21 –chiro absence				4.6 ± 0.4	4.5 ± 0.6	−2.28	High
Enzyme Activity ($x10^{-1}$ μM mL^1 hr^1)							
Chitinase	0.68 ± 0.06	2.26 ± 0.22	+ 231				
βglucosidase	1.37 ± 0.20	5.67 ± 0.42	+ 313	3.35 ± 0.41	3.31 ± 0.64	−1.2	Fibres
Chironomid Larvae							
Biomass ($x10^{-1}$ mg)				4.75 ± 0.27	5.42 ± 0.24	+ 14.1	Frags
Lipid Content (%)				13.1 ± 2.0	14.3 ± 1.7	+ 9.17	High
Chlorophyll–a Concentration ($x10^{-1}$ mg/L)							
+chiro presence	1.14 ± 0.17	3.73 ± 0.27	+ 228				
-chiro absence				1.37 ± 0.31	0.78 ± 0.25	−44.8	PP High
Ecosystem Respiration ($x10^{-1}$ μmol O_2 hr^1)							
+chiro presence	7.65 ± 3.87	10.3 ± 2.60	+ 34.8				
-chiro absence				6.95 ± 0.62	7.83 ± 0.37	+ 12.6	MPs *per se*
				6.95 ± 0.62	8.00 ± 0.35	+ 15.1	PP
				6.95 ± 0.62	8.44 ± 0.43	+ 21.5	PP High
				6.95 ± 0.62	8.98 ± 0.23	+ 29.3	PP High Frags
				6.95 ± 0.62	7.90 ± 0.79	+ 13.7	PP High Fibres
				6.95 ± 0.62	8.99 ± 0.43	+ 29.3	PET Low Frags

Effect sizes are denoted as percentage change between controls and treatment groups. For the control-microplastic comparisons, the specific microplastic treatments that led to the significant effect was noted in the final column. * Comparison with the chironomid-absent control, %change when compared with the chironomid-present control is 40

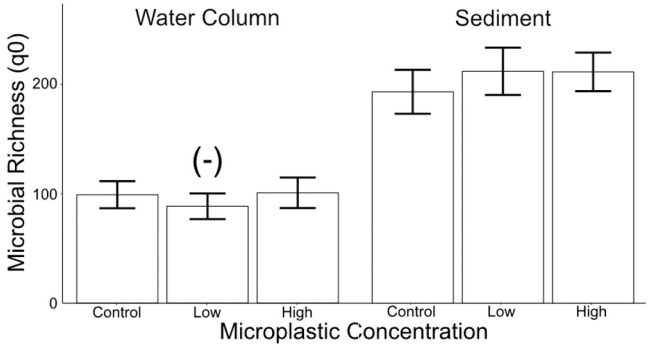

Fig. 2. Effects of chironomid presence on water column and sediment microbial community species richness (Hill diversity index q0). Bars plot the mean of block averages ± 1 SE (n = 12). Treatments that were significantly higher or lower than controls based on analysis of log response ratios (Supplementary file Fig S4a) are denoted with (+) and (-) respectively.

by the high concentration of PP MPs ($F_{3,\ 102}$=2.69, p = 0.050), where the high concentration increased microbial respiration relative to controls (Table 1). Finally, there was a three-way interaction between polymer, shape and concentration on microbial respiration ($F_{2,\ 102}$=3.54, $p < 0.050$). Microbial respiration was increased relative to the controls for high concentrations of PP fragments and fibres, and low concentrations of PET fragments relative to controls (Fig. 5b).

4. Discussion

Our experiment was explicitly designed to assess the effects of exposure to different MP concentrations, shapes and polymers on the structure and function of a freshwater microcosm community. We found support for H1, with the presence of MPs increasing the relative abundance of specific microbial taxa, and for H2, while MP fragments increased microbial enzyme activity. For H3, MPs of PET, a denser polymer, increased microbial respiration but did not have effects on microbial communities or chironomids that differed from those of the remaining polymers. Several additional positive and negative effects of specific MP properties on microbial, consumer and functional responses were observed, often in interaction with MP concentration. However, most of the strongest effects in our experiment were associated with other factors. For example, effect sizes associated with chironomid presence (ranging from ± 4.8 – 313 %) on microbial biodiversity and ecosystem functions were generally greater than those of the MP treatments (± 1.2 – 88.8 %). Similarly, chironomid presence, habitat type and sample month were all stronger drivers of microbial community composition than the MP treatments. Furthermore, many of the effects of MP exposure, including some of those with stronger effect sizes, differed between microcosms with and without the chironomid consumer. Overall, these findings suggest that effects of MPs on freshwater food webs might generally be small relative to those of other environmental drivers (i.e. presence of consumers, habitat characteristics), and also highly variable, dependent on MP concentration, shape and polymer, and interactions with consumers.

Chironomids are known to have strong effects on microbial communities and ecosystem functions, by providing energy sources (e.g. chitin from shedded exoskeletons) or through bioturbation activities that transfer sediment nutrients (e.g. nitrogen and phosphorus) into the water column (Hölker et al., 2015). In the present study, chironomid presence was a key driver of microbial community composition, function and diversity. Furthermore, chironomid presence regulated several microbial responses to MPs, with effects that were apparent when chironomids were absent either not observed or even reversed in their presence. We hypothesise that some of these shifts arise from the strong

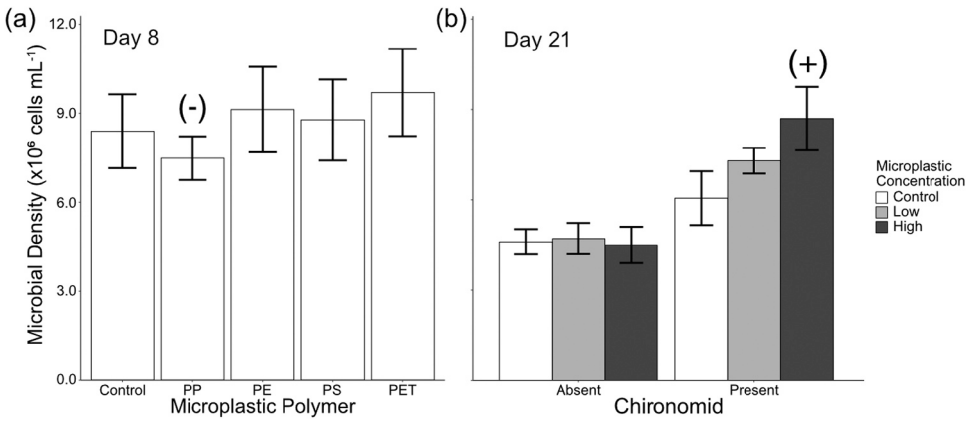

Fig. 3. Effects of (a) MP polymers on microbial abundance after eight days of conditioning and (b) chironomid larval presence and MP concentration on microbial abundance at the end of the experiment (day 21). Bars plot the mean of block averages ± 1 SE (n = 12). Treatments that were significantly higher or lower than controls based on analysis of log response rations (Supplementary file Fig S4b & S4c) are denoted with (+) and (-) respectively.

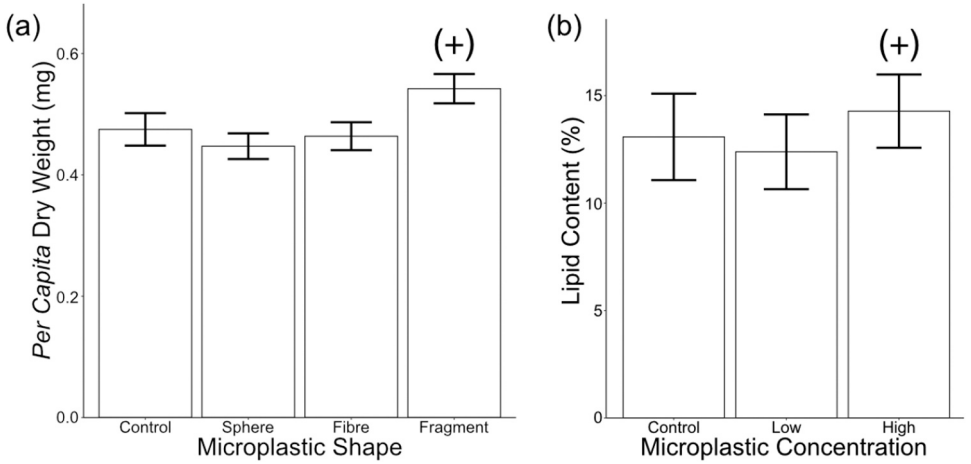

Fig. 4. Effects of (a) MP shape on average chironomid biomass and (b) MP concentration on average chironomid lipid content. Bars plot the mean of block averages ± 1 SE (n = 12). Treatments that were significantly higher or lower than controls based on analysis of log response rations (Supplementary file Fig S4d & S4e) are denoted with (+) and (-) respectively.

environmental effects of chironomid bioturbation activity, which are likely to not only increase nutrient cycling but also suspension of both microbial cells and settled MPs between benthic substrates and the water (Hölker et al., 2015; Malli et al., 2022). For example, resuspension of MP particles supporting microbial biofilms might explain why the effect of the high MP concentration on water column microbial abundance switched from slightly negative to strongly positive when chironomids were present (Fig. 3b). Along the same lines, increased nutrient cycling in the presence of chironomids might have countered the negative effects of MP exposure on chlorophyll-a concentration and ecosystem respiration detected when chironomids were absent (Fig. 5a), by stimulating autotrophs and heterotrophs respectively (Herren et al., 2017). Further research is required to assess these hypotheses, and the potential for interactions between consumers and microbes to alter MP effects on ecosystems more generally.

Our MP particle concentration, shape and polymer treatments affected different aspects of microbial community structure, consumer body condition, and ecosystem functioning. During the initial conditioning period (before chironomid addition), we observed that microbial abundance in the water was reduced in the presence of PP MPs (Fig. 3a). We hypothesise that the abundance of free-living cells in the water column was reduced due to formation and subsequent sedimentation of hetero-aggregates with the MP particles. Hetero-aggregate formation and sedimentation might similarly explain the reduction of water column chlorophyll-a observed at high concentrations of PP MPs in the absence of chironomids (Fig. 5a). Such effects have been observed previously for microalgae exposed to 6.1 particles/mL of PP MPs, lower than the high concentration used in our study (150 particles/mL; Lagarde *et al.*, 2016).

Further MP effects observed in our experiment are likely to reflect differences in particle surface area. Chironomid biomass increased in the presence of MP fragments (Fig. 4a), which had roughness (as indicated by lower circularity) compared to spheres, and hence most likely to support more extensive biofilm growth. Chironomid lipid content was also greatest under the high MP concentration treatment *per se* (Fig. 4b). Huang et al. (2021) previously documented an increased sediment biofilm biomass associated with increasing concentration of MPs, and we hypothesise these increases in chironomid biomass and body condition reflect greater availability of biofilm, increasing the nutrient quality of FPOM for consumers (Cummins, 1974). Additional potential effects related to surface area include increased microbial respiration (Fig. 5b), indicative of increased microbial activity, observed in the presence of PP and PET MPs (at high and low concentrations, respectively), with PP in particular regarded as a relatively brittle polymer known to readily roughen (Arakawa and Takahashi, 1991).

Among microbial and ecosystem responses to MPs, only water column bacterial taxa richness responded to MP exposure independent of chironomid presence (Fig. 2). Our results show that MPs *per se* reduced

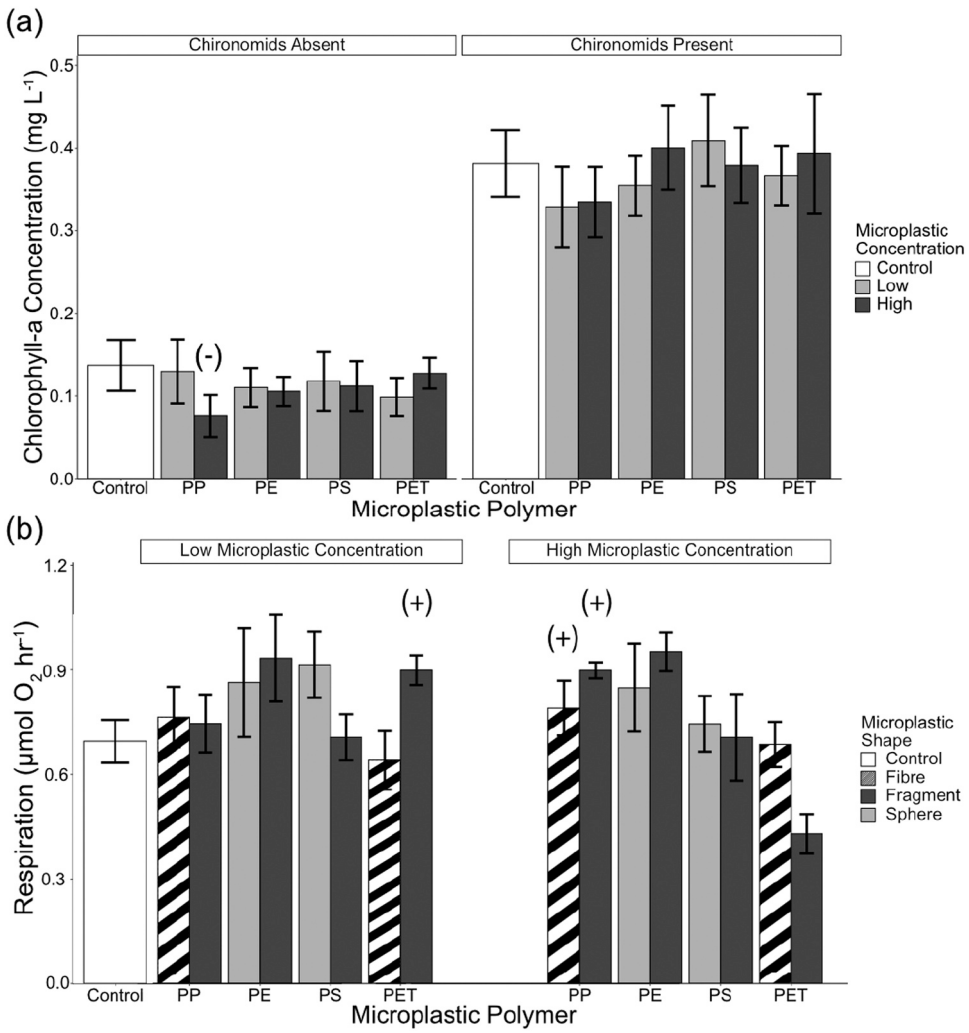

Fig. 5. Effects of (a) chironomid presence, MP polymer and concentration on chlorophyll-a concentration and (b) MP polymer, concentration and shape on ecosystem respiration in microcosms without chironomids. Bars plot the mean of block averages ± 1 SE (n = 12). Treatments that were significantly higher or lower than controls based on analysis of log response rations (Supplementary file Fig S5) are denoted with (+) and (-) respectively.

microbial taxa richness only at the low concentration, indicating that microbial diversity may not react in a monotonic dose-dependent manner to MP exposure. The response of this broad community indicator towards MPs was also associated with the enrichment of certain taxa in the presence of MPs, such as *Flavobacterium* (belonging to Bacteroidota)*, Rhizobiales Incertae Sedis, Pseudomonas, Niveispirillum, Pseudorhodobacter, Phodospirillales, Cellvibrio* (belonging to Pseudomonadota, synonym Proteobacteria) and *Lueolibacter* (belonging to Verrucomicrobiota). A number of these bacterial taxa have been reported previously to be associated with plastic contamination. For example, *Cellvibrio* was shown to be enriched in soil rhizospheres in the presence of plastic residues (Qi et al., 2020), and species of *Pseudomonas* (Pseudomonadota) and *Flavobacterium* (Bacteroidota) may be particularly associated with bacterial biofilms in ponds (McCormick et al., 2014). The increase in some of these bacterial taxa in the presence of MPs may be due to their ability to utilize MPs as habitat or an energy source. Members of the family Gammaproteobacteria including *Pseudomonas* are reported to degrade a wide range of plastic polymers, including all those used in our study (Gambarini et al., 2021), and species of *Flavobacterium* (Phylum Bacteroidota) have been reported to degrade PE (Koutny et al., 2009). The consequences of these shifts in microbial communities in the presence of MPs are worthy of further research attention, given the key role of microbes as basal drivers of multiple ecosystem functions related to nutrient and carbon cycling.

5. Conclusions

Many of the MP effects observed in our experiment were associated with increases in e.g. consumer growth and ecosystem respiration. Such effects are not necessarily positive for wider ecosystem integrity (Frainer et al., 2021), given evidence that increases in activity and productivity of specific organism groups can reduce functional stability, and result in phenomena such as eutrophication (Smith, Tilman and Nekola, 1999). Among MP properties, our results indicate that MPs composed of PP and/or characterised by a greater surface area are most likely to affect freshwater ecosystem properties, suggesting a particular need to reduce prevalence of PP and those polymers that fragment most readily in the environment. However, further research focussed on the cumulative effects of a broader range of MP types, including MP mixture effects, is required to better understand consequences of MP effects on microbial activity and consumer biomass on ecosystem integrity, and underpin more concrete management recommendations.

Freshwater ecosystems are impacted by multiple anthropogenic stressors simultaneously including *inter alia* elevated nutrients, chemical pollution and global warming (Dudgeon et al., 2006). The documented impacts of these stressors on biodiversity and ecosystem functioning have been shown to be greater than most of the MP effects observed in our experiment (Hiltunen et al., 2021; Horton et al., 2018). Similarly, consumer presence had stronger effects on most microbial and

functional responses than the MP treatments in our study. Nevertheless, the frequency and variety of detectable MP effects observed here, associated with a range of MPs representing those most commonly detected in the environment (Burns and Boxall, 2018), might be a cause for concern, especially if they contribute significantly to the existing heavy stressor load impacting freshwaters (Dudgeon et al., 2006). More research on potential interactions between MPs and other anthropogenic stressors, and focussed on multiple trophic levels (microbes, primary producers, consumers), is required to more thoroughly assess the true magnitude of the risk posed by MP pollution, relative to those currently posed by the myriad of other environmental problems on the Earth's heavily degraded freshwater habitats.

CRediT authorship contribution statement

Rachel Hurley: Resources, Funding acquisition. **Stefan Bertilsson:** Writing – review & editing, Resources, Methodology, Conceptualization. **Ze Hui Kong:** Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Brendan G. Mckie:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Tong Liu:** Writing – review & editing, Visualization, Software, Methodology, Investigation, Formal analysis. **Francis J. Burdon:** Writing – review & editing, Methodology, Conceptualization. **Amélie Truchy:** Writing – review & editing, Methodology, Conceptualization. **Martyn Futter:** Writing – review & editing, Funding acquisition, Conceptualization. **Mirco Bundschuh:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Brendan Mckie reports financial support was provided by Swedish Environmental Protection Agency. 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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.117697.

Data availability

Data will be made available on request.

References

Arakawa, K., Takahashi, K., 1991. Relationships between fracture parameters and fracture surface roughness of brittle polymers. Int. J. Fract. 48 (2), 103–114. https://doi.org/10.1007/BF00018393.

Bates, D., et al., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67 (1). https://doi.org/10.18637/jss.v067.i01.

Brennan, A., McLachlan, A.J., Wotton, R.S., 1978. Particulate material and midge larvae (Chironomidae: Diptera) in an upland river. Hydrobiologia 59 (1), 67–73. https://doi.org/10.1007/BF00017606.

Burns, E.E., Boxall, A.B.A., 2018. Microplastics in the aquatic environment: evidence for or against adverse impacts and major knowledge gaps. Environ. Toxicol. Chem. 37 (11), 2776–2796. https://doi.org/10.1002/etc.4268.

Callahan, B.J., et al., 2015. DADA2: High resolution sample inference from amplicon data'. bioRxiv, 024034. https://doi.org/10.1101/024034.

Chao, A., Chiu, C.-H., Jost, L., 2014. Unifying species diversity, phylogenetic diversity, functional diversity, and related similarity and differentiation measures through hill numbers. Annu. Rev. Ecol., Evol., Syst. 45 (45, 2014), 297–324. https://doi.org/10.1146/annurev-ecolsys-120213-091540.

Cox, E.P., 1927. A method of assigning numerical and percentage values to the degree of roundness of sand grains'. J. Paleontol. 1 (3), 179–183.

Cummins, K.W., 1974. Structure and function of stream ecosystems. BioScience 24 (11), 631–641. https://doi.org/10.2307/1296676.

Cummins, K.W., Klug, M.J., 1979. Feeding ecology of stream invertebrates. Annu. Rev. Ecol. Syst. 10 (1), 147–172. https://doi.org/10.1146/annurev.es.10.110179.001051.

Dudgeon, D., et al., 2006. Freshwater biodiversity: Importance, threats, status and conservation challenges. Biol. Rev. Camb. Philos. Soc. 81 (2), 163–182. https://doi.org/10.1017/S1464793105006950.

Frainer, A., et al., 2021. Plant litter decomposition as a tool for stream ecosystem assessment. In: Swan, C.M., Boyero, L., Canhoto, C. (Eds.), The Ecology of Plant Litter Decomposition in Stream Ecosystems. Springer International Publishing, Cham, pp. 483–509. https://doi.org/10.1007/978-3-030-72854-0_21.

Gambarini, V., et al., 2021. Phylogenetic distribution of plastic-degrading microorganisms. In: Kent, A.D. (Ed.), mSystems, 6, pp. e01112–e01120. https://doi.org/10.1128/mSystems.01112-20.

Haggerty, R., et al., 2014. Ecosystem respiration increases with biofilm growth and bed forms: Flume measurements with resazurin. J. Geophys. Res. Biogeosciences 119 (12), 2220–2230. https://doi.org/10.1002/2013JG002498.

Hale, R.C., et al., 2020. A global perspective on microplastics. p. e2018JC014719 J. Geophys. Res.: Oceans 125 (1). https://doi.org/10.1029/2018JC014719.

Herlemann, D.P.R., et al., 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J. 5 (10), 1571–1579. https://doi.org/10.1038/ismej.2011.41.

Herren, C.M., et al., 2017. Positive feedback between chironomids and algae creates net mutualism between benthic primary consumers and producers. Ecology 98 (2), 447–455. https://doi.org/10.1002/ecy.1654.

Hiltunen, M., Vehniäinen, E.R., Kukkonen, J.V.K., 2021. Interacting effects of simulated eutrophication, temperature increase, and microplastic exposure on Daphnia. Environ. Res. 192, 110304. https://doi.org/10.1016/j.envres.2020.110304.

Hoellein, T.J., et al., 2019. Microplastic deposition velocity in streams follows patterns for naturally occurring allochthonous particles. Sci. Rep. 9 (1), 1–11. https://doi.org/10.1038/s41598-019-40126-3.

Hölker, F., et al., 2014. Microbial diversity and community respiration in freshwater sediments influenced by artificial light at night. Philos. Trans. R. Soc. B Biol. Sci. 370. https://doi.org/10.1098/rstb.2014.0130.

Hölker, F., et al., 2015. Tube-dwelling invertebrates: tiny ecosystem engineers have large effects in lake ecosystems. Ecol. Monogr. 85 (3), 333–351. https://doi.org/10.1890/14-1160.1.

Horton, A.A., et al., 2018. Acute toxicity of organic pesticides to Daphnia magna is unchanged by co-exposure to polystyrene microplastics. Ecotoxicol. Environ. Saf. 166, 26–34. https://doi.org/10.1016/j.ecoenv.2018.09.052.

Hossain, M.R., et al., 2019. Microplastic surface properties affect bacterial colonization in freshwater. J. Basic Microbiol. 59 (1), 54–61. https://doi.org/10.1002/jobm.201800174.

Huang, Y., et al., 2021. Effect of microplastics on ecosystem functioning: microbial nitrogen removal mediated by benthic invertebrates. Sci. Total Environ. 754, 142133. https://doi.org/10.1016/j.scitotenv.2020.142133.

Huot, Y., et al., 2007. Does chlorophyll *a* provide the best index of phytoplankton biomass for primary productivity studies? Biogeosciences Discuss. 4 (2), 707–745.

Kong, Z.H., et al., 2023. Comparing effects of microplastic exposure, FPOM resource quality, and consumer density on the response of a freshwater particle feeder and associated ecosystem processes. Aquat. Sci. 85 (3), 70. https://doi.org/10.1007/s00027-023-00964-w.

Koutny, M., et al., 2009. Soil bacterial strains able to grow on the surface of oxidized polyethylene film containing prooxidant additives. Int. Biodeterior. Biodegrad. 63 (3), 354–357. https://doi.org/10.1016/j.ibiod.2008.11.003.

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest package: tests in linear mixed effects models. J. Stat. Softw. 82 (13), 1–26. https://doi.org/10.18637/JSS.V082.I13.

Lucas, R., et al., 2017. A critical evaluation of ecological indices for the comparative analysis of microbial communities based on molecular datasets. FEMS Microbiol. Ecol. 93 (1), fiw209. https://doi.org/10.1093/femsec/fiw209.

Malli, A., et al., 2022. Transport mechanisms and fate of microplastics in estuarine compartments: a review. Mar. Pollut. Bull. 177, 113553. https://doi.org/10.1016/j.marpolbul.2022.113553.

McCormick, A., et al., 2014. Microplastic is an abundant and distinct microbial habitat in an urban river. Environ. Sci. Technol. 48 (20), 11863–11871. https://doi.org/10.1021/es503610r.

McKie, B.G., et al., 2023. Evaluating the properties, fate and individual-to-ecosystem level impacts of contrasting microplastics in freshwaters. Naturvårdsverket, Stockholm.

McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLOS ONE 8 (4), e61217. https://doi.org/10.1371/journal.pone.0061217.

Moënne-Loccoz, Y., et al., 2015. Microorganisms and biotic interactions. In: Bertrand, J.-C., et al. (Eds.), Environmental Microbiology: Fundamentals and Applications: Microbial Ecology. Springer Netherlands, Dordrecht, pp. 395–444. https://doi.org/10.1007/978-94-017-9118-2_11.

Oksanen, J., , 2018. 'vegan: Community Ecology Package.

Qi, Y., et al., 2020. Effects of plastic mulch film residues on wheat rhizosphere and soil properties. J. Hazard. Mater. 387, 121711. https://doi.org/10.1016/j.jhazmat.2019.121711.

Quast, C., et al., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41 (D1), D590–D596. https://doi.org/10.1093/nar/gks1219.

R Core Team, 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ⟨https://www.R-project.org/⟩.

Renes, S.E., et al., 2020. Disturbance history can increase functional stability in the face of both repeated disturbances of the same type and novel disturbances. Sci. Rep. 10 (1), 1–13. https://doi.org/10.1038/s41598-020-68104-0.

Reznick, D., 1983. The structure of guppy life histories: the tradeoff between growth and reproduction. Ecology 64 (4), 862–873. https://doi.org/10.2307/1937209.

Rozman, U., Kalčíková, G., 2022. Seeking for a perfect (non-spherical) microplastic particle – The most comprehensive review on microplastic laboratory research. J. Hazard. Mater. 424, 127529. https://doi.org/10.1016/j.jhazmat.2021.127529.

Scherer, C., et al., 2017. Feeding type and development drive the ingestion of microplastics by freshwater invertebrates. Sci. Rep. 7 (1), 1–9. https://doi.org/10.1038/s41598-017-17191-7.

Scherer, C., et al., 2020. Toxicity of microplastics and natural particles in the freshwater dipteran Chironomus riparius: same same but different? Sci. Total Environ. 711, 134604. https://doi.org/10.1016/j.scitotenv.2019.134604.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9 (7), 671–675. https://doi.org/10.1038/nmeth.2089.

Shepard, R.B., Minshall, G.W., 1984. Role of benthic insect feces in a rocky mountain stream: fecal production and support of consumer growth. Ecology 119–127.

Silva, C.J.M., et al., 2019. Ingestion of small-sized and irregularly shaped polyethylene microplastics affect Chironomus riparius life-history traits. Sci. Total Environ. 672, 862–868. https://doi.org/10.1016/j.scitotenv.2019.04.017.

Sinclair, L., et al., 2015. Microbial community composition and diversity via 16S rRNA gene amplicons: Evaluating the illumina platform. PLoS ONE 10 (2), e0116955. https://doi.org/10.1371/journal.pone.0116955.

Smith, V.H., Tilman, G.D., Nekola, J.C., 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environ. Pollut. 100 (1), 179–196. https://doi.org/10.1016/S0269-7491(99)00091-3.

Steffan, S.A., Dharampal, P.S., 2019. Undead food-webs: Integrating microbes into the food-chain. Food Webs 18, e00111. https://doi.org/10.1016/j.fooweb.2018.e00111.

Truchy, A., et al., 2020. Habitat patchiness, ecological connectivity and the uneven recovery of boreal stream ecosystems from an experimental drought. Glob. Change Biol. 26 (6), 3455–3472. https://doi.org/10.1111/gcb.15063.

Vannote, R.L., et al., 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37 (1), 130–137. https://doi.org/10.1139/f80-017.

Vroom, R.J.E., et al., 2017. Aging of microplastics promotes their ingestion by marine zooplankton. Environ. Pollut. 231, 987–996. https://doi.org/10.1016/j.envpol.2017.08.088.

Wei, Y., Liao, S.-A., Wang, A., 2016. The effect of different carbon sources on the nutritional composition, microbial community and structure of bioflocs. Aquaculture 465, 88–93. https://doi.org/10.1016/j.aquaculture.2016.08.040.

Weinstein, M.M., , 2019. 'FIGARO: An efficient and objective tool for optimizing microbiome rRNA gene trimming parameters'. Available at: https://doi.org/10.1101/610394.

Xu, S., et al., 2023. MicrobiotaProcess: a comprehensive R package for deep mining microbiome. Innovation 4 (2), 100388. https://doi.org/10.1016/j.xinn.2023.100388.

Zhao, S., et al., 2018. Field-based evidence for microplastic in marine aggregates and mussels: implications for trophic transfer. Environ. Sci. Technol. 52 (19), 11038–11048. https://doi.org/10.1021/acs.est.8b03467.