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by Brendan G. McKie[1], Ze Hui Kong[1], Tobias Schmitt[2], Amélie Truchy[1,3], Francis J. Burdon[1,4], Sabrina N. R. Zabalgoitia[1], Martina Stangl[1] Rachel Hurley[5], Martyn N. Futter[1], and Mirco Bundschuh[1,2]

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Förord

Denna rapport med titeln *Evaluating the properties, fate and individual-to-ecosystem level impacts of contrasting microplastics in freshwaters* presenterar resultaten av ett av fem beviljade projekt inom utlysningen Mikroplaster 2018. Forskningsresultaten från denna utlysning syftar till att öka kunskapen om mikroplaster och deras källor, spridningsvägar, ekologiska effekter och konsekvenser och hur åtgärder kan bidra till att reducera dem.

Projektet har finansierats med medel från Naturvårdsverkets miljöforskningsanslag till stöd för Naturvårdsverkets och Havs- och vattenmyndighetens kunskapsbehov.

Denna rapport är författad av Brendan G. McKie, Ze Hui Kong, Tobias Schmitt, Amélie Truchy, Francis J. Burdon, Sabrina N. R. Zabalgoitia, Martina Stangl, Rachel Hurley, Martyn N. Futter och Mirco Bundschuh.

Författarna ansvarar för rapportens innehåll.

Stockholm, februari 2023

Johan Bogren
Tf. Chef för Hållbarhetsavdelningen

Preface

This report entitled *Evaluating the properties, fate and individual-to-ecosystem level impacts of contrasting microplastics in freshwaters* presents the results of one of five funded projects within the call Microplastics 2018. The research results from this call aim to increase knowledge about microplastics and their sources, transmission routes, ecological effects, and consequences and how measures can help reduce them.

The project has been funded with the Swedish Environmental Protection Agency's environmental research grant to support the Swedish Environmental Protection Agency's and the Swedish Agency for Marine and Water Management's knowledge needs.

This report is written by Brendan G. McKie, Ze Hui Kong, Tobias Schmitt, Amélie Truchy, Francis J. Burdon, Sabrina N. R. Zabalgoitia, Martina Stangl, Rachel Hurley, Martyn N. Futter and Mirco Bundschuh.

The authors are responsible for the content of the report.

Stockholm, February 2023

Johan Bogren
Deputy department head
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Contents

1. Summary

1. Microplastic particles (MPs) are fragments, fibres and other shapes derived from plastic polymers in the size range of 1–5 000 µm. Concern about the environmental impacts of MPs and their implications for human wellbeing has never been higher. Unfortunately, growth in the empirical understanding of the dynamics and impacts of MPs lags behind. This hinders the capacity of scientists, managers and policy makers to address public concerns about the true level of risk posed by MPs, and to develop effective management, policy and governance strategies for eliminating or reducing those risks.
2. Research on the behaviour and impacts of MPs in freshwater ecosystems is especially deficient, despite their vulnerability to inputs of plastic waste (e.g. via storm water and other terrestrial runoff), and their capacity to act as key transport pathways through the landscape. This represents a substantial black box in our understanding of the dynamics of MPs from inland to the ocean.
3. In seven research activities (5 mesocosm experiments, 1 field study and 1 literature review) we addressed two broad research questions:
   a) Initial fate and environmental interactions of MP particles in streams, including biofilm formation and sorption of chemical stressors
   b) Ecological impacts of MPs on resource consumption, growth and survival of organisms, and on key ecosystem processes.
4. Among our key results addressing the initial fate and biofilm formation of MP particles, we found that (i) biofilm formation generally made denser particles more buoyant and caused more buoyant particles to sink faster, (ii) biofilms on polystyrene MPs supported more cyanobacteria than other polymers, and (iii) aquatic macrophytes increase MP retention by up to 94 %.
5. Among our key results addressing the ecological impacts of MPs, we found that almost all MP shapes and polymers studied had one or more effects on stream microbial organisms and associated ecosystem processes (e.g. microbial respiration, detritus breakdown), and/or on the life history of a model macroinvertebrate detritivore.
6. We also provide evidence that effects of MPs on microbial organisms can propagate up food-chains to affect consumer growth and fat storage.
7. Some MP impacts were similar to those of naturally occurring organic and inorganic particles, whilst others represented a risk over and above that associated with natural particles.
8. The number of MP impacts detected in our experiments provides sufficient basis for "moving beyond the precautionary principle" when motivating a need for monitoring and management – there is now sufficient evidence that MPs alter key aspects of the functioning of stream benthic food webs to motivate a need for action.
9. Based on our results, we further provide a series of recommendations for monitoring, policy and management targeting MPs, and for future research.

2. Sammanfattning

1. Mikroplastpartiklar är alla fragment, fibrer och andra former som härrör från plastpolymerer i storleksintervallet 1–5 000 µm. Oron för mikroplastpartiklars miljöpåverkan och deras konsekvenser för människors välbefinnande har aldrig varit större. Tyvärr är kunskapen om hur de sprider sig och påverkar miljön bristfällig. Detta begränsar forskare, förvaltare och beslutsfattares möjlighet att dels ta itu med allmänhetens oro över den verkliga risknivå som mikroplastpartiklarna innebär, dels att utveckla effektiva strategier för förvaltning och styrning för att minska riskerna.
2. Forskning på beteende och inverkan av mikroplast i sötvattenekosystem är särskilt bristfällig. Detta trots att dessa ekosystem är sårbara för tillförsel av plastavfall (till exempel via dagvatten och annan markdrift) och att de kan fungera som transportvägar för mikroplastpartiklar genom landskapet. Det utgör en väsentlig kunskapslucka i vår förståelse för partiklarnas dynamik från land till hav.
3. I sju forskningsaktiviteter (5 mesokosmexperiment, 1 fältstudie och 1 litteratursammanställning) tog vi upp två breda forskningsfrågor:
   - i) inledande öde (sedimentering, nedströms transport) av mikroplastpartiklar i vattendrag, inklusive biofilmbildning, samt sorption av kemiska stressfaktorer.
   - ii) Ekologiska effekter på vattendragets ekosystem, inklusive effekter på resurskonsumtion, tillväxt och överlevnad av organismer samt på viktiga ekosystemprocesser.
4. Bland våra nyckelresultat angående det initiala ödet och biofilmbildningen på mikroplastpartiklar, fann vi att (i) biofilmbildning i allmänhet gjorde att partiklar med högre densitet blev mer flytande och gjorde att flytande partiklar sjönk snabbare, (ii) biofilmer på polystyrenpartiklar hade fler cyanobakterier än andra polymerer, och (iii) vattenlevande makrofyter ökar partikelretention med upp till 94 %.
5. Bland våra nyckelresultat angående mikroplastpartiklarnas ekologiska effekter fann vi att nästan alla partikelformer och polymerer som studerades hade en eller flera effekter på (i) mikrobiella organismer och associerade ekosystemprocesser (till exempel mikrobiell respiration, nedbrytning av detritus) och/eller om (ii) överlevnad och kroppskondition (till exempel tillväxt eller fettlagring) för en modell detrivor makroevertebrat.
6. Vi har också dokumenterat bevis för att effekter av mikroplastpartiklar på mikrobiella organismer kan spridas upp i näringskedjorna och påverka andra organismer.
7. Vissa effekter liknade dem för naturligt förekommande organiska och oorganiska partiklar, medan andra representerade en risk utöver den som är förknippad med naturliga partiklar.
8. Antalet effekter av mikroplastpartiklar som upptäckts i våra experiment ger tillräcklig grund för att ”gå bortom försiktighetsprincipen” när de motiverar ett behov av övervakning och åtgärder.
9. Baserat på våra resultat ger vi en rad rekommendationer för mikroplaster gällande övervakning, policy och förvaltning av mikroplaster, samt framtida forskning.

3. Utökad sammanfattning på svenska

Bakgrund

Oron bland allmänheten och forskare för miljöpåverkan från mikroplastpartiklar och deras konsekvenser för människors välbefinnande har aldrig varit högre. Tyvärr är kunskapen om hur mikroplastpartiklar sprider sig och påverkar miljön bristfällig. Detta begränsar forskare, förvaltare och politiska beslutsfattares möjlighet att dels ta itu med allmänhetens oro över den verkliga risknivå som mikroplast ställer, dels att utveckla effektiva strategier för förvaltning och styrning för att minska riskerna. I Sverige har till exempel motiveringen för åtgärder som riktar sig till beslutsfattare till stor del baserats antingen på ”försiktighetsprincipen”, med de vanligaste formerna och källorna till mikroplaster prioriterade på grund av att dessa sannolikt kommer att utgöra den största risken i naturen, eller de som har varit relativt lätta att rikta in sig på i politiken (t ex. mikroplaster i kosmetiska produkter).

Forskning på beteende och inverkan av mikroplastpartiklar i sötvattenekosystem är särskilt bristfällig trots sårbarheten hos dessa ekosystem för tillförsel av plastavfall (till exempel via dagvatten och annan markdrift) och trots att sötvatten ofta fungerar som transportvägar för mikroplastpartiklar genom landskapet. Vikten av rinnande vatten för transport, omvandling och biologiskt upptag av naturligt förekommande partikelformigt organiskt material (POM) är väl etablerad. Det är troligt att vattendragsekosystem spelar en liknande nyckelroll i att reglera mikroplasters öde i miljön, inklusive upptag i födokällor till människor.

POM kretsar genom det rinnande vattnets ekosystem i en sammanlänkad serie av processer, som tillsammans beskrivs som ”partikelbearbetningskedjan” (se figur 1a). Grovt partikelformigt organiskt material (GPOM), inklusive höstlöv, kommer in i bäckar på samma vägar som plastavfall: avrinning från land och genom vindkast. Verkningarna av mikrober och detritivorer reducerar GPOM till fint partikelformigt organiskt material (FPOM, partikel ø ≤ 1 000 µm). FPOM är den huvudsakliga födokällan för ryggradslösa arter som klassificeras som ”samlare”. Samlare tillhör den djurgruppen i sötvatten som mest troligtvis konsumerar mikroplastpartiklar, men är i sin tur ofta föda för rovdjur, inklusive fiskar

Både FPOM och mikroplastpartiklar kännetecknas av en stor partikelyta i jämförelse med volym, vilket gynnar utvecklingen av alger och mikrobiella biofilmer. Sådana biofilmer kan påverka partiklarnas öde, till exempel genom att öka partikelvikten och sannolikheten att de sjunker. Biofilmer kan även bidra till oönskad ”påväxt”, det vill säga ackumulering av mikrobiella och andra organismer på hårda ytor inklusive människans infrastruktur (engelska: biofouling). Utveckling av biofilm ökar emellertid också näringsämneskvaliteten hos POM och därmed sannolikheten att samlare i sin tur konsumeras av andra djur. På samma sätt kan biofilmbildningen på mikroplastpartiklar möjligen öka värdet av partiklar till samlare om de kan smälta alg- och mikrobiologiska biofilmer. Biofilmer kan även ge upphov till negativa effekter på samlarna, om upptaget av föroreningar som adsorberas till mikroplastpartiklar ökar.

Vår förståelse av mikroplastpartiklars dynamik och effekter i förhållande till naturligt förekommande POM är fortfarande mycket begränsad. Denna förståelse är samtidigt helt avgörande för att sätta risker med mikroplast i perspektiv, med tanke på att biota exponeras för en rad naturligt förekommande partiklar från olika källor och ofta i koncentrationer som är mycket högre än de för mikroplastpartiklar.

Projekts syfte och struktur

I det här projektet utvecklade vi ett ramverk för att undersöka effekterna av mikroplastpartiklar i sötvattensekosystem, som är baserat på två allmänna principer: (1) sötvattensmiljöer är fulla av naturligt förekommande POM, som ibland har en hög resurskvalitet (till exempel döda algceller) och ibland en extremt låg resurskvalitet (till exempel jordhärledda kolloider) (2) mikroplastpartiklar representerar en typ av kolrik POM, men med flera biologiskt oönskade egenskaper (till exempel extremt låg nedbrytningshastighet). Vi fokuserar därför på de mikro- och makroorganismgrupper och ekologiska processer som reglerar naturligt förekommande POM i vattendrag. Vi fokuserar även på hur kontaminering av POM-pooler av mikroplastpartiklar påverkar dessa organismer och processer.

I sju forskningsaktiviteter (fem mesokosmexperiment, ett fältförsök och en litteratursammanställning) tog vi upp två breda forskningsfrågor:

i) mikroplastpartiklars inledande öde (sedimentering och nedströms transport) i vattendrag, inklusive biofilmbildning, samt sorption av kemiska stressfaktorer
ii) Ekologiska effekter av mikroplastpartiklar på vattendragets ekosystem, inklusive effekter på resurskonsumtion, tillväxt och överlevnad av organismer, samt på viktiga ekosystemprocesser.

I denna rapport bygger vi på resultat från dessa forskningsaktiviteter för att ta oss an tre övergripande mål: (i) att kvantifiera hur egenskaperna (till exempel storlek, form, biopåväxtpotential) hos olika typer av mikroplastpartiklar reglerar deras öde och effekter i vattendrag, (ii) att utvärdera riskerna förknippade med mikroplastpartiklar i förhållande till naturliga organiska och oorganiska partiklar, och (iii) att identifiera de potentiellt mest skadliga typerna av mikroplastpartiklar i vattendrag som ett hjälpmedel vid riskbedömning.

Metod och resultat: Inledande del och miljöinteraktioner

Vi utförde experiment i artificiella vattendrag för att kvantifiera partiklarnas initiala öde, vilket i slutändan avgör vilka livsmiljöer och arter, inklusive både primära och sekundära konsumenter, som utsätt för den högsta exponeringsrisken.

För att undersöka hur utveckling av biofilmer påverkar hastigheten med vilken partiklarna sjunker, exponerade vi 5 olika polymertyper med biofilmorganismer från ett oligotroft (näringsfattig) eller eutroft (näringsrik) dammekosystem. Därefter mätte vi hur snabbt de olika partiklarna sjönk med eller utan biofilm.

Ett potentiellt hot som utgörs av genomgripande plastföroreningar över hela världen är deras förmåga att binda och transportera hydrofoba organiska föreningar

(HOF) i flodnätverk. HOF är en grupp skadliga kemikalier inklusive olika bekämpningsmedel. Vi genomförde en systematisk litteraturgenomgång för att utvärdera vilka fysiska egenskaper som delas av mikroplastpartiklar och naturliga organiska partiklar, som gynnar sorption av HOC, samt vilka egenskaper som skiljer sig åt. Genomgången syftade vidare till att jämföra de biologiska effekterna av de två partikeltyperna i deras förmåga att fungera som vektorer för HOC och tog därför även hänsyn till faktorer som kemisk sammansättning, externa miljöfaktorer och partikelförekomst.

Bland våra nyckelresultat angående det initiala ödet och biofilmbildningen på mikroplastpartiklar, fann vi att:

1. Mikroplastpartiklar med en hög densitet blev mer flytande när biofilmer utvecklades. Detta beror sannolikt på produktionen av gasbubblor som produceras av Chlorophyta (den dominerande algtypen i partikelbiofilmer) och andra strukturer såsom cyanobakteriella gasvakuoler. Å andra sidan så sjönk plastpartiklar som var annars mycket flytande efter att biofilmer utvecklat, förmodligen eftersom den ökade biomassan i sin tur ökade densiteten jämfört med vattnets densitet.
2. Biofilmer på polystyren mikroplast hade fler cyanobakterier än andra polymerer
3. Vattenlevande växter (makrofyter) ökar mikroplastretention med upp till 94 %.
4. I vår litteraturöversikt fann vi att HOF-exponering i kombination med naturliga POM-källor hade övervägande negativa effekter på biota. Däremot fanns det lika många studier som visade på negativa effekter på biota som studier som visade neutrala och positiva resultat. mikroplastkoncentrationer i miljön är väsentligen försumbara jämfört med mängden POM-material, så även om de är bättre bärare av föroreningar, är deras roll i HOF-transport totalt sett sannolikt försumbar i förhållande till naturliga POM.

Metod och resultat: Ekologiska effekter

För att undersöka de ekologiska effekterna av mikroplastpartiklar i sötvatten så utförde vi tre olika mesokosmförsök på labb och ett i fält. I alla experiment hade vi en modellnäringsväv med detritus i form av GPOM och/eller FPOM, mikrobiella organismer och ibland en representativ samlarart, fjädermyggan *Chironomus riparius*. I experiment med fjädermyggan hade vi även ett tjockt lager med mineralsediment, eftersom *C. riparius* trivs bäst när den har tillgång till sediment för konstruktion av den tub i sedimentet där den lever. Dessa modellekosystem exponerades för olika typer av mikroplastpolymerer (polystyren, polyetenen, polypropen) och former (fragment, sfärer, fibrer). I fältförsöket exponerades systemet för polypropenpartiklar från kirurgiska munskydd. Koncentrationer av mikroplastpartiklar var alltid inom spannet av koncentrationer som hittas i verkligheten. Vi kvantifierade en rad ”ekosystemattribut” som responsvariabler, såsom överlevnad, tillväxt och fettlagring hos fjädermyggan, samt mikrobiell enzymfunktion och respiration. Vi kvantifierade även viktiga ekosystemprocesser som kan påverkas av mikroplastpartiklar, såsom algtillväxt och nedbrytning av detritus.

Nyckelresultat inkluderar:

1. Nästan alla de studerade polymererna och formerna hade en eller flera effekter på mikrobiella organismer och ekosystemprocesser (till exempel mikrobiell respiration, nedbrytning av detritus) och/eller överlevnad och kroppskondition (t. ex. tillväxt eller fettlagring) för samlaren *C. riparius*.
2. Några av dessa effekter översteg de som är förknippade med naturligt förekommande FPOM av låg kvalitet och de flesta upptäcktes trots de relativt låga koncentrationerna av mikroplastpartiklar, i förhållande till de mycket större koncentrationer av andra partiklar i våra mesokosmer.
3. De flesta av de upptäckta effekterna var uppenbara även vid de lägre koncentrationer som användes i vårt experiment (1 000 partiklar per kg sediment, vilket representerar mediankoncentrationen i sediment som observerats över hela världen).
4. Bland de utvärderade formerna påverkade fragment det största antalet responsvariabler. I de flesta fall var dessa effekter associerade med ökad mikrobiell abundans eller aktivitet (enzymaktivitet eller respiration). Detta återspeglar sannolikt hur bra den komplexa ytan av mikroplastfragment är för att stödja biofilmtillväxt.
5. Vi har också dokumenterat bevis för att effekter av mikroplastfragment på mikrobiella organismer kan ha efterföljande effekter på konsumenternas kroppskondition (storlek och fettlagring).
6. Bland polymerer är PET anmärkningsvärt för flertal negativa effekter på ekosystemprocesser (respiration och algproduktivitet), även om effekten på mikrobiell respiration var mindre än effekten av FPOM med låg kvalitet.
7. PE och PP var de polymerer som mest sannolikt påverkar konsumenternas överlevnad, och dessa effekter översteg vad som observerats för referens FPOM partiklar. PP påverkade algproduktiviteten negativt.

Slutsatser och rekommendationer

Vi menar att alla förändringar som är förknippade med närvaron av mikroplastpartiklar representerar en ekologisk förändring som kräver uppmärksamhet från förvaltningen, oavsett om den förändringen representerade en ökning eller minskning av något särskilt ekosystemattribut. Detta beror på att även en ökning av till exempel algproduktivitet eller organismtillväxt i förhållande till kontrollförhållanden kan tyda på en näringsvävskomponent (basresurser eller konsumenter) som är i obalans.

Antalet mikroplasteffekter som upptäckts i våra experiment ger tillräcklig grund för att ”gå bortom försiktighetsprincipen” med motivering av behovet för övervakning och åtgärder. Baserat på våra resultat ger vi vidare en rad rekommendationer för mikroplaster gällande övervakning, miljöförvaltning, policy och styrmedel, samt för framtida forskning. Här sammanfattas rekommendationer inriktad mot övervakning, åtgärder och policy.

Observera att när vi till exempel ger rekommendationer om att minska mängden av vissa mikroplastpartiklar i miljön anger vi inte hur denna minskning kan uppnås. Flera olika möjligheter finns för att uppnå en sådan minskning (minskad plastproduktion, förbättrad avfallshantering, ökad återvinning och/eller återanvändning, effektivare vattenrening) men det ligger utanför detta projekts ram att analysera vilket tillvägagångssätt som är mest lämpligt i en svensk kontext.

1. Prioriteringar för övervakning av mikroplastpartiklar:
   a) Inför övervakning av mikroplastpartiklar i sötvattensekosystem: De många olika effekter (positiva och negativa) som vi dokumenterar belyser behovet av att övervaka transporten och distributionen av mikroplastpartiklar i sötvatten. En övervakning behövs för att identifiera de ekosystem och livsmiljöer som upplever de högsta nivåerna av MP-ackumulering idag.
   b) Fokusera på övervakning av mikroplastpartiklar i sediment/bentiska substrat i vattendrag, där de allra flesta känsliga organismer i vattendrag lever och där det sker viktiga ekosystemprocesser som styr vad som händer med mikroplast i sötvatten.
   c) Övervakning av ackumulering och transport av mikroplastpartiklar bör fokuseras till de livsmiljöer som är kända för att gynna snabb algbiofilmbildning – speciellt ljus- och näringsrika livsmiljöer. Sådana förhållanden kan vara förknippade med ökad transport av mikroplasttyper med en högre densitet (till exempel däckslitage partiklar) som annars skulle förväntas snabbt sjunka.
   d) Särskilt fokus bör ligga på dagvattenhantering, där snabb biofilmtillväxt (till exempel i dammar och våtmarker) kan gör tätare partikeltyper som annars skulle förväntas snabbt sjunka mer flytande.
   e) Övervakning av mikroplastackumulering i makrofytbäddar i sötvatten, eftersom makrofyter ökar retentionen av mikroplastpartiklar med upp till 94 procent.
   f) Våra experiment sattes inte upp för att kunna ge riktlinjer för noll- effekt-koncentrationer, men med tanke på att vi upptäckte multipla effekter vid en koncentration av 1 000 mikroplastpartiklar/kg sediment så kan detektion av sådana koncentrationer i naturen användas som en markör för en habitat som sannolikt redan påverkas av förändringar i mikrobiella processer och möjligen bredare ekosystemfunktioner.

Prioriteringar inom policy och förvaltning:

   a) Fortsätta och utöka nuvarande åtgärder för att minska plastföroreningarna (både makro- och mikroplastpartiklar) i biosfären. Antalet effekter av mikroplast på viktiga ekosystemegenskaper i våra experiment, ofta förknippade med närvaron av mikroplastpartiklar i sig, snarare än specifika polymerer eller former, belyser det akuta behovet av att minska förekomsten av plast i miljön.
   b) Biofilmbildning är allmänt förekommande i naturen, men tjockare, algdominerade biofilmer bildas snabbare i ljusrika, näringsrika livsmiljöer. Sådana förhållanden bör undvikas i samband med stora utsläpps-/ackumuleringspunkter för mikroplastpartiklar (fabriker, dagvattenutlopp med mera) på grund av risken för ökande transporter av annars snabbsjunkande mikroplastpartiklar.
   c) Makrofyter kan planteras vid stora utsläppspunkter av mikroplast (vattenreningsverk, fabriker med mera) för att öka ”filtrering” av mikroplastpartiklar från vattnet. Effektiviteten av denna åtgärd ökar om makrofyterna skördas med jämna mellanrum.
   d) Inga specifika rekommendationer angående interaktioner mellan HOC och mikroplastpartiklar, eftersom ingen polymer var förknippad med en konsekvent större risk än någon annan, eller i jämförelse med naturligt förekommande FPOM.

e) Minska föroreningar från engångsplaster i miljön. Sådan plast har fysiska egenskaper som gynnar en snabbare reduktion ner till fragment[1, 2], som kan stödja omfattande mikrobiella biofilmer med tillhörande knock-on-effekter som observeras här.
f) När det gäller polymerer, fokusera särskilt på att minska PET, PE och PP i miljön. Dessa polymerer var förknippade med ett större antal effekter generellt, inklusive ett större antal negativa effekter, på viktiga ekosystemegenskaper i våra studier.
g) Fokusera även på att minska PS i miljön, för att minska det potentiella bidraget av mikroplastpartiklar till ett ökad tillväxt av cyanobakterier (viktiga eutrofierande och biofouling organismer), inklusive i oligotrofa livsmiljöer.

4. Introduction

4.1 Main research topics and report structure

Here, we report on results from the three-year Swedish EPA financed project *Evaluating the properties, fate and individual-to-ecosystem level impacts of contrasting microplastics in freshwaters.*

We follow the definition of Frias & Nash [3] in regarding all fragments, fibres and other shapes derived from plastic polymers in the size range of 1–5 000 µm as microplastics. We include debris from vehicle tires falling within this size range within our definition of microplastics

In this project, we developed a framework for investigating the impacts of microplastics (MPs) in stream ecosystems that is based on recognition of two general principles:

1. freshwater habitats are full of particulate organic matter (POM, i.e. particles with a high organic carbon content) ranging from high resource quality (e.g. dead algal cells) to extremely low resource quality (e.g. soil derived colloids)
2. MPs, as hydrocarbons, represent a type of carbon-rich POM[4], albeit characterised by several biologically undesirable properties (e.g. extremely high recalcitrance, presence of toxic compounds).

We therefore focus on the micro- and macro-organism groups and ecological processes that regulate naturally occurring POM in streams, and how contamination of POM pools by MPs affects those organisms and processes.

More specifically, our research has three overarching goals:

i) to quantify how the properties (e.g. size, shape, biofouling potential) of different types of microplastics (MPs) regulate their fate and impacts in stream ecosystems,

ii) to evaluate the risks associated with MPs relative to natural organic and inorganic particles,

iii) to identify the potentially most harmful types of MPs in riverine networks as an aid in hazard evaluation.

We begin with a general introduction to the research area, including presentation of the project's theoretical framework. Following this, the report is structured around two broad gaps in the understanding of the behaviour of different types of MPs in freshwater ecosystems in general and stream ecosystems in particular:

i) Initial fate and environmental interactions, addressing the initial fate (sedimentation, downstream transport) of MP particles and their interactions with the environment, including habitats and retention, biofilm formation and sinking, and sorption of chemical stressors.

ii) Ecological impacts including impacts of MPs on resource consumption, growth and survival of organisms, and on biodiversity and key ecosystem processes.

The second section of the report details the seven research activities (RAs – see Table 1 for a catalogue of abbreviations) conducted to address these topics. The RAs consisted of six experiments and one literature review. Each activity is reported with a brief outline of key research questions, overview of methods, and a list of key results and preliminary discussion points. The report concludes with a general overview of the main results, and recommendations for priorities in monitoring, policy and management targeting microplastics.

4.2 Introduction to the research area

Concern about the environmental impacts of MPs and their implications for human health and wellbeing has never been higher, as seen in the rapidly increasing coverage in scientific, traditional and social media[5]. Unfortunately, growth in the empirical understanding of the dynamics and ecological impacts of MPs lags behind[6, 7]. This hinders the capacity of scientists to inform managers and policy makers about the true level of risk posed by MPs to the environment, biodiversity and key ecosystem functions and services, and to develop effective and appropriate management, policy and governance strategies for eliminating or reducing those risks[7, 8], including for aquatic ecosystems in particular, where MP accumulation is worryingly high[9, 10].

Table 1. Catalogue of abbreviations used in this report

Plastic polymers: types & characteristics		Other	
LDPE	Low-density polyethylene	ANOVA	Analysis of Variance*
PA	Polyamide (Nylon)	CPOM	Coarse particulate organic matter
PE	Polyethylene	FPOM	Fine particulate organic matter
PET	Polyethylene terephthalate	HOC	Hydrophobic organic compounds
PP	Polypropylene	LSMF	Landau Stream Mesocosm Facility
PMMA	Poly(methyl methacrylate)	MP	Microplastics
PS	Polystyrene	NOM	Natural organic matter
PVC	Polyvinyl chloride	POM	Particulate organic matter
TWP	Tire wear particles	RA	Research activity
uPVC	unplasticised polyvinyl chloride	%LML	Percent leaf mass loss
ø	Particle diameter	%TSL	Percent tensile strength loss
ρ	Particle density		

* The statistical method for testing differences among treatments in all our experiments.

Vast quantities of plastic enter aquatic ecosystems primarily via terrestrial runoff (e.g. storm water from urban and industrial areas), wastewater or windthrow[11-13]. Some of this plastic, known as “primary MPs” may already be in MP form (particle ø ≤ 5 mm), such as pre-manufactured as beads. Additionally, larger plastic waste particles can be reduced down to microparticle size, due to physicochemical- (including photo- and mechanical degradation) and biodegradation processes, and is then known as “secondary MPs”[6]. The most common types of MPs in the Swedish environment are secondary MPs derived from fibres released initially from clothing in domestic washing machines, and car tire debris[8, 14]

A review overseen by a member of our project team[6] uncovered strong biases in MP research in aquatic ecosystems. This is seen particularly in the overwhelmingly marine emphasis of most (77 %) studies, which have focussed especially on fish, reflecting the importance of marine fisheries in human food supply[6, 11]. Documented impacts include interruption of dietary processes, growth and metabolism (e.g. increased energy expenditure, reduced growth) and altered neurological and genetic function[15-17]. MPs also interact with other stressors[17-19], particularly chemical stressors (e.g. by binding toxins, which may increase toxin transport and/or uptake into the food web), and can increase biofouling (by acting as substrates for excessive biofilm development, and forming aggregates with algal or bacterial cells)[18, 20]. The review also uncovered strong biases in the types of MPs studied, with polystyrene and polyethylene "beads" over-represented in research.[21] In contrast, polypropylene, polyester and polyamide particles are under-represented, despite their widespread detection in the field, as are fibres and fragments, even though these are the most common form of MPs reported in field-collected animals[6, 9].

In contrast with the marine environment, there has been relatively little focus on the behaviour and impacts of MPs in freshwater ecosystems[6, 13]. This is despite the vulnerability of freshwaters to inputs of plastic waste, especially via storm water and other terrestrial runoff, and the potential of stream and river networks to act as key transport pathways through the landscape[7, 22]. Indeed, the importance of running waters for the transport, transformation, and biological uptake of naturally occurring particulate organic matter (POM, see Table 1 for a list of abbreviations used in this report), is well established[23, 24]. It is likely that stream and river networks play a similar key role in regulating the fate of MPs in the environment, including uptake into the human food chain. Unfortunately, the great majority of MP research focussed on freshwaters has focussed on single species in small laboratory microcosms, which is very limited in its capacity to inform on the larger scale fate of MPs in river networks and foodwebs.

The potential for movement, retention, modification and impacts of different types of MPs in freshwater habitats is almost completely unknown, representing a substantial black box in our understanding of the dynamics of MPs from inland to the ocean, and hindering development of credible policy and management.

4.3 Theoretical framework: Microplastics & the "particle processing chain" in streams

Stream and river networks support life and underpin landscape integrity through provision of multiple ecosystem services, including provision of clean fresh water, transport of carbon and nutrients to recipient (lake, estuary, marine) ecosystems, and transformation of carbon and nutrients into biomass, including organisms in the human food supply chain (fish, crayfish, etc.)[25-27]. Streams are major sinks for excess nitrogen (N) and phosphorus (P) due to high algal nutrient uptake and high microbial denitrification rates[28], while the decomposition of terrestrially-derived organic matter is key to landscape-level carbon budgets[29]. Underpinning these ecosystem services is the unique biodiversity of streams, disproportionally high relative to other habitats[30].

Particulate organic detritus cycles through stream food webs in a linked series of processes described collectively as the "particle processing chain"[31] (Figure 1a). Coarse particulate organic matter (CPOM), including autumn shed leaf litter, enters streams by *the same pathways as plastic waste*: overland runoff and windthrow. The actions of microbes and detritivores reduce CPOM to fine particulate organic matter (FPOM, particle ø ≤ 1 000 µm), primarily as detritvore faecal pellets[32], though the FPOM pool also includes pellets produced by other biota (Figure 1a), and micro-aggregates[24]. FPOM contributes substantially to the total organic carbon pool in streams[33, 34], and downstream transport of FPOM has particular importance for longitudinal connectivity in river catchments[23].

FPOM is the main food source for invertebrate species classified as "collectors"[34-36]. Collector gatherers, hereafter "*deposit feeders*", primarily consume FPOM deposited on or within stream substrates, whilst collector "*filterers*" are suspension feeders that sieve suspended POM[36]. These species have morphological adaptations (e.g. head fans or brushes for sieving or sweeping up FPOM) or behaviours (e.g. construction of filtering nets or burrows) for feeding on suspended, deposited or buried FPOM (Fig 1a). Collectors are in turn prominent in the diets of predators, including fish[37, 38].

Filtering and deposit feeding invertebrates in the particle feeding "Collector" guild comprise a large portion of benthic biomass in streams, and are highly efficient at capturing and assimilating FPOM. Accordingly, this guild is highly likely to be affected by the presence of MPs, and to play a key role in the retention and accumulation of MPs in freshwater foodwebs. Despite this, with the exception of some studies on mussels, *freshwater collectors have been little-investigated in MP research.*

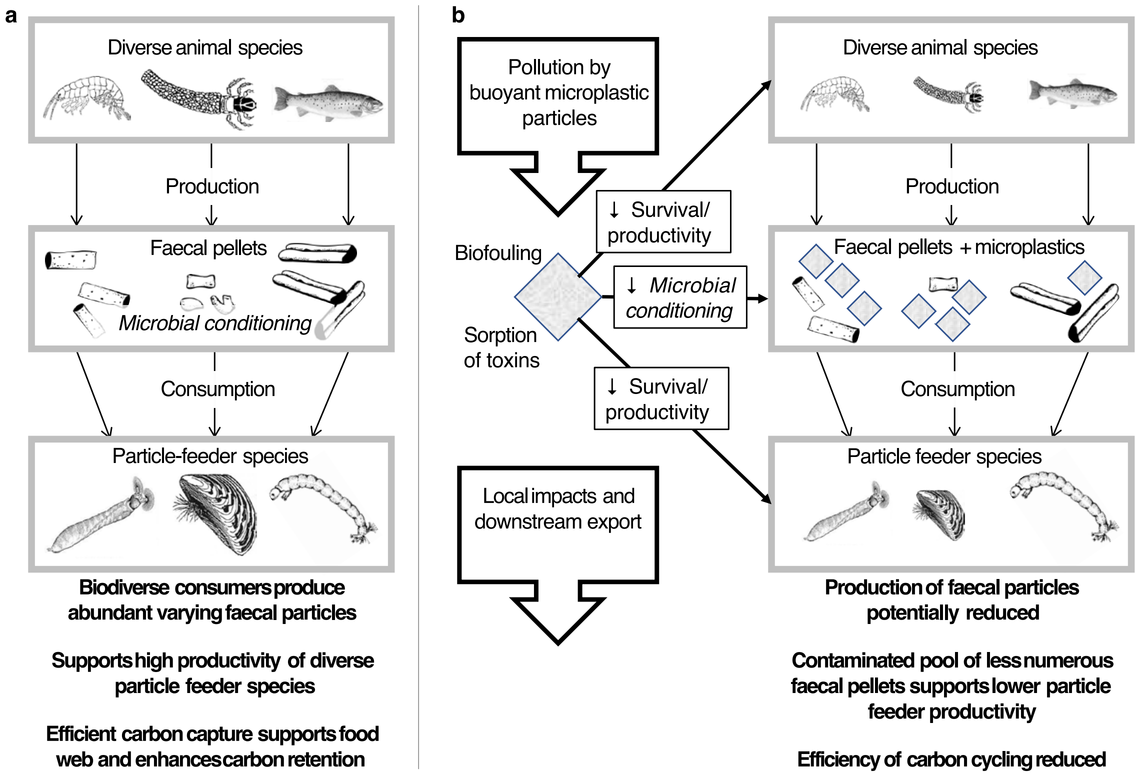

Figure 1. Particles in stream food webs. (A) In a food web uncontaminated by microplastics (MPs), faecal particles produced by diverse animal species (here an amphipod crustacean, caddisfly and salmon fish) are conditioned by microbes, improving nutrient quality prior to consumption by a diverse assemblage of filter feeders (here a blackfly larvae and freshwater mussel) and deposit feeders (here a chironomid midge larvae). This supports productivity of collector organism and their predators, and underpins retention and further cycling of carbon within the freshwater food web. (B) In a food web contaminated by particles with properties including moderate buoyancy, high sorption potential and a tendency to support biofouling, a worst-case scenario might include not only direct effects on collector growth and survival resulting from ingestion of MPs (e.g. increased metabolic costs due to processing and expulsion of MPs and/or toxic effects from adsorbed chemical stressors) but also indirect effects associated with reduced faecal production and/or microbial conditioning (e.g. arising from reduced environmental quality caused by biofouling, chemical adsorption, and general pollution). Together these impacts are likely to reduce the efficiency of carbon retention and cycling, and may affect biodiversity. Buoyant MPs are also likely to be exported downstream, potential impacting lake, estuary and marine habitats

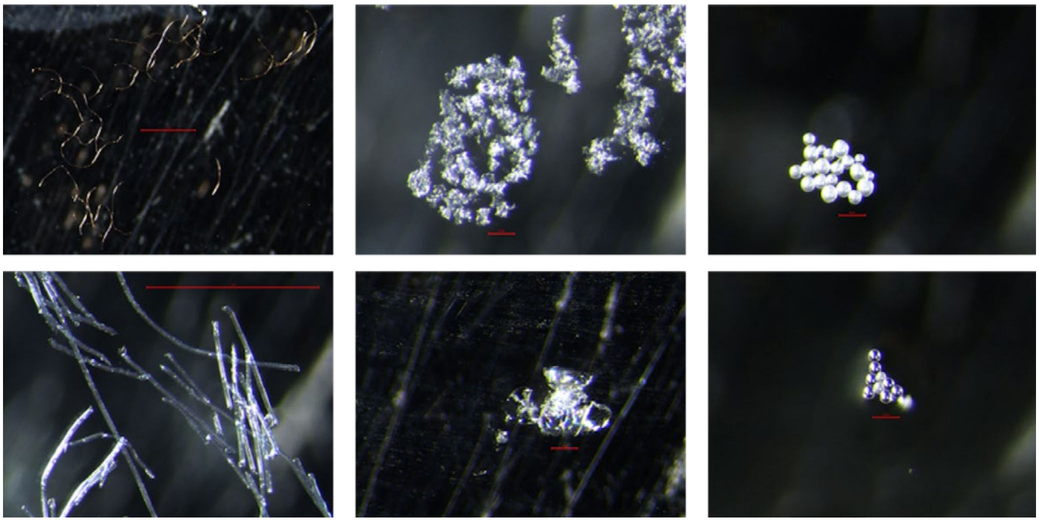

Figure 2. The three main microplastic shapes – fibres, fragments and spheres – illustrated here for four different polymers. Clockwise from top left: Fibres of PET, fragments of PP, spheres of PS, spheres of PE, fragments of PE, fibres of PP (see Table 1 for all polymer abbreviations)

Microplastics and FPOM alike vary substantially (Figure 2) in properties including density, buoyancy, shape, surface area to volume ratio, and surficial interactions with co-contaminants[24]. Different collector species vary in their capacity to capture and process different types of POM, depending on their habitat use, feeding preferences and physiology[39]. These species-specific preferences are likely to regulate the exposure of different collectors to different MP types. Dense particles and fibres are more likely to sediment out or otherwise be entrapped in benthic habitat structures, where they will be more available for consumption by deposit feeders, whereas more buoyant particles will be more available to filterers (Figure 1b). In general, a diverse assemblage of collectors, including both deposit and filter feeders, are more likely to be complementary in the types of FPOM and MP they consume, maximising rates of particle capture and processing[24, 40] (Figure 1a). However, contamination of the FPOM pool by MPs might interrupt these dynamics, by compromising feeding, growth and survival of the most vulnerable species, and/or interactions between consumers and microbes[41] (Figure 1b).

Both FPOM and MPs are characterised by a large surface to volume ratio, which favours adsorption of co-contaminants such as metals or organic chemical stressors[24, 42, 43]. This can alter the bioavailability of these co-contaminants, with the direction depending on the binding strength between contaminant and FPOM/MP. The large surface area-to-volume ratio also potentially favours development of algal and microbial biofilms, and MPs might also form aggregations with e.g. algal cells. Such outcomes can influence the fate of MPs, e.g. by increasing particle (or aggregate) weight and probability of sinking, and might contribute to increased biofouling. However, biofilm development also increases the nutrient quality of POM, and hence the probability of consumption by collectors[24]. In the case of MPs, this might have positive outcomes if collectors consuming MPs are able to digest algal and microbial biofilms whilst expelling the plastic with little energetic cost, or might have negative outcomes if uptake of contaminants adsorbed to the MPs is increased.

Finally, our understanding of the dynamics and impacts of MPs relative to naturally occurring POM remains very limited, but is *absolutely critical* for placing the risk posed by MPs into perspective[44, 45], given that biota are presented with a range of naturally occurring particles from various sources and often at concentrations much higher than MPs.

Concurrent assessment of the properties, transformations (including chemical sorption and biofilm development), and processing of naturally occurring POM and MPs is rarely undertaken. Such assessment, encompassing a range of particle types and across a gradient of POM:MP concentration ratios, is needed to: (i) evaluate how the behaviour of MPs in the functioning of stream food webs differs from that of naturally occurring POM and (ii) identify at which relative concentrations MPs begin to pose the greatest risks to biota and function.

4.4 Research questions

We address the following research questions in a linked series of research activities (RA). Research activities (RA) were conducted using state-of-the-art experimental and laboratory facilities at the three main project partners: Swedish University of Agricultural Sciences (Uppsala), University of Landau (Germany) and Norwegian Institute for Water Research (NIVA).

i) What are the properties of microplastics (MPs) originating from different parent materials, how do they compare with the properties of naturally occurring particulate organic matter (POM), and how are these properties modified through exposure in aquatic habitats? (RAs 1-3)

ii) Which MP properties influence their physical fate in streams (sedimentation, dispersion, aggregation) as well as their uptake by biota with different feeding strategies? (RAs 1-3)

iii) How do MPs impact the growth and survival of individual consumers (RAs 3, 6-7), microbial abundance and diversity (RAs 6–7), and ecosystem functioning (RAs 4-7)?

iv) What is the capacity of MPs to adsorb chemical stressors relative to naturally occurring POM, and how is the growth and survival of consumers affected (RA 3)?

v) How do MPs interact with other stressors and environmental factors in impacting growth and survival of individuals, community composition, and ecosystem functioning (RA 5-6)?

5. Research activities: background, methods, results and discussion

5.1 Overview of MP polymers and statistical approach

In our experiments, we used a range of polymers representing a broad range of uses in the modern world:

HDPE: Plastic toys, bottles, outdoor chairs, pipes, ropes

PS: Disposable plastic cutlery, laboratory/medical items (petri dishes, microplates, etc.)

PP: Plastic furniture, food packaging, synthetic fibres (including face masks)

PET: Bottles, synthetic fibres

TWP: particles worn from vehicle tires

uPVC: used especially in building construction: windows, doors, cladding, roofing etc

With the exception of a literature review, all of our RAs employed manipulative experiments. Accordingly, our main statistical method was analysis of variance, testing for differences and interactions among levels of our experimental factors. Here we report significance levels in the format $p < 0.05$, <0.01 or <0.001.

5.2 Initial fate and environmental interactions

5.2.1 RA-1: Fate and Retention of different Microplastic Particles in Streams

BACKGROUND

Most sources of MP pollution are land based, including tire debris from vehicles and artificial turfs, and diverse polymers used in clothing, packaging and industry. Significant amounts of MPs from these sources ultimately find their way into freshwater ecosystems either during surface run-off events, or via sewage and domestic wastewater treatment plants [46, 47]. Running water habitats in particular are likely to be not only major transportation pathways for MPs from land to oceans, but also key sites for the accumulation and biological transformation of MPs. However, little is known of the transport and retention of different types of MPs in running water ecosystems.

RA-1 took a scaled-up experimental approach to quantify the initial fate of MPs, which ultimately determines the habitats and species, including both primary and secondary consumers, with the highest exposure risk. Two initial MP fate pathways in freshwater networks can be distinguished:

1. Denser MPs may be deposited rapidly, where they might be either consumed by deposit feeders or enter sediment layers.
2. Less dense MPs may pass rapidly through a local stream reach, and are more likely to be transported further downstream, potentially reaching lakes, estuaries or oceans.

In both cases, transport and sedimentation rates are potentially strongly influenced by key stream habitat elements, and in particular the presence of aquatic plants (macrophytes). Two experiments were conducted within RA-1, focussing on how variation in stream habitat structures, including in the density of aquatic plants, alter the initial fate of contrasting types of MPs on entering stream environments.

METHODS EXPERIMENT 1

A setup of twelve metal flume channels (Figure 3), each 350 cm long by 30 cm wide, was established indoors at a research station adjacent to Eußerbach creek, Germany (49°15'15.1"N 7°57'42.2"E). The flumes were prepared with an evenly distributed 3 cm layer of sediment from Eußerbach creek, and water was diverted from the Eußerbach to flow continuously through the flumes at a mean velocity of 0,106–0,114 m/s. Six (i.e. half) of the flumes were additionally planted with a bed of macrophytes, comprised of *Elodea nutalli* (Planch.) collected from a nearby pond (see Figure 4). The macrophytes were always planted in the middle 300 cm of the flumes (i.e. there was always a plant-free stretch at the start and end of the channels). Within this middle section, *E. nutalli* was planted at one of two density treatments: high (100 % –), low (25 % – benthos cover). The channels were left for 7 days, to allow the plants to establish.

Figure 3. Artificial stream channels used in particle fate experiments 1 (indoor metal flumes)

A 50:50 mixture of two of the most commonly found MP polymers (Goßmann et al., 2021) was prepared for release into the channels. The first polymer consisted of finely ground fragments (ø = 66 ± 29 µm) of PS obtained ground plastics produced by from *Arrowplast recycling GMBH* (Landau, Germany). The second consisted of PET fragments from ground plastic bottles produced by MultiPET GmbH (Bamberg Germany). The two polymer types also contrast strongly in density - polystyrene (PS, density ρ = 1.05 g/cm³) and polyethylene terephthalate (PET, ρ = 1.38 g/cm³). A total of 200 000 particles of this mixture was released into 9 of the 12 channels (100 000 fragments per polymer).

In total, there were four experimental treatments, each replicated in three channels:

I) Control channels without any vegetation and without any MP release,

II) Zero plant density channels with MP release

III) 25 % plant density channels with MP release

IV) 100 % plant density channels with MP release

After 25 minutes following MP release, the fate of the MPs was evaluated in three habitats: in the water (two points in the channel, upstream and downstream of the macrophyte beds, were sampled four times before, during and after peak transport, based on a conservative tracer), the sediment (three points: upstream, at the midpoint and downstream from the macrophyte beds) and the macrophytes themselves (three points (at the start, midpoint and end of the macrophyte bed, moving up to downstream).

MPs were quantified after filtration of the respective samples through GF6 glass fibre filters (pore size 1–3 µm) using a vacuum pump. Prior to filtration, MP particles were separated from the samples using different protocols depending on the habitat sampled:

1. **Water samples** contained only water and MP particles and therefore did not require any preparation prior to filtration.
2. **Sediment samples** were first mixed with 25 ml of a saturated sodiumbromide solution. The mixture was shaken for 30 seconds and vortexed for further 30 seconds. The more dense sediment particles drop to the bottom of the beaker, while the MP particles float to the surface. After two hours, supernatant is poured off for filtration,
3. **Plant samples** were washed through a funnel using distilled water. The water containing the MP particles was then then filtered using the vaccuum pump.

MP particles were then counted on the filter papers (PET particles were coloured blue and PS yellow, allowing them to be easily distinguished against a white background). These data were then used to evaluate how the different habitat configurations affected the transport and retention of the two MP types).

KEY RESULTS

- Recovery rates were high for both polymers, ranging between 86.67–100 % for PS and 73.33–93.14 % for PET.

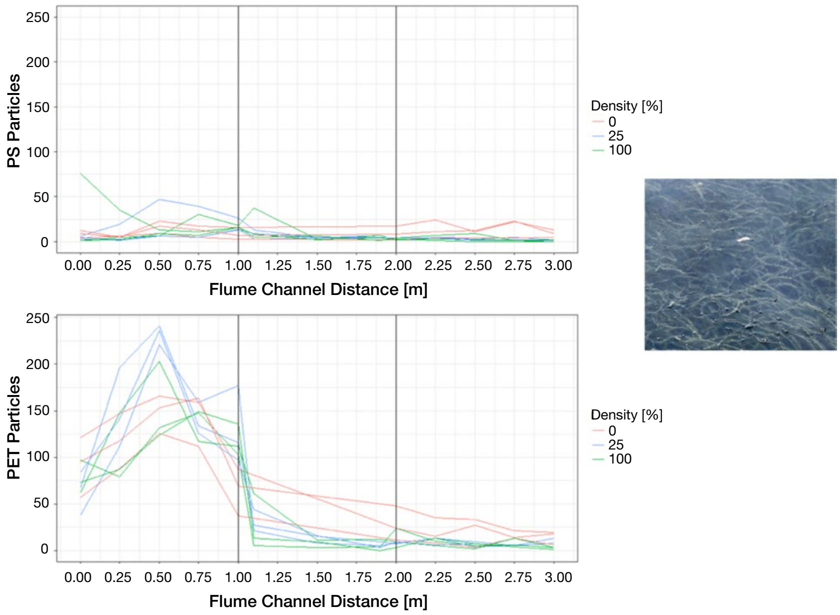

Figure 4. PET and PP particle sedimentation over the length of the experimental flumes. Each line plots data for one flume. The plant density treatments are overlaid with different colours: no plants (red), 25 % cover (blue), 100 % cover (green). The marked area between 1–2 m shows the area allocated to the macrophyte bed (when present). Inset: Elodea nutalli, image: Tobias Schmidt.

- Polystyrene particles were transported further overall in the water column, in line with the similar densities of PS (1.05 g/cm^3) and water (0.999 g/cm^3 at 8°C). In contrast, the higher density PET particles (1.38 g/cm^3) caused them to sink and sediment much faster. Consequently, quantities of PET in the sediment samples were on average more than 6.5 times higher (59.21 particles per sample) than PS (8.95 particles per sample).
- The presence of plants effectively removed MPs of both polymers from the water column (ANOVA both p=0.001), with this effect increasing with increasing plant density. The high-density plant cover treatment increased MP retention by 94 % for PET, and 71 % for PS. This highlights the potential of aquatic plants to act as natural, renewable MP filters (e.g. in constructed wetlands), but also the potential of macrophytes to be hotspots for accumulation of MPs in nature.

5.2.2 RA-2 Biofilms on microplastics: composition and effect on sinking rates

BACKGROUND

When plastic debris enters the aquatic environment, it is colonised by microorganisms within seconds[48]. These biofilm-formations called “plastispheres”[49] are likely to affect the hydrodynamics and consequently the fate of MP particles. However, the species composition of the biofilms is also important – predominantly algal biofilms may differ in sinking rates from predominantly bacterial biofilms, and some types of microbial organisms may be more likely to contribute to subsequent biofouling. Biofouling occurs when excessive growth of any organism (most often microbial, though excessive growth of e.g. macroalgae or small mussels can also cause biofouling) leads to negative effects on the environment (reduced water quality, increased evasion of smelly gasses) and/or infrastructure (blocked pipes, weighed-down boat hulls, etc.)[50]. In freshwaters, the organism group contributing most notably to biofouling are cyanobacteria (which are e.g. the organisms most often implicated in eutrophication)[51, 52].

Differences in biofilm development are also likely to affect the adsorption of additional chemical stressors to the plastisphere. Presently however, relationships between MP type (polymer and shape) and biofilm formation are poorly understood, hindering prediction of what types of MPs are most likely to be affected by biofilm growth, including potentially biofouling biofilms.

RA-2 evaluated how biofilm formation, including taxonomic composition, differed among different MP polymer types, and subsequent consequences for particle sinking rates.

METHODS

RA-2 evaluated MPs derived from a wide variety of polymers. These comprised fragments of un-plasticized polyvinyl chloride (uPVC, ø = 158 ± 35 µg, ρ = 1.4 g/cm^3), recycled polyethylene terephthalate (PET, ø= 119 ± 74, ρ = 1.37 g/cm^3), low-density polyethylene (LDPE, ø = 96 ± 59 µm, ρ = 0.92 g/cm^3) and polystyrene (PS, ø= 66 ± 29, ρ = 1.05 g/cm^3), along with tire wear particles (TWP, ø = 1.155, ρ = 1.15 g/cm^3) obtained from cleaned, ground, used car tires. LDPE and uPVC were obtained from GoodFellow GmBH (Friedberg Germany), TWP from Kurz Karkassenhandel GmBH (Landau Germany), PS from *Arrowplast recycling GMBH* (Landau, Germany) and PET from MultiPET GmbH (Bamberg Germany).

The final particle type used in RA-2 consisted of finely saw-dust particles derived from conifer wood (ρ < 0.5 g/cm^3, ø = 108 ± 53 µm), which served as a surrogate for natural fine particulate organic matter (FPOM).

The five MP types, along with the FPOM controls, were incubated with the two microbial cultures in two experimental runs (1 culture per run) in batch reactors (n = 5 per particle type and run). The culture used during the first run was obtained from a forested eutrophic pond within a nature reserve near Landau (Pfalz) (49°14´18.6"N, 7°59´24.3"E), and the second from an oligotrophic research pond within the Eußerthal Ecosystem Research Station (49°15´15.6"N, 7°57´41.8"E) located on a forest clearing.

Each batch reactor contained c. 500 000 particles (determined based on particle weights) of the respective polymer, 1.5 L nutrition medium (Kuhl and Lorenzen, 1964) and 300 ml of the respective inoculum (pond water samples). The reactors were maintained in a temperature-controlled room at 20°C. Biofilm was allowed to grow and attach to MPs over a cultivation period of four weeks, with continuous stirring 300 rpm to ensure a homogenously particle distribution, and aeration. Three control reactors per run were prepared with inoculum only.

The taxonomic composition of microorganisms colonizing the MPs was characterised based on next generation sequencing of DNA extracted from the biofilms.

To determine the velocities of the aged and non-aged polymers, we conducted a series of nearly 3 000 sinking and buoyancy experiments, on days 14, 18, 21, 24 and 28 of the incubations. A plexiglass column with a diameter of 20 mm and a height of 100 cm was filled with Kuhl-Medium and placed on a platform within a darkroom (Fig 5). For each sinking rate trial, a 10 ml sample was taken from the batch reactors and then added to the setup. Two cross-line lasers (Mini Cross Line

Laser L12R, Suaoki, Japan) were mounted on a stand right beside the cylinder, one 68 cm higher than the other (Figure 5). Time measurement in each trial commenced when the first particle crossed the upper laser and was immediately stopped when the first particle crossing the second laser was detected. Sinking velocity was calculated as the ratio of the sinking distance and the time between the two scattering-events.

Figure 5. Apparatus used in particle buoyancy and sinking rate assessments.

KEY RESULTS

a) Taxonomic composition of the biofilms (analysed separately for eukaryotes and prokaryotes):
   - Eukaryotes were dominated by green algae (chlorophytes), with fungi only significant on FPOM, and, on LDPE cultured with the oligotrophic pond assemblage (Figure 6).
   - Prokaryotes were dominated by proteobacteria, but cyanobacteria, a key agent of buifouling and eutrophication, was significant on some MP types (especially LDPE and PS), especially when incubated with the eutrophic pond inoculum (Figure 6).
   - FPOM also supported significant cyanobacteria growth, but tended to have a more even distribution of prokaryotic taxa than the MPs.
   - Interestingly, tire debris never supported significant cyanobacterial growth (Figure 6).
   - Excessive green algal (Chlorophyta) growth can also contribute to biofouling. All MPs supported high abundances of Chlorophyta, with only LDPE appearing to be a less suitable substrate.

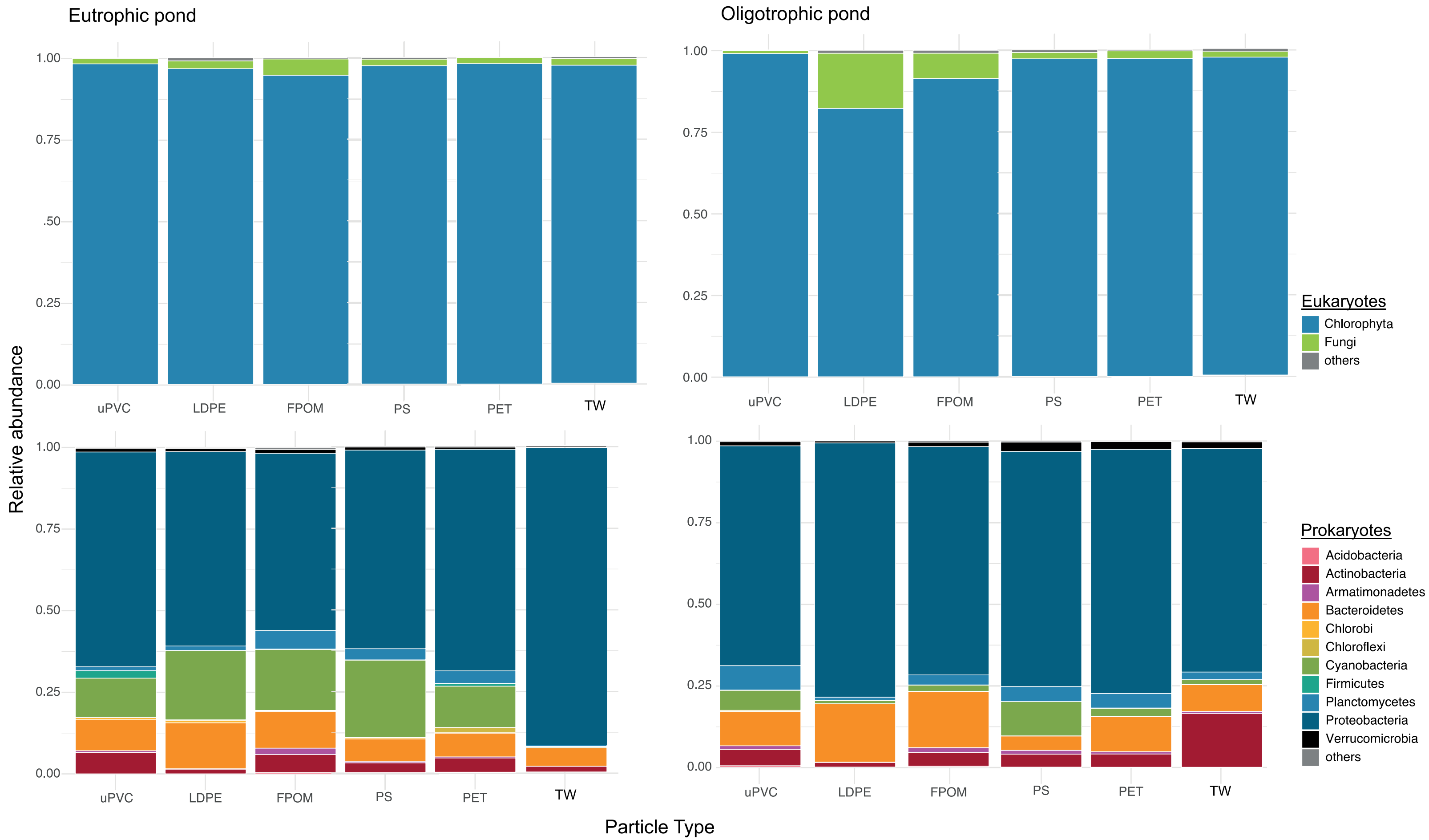

Figure 6. Taxonomic composition of eukaryotic (upper panels) and prokaryotic microbes colonizing different particle types incubated with inoculum from a eutrophic and oligotrophic pond.

b) Biofilm effects on particle sinking:

Biofilm growth significantly influenced the fate of all particles used during the experiment by altering their hydrodynamics and physical properties. The overall patterns were:

- Particles that sank rapidly as unaged controls became more buoyant as biofilms developed. This is likely to reflect the production of gas bubbles by Chlorophyta (the dominant algal type in particle biofilms) and other structures such as cyanobacterial gas vacuoles. Gas bubbles and gas vacuoles contribute to the buoyancy of green algae and cyanobacteria respectively, and can constitute a substantial portion of the biovolume of living algal biofilms[53, 54].
- Particles that were very buoyant as unaged controls conversely sank more rapidly as biofilms developed, presumably as the added biomass increased density of the whole particle-biofilm conglomeration towards that of water.

In detail:

- Control (unaged) FPOM (Figure 7) and LDPE sank relatively slowly, in line with their low densities, as did the denser uPVC (Figure 7). In the case of uPVC, these were small-sized particles with *extensive invaginations*, which easily filled with air-bubbles increasing their buoyancy.
- Sinking rates for FPOM and uPVC increased rapidly with biofilm development (Figure 7), with the exception of uPVC incubated with biofilm from the oligotrophic pond (Figure 7B). LDPE sinking rates were increased only slightly as biofilms developed.
- Control (unaged) TWP and PET (Figure 7) along with PS sank relatively rapidly. Biofilm formation for all three polymers decreased sinking rates markedly (Figure 7).
- Effects of biofilm formation on sinking velocity were similar between the eutrophic and oligotrophic pond inoculum. A notable exception was uPVC, whose sinking rate was increased when incubated with the eutrophic but not oligotrophic pond (Figure 7). We hypothesise that this indicates that the eutrophic but not oligotrophic biofilm effectively blocked off the invaginations characterising the surface of uPVC.

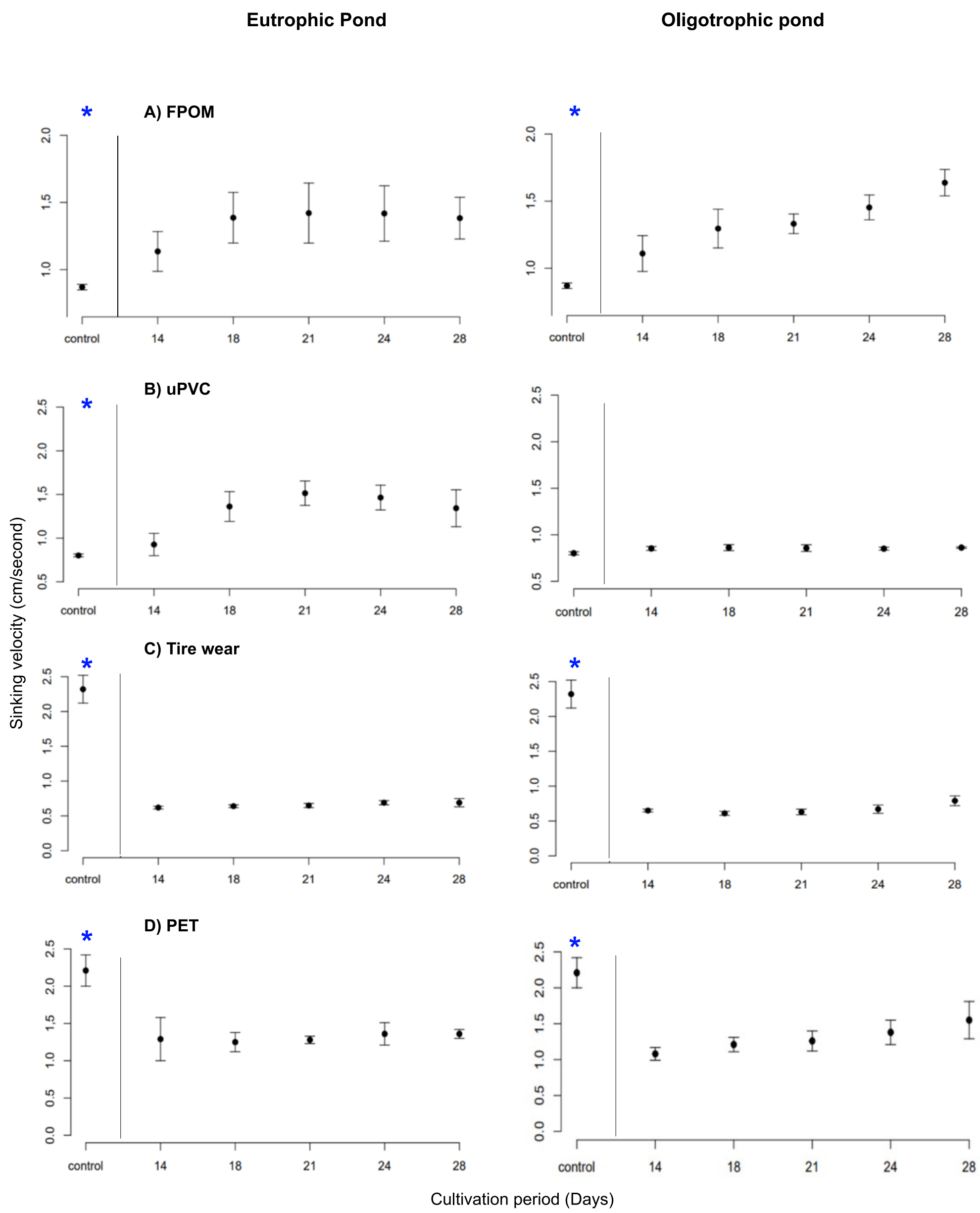

Figure 7. Effects of aging period on sinking velocities of natural FPOM (A) and three plastic polymers (C–D) incubated with biofilm organisms from either a eutrophic (left panel) or oligotrophic (right panel) pond. Data point to the left of the blue lines corresponds to unaged particles, lacking any biofilm growth. *p<0.05 (control compared with remaining treatments).

5.2.3 RA-3 Evaluating the potential of microplastics and natural organic matter for sorption of hydrophobic organic contaminants based on selected properties

BACKGROUND

A potential threat posed by pervasive plastic pollution worldwide relates to their capacity to bind and transport hydrophobic organic compounds (HOCs) in river networks[55]. HOCs are a group of harmful chemicals including pesticides, polycyclic aromatic hydrocarbons etc. that do not mix well with water and will seek other hydrophobic (water-repelling) solid surfaces. Plastics however are not the only particles within aquatic systems capable of carrying or "sorbing" these pollutants[56]. Most comparable are various forms of natural particulate organic matter (POM) in the 0.45µm–1mm size range such as suspended and bottom sediments, soil, bacteria etc. Natural POM is orders of magnitude more abundant than MPs in most freshwater ecosystems [14, 33]. Hence, it is not enough to evaluate the risk that MPs might pose in increasing transport of HOCs by evaluating their capacity to sorb these chemicals. Rather, it is necessary to evaluate the sorption capacity of HOCs relative to that of much more abundant natural organic matter (NOM).

In RA-3, we conducted a systematic literature review to evaluate the physical properties shared by MPs and natural POM that favour the sorption of HOCs, as well as where they differ (published as an MSc thesis[57]). These include size, surface area, porosity and age. Surface area and porosity reflect the availability of sorption sites with which HOCs can interact, so larger values for these properties imply a higher potential for sorption. Size and age play a role in influencing the above two properties, altering the ratio between surface area and volume and state of the particle surface after exposure to elements such as UV radiation and physical abrasion. The review further aimed to compare the biological impacts posed by the two particle types in their capacity to act as vectors for HOCs, and thus also considered factors such as chemical composition, external environmental factors, and particle abundance.

METHODS

The search engines Web of Science, Scopus and Google Scholar were accessed between May and June of 2020. One set of searches focussed on MPs, and a second on NOM. For both sets, the main terms "microplastic*" or "natural organic matter + particle" where combined with "hydrophob*" and "chemical". These terms were further combined with "size", "surface area", "residence time", "equilibrium", "bioaccumulation", "fate", "toxicity" and "freshwater". The final article set was then analysed, and data extracted on MP/NOM particle characteristics including size, shape, surface area, porosity, age and chemistry. Information on combined particle and HOC toxicity to biota were also extracted. The range and median for these properties were calculated. The extracted quantitative data was then summarised and evaluated to arrive at a conclusion about the relative risk of MPs compared with NOM for sorbing and transporting HOCs.

KEY RESULTS

- There are substantially more articles reporting particle properties and sorption potential for MPs than POM (Figure 8).
- Overall, natural particles tended towards larger median surface areas and porosities than MPs. This is likely because they possess very uneven surface textures as a result of their diverse chemical composition and the uneven degradation of less resistant materials over time, leaving an irregular shape.
- As MPs are made up of repeating subunits, they possess more smooth surfaces but are also much more resistant to being eroded over time.
- Based on physical characteristics alone then, it would be assumed that POM materials are better HOC carriers or “vectors”.
- When chemistry is also considered however, MPs are much more favourable because they are more hydrophobic compared with natural particles that both attract and repel water.
- In laboratory experiments, HOC exposure in combination with natural POM sources derived from bottom sediment and soil had predominantly negative effects on biota (Figure 9). In contrast, MP studies having negative compared with neutral or positive effects on biota were more evenly balanced (Figure 9).
- Negative effects of MP largely occurred when organisms consumed particles with sorbed HOCs. Positive effects occurred when organisms avoided consumption of MPs.
- MP concentrations in the environment are essentially negligible when compared to the plethora of POM materials, so although they are better pollutant carriers their role in bulk HOC transport is likely to be negligible relative to NOM.

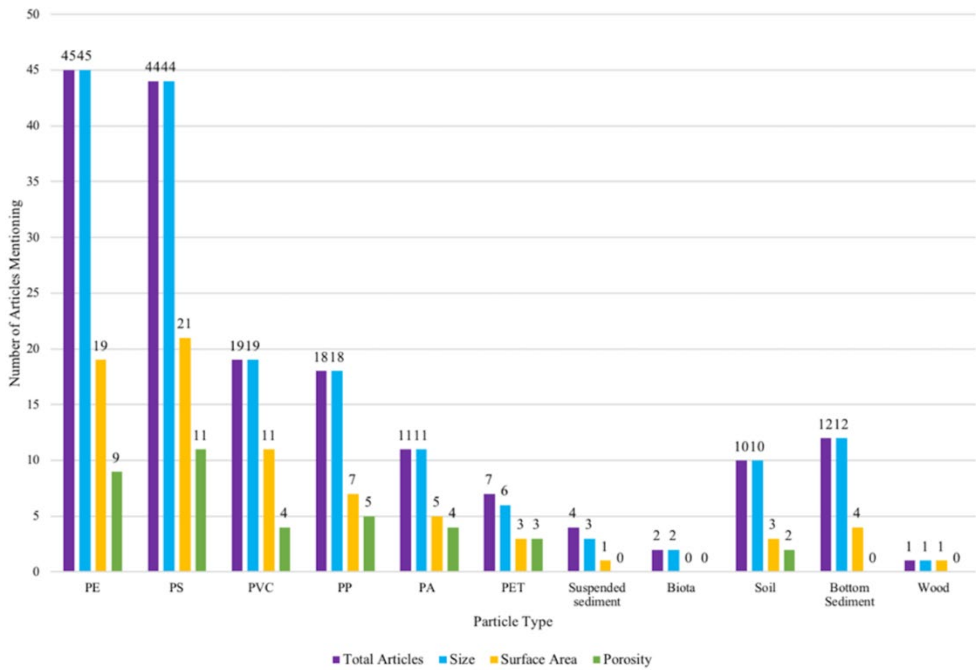

Figure 8. Articles publishing data on three key physical characteristics of microplastic (MP) derived from different polymers: polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), polypropylene (PP), polyamide (PA) and polyethylene terephthalate (PET) as well as particulate organic matter (POM) derived from different naturally occurring materials (suspended sediment, biota, soil, bottom sediment, wood).

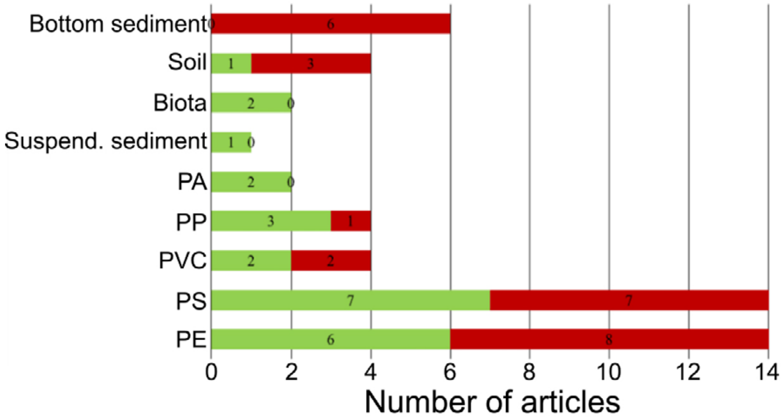

Figure 9. Summary of laboratory experiments investing the effects of hydrophobic organic contaminants (HOCs) in combination with either natural POM derived from different sources (bottom sediment, soil, biota, suspended sediment) or microplastics: Polyamide (PA), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polyethylene (PE) on the mortality or health of biota. The number of studies documenting a negative impact are plotted in red, and those documenting either no effect or a positive effect are shown in green.

5.3 Ecological impacts

5.3.1 RA-4 Microplastics in detrital biofilms: consequences for detrital breakdown

BACKGROUND

Biofilms are often well developed on naturally occurring CPOM, such as that of leaf litter or dead wood. Such surfaces are often notable for the high abundance of hyphomycete fungi, which rapidly extend their thread-like hyphae across and within detrital surfaces, potentially binding additional particles. Contamination of detrital biofilms with MP particles then has potential to affect microbial activity, e.g. by acting as a barrier for hyphae spread, and rates of detritus breakdown.

RA-3 aimed to evaluate how binding of different MP polymers in leaf litter biofilms, and to investigate consequences for microbially-mediated leaf litter decomposition.

METHODS

Leaf disks cut from autumn shed *Alnus glutinosa* L. (Gaertn.) litter were incubated with microbial communities from Rhodenbach creek for 14 days. The discs were then exposed in replicate laboratory microcosms to one of three particle treatments (natural FPOM, PS and PMMA, at six different concentrations (0, 0.18, 1.8, 18, 180, 1 800 particles/mL).

KEY RESULTS:

- Increasing MP concentrations caused an overall reduction in microbial leaf decomposition rates, but with a stronger reduction observed for natural FPOM than the two MP polymers (Figure 10).
- All three particle types showed a strong tendency to be bound in microbial biofilms growing on the leaf litter, and it is likely that slowed decomposition in all three cases reflects physical disruption of microbial growth by the more refractory FPOM/MP particles.

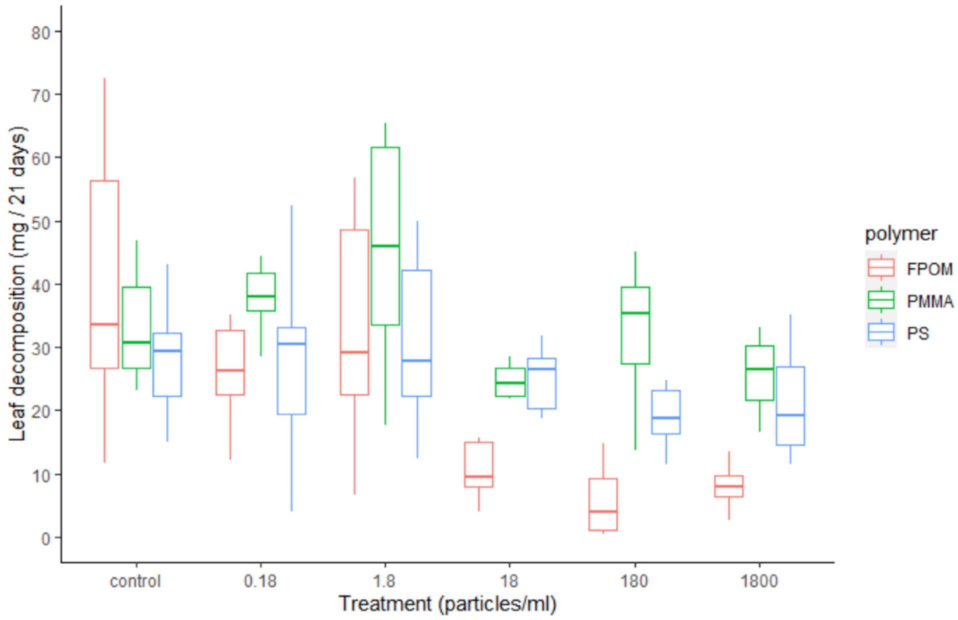

Figure 10. Effect of particle exposure concentration on microbial mediated decomposition of leaf detritus for FPOM, Poly (methyl methacrylate) (PMMA) and polystyrene (PS) particles. Concentration x polymer $p < 0.01$.

5.3.2 RA-5: Comparing effects of microplastic exposure, FPOM resource quality, and consumer density on the response of a freshwater particle feeder and associated ecosystem processes

BACKGROUND AND RESEARCH QUESTIONS

Microplastic contamination of natural FPOM pools have potential to cause "food dilution effects"[58], resulting in reduced growth and survivorship of the particle feeding organisms that normally consume FPOM. Food dilution effects occur when a more labile food resource is mixed with a highly refractory substance that reduces nutrient concentration in the resource pool and increases food handling time[59]. MP exposure can also affect metabolism and other physiological functions[60], which over time might accumulate to impact consumer growth and survival, and ecosystem functioning[61]. On the other hand, MP concentrations are typically much lower than those of naturally occurring FPOM (cf. >1 000 × greater daily transport of FPOM than MPs in Swedish rivers[14, 33]) which might limit the potential for MP exposure to drive food dilution effects.

Negative impacts of MP exposure arising from food dilution or physiological effects are likely to be stronger in consumer populations already experiencing limitations in resource quantity and/or quality (which collectively regulate resource availability to consumers)[62, 63]. The nutrient quality of FPOM is notably low in comparison with other aquatic resources (e.g. algae)[24, 64], but it is normally enhanced through the growth of surficial biofilms on particles [65, 66], which in turn is strongly regulated by substrate characteristics (e.g. refractory C content, C to nutrient ratios). Accordingly, MP exposure that disrupts microbial activities and further dilutes the quality of an already low quality FPOM resource [67, 68] might have particularly strong potential to impact particle feeders. Consumer density is a further key factor regulating resource availability. As consumer density increases, resource limitation is intensified as a consequence of competition, which may lead to negative density dependent effects on individual behaviour, growth and survival[40, 69, 70]. However, the potential for additional environmental drivers such as consumer density and resource quality to regulate the impacts of MP exposure on consumers and associated ecosystem processes remains little assessed, especially in freshwater habitats. This represents a knowledge gap that limits our capacity for assessing the potential impacts of MPs in freshwater ecosystems, relative to those of other key environmental drivers.

METHODS

We conducted a microcosm experiment to evaluate how a realistic (1 400 particles/kg sediment) MP concentration interacts with FPOM resource quality (low vs high nutrient content) and consumer density (10 vs. 20 individuals per microcosm) to affect growth and survival of larval *Chironomus riparius* (Diptera: Chironomidae), a model particle feeder (Figure 13a), and microbial respiration. The MPs consisted of Polyethylene microspheres (Cospheric, 45–53 µm). Each treatment was replicated five times leading to 60 microcosms across five randomised blocks. The low quality FPOM consisted of natural organic particles sourced from a forest stream, while the high-quality resource was heterogeneous lab mixture, comprising ground leaf litter mixed with ground fish food flakes. Chironomids used in the experiment were hatched from eggs, obtained from a well-established laboratory culture. In addition to FPOM, all microcosms contained 80 g of sterile river sand and 150 mL of filtered stream water (see Figure 13 for a similar set-up).

The experiment was maintained for 14 days, after which chironomid growth and survivorship were quantified, as was community respiration (oxygen consumption by all biota in a microcosm), as a measure of community metabolism[71]. For microcosms without chironomids present, community respiration is almost entirely attributable to activity of microbes. For further methodological details, see Kong et al 35.

KEY RESULTS

- MP exposure reduced larval body lengths by 26.7 %, but only under the low consumer density treatment (Figure 11).
- MPs reduced community respiration by 26.2 %, but only in the absence of chironomids, indicating an impact on microbial respiration (Figure 12).
- Low food quality and high consumer density were associated with larger effect sizes, including 53.5–70.2 % reductions in community respiration, chironomid body length and/or body mass.
- These results suggest that effects of contamination of FPOM with MPs at environmentally realistic concentrations on the life histories of particle feeders such as *C. riparius* might be limited, especially relative to effects of resource quality and consumer density.
- However, the reduction in microbial respiration when MPs were present highlights the need for further research addressing MP impacts on microbes, given their key roles in ecosystem functioning.

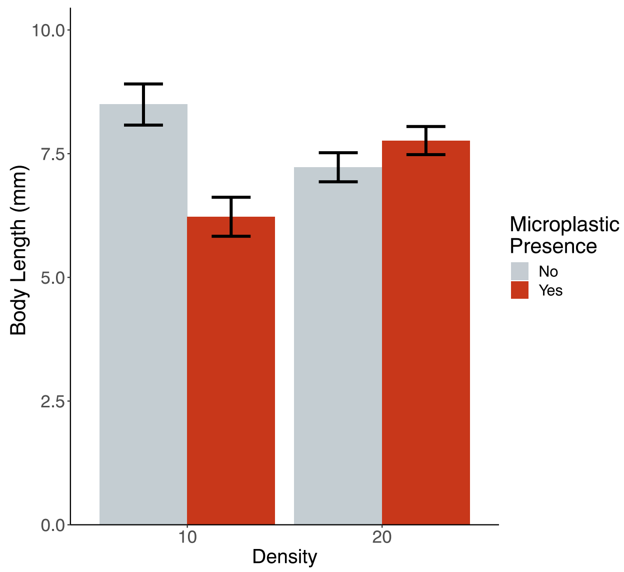

Figure 11. Effects of consumer densities and MP exposure on mean (± 1 SE) chironomid body length. Density x MP interaction p<0.05. Figure after Kong et al. 35.

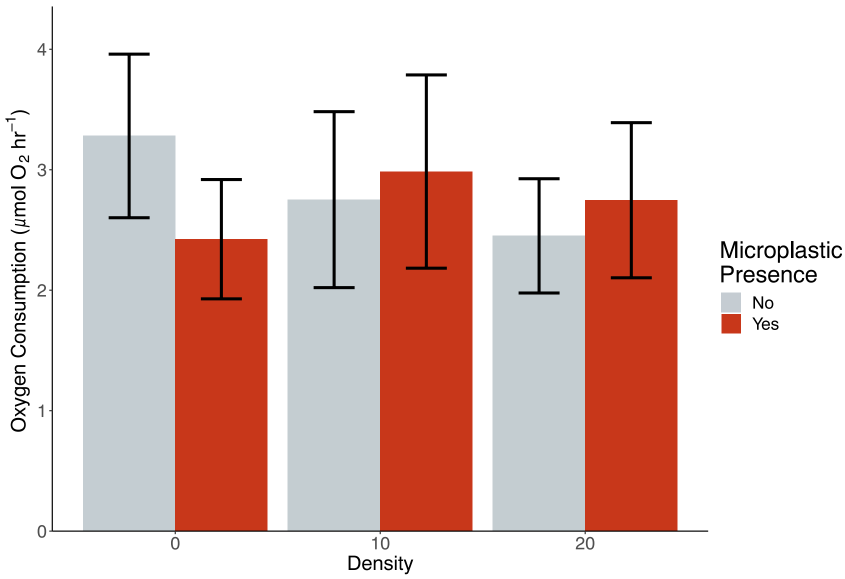

Figure 12. Effects of consumer density and MP exposure on mean (± 1 SE) community respiration. Density x MP interaction p<0.05. Figure after Kong et al. 35.

5.3.3 RA-6 Do impacts of microplastics in stream food webs depend on particle concentration, shape and/or polymer?

BACKGROUND

Microplastics vary enormously in their physical and chemical characteristics (Figures 1–2), including particle size, polymer, shape and colour[72]. These characteristics may influence the fate and effect of MP particles in the environment. For example, the density, size and shape of particles influences their settling rate in water[73]. The growth of biofilms is enhanced with increased surface roughness due to a higher surface area for bacteria to adhere to, and can alter particle densities and sinking rates[74]. Excessive biofilm growth can lead to biofouling[75]. Thus, these physical characteristics of MPs are crucial for understanding not only where different particles are likely to occur, but also their impacts.

Unfortunately, MP research to date has been strongly biased towards a narrow range of commercially available particle types, i.e. polystyrene and polyethylene "beads".[21] In contrast, most MPs recorded in the environment are fibres and fragments made of PE, PET, PA, PP and PS (Burns and Boxall 2018). A major source of MP fibres is synthetic fabric which comprises the majority of modern clothing and textiles (Manshoven et al. 2021), released as microfibres through laundry processes (Napper and Thompson 2016). Irregular MP fragments are generated from the breakdown of larger plastic items, either intentionally (Suzuki et al. 2022) or through abiotic and biotic forces in the environment (Corcoran 2022). In comparison, MP spheres are only intentionally produced for use in industry and cosmetics instead of being generated or released from larger plastic items in the environment.

In RA-6, we conducted an extensive laboratory microcosm experiment to investigate the influence of MP shape (Fragment, Fibre, Sphere), polymer (PE, PET, PP, PS) and concentration (0, 1 000, 50 000 p/kg) on the effects of MP exposure on *Chironomus riparius* larvae¸ microbial communities and enzyme activities, and associated ecosystem processes. The concentrations were chosen to represent the median and highest observed MP sediment concentrations observed worldwide (see Figure 14)

METHODS

The experiment used the same replicate glass microcosms described under RA-5 maintained in a constant temperature cabinet (Figure 14a). Each microcosm contained a layer of sterile river sand, filtered stream water, and FPOM, consisting of the high quality "lab mix" preparation described in Kong et al 35, and used in RA-6. Half the microcosms contained 10 *C. riparius* individuals. MP fragments for all polymers (PE, PET, PP, PS) were produced from pellets or spheres with a mixer ball mill (MM400, Retsch). Stainless steel grinding jars were filled with polymer pellets and stainless steel grinding balls and frozen in liquid nitrogen for 20 mins. The size range for all fragments was 25–63 µm (based on sieving). MP spheres of PS (CPMS-0.96 10–63 µm, Cospheric) and PE were purchased (PSMS-1.07 38–48 µm, Cospheric). MP fibres of PET were produced by collecting loose fibres from the effluent of washing machines loaded with microfibre blankets. MP fibres of PP were cut from surgical masks (Medical Face Mask Type IIR, Zhende medical). Approximate size dimensions for both fibre types were ø ~ 20 µm, length ~ 500 mm. Manufacturers of remaining polymers used in the experiment: LyondellBasell, Netherlands (HDPE), INEOS Styrosolution Germany (PS), Borealis Austria (PP) and Neogroup Lithuania (PET).

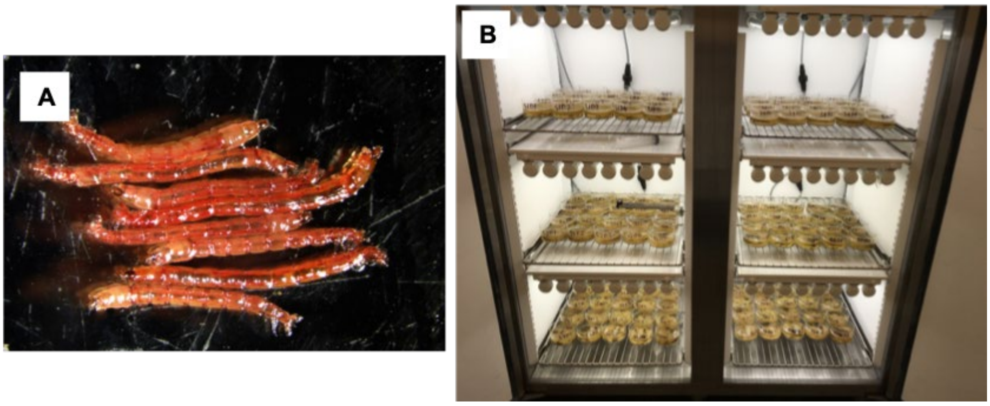

Figure 13. (a) Larval C. *riparius*, our model particle feeder, at the end of the experiment, (b) Experimental microcosms in a controlled environment cabinet. Each microcosm contained a layer of mineral sediment, a layer of FPOM, and filtered stream water. In total the experiment required 180 microcosms.

Two concentrations of MPs were chosen based on global freshwater sediment concentrations (Figure 14). The low concentration at 1 000 p/kg$_{sediment}$ is approximately within the global mean and the high concentration at 50 000 p/kg$_{sediment}$ is significantly higher than 95 % of recorded concentrations (Figure 14). All particles were left to condition for 8 days to allow microbial growth on both FPOM and MP particles, prior to addition of chironomids (to the chironomid present microcosms). Once chironomids were added, the experiment was maintained for a further two weeks.

At the end of the experiment, chironomid mortality, growth and lipid content were quantified, as was community respiration. Additionally, water samples were collected from each microcosm for quantification of microbial responses. Microbial Extracellular enzyme activities (β-glucosidase and chitinase) activities were quantified immediately using methylumbelliferone (MUF)-linked substrates according to Renes et al.[76] Further samples were collected and fixed with 37 % formaldehyde buffered with sodium tetraborate to a final concentration of 2 %. Samples were stained with SYBR Green® and incubated at room temperature for 5 mins. Samples were then filtered through a 20 µm cell strainer to remove any microplastics. Microbial density was determined through flow cytometry using a CytoFLEX flow cytometer (Beckman Coulter). Samples were analysed by loading 100 µL of sample on 96 well plates and using a flow rate of 10 µL/min. SYBR-stained negative controls of milliQ water and samples passed through a 0.22 µm syringe filter were processed to identify and exclude background particles.

A final set of water samples were collected for determination of algal biomass in the water, based on Chlorophyll-a concentrations. The samples were filtered through 0.2 µm 25 mm Whatman® membrane filters, wrapped in aluminium foil and stored in -20 °C. Chlorophyll was extracted from filters using 99.5 % ethanol for 22 hours at 4 °C in the dark. Samples were shaken once about halfway throughout the extraction. Clean filters were also extracted to serve as blanks. After 22 hours, the filters were removed, and the extracts centrifuged at 1 000 gs for 5 mins to sediment any remaining particles. 200 µL of extracted samples and a Chl-a standard series (Sigma-Aldrich, from spinach) were then pipetted into cooled black solid-bottom 96-well plates. Wells were sealed with tape between loading each row to minimise evaporation. Chl-a concentrations were measured using λex/em = 444/680nm in a microplate reader (Hidex Sense).

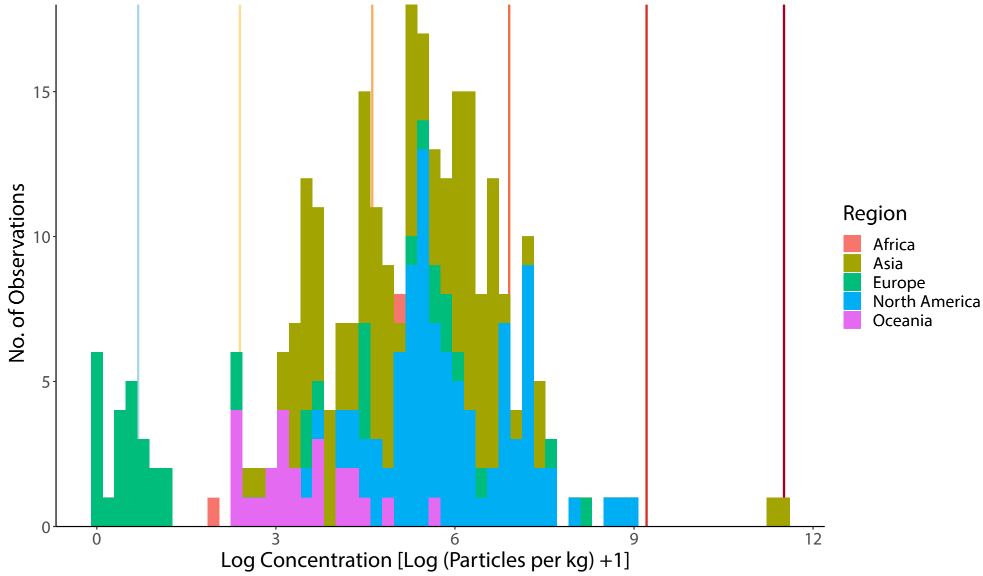

Figure 14. Global distribution of MP sediment concentration observations, based on a survey of the literature. Data comprises of single measures recorded globally. Vertical guide lines have been plotted on a geometric sequence with a ratio of 10 (1, 10 ,100, …).

KEY RESULTS

- Survivorship of our model particle feeder (*C. riparius*) was affected by an interaction between MP polymer and shape (Figure 15) which was near significance ($p = 0.051$). Larval survivorship was reduced (compared with controls) by 8.15 % and 7.23 % when reared with PE and PP fragments respectively but was increased by 9.64 % when reared with PE spheres.
- Activity of the microbial extracellular enzyme ß-Glucosidase was 10.3 % higher than controls in the presence of MP overall. ß-Glucosidase activity was most strongly elevated in the presence of MP fragments (17.5 % higher than controls). (Figure 16).
- Chironomid biomass was 13.9 % greater when reared with MP fragments, regardless of the polymer or concentration (Figure 17).
- Microbial cell density increased with increasing MP concentration but only in the presence of chironomids, by 20.5 % and 43.6 % at the low and high concentrations respectively (Figure 18).
- Chironomid lipid content was increased by 13.9 % when reared at the high MP concentration, regardless of MP polymer or shape (Figure 19).
- Chlorophyll-a concentrations were overall 230 % higher in the presence of chironomids, likely reflecting a fertilizing effect of chironomid waste products (Figure 20). PP tended to reduce chlorophyll-a concentrations overall, with the strongest reduction in high-PP concentration microcosms without chironomids (40.4 % reduction relative to controls).
- Community respiration in microcosms without chironomids was 29.5 % elevated when PE was present but reduced by 19.5 % at the high concentration of PET (Figure 21).

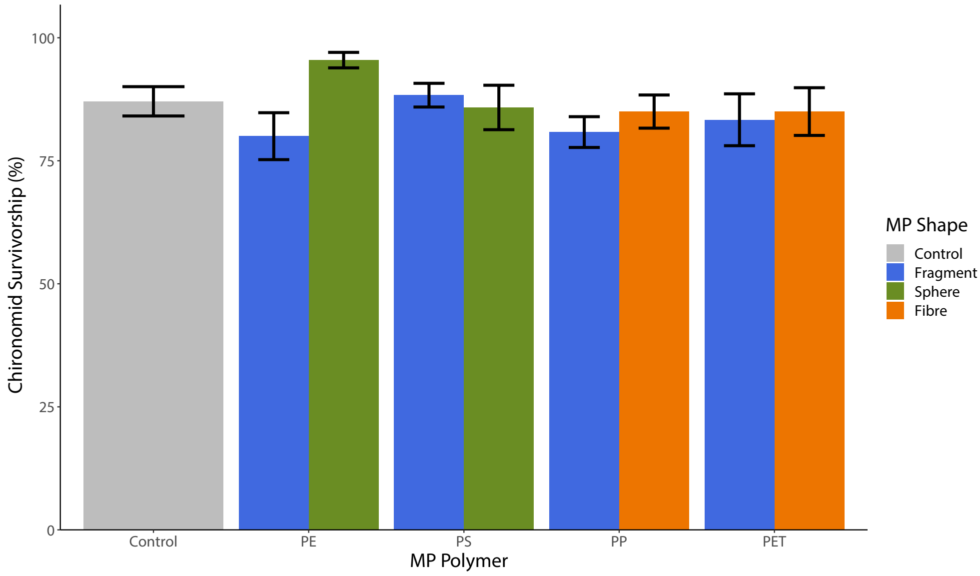

Figure 15. Effects of MP polymer and shape on % survivorship (mean ± SE) of chironomid larva. Data pools across the low and high MP concentration treatments. Polymer x Shape interaction p = 0.051.

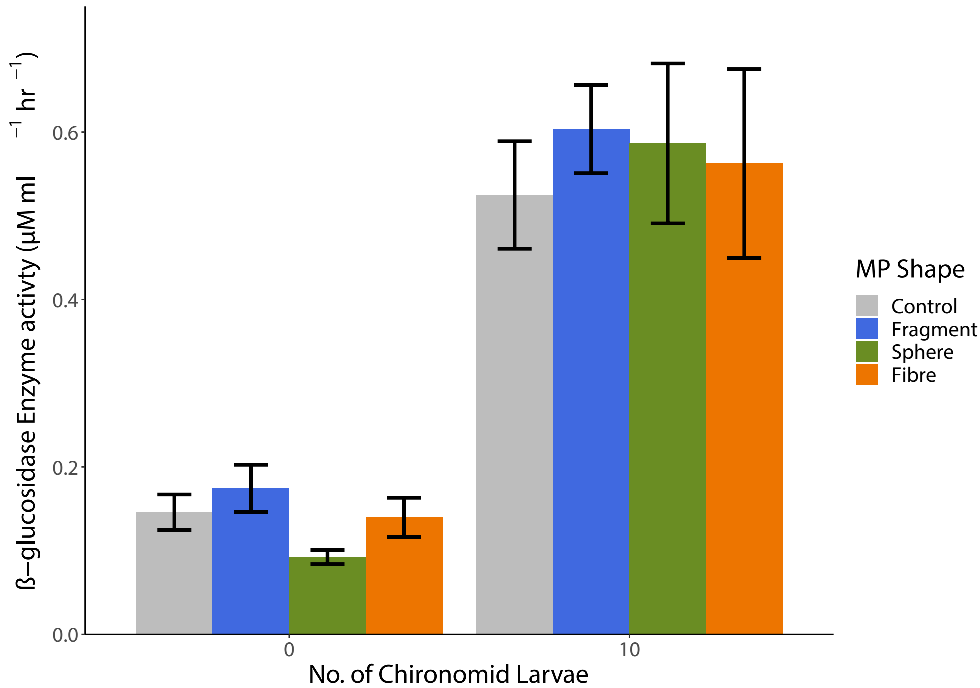

Figure 16. Effects of chironomid density and MP shape on activity of the microbial enzyme ß-Glucosidase (mean ± SE). Data pools across the low and high MP concentration treatments, and across MP polymers. Density x shape interaction $p < 0.05$.

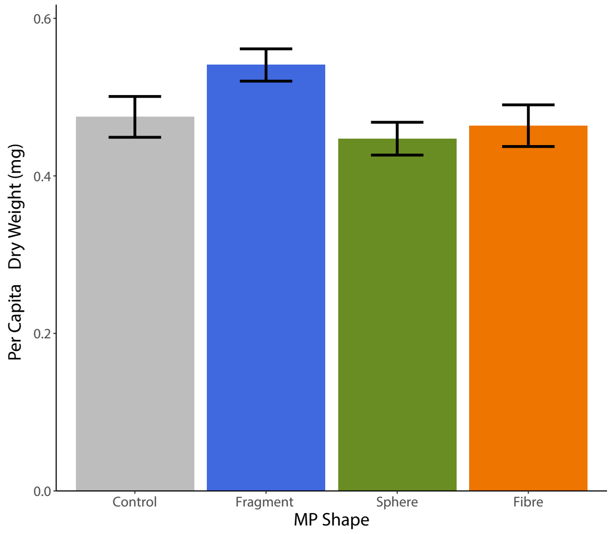

Figure 17. Effects of MP shape on biomass of chironomid larvae (mean ± SE). Data pools across the MP polymers and the low and high concentration treatments. MP Shape $p < 0.01$.

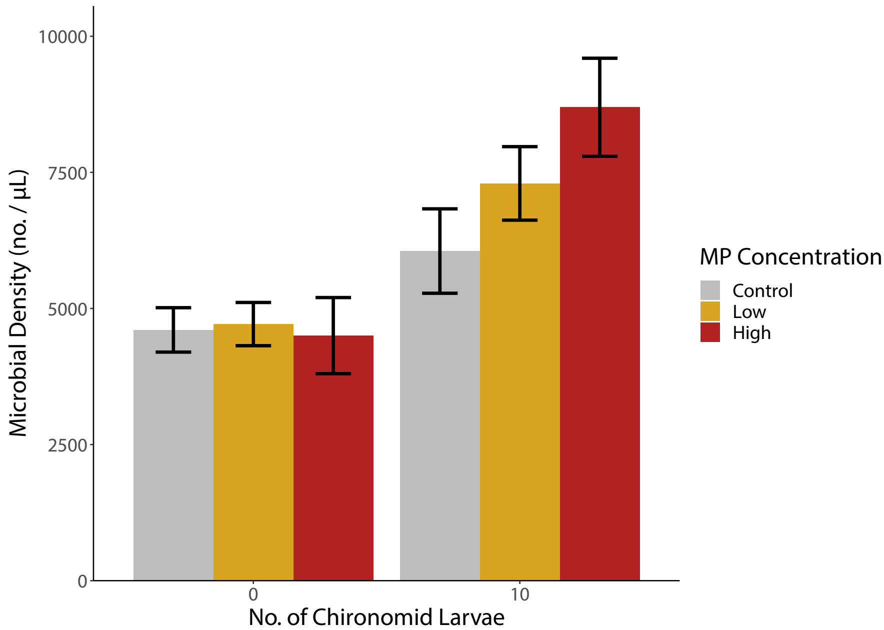

Figure 18. Effects of MP concentration and chironomid density on microbial cell density (mean ± SE). Data pools across the MP polymer and shape treatments. Microbial density x chironomid interaction $p < 0.05$.

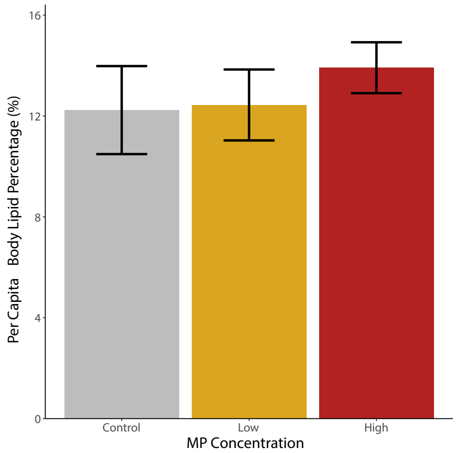

Figure 19. Effects MP concentration on % lipid content of chironomid larvae (mean ± SE). Data pools across the MP polymer and shape, and chironomid density treatments. MP concentration $p < 0.01$.

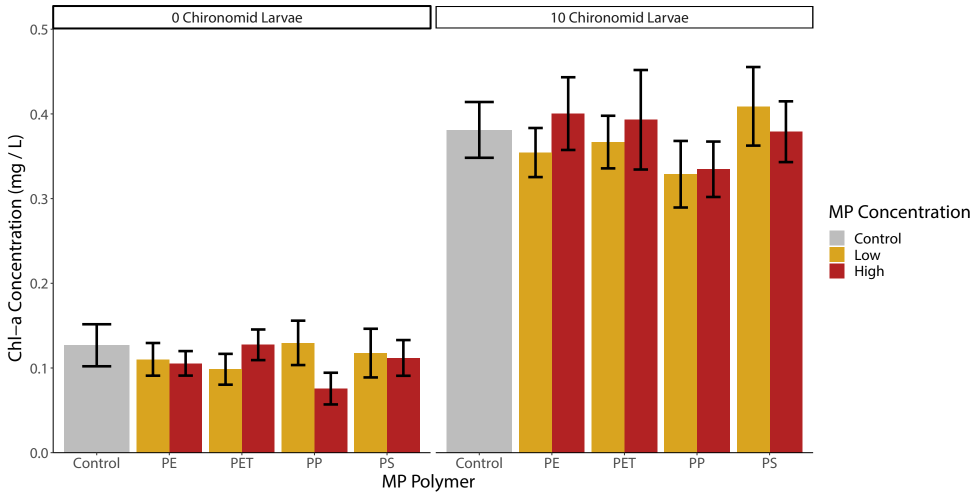

Figure 20. Effects of MP polymer, concentration and chironomid density (separate panels) on concentration of chlorophyll-a (mean ± SE). Data pools across the MP shape treatments. MP presence x MP concentration x Chironmid presence interaction $p < 0.05$.

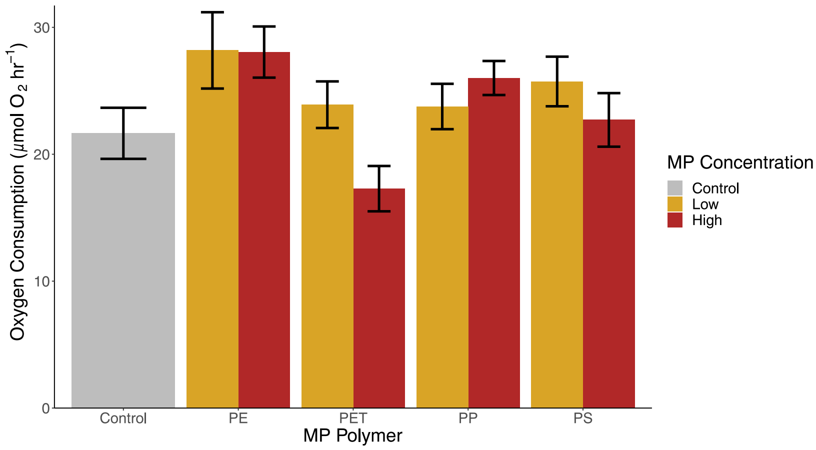

Figure 21. Effects of MP polymer and on microbially-mediated community respiration (mean ± SE). Data presented for zero-density chironomid microcosms only. Polymer x concentration interaction $p < 0.01$.

Results from RA-6 comprised a mixture of outcomes attributable solely to the presence of MPs, and others that were contingent on polymer type and shape. Among polymers, PP and PE had the largest number of contingent effects on sur vival, respiration and algal production. Among shapes, fragments were associated with the largest number of effects , including on e.g. survival and ß-glucosidase activity.

In contrast, the presence of increasing concentrations of MPs *per se* caused increases in bacterial cell density, and this in turn was linked to increased lipid storage in the chironomid consumers. This suggests that the presence of MPs stimulated an increase in bacterial cell productivity, which were then available to consumers leading to a general increase in energy storage.

Several of the MP effects detected in RA 6 can be linked to effects of MPs on microbial activity or abundance, indicative of effects on the formation and activity of microbial biofilms. In some cases, these effects were specific to particular polymer types, whilst in other cases the effects were linked to the quantity of MP particles *per se*.

Some of these findings point towards the potential for MP impacts on microbial organisms to have knock-on effects on consumers. For example, the presence of MP fragments increased ß-glucosidase activity and was also associated with an increase in chironomid biomass. There are two possible interpretations of this result. Firstly, it is possible that the increase in ß-glucosidase activity is simply an indication of increased microbial biofilm availability *per se*, and that this biofilm was consumed by the chironomid larvae preferentially, increasing their growth. The second is that increased ß-glucosidase activity indicates an increased breakdown of more complex C molecules into simpler forms, increasing bioavailability of energy and nutrients to the chironomids.

Similarly, the presence of high concentrations of MP particles was associated with an increase in chironomid lipid content. In both cases, these results might reflect a positive effect of MP presence on biofilm formation.

5.3.4 RA-7 Plastic pollution from surgical face masks: Effects of size and leaching on leaf decomposition and associated functions in a field experiment

BACKGROUND AND RESEARCH QUESTIONS

With the COVID-19 pandemic, surgical face masks became a pervasive element in everyday life. A lack of capacity and awareness for proper disposal made it inevitable that single-use face masks would enter natural ecosystems and thus intensify global plastic pollution (Figure 22). Disposable protective equipment is characterised by its very short lifespan, and primary functioning is compromised after just a few hours[77]. As such, the plastic material in face masks, composed of non-woven poly-propylene, remains mostly unaltered when discarded, with most leaching and reduction to MPs therefore occurring primarily in the environment.

The study of MP impacts in the field is logistically challenging, due to difficulties associated with controlling MP quantities *in situ* at environmentally relevant concentrations. In RA-8 (published as an MSc thesis[78]), we took a novel approach to investigate impacts of facemask-derived polypropylene pollution in an urban pond. We quantified impacts of PP pollution on decomposition of *Alnus glutinosa* leaf litter, enclosed within cotton mesh bags, and associated microbial and fungal parameters

(Figure 23–24). The focus of our study was on decomposition driven by microbes, since the mesh size of the cotton bags was too small for access by macroinvertebrate leaf eating detritivores. We specifically compared impacts of macro- vs microplastic sized PP fragments, and of leached vs unleached PP material. Throughout, we further compared impacts of PP pollution with those of a standard reference material (saw-dust).

METHODS

The field experiment followed a modified leaf litter bag protocol[79] in an urban garden pond in Uppsala, and focussed on decomposition processes mediated by microbes. The litterbags with a mesh size of approximately 0.5mm in diameter (Figure 23A) were constructed from cotton and filled with 3.5g alder (*A. glutinosa*) leaves. Further, three different material treatments with different types of added material (Figure 24) were applied to the litterbags: (1) plastic material was added, (2) saw dust as reference material was added and (3) no material was added (control group). Within the plastic treatment two different plastic particle size treatments (small/microplastic, size 3x3mm, and big/ microplastic, 60 × 60mm) and two leaching treatments (unleached, or pre-leached for 48 hours in milli-Q water) were included. The experiment period lasted seven weeks and included five timepoints at which subsamples of litterbags were retrieved.

Figure 22. Facemask discarded in a pond, the blue polypropylene layer uppermost. Photograph: Martina Stangl.

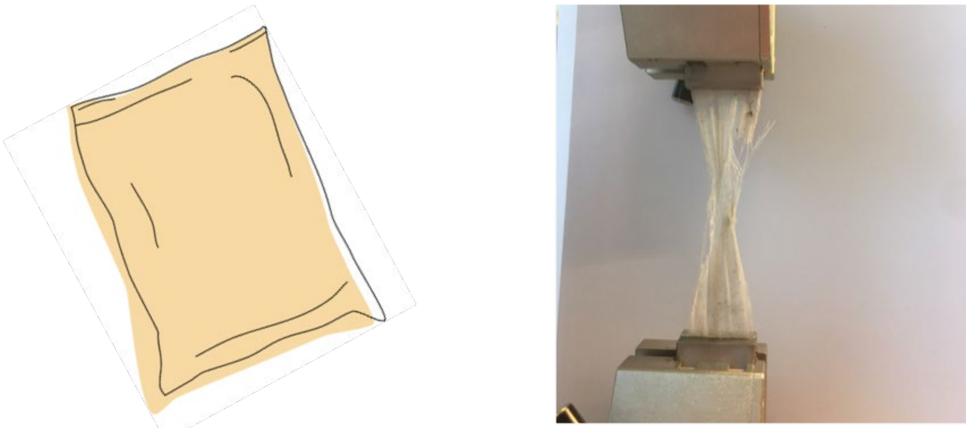

Figure 23. (A) Sketch of cotton mesh bags, and (B) image of tensile strength measurements of a cotton bag taken after the field study. Measurements undertaken with a tensiometer. Images by Martina Stangl.

Three main response variables were quantified: (1) ecosystem respiration as an indicator for the metabolic activity of the organism community, (2) leaf mass loss and (3) tensile strength loss of the cotton bags (Figure 23B). The latter two variables functioned as indicator for the decomposition potential of organic material in the studied ecosystem for substrates differing in lability – the cotton bags consist largely of labile cellulose, whilst the alder leaf litter, whilst rich in N, also contains more refractory C and secondary compounds (e.g. lignin, tannins, phenols).

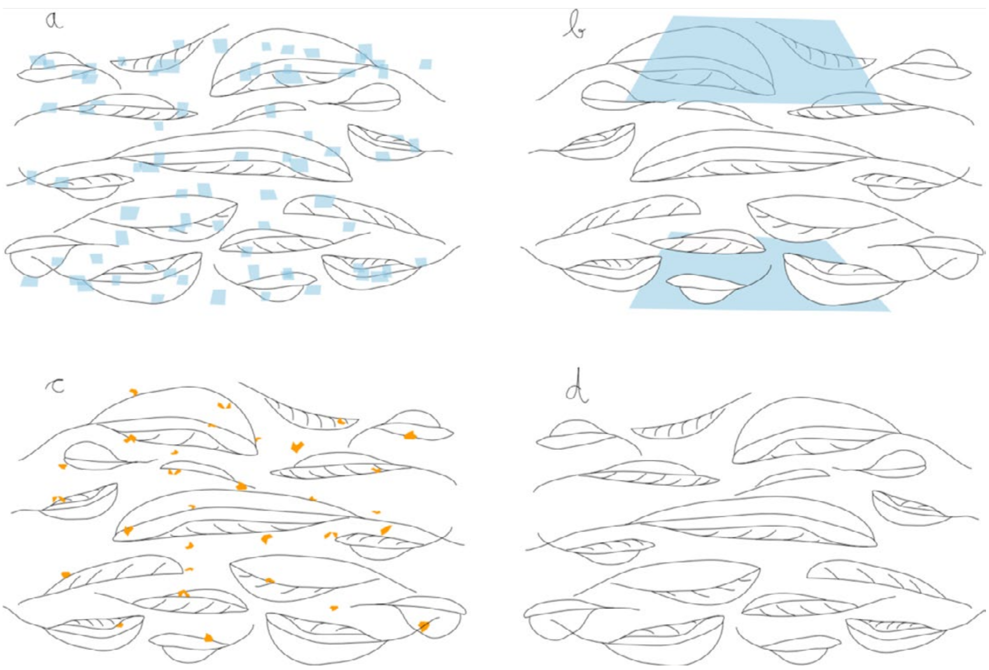

Figure 24. Sketch of how the added material was distributed within the leaves in the cotton bags. (a) illustrates the leached and unleached small plastic treatments, (b) shows the leached and unleached big plastic treatments, (c) illustrates the reference (saw dust) treatment, and (d) shows the control bags with no added material (not to scale). Image by Martina Stangl

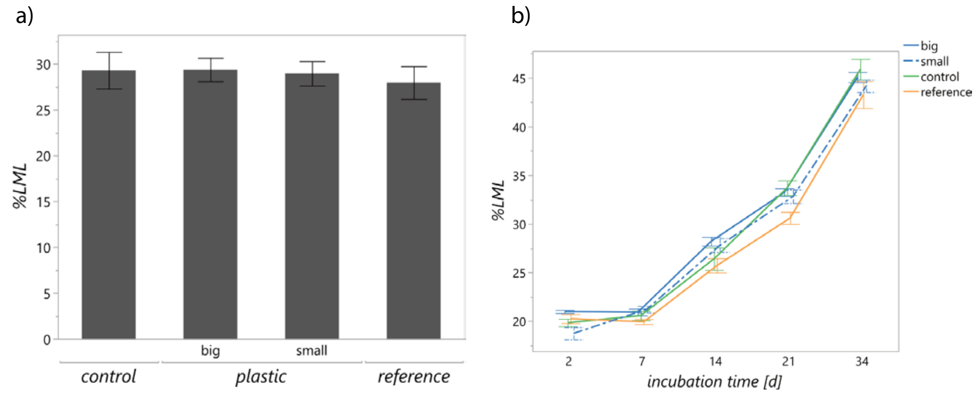

Figure 25. Effect of the presence of big and small MP particles and the reference sawdust on percent leaf mass loss (%LML) (a) over the entire experimental period and (b) on each litter bag retrieval date. Mean ± SE plotted. Material (testing control v plastic v reference) $p < 0.01$, MP size < 0.5, incubation time x MP size $p < 0.05$.

KEY RESULTS:

- Leaf decomposition was affected by significant but small effects of material type. The presence of sawdust reduced percent leaf mass loss by 4.5 % across all retrieval dates, relative to the control.
- In contrast, the presence of small but not large MPs reduced leaf mass loss by only 1 % (Figure 25a). However, the negative effect of small MPs increased with exposure time and was identical to that of the reference material on the last retrieval date (Figure 25b).
- Plastic presence increased tensile strength loss of the cotton bags by 6.4 %, relative to the control, whereas the presence of the reference material reduced tensile strength loss by 8 % (Figure 26a)
- The effect of plastic presence on tensile strength loss was largely driven by leaching of compounds for the PP material, with tensile strength loss increased by 16.7 %, relative to the controls (Figure 26a). This effect was strongest when the cotton bags were incubated with small-sized, unleached particles, with tensile strength loss increased by 26.6 %, relative to controls (Figure 26b).
- Community respiration was also increased by leaching of compounds from the PP material, being 14.3 % higher for communities incubated with unleached plastics than with the controls (Figure 27).

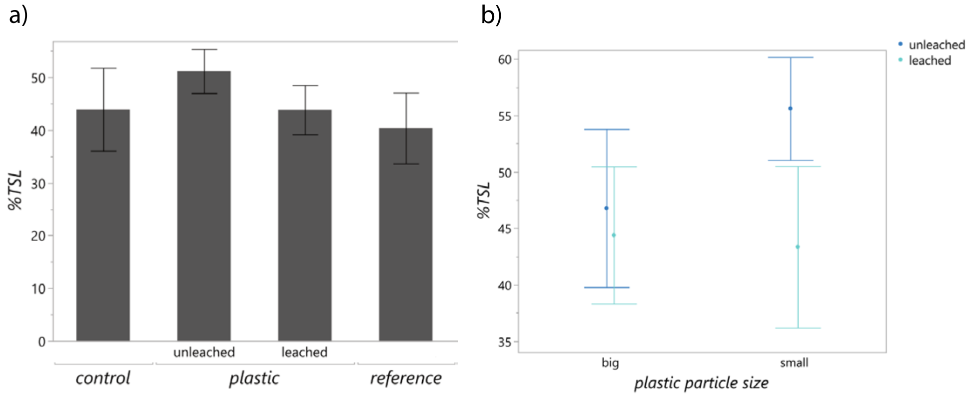

Figure 26. Variation in percent tensile strength loss (%TSL) of the cotton mesh bags: effects of (a) the presence of big and small MP particles and the reference sawdust and (b) plastic particle size and leaching. Mean ± SE plotted. Material (testing control v plastic v reference) $p < 0.05$, Leaching $p < 0.05$, MP leaching x MP size $p < 0.05$.

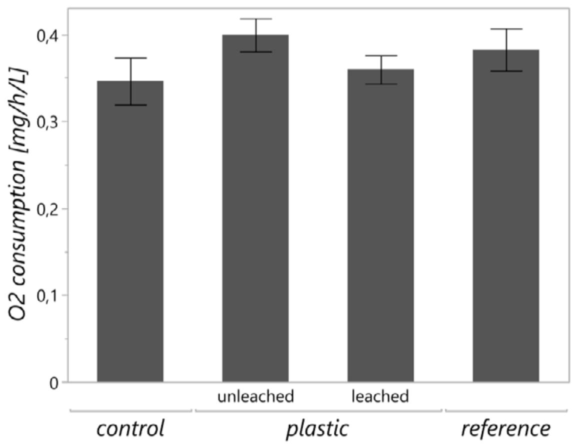

Figure 27. Effects of the presence of big and small MP particles and the reference sawdust on community respiration of microbes growing on leaf litter. Mean ± SE plotted. Leaching $p < 0.05$.

The increases in tensile strength loss of the cellulose cotton bags (which is largely attributable to microbial activity) and microbial respiration caused by exposure to previously unleached PP particles indicates a high bioavailability of leachates for microbial organisms. Such impacts are however likely to be transient and localised in nature but might accumulate with increasing amounts of plastic waste.

Small PP particles slowed down decomposition, but not more than the presence of highly refractory sawdust particles. In both cases, it is likely that the PP and sawdust particles slowed decomposition by acting as barriers to the spread of fungal hyphae through the more complex leaf litter matrix.

Overall, the effects of PP waste from face masks were contingent on particle size and/or leaching. Smaller fragments reduced leaf mass loss more than large fragments, presumably because the smaller fragments were more evenly distributed through the litter pack and had more potential to interrupt microbial activities (e.g. by acting as a physical barrier for fungal hyphae). In contrast, both microbial respiration and tensile strength loss were more affected by whether the plastic particles had been previously leached or not. The stimulation of these variables caused by unleached plastic particles suggests that at least some leachates from face masks have a high bioavailability to microorganisms.

6. Key Results & Recommendations

6.1 Preamble: Understanding microplastic fate and impacts to guide priority-setting in monitoring, policy and management

Up to now, the lack of research focused on ecosystem-level impacts of MPs in general, and in freshwaters in particular, has limited the capacity for scientific knowledge to inform priority setting in monitoring, policy and management. For example, in Sweden motivation for actions addressing MPs have largely been based either on the "precautionary principle", with the most abundant forms and sources of MPs targeted in management on the basis these are likely to pose the greatest risk in nature, or those that have been relatively easy to target in policy (e.g. MPs in cosmetic products).[80]

> Overall, the sheer number of impacts of different MP shapes and polymers detected in our experiments provides sufficient basis for "moving beyond the precautionary principle" when motivating a need for monitoring and management – there is now sufficient evidence that MPs alter key aspects of the functioning of stream benthic food webs to motivate a need for action.

Here, we first provide a summary of key results underpinning our recommendations (section 6.2). This is followed by some recommendations for priority setting for future monitoring, management and policy, and research focussed on MPs (section 6.3). In arriving at our recommendations, we have focussed on:

i) Risks for specific habitats and organisms based on particle fate and environmental interactions, and especially biofilm development.
ii) The risk of significant ecological impacts on microbes and/or the wider trophic web, relative to those associated with naturally occurring particles.

We are unable to make recommendations as to what types of specific management or policy actions are most likely to be effective. This would require integrating our results with, for example, information on sources and quantities of different types of MPs in different environments, or an evaluation regarding the possibilities for reducing production vs increasing recycling. Hence, when we make recommendation to reduce quantities of particular plastics in the environment, we do not specify how that reduction might be achieved. Several different possibilities for achieving such a reduction (reduced production, improved waste disposal, increased recycling and/or reuse, more efficient removal at water treatment plants) but it is beyond the scope of this project to analyse which approach is most suitable in the Swedish context.

We organise our recommendations around the two topics identified above: risk of ecological impacts and risks to specific habitats. In doing so, we regard *any change* associated with the presence of MPs to represent an ecological change that requires management attention, regardless of whether that change represented an increase or decrease in any particular ecosystem attribute. This is because even an increase in, e.g. algal productivity or organism growth relative to control conditions might indicate a food web component (basal resources or consumers) that is out of balance[79, 81]. Increases in productivity, resource processing rates or organism/ enzyme activities are not necessarily positive for environmental integrity. Indeed, the increase in algal biomass associated with excess nutrients in lakes is one well-known example of where an increase in an ecosystem process (i.e. algal productivity) is actually a sign of worsening ecosystem health[82].

6.2 Key results: initial fate, environmental interactions, and ecological impacts

Table 2 provides an overview of our main findings from RAs 1-3 regarding the fate and environmental interactions of different MP polymers. The key results include:

1. Biofilm formation changed the sinking behaviour of almost all types of MP polymer. Specifically, biofilm formation generally made denser particles more buoyant, and caused more buoyant particles (prior to aging) to sink faster.
2. MP retention increases with increasing macrophyte density. At the highest density studied, macrophytes increased MP retention by 94 % for PET, and 71 % for PS.
3. PS is notable for supporting higher levels of cyanobacteria, an important eutrophying and biofouling organism-group, than naturally occurring FPOM. This contrast was strongest in the oligotrophic pond biofilm, where FPOM supported little cyanobacteria. Most MP polymers (except LDPE) support more growth of chlorophyte algae than natural FPOM, but the differences were not great.
4. Effects of interactions between various microplastic polymers and HOC on the growth and survival of biota varied greatly in form (positive, negative or neutral), but the level of impact was almost always less than the largely negative impacts of naturally occurring POM.

Table 3 provides an overview of our main findings from RAs 4-7 regarding the fate and environmental interactions of different MP polymers. The key results include:

1. Detection of multiple impacts of MP contamination of our model freshwater mesocosms: almost all polymers and shapes affected multiple aspects of biodiversity and ecosystem functioning.
2. Furthermore, some of these effects exceeding those associated with naturally occurring, low quality FPOM, and most were detected despite the low concentrations of MP, relative to the orders of magnitude greater concentrations of both mineral and organic particle quantities in our mesocosms.
3. Most of the detected impacts were apparent even at the lower concentrations employed in our experiment (1 000 particles per kg sediment, representing the median sediment concentration observed worldwide).

4. Among the shapes assessed, fragments affected the largest number of properties, and these effects were generally observed for multiple (2–4) polymer types. In most cases, fragment effects were associated with increased microbial abundance or activity (enzyme activity or respiration). This is likely to reflect the suitability of the roughened, complex surface of MP fragments for supporting biofilm growth, with subsequent effects on consumer body condition (size, fat storage).
5. Among polymers, PET is notable for its consistent negative effects on key ecosystem functional indicators (community respiration and algal productivity), though the effect on respiration was smaller than that of low quality FPOM. PP also negatively affected algal productivity.
6. PE and PP were the polymers most likely to effect consumer survival, with this effect being stronger than observed for low quality FPOM.
7. The common positive effect of the presence of microplastics on microbial cell density and enzyme activity and on consumer body size and fat content points towards the potential for MP effects on microbes to propagate through food webs to affect consumer body condition.

Table 2. Summary of main results from RAs 1-3 regarding the fate and environmental interactions of different MP polymers on four different variable sets: (a) effects of biofilm formation on sinking velocity of different polymers and low quality FPOM, (b) effects of aquatic macrophytes on retention of two polymers, (c) differences among polymers in support of cyanobacterial biofilms and (d) effects of interactions between HOCs and different particle types on the mortality/health of biota: comparison among polymers and naturally occurring low quality FPOM. Figures for sinking velocities are mean values. For b-d, an increase in relative effect sizes are indicated by an increasing number of arrows, with positive and negative effects indicated by up and downwards pointing arrows respectively. "—" = no effect. Blank = not assessed.

	Response	Treatment	Low quality FPOM	LDPE	PET	PS	PVC/ uPVC	TWP	PA	PP	PE
(a)	Sinking velocity (cm/s)	No biofilm	0.8	0.38	2.25	1.95	0.8	2.3			
		Biofilm (aged)	1.6	0.35	1.05	0.5	1.45	0.55			
(b)	Retention	Macrophytes			↑↑↑	↑↑					
(c)	Cyanobacteria	Eutrophic	↑↑	↑↑	↑	↑↑↑	↑	—			
		Oligotrophic	—	—	—	↑↑↑	↑	—			
(d)	HOC sorption: Mortality/ Health Biota		↓↓↓			↓↑	↓↑		↑	↓↑	↓↑

Table 3. Summary of main results from RAs 4-7 addressing the ecological impacts of various MP shape and polymer combinations on three ecosystem attributes: (a) invertebrate consumers (the particle feeding chironomid *C. riparius*), (b) microbial responses (c) ecosystem processes. An increase in relative effect sizes is indicated by an increasing number of arrows, with positive and negative effects indicated by up and downwards pointing arrows respectively. Red stars indicates experiment was conducted in microcosms where quantities of mineral sediment organic particles were orders of magnitude greater than the added microplastics. "—" = no effect. Blank = not assessed.

Ecosystem attribute	Response variable	Low quality FPOM	Fragments	Fibres	Spheres
(a) Particle consumer (chironomid)	Survival	—	↓ (PE, PP)	—	↑ (PE)
	Body size	↓↓↓	↑ (PE, PP, PS, PET)	—	↓ (PET)
	Fat content	—	↑ (PE, PP, PS, PET)	↑ (PP, PET)	↑(PE, PS) — (PET)
(b) Microbial responses	Enzyme activity	★	↑ (PE, PP, PS, PET)	—	↓ (PE, PS)
	Cell density	★	↑↑ (PE, PP, PS, PET)	↑↑(PP, PET)	↑↑(PE, PS)
	Community respiration	↓↓↓	↑ (PE, PP, PS) ↓ (PET)	↑ (PP) ↓ (PET)	↑ (PE, PS) ↓ (PET)
(c) Ecosystem processes	Detritus decomposition	↓↓↓	↓ ↓ (PMMA, PS)	↓ (leached PP) ↑ (unleached PP)	
	Algal productivity	★	↓↓ (PET, PP)	↓↓ (PET, PP)	—

6.3 Recommendations: Priorities in monitoring, management and research

Based on our research and with reference to the literature, we present recommendations in separate sections for monitoring, management and policy, and research.

6.3.1 Priorities for monitoring of MPs

1. *Increase monitoring of MPs in freshwater ecosystems.*
   The pervasiveness of MP impacts (positive or negative) on multiple ecosystem properties, highlights the urgent need to monitor the transport and distribution of MPs in freshwaters, in order to identify the ecosystems and habitats experiencing the highest rates of MP accumulation today.
2. *Focus on monitoring of MPs in sediment/benthic substrates and/or organisms in freshwaters, and particularly in streams.*
   Most organisms in stream ecosystems are benthic (e.g. benthic invertebrates, diatoms, detrital microorganisms etc), and it is in benthic habitats that the greatest risk of exposure lies. Our results show that benthic microbes and invertebrates can be affected at the lower exposure concentration we assessed (1 000 MP particles/kg sediment).
3. *Focus monitoring of microplastic accumulation and export from habitats known to favour rapid algal biofilm formation – specifically light and nutrient rich habitats.*
   Such conditions may be associated with increased export of denser MP types (e.g. TWP) that otherwise would be expected to rapidly sink and be retained in the sediment.
4. *A particular focus should be on outlets of storm water drainage systems.*
   Rapid biofilm growth in e.g. water retention ponds and wetlands, might increase buoyancy of denser particle types that would otherwise be expected to rapidly sink.
5. *Flag sediment concentrations of 1 000 MP particles / kg sediment as a marker for a habitat already likely to be experienced altered ecosystem functioning.*
   Our experiments were not set up to be able to give guidelines regarding e.g. no effect concentrations (i.e. we did not expose our test systems to multiple concentrations in order to quantify dose-response relationships). However, given that we detected multiple effects at a concentration of 1 000 MP particles / kg sediment then we suggest that detection of the concentration in nature can be used as to mark habitats where MP contamination may already be altering microbial processes and wider ecosystem function.
6. *Monitoring of MP accumulation in macrophyte beds in freshwaters.*
   Macrophyte stands can increase retention of MPs by up to 94 % during macrophyte growth season, and thus represent a substantial potential exposure point for biota. However, as macrophytes die back during autumn it is likely that a substantial portion of the retained MPs will be released again.

6.3.2 Priorities in policy and management

1. *Continue and extend current measures for reducing plastic pollution in the biosphere.*
   The number of impacts of MPs on key ecosystem properties in our experiments, often associated with the presence of MPs *per se* rather than specific polymers or shapes, highlights the urgent need to reduce the occurrence of plastic (both macro- and microplastics) in the environment *per se*.

   Although our effect sizes were small relative to other pervasive stressors impacting freshwaters (nutrient enrichment, chemical stressors, increasing temperatures etc)[83–86], they represent impacts that likely add to the cumulative stress experienced by freshwaters today[87]. Furthermore, we anticipate that the impacts observed in our experiments will strengthen in the future in line with increasing MP concentrations, as the vast accumulation of macroplastics in the environment increasingly breaks down to microplastic size[88]. Finally, the fact that microbial activities and processes seem particularly vulnerable is of cause for concern, given the fundamental roles microorganisms play in the functioning of the biosphere and in supporting ecosystem services[84, 89–91].
2. *Reduce pollution from single-use plastics in the environment.*
   Single-use plastics often have physical characteristics that favour a more rapid degradation, including down to fragments capable of supporting extensive microbial biofilms with the associated knock on effects observed here[1, 2, 92].

   In a recent review, Chamas *et al.*[1] concluded that a detailed understanding of the fragmentation dynamics is lacking for most MP polymers, but nevertheless highlighted that it is the more disposable, single use forms of plastics that degrade most rapidly. For example, plastics manufactured as films (e.g. shopping bags) and fibres tend to reduce faster than plastics manufactured as beads or more solid forms. Likewise, HDPE manufactured as bottles fragments faster than HDPE manufactured as denser pipes. Chubarenko[2] focussed on mechanical fragmentation of single use plastics in marine shoreline sediments (so called "swash mixing"). They found that single-use plastics from disposable plates (PS) and garbage bags (LDPE) fragmented mechanically faster than that from rigid disposable cups (PP), though fragmentation of all three types were faster than previous observations[2]. Films from plastic bags are notable not only for extremely rapid fragmentation, but also for a tendency in moving water to form complex particles where the film folds repeatedly (up to 8 times) on itself[2]. Such folded particles are characterised by extensive interstitial space and a very high surface area:volume ratio likely to be especially suitable for biofilm formation.
3. *Avoid the light- and nutrient rich conditions that favour rapid biofilm formation in connection with major MP release / accumulation points.*
   Biofilm formation is ubiquitous in nature, but denser, algae dominated biofilms form more rapidly in light-rich, nutrient-rich habitats. Such conditions should be avoided in connection with major MP release / accumulation points (e.g. factories, stormwater outlets, water retention ponds and treatment wetlands etc) due to the risk biofilm formation increasing the buoyancy of otherwise dense MPs. Increased buoyancy for these particles in turn increases the risk of further transport to downstream lake, estuary and marine habitats.

4. *Among polymers, focus especially on reducing PET, PE and PP in the environment.* These polymers were associated with a greater number of impacts generally, including a greater number of negative impacts, on key ecosystem properties in our studies.
5. *Macrophytes might be planted at major MP input points (e.g. water treatment plants, factories, stormwater outputs etc) to increase "sieving" of MPs from the water.* Regular harvesting of macrophytes would facilitate removal of the particles from the freshwater environment.
6. *No specific recommendations regarding interactions between HOCs and MPs.* Based on our literature review[57], we conclude that no polymer was associated with a consistently greater risk than any other, or in comparison to natural occurring FPOM.

6.3.3 Priorities in research

1. *Investigation of the fate and ecological impacts associated with additional polymers, organism groups and environmental conditions, imcluding impacts on biodiversity.* We investigated the impacts of a broad array of MP polymers and shapes in a wide range of settings (short and longer term micro- and mesocosm studies, in a literature review and in the field). Nevertheless, our research has only scratched the surface of the full range of MP types and environmental interactions possible. In our ecological impact studies, we did not assess one of the most common MP types in Sweden (TWP), and only focussed on one type of consumer (particle feeder chironomids). This points to the need for an extension of our research to consider further plastic types (such as TWP), organism groups (e.g. fish) and environmental conditions (variation in temperature, flow, macrophyte communities, other stressors etc). Finally, we were unable to present data on the effects of MPs on biodiversity, and this should be prioritised in future research.
2. *Evaluation of interactions between MPs and additional environmental stressors.* The full extent of cumulative effects of (i) MPs on microbial organisms on other organisms and ecosystem functions and (ii) possible interactions between MPs and other stressors have not been extensively evaluated. There is thus a pressing need for research evaluating the extent to which MP exposure pushes biota and ecosystems that are already affected by other anthropogenic stressors past thresholds beyond which their survival is compromised.
3. *Mapping of MP accumulation in different types of riverine "biotopes" (microhabitats) such as macrophytes, pools, side bars, sediments) in situ.*
   Such mapping is necessary to evaluate where the greatest exposure risks for biota are, and to guide monitoring.
4. *Quantification of MP effects over a wider range of exposure concentrations.* Identification of more specific effect threshold concentrations, including no effect concentrations, requires quantification of our response variables over a broader range of concentrations than was possible for us here.
5. *Further investigation of eutrophying and biofouling potential of different MP shapes and polymers in realistic mesocosms or in situ, with a particular focus on PS.* There is a need to evaluate the association between cyanobacteria and MPs *in situ*, and especially PS which supported the greatest abundances of cyanobacteria in our studies.

6. *Detailed quantification of particle characteristics during aging, how this affects biofilm development and buoyancy, and ultimately the hydraulics of particle transport and retention in freshwater habitats.*
   The dynamics of biofilm growth (development and die-back) can result in periods of varying buoyancy (sinking and floatation) of MP plastics[18], and there is a need to evaluate how such dynamics affect the retention and transport of different polymers in freshwaters.
7. *Modelling relationships between waterborne MP export and downstream exposure concentrations in sediments, aquatic vegetation and other microhabitats, to guide water managers on acceptable suspended concentrations in outlet water from e.g. sewage treatment plants or storm water outlets.*
   Monitoring of MP export as part of recipient control from e.g. water treatment plans or industrial developments is most easily done by measuring concentrations in e.g. outlet water rather than in downstream habitats (since it is uncertain which habitats should be sampled over which spatial extent, given variation in particle transport distances). However, the highest risk of exposure for most organisms in stream ecosystems is on or within benthic substrates. Feasible recipient control thus requires modelling of relationships between waterborne MP export and downstream exposure concentrations in sediments, aquatic vegetation and other microhabitats.
8. *Evaluation of whether increases or decreases in key ecosystem properties associated with MP exposure are equivalent in their consequences for biodiversity and ecosystem function.*
   In this report, we have regarded any change in any ecosystem property, regardless of whether it is positive or negative, associated with MPs as representing a reduction in wider ecosystem integrity. Future research should focus on which changes are associated with the most deleterious outcomes for ecosystem integrity. This includes further multi-trophic research[41], where impacts of microplastics on organisms at one trophic level for organisms at other trophic levels are assessed.
9. *Investigation into the effects of toxic compounds associated with different plastic products in freshwaters.*
   Most experiments conducted in our project worked with particles that had been pre-leached to remove toxic additives, on the basis that such compounds will mostly rapidly leach away in aquatic environments *in situ*. Nevertheless, results from RA-7 demonstrate that leachates can have effects on ecosystem properties in still water habitats, and there is a need for further investigation into the effects of such leachates.
10. *Investigation of fragmentation speeds and trajectories of different plastic products in the environment, to aid more product-specific priority setting in management and policy.*
    Fragments were associated with the strongest impacts in our study, but knowledge of which types of plastic fragment most rapidly remains limited.
11. *Assess impacts of realistic MP mixtures on ecosystem properties to evaluate risks of real-world MP exposure more accurately.*
    Although there is value in studying the effects of different MP polymers and shapes separately, as we have done here, the norm is for MPs to occur as mixtures *in situ*. Mixture effects may well differ from those of single polymers or shapes in isolation.

See appendix 1 for a list of future research activities within the current project that address some of these research needs.

7. Acknowledgements

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9. Appendix

At the time of reporting, research activities within the project were not complete, largely due to the impact of the COVID-19 pandemic. These research activities partly address some of the recommendations for research identified in Section 6.3.3 of the report. Pending research in each of the RAs are listed here.

RA-1: A second particle release experiment was conducted at the Landau Stream Mesocosm Facility (LSMF) at the University of Koblenz-Landau, Campus Landau (Germany). The test facility consists of 16 independent concrete channels (Figure 3), each 45 m in length, 0.4 m width. The channels each contained multiple microhabitats (sediment, macrophytes). In this experiment, an MP mixture consisting of three strongly contrasting polymers and shapes, PS (powder, $\rho = 1.05$ g/cm^3), car tire debris (cylindrical fragments, $\rho = 1.15$ g/cm^3) and PET (irregular fragments, $\rho = 1.37$ g/cm^3), was released into the channels (333.33 particles/L per stream). Each run lasted for 150 mins, after which 34 water samples, 83 sediment samples (14 between, and 27 within each vegetation zone), and 45 macrophyte samples (when present) were collected for each channel. This experiment yielded c. 2000 samples which were not fully processed at the time of writing.

RA-2: Scanning electron micrographs were taken of virgin particles and after 14 and 28 days of "aging" in each culture using an FEI Quanta 250 environmental scanning electron microscope (FEI Company Hillsboro, United States). Particle characteristics and biofilm growth are being quantified using image analysis, which is not complete at the time of writing.

RA-4: High resolution photographs were taken of leaf surfaces to quantify binding of MPs within detrital biofilms, based on image analysis. These analyses were not complete at the time of writing.

RA-6: Microbial samples were preserved for analyses of microbial community composition and analysis, based on DNA sequencing. Bioinformatics on these samples were not complete at the time of writing.

RA-7: Microbial samples were preserved for analyses of microbial community composition and analysis, based on DNA sequencing, and litter samples were preserved for analysis of fungal biomass. These analyses were not complete at the time of writing.

RA-8: is an additional research area, comprising a larger scale stream mesocosm experiment including microorganisms, and consumers representing different trophic groups (algal grazers, detritivores etc.). In this experiment, we investigate interactions between temperature increases projected under global warming, and MP pollution. We will quantify a similar set of variables as in RA 5-7, but with a stronger focus on ingestion of MP particles by consumers, and interactions between MPs and algal food webs. The experiment commenced in February 2023 and will be completed within three months.

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Evaluating the properties, fate and individual-to-ecosystem level impacts of contrasting microplastics in freshwaters

Microplastic particles are fragments, fibres and other shapes derived from plastic polymers. Concern about the environmental impacts of microplastic particles and their implications for human wellbeing has never been higher. Unfortunately, the understanding of the dynamics and impacts of microplastics in the environment lags behind.

Freshwater ecosystems are vulnerable to inputs of plastic waste from storm water and other terrestrial runoff. Despite this, research on the behaviour and impacts of microplastic particles in freshwater ecosystems, and their capacity to act as key transport pathways through the landscape from inland to the ocean, is especially deficient.

This report presents results of one of five funded research projects within the call Microplastics 2018. This project addresses the environmental interaction of microplastic particles in streams and the ecological impacts of microplastic particles on resource consumption, growth and survival of organisms and on key ecosystem processes.

One key result in the project is that almost all microplastic shapes and polymers studied had one or more effects om stream microbial organisms and associated ecosystem processes. The research also provide evidence that effects on microbial organisms can propagate up food-chains to affect consumer growth and fat storage.

The project has been funded by the Swedish EPA's environmental research grant, which aims to fund research in support of the Swedish EPA and the Swedish Marine and Water Authority's knowledge needs.

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